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Assessing the factors driving year-round variation

Keva, Ossi

2017-12

Keva , O , Hayden , B , Harrod , C & Kahilainen , K K 2017 , ' Total mercury concentrations in liver and muscle of European whitefish (*Coregonus lavaretus* (L.)) in a subarctic lake - Assessing the factors driving year-round variation ' , Environmental Pollution , vol. 231 , pp. 1518-1528 . <https://doi.org/10.1016/j.envpol.2017.09.012>

<http://hdl.handle.net/10138/307284>

<https://doi.org/10.1016/j.envpol.2017.09.012>

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Total mercury concentrations in liver and muscle of European whitefish (*Coregonus lavaretus* (L.)) in a subarctic lake - assessing the factors driving year-round variation

Ossi Keva^{a*}, Brian Hayden^{a,b,c}, Chris Harrod^{d,e} & Kimmo K. Kahilainen^{a,b}

*Corresponding author.

E-mail address: ossi.keva@helsinki.fi

^aDepartment of Environmental Sciences, University of Helsinki, P.O.Box 65, FIN-00014, Finland.

^bKilpisjärvi Biological Station, Käsivarrentie 14622, FIN-99490 Kilpisjärvi, Finland.

^cCanadian Rivers Institute, Biology Department, University of New Brunswick, Fredericton, NB, E3B 5A3, Canada.

^dInstituto de Ciencias Naturales Alexander Von Humboldt, Universidad de Antofagasta, Avenida Angamos 601, Antofagasta, Chile.

^eNúcleo Milenio de Salmónidos Invasores, Concepción, Chile

Highlights:

- Year-round variation of THg in fish tissues is poorly understood in subarctic lakes.
- THg concentrations of liver and muscle were highest in winter and lowest in summer.
- Starvation and planktivory increased THg, while growth dilution decreased THg.
- Intra-annual variation of THg in tissues was higher than inter-annual accumulation.
- Bioaccumulation of THg was highest in winter and lowest in summer for both tissues.

Graphical abstract:

22

23 **Abstract:**

24 Subarctic lakes are characterised by extreme seasonal variation in light and temperature which
 25 influences growth, maturation, condition and resource use of fishes. However, our understanding of
 26 how seasonal changes affect mercury concentrations of fishes is limited. We conducted a year-round
 27 study (3 ice-covered months, 3 open-water months) with open-water inter-annual aspect (3 years:
 28 samples from August/September), focusing on total mercury (THg) concentrations and ecological
 29 characteristics of a common freshwater fish, European whitefish (*Coregonus lavaretus* (L.)) from a
 30 subarctic lake. We measured THg concentrations from tissues with fast (liver, n=164) and moderate
 31 (muscle, n=225) turnover rates, providing information on THg dynamics over different temporal
 32 scales. In both tissues, lipid-corrected THg concentrations were highest in winter (liver: 1.70 ± 0.88
 33 $\mu\text{g/g}$, muscle: 0.24 ± 0.05 $\mu\text{g/g}$) and lowest in summer (liver: 0.87 ± 0.72 $\mu\text{g/g}$, muscle: 0.19 ± 0.04 $\mu\text{g/g}$).
 34 THg concentrations increased in winter following the summer-autumn dietary shift to pelagic
 35 zooplankton and starvation after spawning. Whitefish THg concentrations decreased towards
 36 summer, and were associated with consumption of benthic macroinvertebrates and subsequent growth
 37 dilution. Mercury bioaccumulated in both tissues with age, both showing the strongest regression
 38 slopes in winter and lowest in summer. THg concentrations in liver and muscle tissue were correlated
 39 throughout the year, however the correlation was lowest in summer, indicating high metabolism
 40 during somatic growing season in summer and growth dilution. Multiple linear regression models
 41 explained 50% and 55% of the THg variation in liver and muscle both models dominated by
 42 seasonally-variable factors i.e. sexual maturity, $\delta^{13}\text{C}$, and condition factor. The higher level of

43 seasonal variation (21%) in whitefish THg concentration in muscle than the inter-annual
44 accumulation (8%), and seasonally varying bioaccumulation slopes highlights the importance of
45 including seasonal factors in future THg studies.

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49

50 **Capsule:**

51 Strong seasonal variation was observed in THg concentrations and bioaccumulation slopes in
52 muscle and liver tissues, suggesting that the temporal component of sampling should be considered
53 in future THg monitoring and risk assessment programmes.

54 **Keywords:**

55 Bioaccumulation; dietary shift; growth dilution; seasonal variation; starvation

56

57 1. Introduction

58 Atmospheric mercury (Hg) originates from both natural and anthropogenic sources, and
59 concentrations in ecosystems across the globe have increased since the industrial revolution (Pacyna
60 et al., 2010). Atmospheric deposition typically dominates the supply of Hg to Arctic and subarctic
61 lakes lacking direct Hg pollution sources in their catchment (e.g. Downs et al., 1998, Ariya et al.,
62 2015). The Arctic has shown clear, and marked increasing trends in Hg concentrations, e.g. in lake
63 sediments since the 18th century industrial era (Chételat et al., 2015). Hg speciation (e.g. methylation)
64 in aquatic environments occurs through both biotic and abiotic pathways, and via numerous different
65 mechanisms (e.g. Jensen & Jernelöv, 1969; Pak & Bartha, 1998; Celo et al., 2006). In lake
66 ecosystems, sulphur-oxidizing bacteria play key roles in the methylation process, resulting in the
67 production of organic MeHg (e.g. Morel et al., 1998). Both benthic and pelagic primary producers,

68 i.e. periphyton and phytoplankton, uptake inorganic and organic Hg through passive and active
69 transport processes (Mason et al., 1995, 1996; Douglas et al., 2012). Benthic macroinvertebrates and
70 zooplankton consume these primary producers and transfer the Hg to invertebrate feeding fish, which
71 are in turn eaten by higher trophic level consumers such as piscivorous fish. In subarctic lakes, Hg
72 often accumulates faster in the pelagic food web compartment than the littoral (e.g. Kahilainen et al.,
73 2016a; Thomas et al., 2016; Kahilainen et al., 2017), likely reflecting increased trophic transfer
74 efficiency in the pelagic food web. MeHg is especially highly reactive, bioaccumulating in organisms
75 and biomagnifying through the food chain (e.g. Watras & Bloom, 1992; Watras et al., 1998). For
76 example, MeHg is estimated to represent a total proportion of mercury (THg) concentration that
77 ranges from 10% in the water column, to 15% in phytoplankton, 30% in zooplankton and up to 95%
78 in fish muscle (Watras & Bloom, 1992; Watras et al., 1998).

79 Hg in fishes is almost exclusively derived from their diet, where consumed prey is digested and Hg
80 is translocated via blood to the liver and subsequently stored in muscle tissues (e.g. Oliveira Ribeiro
81 et al., 1999; Wang & Wang, 2015). Hg concentrations are generally highest in liver and lower in
82 muscle, and vary among species (e.g. Jernelöv & Lann, 1971; Kahilainen et al., 2016a). In addition,
83 MeHg/THg ratios vary between tissues: e.g. ratios in liver and muscle is typically 40-80 % and >90
84 % respectively (e.g. Bloom et al., 1992; Blank et al., 2013; Madenjian et al., 2016). MeHg has high
85 tendency to form compounds with sulphur groups and bind to sulphur rich amino acids such as
86 methionine and cysteine (Huges, 1957; Kerper et al., 1992). As proteins contain more sulphur than
87 lipids, most Hg (>99%) is located in proteins (e.g. Amlund et al., 2007). In many fish studies, different
88 Hg species are combined and only THg concentrations in muscle are measured, since the proportion
89 of MeHg in fish muscle tissue is often >90% of THg (Downs et al., 1998; Watras et al., 1998;
90 Madenjian et al., 2016).

