Intraspecies variability of fatty acid content and composition of a cosmopolitan benthic invertebrate, *Gammarus lacustris*

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Abstract

Aquatic invertebrates are valuable dietary sources of essential polyunsaturated fatty acids, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), for fish. Phylogeny, diet, and various ecological factors affect the fatty acid composition of aquatic invertebrates. We focused our study on the effect of ecological factors to a cosmopolitan species inhabiting lakes that differed in salinity, temperature, and presence/absence of predators (fish). To avoid the effect of phylogeny, which strongly influences the fatty acid composition of animals, we studied several populations of one cosmopolitan benthic species, *Gammarus lacustris* Sars. We found that differences in fatty acid percentages of *G. lacustris* were mainly affected by differences in their diets. Some populations preferred dinoflagellates, cryptophytes, green algae/cyanobacteria, and bacteria; other populations selected diatoms; and still other populations consumed zooplankton or allochthonous (terrestrial) organic matter.

The salinity and presence/absence of fish affected the contents of EPA and DHA in *G. lacustris*. Populations from saline and fishless lakes had significantly higher contents of EPA and DHA. Thus, stocking of fishless lakes dominated by *G. lacustris* with fish could lead to a decrease in EPA and DHA contents in the gammarids. We propose that some saline and fishless lakes could be used as a source of gammarids for aquaculture fish feeding.

KEYWORDS essential polyunsaturated fatty acids, fish, food quality, mineralization, temperature

Introduction

In aquatic ecology, fatty acids (FAs) are often used as biochemical markers to reconstruct animals' diets (Daly et al. 2010, Mezek et al. 2011, Budge et al. 2012, Kelly and Scheibling 2012, Lau et al. 2012) and as indicators of the food quality of different aquatic items for consumers (e.g., Parrish 2009, Takeuchi 2014, Litz et al. 2017). Some polyunsaturated fatty acids (PUFAs) of n-3 family, eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3), are essential for growth and development of aquatic and terrestrial animals, including humans (e.g., Plourde and Cunnane 2007, Arts and Kohler 2009, Parrish 2009, Pike 2015). The contents of these PUFAs are considered major indicators of food quality (e.g., Ahlgren et al. 2009). EPA and DHA are primarily synthesized d*e novo* by certain algae, mainly diatoms, cryptophytes, and dinoflagellates (Berge and Barnathan 2005, Dijkman and Kromkamp 2006, Kelly and Scheibling 2012, Taipale et al. 2013). Some animals can convert EPA and DHA from their precursor, α-linolenic acid, but at insufficient rates; thus, in general, animals must obtain these molecules directly from their diets (Brett and Müller-Navarra 1997, Gladyshev et al. 2009, Guo et al. 2016). PUFAs are transferred from microalgae to invertebrates and fish and then to humans through food webs (Gladyshev et al. 2013).

Aquatic invertebrates are a major food source for many fish. Various factors such as phylogeny, diet, temperature, salinity, pollutants, immune system function, starvation, parasites, predation, age, and gender affect invertebrate FA content and therefore the nutritive values of invertebrates for their consumers (e.g., Brett et al. 2009, Fokina et al. 2010, Makhutova et al. 2011, Lau et al. 2012, Gladyshev et al. 2015). Thus, studying the effects of these factors is important for pure and applied aquatic ecology.

Phylogeny itself or together with diet is considered a key factor determining FA profiles of aquatic animals (Makhutova et al. 2011, Lau et al. 2012, Gladyshev et al. 2015). Among ecological factors, water temperature is one of the most studied. According to the hypothesis of "homeoviscous adaptation," a decrease in ambient temperature leads to an increase in the percentages of PUFAs with comparatively low melting points to maintain cell membrane fluidity (Stillwell and Wassall 2003, Brett et al. 2009, Koussoroplis et al. 2013). The role of highly unsaturated FAs, such as EPA and DHA in temperature adaptation, is questionable, however, and is still under discussion (Hazel 1995, Gladyshev et al. 2015). The effect of salinity on the FA composition of aquatic animals is believed to manifest in changes of the percentages of omega-3 (n-3) and omega-6 (n-6) PUFAs (Fokina et al. 2010, Sarker et al. 2011, Fonseca-Madriqal et al. 2012). Numerous data on the importance of the influence of diets on FA composition of planktonic and benthic animals have been obtained, mainly under experimental conditions (Weers et al. 1997, Brett et al. 2006, Torres-Ruiz et al. 2010, Gladyshev et al. 2016a). Lau et al. (2013), however, studied FA variation in *Asellus aquaticus* (benthic isopod) across a nutrient gradient by combining a field study and laboratory experiments and showed a strong effect of dietary FAs on the PUFA composition of that isopod. The effect of other factors, for instance parasites or predation, has been much less studied or not studied at all.

We consider that cosmopolitan species inhabiting aquatic ecosystems with a wide range of environmental conditions are the most interesting taxa to study the effect of ecological factors on FA profiles of aquatic animals in natural conditions. Thus, the aim of our study was to reveal the effect of salinity, temperature, diet, and the presence/absence of predators (fish) on the FA content and composition of an aquatic cosmopolite invertebrate. To avoid the effect of phylogeny, which strongly influences FA composition of animals, we studied several populations of the cosmopolitan benthic species, *Gammarus lacustris* Sars.

Methods

Field sampling

Gammarus lacustris was collected in 10 lakes of different regions of Russia: in 4 lakes (Svetloe, Anikino, Shira, and Shunet) in July 2014, in 6 lakes (Svetloe, Anikino, Shira, Shunet, Fyrkal, and Krasnenkoye) in July 2015, in 3 lakes (Matarak, Utichye-1, and Utichye-3) in July 2016, and in 2 lakes (Utichye-1 and Sobachye) in July and in the beginning of August 2017 (Fig. 1, Table 1). The lakes varied in salinity, temperature, and the presence/absence of fish (Table 1). To avoid an unaccounted possible significant effect of FA variability associated with gammarid age, we divided gammarids into 3 size groups: small (0.2–0.5 cm), medium (0.6–0.8 cm), and large (0.9–1.2 cm), which covered all sizes represented in the lakes. Each sample of small-sized animals consisted of 3–21 individuals, medium-sized animals 3–7 individuals, and large-sized animals 1–7 individuals. The number of samples for each size group varied from 1 to 4. The total number of samples (n) of *G. lacustris* for FA analysis was as follows from each lake: Svetloe $n = 18$, Fyrkal $n = 8$, Anikino $n = 16$, Krasnenkoye $n = 10$, Shira $n = 16$, Shunet $n = 18$, Matarak $n = 7$, Utichye-1 $n = 9$, Utichye-3 $n = 7$, and Sobachye $n = 6$. Animals were sampled in the littoral zone using a sweep net. Immediately after sampling, the live animals were placed in beakers with filtered (pore

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size 80 µm) ambient water for 24 h to empty their guts. The animals' body surfaces were then gently wiped with filter paper to remove water, and the animals were weighed and placed in a chloroform:methanol mixture (2:1, v/v) and kept until further analysis at −20 °C.

