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Nutritional quality of littoral macroinvertebrates and pelagic zooplankton in subarctic lakes

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Abstract

Littoral benthic primary production is considered the most important energy source of consumers in subarctic lakes. We analyzed essential fatty acid (EFA) and amino acid (EAA) content of 23 littoral benthic macroinvertebrate taxa as well as cladocerans and copepods from pelagic and littoral habitats of 8–9 subarctic lakes to compare their nutritional quality. Pelagic crustacean zooplankton had significantly higher EFA and total FA content (on average 2.6-fold and 1.6-fold, respectively) than littoral macroinvertebrates in all our study lakes. Specifically, docosahexaenoic acid (DHA), one of the most important EFA for juvenile fish, was almost exclusively found in pelagic copepods. In littoral macroinvertebrates, only *Lymnaea* (Gastropoda), *Eurycercus* (Cladocera), and *Gammarus* (Amphipoda) contained a low amount of DHA, whereas most littoral invertebrate taxa contained moderate amounts of eicosapentaenoic acid (EPA). The difference in DHA content may explain why so many generalist fish shift their diet to pelagic zooplankton at their peak abundance in mid/late-summer. Meanwhile, the differences in EAA content between pelagic zooplankton and littoral invertebrates were much lower than for EFA suggesting a wider availability of EAA in subarctic lakes, except for methionine. In the studied subarctic lakes, EFA and EAA variation in consumers was more related to taxon-specific than lake-specific characteristics. This indicates that climate-induced changes in the abundance and community structure of zooplankton vs. littoral macroinvertebrates will be important parameters in determining the availability of EFA and EAA to juvenile fish, and potentially fish production.

Littoral zones often dominate the primary production in clear oligotrophic lakes (Loeb et al. 1983; Vadeboncoeur et al. 2002, 2003) providing a major food supply for secondary production (Hecky and Hesslein 1995; Vander Zanden et al. 2006). This is particularly evident in many subarctic lakes, where littoral periphyton provides the predominant energy source for consumers (Sierszen et al. 2003; Karlsson and Byström 2005; Eloranta et al. 2010). Over the last decades, there has been speculation about whether the widespread reliance of fish on carbon fixed by littoral primary producers is due to larger magnitudes of periphyton production or more efficient trophic transfer of energy in the littoral than pelagic food webs (Hecky and Hesslein 1995; Vadeboncoeur et al. 2003). The relative importance of the two habitats (pelagic and littoral) for consumers is particularly interesting in northern ecosystems, which are experiencing rapid environmental changes, i.e., rising temperatures and precipitation, increasing terrestrial runoff and potential shifts in primary production toward pelagic dominance (Creed et al. 2018; Hayden et al. 2019). These may have profound effects on lake food webs, especially on the importance and quality of both pelagic and littoral primary producers as well as bacterial contribution to basal resources, available for invertebrate consumers and fish in the higher trophic levels (Ask et al. 2009; Creed et al. 2018; Hayden et al. 2019; Bergström et al. 2020).

Today there is strong evidence that energy transfer efficiency in lake food webs is largely regulated by food quality,

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e.g., the edibility and nutritional quality of primary producers to consumers (e.g., Ahlgren et al. 1990; Müller-Navarra et al. 2000; Taipale et al. 2014). Algae (phytoplankton and periphytic algae) are the primary source of many essential biomolecules, such as amino acids (AAs), fatty acids (FAs), and sterols (Ahlgren et al. 1992; Taipale et al. 2016, 2018), which cannot be adequately synthesized by the consumers de novo (Ketola 1982; Brett and Müller-Navarra 1997; Martin-Creuzburg et al. 2009; but see e.g., Lazzarotto et al. 2015). Furthermore, food quality (particularly polyunsaturated fatty acid, PUFA, content) directly determines the somatic growth, fitness and reproductive success in zooplankton (Brett et al. 2006; Taipale et al. 2011, 2014; Galloway et al. 2014). Alfa-linolenic acid (ALA, 18:3ω3) and linoleic acid (LIN, 18:2ω6) are precursors of physiologically essential fatty acids (EFAs) eicosapentaenoic, docosahexaenoic, and arachidonic acids (EPA, DHA, and ARA) (Arts et al. 2009). However, consumers and especially juvenile fish have negligible ability to bioconvert ALA to DHA and LIN to ARA (Arts et al. 2009; Taipale et al. 2018), and thus ARA (20:4ω6), EPA (20:5ω3) and DHA (22:6ω3) may be considered physiologically essential for fish (Arts et al. 2009; Tocher 2010; Taipale et al. 2018). Juvenile rainbow trout (*Oncorhynchus mykiss*) cannot grow without dietary DHA even though diet would contain high amount of short-chain ω-3 PUFA and EAA (Taipale et al. 2018), emphasizing the importance of synthesis and transfer of DHA in freshwater food webs. The availability of EFAs depends on the phytoplankton community composition, and two current major environmental changes, eutrophication and browning, have been suggested to shift phytoplankton community composition in the direction of species containing less PUFAs, which may downgrade the availability of EFAs in the pelagic food webs (Müller-Navarra et al. 2004; Taipale et al. 2016, 2019; Senar et al. 2019). Littoral periphyton have a lower total lipid and PUFA content compared to pelagic seston (Mariash et al. 2011), most likely because the PUFA-rich flagellated algal taxa, such as cryptophytes, chrysophytes, and dinophytes, are absent from periphyton but often dominate phytoplankton in subarctic lakes (Forsström et al. 2005). Despite the lower total lipid content per unit mass in periphyton compared to phytoplankton, periphyton may still dominate the whole-lake FA pool in shallow and oligotrophic subarctic lakes (Mariash et al. 2014).

AAs have key roles in cellular metabolism, being building blocks for many essential compounds, and are needed in almost all biochemical reactions. AA synthesis is connected to the citric acid cycle and associated processes. Generally, animals lack the ability to produce several AAs needed for protein synthesis and are therefore considered as essential amino acids (EAAs) (Bender 2012; Galili et al. 2016; Ruess and Müller-Navarra 2019). Laboratory feeding studies have shown that crustaceans and fish generally require 10 different EAAs: arginine, methionine, valine, threonine, isoleucine, leucine, lysine, histidine, phenylalanine, and tryptophan (Cowey and Foster 1971; Cowey 1995; NRC 2011). Freshwater

phytoplankton can synthesize all the EAAs and potentially free-living bacteria and microbial endosymbionts in consumer guts and intestines also can produce these (Ahlgren et al. 1992; Peltomaa et al. 2017; Taipale et al. 2018). In aquatic systems phytoplankton are more enriched with AA than macrophytes and, thus, considered as main EAA producers for the food webs (Ruess and Müller-Navarra 2019). However, studies on AA sources, availability and taxon-specific differences in lake food webs are still limited, especially comparing those between pelagic and littoral habitats (cf. Bleakley and Hayes 2017; Peltomaa et al. 2017; Taipale et al. 2018, 2019; Thera et al. 2020).

In subarctic lakes, benthic pathways provide major source of energy to dominant generalist salmonid species, such as Arctic charr, *Salvelinus alpinus*, and European whitefish, *Coregonus lavaretus*, during the whole open water season, while pelagic sources dominate the diet during the peak zooplankton abundance in summer (Tolonen 1998; Eloranta et al. 2010; Hayden et al. 2014; Kahilainen et al. 2016). The current knowledge on FAs in dorsal muscle of whitefish suggest relatively stable annual composition, except lowered concentrations of EFA during physiologically demanding spawning period in winter (Sushchik et al. 2007; Keva et al. 2019).

Littoral habitats not only support adult fish in subarctic lakes, but also provide essential nurseries for juveniles (Kahilainen et al. 2003; Byström et al. 2004). Ontogenetic diet and habitat shifts are typical for generalist fish in subarctic lakes (Kahilainen et al. 2003; Eloranta et al. 2010; Hayden et al. 2014). The timing of these shifts often involves trade-offs: e.g., pelagic habitats can provide abundant and nutritious prey for young fish, but are also exposed and dangerous, in terms of fish predators, compared to the more sheltered littoral (Werner and Gilliam 1984; Langeland et al. 1991). The individual specialization and seasonal diet shifts to different quality prey items of fish results in alterations to their tissue biochemical composition (Keva et al. 2017, 2019; Thomas et al. 2019). The differences in the quality of food resources should be accounted for when evaluating littoral habitats and also for pelagic predatory fish, which may use both habitats for foraging and provide important stabilizing effect on fish populations (Stein et al. 1995; Schindler and Scheuerell 2002; Eloranta et al. 2015; Thomas et al. 2019). Understanding the true value of littoral and pelagic habitats for lake ecosystem processes in terms of quality is particularly important under rapidly changing environmental conditions toward pelagic energy driven food webs in the warming subarctic (Creed et al. 2018; Hayden et al. 2019).

