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ORIGINAL PAPER

Seasonal correlations of elemental and ω3 PUFA composition of seston and dominant phytoplankton species in a eutrophic Siberian Reservoir

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Abstract The elemental and fatty acid composition of seston was studied for 3 years, from May to October, in a small Reservoir. Under comparatively low C:P ratio, multivariate canonical analysis revealed no straightforward simple correlations between phosphorus and single ω3 PUFA species, but complex significant interaction between elemental composition (stoichiometry) of seston and total sestonic ω3 PUFA as a whole. Since sestonic C, P and N were found to originate mostly from phytoplankton, the contents of particulate elements and PUFA were attributed to single species in periods of their pronounced dominance. Phytoplankton species of genera of *Stephanodiscus*, *Peridinium*, *Gomphosphaeria*, *Planktothrix* and *Anabaena* in periods of their pronounced dominance had relatively constant species-specific elemental and PUFA composition. Phytoplankton species significantly differed in their elemental and PUFA composition, as well as in ratios of C:N, N:P, PUFA:P and partly C:P that indicate food quality for zooplankton. Hence, there were no phytoplankton species of clearly high or low nutritional value. All of phytoplankters, or at least detritus, that originated from them, may meet specific elemental and biochemical requirements of specific groups of zooplankton. Dividing phytoplankton on basis of their elemental and biochemical composition, i.e., nutrition quality, into large taxa (cyanobacteria, diatoms, etc.) appeared to be too coarse for assessing nutritional value for zooplankton.

Keywords Seston · Elemental stoichiometry · Polyunsaturated fatty acids · Phytoplankton

Introduction

Interactions at the primary producers–primary consumers interface are known to be of crucial importance for overall ecosystem functioning, because transfers of energy and matter between these two trophic levels are complicated by profound differences in elemental and biochemical composition of photoautotrophs and animals. For pelagic ecosystems many studies of such interactions have focused on elemental and biochemical

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quality of phytoplankton as food for herbivorous zooplankton. On the one hand, C:N:P (especially C:P) stoichiometry is regarded to be the key parameter determining the food quality (Elser et al. 1998; Anderson et al. 2004; Vrede et al. 2004), and on the other hand, some studies show the contents of essential polyunsaturated fatty acids (PUFA) of the $\omega 3$ family as the core characteristic (Muller-Navarra 1995a, b; Von Elert and Stampfl 2000; Bec et al. 2003). A number of authors reported that both elemental and biochemical properties of phytoplankton are important as mutually non-exclusive mechanisms of limitation of zooplankton (Gulati and DeMott 1997; Boersma et al. 2001; Park et al. 2002). Moreover, these factors probably cannot be separated and ‘‘the discussion concerning biochemical versus elemental limitation of living zooplankton will not be relevant, because in high-quality phytoplankton P and $\omega 3$ PUFA go hand in hand’’ (Ahlgren et al. 1997).

Evidence for each of the above points of view is based on field correlations, but also on primarily on laboratory experiments. Field correlations evidently do not prove any mechanism of how phytoplankton quality affects zooplankton growth. Specially designed laboratory experiments are intended to elucidate namely mechanisms of an influence of a given parameter of food quality on somatic and population growth of zooplankton. Nevertheless, translating results of laboratory experiments to natural ecosystems always is faced with uncertainties (Gulati and DeMott 1997; Sterner and Schulz 1998; DeMott et al. 2001). First, laboratory studies are restricted to a limited number of algae and cyanobacteria, which are not necessarily dominant species in natural phytoplankton assemblages. Secondly, in many special-purpose experiments, the contents of elements and fatty acids are far from an ecologically relevant ranges (Brett et al., 2000; Becker and Boersma 2005). Moreover, even short-term mesocosm experiments, let alone laboratory cultures, do not allow for arrivals of new species of phytoplankton and zooplankton from the regional species pools (so-called ‘meta-communities’), while these arrivals could have affected the long-term outcome of the experiment (Hall et al. 2004). Evidently, the inevitable trade-off between mechanism and realism in experiments and field observations require equal attention to both approaches.

Recent reviews on the food quality studies show that field data on PUFA and element contents in natural phytoplankton communities dominated contrasting taxa, e.g., cyanobacteria versus eukaryotic alga would be very important for the analysis of food quality (Gulati and DeMott 1997; Sterner and Schulz 1998). Gulati and DeMott (1997) pointed out that factors that correlate high or low concentrations of PUFA in food with variations in P are crucial in defining food quality in the field, and that these are still poorly investigated. A specific but unanswered question is whether PUFA are generally positively or negatively related to P limitation in algae. Do these two potentially limiting factors covary, or are they independent (Gulati and DeMott 1997)? There are three groups of alternative evidences and hypotheses that PUFA and phosphorus content in phytoplankton (seston) are related to each other (i) positively (Ahlgren et al. 1997; Wacker and Von Elert 2001); (ii) negatively (Sterner and Schulz 1998; Muller-Navarra et al. 2004); and (iii) there is no correlation between these sestonic parameters (Muller-Navarra 1995a; Park et al. 2002). Thus, the first aim of our present work is to make an attempt to elucidate these controversial evidences and study the seasonal correlations between elemental and PUFA contents of seston in a small Siberian eutrophic Reservoir, where contrasting phytoplankton taxa, cyanobacteria and eukaryotic alga, successively dominated.