91 In fish, Hg generally bioaccumulates with increasing size and age (e.g. Downs et al., 1998; Amundsen
92 et al., 2011; Swanson et al., 2011). In species with ontogenetic dietary shifts, Hg concentration can

93 increase as consumers shift to a higher trophic level or switch to Hg-enriched pelagic prey (e.g. Power
94 et al., 2002; Thomas et al., 2016; Kahilainen et al., 2017). Fast growing individuals accumulate
95 muscle tissue faster than Hg, a phenomenon termed growth dilution (e.g. Simoneau et al., 2005; Ward
96 et al., 2010). Furthermore, growth dilution is inversely related to increasing condition factor and
97 individual lipid reserves (e.g. Amlund et al., 2007; Braaten et al., 2014; Kahilainen et al., 2016a). In
98 Arctic and subarctic lakes, many fish species have a lower growth rate, higher longevity and later
99 sexual maturation relative to their equivalents in temperate lakes (Heibo et al., 2005; Blanck &
100 Lamouroux, 2007), increasing the period of Hg bioaccumulation. Furthermore, in seasonally ice-
101 covered systems, condition and lipid reserves of fish are generally the lowest in winter (e.g. Hayden
102 et al., 2014a, 2015). Decreasing lipid content, and potentially also protein loss during starvation, may
103 condense Hg in remaining tissues (e.g. Kahilainen et al., 2016a). In the Hg contamination literature,
104 this phenomenon is termed as starvation (e.g. Cizdziel et al., 2002, 2003; Moreno et al., 2015) and,
105 along with growth dilution, it may play a key role in the seasonal variation in Hg concentrations in
106 cold-water fishes. Such variation may be important factor when considering Hg monitoring programs
107 and human health considerations, as many cold-water fishes play important roles in the year-round
108 diet of indigenous and non-indigenous people in the region (AMAP 2011).

109 The European whitefish (*Coregonus lavaretus* (L.)) is a highly diverse and often the most abundant
110 fish species in subarctic lakes of northern Fennoscandia (Siwertsson et al., 2010; Praebel et al., 2013;
111 Malinen et al., 2014). Most of the lakes are inhabited by a generalist monomorphic whitefish
112 populations using all habitat types, while polymorphic populations are diverged into separate pelagic
113 and benthic morphs (Kahilainen et al., 2007; Harrod et al., 2010; Siwertsson et al., 2010). In the most
114 complex cases, whitefish is ecomorphologically diverged into one of the three main lake habitats,
115 littoral, pelagic or profundal (Kahilainen & Østbye, 2006; Harrod et al., 2010; Kahilainen et al., 2014).
116 The whitefish morphs show many morphological and physiological adaptations to their specific habitat
117 types, where a heritable trait, number of gill rakers, encapsulates most of the variation as a single
118 measurement (Kahilainen et al., 2011b, 2014, 2016). Profundal morphs have the very low amount of

119 short and widely spaced gill rakers suitable for foraging on fine sediments in dark condition
120 characteristic of profundal habitat, whereas littoral and generalist morphs have intermediate number of
121 relatively short gill rakers followed by pelagic morphs with very high number of fine, long and
122 densely spaced gill rakers as adaption to foraging on small zooplankton prey (Kahilainen et al., 2007,
123 2011a, 2017). In both monomorphic and polymorphic lake types, whitefish as the most abundant
124 species is key invertebrate predator and main prey for many piscivores, thus acting as a central node
125 in lake food webs (e.g. Kahilainen & Lehtonen, 2003; Kahilainen et al., 2009, 2011a). The key role
126 of both monomorphic and polymorphic whitefish in the food webs of subarctic lakes has influence
127 on pelagic and benthic energy and Hg flows (Thomas et al., 2016; Kahilainen et al., 2017), but we
128 currently lack of knowledge regarding potential temporal variation in patterns of contaminant
129 bioaccumulation that is likely influenced by seasonality of prey availability, growth, reproduction
130 and condition.

131 To fill this knowledge gap, we undertook a year-round study of THg concentrations in a
132 monomorphic whitefish population, and their putative prey sources in a relatively well-studied
133 subarctic lake, Lake Kilpisjärvi, located in northern Fennoscandia. Here, monomorphic whitefish are
134 known to undergo a dietary shift from littoral benthic macroinvertebrates during winter and spring to
135 pelagic zooplankton in mid to late summer, coinciding with an annual zooplankton bloom (Tolonen,
136 1999; Hayden et al., 2014a). Pelagic prey is generally considered a more important source of Hg, due
137 to often higher MeHg concentrations in zooplankton than littoral benthic prey (Watras et al., 1998;
138 Suchanek et al., 2008). Previous work on whitefish morphs indicated that pelagic zooplanktivorous
139 morphs had much higher THg concentrations and steeper bioaccumulation slopes than benthivorous
140 morphs (Kahilainen et al., 2017). Open-water season dietary shifts from benthic macroinvertebrates
141 to pelagic zooplankton in Arctic fishes such as Arctic charr (*Salvelinus alpinus* (L.)) have been shown
142 to affect THg concentrations in fish liver and muscle tissue (Kahilainen et al., 2016a). Open-water
143 season THg studies of fish muscle has been conducted with many species (e.g. Zhang et al., 2012;
144 Braaten et al., 2014; Moreno et al., 2015; Olk et al., 2016), but we are unaware of any year-round

145 (including ice-covered winter) muscle and liver studies on THg concentrations of fish. Dietary shifts
146 are clearly important in Hg exposure, but we know very little about the seasonal patterns of THg
147 concentrations in whitefish and the main factors affecting any putative changes. To address these
148 questions, we collected data year-round on whitefish growth, sexual maturation, condition and
149 resource use with THg measured from muscle and liver tissues in a subarctic lake in northern Finnish
150 Lapland. Our study was designed to test three hypotheses:

151 **Hypothesis 1 (H1)** We hypothesized that the late summer dietary shift in whitefish from the low THg
152 littoral benthic macroinvertebrates to the high THg pelagic zooplankton (Kahilainen et al., 2016a;
153 2017) likely results in an increase in THg concentrations, and this shift will be evident in
154 metabolically active liver prior to muscle. We also predicted that the THg concentration of liver and
155 muscle will increase during winter due to starvation and subsequently decrease in spring and early
156 summer due to growth dilution.

157 **Hypothesis 2 (H2)** If there are seasonal changes in THg of muscle and liver tissues, we expected to
158 find changes in bioaccumulation slopes and the THg regression slopes between these two tissues.
159 First, we hypothesized that bioaccumulation occurred in both tissues in all months, but that we would
160 report shallower slopes during the summer somatic growing season due to growth dilution. Secondly,
161 we hypothesized that the intra-annual relationships of THg concentration between liver and muscle
162 would be significant year around, but would show a weaker relation in summer, when metabolic
163 activity is higher in both tissues.

164 **Hypothesis 3 (H3)** If season is an important determinant of THg concentrations, we expected to see
165 seasonal-related factors e.g. maturity and stable isotope ratios selected in multiple linear regression
166 models examining the drivers for muscle and liver THg concentrations, in addition to traits related to
167 individual fish size.

