Feeding spectra of *Arctodiaptomus salinus* (Calanoida, Copepoda) using fatty acid trophic markers in seston food in two salt lakes in South Siberia (Khakasia, Russia)

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Abstract During two vegetation seasons (2004–2005), we compared feeding spectra of *Arctodiaptomus salinus* (Calanoida, Copepoda) populations inhabiting two neighboring salt lakes, Shira and Shunet, Khakasia, Russia, using fatty acid (FA) trophic markers. Sestonic FA composition in two lakes moderately differed, whereas levels of diatom FA markers were higher in Lake Shunet and of *Cyanobacteria* and green algae markers in Lake Shira. In general, markers in storage lipids—triacylglycerols (TAG) of *A. salinus*—reflected the differences in sestonic composition of the two lakes. Nevertheless, TAG fraction was also enriched by FA trophic markers of the minor components of seston, which were selectively ingested by the

animals. In Lake Shira, *A. salinus* had significantly higher concentrations of bacterial FA markers in TAG. In Lake Shunet, TAG of *A. salinus* contained significantly higher relative amounts of 18:4 ω 3, 18:5 ω 3 and C22 polyunsaturated fatty acids (PUFA), which indicated marked contribution of cryptophytes or (and) flagellates into the diet. Laboratory experiments showed feeding on *Cryptomonas* and sulfur purple bacteria in Lake Shunet and ciliates and colonial picoplankton in both lakes, and generally confirmed the differences in FA trophic markers in *A. salinus* between the lakes. The two populations of *A. salinus* markedly differed in levels of essential long-chain PUFA, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, although the levels of these FA in seston were quite similar between the two lakes. The higher levels of the essential ω 3 PUFA in *A. salinus* in Lake Shunet may be an adaptive response of the animals to a vertical stratification of physico-chemical conditions and significantly higher salinity levels at the boundary of adjacent bottom layer in this lake.

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Introduction

Calanoid copepods often dominate or co-dominate the zooplankton community in both fresh- and saline

water ecosystems (Wen and Zhi-hui 1999; Jersabek et al. 2001; Swadling et al. 2001; Torke 2001; Tolomeyev 2002; Guerrero et al. 2006). In contrast to cladocera and rotifers, copepods have a higher average age and are slow-growing organisms with relatively low reproductive output (e.g. Sommer and Stibor 2002). To compete with the fast-growing species, they have to either occupy different food niche or to use various trade-offs between the limited food resources and extreme physico-chemical environmental conditions. The dominance of *Arctodiaptomus salinus* in the zooplankton of two Khakasian saline lakes Shira and Shunet (Siberia, Russia) can be considered as an example of the adaptation of copepods to the severe environment conditions. In general, this species is common in saline lakes all over the world, presumably due to its greater salinity tolerance (Guerrero et al. 2006, 2007). The phytoplankton in both lakes is dominated by cyanobacterial species, including a majority of picoplankton forms (Quesada et al. 2002; Degermendzhy et al. 2003) that obviously cannot be considered as an important food source for these calanoids that are adapted to filter-feed on coarse food particles (Kleppel 1993; Sommer and Sommer 2006). However, several physiological and ecological traits of the copepod can provide clues to the understanding of strategies adapted by these calanoids. The filtering rates of copepods in the cold and saline waters, i.e. water of high density, are obviously higher than those of Cladocera and Rotifera. Hence, the calanoids can benefit at low total concentration of food, even though the consumption of small-size particles is much less efficient (Hansen et al. 1994; Bern 1994). Selective feeding based on taste discrimination allows calanoid copepods to avoid undesired food, often of low nutrition quality or toxic species (DeMott 1991; Kurmayer and Juttner 1999). Thus, they can optimize the intake of valuable food even when it is present in the environment in a low concentration. It is known that copepods can withstand periods of food deficiency (summer phytoplankton depression or winter low production) due to accumulated energy reserves in the form of triacylglycerols (TAG) or wax esters (Hagen and Auel 2001). In addition, specific accumulation of PUFAs may be responsible for maintaining cell membrane fluidity at low temperatures (the so-called homeoviscous adaptation), which allows copepods to remain active during the winter and, as a result, to

prolong generation time and life cycle (Kattner et al. 2007; Smyntek et al. 2008; Brett et al. 2009).

Studies on the nutritional requirements and feeding behavior of copepods are highly necessary to understand degree and ways of their adaptation to the given environment. The direct microscopic analysis of gut contents of zooplankton are often constrained by two reasons: (1) some ingested food particles found in the gut are there because they are not assimilated (Porter 1975) so that the presence of food in the gut can present a biased picture; (2) in contrast, some of the phytoplankton species ingested can be easily and quickly digested and thus may not be detected in the gut (Knisley and Geller 1986). Thus, in recent decades, biochemical markers, namely fatty acids, have been widely used to estimate sources and flows of organic matter in various ecosystems and assimilated food items in the biomass of aquatic consumers (Napolitano 1999; Rooker et al. 2006; El-Sabaawi et al. 2009). Most zooplankton species are considered to have weak ability to synthesize fatty acyl groups of their lipids and, hence, have to obtain most part of fatty acids with the food (Goulden and Place 1990; Brett et al. 2006). Meanwhile, the accumulation of storage lipids is sufficient in some zooplankton species, especially in copepods (Hagen and Auel 2001). It has been considered that bulk of fatty acyl chains in TAG originate from food and can reflect the composition of their food consumed during the preceding 3–4 weeks (Bourder and Amblard 1989; Desvillettes et al. 1997).

During this study, our aim was to identify and compare the actual food sources for *A. salinus* in two neighboring saline lakes that contrasted in hydro-physical and chemical conditions as well as in their plankton species composition. We used two approaches to identify the original diet of the copepods. First, we analyzed fatty acid biomarkers in seston and animals in the lakes, and second, we carried out feeding experiments to show the groups and species of microorganisms that were consumed. We addressed four main questions in order to outline how plastic are the calanoid copepod in adapting to biochemical of their food: (1) How and to what extent the fatty acids of *A. salinus* reflect the fatty acid composition of their seston food? (2) How comparable are the fatty acid levels of *A. salinus* between the lakes? (3) Do the differences in feeding spectra explain the variability of fatty acid composition in

copepod populations in the lakes? and (4) Do the different salinity conditions of two lakes explain, at least partly, the variations in fatty acid composition of the two populations of *A. salinus*?

Study site

We carried out the study in two neighboring saline, meromictic lakes Shira and Shunet located in the Republic of Khakasia, Russia. Lakes are situated in steppe zone, which is characterized by a sharply continental climate. Lakes have ice-free period for about 6 months of the year.

