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5 6 7	1	Seasonal changes in European whitefish muscle and invertebrate prey fatty acid
8 9 10	2	composition in a subarctic lake
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22 Key words: Annual; Diet shift; HUFA; n-3/n-6; Spawning; Winter ecology

23 FWB additional key words: Biochemical analyses; Experimental ecology; Fatty acid; Fish;

24 Food web; Fresh waters; Invertebrate; Zooplankton

Contor Review

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25 Summary:

1. Despite extensive research into fish fatty acids (FA) over recent decades, we know little about seasonal changes of fish FA profile and content. Such changes are expected to be large in subarctic lakes, where ambient light and temperature show extreme seasonal variation due to the long cold period and polar night in winter.

2. We studied seasonal changes in the FA profile (mol%) and content (mg/g DW) of sexually mature European whitefish (*Coregonus lavaretus*) muscle in a large and deep subarctic lake located in northern Fennoscandia. We collected fish, zooplankton and benthic macroinvertebrate samples during three ice-covered months, including December (during whitefish spawning), and three open-water months. Fish size, age, sex, stomach content and fullness, as well as gonadosomatic index were also assessed as co-variates.

36 3. Whitefish changed diet from benthic macroinvertebrates to zooplankton from winter to summer. 37 Generally, whitefish somatic growth was slow and most energy was used for gonad growth. 38 Zooplankton had higher total content and different profile of FA compared to benthic 39 macroinvertebrates. Increased zooplanktivory in summer was detected with higher α -linolenic acid 40 (ALA, 18:3n-3) and stearidonic acid (SDA, 18:4n-3) percentage and content as well as increased the 41 ratio of polyunsaturated FAs (PUFAs) of n-3 and n-6 family (n-3/n-6 –ratio) in fish muscle.

4. Whitefish gonadal growth and development occurs during the summer growing season and continues until the initiation of spawning in early winter. We found that the content of physiologically crucial PUFA, eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), and arachidonic acid (ARA, 20:4n-6) decreased by ca. 60% between late summer and the spawning period

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in early winter. After spawning, total FA content of whitefish muscle increased rapidly reaching the maximum recorded level in mid-summer.

5. Seasonal changes in whitefish muscle FA profiles and contents were modified both by available diet and reproductive phase, however, reproductive physiology was clearly a stronger driver of the changes in muscle FA composition. In general, our results suggest a very slow turn-over rate of FA in dorsal muscle of slow growing subarctic whitefish.

1. Introduction

Seasonal variation of light and temperature in subarctic lakes has significant impacts on production and the metabolism of animals (McMeans et al., 2015). Primary production is generally low during the dark polar winter, followed by increased solar irradiance in spring, coupled with nutrient-rich runoff from watershed meltwater, which contribute to a primary production peak (Christoffersen et al., 2008; Lizotte, 2008; Hampton et al., 2017). In the clear water of subarctic lakes, benthic primary production plays a major role in the overall biomass production (Sierzen et al., 2003; Forsström et al., 2013). At an annual scale, benthic macroinvertebrate density remains relatively stable in such lakes, whereas zooplankton typically display a single summer peak coinciding with the late summer phytoplankton boom (Hayden et al., 2014; Hampton et al., 2017).

Light and temperature are key environmental cues for fish, inducing gonadal development and later spawning activities (e.g. Wanzenböck et al., 2012). Gonadal development places extremely high energetic demands on fish, and likely requires a high quality food supply prior to the gonads being grown (e.g. Jobling et al., 1998). At an annual scale, benthic macroinvertebrates are the most important prey for many salmonid species in subarctic lakes (Svenning et al., 2007; Amundsen and Knudsen, 2009; Eloranta et al., 2010; Hayden et al., 2014). However, salmonids show a dietary shift

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from benthic prey to zooplankton during the summer coinciding with the peak zooplankton abundance (Heikinheimo et al., 2000; Eloranta et al., 2010, 2013; Havden et al., 2014). Zooplankton may provide physiologically crucial highly unsaturated fatty acids (HUFA), such as eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6). Fish are generally unable to synthesize these important biomolecules efficiently from precursor molecules: a-linolenic (ALA, 18:3n-3) and linoleic acid (LIN, 18:2n-6) (e.g. Henderson 1996; Tocher et al., 2003), and therefore rely on lower trophic levels for their supply. The current paradigm in ecological FA studies is that EPA and DHA are synthesized only by some phytoplankton taxa (Ahlgren et al., 1992, Gladyshev et al. 2013; Taipale et al., 2013, 2016) and transferred to fish via zooplankton, allowing the growth and functions of delicate and complex organs of fishes, e.g. muscle, eye, brain and gonads (e.g. Watanabe et al., 1989; Arts et al., 2001; Tocher et al., 2003). Salmonids gain both somatic and gonadosomatic mass during summer period, and thus pelagic planktivory has been suggested to be essential for gonadal development (Eloranta et al., 2010; 2013; Hayden et al., 2014).

Pelagic zooplankton, especially copepods tend to have higher EPA and DHA content compared to benthic macroinvertebrates (e.g. Gladyshev et al., 2015; Makhutov et al., 2016), which in turn tend to contain more ARA (e.g. Lau et al., 2012; Hixons et al., 2015). However, the paradigm of nutritional superiority of zooplankton over benthic macroinvertebrates might be an over-simplification and should be considered with caution (Gladyshev et al., 2018). Fish fatty acid (FA) composition integrates different FAs from their foraging habitat and thus can become habitat specific. Therefore, a consumer's FA composition can be considered as a useful proxy of the long-term feeding habitat. For example, studying tissue n-3/n-6 ratios may reveal ecological segregation among different fish species from the same lake (Kuusipalo and Käkelä, 2000; Lau et al., 2012). Due to the trophic retention of FAs, a low n-3/n-6 ratio in fish is often used as an indicator for utilization of littoral or terrestrial resource (benthic macroinvertebrates), whereas a high ratio indicates the contribution of

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pelagic phytoplankton via zooplankton (Kainz et al., 2017; Strandberg et al., 2018). Moreover, in many cases ALA, stearidonic acid (SDA, 18:4n-3) and EPA have been used as pelagic biomarkers, and LIN and ARA as littoral markers (Eloranta et al., 2013; Taipale et al., 2013; Thomas et al., 2019).

In vertebrate cells, FAs are used to build membrane phospholipids, from which FAs are subsequently 96 97 cleaved off, for the synthesis of signaling molecules. When surplus energy is gained, FAs are stored 98 as triacylglycerol, whereas under a negative energy status, FAs are deliberated from lipids for 99 oxidative energy production (Henderson, 1996; Tan et al., 2014; Calder, 2015). The FA-derived structural molecules or energy production are essential for the growth and reproduction of fish. FA 101 utilization rates differ: PUFAs are hydrolyzed from cytosolic lipid droplets more readily than monounsaturated (MUFA) or saturated (SFA) FAs – at the same time short carbon chains are easier to hydrolyze and further oxidize than long carbon chains (Groscolas and Raclot, 1998; Eroldoğan et al., 2013). Fish FA turnover rates vary among tissue types. A dietary change is visible in fish muscle FA composition 1–2 months after a switch to a new diet, and FA turnover rates are faster in juveniles than adults (Jobling et al., 2002; Milardi et al., 2016; Taipale et al., 2018). FA turnover rate in perivisceral fat and the whole carcass is longer, ≥ 3 months (Jobling et al., 2002).

Fish white muscle is usually characterized by large absolute and relative content of EPA, DHA and 44 109 ARA (Łuczyńska et al., 2008; Muir et al., 2014; Gladyshev et al., 2017; Strandberg et al., 2018). Adipose salmonid fish, such as Arctic charr (Salvelinus alpinus (L.)), have large lipid reserves in 46 110 ⁴⁸ 111 muscle and carcass (including skin), whereas in many lean species, such as cod (Gadus morhua L.), ⁵⁰ 51 112 the liver is the most important lipid storage tissue (Jobling et al., 1998; 2008). Luzzana et al. (1996) 53 113 reported that in Lake Maggiore (northern Italy), at the southernmost distribution of European 55 114 whitefish (Coregonus lavaretus (L.)), liver contains twice as much FAs than muscle and perivisceral ⁵⁷ 115 58 adipose tissue is an important energy source for gonad development. However, information on annual 60 116 variation of FA in muscle tissue remains scarce for subarctic whitefish, which often dominates lake

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fish communities in the region. Whitefish hold a key role in food web dynamics, as they support 117 118 important fisheries across their distribution and are sensitive to environmental stressors such as 119 climatic and land use change (Hayden et al. 2017; Thomas et al. 2017).

Subarctic lakes provide excellent opportunities to study the relative importance of reproduction and 12 120 ¹⁴ 121 pelagic dietary shifts on fish muscle FA composition, due to: i) the intense summer growth season 122 and ii) the fact that benthic-derived energy dominates these systems for most of the year except for a 19 123 short summer shift to pelagic-derived energy (Sierzen et al., 2003; Eloranta et al., 2010; Hayden et 21 1 24 al., 2014). Such a shift could be especially important in autumn/winter spawning fishes that must 125 develop their gonad tissues (which can reflect more than 20 % of somatic mass in females (e.g. Rösch 26 126 2000)) during summer and autumn. In the current study, we examined variation in annual dorsal 28 1 27 muscle FA in whitefish inhabiting a well-studied subarctic lake (Hayden et al., 2014; Keva et al., ³⁰ 128 2017). The main motivation for the study was a lack of knowledge on how fish muscle FA 33²129 composition and content vary intra-annually in subarctic lakes, and how dietary resource shifts, fish condition and the reproductive cycle may affect muscle FAs. To seek answers for these questions, we 35 130 37 131 examined two hypotheses:

⁴¹ 132 H1: The strong year-round reliance of whitefish on littoral benthic macroinvertebrates should be 44 133 reflected in muscle FA composition, where littoral markers (e.g. ARA) should dominate FAs for most of the year. However, during, and shortly after the zooplankton dietary shift in late summer, pelagic 46 1 34 ⁴⁸ 135 markers (e.g. LIN, SDA and EPA) should increase in whitefish muscle FA content and profiles 136 (Hayden et al., 2014).

⁵⁴ 55 137 H2: Gonadal development, spawning and overwintering are energetically expensive for fish 56 (Jørgensen et al. 1997; Jobling et al., 1998). Whitefish invest HUFAs into gonad tissues by mobilizing 57 138 58 59 1 3 9 FAs from perivisceral lipids, and to a lesser degree, from muscle lipids (Luzzana et al., 1996; Muir 60

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140 et al., 2014). Gonadal development in whitefish is rapid, and usually occurs in late autumn, just prior 141 to spawning in early winter (e.g. Havden et al., 2014, Keva et al., 2017). Therefore, we hypothesized that content of n-3 HUFA (EPA and DHA) and n-6 HUFA (ARA) in whitefish muscle should be the 142 10 1 4 3 lowest during, and after spawning. In addition, the total FA content of muscle should be the lowest 144 in midwinter, and that the subsequent recovery should be slow as whitefish generally feed at very low rates during winter, due to low water temperatures (Havden et al., 2013, 2014; Keva et al., 2017). 15 145

19 146 2. Materials and methods

2.1 Sampling area and period 23 147

148 Samples for this year-round study were collected in 2011 and 2012 both during ice-covered winter 29 149 months (December, February, May) and ice-free summer months (June, July, September) from a 31 1 50 subarctic lake, Kilpisjärvi (hereinafter Kilpis) located in northern Finland (Fig. S1). Kilpis is an 151 oligotrophic lake with cold, clear and neutral water (detailed water chemistry in Hayden et al., 2014), with a surface area of 37.3 km², a shoreline length of 71.5 km, and maximum and mean depths of 57 ₃₆ 152 m and 19.7 m, respectively. The catchment area (293 km²) mainly consists of subarctic tundra and 38 1 5 3 40 154 human population densities are low (e.g. Hayden et al., 2017).

