Population structure, life cycle and trophic niche of the glacial relict amphipod, *Gammaracanthus lacustris*, in a large boreal lake

Jouni K. Salonen [1]*, Minna Hiltunen [1, 2], Kaisa Figueiredo [2], Pinja Paavilainen [2], Tuula Sinisalo [1], Ursula Strandberg [2], Paula Kankaala [2], Jouni Taskinen [1]

[1] Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland

[2] Department of Environmental and Biological Sciences, University of Eastern Finland, P.O. Box 111, FI-80101 Joensuu, Finland

* Correspondence to: Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland. Tel.: +358 40 805 3874. E-mail: jouni.k.salonen@jyu.fi

Running title

Ecology of *Gammaracanthus lacustris*

Keywords

carnivorous crustacean, fatty acids, food web, stable isotope analysis, vertical distribution

Abstract

1. Ecology of the glacial relict macrocrustacean *Gammaracanthus lacustris*, a rare inhabitant of deep Fennoscandian lakes, is poorly known. We studied the life cycle and trophic position of this cold-stenothermic amphipod in Lake Paasivesi, eastern Finland. The study is based on intensive sampling and analyses of fatty acid composition as well as stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios.
2. Both day and night, the *G. lacustris* population occurred at depths below 25 m at temperatures less than 8 ºC, and the density increased towards the bottom of the lake, where it was 0.4–0.6 individuals/m^3.
3. *G. lacustris* was observed to reach a length of more than 40 mm and live up to four years. The oldest and the largest individuals and females seemed to favour the deepest zones.
4. In October, almost 100 % of females with length at least 25 mm (i.e. females presumably at least one-year-old) had an embryo sack with 20–200 eggs or embryos – the larger the female, the more young it had.
5. The developing eggs of *G. lacustris* had a very high fatty acid content, indicating that the females invest heavily on provisioning their young. Furthermore, the fatty acid composition differed among life stages, and in particular the eggs had a higher proportion of eicosapentaenoic acid (EPA) than juveniles or adults.
6. The stable $\delta^{13}C$ and $\delta^{15}N$ isotope ratios and fatty acid biomarkers of the food web, together with visual observations, indicate that *G. lacustris* is a carnivore that feeds mainly on zooplankton and other relict macrocrustaceans.
7. Due to its high content of essential polyunsaturated fatty acids, *G. lacustris* is a valuable prey for fish and other predators in the food web. However, as a glacial relict with strict habitat requirements the species is vulnerable to global warming and local environmental changes. These facts should be taken into account in the management of lakes and their catchments.

1. Introduction

Gammaracanthus lacustris (Gammaracanthidae) is a relatively large (25–45 mm) glacial relict amphipod, whose current geographical distribution in Northern European lakes originates from the time of the last Ice Age (e.g. Segerstråle, 1956, 1966, 1976). Around 10 000 years ago, the long-running melting process of the glacial ice induced a large drop in the water level of the Yoldia Sea, which once covered a large area of the present Northern Europe and where ancestors of *G. lacustris* occurred. During this process, the present Baltic Sea was formed, and the deepest basins of the ancient Yoldia Sea were re-formed as 'new' lakes. Then, the species which populated the deep basins of Yoldia were trapped in the lakes and either died off or – like *G. lacustris* – adapted to their new, lacustrine habitat. Strong evidence for this current theory is that *G. lacustris* has been found to naturally occur only in lakes which are located below the ancient coastline of the Yoldia Sea and have a maximum depth more than 35 m (e.g. Haapala, 2006; Särkkä, 1976; Spikkeland, Kinsten, Kjellberg, Nilssen, & Väinölä, 2016). In Finland, for example, *G. lacustris* occurs in about twenty lakes (Haapala, 2006; Särkkä, Meriläinen, & Hynynen, 1990; Väinölä & Rockas, 1990).

Cold-stenothermic species, such as *G. lacustris*, can be affected by climate warming in many ways. However, the ecology of *G. lacustris* and their role in lake food webs and ecosystems are poorly known. There is no detailed research on the vertical occurrence of this species, although it is generally suggested to prefer the deepest zones of lakes with temperatures less than 8°C (Bagge, 1992; Bagge, Liimatainen, & Liljaniemi, 1996; Hill, Fürst, & Hammar, 1990; Särkkä et al., 1990). Neither is there knowledge on potential nocturnal migration, a common phenomenon in many aquatic crustaceans (Donner, Lindström, & Lindström, 1987; Lindström & Fortelius, 1990; Næsje, Saksgård, Jensen, & Sandlund, 2003). Not much is known about the reproduction of *G. lacustris*, but males are supposed to mate with females in spring or in summer in the deepest basins (see Hill et al., 1990). The females carry their young in an embryo sack until late autumn or winter, after which the fully developed young *G. lacustris* are released (Bagge et al., 1996; Hill et al., 1990). In Sweden, the life cycle of *G. lacustris* has been suggested to last two years and include only one reproduction (Hill et al., 1990). Knowledge on the vertical distribution, diel migrations and phenology of reproduction of the species is necessary to be able to evaluate the effects of climate change on *G. lacustris*.

Generally, *G. lacustris* is considered as an indicator species, whose occurrence signals good water quality and close to natural environmental conditions (Haapala, 2006; Särkkä, 1976; Väinölä & Rockas 1990). According to stomach content analyses, it feeds on copepods, chironomids and other crustaceans like *Pallaseopsis quadrispinosa* (Hill et al., 1990), and is a prey species for seal and fish like burbot (*Lota lota*), trout (*Salmo trutta*), smelt (*Osmerus eperlanus*) and fourhorn sculpin (*Myoxocephalus quadricornis*) (Auttila et al., 2015; Hammar, Bergstrand, & Enderlein, 1996; Hill et al., 1990). However, to better infer prey-predator connections in *G. lacustris* habitats, more research and alternative methods are needed. Stable isotope ratios of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and recently also hydrogen ($\delta^{2}H$) have been used to determine food web interactions in aquatic ecosystems (Doucett, Marks, Blinn, Caron, & Hungate, 2007; Johannsson et al., 2001; Post, 2002; Sinisalo, Jones, Helle, & Valtonen, 2008), but to our knowledge not for communities including *G. lacustris*.

Lipids have a dual role in food web studies: they are used as biomarkers indicative of dietary origin, but at the same time, they are essential nutritional constituents for organisms. Fatty acids are important components of cell membranes in all organisms, and also have functions in energy storage and signal transduction. All animals can synthesize saturated and monounsaturated fatty acids (SAFA and MUFA) *de novo*, but the ability to synthesize polyunsaturated fatty acids (PUFA) is mostly restricted to primary producers (Glencross, 2009). Furthermore, certain PUFA such as docosahexaenoic acid (DHA, 22:6ω3), eicosapentaenoic acid (EPA, 20:5ω3) and arachidonic acid (ARA, 20:4ω6), are essential for animal nutrition. Thus, either they or their precursors α-linolenic acid (ALA, 18:3ω3) and linoleic acid (LIN, 18:2ω6) need to be obtained from the diet (Glencross, 2009; Goedkoop, Demandt, & Ahlgren, 2007). Consequently the abundance of essential fatty acids may determine the quality of food items to consumers. For example, the benthic gammarid *Gammarus roeselii* benefits from addition of DHA from a cyanobacterial diet (Gergs, Steinberger, Basen, & Martin-Creuzburg, 2014), and growth rate of *Chironomus riparius* larvae is faster when their diet contains both ω3 and ω6 PUFA (Goedkoop et al., 2007).

Fatty acids are rather conservatively assimilated, and can thus be used as biomarkers indicative of dietary origin (Dalsgaard, St. John, Kattner, Müller-Navarra, & Hagen, 2003; Galloway et al., 2014; Tverin et al., 2019). PUFA generally originate from phytoplankton, while detritus and bacteria are rich in SAFA, MUFA and branched fatty acids (Dalsgaard et al., 2003). Furthermore, fatty acid profiles are distinct between phytoplankton classes

(Taipale et al., 2013), which allows their separation in consumer diets (Galloway et al., 2014). For example, diatoms are rich in 16:1ω7, EPA and certain 16PUFA (Taipale et al., 2013), and these fatty acids have been used to track the contribution of diatoms to food webs (Fraser, Sargent, Gamble, & Seaton, 1989; St. John & Lund, 1996). The ratio 18:1ω9/18:1ω7 has been used as an index of carnivory, with higher values (>> 2) indicating a more carnivorous diet (Auel, Harjes, Da Rocha, Stübing, & Hagen, 2002; Falk-Petersen, Hagen, Kattner, Clarke, & Sargent, 2000; Maazouzi, Masson, Izquierdo, & Pihan, 2007; Stevens, Deibel, & Parrish, 2004). Generally, the fatty acid composition of freshwater amphipods has rarely been studied (but see Maazouzi et al., 2007; Makhutova, Kalachova, & Gladyshev, 2003).

We investigated life cycle, reproduction, vertical density, potential vertical migration, length and sex distribution as well as diet and trophic position of *G. lacustris* in several sampling campaigns conducted in Lake Paasivesi (eastern Finland) during the years 2006–2011. Our detailed data acquired with novel methods like analysing fatty acids and stable isotope ratios of carbon and nitrogen of the food web compartments were combined with previously published information on *G. lacustris* in order to better understand its role in lacustrine food webs. Thus, the general goal of this study was to build a comprehensive picture of the ecology of this glacial relict amphipod to assess the prospects of this species in warming climate.

2. Material and methods

The study area, Lake Paasivesi in eastern Finland (Figure 1), has a maximum depth of 74 m, average depth of 21 m, and surface area of 12596 km^2 (Anonymous, 2018; Bagge et al., 1996). It belongs to Lake Saimaa water system, which was part of the ancient Yoldia Sea. The sub-basin is believed to have been created by a fallen meteorite (Pesonen, Kuivasaari, Lehtinen, & Elo, 1999). Lake Paasivesi is an oligotrophic (chl-a 2–3 µg L^{-1}) lake with dissolved organic carbon (DOC) concentration of ca. 8.4 mg L^{-1}. More information on nutrients, chlorophyll *a*, phytoplankton community composition and water colour of the study lake can be found in Hiltunen, Strandberg, Taipale, & Kankaala (2015).