In additional, we tried to relate the elemental stoichiometry and the PUFA pattern of seston to the phytoplankton species composition in periods of pronounced dominance of single species. On the one hand, different phytoplankton species are known to have specific biochemical and elemental composition (Ahlgren et al. 1992) and on the other, the contents of elements and PUFAs are evidently not the sole determinants of the food quality for zooplankton, but also size, shape, cell wall thickness, motility and pliability of phytoplankton, are of key importance (e.g., Gulati and DeMott, 1997; Gladyshev et al. 1999; Mitra and Flynn 2005). All these parameters—chemical, biochemical and morphological—are evidently related to the growing conditions. Algal taxonomic affiliation or "species succession", rather than elemental stoichiometry, may explain a large part of the variability in algal food quality for zooplankton (Brett et al. 2000; Park et al. 2002). However, species composition of natural phytoplankton communities was hardly regarded as a factor that covaried with elemental stoichiometry, in contrast to laboratory studies. It is common knowledge that a low phosphorus supply provides P-deficient microalgae of low nutrition quality. The low quality was often found to be due to a low content of PUFA. Laboratory experiments with monocultures of algae have proven this exhaustively (e.g., Sundbom and Vrede 1997; Von Elert 2002, Acharya et al. 2004, but see also DeMott 1998). The essential question is whether the effect of P-limitation, revealed in laboratory monocultures, is still apparent in complex natural assemblages. Unfortunately, it is not possible to directly determine the relative importance of algal P content and taxonomic affiliation for the dietary quality of natural zooplankton assemblages. This could only be done for a set of data with numbers of observations for the major algal taxa representative of natural lakes (Brett et al. 2000). Moreover, dominance, and even a significant contribution of a phosphorus-deficient microalgal species in natural seston, is unrealistic because such species inevitably will be out-competed and driven to extinction from multi-species community of a natural ecosystem. If a species becomes dominant, it means that all the factors of its multidimensional ecological niche are optimal relative to other competing species, and one may expect, that under such optimal conditions this species should exhibit more or less constant properties, for instance, elemental and PUFA stoichiometry. We hypothesize, that in periods of pronounced dominance of a natural phytoplankton species, this species would have relatively constant ('optimal') species-specific elemental stoichiometry and PUFA content.

The study site

The studied Reservoir is situated 56°03′ N and 92°43′ E at the North-West edge of Krasnoyarsk city in the Bugach river (secondary tributary of the Yenisei river) and locally known as the Bugach pond. The Reservoir has steppe banks and is exposed to a considerable wind action. Its surface area is 0.32 km^2, and its maximum depth is 7 m, and the mean depth is ~2 m. Mean seasonal concentrations of dissolved inorganic phosphorus in 2002, 2003 and 2004 were 0.014 mg l^{-1}, 0.005 mg l^{-1} and 0.008 mg l^{-1}, respectively, and mean concentrations of NH_4–N were 0.163 mg l^{-1}, 0.254 mg l^{-1} and 0.221 mg l^{-1}, respectively. Detailed descriptions of the Reservoir are given elsewhere (Sushchik et al. 2003a, 2004).

Methods

The Reservoir was sampled weekly from May to October, during the vegetation seasons of 2002–2004. Samples were taken from a site near the middle with a Ruttner-like 8 l sampler and were pooled from depths 1–3(4) m. Thermal stratification at the sample site was absent, depth at the station was about 4.5 m.

Phytoplankton sub-samples of 0.15–0.5 l were filtered through "Vladipor" membrane filters (Mytischi, Russia; pore size, 0.75–0.85 μm). The filters were placed in filtered Reservoir water with Lugol's solution. Prior to enumeration in counting chamber, phytoplankton was scraped from filters with scalpel. Microalgae were counted and identified using a Fuchs–Rosenthal counting chamber (0.0032 ml volume) under an inverted microscope at 400× magnification. To calculate the biovolume and wet weight (ww) of algae, they were equated to appropriate geometrical shapes (or their combinations) and relevant sizes were measured using an ocular micrometer.

To assay particulate organic carbon and nitrogen, total phosphorus and fatty acids, 1–1.5 l water sub-samples were pre-screened through 115 μm nylon mesh. Using a smaller seston size fraction, such as <30 μm would have excluded some of the important food source for zooplankton, such as filamentous prokaryotes (DeMott and Gulati 1999).

For organic carbon and nitrogen measurements, seston from these pre-screened 200–300 ml water sub-samples was collected by vacuum filtration onto precombusted GF/F filters (Whatman, USA). The samples for carbon and nitrogen were dried at ambient temperature overnight and stored in desiccator until analysis with a Flash EA 1112 NC Soil/MAS 200 elemental analyzer (NEOLAB LLC, USA).

The particulate P sub-samples of volume of 100–150 ml were filtered onto membrane filters (Vladipor, Mytischi, Russia, pore size 0.75–0.85 μm). The background P content of the filters was preliminarily measured and subtracted from the samples' values. On the day of sampling the samples were analysed directly, by persulphate oxidation followed by the ammonium-molybdate method on a spectrophotometer at 680 nm.

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), 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 was then separated from filters and placed into methanol and frozen.

Details of fatty acid analysis are described in Gladyshev et al. (2000). Lipids were extracted three times with chloroform–methanol (2:1, v/v). After evaporation of the solvent, methyl esters of fatty acids were prepared in a mixture of methanol–sulphuric acid (20:1, v/v) at 90°C for 2 h.

Methyl esters of fatty acids (FAME) were analyzed on a gas chromatograph equipped with a mass spectrometer detector (GCD Plus, Hewlett-Packard, USA) with a capillary HP-FFAP column as described earlier (Sushchik et al. 2004). Identification of peaks of FAMEs and their specific derivatives were carried out as described elsewhere (Sushchik et al. 2003b).

Statistical analysis, Student's *t*-test, one-way ANOVA, canonical correlation analysis (CCA) and correspondence analysis (CA) were carried out conventionally (Campbell 1967; Jeffers 1981), using STATISTICA software, version 6.0 (StatSoft, Inc.).

Results

Seasonal dynamics

Ranges of seasonal variations of sestonic parameters are given in Table 1. The concentration of sestonic C, or particulated organic carbon (POC) during all 3 years had prominent peaks with seasonal maxima in June (Fig. 1). In the second half of summer and autumn POC concentrations varied with more (2002) or less (2004) expressed local maxima.

