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

Subarctic lakes are characterised by extreme seasonal variation in light and temperature which influences growth, maturation, condition and resource use of fishes. However, our understanding of how seasonal changes affect mercury concentrations of fishes is limited. We conducted a year-round study (3 ice-covered months, 3 open-water months) with open-water inter-annual aspect (3 years: samples from August/September), focusing on total mercury (THg) concentrations and ecological characteristics of a common freshwater fish, European whitefish (Coregonus lavaretus (L.)) from a subarctic lake. We measured THg concentrations from tissues with fast [liver n=164] and moderate [muscle, n=225] turnover rates, providing information on THg dynamics over different temporal scales. In both tissues, lipid-corrected THg concentrations were highest in winter (liver: 1.70±0.88 µg/g, muscle: 0.24±0.05 µg/g) and lowest in summer (liver: 0.87±0.72 µg/g, muscle: 0.19±0.04 µg/g). THg concentrations increased in winter following the summer-autumn dietary shift to pelagic zooplankton and starvation after spawning. Whitefish THg concentrations decreased towards summer, and were associated with consumption of benthic macroinvertebrates and subsequent growth dilution. Mercury bioaccumulated in both tissues with age, both showing the strongest regression slopes in winter and lowest in summer. THg concentrations in liver and muscle tissue were correlated throughout the year, however the correlation was lowest in summer, indicating high metabolism during somatic growing season in summer and growth dilution. Multiple linear regression models explained 50% and 55% of the THg variation in liver and muscle both models dominated by seasonally-variable factors i.e. sexual maturity, δ13C, and condition factor. Seasonally varying bioaccumulation slopes and the higher level of intra-annual variation (21%) in whitefish THg concentration in muscle than the inter-annual accumulation (8%) highlight the importance of including seasonal factors in future THg studies.

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

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Suggested reviewers	Michael Power, Hans Fredrik Veiteberg Braaten, Heidi Swanson, Jim Reist, Andrew Muir

Submission Files Included in this PDF

File Name [File Type]

Cover Letter_Keva el al 2017_revised.docx [Cover Letter]

Response Letter_Keva et al 2017.docx [Response to Reviewers]

Manuscript_with changes_Keva et al. 2017_Environ Pollut.docx [Revised Manuscript with Changes Marked]

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Supplementary information for Keva et al.docx [Supporting File]

Dear Editor,

I am honoured to submit our revised manuscript entitled "Total mercury concentrations in liver and muscle of European whitefish (*Coregonus lavaretus* (L.)) in a subarctic lake - assessing the factors driving year-round variation"

We are grateful for the reviewer's invaluable input in commenting our manuscript. We have followed almost all suggestions and issues mentioned in reviewer's comments. We have outlined every change made point by point, please find them in Response letter. If the comments were not followed, we have justified our decision. We believe our manuscript has improved greatly after this major revision and sincerely hope our revised manuscript would be considered to be published in Environmental Pollution.

Sincerely,
Ossi Keva

Response to reviewers' comments

Comments from the editors and reviewers:
-Reviewer 1

This manuscript contributes to the ongoing studies of the Kahilainen team which is investigating mercury concentrations in European whitefish populations. I had previously been sent this paper for review by another journal. That editor made his/her decision before my review was submitted. I also see that little has changed from the earlier submission.

The research conducted by the Kahilainen team is exciting, particularly their findings of different ectomorphs in different lakes with different feeding types and morphology. This paper builds on those studies by examining seasonal changes in feeding and mercury concentration in whitefish in one lake. It is generally informative and am recommending for publication following major revisions. My main issue with the paper is the investigation of mercury concentrations in scales which is detracting from the paper and should be removed or mentioned in a supporting section. In addition, there are gaps in the food web collections and confusions which result in a weaker linking between seasonal variations in mercury in the diet and mercury in various body compartments. Comments are below.

The introduction begins as a generic introduction of mercury in the environment rather than building on the studies of the Kahilainen team and the specific objectives of this study. That is, the fact that whitefish fish may feed in different environments through the year, with implications to mercury concentrations in various tissues. I found the mention of mercury in scales trivial and not worthy of study in terms of metabolism or seasonal study. Presumably once laid down, mercury would not be metabolised or transformed and so the value of whole scale measurements of fish several years old is questionable. Given that mercury concentrations in profundal benthos is about the same concentration as in whitefish muscle, it would seem that whitefish are consuming mainly pelagic zooplankton and littoral benthos but with some profundal benthos.

Reply: We thank the editor and reviewer for the critical and supporting comments. We have now followed the comments almost exclusively, except removal of inter-annual comparisons (2010, 2012 & 2014) that we consider important in comparison to intra-annual results (2011-2012). As part of our major revision, we have totally removed the part of the manuscript detailing our analyses of scales in reflecting the reviewer's comments.

We have added supplementary materials providing invertebrate taxa THg, stable isotopes and C:N ratios as well as detailed dietary table and multiple regression model AIC value table. The introduction is largely rewritten including significant part of our previous studies on whitefish ecology and putative implication on THg.

Regarding the reviewer's comments about profundal benthos – they are of very low abundance in L.Kilpis. Our SIA results indicate that whitefish in the lake largely consume littoral benthos and pelagic zooplankton, limiting support for the idea that most of the whitefish Hg would be derived from profundal benthos. Mercury has tendency to magnify in food chain, and we think the high difference between littoral and pelagic prey items with whitefish muscle is caused by biomagnification. However, our coverage of algae and the wider fish community is limited, to allow for a robust calculation of biomagnification slopes from the food web in the current paper. That could be a very interesting line to follow in subarctic lakes with suitable data.

Since I am familiar with the research conducted by this team, I am curious as to which European whitefish morph type they are investigating and why this lake was picked.

Reply: We apologise for the lack of clarity here. The only whitefish morph in L.Kilpis is the large sparsely rakered whitefish morph (LSR), a generalist morph that consumes both pelagic and benthic prey. We have now clearly indicated the whitefish type in this lake using term monomorphic. In this and previous studies, we have counted number of gill rakers from each whitefish individual in L.Kilpis and there is no sign of multiple morphs in this lake.

Lines 125-130. This would be a good place to bring in the findings on ectomorphs and different feeding types. Any thoughts as to why whitefish switch from consuming benthos to zooplankton in summer.

Reply: This part is now completely rewritten with introduction of whitefish populations in this subarctic region and their resource use. As the monomorphic whitefish populations are the most common type in this region, we consider the current study relevant to assess both intra- and inter-annual patterns of THg concentrations and bioaccumulation. Based on previous studies and prey availability, the generalist whitefish will utilize the most available prey type in each season. The further importance of dietary shift to zooplankton is likely related to high quality of this prey as a source of fatty acids (especially DHA and EPA) that are of crucial importance in many fish somatic and gonadosomatic tissues.

Line 142 mentions that gill rakers are being counted but without explanation as to why. I assume that the same ectomorph is being investigated throughout the year; if not then the seasonal changes may be related to different ectomorphs captured through the year with the methods used.

Reply: We have now revised the introduction including explanation of gill raker counts in whitefish studies. Briefly, number of gill rakers is a heritable trait in whitefish (and other fishes too) that correlates with many phenotypic traits and niche use in general. It is important to count number of gill rakers in monomorphic populations too, as there is still individual variation (often circa range of 10 rakers) that could be correlated to resource use. In current case of L. Kilpis, number of gill rakers did not enter to multiple linear regression models, but it was important to include this trait initially. L. Kilpis has only one morph (LSR whitefish) present.

Lines 143-144. I do not understand why scales were examined. Since a growth layer is laid down each year, and presumably does not change chemically (i.e. mercury not metabolized and lost), the value of this in a seasonal study is questionable. To my mind, this detracts from the paper. Hypothesis 2 has little merit. Hypothesis 3 is not that novel.

Reply: The scale data has now been completely removed and all hypothesis has been revised. We thank reviewer for excellent suggestions for alternative hypothesis 2 that now concerns seasonal bioaccumulation and tissue correlations. We found these results highly interesting and likely important for future THg studies. Hypothesis 3 might be studied partly before, especially in snap-shot studies of single month, but we are unaware of any studies with annual data. Thus we consider H3 as crucially important to discuss the seasonally changing THg concentrations in monomorphic whitefish. Here, we have followed reviewer comments of using AIC as selection criteria and we also tested the interaction terms that are explained in more detail below.

Lines 166-168. What is the conductivity and pH?

Reply: We have added these values to method lines 174 and 175. The annual average of pH is 7.2 and it is stable throughout the year. Conductivity is ca. 3mS m^{-1} through out the whole water column (surface: 3.05 mS m^{-1}; bottom 2.99 mS m^{-1}) year round.

Lines 184:186. The authors should indicate that most of their collections were made in one year, i.e., December 2011 to September 2012 and followed whitefish from winter into the following fall. I am not sure why September 2010 and 2014 sample data are included as they do not illustrate seasonal patterns and creates some confusion. Climate varies from year to year and mercury in lake food webs may be quite different in a cold year than warm. Table 1 gives 2014 month as August.

Reply: We thank reviewer for these comments. We included (in addition to the intra-annual data from 2011-2012), the single month dataset of September 2010 and August 2014 to assess inter-annual concentration. We consider these highly relevant in order to assess intra-annual results with longer term data. We agree that there are climatic variation in this region, and these will affect Hg in food webs. However, Lake Kilpis is a relatively deep lake with large water volume having a buffer effect on extreme air temperatures. The mercury in Arctic and subarctic lake food webs mainly originates from air deposition (AMAP 2011), the annual air Hg concentrations has remained relatively stable (circa 1.4 ng m^{-3}) in the closest measuring station (circa 200 km south of the study area) during the measurement period (1996-2016). The air deposition of Hg is hypothesized to decrease with increasing temperature due to the kinetic properties of bromine and mercury (AMAP 2011). However, differences in precipitation and temperature are most likely crucial to methylation processes in the catchment and therefore may affect THg in food webs. In addition to the bottom up effects, THg of fish may differ as well intra-annually due to the differences in feeding environment and/or the cumulative annual temperature. Taken collectively, we would like to keep September 2010 and August 2014 data in the manuscript since the data show clearly the inter-annual accumulation of THg.

Lines 193-193. Was the plankton mesh size 50 µm which seems very fine. On the other hand, benthos was washed through a 500 µm mesh net which would have lost a lot of epibenthic zooplankton. I do not understand why littoral and profundal benthic samples were not reported separately in table 1 as in table 2. Also, it is unfortunate that zooplankton tows not made in littoral zone as plankton assemblages can be different. I assume the littoral zone was rocky and devoid of macrophytes given that an Ekman dredge was used to collected benthos. These collections are unclear because table 1 separates zooplankton into cladocerans and copepods; benthic zooplankton; benthic macroinvertebrates and includes terrestrial insects and fish whereas table 2 reports mercury in pelagic zooplankton, littoral benthic macroinvertebrates, and profundal benthic macroinvertebrates. For the core seasonal year (December 2011-September 2012), one month is missing for zooplankton with two months with only one replicate sample; one month for littoral benthic invertebrates with only 1-2 replicates which is very small and standard deviations not shown; and there are only two months for profundal benthic invertebrates with a small number of replicates and no standard deviations. Carbon and nitrogen isotopes also were not measured (or reported) in prey which makes for a poor food web study. Overall, the study design for the food web portion is poor with major gaps and shortcomings in the number of collections, replicates, and identification of the taxa analyzed.

Reply: We agree that our invertebrate data could have been more conclusive for THg. However, we considered even a patchy data important than no data at all, when explaining the relatively levels of THg in prey animals as well as stable isotope values. These data are now added as supplement table 1. We used 50 µm mesh size zooplankton net to collect pelagic cladocerans and copepods that are commonly consumed by whitefish in this region, whereas larger mesh of 500 µm was used for benthic macroinvertebrates. In the region, a smaller mesh in benthic sampling cause problems with clogging sediment material. The efficient sampling of large biomass of benthic zooplankton would have required completely different sampling methods e.g. littoral hand netting during peak abundance of benthic cladocerans or potentially a benthic sledge for very fine sediment areas for copepods. Unfortunately, these were not conducted. Table 1 shows invertebrates found from the stomachs of whitefish. Therefore, it is not possible to separate littoral and profundal BMI in this table. Oligochaeta and Chironomids were the only macroinvertebrates found in profundal zone (but they were also found in littoral zone). In contrast to this, table 2 shows our sampled invertebrates from different habitats. Different pelagic ZPL taxa (cladocerans, copepods) were not possible to separate for Hg analysis from the mixture samples. We have now added annual detailed fish diet data as Table S1 and THg, SIA and C:N data of sampled invertebrates from lake (Table 2S) to supplementary data. For some animal taxa occurring through whole benthic slope e.g. Chironomid larvae, we cannot separate them in fish diet for littoral or profundal prey (Table 1S).

Lines 200-206. Why were nets set in deep water set only above bottom and not also nearer the surface; whitefish inferred to have consumed zooplankton which presumably would have been mainly in upper layers during thermal stratification. How long were the nets set and was digestion of stomach contents and issue in gut content identification?

Reply: We thank reviewer for these considerations. The lack of pelagic data is a clear disadvantage of our study. The lack of pelagic data is related to extreme difficulty to sample pelagic habitat in winter, when floating line of the net or separate floats will freeze to overlaying ice-cover. As we started the annual study from winter onwards using benthic gear only, we considered to keep sampling design similar during the whole period. In these cold lakes, whitefish are active and all habitat types are very connected e.g. indicated by a large amount of pelagic zooplankton prey in benthic captured fish in the summer. We agree that full habitat specific annual study would be optimal to test various questions regarding to THg concentration and bioaccumulation, but unfortunately we cannot proceed there with current data. The nets was set for overnight in summer for 10-12h and in winter 24-48h. The decomposition of stomach contents were not an issue in identification as water temperature is low throughout the year.

Line 209-214. Why were both otoliths and scales used to age fish? Explain why gill rakers were counted. Explain why GSI calculated. The liver LSI also would have been a nice addition and why was this not calculated as presumably determined when drying tissues. If available, please include. The authors could also look at percent water content of tissues if they have and seasonal variation. When do whitefish reproduce and how does this affect GSI? How does this index differ between males and females? Why are authors considering year class and not age? Since most data are for 2012 collections, fish were 9 years old.

Reply: Both otoliths and scales were used for age determination to improve the reliability of the determinations. Use of multiple aging structures for whitefish has been documented to increase the accuracy of age determination (revision lines 224-225). Calculation of gill raker number is a standard method in whitefish research as it captures much of the phenotypic variation and is related to resource use. This part is explained in introduction (lines 116-122) and methods (line 229-231). Gonadosomatic index (GSI) is a continuous measure and documents the stage of gonads during the year lowest values in spring (May) and highest during the spawning (December, see table 1). It is important measure related to relative share on energy division to somatic and gonad growth as well as related to overall condition and starvation. While, females will invest more energy to eggs than males to milt, we decided to not present values for both sexes. Here, such separation would have been then applied for all values in table. If sex would have very important determinant of THg, when we would have seen it multiple regression analyses. Sexual dimorphism in whitefish is not pronounced as growth and maturation broadly follows the same patterns.

Unfortunately, we lack the LSI and water content data, but we will keep those in our minds for future studies. Year class approach was presented as the year class 2003 was so dominant during the study years. Please note, that the bioaccumulation along whitefish age is now presented as a figure 3.

Line 238. I really do not see the point of looking at mercury in scales for a seasonal study. Feel detracts from paper without more justification. Findings intuitive. Should be removed.

Reply: The scale data has now been fully removed.

Line 269. I am not a statistician but prefer ACI analyses over stepwise regressions which I believe are an older technique and less commonly used. Part of this does not seem central to the new aspects of the paper and analyses of this type have been done for the European whitefish populations in other lakes in the area. Also, the authors do not discuss interaction terms and their exploration. Under results, length, weight and condition factor are shown by month. The authors also could consider showing the predicted weight at a specific length, e.g., 220 mm to better show seasonal variation in weight and condition factor.

Reply: Thank you for suggesting a better statistical method for model selection. We have now conducted new multiple linear regression models, where the best model is selected using AIC procedure (Table 4). Please see supplementary table S3 for best model selection. In addition, we run the stepwise with the interaction terms (See below Table 1R and 2R), however we are not convinced about the superiority of these interaction models since they include so many variables with relatively little improvement to the coefficient of determination. Therefore, we would prefer to use the ^1 models used now in revised manuscript. However, we are willing to reconsider this if it is a major issue. In L. Kilpis, year class 2003 dominates the samples and we have calculated the somatic mass and condition for this specific year class in Table 1. We think that would be highly standardized way to show increase in somatic mass and change in condition for standardized group.

Table 1R. Forward stepwise model selection for linear multiple regression analysis based on minimum AIC values. The variables in models are: Sexual maturity (SexM), tissue specific stable isotopes values of carbon and nitrogen (d13C & d15N), gonadosomatic index (GSI), condition factor (CF), sampling month (Month), total length (TL). The interaction terms are marked with colon. The selected models are in bold.

Model	AIC
Liver	
{}	-66.9
SexM	-121.0
SexM+d13C	-145.1
SexM+d13C+d15N	-159.9
SexM+d13C+d15N+GSI	-162.9
SexM+d13C+d15N+GSI+SexM:GSI	-169.2
SexM+d13C+d15N+GSI+SexM:GSI+CF	-172.2
SexM+d13C+d15N+GSI+SexM:GSI+CF+CF+SexM	-177.4
SexM+d13C+d15N+GSI+SexM:GSI+CF+CF:SexM+Month	-180.2
SexM+d13C+d15N+GSI+SexM:GSI+CF+CF:SexM+Month+Month:GSI	-185.1
SexM+d13C+d15N+GSI+SexM:GSI+CF+CF:SexM+Month+Month:GSI+Month:CF	-189.5
SexM+d13C+d15N+GSI+SexM:GSI+CF+CF:SexM+Month+Month:GSI+Month:CF+Month:d13C	-191.3
SexM+d13C+d15N+GSI+SexM:GSI+CF+CF:SexM+Month+Month:GSI+Month:CF+Month:d13C+TL	**-192.7**
Muscle	
{}	-991.7
d13C	-1047.6
d13C+SexM	-1089.6
d13C+SexM+CF	-1099.7
d13C+SexM+CF+CF:d13C	-1121.3
d13C+SexM+CF+CF:d13C+d15N	-1132.6
d13C+SexM+CF+CF:d13C+d15N+TL	-1133.5
d13C+SexM+CF+CF:d13C+d15N+TL+Month	-1136.1
d13C+SexM+CF+CF:d13C+d15N+TL+Month+GSI	-1138.2
d13C+SexM+CF+CF:d13C+d15N+TL+Month+GSI+SexM:GSI	-1139.5
d13C+SexM+CF+CF:d13C+d15N+TL+Month+GSI+SexM:GSI+TL:d13C	-1140.9
d13C+SexM+CF+CF:d13C+d15N+TL+Month+GSI+SexM:GSI+TL:d13C+SexM:d13C	-1145.4
d13C+SexM+CF+CF:d13C+d15N+TL+Month+GSI+SexM:GSI+TL:d13C+SexM:d13C+d13C:d15N	-1145.7
d13C+SexM+CF+CF:d13C+d15N+TL+Month+GSI+SexM:GSI+TL:d13C+SexM:d13C+d13C:d15N+Month:d13C	**-1146.4**

Table 2R. Multiple linear regression models explaining THg variation in liver and muscle with interaction terms. Coefficient of determination (adjusted R^2), residual standard error (RSE), F and p-statistics and AIC values are presented for each model. Slope (B), the standard error of the estimate (SE), and the significance indicators (t and p) are presented for each factor selected to the models. The variables in models are: Sexual maturity (SexM), tissue specific stable isotopes values of carbon and nitrogen (d13C & d15N), gonadosomatic index (GSI), condition factor (CF), sampling month (Month), total length (TL). The interaction terms are marked with colon.