Lake Shira (54°30'N and 90°14'E; 354 m above sea level) has a surface area 34.5 km², mean depth—11.2 m, maximum depth—23.3 m. The salinity ranged from 11.5 to 14.5 g l⁻¹ over the depth with the decrease up to 9.5 g l⁻¹ in surface layers in May after the ice melting. The only small river that flows into the lake accounts for 80% of the lake's total annual inflow. The main ecological features of Lake Shira are given elsewhere (Degermendzhy and Gulati 2002; Gulati et al. this volume). Briefly, in the summer period the stratification thermocline is located at the depth about 6 m and average depth of chemocline zone is about 13 m. *Arctodiaoptomus salinus* is the only dominant crustacean present throughout the year in the zooplankton (Zadereev and Tolomeyev 2007). In summer, the biomass of *A. salinus* is about 20 g m⁻². In July, the rotifers *Brachionus plicatilis* and *Hexarthra oxiiuris* develop large populations, reaching up to 50% of total zooplankton biomass. During the stratification period, *A. salinus* has a bimodal vertical distribution with a slight decrease in abundance in the metalimnion (Zadereev and Tolomeyev 2007). The distribution of zooplankton in deeper layers is limited by the anoxic zone at 14–15 m. The phytoplankton community in Lake Shira is dominated by Cyanobacteria *Lyngbya contorta* and by several *Aphanothece* morphotypes. The “microbial loop” comprises heterotrophic nano-flagellates (up to 10 thousand cells ml⁻¹), ciliates *Strombidium* sp. and *Cyclidium* sp., and photosynthetic bacteria *Amoebobacter purpurea* in the chemocline zone.

Lake Shunet (54°36'N and 90°20'S) is also of internal drainage, its surface area is 0.47 km²,

maximum depth is 6.2 m and anoxic zone starts below 5.2 m. The salinity of the water from surface to the depth of 4 m ranges from 14.4 to 19.2 g l⁻¹; it sharply increases up to 40 g l⁻¹ at 5 m and exceeds 60 g l⁻¹ near the bottom. *A. salinus* also dominates in zooplankton with the summer biomass of 2.5–4.0 g m⁻³ with the density of 60–150 ind. l⁻¹. Three rotifer genera, *Keratella*, *Hexarthra* and *Brachionus*, also occur in zooplankton community.

The taxonomic composition of phytoplankton of Lake Shunet was evaluated only in July 2002 when picoplankton *Cyanobacteria Synechocystis* sp. up to 60% (0.12 mg l⁻¹) of total phytoplankton biomass dominated the phytoplankton community (Degermendzhy et al. 2003). *Cyclotella* sp., a diatom, reached up to (0.06 mg l⁻¹), i.e. 30% of total phytoplankton biomass. Green algae dominated by *Dictyosphaerium tetrachotomum* comprised only 10% (0.03 mg l⁻¹) of total phytoplankton biomass. Purple and green phototrophic bacteria were highly abundant (their total density—10⁸ cells ml⁻¹) and formed a thin layer of about 5 cm in the chemocline zone (Rogozin et al. 2005). A diverse microorganism community, comprising ciliates *Strombidium* and *Cyclidium* spp., and autotrophic flagellates *Cryptomonas* sp. (up to 25 * 10⁴ cells l⁻¹) was found above this layer at depth of 5.0–5.2 m.

Methods

Sampling

In both lakes, we sampled during June and July in both 2004 and 2005. Seston in Lake Shira was sampled with a 6-L Ruttner-like sampler from the depths of 3 and 9 m that corresponded to the mean depths of epilimnion and oxygenic hypolimnion zones, respectively. Hypolimnion of Lake Shunet (depth lower than 5 m) was anoxic in June and July of both years, with a complete absence of zooplankton. Hence, we sampled only from the epilimnion (mean depth 2.5 m). The samples were prescreened through 115-μm nylon mesh to remove the most large zooplankton organisms. For fatty acid analyses, seston was collected from 400–600 ml sub-samples by vacuum filtration onto membrane filters (Vladipor, pore size 0.75–0.85 μm), precovered with a layer of BaSO₄ to facilitate the separation of residues. The

filters were dried at 35°C within an hour, and the residue was then separated from filters and placed into chloroform–methanol mixture (2:1, v/v) and frozen.

Zooplankton was sampled with closing plankton net of 115- μ m nylon mesh. In Lake Shira, net was hauled from 5 m to surface and from 10 to 7 m to cover the epilimnion and hypolimnion, respectively. In Lake Shunet, the net was hauled from 5 m to surface to cover whole epilimnion zone in one stretch. The zooplankton samples were washed several times with filtered (0.7–0.8- μ m pore size filter) lake water, and live animals were allowed to swim in this filtered water for several hours to empty their guts. Microscopical analysis showed that the collected zooplankton mainly comprised *A. salinus*, and other crustaceans and rotifers were a small component (ca. 7–12% of total biovolume). The animals were then collected onto a sieve mesh and placed on filter paper for a short while to wipe of surface moisture; they were then transferred into the chloroform–methanol mixture and frozen until the further biochemical analyses.

Biochemical analyses of seston and *Arctodiaptomus*

The procedure for lipid analyses of seston and hydrobionts' biomass is described in detail elsewhere (Sushchik et al. 2003b; Makhutova and Khromechek 2008). Briefly, lipids from seston and *Arctodiaptomus* biomass were extracted by chloroform–methanol (2:1, v/v) three times. To analyze the fatty acid composition of total lipids, part of the total lipid extract was methylated (see description below). Another part of the lipid extract was fractionated by thin-layer chromatography (TLC) on silica gel G with a solvent system for neutral lipids, as described by Sushchik et al. (2003b). The lipid fraction containing triacylglycerols was scrapped from the silica gel plate (see Fig. 1), and the solvent was evaporated before methylation. The methyl esters of fatty acids (FAMES) of total lipids and of the triacylglycerols were prepared in a mixture of methanol–sulfuric acid (20:1, v/v) at 90°C during 2 h. FAMES were analyzed and identified on a gas chromatograph equipped with a mass spectrometer detector (GCD Plus, Hewlett-Packard, USA) using a capillary HP-FFAP column as described by Sushchik et al. (2004).

Fig. 1 Qualitative thin-layer chromatogram of lipids from *A. salinus* biomass collected in epilimnion (*Shi-E*) and hypolimnion (*Shi-H*) of Shira and Shunet (*Shu*) lakes (Khakasia, Russia). *Std* standard reference mixture, *FFA* free fatty acids

Feeding experiments

Experiments were aimed to find out the feeding selectivity of *A. salinus* on plankton organisms in the investigated lakes. While FATM approach differentiates food resources between large microbial groups, the direct measurements allow identifying the species consumed. The study allows interpreting the FATM sources in the animal's food. For instance, domination of picoplankton *Cyanobacteria* in seston and finding the cyanobacterial markers in zooplanktors does not necessarily infer the feeding on them. If these species are not consumed (due to small size), the cyanobacterial markers in zooplanktors may originate from non-dominating but large species or they were accumulated formerly.

