

Speciation leads to divergent methylmercury accumulation in sympatric whitefish

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Abstract Central European lake whitefish (*Coregonus* spp.) colonized Swiss lakes following the last glacial retreat and have undergone rapid speciation and adaptive radiation. Up to six species have been shown to coexist in some lakes, and individual species occupy specific ecological niches and have distinct feeding and reproductive ecologies. We studied methylmercury (MeHg) accumulation in sympatric whitefish species from seven Swiss lakes to determine if ecological divergence has led to different rates of MeHg bioaccumulation. In four of seven lakes, sympatric species had distinctly different MeHg levels, which varied by up to a factor of two between species. Generally, species with greater MeHg levels were smaller in body size and planktivorous, and species with lower MeHg were larger and benthivorous. While modest disparities in trophic position between species might be expected a priori to explain the divergence in MeHg, $\delta^{15}\text{N}$ of

bulk tissue did not correlate with fish MeHg in five of seven lakes. Results of a nested ANCOVA analysis across all lakes indicated that only two factors (species, lake) explained substantial portions of the variance, with species accounting for more variance (52 %) than inter-lake differences (32 %). We suggest that differences in MeHg accumulation were likely caused by diverging metabolic traits between species, such as differences in energy partitioning between anabolism and catabolism, potentially interacting with species-specific prey resource utilization. These results indicate substantial variability in MeHg accumulation between closely related fish species, illustrating that ecological speciation in fish can lead to divergent MeHg accumulation patterns.

Keywords Adaptive radiation · Bioaccumulation ·
Coregonus · Methylmercury · Speciation

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Introduction

Mercury (Hg) is a globally dispersed pollutant that enters aquatic ecosystems via direct atmospheric deposition (Lamborg et al. 2002) and watershed runoff (Swain et al. 1992). A fraction of inorganic Hg undergoes microbial conversion to methylmercury (MeHg), a potent toxin to both humans and wildlife (Wiener et al. 2003, 2006a; Mergler et al. 2007). MeHg enters aquatic food webs at their base through uptake and sequestration by phytoplankton and benthic algae, and bioaccumulates and biomagnifies in food webs (Wiener et al. 2003).

The degree to which MeHg accumulates in individual fish depends on a range of factors, including those at the system and organism levels. System-level factors include differences in the loadings of inorganic Hg to ecosystems (Hammerschmidt and Fitzgerald 2006b; Harris et al. 2007),

and different environmental conditions within ecosystems that influence methylation and MeHg bioavailability (e.g., pH, redox conditions, organic carbon levels and activity of methylating bacteria; Benoit et al. 2003; Wiener et al. 2006b). Organism-level factors include prey MeHg concentration (Hall et al. 1997) and trophic position (TP), which have great influence on MeHg levels in biota due to biomagnification (Wiener et al. 2003). Although fish of the same species might be expected to feed at approximately the same TP, the TP of individuals can vary due to omnivory (Kidd et al. 1995) and ontogenetic dietary shifts (Eagles-Smith et al. 2008). Accumulation of MeHg can also depend on the food source, because bioavailability of MeHg at the base of the food web differs spatially. Eagles-Smith et al. (2008), for example, found that Hg accumulation in fish of Clear Lake, CA, was influenced by foraging habitat due to differing Hg concentrations in pelagic and benthic prey organisms. Such opportunistic foraging can lead to variations in fish MeHg levels even in individuals of the same species (Cabana and Rasmussen 1994; Kidd et al. 1995). Other organism-level factors include metabolic traits, such as MeHg elimination rates from tissue or energy allocation and growth rates, which can vary among fish species (Trudel and Rasmussen 1997). Overall, fish MeHg concentrations can vary substantially among systems (Parkman and Meili 1993), and both spatially and seasonally within systems (Eagles-Smith et al. 2008).

Alpine lake whitefish (*Coregonus* spp.) colonized European sub-alpine lakes after the last glacial retreat (<15,000 years ago). Since then, they have undergone a relatively rapid adaptive radiation resulting in communities of closely related species with highly divergent ecologies, making them a particularly interesting study system for MeHg bioaccumulation. Current intralacustrine diversity of whitefish varies from one to six species, with sympatric species in single large lakes or hydrologically connected lakes generally being each other's closest relatives (Douglas et al. 1999; Hudson et al. 2011). Similar to other lacustrine adaptive radiations (e.g., Schluter 1996; Barluenga et al. 2006; Hudson et al. 2007; Herder et al. 2008; Ohlberger et al. 2008; Seehausen et al. 2008), the species of the Central European whitefish diverge in trophic and reproductive niches along two interacting axes; from benthic to open-water limnetic habitats and from littoral to profundal lake areas (Steinmann 1950; Vonlanthen et al. 2009).

The most distinctive morphological trait differentiating among co-existing whitefish is gill raker number. The number of these projections on the gill arch affects the efficiency of retention for a given prey size (Sanderson et al. 2001). Within *Coregonus* radiations, greater numbers of individual gill rakers are associated with feeding

predominantly on zooplankton and greater utilization of the pelagic niche, and lower gill raker numbers are associated with feeding predominantly on zoobenthos and greater utilization of the benthic niche (Bernatchez 2004; Harrod et al. 2010; Kahilainen et al. 2011). Thus, parallel phenotypic trends can be observed across lakes, with slower-growing, higher gill raker number species co-existing with larger-bodied, low gill raker number species that typically occupy the pelagic and benthic niches, respectively (Steinmann 1950; Douglas and Brunner 2002; Vonlanthen et al. 2009; Hudson et al. 2011). This diversity of feeding strategies can be influenced by the nutrient status of the lake. It has been shown that anthropogenic eutrophication can increase pelagic primary production while reducing benthic primary production (Chandra et al. 2005) and increase the reliance of benthic littoral consumers on pelagic food sources (Vadeboncoeur et al. 2003).

Little is known about how evolutionary diversification through adaptive radiation in consumers in general, and in freshwater fish specifically, might affect contaminant accumulation. Despite the importance of whitefish to the Swiss commercial and recreational fishery (BAFU 2008, 2009), there is also limited information on MeHg levels within and among whitefish species. Given the substantial differences in habitat and feeding ecology, we hypothesized that MeHg accumulation would differ significantly among *Coregonus* spp. in Swiss lakes. We evaluated this hypothesis by examining 16 whitefish species collected across 7 lakes (Fig. 1). The objectives of this study were to (i) determine whether there are differences in MeHg bioaccumulation between sympatric whitefish species, and (ii) examine possible drivers of these differences (e.g., length, lake, isotopic composition, gill raker number). MeHg concentrations were measured in whitefish muscle, and tissue stable isotope ratios of carbon and nitrogen ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and gill raker number were also quantified to draw inferences about life history and TP.