Model	Factor	B± SE	t	p
Liver	d13C	-0.1728± 0.0510	-3.3887	0.0009
adj.R^2=0.591, RSE=0.520	TL	0.0284± 0.0084	3.3646	0.0010
F$_{13,150}$=19.08, p<0.001	CF:TL	-0.0368± 0.0118	-3.1060	0.0023
AIC=-192.7	CF:Month	0.7293± 0.2423	3.0101	0.0031
	GSI:Month	0.0416± 0.0155	2.6816	0.0081
	d^{15}N	0.1795± 0.0760	2.3606	0.0195
	Constant	-4.7929± 2.1128	-2.2686	0.0247
	SexM:CF	0.9957± 0.5137	1.9380	0.0545
	d^{13}C:Month	0.0131± 0.0071	1.8414	0.0675
	GSI	-0.5691± 0.3408	-1.6699	0.0970
	SexM	-0.4289± 0.3648	-1.1758	0.2415
	Month	-0.1702± 0.2546	-0.6684	0.5049
	CF	-1.2791± 2.4729	-0.5173	0.6057
	SexM:GSI	-0.0015± 0.0489	-0.0303	0.9759
Muscle	TL	0.0024± 0.0008	3.1767	0.0018
adj.R^2=0.633, RSE=0.031	d^{13}C:TLmm	0.0001± 0.0000	2.9971	0.0032
F$_{13,153}$=22.97, p<0.001	d^{13}C:SexM	-0.0028± 0.0013	-2.1962	0.0296
AIC=-1164.4	GSI	0.0198± 0.0097	2.0403	0.0430
	SexM	-0.0596± 0.0318	-1.8782	0.0623
	SexM:GSI	-0.0042± 0.0024	-1.7845	0.0763
	d^{13}C:d^{15}N	-0.0059± 0.0035	-1.6720	0.0966
	d^{13}C:Month	0.0009± 0.0005	1.5876	0.1144
	Month	0.0191± 0.0136	1.4012	0.1632
	d^{15}N	-0.1176± 0.0844	-1.3933	0.1655
	d^{13}C:CF	0.0327± 0.0263	1.2444	0.2152
	CF	0.5866± 0.6450	0.9094	0.3645
	d^{13}C	-0.0099± 0.0350	-0.2828	0.7777
	Constant	-0.1502± 0.8453	-0.1777	0.8592

Line 274. The rationale for examining gill raker number is not given but presumably relates to ectomorph. Please explain better.

Reply: Please see introduction (lines 116-122) and methods (lines 229-231).

Line 283. Fish are referred to by year class when age would be more appropriate unless the authors wish to infer something unique about 2003 that contributed to this age group being dominant. With 30 fish caught each time and fish ranging from 1-11 years old, few fish were in each age class for each collection date. Figure 3 could also be shown as mercury age regressions for each sample month with 6 regressions on the figure. It is already know that mercury concentrations increase with fish age and the authors are trying to show that these relationships vary seasonally.

Reply: Many thanks for these comments. Indeed, monthly bioaccumulation regressions with age are now shown as we replaced the year class boxplots. We think this was a major improvement and we have revised hypothesis 2 accordingly.

Line 290. Do the authors mean profundal or littoral benthic macroinvertebrates?

Reply: We mean littoral benthic macroinvertebrates, it is now corrected to line 315.

Line 295. Carbon isotopes did not vary much seasonally with the June 2012 values being very high with a large standard deviation, especially the liver. The variation within months is less than variation between months. Again, I would have liked to see the littoral and profundal macrobenthic values reported separately in Table 1. Ideally dominant taxa would have been shown. Were there variations in diet with fish size and location in the lake?

Reply: The dietary shift of whitefish was clearest in stomach contents, but due to slow turn-over rate of muscle tissue, it is not very clear in SIA. Liver show some trend in lowest carbon value in July when whitefish consumed zooplankton heavily, however variation is too large for statistical significance. Unfortunately, our data was too small to examine reliably the stomach contents for different locations and size classes.

Line 297. Stable isotopes should have been measured in the prey items. Was this done and if not, why?

Reply: We have now added SIA, THg and C:N data of invertebrates to supplementary Table S2.

Line 307. Again the scale measurements add nothing to the paper for me.

Reply: Scale part is now removed.

Lines 310-311. Should the authors retain the extra years (2010 and 2014) can they explore reasons why mercury concentrations were higher in fish in some years than others? Temperature is a common variable that researchers in this field are considering. Some believe warm temperatures cause more mercury to be methylated while others feel cooler springs/summers result in lower growth and hence higher mercury concentrations. Temperature also could have been introduced as a variable affecting lipid concentrations, etc. This section is not clear to me or particularly informative. It is well known mercury concentrations increase in fish with age and that concentrations vary from year to year due to various conditions.

Reply: The reasons for the inter-annual bioaccumulation is discussed in lines 413-424. It is most likely caused by the aging of the whitefish population as the year class 2003 was dominant during the whole study 2010-2014. L. Kilpis is dominated by one large year class which keeps the younger year classes in check for 10-15 years. Regarding the temperature data, we can see some variation in air temperature, but the large water volume of L. Kilpis has stabilizing effect to air temperature variations. Presumably the temperature variation affects to methylation, bioaccumulation and magnification, but we strongly believe that major factor to bioaccumulation from 2010 to 2014 is caused by the aging of whitefish population.

Lines 327-341. An ACI analyses would show fewer significant influencing variables. Also it would more clearly show if month were a significant variable independent of changes in weight and condition factor and feeding. For example, more mercury may be methylated in some months than other.

Reply: We have now used AIC for ranking the different models. For details, please see our reply above.

Discussion: strength lies is the demonstration of seasonal variations in mercury concentrations which can be related to growth and condition factor and season. It is not so clearly shown what influence the changing diets have on this with a small number of replicates, standard deviations often not shown, no stable isotope data reported, and confusions on benthic zooplankton, pelagic zooplankton, littoral and profundal benthic macroinvertebrates. The discussion is very long for the data presented. Integrating this paper more with other research, including papers in review and likely to be published, would be an asset.

In summary, this is a potentially interesting paper but would benefit from a major revision to build on its strengths. The scale data does nothing for me and adds nothing to the paper. Consider adding liver somatic data if have and also a different type of statistical analyses which more clearly shows the predominant influencing variables and consider interactions. The food web portion (zooplankton and benthos) is not well-presented and suffers from data gaps, missing data (stable isotopes, taxa analyzed) and confusions in what is being discussed

Reply: We thank reviewer for critical comments that helped us significantly in major revision. We believe that exclusion of scale sections and addition of monthly bioaccumulation regressions and tissue comparison plots have strengthen the manuscript. We have also added three supplementary tables and one figure composing of prey isotopes, whitefish diet, AIC based model selection and inter-annual autumnal bioaccumulation. We recon these to be likely helpful for the most interested readers. The introduction and discussion parts are significantly revised and we hope our manuscript could be now considered for publication in journal.

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

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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.

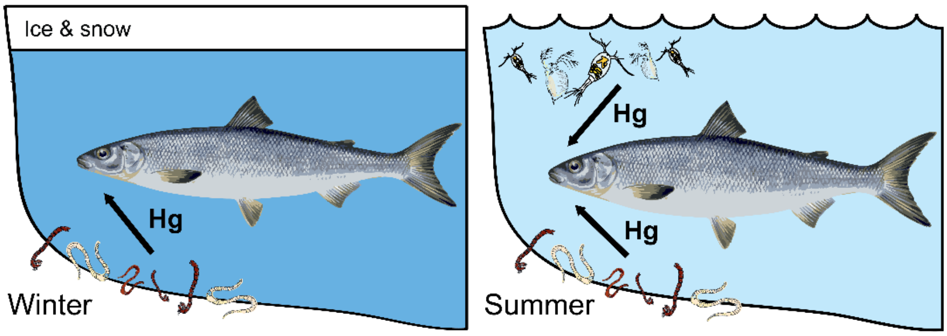
~~-Variation in scale THg was high, and seasonal patterns were less obvious.~~

-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:



Abstract:

Subarctic lakes are characterised by extreme seasonal variation in light and temperature which influences growth, maturation, condition and resource use of fishes. However, our understanding of how seasonal changes affect mercury concentrations of fishes is limited. We conducted a year-round study (3 ice-covered months, 3 open-water months) with open-water inter-annual aspect (3 years: samples from August/September), focusing on total ~~-~~mercury (THg) concentrations and ecological characteristics of a common freshwater fish,~~ the~~ European whitefish (*Coregonus lavaretus* (L.)) from a subarctic lake. We measured THg concentrations from tissues with fast (liver, n=164) and~~167),~~ moderate (muscle, n=225) ~~and slow (scale, n=75)~~ turnover rates, providing information on THg dynamics over different temporal scales. In both~~liver and muscle~~ tissues, lipid-corrected THg concentrations were highest in winter (liver: 1.70±0.88 µg/g, muscle: 0.24±0.05 µg/g) and lowest in summer (liver: 0.87±0.72 µg/g, muscle: 0.19±0.04 µg/g). THg concentrations increased in winter following the summer-autumn dietary shift to pelagic zooplankton and starvation after spawning.~~ in mid-winter.~~ Whitefish THg concentrations decreased towards summer, and were associated with consumption of benthic macroinvertebrates and subsequent growth dilution. ~~THg concentrations recorded from scales were low and displayed high variance, showing the lowest value in May (0.012±0.001 µg/g) and the highest in July (0.016±0.005 µg/g).~~ Mercury bioaccumulated in both~~all~~ tissues with age, both showing the strongest regression slopes in winter and lowest in summer.~~.~~ THg concentrations in liver and muscle tissue were correlated throughout the year, however the correlation was lowest in summer, indicating high metabolism~~whereas scale and muscle/liver only showed significant relations~~ during somatic growing season in summer and growth dilution. Multiple linear regression models ~~revealed that seasonal varying variables i.e. sexual maturity, $\delta^{13}C$, condition factor~~ explained 50% and 55%~~most~~ of the THg variation in liver ~~(50%)~~ and muscle both models dominated by seasonally-variable factors i.e. sexual maturity, $\delta^{13}C$, and condition factor. Seasonally varying bioaccumulation slopes and ~~(55%). A model examining variation in scale THg concentrations only explained 6% of~~ the ~~variation. The~~ higher level of intra-annual~~seasonal~~ variation (21~~-33~~%) in whitefish THg concentration in muscle ~~and scale,~~ than the~~ir~~ inter-annual accumulation (8%) highlight~~-7%), highlights~~ the importance of including~~to include~~ seasonal factors in future THg studies ~~of fish~~.

Capsule:

Strong seasonal~~Seasonal~~ variation was observed in THg concentrations and bioaccumulation slopes~~was higher compared to THg accumulation~~ in muscle and liver~~all~~ tissues, suggesting~~this indicates~~ that the temporal component of sampling should~~seasonal variation needs to~~ be considered in future THg ~~studies as well as in~~ monitoring and risk assessment programmes.

Keywords:

Bioaccumulation; dietary shift; growth dilution; seasonal variation; starvation~~; trophic ecology~~

1. Introduction

Atmospheric mercury (Hg) originates from both natural and anthropogenic sources, and concentrations in ecosystems across the globe have increased since the industrial revolution (Pacyna et al.,~~.~~ 2010). Atmospheric deposition typically dominates the supply of Hg to Arctic and subarctic lakes lacking direct Hg pollution sources in their catchment (e.g. Downs et al., 1998, Ariya et al., 2015).~~.~~ The Arctic has shown clear, and marked increasing trends in Hg concentrations, e.g. in lake sediments since the 18th century industrial era (Chételat et al.,~~. 2015). In nature, Hg largely exists in one of three oxidation states (Hg^0, Hg^{1+}, Hg^{2+}) and in a number of different compounds (such as Cl^-, $S0_4^{2-}$ and CH_3^-) (Ullrich et al. 2001). In the atmosphere, Hg is mainly (98%) present in its highly volatile elemental form (Hg^0), but in surface waters and catchment area the oxidized form (Hg^{2+}) is more abundant (70-90%) (Morel et al. 1998). Hg spreads via ocean currents and global winds and can enter terrestrial or freshwater ecosystems via atmospheric deposition, allowing it to reach typically pristine Arctic or subarctic regions (e.g. Downs et al. 1998, Ariya et al.~~ 2015). Hg speciation (e.g. methylation) in aquatic enviornments occurs through both biotic and abiotic pathways~~, in catchment~~ and ~~lakes,~~ via numerous different mechanisms (e.g. Jensen & Jernelöv, 1969;~~,~~ Pak & Bartha, 1998;~~,~~ Celo et al.,~~.~~ 2006). ~~However, biotic methylation of Hg^{2+} has been recognized as important factor in forming of methylmercury (MeHg, CH_3Hg), which is harmful to organisms due to its neurotoxic and apoptotic properties (e.g. Morel et al. 1998, National Research Council 2000).~~

In lake ecosystems, sulphur-oxidizing bacteria play key roles in the methylation process, resulting in the production of organic MeHg (e.g. Morel et al.,~~.~~ 1998). Both benthic and pelagic primary producers, i.e. periphyton and phytoplankton, uptake inorganic ~~Hg^{2+}~~ and organic ~~Me~~Hg through passive and active transport processes (Mason et al.,~~.~~ 1995, 1996; Douglas et al.,~~.~~ 2012). Benthic macroinvertebrates and zooplankton consume these primary producers and transfer the Hg to invertebrate feeding~~benthivorous and planktivorous~~ fish, which are in turn eaten by higher trophic level consumers such as piscivorous fish. In subarctic lakes, Hg often accumulates faster in the pelagic food web compartment than the littoral (e.g. Kahilainen~~Lavoie~~ et al., 2016a;~~. 2010, Chételat et al. 2011,~~ Thomas et al.,~~.~~ 2016; Kahilainen et al., 2017), likely reflecting increased trophic transfer efficiency ~~and thus increased Hg transfer~~ in the pelagic food web. MeHg is especially highly ~~bio-~~reactive, bioaccumulating in organisms and biomagnifying through the food chain (e.g. Watras & Bloom, 1992;~~,~~ Watras et al.,~~.~~ 1998). For example, MeHg is estimated to represent a total proportion of mercury (THg) concentration that ranges from 10% in the water column, to 15% in phytoplankton, 30% in zooplankton and up to 95% in fish muscle (Watras & Bloom, 1992;~~,~~ Watras et al.,~~.~~ 1998).

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

~~Hg in fishes is derived from their diet – Hg in the items that they consume and digest is translocated via blood to the liver and is subsequently stored in muscle tissues (e.g. Oliveira Ribeiro et al. 1999, Wang & Wang 2015). Hg concentrations are generally highest in liver and lower in muscle, with scale tissues showing the lowest concentrations, but this can vary between species (e.g. Jernelöv & Lann 1971, Červenka et al. 2011, Kahilainen et al. 2016). In addition, MeHg/THg ratios vary between tissues: e.g. ratios in liver and muscle is typically 40-80 % and >90 % respectively (e.g. Bloom et al. 1992, Blank et al. 2013, Madenjian et al. 2016). Scales are composed of mineralized compounds and organic matter such as collagen, to which Hg is likely bound. In northern latitudes, fish scales grow almost exclusively during the summer growing season, and it is likely that Hg is routed to scales at this time. However, Hg metabolism in fish scales is understudied and MeHg/THg ratios have not been documented.~~

In fish, Hg generally bioaccumulates with increasing~~ed~~ size and age (e.g. Downs et al.,~~.~~ 1998;~~,~~ Amundsen et al.,~~.~~ 2011;~~,~~ Swanson et al.,~~.~~ 2011). In species with ontogenetic dietary shifts, Hg concentration can ~~also~~ increase as consumers shift to a higher~~increase their~~ trophic level or switch to ~~utilization of~~ Hg-enriched pelagic prey (e.g. Power et al.,~~.~~ 2002;~~,~~ Thomas et al.,~~.~~ 2016; Kahilainen et al., 2017). Fast growing individuals accumulate muscle tissue faster than Hg, a phenomenon termed growth dilution (e.g. Simoneau et al.,~~.~~ 2005;~~,~~ Ward et al.,~~.~~ 2010). Furthermore, growth dilution is inversely related to increasing condition factor and individual lipid reserves (e.g. Amlund et al.,~~.~~ 2007;~~,~~ Braaten et al.,~~.~~ 2014;~~,~~ Kahilainen et al., 2016a). In~~. 2016). However, in~~ Arctic and subarctic lakes, many fish species have a lower growth rate, higher longevity and later sexual maturation relative to their equivalents in temperate lakes (Heibo et al.,~~.~~ 2005;~~,~~ Blanck & Lamouroux, 2007), increasing the period of Hg bioaccumulation. Furthermore, in seasonally ice-covered systems, condition and lipid reserves of fish are generally the lowest in winter (e.g. Hayden et al., 2014a, 2015). Decreasing lipid content, ~~(~~and potentially also protein loss during starvation, may~~) can~~ condense Hg~~,~~ ~~especially~~ in remaining tissues~~lipid reserves, thus increasing the Hg concentrations~~ (e.g. Kahilainen et al., 2016a). In the Hg contamination literature, this phenomenon is termed as starvation (e.g. Cizdziel et al.,~~.~~ 2002, 2003;~~,~~ Moreno et al.,~~.~~ 2015) and, along with growth dilution, it may play a key role in the seasonal variation in Hg concentrations in cold-water fishes. Such variation may be important factor when considering Hg monitoring programs and human health considerations, as many cold-water fishes play important roles in the year-round diet of ~~people resident in the region, both~~ indigenous and non-indigenous people in the region (AMAP 2011).

The European whitefish (*Coregonus lavaretus* (L.)) is a highly diverse and often the most abundant fish species in subarctic lakes of northern Fennoscandia (Siwertsson et al., 2010; Praebel et al., 2013; Malinen et al., 2014). Most of the lakes are inhabited by a generalist monomorphic whitefish populations using all habitat types, while polymorphic populations are diverged into separate pelagic and benthic morphs (Kahilainen et al., 2007; Harrod et al., 2010; Siwertsson et al., 2010). In the most complex cases, whitefish is ecomorphologically diverged into one of the three main lake habitats, littoral, pelagic or profundal (Kahilainen & Østbye, 2006; Harrod et al., 2010; Kahilainen et al., 2014). The whitefish morphs show many morphological and physiological adaptions to their specific habitat types, where a heritable trait, number of gill rakers, encapsulates most of the variation as a single measurement (Kahilainen et al., 2011b, 2014,~~The European whitefish (*Coregonus lavaretus* (L.)) is the most abundant fish species in many subarctic lakes in Fennoscandia. It plays a significant role in energy and Hg flow within these lakes as it is a central node in lake food webs and the key species in local subsistence fisheries (e.g. Kahilainen et al. 2007, Hayden et al. 2015, Thomas et al. 2016). Whitefish undergo a dietary shift from~~ 2016). Profundal morphs have the very low amount of short and widely spaced gill rakers suitable for foraging on fine sediments in dark condition charactistic of profundal habitat, whereas littoral and generalist morphs have intermediate number of relatively short gill rakers followed by pelagic morphs with very high number of fine, long and densely spaced gill rakers as adaption to foraging on small zooplankton prey (Kahilainen et al., 2007, 2011a, 2017). In both monomorphic and polymorphic lake types, whitefish as the most abundant species is key invertebrate feeding predator and main prey for many piscivores, thus acting as a central node in lake food webs (e.g. Kahilainen & Lehtonen, 2003; Kahilainen et al., 2009, 2011a). The key role of both monomorphic and polymorphic whitefish in the food webs of subarctic lakes has influence on pelagic and benthic energy and Hg flows (Thomas et al., 2016; Kahilainen et al., 2017), but we currently lack of knowledge regarding potential temporal variation in patterns of contaminant bioaccumulation that is likely influenced by seasonality of prey availability, growth, reproduction and condition.