155 Whitefish dominate the fish fauna of Kilpis: they comprise approximately 95% of the total fish 47 156 biomass (Harrod et al., 2010; Malinen et al., 2014). In this region, whitefish populations are often 49 1 5 7 polymorphic, but Kilpis has only a single generalist morph that is the most ubiquitous to the region; 158 the large sparsely rakered (LSR) whitefish (Harrod et al., 2010; Kahilainen et al., 2017). Seven other 53 54 159 fishes inhabit Kilpis: alpine bullhead (Cottus poecilopus Heckel), pike (Esox lucius L.), burbot (Lota 56 160 lota (L.)), minnow (Phoxinus phoxinus L.), brown trout (Salmo trutta L.), Arctic charr and grayling ⁵⁸ 161 (Thymallus thymallus L.) (Kahilainen et al., 2007). In Kilpis, copepods (especially Eudiaptomus

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graciloides Liljeborg and to a smaller degree Cyclops scutifer Sars) dominate the pelagic zooplankton 162 163 community year-round, whereas cladocerans (mainly *Bosmina* sp. and to a smaller degree *Daphnia* sp. and Holopedium gibberum Zaddach) are apparent during the mid- to late-summer months 164 10 165 (Kahilainen et al., 2007; Hayden et al., 2014). The pelagic zooplankton peak typically occurs in late 166 July, whereas densities are lowest in mid-winter (Hayden et al., 2014). The profundal benthos of 15 167 Kilpis largely consists of chironomid larvae, Oligochaeta and Pisidium sp., whereas the shallower 17 168 water littoral benthos is more diverse, and includes several insect larvae (Trichoptera, Plecoptera, 169 Ephemeroptera, Megaloptera, Dytiscidae, Tabanidae) and periphyton-grazing snails (Lymnaea sp., 22 170 Valvata sp.) (Hayden et al., 2014).

26 171 2.2 Sampling methods and measurements

30 172 Fish samples were collected with 240 m long benthic gill net series including seven panels of different mesh sizes (knot-to-knot mesh sizes: 12, 15, 20, 25, 30, 35, 45 mm; net height: 1.8 m) and one multi-32 173 34 174 mesh NORDIC-net (5.25–55 mm; net height 1.5 m) set overnight (10–12h) during the open-water ₃₇ 175 sampling (Jun-12, Jul-12, Sep-12) or for up to two days (24–48h) during the under-ice sampling (Dec-39 176 11, Feb-12, May-12). On capture, all fish were immediately euthanized by cranial concussion, ⁴¹ 177 removed from nets, stored in ice and transported to the laboratory. Pelagic zooplankton were sampled 43 178 through vertical hauls (from depth of 10 m) of a plankton net (diameter 25 cm, mesh size: 50 µm), 46 179 benthic macroinvertebrates were sampled using an Ekman-grab (area: 272 cm²), in shallow littoral 48 180 areas benthic macroinvertebrates were also collected by a kick-net. All invertebrate and fish 50 51 181 individuals were identified to the lowest practical taxonomic level.

54 55 182 Fish total length (± 1 mm) and blotted wet mass (± 0.1 g) were measured, and the Fulton's condition 56 57 183 factor was derived from the formula (Nash et al., 2006): $K=M/TL^3 \times 100$, where K is condition factor, 58 59184 M is mass (g) and TL is total length of fish (cm). Age determination was performed under microscope 60

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using one clear and one burned-and-cracked sagittal otolith immersed under water in a petri-dish and using a microfiche to read ventral scales pressed on a polycarbonate slide (Kahilainen et al., 2003). We used these different bony structures to improve the reliability of aging (Kahilainen et al., 2017). The first left gill arch was dissected and gill rakers were counted under preparation microscope. Gill raker number is a heritable trait in coregonids, and is used for morph identification as they are related to diet, whereby a high number of gill rakers facilitates dietary specialization to zooplankton (Kahilainen et al., 2011a, 2011b).

Sex and maturation level were visually determined from gonads using a 1-7 scale, where values between 1 and 3 represent juveniles and 4 and 7, mature individuals in different maturity stages. Gonads were weighed $(\pm 0.01g)$ and the gonadosomatic index calculated (Hayden et al., 2014): GSI=GM/SM×100, where GSI is gonadosomatic index, GM is the gonad mass (g) and SM is the ³⁰ 196 somatic mass (g). Stomach contents where characterized using a points method (Hynes, 1950), where stomach fullness was visually estimated in scale of 0-10 (0=empty, 10=extended full). Prey items were first identified to the lowest feasible taxonomic level under a dissection microscope and their relative contribution to total fullness was estimated. A piece of dorsal muscle tissue and invertebrate ³⁹₄₀200 samples were freeze-dried (-80°C for 48h), ground to fine powder and frozen (-80°C) for subsequent analysis. We took advantage from previously published stable isotope and total mercury studies (Hayden et al., 2014; Keva et al., 2017) to gain individual values for fish age, sex and maturity stage 46 47 203 to select individuals to FA analyses. We selected six mature individuals (3 male, 3 female) per 49²⁰⁴ sampling month, all from the same dominant year class (2003), and from a similar size class where possible, to minimize potential effects of maturity, age and size on FA composition. Harsh ice-out conditions in Jun-12 resulted in limited sample size and was supplemented with some older and larger individual for FA analyses.

2.3 Fatty acid analysis

 $C_{FAi} = \frac{m_{st}}{m_{sample}} \times \frac{A_{FAi}}{A_{st}} \times \frac{M_{FAi}}{M_{st}} \times \frac{CF_{FAi}}{CF_{st}}$

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Freeze-dried samples were ground to fine powder and weighed $(10\pm 1 \text{ mg})$ into tin cups, which were

subsequently placed into test tubes (10 ml). Each sample was spiked with an internal standard (free

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where C_{FAi} is the content of individual FA (µg/mg) in the sample, m_{st} and m_{sample} are the masses of 60 2 3 0

12 211 FA 13:0) which was used in calculating FA content ($\mu g/mg$) in the sample (formula 1). The sample 14212 and internal standard were mixed into 2 ml of 1% methanolic H_{S2}O₄ supplemented with 1ml hexane, 16 213 and the solution heated under nitrogen atmosphere in capped vials in a heat block at 95 °C for 120 19 214 min. After cooling of the tubes, water (1.5 ml) and hexane (4 ml) were added, and subsequently 21 215 generated FA methyl esters (FAMEs) were extracted into hexane. FAME solutions were dried on ²³₂₄216 NaS₂O₄, concentrated under nitrogen flow, and the hexane volume adjusted to 1 ml. Samples were ²⁵ 26 217 stored at -80 °C until analyzed with a GC-2010 Plus gas chromatograph (Shimadzu Scientific 28 2 1 8 Instruments, Kyoto, Japan) equipped with an auto injector (AOC-20i) and a flame ionization detector 30 219 (FID). The quantification was based on the FID responses, and the peak areas were integrated using ³² 33 220 GCsolution software (version 2.41.00, Shimadzu). The structures of the 80 FAs detected were 34 35 221 identified based on their mass spectrum recorded by Shimadzu GCMS-QP2010 Ultra (Shimadzu) 37 222 with mass selective detector (MSD). In the GC-FID and GC-MSD, the FAMEs were ³⁹ 223 chromatographed using a similar capillary column (Zebron XB-wax, length 30 m, diameter 0.25 mm, 41 42 224 film thickness 0.25 µm; Phenomenex, Torrence CA, USA). FA molar percentages (mol%) were 44 2 25 calculated as the ratio of FA peak area to the peak areas of all FAs adjusted with the theoretical 46 47 226 correction factors for FID (Ackman, 1992). Sample FA content was calculated with the following 48 49 227 formula (1) based on the assumption that the FID corrected ratio of each unknown FA amount to its peak area equals to the FID corrected ratio of the known amount of the standard FA to its peak area: 51 228

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internal standard FA (13:0) and the dried sample weighed into the tin cup (mg) respectively. A_{FAi} and 231 A_{st} are the integrated peak areas of FA_i and the standard FA, respectively. M_{FAi} and M_{st} represent the 232 molecular mass of FA_i and the standard FA (13:0). CF_{FAi} and CF_{st} are the corresponding theoretically 233 234 calculated and experimentally confirmed correction factors for the slightly different FID responses 13 235 of different FA structures. After these calculations we sorted FAs by their mean mol% contribution 15 2 3 6 and selected FAs higher than 0.5 mol% for later analysis without normalizing the data to 100% (as ¹⁷ 237 done previously by Luzzana et al., 1996; Hessen and Leu, 2006). This subset of FAs was used in all 20 238 further data analysis and cataloging. In addition, analyzed FAs were grouped into SFA, MUFA, PUFA, n-3 PUFA, n-6 PUFA, and also the dimethyl acetals derived from phospholipid alkenyl chains 22 2 3 9 ²⁴ 240 (DMAs) were included in the analyses. The ratios of n-3/n-6, unsaturated to saturated FAs ²⁶ 27 241 (UFA/SFA) and the sum of all FAs (Tot-FA) were calculated.

2.4 Statistical analysis 31 2 4 2

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34 ₃₅ 243 Differences in fish background ecological data (variables described in Sampling methods and 36 37 244 measurements) and FA between sexes were tested by month with T-test or Mann-Whitney U-test 38 ³⁹ 245 when appropriate. For the FA mol% data, we used permutational analysis of variance 40 41 42 246 (PERMANOVA) based on a Bray-Curtis distance matrix to test the most important variables driving 43 44 2 47 dissimilarities. We used non-metric multidimensional scaling (nMDS) ordinations based on the Bray-45 ⁴⁶ 248 Curtis distance matrix to illustrate the PERMANOVA results. We used SIMPER (similarity 47 ⁴⁸/₁₀ 249 percentage test) as a *post-hoc* means to characterize differences observed in the PERMANOVA 49 50 results. Additionally, to test the differences of individual FA percentage (mol%) and content (mg/g 51 250 52 53 2 5 1 DW) between sampling months in fish or between invertebrate habitats, we used Analysis of Variance 54 ⁵⁵ 252 56 (ANOVA) with Bonferroni corrected t-tests (here-after Bonferroni test) for *post-hoc* comparisons. If 57 58 253 the assumption of normality (Shapiro-Wilk's test) or homogeneity (Levene's test) was violated, we 59 60 2 5 4 used repeated Welch's t-test (W-ANOVA) with Games Howell post-hoc tests. For hypothesis 1, we

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examined the difference in FA quality and quantity between fish caught in September and fish from 255 256 the previous months to reveal the effects of the shift from a benthic to pelagic diet on whitefish muscle FA composition. For hypothesis 2, we focused on the possible FA differences between the fish caught 257 in December and previous and following months to reveal how spawning, and subsequent 10258 ¹² 259 physiological recovery affected whitefish muscle FA composition. In all statistical tests, we used an 15 260 alpha level of 0.05 to test null hypothesis. All statistical analyses were conducted using R through 17 261 RStudio version 3.4.1. with base and/or vegan packages (R Core Team, 2017; Oksanen et al., 2018).