Here, we compared nutritional quality, EFA and EAA content, of littoral zooplankton and benthic macroinvertebrates as well as pelagic zooplankton in 8–9 subarctic lakes with different water quality in Finnish Lapland. The past work by Lau et al. (2012) from boreal lakes indicates strong interspecific differences in FA composition of macroinvertebrates and zooplankton. We expect to find similar interspecific EFA and EAA differences in subarctic lakes, although comprehensive studies of AA composition of aquatic consumers are currently lacking.

To better understand which biomolecules may start to restrict the growth of subarctic fish, we compared the EFA and EAA content of macroinvertebrates and pelagic zooplankton with the optimum levels for juvenile salmonid fish growth, derived from aquaculture studies. Although the values obtained from farmed fish may not fully correspond to natural conditions, we believe that the comparison is indicative and useful. We hypothesize that zooplankton are higher quality food (per unit dry mass) compared to macroinvertebrates.

Methods

Study sites and the sample collection

The study was conducted between 66°05′ and 69°03′N and 20°49′ and 27°07′E in Finnish Lapland (Fig. 1), where we sampled nine lakes along climatic (open-water season air temperature +3.2°C, +60 mm precipitation (maximum difference among lakes)) and water quality gradient (Table 1). The chemical parameters were measured from composite epilimnetic water samples from the pelagic, which were collected in August–September during years 2009–2013 by Hayden et al. (2017) or regional monitoring programs (Lapland Centre for Economic Development Transport and Environment). According to mean phosphorus concentration and Secchi depth (Carlson 1977) of the lakes, six of them are oligotrophic (Kilpis-, Muddus-, Oiko-, Paadar-, Rattos-, and Vastusjärvi), two are mesotrophic (Jerisjärvi and Särkijärvi) and one lake is eutrophic (Rattosjärvi). Temperature and precipitation data were obtained from eight weather stations located evenly across the study area, whereas water quality data of regional monitoring programs were obtained from Finnish environmental administration monitoring database HERTTA (https://www.syke.fi/en-US/Open_information).
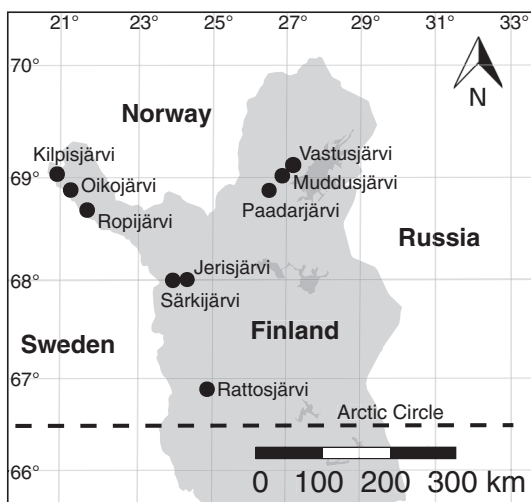

Fig. 1. Location of the subarctic study lakes in Finnish Lapland denoting where zooplankton and benthic macroinvertebrate samples were gathered. Latitude and longitude refer to north and east.

All the lakes contained three principal habitat types (pelagic, profundal, littoral). The proportion of littoral habitat (benthic areas situated shallower than the theoretical compensation depth, which was determined from light attenuation curves (Hayden et al. 2017)) varied from 14% to 84% of lake surface area. All lakes have a multispecies fish communities varying from salmonid to percid and finally cyprinid dominated fish fauna with increasing temperature and productivity from north toward south along the latitudinal gradient (Hayden et al. 2017). There is also a massive increase in fish abundance (ca. 300-fold) and biomass (ca. 50-fold) in all three habitat types (littoral, pelagic, profundal) along the gradient. The abundance, and possibly also diversity, of littoral benthic macroinvertebrates increase along the temperature and productivity gradient (Hayden et al. 2017).

We used qualitative kick-net sampling (mesh size 0.5 mm) to collect benthic macroinvertebrates from littoral areas, and all the lakes were sampled between 15th and 25th of August 2017. The samples were collected from several sites around the lake and by covering both rocky and vegetated shore types if such existed. To obtain enough material for FA and AA analyses (2–4 mg dry weight (DW)), the animals were pooled at a taxonomic level, varying from class (e.g., Oligochaeta) to species (e.g., *Asellus aquaticus*). Littoral macroinvertebrate FA composition was quantified from eight lakes and AA composition from nine lakes. Lake Oikojärvi lacked macroinvertebrate FA data. Macroinvertebrate samples were frozen at −20°C immediately after the identification (maximum of 10 d), followed by transportation to laboratory, where they were stored in −80°C prior to freeze-drying (with ALPHA 1-4 LD plus, Christ) at −70°C for 48 h.

We also collected data for FA and AA of pelagic and littoral crustacean zooplankton from the same nine study lakes as for benthic macroinvertebrates (Table 2). The lakes were sampled by vertical net tows (50–200 μm mesh size) from hypolimnion to epilimnion in the pelagic, and by horizontal net tows from a boat at the same littoral sites as where the macroinvertebrates were sampled. Zooplankton were always sampled during the same day as macroinvertebrates. Crustacean zooplankton were identified and separated into two taxonomic groups, cladocerans and copepods, when their abundance was high enough for the separation (from four lakes, Table 2). Bulk zooplankton samples consisted of both cladocerans and copepods in littoral and pelagic habitats.

FA analyses

Freeze-dried benthic invertebrate samples (mean ± SD of 1.7 ± 0.7 mg DW and ranging from 0.1 to 3.0 mg DW) were pulverized with a mortar and pestle and analyzed for FAs as follows. We extracted lipids twice with 2 : 1 (by vol) chloroform : methanol following Folch et al. (1957) using heneicosanoic acid (21 : 0) as an internal standard. For gas chromatography (GC), we derivatized the FAs into fatty acid methyl esters (FAMEs) using an acid catalyzed

Table 1. Physical and chemical characteristics of the nine subarctic study lakes and the catchment areas. Measured variables include: lake surface area (Area), latitude north (Lat.°N), longitude east (Long.°E), altitude of lake (Alt.), mean open-water season precipitation (Precip) and air temperature (Temp.) in June–September 1981–2010, compensation depth (Comp.D), mean depth (Mean.D), Secchi depth (Sec.D), maximum depth (Max.D), littoral zone proportion of total lake surface area (Litt), mean total phosphorus (TP) and nitrogen (TN) concentrations, mean dissolved organic carbon concentrations (DOC), mean water color (Col.). The data represents late-summer/early autumn and is based on own sampling and HERTTA database of Finnish Environmental Administration (https://www.syke.fi/en-US/Open_information). N.

Lake	Kilpis-	Muddus-	Paadar-	Vastus-	Oiko-	Ropi-	Jeris-	Särki-	Rattosjärvi
Area (km^2)	37.3	48.0	21.0	4.3	1.2	1.3	23.5	5.0	4.1
Lat. °N	69.0	69.0	68.9	69.0	68.5	68.4	67.6	67.5	66.5
Long. °E	20.5	26.5	26.5	27.1	21.1	21.4	24.1	23.6	24.5
Alt. (m a.s.l.)	473.0	146.0	144.0	146.0	448.0	399.0	258.0	261.0	118.0
Precip. (mm)	197.0	236.0	234.0	239.0	218.0	240.0	256.0	256.0	257.0
Temp. (°C)	8.4	10.0	10.1	9.8	8.7	9.0	10.5	10.5	11.6
Comp.D (m)	10.0	7.0	5.0	3.0	5.0	5.0	3.5	1.5	1.5
Mean.D (m)	19.4	8.5	11.7	2.7	3.1	6.9	3.4	5.1	2.1
Sec.D. (m)	9.5	4.5	3.5	2.0	Na = 2.5	3.3	Na = 2.0	2.3	0.8
Max.D (m)	57.0	73.0	56.0	15.0	10.0	20.0	12.0	14.0	6.8
Litt (%)	29.0	59.0	38.0	69.0	84.0	54.0	59.0	14.0	32.0
TP (μg L^{-1})	4.0	5.0	6.0	6.0	7.0	10.0	25.0	25.0	47.0
TN (μg L^{-1})	120.0	160.0	160.0	210.0	215.0	273.0	550.0	520.0	520.0
DOC (mg L^{-1})	2.8	5.7	6.1	5.7	5.1	5.4	6.1	5.3	12.2
Col. (mg Pt L^{-1})	4.7	21.7	26.7	25.0	40.0	35.0	30.0	28.3	90.0

transesterification reaction with 1% H_2SO_4 in methanol while heating in 90°C in a heat block for 90 min. We dissolved the produced FAMEs in hexane and analyzed them with a gas chromatograph-mass spectrometer (GC–MS, Agilent 6890N and 5973N, Agilent Technologies). The column was Agilent DB–23 (60 m × 0.25 mm × 0.15 μm) and helium was used as a carrier gas with an average velocity of 28 cm s^{-1}. The oven temperature was 50°C for 1 min, then raised 15°C min^{-1} to 150°C, followed by 1.5°C min^{-1} to 210°C, and 3°C min^{-1} to 230°C, which was held for 5 min. Run time for a sample was 59 min. We calculated the FA contents using calibration curves based on known solutions of a standard mixture (GLC-538, Nu-Chek Prep). The internal standard recovery was 93.8 ± 27.5% (mean ± SD). We used MSD ChemStation E.02.01.1177 (Agilent Technologies) for peak identification and integration of the chromatograms.

