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**Planktivorous vendace (*Coregonus albula*) utilize algae-derived fatty acids
for biomass increase and lipid deposition**

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Running head: Fatty acid accumulation in vendace

Abstract

Spatial and seasonal variation in the abundance and composition of phytoplankton and other basal resources (bacteria, terrestrial detritus) influence the availability of essential polyunsaturated fatty acids (PUFA) for upper trophic levels in lake food webs. We studied accumulation, composition, and content of fatty acids in planktivorous vendace (*Coregonus albula*) muscle tissue during the summer feeding period (May to late September) in six boreal lakes. Vendace muscle fatty acid content increased from ~17.7 mg g⁻¹ DW to ~28.1 mg g⁻¹ DW from spring to summer, corresponding to a ~ 59 % increase in total fatty acids. PUFA accounted for 45-65 % of all fatty acids, and the most abundant fatty acid was docosahexaenoic acid (DHA, 22:6n-3). The DHA content remained relatively constant throughout the sampling period (~7-8 mg g⁻¹ DW), but the proportion of DHA decreased from ~40 % in spring to ~30 % in summer and autumn. The decrease in DHA proportion was due to accumulation of other fatty acids, primarily C₁₈ PUFA (predominantly synthesized by algae) and monounsaturated fatty acids. A similar fatty acid accumulation pattern was observed in lipid storing copepods, an important prey for planktivorous vendace. The abundance of bacterial and terrestrial biomarker fatty acids in vendace muscle and prey items was low (<5% and <0.1%, respectively). The increase in weight and the deposition of internal energy stores in vendace were achieved predominantly by algae-based food chain. This has ecological implications, as vendace requires sufficient lipid stores for reproduction in autumn and survival over winter.

Key words: algal biomarkers, bacterial biomarkers, dissolved organic carbon, pelagic food web, large lakes

Introduction

Planktivorous fish in boreal lakes experience high seasonal variability in prey availability. The ability to store energy in the form of lipid deposits is crucial for winter survival when zooplankton prey is less abundant (Ågren, Muje, Hänninen, Herranen & Penttilä, 1987; Lahti & Muje, 1991; Linko, Rajasilta & Hiltunen, 1992). Reproductive success in late autumn is also highly dependent on lipid gain during the previous summer (Ågren et al., 1987; Lahti & Muje, 1991; Linko et al., 1992). For reproduction, the abundance of polyunsaturated fatty acids (PUFA) from the n-6 and n-3 families have been shown to be particularly important (Sargent, Bell, McEvoy, Tocher & Estevez, 1999). Animals need PUFA for various physiological processes, e.g. maintaining membrane fluidity and as precursors of local tissue hormones, but most organisms lack the ability to biosynthesize these molecules *de novo* and therefore must obtain them from their diet (Glencross, 2009). In aquatic food webs, algae are the major source of n-3 and n-6 PUFA (Ahlgren, Lundstedt, Brett & Forsberg, 1990; Brett, Kainz, Taipale & Seshan, 2009; Taipale et al., 2013, 2016). Although some fish species are able to synthesize the long chained PUFA, eicosapentaenoic acid (EPA 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA 20:4n-6), from shorter C₁₈ PUFA, this process is generally regarded as inefficient (Tocher, 2003). Dietary EPA and DHA are shown to be important for optimal health and somatic growth of zooplankton and fish (Ahlgren et al., 1990; Mairesse, Thomas, Gardeur & Brun-Bellut, 2007). In aquatic environments, algae (phytoplankton and attached algae) are the main producers of EPA and DHA, but different algal classes have very distinct fatty acid compositions and they differ in their EPA and DHA content (Ahlgren et al., 1990; Taipale et al. 2013; Galloway & Winder, 2015). For example, cryptophytes and dinoflagellates are able to biosynthesize EPA and DHA, whereas green algae and cyanobacteria contain predominantly the shorter chained C₁₈ PUFA (α -linolenic acid, ALA, 18:3n-3). Thus, the phytoplankton community composition

strongly influences the food web processes and availability of PUFA to upper trophic level consumers (Brett & Müller-Navarra, 1997; Müller-Navarra, Brett, Liston & Goldman, 2000; Galloway & Winder, 2015; Strandberg et al., 2015b; Taipale et al., 2016).

The knowledge on fatty acids in different basal resources can be applied for tracing the pathways of autochthonous and allochthonous organic carbon flow in lake ecosystems (Desvillettes, Bourdier, Amblard & Barth, 1997; Galloway et al., 2014). Although consumer-specific differences in lipid digestion and metabolism will modify the fatty acids at different trophic levels, specific fatty acids may be traced through several trophic levels (Gladyshev et al., 2011; Strandberg et al., 2015a). Odd-chained and/or branched fatty acids are typically characterized as actinobacterial, whereas long-chain (≥ 24 carbon) saturated fatty acids are considered to originate from detrital terrestrial particulate organic matter (t-POM) (Desvillettes et al. 1997; Taipale, Kankaala, Hämäläinen & Jones, 2009; Galloway et al., 2014). Similarly, low n-3/n-6 ratio (< 1) in zooplankton has been suggested to indicate an allochthonous diet (Brett et al., 2009; Taipale, Kainz & Brett, 2015). In addition to dietary differences in FA composition, zooplankton fatty acid composition is taxon-specific (Persson & Vrede, 2006, Smyntek, Teece, Schulz & Storch, 2008; Hiltunen, Strandberg, Taipale & Kankaala, 2015). Variation in the importance of autotrophic (algal) vs. heterotrophic (bacterial) production at the base of the food web may also influence the fatty acid composition and lipid accumulation of planktivorous fish.