Simultaneously with sampling of *G. lacustris*, we measured water temperature and took samples for analyses of salinity of the water from the lakes. Salinity of the water in Lake Sobachye was not measured, so we used data from the literature. To determine salinity, the previously filtered water (pore size 130 µm) was evaporated by heating and then combusted at 450 °C to a constant weight. The obtained ash content was used as a measure of salinity. Additionally, we sampled *G. lacustris* for analyses of total organic carbon, nitrogen, and moisture. Phytoplankton samples were collected in the littoral zone in Lakes Svetloe and Anikino in July 2015, in Lakes Shira, Shunet, Fyrkal, Krasnenkoye, Matarak, Utichye-1, and Utichye-3 in July 2016, and in Lake Sobachye in August 2014–2017. The samples were filtered through "Vladipor" membrane filters (Mytischi, Russia; pore size, 0.75–0.85 µm) and then placed in filtered lake water with Lugol's solution. Microalgae were identified and counted using a Fuchs-Rosenthal counting chamber (3.2 µL volume) under an inverted microscope at 400× magnification.

Fatty acid analysis

The methods of lipid extraction, transesterification (methylation) of the lipid extracts, and purification of methyl esters were described by Christie (2003). Briefly, lipids from the samples were extracted with chloroform:methanol (2:1, v/v) 3 times simultaneously with mechanical homogenization of the tissues with glass beads. Before extraction, a defined volume of an internal standard solution (a solution of free 19:0 in chloroform, 0.5 mg/mL; Sigma-Aldrich, St. Louis, MO, USA) was added to the samples. The volume of the internal standard solution added to the samples depended on the lipid content and weight of the

samples and corresponded to 1 mL per 1 g of wet weight (ww) of animal tissues. The combined lipid extracts were filtered, dried by passing through anhydrous Na_2SO_4 layer, and evaporated at 35 °C. FA methyl esters (FAMEs) were prepared in a mixture of methanol:sulfuric acid (20:1, v/v) at 90 °C for 2 h as previously described (Makhutova et al. 2012). FAMEs were analyzed on a gas chromatograph (GC) equipped with a mass spectrometer detector (model 6890/5975C, Agilent Technologies, Santa Clara, CA, USA) and a 30 m long × 0.25 mm internal diameter HP-FFAP capillary column (Agilent Technologies, Santa Clara, CA, USA). The GC temperature program was as follows: from 100 to 190 °C at 3 °C/min, 5 min isothermally, then to 230 °C at 10 °C/min, and 20 min isothermally. Other instrument conditions were as described elsewhere (Gladyshev et al. 2014). FAME peaks were identified by their mass spectrum compared to those in a database (NIST-2005, Gaithersburg, MD, USA) and to those of available authentic standards (Sigma-Aldrich, St. Louis, MO, USA).

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Carbon, nitrogen, and moisture analysis

The samples of *G. lacustris* (50–150 mg ww) for total organic carbon, nitrogen, and moisture were dried until constant weight at 70 °C. Samples were then weighed to measure their moisture and stored in a desiccator until analysis with a Flash EA 1112 NC Soil/MAS 200 elemental analyzer (ThermoQuest, Italy). Calibration curves for the elemental analyzer were built using aspartic acid and standard reference soil samples (ThermoQuest, Italy). The numbers of samples for carbon, nitrogen, and moisture of *G. lacustris* were similar to the numbers of samples for FAs.

Statistical analyses

Standard errors (SE) and one-way ANOVA with Tukey LSD *post hoc* test were calculated conventionally and applied for normally distributed variables (Campbell 1967, Brown 2005). When not normally distributed, a Kruskal-Wallis test with multiple comparisons of mean ranks was applied. To reveal differences in the content of some FAs of *G. lacustris* inhabiting lakes with different temperatures, salinity, and presence/absence of fish, a Mann-Whitney *U* test was used. We considered lakes with salinity >3000 mg/L as saline lakes (Shunet, Shira, Krasnenkoye, Utichye-1, and Utichye-3) and <3000 mg/L as freshwater lakes (Svetloe, Fyrkal, Anikino, and Matarak; Hammer 1986). To reveal differences in the FA composition of *G. lacustris* inhabiting different lakes, we used multivariate discriminant analysis (MDA), a method of linear modelling to classify observations using *a priori* known groups (Legendre and Legendre 1998). All calculations were carried out using STATISTICA 9.0 software (StatSoft, Inc., Tulsa, OK, USA).

Results

Water temperature, salinity, and the presence/absence of fish were recorded for all study lakes (Table 1). Five lakes (Shira, Shunet, Krasnenkoye, Utichye-1, and Svetloe) were fishless and, except Lake Svetloe, warm and saline. The other 5 lakes contained fish and were warm and freshwater, except Lake Utichye-3, which was saline, and Lake Sobachye, which was cold (Table 1). Lake Anikino is a warm lake, but in 2015 a hot June was followed by cool weather, which persisted until the end of the summer, and therefore the water temperature of Lake Anikino on the sampling dates in 2015 was below normal (Table 1).

Algae from 3 main taxa, Cyanophyta, Chlorophyta, and Bacillariophyta, dominated the phytoplankton in all lakes except Lakes Svetloe and Sobachye, where the major taxa were Dinophyta, Chrysophyta, and Bacillariophyta (Table 1).

Percentages of prominent FAs were calculated for all lakes (Table 2). Populations of *G. lacustris* from Lakes Shunet, Fyrkal, and Utichye-1 had high percentages of FA markers of bacteria (18:1n-7, 15:0, and 17:0) while populations from Lakes Sobachye and Svetloe had lowest percentages of these FAs. *G. lacustris* from Lakes Utichye-3 and Krasnenkoye had a high percent of FA markers of diatoms: 16:1n-7, 16:2n-4, 16:3n-4 and 20:5n-3. Additionally, high percentages of the physiologically important 20:5n-3 were found in the populations from Lakes Sobachye, Shunet, and Anikino while low percentages of EPA were found in the populations from Lakes Svetloe, Utichye-1, Fyrkal, and Matarak. A high level of linoleic acid (LA, 18:2n-6), which is known as a marker of green algae, cyanobacteria, and even terrestrial vegetation, was a characteristic of *G. lacustris* from Lakes Svetloe and Fyrkal. Other markers of green algae and cyanobacteria (18:3n-3 and 18:3n-6) were observed in *G. lacustris* from Lakes Krasnenkoye and Anikino, and a population of *G. lacustris* from cold Lake Svetloe had the highest percent of 18:4n-3. *G. lacustris* from Lakes Shira, Shunet, and Sobachye contained high percentages of physiologically important 22:6n-3; percentages of 22:6n-3 in gammarids from these lakes were about twice as high as in *G. lacustris* inhabiting the other lakes. The highest percent of the physiologically most important FA of the n-6 family, 20:4n-6, was found in *G. lacustris* from Lake Sobachye followed by Lake Fyrkal.

