Effect of temperature on contents of essential highly unsaturated fatty acids in freshwater zooplankton

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ABSTRACT

In 11 lakes from cold and warm regions we studied the content of highly unsaturated fatty acids (HUFA) in seston (<130 μm) and crustacean zooplankton using gas chromatography–mass spectrometry. An increase of temperature correlated with a decrease of HUFA content in zooplankton. A multivariate canonical correlation analyses revealed, that the decrease of HUFA content was related with a decrease of per cent of copepods in zooplankton communities, which are known to have higher HUFA levels in their biomass, than cladocerans. This means that temperature primarily affected the HUFA levels indirectly, via changing of taxonomic structure of zooplankton community, while the homeoviscous adaptation of zooplankton individuals had comparatively lower importance. As found, water temperature was better predictor of HUFA contents of zooplankton, than the fatty acid composition of seston. Thus, it can be predicted, that a probable climate warming will decrease the content of the essential HUFA in freshwater zooplankton with possible negative consequences for animals of higher trophic levels.

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Introduction

In last decades highly unsaturated fatty acids of the ω3 family (HUFA) have come to be recognized as biochemicals of a high physiological importance for animals at all trophic levels, including plankton invertebrates, fish and humans (Müller-Navarra 1995; Arts et al. 2001; Wacker and Von Elert 2001). Animals do not have desaturases to insert double bonds in fatty acid molecules in ω3-position, therefore α-linolenic acid (ALA; 18:3ω3) is the essential nutrient in animal nutrition (Bell and Tocher 2009; Lands 2009). Physiologically most important are such HUFAs as eicosapentaenoic acid (20:5ω3, EPA), and docosahexaenoic acid (22:6ω3, DHA). Although animals, including humans, can to a varying degree desaturate and elongate the essential ALA to EPA and DHA, the consensus is that the degree of conversion in general cannot meet the physiological requirements and animals must obtain these

HUFAs from food (Goulden and Place 1990; Plourde and Cunnane 2007). Among organisms in the biosphere, algae, and, in particular, diatoms, cryptophytes, and dinoflagellates, rather than vascular plants, can *de novo* synthesize high amounts of EPA and DHA (Harwood 1996; Tocher et al. 1998). Once synthesized at the level of primary producers HUFAs are transferred and can accumulate, at progressively higher trophic levels, in the biomass of aquatic organisms. Therefore, aquatic ecosystems play the unique role in the biosphere as the principal source of the long-chain ω-3 HUFA for animals that might include some inhabitants of terrestrial ecosystems (Gladyshev et al. 2009).

Production of HUFAs in aquatic ecosystems and their export to terrestrial ecosystems are evidently affected by many factors, including anthropogenic pollution. For instance, eutrophication (enhancing of total phosphorus concentrations) favors cyanobacteria, which contain no HUFA, and thereby decreases HUFA production in lakes (Müller-Navarra et al. 2004). Another potential hazard which may reduce the production of HUFA is climate warming. HUFA synthesis by microalgae is known to inversely depend on temperature (e.g., Jiang and Gao 2004; Guschina and Harwood 2009). Besides algae, all poikilothermic aquatic organisms, including zooplankton, are believed to increase the pro-

Fig. 1. Map of sampling regions.

portion of low-melting HUFA in their lipids to maintain proper cell membrane fluidity at low temperatures, which is known as the homeoviscous adaptation (Farkas 1979; Maazouzi et al. 2008; Brett et al. 2009; Guschina and Harwood 2009).

Increases of HUFA levels in zooplankton with decrease of water temperature were found in laboratory experiments (Farkas 1979; Schlechtriem et al. 2006; Masclaux et al. 2009) and also in some lakes as results of inter-annual and (or) seasonal temperature variability (Farkas and Herodek 1964; Maazouzi et al. 2008; Gladyshev et al. 2010). However, data on short-term laboratory experiments and also data on seasonal variations within a single ecosystem (community) may not be decisive for a conclusion on a possible effect of global warming on HUFA production in aquatic ecosystems. Indeed, Kattner and Hagen (2009) did not find significant differences in latitudinal distribution of EPA and DHA levels in marine zooplankton. The cited authors concluded that global warming could have an impact on the flux of energy to higher trophic levels, rather than affect food quality regarding the content of essential fatty acids. Is this true for freshwater ecosystems? To answer this question a comparative study of the latitudinal distribution of HUFA levels in freshwater zooplankton is needed. In contrast to marine zooplankton, where copepods dominate, freshwater zooplankton is composed mainly by two groups, copepods and cladocerans, which significantly differ in HUFA levels (Persson and Vrede 2006; Smyntek et al. 2008; Brett et al. 2009; Kainz et al. 2009; Ravet et al. 2010).

At present there is more confusion than consensus about the responses of freshwater plankton communities to climate warming (Schindler et al. 2005). Some authors found, that climate warming favored cladocerans, rather than copepods and rotifers (Hampton et al. 2008; Thompson et al. 2008; Visconti et al. 2008), while others drew opposite conclusions (Winder and Schindler 2004; Blenckner et al. 2007; Dupuis and Hann 2009). In any case, climate warming was found to affect the proportions of copepods and cladocerans in lakes and reservoirs, but probable consequences of these changes for production of the essential HUFA in freshwater ecosystems are unknown yet.