Vendace (*Coregonus albula* (L.)) is a small (total length 10-25 cm) zooplanktivorous coregonid inhabiting large to medium-sized boreal lakes, and is the most important commercial fish in these lakes (Viljanen, 1986; Marjomäki, Keskinen & Karjalainen, 2016). Vendace is abundant in oligo-mesotrophic lakes with low to moderate humic content and well-oxygenated cooler water layers (Dembiński, 1971; Rask, Viljanen, & Sarvala, 1999; Winfield, Fletcher & James, 2004). It is generally not present in small shallow lakes with

high terrestrial dissolved organic carbon (DOC) concentration due to the absence of a well-oxygenated hypolimnion. Weight gain and biomass increase of planktivorous fish is strongly related to prey availability and abundance. In boreal lakes, the main feeding period for planktivorous vendace is summer, when zooplankton (copepods and cladocerans) are abundant (e.g. Hamrin, 1983; Viljanen, 1983; Helminen, Sarvala & Hirvonen, 1990). During the feeding season, vendace accumulate lipids in their tissues to ensure spawning in autumn and survival over winter (Ågren et al., 1987; Lahti & Muje, 1991; Linko et al., 1992). Almost all vendace spawn for the first time during their second autumn (age 1+) and in intensively harvested vendace populations, up to 80% of all spawners belong to this age group (Huusko & Hyvärinen, 2005; Karjalainen et al., 2016).

We studied the composition and accumulation of fatty acids in pelagic food chains in large (>100 km²) boreal lakes in Eastern Finland with moderate (5 – 10 mg C L⁻¹) DOC concentrations (Hiltunen et al., 2015; Strandberg et al., 2015a,b). Studies on fatty acids in phyto- and zooplankton in these lakes have been previously published (see Hiltunen et al., 2015; Strandberg et al., 2015a,b). Here we focus on seasonal changes in vendace fatty acid composition and content during the feeding period before autumn spawning. We hypothesized that 1) the seasonal changes in phytoplankton community composition, and concurrent changes in zooplankton fatty acid composition, modify fatty acid composition of planktivorous vendace, and 2) resources of allochthonous origin play a minor role in vendace nutrition leading to low abundance of terrestrial and bacterial fatty acid biomarkers in vendace.

Materials and methods

Pelagic food webs were studied in six large (area >100 km²) lake basins in eastern Finland. In 2011 we sampled lakes Kallavesi, Suvasvesi, Orivesi, Paasivesi, and Pyhäselkä. Each lake

was sampled 3 times: late May-early June (spring samples), late July- early August (summer samples), and at the end of September (autumn samples). Additional samples were collected from Lake Karjalan Pyhäjärvi in August 2012. Due to the lack of samples, data from the latter lake is presented separately and excluded from detailed analysis. All the lakes are hydrologically connected within the Vuoksi water course (see Hiltunen et al., 2015, for a map of the sampling area). The studied lake basins are oligotrophic or mesotrophic with total phosphorus concentrations varying between ca. 5 - 12 $\mu\text{g L}^{-1}$ and chlorophyll *a* concentration ca. 2 – 6 $\mu\text{g L}^{-1}$ (Table 1). The concentration of dissolved organic carbon (DOC) ranged from ca. 5 to 10 mg C L^{-1} .

For phyto- and zooplankton sampling, laboratory methods and detailed results see Hiltunen et al. (2015) and Strandberg et al. (2015b). The fish samples here are the same as in Strandberg et al. (2015a). Vendace were caught by gill netting (mesh sizes 13-14, 16-17 and 15-18 mm) or by trawling (mesh sizes 12 or 13 mm, trawl bottom 5 mm). For each sampling day and site, at least three random fish were taken for analyses and the weight and length of the fish were measured (total $n=52$). Although not systematically determined, presumably all caught individuals were mature based on their size (see Table 2, Lahti & Muje, 1991, Karjalainen et al., 2016). Replicates of dorsal muscle tissue were dissected and stored in -70 °C until fatty acid analyses. We also opportunistically analyzed roe samples. Roe samples were collected from lakes Paasivesi ($n=2$), Kallavesi ($n=1$), Orivesi ($n=1$), Pyhäselkä ($n=2$), and Suvasvesi ($n=1$), and stored in -70 °C until further analysis.