The percentages of total n-3 PUFAs in *G. lacustris* from all habitats studied were higher than the percentages of total n-6 PUFAs (Table 2); however, the n-3/n-6 ratio was variable. *G. lacustris* from Lake Krasnenkoye had the highest n-3/n-6 ratio due to a high percent of EPA and low percentages of arachidonic acid (ARA, 20:4n-6) and LA in this population. *G. lacustris* from Lakes Shira, Shunet, and Utichye-3 had medium values for the n-3/n-6 ratio due to high or medium percentages of DHA and EPA, and medium percentages of ARA and LA. *G. lacustris* from Lakes Svetloe and Fyrkal had low values of this ratio due to low percentages of EPA and DHA and high percentages of LA and/or ARA.

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Примечание [JEF8]: Table 2 is cited here as the compilation of FA information, so I deemed the numerous other Table 2 citations in the paragraph as unnecessary. Please review

High total FA content was found in *G. lacustris* from 3 fishless lakes, Svetloe, Krasnenkoye, and Utichye-1, while low total FA content was found in *G. lacustris* from lakes that contained fish, Sobachye, Anikino, Fyrkal, Matarak, and Utichye-3, and one fishless lake, Shira (Table 2). Among fishless lakes, high total FA content was found in lakes with low salinity, whereas medium FA content was found in lakes with high and medium salinity (Table 2).

The moisture of *G. lacustris* varied from 73.4% to 82.7% (Table 2). Total organic carbon and nitrogen of *G. lacustris* varied from 34.5% to 43.7% of dry weight and from 7.6% to 9.1% of dry weight, respectively (Table 2).

The percentages of 18 FAs were used in an MDA (Table 3, Fig. 2) that showed root 1 and root 2 were statistically significant (Table 3). MDA also showed that the most significant differences in the first canonical root were observed between populations of *G. lacustris* from cold freshwater Lake Svetloe and warm saline Lake Krasnenkoye (Table 3, Fig. 2). The differences were caused primarily by a high percent of 18:2n-6 in *G. lacustris* from Lake Svetloe and a high percent of 20:5n-3 in *G. lacustris* from Lake Krasnenkoye (Tables 2 and 3). The second canonical root reflected the differences between *G. lacustris* inhabiting warm saline fishless Lake Utichye-1 and cold freshwater fishless Lake Svetloe (Table 3, Fig. 2). The differences were primarily due to high percentages of 18:4n-3 and 20:4n-3 in the population from Lake Svetloe and high percentages of 18:1n-7 and 17:0 in the population from Lake Utichye-1 (Tables 2 and 3). Additionally, the MDA showed the similarity of FA percentages of *G. lacustris* inhabiting all fish lakes, Sobachye, Anikino, Fyrkal, Matarak, and Utichye-3, and the similarity of FA percentages of *G. lacustris* inhabiting 3 saline warm fishless lakes, Shira, Shunet, and Krasnenkoye (Fig. 2).

To identify the most variable and most constant FAs of *G. lacustris*, we calculated the 10th and 90th percentiles and, additionally, the minimum, the median, and the maximum

values for the percentages of each fatty acid (Table 4). The most constant FAs, in which 80% of the data had a narrow range, were 16:0, 18:0, 18:1n-9, 18:1n-7, and 20:5n-3 (Table 4). High variability in percentages was found for ai17:0, 16:2n-4, 16:3n-4, 18:4n-3, 20:4n-3, and 22:5n-6 (Table 4). DHA percentages showed higher variability than EPA percentages, whereas the variabilities of EPA and DHA contents were similar and comparably high (Table 4).

Contents of EPA and DHA in *G. lacustris* inhabiting the fishless lakes were significantly higher than those in *G. lacustris* from lakes that contained fish (Table 5). The populations from the saline lakes had significantly higher contents of EPA and DHA than populations from the freshwater lakes; however, the EPA and DHA contents in populations from the cold and the warm lakes were not significantly different (Table 5).

Discussion

The intraspecific differences in the FA composition of *G. lacustris* inhabiting lakes that differed in salinity, temperature, food composition, and the presence/absence of fish were probably caused by differences in their diets. Most likely, *G. lacustris* from Lakes Utichye-3, and Krasnenkoye consumed diatoms in greater proportions than the other populations because the individuals had higher percentages of the diatom biomarkers (16:1n-7, 16:2n-7, 16:2n-4, etc.). *G. lacustris* from Lake Svetloe preferred cryptophytes and/or dinoflagellates rich in 18:4n-3 and green algae and/or cyanobacteria rich in 18:2n-6; the population from Lake Fyrkal also preferred green algae/cyanobacteria, but of other species rich in 18:3n-3. Indeed, many gammarids are believed to be herbivores, and their major food sources are benthic unicellular microalgae (Gladyshev et al. 2000, Biandolino and Prato 2006, Platvoet et al. 2006, Mirzajani et al. 2011, Michel et al. 2015). The diets of *G. lacustris* from Lakes Shunet, Fyrkal, and Utichye-1 contained bacteria in greater proportions than in the other populations.

The populations studied could consume algae and bacteria directly from benthic biofilms and detritus and indirectly through trophic chains consuming small animals whose food was algae and bacteria.

Along with 18:2n-6, 20:4n-6 is considered a marker of allochthonous organic matter of comparatively low nutritive value (Gladyshev et al. 2017). Arachidonic acid is common in biomass of terrestrial insects and other terrestrial invertebrates (van Dooremalen et al. 2009, Fontaneto et al. 2011). The highest percentage of 20:4n-6 in *G. lacustris* from northern oligotrophic Lake Sobachye and rather high percentage of 18:2n-6 together with comparatively low percentages of the other algal markers probably indicate a high contribution of allochthonous organic matter (e.g., small terrestrial insects) in the diet of this population of gammarids. According to the literature, gammarids can feed on animals; thus predation, including cannibalism, is considered a common feeding strategy for amphipods (MacNeil et al. 1999, Wilhelm and Schindler 1999, MacNeil and Platvoet 2005, Biandolino and Prato 2006). In our study, we observed the remains of copepods, specifically *Arctodiaptomus salinus*, in the gut contents of *G. lacustris* from Lake Shira (Fig. 3).