Thus, the aim of our work was to study and compare HUFA contents in plankton of lakes from different regions with contrasting summer water temperature to evaluate a possible effect of temperature on levels of the essential compounds in freshwater ecosystems. Like some other authors (Persson and Vrede 2006), we chose a sampling strategy which entailed sampling several lakes once or a few times, rather than an extensive time-series for a few lakes. This strategy is believed to give an opportunity to draw some general conclusions (Persson and Vrede 2006), including predictions of consequences of climate warming. The same approach was used when studying latitudinal temperature effect on HUFA content in marine zooplankton (Kattner and Hagen 2009).

Methods

Site description

Samples of zooplankton were taken during two summers in 11 lakes, located in 4 regions (Fig. 1 and Table 1). The lakes have different surface area, ranging from 0.1 km² (Karovoye Lake) to 77 km² (Kurilskoye Lake) and different maximum depth, from 1.5 m in Kilometrovoye Lake to 316 m in Kurilskoye Lake (Table 1). Lakes, located in Kamchatka peninsula, in Ergaki mountains and in Bol-

Table 1
Description of studied lakes: surface area (A, km²), average depth (h_{av}, m), maximum depth (h_{max}, m), Secchi disk transparency (S, m) and pH.

Region Lake	Location	A	h_{av}	h_{max}	S	pH
Kamchatka peninsula						
Kurilskoye	51°27′N 157°05′E	77	195	316	7.8	6.8–7.2
Ergaki mountains						
Oiskoye	52°50′N 93°15′E	0.6	6	25	4.5	7.1
Karovoye	52°49′N 93°20′E	0.1	3	9	>9.0	7.1
Bolshezemelskaya tundra						
Bolshoi Kharbei	67°33′N 62°53′E	21.3	4.6	18.5	2.7	6.4
Golovka	67°36′N 62°55′E	3.1	1.8	12.0	1.0	8.0
Dvaozera	67°35′N 62°53′E	0.2	≈1	n.d.	n.d.	n.d.
Kilometrovoye	67°36′N 62°52′E	1	<1	1.5	1.0	8.6
Republic of Belarus						
Obsterno	55°37′N 27°27′E	9.9	5.3	12	3.65	7.6
Nobisto	55°37′N 27°24′E	3.7	1.2	2.8	2.3	7.6
Gorushka	55°38′N 27°20′E	0.2	3.0	6.9	1.7	7.8
Lukomlskoye	54°39′N 29°06′E	36.7	6.5	11.5	3.0	7.1

n.d. – no data.

shezemelskaya tundra are oligotrophic, while lakes, located in Republic of Belarus are meso- and eutrophic. Lukomlskoye Lake is the reservoir-cooler for Lukomlskaya thermal power station. There was no temperature stratification in all the lakes in the sampling sites, except one case: Kurilskoye Lake in August. All lakes had circum-neutral pH (Table 1). Kurilskoye Lake was sampled three times, in June, July and August, 2008, at a site near center of the lake. Two samples, from littoral and pelagial (near center) were taken from each of four Belarusian lakes, because pelagic and littoral plankton communities are known to be significantly different, and water temperature may also differ (see below). Two samples, in center and in bay, were also taken in one lake of Bolshezemelskaya tundra, Bolshoi Kharbei, and in one lake of Ergaki, Oiskoye. From all other lakes, which were comparatively small and (or) had no macrophyte-covered littoral, one sample at a central site was taken.

Seston sample collection and pre-treatment

Samples were taken with a Ruttner-like sampler and were pooled from surface down to ∼0.5 m off the bottom. In shallow sites, such as littoral, with depth about 1 m, samples were taken by a bucket. Seston fractions were pre-screened through the 130 μm nylon mesh. For following fatty acid analyses 400–2900 mL of the pre-screened pooled sub-samples were collected by vacuum filtration onto membrane filters (Vladipor, Mytishchi, pore size 0.75–0.85 μm), pre-covered with a layer of $BaSO_4$ to facilitate the separation of residues. The filters were dried at ∼35 °C for about 30 min, and the residues were then separated from filters and placed into chloroform–methanol and frozen at −20 °C.

Zooplankton sample collection and pre-treatment

In deep lakes samples were taken by upward towing of plankton nets. In shallow lakes and in littoral samples were taken with a bucket and filtered through plankton nets. At least 300 l of water were filtered for each sample. Mesh size of plankton nets was 130 μm, except Kurilskoye Lake and Belarusian lakes, where 70 μm mesh was used. Each zooplankton sample, concentrated in plankton net, was separated into subsamples for zooplankton counting and fatty acid and organic carbon analysis. The zooplankton subsamples were fixed with 4% formalin. Zooplankton from the subsamples for fatty acid analyses was collected by vacuum filtration and stored, like seston.

Zooplankton analysis

Zooplankton, namely metazooplankton >130 μm, Copepoda and Cladocera, were identified and counted under a stereomicroscope, and measured using an ocular micrometer. Size was converted to wet weight using conventional species coefficients (Balushkina and Winberg 1979).