To fill this knowledge gap, we undertook a year-round study of THg concentrations in a monomorphic whitefish population, and their putative prey sources in a relatively well-studied subarctic lake, Lake Kilpisjärvi, located in northern Fennoscandia. Here, monomorphic whitefish are known to undergo a dietary shift from littoral benthic macroinvertebrates during winter and spring to pelagic zooplankton in mid to late summer, coinciding with an annual zooplankton bloom (Tolonen, 1999;~~,~~ Hayden et al., 2014a)~~. 2014, Kahilainen et al. 2016).~~ Pelagic prey is generally considered a more important source of Hg, due to often higher MeHg concentrations in zooplankton than littoral benthic prey (Watras et al.,~~.~~ 1998;~~,~~ Suchanek et al.,~~.~~ 2008). Previous work on whitefish morphs indicated that pelagic zooplanktivorous morphs had much higher THg concentrations and steeper bioaccumulation slopes than benthivorous morphs (Kahilainen et al., 2017). Open-water season dietary shifts from benthic macroinvertebrates to pelagic zooplankton in Arctic fishes such as Arctic charr (*Salvelinus alpinus* (L.)) have been shown to affect THg concentrations in fish liver and muscle tissue (Kahilainen et al., 2016a). Open-water season THg studies of fish muscle has been conducted with many species (e.g. Zhang et al.,~~.~~ 2012;~~,~~ Braaten et al.,~~.~~ 2014;~~,~~ Moreno et al., 2015;~~,~~ Olk et al.,~~.~~ 2016), but we are unaware of any year-round (including ice-covered winter) muscle and liver~~multi-tissue~~ studies on THg concentrations of fish. Dietary shifts are clearly important in Hg exposure, but we know very little about the seasonal patterns of THg concentrations in whitefish and the main factors affecting any putative changes. To address these questions~~knowledge gaps~~, we collected data year-round on whitefish growth, sexual maturation, condition and resource use with THg measured from muscle and liver~~different~~ tissues in a subarctic lake in northern Finnish Lapland.~~ To assess intra- and inter-annual bioaccumulation patterns, we examined a suite of factors (sampling month, age, total length, condition factor, sex, sexual maturity, GSI, gillraker number, δ^{13}C, δ^{15}N) driving variation in THg concentration from different tissues (liver, muscle, scale) over the study period.~~ Our study was designed to test three hypotheses:

Hypothesis 1 (H1) We hypothesized that the late summer dietary shift in whitefish from the low THg littoral benthic macroinvertebrates to the high THg pelagic zooplankton (Kahilainen et al., 2016a; 2017) likely results in an increase in THg concentrations, and this shift will be evident in metabolically active liver prior to muscle. We also predicted that the THg concentration of liver and muscle will increase during winter due to starvation and subsequently decrease in spring and early summer due to growth dilution.~~ The metabolic dynamics of scales act at a slower rate than liver or muscle tissues, and therefore we hypothesized the THg concentrations of scales would not show as pronounced seasonal variation as other tissues.~~

Hypothesis 2 (H2) If there are seasonal changes in THg of muscle and liver tissues, we expected to find changes in bioaccumulation slopes and ~~Due to~~ the THg regression slopes between these two tissues. First, we hypothesized that bioaccumulation occurred in both tissues in all months, but that we would report shallower slopes during the summer somatic growing season due to growth dilution. Secondly~~comparatively similar metabolic dynamics of Hg in whitefish liver and muscle~~, we hypothesized that the intra-annual ~~and combined annual~~ relationships of THg concentration between liver and muscle would be significant year around, but would show a weaker relation in summer, when metabolic activity is higher in both tissues.~~stronger than between liver and scale or muscle and scale.~~

Hypothesis 3 (H3) If season is an important determinant of THg concentrations, we~~We~~ expected to see seasonal-~~find season~~ related factors e.g. maturity and stable isotope ratios selected in multiple linear regression models examining the drivers for muscle and liver THg concentrations, in addition to traits related to individual fish size. ~~in addition to fish size related traits. The Hg metabolism of scales is unknown, and we predicted, following the other tissue bioaccumulation patters, that fish size related traits should be positively related to scale THg concentrations.~~

2. Materials and methods

2.1 Study area

This study was conducted in a subarctic L~~l~~ake~~,~~ Kilpisjärvi (hereafter L. Kilpis), located in northern Fennoscandia (69°03'N, 20°49'E; 473 m above sea level; Fig. 1).~~; e.g. Hayden et al 2014).~~ L. Kilpis is a relatively large (surface area 37.3 km^2, shoreline 71.5 km), oligotrophic (Tot-P < 5 µg l^{-1}, Tot-N < 150 µg l^{-1}, chlorophyll-a < 2 µg l^{-1}), neutral (pH 7.2, conductivity 3.0 mS m^{-1}), clear water (Secchi and compensation depth 10 and 14 m, DOC 2.8 µg l^{-1}) and deep (maximum and average depths 57 m and 19.4 m) headwater lake (Kahilainen et al.~~,~~ 2007;~~;~~ Hayden et al., 2014a; Kahilainen et al., 2017~~.~~

~~2014~~). The average annual air temperature of the region is -2.3 °C and precipitation is 450 mm y^{-1}, of which ca. 60% falls as a snow. The year-round average water column temperature lake water varies from 0.4-10°C (Hayden et al., 2014a; 2014b).~~snow.~~ Ice cover is present on the lake from mid-November until mid-June and may reach a thickness of 1 m in late winter (Lei et al.,~~.~~ 2012). The L. Kilpis catchment (293 km^2) consists of subarctic mountain birch (*Betula* sp.) surrounding the lake, whereas areas with elevations above 600 m a.s.l. are Arctic tundra. The proportion of peatland in the catchment is low. There are no direct sources of Hg (e.g. volcanos, mines, factories) in the vicinity, suggesting that the principal source of Hg to the lake and catchment over historical and contemporary timelines has been atmospheric deposition.

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

2.2 Sample period and sampling methods

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

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

Fish were collected using gillnets fished in series including seven 1.8 m high and 30 m long nets (knot-to-knot mesh sizes: 12, 15, 20, 25, 30, 35, 45 mm), supplemented with one 1.5 m high and 27 m long Nordic multimesh gillnet (5.25-55 mm). Gillnet series were set in benthic habitat at depths 2-15 m overnight (summer: 10-12h, winter: 24-48h). Fish were immediately euthanized by cerebral concussion at the sampling site. After immediate transport to the laboratory, total length and mass~~weight~~ of whitefish were measured to the nearest mm and 0.1 g. Fulton's condition factor (K-) was calculated 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.~~, and scales were also assessed for THg concentrations.~~ Individual whitefish age was determined from the combined~~joint~~ use of clear~~ed~~, burned and cracked otoliths under a binocular microscope as well as unregenerated scales pressed on polycarbonate slides and viewed using a microfiche reader (Kahilainen et al.,~~.~~ 2003). The join use~~Year class~~ of otoliths and scales was used to improve the accuracy of aging (Kahilainen et al., 2017). Whitefish populations in L. Kilpis are typically dominated~~fishes were determined~~ by single~~subtraction of capture~~ 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.~~age.~~ 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:~~ 1978):~~

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

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

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

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

2.4 Total mercury analysis

THg concentrations (µg g^{-1} d.w.) were analysed from the freeze-dried ZPL (n=17), BMI (n=20), liver (n=167) and muscle (n=225) samples~~, as well as air-dried non-regenerated scales (n=75)~~ by atom absorption spectrometry using a direct Hg analyser (Milestone DMA 80). We had a target fish sample size for each month of ~~ca.~~ 30 individuals representing the total length and age distribution of the population (Table 1) and all invertebrate samples containing enough tissue were analysed. From each sample, two duplicates (20-30 mg) were analysed when material was not limited due to low sample mass - as was the case with some liver samples and almost all invertebrate samples. Average~~The~~ relative difference (RSD) between duplicates of liver (n=113 pairs), muscle (n=223 pairs~~), scale (n=71 pairs~~) and invertebrates (n=2 pairs) was 1.1, 1.3~~, 7.1~~ and 11.2%, respectively. At the start and end of each run, samples of certified reference material (DORM-4; 0.410 ± 0.055 µg g^{-1}; National Research Council Canada) were combusted. The average and recovery-% of the certified reference material (n=~~ = ~~66) was 0.408 ± 0.011 (SD) and 99.6% respectively. Blank control samples (grand mean ± SD: 0.001 ± 0.001, n=~~ = ~~113) were added both at the end of each run, as well as between different tissues and taxa. Run specific blank THg values was subtracted from analysed sample THg values to avoid instrumental error.~~,~~ The mean of the blank adjusted duplicate THg values was later lipid-corrected.

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

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

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

2.5 Statistical methods

Examination of seasonal changes of Hg concentrations in whitefish tissues (H1) and all supporting analyses of variance were conducted with non-parametric tests (Kruskal-Wallis H-test with post hoc: Mann-Whitney U-test, or if the assumption of homogeneity of variances was violated, we used repeated Welch's t-tests with the Games-Howell post-hoc test). The seasonal bioaccumulation and~~The~~ relationships between Hg concentrations in liver and muscle~~of different tissues~~ (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,~~employing a~~ backward and both direction stepwise selection procedure, selecting the best model based on minimum AIC values. Here, we first checked for auto-correlation and selected variables with $R^2 < 0.7$ (sampling month, total length, condition factor, sex, sexual maturity, GSI, gillraker number, $\delta^{13}C$, $\delta^{15}N$) for inclusion in the model. Significance~~A 95% confidence~~ level ($p < 0.05$) was used ~~as entry limit in the multiple linear regression analysis, as well as an indicator of statistical significance~~ 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).~~).~~

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~~s~~, all~~ of them~~ 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~~than~~ 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~~were the~~ highest (n=24) and stomach fullness (0.4)~~ the~~ 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 ~~the ~~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~~consumption~~ of trophic level~~BMI~~ was also evident in ~~relative stable ~~muscle δ^{15}N and values (Welch's ANOVA: $F_{7, 217} = 2.49$, $p = 0.017$), with pairwise comparisons showing the highest values in winter (Table 1). The strong annual reliance on littoral BMI~~dietary shift~~ was also evident from relatively similar muscle δ^{13}C values (Welch's ANOVA: $F_{7, 217} = 5.54$, $p<0.001$), with values showing a slight ~~being relatively ~~^{13}C depletion~~ed~~ in winter and ^{13}C enrichment at early~~enriched in~~ summer (Table 1).

3.2 H1 - Annual mercury concentrations in invertebrates and whitefish tissues

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

THg concentrations varied seasonally both in liver (Welch's ANOVA: $F_{5, 158} = 5.29$, $p < 0.001$) and muscle tissue (Kruskal-Wallis: $H_{7, 217} = 41.87$, $p < 0.001$). The seasonal changes showed a similar pattern in both liver and muscle tissues (Table 2, Fig. 2). The highest THg concentrations (liver: 1.70 ± 0.88 µg g^{-1}, muscle: 0.24 ± 0.05 µg g^{-1}) were found in mid-winter under thick ice (February 2012) and the lowest (liver: 0.87 ± 0.72 µg g^{-1}, muscle: 0.19 ± 0.04 µg g^{-1}) in open-water summer (June-July 2012). ~~However, seasonal variation in scales was less obvious (Welch's ANOVA: $F_{7, 67} = 2.17$, $p = 0.048$), with post-hoc tests (Table 2) only highlighting significant differences between May 2012 (0.012 ± 0.001 µg g^{-1}) and August 2014 (0.016 ± 0.002 µg g^{-1}).~~

We estimated the annual accumulation of THg (0.01-0.02 µg g^{-1}) in muscle tissue by comparing samples from September 2010 and August 2014 (Table 2): post-hoc tests indicated that THg concentrations were higher in 2014 (0.23 ± 0.05 µg g^{-1}) than in 2010 (0.17 ± 0.04 µg g^{-1}). ~~THg accumulation with age (Fig. 3) was also evident among year classes (2002-2011) in both muscle (Kruskal-Wallis: $H_{8, 154} = 63.64$, $p < 0.001$) and liver tissues (Welch's ANOVA: $F_{8, 152} = 10.12$, $p < 0.001$). For example, muscle THg concentrations of fish from the 2003 year class were statistically higher than for those from the 2009 and 2010 year classes (post hoc: Mann-Whitney U-test: p<0.001 in both cases), while liver THg concentrations of the 2003 year class were higher than that recorded from the 2008-2011 year classes (post hoc: Games-Howell: p<0.001 in all cases).~~

3.3 H2 - Seasonally varying bioaccumulation and relationships~~The relationship~~ of ~~mercury concentration between different tissues~~

THg ~~concentrations~~ in liver and muscle tissue ~~were circa 4-7 times higher than those from muscle, and 50-100 times higher than in scales in all sampling months. This was evident both from tissue mean THg concentrations and the slopes of linear regression equations based on THg concentrations in different tissues (Tables 2 and 3). Regressions of THg concentrations between liver and muscle or muscle and scale (Table 3, Fig. 4) were both statistically significant when data were pooled at an annual level, but differed in their predictive power (liver-muscle: adj. $r^2 = 0.53$, $p < 0.001$, muscle-scale: adj. $r^2 = 0.10$, $p = 0.015$). Liver-muscle regressions were statistically significant throughout the year (however r^2 values of the slopes were highest in June and lowest in July), whereas the liver-scale and muscle-scale regression were significant only in July-September (Table 3).~~

Bioaccumulation of THg by age varied seasonally in both tissues showing the highest slopes and the 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).

3.4 H3 - Results of multiple linear regression analysis

The best~~Backwards~~ stepwise multiple regression models~~examining~~ explained 50~~%, 55~~% and 55~~6~~% of the variation in THg concentration in liver, muscle ~~and scale ~~respectively (Table 3, Table S3). Both models included the exact same ecological~~4). The models indicated that seven, six and one~~ variables explaining the ~~explained ~~variation in ~~liver, muscle and scale ~~THg concentrations.~~, respectively. Models for liver and muscle were generally similar, with the exception that sampling month was conversely correlated in these models and that the liver-model also included GSI.~~ Sexual maturity, δ^{15}N and total length were~~was~~ positively ~~cor~~related to THg concentrations in both liver and muscle models, whereas δ^{13}C and condition factor was inversely ~~cor~~related to the concentrations. In both models, sexual maturity, δ^{13}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.~~factors explaining the THg concentrations. The model examining scale THg concentrations performed poorly, and only included condition factor, which was inversely correlated with scale THg concentrations. The poor quality of the scale-model was probably caused by the lack of variability in size and age data, as only fish from the 2003 year class were analyzed.~~

4. Discussion

4.1 Main results

We found evidence for year-round variation in THg concentrations in whitefish liver and~~,~~ muscle ~~and scale ~~tissues. As we hypothesized (H1), annual THg concentration of liver and muscle were ~~the~~ highest in winter and ~~the ~~lowest in open-water summer months. In addition, strength and significance of bioaccumulation and the~~there was a clear~~ positive relationship of THg concentration between muscle and liver peaked~~tissues~~ in winter and were shallow or non-significant in summer~~individual fish, whereas similar relationship between scale and muscle was much weaker~~ (H2). The seasonally related variables, such as sexual maturity, δ^{13}C, and condition factor, included in the multiple linear regression models supported hypothesis H3, i.e. that starvation and zooplanktivory increased THg concentration and growth dilution lowered it.

4.2 Seasonal variation in mercury concentrations (H1)

We found strong seasonality of THg concentrations, where liver and muscle showed~~ in all tissues, showing~~ maximum differences of 49% ~~%, 21% and 33% in liver, muscle ~~and 21% in monthly comparisons~~scale~~, respectively. Here, both~~liver and muscle~~ tissues showed similar patterns, following a sine-curve peaking in winter and reaching the minimum~~falling~~ in summer.~~, whereas scales showed reverse patterns, and included much more noise.~~ These changes were related to consistent year-round changes in several~~various~~ measures of whitefish ecology (e.g. resource use, maturation and condition).~~) of whitefish ecology.~~ Whitefish showed a seasonal shift in diet in the summer, changing from a BMI~~-dominated~~ diet to a pelagic ZPL-dominated diet, evident from both stomach contents and liver δ^{13}C values, which became increasingly ^{13}C depleted. In L. Kilpis, we showed that THg concentrations in pelagic ZPL were higher than littoral BMI and~~:~~ other studies have ~~also ~~shown that pelagic ZPL may~~can also~~ have a higher MeHg/THg ratio than ~~that shown by ~~littoral BMI (e.g. Watras et al.~~,~~ 1998;~~,~~ Suchanek et al.~~,~~ 2008). In light of this, we suggest that the reported whitefish dietary shift to pelagic ZPL contributes to an increasing trend of THg levels of liver and muscle from late summer onwards. This is further supported by results of recent study showing higher THg concentrations on zooplanktivorous than benthivorous whitefish morphs in a series of subarctic lakes (Kahilainen et al., 2017). Hg turnover is faster in liver than in muscle (Oliveira Ribeiro et al.~~,~~ 1999) and this may explain our observation that Hg concentrations increased slightly faster in liver than in muscle following the diet shift to pelagic ZPL. The open water season dietary shift of Arctic charr in nearby subarctic Lake Galggojavri from BMI to pelagic ZPL has been found to increase fish THg concentrations in liver towards autumn (Kahilainen et al.~~,~~ 2016). Similarity of diet and THg patterns in whitefish and Arctic charr during open-water season suggest generality of our findings, at least locally.

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

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

The rate of increase in THg values in both liver and muscle slowed after sexual maturation. ~~In L. Kilpis whitefish, THg concentrations increased with age in both liver and muscle tissues. This pattern has been recorded also in many other studies using muscle tissues of subarctic salmonids (e.g. Amundsen et al. 2011, Swanson et al. 2011), but is less commonly reported from liver tissues. The pairwise comparison of scale THg concentrations over sampling months revealed high variation with the lowest value in May under ice and the highest value in July during intensive growing period of scales and other tissues (Tolonen 1998). Despite only single significant comparison, there was a weak indication of slow THg accumulation from 2010 to 2014.~~

~~The rate of increase in THg values in both liver and muscle slowed after sexual maturation.~~ The ontogenetic dietary shift from ZPL to BMI at an early age in whitefish (Tolonen, 1998) and decreased somatic growth after maturation might explain the slowing THg accumulation. We found ~~As supporting the age-correlated THg accumulation, we found~~ that population-level mean THg concentrations in whitefish muscle ~~and scale~~ increased by~~with rate of circa~~ 8~~ and 7~~ % per year, between September 2010 and August 2014. This value is~~, respectively. These values are~~ indeed circa 2.5~~up to three~~ times lower that observed intra-annual variation during ~~than found for muscle and scale in monthly comparisons in~~ 2011-2012. Both observations strongly reflect the dominance of the single mature 2003 ~~During all sampling periods, the strong~~ year-class, with limited annual somatic growth explaining low inter-annual bioaccumulation and strong investment on gonads causing intra-annual variation of muscle THg. In ~~2003 was the most abundant, therefore the annual slight increase of THg concentrations could be explained with general aging of the whitefish population. Also in~~ previous studies of L. Kilpis whitefish, ~~,~~the dominance of a single year-class has been documented in diffent decades~~L. Kilpis~~ (Tolonen, 1998;~~,~~ Harrod et al.,~~.~~ 2010) and such patterns of year-class dominance and generally high age of fish is typical in Arctic and subarctic lakes (Rolls et al., 2017). Taken collectively, our results of intra- and inter-annual patterns of THg should be thus the most valid for fish populations consisting of mature fish investigating their energy mostly to gonad growth instead of somatic growth, a pattern typical for a range of fish species. ~~.~~

4.3 Seasonal bioaccumulation and mercury~~Mercury~~ metabolism between liver and~~,~~ muscle ~~and scale~~ (H2)

In L. Kilpis whitefish, THg concentrations increased with age in both liver and muscle tissue. This pattern has been recorded also in many other studies using muscle tissues of subarctic salmonids (e.g. Amundsen et al. 2011, Swanson et al. 2011), but is less commonly reported from liver tissue. A previous study by Kahilainen et al. (2017), showed that THg concentrations in muscle generally increased with age in different European whitefish morphs, but the regression slopes were the most steep for pelagic morphs (range 0.038-0.103) and shallow for benthic whitefish morphs (0.017-0.020). This study also assessed bioaccumulation in three monomorphic whitefish population, which displayed relatively shallow slopes (0.020-0.025). These results corroborates our findings here, as we found shallow, or even non-significant bioaccumulation slopes during the summer growing season for both liver and muscle with some time-lag related to faster metabolic rate of former than later (e.g. Oliveira-Ribeiro et al., 1999; Hayden et al., 2014a; Kahilainen et al., 2016a). Interestingly, the bioaccumulation slopes of both tissues were clearly steeper during the ice-covered winter, most likely driven by the older mature individuals which had higher relative difference between winter and summer THg concentrations compared to the younger immature individuals. This could be explained by the stronger response of older fish to spawning, which is likely due to the fact that only six years or older individuals were sexually mature, and this was the group driving the changes in bioaccumulation seasonally.