²¹₂₂262 3. Results

25 263 3.1 Basic ecological metrics

28 We first examined potential differences in background ecological data between sexes: we found that 29 264 30 31 265 the only factor that differed was GSI, with females continually having GSI values 5-10 times higher 32 ³³ 266 than males (Table S1). In the pooled ecological background data, the whitefish we examined were 34 35 ₃₆ 267 similar in age (mean \pm sd: 9.2 \pm 1.6) and size (TL: 29.2 \pm 3.8 cm, mass: 197.1 \pm 110.0 g, condition factor: 37 38 268 0.74±0.07, gill rakers: 24±2) throughout the study (Table 1; Table S1), apart from the individuals 39 40 269 caught in June. These individuals were older (11.1 ± 3.4) and larger (TL: 34.7\pm6.7 cm, mass: 41 42 ^{→2} 43 270 356.7 ± 206.3 g, condition factor: 0.74 ± 0.12 , gill rakers: 25 ± 1) compared to the fish caught in the other 44 45 271 months, and reflect issues with limited sample sizes following sampling immediately after ice break-46 ⁴⁷ 272 up. GSI was stable from February to July and increased progressively towards the December 48 50²⁷³ 49 spawning period (Table 1). Gill raker number remained stable during the whole season (Table 1). 51 52 274 Condition factor was highest in September and lowest in February, but we did not find statistical 53 54 275 differences (Table 1). Stomach fullness was lowest under ice, *i.e.* during and after spawning (Dec-55 ⁵⁶-7276 May: 1.2 ± 1.5) and highest in the open-water season (Jun-Sep: 4.7 ± 1.1) (Table 1). Whitefish largely 57 58 59 277 consumed benthic prey, especially Pisidium sp. and Chironomid larvae, which were present in the 60

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stomachs throughout the year. However, in June and September, littoral *Eurycercus* sp. and pelagic
zooplankton (e.g. *Bosmina* sp. and Calanoida) made the largest relative contribution to whitefish diet
(Table 1).

We found very small differences in FAs between whitefish sexes i.e. six differences out of 138 potential comparisons (Table S2). In addition, PERMANOVA indicated that sampling month was the only important variable ($r^2=0.648$, p<0.01) explaining dissimilarities among whitefish FA profiles. Sex ($r^2=0.003$, p=0.887) or the month*sex interaction ($r^2=0.069$, p=0.302) were clearly nonsignificant (Table S3). Therefore, we pooled the two sexes together in all subsequent statistical analysis.

Ordination of FA profiles showed that invertebrates (classified by both taxa and habitat) were clearly 30 288 differentiated from fish (Fig. 1; PERMANOVA: Table S3). Due to low sample size of invertebrates 32 289 by taxa and month, the invertebrate data was pooled into to three habitat groups (pelagic zooplankton, ³⁴ 290 littoral benthic macroinvertebrates, profundal benthic macroinvertebrates). Habitat (r²=0.240, 37 291 p=0.003) was the most important variable for explaining the dissimilarities between invertebrate FA profiles, with neither month ($r^2=0.087$, p=0.880), nor the habitat*month interaction ($r^2=0.089$, p=1.0) 39 2 9 2 ⁴¹ 293 affecting FA profiles (Table S3). SIMPER, which indicated that 70-80% of the FA dissimilarity 44 294 within fish and invertebrates was associated with habitat (Table 2) was explained by: 14:0, 16:0, 46 295 16:1n-7, 18:1n-7 18:1n-9, ARA, EPA and DHA. Similarly, SIMPER results for Fish FA profile data ⁴⁸ 296 based on sampling month (Table 2) indicated that 70-80% of the dissimilarity was explained by: 14:0, ⁵⁰ -1 297 16:0, 18:0, 16:1n-7, 18:1n-9 ARA, EPA, DHA, but in some cases both LIN and SDA also contributed 53 298 to dissimilarities.

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10 11 301 Invertebrate groups showed differences in mol% among the FA structural categories (ANOVA/W-12 13 302 ANOVA: SFA, F_{2.12.3}=4.2, p=0.004; MUFA, F_{2.19.9}=14.5, p<0.001; PUFA, F_{2.36}=4.4, p=0.02). SFA 14 15 303 and PUFA were highest in pelagic zooplankton (post-hoc tests: p<0.05; Table S4), whereas MUFA 16 17 18 304 showed lower contribution in pelagic zooplankton (15.2±3.8 mol%) than in littoral benthic 19 20 305 macroinvertebrates (30.2±10.4 mol%) (post-hoc tests: p<0.05; Table S4). The FA profile of benthic 21 22 306 macroinvertebrates was relatively similar between habitats, but MUFA was higher in littoral benthic 23 ²⁴₂₅ 307 macroinvertebrates compared to profundal benthic macroinvertebrate (19.9±5.6 mol%) (post-hoc 26 27 308 test: p<0.05; Table S4). DHA percentage was clearly highest in pelagic zooplankton (5.9±4.5% 28 29 309 mol%), and ARA contribution was the highest in profundal benthic macroinvertebrates (2.2 ± 1.2) 30 ³¹ 310 mol%), a difference highlighted by SIMPER (Table 2; Table 4S).

³⁵₃₆ 311 The n-3/n-6 ratio was the most important FA marker highlighting differences among invertebrate 37 38 312 groups (Fig. S2; Table S6), being around 80% higher in pelagic zooplankton (2.43) compared to 39 40 3 1 3 littoral and profundal benthic macroinvertebrates (1.38). The mean of Tot-FA (171.5 mg/g DW) was 41 42 43 314 >100%, SFA (61.1 mg/g DW) and n-6 PUFA (22.1 mg/g DW) were >200% higher, PUFA (75.6 mg/g 44 45 315 DW) was >300% higher and n-3 PUFA (53.5 mg/g DW) was >400% higher in pelagic zooplankton 46 47 316 compared to benthic macroinvertebrate habitat groups. SIMPER and ANOVA results showed that 48 ⁴⁹ 317 EPA, DHA, ARA, 14:0, ALA, and 22:5n-6 were also clearly higher in zooplankton (Table 2; Fig. S2; 50 51 52 318 Table S6). However, variation (±SD) in pelagic zooplankton was relatively high due to seasonal 53 54 319 changes in the FA content (Fig. S3-S5; Table S6), and therefore statistical differences in ANOVA 55 56 320 was not found besides in n-3/n-6 -ratio.

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⁶⁰ 321 In pelagic zooplankton, ALA, SDA and n-3/n-6 ratios all varied among months (ANOVA/W-

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322 ANOVA: ALA, F_{5.30}=5.5, p=0.001; SDA, F_{5.30}=4.2, p=0.005; n-3/n-6 F_{5.30}=8.5, p<0.001), and all 323 were highest in September (Fig. S2). Moreover, whitefish muscle SDA content varied among months (ANOVA: $F_{5,30}=6.8$, p<0.001; Fig. S2; Table S7) and were ca. twice as high in September (0.32±0.14) 324 10 3 2 5 mg/g DW) than in the other months (pooled average: 0.13±0.04 mg/g DW). Moreover, n-3/n-6 ratio 12 326 in whitefish muscle was found to be highest in September (3.91±0.33) and lowest in December 15 327 (2.72±0.48) (Fig. 2; Table S7).

19 328 3.3 H2 Whitefish muscle FA profile and content during the spawning at December

22 23 329 Whitefish FA profile varied seasonally: December was particularly distinct (Fig. 1; Fig. 2; Fig. S2; 24 25 3 30 Table 2, Table S5). The relative percentages of each FA category differed considerably between 26 ²⁷ 331 28 months (ANOVA/W-ANOVA: SFA, F_{5.13.5}=11.4, p<0.001; MUFA, F_{5.13.8}=5.1, p=0.01; PUFA, 29 ₃₀²332 F_{5,13.7}=8.1, p<0.001; n-3 PUFA, F_{5,30}=16.8, p<0.001; n-6 PUFA, F_{5,30}=18.9, p<0.001). SFA and 31 32 333 MUFA percentages were highest in December (45.6±4.1 mol%) and decreased towards summer -33 ³⁴ 334 35 reaching the lowest value recorded in June $(31.9\pm1.3 \text{ mol}\%)$ (*post-hoc* tests: p<0.01; Table S5). 36 37 335 Conversely, the lowest percentage of n-3 PUFA ($17.9\pm6.8 \text{ mol}\%$) and n-6 PUFA ($7.0\pm1.4 \text{ mol}\%$) 38 39 336 was found in December, and both FA classes increased towards the following summer (post-hoc tests: 40 41 337 p<0.01 in all cases). Only DMA 16:0 remained static (~0.6 mol%) across the whole sampling period 42 43 44 338 (Fig. S2; Table S5). In addition, UFA/SFA and n-3/n-6 -ratios differed among the months 45 46 3 39 (ANOVA/W-ANOVA: F_{5,13,2}=32.3, p<0.001; F_{5,30}=8.5, p<0.001, respectively) being highest in 47 48 3 4 0 September (1.6 \pm 0.1 and 3.6 \pm 0.3) and lowest in December (0.9 \pm 0.2 and 2.5 \pm 0.5) (post-hoc tests: 49 ⁵⁰ 341 51 p<0.01; Table S5).

⁵⁴ 342 To summarize the detailed FA profile data of whitefish muscle, 16 of the 24 selected FAs showed 56 57 343 differences in their percentages in December compared to the other months (Fig. S2; Table S5). 58 59 344 SIMPER and ANOVA results showed that only some n-3 PUFAs (ALA, SDA, 20:4n-3, EPA, DHA) 60

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and individual n-6 PUFAs (ARA, 22:5n-6) decreased from September to December, after which they 345 346 increased towards summer (Table 2; Fig. S2; Table S5). In contrast, SFAs (14:0, 16:0, 18:0) and n-7 and n-9 MUFAs (16:1n-9, 24:1n-9, 18:1n-9) increased in their percentages from September to 347 10 3 4 8 December, and after that decreased towards February (Table 2; Fig. S2; Table S5).

14 3 4 9 Mean whitefish muscle total-FA content were almost 25% lower in December (15.36±3.02 mg/g 15 17 350 DW) than in the other months (pooled: 22.31 ± 5.92 mg/g DW), but the difference was not statistically 18 19 351 significant (Fig. 2; Table S7). Moreover, PUFA, n-3 PUFA, n-6 PUFA content and UFA/SFA and n-20 21 3 5 2 3/n-6 ratios (from content data) showed intra-annual variation (ANOVA/W-ANOVA: PUFA, 22 ²³₂₄ 353 F_{5.30}=7.2, p<0.001; n-3 PUFA, F_{5.13,5}=29.2, p<0.001; n-6 PUFA, F_{5.30}=5.7, p=0.001; UFA/SFA, ²⁶ 354 F_{5.12.7}=15.4, p<0.001; n-3/n-6, F_{5.30}=7.9, p<0.001). In December, PUFA (5.2±1.0 mg/g DW), n-3 28 3 5 5 PUFA (3.9 \pm 0.9 mg/g DW), n-6 PUFA (1.4 \pm 0.1 mg/g DW) contents and UFA/SFA –ratio (1.3 \pm 0.3) ³⁰ 356 were at the lowest levels recorded during the study (*post-hoc* tests in all cases p<0.05; Table S7). 33 357 PUFA content were around 60% lower in December (5.2 mg/g DW) than in other months (pooled average: 10.8 mg/g DW). SFA and MUFA content in fish muscle were stable throughout the year, 35 358 37 359 yet showing generally the lowest content in February, despite 24:1n-9 which was lowest in September 40 360 (Fig. 2; Table S7). Eight of the most abundant FAs contributed >75 % of the total FAs, and of these, 42 361 three (ARA, EPA, DHA) showed differences in content among months (ANOVA/W-ANOVA: 44 362 p<0.05 in all cases; Fig. 2; Fig. S2; Table 2; Table S7), and had the lowest content in December (*post-*⁴⁶..., 363 *hoc* tests in all cases p < 0.05).

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4. Discussion

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4.1 Main results

In our study conducted across a single annual cycle, we found that phytoplankton-zooplankton related 11 366 13 367 markers (ALA, SDA and n-3/n-6) reached their highest percentages and ratio (respectively) in 368 whitefish muscle tissue in September, approximately one to two months after the whitefish underwent 18 369 a dietary shift to zooplankton (H1). In December, just prior to spawning, percentages and content of 20 3 7 0 whitefish muscle HUFA (EPA, DHA and ARA) were the lowest, whereas in mid-summer they were ²² 371 higher (H2), emphasizing major biochemical differences during spawning time compared to summer.