An experimental study on the diet of *G. lacustris* from Lake Shira also revealed consumption of the rotifer *Brachionus plicatilis* and the copepod *A. salinus* by these gammarids (Yemelyanova et al. 2002). The calanoid copepod *A. salinus* has high percentages of DHA (up to 17% of total FAs) and is a dominant zooplankton species in lakes Shira and Shunet (Tolomeev et al. 2010). Our previous work demonstrated that seston (primarily phytoplankton) and benthic biofilms (primarily phytobenthos) from Lake Shira were poor in DHA while the bodies of *G. lacustris* had high percentages (8.6%) of DHA (Makhutova et al. 2003). Based on the literature and our observations, we hypothesize that *G. lacustris* from Lakes Shira and Shunet had high contents of DHA because they consumed *A. salinus*.

Salinity affects the activity of enzymes involved in the synthesis of PUFAs, leading to an increase in the DHA/EPA and polyunsaturated FAs/saturated FAs (PUFA/SFA) ratios in marine fish and invertebrates (Guermazi et al. 2008, Fokina et al. 2010, Tolomeev et al. 2010, Sarker et al. 2011, Fonseca-Madriqal et al. 2012, Dantagnan et al. 2013). Thus, another reason for high percentages of DHA in the populations of *G. lacustris* from saline lakes Shira and Shunet might be an adaptation of this species to high salinity. In the present study, however, the maximum and the minimum values for the DHA/EPA ratio were found in populations from saline lakes Shira and Krasnenkoye, respectively. Additionally, the PUFA/SFA ratios were comparable in the populations from saline and freshwater lakes. Thus, we propose the high percentages of DHA in *G. lacustris* inhabiting saline lakes Shira and Shunet could be explained by their diet rather than by an adaptation to salinity.

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The effect of another factor, temperature, on FA percentages of *G. lacustris* was also not revealed. According to Tukey HSD *post hoc* test results (Table 2), the population of *G. lacustris* from cold lake, Lake Svetloe, significantly differed from the other populations only in the percentages of 18:4n-3, which is one of the algal markers. Additionally, relatively high percentages of 14:0 and 18:2n-6 in the population from the cold lake compared to the other populations obviously indicated the specific composition of their diet. The population from another cold lake, Lake Sobachye, did not differ significantly from warm water populations in FA percentages. High percentages of short-chain saturated FAs and long-chain PUFAs are thought to enhance cell membrane fluidity at low temperatures (Stillwell and Wassall 2003, Maazouzi et al. 2008, Smyntek et al. 2008, Arts and Kohler 2009, Brett et al. 2009, Sperfeld and Wacker 2012, Koussoroplis et al. 2013, Dodson et al. 2014, Gladyshev et al. 2015). We did not observe higher percentages of these FAs in the populations of *G. lacustris* from the cold lakes than in the other populations.

The presence/absence of fish in the lakes probably affected the percentages of prominent FAs. The results of the MDA indicated the similarity of FA percentages of *G. lacustris* from all fish lakes and differences between FA percentages of *G. lacustris* from fishless lakes. In fishless lakes, gammarids could probably selectively consume preferable and diverse food items from throughout the lakes, whereas in fish lakes, the presence of predators restricted the selective feeding of gammarids to habitats where they could hide from fish.

Statistical analysis of all the data on FA percentages of the gammarids studied allowed us to determine the most and least variable FAs. The ranges of the percentages of the most consistent FAs are probably characteristics of crustacea in general and gammarids specifically. This means that for *G. lacustris*, percentages of 16:0, 18:0, 18:1n-9, and 20:5n-3, which mainly are part of membrane phospholipids, are stable, and any healthy population of this species will have similar percentages of these FAs. The most variable FAs were mainly algae and bacteria markers and some long-chain PUFAs, which could originate from animal diet and might also be synthesized by gammarids. In contrast to the constant FAs, most of the variable FAs are used for energy (e.g., Leonard et al. 2004). The high variability of FA food markers in *G. lacustris* confirms the hypothesis that differences in the FA percentages of the populations studied were mainly caused by differences in their diets.

Note that the level of physiologically important n-3 PUFA, specifically EPA, in *G. lacustris* was more stable than the level of another physiologically important n-3 PUFA, DHA. Although DHA is considered a characteristic of Gammaridae (Makhutova et al. 2016), *G. lacustris* physiological needs for DHA are likely lower than for EPA. High and stable percentages of EPA in the gammarids, however, can be explained by high availability of EPA in food items in the lakes. In this study, we did not directly measure the FA composition of food sources of gammarids, but the dominance of Bacillariophyta in the lakes usually rich in

EPA (Dijkman and Kromkamp 2006, Kelly and Scheibling 2012, Taipale et al. 2013) suggests high availability of EPA in the food webs of these lakes.

The content of EPA and DHA in benthic invertebrates is used as an indicator of their food quality for fish (e.g., Ahlgren et al. 2009, Makhutova et al. 2011, 2016, Gladyshev et al. 2015, 2016b). Gammarids are favorable food items for many fish (MacNeil et al. 1999, Zuev et al. 2011) and a valuable source of essential EPA and DHA (Sushchik et al. 2003, 2007, Makhutova et al. 2011, 2016). In the present study, however, populations of *G. lacustris* had highly variable EPA and DHA content. The gammarids from fishless and saline lakes had significantly higher contents of these essential PUFAs than gammarids inhabiting freshwater lakes that contained fish.

Three different mechanisms may be responsible for the high contents of EPA and DHA in the populations from the fishless lakes: (1) gammarids consume food with high proportions of EPA and DHA, but in low quantity, and do not accumulate storage lipids; (2) gammarids consume food with low proportions of EPA and DHA, but in high quantity, and accumulate storage lipids; (3) gammarids consume food with high proportions of EPA and DHA in high quantity and accumulate storage lipids. All 3 mechanisms were observed in the studied lakes. The populations from Lakes Shira and Shunet had relatively low contents of total FAs (9.8 and 11.6 mg/g ww, respectively) but notably high percentages of EPA together with DHA (18.7% and 19.5%, respectively). The population from Lake Svetloe had a high content of total FAs (17.6 mg/g ww) but the lowest percentages of EPA and DHA (11.6%). Finally, the population from Lake Krasnenkoye had a high content of total FAs (16.8 mg/g ww) and relatively high percentages of EPA and DHA (16.3%).