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

~~The relationship between the THg values of liver and muscle tissues were stronger than that seen between muscle and scale, probably due to inherent metabolic links between these tissues (Oliveira Ribeiro et al. 1999, Sinnatamby et al. 2008). There was little evidence of any relationship between THg values of liver and scale, partly reflecting the large amount of between-individual variation seen in liver THg values, but also the fact that there is little direct metabolic link between these tissues (Sinnatamby et al. 2008). Growth of whitefish scales in L. Kilpis starts in July (Tolonen 1998), possibly explaining the connection between THg values of scale and muscle as well as scale and liver in July. In other months, the scale-muscle regressions explained far less variation or were non-significant, suggesting that transport of Hg to scales primarily occurs during the main growth period.~~

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

We found that a high proportion of the variation (50% and 55%) in THg concentration in liver and muscle ~~and liver~~ was explained through multiple linear regression analyses.~~, however, the models were less successful at explaining THg concentration in scales.~~ Previous studies employing regression analyses to explain THg concentrations in whitefish have frequently documented that fish size and age are important factors affecting Hg concentration (e.g. Moreno~~Amundsen~~ et al., 2015; Thomas~~.~~ ~~2011, Swanson~~ et al., 2016; Kahilainen et al., 2017).~~. 2011).~~ Surprisingly total length, which was ~~auto-~~correlated with age and mass~~weight~~, was a relatively poor predictor of liver and muscle THg concentrations in L. Kilpis. This most likely reflect the low investment to somatic growth of single year-class 2003 dominated whitefish population, where most fish are close to their maximum length.~~.~~ However, the inclusion of THg in liver and muscle~~multiple tissues~~ and factors related to temporal variation such as sampling month, stable isotopes and sexual maturity have been examined to a far lesser degree. In this study, all of these factors describing year-round variation were highly important factors included in liver and muscle models and are discussed below.

In L. Kilpis, both muscle and liver THg values were inversely related with tissue specific δ^{13}C values, which likely reflects increased autumnal consumption of ^{13}C depleted pelagic ZPL containing more THg than littoral BMI (Kahilainen et al., 2016a; Thomas~~. 2016, Karimi~~ et al.,~~.~~ 2016; Kahilainen et al., 2017). Liver and muscle THg values were negatively related with condition factor, which can be used as supporting evidence for starvation and growth dilution (Cizdziel et al.,~~.~~ 2002, 2003;~~,~~ Evans et al. 2015). Condition factor does reflect spawning when gonads~~, which removes eggs~~ with low THg concentration are removed from the fish body, potentially further condensing Hg in whitefish muscle (Madenjian et al.,~~.~~ 2016). Increased δ^{15}N values have been linked to metabolic-stress associated with starvation (Moreno et al.,~~.~~ 2015) in some taxa. Here, we found slight seasonal changes in muscle and liver δ^{15}N values showing the highest values in winter and lowest in autumn. Therefore, the positive correlation of δ^{15}N and THg values in muscle and liver model possibly reflects winter starvation, when fish use protein reserves from both liver and muscle. Positive correlation between~~correlations of~~ sexual maturity and THg concentrations in both liver and muscle models indicate a~~are obviously related to the~~ high significance of gonad~~egg~~ development and spawning to the THg concentrations. Spawning may be related to starvation in whitefish, due to the high cost of gonad investment and low prey abundance during winter spawning period (Hayden et al., 2014a). In the liver model, the negative relationship between GSI and THg supports this idea; whitefish GSI was lowest and THg was highest ~~immediately~~ after spawning in February-May, when we also reported the highest THg concentrations. In the muscle model, the opposite correlation between GSI and THg (positive) could be explained by random effect in the model since we found no correlation between GSI and muscle THg through simple linear regression analysis: in addition GSI had low significance in the multiple linear regression model explaining the variation in muscle THg. Sampling month significantly affected THg concentration, but the effect was positive in the liver model and negative in the muscle model, likely indicating that Hg is translocated faster in liver than in muscle. This could be explained by the different turnover times of these tissues, meaning that ~~the~~ late summer derived Hg ~~is~~ can be measured faster in liver (early winter) than in muscle (mid-winter). Therefore, the positive correlation between sampling month and liver THg could be explained by the high THg values in early winter (December). Most likely, the negative correlation between muscle THg concentrations and sampling month was driven by the high THg concentrations in mid-winter (February) and low concentrations in summer (June-July).

~~In contrast to our expectations, we found a weak negative relation between condition factor and scale THg (H3). The paucity of data detailing tissue turnover rate for whitefish scales makes interpreting the ecological meaning of this correlation difficult. Despite having a large number of ecological variables in our dataset, we were unable to predict scale THg concentrations with sufficient reliability due to limited age and size range. Despite the fact that scale and muscle THg concentrations are correlated and both accumulate inter-annually, there is evident further need to include more year-classes in scale THg analyses to test bioaccumulation with size and age.~~

4.5 Monitoring and human health

An interesting aspect of our results was that intra-annual variability in THg concentrations of whitefish exceeded inter-annual variation, evident also in multiple linear regression analyses, where seasonal factors indicating diet ($\delta^{13}C$) and condition were generally more important than fish total length. As the year-round maximum variation of muscle tissue (~~in different tissues~~ 21%) is surprisingly high~~-49%,~~ compared to ~~7-8% in~~ inter-annual (8%) accumulation in muscle, and that bioaccumulation slopes varied from non-significant or shallow in summer to highly steep and highly significant in winter, we suggest that such seasonal variation needs to be considered in future studies and especially in any long-term THg monitoring program. This is particularly important as the aims of Hg monitoring programs are typically related to human health (AMAP 2011). Primarily, the sampling month should be standardized but since the annual anomalities, the seasonal succession (e.g. temperature build up) should be quantified as well since they might affect on THg of fish.

Whitefish is the most important target fish of local ~~people~~ fisheries year-round and represent a stable proportion of ~~their~~ subsistence diet of native and non-native people (Thomas et al. 2016; Kahilainen et al., 2017). Although THg levels in all our fish samples were below national health limits (i.e. 0.5 µg g^{-1} wet mass~~weight~~; approx. 2.0-2.5 µg g^{-1} dry mass~~weight~~), the year-round patterns observed for whitefish may be relevant in other systems e.g. in other autumn or winter spawning fish such as many salmonids (Arctic charr, brown trout, lake trout, vendace) with putative winter starvation after reproduction. In spring spawning species, patterns could be different as the summer growing season starts immediately or soon after their reproduction, but additional year-round studies are needed to test this. For example, an annual variation of 21% would create a potential for THg values to exceed health limit guidelines and regional fish consumption regulations. Furthermore, seasonal changes of THg concentrations and bioaccumulation slopes in fish may lead to increased risk to human health in regions, where monitoring is restricted to low THg months i.e. mid summer. Depending on the aims of human health monitoring, both summer and winter sampling may be advisable as subsistence fishing is very common across Arctic and subarctic lakes in both seasons.

4.6 Conclusions

We revealed clear seasonal changes in the concentration and bioaccumulation slopes~~concentrations~~ of THg in whitefish muscle and liver tissues. The results indicated that both starvation and growth dilution drive seasonal changes in THg concentrations in both tissues. Our data also provides new evidence for the role of pelagic diet shifts on increasing THg concentrations in both muscle and liver. ~~We found that the THg concentrations of scales could be affected by this diet shift occurring during the main growth period of scales.~~ The seasonal changes in diet and condition were generally more important factors than fish length explaining THg concentrations of whitefish muscle and liver~~different~~ tissues ~~of fish~~. The intra-annual variation in THg concentrations was higher than inter-annual bioaccumulation, in addition we found that bioaccumulation varied seasonally being highest in winter and low or non-significant in summer. Therefore, ~~over years, therefore~~ it is essential to consider seasonal factors in future studies and Hg monitoring programs.

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Supplementary data

Supplementary data to this article can be found online at:

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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 ± SD values are presented, for different prey groups mean percentage contribution is presented. Variables marked with * indicate~~present~~ year class 2003 whitefish data. Superscript with small letters$^{a\text{-}h}$ 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, Simulidae, 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 ± 2.1	6.4 ± 2.5	7.3 ± 2.4	6.2 ± 3.2	5.8 ± 4.6	5.6 ± 3.9	^{a}8.1 ± 2.4	7.4 ± 4.2
Total length (mm)	247 ± 50	245 ± 52	248 ± 49	221 ± 71	227 ± 100	210 ± 94	269 ± 50	234 ± 83
Total mass~~weight~~ (g)	133.0 ± 89.1	117.1 ± 55.7	117.8 ± 50.6	97.5 ± 76.7	141.8 ± 194.3	106.8 ± 95.2	165.0 ± 55.1	126.1 ± 93.7
Somatic mass		^{g}111.2 ±50.1	^{g}117.2 ± 50.3	^{g}97.2 ± 76.3	140.3 ± 191.4	^{g}105.5 ± 93.4	^{bcdf}162.0±54.2	
Somatic mass* (g)		^{g}138.3 ± 26.5	145.7 ± 30.7	157.6 ± 24.1	^{g}123.0 ± 26.8	182.61 ± 51.2	^{be}185.5 ± 25.8	
Condition factor	^{def}0.75 ± 0.10	^{g}0.69 ± 0.07	^{g}0.70 ± 0.07	^{ag}0.67 ± 0.07	^{ag}0.65 ± 0.10	^{ag}0.67 ± 0.11	^{bcdefh}0.78±0.05	^{f}0.71 ± 0.08
Condition factor*	0.75 ± 0.04	^{g}0.72 ± 0.05	^{g}0.71 ± 0.09	0.72 ± 0.04	0.63 ± 0.08	0.75 ± 0.07	^{bc}0.79 ± 0.05	0.74 ± 0.06
Sexual maturity	^{cg}2.53 ± 0.94	3.03 ± 1.54	^{adef}4.43 ± 2.56	^{c}2.40 ± 1.57	^{c}2.39 ± 1.79	^{c}2.30 ± 2.09	^{a}3.41 ± 1.18	2.91 ± 1.51
GSI~~Gonadosomatic index~~		^{d}2.9 ± 4.7	^{g}0.4 ± 0.3	^{bg}0.2 ± 0.2	^{g}0.5 ± 0.6	^{g}0.6 ± 1.2	^{cdef}1.7 ± 1.6	
Gillraker count	25.2 ± 1.2	25.0± 2.1	24.3 ± 2.0	24.2 ± 1.6	23.9 ± 1.5	24.0 ± 1.7	24.4 ± 1.6	24.1 ± 2.0
C:N Liver		^{df}4.54~~5~~ ± 0.64~~6~~	^{f}4.35~~5~~ ± 0.72~~7~~	^{b}4.14~~1~~ ± 0.23~~2~~	4.15~~2~~ ± 0.32~~3~~	^{bc}4.04 ± 0.35 ~~± 0.4~~	4.22~~2~~ ± 0.19~~2~~	
C:N Muscle	3.20~~2~~ ± 0.04~~0~~	3.21~~2~~ ± 0.04~~0~~	3.20~~2~~ ± 0.06~~1~~	3.20~~2~~ ± 0.05~~1~~	3.22~~2~~ ± 0.05~~1~~	^{h}3.22~~2~~ ± 0.05~~1~~	^{h}3.22~~2~~ ± 0.05~~1~~	^{fg}3.18~~2~~ ± 0.05~~1~~
δ ^{13}C Liver (lipid free)		-23.5 ± 1.7	-23.7 ± 2.1	-23.7 ± 1.8	-23.3 ± 8.6	-24.9 ± 1.9	-23.8 ± 1.4	
δ^{13}C muscle (lipid free)	-24.6 ± 2.9	^{e}-25.0 ± 1.7	^{e}-25.2 ± 1.4	-24.6 ± 1.5	^{bch}-22.1 ± 3.0	-24.3 ± 1.2	-24.6 ± 1.2	^{e}-25.1 ± 1.5
δ^{15}N Liver		8.0 ± 0.6	^{g}8.2 ± 0.6	^{g}8.3 ± 0.7	^{g}8.3 ± 0.8	^{g}8.1 ± 0.5	^{cdef}7.5 ± 0.5	
δ^{15}N Muscle	^{b}8.4 ± 0.5	^{a}8.7 ± 0.3	8.6 ± 0.3	8.6 ± 0.4	8.5 ± 0.4	8.5 ± 0.4	8.6 ± 0.3	8.6 ± 0.4
Stomach fullness	^{bf}3.8 ± 1.6	^{acdefgh}0.4±0.9	^{bfg}2.3 ± 2.6	^{bf}3.3 ± 2.5	^{b}4.0 ± 1.6	^{abcdh}5.4 ± 1.9	^{bc}4.6 ± 1.7	^{bf}3.7 ± 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		

Table 2. THg concentrations (μg g^{-1} d.w.) ± SD (n) of liver an muscle~~different~~ tissues of whitefish and invertebrates by sampling months and years. Superscript with capital letters^{A-H} presented before THg means of different tissues indicates 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). Superscript small letters^{a-c} in grand mean row indicates statistical differences between corresponding $^{a-c}$invertebrate group.

	Liver	Muscle	~~Scale~~	^{a}ZPL$_{pelagic}$	^{b}BMI$_{littoral}$	^{c}BMI$_{profundal}$
^{A}Sep-10		^{BCEH}0.17±0.04 (30)	~~0.012±0.002 (10)~~			
^{B}Dec-11	^{F}1.56±0.82 (29)	^{A}0.22±0.04 (30)	~~0.014±0.003 (11)~~	0.040 (1)	0.050 (2)	0.573 (2)
^{C}Feb-12	^{EF}1.70±0.88 (30)	^{AEF}0.24±0.05 (30)	~~0.013±0.003 (11)~~	0.061 (1)	0.057 (2)	
^{D}May-12	1.39±0.99 (30)	0.22±0.06 -(30)	~~^{H}0.012±0.001 (4)~~	0.065±0.006 (3)	0.055 (2)	
^{E}June-12	^{C}0.87±0.72 (17)	^{AB}0.20±0.06 (18)	~~0.015±0.005 (3)~~		0.057 (1)	
^{F}July-12	^{BACG}0.88±0.42 (30)	^{B}0.19±0.04 (30)	~~0.016±0.005 (11)~~	0.060±0.002 (3)	0.036 (2)	
^{G}Sep-12	^{F}1.29±0.57 (28)	0.20±0.04 –(29)	~~0.013±0.002 (11)~~	0.067±0.002 (3)		0.319 (2)
^{H}Aug-14		^{A}0.23±0.05 (28)	~~^{D}0.016±0.002 (14)~~	0.084±0.005 (6)	0.027±0.010 (5)	0.205 (2)
Grand mean	1.31±0.81 (164)	0.21±0.05 (225)	~~0.014±0.003 (75)~~	^{b}0.070±0.013 (17)	^{a}0.042±0.014 (14)	0.366±0.356 (6)

Table 3. Multiple~~Linear regression equations of the relationships between THg concentrations of different tissues by month, full year regression equations is presented as a grand mean in each row. Statistically significant (p<0.05) equations are shown in bold.~~

	~~Liver$_y$-Muscle$_x$~~	~~Liver$_y$-Scale$_x$~~	~~Muscle$_y$- Scale$_x$~~
~~Dec-11~~	~~**y=11.77x-1.00 adj. r^2=0.37**~~ ~~**$F_{1,27}$=17.11 p<0.001**~~	~~y=28.62x+1.47 adj. r^2= -0.10~~ ~~$F_{1,9}$=0.06 p=0.81~~	~~y=3.10x+0.20 adj. r^2=-0.02~~ ~~$F_{1,9}$=0.80 p=0.40~~
~~Feb-12~~	~~**y=13.32x-1.55 adj. r^2=0.60**~~ ~~**$F_{1,28}$=43.53 p<0.001**~~	~~y=32.00x+1.60 adj. r^2=-0.10~~ ~~$F_{1,9}$=0.10 p=0.76~~	~~y=8.87x+0.15 adj. r^2=0,22~~ ~~$F_{1,9}$=3.82 p=0.08~~
~~May-12~~	~~**y=12.13x-1.30 adj r^2=0.54**~~ ~~**$F_{1,28}$=34.68 p<0.001**~~		
~~Jun-12~~	~~**y=10.51x-1.19 adj. r^2=0.76**~~ ~~**$F_{1,15}$=50.86 p<0.001**~~		
~~Jul-12~~	~~**y=5.52x-0.17 adj. r^2=0.18**~~ ~~**$F_{1,28}$=7.43 p=0.01**~~	~~**y=74.86x+0.02 adj. r^2=0.60**~~ ~~**$F_{1,9}$=15.90 p=0.003**~~	~~**y=6.41x+0.09 adj. r^2=0.78**~~ ~~**$F_{1,9}$ =37.17 p<0.001**~~
~~Sep-12~~	~~**y=8.560x-0.486 adj. r^2=0.24**~~ ~~**$F_{1,26}$=9.41 p=0.005**~~	~~y=84.95x+0.13 adj. r^2=-0.02~~ ~~$F_{1,9}$=0.84 p=0.38~~	~~**y=14.51x+0.01 adj. r^2=0.32**~~ ~~**$F_{1,9}$ =5.75 p=0.04**~~
~~Grand mean~~	~~**y=11.70x-1.19 adj. r^2=0.53**~~ ~~**$F_{1,162}$=182.79 p<0.001**~~	~~y=93.89x+1.08 adj. r^2=0.01~~ ~~$F_{1,49}$=1.29 p=0.26~~	~~**y=5.23x+0.16 adj. r^2=0.10**~~ ~~**$F_{1,49}$=6.39 p=0.015**~~

~~Table 4. The results of stepwise (backward selection) multiple~~ linear regression models explaining THg variation in liver and muscle.~~analysis.~~ Coefficient of determination (adjusted R^2) and residual standard error (RSE~~of the estimate (SE~~) are presented for each model. Slope (B), the standard error of the estimate (SE), ~~and~~ the~~statistical~~ significance indicators (t and p) and AIC values are presented for each factor selected to the models. The results of AIC stepvise procedure are presented in Table S3.