4.2 H1 Whitefish dietary shift from benthic macroinvertebrate to zooplankton can be

³⁰ 373 detected with FA biomarkers

Seasonal changes in zooplankton biomass volume and composition are associated with shifts in fish 34 374 ³⁶ 375 foraging behavior. Various empirical studies using stomach content and stable isotope analyses have 39 376 shown that generalist salmonids undergo seasonal diet shifts in subarctic lakes (Amundsen and 41 377 Knudsen, 2009; Eloranta et al., 2010; Kahilainen et al., 2016). During the ice-covered period, when 43 378 pelagic zooplankton densities are low, generalist fishes typically feed on benthic macroinvertebrates. 379 Moreover, feeding activity (stomach fullness) has been usually reported to be the highest in summer 48 380 and the lowest in winter (Svenning et al., 2007; Hayden et al., 2015) – as seen here. However, feeding 50 381 activity does continue during the long period of ice cover, but this has traditionally been related to ⁵² 382 maintenance metabolism only. Increased feeding activity and energy gain during summer result in a 54 55 383 growing season for most fish, which is reflected in higher condition indices in summer than in winter 57 384 (Le Cren, 1951; Tolonen, 1999). Eloranta et al. (2013) found in their snap-shot summer-winter field ⁵⁹ 385 study, that Arctic charr muscle contained more FAs in summer than in winter, suggesting that it was

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caused by summer-time zooplanktivory and overall high feeding activity. Whitefish muscle tissue is 386 387 much leaner than that of Arctic charr and we observed relatively stable Tot-FA, n-3 PUFA and n-6 PUFA content outside the spawning period. This highlights the conservative nature of muscle FA 388 10 3 8 9 composition and the major energy demand of gonadal development. Studies from aquaculture (e.g. 11 ¹² 390 Turchini et al. 2003; Suomela et al. 2017) have revealed that the consumption of fish feed provided 13 14 15 391 in excess can modify muscle FA composition over a period of 1-2 months during the growing season. 16 17 392 We did not find similar FA signature turnover rates in the current study, most likely due to limited 18 19 393 prey resources and slow growth rate of whitefish, which gain only minor somatic growth during the 20 21 22 394 growing season (Hayden et al., 2014; Keva et al., 2017). Previous whitefish studies have shown a 23 24 3 95 clear dietary shift from benthic macroinvertebrates to zooplankton using SCA, but with stable isotope 25 26 3 9 6 analysis of whitefish muscle, the shift was undetectable suggesting a very long turnover-time of 27 ²⁸ 29 397 muscle tissue in such cold-water lake (Hayden et al., 2014; Thomas and Crowther, 2015). 30 31 398 Collectively, this may indicate that turnover-times of stable isotopes and FAs derived from 32 33 399 aquaculture environments using optimal diets, excess feeding and lack of predation may not extend 34 ³⁵ 400 to wild populations in resource-limited subarctic lakes. 36

40³401 39 Despite the relative stability of FA composition outside the spawning period, increased 41 42 402 zooplanktivory during late summer was highlighted by some FA markers. In this study pelagic 43 44 403 zooplankton contributed less in whitefish stomach content than littoral zooplankton in summer (i.e. 45 46 ... 404 Eurycercus sp.). In literature, FA data of littoral Eurucercus is scarce, but some studies suggest it to 47 48 49 405 contain significant amounts of ALA and HUFAs (Smirnov 2017), therefore being potentially 50 nutritionally valuable for fish and closely similar to pelagic zooplankton. In the current study, dietary 51 406 52 ⁵³ 407 related changes in whitefish muscle FA composition were only observable during late summer - an 54 ⁵⁵ 56 408 observation that is consistent with the previous findings from slow growing subarctic fish (e.g. 57 Milardi et al., 2016). SDA and ALA were at their highest percentages in whitefish muscle in 58 409 59 60 4 1 0 September. This was in line with the dietary hypothesis, since zooplankton were also rich in these

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411 FAs which have been previously reported to be higher in zooplankton than in benthic 412 macroinvertebrates (e.g. Eloranta et al., 2013). Moreover, SFA 14:0 increased from June, reaching the highest content in December, but this change was not statistically significant due to high variance. 413 10414 However, 14:0 is a potential pelagic biomarker, typically high in diatoms, which are digested by ¹²415 zooplankton and later by fish (e.g. Taipale et al., 2016; Thomas et al., 2019). The n-6 PUFAs reached 15 416 their maximum content in June and decreased steadily towards December, while n-3 PUFA content 17417 was relatively stable before fast decline during the spawning season in December. These trends result ¹⁹418 in both the seasonal maximum in the n-3/n-6 ratio in September and the minimum in December, and ⁻ 419 may originate from the interacting and combined effects of the dietary shift in mid-summer and 24 4 20 gonadal investment in late autumn–early winter (Kainz et al., 2017; Strandberg et al., 2018).

²⁸ 421 4.3 H2 Energy investment to gonads affects the quantity and quality of whitefish muscle

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35 423 Gonads include elevated content of lipids with HUFAs (especially EPA, DHA, ARA), which are 36 37 essential for gonadal development, and are relocated from different tissues and organs to the gonads 38 4 2 4 39 40 4 2 5 (Luzzana et al., 1996; Jobling et al., 1998; Muir et al., 2014). Muir et al. (2014) found that female 41 42 43 426 lake whitefish (Coregonus clupeaformis Mitchill) condition did not affect egg FA content and 44 45 427 therefore concluded that FA content of eggs is highly conserved. They demonstrated that total FA 46 content in lake whitefish eggs were 3-4 times higher than in the muscle tissue. In addition, Strandberg 47 428 48 ⁴⁹ 429 et al. (2018) suggested that in the autumn-spawning pelagic zooplanktivore vendace (Coregonus 50 51 ₅₂ 430 albula), reproduction costs can be such to affect muscle FA composition up to the late spring in the 53 54 4 3 1 following year. Sushchik et al. (2007) suggested that spawning was the main factor driving the 55 56 4 3 2 seasonal changes i.e. EFA depletion during the spawning of riverine Siberian grayling (Thymallys 57 ⁵⁸ 433 arcticus).

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In the present study, whitefish muscle Tot-FA, n-3 PUFA and n-6 PUFA content were lowest in 434 435 December during spawning, but these were already recovered almost fully in February, reaching a maximum in mid-summer. Therefore, we conclude that gonadal development requires large amounts 436 10 4 3 7 of energy and HUFAs (e.g. EPA, DHA and ARA), especially directly prior to spawning when most 11 ¹²438 of the gain in gonadal mass is concentrated (Jobling et al., 1998). We did not find major differences 13 14 15 439 in female and male muscle FA content nor profile seasonally, suggesting approximately similar 16 qualitative gonad investment or high energy costs associated with spawning (or a combination of 17 440 18 ¹⁹ 441 these factors). Surprisingly, the highest content of MUFA and SFA were observed during spawning 20 21 ⁻ 442 and lowest right after, even more unexpectedly, a rapid increase in PUFAs was recorded after 23 24 4 4 3 spawning. The high MUFAs and SFAs could be explained by the assimilation of perivisceral fat and 25 26 4 4 4 translocation of storage fats from adipose tissue (mainly MUFAs and SFAs) to liver and muscular 27 ²⁸ 445 cells allowing the production of different lipid classes (Jobling et al., 1998). This is supported by the 29 30 31 446 observation of elevated C:N ratio in whitefish liver in December (Keva et al., 2017), as FAs are high 32 33 4 47 in carbon and low in nitrogen. However, the reasons for the relatively rapid and major increment of 34 ³⁵448 important HUFAs (+100-200 %) after spawning and during the time of low feeding activity remains 36 37 ₃₈ 449 unclear. We suggest that this may reflect an increased rate of lipid mobilization from other tissues, 39 increased HUFA synthesis, increased feeding activity beginning in the spring, or most likely a 40 4 5 0 41 ⁴² 451 combination of these factors. 43

46 .7 452 In addition to the hypothesis we examined, we found that whitefish FA profiles reacted incrementally ₄₉ 453 (or decreased) relative to other FA content. In our data, this trivial mathematical phenomenon related on the dependency of proportional variables, is particularly seen in whitefish muscle 16:0 where 51 4 5 4 ⁵³ 455 mol% data showed significant increases from September to December while 16:0 content showed no ⁵⁵ 56 456 significant variation. Without the content data, we would not be able to identify whether this trend 58 4 57 was driven by the changes in other FA content or simply by changes in 16:0 content. Therefore, we

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argue that especially in FA studies recording temporal changes in FA quality, concentration-based 458 analyses should be preferred as previously suggested (e.g. Gladyshev et al., 2018). 459

4.4 Conclusions

We showed that whitefish muscle contained ~60% less of the physiologically bioactive HUFA (EPA, DHA and ARA) during spawning (mean±sd: 0.83±0.15, 2.25±0.71, 0.68±0.08 mg/g DW), compared to other months (pooled average: 1.97±0.01, 5.84±0.08, 1.51±0.02 mg/g DW). We showed that seasonal variation in FA content was associated with annual dietary shifts and more closely with spawning. The recorded season based FA variation in fish muscle was at similar or even higher level to that reported in FA content across a gradient of lake productivity from oligo-, meso- to eutrophic lakes (Taipale et al., 2016). This underlines a pressing need to include seasonality in future studies and monitoring programs using FAs, at least in northern latitudes. As a future perspective, we suggest that researchers undertake year-round FA comparisons of low and high lipid-content fishes with different spawning times, preferably along a gradient of growth rates to test generality of current Lien study results.

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SEASONAL VARIATION IN WHITEFISH MUSCLE FATTY ACID COMPOSITION

Table 1. Ecological characteristics (sample size; age; body size; condition factor, gill raker number, 668 gonadosomatic index; GSI and diet) of whitefish. For each continuous variable monthly mean \pm SD 669 670 values and ANOVA statistics are presented if ANOVA p<0.05. Bold superscript numbers before the 671 mean values indicate statistical difference (Bonferroni corrected t-tests, adj.p<0.05) against the 10 672 indicated months. Stomach fullness, number of empty stomachs and detailed stomach content for 11 12 673 different prey groups are presented as mean percentage contributions. Abbreviations l., n. and p. after $^{13}_{14}674$ invertebrate orders indicates larva, nymph and pupa respectively.