Concentration of sestonic N (particulate organic nitrogen, PON) in general followed the dynamics of POC, except in 2004, when a gradual increase of PON concentrations occurred from the beginning of July, and seasonal maximum occurred in October (Fig. 1). Sestonic P (particulated organic phosphorus, POP) also reached seasonal maxima in June, while in July–October

Table 1 Mean values ± standard errors and ranges (in parentheses) of seasonal variations of sestonic parameters in May–October 2002–2004 in Bugach Reservoir. Samples were collected in the period May to October in 2002–2004

Parameter	mean ± SE (max–min)	Number of observations
C (mg l^{-1})	6.8 ± 0.4 (2.9–15.6)	55
N (mg l^{-1})	1.7 ± 0.2 (0.4–5.7)	47
P (mg l^{-1})	0.12 ± 0.01 (0.04–0.22)	52
C:N (mol)	5.5 ± 0.4 (2.7–16.0)	47
C:P (mol)	126 ± 8.8 (20–269)	52
N:P(mol)	35.4 ± 3.1 (7.8–93.7)	44
ALA (μg l^{-1})	19.4 ± 2.0 (2.6–52.7)	56
EPA (μg l^{-1})	16.0 ± 1.9 (2.7–86.4)	56
DHA (μg l^{-1})	8.7 ± 1.1 (0.0–39.1)	56
$\Sigma\omega$3-PUFA (μg l^{-1})	71.2 ± 7.8 (15.7–395.6)	56
EPA:C (μg mg^{-1})	2.8 ± 0.3 (0.2–14.3)	55
$\Sigma\omega$3-PUFA:C (μg mg^{-1})	11.9 ± 1.4 (1.7–65.3)	55
Phytoplankton (mg ww l^{-1})	23.9 ± 4.8 (2.3–206.1)	55

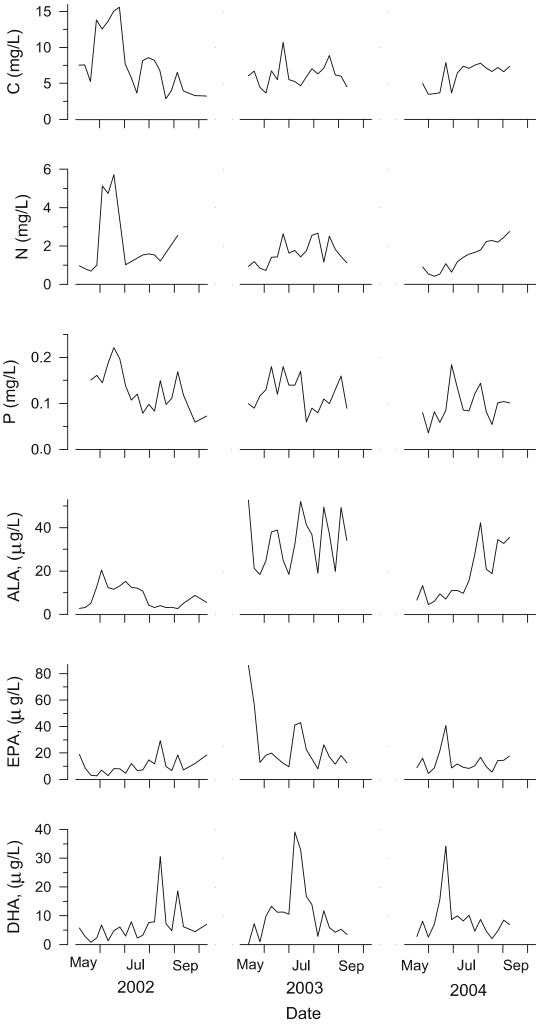

Fig. 1 Seasonal dynamics of sestonic elements and $\omega3$ PUFA species in Bugach Reservoir

variations of POP concentrations in general did not coincide with those of POC and PON (Fig. 1).

Concentrations of the main sestonic PUFAs did not reveal any general pattern. In 2002, the α-linolenic acid (ALA, 18:3$\omega3$) had seasonal maximum in early June and a decrease thereafter (Fig. 1). In 2003 ALA had several similar peaks at each month. In 2004, the concentration of ALA increased to reach the seasonal maximum in early August.

Eicosapentaenoic acid (EPA, 20:5$\omega3$) had annual maxima in August 2002, in May 2003 and in June 2004 (Fig. 1).Docosahexaenoic acid (DHA, 22:6$\omega3$) had seasonal maxima in August, July and June of 2002, 2003 and 2004, respectively (Fig. 1). DHA had the higher variations than the other PUFAs (Table 1).

The contents of the three PUFA species normalized to sestonic carbon (Fig. 2) had seasonal dynamics similar to that of their concentrations (Fig. 1), except for the ratio of ALA:C that showed an annual maximum in July 2002, and the ratio of EPA:C with annual maximum in October 2002.

Sestonic C:N ratio in general tended to decrease from May–June to October, while the C:P ratio had maxima in July–August in all 3 years (Fig. 3). The N:P ratios in different years had maxima in the beginning, middle or at the end of summer (Fig. 3).

The sum of sestonic $\omega3$-PUFA, besides ALA, EPA and DHA, included also stearidonic acid (SA, 18:4$\omega3$), eicosatetraenoic acid (ETA, 20:4$\omega3$) and some others. The seasonal dynamics of the ratio of $\omega3$-PUFA:P had no general pattern: in 2002 an annual maximum occurred in October, in 2003 in May, and in 2004—in June (Fig. 3). Dynamics of the ratio of $\omega3$-PUFA:C were similar to those of $\omega3$-PUFA:P (Fig. 3).

The dynamics of phytoplankton biomass and species composition differed between years (Figs. 4, 5). *Stephanodiscus* sp., peaked in May

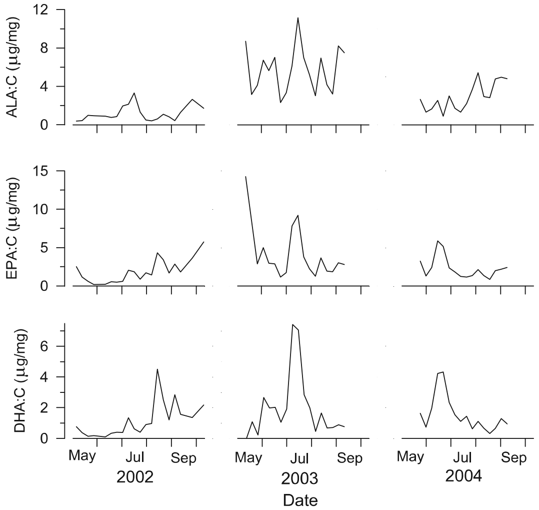

Fig. 2 Seasonal dynamics of the content of selected sestonic $\omega3$ PUFA species normalized to particulate organic carbon in Bugach Reservoir