Tissue	Factor	B ± SE	t	p
Liver ~~R^2=0.50, SE=0.577~~	Sexual maturity	0.241 ± 0,042	5.753	<0.001
adj.R^2=~~$F_{7,\ 156}$ = 24.~~0.50, RSE=~~, p<~~0.577~~001~~	δ^{13}C liver	-0.120 ± 0.024	-4.928	<0.001
$F_{7,\ 156}$=24.0, p<0.001	Condition factor	-2.582 ± 1.092	-2.997	0.003
AIC=-172.67	Constant	-2.985 ± 1.092	-2.733	0.007
	GSI	-0.060 ± 0.022	-2.677	0.008
	δ^{15}N liver	0.197 ± 0.083	-2.677	0.019
	Total length	0.003 ± 0.001	2.367	0.020
	Sampling month	0.034 ± 0.016	2.100	0.037
Muscle ~~R^2= 0.55, SE=0.034~~	δ^{13}C muscle	-0.013 ± 0.002	-8.225~~137~~	<0.001
adj.R^2=0.55, RSE=0.034~~$F_{6,160}$=34.261, p<0.001~~	Condition factor	-0.211~~197~~ ± 0.046	-4.570~~303~~	<0.001
$F_{7,159}$=30.18, p<0.001	Total length	0.0003 ± 0.0001	3.945~~846~~	0.003~~2~~
AIC=-1118.82	~~Sexual maturity~~Sampling month	-0.003~~8~~ ± 0.001~~2~~	-2.974~~523~~	0.004~~013~~
	~~Sampling month~~Sexual maturity	~~-~~0.006~~2~~ ± 0.003~~0~~	~~-~~2.274~~490~~	0.02~~1~~4
	Constant	-0.172~~190~~ ± 0.080	-2.147~~360~~	0.033~~019~~
	δ^{15}N muscle	0.016~~8~~ ± 0.008	2.088~~168~~	0.038~~2~~
~~**Scale** R^2=0.06, SE=0.003~~	GSI~~Constant~~	0.002~~6~~ ± 0.0014	1.746~~6.093~~	~~<~~0.082~~001~~
~~$F_{1,\ 49}$= 8.0, p<0.001~~	~~Condition factor~~	~~-0.016 ± 0.006~~	~~-2.828~~	~~0.007~~

Figure legends

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

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

Figure 3. Linear regression models showing THg bioaccumulation in whitefish liver (A-F) and muscle (G-L) tissues. Dashed lines represent 95% confidence intervals. ~~Variation in whitefish mercury concentration of liver (A) and muscle (B) shown for 2002-2011 year classes (separated with dashed vertical lines). The sampling months (December$_{2011}$-September$_{2012}$) are marked with the capital letters (D, F, M, J, J and S) for each year class. Bold horizontal lines indicate the median values, the boxes represent first and third quartile and whiskers represents minimum and maximum values. Outliers (black circles) are presented if there are data points smaller or larger than the difference between first and third quartile.~~

Figure 4. Linear regression models~~regressions~~ with 95% confidence intervals (dashed lines) illustrating seasonally changing THg relationship between liver and muscle tissue~~different tissues~~ of whitefish in December 2011 (A), February 2012 (B), May 2012 (C), June 2012 (D), July 2012 (E) and September 2012 (F).~~. For linear regression equations see Table 3.~~

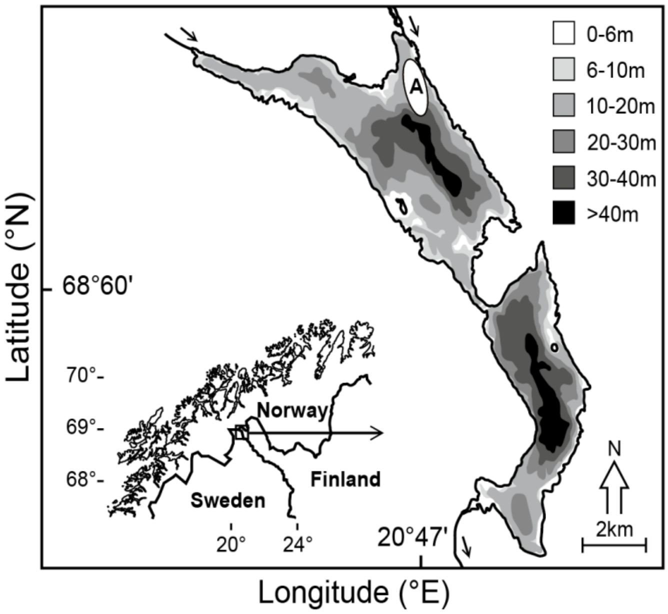
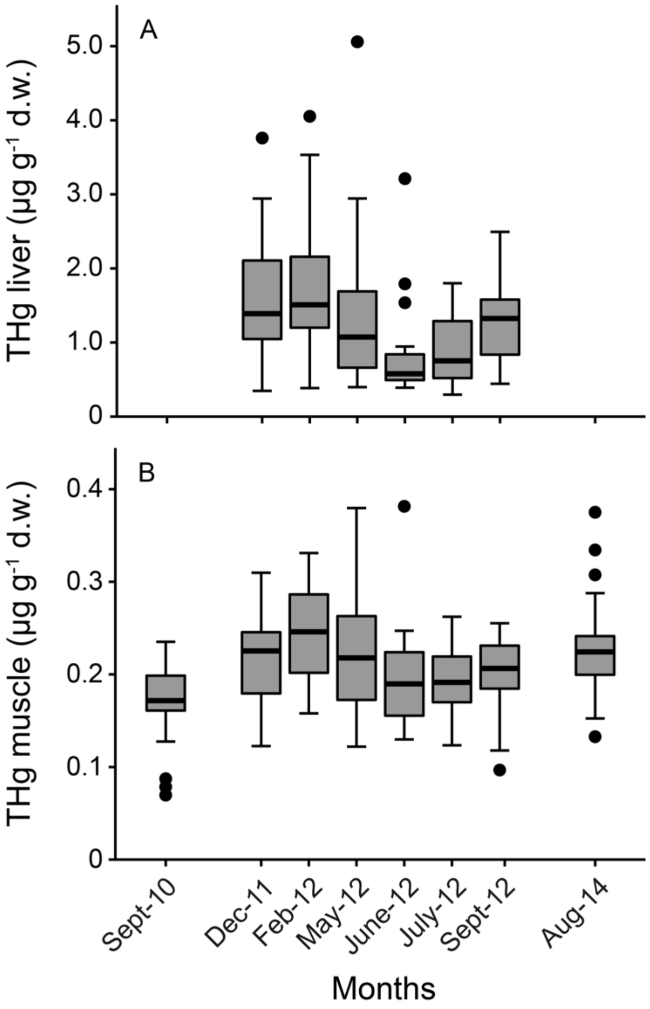

0-6m
6-10m
10-20m
20-30m
30-40m
>40m
A
N
2km
Latitude (°N)
68°60'
70°-
69°-
68°-
Norway
Finland
Sweden
20°
24°
20°47'
Longitude (°E)

Fig. 1.


A
THg liver (µg g⁻¹ d.w.)
5.0
4.0
3.0
2.0
1.0
0
B
THg muscle (µg g⁻¹ d.w.)
0.4
0.3
0.2
0.1
0
Sept-10
Dec-11
Feb-12
May-12
June-12
July-12
Sept-12
Aug-14
Months

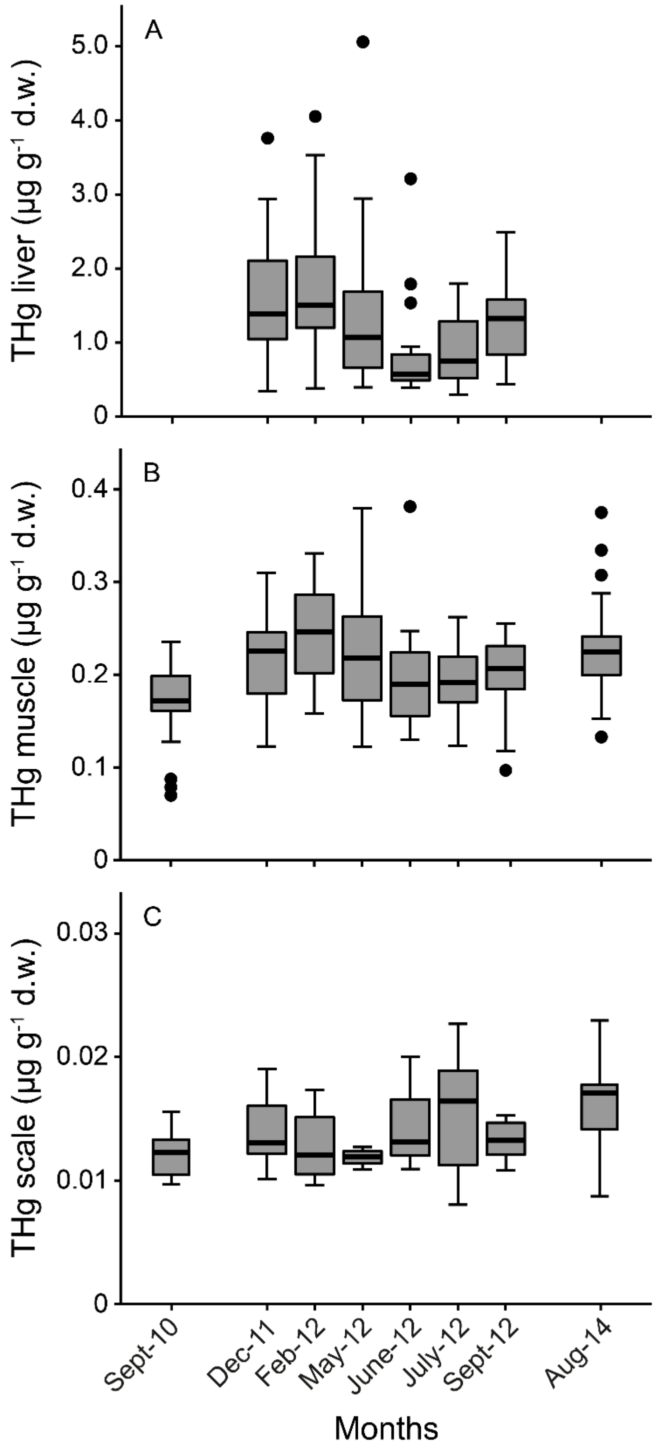
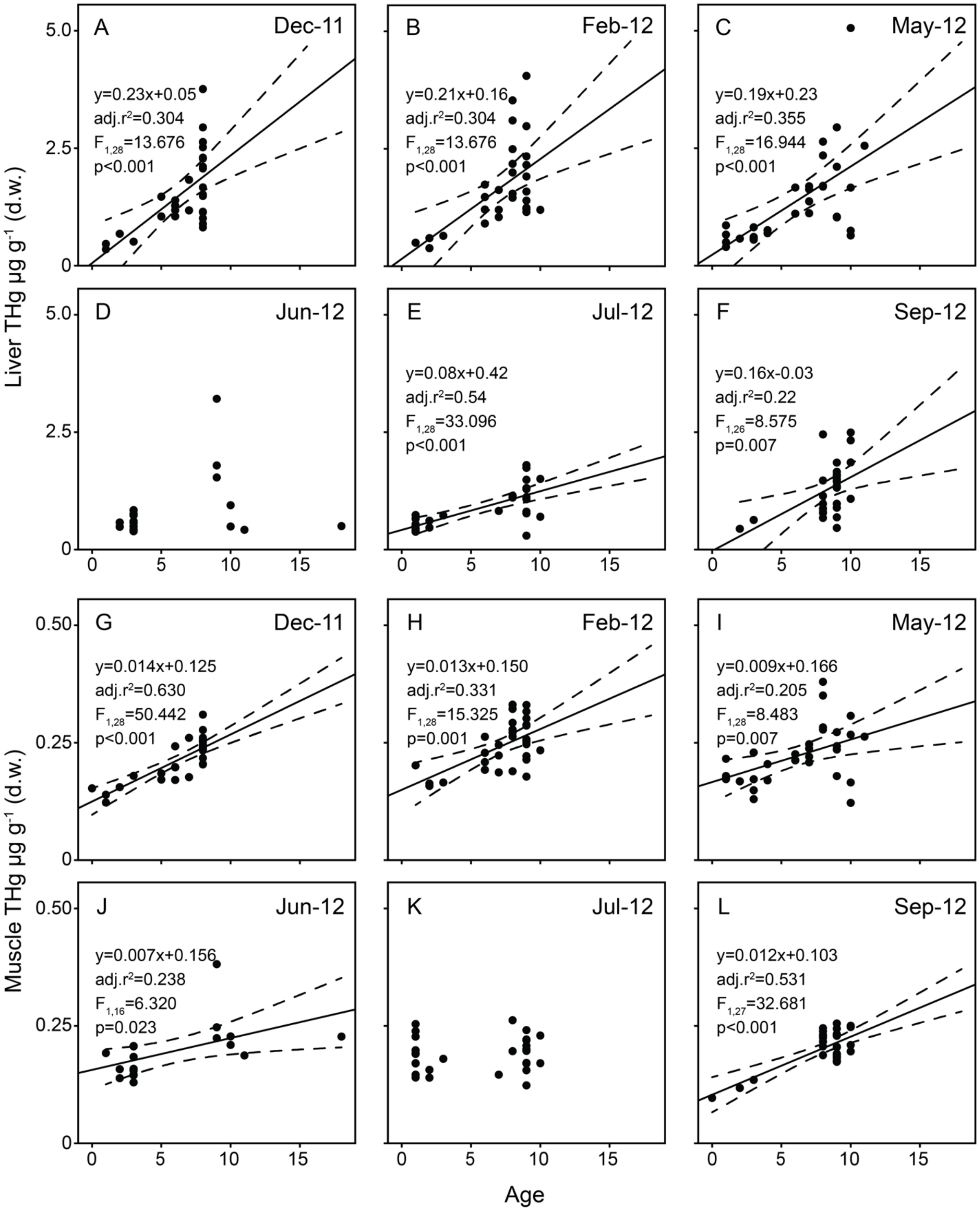

A
THg liver (μg g^{-1} d.w.)
0
1.0
2.0
3.0
4.0
5.0
B
THg muscle (μg g^{-1} d.w.)
0
0.1
0.2
0.3
0.4
C
THg scale (μg g^{-1} d.w.)
0
0.01
0.02
0.03
Sept-10
Dec-11
Feb-12
May-12
June-12
July-12
Sept-12
Aug-14
Months

Fig. 2.


Liver THg µg g^{-1} (d.w.)
A
Dec-11
y=0.23x+0.05
adj.r^2=0.304
$F_{1,28}$=13.676
p<0.001
B
Feb-12
y=0.21x+0.16
adj.r^2=0.304
$F_{1,28}$=13.676
p<0.001
C
May-12
y=0.19x+0.23
adj.r^2=0.355
$F_{1,28}$=16.944
p<0.001
D
Jun-12
E
Jul-12
y=0.08x+0.42
adj.r^2=0.54
$F_{1,28}$=33.096
p<0.001
F
Sep-12
y=0.16x-0.03
adj.r^2=0.22
$F_{1,26}$=8.575
p=0.007
5.0
2.5
0
0
5
10
15
Muscle THg µg g^{-1} (d.w.)
G
Dec-11
y=0.014x+0.125
adj.r^2=0.630
$F_{1,28}$=50.442
p<0.001
H
Feb-12
y=0.013x+0.150
adj.r^2=0.331
$F_{1,28}$=15.325
p=0.001
I
May-12
y=0.009x+0.166
adj.r^2=0.205
$F_{1,28}$=8.483
p=0.007
J
Jun-12
y=0.007x+0.156
adj.r^2=0.238
$F_{1,16}$=6.320
p=0.023
K
Jul-12
L
Sep-12
y=0.012x+0.103
adj.r^2=0.531
$F_{1,27}$=32.681
p<0.001
0.50
0.25
0
Age

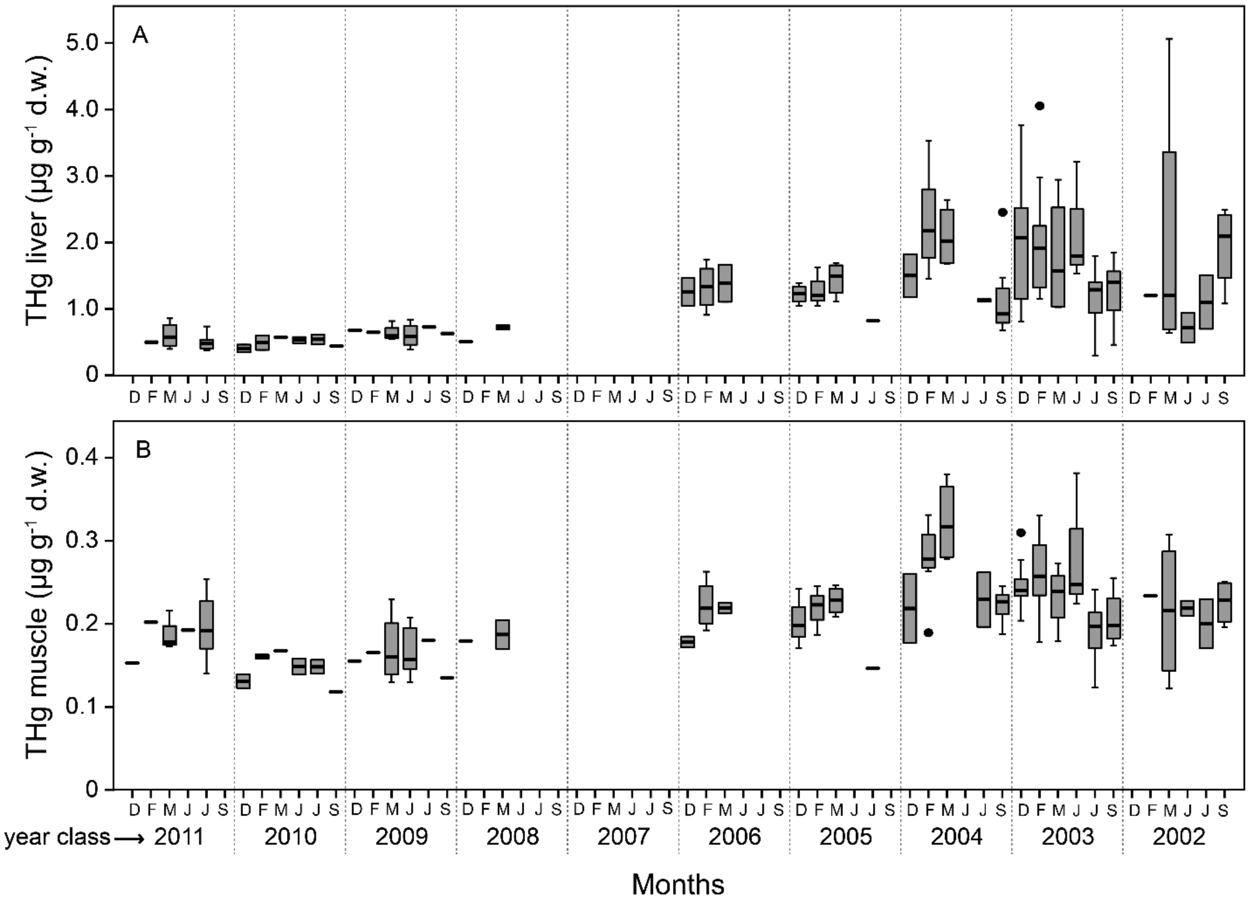
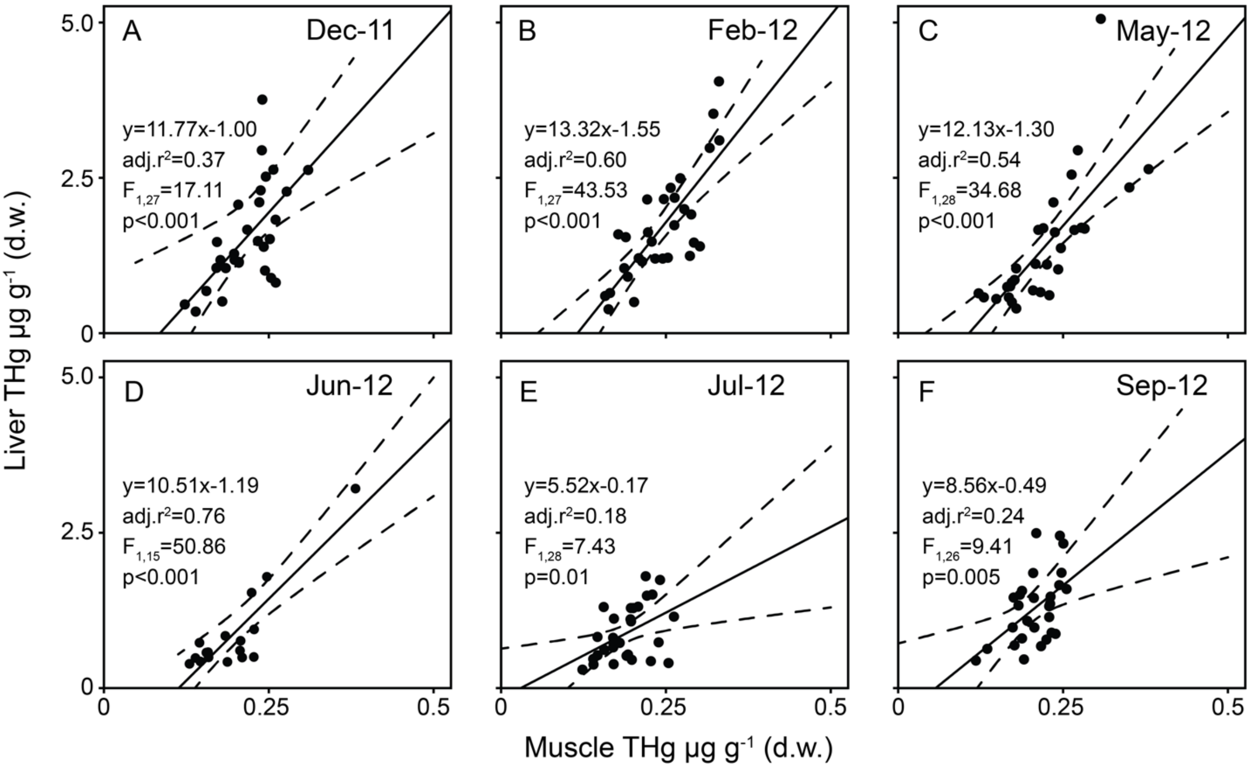

A
THg liver (μg g⁻¹ d.w.)
0
1.0
2.0
3.0
4.0
5.0
B
THg muscle (μg g⁻¹ d.w.)
0
0.1
0.2
0.3
0.4
D F M J J S
year class ⟶ 2011
2010
2009
2008
2007
2006
2005
2004
2003
2002
Months

Fig. 3.