Feeding of *A. salinus* on natural seston of the lakes Shira and Shunet was conducted in the laboratory using the flow-through method (Gladyshev et al. 1999). Three paired glass chambers of 50 ml each with continuously pumped-through lake water were used to find out feeding selection on phytoplankton and protozoa species (Fig. 2). Each experimental chamber contained 50–60 individuals of *A. salinus*, while the controls contained no animals. Chambers have a removable bottom made of net with 80- μ m mesh to prevent the zooplankton from escaping the chamber and to assist in transferring the animals into the chamber at the start of the experiment. Viscosity of surface film formed on the net bottom prevents the

Fig. 2 The scheme of flow-through method (modified from Gladyshev et al. 1999). 1 Lake water taken from 5, 5.2 m in Lake Shunet and from 12 m in Lake Shira for three experiments, 2 feeding water filtered from zooplankton through net (mesh size 80 µm), 3 shaker, 4 connecting tubes, 5 peristaltic pump, 6, 7 experimental and control chambers with the net bottoms. Viscosity of surface film formed on the net

bottom prevents the water from running out entirely but allows the dosed flow, 8 copepods, 9, 10 experimental and control collecting vessels with fixing Lugol's solution, 11 10-ml sample for protozoa counting, 12 50-ml sample filtered through the membrane filter for phytoplankton counting under epifluorescent microscope

uncontrolled water flow, but allows only the flow driven by a peristaltic pump. After passing the chambers, the water with seston particles flows into collecting vessels wherein the seston was fixed immediately with Lugol's solution, to prevent changes in cells number due to either growth or lysis. Flow rate of the water was 200 ml day⁻¹.

Lake seston used in experiments was taken from 5.0- to 5.2-m stratum in Lake Shunet and from 12 m in Lake Shira. These strata in the two lakes, respectively, had very diverse species composition of microorganisms, observed during preliminary microscopic analysis. In the first of two experiments conducted with the seston of Lake Shunet, we used the water from 5.0 m depth, where ciliates and flagellates were abundant; the second experiment was conducted with seston from 5.2 m depth, and 10× diluted with lake water from 3 m. The objective of this experiment was to measure feeding of *A. salinus* on sulfur purple bacteria. This resource might be important for calanoids in meromictic lakes as it has been reported by Overmann et al. (1999). We did not use natural concentrations of bacteria because of their very high density. However, preliminary observations revealed that the calanoids can exploit this food resource by either migrating into the “purple layer” for a short time or when bacteria are brought into the upper layers due to turbulence. Feeding of *A. salinus* in Lake Shira was studied using seston from 12 m, as

done previously on the seston from epilimnion zone by Tolomeyev (2002).

Experiments lasted 3 days, and samples for phytoplankton and protozoa counting (50 and 10 ml, respectively) were taken daily. Seston was concentrated on the membrane filters (Vladipor, pore size 0.75–0.85 µm) by filtration. Algae on filters were counted under epifluorescent microscope Axioscop 40 (Carl Zeiss). Protozoa organisms were counted in a Fuchs-Rosenthal chamber without the concentrating treatment. During the experiment, the lake water in the feed vessel was refreshed daily.

If the density of the certain species considerably decreased in the experimental chambers compared with the controls, this decrease was assumed to be caused by their consumption by *A. salinus*. For each consumed species, daily ration was calculated as the mean of rations obtained each day during 3-day experiment. Daily ration was calculated as follows:

$$R = (N_c - N_e) \cdot w \cdot F/B,$$

where N_c and N_e are concentrations of the food items (cells ml⁻¹) in control and experimental collecting vessels, respectively; w is mean wet weight of food cells (mg) calculated from biovolume using the conversion factor of 1.0 (e.g. Gosselain et al. 2000), F —daily flow volume (ml), B —total wet biomass (mg) of *A. salinus* in the experimental chamber.

Standard and multivariate statistics

Student's *t*-test and paired *t*-test were carried out conventionally to compare FA profiles of seston and *Arctodiaptomus* in two lakes. Principal component analysis (PCA) using Canoco software, version 4.5 (Leps and Smilauer 2003) was performed to elucidate main factors contributing in variability of FA composition of the animals.

Results

Fatty acids in seston

A total of 43 fatty acids were identified in all seston samples from both lakes, and levels of main FA are

given in Table 1. In general, levels of saturated and monounsaturated acids were slightly higher in Lake Shunet than those in Lake Shira. FA percentages in seston of the upper and lower layers in Lake Shira were quite different: levels of PUFA fraction in the upper layer were smaller than those of saturated acids. The vertical distribution of sestonic fatty acids in Lake Shira had been earlier recorded (in 2001), and appreciable variation in most prominent fatty acids, e.g., saturated FA, 16:0 and 18:3, with the depth had been found (Fig. 3). In Lake Shira, epilimnion and hypolimnion layers were almost equal in their size, while in Lake Shunet the epilimnion zone was rather thick and comprised most part of water column. Therefore, we used different sampling procedures for seston samples in these two lakes (see “Methods”). For comparing the

Table 1 Fatty acid composition (% of the total) of seston in lakes Shira and Shunet (Khakasia, Russia), 2005

FA	Shira				Shunet					
	30 Jun 3 m	30 Jun 9 m	10 Jul 3 m	10 Jul 9 m	2 Jul 2.5 m	2 Jul 2.5 m	2 Jul 2.5 m	7 Jul 2.5 m	7 Jul 2.5 m	7 Jul 2.5 m
14:0	8.4	7.2	6.1	6.8	12.5	12.6	13.0	17.2	8.6	13.5
16:0	30.3	25.4	32.0	27.3	32.4	26.5	31.2	26.8	30.3	25.4
18:0	14.4	8.3	21.3	13.5	8.7	13.3	8.7	8.8	30.5	11.1
Other SAFA	5.6	4.5	16.1	3.6	4.0	1.7	3.0	2.8	1.8	2.9
Total SAFA	58.7	45.4	75.5	51.2	57.6	54.1	55.9	55.6	71.2	52.9
Total BFA	4.0	3.9	2.5	1.7	1.4	1.5	0.8	3.6	1.5	3.8
16:1 ω 7	8.7	9.3	5.5	7.7	12.1	15.1	14.1	18.6	11.1	14.3
18:1 ω 9	14.3	12.0	8.7	17.5	16.7	14.7	16.0	12.5	10.2	14.5
Other MUFA	3.2	4.9	3.8	2.6	4.0	5.9	5.6	5.0	2.2	4.2
Total MUFA	26.2	26.2	18.0	27.8	32.8	35.7	35.7	36.1	23.5	33.0
16:2 ω 6	ND	0.1	ND	ND	ND	ND	1.3	ND	ND	ND
16:2 ω 4	1.3	1.5	ND	1.5	1.6	1.3	ND	0.2	0.3	0.6
16:3 ω 3	1.2	1.9	ND	2.1	ND	ND	ND	ND	0.1	0.3
18:2 ω 6	2.3	3.1	1.6	2.9	2.2	2.5	2.2	1.4	1.1	2.1
18:3 ω 3	3.6	7.3	2.4	6.0	1.7	1.0	1.6	1.1	0.7	1.5
18:4 ω 3	ND	1.3	ND	1.3	ND	1.1	ND	ND	0.3	1.8
20:4 ω 6	0.1	0.2	ND	ND	ND	ND	ND	ND	ND	ND
20:3 ω 3	0.1	0.4	ND	0.5	ND	ND	ND	ND	ND	ND
20:4 ω 3	0.2	0.5	ND	0.3	0.3	0.1	0.3	ND	ND	0.3
20:5 ω 3	1.6	5.1	ND	3.4	1.4	1.1	1.3	1.4	0.8	1.9
22:5 ω 3	0.0	0.4	ND	ND	ND	ND	ND	ND	ND	ND
22:6 ω 3	0.3	1.3	ND	0.5	0.7	1.1	0.3	ND	0.2	0.7
Total PUFA	10.7	23.1	4.0	18.5	7.9	8.2	7.0	4.1	3.5	9.2