Collection and analysis of horizontal/vertical density samples

To study the vertical distribution of *G. lacustris*, samples were horizontally collected on the 4 October 2006 and 3 October 2007 from the depths of 1 m and 5–60 m (5 m intervals), and on the 8 October 2008 from 30–65 m (5 m intervals), using Hydro-Bios GmbH Multi Plankton Sampler MultiNet Type Midi device. The device, which was computer-navigated from the research vessel R/V Muikku (see Jurvelius et al., 2008), contains a steel frame (50 x 50 cm) equipped with 5 separate sample nets (length 250 cm), each of them having a detachable sample pot (mesh 100–500 μm) at the end of the net. At a towing speed of 1 m/s, the device was first lowered down to the deepest water layer, in which the first sample was taken by opening the first net for 3 min. Then, the net with the first sample was closed, the apparatus was moved up to the depth of the next sample and the second net was opened for the next sample. Finally, after five samples, the device was lifted up, the nets were washed from the outside, and the sample pots were replaced with new ones. The organisms in the samples were then moved to separate plastic pots and stored in 80 % ethyl alcohol. Three replicate samples were taken from each depth, and each year the sampling was conducted between 10:00–16:00 at the same location, above the deepest part of the lake (Figure 1). The volume of water passing through each sample was automatically measured by the device, and the vertical profiles of oxygen and chlorophyll concentration, as well as temperature and light were also measured at the time of sampling with a CTD sounder (SBE 19 Seacat Profiler; Sea-Bird Electronics Inc., Bellevue, WA, USA and Licor LI-193SA Spherical Quantum Sensor, Li-Cor Biosciences, Lincoln, NE, USA).

In 2007, additional samples (only from depth zones 45–60 m) were collected as described above from two other locations in the basin (Figure 1) for spatial density difference analysis. Furthermore, samples were collected at night to investigate the potential nocturnal vertical migration of *G. lacustris*. This was conducted at the same time in the same main location and depths as the 2006 and 2007 daylight sampling, but in the darkness between 19:00-23:00.

As the Multi Plankton Sampler device could not be used to collect samples near the bottom of the basin (i.e. below 65 m) due to the risk of breaking the device, samples were also vertically collected with a Hydro-Bios GmbH WP2 device in 8 October 2008. This device, which consists of a 95 cm long net (200 μm in mesh and 0.57 m in diameter) was first let down to the bottom of the lake. Then, the device was left to rest on bottom for about 15 min to steady the potential disturbance the fallen device caused, after which the net was lifted up slowly. The net was closed in the depth of 30 m. Then, the net was lifted up and the sample was

handled as the horizontal samples. A total of 11 vertical samples were taken from the same location as the annual horizontal samples (Figure 1). These samples were used to estimate the density of *G. lacustris* near bottom, and by combining this result with the results of horizontal samples it was then possible create a comprehensive estimate on the number of *G. lacustris* in Lake Paasivesi.

In the laboratory, the length (distance between tail and eyes with an accuracy of 0.5 mm) of each *G. lacustris* was first measured using either laminated graph paper or ocular lens of microscope. Then, the sex of each individual was determined (see Gledhill, Sutcliffe, & Williams, 1993). Furthermore, the egg sack of each gravid female was opened and the number of eggs/embryos in the sack was counted. However, neither their sex nor the number of embryos of *G. lacustris* sampled in 2006 could be defined due to the weak condition of the samples.

Additionally, 35 *G. lacustris* individuals were collected from Lakes Paasivesi (14 ind.), Kallavesi (1 ind.), Pyhäselkä (3 ind.) and Suvasvesi (17 ind.) using the Multi Plankton Sampler in 2011. Each of these lakes is a part of the lake system of Lake Saimaa, which consists of several large inter-connected basins (including the main study area, Lake Paasivesi). These individuals were weighed, and then measured as described above but also freeze-dried and weighed again to determine the length-weight regressions for *G. lacustris*. These equations were then used to transform the density estimates of *G. lacustris* (where all individuals where measured for length) to dry and wet weight biomass.

Collection and analysis of fatty acid samples

G. lacustris samples for the analysis of fatty acid content and composition were collected on 2 August and 26 September 2011 from the same location (Figure 1) as the 2006–2008 samples with several horizontal ca. 5 min tows at depths of 30 to 60 m, using the same Multi Plankton Sampler (mesh size 100 µm) device. Samples were frozen and stored at -70 °C until analysis. Prior to fatty acid analysis, the samples were briefly thawed, the length of the animals was measured, and they were sorted to juveniles (length < 23 mm), gravid (egg-bearing) females, other adults (males and females without eggs), and the eggs. Then the samples were freeze-dried, ground, and a subsamples of 1–3 mg (5–7 per group) were analysed for fatty acids. Fatty acid methyl esters (FAMEs) were prepared with the method

described in Hiltunen, Honkanen, Taipale, Strandberg, & Kankaala (2017). Briefly, lipids were extracted with 2:1 chloroform-methanol and the fatty acids were transesterified with an acid-catalyzed reaction. FAMES were analysed with a gas chromatograph equipped with a mass-selective detector (GC-MS, Agilent 6890N and 5973N). The column was Agilent DB-23 (30 m x 0.25 mm x 0.25 μm) and helium was used as a carrier gas with an average velocity of 36 cm s^{-1}. The oven temperature program was as follows: first 50 °C for 1 min, then raised 15 °C min^{-1} to 150 °C, then 1.5 °C min^{-1} to 190 °C, and finally 2 °C min^{-1} to 210°C, where it was held for 12 min. Peak identification was based on retention times of known compounds in the standards GLC 68-D (Nu-chek Prep.), Menhaden oil (Larodan), Supelco 37 mix (Sigma Aldrich), 24:5ω3 (Larodan), and mass spectra. Quantification was based on a concentration series of the standard mixture GLC 68-D (Nu chek Prep.), and the recovery of an internal standard (21:0). In our analysis, we have also included published values of fatty acids in seston, zooplankton, and fish (Hiltunen et al., 2015; Strandberg et al., 2015) collected at the same time from Lake Paasivesi as the *G. lacustris* samples.

Collection and analysis of stable isotope samples

Samples of *G. lacustris*, zooplankton, and fish for analysis of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were collected from Lake Paasivesi on 3 September 2008. *G. lacustris* was sampled with horizontal tows from 45 m depth as described above. The life stages or sexes were not separated for SIA. Zooplankton was sampled with vertical net tows (mesh size 100 μm). Fish were caught with series of nets with various mesh sizes (8, 10, 12, 14, 16, 18, 22, 35, 45, 55 mm). The samples were sorted, dried, and ground, and subsamples of approximately 0.6 mg were weighed in tin cups for analysis of $\delta^{13}C$, $\delta^{15}N$, %C, and %N which was conducted on a Carlo-Erba Flash 1112 series Element Analyzer connected to a Thermo Finnigan Delta Plus Advantage IRMS at the University of Jyväskylä, Finland. Dried and homogenized fish muscle was used as an internal laboratory working standard. The results are expressed as δ‰ relative to the international standards, atmospheric nitrogen for N and Pee Dee belemnite for C. We have also included published stable isotope values of *G. lacustris* from Lake Paasivesi sampled in September 2010 (Auttila et al., 2015), in addition to seston, zooplankton and fish (Strandberg et al., 2015) in our analysis (see Supporting Information Table S1). For examining diet sources and food web position of *G. lacustris*, the $\delta^{13}C$ values of crustaceans were lipid-corrected ($\delta^{13}C_{lc}$) according to their C/N ratio by the equation presented by Syväranta & Rautio (2010):

$$\delta^{13}C_{lc} = \delta^{13}C_{bulk} + 7.95 \times ((C{:}N_{bulk} - 3.8)/C{:}N_{bulk})$$

The equation base on bulk and lipid extracted $\delta^{13}C$ values cladocerans and copepods, collected from boreal and subarctic lakes, C/N ratio varying from 3.8 to 19.3. We assumed that the equation is also valid for *G. lacustris* and opossum shrimp *Mysis relicta* within the same C/N range in Lake Paasivesi. For fish, the $\delta^{13}C$ values were lipid-corrected with to equation by Kiljunen et al. (2006), which is based on extensive sampling of 14 European fish species. The uncorrected stable isotope values and C/N ratios are available in Supporting Information Table S1.

Statistics

The statistical analyses were carried out with IBM SPSS Statistics version 24 and PRIMER 6 with PERMANOVA+ add-on. Non-parametric Spearman rank correlation analysis was used to investigate the potential connection between sample depth and density, length and biomass of *G. lacustris*, and between the length of egg-carrying female and the number of embryos. The potential differences in the proportion of males/females in different depth zones were analysed using Chi-Square Test, and non-parametric Mann-Whitney U Test (as the criteria for parametric tests were not met) was used to investigate the potential differences in median length of female and male *G. lacustris*. Mann-Whitney U Test was also used to analyse the potential differences in the median density of *G. lacustris* between different years in certain depth, while the potential differences in mean density in the same depths of different areas were analysed using one-way analysis of variance (1-ANOVA). Wilcoxon signed-rank test was used to investigate the potential differences in relative density distribution of *G. lacustris* between day and night samples.