168

169 2. Materials and methods

170 2.1 Study area

171 This study was conducted in a subarctic Lake Kilpisjärvi (hereafter L. Kilpis), located in northern
172 Fennoscandia (69°03'N, 20°49'E; 473 m above sea level; Fig. 1). L. Kilpis is a relatively large (surface
173 area 37.3 km², shoreline 71.5 km), oligotrophic (Tot-P < 5 µg l⁻¹, Tot-N < 150 µg l⁻¹, chlorophyll-a <
174 2 µg l⁻¹), neutral (pH 7.2, conductivity 3.0 mS m⁻¹), clear water (Secchi and compensation depth 10
175 and 14 m, DOC 2.8 µg l⁻¹) and deep (maximum and average depths 57 m and 19.4 m) headwater lake
176 (Kahilainen et al., 2007; Hayden et al., 2014a; Kahilainen et al., 2017). The average annual air
177 temperature of the region is -2.3 °C and precipitation is 450 mm y⁻¹, of which ca. 60% falls as a snow.
178 The year-round average water column temperature lake water varies from 0.4-10°C (Hayden et al.,
179 2014a; 2014b). Ice cover is present on the lake from mid-November until mid-June and may reach a
180 thickness of 1 m in late winter (Lei et al., 2012). The L. Kilpis catchment (293 km²) consists of
181 subarctic mountain birch (*Betula* sp.) surrounding the lake, whereas areas with elevations above 600
182 m a.s.l. are Arctic tundra. The proportion of peatland in the catchment is low. There are no direct
183 sources of Hg (e.g. volcanos, mines, factories) in the vicinity, suggesting that the principal source of
184 Hg to the lake and catchment over historical and contemporary timelines has been atmospheric
185 deposition.

186 L. Kilpis has a relatively simple fish community, of which monomorphic whitefish is the dominant
187 species, contributing ca. 90% to the total fish community by abundance, with an estimated density of
188 ca. 80 individuals ha⁻¹ (Harrod et al., 2010; Malinen et al., 2014). The generalist whitefish morph in
189 L. Kilpis is large sparsely rakered whitefish (LSR) inhabiting all lake habitats using both pelagic and
190 benthic prey resources (Kahilainen et al., 2007). Other fish species in L. Kilpis are alpine bullhead
191 (*Cottus poecilopus* (Heckel)), Arctic charr, burbot (*Lota lota* (L.)), grayling (*Thymallus thymallus*
192 (L.)), minnow (*Phoxinus phoxinus* (L.)), pike (*Esox lucius* (L.)) and brown trout (*Salmo trutta* (L.))
193 (Kahilainen et al., 2007).

194 2.2 Sample period and sampling methods

195 Samples were collected over a total of eight sampling periods to assess both inter- and intra-annual
196 THg concentrations and bioaccumulation: September 2010, December 2011, February 2012, May
197 2012, June 2012, July 2012, September 2012 and September 2014. Samples collected in December,
198 February and May were from the period when the lake was ice-covered (ice thickness range: 12-85
199 cm) and other months represent the open-water season. Hayden et al. (2014a) used stomach content
200 in addition to carbon and nitrogen stable isotope ratios from this period to show that whitefish
201 predominantly feed on littoral benthic macroinvertebrates (BMI) and pelagic zooplankton (ZPL) is
202 used as a significant prey only during the late summer. Here, we re-examined samples from the same
203 invertebrates and fish to assess how such dietary shifts, as well as other putative seasonal and life-
204 history factors affect Hg concentrations in whitefish.

205 ZPL samples were collected with a plankton net (mesh size: 50 μm , diameter: 25 cm) by vertical
206 hauls through 0-20 m to gain sufficient material for stable isotope analysis (SIA) and THg analysis.
207 Composite zooplankton samples included both cladocerans and copepods and were stored in plastic
208 vials and frozen ($-20\text{ }^{\circ}\text{C}$). BMI samples were collected with an Ekman grab (272 cm^2) from littoral
209 (1 m) and profundal (20 m) habitats, sieved through 500 μm mesh net and identified to the lowest
210 feasible taxon, stored to plastic vials and frozen ($-20\text{ }^{\circ}\text{C}$). After initial freezing to $-20\text{ }^{\circ}\text{C}$, both ZPL
211 and BMI samples were freeze-dried (-75°C , 48 hours) for SIA and THg analyses.

212 Fish were collected using gillnets fished in series including seven 1.8 m high and 30 m long nets
213 (knot-to-knot mesh sizes: 12, 15, 20, 25, 30, 35, 45 mm), supplemented with one 1.5 m high and 27
214 m long Nordic multimesh gillnet (5.25-55 mm). Gillnet series were set in benthic habitat at depths 2-
215 15 m overnight (summer: 10-12h, winter: 24-48h). Fish were immediately euthanized by cerebral
216 concussion at the sampling site. After immediate transport to the laboratory, total length and mass of
217 whitefish were measured to the nearest mm and 0.1 g. Fulton's condition factor (K) was calculated
218 for each individual following Nash et al. (2006):

$$K = \frac{M}{TL^3} \times 100 ,$$

where M (g) is mass and TL (cm) is total length of fish.

Both sagittal otoliths and circa 50-100 ventral scales between the pelvic and anal fins were taken from each fish for age determination. Individual whitefish age was determined from the combined use of clear, burned and cracked otoliths under a binocular microscope as well as scales pressed on polycarbonate slides and viewed using a microfiche reader (Kahilainen et al., 2003). The joint use of otoliths and scales was used to improve the accuracy of aging (Kahilainen et al., 2017). Whitefish populations in L. Kilpis are typically dominated by single year class for 10-15 years (Tolonen, 1999), and in current study the dominant year-class during all sampling years comprised of fish that hatched in 2003. The number of gillrakers (range 19-29), including small rudimentary rakers located at both ends of the first brachial gill arch, were counted under a preparation microscope. The number of gill rakers is a heritable trait in whitefish used to define different morphs and related to overall phenotype of whitefish individual as well as the main resource use patterns (Kahilainen et al., 2011a, 2011b). In L. Kilpis whitefish population is monomorphic, but the number of gill rakers could potentially be related to individual dietary specialization and thus THg concentration. Sex was determined (1 = female, 2 = male, 3 = juvenile) visually from gonads. If gonads were underdeveloped (sex = 3), sexual maturity was coded as 0, otherwise sexual maturity was defined with scale from 1 to 7, where 0-3 represents juveniles and 4-7 mature individuals at different stages of maturity (Bagenal 1978). In the most intensive sampling period of 2011-2012, both gonads were weighed (± 0.01 g) and the gonadosomatic index was calculated (Bagenal, 1978) to gain continuous proxy for gonad investment and level of sexual maturity:

$$GSI = \frac{GM}{SM} \times 100 ,$$

where GSI is gonadosomatic index, GM is the mass of gonads (g), SM is somatic mass (g).

242 Whitefish stomachs were dissected from the oesophagus to the pyloric caeca and prey items were
243 placed into a Petri dish. Stomach fullness was estimated visually using a modified points method
244 (Swynnerton & Worthington 1940). Here, stomach fullness was assessed using a scale from 0 (empty)
245 to 10 (fully distended). Prey items were identified to the lowest feasible taxonomic level and their
246 relative share of total fullness was estimated. A sample of liver and white dorsal muscle were taken
247 from each fish, separately stored in 2 ml plastic vials, frozen at -20°C and subsequently freeze-dried
248 (-75°C for 48h) prior to preparation for SIA and THg analysis.

249 Freeze-dried samples of liver and muscle were ground to a fine powder, and weighed (ca. 0.5 mg)
250 into tin cups. Stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), in addition to the elemental
251 ratio of carbon and nitrogen (C:N), were analysed through an elemental analyser connected to
252 continuous flow isotope ratio mass spectrometer. Analytical error for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was 0.1 ‰.
253 Fish $\delta^{13}\text{C}$ values were arithmetically lipid-corrected using sample-specific C:N ratios of either muscle
254 (Kiljunen et al., 2006) or liver (Logan et al., 2008) samples.