For zooplankton FA analyses, freeze-dried zooplankton samples (mean ± SD of 0.9 ± 0.3 mg DW and ranging from 0.1 to 1.4 mg DW) were pulverized with a mortar and pestle. The zooplankton lipid extraction and FA methylation methods were identical with benthic macroinvertebrate samples except the used internal standard was 1,2-dinonadecanoyl-sn-glycero-3-phosphatidylcholine (Larodan, Malmö, Sweden). The produced FAMEs were analyzed in hexane using a gas chromatograph attached to a mass spectrometer (GC-2010 Plus and QP-2010 Ultra, Shimadzu) with Zebron ZB-FAME column (30 m + 5 m guardian × 0.25 mm × 0.2 μm). At the begin of the each GCMS run, the oven temperature was held at 50°C for 1 min, followed by raising 10°C min^{-1} to 130°C, 7°C min^{-1} to 180°C, 2°C min^{-1} to 200°C and was held there for 3 min which after the oven temperature was raised 10°C min^{-1} to 260°C. The injector temperature was 270°C and the interface 250°C. Total column flow was 27.5 mL min^{-1} and linear velocity 36.3 cm s^{-1}. Fatty acid identification was based on mass spectra and the FA content calculations were done based on four point standard mixture calibration curves (GLC-566c, Nu-Chek Prep) with GCMS solution version 4.42 (Shimadzu). The total FA content (μg mg^{-1} DW) of a sample was calculated by summing up all the identified FAs, and the ω3 and ω6 contents by summing up all the ω3- and ω6-FAs. EFA content was calculated by summing up 18:2ω6, 20:4ω6, 18:3ω3, 18:4ω3, 20:5ω3 and 22:6ω3. The internal standard recovery was 104.6 ± 18.5% (mean ± SD).

Different GC–MS methods were used for macroinvertebrates and zooplankton due to samples being analyzed at different locations. Macroinvertebrates were analyzed in the University of Eastern Finland (Joensuu), while the zooplankton samples were analyzed in the University of Jyväskylä (Jyväskylä). Analytical variation between the methods is likely negligible because the extractions and methylations were identical and, in both methods, external standards were used for correcting the GC–MS response and internal standard was used to adjust the measured FA content.

AA analyses

For the macroinvertebrate and zooplankton AA analyses, freeze-dried samples (mean ± SD of 0.8 ± 0.3 mg DW and

Table 2. Appearance of the total of 23 collected benthic invertebrate taxa within and among the study lakes, together with pelagic and littoral bulk zooplankton and pelagic cladocerans and copepods. Numbers denote the mean essential fatty acids/essential amino acids (EFA/EAA) contents ($\mu g\ mg^{-1}$ DW) of the taxa listed. The mean EFA is the sum of $18:2\omega6$ (LIN), $20:4\omega6$ (ARA), $18:3\omega3$ (ALA), $18:4\omega3$ (SDA), $20:5\omega3$ (EPA), and $22:6\omega3$ (DHA) divided by the count. The mean EAA is the sum of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine divided by the count. Abbreviations: Nd, no data; (*), bulk samples. More detailed information of the EFA and EAA contents are given in the Table S1. The lakes are in ascending order of average total phosphorus concentration.

	Lake								
Taxon	Kilpis-	Muddus-	Paadar-	Vastus-	Oiko-	Ropi-	Jeris-	Särki-	Rattosjärvi
Crustacean macroinvertebrates									
Amphipoda									
Gammarus sp.									16.4/290.0
Isopoda									
Asellus aquaticus							7.7/113.7	12.6/129.4	15.3/143.0
Crustacean zooplankton									
Pelagic zooplankton*	52.9/149.0	33.8/154.5	26.6/172.1	23.3/nd	41.5/170.1		26.5/nd	27.6/185.1	23.2/168.9
Cladocera (pelagic)	27.2/164.8		Nd/142.5			19.9/144.6	24.3/170.6	31.3/188.4	27.5/176.1
Copepoda (pelagic)	89.1/153.8		42.5/182.6	30.0/31.2	61.6/182.3		29.6/172.6	52.0/190.1	
Littoral zooplankton*	87.5/151.8	29.4/172.5		20.1/nd	29.5/168.3	28.2/156.0	40.5/176.2	31.8/173.6	24.3/165.6
Eurycercus sp.	15.9/120.6		23.9/143.4	11.8/73.2	Nd/136.5	15.0/144.0	10.8/141.7	31.3/188.4	27.5/176.1
Insecta									
Coleoptera									
Dytiscidae	20.8/146.9		8.4/132.2	13.4/130.0	Nd/133.2		29.1/111.3	15.3/106.1	0.3/117.2
Diptera									
Ceratopogonidae			29.8/140.0						
Chironomidae	6.2/59.9	5.9/133.0	1.5/168.6	14.0/162.1	Nd/166.2	8.3/149.9	14.5/116.3	12.3/138.5	15.8/104.6
Tabanidae			Nd/108.2						
Anisoptera				4.7/138.9					
Ephemeroptera	7.5/167.3		29.8/107.3	23.4/106.0		41.4/135.3	28.3/128.8	37.9/131.6	15.5/141.5
Hemiptera									
Corixidae	11.5/154.8		12.3/155.1	14.4/143.2	Nd/137.8				
Gerridae									8.9/127.8
Megaloptera									
Sialis sp.			21.4/133.9	2.4/121.4				21.4/93.8	15.8/107.4
Plecoptera			25.9/117.1	29.3/151.1				33.9/119.3	
Trichoptera	17.1/161.2	23.2/132.0	35.9/105.9	5.7/112.9	Nd/151.5	22.5/102.4	13.0/145.1	40.5/140.0	30.0/129.2
Annelida									
Hirudinea								4.6/142.0	0.1/127.9

(Continues)

Table 2. Continued

Taxon		Kilpis-	Muddus-	Paadar-	Vastus-	Oiko-	Ropi-	Jeris-	Särki-	Rattosjärvi
						Lake				
Oligochaeta		4.9/73.9	10.6/149.1	5.7/109.4	10.0/128.2	Nd/132.1	4.1/283.5	8.7/158.7	1.7/158.8	1.2/160.1
Mollusca										
Gastropoda										
Lymnaea sp.			4.7/nd	7.0/108.6			6.9/208.4	5.3/127.9	8.2/136.4	1.9/156.4
Valvata sp.				4.9/64.8						
Sphaeriidae										
Pisidium sp.			3.5/165.7							
Sphaerium sp.								2.1/122.8	5.5/96.2	
Nematoda							26.6/nd			
Arachnida										
Trombidiformes										
Hydracarina				34.8/119.6			20.6/157.2	32.0/85.5	36.3/141.1	3.0/128.9

ranging from 0.1 to 1.4 mg DW) were pulverized with a mortar and pestle. Proteins were hydrolyzed with 1 mL of 6 M HCl at 110°C for 20 h for AAs analysis. Each sample was spiked with internal standard (Norvaline). Invertebrate AAs were run as their propyl chloroformates using EZ:faast kit for preparation (Phenomenex). Samples were run with a GC–MS (GC-2010 Plus and QP-2010 Ultra, Shimadzu) using ZB-AAA column (9.5 m $\times$ 0.25 μm $\times$ 0.25 mm) and with the following temperature program: temperature was raised from the initial 110°C to 320°C at the rate of 30°C min^{-1}, after which it was held for 7 min at 320°C. Injection temperature was 300°C and the interface 290°C. Total column flow was 2.4 mL min^{-1} and linear velocity 71.2 cm s^{-1}. AA identification was based on specific ions included in the EZ:faast library. For quantification, we used four-point calibration curves derived from Sigma-Aldrich AA-S-18 standard mixture supplemented with norvaline. The internal standard recovery was 92.0 $\pm$ 33.1% (mean $\pm$ SD). We used GCMS solution version 4.42 (Shimadzu) for the identification and calculation of AA content. Due to the properties of the EZ: faast kit, we were able to analyze eight EAAs (valine, leucine, isoleucine, threonine, methionine, phenylalanine, lysine, and histidine), which represent the EAAs in this study, but not arginine or tryptophan. In addition to EAAs, we were able to quantify two conditionally EAAs (glycine and proline) and seven non-EAAs (alanine, serine, asparagine, glutamic acid, ornithine, glycine-proline, and tyrosine). Sum of these AAs represent the total AAs in this study.