The best fit model to estimate *G. lacustris* dry weight (DW) from length was a power function:

$$Dry\ weight(g) = 4.935 \times 10^{-6} \times length(mm)^{2.668}$$

($R^2 = 0.884$, n = 35)

while for fresh weight it was:

$$Fresh\ weight(g) = 2.0 \times 10^{-5} \times length(mm)^{2.808}$$

(R^2 = 0.932, n = 35)

The weights together with the density data of length-measured individuals were used for estimating the biomass of *G. lacustris* in the lake. Differences in fatty acid content between life stages of *G. lacustris* were analysed with one-way analysis of variance (1-ANOVA) and in the fatty acid percent composition (including all the 41 fatty acids analysed) with permutational multivariate analysis of variance PERMANOVA (Anderson, Gorley, & Clarke, 2008). Differences in the proportion of individual fatty acids (with abundance > 1 %) among life stages was tested with the non-parametric Kruskal-Wallis H-test with post-hoc pair-wise comparisons. PERMANOVA was run with unrestricted permutation of raw data and type III sum of squares. Non-metric multidimensional scaling (NMS) was used to illustrate the differences in fatty acid composition between *G. lacustris* life stages and other components of the food webs. The multivariate analysis methods used Euclidean distance as the distance measure. We also used t-test to compare our carbon and nitrogen isotope values with the results of others.

3. Results

Vertical distribution, population structure and biomass

All *G. lacustris* individuals (n = 575) in daylight samples were caught in the water layers below 25 m where temperature ranged from 6–8 °C during the samplings in October. The mean (± S.E.) density of *G. lacustris* in the horizontally collected 30–65 m samples was 0.17 ± 0.02 ind./m^3. For the vertically towed samples, i.e. from the bottom of the lake (which was 67 m) to the depth of 30 m, the mean density *G. lacustris* was slightly higher; 0.19 ± 0.04 ind./m^3 (range 0.11–0.42 ind./m^3, n = 11). Based on these values, the density in the bottom layer that was not accessible for the Multi Plankton Sampler (65–67 m) was assessed to be 0.575 ind./m^3. As a side note, the densities of two other relict crustacean species, *P. quadrispinosa* (n = 38) and *Monoporeia affinis* (n = 144), in the horizontally collected 30–65 m samples were much lower, 0.012 ± 0.002 ind./m^3 and 0.046 ± 0.008 ind./m^3, respectively.

However, and in contrast to *G. lacustris*, some *P. quadrispinosa* and *M. affinis* individuals were also found in 1–25 m water layers.

The mean density ($n_{samples}$ = 121, r_s = 0.867, $p < 0.001$) and length ($n_{individuals}$ = 575, r_s = 0.128, p = 0.002) of *G. lacustris* increased substantially towards the deepest water layers (Table 1, Figure 2). Not surprisingly, the greatest biomasses of *G. lacustris* (dry weight 10–14 mg/m^3) were also found close to the bottom at the depths of 55–65 m (Table 1), and there was a positive correlation between sample depth and the dry weight biomass of *G. lacustris* ($n_{samples}$ = 121, r_s = 0.863, $p < 0.001$). No significant differences in median density of *G. lacustris* between the three different sampling years were found in any depth zone (Mann-Whitney U Test, p > 0.11 always). Nor were there significant differences between mean densities of *G. lacustris* sampled in 2007 from the three separate locations (Figure 1) in any depth zone (1-ANOVA, p > 0.173 in all).

In the night-time samples, there were a total of 317 *G. lacustris* individuals. More than 99 % of them were caught below 25 m depth (Figure 2); the exceptions were single individuals at the depths 1 m, 15 m and 25 m. The mean (± S.E.) density in the depth of 30–60 m was similar to daylight samples, 0.18 ± 0.04 ind./m^3, and again the density greatly increased towards the bottom of the lake (Figure 2). Thus, there were no differences in the relative distribution of *G. lacustris* in the water column between day and night (Wilcoxon W = 27, p = 0.959).

The length of sampled *G. lacustris* varied between 15.5 and 40.5 mm. According to the length distribution (Figure 3), the individuals with length less than 25 mm were juveniles living their first year, while individuals of 25–32 mm in length were probably two-years-old. The other 67 individuals (12 % of all individuals) were longer than 32 mm, and there were two small peaks in the length distribution among these large individuals (Figure 3). Thus, it seems likely that *G. lacustris* lives up to four years in Lake Paasivesi.

The mean (± S.E.) length of females (n = 240), 24.8 ± 0.33 mm, was greater than that of males (n = 166), 23.9 ± 0.37 mm (Mann-Whitney U Test, Z = -2.429, p = 0.015). There was also a difference in the frequency of occurrence of sexes between different depth zones (Chi-Square Test, χ^2_1 = 5.972, p = 0.015). At 50–65 m water depths there were more females (61 %) than males, while at 35–45 m water depths males were the majority (65 %). Forty-nine

percent of the sampled females (29 % of all individuals) were gravid, having an embryo sack, which included between 20–205 (mean ± S.E. 92 ± 3) eggs/embryos per individual. A significant positive correlation was found between the number of embryos and the length of the female ($n = 117$, $r_s = 0.556$, $p < 0.001$); the larger the female, the more embryos in the sack. Furthermore, fewer than 2 % of females smaller than 25 mm were gravid, while more than 96 % of females of the length group 25–32 mm were gravid. Moreover, all 17 females larger than 32 mm had a sack. There were remarkable differences in the developmental status of the embryos in the sacks between individual females both in September and October, as both undeveloped eggs but also highly developed small individuals were found. Using the density and biomass estimates and measured volumes of different depth zones, the total number of *G. lacustris* in Lake Paasivesi can be estimated as 50 000 000 (S.E. ± 7 000 000) individuals with total biomass of 1500 ± 200 kg dry weight.

Fatty acid content and composition of G. lacustris

In total 41 fatty acids (with iso- and anteiso-branched fatty acids, VLC-PUFA, and isoprenoids and dimethylacetals pooled) were found in the *G. lacustris* samples, but most were present in low concentration (Table S2). Only 11 fatty acids were found with a contribution of > 1 % (Table 2). Total fatty acid content differed among life stages of *G. lacustris* ($F_{3,19} = 5.056$, $p = 0.01$); the content was higher in eggs than in gravid females or juveniles (Figure 4, Tukey HSD, $p \leq 0.05$). Also, the fatty acid percent composition differed among life stages of *G. lacustris* (Figure 5, pseudo-$F_{3,19} = 5.9599$, $p < 0.001$) so that the composition of eggs differed from all other life stages ($p < 0.025$), and the composition of juveniles was different to gravid females ($p = 0.02$) but not to other adults ($p = 0.09$). For example, eggs had significantly higher proportions of EPA, and almost significantly higher proportion of ARA ($p = 0.067$) and DHA ($p = 0.05$) in comparison with the gravid females and other adults (Table 2). One juvenile *G. lacustris* in particular had a very high proportion of DHA (32 %), together with a low total fatty acid content (36 µg mg DW^{-1}) compared to other juveniles (60–200 µg mg DW^{-1}). Interestingly, all life stages of *G. lacustris* had a much higher proportion of MUFA, especially 16:1ω7 and 18:1ω9, than their potential prey zooplankton, which results in clear separation of *G. lacustris* from the other taxa in the NMS ordination (Figure 5, Table 2). Furthermore, *G. lacustris* had a lower proportion (< 1 %) of ALA, and stearidonic acid (SDA, 18:4ω3) compared to seston, zooplankton, or fish (4–26 %, Strandberg et al., 2015). We also found very-long-chain PUFA (VLC-PUFA, > C22) in four

G. lacustris individuals, forming up to 5 % of their total fatty acids. An estimate of EPA and DHA in the adult and juvenile *G. lacustris* (the eggs were excluded because their weight was not measured), and thus the EPA and DHA available for higher trophic levels (as standing stock in autumn), ranged between 0.002–0.3 mg/m^3, being highest in the deepest part of the lake (Table 1).

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios of the food web compartments

The $\delta^{13}C$ values in *G. lacustris* originating from Lake Paasivesi were very similar between our study (-27.2 ± 0.3 ‰, mean ± S.E.) and that of Auttila et al. (2015) (-27.0 ± 0.1 ‰) and did not differ significantly (t = -0.752, p = 0.499). The same applies to $\delta^{15}N$ values: 12.0 ± 0.5 ‰ and 12.3 ± 0.1 ‰, in our study and in Auttila et al. (2015), respectively (t = -0.595, p = 0.590). Thus, we pooled the data for subsequent stable isotope biplot food web analysis (Figure 6), where *G. lacustris* evidently groups together with the fish in Lake Paasivesi. In particular, the $\delta^{15}N$ values of *G. lacustris* (> 10 ‰) were very similar to planktivorous (smelt) and piscivorous (perch, pike, pikeperch) fish, while crustacean zooplankton (copepods and cladocerans) generally had much lower $\delta^{15}N$ values (Figure 6). The lipid-corrected $\delta^{13}C$ values of *G. lacustris* were similar to cladocerans and cyclopoids, but 2–2.7 ‰ more enriched compared to those of *M. relicta* and calanoid copepods (see also Fig. S1 for the biplot without lipid correction and Table S1 for SI values and C/N ratios of these species).

Direct observations on feeding of G. lacustris

During the field sampling, many *G. lacustris* individuals were observed to capture opossum shrimp *M. relicta* within minutes when placed together in a pot. *G. lacustris* consumed the soft parts of *M. relicta*, leaving an empty shell.

4. Discussion

Our results clearly indicate that *G. lacustris* prefers the deepest water layers, as fewer than 1 % of individuals were found above 30 m and the density of occurrence increased greatly towards the bottom of Lake Paasivesi. Furthermore, water temperature changed remarkably at 30 m depth, remaining below 8 °C at depths occupied by these amphipods, while

elsewhere it was 9–12 °C. This is consistent with the observations of Hill et al. (1990) that *G. lacustris* inhabits water layers where temperature does not exceed 8°C. Consequently, *G. lacustris* can be classified as a cold-stenothermic species whose distribution is restricted to cold hypolimnetic waters of deep lakes, and their inability to migrate between water bodies – as suggested by Särkkä et al. (1990) and Väinölä, Vainio, & Palo (2001) – apparently derives from these habitat restrictions. At the population level, gravid females and larger adult and likely mature individuals were found in the deepest zones. This preference probably indicates a safer habitat (fewer visually predatory fish) in the deeper than the shallower layers. It is also possible that near the bottom there are more optimal prey items, such as chironomid or *Chaoborus* spp. larvae, for adult *G. lacustris* (Hill et al., 1990).