In contrast to the 3 different mechanisms that regulated contents of EPA and DHA in the populations from fishless lakes, in fish lakes, predation seemed to be the principal determinant of the PUFAs content. The gammarids likely consumed food with low, medium,

or even high proportions of EPA and DHA, but in low quantity. The populations from Lakes Fyrkal, Anikino, Matarak, and Sobachye had low contents of total FAs (8.5, 8.1, 8.1, and 4.2 mg/g ww, respectively) and low (12.7%), medium (15.9% and 14.4%), and high (19.3%) percentages of EPA and DHA. To date we have only a speculative explanation of this finding. In the presence of fish, gammarids could not move freely in water column searching for biochemically valuable food items, but instead they searched for shelters to avoid predators and could not actively forage during hiding. In the fishless lakes, however, populations of *G. lacustris* inhabited both the littoral and the pelagic zones and moved freely in open water areas, whereas in the fish lakes, the gammarids inhabited only the littoral zone, hiding among macrophyte beds, tangles of roots of higher plants, and stones. Additionally, fish could affect the gammarids through trophic chains (J. Vrba, 2017 10th Symposium for European Freshwater Sciences, pers. comm.). Planktivorous fish consumed zooplankton and probably deprived gammarids of a valuable food source. In fishless lakes, gammarids could feed on zooplankton, evidenced by the example of *G. lacustris* from Lake Shira (Fig. 3). Evidently, *G. lacustris* inhabiting fish lakes was limited both in quantity and diversity of food compared to free-swimming gammarids from the fishless lakes. By comparison, lower total FA content in *G. lacustris* from fish lakes may be also due to higher metabolic rates as a reaction to the presence of alarming substances (kairomones) from fish.

The high content of EPA and DHA in gammarids from saline lakes could be explained by the high content of total FAs and high percentages of EPA and DHA (Lake Krasnenkoye) and by high percentages of EPA and DHA while the contents of total FAs were relatively low (Lakes Shira, Shunet, and Utichye-3). Not all populations from the saline lakes had high contents of EPA and DHA, however. The contents of these PUFAs in *G. lacustris* from saline Lake Utichye-1 (1.08 mg/g EPA and 0.42 mg/g DHA) were close to those of *G. lacustris* from freshwater lakes.

Thus, if *G. lacustris* is considered a source of physiologically important EPA and DHA for fish, the most valuable populations are those that inhabit saline fishless lakes. If true, then increasing freshwater salinization, decadal trends of which have been observed around the world (e.g. Dugan et al. 2017, Kaushal et al. 2018), may not lead to a decrease but rather to an increase in the food quality of the gammarids that thrive in saline lakes. We propose some saline small lakes may be used for growing gammarids for aquaculture fish feeding. Stocking fish in fishless lakes dominated by *G. lacustris* may result in lower food quality of the gammarids. *G. lacustris* inhabiting fishless lakes moves freely in open water areas and in the littoral zone. Harvesting gammarids from such lakes and using them for aquaculture fish feeding could be economically efficient. Alternatively, fishless lakes may be used for stocking sterile fish (e.g., triploid rainbow trout) for fattening for a short period, followed by harvesting all of the stocked fish. The calculation of economic efficiency was beyond the scope of our study, but we propose that using saline and fishless lakes as a source of gammarids for fish feeding may be highly beneficial.

In the present study, we performed only pairwise comparisons between fish/fishless, cold/warm, and saline/fresh lakes. When more data are obtained, interactive effects of these factors may be revealed, for instance by factorial ANOVA or/and by redundancy analysis.

Our field study showed variability of FA percentages and EPA and DHA contents in benthic invertebrate *G. lacustris*, an important food source for fish. Differences in FA percentages of the populations were primarily caused by the variability of FA food markers. Thus, among the factors we investigated, food composition of the populations was probably the principal factor affecting the FA composition of the gammarids. According to the contents of physiologically important EPA and DHA, the populations from saline fishless lakes had higher nutritional value than the populations from freshwater lakes and lakes with fish.

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Figure 1. Sampling regions. Numerals indicate the number of lakes sampled.

Примечание [JEF12]: All in Russia?

Примечание [U13]: Yes, all in Russia

Figure 2. Multivariate discriminant analysis of fatty acid composition of *Gammarus lacustris* from lakes: Svetloe (s), Shira (h), Shunet (u), Anikino (a), Krasnenkoye (k), Fyrkal (f) Matarak (m), Utichye-1 (y1), Utichye-3 (y3), and Sobachye (d).

Figure 3. Remains of copepods in gut content of *Gammarus lacustris* from Lake Shira. (a) parts of cephalothorax, (b) mandible, (c) swimming legs, and (d) furca.

Примечание [JEF14]: Please relabel figure with lowercase letters

Примечание [U15]: Done (see the added file with fig 3)

Table 1. Description of the lakes studied: location, surface area (A, in km^2), maximum depth (h_{max}, in m), temperature (t, in °C), total mineralization (M, in mg L^{-1}), the presence/absence of fish, and dominating phytoplankton taxa.

Region, lake	Location	A	h_{max}	t °C				M			Fish	Phytoplankton
				2014	2015	2016	2017	2014	2015	2016		
Ergaki Mountains												
Svetloe	52°48'N, 93°25'E	0.48[a]	24[a]	18	15	n.d.	n.d.	10	11	n.d.	–	Din Chry Bac
Putorana Plateau												
Sobachye	69°01′N, 91°05′E	99.0[b]	162[b]	n.d.	n.d.	n.d.	≤10		13-42[f]		+	Bac Chry Din
Khakasia												
Fyrkal	54°63'N, 89°81'E	13.0	n.d.	n.d.	24	n.d.	n.d.	n.d.	200	n.d.	+	Cya Chl Bac
Krasnenkoye	54°44'N, 90°34'E	0.11	n.d.	n.d.	23	n.d.	n.d.	n.d.	3670	n.d.	–	Cya Bac
Shira	54°30'N, 90°11'E	35.9[c]	23[c]	23	25	n.d.	n.d.	16000	15610	n.d.	–	Cya
Shunet	54°25'N, 90°13'E	0.47[c]	6[c]	21	24	n.d.	n.d.	16460	17820	n.d.	–	Cya Bac
Matarak	54°24'N, 90°11'E	0.79[d]	n.d.	n.d.	n.d.	25	n.d.	n.d.	n.d.	652	+	Chl Cya Din Bac
Utichye-1	54°28'N, 90°25'E	0.45[d]	2[e]	n.d.	n.d.	27	n.d.	n.d.	n.d.	4909	–-	Cya Chl
Utichye-3	54°30'N, 90°27'E	1.40[d]	3[e]	n.d.	n.d.	26	n.d.	n.d.	n.d.	6322	+	Bac Cya
Tyumen area												
Anikino	56°11'N, 69°43'E	0.33	2	25	18	n.d.	n.d.	1097	998	n.d.	+	Cya Chl Bac

Cya = Cyanobacteria, Chl = Chlorophyta, Bac = Bacillariophyta, Chry = Chrysophyta, Din = Dinophyta, n.d. = no data
[a]Anishchenko et al. 2015; [b]Gladyshev et al. 2017; [c]Gladyshev et al. 2015; [d]Parnachev and Degermendzhy 2002; [e]Parnachev et al. 1999; [f]Zadelenov et al. 2017.