	² Feb-12	⁵ May-12	⁶ Jun-12	⁷ Jul-12	⁹ Sep-12	¹² Dec-11	ANOVA
N	6	6	6	6	6	6	
Age	9±0	9±0	¹² 11.2±3.4	9±0	9±0	⁶ 8±0	F _{5,30} =3.35
Total length (mm)	6274±20	6288±19	2,5,7,9,12347±67	6282±6	6286±8	6272±15	$F_{5,30}=5.07$
fotal mass (g)	6145±28	6177±40	^{2,5,7,9,12} 356±206	6166.43±14	6187±16	6150.23±34	$F_{5,30}=4.90$
GSI	¹² 0.48±0.39	120.48 ± 0.34	¹² 0.93±0.61	¹² 0.78±0.42	2.91±1.68	2,5,6,76.32±5.59	$F_{5,30}=5.52$
Gill raker count	23±2	24±1	25±1	24±2	25±1	27±2	$F_{5,30}=2.91$
Condition factor	0.70 ± 0.04	0.73±0.04	0.74±0.13	0.74 ± 0.04	0.80 ± 0.04	0.74 ± 0.04	$F_{5,30}=1.45$
Stomach fullness	6,7,90.83±1.17	^{7,9} 2.00±2.10	^{2,12} 4.33±0.52	^{2,12} 4.50±0.55	^{2,5,12} 5.17±1.72	^{6,7,9} 0.83±0.98	F _{5,30} =13.39
Empty stomachs	3	2	0	0	0	3	
Pelagic							
zooplankton	0.0	0.0	0.0	3.7	4.8	0.0	
Bosmina sp.	0.0	0.0	0.0	3.7	0.0	0.0	
Calanoida	0.0	0.0	0.0	0.0	3.2	0.0	
Copepoda	0.0	0.0	0.0	0.0	1.6	0.0	
Benthic							
zooplankton	0.0	41.7	0.0	50.0	48.4	0.0	
<i>Eurycercus</i> sp.	0.0	0.0	0.0	50.0	48.4	0.0	
Megacyclops	0.0	41.7	0.0	0.0	0.0	0.0	
Benthic							
macroinvertebrates	100.0	26.7	100.0	31.5	12.9	100.0	
Chironomid l.	70.0	15.8	18.5	5.6	0.0	60.0	
Chironomid p.	0.0	0.0	50.0	9.3	0.0	0.0	
Ephemeroptera n.	0.0	0.0	0.0	0.0	0.0	20.0	
<i>Lymnaea</i> sp.	0.0	0.0	26.5	7.4	1.6	0.0	
Pisidium sp.	30.0	10.0	1.2	5.6	11.3	20.0	
Plecoptera n.	0.0	0.0	3.8	0.0	0.0	0.0	
Trichoptera l.	0.0	0.8	0.0	3.7	0.0	0.0	
Terrestrial insects	0.0	0.0	0.0	13.0	33.9	0.0	
Geometrid moth	0.0	0.0	0.0	0.0	11.3	0.0	
Other insects	0.0	0.0	0.0	13.0	22.6	0.0	
Fish	0.0	31.6	0.0	0.0	0.0	0.0	
Whitefish eggs	0.0	31.6	0.0	0.0	0.0	0.0	
Other	0.0	0.0	0.0	1.8	0.0	0.0	
Corixidae	0.0	0.0	0.0	1.8	0.0	0.0	
SUM (04)	100.0	100.0	100.0	100.0	100.0	100.0	

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Running head: SEASONAL VARIATION IN WHITEFISH MUSCLE FATTY ACID COMPOSITION 1

675 Table 2. SIMPER results of FA profile data. Columns separated with dashed lines indicate pairwise SIMPER tests between whitefish (LSR) and 676 invertebrates: zooplankton (ZPL), benthic macroinvertebrates (BMI) grouped by habitats (upper section of the table) and subsequent months (lower 677 section of the table). The total amount of dissimilarity (%) between groups is shown in the first underlined row in parentheses. FAs are ordered 678 from the most to the least significant driver to total dissimilarity, dis.sum indicates cumulative sum in total dissimilarity. FA means from the tested 679 groups are presented in the means column, corresponding with the group order in the underlined header.

LS	RZPL (38.3	<u>%)</u>	LSRpi	rofundal BMI	(44.3%)	LSR	littoral BMI (4	45.6%)	ZPLpi	ofundal BMI	(41.2%)	ZPL1	ittoral BMI (43.0%)	profunda	llittoral BMI	(36.4%)
FA	means	dis.sum	FA	means	dis.sum	FA	means	dis.sum	FA	means	dis.sum	FA	means	dis.sum	FA	means	dis.sum
14:0	2.916.6	0.21	DHA	19.31.3	0.27	DHA	19.30.9	0.25	14:0	16.64.1	0.23	14:0	16.53.6	0.21	16:0	9.814.3	0.21
DHA	19.35.9	0.42	16:0	25.89.8	0.51	16:0	25.114.3	0.41	16:0	15.99.8	0.39	16:1n-7	6.014.0	0.35	16:1n-7	6.314.0	0.40
16:0	25.115.9	0.57	EPA	7.93.6	0.58	16:1n-7	2.614.0	0.56	DHA	5.91.3	0.48	16:0	15.914.3	0.47	18:1n-9	5.68.3	0.50
ARA	5.81.6	0.63	16:1n-7	2.66.3	0.63	EPA	7.93.3	0.63	18:1n-7	1.55.2	0.54	DHA	5.90.9	0.55	LIN	4.14.7	0.57
EPA	7.95.1	0.69	ARA	5.82.2	0.69	ARA	5.81.2	0.69	16:1n-7	6.06.3	0.6	18:1n-7	1.56.1	0.62	18:1n-7	5.26.1	0.63
16:1n-7	2.66.0	0.74	18:1n-9	5.25.6	0.73	18:1n-9	5.28.3	0.75	EPA	5.13.6	0.66	18:1n-9	5.28.3	0.69	EPA	3.63.3	0.69
ALA	1.84.1	0.78	18:1n-7	2.85.2	0.77	18:1n-7	2.86.1	0.80	18:1n-9	5.25.6	0.72	EPA	5.13.3	0.75	18:0	4.04.2	0.72
18:0	5.83.2	0.82	LIN	2.24.1	0.81	LIN	2.24.7	0.83	ALA	4.11.34	0.77	LIN	4.04.7	0.80	14:0 <i>iso</i>	2.92.0	0.76
Dec-1	1 Feb-12 (2	3.6%)	Feb-	12Mar-12 (6	.9%)	Mar	12Jun-12 (9	.2%)	Jun	·12Jul-12 (8.	<u>9%)</u>	<u>Jul-1</u>	2Sep-12 (9	.4%)	Sep-1	2 Dec-11 (2	1.0%)
FA	means	dis.sum	FA	means	dis.sum	FA	means	dis.sum	FA	means	dis.sum	FA	means	dis.sum	FA	means	dis.sum
DHA	9.923.0	0.31	DHA	23.024.5	0.26	DHA	24.521.0	0.25	DHA	21.019.7	0.17	DHA	19.717.4	0.20	DHA	17.49.9	0.22
16:0	30.624.5	0.45	EPA	8.18.4	0.38	18:1n-9	3.96.1	0.38	18:1n-9	6.14.9	0.28	16:0	24.822.8	0.34	16:0	22.830.6	0.43
EPA	4.28.1	0.54	16:0	24.524.3	0.48	EPA	8.48.7	0.47	16:0	23.524.8	0.37	EPA	9.48.4	0.43	EPA	8.44.2	0.55
18:1n-9	7.44.0	0.62	ARA	6.46.7	0.55	16:1n-7	1.83.0	0.57	16:1n-7	3.02.8	0.46	16:1n-7	2.83.6	0.51	18:1n-9	4.87.4	0.62
14:0	5.42.5	0.70	14:0	2.51.9	0.61	16:0	24.323.5	0.65	14:0	1.62.8	0.53	ARA	6.114.9	0.58	14:0	3.25.4	0.68
ARA	3.46.4	0.76	16:1n-7	1.51.8	0.67	LIN	1.82.7	0.70	EPA	8.79.4	0.60	18:1n-9	4.94.8	0.63	18:0	5.37.5	0.74
18:0	7.55.7	0.81	18:1n-7	2.22.5	0.71	14:0	1.91.6	0.74	ARA	7.06.1	0.66	SDA	0.91.5	0.68	ARA	4.93.4	0.79
16:1n-7	3.21.5	0.85	18:1n-9	4.03.9	0.75	ARA	6.77.0	0.78	LIN	2.72.2	0.71	18:1n-7	2.83.2	0.72	16:1n-7	3.63.2	0.82

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1 2 3	
4 5 681 6	Figure captions
8 682 9	Figure 1. nMDS biplot of whitefish and invertebrate FA profile data. Whitefish are shown as circles
¹⁰ 683	and month by different intensity of shading by gray scale. Invertebrate groups and habitats are
12 13 684	presented with different marker shapes, with the shading of smaller overlaying circles indicating
15 685 16	sampling month. The most important fatty acids corresponding to 70-80% of the total dissimilarities
17 18 18	between groups were identified using SIMPER results (Table 2) and they are presented as light gray
19 20 687 21	text.
22 23 688	Figure 2. Boxplots of whitefish muscle Total FA and PUFA content (mg/g DW) (A–B), UFA/SFA
24 25 689 26	and n-3/n-6 -ratios (C-D) and content of eight most abundant FAs from the lowest to the highest
²⁷ 690 28	contribution (E-L). Note the differences in y-axis scales in figures A, B, C, D, E-J, K-L. Bold
29 30 691	horizontal lines indicate median values, the box indicate first and third quartile and whiskers indicate
32 692 33	present minimum and maximum values unless outliers (open circles) are displayed (distance from
34 693 35 36	median > 1.5*interquartile range).
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SEASONAL VARIATION IN WHITEFISH MUSCLE FATTY ACID COMPOSITION



SEASONAL VARIATION IN WHITEFISH MUSCLE FATTY ACID COMPOSITION



APPENDIX: SEASONAL VARIATION IN WHITEFISH MUSCLE FATTY ACID COMPOSITION 1

Supplementary material for: "Seasonal changes in European whitefish muscle and

invertebrate prey fatty acid composition in a subarctic lake"

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This file contains 7 supplementary tables and 6 figures

Table S1 Whitefish basic ecological metrics (age, size, GSI, condition factor, gill rakers) by month and sex. Bold mean±SD (n) values indicate statistical difference between sexes in month columns separated with dashed vertical lines. T-test (normal font) or Mann Whitney U-test (*italics*) were used to test the differences in the distributions.

Table S2 Whitefish muscle FA content (mg/g DW) by month and sex. Bold mean±SD (n) values indicate statistical difference between sexes in month columns separated with dashed vertical lines. T-test (normal font) or Mann Whitney U-test (*italics*) was used to test the differences in the distributions.

Table S3 PERMANOVA results of invertebrate habitat group and whitefish muscle FA profile. Dissimilarities in FA profiles were compared against habitat, month, habitat*month (upper section of the table) and in a dataset only including fish data (lower section of the table) month, sex and month*sex.

Table S4 Invertebrate mean±SD molar percent (mol%) of the selected FAs (contributing >0.5 of total LSR FA mol%). ANOVA (^A) or Welch ANOVA (^W) statistics (F, df between groups and df) and the corresponding p-value are shown in bold in the following column (p_{anova}) where asterisk (*) equals to p<0.001. Pairwise Bonferroni-corrected T-tests or Games Howell tests are shown in the final column where adj.p<0.05, where numbers indicate sampling months according to the head row.

Table S5 Whitefish mean±SD molar percent values (mol%) of the FAs contributing >0.5 of total by month. ANOVA (^A) or Welch ANOVA (^W) statistics (F, df between groups and df) and the corresponding p-value are shown in bold in the following column (p_{anova}) where asterisk (*) equals to p<0.001. Pairwise Bonferroni-corrected T-tests or Games Howell tests are shown in final column (adj.p<0.05), where numbers indicate sampling months according to the header row.

Table S6 Invertebrate mean±SD content (mg/g DW) of the selected FAs (contributing >0.5 of total LSR FA mol%). ANOVA (^A) or Welch ANOVA (^W) statistics (F, df between groups and df) and the corresponding p-value are shown in bold in the following column (p_{anova}) where asterisk (*) equals to p<0.001. Pairwise Bonferroni-corrected T-tests or Games Howell tests are shown in the final column (adj.p <0.05), where numbers indicate sampling months according to the head row.

Table S7 Whitefish mean \pm SD content (mg/g DW) of the FAs contributing >0.5 of molar percent total by month. ANOVA (^A) or Welch ANOVA (^W) statistics (F, df between groups and df) and the corresponding p-value are shown in bold in the following column (p_{anova}) where asterisk (*) equals to p<0.001. Pairwise Bonferroni-corrected T-tests or Games Howell tests are shown in the final column (adj.p<0.05), where numbers indicate sampling months according to the head row.

Figure S1 Bathymetric map of Lake Kilpisjärvi located in Northern Fennoscandia. The sampling site is marked with an ellipse labelled A. Different shades of grey indicate the different depth zones of the lake and indicates river flow directions.