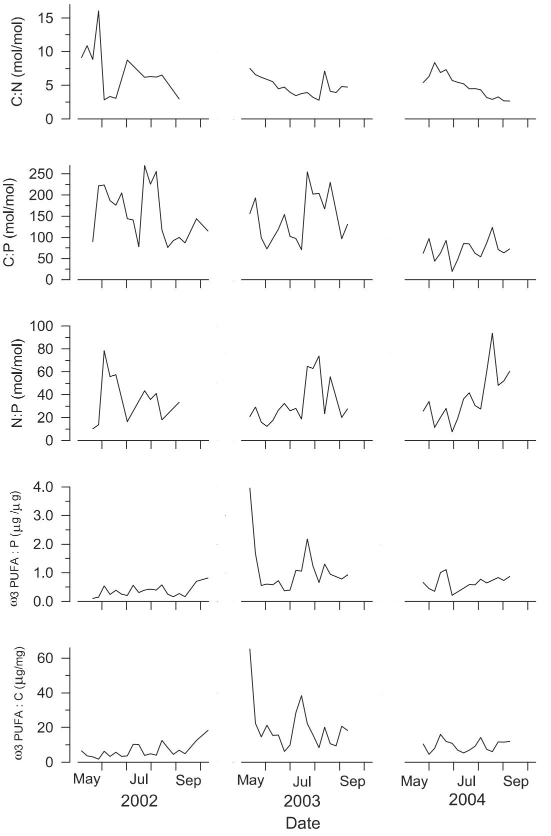

Fig. 3 Seasonal dynamics of elemental ratios (mol/mol) and of the ration of sestonic ω3 PUFAs normalized to particulate phosphorus or particulate carbon in Bugach Reservoir

2002 and 2003. This genus could not be differentiated to the species level with a light microscope, but in a previous study *Stephanodiscus hantzschii* Grun. and *Stephanodiscus minutulus* (Kutz) Cleve and Moller were identified using SEM (Sushchik et al. 2004). In May 2003, the diatoms contributed to had their seasonal maxima of phytoplankton biomass. In May of 2004 the spring peak of diatoms was omitted. At the end of May—beginning of July in 2002 and 2003 the cyanobacteria, species *Planktothrix agardhii* (Gom.) Anagn. and *Anabaena flos-aquae* (Lyngb.) Breb., dominated and contributed to phytoplankton biomass maxima. *Peridinium* sp. dominated in August–September of 2002 and in June–July of 2004. *Gomphosphaeria lacustris* Chod. was the dominant cyanobacterium in September of 2003 and in August–September of 2004 (Figs. 4, 5).

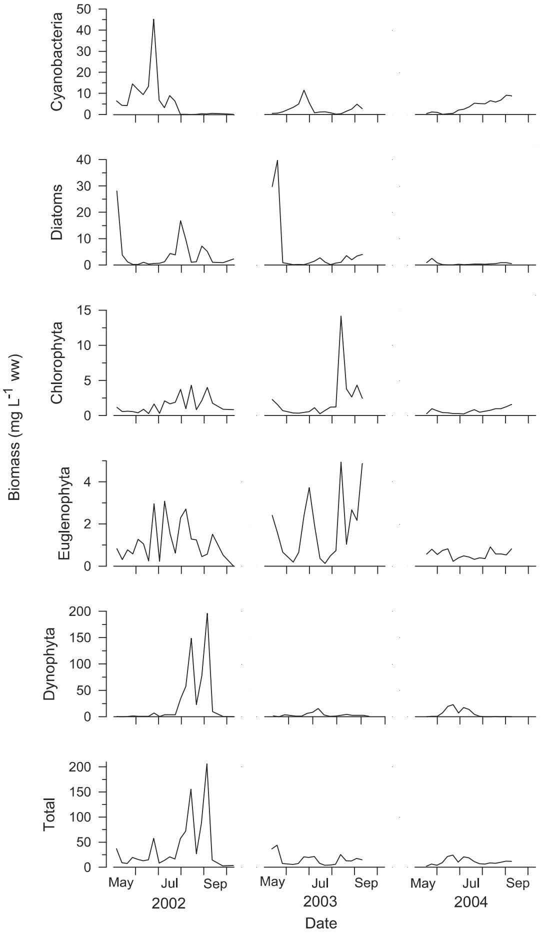

Fig. 4 Seasonal dynamics of biomass of major phytoplankton groups (wet weight) in Bugach Reservoir

Canonical correlations of sestonic parameters

There were statistically weak product-moment pair correlation (maximum $r^2 = 0.34$) between any element and any ω3 PUFA's species, or their sum. Multivariate statistical analysis was used to find possible relation between the elemental composition and the stoichiometry on the one hand and the PUFAs composition of seston on the other hand. The first analysis (matrix No. 1) included *log*-transformed data on concentrations (mg l^{-1}) of particulate carbon, nitrogen and phosphorus as variables within the left set (independent canonical variable) and concentrations (µg l^{-1}) of ALA, EPA, DHA and the sum of ω3-PUFAs as variables within the right set (dependent canonical variable). The results are given in

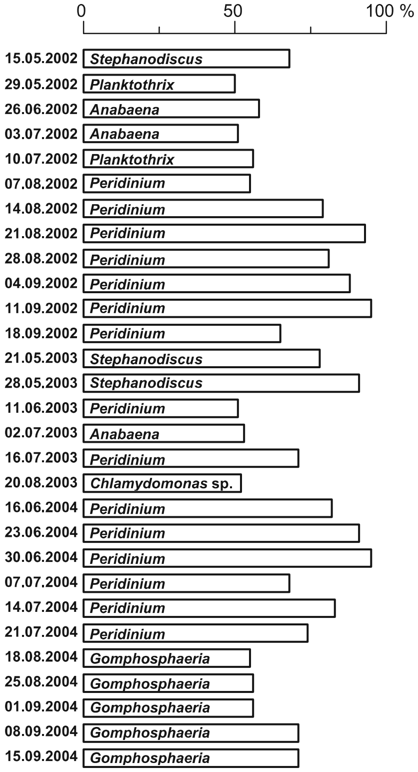

Fig. 5 Dominant phytoplankton species in Bugach Reservoir: per cent of total biomass of phytoplankton. Only dates, when the dominant species comprised >50% of the total biomass are depicted

Table 2 Results of canonical correlation analysis of matrix No. 1 of *log*-transformed concentrations of elements and PUFAs in the seston of Bugach Reservoir. Samples were collected in the period May–October in 2002–2004