Table 2. Mean values of prominent fatty acids (FA; % of the total), total fatty acids (mg/g wet weight), moisture, total organic carbon (C), and nitrogen (N) of *Gammarus lacustris* from the lakes studied. Means in lines labeled with the same letter are not significantly different at $P < 0.05$ after Tukey HSD *post hoc* test (normal distribution, standard errors [SE] are given) or Kruskal-Wallis test with multiple comparisons of mean ranks (nonnormal distribution [SE] are omitted). Fish/M = the presence or absence (+ or –) of fish and average total mineralization (mg/L) of the lakes.

	Svetloe	Sobachye	Fyrkal	Anikino	Krasnenkoye	Shira	Shunet	Matarak	Utichye-1	Utichye-3

Fish/M	−/11	+/13–42	+/200	+/1048	−/3670	−/15805	−/17140	+/652	−/4909	+/6322
12:0	1.1 (0.1)^{A}	0.2 (0.0)^{B}	1.0 (0.2)^{AB}	0.6 (0.1)^{BC}	1.0 (0.1)^{AB}	0.9 (0.1)^{AB}	0.7 (0.1)^{AB}	1.2 (0.4)^{AC}	0.7 (0.1)^{AB}	1.1 (0.3)^{AB}
14:0	3.7 (0.3)^{A}	0.7 (0.1)^{C}	1.8 (0.2)^{BC}	2.3 (0.3)^{BD}	3.6 (0.1)^{AD}	2.0 (0.2)^{BC}	2.2 (0.2)^{BC}	2.7 (0.4)^{AB}	2.8 (0.3)^{AB}	2.6 (0.5)^{AB}
15:0	0.5^{A}	0.4 (0.0)^{A}	0.8^{ABC}	0.7^{AC}	1.0^{BC}	0.7^{AC}	1.3^{B}	0.8^{ABC}	0.8 (0.0)^{BC}	0.7^{ABC}
16:0	16.3 (0.3)^{AC}	14.6 (0.5)^{C}	16.8 (0.6)^{ABC}	17.1 (0.2)^{ABC}	16.9 (0.5)^{ABC}	16.2 (0.7)^{AC}	15.6 (0.5)^{AC}	18.2 (1.1)^{AB}	16.6 (0.8)^{ABC}	19.3 (0.3)^{B}
17:0	0.6 (0.0)^{A}	0.8 (0.0)^{ACE}	1.5 (0.0)^{D}	1.2 (0.1)^{BD}	0.8 (0.0)^{AC}	1.0 (0.0)^{BC}	1.1 (0.1)^{BC}	1.2 (0.1)^{BDE}	1.4 (0.0)^{D}	0.9 (0.0)^{AB}
18:0	3.4 (0.2)^{AB}	3.7 (0.2)^{AEF}	4.9 (0.3)^{CDF}	4.2 (0.2)^{ABC}	3.1 (0.1)^{BE}	4.4 (0.4)^{AC}	3.7 (0.2)^{ABD}	5.3 (0.1)^{CF}	4.5 (0.2)^{AF}	4.5 (0.2)^{AEF}
20:0	0.3 (0.0)^{ABCD}	0.2 (0.0)^{ABCD}	0.4 (0.0)^{CD}	0.3 (0.0)^{BCD}	0.2 (0.0)^{A}	0.3 (0.0)^{ABCD}	0.2 (0.0)^{ABD}	0.3 (0.0)^{D}	0.2 (0.0)^{ABCD}	0.3 (0.0)^{ABCD}
22:0	0.2^{A}	0.3^{AB}	0.4^{B}	0.4^{B}	0.2^{A}	0.4^{AB}	0.3^{AB}	0.3^{AB}	0.2^{AB}	0.3^{AB}
i15:0	0.4^{AC}	0.2 (0.0)^{C}	0.7^{AB}	0.6^{AB}	0.5^{AB}	0.5^{AB}	0.5^{AB}	0.6^{AB}	0.9 (0.1)^{B}	0.4^{AC}
ai15:0	0.3 (0.0)^{AB}	0.1 (0.0)^{B}	0.4 (0.0)^{ABC}	0.2 (0.0)^{B}	0.3 (0.0)^{ABC}	0.4 (0.1)^{AC}	0.4 (0.0)^{AC}	0.5 (0.1)^{C}	0.5 (0.0)^{C}	0.2 (0.0)^{AB}
i17:0	0.3 (0.0)^{A}	0.3 (0.0)^{AEG}	0.6 (0.0)^{BD}	0.5 (0.0)^{CDF}	0.4 (0.0)^{AC}	0.7 (0.0)^{B}	0.5 (0.0)^{CDE}	0.7 (0.1)^{BF}	0.7 (0.0)^{B}	0.5 (0.0)^{CDFG}
ai17:0	0.2^{ABC}	0^{BC}	0.3^{A}	0.02^{B}	0.2^{AB}	0.3^{AC}	0.2^{A}	0.1^{AB}	0.2^{AB}	0^{BC}
16:1n-9	0.4^{ABC}	0.3^{C}	0.4^{BC}	0.7^{AD}	0.8^{ABD}	0.5^{ABCD}	0.4^{ABCD}	0.3^{C}	0.9^{D}	0.3^{C}
16:1n-7	8.6 (0.5)^{AB}	7.3 (1.1)^{AB}	6.1 (0.5)^{A}	10.5 (1.2)^{BD}	10.4 (0.8)^{BCD}	6.4 (0.6)^{A}	8.6 (0.6)^{AB}	7.8 (0.4)^{AB}	6.6 (0.4)^{AC}	13.9 (0.3)^{D}