Fatty acid analysis

For fatty acid analyses, organic carbon of a single seston sample and zooplankton sample approximately ranged in 1–5 mg and 7–25 mg, respectively. Lipids were extracted with 5 ml of chloroform–methanol (2:1, v/v) three times. After evaporation of the solvent, methyl esters of fatty acids (FAMEs) were prepared in a mixture of methanol–concentrated sulphuric acid (20:1, v/v) at 85 °C for 2 h. Subsequently, the trans-esterification was stopped by adding 2 ml of distilled water, and FAMEs were extracted two times with 3 ml of hexane. The hexane lipid fraction was roto-evaporated to dryness and resuspended in 15–20 μl of hexane and 1–2 μl of a sample was injected. FAMEs were analysed and identified using a gas chromatograph–mass spectrometer (model 6890/5975C, "Agilent Technologies", USA). The FAMES were quantified according to the peak area of internal standard, nonadecanoic acid, which solution of 0.5 mg/ml was added in a fixed volume (30 μl) prior the extraction. Peaks of FAMEs were identified by their retention time and mass spectra, comparing to those in the data base (Hewlett-Packard, USA; "Agilent Technologies", USA) and to those of available authentic standards (Sigma, USA). Each sample of fatty acids was analysed in a single replicate. Replicate injections of standards indicated that analytical precision was <0.6%. More detailed descriptions of the analyses are given elsewhere (Gladyshev et al. 2000; Sushchik et al. 2003a).

Organic carbon analysis

For organic carbon measurements seston and zooplankton from subsamples were collected by vacuum filtration onto precombusted GF/F filters (Whatman, USA). The filters with residues were dried at ambient temperature overnight and stored in a desiccator until analysis. The samples were analysed with a Flash EA 1112 NC Soil/MAS 200 elemental analyzer (Neolab LLC, USA) (Gladyshev et al. 2007). The calibration curves for the elemental analyzer were generated using aspartic acid and standard soil reference material (Neolab LLC, USA). Carbon sample of zooplankton of the Kurilskoye Lake in June was not available.

Statistical analysis

Statistical analysis, product-moment (Pearson) correlation coefficients and canonical correlation analysis (CCA) were carried out conventionally (Campbell 1967; Jeffers 1981), using STATISTICA software, version 9 (StatSoft Inc., Tulsa, OK, USA).

Results

Water temperature during sampling varied from 1.9 °C in Kurilskoye Lake in June to 28.2 °C in littoral of Lukomlskoye Lake (Table 2). Since there was no temperature stratification in all the lakes in the sampling sites, except Kurilskoye Lake in August, the temperature values (Table 2) represented averages for water column, because seston and zooplankton samples were also pooled from the entire water column.

Levels (per cents of the total FAs) of EPA in seston varied from 1.1% in Kurilskoye Lake in August to 9.7% in Kurilskoye Lake in June (Table 2). Levels of DHA in seston were from 0.0% in Kurilskoye Lake in August to 3.1% in pelagial of Gorushka Lake (Table 2). Content of EPA per organic carbon in seston ranged from 0.7 mg g^{-1} in Kilometrovoye Lake to 15.0 mg g^{-1} in Kurilskoye Lake in June (Table 2). Values of DHA/C in seston were from 0.0 mg g^{-1} in Kurilskoye Lake in August to 3.0 mg g^{-1} in Kurilskoye Lake in June (Table 2).

Levels of EPA in zooplankton ranged from 6.5% in center of Oiskoye lake and in littoral of Lukomlskoye Lake to 13.1% in Karovoye Lake (Table 3). Levels of DHA were from 1.5% in littoral of Lukomlskoye Lake to 12.9% in bay of Bolshoi Kharbei Lake (Table 3). Content of EPA per organic carbon in zooplankton biomass varied from 0.7 mg g^{-1} in littoral of Lukomlskoye Lake to 96.1 mg g^{-1} in Kurilskoye Lake in July (Table 3). Values of DHA/C ranged from 3.1 mg g^{-1} in littoral of Lukomlskoye Lake to 75.6 mg g^{-1} in Kurilskoye Lake in July (Table 3). Minimum value of per cent of copepods in biomass of zooplankton, 1.8%, was in Dvaozera Lake, and maximum value, 92.7%, was in Kurilskoye Lake in July (Table 3). Biomass of copepods ranged from 12 mg m^{-3} of wet weight in littoral of Lukomlskoye Lake to 1399 mg m^{-3} in pelagial of Gorushka lake (Table 4). Biomass of cladocerans was from 1 mg m^{-3} in Kurilskoye Lake to 1397 mg m^{-3} in littoral of Nobisto Lake (Table 4). Common

Table 2
Water temperature (t, °C), levels (percent of total FAs) of eicosapentaenoic and docosahexaenoic acids and their concentration per organic carbon (mg g^{-1}) in seston in the studied lakes, 2008 (Kurilskoye) and 2009 (the other lakes). EPA and DHA were quantified as equivalents of nonadecanoic acid.