Essential fatty and AA requirements for juvenile salmonids

We used threshold factors of EFAs and EAAs for rainbow trout and Atlantic salmon (*Salmo salar*) to evaluate the nutritional value of zooplankton and macroinvertebrates for salmonid fish at optimal conditions and to better understand which biomolecule may start to restrict optimal health and growth of subarctic salmonid fish. We used rainbow trout as a reference species, since it is among the most extensively studied salmonid species in aquaculture, although not found from our study lakes. Atlantic salmon is not found from our study lakes either, but is found from the same watersheds and represent a salmonid species common in this subarctic region. The threshold values were given by Food and Agriculture Organization of the United Nations (FAO, http://www.fao.org/home/en/, 11.9.2019), which were also used as reference values in laboratory experiments of FA and AA composition of rainbow trout by Taipale et al. (2018). These reference values should be considered minimum requirements for optimal growth in aquaculture environment. However, these values may differ from the requirements under natural conditions and should therefore only be considered as indicative. We were able to analyze eight of the 10 EAAs for juvenile salmonid fish (*see* previous paragraph) and all the EFAs (18:2ω6, 20:4ω6, 18:3ω3, 18:4ω3, 20:5ω3 and 22:6ω3) given by FAO.

Statistical analyses

We used nonmetric multidimensional scaling (NMDS) to examine and visualize the variation of FA and AA composition of different taxa. Data was expressed as percentages of the total mass of FAs and AAs. To detect differences in FA and AA composition between taxa, lakes and trophic states, we used permutational multivariate ANOVA (PERMANOVA) with permutation of residuals under restricted model and Type III sums of squares. We operated the multivariate statistical methods on Euclidean distances without transforming the data (Happel et al. 2017). We conducted PERMANOVA with lake and taxon as fixed factors. Pairwise comparisons among all pairs of levels of a given factor were obtained by separate runs of PERMANOVA, and the *p*-values were obtained using permutations (Anderson et al. 2008). The variation explained by different factors was assessed from the estimates of components of variation (ECV) of each factor. If ECV of a factor was negative, it was set to zero and removed, and an estimate for the remaining factor was recalculated (Fletcher and Underwood 2002). We also conducted centroid distance-based tests for homogeneity of multivariate dispersions (PERMDISP) alongside PERMANOVA to clarify the nature of multivariate effects on the basis of a chosen resemblance measures, i.e., whether the groups differ from location (between-group variation) or dispersion (within-group variation) in multivariate space. Moreover, we conducted cluster analysis with taxonomic group averages for the mean macroinvertebrate, cladoceran and copepod FA and AA composition. We used dendrogram dissimilarity level of 0.6 (60% dissimilarity among the clusters) for NMDS plots. These analyses were conducted with PRIMER 6 (version 6.1.15) and PERMANOVA (version 1.0.5) (PRIMER-E). For testing differences in the most essential individual FAs, AAs or FA and AA groups (such as ω3, EPA, EFA, EAA) between taxa and lakes, we used Welch's ANOVA with Games–Howell post hoc tests (due to unequal variances) for pairwise comparisons in SPSS (version 23.0.0.2, IBM). Only the eight most abundant macroinvertebrate taxa were selected for ANOVA tests, where the bulk samples of a single taxon from a particular lake comprised replicates. Graphs were performed with ggplot2 library module (Wickham 2016) in R version 3.4.3. (R Core Team 2017). Significance limit was set to alpha level of 0.05. If not otherwise noted, all the descriptive statistics in the main text are mean ± SD.

Results

Collected taxa

This study encompassed a total of 23 benthic macroinvertebrate taxa (Table 2). The average number of collected taxa (± SD) per lake was 10 ± 4. The least taxa were caught from Lakes Kilpisjärvi and Oikojärvi (three and six, respectively), which were the coldest and least productive lakes in the dataset (Table 1). The greatest number of taxa were caught from Paadarjärvi and Särkijärvi (14 from both). The number of caught taxa had a negative relationship with the altitude of the lake (Pearson's correlation coefficient, $R = -0.820$, $p < 0.01$) and positive relationship with the mean open-water season air temperature (Pearson's $R = 0.840$, $p < 0.01$). Also, temperature was negatively correlated with the altitude (Pearson's $R = -0.800$, $p < 0.01$). Ephemeroptera, Oligochaeta and Chironomidae were present in most lakes. There were eight taxa which were caught only from one lake (Table 2). In general, the number of individuals of each benthic macroinvertebrate taxon varied from about tens to hundreds of individuals.

Cladocerans and copepods were found in both pelagic and littoral habitats. Cladoceran *Eurycercus* was found only in the littoral kick net samples and was therefore classified as a benthic cladoceran. Zooplankton collected with plankton net were abundant (> 100 individuals per lake and sample).

FA and AA composition and content of benthic macroinvertebrates

We identified a total of 42 different FAs, of which the most abundant were 16:0, 18:1ω9, 16:1ω7, 20:5ω3 (EPA), and 18:3ω3 (ALA) (Table S1). We identified a total of 17 AAs, from which the most abundant were alanine, leucine, valine and lysine (Table S1). The NMDS ordination with taxon and lake as factors did not show clear distinctions between the benthic macroinvertebrate FA compositions (Fig. S1). Also, PERMANOVA did not reveal significant differences in FA compositions among the taxa and between the lakes (Table S2). Taxon explained 28.7% of the invertebrate FA composition, and there was a significant dispersion effect (PERMDISP, deviations from centroids: $F_{2, 84} = 4.13$, $P_{perm} = 0.032$) indicating that the differences were within the groups. The explained variation by lake and lake × taxon were assumed zero due to negative ECV values. NMDS ordination did not reveal clear distinction in macroinvertebrate AA composition (Fig. S2), and there were no significant differences among the taxa and lakes according to PERMANOVA (Table S2).