Laboratory analyses

Vendace muscle and roe samples were freeze-dried prior to lipid analyses. Muscle tissue of each fish was analyzed in duplicate and the mean value was used. Only one roe sample per fish was analyzed. Total lipids were extracted twice with chloroform:methanol (2:1). Fatty acids were transmethylated by adding methanolic sulfuric acid (1 % v/v) and heating for 16 h

at 50°C. The fatty acid methyl esters were extracted twice with *n*-hexane/diethyl ether (1:1 by vol), evaporated to dryness and suspended into *n*-hexane. The fatty acid methyl esters of vendace samples were analyzed with a gas chromatograph (Agilent 6890N) equipped with a mass spectrometer (Agilent 5973N). The GC method was as follows: inlet temperature 250 °C, splitless injection, initial oven temperature 60 °C maintained for 1.5 min, after which oven temperature ramped 10 °C/min to 100 °C, and then 2 °C/min to 140 °C, followed by 1 °C/min increase to 180 °C and finally 2 °C/min to 210 °C. The final oven temperature was maintained for 6 minutes. Helium was used as the carrier gas with an average velocity of 36 cm/sec. The column was an Agilent DB-23 (30 m x 0.25 mm x 0.15 µm). Peak identification was based on mass spectra and standard FAME mixes (37 FAME mix from Sigma-Aldrich and GLC-68D from Nu-Chek Prep). The GLC-68D reference standard was used for the calibration curve. For fatty acids not present in the reference standard, the calibration curve was calculated based on the structurally most similar fatty acid.

Statistical analyses

Fulton condition index of fish (K) was calculated as follows: $K=W/L^3$, where W = weight (g) and L = length (cm), a scaling factor of 100 was applied to bring the value close to 1. Multidimensional scaling (MDS) was used to visualize fatty acid profiles among vendace, and permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was used to test for differences in vendace fatty acid composition between different seasons (fixed factor) and lake basins (random factor). Vendace sampled from Karjalan Pyhäjärvi only in August 2012 were excluded from the analyses. Prior to the multivariate analyses, the fatty acid data was arcsine square root transformed. Ordination was based on Euclidean distances, where samples close to another are compositionally more similar than samples further apart. Goodness of fit for the ordination is expressed as the stress value. The lower the stress, the better objects are fitted in the multidimensional space. The significance of PERMANOVA

analyses was determined using unrestricted permutation of the raw data (9999 permutations) with type III sums of squares, (Anderson, Gorley & Clarke, 2008). Multivariate analyses (MDS, PERMANOVA) were done with PRIMER 6.0 with the PERMANOVA + add on. Other statistical analyses were done with IBM SPSS Statistics 23 program package. Correlations between the total fatty acid content and the proportions of PUFA, monounsaturated fatty acids (MUFA), BAFA and the n-3/n-6 ratio were evaluated, and bootstrapping (1000 bootstrap samples) was applied to estimate the confidence interval (95 % biased corrected) for the correlation coefficient. Seasonal differences (spring, summer, autumn) in the relative proportions of different fatty acid classes in seston, zooplankton and vendace was investigated with ANOVA using Games-Howell post hoc test. Seasonal differences in total fatty acid content of vendace were tested with Wilcoxon signed rank test and proportional differences of specific fatty acids between vendace muscle and roe were analyzed with Mann-Whitney U-test.

Results

The total amount of fatty acids in the vendace muscle tissue increased significantly from 17.7 mg g⁻¹ DW in spring to 28.1 mg g⁻¹ DW in summer in accordance with the simultaneous increase in the condition index (Wilcoxon signed rank test, $p < 0.05$, Table 2, Figure 1). The fatty acid content of vendace in autumn did not differ from that of summer samples. The most abundant fatty acids in the vendace dorsal muscle were 14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-3, 18:4n-3, 20:4n-6 (ARA), 20:5n-3 (EPA), 22:5n-6, 22:5n-3 and 22:6n-3 (DHA), accounting for >89% of all fatty acids (Supplement 1). Polyunsaturated fatty acids were the most abundant group of fatty acids, accounting for 45-65% of all fatty acids. Of these, DHA and EPA were the most abundant ones accounting for ~ 34% and ~ 9% of all fatty acids, respectively. Saturated fatty acids (SFA) accounted for ~33% of all fatty acids

during all seasons. The general fatty acid composition of vendace did not differ between the lake basins (PERMANOVA, Pseudo- $F_{5,46} = 1.759$, $P = 0.1$).

The seasonal differences in the fatty acid composition, especially between spring and summer/autumn (Fig. 2), of vendace were significant (PERMANOVA, Pseudo- $F_{2,49} = 13.224$, $P < 0.001$). The proportion of C_{22} PUFA decreased in vendace muscle as the total fatty acid content increased ($r = -0.842$, $P < 0.001$, 95 % CI $-0.896 - -0.782$; Fig. 3). This was mainly due to decreasing proportion of DHA, from $\sim 40 \pm 4$ % in spring, to 29 ± 7 % in summer and 32 ± 4 % in autumn, although the DHA content in vendace muscle remained at the same level (spring 6.9 ± 1.5 , summer 7.3 ± 1.6 , autumn 8.0 ± 1.2 mg g^{-1} DW). The seasonal increase in the total fatty acid content in the muscle tissue was mainly due to accumulation of monounsaturated fatty acids (sum of C_{14} , C_{16} and C_{18} MUFA), C_{18} and C_{20} PUFA, where proportions correlated positively with increasing fatty acid content (Fig. 3). The n-3/n-6 PUFA ratio in vendace muscle varied between 3.2 and 10.0 (mean 6.0 ± 1.3), decreasing as total fatty acid content increased ($r = -0.584$, $P < 0.001$).