Figure S2 Invertebrate (A, B) and whitefish (C, D) mean mol% and content data, respectively, of the selected FAs. Grey hatching in the bars indicates A, B: different invertebrate groups (benthic algae, littoral benthic macroinvertebrates, profundal benthic macroinvertebrates and pelagic zooplankton); C, D: different months. The vertical line separates the eight most abundant FAs in whitefish muscle contributing >75% of all FAs and is provided to aid visual separation of FAs.

Figure S3 Pelagic zooplankton mean mol% and content (mg/g DW) of the selected FAs by month (A and B respectively). The vertical line is included only to aid visual sorting of eight most abundant FAs according to whitefish muscle FA profiles.

Figure S4 Littoral benthic macroinvertebrates mean mol% and content (mg/g DW) of the selected FAs by month (A and B respectively). The vertical line is included only to aid visual sorting of eight most abundant FAs according to whitefish muscle FA profiles.

Figure S5 Profundal benthic macroinvertebrates mean mol% and content (mg/g DW) of the selected FAs by month (A and B respectively). The vertical line is included only to aid visual sorting of eight most abundant FAs according to whitefish muscle FA profiles.

Figure S6 nMDS biplot of invertebrate (A) and whitefish muscle (B) FA profile data. Invertebrate groups are presented with colors and habitats with rasters (A). Whitefish are grouped by month indicated with gray shades, circles = females, squares = males. The most important fatty acids corresponding to 70-80% of the total dissimilarities between groups were identified using SIMPER results (Table 2) and they are presented as light gray text.

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Table S1

	Dec-11		Feb	-12	May	-12	
	Female (3)	Male (3)	Female (3)	Male (3)	Female (3)	Male (3)	
Age	8±0	8±0	9±0	9±0	9±0	9±0	
Total length (mm)	282±15	262±5	273±8	275±31	283±17	294±23	
Total mass (g)	173±35	127±5	141±11	150±43	162±33	192±47	
GSI	11.39±1.03	1.25±0.13	0.77±0.32	0.18±0.14	0.77±0.19	0.19±0.03	
Gill raker count	25.67±1.53	27.33±1.53	23.67±0.58	23±3.46	24±1.73	23±1	
Condition factor	0.77±0.04	0.71±0.02	0.69±0.05	0.71±0.04	0.71±0.03	0.75±0.04	
	Jun-12		Jul-	12	Sep-12		
	Female (4)	Male (2)	Female (3)	Male (3)	Female (3)	Male (3)	
Age	12±4	9±0	9±0	9±0	9±0	9±0	
Total length (mm)	388±21	264±6	280±8	284±3	288±6	284±11	
Total mass (g)	479±105	112±26	169±21	164±6	186±16	188±20	
GSI	1.29±0.33	0.22±0.01	1.1±0.31	0.46±0.2	4.39±0.36	1.42±0.58	
Gill raker count	24.75±1.26	24±1.41	23.33±3.06	24.33±1.53	24.67±1.15	25.67±1.53	
Condition factor	0.81±0.08	0.61±0.1	0.77±0.05	0.71±0.02	0.78±0.03	0.82±0.04	
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3 December-11 February-12 May-12 June-12 June-12 July-12 4 Female (3) Male (3) Female (3) Male (3) Female (3) Male (3) Female (4) Male (2) Female (3) Male (3) Female (3) <td< th=""><th></th></td<>	
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12 MUFA 3.07±0.91 3.34±2.09 2.09±0.65 2.24±0.55 1.97±0.51 2.76±0.03 3.69±1.39 2.64±1.24 2.94±1.87 2.59±1.14 2.1	±0.92 4.46±1.40
13 16:1n-5 0.13±0.03 0.12±0.04 0.12±0.05 0.11±0.03 0.09±0.01 0.14±0.02 0.11±0.02 0.10±0.02 0.15±0.09 0.17±0.12 0.1	:0.04 0.18±0.03
14 16:1n-7 0.46±0.23 0.51±0.36 0.20±0.09 0.34±0.17 0.20±0.09 0.47±0.04 0.73±0.38 0.45±0.42 0.64±0.53 0.42±0.19 0.4	±0.23 1.04±0.31
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20 PUFA 5.01±0.71 5.42±1.32 12.92±2.71 11.55±2.47 13.81±2.11 14.22±1.79 15.45±2.66 12.46±0.99 11.03±4.39 13.48±6.12 9.6	±4.00 13.99±2.19
21 n-3/n-6 2.68±0.34 2.75±0.68 3.60±0.39 3.72±0.42 3.85±0.23 3.60±0.25 3.18±0.53 3.30±0.21 3.37±0.24 3.89±0.30 4.1	<u>c</u> 0.30 3.70±0.21
22 n-6 PUFA 1.36±0.09 1.44±0.20 2.83±0.69 2.43±0.33 2.86±0.58 3.08±0.23 3.77±0.96 2.90±0.37 2.54±1.06 2.75±1.25 1.8	2.98±0.50
23 LIN 0.40±0.08 0.42±0.18 0.38±0.16 0.38±0.10 0.34±0.07 0.5±0.03 0.80±0.44 0.52±0.22 0.51±0.32 0.46±0.24 0.46±0.24	_0.18 0.64±0.11
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28 n-3 PUFA 3.65±0.64 3.98±1.20 10.09±2.06 9.12±2.14 10.95±1.53 11.14±1.56 11.69±1.87 9.55±0.62 8.48±3.33 10.73±4.88 7.7	<u>-</u> 3.31 11.01±1.71
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32 EPA 0.81±0.07 0.85±0.23 2.08±0.64 1.86±0.37 2.05±0.54 2.42±0.95 2.69±0.59 2.08±0.06 2.06±0.67 2.59±1.07 1.8	<u>-0.92</u> 2.70±0.50
35 22:5n-3 0.23±0.03 0.22±0.09 0.53±0.18 0.51±0.12 0.62±0.15 0.73±0.17 0.86±0.35 0.68±0.25 0.54±0.20 0.57±0.21 0.4	20.14 0.69±0.18
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38 UFA/SFA 1.20±0.06 1.36±0.50 2.44±0.18 2.35±0.30 2.76±0.01 2.38±0.19 2.73±0.15 2.54±0.00 2.39±0.03 2.33±0.06 2.2	2.23±0.26
39 Tot-FA 14.9±0.10 15.82±4.71 21.36±5.21 19.71±3.65 21.63±3.58 24.26±2.06 26.36±5.37 21.19±3.13 19.92±8.91 23.04±10.28 16.8	<u>:6.83 27.06±5.58</u>

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Table S3

Model: (Invert. +Fish data)	Df	Sums of sqs	Mean sqs	F-model	R ²	р
Habitat	3	2.84	0.947	26.9059	0.525	0.001
Month	5	0.275	0.055	1.5612	0.051	0.075
Habitat:Month	15	0.502	0.033	0.9507	0.093	0.575
Residuals	51	1.795	0.035		0.332	
Total	74	5.411			1	
Model: (Invert. data)	Df	Sums of sqs	Mean sqs	F-model	R ²	р
Habitat	2	0.677	0.339	4.3272	0.24	0.003
Month	5	0.246	0.049	0.63	0.087	0.88
Habitat:Month	10	0.251	0.025	0.3205	0.089	1
Residuals	21	1.643	0.078		0.583	
Total	39	2.817			1	
Model: (Fish data)	Df	Sums of sqs	Mean sqs	F-model	R ²	р
Month	5	0.279	0.056	11.1068	0.648	0.001
Sex	1	0.001	0.001	0.2713	0.003	0.887
Month:Sex	5	0.03	0.006	1.1765	0.069	0.302
Residuals	24	0.121	0.005		0.28	
Total	35	0.431			1	

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Table S4

3	Invertebrate mol%	¹ BMI_littoral (21)	² BMI_profundal (12)	³ ZPL_pelagial (6)	Benthic algae (1)	ANOVA	p _{anova}	post-hoc tests
4	SFA	25.45±7.94	22.7±7.24	38.16±11.65	27.33	^w F _{2,12.3} =4.200	0.04	2-3
5	14:0 <i>iso</i>	1.95 ± 1.48	2.94±1.28	1.18±0.50	2.83	$^{W}F_{2,21.4}=8.913$	*	2-3
6 7	14:0	3.60±1.00	4.05 ± 1.78	16.57±6.01	5.72	${}^{W}F_{2,10.1}=13.187$	*	1-3, 2-3
/ 8	15:0	1.40 ± 0.85	1.99±0.72	1.30±0.37	1.80	${}^{W}F_{2,19.8}=3.758$	0.04	2-3
9	16:0	14.29±8.13	9.76±8.07	15.91±4.71	15.56	^w F _{2,17.3} =2.064	0.16	
10	18:0	4.21±1.77	3.96±1.65	3.21±1.32	1.43	${}^{W}F_{2,15,2}=1.109$	0.36	
11	MUFA	30.22±10.36	19.89±5.59	15.22±3.80	28.11	^w F _{2,19.9} =14.469	*	1-2, 1-3
12	16:1n-9	0.73±0.35	0.71±0.14	0.70±0.16	1.01	${}^{W}F_{2,15.6}=0.054$	0.95	
13	16:1n-7	13.98±7.10	6.33±3.73	5.98±1.96	15.31	^w F _{2,22} =10.731	*	1-2, 1-3
14	16:1n-5	0.66±0.23	1.27±0.36	0.79±0.21	0.58	^w F _{2,13.7} =13.201	*	1-2, 2-3
15	18:1n-9	8.26 ± 4.98	5.62±3.30	5.21±1.67	7.51	^w F _{2,21.3} =2.837	0.08	
16	18:1n-7	6.11±2.67	5.17±2.23	1.53±0.27	2.71	${}^{A}F_{2,36}$ =8.935	0.001	1-3, 2-3
17 18	24:1n-9	0.47 ± 0.49	0.79±0.30	1.02±0.35	0.99	${}^{W}F_{2,14.7}=5.089$	0.02	1-3
19	PUFA	15.02±6.26	16.89±5.29	25.15±13.31	16.34	^w F _{2,36} =4.377	0.02	1-3
20	n-3/n-6	1.65±2.19	1.35±0.35	2.35±0.38	2.13	${}^{W}F_{2,16.8}=13.969$	*	2-3
21	n-6 PUFA	6.77±3.54	7.48±3.10	7.42±3.79	5.22	${}^{A}F_{2,36}=0.194$	0.825	
22	LIN	4.73±3.54	4.11±3.02	4.02±2.07	3.33	^w F _{2,17} =0.226	0.8	
23	ARA	1.21±1.06	2.22±1.20	1.58±0.87	0.38	${}^{W}F_{2,14.6}=2.846$	0.09	
24	22:4n-6	0.43 ± 0.4	0.56±0.31	0.30±0.09	0.98	${}^{W}F_{2,22.1}=4.138$	0.03	2-3
25	22:5n-6	0.40±0.43	0.58±0.31	1.52±0.84	0.54	${}^{W}F_{2,11.9}=5.04$	0.03	1-3
26	n-3 PUFA	8.24±4.04	9.41±2.54	17.73±9.73	11.12	${}^{W}F_{2,11.8}=2.762$	0.1	
2/ วง	ALA	1.67±0.99	1.39 ± 0.54	4.11±2.27	4.00	^w F _{2,11.8} =4.268	0.04	
20 79	SDA	1.01 ± 0.80	1.60 ± 0.78	1.32±1.52	1.60	${}^{W}F_{2,11.7}=2.012$	0.18	
30	20:4n-3	0.34±0.28	0.56±0.21	0.64±0.24	0.57	${}^{W}F_{2,14.1}=4.571$	0.03	1-2
31	EPA	3.25±2.66	3.61±2.33	5.09±3.07	2.01	^w F _{2,13} =0.850	0.45	
32	22:5n-3	1.05 ± 1.78	0.94 ± 0.65	0.64 ± 0.25	0.76	${}^{W}F_{2,23,2}=1.302$	0.29	
33	DHA	0.93±0.94	1.31±0.78	5.91±4.45	2.18	^w F _{2,11.3} =4.058	0.05	
34	DMA	0.28±0.34	0.46 ± 0.29	0.19±0.10	0.23	${}^{W}F_{2,21.9}=4.077$	0.03	2-3
35	DMA16:0	0.28±0.34	0.46 ± 0.29	0.19±0.10	0.23	^w F _{2,21.9} =4.077	0.03	2-3
36	UFA/SFA	1.99±0.99	1.73±0.47	1.22±0.65	1.63	$^{W}F_{2,14.4}=2.452$	0.12	
3/	Tot-FA	70.96±13.78	59.94±12.71	78.73±5.67	72.01	$^{W}F_{2,20.1}=9.303$	*	2-3