Another potential reason for the occurrence of females in the deepest water layers in October is the approaching season of releasing their young. Almost all adult (i.e. length more than 25 mm) females had highly developed small individuals in their embryo sack. The release of offspring near the bottom may enable freshly released young to bury themselves in the sediment to avoid predators or cannibalism. On the other hand, smaller, i.e. juvenile *G. lacustris* individuals were collected more often in the upper water layers than were older ones, and this may also serve as a mechanism for avoiding cannibalism. For example, adult *G. lacustris* have been reported to prey on *P. quadrispinosa*, another relict amphipod with a length of 15–20 mm (Hill et al., 1990).

We found *G. lacustris* to live at least three years, maybe even four years in Lake Paasivesi. Life span of another glacial relict amphipod, *M. affinis*, may similarly last up to four years (Leonardsson, Sörlin, & Samberg, 1988). In contrast, Hill et al. (1990) noticed individuals in a Swedish *G. lacustris* population to live for only two years. That population was introduced, and the study lake was smaller and shallower than Lake Paasivesi. Hill et al. (1990) also reported that *G. lacustris* only reproduces once, while our data suggests that the older and larger individuals continue to reproduce after their first reproduction in their second summer, and also produced more young than the two-year-olds. However, we could not follow the same individuals for several years to confirm these assumptions. Nevertheless, results of the present study and that by Hill et al. (1990) suggest that the life history of *G. lacustris* may differ considerably between lakes.

No sign of diel vertical migration in *G. lacustris* was found, although this behaviour is common in other relict crustaceans, like *M. affinis* (Donner et al., 1987; Lindström & Fortelius, 1990) and *M. relicta* (Næsje et al., 2003). Additionally, there were generally no density or length differences between *G. lacustris* in samples collected from the same depth zones, but either in different years or from different locations in the basin. Furthermore, almost all the females larger than 25 mm (i.e. presumably at least two-year-old) collected during the three sampling years were gravid, suggesting that the population reproduces roughly at the same frequency in each year. The *G. lacustris* population of Lake Paasivesi seems to be stable diurnally and among years with respect to density and reproduction frequency, and are evenly distributed spatially throughout the bottom-layers of the lake basin.

Fatty acid content of *G. lacustris* eggs was very high, indicating that the females invest heavily on provisioning their young. The high fatty acid content provides energy for the developing eggs/embryos during a time period of several months while present in the egg sack of their mother (Bagge et al., 1996; Hill et al., 1990). Furthermore, the fatty acid composition of *G. lacustris* differed among life stages, and the developing eggs in particular had a higher proportion of EPA than juveniles or adults. EPA and ARA are considered essential fatty acids in animal nutrition (Glencross, 2009), and thus may be in high demand during embryonic development. EPA and ARA are important components of cell membranes, and are needed in the production of eicosanoids, local hormones that are involved in reproductive processes, including hatching control (Glencross, 2009). Total fatty acid content of the juveniles and adults was very variable, probably reflecting differences in nutritional condition of individuals. One juvenile individual had a very low total fatty acid content, which together with a high percent contribution of DHA indicates starvation (Schlechtriem, Arts, & Johannsson, 2008). We observed large variation in the number of eggs/embryos that adult females were carrying. As females invest large amounts of lipids into their eggs, their nutritional condition likely affects the number of young they are able to produce.

Interestingly, *G. lacustris* had a much higher proportion of monounsaturated fatty acids (MUFA) than their potential prey, zooplankton. All animals are able to synthesize the MUFA 16:1ω7 and 18:1ω9 *de novo* (Glencross, 2009), and thus they are ubiquitous and cannot be used as specific biomarkers indicating dietary origin. However, 18:1ω9 is also elevated in the fatty acids of opossum shrimp *Mysis relicta* (Hiltunen et al., 2015; Hinderer, Jude, Schaeffer, Warner, & Scavia. 2012) and phantom midge larvae *Chaoborus* spp. (Hiltunen et al., 2015).

We found experimentally that *G. lacustris* will readily attack *M. relicta*. Furthermore, *M. relicta* and *Chaoborus* spp. are both present near the bottom where large *G. lacustris* reside as observed from our samples and also reported in previous studies (e.g. Bagge, 1992; Horppila et al., 2003; Næsje et al., 2003). These species may be important prey for *G. lacustris*, and explaining the high 18:1ω9 content. The fatty acid 18:1ω9 is abundant in the lipids of carnivorous crustaceans of the polar region, and may also be the dominant fatty acid in benthic amphipods scavenging on carcasses (Albers, Kattner, & Hagen, 1996; Nyssen, Brey, Dauby, & Graeve, 2005; Scott, Falk-Petersen, Gulliksen, Lønne, & Sargent, 2001; Mohan, Connelly, Harris, Dunton, & McClelland, 2016). The ratio 18:1ω9/18:1ω7 has been used as an index of carnivory, with higher values indicating a more carnivorous diet (Falk-Petersen et al., 2000; Stevens et al., 2004). In our study, *G. lacustris* had a very high 18:1ω9/18:1ω7 ratio (> 7) compared to pelagic zooplankton (≤ 2.5), indicating a carnivorous diet. In comparison, previous studies on freshwater littoral amphipods have found similar or slightly lower levels of 18:1ω9 (Makhutova et al., 2003; Maazouzi et al., 2007). However, *G. lacustris* in our study had significantly higher proportions of 16:1ω7 than either *Gammarus lacustris* (Makhutova et al., 2003) or *Dikerogammarus villosus* (Maazouzi et al., 2007). 16:1ω7 is a characteristic fatty acid of diatoms (Taipale et al., 2013); other diatom fatty acid biomarkers were found in *G. lacustris* (16:2ω4, 16:3ω4, 18:4ω4), suggesting that it either feeds on diatoms or preys on zooplankton that feed on diatoms, the latter being the more likely explanation. Furthermore, *G. lacustris* had low levels of C18PUFA compared to C20-22PUFA, which is consistent with a predatory diet (Strandberg et al., 2015).

The biomass of *G. lacustris* was somewhat lower than that of hypolimnetic zooplankton in other large lakes of the same region (> 8 mg C/m^3; Rahkola-Sorsa, 2008). However, the high density of *G. lacustris* near the lake bottom suggests that they could offer an important food source for benthic fish. Remains of *G. lacustris* has been found in stomachs of fish and seals (Auttila et al., 2015; Hill et al., 1990) and the species may even be the principal prey for smelt, burbot and fourhorn sculpin (Bagge, 1992; Bagge & Hakkari, 1982; Hammar et al., 1996). We provide new information on the nutritional value of this glacial relict amphipod. Our results on fatty acids in *G. lacustris* indicate that they are good quality prey items for fish and other predators. They have a high content of fatty acids, and also contain EPA and DHA, which are essential components of fish nutrition (Glencross, 2009). The percent contribution of EPA and DHA is lower in *G. lacustris* than in zooplankton, but presumably their bigger size makes them an attractive food source. Gravid females are especially rich in lipids and

essential fatty acids. Even when excluding the eggs from the calculations, we estimated that up to ~0.3 mg EPA+DHA /m^3 (as standing stock) is available for higher trophic levels through *G. lacustris*, which is of the same magnitude as through hypolimnetic copepods in the same region (~0.4 mg EPA+DHA/m^3, Hiltunen et al., 2015; Rahkola-Sorsa, 2008). Thus, our results together with previous research indicates that *G. lacustris* has an important role in the pelagic food web of lakes where it occurs.

The high $\delta^{15}N$ values (> 10 ‰), similar to that of sampled fish, suggest a high trophic position of *G. lacustris* in the food web of Paasivesi. In general, the $\delta^{15}N$ value increases 2–5 ‰ in each trophic step, whereas $\delta^{13}C$ values exhibit much lower trophic enrichment, and thus prey and predator values are typically similar (Post, 2002). The lipid-corrected $\delta^{13}C$ values of *G. lacustris* were similar to cladocerans and cyclopoid copepods. However, it is unlikely that cladocerans are an important dietary source for *G. lacustris*, because they are mainly found in the upper parts of the water column and the $\delta^{15}N$ distance between the taxa is > 8 ‰. Copepods, and especially the glacial relict species *Limnocalanus macrurus*, prefer colder and deeper water layers (Rahkola-Sorsa, 2008), similar to *G. lacustris*. The lipid-corrected $\delta^{13}C$ values of calanoid copepods and *M. relicta* were 2–2.7 ‰ lower (but see Fig. S1). However, the $\delta^{13}C$ values of the copepod species, *L. macrurus*, were much lower (2.7 ‰) than in *G. lacustris*, suggesting that *G. lacustris* does not substantially feed on *L. macrurus*. The low $\delta^{13}C$ values of *L. macrurus* could potentially be due to endogenous lipid synthesis and modification by this wax-ester storing glacial-relict copepod, as also suggested for Arctic marine copepods by Mohan et al. (2016). In contrast, the fatty acid results imply that *G. lacustris* indeed feeds on *L. macrurus*, as we also found VLC-PUFA in some *G. lacustris*. Our previous research (Hiltunen, Strandberg, Keinänen, Taipale, & Kankaala, 2014; Hiltunen et al., 2015) shows that these fatty acids are generally only found in *L. macrurus*, and not in other zooplankton taxa or seston. As the highest values for VLC-PUFA were detected in juvenile *G. lacustris*, it seems that *L. macrurus* is mainly consumed by the juveniles, and adults use different food sources. We cannot completely rule out that VLC-PUFA originate from metabolic modifications, as these may be intermediate products in DHA synthesis (Monroig, Tocher, & Navarro, 2013). However, we do not think it is probable, because the variation in VCL-PUFA in juveniles were high and VLC-PUFA were relatively abundant (1.3 ± 0.9 % of all fatty acids), suggesting dietary origin. Unfortunately, the life stages were not separated for SI analysis, and thus we cannot explore this potential ontogenetic diet shift

further. Another explanation for the high $\delta^{15}N$ values of *G. lacustris*, compared to those found in predatory fish (pike, perch and pikeperch), could be due to feeding on fish carcasses on the lake bottom. This could also result in the $\delta^{13}C$ values observed in *G. lacustris* when combined with feeding on *L. macrurus* with low $\delta^{13}C$ values. Another relict amphipod, *P. quadrispinosa*, which is a known prey species for *G. lacustris* (Hill et al., 1990), was also found to co-occur with *G. lacustris* in Lake Paasivesi. These facts together with our results for both stable isotopes and fatty acid biomarkers point to *G. lacustris* being an opportunistic predator that feeds on zooplankton (especially on copepods), other macrocrustaceans, and potentially also on fish carcasses. *G. lacustris* may thus not only be a prey item (Bagge, 1992; Bagge & Hakkari, 1982; Hammar et al., 1996), but also a potential competitor for certain fish species.