Proportion of solely bacterial fatty acids (BAFA, branched or odd-chain) in vendace muscle varied from less than 0.5 to 4 % and correlated positively with the total fatty acid content of fish ($r = 0.864$, $P < 0.001$, Fig 3). Low proportions (≤ 0.5 %) of isoprenoid fatty acids, 4,8,12- trimethyltridecanoic (4,8,12-TMTD) and 3,7,11,15-tetramethylhexadecenoic (phytanic) acids were also detected. Trace values of very long chained PUFA (≥ 24 carbons) were found in the summer samples (mean of all summer samples 0.1%). Traces (< 0.1 %) of lignoceric acid (24:0) was detected in the muscle of only 15% of the studied fish, while in most fish 24:0 was not found at all.

The most abundant fatty acids in vendace roe, sampled in autumn, were generally the same as in the muscle of adult fish (Supplement 2). PUFA accounted for about 48 % of all fatty acids in the roe, but the proportion of DHA (~ 16 %) was significantly lower (Mann-

Whitney U test, $P < 0.001$) than what was analyzed from the muscle tissue in autumn. The total fatty acid content in roe was about 10 times higher than total fatty acid content of the muscle tissue in the autumn. ($279 \pm 35 \text{ mg g}^{-1} \text{ DW}$). The mean DHA content of roe was $\sim 44 \pm 8 \text{ mg g}^{-1} \text{ DW}$.

Discussion

The content and composition of fatty acids in vendace muscle tissue changed significantly from spring to autumn. The low fatty acid content in spring can be explained by depletion of internal energy stores during spawning in autumn and by limited food availability and in winter (Ågren et al., 1987). Summer is the main feeding period for vendace, and the 1.6-fold increase in total fatty acid content from spring to summer reflects the accumulation of lipids and building of internal energy stores in preparation for reproduction and overwintering (Ågren et al., 1987; Lahti & Muje, 1991). Fatty acids that most contributed to the increase in total fatty acid content were MUFA (particularly 16:1n-7, 18:1n-9) and C₁₈ PUFA (18:2n-6, 18:3n-3, 18:4n-3), whereas long chain polyunsaturated DHA declined from ca. 40 % in spring to 30 % in summer. Similar trends of declining proportion of C₂₂ PUFA and increasing proportion of C₁₈ PUFA was also seen in calanoid copepods (*Limnocalanus macrurus*, *Eudiaptomus* spp.) in the study lakes (Hiltunen et al., 2015, Table 3).

The seasonal trends in fatty acid composition of vendace and zooplankton corresponded with changes in the phytoplankton community composition (Strandberg et al., 2015b). In seston (fraction 0.22 – 50 µm), the most abundant group of fatty acids were the C₁₈ PUFA, $\sim 30 \%$ of all fatty acids during all the seasons (Strandberg et al., 2015b, Table 3). C₁₈ PUFA is common in a wide variety of algal groups, including cryptophytes and chrysophytes, as well as green algae and cyanobacteria (Taipale et al., 2013), thus explaining the abundance and availability of C₁₈ PUFA in the pelagic food chain throughout the sampling season.

Cryptophytes and diatoms were the most abundant phytoplankton groups in all the lakes and seasons, but the proportion of dinoflagellates decreased and the proportion of cyanobacteria and green algae increased from spring to fall (Strandberg et al., 2015b). Dinoflagellates are abundant source of DHA and the decrease in the abundance of dinoflagellates from spring to summer coincided with the decrease of DHA in seston (Strandberg et al., 2015b) as well as in zooplankton and vendace (Table 3), thus, supporting our first hypothesis.

Zooplankton are an important link for the transfer of energy and nutrients from producers to upper trophic level consumers. In pelagic food webs, EPA and DHA originating from seston accumulate in zooplankton and fish (Kainz, Arts & Mazumder, 2004; Strandberg et al., 2015a). The general fatty acid composition of vendace muscle tissue was characterized by a high proportion of PUFA (45-65 % of all fatty acids), which was similar in magnitude to copepod zooplankton, but greater than in cladocerans and seston (Hiltunen et al., 2015; Strandberg et al. 2015a, Table 3). Taxonomic differences in fatty acid composition between cladocerans and copepods might be an important factor determining the transfer of fatty acids to vendace. Cladocerans are known to efficiently retain C₂₀ PUFA (EPA and ARA), but almost completely lack C₂₂ PUFA, whereas copepods are abundant in DHA (Persson & Vrede, 2006; Smyntek et al., 2008; Hiltunen et al., 2015).

Stomach content analyses have indicated that vendace predominately prey on cladocerans in summer (Hamrin, 1983; Viljanen, 1983), corresponding with the decline of DHA proportion in vendace from spring to summer. The dominance of cladocerans in vendace diet in summer was also observed in our study lakes, Pyhäselkä and Kallavesi, where ~ 90% of prey remains in vendace guts (n=13) consisted of cladocerans (Viljanen & Lehtovaara, unpublished). In autumn, vendace diet has been suggested to shift more to copepods (Northcote & Hammar, 2006). This shift could be expected to increase the proportion and content of DHA in the muscle; however, we did not observe any such pattern. Allocation of