SAFA saturated fatty acids, BFA branched fatty acids (include i14:0, i15:0, ai15:0, i16:0, i17:0, ai17:0), MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids, ND not detected

Fig. 3 Depth distribution of seston fatty acid markers (% of the total) in Lake Shira (Khakasia, Russia), 2001, August. SAFA saturated fatty acids, bacterial sum of branched and straight odd-numbered FA

main FAs in seston of the two lakes, we calculated their average fatty acid values of samples from the upper and lower layer from Lake Shira. The average values in Lake Shira were compared with those for 2.5-m samples from Lake Shunet (Fig. 4). The

percentages in total FAs of some fatty acids (14:0, 16:1 ω 7, 16:3 ω 3 and 18:3 ω 3) in seston from the two lakes significantly differed. According to biomarker approach (Napolitano 1999; Brett et al. 2009), the higher levels of FA 14:0 and 16:1 ω 7 in Lake Shunet

Fig. 4 Major saturated, monoenic and polyenoic FA (% of the total) in seston of Shira and Shunet lakes (Khakasia, Russia), 2005. The data are means of values from Table 1, bars are standard errors. Asterisks denote statistically significant differences after Student's *t*-test ($P < 0.05$)

indicated higher contribution of the diatom species in seston, while the higher levels of FA 18:3ω3 in Lake Shira were due both to *Oscillatoriales* and green algae in pelagic seston. Higher levels of FA 16:3ω3 also implied greater role of green algae in Shira. Apart from the mentioned FA, the averaged levels of other FA in seston of the lakes did not significantly differ.

Fatty acids in total lipids of *A. salinus*

Based on prominent fatty acids identified in samples of total lipids of *A. salinus* (Tables 2, 3), it is clear that fatty acid profiles of the animal body were much more diverse than those of seston in these two lakes. The animals had numerous branched C14–17 saturated and monoenic acids and PUFAs with C16–C22 carbon atom chains. The dominant FA in the copepods were the same in both lakes and comprised 16:0, 18:1ω9, 20:5ω3, 22:6ω3 and 16:1ω7 (Tables 2, 3). In Lake Shira, the averages of FA levels in *A. salinus* samples of epilimnion (0–5 m) and hypolimnion (7–10 m) did not significantly differ during the two study years (Student's *t*-test: the smallest $P = 0.072$) (Fig. 5). This information enabled us to pool the FA data for epilimnion and hypolimnion of Lake Shira to compare with column samples from Lake Shunet. The mean percentages of the most essential PUFA in *A. salinus* differed significantly between the populations of this calanoid inhabiting these neighboring lakes (Student's *t*-test ranged from 2.8 to 4.6, $P < 0.05$, $v = 18$) (Fig. 6). *A. salinus* in Lake Shira contained more 18:3ω3, but the population of this calanoid in Lake Shunet was richer in 18:2ω6, 18:4ω3, 20:5ω3 (eicosapentaenoic, EPA) and 22:6ω3 (docosahexaenoic, DHA) (Fig. 6).

Fatty acids in triacylglycerols of *A. salinus*

Thin-layer chromatography of lipids of *A. salinus* from both lakes showed strong dominance of triacylglycerols (Fig. 1). Polar lipids, mainly phospholipids, sterols and free fatty acids were also prominent. Wax esters were in negligible amounts.

Fatty acid profiles of triacylglycerols were the similar to those of total lipids. The percentage of several FAs, however, changed to some degree (Fig. 7). In the Shira's population, the TAG fraction was slightly richer in ω6 and ω3 C18 PUFA and lower in C22 PUFA compared with the total lipids. In Lake Shunet, TAG and total lipids differed markedly. The average FA percentages of 14:0, 16:1ω7 and 18:4ω3 were higher and 20:5ω3 and 22:6ω3 were lower in TAG fraction than those in total lipids (Tables 2, 3; Figs. 6, 7).

Mean percentage values of 21 FA of TAG fraction differed significantly between the populations from the lakes (Student's *t*-test ranged from 2.7 to 8.5,

Table 2 Prominent fatty acids (% of the total) in total lipids of bodies of *A. salinus*, collected in Lake Shira (Khakasia, Russia) in 2004–2005

FA	2004						2005					
	22 Jun e	22 Jun h	30 Jun e	30 Jun h	2 Jul e	2 Jul h	30 Jun e	30 Jun h	5 Jul e	5 Jul h	10 Jul e	10 Jul h
14:0	8.7	6.5	6.5	5.8	10.4	8.4	3.9	4.3	3.7	5.0	5.3	5.0
16:0	28.9	27.7	24.9	23.2	30.3	25.4	15.2	14.8	14.6	15.8	20.7	17.7
18:0	3.7	5.8	7.1	2.9	5.1	4.3	3.7	1.9	3.5	3.5	7.4	4.3
Other SAFA	4.3	4.4	3.7	3.0	4.7	3.3	3.3	2.4	2.4	2.4	2.9	2.1
Total SAFA	45.6	44.4	42.2	34.9	50.5	41.4	26.1	23.4	24.2	26.7	36.3	29.1
Total BFA	8.5	7.3	7.5	6.2	8.4	8.0	5.1	4.5	3.9	5.4	6.6	5.2
16:1 ω 7	10.9	9.3	10.7	10.2	12.2	12.4	7.7	8.4	6.3	10.0	8.3	8.5
18:1 ω 9	16.9	15.1	13.1	17.7	11.3	12.5	11.6	10.6	7.9	9.3	18.0	13.8
Other MUFA	0.6	0.3	2.7	1.0	1.7	1.7	5.1	5.8	5.6	6.8	4.2	3.1
Total MUFA	28.4	24.7	26.5	28.9	25.2	26.6	24.4	24.8	19.8	26.1	30.5	25.4
16:3 ω 3	1.4	0.7	1.4	1.6	1.4	1.8	0.9	2.0	1.1	1.7	ND	2.0
18:2 ω 6	2.7	2.2	3.4	4.5	2.1	3.0	3.1	3.0	2.5	2.7	2.7	2.9
18:3 ω 3	4.9	3.0	5.4	7.2	3.5	6.0	5.1	7.6	5.2	6.5	7.1	7.1
18:4 ω 3	2.4	1.3	2.6	3.5	2.0	3.4	2.9	4.3	3.1	4.1	1.8	3.7
20:4 ω 6	0.3	0.7	0.7	0.7	0.4	0.5	1.5	1.0	1.7	1.4	2.0	2.7
20:4 ω 3	0.3	0.6	0.5	0.5	0.3	0.4	1.0	0.7	1.2	0.7	0.8	0.7
20:5 ω 3	3.0	5.6	4.9	6.4	2.7	4.7	9.7	11.7	12.4	10.5	4.2	8.7
22:5 ω 6	0.1	0.6	0.4	0.2	0.2	0.2	1.8	1.1	2.3	0.9	0.9	1.0
22:6 ω 3	0.5	6.1	2.1	2.6	0.9	1.2	14.5	10.8	17.8	9.4	4.4	8.2
Other PUFA	2.0	2.7	2.2	2.5	2.2	2.8	2.9	3.6	3.2	3.0	2.3	2.9
Total PUFA	17.6	23.5	23.6	29.7	15.7	24.0	43.4	45.8	50.5	40.9	26.2	39.9

e epilimnion (0–5 m), *h* hypolimnion (7–10 m)