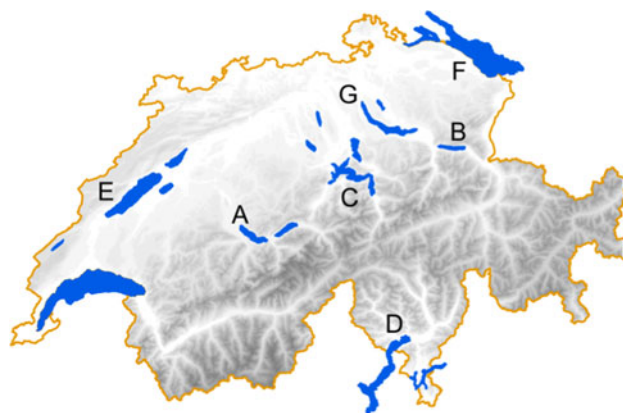


Fig. 1 Sampling sites: **a** Lake Thun, **b** Lake Walen, **c** Lake Lucerne, **d** Lake Maggiore, **e** Lake Neuchatel, **f** Lake Constance, **g** Lake Zurich

Methods

Study sites

The lakes of Switzerland were carved by Alpine glaciers or dammed by moraines left behind by retreating glaciers. The resulting bathymetry of Swiss lakes is correspondingly diverse, ranging from fjord-like, steep-walled basins (e.g., Lake Lucerne) to lowland lakes with extensive littoral zones (e.g., Lake Neuchatel) (BUWAL 1994). Swiss lakes were originally oligotrophic, but in the twentieth century have received increased anthropogenic inputs of nutrient from agricultural runoff and domestic and industrial waste waters. Nutrient loadings peaked around the 1970s, when several lakes contained over 100 μg total phosphorus per liter (e.g., Lakes Zurich, Greifen, and Baldegg). Other lakes were not impacted as severely and did not exceed 30 μg $\text{P}_{\text{tot}}/\text{L}$ (e.g., Lakes Thun and Walen; Table 1). Comprehensive treatment of waste water and extensive restoration efforts are returning many lakes to near-pre-industrial oligo- to mesotrophic status. For this study, lakes were selected to represent a range of potential environmental forcings such as current and historic trophic status, depth, and geographic region (Table 1). Each of the lakes has at least two sympatric species of whitefish.

Fish sampling and sample preparation

Fish were sampled with gill nets between 2004 and 2007 on their respective spawning grounds, when possible. Generally, fish from a given lake were collected during field campaigns over the course of several days (Table 2). In Lake Thun and Walen, samples were collected over multiple campaigns. Total Hg concentrations in fish did not differ significantly among campaigns (see footnote in Table 2), and thus samples were pooled for a given species. A total of 174 fish were sampled for this study (Table 2), fork length was measured, and gill rakers were counted. Scales were removed from some fish for age determination (Vonlanthen et al. 2009). Skinless white muscle tissue was removed from the left anterior dorsal fillet above the lateral line promptly after capture. Tissue samples were frozen (≤ -20 °C) and either freeze-dried or oven-dried at 80 °C for 48 h prior to homogenization and analysis.

Whitefish species at the extremes of the limnetic-benthic and the shallow-deep axes of divergence were chosen for analysis, where possible, based on their morphology (i.e., feeding ecotype and growth) and spawning ecology (i.e., depth and time; Table 3). In Lakes Thun and Lucerne, an intermediate species also was sampled, and in Lake Maggiore two non-native whitefish species that had been introduced in the nineteenth and twentieth century were

Table 1 Physiochemical characteristics of the study lakes and whitefish species sampled: maximum and mean depth and drainage basin and lake surface area show the morphological characteristics of the lakes (BUWAL 1994)

Lake	Species	Depth (m)		Area (km ²)		P_{tot} ($\mu\text{g L}^{-1}$)	
		Max	Mean	Basin	Lake	2009	Max
Thun	Brienzzig (<i>C. albellus</i>)	217	135	2,490	48	4	23
	Balchen (<i>C. sp.</i> "balchen")						
	Kropfer (<i>C. alpinus</i>)						
Walen	Albeli (<i>C. heglingsus</i>)	145	103	1,061	24	3	29
	Felchen (<i>C. duplex</i>)						
Lucerne	Albeli (<i>C. zugensis</i>)	214	104	1,831	114	4	30
	Schwebbalchen (<i>C. sp.</i> "schwebbalchen")						
	Balchen (<i>C. sp.</i> "bodenbalchen")						
Maggiore	Bondella (<i>C. sp.</i> "bondella")	372	178	6,386	212	10	35
	Lavarello (<i>C. sp.</i> "lavarello")						
Neuchatel	Bondelle (<i>C. candidus</i>)	152	64	2,670	218	7	59
	Palée (<i>C. palaea</i>)						
Constance	Gangfisch (<i>C. macrophthalmus</i>)	254	100	21,237	539	6	87
	Sandfelchen (<i>C. arenicolus</i>)						
Zurich	Albeli (<i>C. heglingsus</i>)	136	51	1,740	88	23	101
	Felchen (<i>C. duplex</i>)						

Total phosphorus concentrations (P_{tot}) show the current trophic status (2009) and the extent of former eutrophication (maximum measured since 1960) (BAFU, unpublished data)

Table 2 Number of individual whitefish used for analysis of physiological traits (fork length, gill raker number, age), total mercury (HgT), methylmercury (MeHg), isotopic composition ($\delta^{15}\text{N}/\delta^{13}\text{C}$), and sampling date

Lake	Species	Length	Gill raker	Age	HgT	MeHg	$\delta^{15}\text{N}$ $\delta^{13}\text{C}$	Sampling time
Thun	<i>C. albellus</i>	10	10	10	10	4	5	Sep/Dec 05 ^a
	<i>C. sp. "balchen"</i>	10	10	10	10	4	10	Dec 05
	<i>C. alpinus</i>	10	10	10	10	–	6	Sep/Dec 05, Aug 06 ^a
Walen	<i>C. heglings</i>	20	19	15	20	4	15	Dec 04, Aug 05 ^a
	<i>C. duplex</i>	15	15	10	15	4	10	Dec 04, Aug/Dec 05 ^a
Lucerne	<i>C. zugensis</i>	10	10	–	10	–	10	Nov–Dec 07
	<i>C. sp. "schwebbalchen"</i>	8	7	–	8	–	8	Nov–Dec 07
	<i>C. sp. "bodenbalchen"</i>	11	11	–	11	–	11	Nov–Dec 07
Maggiore	<i>C. sp. "bondella"</i>	10	10	9	10	–	10	Dec 06
	<i>C. sp. "lavarello"</i>	10	10	10	10	–	10	Dec 06
Neuchatel	<i>C. candidus</i>	9	9	9	9	–	9	Dec 05–Jan 06
	<i>C. palaea</i>	10	10	10	10	–	10	Dec 05–Jan 06
Constance	<i>C. macrophthalmus</i>	10	10	–	10	–	10	Dec 04
	<i>C. arenicolus</i>	10	5	–	10	–	10	Dec 04
Zurich	<i>C. heglings</i>	11	11	–	11	–	11	Dec 04
	<i>C. duplex</i>	10	10	–	10	–	10	Dec 04
Total		174	167	93	174	16	155	