Total fatty acid content of the benthic macroinvertebrates ranged from 4.4 to 154.5 µg mg^{-1} dry weight (DW) while the mean (± SD) was 54.4 ± 35.5 µg mg^{-1} DW (Table S1, Fig. 2a). The mean (± SD) ω3 content of benthic macroinvertebrates was 9.2 ± 3.2 µg mg^{-1} DW and the mean (± SD) ω3:ω6 ratio 1.4 ± 1.2, with neither variable differing among the lakes (Welch's ANOVA for ω3: $F_{7, 16.0} = 1.58$, $p = 0.212$, and for ω3:ω6 ratio: $F_{7, 17.0} = 0.78$, $p = 0.610$). However, both ω3 contents (Welch's ANOVA, $F_{7, 16.5} = 4.54$, $p < 0.01$) and ω3:ω6 ratios (Welch's ANOVA, $F_{7, 17.4} = 5.51$, $p < 0.01$) differed among the taxa. Ephemeroptera (taxon with the highest ω3 and ω3:ω6 values) differed significantly from all the other benthic macroinvertebrate taxa in ω3 content except *Eurycercus*, Hydracarina and Trichoptera according to Games–Howell post hoc test. In terms of ω3:ω6, Ephemeroptera differed significantly from all the taxa except *Eurycercus* and *Lymnaea* (Fig. 3a). The mean (± SD) EPA and DHA contents of benthic macroinvertebrates were 5.1 ± 4.1 and 0.4 ± 0.7 µg mg^{-1} DW, respectively, and the lakes did not differ from each other. The
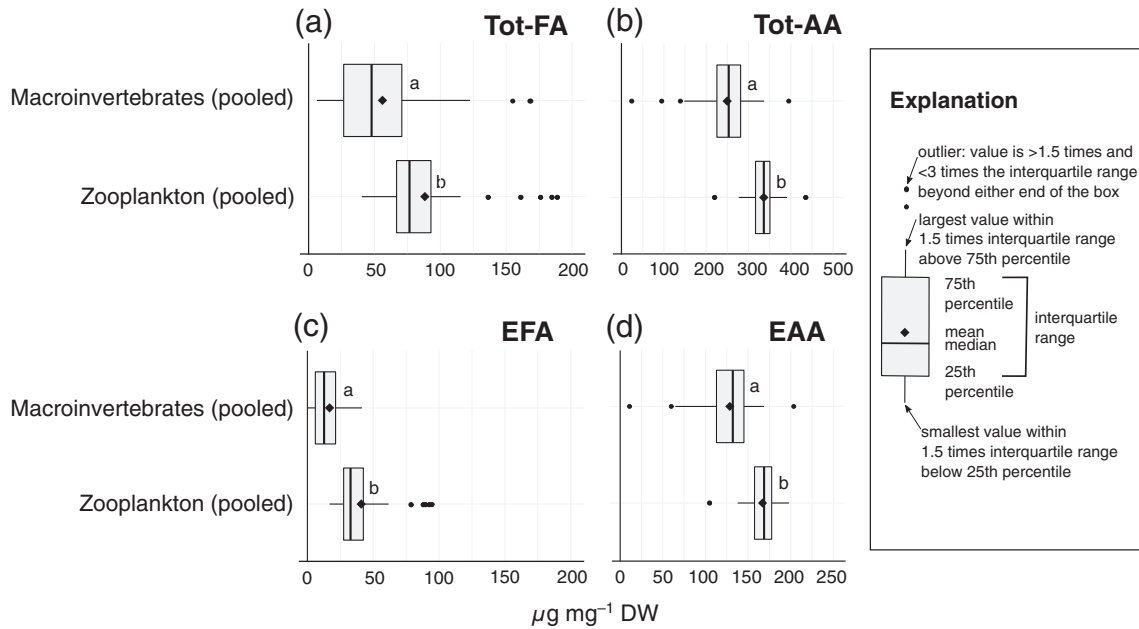
Fig. 2. Boxplot of the benthic macroinvertebrate and zooplankton total fatty acid (Tot-FA, **a**), total amino acid (Tot-AA, **b**), essential fatty acid (EFA; **c**) and essential amino acid (EAA; **d**) contents in the study lakes. Letters denote statistically significant differences (Games–Howell post hoc test, b > a).
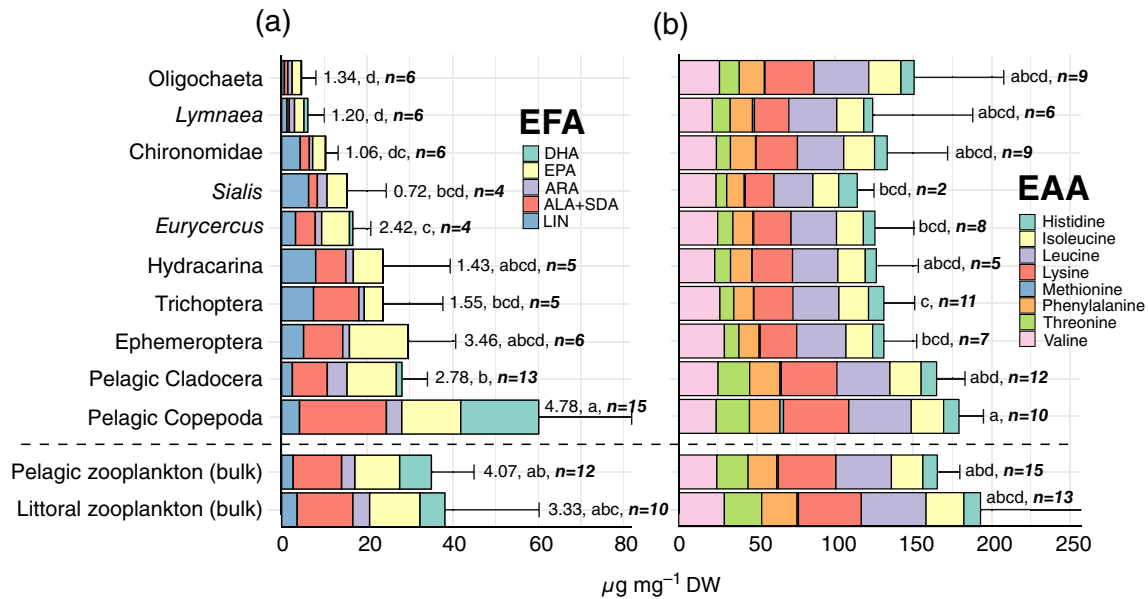

Fig. 3. The mean EFA (**a**) and EAA (**b**) distribution among the eight most abundant invertebrate taxa and littoral and pelagic zooplankton caught from the study lakes. Abbreviations denote the following: EPA; 20:5ω3, DHA; 22:6ω3, LIN; 18:2ω6, ALA + SDA; 18:3ω3 + 18:4ω3, ARA; 20:4ω6. The mean ω3:ω6 ratios of each taxa are indicated next to the bars in panel a. Error bars denote the standard deviation of the average EFA and EAA content, and the letters denote between-taxa differences in the sum of EFA and EAA according to Games–Howell post hoc test (d>c>b>a). Sample size (*n*) is indicated in each bar.

content of EPA in benthic macroinvertebrates differed significantly among the taxa (Welch's ANOVA, $F_{7, 16.7} = 7.13$, $p < 0.001$). Ephemeroptera had significantly higher EPA content than any other benthic macroinvertebrate taxa (according to Games–Howell post hoc), excluding Hydracarina and *Eurycercus* (Fig. 2a). In benthic macroinvertebrates, DHA content was the

highest in *Gammarus* (1.7 μg mg^{-1} DW), *Lymnaea* (0.8 ± 0.7 μg mg^{-1} DW) and *Eurycercus* (0.5 ± 0.4 μg mg^{-1} DW), but due to lack of DHA in several taxa (and thus several zero variances) we did not perform statistical testing.

Total AA content of benthic macroinvertebrates ranged from 94.9 to 581.9 μg mg^{-1} DW while the mean (± SD) was

259.1 ± 65.6 µg mg^{-1} DW (Fig. 2b). The mean ($\pm$ SD) EAA content of benthic macroinvertebrates was 132.0 ± 36.0 µg mg^{-1} DW. There were no significant differences in average macroinvertebrate EAA (Welch's ANOVA, $F_{8, 22.7} = 1.23$, $p = 0.328$ or total AA ($F_{8, 23.1} = 0.84$, $p = 0.579$) contents among the lakes. The mean ($\pm$ SD) benthic macroinvertebrate EFA content was 15.2 ± 11.2 µg mg^{-1} DW, and lakes did not differ significantly from each other (Welch's ANOVA, $F_{6, 15.9} = 1.48$, $p = 0.249$). Macroinvertebrate taxa differed significantly in their EFA content (Welch's ANOVA, $F_{7, 17.1} = 7.19$, $p < 0.001$), but not in EAAs (Welch's ANOVA, $F_{7, 18.5} = 0.54$, $p = 0.709$) nor total AAs ($F_{7, 18.2} = 0.50$, $p = 0.824$) (Fig. 3a,b).