	Left set	Right set
No. of variables	3	4
Variance extracted (%)	100	90.7
Total redundancy (%)	40.7	15.3
Number of valid cases	43	
	Root 1	Root 2
Eigenvalues	0.619	0.173
Canonical *R*	0.787	0.416
Chi-square	44.3	7.6
d.f.	12	6
P	<0.001	>0.05
Factor loadings		
Left set		
N	−0.931	0.048
C	−0.778	−0.621
P	−0.621	0.002
Right set		
ALA	−0.338	0.760
EPA	0.077	0.834
DHA	−0.032	0.576
$\Sigma\omega3$-PUFA	−0.436	0.872

Table 2 and depicted in Fig. 6. The overall canonical correlation coefficient, R_1, pertaining to the first canonical root, was high (0.787) and statistically significant ($P < 0.001$). Values of the extracted variance and total redundancy, especially by the left set, also indicated a strong relationship between the two sets of variables, i.e., between concentrations of C, N, and P and PUFAs in seston. Regarding factor loadings (Table 2), N showed the highest association with the left canonical variable, while C and P contributed with less significance. The right canonical variable was more common with $\Sigma\omega3$-PUFA and less with ALA. Contributions of EPA and DHA were comparatively very low. Thus, the significant canonical correlation R_1 between the two sets of variables, i.e., concentrations of elements and PUFAs in seston, was mostly due to a positive interaction between N and $\Sigma\omega3$-PUFA (Table 2, Fig. 6). The second canonical root was insignificant (Table 2, Fig. 6).

Matrix No. 2 (Table 3, Fig. 6) comprised *log*-transformed molar ratios of the elements in seston as the left set (independent canonical variable) and mass ratios of sestonic PUFAs to sestonic carbon (μg mg^{-1}) and to phosphorus (μg μg^{-1}) as the right set (dependent canonical variable). The extracted variance and total redundancy indicated very strong relations between the two sets. Indeed, canonical correlations pertained to both roots, R_1 and R_2, were high and statistically significant. In the left set of the first canonical root, N:P ratio had the highest factor loading. In the right set ALA:C had the maximum absolute value of the loading, while DHA:C, $\Sigma\omega3$-PUFA:C and EPA:C had successively lower values. Thus, the first and highest canonical correlation, R_1, was mainly due to a negative interaction between N:P and ALA:C, and to a smaller extent—due to a positive interaction between N:P and DHA:C. The second canonical correlation, R_2, pertained to the second root, was provided primary by the negative interaction between C:P and ALA:C (Table 3).

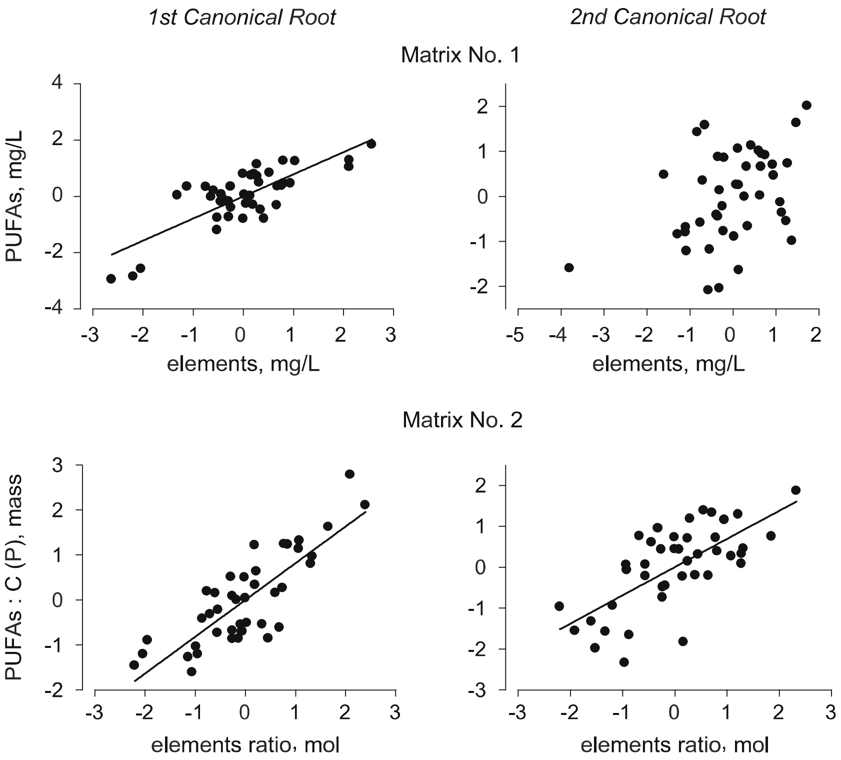

Fig. 6 Plots of correlations between the two canonical variables of the first and second canonical roots for two data matrices (see text for details). Solid lines represent linear approximations. Note that R_2 for matrix No. 1 is insignificant. Matrix No. 1 includes *log*-transformed concentrations of sestonic N, C and P as *x*-axis variables and ALA, EPA, DHA and $\Sigma\omega3$-PUFA as *y*-axis variables; matrix No. 2—*log*-transformed molar sestonic ratios C:P, C:N and N:P as *x*-axis variables and mass ratios of sestonic $\Sigma\omega3$-PUFA:C and $\Sigma\omega3$-PUFA:P as *y*-axis variables. Samples were collected in the period May to October in 2002–2004 in Bugach Reservoir

Table 3 Results of canonical correlation analysis of matrix No. 2 of *log*-transformed molar ratios of elements in seston and mass ratios of sestonic PUFAs to sestonic carbon and phosphorus in Bugach Reservoir. Samples were collected in the period May to October in 2002–2004

	Left set	Right set
No. of variables	3	5
Variance extracted (%)	100	59.9
Total redundancy (%)	45.9	21.9
Number of valid cases	42	
	Root 1	Root 2
Eigenvalues	0.667	0.476
Canonical *R*	0.817	0.690
Chi-square	73.1	32.9
d.f.	15	8
P	<0.001	<0.001
Factor loadings		
Left set		
C:P	−0.441	−0.765
C:N	0.478	0.248
N:P	0.726	−0.687
Right set		
ALA:C	−0.527	0.576
EPA:C	0.328	0.128
DHA:C	0.431	0.080
$\Sigma\omega3$-PUFA:C	−0.058	0.176
$\Sigma\omega3$-PUFA:P	−0.372	−0.176