^a Fish HgT of a given species did not differ significantly among sampling dates (ANOVA, $p > 0.05$)

Table 3 Characteristics, total mercury concentrations, and isotopic composition of the analyzed whitefish species: significantly different averages of sympatric species are denoted with different letters (ANOVA or Kruskal–Wallis test, $p < 0.05$)

Lake	Species	Spawning depth	Gill raker number	Ecotype	Fork length (mm)	Mercury ($\mu\text{g g}^{-1}$ ww)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Thun	<i>C. albellus</i>	Profundal	36.5 ^a ± 5.1	HGR	272 ^a ± 10	0.047 ^{a,b} ± 0.010	8.1 ± 0.3	–30.9 ± 0.2
	<i>C. sp. "balchen"</i>	Littoral	29.0 ^b ± 3.3	LGR	366 ^b ± 7	0.036 ^b ± 0.008	8.1 ± 0.6	–29.5 ± 1.8
	<i>C. alpinus</i>	Profundal	20.3 ^c ± 2.5	VLGR	298 ^c ± 24	0.053 ^a ± 0.012	7.9 ± 1.2	–29.8 ± 1.6
Walen	<i>C. heglings</i>	Profundal	35.2 ^a ± 1.7	HGR	223 ^a ± 12	0.105 ^a ± 0.019	7.1 ± 0.4	–31.8 ± 0.6
	<i>C. duplex</i>	Littoral	26.5 ^b ± 2.6	LGR	398 ^b ± 44	0.063 ^b ± 0.013	7.7 ± 2.1	–31.6 ± 0.6
Lucerne	<i>C. zugensis</i>	Profundal	37.5 ^a ± 2.8	HGR	230 ^a ± 20	0.054 ^a ± 0.016	9.3 ^{a,b} ± 0.6	–30.9 ^a ± 0.4
	<i>C. sp. "schwebbalchen"</i>	Sublittoral	32.3 ^b ± 3.7	MGR	317 ^b ± 21	0.038 ^b ± 0.007	8.9 ^b ± 0.7	–30.5 ^b ± 0.2
	<i>C. sp. "bodenbalchen"</i>	Littoral	28.5 ^c ± 2.1	LGR	414 ^c ± 13	0.036 ^b ± 0.006	9.6 ^a ± 0.5	–31.3 ^c ± 0.2
Maggiore	<i>C. sp. "bondella"</i>	Littoral	30.7 ^a ± 3.2	MGR	341 ^a ± 30	0.078 ± 0.019	9.9 ^a ± 0.4	–26.5 ± 0.8
	<i>C. sp. "lavarello"</i>	Profundal	35.7 ^b ± 2.5	HGR	273 ^b ± 22	0.068 ± 0.010	10.9 ^b ± 0.7	–26.3 ± 0.7
Neuchatel	<i>C. candidus</i>	Profundal	33.3 ^a ± 2.1	MGR	281 ^a ± 22	0.044 ^a ± 0.007	13.6 ^a ± 0.6	–31.1 ± 0.2
	<i>C. palaea</i>	Littoral	27.1 ^b ± 0.9	LGR	400 ^b ± 27	0.037 ^b ± 0.006	12.8 ^b ± 0.4	–31.5 ± 0.7
Constance	<i>C. macrophthalmus</i>	Sublittoral	37.0 ^a ± 3.6	HGR	331 ^a ± 14	0.048 ± 0.011	13.7 ± 0.5	–31.3 ^a ± 0.2
	<i>C. arenicolus</i>	Littoral	26.4 ^b ± 2.7	LGR	519 ^b ± 34	0.040 ± 0.007	14.0 ± 0.4	–31.8 ^b ± 0.3
Zurich	<i>C. heglings</i>	Profundal	36.3 ^a ± 2.2	HGR	298 ^a ± 18	0.078 ± 0.019	15.0 ^a ± 0.4	–30.7 ^a ± 0.5
	<i>C. duplex</i>	Littoral	29.3 ^b ± 2.0	LGR	458 ^b ± 40	0.066 ± 0.023	13.7 ^b ± 0.5	–31.6 ^b ± 0.7
All lakes			31.5 ± 5.5		335 ± 86	0.059 ± 0.025	10.7 ± 2.8	–30.5 ± 1.8

Spawning depth and feeding ecotype are described by Hudson et al. (2011): very low gill raker (VLGR; <25), low gill raker (LGR; 25–30), medium gill raker (MGR; 31–35); high gill raker (HGR; >35)

studied. Species were categorized into four feeding ecotypes according to Hudson et al. (2011): those with very low gill raker number (VLGR; <25), low gill raker (LGR;

25–30), medium gill raker (MGR; 31–35), and high gill raker (HGR; >35). Species and fish characteristics are shown in Table 3.

Total mercury and methylmercury

Total Hg (HgT) concentration in fish muscle was measured by thermal decomposition, amalgamation, and detection by atomic absorption spectrophotometry following USEPA method 7473. The instrument was calibrated with NRC/CNRC reference materials (MESS-3 and DORM-3). For consistency with the broader literature on mercury in fish, concentrations were expressed as wet weight concentrations (ww), using an estimated muscle moisture content of 80 % (Bank et al. 2007; Muir et al. 2010). Water content was not measured in these fish; however, using a uniform moisture content is justified because moisture content in whitefish has been shown to vary by only a few percent among fish (Muir et al. 2010), which is within the uncertainty of our Hg measurements.

MeHg is typically the major form of Hg in fish muscle tissue (>95 %; Bloom 1992). Therefore, MeHg levels were measured only in a subset of whitefish muscle samples to confirm MeHg predominance. MeHg was determined in 16 fish from two lakes having two whitefish species each (Table 2). Dried tissue was digested with dilute HNO₃ (Hammerschmidt and Fitzgerald 2006a) and both MeHg and HgT were measured to determine the proportion of HgT as MeHg. MeHg was quantified by flow-injection, gas-chromatographic cold-vapor atomic fluorescence spectrometry (CVAFS; Bloom 1989; Tseng et al. 2004) and HgT by dual amalgamation CVAFS (Fitzgerald and Gill 1979). Sample Hg measurements were calibrated against solutions traceable to the U.S. National Institute of Standards & Technology and all measurements of MeHg and HgT in NRC/CNRC reference materials TORT-2 and DORM-3 ($n = 3$ each) were within their respective certified ranges. Recovery of known additions from digested tissue matrixes averaged 96 % for both MeHg and HgT ($n = 2$ each). Method precision, estimated from subsamples of three fish that were digested in duplicate averaged 5.0 and 6.4 relative percent difference for MeHg and HgT, respectively.