The earlier research on *G. lacustris* (e.g. Haapala, 2006; Särkkä, 1972, 1976; Väinölä & Rockas, 1990) determined the occurrence of this species in certain lakes, while our current results provide comprehensive knowledge on the ecology of *G. lacustris*. Probably the first ever published data on the vertical occurrence and biomass of *G. lacustris* indicates that (1) the whole population occurs in the deepest basins, and (2) has no diurnal vertical migration. Our study also found that (3) *G. lacustris* may live up to 4 years, (4) reproduce more than once, and (5) the largest females produce the largest numbers of offspring. Furthermore, our novel data on the fatty acid composition, and stable isotope ratios show that (6) *G. lacustris* is a potentially important source of fatty acids for fish, and (7) the trophic level of the carnivorous *G. lacustris* is relatively high.

In the assessment of threatened species in Finland (2000) *G. lacustris* was classified as 'near threatened' following the IUCN categories. The occurrence of *G. lacustris* and other glacial relict crustaceans increases the diversity of the communities in Fennoscandian large lakes. Their marine origin and preference for cold water makes them more vulnerable than other taxa to the observed increase in water temperatures due to climate change. In addition to higher water temperatures (O'Reilly et al., 2015), the potential consequences of climate change also include changes in lake phenology, stratification patterns, and biotic communities that may impact food web structure (Shimoda et al., 2011). The species with prominent vertical migrations, such as *L. macrurus* (Rahkola et al., 1999) and *M. relicta* (Næsje et al., 2003), will directly encounter higher temperatures in the epilimnetic waters, while *G. lacustris* is relatively safe as long as their deep habitat remains cold throughout the

year. However, these strict habitat requirements likely prevent *G. lacustris* to naturally migrate between water bodies, leaving the populations vulnerable to local environmental changes. Illustrating this, Spikkeland et al. (2016) and Väinölä & Rockas (1990) have reported several lakes where *G. lacustris* is no longer found although it previously occurred there. Thus, the strict habitat requirements of cold-stenothermic glacial relict species should be always taken into account in the management plans of lakes and their catchments susceptible to fast global warming in the boreal area.

Acknowledgements

We thank Aslak Eronen, Felix Luukkanen, Tuomo Sjönberg, Otso Valta, Jouni Voipio, Sean Yeung, Ville Vesakoski, Jussi Vesamäki, and the crew on R/V Muikku – especially the captain Matti Jalkanen – for valuable help during the field sampling and investigation of the samples. We thank Miina Auttila for providing the stable isotope data on *G. lacustris*, and Timo J. Marjomäki for his help with the statistical analyses. This study was financially supported by the Academy of Finland grant (#139786) to Paula Kankaala, Oscar Öflunds Stiftelse grant to Jouni K. Salonen and Minna Hiltunen, Olvi Foundation, Societas Biologica Fennica Vanamo and the Emil Aaltonen Foundation grants to Jouni K. Salonen, and Elma, Eino & Veikko Jumppanen Foundation and Raija & Ossi Tuuliainen Foundation grants to Jouni Taskinen.

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors have no conflict of interest to declare.

References

Albers, C. S., Kattner, G., & Hagen, W. (1996). The compositions of wax esters, triacylglycerols and phospholipids in Arctic and Antarctic copepods: evidence of energetic adaptations. *Marine Chemistry*, 55, 347–358.

Anderson, M. J., Gorley, R. N., & Clarke, K. R. (2008). Permanova+ for Primer: guide to software and statistical methods. Plymouth, Devon: Primer-E Ltd.

Anonymous. (2018). The database of Finland's environmental administration. http://www.ymparisto.fi/fi-FI/Kartat_ja_tilastot/Tietojarjestelmat. Accessed 27.11.2018.

Auel, H., Harjes, M., Da Rocha, R., Stübing, D., & Hagen, W. (2002). Lipid biomarkers indicate different ecological niches and trophic relationships of the Arctic hyperiid amphipods *Themisto abyssorum* and *T. libellula*. *Polar Biology*, 25, 374–383.

Auttila, M., Sinisalo, T., Valtonen, M., Niemi, M., Viljanen, M., Kurkilahti, M., & Kunnasranta, M. (2015). Diet composition and seasonal feeding patterns of a freshwater ringed seal (*Pusa hispida saimensis*). *Marine Mammal Science*, 31, 45–65.

Bagge, P. (1992). The macrocrustaceans (Branchiura and Peracarida) in Lake Saimaa. *Joensuun yliopisto, Karjalan tutkimuslaitoksen julkaisuja*, 103, 117–128.

Bagge, P., & Hakkari, L. (1982). The food and parasites of fish in some deep basins of northern L. Päijänne. *Hydrobiologia*, 86, 61–65.

Bagge, P., Liimatainen, H.-M., & Liljaniemi, P. (1996). Comparison of sampling methods for semipelagic animals in two deep basins of Lake Saimaa. *Hydrobiologia*, 322, 293–300.

Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., & Hagen, W. (2003). Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology*, 46, 225–340.

Donner, K. O., Lindström, A., & Lindström, M. (1987). Seasonal variation in the vertical migration of *Pontoporeia affinis* (Crustacea, Amphipoda). *Annales Zoologici Fennici*, 24, 305–313.

Doucett, R. R., Marks, J. C., Blinn, D. W., Caron, M., & Hungate, B. A. (2007). Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. *Ecology*, 88, 1587–1592.

Falk-Petersen, S., Hagen, W., Kattner, G., Clarke, A., & Sargent, J. (2000). Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. *Canadian Journal of Fisheries and Aquatic Sciences*, 57 (S3), 178–191.

Fraser, A. J., Sargent, J. R., Gamble, J. C., & Seaton, D. D. (1989). Formation and transfer of fatty acids in an enclosed marine food chain comprising phytoplankton, zooplankton and herring (*Clupea harengus* L.) larvae. *Marine Chemistry*, 27, 1–18.

Galloway, A. W. E., Taipale, S. J., Hiltunen, M., Peltomaa, E., Strandberg, U., Brett, M. T., & Kankaala, P. (2014). Diet-specific biomarkers show that high-quality phytoplankton fuels herbivorous zooplankton in large boreal lakes. *Freshwater Biology*, 59, 1902–1915.

Gergs, R., Steinberger, N., Basen, T., & Martin-Creuzburg, D. (2014). Dietary supply with essential lipids affects growth and survival of the amphipod *Gammarus roeselii*. *Limnologica*, 46, 109–115.

Glencross, B. D. (2009). Exploring the nutritional demand for essential fatty acids by aquaculture species. *Reviews in Aquaculture*, 1, 71–124.

Gledhill, T., Sutcliffe, D. W., & Williams, W. D. (1993). British Freshwater Crustacea Malacostraca: A Key with Ecological Notes. Ambleside, Cumbria: Freshwater Biological Association.

Goedkoop, W., Demandt, M., & Ahlgren, G. (2007). Interactions between food quantity and quality (long-chain polyunsaturated fatty acid concentrations) effects on growth and development of *Chironomus riparius*. *Canadian Journal of Fisheries and Aquatic Sciences*, 64, 425–436.

Haapala, A. (2006). Observations on relict crustacean fauna in the water district of Lake Myhinjärvi, North Savo, Finland. *Memoranda Societatis pro Fauna et Flora Fennica*, 82, 24–29.

Hammar, J., Bergstrand, E., & Enderlein, O. (1996). Why do juvenile fourhorn sculpin, *Triglopsis quadricornis*, appear in the pelagic habitat at night? *Environmental Biology of Fishes*, 46, 185–195.

Hiltunen, M., Strandberg, U., Keinänen, M., Taipale, S., & Kankaala, P. (2014). Distinctive lipid composition of the copepod *Limnocalanus macrurus* with a high abundance of polyunsaturated fatty acids. *Lipids*, 49, 919–932.

Hiltunen, M., Strandberg, U., Taipale, S. J., & Kankaala, P. (2015). Taxonomic identity and phytoplankton diet affect fatty acid composition of zooplankton in large lakes with

differing dissolved organic carbon concentration. *Limnology and Oceanography*, 60, 303–317.

Hiltunen, M., Honkanen, M., Taipale, S., Strandberg, U., & Kankaala, P. (2017). Trophic upgrading via the microbial food web may link terrestrial dissolved organic matter to *Daphnia*. *Journal of Plankton Research*, 39, 861–869.

Hinderer, J. L. M., Jude, D. J., Schaeffer, J. S., Warner, D. M., & Scavia, D. (2012). Lipids and fatty acids of *Mysis diluviana* in lakes Michigan and Huron, 2008. *Journal of Great Lakes Research*, 38 (2), 93–97.

Hill, C., Fürst, M., & Hammar J. (1990). Introduction of the amphipods *Pallasea quadrispinosa* and *Gammaracanthus lacustris* into lakes in northern Sweden. *Annales Zoologici Fennici*, 27, 241–244.