Dominant phytoplankton species and sestonic stoichiometry and PUFA parameters

Data on periods of pronounced (>50% of total biomass) dominance of single species were analyzed to examine the natural stoichiometry and PUFA content of particular phytoplankton taxa. Dates and species, included in the following analysis are given in Fig. 5. Average stoichiometry parameters and their standard errors for the dominant species, except *Chlamydomonas* sp., which represented a single case, were calculated (Fig. 7). The periods of dominance of single species, as independent variables, explained a substantial and statistically significant part of the variance of all the sestonic stoichiometry parameters as dependent variables, except for C:P, as is revealed by the one-way ANOVA (Table 4). $\Sigma\omega3$-PUFA:C had a comparatively modest, while significant influence of the independent variable, but the other sestonic parameters, C:N, N:P and $\Sigma\omega3$-PUFA:P were strongly influenced by the phytoplankton species: >68% of the variability of these

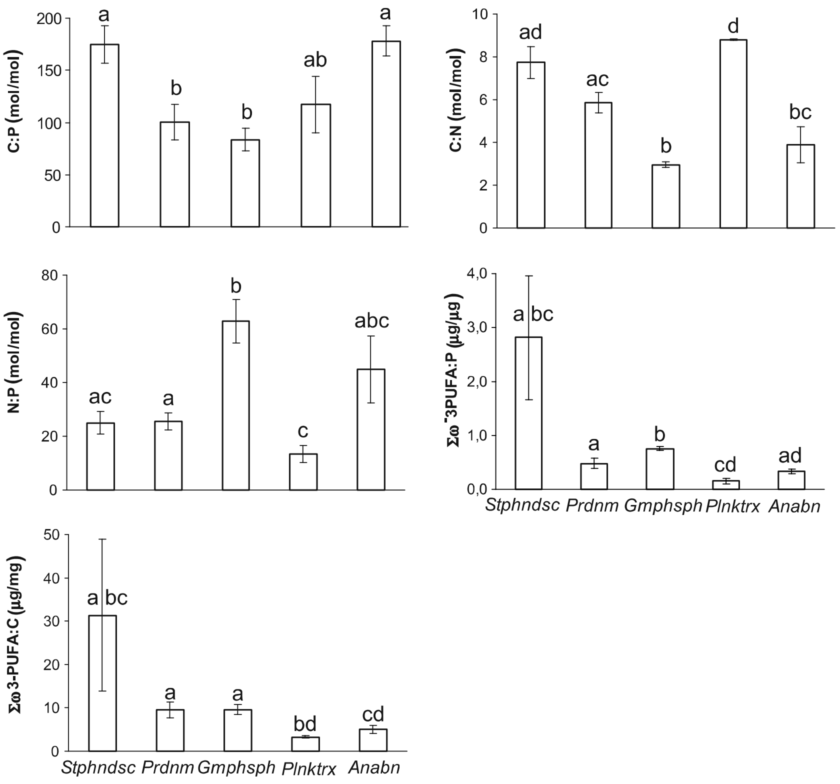

Fig. 7 Average values of elemental and PUFA ratios of seston for dates of pronounced dominance of some phytoplankton species in the period May to October in 2002–2004 in Bugach Reservoir. Bars represent standard errors. Bars labeled with the same letter are not significantly different at $P < 0.05$ after Student's *t*-test

Table 4 Results of ANOVA comparing sestonic stoichiometry in the periods of the pronounced dominance of *Stephanodiscus*, *Anabaena*, *Planktothrix*, *Peridinium* and *Gomphosphaeria* as independent variables (between levels d.f. = 4) in Bugach Reservoir. Samples were collected in the period May to October in 2002–2004

Dependent variable	Sum of squares	Variance explained (%)	d.f.	*F*	*P*
C:P	87544.6	30.7	21	2.33	>0.05
C:N	105.8	71.6	18	11.36	<0.001
N:P	9072.2	68.7	17	9.33	<0.001
$\Sigma\omega$3-PUFA:C	3953.9	38.3	22	3.41	<0.05
$\Sigma\omega$3-PUFA:P	14.7	71.9	21	13.45	<0.001

parameters was explained by the dominance of given taxa (Table 4).

The highest C:P ratio was characteristic of seston, dominated by *Stephanodiscus* sp. and *Anabaena*, while *Planktothrix*, *Peridinium* and especially *Gomphosphaeria* provided significantly lower ratio (Fig. 7). The maximum C:N ratio was provided by *Planktothrix* and also by *Stephanodiscus*, although the latter differed from *Peridinium* insignificantly. The lowest sestonic C:N ratio, like that of C:P, was due to the dominance of *Gomphosphaeria* and differed significantly from that of other species, except *Anabaena*. *Gomphosphaeria* also provided the highest N:P ratio and did not differ significantly from that of *Anabaena*. The lowest $\Sigma\omega$3-PUFA:P ratio, as well as $\Sigma\omega$3-PUFA:C ratio, pertained to periods of dominance of *Planktothrix* and did not differ significantly from that of *Anabaena*. Maximum values of the PUFAs' sestonic ratios in periods of dominance of *Stephanodiscus* were statistically insignificant due to high variability (Fig. 7).

Using correspondence analysis, the elemental stoichiometry and PUFA content of seston in the periods of dominance of single phytoplankton species were represented in a two-dimensional space (Fig. 8). The first dimension explained 65.0% of inertia (of total Chi-square value) of the data set, and the second dimension reproduced 30.5%. Chi-square values for both dimensions and the total Chi-square were significant ($P < 0.001$).

The first dimension (Fig. 8) demonstrated large difference between *Planktothrix* and *Stephanodiscus* on the one hand and *Gomphosphaeria*, on the other. *Anabaena* and *Peridinium* had an intermediate position. These positions of algal species in the first dimension were provided mostly by difference in their C:N and N:P stoichiometry (Fig. 8). Nevertheless, C:N position was very close to those of $\Sigma\omega3$-PUFA:P, $\Sigma\omega3$-PUFA:C and C:P, successively, which also contributed to the difference versus N:P. The second dimension, although comparatively less substantial but also significant, indicated the largest difference between *Stephanodiscus* and *Planktothrix* primarily due to the difference between their C:N and $\Sigma\omega3$-PUFA:P. However, two comparatively close groups of parameters could be separated in the second dimension: C:N, C:P and N:P versus $\Sigma\omega3$-PUFA:P and $\Sigma\omega3$-PUFA:C (Fig. 8).