18:1n-9	21.9 (0.7)^{AB}	23.2 (0.6)^{BF}	18.2 (0.6)^{CD}	13.3 (0.5)^{E}	17.3 (1.3)^{CD}	19.1 (0.8)^{AC}	16.6 (0.4)^{CD}	18.7 (0.4)^{ACF}	24.0 (1.0)^{B}	14.1 (0.2)^{DE}
18:1n-7	3.8 (0.1)^{A}	5.5 (0.2)^{B}	6.2 (0.2)^{B}	5.9 (0.3)^{B}	4.0 (0.1)^{A}	5.4 (0.1)^{B}	5.9 (0.1)^{B}	5.6 (0.1)^{B}	5.7 (0.1)^{B}	5.8 (0.1)^{B}
16:2n-4	0.6^{ABC}	0.1^{CD}	0.1^{CD}	0.6^{ACD}	1.3^{B}	0.3^{ACD}	0.7^{AB}	0.2^{ACD}	0.1^{D}	1.1^{AB}
16:3n-4	0.5^{AB}	0.1^{ACD}	0.03^{C}	0.9^{AB}	1.1^{B}	0.2^{AC}	0.8^{BD}	0.2^{ACD}	0.05^{C}	1.2^{B}
18:2n-6	9.8 (0.4)^{A}	7.0 (0.3)^{EF}	8.0 (0.4)^{AF}	5.7 (0.5)^{CDE}	3.6 (0.3)^{B}	4.1 (0.3)^{B}	4.6 (0.2)^{BC}	6.6 (0.4)^{DF}	5.5 (0.2)^{BDE}	4.5 (0.3)^{BD}
18:3n-6	0.5^{ABC}	0.3^{AC}	0.4^{CD}	0.6^{ABC}	0.8^{B}	0.3^{AC}	0.4^{ACD}	0.6^{BD}	0.3^{CD}	0.5^{ABC}
18:3n-3	2.6^{ACD}	1.6^{BD}	3.4^{AC}	4.2^{C}	4.8^{C}	2.2^{ABE}	1.6^{B}	1.7^{BD}	3.6^{CE}	1.6^{BD}
18:4n-3	2.2 (0.2)^{A}	0.3 (0.0)^{C}	0.4 (0.0)^{C}	0.6 (0.1)^{C}	1.0 (0.0)^{BC}	1.1 (0.1)^{B}	1.2 (0.1)^{B}	1.1 (0.1)^{BC}	0.7 (0.1)^{BC}	0.8 (0.0)^{BC}
20:4n-6	3.3 (0.2)^{AB}	8.0 (0.2)^{F}	6.6 (0.3)^{DF}	5.5 (0.4)^{DE}	2.6 (0.2)^{A}	5.1 (0.5)^{CD}	3.9 (0.3)^{ABC}	4.8 (0.3)^{BD}	4.5 (0.3)^{BCE}	4.2 (0.2)^{ABCE}
20:3n-3	0.3^{AB}	0.2^{BD}	0.4^{ACD}	0.5^{C}	0.5^{C}	0.4^{A}	0.2^{BD}	0.2^{B}	0.4^{ACD}	0.2^{B}
20:4n-3	0.4 (0.0)^{A}	0.0 (0.0)^{D}	0.2 (0.0)^{CD}	0.2 (0.0)^{CD}	0.3 (0.0)^{ACE}	0.6 (0.1)^{B}	0.3 (0.0)^{AC}	0.2 (0.0)^{CD}	0.2 (0.0)^{CE}	0.1 (0.0)^{DE}
20:5n-3	8.3 (0.4)^{A}	14.0 (0.4)^{D}	9.7 (0.4)^{AC}	12.1 (0.4)^{BDE}	13.6 (0.7)^{D}	11.0 (0.5)^{BC}	12.7 (0.2)^{BD}	10.3 (0.9)^{ACE}	8.6 (0.3)^{A}	12.8 (0.4)^{BDE}
22:5n-6	0.5 (0.0)^{AC}	0.4 (0.0)^{ABC}	0.7 (0.1)^{A}	0.6 (0.1)^{A}	0.2 (0.0)^{B}	0.6 (0.0)^{A}	0.6 (0.0)^{A}	0.2 (0.0)^{BC}	0.2 (0.1)^{BC}	0.1 (0.0)^{B}
22:5n-3	0.5 (0.0)^{A}	1.5 (0.1)^{D}	1.1 (0.1)^{BCD}	1.2 (0.1)^{CD}	0.6 (0.0)^{AB}	0.9 (0.1)^{B}	0.9 (0.1)^{BC}	0.7 (0.1)^{AB}	0.7 (0.1)^{AB}	0.9 (0.1)^{BC}
22:6n-3	3.3^{AC}	5.3^{C}	2.9^{AC}	3.8^{AC}	2.7^{A}	7.8^{C}	6.8^{C}	4.1^{AC}	3.2^{AC}	3.8^{AC}
Total FA	17.6 (0.9)^{A}	4.2 (0.3)^{C}	8.5 (1.0)^{BC}	8.1 (0.9)^{BC}	16.8 (0.9)^{AD}	9.8 (1.7)^{BC}	11.6 (1.3)^{BD}	8.1 (0.6)^{BC}	13.0 (1.7)^{AB}	10.5 (0.6)^{BCD}
BFA	2.5 (0.1)^{A}	2.0 (0.1)^{A}	5.1 (0.3)^{CD}	3.7 (0.3)^{B}	4.1 (0.2)^{BD}	4.0 (0.3)^{BD}	5.8 (0.3)^{C}	4.4 (0.6)^{BD}	5.1 (0.2)^{CD}	3.2 (0.2)^{AB}
DFA	13.6 (0.6)^{ABCD}	8.3 (1.3)^{AC}	8.1 (0.7)^{A}	14.6 (1.9)^{BCD}	17.1 (1.1)^{BD}	9.1 (0.9)^{A}	12.6 (1.0)^{AB}	10.9 (0.7)^{AB}	9.7 (0.6)^{AC}	19.0 (0.9)^{D}
G-C FA	13.1^{A}	8.8^{AB}	12.0^{A}	10.8^{A}	10.0^{AB}	6.7^{B}	6.7^{B}	8.9^{AB}	9.7^{AB}	6.7^{B}