FA and AA composition and content of zooplankton

From zooplankton, we identified a total of 42 different FAs, from which the most abundant were 16:0, 20:5ω3 (EPA),

18:1ω9, 22:6ω3 (DHA), 18:4ω3 (SDA) and 18:3ω3 (ALA) (Table S1). We identified a total of 15 AAs, from which the most abundant were aspartic acid, glutamic acid, lysine and leucine (Table S1). The NMDS ordination of zooplankton FA composition with lake and taxon (pelagic and littoral bulk zooplankton treated as taxon group) as factors showed clear distinctions between pelagic cladocerans and copepods and pelagic and littoral bulk zooplankton samples (Fig. S2). Also, PERMANOVA revealed significant differences in FAs among the four zooplankton taxon groups and among the lakes (Tables S3 and S4). Taxon explained 32.4% of the variation in zooplankton FA, lake explained 32.4% and these two also had significant interaction, which explained 28.3% of the total variation in zooplankton FA. PERMDISP revealed that the differences among the lakes were due to significant within-lake variation (deviations from centroids: $F_{8, 41} = 4.30$,
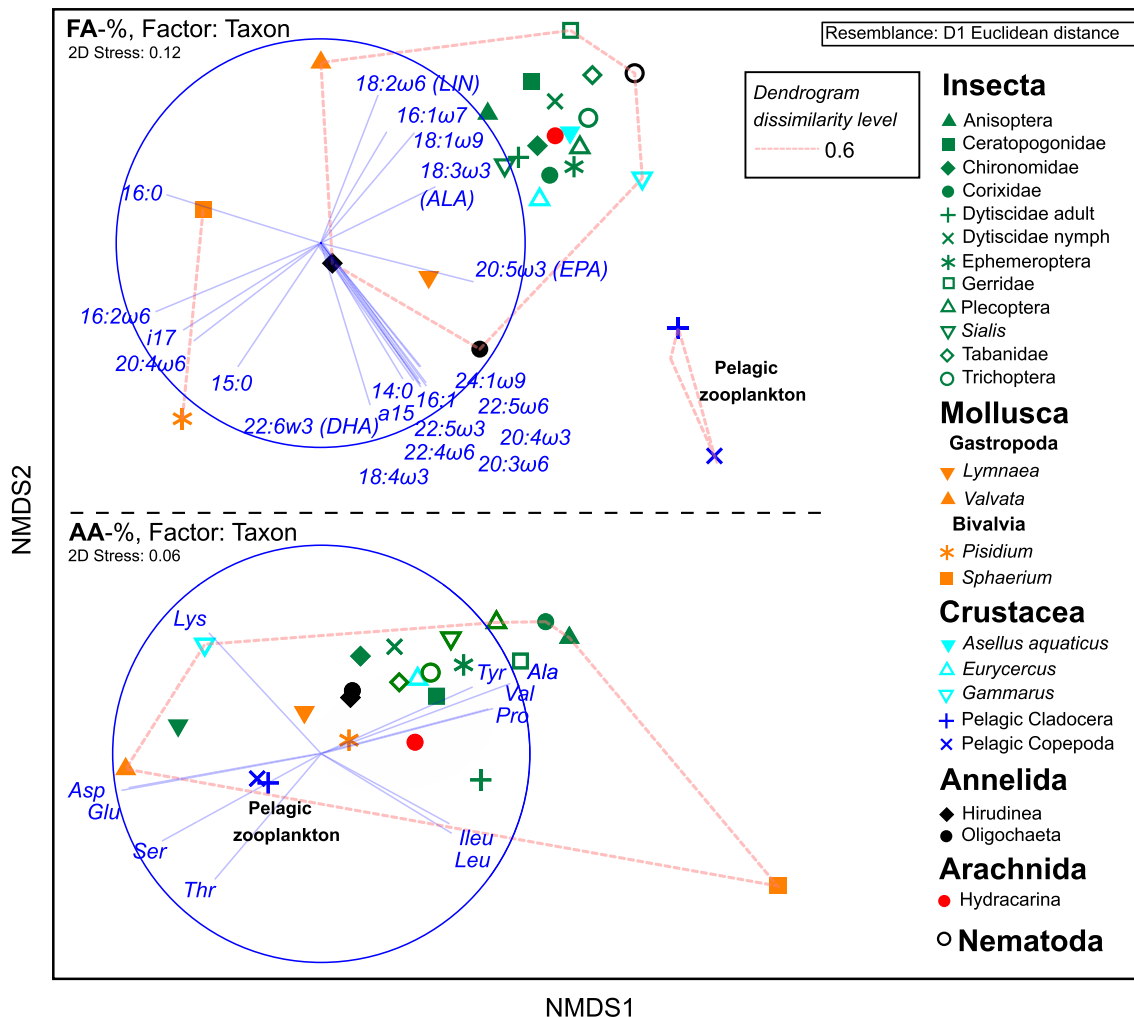

Fig. 4. Nonmetric multidimensional scaling (NMDS) ordination of the mean macroinvertebrate and zooplankton fatty acid and amino acid compositions using taxon as a factor. Fatty and amino acids that correlated strongly (Pearson's $R > 0.6$) with either of the axis have been presented as blue direction vectors. The areas connected by the red lines indicate cluster analysis with dendrogram dissimilarity level of 0.6 (60% dissimilarity among the three clusters). Amino acids are abbreviated as follows: Alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), isoleucine (Ileu), leucine (Leu), lysine (Lys), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr), and valine (Val).

Table 3. Dietary nutrient requirement of juvenile salmonid fish based on the recommendation of Food and Agriculture Organization of the United Nations (FAO 2019) for rainbow trout (*Oncorhynchus mykiss*), denoted as Rt, and Atlantic salmon (*Salmo salar*), denoted as As, in comparison with average (mean ± SD) biochemical content of zooplankton and benthic invertebrates from the study lakes. Essential fatty acid abbreviations: linoleic acid (LIN), arachidonic acid (ARA), alfa-linolenic acid (ALA), stearidonic acid (SDA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Nd, no data (not measured). Bolding indicates values reaching requirement limit.

	Requirement for juvenile salmonids Rt/As	Pelagic Cladocera	Pelagic Copepoda	Pelagic zooplankton (bulk)	Littoral zooplankton (bulk)	Littoral macroinvertebrates
EAA, μg mg^{-1} DW						
Arginine	20.0/20.0	Nd	Nd	Nd	Nd	Nd
Histidine	7.0/7.0	**9.9 ± 1.4**	**9.8 ± 1.1**	**9.2 ± 1.2**	**10.6 ± 6.1**	**9.4 ± 6.4**
Isoleucine	8.0/8.0	**19.9 ± 2.6**	**20.7 ± 3.2**	**20.1 ± 2.3**	**24.2 ± 15.5**	**18.2 ± 4.9**
Leucine	14.0/14.0	**33.9 ± 5.5**	**39.9 ± 3.1**	**35.4 ± 4.0**	**41.4 ± 25.2**	**30.3 ± 8.1**
Lysine	18.0/18.0	**35.5 ± 3.8**	**41.7 ± 5.0**	**36.8 ± 5.2**	**40.2 ± 24.3**	**24.6 ± 9.9**
Methionine	10.0/10.0	0.9 ± 0.9	2.3 ± 1.1	1.0 ± 0.6	0.9 ± 0.7	0.5 ± 0.2
Phenylalanine	12.0/12.0	**19.3 ± 2.8**	**19.5 ± 2.0**	**18.4 ± 2.4**	**22.6 ± 14.9**	**13.3 ± 4.5**
Threonine	8.0/8.0	**20.2 ± 3.6**	**21.3 ± 2.7**	**19.9 ± 2.2**	**23.9 ± 13.3**	**9.9 ± 4.0**
Tryptophan	2.0/2.0	Nd	Nd	Nd	Nd	Nd
Valine	13.0/13.0	**25.1 ± 1.9**	**24.0 ± 2.7**	**24.4 ± 2.1**	**29.2 ± 18.3**	**25.8 ± 6.4**
EFA, μg mg^{-1} DW						
18:2ω6 (LIN)	8.0/na	4.1 ± 3.7	4.2 ± 2.4	2.7 ± 1.3	3.6 ± 2.5	4.1 ± 3.7
20:4ω6 (ARA)	5.0/na	4.6 ± 1.2	3.6 ± 2.5	3.1 ± 1.1	3.9 ± 1.1	1.6 ± 1.2
18:3ω3 + 18:4ω3 (ALA + SDA)	10.0/na	8.2 ± 2.4	**20.3 ± 11.4**	**11.3 ± 4.8**	**13.0 ± 11.8**	4.1 ± 4.5
20:5ω3 (EPA)	10.0/5.0	**11.5 ± 2.2**	**13.8 ± 6.3**	**10.5 ± 1.4**	**11.8 ± 2.1**	**5.1 ± 4.1**
22:6ω3 (DHA)	5.0/5.0	1.3 ± 0.3	**13.8 ± 6.3**	**7.4 ± 4.9**	**5.8 ± 7.5**	0.2 ± 0.6

P_{perm} < 0.01), whereas the differences among taxa were in their locations in the multivariate space ($F_{3,\,46}$ = 2.50, P_{perm} = 0.13). Particularly copepods and cladocerans differed in their locations in the multivariate space (Fig. S2). Higher proportions of long-chain C22 PUFAs in copepods separated them from the cladocerans (Fig. S2), and higher C22 PUFAs in zooplankton on average also separated them from the benthic macroinvertebrates (Fig. 4). Also, higher fraction of copepods than cladocerans in some of the bulk zooplankton samples likely explain the rather high dispersion in the multivariate space (Fig. S2).