Lake (date)	t	EPA, %	DHA, %	EPA/C	DHA/C
Kurilskoye					
5 June	1.9	9.7	2.0	15.0	3.0
1 July	3.6	8.9	1.8	10.8	2.2
15 August	5.5	1.1	0.0	1.8	0.0
Oiskoye					
Center (31 August)	10.3	4.2	2.2	3.5	1.9
Bay (4 September)	9.9	2.4	1.2	2.5	1.2
Karovoye (1 September)	11.0	3.8	2.6	3.7	2.6
Bolshoi Kharbei (30 July)					
Center	14.0	4.4	0.9	2.9	0.6
Bay	14.7	2.9	1.0	1.9	0.6
Golovka (29 July)	14.6	3.6	0.8	1.0	0.2
Dvaozera (3 August)	16.1	3.5	1.4	2.1	0.8
Kilometrovoye (1 August)	16.7	1.8	0.7	0.7	0.3
Obsterno (12 July)					
Pelagial	20.4	5.2	1.4	3.1	0.9
Littoral	20.4	3.6	1.2	2.5	0.8
Nobisto (13 July)					
Pelagial	20.2	3.7	2.4	1.6	1.1
Littoral	20.9	2.9	1.6	2.6	1.5
Gorushka (17 July)					
Pelagial	22.1	4.9	3.1	2.2	1.4
Littoral	22.1	4.0	1.7	1.6	0.7
Lukomlskoye (15 July)					
Pelagial	27.5	7.0	1.5	4.4	0.9
Littoral	28.2	9.1	1.2	4.1	0.6

dominant genera in many lakes were *Eudiaptomus*, *Cyclops*, *Daphnia* and *Diaphanosoma* (Table 4).

CCA (Table 5 and Fig. 2) revealed significant overall canonical correlation between log-transformed water temperature, EPA and DHA per cent levels in seston, per cent of copepods in biomass of zooplankton community (independent variables) and EPA and DHA per cent levels in zooplankton (dependent variables). Canonical correlation coefficient, R_1, pertaining to the first canonical root, was high and statistically significant, and the coefficient of second root, R_2, was comparatively low and statistically insignificant (Table 5).

In the set of independent variables per cent of copepods had the lowest negative factor loading for the first canonical root, while in the set of dependent variables DHA level in zooplankton had the lowest negative loading (Table 5). Thus, the highest canonical correlation, R_1, between the two sets of variables was mostly due to interactions between per cent of copepods in the zooplankton communities and per cent of DHA in the total FAs of zooplankton. All the other independent variables, i.e., water temperature and HUFA levels in seston, gave significantly less contribution to the variability of dependent variables, HUFA levels in zooplankton (Table 5).

Table 3
Levels (percent of total FAs) of eicosapentaenoic and docosahexaenoic acids and their concentration per organic carbon (mg g^{-1}) and per cent of copepods in biomass of the mesozooplankton in the studied lakes, 2008 (Kurilskoye) and 2009 (the other lakes). EPA and DHA were quantified as equivalents of nonadecanoic acid.

Lake (date)	EPA, %	DHA, %	EPA/C	DHA/C	Copepods, %
Kurilskoye					
5 June	10.2	12.3	n.d.	n.d.	91.7
1 July	9.8	12.4	96.1	75.6	92.7
15 August	6.6	11.6	64.1	36.4	61.0
Oiskoye					
Center (31 August)	6.5	6.9	16.2	15.3	72.3
Bay (4 September)	7.3	4.7	56.0	47.0	34.2
Karovoye (1 September)	13.1	8.7	19.2	53.8	10.9
Bolshoi Kharbei					
Center (30 July)	10.7	7.6	10.4	14.7	29.4
Bay (30 July)	8.4	12.9	19.6	12.7	64.1
Golovka (29 July)	8.7	6.3	4.0	5.4	29.2
Dvaozera (3 August)	12.8	2.5	1.7	8.8	1.8
Kilometrovoye (1 August)	8.9	5.3	3.5	5.8	30.0
Obsterno (12 July)					
Pelagial	9.2	7.0	8.6	11.2	38.5
Littoral	8.8	2.1	1.8	7.4	11.4
Nobisto (13 July)					
Pelagial	8.2	5.1	4.9	7.8	27.0
Littoral	10.3	3.7	4.5	12.6	25.3
Gorushka (17 July)					
Pelagial	8.1	9.8	13.7	11.4	57.5
Littoral	8.7	7.0	8.4	10.4	32.3
Lukomlskoye (15 July)					
Pelagial	10.7	3.4	2.8	9.0	28.0
Littoral	6.5	1.5	0.7	3.1	16.8

n.d. – no data.

Table 4
Biomass (*B*, mg m^{-3}) and dominant species composition of mesozooplankton in the studied lakes (2008–2009).