Liver THg µg g^{-1} (d.w.)
Muscle THg µg g^{-1} (d.w.)
A
Dec-11
y=11.77x-1.00
adj.r^2=0.37
$F_{1,27}$=17.11
p<0.001
B
Feb-12
y=13.32x-1.55
adj.r^2=0.60
$F_{1,27}$=43.53
p<0.001
C
May-12
y=12.13x-1.30
adj.r^2=0.54
$F_{1,28}$=34.68
p<0.001
D
Jun-12
y=10.51x-1.19
adj.r^2=0.76
$F_{1,15}$=50.86
p<0.001
E
Jul-12
y=5.52x-0.17
adj.r^2=0.18
$F_{1,28}$=7.43
p=0.01
F
Sep-12
y=8.56x-0.49
adj.r^2=0.24
$F_{1,26}$=9.41
p=0.005
0
2.5
5.0
0
0.25
0.5

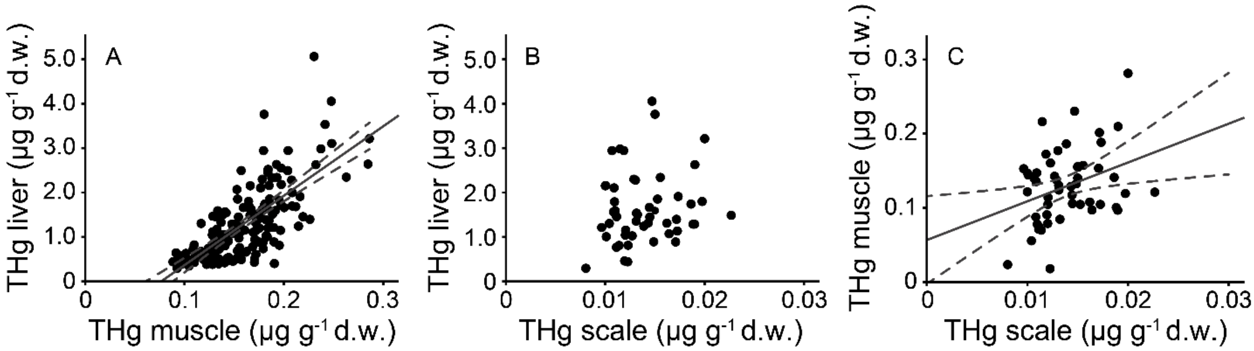

A
THg liver (µg g^{-1} d.w.)
5.0
4.0
3.0
2.0
1.0
0
0
0.1
0.2
0.3
THg muscle (µg g^{-1} d.w.)
B
THg liver (µg g^{-1} d.w.)
5.0
4.0
3.0
2.0
1.0
0
0
0.01
0.02
0.03
THg scale (µg g^{-1} d.w.)
C
THg muscle (µg g^{-1} d.w.)
0.3
0.2
0.1
0
0
0.01
0.02
0.03
THg scale (µg g^{-1} d.w.)

Fig. 4.

Highlights:

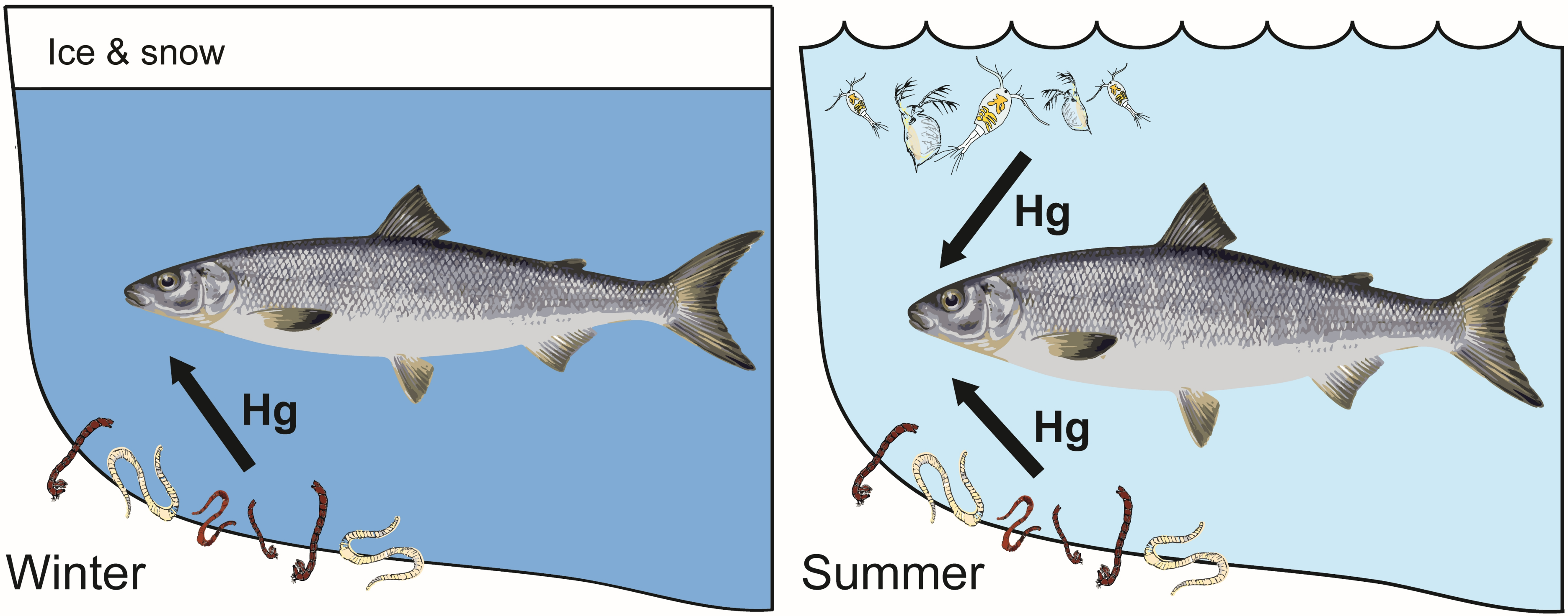
-Year-round variation of THg in fish tissues is poorly understood in subarctic lakes.

-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.


Ice & snow
Hg
Winter
Hg
Hg
Summer

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

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[e]Núcleo Milenio de Salmónidos Invasores, Concepción, Chile

Highlights:

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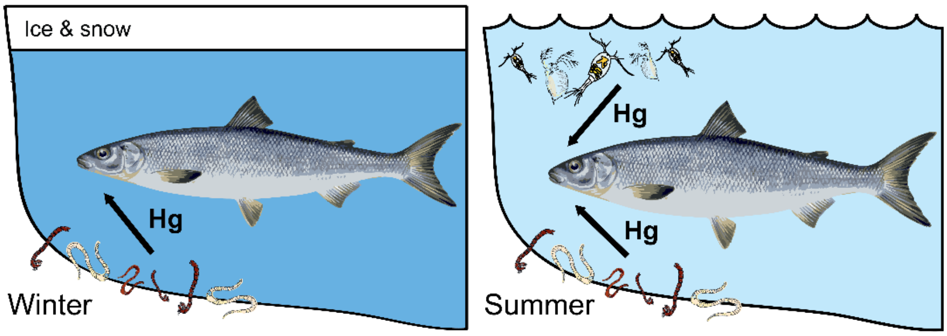
-Starvation and planktivory increased THg, while growth dilution decreased THg.
-Intra-annual variation of THg in tissues was higher than inter-annual accumulation.
-Bioaccumulation of THg was highest in winter and lowest in summer for both tissues.

Graphical abstract:



Abstract:

Subarctic lakes are characterised by extreme seasonal variation in light and temperature which influences growth, maturation, condition and resource use of fishes. However, our understanding of how seasonal changes affect mercury concentrations of fishes is limited. We conducted a year-round study (3 ice-covered months, 3 open-water months) with open-water inter-annual aspect (3 years: samples from August/September), focusing on total mercury (THg) concentrations and ecological characteristics of a common freshwater fish, European whitefish (*Coregonus lavaretus* (L.)) from a subarctic lake. We measured THg concentrations from tissues with fast (liver, n=164) and moderate (muscle, n=225) turnover rates, providing information on THg dynamics over different temporal scales. In both tissues, lipid-corrected THg concentrations were highest in winter (liver: 1.70±0.88 µg/g, muscle: 0.24±0.05 µg/g) and lowest in summer (liver: 0.87±0.72 µg/g, muscle: 0.19±0.04 µg/g). THg concentrations increased in winter following the summer-autumn dietary shift to pelagic zooplankton and starvation after spawning. Whitefish THg concentrations decreased towards summer, and were associated with consumption of benthic macroinvertebrates and subsequent growth dilution. Mercury bioaccumulated in both tissues with age, both showing the strongest regression slopes in winter and lowest in summer. THg concentrations in liver and muscle tissue were correlated throughout the year, however the correlation was lowest in summer, indicating high metabolism during somatic growing season in summer and growth dilution. Multiple linear regression models explained 50% and 55% of the THg variation in liver and muscle both models dominated by seasonally-variable factors i.e. sexual maturity, $\delta^{13}C$, and condition factor. Seasonally varying bioaccumulation slopes and the higher level of intra-annual variation (21%) in whitefish THg concentration in muscle than the inter-annual accumulation (8%) highlight the importance of including seasonal factors in future THg studies.

Capsule:

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

Keywords:

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

1. Introduction

Atmospheric mercury (Hg) originates from both natural and anthropogenic sources, and concentrations in ecosystems across the globe have increased since the industrial revolution (Pacyna et al., 2010). Atmospheric deposition typically dominates the supply of Hg to Arctic and subarctic lakes lacking direct Hg pollution sources in their catchment (e.g. Downs et al., 1998, Ariya et al., 2015). The Arctic has shown clear, and marked increasing trends in Hg concentrations, e.g. in lake sediments since the 18th century industrial era (Chételat et al., 2015). Hg speciation (e.g. methylation) in aquatic enviornments occurs through both biotic and abiotic pathways, and via numerous different mechanisms (e.g. Jensen & Jernelöv, 1969; Pak & Bartha, 1998; Celo et al., 2006). In lake ecosystems, sulphur-oxidizing bacteria play key roles in the methylation process, resulting in the production of organic MeHg (e.g. Morel et al., 1998). Both benthic and pelagic primary producers, i.e. periphyton and phytoplankton, uptake inorganic and organic Hg through passive and active transport processes (Mason et al., 1995, 1996; Douglas et al., 2012). Benthic macroinvertebrates and zooplankton consume these primary producers and transfer the Hg to invertebrate feeding fish, which are in turn eaten by higher trophic level consumers such as piscivorous fish. In subarctic lakes, Hg often accumulates faster in the pelagic food web compartment than the littoral (e.g. Kahilainen et al., 2016a; Thomas et al., 2016; Kahilainen et al., 2017), likely reflecting increased trophic transfer efficiency in the pelagic food web. MeHg is especially highly reactive, bioaccumulating in organisms and biomagnifying through the food chain (e.g. Watras & Bloom, 1992; Watras et al., 1998). For example, MeHg is estimated to represent a total proportion of mercury (THg) concentration that ranges from 10% in the water column, to 15% in phytoplankton, 30% in zooplankton and up to 95% in fish muscle (Watras & Bloom, 1992; Watras et al., 1998).

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

In fish, Hg generally bioaccumulates with increasing size and age (e.g. Downs et al., 1998; Amundsen et al., 2011; Swanson et al., 2011). In species with ontogenetic dietary shifts, Hg concentration can increase as consumers shift to a higher trophic level or switch to Hg-enriched pelagic prey (e.g. Power et al., 2002; Thomas et al., 2016; Kahilainen et al., 2017). Fast growing individuals accumulate muscle tissue faster than Hg, a phenomenon termed growth dilution (e.g. Simoneau et al., 2005; Ward et al., 2010). Furthermore, growth dilution is inversely related to increasing condition factor and individual lipid reserves (e.g. Amlund et al., 2007; Braaten et al., 2014; Kahilainen et al., 2016a). In Arctic and subarctic lakes, many fish species have a lower growth rate, higher longevity and later sexual maturation relative to their equivalents in temperate lakes (Heibo et al., 2005; Blanck & Lamouroux, 2007), increasing the period of Hg bioaccumulation. Furthermore, in seasonally ice-covered systems, condition and lipid reserves of fish are generally the lowest in winter (e.g. Hayden et al., 2014a, 2015). Decreasing lipid content, and potentially also protein loss during starvation, may condense Hg in remaining tissues (e.g. Kahilainen et al., 2016a). In the Hg contamination literature, this phenomenon is termed as starvation (e.g. Cizdziel et al., 2002, 2003; Moreno et al., 2015) and, along with growth dilution, it may play a key role in the seasonal variation in Hg concentrations in cold-water fishes. Such variation may be important factor when considering Hg monitoring programs and human health considerations, as many cold-water fishes play important roles in the year-round diet of indigenous and non-indigenous people in the region (AMAP 2011).

The European whitefish (*Coregonus lavaretus* (L.)) is a highly diverse and often the most abundant fish species in subarctic lakes of northern Fennoscandia (Siwertsson et al., 2010; Praebel et al., 2013; Malinen et al., 2014). Most of the lakes are inhabited by a generalist monomorphic whitefish populations using all habitat types, while polymorphic populations are diverged into separate pelagic and benthic morphs (Kahilainen et al., 2007; Harrod et al., 2010; Siwertsson et al., 2010). In the most complex cases, whitefish is ecomorphologically diverged into one of the three main lake habitats, littoral, pelagic or profundal (Kahilainen & Østbye, 2006; Harrod et al., 2010; Kahilainen et al., 2014). The whitefish morphs show many morphological and physiological adaptions to their specific habitat types, where a heritable trait, number of gill rakers, encapsulates most of the variation as a single measurement (Kahilainen et al., 2011b, 2014, 2016). Profundal morphs have the very low amount of short and widely spaced gill rakers suitable for foraging on fine sediments in dark condition charactistic of profundal habitat, whereas littoral and generalist morphs have intermediate number of relatively short gill rakers followed by pelagic morphs with very high number of fine, long and densely spaced gill rakers as adaption to foraging on small zooplankton prey (Kahilainen et al., 2007, 2011a, 2017). In both monomorphic and polymorphic lake types, whitefish as the most abundant species is key invertebrate feeding predator and main prey for many piscivores, thus acting as a central node in lake food webs (e.g. Kahilainen & Lehtonen, 2003; Kahilainen et al., 2009, 2011a). The key role of both monomorphic and polymorphic whitefish in the food webs of subarctic lakes has influence on pelagic and benthic energy and Hg flows (Thomas et al., 2016; Kahilainen et al., 2017), but we currently lack of knowledge regarding potential temporal variation in patterns of contaminant bioaccumulation that is likely influenced by seasonality of prey availability, growth, reproduction and condition.

To fill this knowledge gap, we undertook a year-round study of THg concentrations in a monomorphic whitefish population, and their putative prey sources in a relatively well-studied subarctic lake, Lake Kilpisjärvi, located in northern Fennoscandia. Here, monomorphic whitefish are known to undergo a dietary shift from littoral benthic macroinvertebrates during winter and spring to pelagic zooplankton in mid to late summer, coinciding with an annual zooplankton bloom (Tolonen, 1999; Hayden et al., 2014a). Pelagic prey is generally considered a more important source of Hg, due to often higher MeHg concentrations in zooplankton than littoral benthic prey (Watras et al., 1998; Suchanek et al., 2008). Previous work on whitefish morphs indicated that pelagic zooplanktivorous morphs had much higher THg concentrations and steeper bioaccumulation slopes than benthivorous morphs (Kahilainen et al., 2017). Open-water season dietary shifts from benthic macroinvertebrates to pelagic zooplankton in Arctic fishes such as Arctic charr (*Salvelinus alpinus* (L.)) have been shown to affect THg concentrations in fish liver and muscle tissue (Kahilainen et al., 2016a). Open-water season THg studies of fish muscle has been conducted with many species (e.g. Zhang et al., 2012; Braaten et al., 2014; Moreno et al., 2015; Olk et al., 2016), but we are unaware of any year-round (including ice-covered winter) muscle and liver studies on THg concentrations of fish. Dietary shifts are clearly important in Hg exposure, but we know very little about the seasonal patterns of THg concentrations in whitefish and the main factors affecting any putative changes. To address these questions, we collected data year-round on whitefish growth, sexual maturation, condition and resource use with THg measured from muscle and liver tissues in a subarctic lake in northern Finnish Lapland. Our study was designed to test three hypotheses:

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

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

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

2. Materials and methods

2.1 Study area

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

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

2.2 Sample period and sampling methods

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

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

Fish were collected using gillnets fished in series including seven 1.8 m high and 30 m long nets (knot-to-knot mesh sizes: 12, 15, 20, 25, 30, 35, 45 mm), supplemented with one 1.5 m high and 27 m long Nordic multimesh gillnet (5.25-55 mm). Gillnet series were set in benthic habitat at depths 2-15 m overnight (summer: 10-12h, winter: 24-48h). Fish were immediately euthanized by cerebral concussion at the sampling site. After immediate transport to the laboratory, total length and mass of whitefish were measured to the nearest mm and 0.1 g. Fulton's condition factor (K) was calculated 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 unregenerated scales pressed on polycarbonate slides and viewed using a microfiche reader (Kahilainen et al., 2003). The join 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).