255 2.4 Total mercury analysis

256 THg concentrations ($\mu\text{g g}^{-1}$ d.w.) were analysed from the freeze-dried ZPL (n=17), BMI (n=20), liver
257 (n=167) and muscle (n=225) samples by atom absorption spectrometry using a direct Hg analyser
258 (Milestone DMA 80). We had a target fish sample size for each month of 30 individuals representing
259 the total length and age distribution of the population (Table 1) and all invertebrate samples
260 containing enough tissue were analysed. From each sample, two duplicates (20-30 mg) were analysed
261 when material was not limited due to low sample mass - as was the case with some liver samples and
262 almost all invertebrate samples. Average relative difference (RSD) between duplicates of liver (n=113
263 pairs), muscle (n=223 pairs) and invertebrates (n=2 pairs) was 1.1, 1.3, 7.1 and 11.2%, respectively.
264 At the start and end of each run, samples of certified reference material (DORM-4; $0.410 \pm 0.055 \mu\text{g}$
265 g^{-1} ; National Research Council Canada) were combusted. The average and recovery-% of the certified
266 reference material (n=66) was 0.408 ± 0.011 (SD) and 99.6% respectively. Blank control samples

267 (grand mean $\pm$ SD: 0.001 ± 0.001 , $n=113$) were added both at the end of each run, as well as between
268 different tissues and taxa. Run specific blank THg values was subtracted from analysed sample THg
269 values to avoid instrumental error. The mean of the blank adjusted duplicate THg values was later
270 lipid-corrected.

271 Hg binds mainly to proteins (e.g. Amlund et al., 2007) and therefore seasonal changes in lipid reserves
272 in muscle and liver tissues can affect Hg concentrations (Kahilainen et al., 2016a). C:N ratio is a
273 useful proxy for lipid content in tissues (Fagan et al., 2011; Hoffman et al., 2015). A C:N ratio of ca.
274 three represents pure protein, with values above three indicate increasing concentrations of lipids.
275 Whitefish usually have lower lipid concentrations, and display less seasonal variation, in muscle
276 rather than liver tissues (Hayden et al., 2014a; 2015). However, THg concentrations were
277 arithmetically lipid-corrected using C:N ratios (Kahilainen et al., 2016a) to minimize the effects of
278 seasonally varying lipid concentrations on the measured THg concentrations clarifying the effects of
279 other seasonally varying factors:

$$280 \quad TotHg_{Lipid-corrected} = \frac{C:N_{sample}}{3.2} \times TotHg_{raw} ,$$

281 where $TotHg_{Lipid-corrected}$ is the C:N corrected THg value ($\mu\text{g g}^{-1}$ d.w.), $C:N_{sample}$ is the C:N ratio
282 of sample individual, 3.2 is the minimum seasonal average of the measured C:N ratios and $TotHg_{raw}$
283 is measured total mercury value ($\mu\text{g g}^{-1}$ d.w.). $TotHg_{Lipid-corrected}$ (hereafter THg) values was used
284 in all subsequent statistical analysis.

285 2.5 Statistical methods

286 Examination of seasonal changes of Hg concentrations in whitefish tissues (H1) and all supporting
287 analyses of variance were conducted with non-parametric tests (Kruskal-Wallis H-test with post hoc:
288 Mann-Whitney U-test, or if the assumption of homogeneity of variances was violated, we used
289 repeated Welch's t-tests with the Games-Howell post-hoc test). The seasonal bioaccumulation and
290 relationships between Hg concentrations in liver and muscle (H2) were tested with linear regression

analysis. From data collected during the intensive 2011-2012 sampling period, we examined the factors explaining variation in THg concentrations from the different tissues (H3) using multiple linear regression analyses, where we tested forward, backward and both direction stepwise selection procedure, selecting the best model based on minimum AIC values. Here, we first checked for autocorrelation and selected variables with $R^2 < 0.7$ (sampling month, total length, condition factor, sex, sexual maturity, GSI, gillraker number, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$) for inclusion in the model. Significance level ($p < 0.05$) was used in all other analysis. Statistical analyses were conducted using SPSS 23 (IBM Corp., Armonk, NY, USA) and R (RStudio 0.99.892; R Core Team, 2016) using MOSS package (Venables & Ripley, 2002).

300

3. Results

3.1 Year-round patterns in whitefish ecological characteristics

Somatic mass (Kruskal-Wallis: $H_{5, 161} = 23.30$, $p < 0.001$), condition factor (Welch's ANOVA: $F_{7, 221} = 7.80$, $p < 0.001$) and GSI (Welch's ANOVA: $F_{5, 161} = 6.76$, $p < 0.001$) showed seasonal variation, all increasing towards autumn (Table 1). The dominant year class (2003) showed similar seasonal pattern in somatic mass (Kruskal-Wallis: $H_{5, 55} = 22.81$, $p < 0.001$) and condition factor (Kruskal-Wallis: $H_{7, 84} = 20.79$, $p < 0.001$) to the whole population (Table 1). The number of empty stomachs (Table 1) and stomach fullness (Welch's ANOVA: $F_{7, 217} = 18.86$, $p < 0.001$) varied between seasons. The number of empty stomachs was highest ($n=24$) and stomach fullness (0.4) lowest in ice-covered December just prior to spawning, whereas no empty stomachs were found in July-September when the average stomach fullness (5.4-4.6) was highest (Table 1). Stomach content analysis showed that for much of the year, whitefish largely consumed BMI, but that the prevalence of zooplankton in the diet increased in late summer (Table 1, Table S1). The year-round similarity of trophic level was also evident in muscle $\delta^{15}\text{N}$ and values (Welch's ANOVA: $F_{7, 217} = 2.49$, $p = 0.017$), with pairwise comparisons

315 showing the highest values in winter (Table 1). The strong annual reliance on littoral BMI was also
316 evident from relatively similar muscle $\delta^{13}\text{C}$ values (Welch's ANOVA: $F_{7, 217} = 5.54$, $p < 0.001$), with
317 values showing a slight ^{13}C depletion in winter and ^{13}C enrichment at early summer (Table 1).

318 3.2 H1 - Annual mercury concentrations in invertebrates and whitefish tissues

319 THg concentrations varied (Welch's ANOVA: $F_{2, 34} = 13.09$, $p < 0.001$) between the different putative
320 whitefish prey groups (Table 2). ZPL displayed higher THg concentrations than littoral BMI (0.070
321 ± 0.013 and $0.042 \pm 0.014 \mu\text{g g}^{-1}$ respectively; Table 2). The mean THg of profundal BMI ($0.366 \pm$
322 $0.356 \mu\text{g g}^{-1}$) were circa five times higher than the concentrations in ZPL, but the difference was not
323 statistically significant, reflecting high variation in the former group. Due to the low sample number,
324 the taxa specific seasonal THg, stable isotope and C:N values did not allow statistical testing (Table
325 S2)

326 THg concentrations varied seasonally both in liver (Welch's ANOVA: $F_{5, 158} = 5.29$, $p < 0.001$) and
327 muscle tissue (Kruskal-Wallis: $H_{7, 217} = 41.87$, $p < 0.001$). The seasonal changes showed a similar
328 pattern in both liver and muscle tissues (Table 2, Fig. 2). The highest THg concentrations (liver: 1.70
329 $\pm 0.88 \mu\text{g g}^{-1}$, muscle: $0.24 \pm 0.05 \mu\text{g g}^{-1}$) were found in mid-winter under thick ice (February 2012)
330 and the lowest (liver: $0.87 \pm 0.72 \mu\text{g g}^{-1}$, muscle: $0.19 \pm 0.04 \mu\text{g g}^{-1}$) in open-water summer (June-
331 July 2012). We estimated the annual accumulation of THg (0.01 - $0.02 \mu\text{g g}^{-1}$) in muscle tissue by
332 comparing samples from September 2010 and August 2014 (Table 2): post-hoc tests indicated that
333 THg concentrations were higher in 2014 ($0.23 \pm 0.05 \mu\text{g g}^{-1}$) than in 2010 ($0.17 \pm 0.04 \mu\text{g g}^{-1}$).