Discussion

Ranges of concentrations of POC in Bugach Reservoir(Table 1) were significantly higher than those for oligotrophic (Andersen and Hessen 1991; Kreeger et al. 1997) and mesotrophic water bodies (Boersma et al. 2001; Wacker and Von Elert 2001). In general, they were comparable with data for eutrophic, cyanobacteria-dominated lakes (DeMott and Gulati 1999; Hessen et al. 2005), except for very low values reported for more fine pre-screened (mesh size 30–60 μm) seston (Urabe 1993; Kreeger et al. 1997; DeMott et al. 2001). Evidently, Bugach Reservoir had seston carbon levels far above the zooplankton starvation threshold (e.g., Schulz and Sterner 1999, but see DeMott et al. 2004).

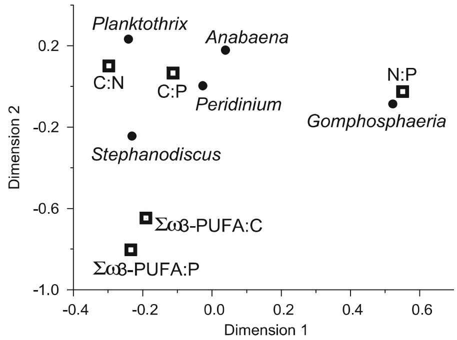

Fig. 8 Results of correspondence analysis of elements and PUFAs' stoichiometry (squares) on dates when some phytoplankton species (circles) were dominant in the period May to October in 2002–2004 in Bugach Reservoir, represented in a two-dimensional space, reproduced 95.5% of total inertia

The sestonic carbon in Bugach Reservoir believed to mainly comprise phytoplankton. Firstly, visual microscope examination revealed, that seston consists to a large extent of apparently live phytoplankton, and contains only a negligible per cent of contaminants: small protozoans, rotifers, and nauplii, as reported by some other authors (Ahlgren et al. 1997; DeMott and Gulati 1999). Secondly, workers who used no pre-screening found out that zooplankton comprised on the average 4.2% of total sestonic POC (Hessen et al. 2003). Contamination of the seston with zooplankton is, therefore, not considered likely to affect the results of elementary analysis of the seston fraction (Hessen et al. 2005). Secondly, a contamination of the seston in Bugach Reservoir by allochthonous organic matter also is believed to be of a modest degree. Based on an equation (inequality) from Hessen et al. (2003), that relate seston carbon and algal volume, seston carbon will be directly proportional to phytoplankton carbon if phytoplankton biomass $>>0.8$ mg l^{-1} ww. In our study the average value of phytoplankton biomass, and even the minimum value is high enough, and according to Hessen et al. (2003), in the studied period in Bugach Reservoir phytoplankton and autochthonous detritus that is derived from their biomass comprised more than 50% of the seston. As to detritus freshly derived from phytoplankton, it can be of even higher food value for zooplankton, than phytoplankton itself, especially those of inedible size and shape (Gulati et al. 2001). Consequently, for the following consideration we regard sestonic elemental and PUFA composition, measured in our study, as pertaining primarily to phytoplankton.

Concentrations of sestonic N and P, as that of POC, in Bugach Reservoir (Table 1) are significantly higher than those in mesotrophic (Muller-Navarra 1995a; Kreeger et al. 1997) and also in eutrophic lakes (Kreeger et al. 1997) if comparatively fine prescreening is used, but are practically equal to those in samples from cyanobacteria-dominated eutrophic lakes with comparable prescreening (DeMott and Gulati 1999; Hessen et al. 2005). The range of values of POP in Bugach Reservoir exceed the threshold level for total phosphorus of 0.02 mg l^{-1}, which is regarded as the border-line between oligotrophic and eutrophic waters (Muller-Navarra et al. 2004).

Ranges of C:P in Bugach Reservoir are significantly lower than those in oligotrophic (Elser et al. 1998; Carrillo et al. 2001 Park et al. 2004) and mesotrophic water bodies (Urabe and Watanabe 1992; Ahlgren et al. 1997; Boersma et al. 2001), and also somewhat lower, at least in minimum, than in eutrophic lakes (Urabe 1993; Kreeger et al. 1997; DeMott and Gulati 1999). Differences in pre-screening may be important for the comparisons, but Muller-Navarra et al. (2000) found no quantitative difference in C:P in seston, and seston < 30 μm. Ranges of C:N in Bugach Reservoir are lower than those for mesotrophic water bodies (Urabe and Watanabe 1992; Kreeger et al. 1997) and eutrophic lake (Kreeger et al. 1997), but the seasonal maximum value is comparable with that of a mesotrophic lake (Ahlgren et al. 1997) and higher than that of eutrophic lakes (Urabe 1993; DeMott and Gulati 1999; DeMott et al. 2001), and even higher than that of an oligotrophic (humic) lake (Andersen and Hessen 1991). Range of N:P in Bugach Reservoir is wider, than that reported for oligotrophic (Carrillo et al. 2001), mesotrophic and eutrophic lakes (Kreeger et al. 1997). Thus, seston of Bugach Reservoir has a comparatively low C:P ratios, which are closer to that of eutrophic lakes. No such tendency is revealed for C:N and N:P ratio.

As known, limiting C:P threshold for zooplankton, namely daphnids is >300 (e.g., Sterner and Schulz 1998; Boersma 2000; Becker and Boersma 2003). Hence, seston in Bugach Reservoir regarding C:P ratio in general meets qualitative stoichiometric requirements of daphnids. Other important zooplankton taxa, bosminids, chydorids, sidids and adult copepods, are reported to be less sensitive to high C:P ratios (Andersen and Hessen 1991; Sterner and Schulz 1998; Schulz and Sterner 1999). Even if to take the lower limiting threshold for daphnids as C:P >100 (DeMott et al. 1998; Hall et al., 2004), which may also apply to such phosphorus-sensitive groups as rotifers and copepod nauplii (Stich 1991; Carrillo et al. 2001; Villar-Argaiz and Sterner 2002), the seston of Bugach appeared to be of very high elemental quality during about half of the season. The N:P ratio also seemed to be in general of a favorable value for herbivorous zooplankton during most of the season, probably except for copepod nauplii (Carrillo et al. 2001).