DHA to reproduction may also partly explain why copepod diet was not clearly reflected in the muscle fatty acid composition (Dabrowski, 1982; Pickova, Kiessling, Pettersson & Dutta, 1999; Tocher, 2003). Nevertheless, the high proportion of DHA (on average 34 % of all fatty acids) in vendace muscle suggests that copepods are an important prey item. Alternatively, vendace may have elongated and desaturated shorter chain n-3 PUFA (e.g. 18:3n-3 and/or 20:5n-3) to produce DHA. Coregonid *Coregonus lavaretus maraena* has been shown to biosynthesize DHA from shorter chained analogues (Watanabe et al., 1989), suggesting that vendace may also elongate shorter chained fatty acids to DHA. However, it remains questionable if this metabolic pathway would be efficient enough to result in high proportions of DHA, analyzed in vendace muscle (Kaitaranta, 1980; Muje, Ågren, Lindqvist & Hänninen, 1989). Additionally, the very long chained polyunsaturated fatty acids (C₂₄-C₂₆ PUFA), which are specific biomarkers for the large copepod *Limnocalanus macrurus* (Hiltunen, Strandberg, Keinänen, Taipale & Kankaala, 2014), were found in vendace muscle, demonstrating a clear dietary link between the copepod and vendace.

Reproductive success is highly dependent on lipid reserves in many fish because the formation of gonads is very energy intensive and the produced eggs need to be provisioned with lipids (Marshall, Yaragina, Lambert & Kjesbu, 1999; Pickova et al., 1999, Tocher, 2003). The fatty acid content of vendace roe was high (279±35 mg g⁻¹) demonstrating that reproduction requires substantial allocation of lipids, particularly in females (Lahti & Muje, 1991). About 50% of fatty acids in roe were PUFA, and although the proportion of DHA in roe was lower than in the muscle tissue, due to the high total fatty acid content, the amount of DHA in roe was about 6 times higher than in the muscle tissue.

We could not find any between-lake differences in the fatty acid composition and content of vendace. This may be partly due to low number of analyzed individuals per lake, but presumably also due to the similarity of the sampled lakes, which are interconnected in the

Vuoksi water course (Table 1). Although some differences in phytoplankton community structure in relation to DOC concentration was observed in the study lakes, taxonomic identity was the most important determinant (62%) of zooplankton fatty acid composition and the role of environmental factors was minor (Hiltunen et al., 2015). The proportion of DHA was much lower (~ 13%, Linko et al., 1992) in vendace of mesotrophic Säkylän Pyhäjärvi (SW Finland) compared to vendace in our study lakes suggesting lower availability of DHA from phytoplankton via zooplankton to vendace. Lake trophic state and increased nutrient concentrations with concurrent changes in phytoplankton community composition is connected to decreasing EPA and DHA in a predatory fish, the Eurasian Perch (*Perca fluviatilis*, Taipale et al., 2016). In contrast to our study lakes, Lake Säkylän Pyhäjärvi has suffered from eutrophication and experiences occasionally cyanobacteria blooms (Ventelä et al., 2011), which may influence the availability of DHA to vendace.

Phytoplankton are the ultimate source of PUFA in pelagic food web, but other basal resources may be important in providing energy and specific fatty acids. For instance, branched and odd-chained fatty acids are thought to originate from bacterial production (Taipale et al., 2009), which utilize both phytoplankton-derived and terrestrial-origin DOC as a carbon source (e.g. Simon, Cho & Azam, 1992; Pérez & Sommaruga, 2006). The proportion of BAFA in the seston was ~ 2% (Strandberg et al., 2015b) and in zooplankton ~1-5% (Hiltunen et al., 2015), indicating that bacterial fatty acids are incorporated into the pelagic food web but have a low contribution. This suggests that BAFA are not markedly utilized for biomass increase and lipid deposition in vendace. However, the proportion of BAFA increased with increasing lipid content in vendace, indicating direct dietary input. Since fatty acid content in actinobacteria is relatively low (Taipale et al., 2014), it is not surprising that the proportion of BAFA in vendace was minimal (0.5-4%) in comparison to algae-derived PUFA. In an oligotrophic clear-water alpine lake, Lake Annecy (area 28 km²),

whitefish (*Coregonus lavaretus*) larvae contained similar proportions (2-4 %) of BAFA (Perga, Bec & Anneville, 2009). Various fatty acid biomarkers have been applied to estimate autochthonous and allochthonous sources of fatty acids in aquatic biota. Increased proportions of n-6 PUFA (e.g. 18:2n-6) and lower n-3/n-6 ratio may indicate the abundance of terrestrially derived fatty acids, as n-6 PUFA are generally more abundant in terrestrial than aquatic environments (Hixson, Sharma, Kainz, Wacker & Arts, 2015). A low n-3/n-6 ratio (< 1) in fish and zooplankton may indicate a significant contribution of terrestrial fatty acids (Ahlgren, Sonesten, Boberg & Gustafsson, 1996; Taipale et al., 2015). In the current study, the mean n-3/n-6 ratio in vendace was high; varying from 3 to 10, suggesting that allochthonous resources would not directly contribute to vendace diet. Another suggested indicator for terrestrial fatty acids is lignoceric acid (C24:0; Perga et al., 2009). In the current study only 15% of the studied fish contained trace levels of 24:0 ($< 0.1\%$ of total fatty acids). Thus, our second hypothesis was supported. In Lake Annecy, zooplankton and whitefish larvae contained higher, but still small, proportions of 24:0 (0.5-2.9% of total fatty acids; Perga et al., 2009). However, laboratory feeding experiments indicate that 24:0 is not efficiently assimilated by cladocerans or copepods, suggesting that this fatty acid is a poor indicator of terrestrial contribution in consumer diets (Taipale et al., 2015; Taipale et al., unpublished).