N and C isotopic composition

Carbon and nitrogen isotopes are commonly measured to investigate the structure and dynamics of food webs (Vander Zanden and Rasmussen 2001). The nitrogen isotopic composition ($\delta^{15}\text{N}$) of organisms tends to increase at a fairly constant degree between dietary trophic transfers (enrichment factor $\Delta\delta^{15}\text{N}_{\text{TP}} = 3.0\text{--}3.5$ ‰ per TP; Post 2002) and is often used to quantify relative TP. At the same time, ecosystem-level factors (e.g., differences in $\delta^{15}\text{N}$ at the base of the food web; Cabana and Rasmussen 1996; Popp et al. 2007) can interfere with the straightforward use of bulk nitrogen isotopes for quantifying TP. Terrestrial

organic matter entering a lake (allochthonous) generally has a greater $\delta^{13}\text{C}$ (i.e., less negative) than organic matter produced within the lake by primary producers (autochthonous; Kidd et al. 2001). Because $\delta^{13}\text{C}$ undergoes small changes during trophic transfer (<1 ‰ per TP), $\delta^{13}\text{C}$ of fish tissue can provide information about differences in carbon sources to the fish (Vander Zanden and Rasmussen 2001), and associated information about foraging locations.

Stable isotopes of C and N were measured on the majority of fish muscle samples ($n = 155$; Table 2). Aliquots (0.3–0.4 mg) of dried fish tissue were measured for N and C isotopic composition with an elemental analyzer (CE Instruments NC2500) coupled to an isotope-ratio mass spectrometer (GV Instruments IsoPrime). Sample isotope values were determined in units of per mil (‰) with standard δ -notation relative to atmospheric N₂ and the international V-PDB for carbon:

$$\delta^{15}\text{N}_{\text{sample}} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{air}}} \right) - 1 \right] \times 1,000$$

$$\delta^{13}\text{C}_{\text{sample}} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{PDB}}} \right) - 1 \right] \times 1,000$$

R denotes the ratio of $^{15}\text{N}:^{14}\text{N}$ and $^{13}\text{C}:^{12}\text{C}$, respectively. Every tenth sample was measured in triplicate, resulting in an average SD of 0.07 ‰ and a CV of 0.5 %. Peptone and atropine were used as internal standards (two each after every twelfth sample) and reproducibility was ± 0.15 ‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Whitefish in Swiss lakes have varying lipid contents (Zennegg et al. 2003), causing more negative $\delta^{13}\text{C}$ values in tissue of fattier fish. To more purely reflect the signature of dietary carbon, bulk $\delta^{13}\text{C}$ data were lipid normalized (Kiljunen et al. 2006).

Data analysis

One-way analyses of variance (ANOVAs) were conducted to test for differences in HgT concentration between species in each lake. As described below, HgT is representative of MeHg in muscle of whitefish. For lakes with three species of whitefish, ANOVA tests were followed by a Tukey–Kramer post hoc test. The same set of analyses was conducted to test for differences in fork length, gill raker number, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively. When ANOVA assumptions of homogeneity were still violated after log-transformation, non-parametric Kruskal–Wallis test was used followed by pair-wise comparisons with the Mann–Whitney test for lakes with more than two species. Level of significance was determined at $\alpha = 0.05$, but was Bonferroni corrected for multiple comparisons with the Mann–Whitney test. Linear least squares regression was used to examine the influence of fish length on HgT concentrations. To explore the relationship between

HgT and the potential predictors (lake, species, length, gill raker number and isotopic composition) across all study lakes, an ANCOVA was used. The factor ‘species’ was nested in ‘lake’. Statistical analyses were performed with SPSS Statistics 17.0, with the exception of the nested ANCOVA, which was conducted with R 2.14.1.

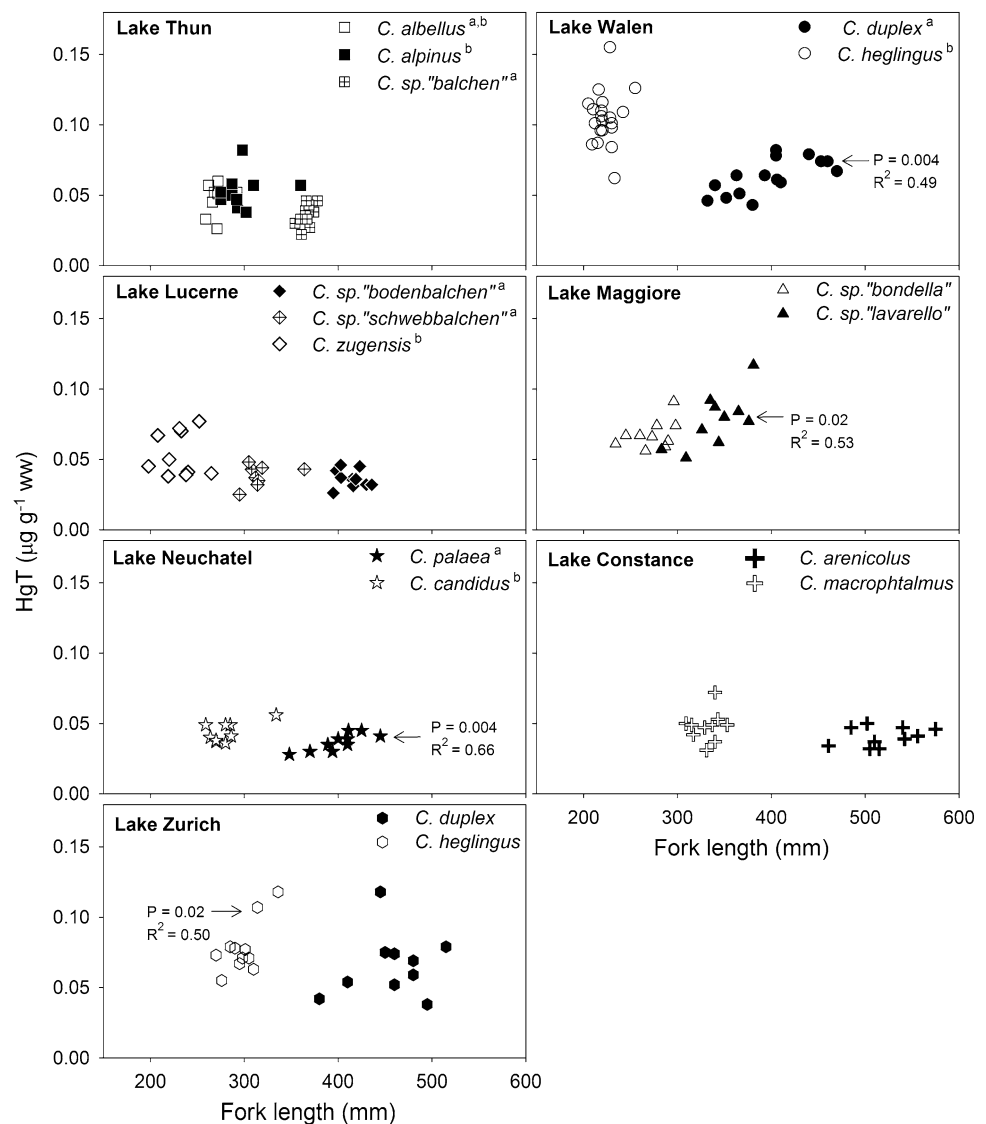
Results

Nearly all of the Hg measured in *Coregonus* spp. muscle tissue was present as MeHg (MeHg averaged 98 ± 3 % of HgT; entire data set available in Online Resource 1). Thus HgT is a good proxy for MeHg in whitefish muscle, and the term MeHg is used throughout the remaining sections. Among all study lakes and whitefish species, MeHg concentrations in individual fish varied by nearly one order of magnitude (0.022 – 0.155 $\mu\text{g g}^{-1}$ ww), and species-specific

averages differed by a factor of ~ 3 (0.036 – 0.105 $\mu\text{g g}^{-1}$; Table 3). MeHg concentrations exhibited considerable variability within some species (e.g., relative SD = 35 % for *C. duplex* in Lake Walen), while concentrations were within a fairly narrow range in other species.