APPENDIX: SEASONAL VARIATION IN WHITEFISH MUSCLE FATTY ACID COMPOSITION 7

Table S5

3 LSR mol%	12 Dec-11 (6)	² Feb-12 (6)	⁵ May-12 (6)	⁶ Jun-12 (6)	⁷ Jul-12 (6)	⁹ Sep-12 (6)	ANOVA	p _{anova}	<i>post-hoc</i> tests
SFA SFA	45.6±4.06	34.29±1.78	33.23±1.64	31.87±1.31	34.48±0.8	33.51±2.88	$^{W}F_{5,13.5}=11.44$	*	12-2, 12-5, 6-7, 12-6, 12-7, 12-9
6 14:0 <i>iso</i>	1.00 ± 0.17	0.82 ± 0.24	0.65±0.13	0.68 ± 0.14	0.72 ± 0.36	1.17±0.56	${}^{W}F_{5,13.7}=3.686$	0.03	12-5, 12-6
7 14:0	5.44 ± 2.88	2.46 ± 0.47	1.90 ± 0.65	1.61 ± 0.54	2.77 ± 0.46	3.17±0.59	${}^{W}F_{5,13.9}=6.271$	*	5-9, 6-7, 6-9
8 15:0	1.03 ± 0.11	0.82±0.16	0.72±0.15	0.64±0.13	0.87 ± 0.29	1.11±0.33	AF5,30=4.271	0.005	5-9, 6-9
9 16:0	30.59±3.23	24.46 ± 1.02	24.28±1.36	23.53±1.04	24.77±1.13	22.8±2.13	$^{W}F_{5,13.8}=5.061$	0.01	12-2, 12-5, 12-6, 12-7, 12-9
10 18:0	7.54 ± 0.94	5.73±0.39	5.69±0.20	5.41±0.33	5.34 ± 0.42	5.26 ± 0.34	$^{W}F_{5,13.6}=6.343$	*	12-2, 12-5, 12-6, 12-7, 12-9
11 MUFA	17.38±4.3	10.05 ± 1.45	10.17 ± 1.51	13.3±3.24	12.66±2.29	13.58 ± 2.28	${}^{W}F_{5,13.8}=5.056$	0.01	12-2, 12-5, 2-9
12 16:1n-9	1.08 ± 0.31	0.61±0.13	0.59±0.11	0.48 ± 0.09	0.73 ± 0.09	0.65±0.12	$^{W}F_{5,13.9}=5.942$	*	12-2, 12-5, 12-6, 6-7,
13 16:1n-7	3.17±1.13	1.54 ± 0.71	1.75±0.70	2.97±1.33	2.82 ± 1.08	3.61±1.23	$^{A}F_{5,30}=3.61$	0.011	2-9
14 16:1n-5	0.84 ± 0.11	0.65 ± 0.10	0.62±0.12	0.55±0.13	0.82 ± 0.19	0.75±0.22	$^{A}F_{5,30}=3.592$	0.012	12-6
15 16 ^{18:1n-9}	7.44 ± 2.08	4.04 ± 0.44	3.90±0.51	6.14±1.75	4.88 ± 1.08	4.84±0.52	$^{W}F_{5,13.6}=5.643$	*	12-2, 12-5, 5-9
17 18:1n-7	3.78 ± 1.22	2.18 ± 0.47	2.45 ± 0.45	2.43±0.52	2.79 ± 0.65	3.24±0.6	$^{W}F_{5,13.9}=3.23$	0.04	2-9
18 ^{24:1n-9}	1.07 ± 0.22	1.03 ± 0.20	0.87 ± 0.18	0.74±0.27	0.62±0.08	0.50 ± 0.11	$^{A}F_{5,30}=8.75$	*	12-7, 12-9, 2-7, 2-9, 5-9
19 PUFA	24.93±8.17	46.38±3.14	48.71±3.79	46.97 ± 2.68	44.81±2.37	40.65 ± 6.75	$^{W}F_{5,13.7}=8.081$	*	12-2, 12-5, 12-6, 12-7, 12-9
20 n-3/n-6	2.49 ± 0.45	3.38 ± 0.32	3.44±0.25	2.94±0.40	3.36±0.36	3.63±0.28	$^{A}F_{5,30}=8.471$	*	12-2, 12-5, 12-7, 12-9, 6-9
21 n-6 PUFA	7.01±1.36	10.62±0.9	10.97 ± 0.44	11.98 ± 0.89	10.33±0.87	8.77±1.27	$^{W}F_{5,13.5}=12.308$	*	12-2, 12-5, 12-6, 12-7, 5-9, 6-7, 6-9
22 LIN	2.28 ± 0.22	1.81±0.23	1.82 ± 0.28	2.73±0.94	2.17±0.36	2.23±0.65	$^{W}F_{5,13.7}=3.515$	0.03	12-2, 12-5
23 ARA	$3.44{\pm}1.02$	6.37±0.79	6.65±0.56	7.01±0.51	6.11±0.63	4.90±0.72	$^{W}F_{5,13.9}=13.882$	*	12-2, 12-5, 12-6, 12-7, 2-9, 5-9, 6-9, 7-9
24 22:4n-6	0.37 ± 0.10	0.49 ± 0.06	0.59 ± 0.11	0.65±0.21	0.47 ± 0.12	0.46±0.14	$^{A}F_{5,30}=3.513$	0.013	12-6
25 22:5n-6	0.93 ± 0.40	1.95 ± 0.27	1.91±0.38	1.59 ± 0.36	1.58 ± 0.25	1.17±0.27	^A F _{5,30} =9.255	*	12-2, 12-5, 12-6, 12-7, 2-9, 5-9
20 27 n-3 PUFA	17.93 ± 6.84	35.75 ± 2.74	37.74±3.47	34.99±3.03	34.48±2.21	31.88±5.58	^w F _{5,13.8} =7.236	*	12-2, 12-5, 12-6, 12-7, 12-9
28 ALA	1.60 ± 0.26	1.49 ± 0.20	1.52 ± 0.25	1.93±0.27	1.83 ± 0.37	2.23±0.39	^A F _{5,30} =5.527	0.001	12-9, 2-9, 5-9
29 SDA	0.73 ± 0.24	0.74 ± 0.34	0.59 ± 0.21	0.61±0.22	0.87 ± 0.52	1.46 ± 0.62	${}^{A}F_{5,30}=4.159$	0.005	12-9, 2-9, 5-9, 6-9
30 20:4n-3	0.51±0.13	0.68 ± 0.11	0.58 ± 0.08	0.43 ± 0.10	0.78 ± 0.11	0.70 ± 0.07	${}^{A}F_{5,30}=10.241$	*	12-7, 12-9, 2-6, 5-7, 6-7, 6-9
31 EPA	4.23±1.47	8.10±0.23	8.42±2.03	8.71±0.88	9.35±1.14	8.37±1.47	${}^{W}F_{5,12.3}=8.5$	*	12-2, 12-5, 12-6, 12-7, 12-9
32 22:5n-3	0.97 ± 0.41	1.79±0.13	2.14±0.33	2.29 ± 0.47	1.90 ± 0.23	1.71±0.21	${}^{A}F_{5,30}=12.519$	*	12-2, 12-5, 12-6, 12-7, 12-9
33 DHA	9.89 ± 4.86	22.95 ± 2.62	24.49 ± 3.07	21.03±2.8	19.74±1.7	17.41±4.19	${}^{W}F_{5,13.7}=8.148$	*	12-2, 12-5, 12-6, 12-7, 5-7, 5-9
34 DMA	0.61±0.12	0.62 ± 0.15	0.75±0.21	0.80±0.21	0.64 ± 0.10	0.75 ± 0.22	${}^{A}F_{5,30}=1.368$	0.264	
35 DMA16:0	0.61 ± 0.12	0.62 ± 0.15	0.75±0.21	0.80 ± 0.21	0.64 ± 0.10	0.75 ± 0.22	${}^{A}F_{5,30}=1.368$	0.264	
UFA/SFA	0.94 ± 0.22	1.65 ± 0.15	1.78 ± 0.15	1.90±0.13	1.67 ± 0.05	1.62 ± 0.12	$^{W}F_{5,13,2}=15.147$	*	12-2, 12-5, 12-6, 12-7, 12-9, 2-6, 6-7, 6-9,
37 Tot-FA	88.52±2.01	91.33±2.67	92.86±1.61	92.94±1.14	92.58±1.36	88.49±9.28	$^{w}F_{5,13.7}=4.301$	0.01	12-5, 12-6, 12-7