n-3/n-6	1.2 (0.1)^{A}	1.4 (0.0)^{AEF}	1.1 (0.1)^{A}	1.8 (0.1)^{BE}	3.2 (0.3)^{D}	2.2 (0.1)^{BC}	2.3 (0.1)^{C}	1.4 (0.1)^{AEF}	1.5 (0.0)^{AEF}	2.0 (0.1)^{BCF}
DHA/EPA	0.4 (0.02)^{A}	0.4 (0.02)^{A}	0.3 (0.02)^{AD}	0.3 (0.03)^{AD}	0.2 (0.01)^{D}	0.7 (0.04)^{B}	0.5 (0.02)^{C}	0.4 (0.02)^{A}	0.4 (0.02)^{A}	0.3 (0.01)^{AD}
PUFA/SFA	1.3 (0.04)^{AB}	1.9 (0.1)^{C}	1.3 (0.05)^{AB}	1.3 (0.04)^{A}	1.3 (0.03)^{AB}	1.4 (0.1)^{A}	1.4 (0.04)^{A}	1.1 (0.13)^{AB}	1.1 (0.06)^{B}	1.1 (0.05)^{AB}
Moisture, %	81.4 (0.6)	82.7 (0.4)	75.4	80.8 (0.5)	77.3 (1.4)	79.2 (1.5)	78.0 (0.7)	73.4 (0.6)	74.7 (0.4)	74.8 (0.4)
C, % dw	41.5 (2.6)	35.1 (0.4)	36.5 (0.3)	38.1 (0.9)	43.7 (3.1)	37.2 (0.9)	39.2 (1.4)	34.5 (0.6)	38.1 (0.2)	37.1 (0.3)
N, % dw	7.6 (0.5)	8.6 (0.2)	8.1 (0.2)	8.1 (0.1)	8.5 (0.5)	8.1 (0.3)	7.8 (0.2)	7.9 (0.1)	9.1 (0.1)	8.0 (0.1)

BFA = FA markers of bacteria (i13:0, ai13:0, 13:0, 13:1, i15:0, ai15:0, i15:1, 15:0, 15:1, i17:0, ai17:0, i17:1, 17:0, 17:1); DFA = FA markers of diatoms (14:0, 16:1n-7, 16:2n-7; 16:2n-4, 16:3n-4, 16:4n-1); G-C FA = FA markers of green algae and cyanobacteria (16:2n-6, 16:3n-6, 16:3n-3, 16:4n-3, 18:2n-6, 18:3n-3, 18:3n-6); DHA = 22:6n-3; EPA = 20:5n-3; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids.

Table 3. Results of multivariate discriminant analysis of fatty acid composition of *Gammarus lacustris* inhabiting the lakes studied.

Subject of analysis (parameter, lakes, fatty acids)	Root 1	Root 2
Canonical R	0.964	0.947
Chi-square	1071	808
Degrees of freedom	162	136
P	0.000000	0.000000
Canonical mean values		
Svetloe	4.859	−4.775
Shira	−2.912	−1.184
Shunet	−3.909	−1.019
Anikino	0.738	1.216
Krasnenkoye	−5.392	−1.556
Fyrkal	3.197	2.260
Matarak	1.923	3.543
Utichye-1	−0.589	4.319
Utichye-3	2.205	2.865
Sobachye	3.741	2.924
Structural factor coefficients		
12:0	0.035	−0.072
14:0	0.020	−0.148
16:0	0.028	0.073
17:0	−0.071	0.305
18:0	0.033	0.165
20:0	0.096	0.043
ai15:0	−0.093	0.040
i17:0	−0.130	0.241
16:1n-7	0.005	−0.005
18:1n-9	0.116	−0.061
18:1n-7	−0.078	0.360
18:2n-6	0.394	−0.181
18:4n−3	0.077	−0.361
20:4n-6	0.096	0.238
20:4n-3	−0.100	−0.301
20:5n-3	−0.189	0.095
22:5n-6	0.006	−0.089
22:5n-3	−0.008	0.197

Table 4. Values of the minimum, median, maximum and 10th and 90th percentiles for each fatty acid ~~proportion~~percentages (% of the total) and EPA (20:5n-3*) and DHA (22:6n-3*) contents (mg/g ww). The length of row for each fatty acid is 115.

FA	Min	Median	Max	Percentiles	
				10^{th}	90^{th}
12:0	0.11	0.75	3.70	0.22	1.46
14:0	0.00	2.48	7.24	0.75	4.07
15:0	0.33	0.71	1.87	0.43	1.31
16:0	12.68	16.43	24.20	14.37	19.34
17:0	0.42	0.96	1.95	0.55	1.49
18:0	2.24	4.10	9.41	2.88	5.21
20:0	0.05	0.24	0.62	0.14	0.4
22:0	0.00	0.25	1.01	0.15	0.51
i15:0	0.12	0.48	1.34	0.31	0.86
ai15:0	0.09	0.31	1.14	0.16	0.54
i17:0	0.18	0.51	1.23	0.27	0.78
ai17:0	0.00	0.15	0.86	0.00	0.41
16:1n-9	0.14	0.43	1.80	0.26	0.91
16:1n-7	2.85	8.00	16.55	4.72	13.64
18:1n-9	10.14	18.06	28.94	13.04	24.02
18:1n-7	3.03	5.48	7.52	3.74	6.53
16:2n-4	0.00	0.39	1.72	0.06	1.28
16:3n-4	0.00	0.35	2.32	0.00	1.37
18:2n-6	2.39	5.60	14.02	3.46	9.22
18:3n-6	0.12	0.43	1.36	0.22	0.81
18:3n-3	1.07	2.48	8.17	1.35	5.08
18:4n-3	0.18	0.86	3.97	0.36	2.1
20:4n-6	1.73	4.38	8.58	2.53	7.46
20:3n-3	0.12	0.30	0.81	0.21	0.53
20:4n-3	0.00	0.27	0.99	0.12	0.51
20:5n-3	6.04	11.47	17.03	7.93	14.22
22:5n-6	0.00	0.45	1.83	0.14	0.82
22:5n-3	0.23	0.77	1.78	0.44	1.46
22:6n-3	1.62	3.90	12.47	2.42	7.87
20:5n-3*	0.19	1.12	3.10	0.56	2.03
22:6n-3*	0.08	0.45	1.57	0.24	0.79

Примечание [U16]: I added "percentages (% of the total)" to clarify that all FAs are shown as percentages, but two FAs (with asterisks) are additionally shown as mg/g ww

Примечание [JEF17]: What does the asterisk indicate?

Table 5. Average values of EPA and DHA (mg/g ww [SE]) of *Gammarus lacustris* inhabiting fish lakes (Fish) number of samples, n = 44~~55~~; fishless lakes (No Fish), n = 71; warm lakes (Warm), n = 91; cold lakes (Cold), n = 24; saline lakes (Saline), n = 60; freshwater lakes (Fresh), n = 55; and significance of differences (p values) after Mann-Whitney U test (*significant values).

	Fish	No Fish	p value	Warm	Cold	p value	Saline	Fresh	p value
EPA	0.93 (0.06)	1.41 (0.07)	0.0000*	1.23 (0.07)	1.21 (0.09)	0.5938	1.40 (0.09)	1.04 (0.06)	0.0054*
DHA	0.29 (0.01)	0.6 (0.03)	0.0000*	0.48 (0.03)	0.49 (0.04)	0.4594	0.58 (0.03)	0.37 (0.02)	0.0000*

Примечание [U18]: I found my mistake