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

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

2.4 Total mercury analysis

THg concentrations (µg g^{-1} d.w.) were analysed from the freeze-dried ZPL (n=17), BMI (n=20), liver (n=167) and muscle (n=225) samples by atom absorption spectrometry using a direct Hg analyser (Milestone DMA 80). We had a target fish sample size for each month of 30 individuals representing the total length and age distribution of the population (Table 1) and all invertebrate samples containing enough tissue were analysed. From each sample, two duplicates (20-30 mg) were analysed when material was not limited due to low sample mass - as was the case with some liver samples and almost all invertebrate samples. Average relative difference (RSD) between duplicates of liver (n=113 pairs), muscle (n=223 pairs) and invertebrates (n=2 pairs) was 1.1, 1.3 and 11.2%, respectively. At the start and end of each run, samples of certified reference material (DORM-4; 0.410 ± 0.055 µg g^{-1}; National Research Council Canada) were combusted. The average and recovery-% of the certified reference material (n=66) was 0.408 ± 0.011 (SD) and 99.6% respectively. Blank control samples (grand mean ± SD: 0.001 ± 0.001, n=113) were added both at the end of each run, as well as between different tissues and taxa. Run specific blank THg values was subtracted from analysed sample THg values to avoid instrumental error. The mean of the blank adjusted duplicate THg values was later lipid-corrected.

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

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

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

2.5 Statistical methods

Examination of seasonal changes of Hg concentrations in whitefish tissues (H1) and all supporting analyses of variance were conducted with non-parametric tests (Kruskal-Wallis H-test with post hoc: Mann-Whitney U-test, or if the assumption of homogeneity of variances was violated, we used repeated Welch’s t-tests with the Games-Howell post-hoc test). The seasonal bioaccumulation and 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 auto-correlation and selected variables with $R^2 < 0.7$ (sampling month, total length, condition factor, sex, sexual maturity, GSI, gillraker number, $\delta^{13}C$, $\delta^{15}N$) 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).

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 $\delta^{15}N$ and values (Welch's ANOVA: $F_{7,217} = 2.49$, $p = 0.017$), with pairwise comparisons showing the highest values in winter (Table 1). The strong annual reliance on littoral BMI was also evident from relatively similar muscle δ^{13}C values (Welch's ANOVA: $F_{7,217} = 5.54$, $p<0.001$), with values showing a slight ^{13}C depletion in winter and ^{13}C enrichment at early summer (Table 1).

3.2 H1 - Annual mercury concentrations in invertebrates and whitefish tissues

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

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

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

Bioaccumulation of THg by age varied seasonally in both tissues showing the highest slopes and the 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).

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, $\delta^{15}N$ and total length were positively related to THg concentrations in both liver and muscle models, whereas $\delta^{13}C$ and condition factor was inversely related to the concentrations. In both models, sexual maturity, $\delta^{13}C$ 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.

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

4.2 Seasonal variation in mercury concentrations (H1)

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

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

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

The rate of increase in THg values in both liver and muscle slowed after sexual maturation. The ontogenetic dietary shift from ZPL to BMI at an early age in whitefish (Tolonen, 1998) and decreased somatic growth after maturation might explain the slowing THg accumulation. We found that population-level mean THg concentrations in whitefish muscle increased by 8 % per year, between September 2010 and August 2014. This value is indeed circa 2.5 times lower that observed intra-annual variation during 2011-2012. Both observations strongly reflect the dominance of the single mature 2003 year-class, with limited annual somatic growth explaining low inter-annual bioaccumulation and strong investment on gonads causing intra-annual variation of muscle THg. In previous studies of L. Kilpis whitefish, the dominance of a single year-class has been documented in diffent decades (Tolonen, 1998; Harrod et al., 2010) and such patterns of year-class dominance and generally high age of fish is typical in Arctic and subarctic lakes (Rolls et al., 2017). Taken collectively, our results of intra- and inter-annual patterns of THg should be thus the most valid for fish populations consisting of mature fish investigating their energy mostly to gonad growth instead of somatic growth, a pattern typical for a range of fish species.

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

In L. Kilpis whitefish, THg concentrations increased with age in both liver and muscle tissue. This pattern has been recorded also in many other studies using muscle tissues of subarctic salmonids (e.g. Amundsen et al. 2011, Swanson et al. 2011), but is less commonly reported from liver tissue. A previous study by Kahilainen et al. (2017), showed that THg concentrations in muscle generally increased with age in different European whitefish morphs, but the regression slopes were the most steep for pelagic morphs (range 0.038-0.103) and shallow for benthic whitefish morphs (0.017-0.020). This study also assessed bioaccumulation in three monomorphic whitefish population, which displayed relatively shallow slopes (0.020-0.025). These results corroborates our findings here, as we found shallow, or even non-significant bioaccumulation slopes during the summer growing season for both liver and muscle with some time-lag related to faster metabolic rate of former than later (e.g. Oliveira-Ribeiro et al., 1999; Hayden et al., 2014a; Kahilainen et al., 2016a). Interestingly, the bioaccumulation slopes of both tissues were clearly steeper during the ice-covered winter, most likely driven by the older mature individuals which had higher relative difference between winter and summer THg concentrations compared to the younger immature individuals. This could be explained by the stronger response of older fish to spawning, which is likely due to the fact that only six years or older individuals were sexually mature, and this was the group driving the changes in bioaccumulation seasonally.

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

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

We found that a high proportion of the variation (50% and 55%) in THg concentration in liver and muscle was explained through multiple linear regression analyses. Previous studies employing regression analyses to explain THg concentrations in whitefish have frequently documented that fish size and age are important factors affecting Hg concentration (e.g. Moreno et al., 2015; Thomas et al., 2016; Kahilainen et al., 2017). Surprisingly total length, which was correlated with age and mass, was a relatively poor predictor of liver and muscle THg concentrations in L. Kilpis. This most likely reflect the low investment to somatic growth of single year-class 2003 dominated whitefish population, where most fish are close to their maximum length. However, the inclusion of THg in liver and muscle and factors related to temporal variation such as sampling month, stable isotopes and sexual maturity have been examined to a far lesser degree. In this study, all of these factors describing year-round variation were highly important factors included in liver and muscle models and are discussed below.

In L. Kilpis, both muscle and liver THg values were inversely related with tissue specific $\delta^{13}C$ values, which likely reflects increased autumnal consumption of ^{13}C depleted pelagic ZPL containing more THg than littoral BMI (Kahilainen et al., 2016a; Thomas et al., 2016; Kahilainen et al., 2017). Liver and muscle THg values were negatively related with condition factor, which can be used as supporting evidence for starvation and growth dilution (Cizdziel et al., 2002, 2003; Evans et al. 2015). Condition factor does reflect spawning when gonads with low THg concentration are removed from the fish body, potentially further condensing Hg in whitefish muscle (Madenjian et al., 2016). Increased $\delta^{15}N$ values have been linked to metabolic-stress associated with starvation (Moreno et al., 2015) in some taxa. Here, we found slight seasonal changes in muscle and liver $\delta^{15}N$ values showing the highest values in winter and lowest in autumn. Therefore, the positive correlation of $\delta^{15}N$ and THg values in muscle and liver model possibly reflects winter starvation, when fish use protein reserves from both liver and muscle. Positive correlation between sexual maturity and THg concentrations in both liver and muscle models indicate a high significance of gonad development and spawning to the THg concentrations. Spawning may be related to starvation in whitefish, due to the high cost of gonad investment and low prey abundance during winter spawning period (Hayden et al., 2014a). In the liver model, the negative relationship between GSI and THg supports this idea; whitefish GSI was lowest and THg was highest after spawning in February-May, when we also reported the highest THg concentrations. In the muscle model, the opposite correlation between GSI and THg (positive) could be explained by random effect in the model since we found no correlation between GSI and muscle THg through simple linear regression analysis: in addition GSI had low significance in the multiple linear regression model explaining the variation in muscle THg. Sampling month significantly affected THg concentration, but the effect was positive in the liver model and negative in the muscle model, likely indicating that Hg is translocated faster in liver than in muscle. This could be explained by the different turnover times of these tissues, meaning that late summer derived Hg can be measured faster in liver (early winter) than in muscle (mid-winter). Therefore, the positive correlation between sampling month and liver THg could be explained by the high THg values in early winter (December). Most likely, the negative correlation between muscle THg concentrations and sampling month was driven by the high THg concentrations in mid-winter (February) and low concentrations in summer (June-July).

4.5 Monitoring and human health

An interesting aspect of our results was that intra-annual variability in THg concentrations of whitefish exceeded inter-annual variation, evident also in multiple linear regression analyses, where seasonal factors indicating diet ($\delta^{13}C$) and condition were generally more important than fish total length. As the year-round maximum variation of muscle tissue (21%) is surprisingly high compared to inter-annual (8%) accumulation in muscle, and that bioaccumulation slopes varied from non-significant or shallow in summer to highly steep and highly significant in winter, we suggest that such seasonal variation needs to be considered in future studies and especially in any long-term THg monitoring program. This is particilarly important as the aims of Hg monitoring programs are typically related to human health (AMAP 2011). Primarily, the sampling month should be standardized but since the annual anomalities, the seasonal succession (e.g. temperature build up) should be quantified as well since they might affect on THg of fish. Whitefish is the most important target fish of local fisheries year-round and represent a stable proportion of subsistence diet of native and non-native people (Thomas et al. 2016; Kahilainen et al., 2017). Although THg levels in all our fish samples were below national health limits (i.e. 0.5 µg g^{-1} wet mass; approx. 2.0-2.5 µg g^{-1} dry mass), the year-round patterns observed for whitefish may be relevant in other systems e.g. in other autumn or winter spawning fish such as many salmonids (Arctic charr, brown trout, lake trout, vendace) with putative winter starvation after reproduction. In spring spawning species, patterns could be different as the summer growing season starts immediately or soon after their reproduction, but additional year-round studies are needed to test this. For example, an annual variation of 21% would create a potential for THg values to exceed health limit guidelines and regional fish consumption regulations. Furthermore, seasonal changes of THg concentrations and bioaccumulation slopes in fish may lead to increased risk to human health in regions, where monitoring is restricted to low THg months i.e. mid summer. Depending on the aims of human health monitoring, both summer and winter sampling may be advisable as subsistence fishing is very common across Arctic and subarctic lakes in both seasons.

4.6 Conclusions

We revealed clear seasonal changes in the concentration and bioaccumulation slopes of THg in whitefish muscle and liver tissues. The results indicated that both starvation and growth dilution drive seasonal changes in THg concentrations in both tissues. Our data also provides new evidence for the role of pelagic diet shifts on increasing THg concentrations in both muscle and liver. The seasonal changes in diet and condition were generally more important factors than fish length explaining THg concentrations of whitefish muscle and liver tissues. The intra-annual variation in THg concentrations was higher than inter-annual bioaccumulation, in addition we found that bioaccumulation varied seasonally being highest in winter and low or non-significant in summer. Therefore, it is essential to consider seasonal factors in future studies and Hg monitoring programs.

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Supplementary data

Supplementary data to this article can be found online at:

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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 ± 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$^{a\text{-}h}$ 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, Simulidae, 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 ± 2.1	6.4 ± 2.5	7.3 ± 2.4	6.2 ± 3.2	5.8 ± 4.6	5.6 ± 3.9	^{a}8.1 ± 2.4	7.4 ± 4.2
Total length (mm)	247 ± 50	245 ± 52	248 ± 49	221 ± 71	227 ± 100	210 ± 94	269 ± 50	234 ± 83
Total mass (g)	133.0 ± 89.1	117.1 ± 55.7	117.8 ± 50.6	97.5 ± 76.7	141.8 ± 194.3	106.8 ± 95.2	165.0 ± 55.1	126.1 ± 93.7
Somatic mass		^{g}111.2 ±50.1	^{g}117.2 ± 50.3	^{g}97.2 ± 76.3	140.3 ± 191.4	^{g}105.5 ± 93.4	^{bcdf}162.0±54.2	
Somatic mass* (g)		^{g}138.3 ± 26.5	145.7 ± 30.7	157.6 ± 24.1	^{g}123.0 ± 26.8	182.61 ± 51.2	^{be}185.5 ± 25.8	
Condition factor	^{def}0.75 ± 0.10	^{g}0.69 ± 0.07	^{g}0.70 ± 0.07	^{ag}0.67 ± 0.07	^{ag}0.65 ± 0.10	^{ag}0.67 ± 0.11	^{bcdefh}0.78±0.05	^{f}0.71 ± 0.08
Condition factor*	0.75 ± 0.04	^{g}0.72 ± 0.05	^{g}0.71 ± 0.09	0.72 ± 0.04	0.63 ± 0.08	0.75 ± 0.07	^{bc}0.79 ± 0.05	0.74 ± 0.06
Sexual maturity	^{cg}2.53 ± 0.94	3.03 ± 1.54	^{adef}4.43 ± 2.56	^{c}2.40 ± 1.57	^{c}2.39 ± 1.79	^{c}2.30 ± 2.09	^{a}3.41 ± 1.18	2.91 ± 1.51
GSI		^{d}2.9 ± 4.7	^{g}0.4 ± 0.3	^{bg}0.2 ± 0.2	^{g}0.5 ± 0.6	^{g}0.6 ± 1.2	^{cdef}1.7 ± 1.6	
Gillraker count	25.2 ± 1.2	25.0 ± 2.1	24.3 ± 2.0	24.2 ± 1.6	23.9 ± 1.5	24.0 ± 1.7	24.4 ± 1.6	24.1 ± 2.0
C:N Liver		^{df}4.54 ± 0.64	^{f}4.35 ± 0.72	^{b}4.14 ± 0.23	4.15 ± 0.32	^{bc}4.04 ± 0.35	4.22 ± 0.19	
C:N Muscle	3.20 ± 0.04	3.21 ± 0.04	3.20 ± 0.06	3.20 ± 0.05	3.22 ± 0.05	^{h}3.22 ± 0.05	^{h}3.22 ± 0.05	^{fg}3.18 ± 0.05
δ ^{13}C Liver (lipid free)		-23.5 ± 1.7	-23.7 ± 2.1	-23.7 ± 1.8	-23.3 ± 8.6	-24.9 ± 1.9	-23.8 ± 1.4	
δ^{13}C muscle (lipid free)	-24.6 ± 2.9	^{e}-25.0 ± 1.7	^{e}-25.2 ± 1.4	-24.6 ± 1.5	^{bch}-22.1 ± 3.0	-24.3 ± 1.2	-24.6 ± 1.2	^{e}-25.1 ± 1.5
δ^{15}N Liver		8.0 ± 0.6	^{g}8.2 ± 0.6	^{g}8.3 ± 0.7	^{g}8.3 ± 0.8	^{g}8.1 ± 0.5	^{cdef}7.5 ± 0.5	
δ^{15}N Muscle	^{b}8.4 ± 0.5	^{a}8.7 ± 0.3	8.6 ± 0.3	8.6 ± 0.4	8.5 ± 0.4	8.5 ± 0.4	8.6 ± 0.3	8.6 ± 0.4
Stomach fullness	^{bf}3.8 ± 1.6	^{acdefgh}0.4±0.9	^{bfg}2.3 ± 2.6	^{bf}3.3 ± 2.5	^{b}4.0 ± 1.6	^{abcdh}5.4 ± 1.9	^{bc}4.6 ± 1.7	^{bf}3.7 ± 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		

Table 2. THg concentrations (µg g^{-1} d.w.) ± SD (n) of liver an muscle tissues of whitefish and invertebrates by sampling months and years. Superscript with capital letters^{A-H} presented before THg means of different tissues indicates 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). Superscript small letters^{a-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±0.04 (30)			
^{B}Dec-11	^{F}1.56±0.82 (29)	^{A}0.22±0.04 (30)	0.040 (1)	0.050 (2)	0.573 (2)
^{C}Feb-12	^{EF}1.70±0.88 (30)	^{AEF}0.24±0.05 (30)	0.061 (1)	0.057 (2)	
^{D}May-12	1.39±0.99 (30)	0.22±0.06 (30)	0.065±0.006 (3)	0.055 (2)	
^{E}June-12	^{C}0.87±0.72 (17)	^{AB}0.20±0.06 (18)		0.057 (1)	
^{F}July-12	^{BCG}0.88±0.42 (30)	^{B}0.19±0.04 (30)	0.060±0.002 (3)	0.036 (2)	
^{G}Sep-12	^{F}1.29±0.57 (28)	0.20±0.04 (29)	0.067±0.002 (3)		0.319 (2)
^{H}Aug-14		^{A}0.23±0.05 (28)	0.084±0.005 (6)	0.027±0.010 (5)	0.205 (2)
Grand mean	1.31±0.81 (164)	0.21±0.05 (225)	^{b}0.070±0.013 (17)	^{a}0.042±0.014 (14)	0.366±0.356 (6)

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

Tissue	Factor	B ± SE	t	p
Liver	Sexual maturity	0.241 ± 0,042	5.753	<0.001
adj.R^2=0.50, RSE=0.577	δ^{13}C liver	-0.120 ± 0.024	-4.928	<0.001
$F_{7,\,156}$=24.0, p<0.001	Condition factor	-2.582 ± 1.092	-2.997	0.003
AIC=-172.67	Constant	-2.985 ± 1.092	-2.733	0.007
	GSI	-0.060 ± 0.022	-2.677	0.008
	δ^{15}N liver	0.197 ± 0.083	-2.677	0.019
	Total length	0.003 ± 0.001	2.367	0.020
	Sampling month	0.034 ± 0.016	2.100	0.037
Muscle	δ^{13}C muscle	-0.013 ± 0.002	-8.225	<0.001
adj.R^2=0.55, RSE=0.034	Condition factor	-0.211 ± 0.046	-4.570	<0.001
$F_{7,159}$=30.18, p<0.001	Total length	0.0003 ± 0.0001	3.945	0.003
AIC=-1118.82	Sampling month	-0.003 ± 0.001	-2.974	0.004
	Sexual maturity	0.006 ± 0.003	2.274	0.024
	Constant	-0.172 ± 0.080	-2.147	0.033
	δ^{15}N muscle	0.016 ± 0.008	2.088	0.038
	GSI	0.002 ± 0.001	1.746	0.082

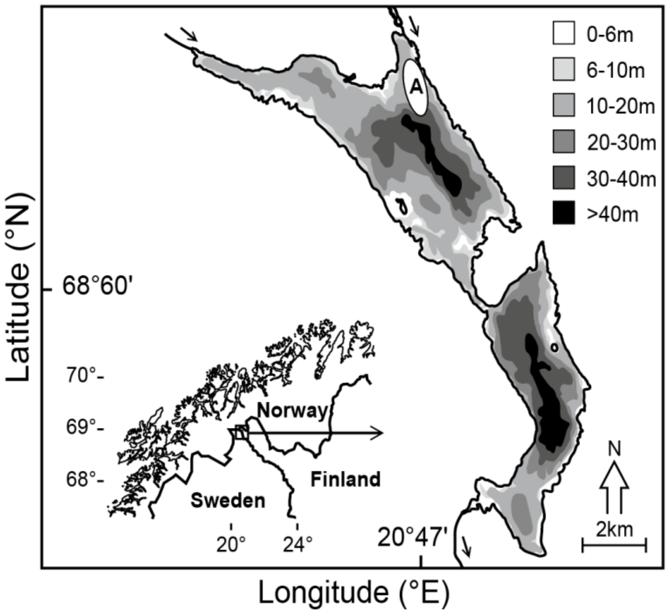
Figure legends

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

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

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

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


0-6m
6-10m
10-20m
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30-40m
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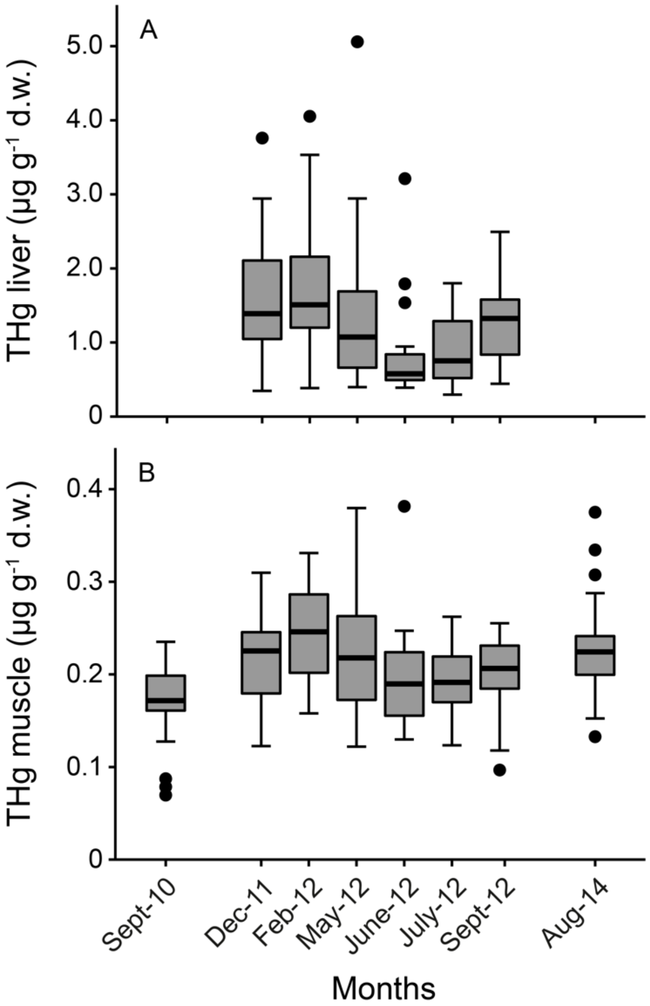
A
Latitude (°N)
68°60'
70°-
69°-
68°-
Norway
Finland
Sweden
20°
24°
20°47'
N
2km
Longitude (°E)

Fig. 1.