334

335 3.3 H2 - Seasonally varying bioaccumulation and relationships of THg in liver and muscle tissue

336 Bioaccumulation of THg by age varied seasonally in both tissues showing the highest slopes and the
337 strongest significances in winter and the lowest or non-significant slopes in summer (Fig. 3). In liver

the non-significant bioaccumulation was found just after the ice-break in June and, in July it was evident in muscle as well (Fig. 3). The regressions of THg concentrations between liver and muscle were statistically significant throughout the whole year, showing the steepest slopes in mid-winter, decreasing towards summer and recovering again towards autumn (Fig. 4). These regression plots show signs of THg enrichment via starvation in winter, with overall high values in February and May, followed by low concentrations in June and July suggesting growth dilution during summer growing season (Fig. 4).

345

3.4 H3 - Results of multiple linear regression analysis

The best stepwise multiple regression models explained 50% and 55% of the variation in THg concentration in liver, muscle respectively (Table 3, Table S3). Both models included the exact same ecological variables explaining the variation in THg concentrations. Sexual maturity, $\delta^{15}\text{N}$ and total length were positively related to THg concentrations in both liver and muscle models, whereas $\delta^{13}\text{C}$ and condition factor was inversely related to the concentrations. In both models, sexual maturity, $\delta^{13}\text{C}$ and condition factor were most significant explanatory factors of the THg concentrations. The main difference between the models was that sampling month and GSI was conversely related in these models.

355

4. Discussion

4.1 Main results

We found evidence for year-round variation in THg concentrations in whitefish liver and muscle tissues. As we hypothesized (H1), annual THg concentration of liver and muscle were highest in winter and lowest in open-water summer months. In addition, strength and significance of

361 bioaccumulation and the positive relationship of THg concentration between muscle and liver peaked
362 in winter and were shallow or non-significant in summer (H2). The seasonally related variables, such
363 as sexual maturity, $\delta^{13}\text{C}$, and condition factor, included in the multiple linear regression models
364 supported hypothesis H3, i.e. that starvation and zooplanktivory increased THg concentration and
365 growth dilution lowered it.

366 4.2 Seasonal variation in mercury concentrations (H1)

367 We found strong seasonality of THg concentrations, where liver and muscle showed maximum
368 differences of 49% and 21% in monthly comparisons, respectively. Here, both tissues showed similar
369 patterns, following a sine-curve peaking in winter and reaching the minimum in summer. These
370 changes were related to consistent year-round changes in several measures of whitefish ecology (e.g.
371 resource use, maturation and condition). Whitefish showed a seasonal shift in diet in the summer,
372 changing from a BMI diet to a pelagic ZPL-dominated diet, evident from both stomach contents and
373 liver $\delta^{13}\text{C}$ values, which became increasingly ^{13}C depleted. In L. Kilpis, we showed that THg
374 concentrations in pelagic ZPL were higher than littoral BMI and other studies have shown that pelagic
375 ZPL may have a higher MeHg/THg ratio than littoral BMI (e.g. Watras et al., 1998; Suchanek et al.,
376 2008). In light of this, we suggest that the reported whitefish dietary shift to pelagic ZPL contributes
377 to an increasing trend of THg levels of liver and muscle from late summer onwards. This is further
378 supported by results of recent study showing higher THg concentrations on zooplanktivorous than
379 benthivorous whitefish morphs in a series of subarctic lakes (Kahilainen et al., 2017). Hg turnover is
380 faster in liver than in muscle (Oliveira Ribeiro et al., 1999) and this may explain our observation that
381 Hg concentrations increased slightly faster in liver than in muscle following the diet shift to pelagic
382 ZPL. The open water season dietary shift of Arctic charr in nearby subarctic Lake Galggojavri from
383 BMI to pelagic ZPL has been found to increase fish THg concentrations in liver towards autumn
384 (Kahilainen et al., 2016). Similarity of diet and THg patterns in whitefish and Arctic charr during
385 open-water season suggest generality of our findings, at least locally.

386 Starvation has been suggested to increase fish Hg concentrations in winter when water temperature,
387 fish activity and condition all decrease (e.g. Cizdziel et al., 2002, 2003; Moreno et al., 2015).
388 However, seasonal changes in lipid concentrations may also have an impact (Kahilainen et al.,
389 2016a). In the present study, THg concentrations were lipid corrected to minimize the effect of
390 seasonal lipid changes in tissues that were evident in C:N values of liver tissue, but not in muscle
391 tissue. When comparing the individuals of the 2003 year class, consisting entirely of mature fish, we
392 found that condition factor and somatic mass were lowest in winter (excluding June, a month with
393 limited sample size), reflecting spawning and subsequent starvation in February. The gonads of lake
394 whitefish (*Coregonus clupeaformis*), a North American sister species to *C. lavaretus*, contain very
395 little Hg and it is likely that mature fish instead store Hg in muscle tissue prior to spawning
396 (Madenjian et al., 2016). Assuming the same pattern in its closely-related, and ecological equivalent
397 sister species, the high THg concentration we reported from February can be partly related to the
398 post-spawning period and increased muscle storage of Hg. Although our Hg data were lipid corrected,
399 we were still able to define starvation affecting seasonal changes of THg concentrations, therefore
400 loss of protein might also have a role.

401 Growth dilution has been suggested to result in reduced Hg concentrations during periods when fish
402 rapidly gain somatic mass (e.g. Doyon et al., 1998; Simoneau et al., 2005; Braaten et al., 2014). The
403 2003 year class increased in somatic mass from winter (December 2011) to autumn (September
404 2012), a period during which THg concentrations fell. This suggests that growth dilution, even with
405 slow growth rates, can explain decreasing THg concentrations in both liver and muscle tissues of
406 sampled whitefish. In addition, increased excretion during summer could also explain the decrease of
407 THg concentrations from winter to summer in liver and muscle, but we were unable to test this with
408 our study design.

409 The rate of increase in THg values in both liver and muscle slowed after sexual maturation. The
410 ontogenetic dietary shift from ZPL to BMI at an early age in whitefish (Tolonen, 1998) and decreased

411 somatic growth after maturation might explain the slowing THg accumulation. We found that
412 population-level mean THg concentrations in whitefish muscle increased by 8 % per year, between
413 September 2010 and August 2014. This value is indeed circa 2.5 times lower than observed intra-
414 annual variation during 2011-2012. Both observations strongly reflect the dominance of the single
415 mature 2003 year-class, with limited annual somatic growth explaining low inter-annual
416 bioaccumulation and strong investment on gonads causing intra-annual variation of muscle THg. In
417 previous studies of L. Kilpis whitefish, the dominance of a single year-class has been documented in
418 different decades (Tolonen, 1998; Harrod et al., 2010) and such patterns of year-class dominance and
419 generally high age of fish is typical in Arctic and subarctic lakes (Rolls et al., 2017). Taken
420 collectively, our results of intra- and inter-annual patterns of THg should be thus the most valid for
421 fish populations consisting of mature fish investing their energy mostly to gonad growth instead
422 of somatic growth, a pattern typical for a range of fish species.