Horppila, J., Liljendahl-Nurminen, A., Malinen, T., Salonen, M., Tuomaala, A., Uusitalo, L., & Vinni, M. (2003). *Mysis relicta* in a eutrophic lake: consequences of obligatory habitat shifts. *Limnology and Oceanography*, 48, 1214–1222.

Johannsson, O. E., Leggett, M. F., Rudstam, L. G., Servos, M. R., Mohammadian, M. A., Gal, G., … Hesslein, R. H. (2001). Diet of *Mysis relicta* in Lake Ontario as revealed by stable isotope and gut content analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 58, 1975–1986.

Jurvelius, J., Knudsen, F. R., Balk, H., Marjomäki, T. J., Peltonen, H., Taskinen, J., … Viljanen, M. (2008). Echo-sounding can discriminate between fish and macroinvertebrates in fresh water. *Freshwater Biology*, 53, 912–923.

Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., & Jones, R. I. (2006) A revised model for lipid-normalizing δ13C values from aquatic organisms, with implications for isotope mixing models. *Journal of Applied Ecology*, 43, 1213–1222.

Leonardsson, K., Sörlin, T., & Samberg, H. (1988). Does *Pontoporeia affinis* (Amphipoda) optimize age at reproduction in the Gulf of Bothnia? *Oikos*, 52, 328–336.

Lindström, M., & Fortelius, W. (1990). Some factors affecting the horizontal migration of *Pontoporeia affinis* (Crustacea, Amphipoda) in laboratory conditions. *Annales Zoologici Fennici*, 27, 309–312.

Maazouzi, C., Masson, G., Izquierdo, M. S., & Pihan, J. C. (2007). Fatty acid composition of the amphipod *Dikerogammarus villosus*: feeding strategies and trophic links. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 147, 868–875.

Makhutova, O. N., Kalachova, G. S., & Gladyshev, M. I. (2003). A comparison of the fatty acid composition of *Gammarus lacustris* and its food sources from a freshwater reservoir, Bugach, and the saline Lake Shira in Siberia, Russia. *Aquatic Ecology*, 37, 159–167.

Mohan, S. D., Connelly, T. L., Harris, C. M., Dunton, K. H., & McClelland, J. W. (2016). Seasonal trophic linkages in Arctic marine invertebrates assessed via fatty acids and compound-specific stable isotopes. *Ecosphere*, 7(8), 1–21.

Monroig Ó, Tocher, D. R., & Navarro, J. C. (2013). Biosynthesis of polyunsaturated fatty acids in marine invertebrates: recent advances in molecular mechanisms. *Marine Drugs*, 11, 3998–4018.

Næsje, T. F., Saksgård , R., Jensen, A. J., & Sandlund, O. T. (2003). Life history, habitat utilisation, and biomass of introduced *Mysis relicta*. *Limnologica*, 33, 244–257.

Nyssen, F., Brey, T., Dauby, P., & Graeve, M. (2005). Trophic position of Antarctic amphipods – enhanced analysis by a 2-dimensional biomarker assay. *Marine Ecology Progress Series*, 300, 135–145.

O'Reilly, C. M., Sharma, S., Gray, D. K., Hampton, S. E., Read, J. S., Rowley, R. J., … Zhang, G. (2015). Rapid and highly variable warming of lake surface waters around the globe. *Geophysical Research Letters*, 42, 10773–10781.

Pesonen, L. J., Kuivasaari, T., Lehtinen, M. & Elo, S. (1999). Paasselkä: a new meteorite impact structure in eastern Finland. *Meteoritics & Planetary Science*, 34 (S4), A90–A91.

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83, 703–718.

Rahkola-Sorsa, M. (2008). The structure of zooplankton communities in large boreal lakes, and assessment of zooplankton methodology. PhD dissertation, University of Joensuu, Finland.

Rahkola, M., Avinski, V., Holopainen, A.-L., Jurvelius, J., Karjalainen, J., & Viljanen, M. (1999). Interacting in the dark: a study of the diel vertical migrations of pelagic plankton and fish in Lake Ladoga. *Boreal Environment Research*, 4, 245–255.

Schlechtriem, C., Arts, M. T., & Johannsson, O. E. (2008). Effect of long-term fasting on the use of fatty acids as trophic markers in the opossum shrimp *Mysis relicta* – a laboratory study. *Journal of Great Lakes Research*, 34, 143–152.

Scott, C. L., Falk-Petersen, S., Gulliksen, B., Lønne, O.-J., & Sargent, J. R. (2001). Lipid indicators of the diet of the sympagic amphipod *Gammarus wilkitzkii* in the Marginal Ice Zone and in open waters of Svalbard (Arctic). *Polar Biology*, 24, 572–576.

Segerstråle, S. G. (1956). The distribution of glacial relicts in Finland and adjacent Russian areas. *Societas Scientiarum Fennica, Commentationes Biologicae*, 15(18), 1–35.

Segerstråle, S. G. (1966). Adaptational problems involved in the history of the glacial relicts of Eurasia and North America. *Revue Roumaine de Biologie, Série de Zoologie*, 11, 59–66.

Segerstråle, S. G. (1976). Proglacial lakes and the dispersal of glacial relicts. *Societas Scientiarum Fennica, Commentationes Biologicae*, 83, 1–15.

Shimoda, Y., Azim, M. E., Perhar, G., Ramin, M., Kenney, M. A., Sadraddini, S., ... Arhonditsis, G. B. (2011). Our current understanding of lake ecosystem response to climate change: what have we really learned from the north temperate deep lakes? *Journal of Great Lakes Research*, 37, 173–193.

Sinisalo, T., Jones, R. I., Helle, E., & Valtonen, E. T. (2008). Changes in diets of individual Baltic ringed seals (*Phoca hispida botnica*) during their breeding season inferred from stable isotope analysis of multiple tissues. *Marine Mammal Science*, 24, 159–170.

Spikkeland, I., Kinsten, B., Kjellberg, G., Nilssen, J. P., & Väinölä, R. (2016). The aquatic glacial relict fauna of Norway – an update of distribution and conservation status. *Fauna norvegica*, 36, 51–65.

St. John, M. A., & Lund, T. (1996). Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. *Marine Ecology Progress Series*, 131, 75–85.

Stevens, C. J., Deibel, D., & Parrish, C. C. (2004). Species-specific differences in lipid composition and omnivory indices in Arctic copepods collected in deep water during autumn (North Water Polynya). *Marine Biology*, 144, 905–915.

Strandberg, U., Hiltunen, M., Jelkänen, E., Taipale, S. J., Kainz, M. J., Brett, M. T., & Kankaala, P. (2015). Selective transfer of polyunsaturated fatty acids from phytoplankton to planktivorous fish in large boreal lakes. *Science of the Total Environment*, 536, 858–865.

Syväranta, J. & Rautio, M. (2010). Zooplankton, lipids and stable isotopes: importance of seasonal, latitudinal, and taxonomic differences. *Canadian Journal of Fisheries and Aquatic Sciences*, 67, 1721–29.

Särkkä, J. (1972). The bottom macrofauna of the oligotrophic Lake Konnevesi, Finland. *Annales Zoologici Fennici*, 9, 141–146.

Särkkä, J. (1976). Records of relict crustaceans in lakes drained by the river Kymijoki, Finland. *Annales Zoologici Fennici*, 13, 44–47.

Särkkä, J., Meriläinen J. J., & Hynynen J. (1990). The distribution of relict crustaceans in Finland: new observations and some problems and ideas concerning relicts. *Annales Zoologici Fennici*, 27, 221–225.

Taipale, S., Strandberg, U., Peltomaa, E., Galloway, A. W. E., Ojala, A., & Brett, M. T. (2013). Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. *Aquatic Microbial Ecology*, 71, 165–178.

Tverin, M., Espanza-Salas, R., Strömberg, A., Tang, P., Kokkonen, I., Herrero, A., … Lundström, K. (2019). *PLoS ONE*, 14, e0208694.

Veriö, A. (1990). Vertical movements at some faults in Finland. Geological Survey of Finland – Nuclear Waste Disposal Research. Working Report 4 - 90.

Väinölä, R., & Rockas, H. (1990). New distributional data on ’glacial relict’ crustaceans. *Annales Zoologici Fennici*, 27, 215–220.

Väinölä, R., Vainio, J. K., & Palo, J. U. (2001). Phylogeography of “glacial relict” *Gammaracanthus* (Crustacea, Amphipoda) from boreal lakes and the Caspian and White seas. *Canadian Journal of Fisheries and Aquatic Sciences*, 58, 2247–2257.

Table 1. Mean density, length, biomass, and EPA+DHA (with standard errors of mean) of *Gammaracanthus lacustris* in daylight samples horizontally collected from Lake Paasivesi in October 2006, 2007 and 2008 (n = 575). Carbon weight was estimated by applying the mean proportion of carbon (45 %) of dry weight obtained from elemental analyses of SI (see methods for details). No individuals were caught in the depth zones of 1, 5, 10, 15, 20 and 25 m.

			Total biomass (mg/m^3)			
Depth (m)	Density (ind./m^3)	Length (mm)	Fresh weight	Dry weight	Carbon weight	EPA + DHA †
30	0.01 ± 0.00	20.8 ± 0.75	0.60 ± 0.40	0.10 ± 0.06	0.04 ± 0.03	0.002 ± 0.002
35	0.03 ± 0.01	22.3 ± 1.55	3.90 ± 1.93	0.62 ± 0.31	0.28 ± 0.14	0.015 ± 0.008
40	0.02 ± 0.01	22.4 ± 2.00	2.66 ± 1.09	0.42 ± 0.17	0.19 ± 0.08	0.010 ± 0.004
45	0.06 ± 0.02	22.6 ± 0.76	7.97 ± 2.71	1.27 ± 0.43	0.57 ± 0.19	0.030 ± 0.010
50	0.13 ± 0.03	25.0 ± 0.72	25.9 ± 7.44	4.06 ± 1.15	1.82 ± 0.51	0.096 ± 0.027
55	0.32 ± 0.06	25.5 ± 0.41	64.1 ± 14.2	10.0 ± 2.19	4.49 ± 0.98	0.238 ± 0.052
60	0.37 ± 0.05	25.7 ± 0.36	76.1 ± 10.7	11.9 ± 1.66	5.35 ± 0.74	0.283 ± 0.039
65	0.42 ± 0.10	26.2 ± 0.67	88.0 ± 20.1	14.1 ± 2.88	6.33 ± 1.29	0.335 ± 0.068
Total	0.17 ± 0.02	25.3 ± 0.23	32.5 ± 4.64	5.12 ± 0.72	2.29 ± 0.32	0.126 ± 0.137

† EPA + DHA – the sum of eicosapentaenoic acid (20:5ω3) and docosahexaenoic acid (22:6ω3)

Table 2. The percent contribution of the most abundant fatty acids (> 1 %), SAFA, MUFA, PUFA, and the biomarker ratios in *G. lacustris* collected in August and September 2011. Included also the results from the non-parametric Kruskal-Wallis H-test: different letters indicate significant differences in post-hoc pairwise comparisons.