$P < 0.05$, $v = 18$) (Fig. 7). TAG fraction of copepods from Lake Shira had significantly higher values of i14:0, ai15:0, 15:0, i16:0, ai17:0, 17:0, 16:2 ω 4, 16:3 ω 3, 18:1 ω 9, 18:3 ω 3 and 20:3 ω 3. Hence, FA trophic markers of bacteria, green algae and *Cyanobacteria* were prominent in the population of Lake Shira. However, copepods from Lake Shunet contained more 14:0, 16:1 ω 7, 18:2 ω 6, 18:4 ω 3, 18:5 ω 3, 22:5 ω 6, 22:5 ω 3 and 22:6 ω 3 (Fig. 7). Thus, the population of this latter case was rich in diatom, cryptophyte and flagellate FA trophic markers.

Similarity of FA levels in TAG fraction of *A. salinus* from lakes Shira and Shunet were tested by the principle component analysis (PCA). Distribution of samples is shown on the distance biplot diagram (Fig. 8). The first two principal axes accounted for 74.4% of total variation of FA. Samples from Lake Shunet were quite similar in TAG and formed two neighboring groups for the

2 years, but distinctly separated from the Lake Shira samples, which greatly varied both within the years and depth. High dissimilarity of Lake Shira samples was mainly due to pronounced variations in bacterial FA (i14:0, ai15:0, 15:0, i16:0, ai17:0, 17:0) in the epilimnion samples in 2004.

Feeding experiments

In experiments with the copepods and seston from Lake Shira, the significant decrease in density of colonial picoplankton *Aphanothece* in the experimental vessels compared with that in the control vessels was observed (Table 4). In the experiments with the Shunet's plankton, experimental and control vessels were differed in purple bacteria, *Cryptomonas salina* and *Cyclidium* sp. (Table 4). Thus, the consumption of these four groups of plankton of two lakes was experimentally confirmed. However, we assume that

Table 3 Prominent fatty acids (% of the total) in total lipids of bodies *A. salinus*, collected in Lake Shunet (Khakasia, Russia), 2004–2005, 0–5 m

FA	2004			2005				
	29 Jun	1 Jul	1 Jul	2 Jul	2 Jul	2 Jul	7 Jul	7 Jul
14:0	6.2	7.5	4.9	8.0	7.9	7.8	7.9	9.4
16:0	19.0	20.6	17.2	14.2	15.1	15.3	13.8	16.2
18:0	4.4	5.4	4.7	2.8	2.6	2.4	2.7	3.0
Other SAFA	3.3	4.1	3.5	1.6	1.9	2.0	2.2	2.7
Total SAFA	32.9	37.6	30.3	26.6	27.5	27.5	26.6	31.3
Total BFA	3.5	4.7	3.4	2.9	2.9	2.5	3.7	4.3
16:1 ω 7	12.2	13.4	11.7	8.7	9.1	9.2	9.0	9.6
18:1 ω 9	9.2	8.5	8.7	8.3	6.9	6.6	7.7	7.7
Other MUFA	1.9	2.3	2.6	2.6	4.1	3.7	2.9	3.7
Total MUFA	23.3	24.2	23.0	19.6	20.1	19.5	19.6	21.0
16:3 ω 3	0.2	0.2	0.2	0.4	0.4	0.4	0.5	0.5
18:2 ω 6	3.5	3.4	3.5	4.6	4.3	4.9	4.4	4.4
18:3 ω 3	3.2	3.0	3.3	4.8	4.6	5.0	4.5	4.1
18:4 ω 3	4.5	3.2	3.7	5.7	5.1	5.4	5.3	4.5
20:4 ω 6	1.0	0.8	1.2	0.9	1.0	0.8	1.4	1.3
20:4 ω 3	1.0	1.3	1.5	0.6	0.6	0.7	0.7	0.6
20:5 ω 3	10.7	9.1	11.8	9.9	9.8	10.2	10.2	8.4
22:5 ω 6	1.2	0.9	1.1	1.9	1.8	1.7	1.7	1.4
22:6 ω 3	11.3	7.8	12.3	17.1	16.7	16.7	16.3	12.3
Other PUFA	3.7	3.6	4.4	3.6	3.4	3.1	3.8	4.0
Total PUFA	40.3	33.3	43.0	49.5	47.7	48.9	48.8	41.5

all ciliates were eaten up since no individuals were observed in the collecting vessels at the end of the experiment. The lack of significance (*t*-statistics) was mainly due to the low density of ciliates in samples. The ability of *A. salinus* to feed on purple bacteria is explained by their aggregation in the colonies of two or four cells. Feeding on the other components of seston (heterotrophic nanoflagellates and unicellular cyanobacterial picoplankton of *Synechocystis* genera) was not detected in the experiments. Poor grazing on picoplankton is obviously related to their small cell size, while heterotrophic nanoflagellates may be rejected via taste discrimination. Daily rations on the consumed species, which included purple bacteria, *Cryptomonas salina* and *Cyclidium* sp., amounted to 18.7, 6.2 and 1.0% of *A. salinus* body's wet weight,

respectively. The lowest *Aphanothece* contributed only 0.4%. The total rations of copepods in the lakes on the counted planktonic groups were rather low. This study implies that the undetected sources (detritus) comprise a great part of the food.

Discussion

The fatty acid profiles of storage lipids, triacylglycerols or wax esters have widely been used to elucidate trophic interactions of various copepod species in both marine and freshwater ecosystems (Persson and Vrede 2006; Kattner et al. 2007; Caramujo et al. 2008). The combination of fatty acid trophic markers (FATM) and stable isotopes has proven useful in deciphering trophic relations among various plankton and benthos groups (Rooker et al. 2006; Gladyshev 2009), including calanoid copepods (El-Sabaawi et al. 2009). However, published data on brackish water species of calanoid copepods and FATM in their tissues are extremely scarce (Guerrero et al. 2007). Our data confirm the results of Guerrero et al. 2007 that brackish water species of calanoids accumulate large quantities of TAG, but not wax esters, in contrast to most marine calanoid species. Hence, we used fatty acid composition of TAG to investigate food sources of *Arctodiaptomus salinus* in lakes Shira and Shunet.