423 4.3 Seasonal bioaccumulation and mercury metabolism between liver and muscle (H2)

424 In L. Kilpis whitefish, THg concentrations increased with age in both liver and muscle tissue. This
425 pattern has been recorded also in many other studies using muscle tissues of subarctic salmonids (e.g.
426 Amundsen et al. 2011, Swanson et al. 2011), but is less commonly reported from liver tissue. A
427 previous study by Kahilainen et al. (2017), showed that THg concentrations in muscle generally
428 increased with age in different European whitefish morphs, but the regression slopes were the most
429 steep for pelagic morphs (range 0.038-0.103) and shallow for benthic whitefish morphs (0.017-
430 0.020). This study also assessed bioaccumulation in three monomorphic whitefish population, which
431 displayed relatively shallow slopes (0.020-0.025). These results corroborate our findings here, as we
432 found shallow, or even non-significant bioaccumulation slopes during the summer growing season
433 for both liver and muscle with some time-lag related to faster metabolic rate of former than later (e.g.
434 Oliveira-Ribeiro et al., 1999; Hayden et al., 2014a; Kahilainen et al., 2016a). Interestingly, the
435 bioaccumulation slopes of both tissues were clearly steeper during the ice-covered winter, most likely

436 driven by the older mature individuals which had higher relative difference between winter and
437 summer THg concentrations compared to the younger immature individuals. This could be explained
438 by the stronger response of older fish to spawning, which is likely due to the fact that only six years
439 or older individuals were sexually mature, and this was the group driving the changes in
440 bioaccumulation seasonally.

441 The relationship between the THg values of liver and muscle tissues was evident during the whole
442 season highlighting the strong metabolic link between these two tissues (Oliveira Ribeiro et al., 1999;
443 Sinnatamby et al., 2008). However, the strength of the link between the THg values of these tissues
444 altered during season highlighting the difference in turn over times between these tissues (Hayden et
445 al., 2014a; Kahilainen et al., 2016a). The data examination revealed that the THg concentrations in
446 liver decreased relatively more compared to muscle towards summer. This could be explained by the
447 slightly faster turnover time of liver tissue THg to muscle, which is consistent with the previous
448 laboratory studies (e.g. Oliveira Ribeiro et al., 1999). Generally, the liver-muscle relationship seems
449 to follow water temperature related metabolic activity and support other evidence growth dilution
450 during the summer and starvation in winter.

451

452 4.4 Factors explaining variation in mercury concentration in whitefish (H3)

453 We found that a high proportion of the variation (50% and 55%) in THg concentration in liver and
454 muscle was explained through multiple linear regression analyses. Previous studies employing
455 regression analyses to explain THg concentrations in whitefish have frequently documented that fish
456 size and age are important factors affecting Hg concentration (e.g. Moreno et al., 2015; Thomas et
457 al., 2016; Kahilainen et al., 2017). Surprisingly total length, which was correlated with age and mass,
458 was a relatively poor predictor of liver and muscle THg concentrations in L. Kilpis. This most likely
459 reflect the low investment to somatic growth of single year-class 2003 dominated whitefish
460 population, where most fish are close to their maximum length. However, the inclusion of THg in

461 liver and muscle and factors related to temporal variation such as sampling month, stable isotopes
462 and sexual maturity have been examined to a far lesser degree. In this study, all of these factors
463 describing year-round variation were highly important factors included in liver and muscle models
464 and are discussed below.

465 In L. Kilpis, both muscle and liver THg values were inversely related with tissue specific $\delta^{13}\text{C}$ values,
466 which likely reflects increased autumnal consumption of ^{13}C depleted pelagic ZPL containing more
467 THg than littoral BMI (Kahilainen et al., 2016a; Thomas et al., 2016; Kahilainen et al., 2017). Liver
468 and muscle THg values were negatively related with condition factor, which can be used as supporting
469 evidence for starvation and growth dilution (Cizdziel et al., 2002, 2003; Evans et al. 2015). Condition
470 factor does reflect spawning when gonads with low THg concentration are removed from the fish
471 body, potentially further condensing Hg in whitefish muscle (Madenjian et al., 2016). Increased $\delta^{15}\text{N}$
472 values have been linked to metabolic-stress associated with starvation (Moreno et al., 2015) in some
473 taxa. Here, we found slight seasonal changes in muscle and liver $\delta^{15}\text{N}$ values showing the highest
474 values in winter and lowest in autumn. Therefore, the positive correlation of $\delta^{15}\text{N}$ and THg values in
475 muscle and liver model possibly reflects winter starvation, when fish use protein reserves from both
476 liver and muscle. Positive correlation between sexual maturity and THg concentrations in both liver
477 and muscle models indicate a high significance of gonad development and spawning to the THg
478 concentrations. Spawning may be related to starvation in whitefish, due to the high cost of gonad
479 investment and low prey abundance during winter spawning period (Hayden et al., 2014a). In the
480 liver model, the negative relationship between GSI and THg supports this idea; whitefish GSI was
481 lowest and THg was highest after spawning in February-May, when we also reported the highest THg
482 concentrations. In the muscle model, the opposite correlation between GSI and THg (positive) could
483 be explained by random effect in the model since we found no correlation between GSI and muscle
484 THg through simple linear regression analysis: in addition GSI had low significance in the multiple
485 linear regression model explaining the variation in muscle THg. Sampling month significantly
486 affected THg concentration, but the effect was positive in the liver model and negative in the muscle

487 model, likely indicating that Hg is translocated faster in liver than in muscle. This could be explained
488 by the different turnover times of these tissues, meaning that late summer derived Hg can be measured
489 faster in liver (early winter) than in muscle (mid-winter). Therefore, the positive correlation between
490 sampling month and liver THg could be explained by the high THg values in early winter (December).
491 Most likely, the negative correlation between muscle THg concentrations and sampling month was
492 driven by the high THg concentrations in mid-winter (February) and low concentrations in summer
493 (June-July).

494 4.5 Monitoring and human health

495 An interesting aspect of our results was that intra-annual variability in THg concentrations of
496 whitefish exceeded inter-annual variation, evident also in multiple linear regression analyses, where
497 seasonal factors indicating diet ($\delta^{13}\text{C}$) and condition were generally more important than fish total
498 length. As the year-round maximum variation in the tissues (21%), is surprisingly high compared to
499 inter-annual (8%) accumulation in muscle, and that bioaccumulation slopes varied from non-
500 significant or shallow in summer to highly steep and highly significant in winter, we suggest that such
501 seasonal variation needs to be considered in future studies and especially in any long-term THg
502 monitoring program. This is particularly important as the aims of Hg monitoring programs are
503 typically related to human health (AMAP 2011). Primarily, the sampling month should be
504 standardized but since the annual anomalies, the seasonal succession (e.g. temperature build up)
505 should be quantified as well since they might affect on THg of fish. Whitefish is the most important
506 target fish of local fisheries year-round and represent a stable proportion of subsistence diet of native
507 and non-native people (Thomas et al. 2016; Kahilainen et al., 2017). Although THg levels in all our
508 fish samples were below national health limits (i.e. $0.5 \mu\text{g g}^{-1}$ wet mass; approx. $2.0\text{-}2.5 \mu\text{g g}^{-1}$ dry
509 mass), the year-round patterns observed for whitefish may be relevant in other systems e.g. in other
510 autumn or winter spawning fish such as many salmonids (Arctic charr, brown trout, lake trout,
511 vendace) with putative winter starvation after reproduction. In spring spawning species, patterns

512 could be different as the summer growing season starts immediately or soon after their reproduction,
513 but additional year-round studies are needed to test this. For example, an annual variation of 21%
514 would create a potential for THg values to exceed health limit guidelines and regional fish
515 consumption regulations. Furthermore, seasonal changes of THg concentrations and bioaccumulation
516 slopes in fish may lead to increased risk to human health in regions, where monitoring is restricted to
517 low THg months i.e. mid summer. Depending on the aims of human health monitoring, both summer
518 and winter sampling may be advisable as subsistence fishing is very common across Arctic and
519 subarctic lakes in both seasons.