		Other	Gravid		Kruskal-Wallis	
	Juveniles	adults	females	Eggs	*H*	*p*
14:0	3.4 ± 0.6^{ab}	4.1 ± 0.3^{a}	3.8 ± 0.1^{a}	2.5 ± 0.1^{b}	9.050	**0.029**
16:0	19.5 ± 0.4^{a}	16 ± 0.6^{b}	17.4 ± 0.5^{b}	18.2 ± 0.6^{ab}	12.053	**0.007**
16:1ω7	23.1 ± 0.4^{ab}	30.5 ± 2.5^{a}	26.1 ± 1.6^{a}	11.8 ± 1.6^{b}	12.168	**0.007**
18:1ω9	19.2 ± 0.7^{a}	23.1 ± 1.4^{b}	27.5 ± 1.6^{c}	28.6 ± 1.2^{c}	14.934	**0.002**
18:1ω7	2.2 ± 0.2	2.5 ± 0.2	2.6 ± 0.1	2.5 ± 0.1	2.181	0.536
18:2ω6 (LIN)	1.1 ± 0.1^{a}	0.9 ± 0.2^{ab}	0.5 ± 0.0^{b}	1.2 ± 0.2^{ab}	8.047	**0.045**
20:4ω6 (ARA)	1.3 ± 0.3	1.1 ± 0.2	1.1 ± 0.1	2.2 ± 0.1	7.171	0.067
22:5ω6	0.9 ± 0.2^{a}	0.4 ± 0.1^{b}	0.4 ± 0.0^{b}	1.1 ± 01^{a}	15.685	**0.001**
20:5ω3 (EPA)	7.3 ± 1.3^{a}	7.3 ± 0.2^{a}	7.2 ± 0.3^{a}	10.3 ± 0.1^{b}	8.907	**0.031**
22:6ω3 (DHA)	13.4 ± 4.1	7.8 ± 1.3	7.6 ± 0.4	13.1 ± 0.8	7.814	0.500
VLC-PUFA	1.3 ± 0.9	0.2 ± 0.2	0.0 ± 0.0	0.1 ± 0.1	2.461	0.482
SAFA †	24.1 ± 1.0	21.1 ± 0.6	22.3 ± 0.6	22.1 ± 0.4	5.935	0.115
MUFA ‡	45.4 ± 4.5^{a}	57.1 ± 2.1^{b}	57.3 ± 0.6^{b}	44.7 ± 0.6^{a}	15.789	**0.001**
PUFA §	27.6 ± 5.4^{ab}	19.8 ± 1.5^{a}	18.7 ± 0.8^{a}	31.0 ± 0.7^{b}	11.923	**0.008**
BrFA ¶	0.4 ± 0.1^{a}	0.4 ± 0.1^{a}	0.8 ± 0.2^{a}	1.4 ± 0.1^{b}	12.105	**0.007**
ω3/ω6	8.1 ± 0.7^{a}	9.4 ± 0.7^{a}	8.6 ± 0.5^{a}	6.0 ± 0.2^{b}	12.237	**0.007**
18:1ω9/18:1ω7	8.9 ± 0.6	9.5 ± 0.8	10.4 ± 0.4	11.8 ± 1.1	6.148	0.105
n	6	7	5	5		

† SAFA - Saturated fatty acids
‡ MUFA - Monounsaturated fatty acids
§ PUFA - Polyunsaturated fatty acids
¶ BrFA - Iso- and anteiso-branched fatty acid

Figure captions

Figure 1. The location of Lake Paasivesi in Finland, northern Europe, and the depth zones of the lake (Veriö, 1990). The white areas in the deepest zone illustrate the sampling locations; the most southern area (start point 62°08.347'N & 29°25.045'E, end point 62°08.347'N & 29°26.901'E) was the main sampling area (2006–2008, 2011), while the two others (62°08.472'N & 29°25.045'E – 62°08.472'N & 29°26.901'E and 62°09.005'N & 29°24.131'E – 62°08.485'N & 29°25.792'E) were sampled only in 2007.

Figure 2. The temperatures measured in 4 October 2006, 3 October 2007 and 8 October 2008 (left), and the relative mean density (± S.E.) of *G. lacustris* both in day and night in 4 October 2006 and 3 October 2007 (right, combined data) in the water layers of Lake Paasivesi. More environmental information on the study lake (e.g. vertical profiles of oxygen, chlorophyll and light) are available from authors and in the study by Hiltunen et al. (2015).

Figure 3. Length distribution of *G. lacustris* individuals (total n = 575) in daylight samples collected from Lake Paasivesi in October 2006, 2007 and 2008, the lengths rounded to the nearest whole number.

Figure 4. Total fatty acid content of *G. lacustris* in different life stages (n = 5–7 per stage; see Table 2) collected from Lake Paasivesi in August and September 2011. Different letters denote significant differences in ANOVA post hoc test (Tukey HSD).

Figure 5. Fatty acid percent composition of *G. lacustris* life stages (this study) in grey, and seston, zooplankton, and vendace (Hiltunen et al., 2015; Strandberg et al., 2015) in white. All organisms originate from Lake Paasivesi. The fatty acids that most influenced the ordination are depicted as vectors. The stress value for the 2-dimendional ordination was 0.1. EPA = eicosapentaenoic acid (20:5ω3), ARA= arachidonic acid (20:4ω6), ALA = α-linolenic acid (18:3ω3), SDA = stearidonic acid (18:4ω3), LIN = linoleic acid (18:2ω6), DHA = docosahexaenoic acid (22:6ω3).

Figure 6. Stable isotope values (mean ± S.E.) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in *G. lacustris* (current study and Auttila et al., 2015), and seston, zooplankton and fish (current study and Strandberg et al., 2015), all originating from Lake Paasivesi. Cladocerans include samples from *Bosmina* spp., *Daphnia* spp., *Limnosida frontosa*, and pooled cladocerans. . The $\delta^{13}C$ values of crustaceans are lipid-corrected according to Syväranta & Rautio (2010) and those of fish according to Kiljunen et al. (2006). See Supporting Information Table S1 for more information on the samples and Figure S1 for biplot without lipid correction.

Figure S1. Stable isotope values (mean ± S.E.) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) without lipid correction in *G. lacustris* (current study and Auttila et al., 2015), and seston, zooplankton and fish (current study and Strandberg et al., 2015), all originating from Lake Paasivesi. Cladocerans include samples from *Bosmina* spp., *Daphnia* spp., *Limnosida frontosa*, and pooled cladocerans. See Figure 6 for lipid-corrected values.

Supporting Information Table S1. Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in the pelagic food web of Lake Paasivesi. No lipid correction of $\delta^{13}C$ values (c.f. Fig. 6 and Supporting Information Fig. S1).

Taxa	Date	$\delta^{13}C$	$\delta^{15}N$	C:N	Study
Seston	August 2011	-27.5	0.9	16.9	Strandberg et al. 2015
Seston	September 2011	-27.5	1.9	22.9	Strandberg et al. 2015
Cladocera	August 2011	-29.6	1.3	4.6	Strandberg et al. 2015
Cladocera	August 2011	-29.6	2.3	4.5	Strandberg et al. 2015
Bosmina spp.	September 2008	-29.9	4.2	6.4	Current study
Bosmina spp.	September 2008	-30.3	4.7	5.4	Current study
Daphnia spp.	September 2008	-28.6	4.9	6.9	Current study
Limnosida frontosa	September 2008	-29.1	5.2	6.3	Current study
Cyclopoida	September 2008	-31.0	8.4	5.7	Current study
Cyclopoida	September 2008	-29.0	7.0	6.7	Current study
Cyclopoida	September 2008	-30.3	9.3	7.6	Current study
Cyclopoida	September 2008	-30.2	7.3	6.4	Current study
Cyclopoida	September 2008	-29.8	6.2	5.9	Current study
Cyclopoida	August 2011	-30.1	7.5	4.4	Strandberg et al. 2015
Eudiaptomus spp.	August 2011	-29.4	4.9	4.1	Strandberg et al. 2015
Eudiaptomus spp.	August 2011	-29.3	4.8	4.0	Strandberg et al. 2015
Eudiaptomus spp.	September 2008	-31.2	8.3	5.1	Current study
Eudiaptomus spp.	September 2008	-31.7	8.1	4.9	Current study
Eudiaptomus spp.	September 2008	-30.5	7.1	4.9	Current study
Heterocope spp.	September 2008	-30.7	8.4	4.8	Current study
Heterocope spp.	September 2008	-30.6	7.9	4.4	Current study
Heterocope spp.	September 2008	-31.1	8.2	4.7	Current study
Heterocope spp.	September 2008	-31.0	7.8	4.6	Current study
Heterocope spp.	September 2008	-31.0	8.0	4.8	Current study
Heterocope spp.	August 2011	-29.6	7.5	4.4	Strandberg et al. 2015
Heterocope spp.	August 2011	-29.4	7.3	4.2	Strandberg et al. 2015
Heterocope spp.	August 2011	-29.4	7.5	4.3	Strandberg et al. 2015
Heterocope spp.	August 2011	-29.3	7.1	4.2	Strandberg et al. 2015
Heterocope spp.	August 2011	-29.3	7.5	4.2	Strandberg et al. 2015
Heterocope spp.	September 2011	-29.5	8.1	4.2	Strandberg et al. 2015
Limnocalanus macrurus	August 2011	-33.2	9.7	6.0	Strandberg et al. 2015
Limnocalanus macrurus	August 2011	-32.7	9.8	5.8	Strandberg et al. 2015
Limnocalanus macrurus	August 2011	-32.3	9.5	5.7	Strandberg et al. 2015
Limnocalanus macrurus	August 2011	-32.2	9.4	5.5	Strandberg et al. 2015
Limnocalanus macrurus	September 2011	-33.1	10.5	7.3	Strandberg et al. 2015
Mysis relicta	September 2008	-29.8	12.1	3.8	Current study
Mysis relicta	September 2008	-29.8	7.3	4.2	Current study
Mysis relicta	September 2008	-29.7	9.8	4.0	Current study
Mysis relicta	September 2008	-29.8	7.6	4.1	Current study
Mysis relicta	September 2008	-29.1	11.2	3.4	Current study
Mysis relicta	September 2008	-29.2	7.7	4.0	Current study