FATM in TAG and feeding experiments as indicators of the diet of copepods

Use of FATM can provide reliable information on assimilated food, although the interpretation of the food sources is possible only at the level of large taxonomic groups. Moreover, FA footprints of some higher taxa, for instance, of green algae and *Cyanobacteria* or of diatoms and Gram-positive bacteria (including phototrophic purple bacteria), are quite similar. Therefore, a deeper understanding of trophic relations can be achieved if FATM analyses are supplemented with information on microplankton in seston and data from feeding experiments. We want to explore the trophic relationship of *A. salinus* based on known FATM for different microplankton groups and using information on species composition of lake seston and the results from feeding experiments (see data in Table 5).

Fig. 5 Average FA levels (% of the total) in total lipids of *A. salinus*, inhabiting epilimnion (0–5 m, $n = 6$) and hypolimnion (7–10 m, $n = 6$) of Shira lake (Khakasia, Russia) in 2004–2005. Note that there were no statistically significant differences for any FA after Student's t -test (minimal $P = 0.072$)

Fig. 6 Average essential PUFA's levels (% of the total) in total lipids of seston and *A. salinus* in Shira and Shunet lakes (Khakasia, Russia), 2004–2005. See text for details

Seston of Lake Shira had the increased levels of two fatty acids, 18:3 ω 3 and 16:3 ω 3. These FAs are typically synthesized by green algae (Thompson, 1996) and have been used as green alga markers in a freshwater lake (Leveille et al. 1997). However, it is known that 18:3 ω 3 is also synthesized by *Cyanobacteria*, especially filamentous species (Gugger et al. 2002). Moreover, Sushchik et al. (2003a) have shown in a freshwater reservoir that 18:3 ω 3 indicates dominance of both green algae and *Cyanobacteria*. We have noted that in summer period *Cyanobacteria* *Lyngbya contorta*, a non-identified small-sized

cyanobacterial species, and green algae of *Dictyosphaerium* genus dominate phytoplankton in Lake Shira (Gaevisky et al. 2002). Green alga *Botryococcus* sp. usually develops in littoral part and moves to open water, while it can easily be distinguished due to highly specific fatty acid composition (Kalacheva et al. 2002). Hence, higher levels of 16:3 ω 3 in Lake Shira were likely provided by green algae, while both greens and *Cyanobacteria* might be responsible for the increased levels of 18:3 ω 3. During this investigation, feeding experiments supported the consumption of colonial *Cyanobacterium Aphanothece* sp. in Lake

Shira (Tables 4, 5). We also observed earlier in similar feeding experiments in Lake Shira (Tolomeyev 2002) that *A. salinus* feeds on another *Cyanobacterium*, *Lyngbya contorta*, and dominant green algae, *D. tetrachotomum*.

In Lake Shunet, FATM 16:3 ω 3 in seston was scarce (Fig. 4). Generally, it corresponded to the species composition, where green algae represented by *D. tetrachotomum* were <10% of total phytoplankton biomass (Degermendzhy et al. 2003). Thus, 18:3 ω 3 found in seston seems to have its origin from cyanobacterial picoplankton. This fatty acid is also found in TAG of *A. salinus*. However, laboratory experiments demonstrated inability of animals to feed on cyanobacterial picoplankton obviously due to the small size of cells. Thus, this fatty acid (18:3 ω 3) in TAG of *A. salinus* must have its origin from other microorganisms in Lake Shunet.

Fatty acids 14:0 and 16:1 ω 7 can be used as markers of diatom summer populations. Commonly, 20:5 ω 3 and ω 7, ω 4 and ω 1 C16 PUFAs are considered as diatom markers (Brett et al. 2009). However, Sushchik et al. (2004) have demonstrated that summer diatom populations can be low in PUFA compared with the spring ones, while 14:0 and 16:1 ω 7 together are good predictors of both summer and spring populations of diatoms (Sushchik et al.

2003a). Diatoms are reported in both lakes in summer (Gaevsky et al. 2002), with lower occurrence in L. Shira, that is in good accord with the lower percentages of FATM 14:0 and 16:1 ω 7, observed in our study. The poor contribution of summer diatoms to the daily ration of *A. salinus* in L. Shira is also supported by the feeding experiments in an earlier study (Tolomeyev 2002), which shows that the only diatom species, *Cyclotella tuberculata*, in Lake Shira is excluded from the ration of *A. salinus*.

We also observed *A. salinus* in Lake Shira to contain more branched C14–17 and odd-numbered straight C15 and C17 acids (Fig. 7) that are commonly accepted as FATM of heterotrophic Gram-positive bacteria that form the “microbial loop” (e.g., Ederington et al. 1995; Stevens et al. 2004). However, it is quite doubtful that *Arctodiaptomus* can effectively filter bacterioplankton (Kleppel 1993). It is very likely that such markers come from another food pathway for the calanoid, i.e. via the bacteria associated with detritus or via protozoa organisms feeding on bacteria. It is, however, interesting that in our experiments *A. salinus* did not graze upon small cells of picoplankton, but instead it showed in both lakes a strong preference in consuming ciliates present in the food suspension (Table 4). Moreover, our study supports some earlier studies, which

Fig. 7 Average FA levels (% of the total) in triacylglycerols of *A. salinus*, inhabiting Lake Shira ($n = 12$) and Lake Shunet ($n = 8$) (Khakasia, Russia) in 2004–2005. Statistically significant differences between the means after Student's *t*-test: * $P < 0.05$; # $P < 0.01$; + $P < 0.001$

Fig. 8 PCA distance biplot showing similarity of the samples of *Arctodiaptomus salinus* varied within Shira and Shunet lakes, years (2004, 2005) and ecological zones. The length of the FA vectors (not drawn, but arrows connecting the origin with the FA cross-marks) reflect the contribution of FA to the ordination subspace definition. *Lake Shunet*: □ - 2004, ■ - 2005. *Lake Shira*: △ - epilimnion zone and ▲ - hypolimnion zone, 2004; ○ - epilimnion zone and; ● - hypolimnion zone, 2005

document feeding on ciliates by the calanoid copepods, in both freshwater (e.g., Desvillettes et al. 1997; Kagami et al. 2002) and marine ecosystems (Ederington et al. 1995). Notably, levels of bacterial FATM in the samples of L. Shira were quite high and varied greatly both spatially and temporally (Fig. 8).

The sestonic fatty acids are vertically highly stratified (Fig. 3), depending on the depth distribution of different algae, bacteria and protozoa taxa that contain these acids (Kopylov et al. 2002; Gaevsky et al. 2002). In contrast, the FATM in the copepods from epilimnion and hypolimnion of L. Shira are similar so that we can assume that the grazer population of *A. salinus* actively migrate to certain specific depths and feed on the particular plankton species or detritus. Both foraging migration and diel vertical migration of zooplankton are widely reported in natural ecosystems, the latter as an avoidance reaction to the visual predation by fish (Lampert et al. 2003). However, in the absence of fish in both lakes,

we can safely assume that the migration of calanoids in our saline lakes must be triggered by factors other than predation. Thus, foraging migration is the main explanation for vertical movements of *A. salinus* in these two lakes. For Lake Shira, it should be emphasized that fatty acids 18:3ω3, 20:5ω3 and 22:6ω3 all of which were abundant in TAG of *A. salinus* from both epilimnion and hypolimnion zones were primarily detected only in hypolimnion seston (Fig. 3; Table 1). Thus, the epilimnion population of *A. salinus* must migrate to these deeper waters to maintain in their bodies the observed levels of these acids. This argument, however, is not supported by Tolomeyev and Zadereev (2005) who found that diel vertical migration of *A. salinus* in Lake Shira is weakly pronounced due to the absence of predators for zooplankton. Nevertheless, the difference between day and nighttime distribution profiles of *A. salinus* does comprise its lower day-time density in the upper (1 m) layer probably due to a negative phototactic response to intensive solar radiation. Moreover, the absence of a pronounced diel descent to deeper layers does not rule out a non-synchronous migration. A non-synchronous migration might be caused by physiological conditions of individuals. Apparently, physiological deficiency of essential acids 18:3ω3, 20:5ω3 and 22:6ω3 in *A. salinus* bodies could act as a trigger for individuals in epilimnion to descend into hypolimnion in pursuit of high-quality food sources.