Lake	Copepoda		Cladocera	
	B	Dominant species	*B*	Dominant species
Kurilskoye				
June	47	*Cyclops scutifer*	2	*Daphnia longiremis*
July	93	*Cyclops scutifer*	1	*Daphnia longiremis*
August	136	*Cyclops scutifer*	1	*Daphnia longiremis*
Oiskoye				
Center	141	*Cyclops abyssorum*	20	*Daphnia longispina*
Bay	106	*Arctodiaptomus* sp.	17	*Daphnia longispina*
Karovoye	60	*Acanthodiaptomus denticornis*	486	*Daphnia longispina*
Bolshoi Kharbei				
Center	117	*Cyclops scutifer*	230	*Daphnia longiremis*
Bay	578	*Eudiaptomus gracilis*	110	*Daphnia longiremis*
Golovka	397	*Eudiaptomus graciloides*	611	*Daphnia longiremis*
Dvaozera	18	*Eudiaptomus graciloides*	811	*Bosmina longispina*
Kilometrovoye	183	*Eudiaptomus gracilis*	422	*Daphnia galeata*
Obsterno				
Pelagial	264	*Eudiaptomus graciloides*	421	*Diaphanosoma brachyurum*
Littoral	130	*Mesocyclops leuckarti*	851	*Ceriodaphnia pulchella*
Nobisto				
Pelagial	90	*Acanthocyclops viridis*	443	*Ceriodaphnia pulchella*
Littoral	87	*Eucyclops macruroides*	1397	*Polyphemus pediculus*
Gorushka				
Pelagial	1399	*Eudiaptomus graciloides*	1018	*Diaphanosoma brachyurum*
Littoral	316	*Eudiaptomus graciloides*	1000	*Ceriodaphnia pulchella*
Lukomlskoye				
Pelagial	132	*Thermocyclops oithonoides*	339	*Diaphanosoma brachyurum*
Littoral	12	*Mesocyclops leuckarti*	66	*Bosmina crassicornis*

There were no significant pair correlation between log-transformed values of the water temperature and HUFA levels in seston. In contrast, there was negative correlation between the water temperature and DHA level in zooplankton, product-moment correlation coefficient $r = -0.61$, number of pairs $n = 19$, $p < 0.01$. There was also positive pair correlation between per cent of copepods in zooplankton community and DHA level in zooplankton: $r = 0.70$, number of pairs $n = 19$, $p < 0.001$.

Table 5
Results of canonical correlation analysis of log-transformed water temperature (*t*, °C), EPA and DHA per cent levels in seston, per cent of copepods in biomass of zooplankton community and EPA and DHA per cent levels in zooplankton of the studied lakes (2008–2009).

	Independent variables	Dependent variables
No. of variables	5	3
Variance extracted (%)	62.1	100
Total redundancy (%)	28.8	50.0
Number of valid cases		18
	Root 1	**Root 2**
Eigenvalues	0.843	0.412
Canonical *R*	0.918	0.642
Chi-square	31.35	8.18
d.f.	15	8
p	0.0079	0.4164
Factor loadings		
Independent variables		
t	0.383	0.800
Copepods	−0.939	−0.092
EPA seston	0.021	−0.382
DHA seston	−0.185	−0.302
EPA + DHA seston	−0.031	−0.387
Dependent variables		
EPA zooplankton	0.485	−0.621
DHA zooplankton	−0.783	−0.575
EPA + DHA zooplankton	−0.387	−0.849

Per cent levels of EPA and DHA were the qualitative characteristic, reflected the FA compositions of seston and zooplankton. To study quantitative relation, contents of HUFA per mass unit of organic carbon were used. Thus, the second CCA included the water temperature, the per cent of copepods, contents of HUFA (EPA and DHA) per organic carbon (C) in seston as independent variables, and HUFA per carbon contents in zooplankton as dependent variables (Table 6 and Fig. 3). Canonical correlation, R_1, pertaining to the first canonical root, was high and significant due to roughly equal positive interaction between the per cent of copepods with DHA/C, on the one hand, and negative interaction between the per cent of copepods and EPA/C, on the other hand (Table 6). Canonical corre-
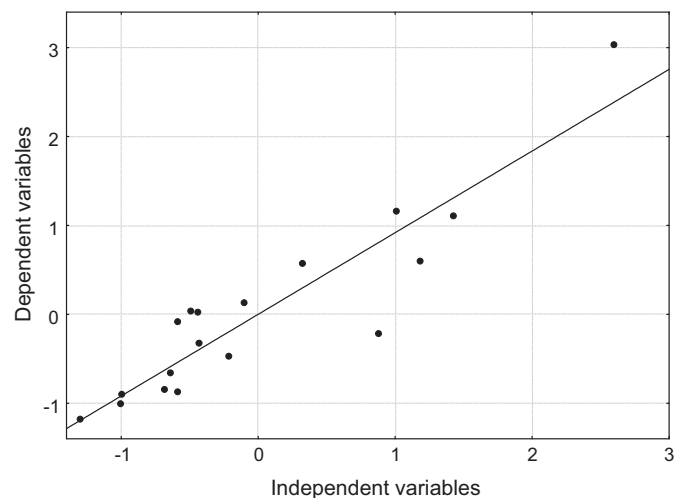
Fig. 2. Plots of correlations between the two sets of log-transformed canonical variables of the first canonical root (see Table 5): per cent of copepods in biomass of zooplankton community, water temperature, EPA and DHA and their sum per cent levels in seston (independent variables) vs. EPA and DHA and their sum per cent levels in zooplankton (dependent variables) in the studied lakes (2008–2009): dots – experimental data; line – linear approximation.
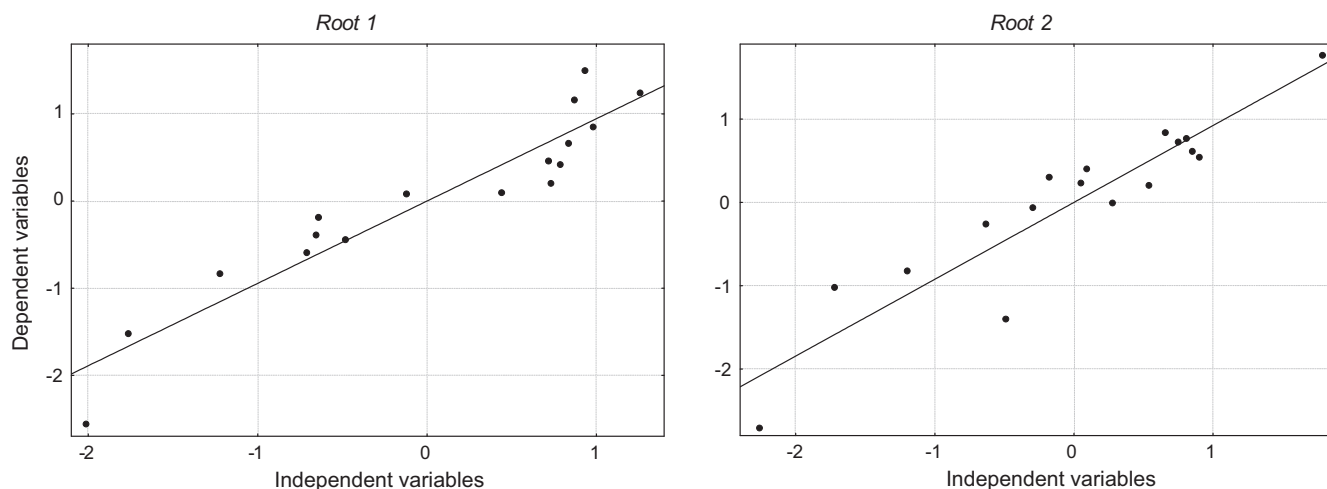
Fig. 3. Plots of correlations between the two sets of log-transformed canonical variables of the first and second canonical roots: water temperature, per cent of copepods in biomass of zooplankton community, EPA/C ($mg\,g^{-1}$) and DHA/C ($mg\,g^{-1}$) and their sum in seston (independent variables) vs. EPA/C ($mg\,g^{-1}$) and DHA/C ($mg\,g^{-1}$) and their sum in zooplankton (dependent variables) in the studied lakes (2008–2009): dots – experimental data, line – linear approximation. EPA and DHA were quantified as equivalents of nonadecanoic acid.