MeHg concentrations differed significantly between sympatric whitefish species in Lakes Walen, Thun, Neuchatel, and Lucerne (Fig. 2). The within-lake difference was most pronounced in Lake Walen, where the average concentration in *C. heglingus* was 67 % greater than in *C. duplex*. All sympatric species had distinctly different gill raker numbers (Table 3), and in all lakes with significant intralacustrine differences in fish MeHg, species with the greatest gill raker number (i.e., more planktivorous) had the highest MeHg levels and those with lowest gill raker number (i.e., more benthivorous) had the lowest MeHg. One exception is *C. alpinus* in Lake Thun, a small profundal benthivore (VLGR), which exhibited the highest

Fig. 2 Mercury concentration as a function of fish fork length. Empty symbols represent the species with highest gill raker number in the respective lake, filled symbols represent the species with lowest gill raker number, and crossed symbols represent species with intermediate gill raker number. Significantly different averages of sympatric species HgT concentrations are denoted with different letters (ANOVA, $P < 0.05$). Linear relationships between MeHg and $\delta^{15}\text{N}$ within species were not significant ($P > 0.05$), except where noted



concentrations among the three whitefish species in the lake. In Lake Lucerne, the intermediate ecotype (MGR *C. sp.* “*schwebbalchen*”) also had intermediate MeHg concentrations that differed significantly from HGR *C. zugensis* but not from LGR *C. sp.* “*bodenbalchen*”. Within-lake differences in MeHg concentrations between sympatric species in Lakes Constance, Maggiore and Zurich were not significant.

Within all study lakes, the size of sympatric species differed significantly (Table 3), and in six of seven lakes MeHg concentrations were greater in the smaller species. The species with the highest MeHg levels overall, *C. heglungus* in Lake Walen, also was the smallest. Within a species, fish size is often found to be positively correlated with Hg concentrations and to explain a substantial portion of intraspecific variance in MeHg (Trudel and Rasmussen 1997; Kidd et al. 2003; Wiener et al. 2006a; Bank et al. 2007). However, within many of the studied species, MeHg concentrations were poorly correlated with fork length among individuals (only 4 of the 16 species had significant positive correlations; Table 4; Fig. 2). Although MeHg concentrations are commonly associated with fish age (e.g., Ward et al. 2010), the age data for the studied whitefish were not sufficient to allow quantitative comparisons or test associations with MeHg.

While $\delta^{15}\text{N}$ had limited variation within some species (range of 0.8 ‰ in *C. albellus*), it varied considerably within others (range of 7.1 ‰ in *C. duplex*; Fig. 3). This

Table 4 Regressions analysis results of fish total mercury ($\mu\text{g g}^{-1}$ ww) versus fork length (mm): P values depict the significance of the regression and R^2 the coefficient of determination

Lake	Species	P	R^2	β ($\mu\text{g g}^{-1} \text{mm}^{-1}$)
Thun	<i>C. albellus</i>	0.1	0.3	
	<i>C. sp.</i> “ <i>balchen</i> ”	0.5	0.07	
	<i>C. alpinus</i>	0.6	0.03	
Walen	<i>C. heglungus</i>	0.004	0.5	0.00020
	<i>C. duplex</i>	0.7	0.01	
Lucerne	<i>C. zugensis</i>	0.7	0.02	
	<i>C. sp.</i> “ <i>schwebbalchen</i> ”	0.4	0.2	
	<i>C. sp.</i> “ <i>bodenbalchen</i> ”	0.9	0.00	
Maggiore	<i>C. sp.</i> “ <i>bondella</i> ”	0.2	0.2	
	<i>C. sp.</i> “ <i>lavarello</i> ”	0.02	0.5	0.00046
Neuchatel	<i>C. candidus</i>	0.004	0.7	0.00019
	<i>C. palaea</i>	0.09	0.4	
Constance	<i>C. macrophthalmus</i>	0.4	0.08	
	<i>C. arenicolus</i>	0.7	0.03	
Zurich	<i>C. heglungus</i>	0.7	0.03	
	<i>C. duplex</i>	0.02	0.5	0.00071

For significant regressions the coefficient of the linear model is given ($\text{HgT} = \beta_0 + \beta \times \text{fish length}$)

suggests that fish of the same species could have been feeding at TPs that were as much as two levels apart in some lakes, assuming $\Delta\delta^{15}\text{N} = 3.0\text{--}3.5$ ‰ per TP (Post 2002), although a potential role of intra-lake variations in baseline $\delta^{15}\text{N}$ (Cabana and Rasmussen 1996) cannot be ruled out. The $\delta^{15}\text{N}$ of sympatric whitefish species differed significantly within four lakes (Lakes Lucerne, Maggiore, Neuchatel, and Zurich; Table 3) with species averages differing by 0.7–1.3 ‰. In Lakes Lucerne and Neuchatel, the species with significantly higher MeHg levels (*C. zugensis* and *C. candidus*, respectively) also had higher $\delta^{15}\text{N}$; this pattern was not observed in either Lakes Thun or Walen. Across all species within individual lakes, MeHg was not significantly correlated with $\delta^{15}\text{N}$ except in Lake Maggiore ($P = 0.01$; $R^2 = 0.31$), and only in two cases significant correlations were noted for individual species (Fig. 3).

The intra-species variation of $\delta^{13}\text{C}$ again was small in some species (minimal range of 0.4 ‰ in *C. sp.* “*schwebbalchen*”) but substantial in others (maximal range of 6.4 ‰ in *C. sp.* “*balchen*”; Fig. 4). The $\delta^{13}\text{C}$ of sympatric species were significantly different in three lakes (Lakes Constance, Lucerne and Zurich; Table 3) with averages differing by 0.5–0.9 ‰ between species. However, unlike gill raker number, which differed significantly between all sympatric species, $\delta^{13}\text{C}$ was not distinct in all lakes between benthivorous and pelagic-feeding species (Table 3). Of the four lakes in which sympatric species differed significantly in MeHg, only the species in Lake Lucerne had distinctly different $\delta^{13}\text{C}$. Similar to $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ was not a strong predictor of MeHg across all species within individual lakes, except in Lake Constance ($P = 0.04$; $R^2 = 0.22$); in some instances MeHg levels in individual species were inversely correlated with $\delta^{13}\text{C}$ (Fig. 4).