In addition to the higher levels of FATM of diatoms, the population of *A. salinus* in Lake Shunet had significantly higher percentages of 18:4ω3, 18:5ω3 and C 22 polyenoic acids in TAG (Figs. 7, 8), which can be considered as markers of organic matter originated from autotrophic flagellates (Desvillettes et al. 1997). This group also contains high levels of 18:3ω3 that can explain the relatively higher content of this acid (18:3) in the animals' bodies than in seston (Fig. 7). As already discussed, *A. salinus* seems to have limited opportunities to acquire 18:3ω3 from scarce green algae or difficult to filter plankton such as cyanobacterial picoplankton in Lake Shunet.

Fatty acid 18:5ω3 is known to be synthesized by dinoflagellates and is used as their FATM if found in zooplankton (Napolitano 1999). Another likely source of its occurrence in zooplankton is retroconversion of ω3 C20 PUFA, i.e. shortening of a FA

Table 4 Mean concentrations of phytoplankton and protozoa species in the control (*Nc*) and experimental (*Ne*) collecting vessels in the experiments on *Arctodiaptomus salinus* feeding conducted by flow-through method

Phytoplankton and protozoa species in seston	Weight of a cell ($\cdot 10^{-12}$ g)	Concentrations in the collecting vessels ($\cdot 10^3 \pm$ SE, cells ml $^{-1}$, $n = 3$)		T	P	$R \pm$ SE (%)
		N_c (without animals)	N_e (with animals)			
Lake Shira						
<i>Aphanothece</i> sp. (colonial)	1.36	67.5 $\pm$ 12.7	16.0 $\pm$ 8.2	18.4	0.002*	0.4 $\pm$ 0.03
<i>Lyngbia contorta</i>	2.25	114.1 $\pm$ 34.9	131.8 $\pm$ 24.5	1.20	0.265	–
Unicellular pico cyanobacteria	0.52	616.4 $\pm$ 127.1	708.5 $\pm$ 120.1	1.8	0.102	–
<i>Strombidium</i> spp.	1,000	0.052 $\pm$ 0.110	0	1.42	0.193	0.03 $\pm$ 0.03
<i>Cyclidium</i> spp.	2,000	0.045 $\pm$ 0.070	0	1.92	0.092	0.02 $\pm$ 0.02
Heterotrophic nanoflagellates	80	0.295 $\pm$ 0.162	0.179 $\pm$ 0.314	1.08	0.311	–
Lake Shunet						
<i>Sulfur purple bacteria</i>	4.19	793.5 $\pm$ 157.7	193.0 $\pm$ 80.3	19.03	0.000*	18.7 $\pm$ 0.02
<i>Synechocystis</i> sp.	0.52	762.4 $\pm$ 32.0	653.8 $\pm$ 98.4	2.02	0.078	–
<i>Cryptomonas salina</i>	600	1.909 $\pm$ 0.806	0.568 $\pm$ 0.130	4.35	0.002*	6.2 $\pm$ 2.05
<i>Cyclidium</i> spp.	2,000	0.070 $\pm$ 0.015	0	11.07	0.000*	1.0 $\pm$ 0.01
<i>Strombidium</i> spp.	57,000	0.085 $\pm$ 0.062	0	3.33	0.020	38.2 $\pm$ 5.31
Heterotrophic nanoflagellates	100	0.079 $\pm$ 0.003	0.125 $\pm$ 0.117	1.20	0.264	–

Significant decrease in density in experimental collecting vessels compared to the control ones supported the consumption. The significance was estimated by paired *t*-test. Asterisk indicated 95% level. Daily rations (*R*, % of the body wet weight) for the consumed species (or groups) were calculated

carbon chain in two carbon atoms (Von Elert 2002). There could be two explanations for *A. salinus* in L. Shunet having unusually high levels of 18:5 ω 3 (ca. 1%) in TAG. First, *A. salinus* in L. Shunet might derive this acid together with C22 ω 3 PUFAs from *Cryptomonas salina*, a flagellate which is highly concentrated in relatively thin layer adjacent to chemocline near lake bottom (Khromechek et al. this volume). Interestingly, we did not find this FATM in any of pelagic seston samples from our two lakes. We took samples from the middle of the water column, while the autotrophic and heterotrophic flagellates mostly inhabited the specific thin layer near bottom. Finding these FA in the copepod tissues confirms the speculation on their non-synchronous individual migrations and feeding on flagellates in this layer. The highly selective feeding of the *Arctodiaptomus* populations on *C. salina* is also confirmed in this laboratory study. In addition, the calanoid population of L. Shunet showed higher content of linoleic acid in TAG, which some authors (El-Saabawi et al. 2009) relate with feeding on dinoflagellates in copepods. A second explanation of unusually high levels of

18:5 ω 3 in *Arctodiaptomus* TAG is that the significantly higher content of DHA of total lipids in L. Shunet's copepod populations (Fig. 6) might result in more active retroconversion and accumulation of 18:5 ω 3 in TAG of *A. salinus* in L. Shunet. However, as the physiological importance of DHA and other long-chain PUFA is very high, their retroconversion in 18:5 ω 3 is unlikely.

Essential PUFAs of *Arctodiaptomus* as a result of physiological adaptation to the lake conditions

Fatty acid trophic markers and the feeding experiments, together, indicate that nutritional spectra of the calanoid populations inhabiting the two neighboring brackish lakes differ partly due to different composition of lakes' seston (Table 5). However, the two *Arctodiaptomus* populations markedly differed in levels of essential long-chain PUFA, EPA and DHA, although the levels of these FA in seston in both lakes were quite similar (Fig. 6). In general, both the grazer populations were highly enriched in the essential ω 3 PUFA, but more so in L. Shunet, compared with

Table 5 Taxonomic groups of plankton and their contribution to the diet of *A. salinus* in lakes Shira and Shunet (Khakasia, Russia) based on analysis of fatty acid trophic markers in triacylglycerols and feeding experiments