lation, pertained to the second canonical root, R_2 (which is known to be orthogonal to the first one), was also significant and approximately as high as that of R_1 (Table 6). The highest contribution to the second canonical correlation was made by negative interaction between water temperature and (EPA + DHA)/C zooplankton (Table 6). The other independent variables, HUFA/C contents of seston, gave significantly less contribution to the variability of the dependent variables, HUFA/C contents of zooplankton (Table 6).

Among the sestonic HUFA, log-transformed EPA/C had the highest pair correlation with log-transformed water temperature: $r = -0.58$, $n = 19$, $p < 0.01$. For zooplankton, (EPA + DHA)/C had the highest correlation with the temperature: $r = -0.84$, $n = 19$, $p < 0.001$ (Fig. 4). Pair correlation between the per cent of copepods and water temperature was comparatively small ($r = -0.46$).

Table 6

Results of canonical correlation analysis of log-transformed water temperature (t, °C), per cent of copepods in biomass of zooplankton community, HUFA (EPA and DHA) to organic carbon (C) ratios ($mg\,g^{-1}$) in seston (independent variables) and HUFA to C ratios in biomass of zooplankton (dependent variables) of the studied lakes (2008–2009). EPA and DHA were quantified as equivalents of nonadecanoic acid.

	Independent variables	Dependent variables
No. of variables	5	3
Variance extracted (%)	71.3	100
Total redundancy (%)	59.0	84.6
Number of valid cases		17
	Root 1	Root 2
Eigenvalues	0.893	0.851
Canonical R	0.945	0.923
Chi-square	48.5	22.9
d.f.	15	8
p	0.00002	0.00356
Factor loadings		
Independent variables		
t	0.081	0.885
Copepods	0.756	−0.496
EPA/C seston	−0.497	−0.506
DHA/C seston	−0.440	−0.695
(EPA + DHA)/C seston	−0.513	−0.583
Dependent variables		
EPA/C zooplankton	−0.237	−0.960
DHA/C zooplankton	0.228	−0.974
(EPA + DHA)/C zooplankton	−0.040	−0.998

Discussion

We found significant inverse relation between water temperature and content of HUFA in zooplankton in lakes from warm (Belarus) and cold (tundra, mountains in Siberia and Kamchatka) regions. Our finding means that the temperature explained 84% of variability of the content of EPA + DHA per organic carbon, while all the other factors, such as accuracy, precision, morphometry and trophy, provided only 16% of the variability. Thus, the used sampling protocol (sampling several lakes once or a few times), like in the work of Persson and Vrede (2006), allows making more general conclusion than if one lake had been sampled repeatedly over the season.