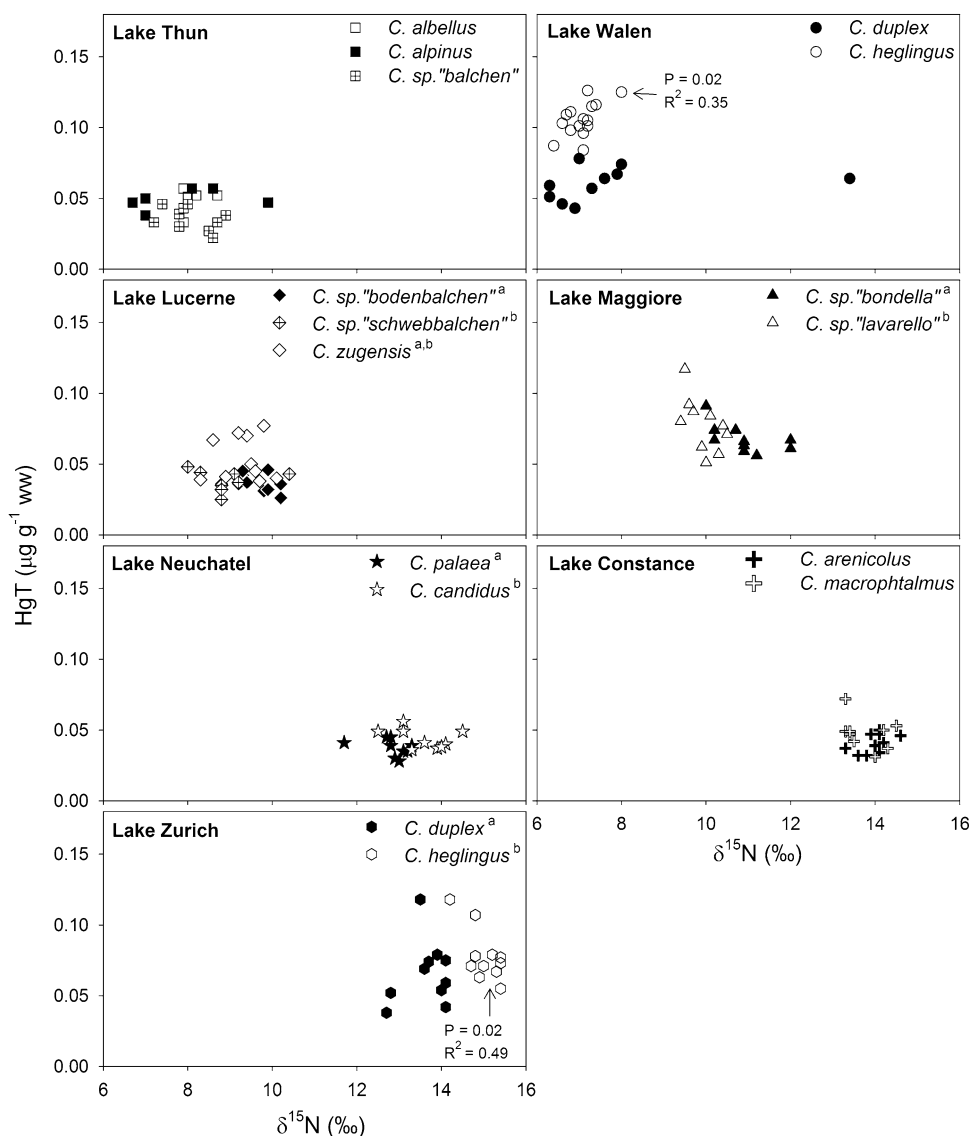
The nested ANCOVA ($R^2 = 0.82$) identified lake and species as the two main predictors of whitefish MeHg, with species accounting for a larger portion of the variance (52 %) than lake (32 %) (Table 5). While fish length and $\delta^{13}\text{C}$ emerged as significant covariates, the variance explained by these two variables was negligible (<1 %). Gill raker number and $\delta^{15}\text{N}$ were not significant covariates.

Discussion

Intralacustrine divergence in mercury bioaccumulation

In four of the seven lakes (Walen, Thun, Lucerne and Neuchatel), ecological divergence between sympatric whitefish species was associated with significant differences in MeHg bioaccumulation. Ecological speciation associated with the adaptive radiation of whitefish has led

Fig. 3 Mercury concentration as a function of fish $\delta^{15}\text{N}$ (‰). Empty symbols represent the species with highest gill raker number in the respective lake, filled symbols represent the species with lowest gill raker number, and crossed symbols represent species with intermediate gill raker number. Significantly different averages in $\delta^{15}\text{N}$ of sympatric species are denoted with different letters (ANOVA, $P < 0.05$). Linear relationships between MeHg and $\delta^{15}\text{N}$ within species were not significant ($P > 0.05$), except where noted