Our results indicate that the commercially important planktivorous fish, vendace, is dependent on the algae-based food chain for deposition of internal energy stores in summer. Compared to algal fatty acids, the proportion of terrestrial and microbial fatty acid biomarkers were low suggesting that the utilization of terrestrial resources in pelagic food webs of large boreal lakes is limited. The importance of algae-derived fatty acids in biomass increase and fatty acid accumulation in vendace is ecologically significant because the survival of coregonids in winter depends on body condition and presence of sufficient lipid

stores (Pangle, Sutton, Kinnunen & Hoff, 2004). Additionally, reproductive success is linked with the accumulation of these lipids, particularly DHA (Sargent et al., 1999).

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Table 1. Location, mean depth and total lake area of the studied lakes as well as mean ($\pm$ SD) water color, Secchi depth, and concentrations of DOC, total nitrogen, total phosphorus, and chlorophyll *a* at the sampling sites in six lake basins in the eastern Finland. The values for color are from 1 m depth and the others pooled for 0-4 m depth sampled in May-June, early August and late September 2011, with the exception of Lake Karjalan Pyhäjärvi, which was sampled in August 2012.

Lake basin	Coordinates	Mean depth (m)	Surface area (km ²)	Colour (mg Pt L ⁻¹)	Secchi depth (m)	DOC (mg L ⁻¹)	totN (μg L ⁻¹)	totP (μg L ⁻¹)	Chl <i>a</i> (μg L ⁻¹)
Karjalan Pyhäjärvi	61°48'N; 29°52'E	8.8	247	15	5.1 $\pm$ 0.3	5.3 $\pm$ 0.5	228 $\pm$ 20	4.9 $\pm$ 0.9	2.2 $\pm$ 0.3
Orivesi	62°10'N; 29°43'E	9	601	28 $\pm$ 10	2.1 $\pm$ 0.3	7.0 $\pm$ 0.3	304 $\pm$ 32	11.8 $\pm$ 1.0	6.3 $\pm$ 2.6
Paasivesi	62° 8'N; 29°26'E	21	124	43 $\pm$ 6	2.5 $\pm$ 0.5	8.4 $\pm$ 0.4	349 $\pm$ 27	5.7 $\pm$ 3.2	2.0 $\pm$ 1.1
Suvasvesi	62°40'N; 28°12'E	10.4	233	32 $\pm$ 3	3.1 $\pm$ 0.5	8.8 $\pm$ 0.4	490 $\pm$ 42	10.0 $\pm$ 7.3	2.8 $\pm$ 1.0
Pyhäselkä	62°28'N; 29°48'E	8.8	361	57 $\pm$ 6	1.9 $\pm$ 0.0	9.5 $\pm$ 0.5	399 $\pm$ 48	9.3 $\pm$ 0.6	1.5 $\pm$ 0.8
Kallavesi	62°49'N; 27°52'E	8.6	478	45 $\pm$ 5	2.3 $\pm$ 0.6	10.2 $\pm$ 0.2	639 $\pm$ 107	12.1 $\pm$ 7.1	3.4 $\pm$ 1.3

Table 2. Length, weight and fatty acid content of vendace sampled from five large boreal lakes in spring (n=15), summer (n=18) and autumn (n=15). Values are mean ($\pm$ SD). Approximate lipid content (Lipid %) in the muscle tissue was calculated assuming that fatty acids comprise 70 % of all lipids in fish muscle (Ahlgren et al. 1996). The fatty acid content of the muscle tissue and the weight of vendace as well as condition index (Fulton's K) were significantly lower in spring than in summer or autumn (marked with +; Wilcoxon signed rank test, $P < 0.05$). The length of vendace did not differ between seasons.

	Length (mm)	Weight (g)	FA content (mg g ⁻¹ DW)	Lipid (%)	Condition index (K)
Spring	139 $\pm$ 25	17.2 $\pm$ 9.6 ⁺	17.7 $\pm$ 4.3 ⁺	2.5	0.58 $\pm$ 0.07 ⁺
Summer	148 $\pm$ 13	23.0 $\pm$ 6.1	28.1 $\pm$ 13.1	4.0	0.69 $\pm$ 0.07
Autumn	146 $\pm$ 19	23.6 $\pm$ 11.0	25.7 $\pm$ 6.1	3.7	0.70 $\pm$ 0.07

Table 3. Average ($\pm$ SD) proportion (%) of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (separately for C₁₈, C₂₀ and C₂₂ PUFA) in seston (0.22 – 50 μ m) PLFA (from Strandberg et al., 2015b), and total fatty acids of major zooplankton taxa (from Hiltunen et al., 2015) and in vendace during spring, summer and autumn in five lakes (names in Table 1) in Eastern Finland (n = 21 – 48 in each). Different letters (a-c) denote significant between-season difference in the relative proportion of the specific FA class within each group/taxa (ANOVA, Games-Howell post hoc test).