520 4.6 Conclusions

521 We revealed clear seasonal changes in the concentration and bioaccumulation slopes of THg in
522 whitefish muscle and liver tissues. The results indicated that both starvation and growth dilution drive
523 seasonal changes in THg concentrations in both tissues. Our data also provides new evidence for the
524 role of pelagic diet shifts on increasing THg concentrations in both muscle and liver. The seasonal
525 changes in diet and condition were generally more important factors than fish length explaining THg
526 concentrations of different tissues of fish. The intra-annual variation in THg concentrations was
527 higher than inter-annual bioaccumulation over years, in addition we found that bioaccumulation
528 varied seasonally being highest in winter and low or non-significant in summer. Underestimating the
529 impact of seasonality on Hg dynamics of fish has the potential to affect conclusions regarding health
530 risk and therefore it is essential to consider seasonal factors in future studies and Hg monitoring
531 programs.

532

533 Acknowledgements

534 We thank Elina Nystedt, Esa Tulisalo, Grace Hayden, Jaana Marttila, Kalevi Laurila, Olli Saari, Oula
535 Kalttopää, Stephen Thomas and Tommi Malinen for their valuable help in laboratory and fieldwork.
536 Kilpisjärvi Biological Station and Department of Environmental Sciences provided the field and

laboratory facilities. Financial support came from Academy of Finland (1140903 and 1268566 to KKK) and MSc thesis stipends to OK from University of Helsinki Alumni Association Fund and Societas Biologica Fennica Vanamo. CH was supported by Fondecyt 1151515 and the Núcleo Milenio de Salmónidos Invasores.

Supplementary data

Supplementary data to this article can be found online at: URL

Table 1S. Detailed stomach content table.

Table 2S. Total mercury (THg), stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and C:N values $\pm$ SD of littoral, pelagic and profundal invertebrates among months.

Table 3S. Stepwise model selection for linear multiple regression analysis based on minimum AIC values.

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Table 1. Ecological characteristics (sample size; age; body size; somatic mass; condition; sexual maturity; gonadosomatic index, GSI; gillraker count; C:N ratios; stable isotopes and diet) of whitefish. For each continuous variable, mean $\pm$ SD values are presented, for different prey groups mean percentage contribution is presented. Variables marked with * indicate year class 2003 whitefish data. Superscript with small letters^{a-h} presented before mean values indicate statistical difference between corresponding mean value (a=Sep-10, b=Dec-11, c=Feb-12, d=May-12, e=Jun-12, g=Sep-12, h=Aug-14). Pelagic zooplankton are divided into cladocera (*Bosmina* sp. and *Holopedium gibberum*) and copepoda (Calanoida and Cycloida), benthic ZPL indicates benthic zooplankton groups (*Eurycercus* sp., *Megacyclops* sp.). Benthic macroinvertebrates (BMI) includes Chironomidae, Ephemeroptera, *Lymnaea* sp., *Pisidium* sp., Plecoptera, Simuliidae, Trichoptera and *Valvata* sp. Fish include whitefish eggs and alpine bullhead, whereas the other group includes macrophyte parts, Corixidae, Hydracarina, Tabanidae and *Polyphemus pediculus*.

	^a Sep-10	^b Dec-11	^c Feb-12	^d May-12	^e Jun-12	^f Jul-12	^g Sep-12	^h Aug-14
Whitefish (n)	30	30	30	30	18	30	29	27
Age	^g 6.2 $\pm$ 2.1	6.4 $\pm$ 2.5	7.3 $\pm$ 2.4	6.2 $\pm$ 3.2	5.8 $\pm$ 4.6	5.6 $\pm$ 3.9	^a 8.1 $\pm$ 2.4	7.4 $\pm$ 4.2
Total length (mm)	247 $\pm$ 50	245 $\pm$ 52	248 $\pm$ 49	221 $\pm$ 71	227 $\pm$ 100	210 $\pm$ 94	269 $\pm$ 50	234 $\pm$ 83
Total mass (g)	133.0 $\pm$ 89.1	117.1 $\pm$ 55.7	117.8 $\pm$ 50.6	97.5 $\pm$ 76.7	141.8 $\pm$ 194.3	106.8 $\pm$ 95.2	165.0 $\pm$ 55.1	126.1 $\pm$ 93.7
Somatic mass	^g 111.2 $\pm$ 50.1	^g 117.2 $\pm$ 50.3	^g 97.2 $\pm$ 76.3	140.3 $\pm$ 191.4	^g 105.5 $\pm$ 93.4	^{bcd} 162.0 $\pm$ 54.2		
Somatic mass* (g)	^g 138.3 $\pm$ 26.5	145.7 $\pm$ 30.7	157.6 $\pm$ 24.1	^g 123.0 $\pm$ 26.8	182.61 $\pm$ 51.2	^{be} 185.5 $\pm$ 25.8		
Condition factor	^{def} 0.75 $\pm$ 0.10	^g 0.69 $\pm$ 0.07	^g 0.70 $\pm$ 0.07	^{ag} 0.67 $\pm$ 0.07	^{ag} 0.65 $\pm$ 0.10	^{ag} 0.67 $\pm$ 0.11	^{bcd} 0.78 $\pm$ 0.05	^f 0.71 $\pm$ 0.08
Condition factor*	0.75 $\pm$ 0.04	^g 0.72 $\pm$ 0.05	^g 0.71 $\pm$ 0.09	0.72 $\pm$ 0.04	0.63 $\pm$ 0.08	0.75 $\pm$ 0.07	^{bc} 0.79 $\pm$ 0.05	0.74 $\pm$ 0.06
Sexual maturity	^{ce} 2.53 $\pm$ 0.94	3.03 $\pm$ 1.54	^{adef} 4.43 $\pm$ 2.56	^c 2.40 $\pm$ 1.57	^c 2.39 $\pm$ 1.79	^c 2.30 $\pm$ 2.09	^a 3.41 $\pm$ 1.18	2.91 $\pm$ 1.51
GSI		^d 2.9 $\pm$ 4.7	^g 0.4 $\pm$ 0.3	^{bg} 0.2 $\pm$ 0.2	^g 0.5 $\pm$ 0.6	^g 0.6 $\pm$ 1.2	^{cdef} 1.7 $\pm$ 1.6	
Gillraker count	25.2 $\pm$ 1.2	25.0 $\pm$ 2.1	24.3 $\pm$ 2.0	24.2 $\pm$ 1.6	23.9 $\pm$ 1.5	24.0 $\pm$ 1.7	24.4 $\pm$ 1.6	24.1 $\pm$ 2.0
C:N Liver		^d 4.5 $\pm$ 0.6	^f 4.5 $\pm$ 0.7	^b 4.1 $\pm$ 0.2	4.2 $\pm$ 0.3	^{bc} 4.0 $\pm$ 0.4	4.2 $\pm$ 0.2	
C:N Muscle	3.2 $\pm$ 0.0	3.2 $\pm$ 0.0	3.2 $\pm$ 0.1	3.2 $\pm$ 0.1	3.2 $\pm$ 0.1	^h 3.2 $\pm$ 0.1	^h 3.2 $\pm$ 0.1	^{fg} 3.2 $\pm$ 0.1
$\delta^{13}\text{C}$ Liver (lipid free)		-23.5 $\pm$ 1.7	-23.7 $\pm$ 2.1	-23.7 $\pm$ 1.8	-23.3 $\pm$ 8.6	-24.9 $\pm$ 1.9	-23.8 $\pm$ 1.4	
$\delta^{13}\text{C}$ muscle (lipid free)	-24.6 $\pm$ 2.9	^e -25.0 $\pm$ 1.7	^e -25.2 $\pm$ 1.4	-24.6 $\pm$ 1.5	^{bch} -22.1 $\pm$ 3.0	-24.3 $\pm$ 1.2	-24.6 $\pm$ 1.2	^e -25.1 $\pm$ 1.5
$\delta^{15}\text{N}$ Liver		8.0 $\pm$ 0.6	^g 8.2 $\pm$ 0.6	^g 8.3 $\pm$ 0.7	^g 8.3 $\pm$ 0.8	^g 8.1 $\pm$ 0.5	^{cdef} 7.5 $\pm$ 0.5	
$\delta^{15}\text{N}$ Muscle	^b 8.4 $\pm$ 0.5	^a 8.7 $\pm$ 0.3	8.6 $\pm$ 0.3	8.6 $\pm$ 0.4	8.5 $\pm$ 0.4	8.5 $\pm$ 0.4	8.6 $\pm$ 0.3	8.6 $\pm$ 0.4
Stomach fullness	^{bf} 3.8 $\pm$ 1.6	^{acdefgh} 0.4 $\pm$ 0.9	^{bfg} 2.3 $\pm$ 2.6	^{bf} 3.3 $\pm$ 2.5	^b 4.0 $\pm$ 1.6	^{abcdh} 5.4 $\pm$ 1.9	^{bc} 4.6 $\pm$ 1.7	^{bf} 3.7 $\pm$ 1.7
Empty stomachs (n)	1	24	12	8	1	0	0	1
Cladocera	5.4				8.3	49.6	2.6	34.0
Copepoda	35.8		32.4	26.0	0.7	0	4.0	3.0
Benthic ZPL	26.1	7.7		20.0		24.9	60.3	21.4
BMI	22.2	92.3	64.4	50.1	91.0	19.8	16.9	30.1
Terrestrial insects	8.6					5.3	16.2	11.5
Fish			3.2	3.9				
Other	1.9					0.4		