However, correlations do not necessarily point to causal relationships. Indeed, in our study, besides temperature, CCA revealed positive relation between the per cent of copepods and DHA content in zooplankton, but negative relation with EPA content (Table 5). In the studied lakes zooplankton with more than 90% copepods had about 10% of EPA and more than 12% of DHA (Table 3). Our data are close to the generalization of Brett et al. (2009) that omnivorous and carnivorous freshwater copepods have about 10% of EPA and about 20% of DHA, while herbivorous freshwater cladocerans have on average 15% of EPA and 2% of DHA levels in their biomass.
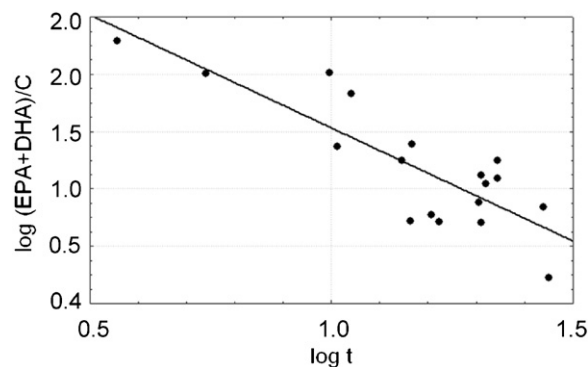
Fig. 4. Plot of correlations between the log-transformed content of sum of eicosapentaenoic and docosahexaenoic acids per organic carbon, (EPA + DHA)/C ($mg\,g^{-1}$) vs. water temperature, t, in the studied lakes (2008–2009): dots – experimental data, line – linear approximation. EPA and DHA were quantified as equivalents of nonadecanoic acid.

The differences in HUFA levels in biomass of cladocerans and copepods are related with their ability to synthesize these long-chain fatty acids by conversion from short-chain precursors, such as absolutely essential α-linolenic acid, 18:3ω3. Cladocerans, e.g., *Daphnia magna*, *Daphnia pulex* and *Daphnia galeata* had negligible ability to the conversion (Goulden and Place 1990; Von Elert 2002). In contrast, many copepod species are known to synthesize substantial amounts of EPA and, especially, DHA from dietary 18:3ω3 (Desvilettes et al. 1997; Nanton and Castell 1998; Anderson and Pond 2000; Caramujo et al. 2008; but see Von Elert and Stampfl 2000).

Seston HUFA content also contributed to the HUFA content of zooplankton, although lower, than the temperature and the per cent of copepods. The content of HUFA in seston, especially, that of EPA, had significant negative correlation with the water temperature, although this correlation was lower, than that for HUFA content in zooplankton. Decrease of EPA and other polyunsaturated fatty acids in seston in response to increase of temperature is well known (e.g., Sushchik et al. 2003b; Gladyshev et al. 2010). Also dependence of HUFA levels of zooplankton on that of their diet, seston, is evident (Kainz et al. 2004; Rossi et al. 2006; Smyntek et al. 2008). Ravet et al. (2010) remarked that despite the apparent effects of seston composition on the fatty acid composition of zooplankton, the fatty acid composition of zooplankton also differed systematically from that of potential dietary items. Moreover, the sampling protocol, used in our study, when the different lakes were sampled once or twice, affected appearance of these tendencies. Indeed, seston fatty acid composition is known to vary more rapidly and frequently, than that of zooplankton (e.g., Ravet et al. 2010), and these fluctuations could noise the correlations. Persson and Vrede (2006) using the same sampling protocol also did not find any statistical relationship between seston and zooplankton fatty acid composition. In any case, in our study water temperature appeared to be better predictor of HUFA contents of zooplankton, than the fatty acid composition of seston.

Hessen and Leu (2006) supposed that higher levels of HUFA in zooplankton of Arctic lakes compared to temperate lakes might be due to lower water temperature. These findings seem to be in a good agreement with the data of many authors, who studied natural freshwater zooplankton communities and interpreted this reverse temperature–HUFA relation on the basis of the hypothesis of homeoviscous adaptation (Farkas and Herodek 1964; Maazouzi et al. 2008; Gladyshev et al., 2010). The hypothesis of homeoviscous adaptation was supported by some laboratory experiments with a number zooplankton species. Farkas (1979) found that copepods, exposed to cold, nearly doubled the level of DHA in their phospholipids. Schlechtriem et al. (2006) reported that *D. pulex*, adapted to 11 °C, had level of EPA 12.7%, while specimens, adapted to 22 °C, had 3.1% of EPA. Masclaux et al. (2009) on the basis of experiments with *D. magna* and *Simocephalus vetulus* concluded that dietary EPA was important for maintaining membrane fluidity of cladocerans at low temperatures. In contrast, *D. magna* exposed to cold, did not have elevated levels of polyunsaturated FA (Farkas et al. 1984). Moreover, Taipale et al. (2009) reported that in a field study water temperature did not correlate significantly with HUFA level in *Daphnia longispina*. The data of Taipale et al. (2009) on freshwater zooplankton seem to be in general agreement with finding of Kattner and Hagen (2009) that marine copepods in arctic and tropical oceans had about equal levels of HUFA. Thus, some authors questioned the importance of the mechanism of membrane fluidity regulation for explanation of HUFA variations in zooplankton (Sargent et al. 1995; Kattner et al. 2007; Kattner and Hagen 2009).

Our present data seem to give an opportunity to explain the above contradictions. Indeed, the best predictor of the HUFA levels and contents of zooplankton appeared to be per cent of copepods in zooplankton community, while water temperature contributed substantially only in the second canonical root (Tables 5 and 6). This means that temperature primarily affected the HUFA levels indirectly, via changing of taxonomic structure of zooplankton community, while the homeoviscous adaptation of zooplankton individuals had comparatively lower importance. There is a basic difference between copepods and cladocerans: copepods are active in winter and summer and could increase the level of HUFA when exposed to cold, while cladocerans spend winter in resting stage and are active only in the summer, and are not able to increase HUFA level in response to cooling (Farkas 1979; Farkas et al. 1984).