Mysis relicta	September 2008	-29.4	7.4	3.9	Current study
Mysis relicta	September 2008	-29.5	7.8	4.0	Current study
Mysis relicta	September 2008	-29.1	8.1	3.6	Current study
Mysis relicta	September 2008	-29.1	7.6	3.8	Current study
Mysis relicta	September 2008	-28.8	8.4	3.5	Current study
Mysis relicta	September 2008	-29.3	10.4	4.0	Current study
Gammaracanthus lacustris	September 2008	-30.0	12.3	6.2	Current study
Gammaracanthus lacustris	September 2008	-31.2	10.5	6.0	Current study
Gammaracanthus lacustris	September 2008	-30.1	12.3	6.6	Current study
Gammaracanthus lacustris	September 2008	-28.2	13.1	4.4	Current study
Gammaracanthus lacustris	September 2010	-29.9	12.2	6.0	Auttila et al. 2015
Gammaracanthus lacustris	September 2010	-29.4	12.3	5.6	Auttila et al. 2015
Gammaracanthus lacustris	September 2010	-29.5	12.2	5.6	Auttila et al. 2015
Gammaracanthus lacustris	September 2010	-30.1	11.6	6.5	Auttila et al. 2015
Gammaracanthus lacustris	September 2010	-28.7	12.3	4.8	Auttila et al. 2015
Gammaracanthus lacustris	September 2010	-29.4	12.2	5.5	Auttila et al. 2015
Gammaracanthus lacustris	September 2010	-28.7	12.6	4.6	Auttila et al. 2015
Gammaracanthus lacustris	September 2010	-28.6	12.7	4.8	Auttila et al. 2015
Gammaracanthus lacustris	September 2010	-28.6	12.3	4.4	Auttila et al. 2015
Gammaracanthus lacustris	September 2010	-28.9	12.7	5.2	Auttila et al. 2015
Coregonus albula	August 2011	-27.2	9.2	3.3	Strandberg et al. 2015
Coregonus albula	August 2011	-28.7	9.2	3.4	Strandberg et al. 2015
Coregonus albula	August 2011	-27.6	8.8	3.2	Strandberg et al. 2015
Coregonus albula	August 2011	-27.9	9.5	3.3	Strandberg et al. 2015
Coregonus albula	August 2011	-29.0	11.9	3.4	Strandberg et al. 2015
Coregonus albula	September 2011	-27.3	9.1	3.3	Strandberg et al. 2015
Coregonus albula	September 2011	-29.1	8.6	3.3	Strandberg et al. 2015
Coregonus albula	September 2011	-28.5	9.3	3.3	Strandberg et al. 2015
Coregonus albula	September 2008	-29.0	9.7	3.4	Current study
Coregonus albula	September 2008	-28.9	9.5	3.2	Current study
Coregonus albula	September 2008	-28.8	10.0	3.3	Current study
Coregonus albula	September 2008	-28.7	9.3	3.2	Current study
Coregonus albula	September 2008	-28.9	9.6	3.3	Current study
Osmerus eperlanus	September 2008	-29.0	11.5	2.6	Current study
Osmerus eperlanus	September 2008	-28.4	11.8	3.3	Current study
Osmerus eperlanus	September 2008	-29.3	11.2	3.5	Current study
Osmerus eperlanus	September 2008	-28.8	11.5	3.2	Current study
Osmerus eperlanus	September 2008	-28.4	11.0	3.2	Current study
Perca fluviatilis	September 2008	-26.9	11.6	3.2	Current study
Perca fluviatilis	September 2008	-25.1	11.2	3.2	Current study
Perca fluviatilis	September 2008	-24.6	10.9	3.2	Current study
Perca fluviatilis	September 2008	-26.8	12.7	3.2	Current study
Esox lucius	September 2008	-26.0	12.0	3.2	Current study
Esox lucius	September 2008	-26.0	11.8	3.2	Current study
Esox lucius	September 2008	-23.3	11.0	3.2	Current study
Sander lucioperca	September 2008	-26.6	12.4	3.2	Current study
Sander lucioperca	September 2008	-27.7	12.5	3.2	Current study
Sander lucioperca	September 2008	-27.6	12.8	3.2	Current study

Sander lucioperca	September 2008	-27.4	11.6	3.2	Current study
Sander lucioperca	September 2008	-27.4	11.6	3.2	Current study

Supporting Information Table S2. Fatty acid percent composition (%) and total fatty acid content of *G. lacustris* collected in Lake Paasivesi in August and September 2011.

	Juveniles		Adults		Gravid females		Eggs	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
12:0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0
14:0	3.4	0.6	4.1	0.3	3.8	0.1	2.5	0.1
15:0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
16:0	19.5	0.4	16.0	0.6	17.4	0.5	18.2	0.6
17:0	0.2	0.1	0.2	0.0	0.1	0.0	0.3	0.0
18:0	0.8	0.1	0.8	0.1	0.8	0.0	0.8	0.1
20:0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Σ SAFA	**24.1**	**1.0**	**21.1**	**0.6**	**22.3**	**0.6**	**22.1**	**0.4**
4,8,12 TMTD	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phytanate	0.4	0.2	0.5	0.1	0.1	0.0	0.0	0.0
DMA	0.8	0.2	0.2	0.1	0.3	0.0	0.2	0.0
Σ Isoprenoids and dimethylacetals	**1.3**	**0.2**	**0.7**	**0.2**	**0.4**	**0.1**	**0.2**	**0.0**
14:1ω5	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0
16:1ω9	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0
16:1ω7	23.1	4.0	30.5	2.5	26.1	1.6	11.8	1.6
16:1ω5	0.1	0.0	0.1	0.0	0.1	0.0	0.2	0.0
17:1ω7	0.1	0.0	0.2	0.0	0.2	0.0	0.5	0.0
18:1ω9	19.2	0.7	23.1	1.4	27.5	1.6	28.6	1.2
18:1ω7	2.2	0.2	2.5	0.2	2.6	0.1	2.5	0.1
18:1ω5	0.0	0.0	0.1	0.0	0.1	0.0	0.3	0.0

19:1ω	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
20:1ω11	0.0	0.0	0.1	0.0	0.1	0.1	0.2	0.0
20:1ω9	0.2	0.1	0.2	0.0	0.2	0.0	0.4	0.0
20:1ω7	0.1	0.0	0.2	0.0	0.2	0.0	0.2	0.0
22:1ω9	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
24:1ω9	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Σ MUFA	**45.4**	**4.5**	**57.1**	**2.1**	**57.3**	**0.6**	**44.7**	**0.6**
16:2ω4	0.3	0.1	0.5	0.1	0.3	0.1	0.2	0.0
16:3ω4	0.3	0.1	0.6	0.1	0.4	0.1	0.2	0.0
18:2ω6	1.1	0.1	0.9	0.2	0.5	0.0	1.2	0.2
18:3ω3 (ALA)	0.5	0.1	0.3	0.1	0.3	0.1	0.6	0.0
18:4ω4	0.1	0.0	0.2	0.0	0.1	0.0	0.1	0.0
18:4ω3	0.3	0.1	0.3	0.1	0.2	0.0	0.1	0.0
20:2ω6	0.4	0.0	0.3	0.0	0.4	0.1	0.7	0.1
20:4ω6 (ARA)	1.3	0.3	1.1	0.2	1.1	0.1	2.2	0.1
20:3ω3	0.2	0.1	0.0	0.0	0.1	0.0	0.2	0.0
20:4ω3	0.2	0.0	0.2	0.1	0.1	0.0	0.2	0.0
20:5ω3 (EPA)	7.3	1.3	7.3	0.2	7.2	0.3	10.3	0.1
22:4ω6	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
22:5ω6	0.9	0.2	0.4	0.1	0.4	0.0	1.1	0.1
22:5ω3 (DPA)	0.3	0.1	0.2	0.0	0.3	0.0	1.0	0.1
22:6ω3 (DHA)	13.4	4.1	7.8	1.3	7.6	0.4	13.1	0.8
VLC-PUFA	1.3	0.9	0.2	0.2	0.0	0.0	0.1	0.1
Σ PUFA	**27.6**	**5.4**	**19.8**	**1.5**	**18.7**	**0.8**	**31.0**	**0.7**
Σ i/ai-branched	**0.4**	**0.1**	**0.4**	**0.1**	**0.8**	**0.2**	**1.4**	**0.1**
ω3/ω6	8.1	0.7	9.4	0.7	8.6	0.5	6.0	0.2
18:1ω9/18:1ω7	8.9	0.6	9.5	0.8	10.4	0.4	11.8	1.1
Tot. FA (µg mg DW^{-1})	120.3	27.9	212.1	41.1	157.2	16.2	300.7	34.7