Total fatty acid content of the zooplankton ranged from 40.4 to 202.5 μg mg^{-1} DW while the mean (± SD) was 88.9 ± 36.5 μg mg^{-1} DW (Table S1, Fig. 2a). The mean (± SD) ω3 content of zooplankton was 33.9 ± 18.9 μg mg^{-1} DW (3.7 times higher than in macroinvertebrates), and the mean (± SD) ω3:ω6 ratio 3.7 ± 1.0 (2.4 times higher than in macroinvertebrates), and there were significant differences among the lakes (Welch's ANOVA for ω3: $F_{8,\,13.5}$ = 11.33, p < 0.01, and for ω3:ω6 ratio: $F_{8,\,12.1}$ = 7.10, p < 0.01). Särkijärvi differed significantly from Ropi- and Vastusjärvi with higher average ω3 content, and Muddusjärvi differed significantly from Vastusjärvi with higher average ω3:ω6 ratio (Games–Howell post hoc test). Both ω3 contents (Welch's ANOVA, $F_{3,\,21.3}$ = 10.22, p < 0.01) and ω3:ω6 ratios (Welch's ANOVA, $F_{3,\,24.3}$ = 21.55, p < 0.01)

differed significantly between pelagic cladocerans and copepods (the mean ω3:ω6 ratios of 2.8 ± 0.5 and 4.9 ± 0.7, respectively) according to Games–Howell post hoc test (Fig. 3).

NMDS ordination did not reveal as clear distinction in the zooplankton AA composition as in their FA composition (Fig. S2), although PERMANOVA revealed significant differences among the taxon groups and lakes (Table S3). PERMDISP did not reveal significant dispersion effect for the factors, indicating differences in their locations. Pelagic copepods differed significantly from the other zooplankton groups (PERMANOVA, pairwise comparisons; Table S4). Higher methionine, lysine, tyrosine and alanine content distinguished copepods from cladocerans (Fig. S2, Table 3). Similarly as for the FAs, taxon explained 32.0% of the zooplankton AAs, lake explained 32.0% and these two also had significant interaction, which explained 28.0% of the estimate component variation (ECV).

Total AA content of the zooplankton ranged from 219.9 to 435.3 μg mg^{-1} DW while the mean (± SD) was 336.1 ± 35.7 μg mg^{-1} DW, which is 1.3 times higher than in the macroinvertebrates (Fig. 2b). The mean (± SD) EAA content of all the zooplankton groups was 175.1 ± 60.7 μg mg^{-1} DW, which is also 1.3 times higher than in the macroinvertebrates (Fig. 2b), and there were no significant differences among the four zooplankton groups (Welch's ANOVA, $F_{3,\,24.3}$ = 2.10, p < 0.126).

Nutritional quality of benthic macroinvertebrates and zooplankton for juvenile salmonids

Pelagic cladocerans had 1.8 times higher EFA content than the littoral macroinvertebrates, whereas pelagic copepods had clearly the highest EFA content, 3.9 times higher than the macroinvertebrates, and all the groups differed significantly from each other (Welch's ANOVA, $F_{2, 18.8} = 32.0$, $p < 0.01$; in all pairwise tests $p < 0.05$). On average, pooled zooplankton had 2.6 ± 0.7 times higher EFA (Welch's ANOVA, $F_{1, 162.2} = 103.7$, $p < 0.01$) but only 1.3 ± 0.4 times higher EAA (Welch's ANOVA, $F_{1, 163.8} = 36.6$, $p < 0.01$) content than the littoral macroinvertebrates (Fig. 2c,d, Table S1).

The average EFA content in analyzed littoral macroinvertebrate taxa was mostly lower than those estimated as optimum levels for growth of juvenile salmonids (Table 3). However, benthic macroinvertebrates exceeded the reported threshold value of EPA (5.0 μg mg^{-1} DW) for Atlantic salmon (Table 3). It is noteworthy that there was relatively large variation in the average macroinvertebrate values of EFAs. Contrary to macroinvertebrates, EFAs, EPA, DHA, and ALA + SDA reached (all ω-3 FAs) the threshold levels for optimal growth of juvenile salmonids in both bulk pelagic and littoral zooplankton samples and in copepods (Table 3). Pelagic cladocerans, instead, had relatively low DHA content, which did not exceed the threshold levels. Particularly the highly important DHA for juvenile salmonids was on average 35 times higher in the bulk zooplankton than in the littoral macroinvertebrates.

Of the measured EAAs, seven out of eight exceeded the threshold levels for optimal growth of juvenile salmonids in all zooplankton groups and macroinvertebrates (Table 3). Methionine content in both zooplankton and macroinvertebrates was clearly below the optimum levels. However, all the zooplankton groups had significantly higher average EAA content than macroinvertebrates (Welch's ANOVA, $F_{4, 31.0} = 16.90$, $p < 0.01$; Table S1), but zooplankton groups did not differ from each other.

Discussion

Principal findings of the study

Our study demonstrates that zooplankton and benthic macroinvertebrates, two major food resources for most fish in the subarctic region, differ more in their EFA than EAA content. Particularly the content of DHA in zooplankton (especially copepods) clearly separated them from benthic macroinvertebrates as a higher quality food. Although we found significantly higher total EAA content in zooplankton than macroinvertebrates, the EAA composition was more similar than EFA composition between the taxonomic groups. Furthermore, the EAA content in both groups was high enough for individual EAAs to exceed most of the optimal levels for juvenile salmonid growth. Benthic macroinvertebrates in the littoral areas rely on benthic algae and coarse particulate organic matter (CPOM) as energy source, and provide the major energy

source for many fish in subarctic lakes (e.g., Sierszen et al. 2003; Karlsson and Byström 2005; Hayden et al. 2014; Berezina et al. 2018). Secondary production by benthic invertebrates is often much higher and annually more stable than pelagic zooplankton production in subarctic lakes (Kahilainen et al. 2003, 2005), but former may not provide sufficient DHA for somatic and gonadosomatic growth of fish (Thomas et al. 2019). Particularly generalist salmonids, such as Arctic charr and whitefish, are prone to supplement their diet with zooplankton during the peak abundance period (Hayden et al. 2014; Keva et al. 2019). Warming climate and increasing productivity likely increase the pelagic energy sources in subarctic potentially contributing to nutritional quality of food webs.

Benthic macroinvertebrate FA and AA composition in comparison to zooplankton

Our results indicate that taxon is the principal determinant of FA and AA composition of benthic invertebrates, although the percentages of explained variation by taxon were clearly lower in our data set compared to, e.g., findings by Lau et al. (2012), who studied invertebrate FAs in boreal lakes. However, most of the taxa in our data set were identified either to order- or family-level, which likely hindered distinction of potential differences between genera or species. This also likely explains the significant within-taxon variation (dispersion effect) of the macroinvertebrate FA composition, indicating that our samples consisted of a variety of species with different functional feeding groups (Cummins 1973). Of the zooplankton, cladocerans and copepods clearly separated from each other particularly due to their FA composition, although taxon explained only 32% of the variation in the zooplankton FA and AA composition.

Lake-specific variation in macroinvertebrate and zooplankton FAs and AAs was mostly related to within-lake variation, not between-lake variation, which means that in some lakes the samples were more dispersed in the multivariate space. This is partly due to an uneven number of caught taxa from the lakes. However, the only eutrophic (also the warmest) Lake Rattosjärvi distinguished from other lakes in the zooplankton FA and AA compositions (relatively less C22 PUFAs than in the other lakes), but this is likely partly explained by lack of copepod samples from that lake. Since our data set covered only one eutrophic lake, one must be conservative in interpreting the results, and therefore a closer look at the differences between the lakes was not attempted in this study. Larger and more comprehensive data sets are needed to see potential differences among lake productivity types.

Macroinvertebrate and zooplankton AA composition and quantity among the lakes and taxa were relatively invariable compared to FAs, which supports the similar findings from other studies and other invertebrate taxa (Cowey and Corner 1963; Cowgill et al. 1986; Guisande et al. 2000; Kolmakova et al. 2013). This was particularly evident when we compared the average FA and AA composition of the taxa with

cluster analysis: pelagic zooplankton and benthic macro-invertebrates formed different clusters for the FAs, but not for the AAs. This may be the result of the fact that the AA composition in animals is typically under strict homeostatic control (Bröer and Bröer 2017).