764 Table 2. THg concentrations ($\mu\text{g g}^{-1}$ d.w.) $\pm$ SD (n) of liver and muscle tissues of whitefish and
765 invertebrates by sampling months and years. Superscript with capital letters^{A-H} presented before THg
766 means of different tissues indicates statistical difference between corresponding mean value (A=Sep-
767 10, B=Dec-11, C=Feb-12, D=May-12, E=Jun-12, G=Sep-12, H=Aug-14). Superscript small letters^{a-}
768 ^c in grand mean row indicates statistical differences between corresponding ^{a-c} invertebrate group.

	Liver	Muscle	^a ZPL _{pelagic}	^b BMI _{littoral}	^c BMI _{profundal}
^A Sep-10		^{BCEH} 0.17 $\pm$ 0.04 (30)			
^B Dec-11	^F 1.56 $\pm$ 0.82 (29)	^A 0.22 $\pm$ 0.04 (30)	0.040 (1)	0.050 (2)	0.573 (2)
^C Feb-12	^{EF} 1.70 $\pm$ 0.88 (30)	^{A^{EF}} 0.24 $\pm$ 0.05 (30)	0.061 (1)	0.057 (2)	
^D May-12	1.39 $\pm$ 0.99 (30)	0.22 $\pm$ 0.06 (30)	0.065 $\pm$ 0.006 (3)	0.055 (2)	
^E June-12	^C 0.87 $\pm$ 0.72 (17)	^{AB} 0.20 $\pm$ 0.06 (18)		0.057 (1)	
^F July-12	^{BCG} 0.88 $\pm$ 0.42 (30)	^B 0.19 $\pm$ 0.04 (30)	0.060 $\pm$ 0.002 (3)	0.036 (2)	
^G Sep-12	^F 1.29 $\pm$ 0.57 (28)	0.20 $\pm$ 0.04 (29)	0.067 $\pm$ 0.002 (3)		0.319 (2)
^H Aug-14		^A 0.23 $\pm$ 0.05 (28)	0.084 $\pm$ 0.005 (6)	0.027 $\pm$ 0.010 (5)	0.205 (2)
Grand mean	1.31 $\pm$ 0.81 (164)	0.21 $\pm$ 0.05 (225)	^b 0.070 $\pm$ 0.013 (17)	^a 0.042 $\pm$ 0.014 (14)	0.366 $\pm$ 0.356 (6)

769

770 Table 3. Multiple linear regression models explaining THg variation in liver and muscle. Coefficient
771 of determination (adjusted R^2) and residual standard error (RSE) are presented for each model. Slope
772 (B), the standard error of the estimate (SE), the significance indicators (t and p) and AIC values are
773 presented for each factor selected to the models. The results of AIC stepwise procedure are presented
774 in Table S3.

775

Tissue	Factor	B $\pm$ SE	t	p
Liver adj. R^2 =0.50, RSE=0.577 $F_{7, 156}=24.0$, $p<0.001$ AIC=-172.67	Sexual maturity	0.241 $\pm$ 0.042	5.753	<0.001
	$\delta^{13}\text{C}$ liver	-0.120 $\pm$ 0.024	-4.928	<0.001
	Condition factor	-2.582 $\pm$ 1.092	-2.997	0.003
	Constant	-2.985 $\pm$ 1.092	-2.733	0.007
	GSI	-0.060 $\pm$ 0.022	-2.677	0.008
	$\delta^{15}\text{N}$ liver	0.197 $\pm$ 0.083	-2.677	0.019
	Total length	0.003 $\pm$ 0.001	2.367	0.020
	Sampling month	0.034 $\pm$ 0.016	2.100	0.037
Muscle adj. R^2 =0.55, RSE=0.034 $F_{7, 159}=30.18$, $p<0.001$ AIC=-1118.82	$\delta^{13}\text{C}$ muscle	-0.013 $\pm$ 0.002	-8.225	<0.001
	Condition factor	-0.211 $\pm$ 0.046	-4.570	<0.001
	Total length	0.0003 $\pm$ 0.0001	3.945	0.003
	Sampling month	-0.003 $\pm$ 0.001	-2.974	0.004
	Sexual maturity	0.006 $\pm$ 0.003	2.274	0.024
	Constant	-0.172 $\pm$ 0.080	-2.147	0.033
	$\delta^{15}\text{N}$ muscle	0.016 $\pm$ 0.008	2.088	0.038
	GSI	0.002 $\pm$ 0.001	1.746	0.082

777 Figure legends

778 Figure 1. Map of L. Kilpis located in northern Fennoscandia. Depth contour areas are presented with
779 different shades of grey and arrows shows afferent and efferent rivers. All samples were collected
780 from area A marked with ellipse.

781 Figure 2. Box-Whisker plots showing seasonal variation in whitefish mercury concentration in liver
782 (A), muscle (B). Bold horizontal lines indicate the median value, the boxes represent first and third
783 quartile and whiskers represents minimum and maximum values. Outliers (black circles) are
784 presented if there are data points smaller or larger than the difference between first and third quartile.

785 Figure 3. Linear regression models showing THg bioaccumulation in whitefish liver (A-F) and
786 muscle (G-L) tissues. Dashed lines represent 95% confidence intervals.

787 Figure 4. Linear regression models with 95% confidence intervals (dashed lines) illustrating
788 seasonally changing THg relationship between liver and muscle tissue of whitefish in December 2011
789 (A), February 2012 (B), May 2012 (C), June 2012 (D), July 2012 (E) and September 2012 (F).

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793 Fig. 1.

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800 Fig. 4.