FA class	Group/taxa	Spring	Summer	Autumn
SFA	Seston	26.6 $\pm$ 4.0 ^a	36.9 $\pm$ 6.8 ^b	29.7 $\pm$ 9.1 ^{ab}
	Cladocera	36.8 $\pm$ 3.9 ^a	36.5 $\pm$ 4.9 ^a	30.3 $\pm$ 2.0 ^b
	<i>Limnocalanus</i>	30.2 $\pm$ 4.9 ^a	12.1 $\pm$ 3.2 ^b	14.0 $\pm$ 3.3 ^b
	<i>Eudiaptomus</i>	31.3 $\pm$ 4.7 ^a	40.4 $\pm$ 6.1 ^b	28.1 $\pm$ 1.6 ^a
	Cyclopoida	35.6 $\pm$ 5.4 ^a	45.3 $\pm$ 7.1 ^b	29.8 $\pm$ 8.2 ^a
	Vendace	33.6 $\pm$ 3.8	33.0 $\pm$ 2.6	32.6 $\pm$ 2.3
MUFA	Seston	16.4 $\pm$ 6.3	19.0 $\pm$ 2.2	20.8 $\pm$ 3.9
	Cladocera	24.2 $\pm$ 6.3	24.0 $\pm$ 4.2	27.6 $\pm$ 4.8
	<i>Limnocalanus</i>	12.9 $\pm$ 3.7	15.0 $\pm$ 3.0	15.9 $\pm$ 2.0
	<i>Eudiaptomus</i>	13.9 $\pm$ 3.0 ^a	7.6 $\pm$ 0.6 ^b	9.5 $\pm$ 1.4 ^c
	Cyclopoida	13.8 $\pm$ 3.2	10.6 $\pm$ 4.2	14.0 $\pm$ 6.6
	Vendace	7.2 $\pm$ 2.2 ^a	12.2 $\pm$ 4.0 ^b	10.8 $\pm$ 2.8 ^b
C ₁₈ PUFA	Seston	29.5 $\pm$ 7.0	30.5 $\pm$ 5.1	31.4 $\pm$ 5.4
	Cladocera	18.8 $\pm$ 3.6	19.0 $\pm$ 3.6	17.7 $\pm$ 5.1
	<i>Limnocalanus</i>	7.5 $\pm$ 1.6 ^a	14.2 $\pm$ 2.9 ^b	17.1 $\pm$ 2.1 ^c
	<i>Eudiaptomus</i>	16.6 $\pm$ 4.3 ^a	16.2 $\pm$ 4.2 ^a	27.9 $\pm$ 3.8 ^b
	Cyclopoida	16.5 $\pm$ 6.0 ^a	15.7 $\pm$ 3.1 ^a	23.0 $\pm$ 9.5 ^b
	Vendace	2.9 $\pm$ 2.4 ^a	7.2 $\pm$ 3.5 ^b	5.8 $\pm$ 2.0 ^b
C ₂₀ PUFA	Seston	16.1 $\pm$ 2.5 ^a	7.1 $\pm$ 3.9 ^b	10.6 $\pm$ 2.4 ^b
	Cladocera	15.7 $\pm$ 5.6	16.9 $\pm$ 6.1	19.6 $\pm$ 2.1
	<i>Limnocalanus</i>	19.1 $\pm$ 4.6 ^a	25.3 $\pm$ 2.1 ^b	23.6 $\pm$ 1.4 ^b
	<i>Eudiaptomus</i>	13.2 $\pm$ 2.2	13.0 $\pm$ 2.3	13.6 $\pm$ 1.0
	Cyclopoida	13.1 $\pm$ 2.9 ^a	9.5 $\pm$ 2.7 ^b	13.2 $\pm$ 2.7 ^a
	Vendace	13.4 $\pm$ 1.7 ^a	15.1 $\pm$ 1.6 ^b	15.5 $\pm$ 1.7 ^b
C ₂₂ PUFA	Seston	9.5 $\pm$ 3.6 ^a	2.4 $\pm$ 2.7 ^b	2.8 $\pm$ 1.7 ^b
	Cladocera	0.7 $\pm$ 0.4	0.5 $\pm$ 0.6	0.4 $\pm$ 0.7
	<i>Limnocalanus</i>	28.5 $\pm$ 3.3 ^a	23.2 $\pm$ 3.4 ^b	23.2 $\pm$ 2.8 ^b
	<i>Eudiaptomus</i>	19.5 $\pm$ 4.6 ^a	19.5 $\pm$ 2.2 ^a	13.7 $\pm$ 2.1 ^b

	Cyclopoida	16.9 ± 3.6	15.7 ± 2.6	14.8 ± 3.0
	Vendace	42.4 ± 4.1 ^a	31.3 ± 6.8 ^b	34.5 ± 4.2 ^b
BAFA	Seston	1.5 ± 0.9	2.2 ± 1.1	2.3 ± 0.8
	Cladocera	4.2 ± 1.8	4.6 ± 1.1	5.0 ± 0.3
	<i>Limnocalanus</i>	3.4 ± 1.8 ^a	1.2 ± 0.7 ^b	1.2 ± 0.3 ^b
	<i>Eudiaptomus</i>	5.0 ± 1.6 ^a	3.4 ± 0.7 ^b	4.3 ± 0.5 ^a
	Cyclopoida	5.4 ± 2.6	5.4 ± 1.3	4.8 ± 1.8
	Vendace	0.9 ± 0.4	1.5 ± 0.9	1.0 ± 0.3

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FIGURE LEGENDS

Figure 1. Mean content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), C₁₈, C₂₀ and C₂₂ polyunsaturated fatty acids (PUFA) and bacterial fatty acids (BAFA) during different seasons in the muscle of vendace from different lake basins (n=3-5 in each lake and season). Error bars represent SD of the total FA content.