787



788

789 Fig. 2.

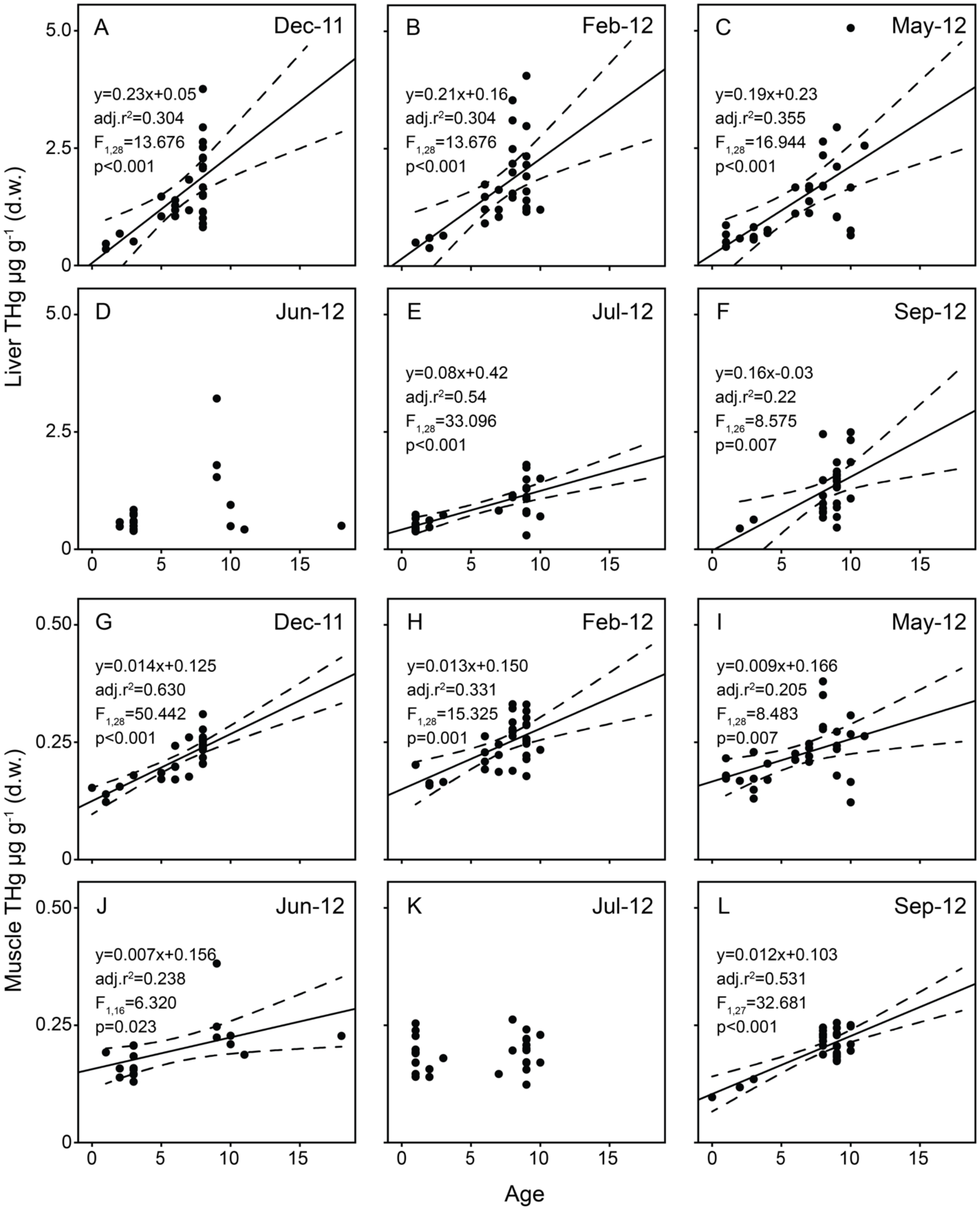

Liver THg µg g-1 (d.w.)
Muscle THg µg g-1 (d.w.)
Age
5.0
2.5
0
0.50
0.25
0
5
10
15
A
Dec-11
y=0.23x+0.05
adj.r2=0.304
F1,28=13.676
p<0.001
B
Feb-12
y=0.21x+0.16
adj.r2=0.304
F1,28=13.676
p<0.001
C
May-12
y=0.19x+0.23
adj.r2=0.355
F1,28=16.944
p<0.001
D
Jun-12
E
Jul-12
y=0.08x+0.42
adj.r2=0.54
F1,28=33.096
p<0.001
F
Sep-12
y=0.16x-0.03
adj.r2=0.22
F1,26=8.575
p=0.007
G
Dec-11
y=0.014x+0.125
adj.r2=0.630
F1,28=50.442
p<0.001
H
Feb-12
y=0.013x+0.150
adj.r2=0.331
F1,28=15.325
p=0.001
I
May-12
y=0.009x+0.166
adj.r2=0.205
F1,28=8.483
p=0.007
J
Jun-12
y=0.007x+0.156
adj.r2=0.238
F1,16=6.320
p=0.023
K
Jul-12
L
Sep-12
y=0.012x+0.103
adj.r2=0.531
F1,27=32.681
p<0.001

Fig. 3.

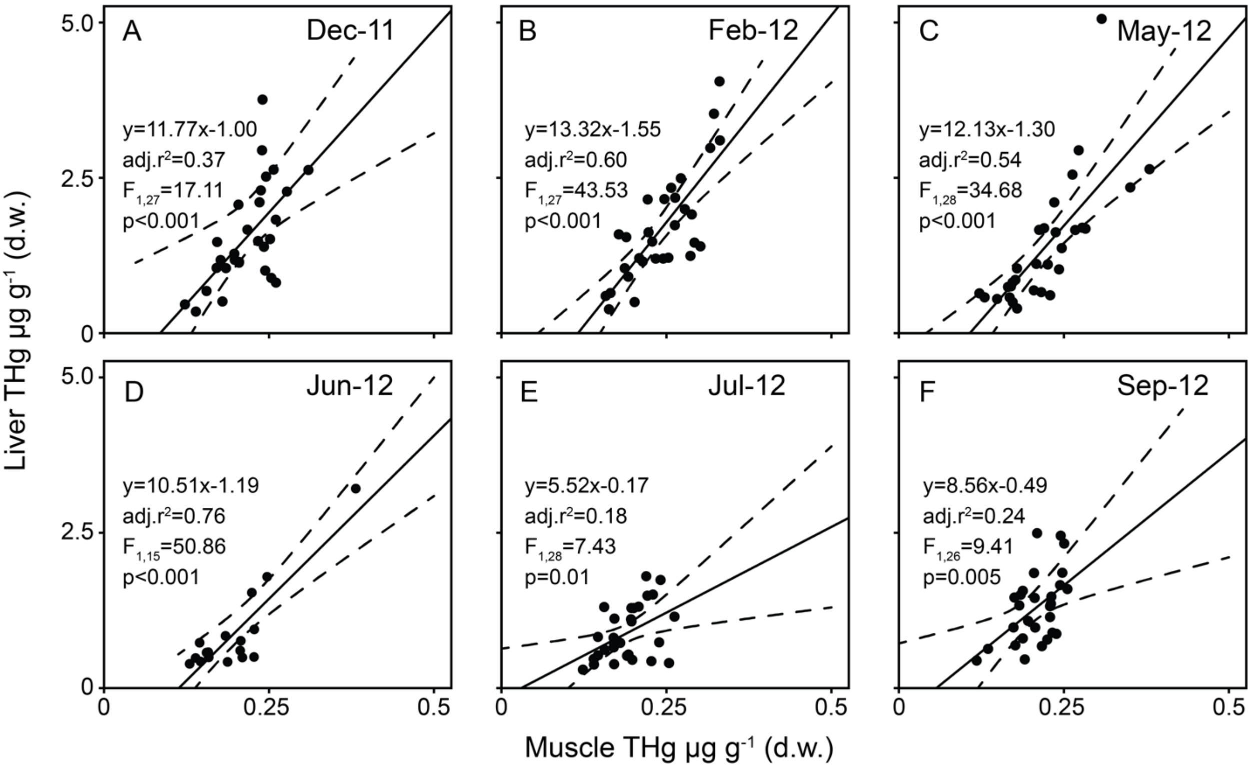

A
Dec-11
y=11.77x-1.00
adj.r²=0.37
F1,27=17.11
p<0.001
B
Feb-12
y=13.32x-1.55
adj.r²=0.60
F1,27=43.53
p<0.001
C
May-12
y=12.13x-1.30
adj.r²=0.54
F1,28=34.68
p<0.001
D
Jun-12
y=10.51x-1.19
adj.r²=0.76
F1,15=50.86
p<0.001
E
Jul-12
y=5.52x-0.17
adj.r²=0.18
F1,28=7.43
p=0.01
F
Sep-12
y=8.56x-0.49
adj.r²=0.24
F1,26=9.41
p=0.005
Liver THg µg g-1 (d.w.)
Muscle THg µg g-1 (d.w.)
0
2.5
5.0
0
0.25
0.5

Fig. 4.

Supplementary information:

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

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Table 1S. Detailed stomach content table. Identified prey items are separated to pelagic zooplankton (pelagic ZPL), benthic zooplankton (benthic ZPL), benthic macroinvertebrates, terrestrial insects, fish and others. Mean percentage contribution is presented for each prey item and group. The bolded groups and values are summarized percentages for each group.

	Sep-10	Dec-11	Feb-12	May-12	Jun-12	Jul-12	Sep-12	Aug-14
N=	30	30	30	30	18	30	29	28
Empty stomachs	1	24	12	8	1	0	0	1
Stomach fullness	3.8	0.4	2.3	3.3	4.0	5.4	4.6	3.7
pelagic ZPL	**41.2**	**-**	**32.4**	**26.0**	**9.0**	**49.6**	**6.6**	**36.9**
Bosmina sp.	5.0	-	-	-	8.3	49.6	2.6	18.3
Holopedium gibberum	-	-	-	-	-	-	-	15.7
Cladocera unident.	0.5	-	-	-	-	-	-	-
Calanoida	-	-	-	11.2	-	-	3.6	-
Other Copepoda	35.7	-	32.4	14.8	0.7	-	0.4	3.0
Benthic ZPL	**26.1**	**7.7**	**-**	**20.0**	**-**	**24.9**	**60.3**	**21.4**
Eurycercus lamellatus	21.2	-	-	-	-	24.9	60.3	21.4
Megacyclops	5.0	7.7	-	20.0	-	-	-	-
Benthic macroinvertebrates	**22.2**	**92.3**	**64.4**	**50.1**	**91.0**	**19.8**	**16.9**	**30.2**
Chironnomid larvae	10.9	60.0	57.3	34.5	16.4	3.0	4.3	6.6
Chironomid pupae	-	-	-	1.0	63.2	12.4	-	18.3
Ephemeroptera	-	7.7	-	-	-	-	-	-
Lymnaea sp.	2.1	7.7	-	0.7	9.6	1.2	2.3	1.0
Pisidium sp.	5.9	16.9	7.1	2.7	0.4	2.3	10.2	3.9
Plecoptera nymph	-	-	-	2.0	1.4	-	-	-
Plecoptera pupae	-	-	-	6.1	-	-	-	-
Simulidae	-	-	-	-	-	0.3	-	-
Tricoptera larvae	0.1	-	-	-	-	0.6	-	-
Valvata sp.	3.2	-	-	3.1	-	-	0.1	0.4
Terrestrial insects	**8.6**	**-**	**-**	**-**	**-**	**5.3**	**16.2**	**11.5**
Geometrid moth	-	-	-	-	-	-	9.4	-
Other terrestrial insects	8.6	-	-	-	-	5.3	6.8	11.5
Fish	**-**	**-**	**3.2**	**3.9**	**-**	**-**	**-**	**-**
Whitefish eggs	-	-	3.2	3.9	-	-	-	-
Other	**1.9**	**-**	**-**	**-**	**-**	**0.4**	**-**	**-**
Macrophyte	1.6	-	-	-	-	-	-	-
Corixidae	-	-	-	-	-	0.3	-	-
Hydracarina	-	-	-	-	-	0.1	-	-
Polyphemus pediculus	0.3	-	-	-	-	-	-	-
SUM	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Table 2S. Total mercury (THg), stable isotopes ($\delta^{13}C$, $\delta^{15}N$) and C:N values ±SD of littoral, pelagic and profundal invertebrates among months. Sample sizes are presented in parenthesis. The last column summarizes the grand mean for each row.

THg	Dec-11	Feb-12	May-12	Jun-12	Jul-12	Sep-12	Aug-14	SUM
Pelagic ZPL	0.040 (1)	0.061 (1)	0.065 ± 0.006 (3)		0.060 ± 0.002 (3)	0.067 ± 0.002 (3)	0.084 ± 0.005 (6)	0.070 ± 0.013 (17)
Littoral Oligochaeta	0.044±0.004 (3)	0.061 (1)	0.058 (1)		0.040 ± 0.005 (3)		0.037 ± 0.007 (2)	0.045 ± 0.009 (10)
Littoral Chironomidae	0.056 (1)	0.053 (1)	0.053 (1)	0.057 (1)	0.032 (1)			0.050 ± 0.010 (5)
Littoral Plecoptera							0.015 (1)	0.015 (1)
Littoral Trichoptera							0.020 (1)	0.020 (1)
Littoral Tipulidae							0.026 (1)	0.026 (1)
Profundal Oligochaeta	0.998 (1)					0.527 (1)	0.339 (1)	0.621 ± 0.340 (3)
Profundal Chironomidae	0.150 (1)					0.110 (1)	0.072 (1)	0.111 ± 0.039 (3)

$\delta^{13}C$	Dec-11	Feb-12	May-12	Jun-12	Jul-12	Sep-12	Aug-14	SUM
Pelagic ZPL	-32.2 (1)	-30.5 (1)	-30.1 ± 0.3 (3)		-33.3 ± 0.2 (3)	-31.6 ± 0.5 (3)	-31.6 ± 0.1 (6)	-31.6 ± 1.1 (17)
Littoral Oligochaeta	-16.6 (1)	-15.7 (1)	-15.3 (1)		-16.8 (1)		-21.3 (1)	-17.1 ± 2.4 (5)
Littoral Chironomidae	-17.4 (1)	-16.3 (1)	-16.4 (1)	-20.3 (1)	-16.5 (1)		-20.7 (1)	-17.9 ± 2.0 (6)
Littoral Plecoptera							-19.9 (1)	-19.9 (1)
Littoral Trichoptera							-20.5 (1)	-20.5 (1)
Littoral Tipulidae							-21.6 (1)	-21.6 (1)
Profundal Oligochaeta	-28.8 (1)					-26.7 (1)	-26.6 (1)	-27.4 ± 1.3 (3)
Profundal Chironomidae	-26.8 (1)					-29.6 (1)	-29.9 (1)	-28.8 ± 1.7 (3)

$\delta^{15}N$	Dec-11	Feb-12	May-12	Jun-12	Jul-12	Sep-12	Aug-14	SUM
Pelagic ZPL	6.3 (1)	3.9 (1)	2.9 ± 0.2 (3)		4.5 ± 0.3 (3)	4.0 ± 0.5 (3)	4.5 ± 0.2 (6)	4.2 ± 0.8 (17)
Littoral Oligochaeta	2.9 (1)	3.3 (1)	3.5 (1)		3.1 (1)		2.6 (1)	3.1 ± 0.3 (5)
Littoral Chironomidae	3.6 (1)	4.7 (1)	4.7 (1)	2.6 (1)	1.7 (1)		2.0 (1)	3.2 ± 1.3 (6)
Littoral Plecoptera							2.8 (1)	2.8 (1)
Littoral Trichoptera							2.2 (1)	2.2 (1)
Littoral Tipulidae							1.6 (1)	1.6 (1)
Profundal Oligochaeta	8.0 (1)					7.6 (1)	6.5 (1)	7.4 ± 0.8 (3)
Profundal Chironomidae	7.5 (1)					6.9 (1)	6.8 (1)	7.1 ± 0.4 (3)

C:N	Dec-11	Feb-12	May-12	Jun-12	Jul-12	Sep-12	Aug-14	SUM
Pelagic ZPL	11.1 (1)	8.7 (1)	5.4 ± 0.0 (3)		6.0 (1)	5.9 ± 0.1 (3)	4.7 ± 0.1 (6)	5.9 ± 1.7 (15)
Littoral Oligochaeta	5.2 (1)	4.8 (1)	4.8 (1)		4.9 (1)		4.9 (1)	4.9 ± 0.2 (5)
Littoral Chironomidae	5.2 (1)	5.4 (1)	5.4 (1)	5.4 (1)	5.6 (1)		5.5 (1)	5.4 ± 0.2 (6)
Littoral Plecoptera							4.4 (1)	4.4 (1)
Littoral Trichoptera							5.4 (1)	5.4 (1)
Littoral Tipulidae							5.1 (1)	5.1 (1)
Profundal Oligochaeta	5.3 (1)					4.2 (1)	4.2 (1)	4.6 ± 0.6 (3)
Profundal Chironomidae	5.4 (1)					6.1 (1)	5.9 (1)	5.8 ± 0.4 (3)

Table 3S. Stepwise model selection for linear multiple regression analysis based on minimum AIC values. The variables in models are: Sexual maturity (SexM), tissue specific stable isotopes values of carbon and nitrogen (d13C & d15N), gonadosomatic index (GSI), condition factor (CF), sampling month (Month), total length (TL). The selected models are in bold.

Model	AIC
Liver	
{}	-66.90
SexM	-121.03
SexM+d13C	-145.12
SexM+d13C+d15N	-159.94
SexM+d13C+d15N+GSI	-162.85
SexM+d13C+d15N+GSI+Month	-166.28
SexM+d13C+d15N+GSI+Month+CF	-168.97
SexM+d13C+d15N+GSI+Month+CF+TL	**-172.67**
SexM+d13C+d15N+GSI+Month+CF+TL+sex	-171.09
SexM+d13C+d15N+GSI+Month+CF+TL+sex+grcount	-169.33
Muscle	
{}	-991.66
d13C	-1047.62
d13C+sexM	-1089.56
d13C+sexM+CF	-1099.73
d13C+sexM+CF+TL	-1111.40
d13C+sexM+CF+TL+Month	-1114.82
d13C+sexM+CF+TL+Month+d15N	-1117.65
d13C+sexM+CF+TL+Month+d15N+GSI	**-1118.82**
d13C+sexM+CF+TL+Month+d15N+GSI+grcount	-1117.94
d13C+sexM+CF+TL+Month+d15N+GSI+grcount+sex	-1116.43