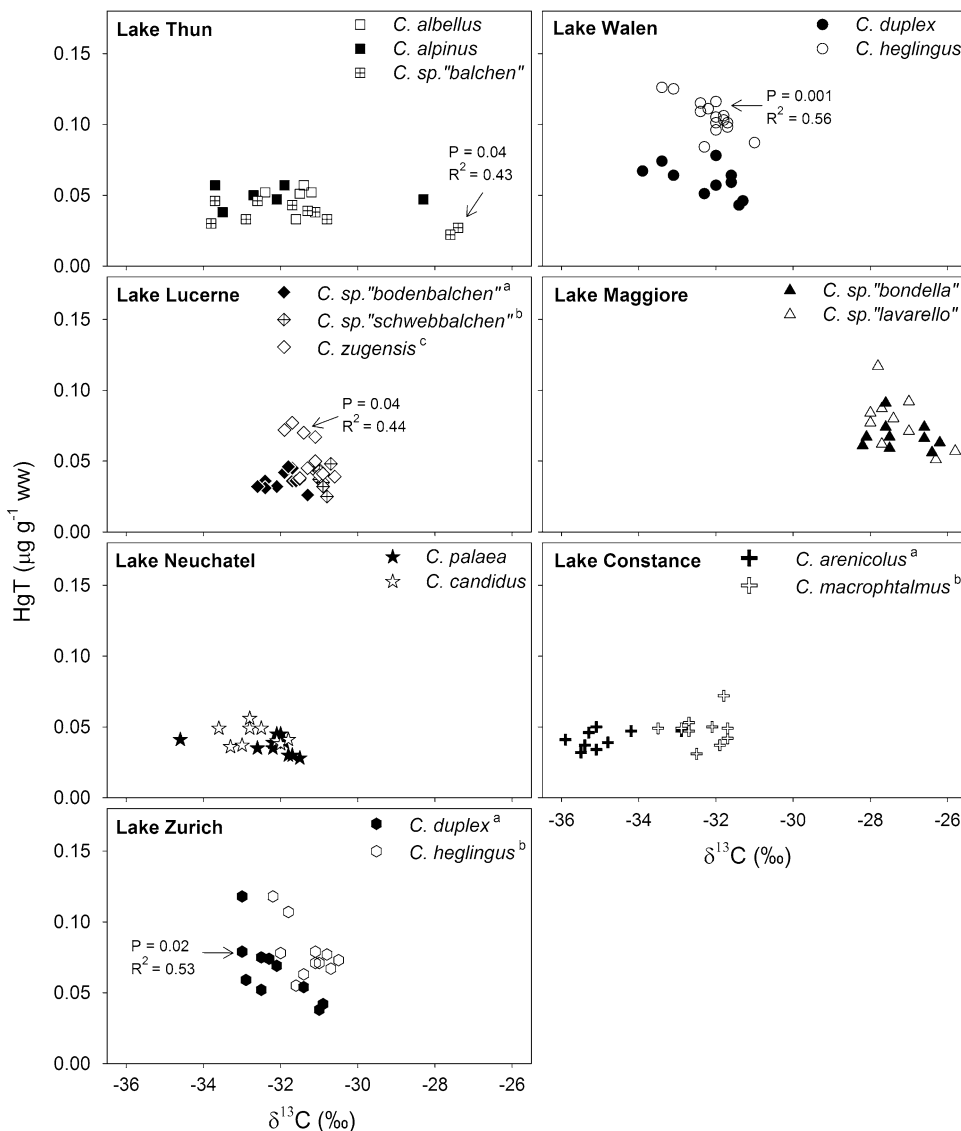


to multiple disparities in life-history traits and ecological niche use, and likely other physiological traits, all of which have the potential to contribute to the observed differences in MeHg accumulation. Because fish accumulate MeHg almost exclusively from their diet (Hall et al. 1997), the observed differences in steady-state MeHg concentration between fish could have been caused by differences in TP and bioavailability of MeHg at the base of the food web in which the whitefish species feed. Differences in either metabolism (e.g., the allocation of energy between synthesis and maintenance of biomass; Ward et al. 2010) or MeHg excretion rates (Trudel and Rasmussen 1997) can also influence MeHg concentrations in fish tissue. The potential contributions of these factors to whitefish MeHg levels are explored below.

In all lakes that had significant inter-species differences in whitefish MeHg concentrations, the species with greater gill

raker number had higher MeHg levels than the species with lower gill raker number. *C. alpinus* in Lake Thun, however, is an exception. Also, the species with deeper spawning habitats had higher MeHg levels than the species with shallower spawning habitats, with the exception of the introduced species pair of Lake Maggiore. Apart from *C. alpinus*, these observations are consistent with a potential role for different foraging habitats—and, consequently, differences in MeHg concentrations among dominant prey organisms—contributing to the observed differences in MeHg concentration between white fish species. Kidd et al. (2003) reached this conclusion in a study of Hg accumulation in the radiation of cichlid fishes in Lake Malawi. They found that the pelagically feeding species had higher Hg concentrations than benthic species and attributed the difference partly to MeHg inputs at the base of the respective food webs. Furthermore, per-trophic-level accumulation rates of the

Fig. 4 Mercury concentration as a function of fish $\delta^{13}\text{C}$ (‰). Empty symbols represent the species with highest gill raker number in the respective lake, filled symbols represent the species with lowest gill raker number, and crossed symbols represent species with intermediate gill raker number. Significantly different averages in $\delta^{13}\text{C}$ of sympatric species are denoted with different letters (ANOVA, $P < 0.05$). Linear relationships between MeHg and $\delta^{13}\text{C}$ within species were not significant ($P > 0.05$), except where noted



pesticide DDT also were higher in pelagic than in benthic cichlids (Kidd et al. 2001). Interestingly, although the distinct differences in gill raker numbers among the sympatric species suggest strongly divergent feeding habitats in this study (benthic vs. pelagic), carbon isotopic compositions largely overlap and did not offer additional support for this explanation. In addition, $\delta^{13}\text{C}$ explained only a negligible fraction of the variance in the ANCOVA across all lakes.

While all whitefish species are considered to feed at approximately the same TP (i.e. TP = 3, secondary consumer), individual whitefish species can have very distinct diets (Kahilainen et al. 2007; Harrod et al. 2010). It has been shown that among sticklebacks (*Gasterosteus* spp.), another taxon that has adaptively radiated in postglacial lakes, planktivorous species tend to occupy a slightly higher TP (i.e., a fraction of one TP) compared to closely related benthivorous species (Matthews et al. 2010). In sticklebacks,

this difference in TP is attributed to longer pelagic food chains with planktivorous ecotypes preying on carnivorous zooplankton. Even a fractional difference in TP can lead to

Table 5 Results of ANCOVA on whitefish MeHg across all study lakes: degrees of freedom (df), F-statistics (F), significance level (P), and proportional variance explained by the factors and covariates (η^2)

Variable	df	F	P	η^2 (%)
Lake	6	6.8	0.006	32
Species ^a	9	10	<0.001	52
Length	1	7.3	0.007	<1
Gill raker	1	0.69	0.4	<1
$\delta^{15}\text{N}$	1	0.12	0.7	<1
$\delta^{13}\text{C}$	1	6.2	0.01	<1

R^2 of the model is 0.82

^a The factor 'Species' is nested in 'Lake'

relatively substantial differences in MeHg levels in fish (e.g., Bank et al. 2007), and could conceivably contribute to the difference we observed among sympatric whitefish species.