Figure 2. Multidimensional scaling ordination of fatty acid proportions (42 fatty acids) in the dorsal muscle of vendace in different lake basins and seasons. Euclidean distance was used for the scaling (2D stress: 0.05). Seasonal differences of the fatty acid composition of vendace is statistically significant (see text), specifically the spring samples differ from summer and autumn samples (results of the PERMANOVA post hoc test in the figure).

Figure 3. Correlation (with 95 % confidence intervals) of the total fatty acid content of vendace muscle and the proportion of A) MUFA (mainly C₁₆ and C₁₈), B) C₁₈ PUFA, C) C₂₂PUFA, and D) BAFA. Note the different scales on the y-axis.

Figure 1.

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608 Figure 2.

Figure 3.

Supplement 1. Fatty acid profile of vendace muscle (% of all fatty acids). Samples from 5 lake basins were combined for each season: spring (n=15), summer (n=18), fall (n=15), because no significant differences were found in the fatty acid profile between lakes (PERMANOVA, $P=0.178$). See the text for details. Samples from Lake Karjalan Pyhäjärvi are presented separately due to lack of samples from spring and fall (n=4).

	Spring		Summer		Fall		Karjalan Pyhäjärvi	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	1.0	0.5	2.0	1.3	1.7	0.8	3.6	1.0
4.8.12-TMTD	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1
i15:0	0.0	0.0	0.2	0.2	0.1	0.1	0.3	0.1
ai15:0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1
15:0	0.3	0.2	0.3	0.2	0.3	0.1	0.3	0.1
i16:0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.0
16:0	24.8	3.3	23.6	3.3	24.6	2.2	25.6	2.0
16:1n-9	0.1	0.0	0.2	0.1	0.1	0.1	0.4	0.1
16:1n-7	0.7	0.4	2.4	1.4	1.9	0.7	2.4	1.0
16:1n-5	0.2	0.1	0.3	0.1	0.2	0.1	0.4	0.1
i17:0	0.1	0.1	0.2	0.1	0.1	0.0	0.2	0.1
ai17:0	0.1	0.1	0.2	0.2	0.1	0.0	0.3	0.1
16:2n-4	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
17:0	0.4	0.2	0.4	0.1	0.4	0.1	0.4	0.1
Phytanate	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
17:1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
18:0	7.0	0.9	6.2	1.2	5.7	0.4	4.6	0.5
18:1n-9	4.7	1.6	6.9	2.5	6.4	1.9	6.7	1.4
18:1n-7	1.6	0.4	2.3	0.6	2.1	0.4	2.6	0.4
18:2n-6	1.1	0.7	2.0	1.0	1.7	0.6	2.2	0.7
18:3n-6	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1
18:3n-3	1.3	0.8	2.8	1.1	2.5	0.9	3.0	1.1
18:4n-3	0.5	0.9	2.0	1.5	1.5	0.7	4.0	1.7
20:0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
20:1n-9	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.0
20:2n-6	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.0
20:4n-6	5.0	1.0	4.5	0.7	4.9	1.1	4.5	0.3
20:3n-3	0.0	0.0	0.1	0.1	0.0	0.1	0.2	0.0
20:4n-3	0.3	0.3	0.7	0.5	0.6	0.3	1.0	0.1
20:5n-3	8.1	1.3	9.9	1.3	10.0	1.3	8.7	0.7
22:4n-6	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
22:5n-6	1.8	0.65	1.8	0.6	1.5	0.5	1.5	0.3
22:5n-3	0.9	0.4	1.3	0.4	1.0	0.3	1.3	0.1
22:6n-3	39.7	4.3	28.9	7.1	32.0	4.1	25.2	6.3
24:1n-9	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.0

Supplement 2. Fatty acid composition of vendace roe (n=7). Only the quantitatively most important fatty acids are presented (mean proportion > 1%). These 14 fatty acids account for 92.0-95.6% of all fatty acids in the roe. Values are mean ($\pm$ SD).

Fatty acid	w%	Fatty acid	w%	Fatty acid	w%
14:0	2.81 (0.49)	18:2n-6	3.81 (1.10)	22:5n-3	1.94 (0.13)
16:0	17.85 (1.63)	18:3n-3	5.53 (1.05)	22:6n-3	16.31 (3.17)
18:0	2.01 (0.27)	18:4n-3	4.34 (1.01)	n3/n6	4.04 (0.69)
16:1n-7	5.27 (1.12)	20:4n-6	4.85 (0.70)	sumSFA	22.66 (1.49)
18:1n-9	13.22 (3.18)	20:4n-3	1.97 (0.45)	sumMUFA	22.36 (4.35)
18:1n-7	3.87 (0.47)	20:5n-3	9.89 (0.71)	sumPUFA	48.65 (4.07)