As found in present study, water temperature had both direct and indirect effect on the HUFA content. Indirect effect appeared to be due to the change of community structure. We found a negative correlation between water temperature and per cent of copepods in zooplankton community, but it was comparatively low. Many authors reported that increase of water temperature favored cladocerans, rather than copepods (Schindler et al. 2005; Hampton et al. 2008; Thompson et al. 2008; Visconti et al. 2008), and domination of copepod in cold (arctic and subarctic) lakes is common (Rautio 2001; O'Brien et al. 2004; Dubovskaya et al. 2010). In contrast, some other authors found enhanced summer biomass of copepods in European lakes after mild winters (positive North Atlantic Oscillation indices) (Blenckner et al. 2007), or no effect of warming on copepods in mesocosm experiments (Strecker et al. 2004). It should be noted, that mesocosm experiments give only short-term direct effect of temperature on zooplankton. Effects of climate warming on freshwater zooplankton are believed to depend on location and lake morphometry (Schindler et al. 2005) and thereby should be studied on a number of different lakes. Moreover, there may be indirect effect of temperature on zooplankton. For instance, correlation between zooplankton biomass, dominated by copepods, with water temperature in Subarctic Sweden lakes along a 6 °C air temperature gradient was comparatively weak, but variations in copepod biomass were better explained by biomass of bacterioplankton and ciliates (Jansson et al. 2010). In turn, bacterioplankton biomass along the gradient was controlled by an indirect effect of the climate, i.e., air temperature controlled terrestrial production, which in turn set limits for the export of organic carbon and nutrients, which then determined planktonic biomass in the lakes (Jansson et al. 2010). This finding on the indirect effect of temperature on copepod biomass seems to be useful for better explanation of some our results. Indeed, there was no high correlation between the water temperature and the per cent of copepods in our study, but the superposition of these two independent variables gave very high and significant canonical correlation with HUFA levels and contents in zooplankton. In other words, water temperature and the per cent of copepods in complex gave better prediction of the HUFA contents in zooplankton than any one of them separately.

Kattner and Hagen (2009) when predicting probable effects of global warming on lipid production of zooplankton in oceans, used the same approach, as in our work, i.e., they compared zooplankton from cold and warm regions. They concluded that global warming "could have a tremendous impact on the food quantity and energy flux to higher trophic levels in polar oceans. In contrast, food quality will probably be less affected concerning essential fatty acids, since temperate and tropical species are also rich in these components. The occurrence of essential fatty acids, such as EPA and DHA, is probably less impacted by global changes, since they are structural components of all marine bio-membranes, largely independent of latitudinal distribution" (Kattner and Hagen 2009, p. 273). According to our data, in freshwater zooplankton communities, in contrast to marine environments, an increase of water temperature will provide a decrease of food quality concerning HUFA contents. Because lakes and reservoirs have global perimeter about two orders of magnitude higher, than the length of ocean shoreline, freshwater ecosystems are the main source of essential HUFA for terrestrial

ecosystems (Gladyshev et al. 2009). Hence, the probable decrease of HUFA levels in freshwaters is believed to have global negative consequences.

Content of HUFA, especially of DHA, in biomass of zooplankton as food are known to be of key importance for the development of fish larvae (Sargent et al. 1999; Copeman et al. 2002; Vizcaino-Ochoa et al. 2010). Namely copepods, which have the ability to either selectively retain certain HUFA from the diet or to synthesize them, are known to be nutritionally beneficial to optimize growth, survival and metamorphosis of fish larvae and to reduce the incidence of disease (Caramujo et al. 2008). Although in our study the per cent of copepods correlated significantly with DHA content in zooplankton, water temperature had the higher absolute value of the pair coefficient of correlation with HUFA content in zooplankton biomass, (EPA + DHA)/C (Fig. 4). This means, that under higher water temperature HUFA contents in zooplankton will decrease with possible negative consequences for higher trophic levels, first of all – for fish larvae. Thus, one may expect a negative effect of possible climate warming on HUFA content in fish in freshwater ecosystems because of decreasing of food quality, namely decreasing of HUFA contents in zooplankton. Indeed, in European lakes climate warming is believed to stimulate a shift from coregonids and other salmonids to percids, and from percids to cyprinids (Jeppesen et al. 2010), and salmonids have significantly higher HUFA content in their biomass compare to that of percides and cyprinids (Gladyshev et al. 2009).

Thus, studying lakes, different in location and morphometry, we found pronounced direct and indirect effect of temperature on HUFA content in zooplankton biomass. In general, the HUFA content decreased with increase of temperature, primarily due to a decrease of per cent of copepods in zooplankton communities, which are known to have significantly higher HUFA, especially DHA levels, than cladocerans. Besides, a temperature increase seems to decrease HUFA levels in zooplankton individuals, likely through the homeoviscous adaptation. Thereby, one may expect that global warming will likely decrease HUFA content in freshwater zooplankton with possible negative consequences for organisms of higher trophic levels.

Acknowledgements

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