However, the two whitefish species in Lake Walen, which had the greatest inter-species differences in MeHg concentrations, did not differ significantly in $\delta^{15}\text{N}$. $\delta^{15}\text{N}$ also did not vary significantly among the three species in Lake Thun, nor was it generally a consistent predictor within either lakes or species (Fig. 3). Therefore, variation in $\delta^{15}\text{N}$ does not appear to explain the observed divergent bioaccumulation of MeHg. In the ANCOVA, $\delta^{15}\text{N}$ was not a significant predictor of MeHg. This is not entirely surprising, however, considering that a major limitation of using $\delta^{15}\text{N}$ as a proxy for TP is that the $\delta^{15}\text{N}$ at the base of food webs ($\delta^{15}\text{N}_{\text{base}}$) can differ considerably, for example, between lakes (Cabana and Rasmussen 1996) or even between pelagic and benthic food webs within the same lake (Kidd et al. 2003; Matthews and Mazumder 2003). Furthermore, in some systems $\delta^{15}\text{N}$ has been shown to be a poor indicator for fish MeHg, namely when comparing fish at fine scales of trophic differentiation (Swanson et al. 2006), or in highly omnivorous fish relying on both pelagic and benthic sources (Greenfield et al. 2001), presumably due to variable prey MeHg concentrations. $\delta^{15}\text{N}$ of whitefish in Scandinavia (*Coregonus lavaretus*) has been shown to vary across morphs, with profundal morphs being enriched due to the signal of their prey (Harrod et al. 2010), thus complicating the deduction of TP from $\delta^{15}\text{N}$. Results from several studies suggest that the relationship between MeHg (or HgT) and $\delta^{15}\text{N}$ can be fairly constant across diverse ecosystems (e.g., Kidd et al. 1995, 2003; Senn et al. 2010), with $\Delta \log \text{HgT} \sim 0.2 \times \Delta \delta^{15}\text{N}_{\text{TP}}$, or $\Delta \log \text{HgT} \sim 0.7 \times \Delta \text{TP}$, assuming a trophic enrichment factor of $\Delta \delta^{15}\text{N}_{\text{TP}} = 3.5 \text{‰}$. Based on this relationship, the inter-species differences in whitefish MeHg levels observed in Lake Walen could be explained by a difference of 1.1 ‰ in $\delta^{15}\text{N}_{\text{base}}$ between the pelagic and benthic food webs. This would be equivalent to *C. heglungus* feeding at approximately one-third of a TP higher than *C. duplex*. Such differences in baseline $\delta^{15}\text{N}$ are plausible; for example, $\delta^{15}\text{N}_{\text{base}}$ differed by 1.5 and 1.0 ‰ between Scandinavian profundal and pelagic and between pelagic and littoral whitefish morphs, respectively, (Harrod et al. 2010) and 0.9 ‰ between pelagic and benthic food webs in Lake Malawi (Kidd et al. 2003). Matthews and Mazumder (2003) found that $\delta^{15}\text{N}_{\text{base}}$ varied by 2.4 ‰ for zooplankton within the same lake, but could not entirely exclude differences in TP as contributing to the isotopic difference. Thus, it remains a possibility that differences in TP between ecotype pairs played a role in the differences in MeHg levels of whitefish, and that these TP differences were masked by differences in $\delta^{15}\text{N}_{\text{base}}$ between food webs.

A major contributor to the observed MeHg differences between sympatric species in some of the lakes could be

diverging metabolisms of the individual species, in particular energy partitioning between anabolism and catabolism, resulting in different steady-state concentrations in the tissue. Indeed, the results of the ANCOVA indicate that species is the most important predictor of MeHg levels, explaining most of the variance. This is consistent with observations in some temperate lakes in North America where ‘normal’ lake whitefish (*C. clupeaformis*) coexist with a ‘dwarf’ form that has much slower growth rates (Fenderson 1964). The normal and dwarf ecotypes feed predominantly on benthic prey and zooplankton, respectively (Bernatchez et al. 1999), broadly similar to patterns of species divergence seen in Swiss lakes with two co-existing whitefish species. HgT bioaccumulation rates of the dwarf pelagic-feeding form in Sérigny Lake, Canada, were 50 % greater than those of normal benthic-feeding ecotypes, although prey HgT concentrations were similar for both types (Doyon et al. 1998). Difference in HgT concentrations between the Sérigny Lake whitefish types were attributed to faster metabolic rates and slower growth rates of ‘dwarf’ whitefish. St-Cyr et al. (2008) found that differences in metabolism and growth may be due to relative over-expression of genes associated with enhanced activity and metabolism and simultaneous down-regulation of genes associated with growth in dwarf relative to normal whitefish. Trudel et al. (2001) found the energy conversion efficiency of ‘dwarf’ whitefish to be two to three times lower than that of their normal sized relatives. It is therefore reasonable to suggest that the smaller whitefish species in Swiss lakes also have higher metabolic rates compared to the larger sized species, and that this difference may contribute to the greater MeHg accumulation in the pelagic whitefish. Indeed, the largest observed difference in mean MeHg concentrations between sympatric species in Swiss lakes (Lake Walen, 0.11 vs. 0.063 $\mu\text{g g}^{-1}$ ww), was of comparable magnitude to that found for dwarf and normal lake whitefish in Canada (Doyon et al. 1998). Also, higher metabolic rates in smaller whitefish species can explain the high HgT concentrations in *C. alpinus* in Lake Thun, which is an extreme benthivore but of small-size.

Thus, while we cannot rule out interactive effects of different foraging habitats or small differences in TP, our results are most consistent with divergent metabolism among whitefish species (small, slow-growing vs. large, fast-growing) as the major driver to the interspecies differences in MeHg levels within individual lakes.

Loss of bioaccumulation disparity due to eutrophication

The species pairs from Lakes Constance, Maggiore and Zurich exhibited no inter-species difference in MeHg. We suggest that lake characteristics could influence whether or

not sympatric species exhibit strong differences in MeHg content. In some Central European lakes, whitefish have recently experienced a collapse in genetic diversity due to anthropogenic eutrophication (Vonlanthen et al. 2012; Hudson et al. unpublished data). In lakes experiencing intense eutrophication, the hypolimnion was seasonally depleted in oxygen (Gachter 1987). In these systems, deep-water niches became increasingly unsuitable for whitefish for feeding and spawning. As a consequence, population sizes decreased due to reduced reproductive success (Mueller and Stadelmann 2004). In addition, as aquatic habitats became more ecologically homogeneous, levels of divergent natural selection among species' niches, potentially responsible for the majority of reproductive isolation among evolutionarily young species, weakened. This therefore could lead to speciation reversal, that is, to an increase in gene flow among sympatric species and hence to a narrowing of genetic and phenotypic disparity (Ruffli 1978; Vonlanthen et al. 2012; Seehausen 2006; Taylor et al. 2006).

Interestingly, the severity of speciation reversal, predicted by the level of historic organic pollution, is reflected in the MeHg disparity among species within lakes. It is possible that when the divergent natural selection was weakened because ecological niches in the lake were altered by eutrophication (Vonlanthen et al. 2012; Hudson et al. unpublished data), the original divergence in MeHg accumulation between species was also narrowed through increased gene flow. As an illustrative example, *C. heglingus* and *C. duplex* from Lake Zurich belong to the same genetic clade as *C. heglingus* and *C. duplex* in Lake Walen (Hudson et al. 2011). Yet, in Lake Walen, which did not experience eutrophication, the co-existing species exhibit the largest difference in MeHg concentrations, while in Lake Zurich, which had been highly eutrophic, the species are indistinguishable in their MeHg concentrations. Lake Constance had been the most heavily polluted of the lakes studied, and, similar to Lake Zurich, the studied species pair did not exhibit differences in their MeHg concentrations. Lake Maggiore cannot be evaluated in a similar fashion, because its whitefish are non-native, and were introduced to the lake between 1880 and 1950 (Steinmann 1951; Grimaldi 1972).

Other studies have suggested that eutrophication can lower MeHg concentration in fish due to biodilution of MeHg in the planktonic food web (Pickhardt et al. 2002; Kehrig et al. 2009). Our observations with whitefish may serve as initial evidence for an unexplored mechanism by which anthropogenic eutrophication can alter pollutant accumulation rates within food webs, namely by causing shifts in suitable habitats and concomitant changes in the feeding ecology and the metabolic activity of certain fish species, though this hypothesis needs further testing.

Similar principles may apply in species of other aquatic adaptive radiations, such as salmon, stickleback or African cichlids, and may also extend to other contaminants.

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