Group	Typical FATM	FATM found in TAG of <i>A. salinus</i> **—high; *—low, ND—not detected		Species found in the lake's seston. (+) consumed species, (–) not consumed; without sign—not studied or consumption was not statistically proved	
		Shira	Shunet	Shira	Shunet
Chlorophyceae	High level of 18:3 ω 3 (ca. 20–40%), ω 3 and ω 6 C16 PUFAs (Viso and Marty 1993)	**	*	+ <i>Dictyosphaerium tetrachotomum</i> + <i>Oocystis lacustris</i> <i>Botryococcus braunii</i>	<i>D.tetrachotomum</i> <i>Chlamydomonas</i> sp.
Cyanobacteria	18:3 ω 3 (or 18:3 ω 6) or 16:0 and 16:1 ω 7c (Gugger et al. 2002)	**	*	+ <i>Lyngbya contorta</i> + <i>Aphanothece</i> sp. (colonial) – Unicellular pico-cyanobacteria	– <i>Synechocystis</i> sp. <i>Gleocapsa</i> sp.
Diatoms	ω 7, ω 4, ω 1 C16 PUFAs, 20:5 ω 3 Ratio of 16:1 ω 7/16:0 > 1, high levels of 16:1 ω 7 and 14:0 (Dijkman and Kromkamp 2006)	*	**	– <i>Cyclotella tuberculata</i>	<i>Cyclotella</i> sp. <i>Nitzschia acicularis</i>
Cryptophytes	High levels of both 18:3 ω 3 and 18:4 ω 3, and both 20:5 ω 3 and 22:6 ω 3 (Brett et al. 2009)	*	**	Not found	+ <i>Cryptomonas salinus</i>
Dinophyceae	18:5 ω 3, 22:6 ω 3 and C22 PUFA (Napolitano 1999)	*	**	Not found	Not found
Protozoans	ω 6 PUFA (Desvillettes et al. 1997) 20:2 ω 6, 20:3 ω 6, 20:4 ω 6 (Green and Scow 2000)	*	**	+ <i>Strombidium</i> spp. + <i>Cyclidium</i> spp. – Heterotrophic flagellates (genera <i>Bodo</i> , <i>Monosiga</i> , <i>Cafeteria</i>)	+ <i>Strombidium</i> spp. <i>Cyclidium</i> spp. – Heterotrophic flagellates
Gram-positive bacteria (heterotrophic)	Branched C 15–17 SFA and MUFA (Napolitano 1999)	**	*	Not studied	Not studied
Gram-negative bacteria (heterotrophic, chemoautotrophic, photosynthetic)	C18 and 16 MUFAs, a high level of 18:1 ω 7 (Napolitano 1999; Imhoff 2003)	*	*	Not studied	+ <i>Lamprocystis purpurea</i>
Methane oxidizing bacteria	16:1 ω 8c, 18:1 ω 8c, 16:1 ω 5 (Green and Scow 2000)	ND	ND	Not studied	Not studied
Consumption was checked in the feeding experiments including previous data (Tolomeyev 2002)					

these fatty acids in seston. This higher enrichment of the diaptomid population in L. Shunet could be an adaptive response to higher salinity levels in the near bottom layer in the lake.

Note that differences in feeding spectra of populations of *A. salinus* in two lakes revealed by FATM in storage lipids and the experiments mirror the FA composition of total lipids, principally in $\omega 3$ long-chain PUFA (Fig. 6). This is not surprising because total lipids of the animals comprise greater part of TAG (Fig. 1). However, the distribution of the PUFAs of key physiological importance, i.e. EPA and DHA in different lipid fractions, differs between L. Shira and L. Shunet populations. Whereas the percentages of EPA and DHA in total lipids and TAG are similar in L. Shira's population, they are markedly higher in total lipids in L. Shunet animals than the FA in TAG fraction. Certainly, in L. Shunet, the bulk of long-chain PUFA in the calanoids originates from polar lipids, the structural components of cell membranes. Thus, we can speculate about the accumulation of long-chain $\omega 3$ PUFAs in polar lipids of *A. salinus* in L. Shunet. Probably, the plankton community comprised of dinoflagellates, cryptophytes and diatoms, inhabiting the layers adjacent to chemocline in the lake, provides the copepods with the essential $\omega 3$ PUFA (18:4 $\omega 3$, 20:5 $\omega 3$ and 22:6 $\omega 3$).

The ability of various species of copepods to synthesize EPA and DHA from C18 PUFA as well as their ability to biochemically convert EPA into DHA is still very controversial. For instance, according to Caramujo et al. (2008) harpacticoid copepods can convert short-chain precursors into long-chain and more unsaturated EPA and DHA; also Persson and Vrede (2006) provide a similar indirect evidence for several plankton freshwater calanoids. On the other hand, Bell et al. (2007) show that four marine calanoid species possess little capacity to convert 18:3 $\omega 3$ into C20-22 PUFA. We too could not conclude from our data whether the population of *A. salinus* in L. Shunet has a greater capacity to synthesize DHA and EPA from the food precursors than its population in L. Shira. Meanwhile, it is unlikely that two genetically and geographically close populations of the calanoid under study significantly differ in their ability to synthesize DHA and EPA. We suggest that higher DHA and EPA levels in the membrane structural lipids of L. Shunet's

population result from their specific retention from food sources and their subsequent accumulation in animal's tissues.

The increased levels of DHA and EPA of the copepods in L. Shunet might also be related to adaptation of the population to significantly higher salinity in chemocline layer. Salinity of water column in L. Shunet is about 14 g l^{-1} in upper layers, but near chemocline zone it increases sharply to 40 l^{-1} at 5 m depth. The changes in PUFA percentages seem to be involved in the osmoregulation of crustaceans through modulation of Na^+/K^+ -ATPase activity and (or) direct membrane permeability to water and ions as reported by Palacios and Racotta (2007). Although the effect of salinity on fatty acid composition of aquatic animals is far from clear, there is evidence that membrane lipids in the freshwater environment should contain more saturated acids to prevent more intensive loss of various salt ions in comparison with that in saline waters. This is in accord with the observation of generally lower PUFA content, especially of $\omega 3$ family, in freshwater than in marine fish (Ahlgren et al. 1994). Evidently, the copepods do not stay continuously in the relatively high-salinity deep layer, even though they must tolerate chemical conditions prevailing here while they feed on specific community there.

Conclusion

Combined use of FATM approach and direct feeding experiments revealed that the differences of feeding spectra *A. salinus* were driven by both different seston FA composition and selective feeding. In Lake Shira, *Arctodiaptomus salinus* preferably consumed green algae, *Cyanobacteria* and heterotrophic bacteria. In L. Shunet, feeding spectra of this calanoid copepod comprised diatoms, cryptophytes, purple bacteria and ciliates. In addition to the different feeding spectra, the two *Arctodiaptomus* populations markedly differed in their body levels of essential long-chain PUFA, EPA and DHA. We presume that this difference is partly explained by FA composition of consumed food and the necessity to adapt to specific physico-chemical conditions of the lakes. The higher levels of the essential $\omega 3$ PUFA in *A. salinus* in Lake Shunet could be considered as an adaptive response of the animals to a higher salinity

level in the chemocline zone where food levels are high. Thus, the chemocline zone of L. Shunet seems to play an important role in life strategy of the copepods dually controlling their fatty acid composition—through both the specific food sources and the specific higher salinity condition.

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