APPENDIX: SEASONAL VARIATION IN WHITEFISH MUSCLE FATTY ACID COMPOSITION

Table S6

5	Invertebrate (mg/g DW)	¹ BMI_littoral (21)	² BMI_profundal (12)	³ ZPL_pelagial (6)	Benthic algae (1)	ANOVA	p _{anova}	post-hoc tests
6	SFA	27.00±25.00	27.48±28.19	61.05±32.89	1.33	^w F _{2,12.4} =2.716	0.11	
7	14:0 <i>iso</i>	2.10±3.63	2.31±2.25	1.57±0.77	0.12	^w F _{2,22.6} =0.608	0.55	
8	14:0	3.08 ± 2.92	4.29±4.54	22.87±13.79	0.23	$^{W}F_{2,10.4}=5.936$	0.02	1-3, 2-3
9	15:0	1.66±2.64	1.83±1.59	1.98 ± 0.86	0.08	^w F _{2,21.3} =0.116	0.89	
10	16:0	14.94±16.38	15.13±18.72	27.98±15.23	0.80	$^{W}F_{2,13.8}=1.691$	0.22	
12	18:0	5.22±5.21	3.92 ± 2.39	6.65 ± 3.08	0.09	${}^{W}F_{2,14.9}=1.848$	0.19	
13	MUFA	30.38±21.70	27.19±26.40	34.54 ± 23.45	1.61	^w F _{2,13} =0.172	0.84	
14	16:1n-9	0.96±1.36	0.77±0.67	1.38 ± 0.98	0.05	${}^{W}F_{2,14.1}=0.93$	0.42	
15	16:1n-7	10.92±8.61	8.49±8.98	11.64 ± 8.55	0.78	${}^{W}F_{2,13.5}=0.353$	0.71	
16	16:1n-5	0.7 ± 0.69	1.60±1.97	1.42 ± 0.80	0.03	${}^{W}F_{2,12}=2.733$	0.11	
17	18:1n-9	9.29±7.76	9.18±11.71	12.77±9.54	0.47	${}^{W}F_{2,12.3}=0.334$	0.72	
18	18:1n-7	7.18±5.99	5.34±3.94	3.61±2.39	0.17	$^{W}F_{2,19.9}=2.367$	0.12	
19	24:1n-9	1.33±2.66	1.82 ± 2.08	3.72±1.80	0.10	$^{W}F_{2,15.7}=3.304$	0.06	
20	PUFA	22.62±26.15	24.6±21.61	75.66±62.41	1.14	${}^{W}F_{2,11.7}=1.954$	0.19	
21	n-3/n-6	1.74 ± 2.40	1.38 ± 0.32	2.43±0.49	2.13	${}^{W}F_{2,14.1}=10.989$	*	2-3
22	n-6 PUFA	8.05±6.92	10.93 ± 10.29	22.12±19.14	0.36	$^{W}F_{2,10.7}=1.712$	0.23	
24	LIN	4.54±3.68	6.26±7.58	10.6±9.40	0.21	${}^{W}F_{2,10.3}=1.307$	0.31	
25	ARA	1.60 ± 1.82	2.84 ± 2.04	4.88 ± 4.06	0.03	${}^{W}F_{2,11.3}=2.821$	0.1	
26	22:4n-6	0.88 ± 1.49	0.80 ± 0.62	0.99±0.72	0.08	${}^{W}F_{2,15.7}=0.149$	0.86	
27	22:5n-6	1.03 ± 1.94	1.03 ± 1.22	5.65 ± 5.03	0.05	${}^{W}F_{2,11.7}=2.358$	0.14	
28	n-3 PUFA	14.57±20.92	13.67±11.46	53.54±43.65	0.78	${}^{W}F_{2,11.9}=2.293$	0.14	
29	ALA	2.06±2.61	1.98 ± 2.13	10.87 ± 10.15	0.24	${}^{W}F_{2,11.4}=2.138$	0.16	
30	SDA	1.65 ± 3.01	1.65 ± 1.48	4.24 ± 7.64	0.10	^w F _{2,11.7} =0.32	0.73	
31	20:4n-3	0.71±1.55	0.78±0.73	1.71 ± 0.98	0.04	${}^{W}F_{2,14.6}=2.253$	0.14	
32 33	EPA	4.47±6.68	5.76±5.38	15.18 ± 12.04	0.14	${}^{W}F_{2,12}=2.085$	0.17	
34	22:5n-3	3.44±8.43	1.46 ± 1.81	2.04 ± 1.24	0.06	${}^{W}F_{2,21.7}=0.704$	0.51	
35	DHA	2.24±4.43	2.03±1.81	19.49 ± 14.88	0.19	$^{W}F_{2,11.5}=3.878$	0.05	
36	DMA	0.27±0.41	0.33±0.23	0.31±0.16	0.01	^w F _{2,19.6} =0.131	0.88	
37	DMA16:0	0.27±0.41	0.33±0.23	0.31±0.16	0.01	^w F _{2,19.6} =0.131	0.88	
38	UFA/SFA	2.40±1.13	2.25±0.65	1.74±0.95	2.07	${}^{W}F_{2,13.8}=0.985$	0.4	
39 40	Tot-FA	80.27±68.42	79.60±74.60	171.56±114.80	4.09	${}^{w}F_{2,11.8} = 1.689$	0.23	

APPENDIX: SEASONAL VARIATION IN WHITEFISH MUSCLE FATTY ACID COMPOSITION 9

Table	S7
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2	Table S7									
3	LSR (mg/g DW)	¹² Dec-11 (6)	² Feb-12 (6)	⁵ May-12 (6)	⁶ Jun-12 (6)	⁷ Jul-12 (6)	⁹ Sep-12 (6)	ANOVA	p _{anova}	post-hoc tests
4	SFA	6.86±1.87	6.03±1.25	6.42±1.00	6.67±1.37	6.34±2.56	6.74±2.70	AF5,30=0.157	0.976	
5	14:0iso	0.12 ± 0.04	0.11 ± 0.01	0.10±0.03	0.11±0.02	$0.10{\pm}0.05$	0.16 ± 0.05	^w F _{5,13.2} =1.502	0.25	
6 7	14:0	0.71 ± 0.60	0.34 ± 0.10	0.28±0.10	0.25 ± 0.04	0.40 ± 0.16	0.49 ± 0.17	$^{W}F_{5,12.8}=3.524$	0.03	
/ 8	15:0	0.14 ± 0.05	0.12 ± 0.02	0.12±0.03	0.11 ± 0.01	0.14 ± 0.06	0.18 ± 0.06	$^{W}F_{5,13,2}=1.838$	0.17	
9	16:0	4.52±0.98	4.23±0.90	4.60±0.77	$4.84{\pm}1.08$	4.52 ± 1.87	4.55±1.74	AF5,30=0.135	0.983	
10	18:0	1.37 ± 0.30	1.22 ± 0.28	1.32±0.17	1.36±0.29	1.20 ± 0.48	1.36 ± 0.76	${}^{W}F_{5,13.6}=0.246$	0.93	
11	MUFA	3.20±1.45	2.17±0.54	2.37±0.54	3.34±1.33	2.77 ± 1.40	3.29±1.66	$^{W}F_{5,13.5}=1.326$	0.31	
12	16:1n-9	0.16±0.10	0.10 ± 0.03	0.11±0.03	0.10 ± 0.02	0.13 ± 0.05	0.12 ± 0.04	^w F _{5,13.8} =0.973	0.47	
13	16:1n-7	0.48 ± 0.27	0.27 ± 0.14	0.34±0.16	0.64 ± 0.38	0.53 ± 0.37	0.75 ± 0.40	^w F _{5,13.6} =2.296	0.1	
14	16:1n-5	0.12±0.03	0.11±0.03	0.12±0.03	0.11 ± 0.02	0.16 ± 0.10	0.14 ± 0.05	$^{W}F_{5,13.4}=0.717$	0.62	
15	18:1n-9	1.41 ± 0.74	0.85 ± 0.21	0.90±0.21	1.59 ± 0.75	1.11±0.63	1.25 ± 0.74	$^{W}F_{5,13.3}=1.611$	0.22	
16	18:1n-7	0.70±0.31	0.46 ± 0.15	0.57±0.16	0.62±0.23	0.61±0.27	0.83 ± 0.45	${}^{W}F_{5,13.8}=1.035$	0.44	
1/	24:1n-9	0.32 ± 0.08	0.36 ± 0.07	0.33±0.05	0.29±0.04	0.23 ± 0.09	0.19 ± 0.05	$^{A}F_{5,30}=5.735$	0.001	12-9, 2-7, 2-9, 5-9
19	PUFA	5.21±0.97	12.23±2.44	14.02±1.76	14.46 ± 2.61	12.25 ± 4.95	11.8 ± 3.75	$^{A}F_{5,30}=7.229$	*	12-2, 12-5, 12-6, 12-7, 12-9
20	n-3/n-6	2.72 ± 0.48	3.66±0.37	3.73±0.26	3.22±0.43	3.63±0.38	3.91±0.33	$^{A}F_{5,30}=7.902$	*	12-2, 12-5, 12-7, 12-9
21	n-6 PUFA	1.40 ± 0.14	2.63 ± 0.53	2.97±0.41	3.48±0.88	2.64±1.04	2.42 ± 0.82	$^{A}F_{5,30}=5.708$	0.001	12-5, 12-6
22	LIN	0.41±0.12	0.38±0.12	0.42 ± 0.10	0.71±0.39	0.48±0.25	0.52±0.19	${}^{A}F_{5,13.8}=0.97$	0.47	
23	ARA	0.68 ± 0.08	1.55 ± 0.33	1.77±0.29	2.02 ± 0.38	1.54 ± 0.56	1.35 ± 0.47	${}^{A}F_{5,30}=8.431$	*	12-2, 12-5, 12-6, 12-7
24	22:4n-6	0.09 ± 0.01	0.15±0.03	0.19 ± 0.04	0.24 ± 0.11	0.14 ± 0.04	0.18±0.15	^w F _{5,13.2} =9.259	*	12-2, 12-5
25	22:5n-6	0.22 ± 0.07	0.55 ± 0.08	0.59±0.10	0.52 ± 0.06	0.48±0.23	0.37±0.12	$^{A}F_{5,30}=7.644$	*	12-2, 12-5, 12-6, 12-7
26	n-3 PUFA	3.82 ± 0.88	9.61±1.95	11.04±1.39	10.97 ± 1.84	9.61±3.94	9.38±2.96	$^{W}F_{5,13.5}=29.222$	*	12-2, 12-5, 12-6, 12-7, 12-9
2/	ALA	0.28 ± 0.05	0.31±0.10	0.34 ± 0.08	0.48 ± 0.16	0.40 ± 0.21	0.57 ± 0.37	^w F _{5,13.3} =2.31	0.1	
20	SDA	0.13 ± 0.05	0.14 ± 0.04	0.13 ± 0.03	0.14 ± 0.03	0.16 ± 0.07	0.32 ± 0.14	$^{A}F_{5,30}=6.842$	*	12-9, 2-9, 5-9, 6-9, 7-9
30	20:4n-3	0.10 ± 0.02	0.17 ± 0.05	0.15 ± 0.03	0.12 ± 0.01	0.20 ± 0.08	0.21±0.12	^w F _{5,12.7} =4.338	0.02	12-5
31	EPA	0.83±0.15	1.97 ± 0.48	2.23 ± 0.72	2.48 ± 0.55	2.33±0.85	2.29 ± 0.80	$^{A}F_{5,30}=5.428$	0.001	12-5, 12-6, 12-7, 12-9
32	22:5n-3	0.22 ± 0.06	0.52 ± 0.14	0.68 ± 0.15	0.80±0.31	0.56 ± 0.18	0.56 ± 0.21	${}^{A}F_{5,30}=6.206$	*	12-5, 12-6
33	DHA	2.25±0.71	6.50 ± 1.29	7.52 ± 0.75	6.95±0.93	5.96 ± 2.67	5.43 ± 1.64	$^{W}F_{5,13.7}=31.282$	*	12-2, 12-5, 12-6, 12-9
34	DMA	0.09 ± 0.02	0.11 ± 0.04	0.14 ± 0.04	0.16±0.06	0.12 ± 0.05	0.14 ± 0.05	$^{A}F_{5,30}=2.239$	0.076	
35	DMA16:0	0.09 ± 0.02	0.11 ± 0.04	0.14 ± 0.04	0.16 ± 0.06	0.12 ± 0.05	0.14 ± 0.05	${}^{A}F_{5,30}=2.239$	0.076	
36	UFA/SFA	1.28 ± 0.33	2.40 ± 0.22	2.57 ± 0.24	2.67 ± 0.15	2.36 ± 0.05	2.26±0.19	${}^{W}F_{5,12.7}=15.418$	*	12-2, 12-5, 12-6, 12-7, 12-9, 6-9
3/ २०	Tot-FA	15.36±3.02	20.54±4.13	22.94±2.98	24.63±5.14	21.48±8.77	21.97±7.88	^A F _{5,30} =1.795	0.144	



APPENDIX: SEASONAL VARIATION IN WHITEFISH MUSCLE FATTY ACID COMPOSITION 11

Coostor Review

Fig. S2





APPENDIX: SEASONAL VARIATION IN WHITEFISH MUSCLE FATTY ACID COMPOSITION



Fig. S6



Figure 2. Boxplots of whitefish muscle Total FA and PUFA content (mg/g DW) (A–B), UFA/SFA and n-3/n-6 – ratios (C–D) and content of eight most abundant FAs from the lowest to the highest contribution (E–L). Note the differences in y-axis scales in figures A, B, C, D, E-J, K-L. Bold horizontal lines indicate median values, the box indicate first and third quartile and whiskers indicate present minimum and maximum values unless outliers (open circles) are displayed (distance from median > 1.5*interquartile range).

156x112mm (300 x 300 DPI)



Figure 1. nMDS biplot of whitefish and invertebrate FA profile data. Whitefish are shown as circles and month by different intensity of shading by gray scale. Invertebrate groups and habitats are presented with different marker shapes, with the shading of smaller overlaying circles indicating sampling month. The most important fatty acids corresponding to 70-80% of the total dissimilarities between groups were identified using SIMPER results (Table 2) and they are presented as light gray text.

175x87mm (300 x 300 DPI)