Higher proportions of DHA in zooplankton mainly separated them from the macroinvertebrates, which had relatively more LIN and ALA. Also, taxa in the phylum Mollusca were distinguished from the other macroinvertebrates mainly by higher proportions of 16:0, 16:2ω6 and ARA. Ephemeroptera was distinguished from other benthic invertebrate taxa with a relatively high total EFA content and clearly the highest ω3:ω6 ratio. Then, e.g., *Lymnaea*, had high proportions of EFA of their total FAs, but a relatively low total FA content. The feeding behavior may partly explain the observed differences in EFA composition: taxa with higher ω3:ω6 tend to actively feed on algae such as the two previously mentioned grazers, while taxa with lower ω3:ω6, such as Chironomidae and Oligochaeta, consist of more opportunistic deposit-feeding species. The low ω3:ω6 values of the predatory *Sialis* (0.7) are consistent with the findings by Lau et al. (2012) (0.8) from oligotrophic boreal lakes, but overall the average of macroinvertebrate ω3:ω6 ratios appeared slightly higher in our study lakes (1.4 vs. 1.1, although not statistically tested). This might be due to the more northern location of our study lakes with different biological and chemical characteristics, such as different algal communities and lower DOC concentrations. Littoral and pelagic bulk zooplankton and pelagic copepods clearly had higher ω3:ω6 ratios and EFA and EAA content compared to benthic macroinvertebrates. Particularly high amounts of DHA were evident in the bulk zooplankton samples and in the copepods. Pelagic cladocerans, instead, had negligible amounts of DHA compared to copepods (and bulk zooplankton), which is commonly known from different climate regions (e.g., Persson and Vrede 2006; Arts et al. 2009; Hiltunen et al. 2016), but contained still more DHA than macroinvertebrates. Also, higher total EFA and EAA content in pelagic cladocerans compared to littoral benthic-grazing cladoceran *Eurycercus* was evident, which is likely explained by different principal food sources (phytoplankton and periphyton).

Different quality between littoral and pelagic resources—implications for habitat use by fish

Our results indicate that benthic macroinvertebrates are not as good diet for fish to cover their nutritional demand, especially for DHA, as zooplankton. The average content of DHA per dry mass in benthic invertebrates, was much below the optimal levels for juvenile salmonids (rainbow trout and Atlantic salmon) determined in aquaculture experiments. The only benthic macroinvertebrate taxa containing any amount of DHA were *Gammarus*, *Lymnaea* and *Eurycercus*, which are all commonly selected taxa by many benthic feeding fish in subarctic lakes (Eloranta et al. 2015; Thomas et al. 2017). To get same amount of essential nutrients, especially DHA, from macroinvertebrates and zooplankton, fish have to select DHA

containing macroinvertebrate taxa and consume them to a greater extent than zooplankton or elongate DHA from precursor FAs.

Fish have varying capacity to convert ALA to EPA and DHA, and LIN to ARA which varies during the ontogeny of fish and also among species (e.g., Yang and Dick 1994; Tocher 2010; Ishikawa et al. 2019). Aquaculture studies thus far indicate that juvenile freshwater fish can satisfy their EFA requirements by C18 PUFA (LIN and/or ALA) at around 1% of their diet dry weight, but there is evidence that DHA is paramount for freshwater fish larvae and fry (Tocher 2010 and references therein). Our results show that precursor FA (LIN and ALA) as well as EPA content were relatively high in the benthic invertebrates, which indicates that juvenile and adult fish may satisfy their energy and nutritional demands by feeding on these taxa during most of the year as observed in many annual studies of salmonids in subarctic lakes (Amundsen and Knudsen 2009; Eloranta et al. 2010; Hayden et al. 2014). Also, the reported threshold values for optimal growth were different in the two salmonid species, as the EPA value for Atlantic salmon was half of the value reported for rainbow trout. This indicates interspecific variation in the requirements of essential biomolecules. Standard deviations of the mean EFA and EAA values in macroinvertebrates were also relatively high, demonstrating the differences in the content of EFAs and EAAs among macroinvertebrate taxa. Moreover, the given minimum values for juvenile salmonids are based on experimental studies for aquaculture and should be considered as recommendations for optimal growth, which can take place under conditions that are rarely met in any natural settings (NRC 2011 and references therein).

Temporal variation in species abundance and development within a year, which we did not account for in this study, likely affects the average EFA and EAA contents in invertebrates (Hayden et al. 2014). Such changes are likely since lipid storages of seston and zooplankton (especially copepods) will decrease from autumn to later winter (Grosbois et al. 2017; Schneider et al. 2017), and whitefish dorsal muscle in a subarctic lake show lowered DHA content in midwinter (Keva et al. 2019). Subarctic lakes experience pronounced seasonality in weather conditions and dynamics of lake communities in contrasting ice-covered and open-water seasons with potential importance to EFAs and EAAs, which should be studied at food web level in the future (*see* Keva et al. 2019; Thomas et al. 2019). DHA is important for fish, and its low content in the benthic invertebrates is a prominent reason for shift to pelagic resources during their peak abundance in summer (Hayden et al. 2014; Keva et al. 2019).

Copepods, which are often the most abundant zooplankton taxa in subarctic lakes (e.g., Tolonen 1998; Primicerio and Klemetsen 1999; Rautio et al. 2011; Skoglund et al. 2013) had a high DHA content compared to cladocerans, which are more rich in EPA (Arts et al. 2009; Hiltunen et al. 2016). However, according to our results the large-sized littoral cladoceran

Eurycercus appears to be alternative source of DHA. *Eurycercus* is actively selected by benthic whitefish and many other benthivores during the peak abundance in August and September (Kahilainen et al. 2003; Hayden et al. 2014; Thomas et al. 2017). Littoral bulk zooplankton samples contained relatively high amounts of DHA and these samples likely consist of both copepods and cladocerans. Zooplankton communities in subarctic lakes tend to vary temporally so that the relative proportions and absolute numbers of copepods are highest in winter, spring and early summer, whereas the proportion of cladocerans increase toward the autumn (Tolonen 1998; Kahilainen et al. 2005; Hayden et al. 2014). Fish species hatching in spring, such as many salmonids, tend to feed on zooplankton for the first months during the season when zooplankton community is initially dominated by copepods (Skoglund and Barlaup 2006; Pothoven and Nalepa 2008). While our study did not specifically account for littoral copepods, these are potentially very important source of DHA for fish fry development (Caramujo et al. 2008; Müller-Navarra 2008; Tocher 2010).

Methionine is a sulfur AA, which is usually the first growth limiting EAA in many fish diets (e.g., Kim et al. 1992; Furuya et al. 2004; Gibson Gaylord et al. 2007). It is, thus, an important AA in aquaculture and indicates the nutritional quality of food (Conceição et al. 2003; Li et al. 2009; Yang et al. 2010). Cowey et al. (1992) studied rainbow trout and found that methionine concentrations of 0.76% of diet were sufficient for maximal growth, but higher concentrations were needed to avoid abnormalities in eye lens development. The average methionine concentrations of both littoral invertebrates and pelagic zooplankton were well below the minimum requirements, but zooplankton had on average 2.5 times higher methionine content than macroinvertebrates. Although the importance of methionine is well recognized in aquaculture, less is known about its abundance and pathways in lake food webs. Pelagic copepods had clearly the highest methionine content in this study. Similar to our study, Kolmakova et al. (2013) found relatively low (0.4%) mean methionine percentage of the total AAs of benthic invertebrates in a large Siberian river. Considering the importance of methionine for the growth of farmed fish, its abundance, temporal variation and pathways in lake food webs and its potential to limit growth in nature warrants further investigations.

Conclusions

Low average content of DHA in littoral macroinvertebrates and low methionine content in both pelagic zooplankton and littoral macroinvertebrates in all lakes was prominent in our data. The differences in EFAs, especially in highly important DHA, between zooplankton (particularly copepods) and macroinvertebrates were more prominent than the differences in EAAs. This indicates that fish can get a wide range and rather high amounts of EAAs from both macroinvertebrates and zooplankton, but copepods are superior sources of DHA compared to benthic macroinvertebrates. More comprehensive studies consisting FA and AA data from basal resources to top predators are needed to better understand the pathways of these essential molecules and whether these are truly limiting for consumer growth in subarctic lakes. Also, the influence of temporal variation in the availability of resources on consumer growth and reproduction may be especially pronounced in seasonally fluctuating subarctic environments, and studies focusing on several nutritional traits of consumer resources are needed to better understand the interplay of food quantity and quality. Moreover, studies accounting for environmental gradients, such as climate and land-use, are needed to understand how cumulative stressors may determine the quality changes of primary consumers in the subarctic lake food webs, which are exposed to rapid environmental changes.

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Conflict of Interest

None declared.