Concentrations of the essential EPA and other PUFA in Bugach Reservoir (Table 1) were higher, both as μg l^{-1} and μg mg C^{-1}, than those in oligo- and meso-trophic lakes (Muller-Navarra 1995a; Leveille et al. 1997; Wacker and Von Elert 2001; Park et al. 2004), especially for more fine pre-screened samples, but comparable with those of a mesotrophic lake (Ahlgren et al. 1997), an estuary (Mannino and Harvey 1999) and an eutrophic pond (Muller-Navarra et al. 2000). However, the minimum value of EPA:C in Bugach Reservoir (Table 1) was significantly lower, than the border between oligotrophy and eutrophy, 2μg EPA mg^{-1} C, determined by Muller-Navarra et al. (2004). Seasonal dynamics of PUFA concentrations in Bugach Reservoir (Figs. 1, 3) did not reveal any general pattern, like those in mesotrophic Lake Erken (Ahlgren et al. 1997), while in oligotrophic lake Lac Pavin prominent spring and autumn peaks of PUFA occurred (Leveille et al. 1997).

In general, both for concentrations and for ratios (stoichiometry), relations found between elemental composition versus PUFA composition of seston (phytoplankton) are quite good (Tables 2 and 3, Fig. 6). For concentrations, the relation between the elemental and the biochemical composition are provided mainly due to positive interactions between sum of $\omega3$ PUFA and nitrogen, rather than phosphorus, in contrast to other studies (Ahlgren et al. 1997; Wacker and Von Elert 2001; Muller-Navarra et al. 2004). In our study ALA, EPA and DHA contributed successively less in the overall relations with the elemental concentrations (Table 2). Thus, in contrast to the finding of Ahlgren et al. (1997), sestonic P as a single element in Bugach Reservoir is not a good predictor of content of any $\omega 3$ PUFA species, nor their sum in living phytoplankton. This discrepancy may be due to higher concentration of phosphorus in Bugach Reservoir compared to those in the mesotrophic lake, studied by Ahlgren et al. (1997). Nevertheless, a good correlation of EPA with sestonic N, rather than P, is reported by Muller-Navarra (1995a) also for a mesotrophic lake. Wacker and Von Elert (2001) found a correlation between ALA and PON, but a stronger correlation of ALA and POP.

The strong canonical correlation between elemental ratios versus PUFAs:C ratios (Fig. 6, Table 3) is primary provided by a high negative interaction between N:P and ALA:C, while positive interactions between N:P and DHA:C, and also negative interaction of C:P and ALA:C are less important. Thus, influence of the particulate phosphorus itself on PUFAs composition of seston (phytoplankton) of Bugach Reservoir is not of primary importance. N:P ratio have both positive and negative correlation with different PUFAs. In general, the overall PUFA composition of seston goes hand in hand with the elemental composition (C:N:P stoichiometry) as a whole, rather than with a single element.

The above conclusion, based on the CCA, is independently supported by results of CA and ANOVA (Fig. 8, Table 4). Indeed, differences between dominant phytoplankton taxa are provided first of all by their differences in C:N and N:P ratios, and only in the second dimension—by differences in C:N and $\Sigma\omega 3$-PUFA:P ratios, and ANOVA reveal no significant difference in C:P of the phytoplankton species.

The discrepancies of our findings with data of other authors (Ahlgren et al. 1997; Wacker and Von Elert 2001; Muller-Navarra et al. 2004) may be due to more complex causes, than the differences in the trophic status of the studied water bodies along. One of such probable causes may be the taxonomic affiliation of dominant species. Dominance of a specific species evidently depends on a variety of ecological factors, rather than solely on phosphorus or nitrogen loading. And when a species meets the complex of optimal factors and becomes the dominant, it, in turn, determines the seston quality, including the elemental and biochemical ratios. ANOVA reveals that intraspecies variations of elemental and PUFA ratios of the dominant phytoplankton species in the study period are significantly lower than interspecies variations. Exceptions to this pattern are C:P and, to a certain degree, $\Sigma\omega 3$ PUFA:C ratios (Table 4). Our hypothesis, that phytoplankton species in periods of their pronounced dominance should have specific (‘optimal’) and relatively constant elemental and PUFA composition seemed to be supported by these findings. Only *Stephanodiscus*, which really represented a mixture of two species, undistinguishable under light microscope, had too high ‘intraspecies’ variability in the ratios of PUFA to elements (Fig. 7). Thus, ‘phosphorus-rich (poor)’ or ‘PUFA-rich (poor)’, etc., seston seems to exist in natural ecosystems inseparably from specific phytoplankton species, dominated under specific environmental conditions, in contrast to laboratory monocultures, where, for instance, P-limited algae with a wide C:P range can survive. In other words, we consider that changes of elemental and PUFA contents in natural ecosystems in general are accompanied with respective changes of phytoplankton species composition, rather than with a decrease or increase of cellular contents of elements and PUFAs in single species.

Our data on the periods of pronounced dominance of single species confirm, that there are no straightforward covariations between stoichiometric elemental ratios and PUFA content in phytoplankton (seston) in terms of their nutritional quality for zooplankton (Fig. 7). For instance, the diatom *Stephanodiscus*, which is known to be of very high nutritional quality regarding the high PUFA content, especially EPA, has a comparatively high C:P, and thereby may be unfavorable food item for many zooplankton species with the limiting threshold C:P > 100, mentioned above. The cyanobacterium *Gomphosphaeria* has a comparatively high PUFA content, similar to that of *Peridinium*, very favorable for zooplankton C:P ratio, but high N:P ratio. The cyanobacterium *Planktothrix* is poor in PUFA and C:P, but very favorable in N:P. Thus, considering the entire zooplankton community, rather than a few species (or even populations) of *Daphnia*, the widespread opinion, that, for instance, diatoms are valuable food items for zooplankton, while cyanobacteria are nutritional objects of a low value, should be revised. As found in the present study, and in some other studies (e.g., Gulati et al. 2001; Sushchik et al. 2004) there are biochemically valuable species of cyanobacteria and species of diatoms of a poor biochemical quality. Moreover, the dominant phytoplankton species differ significantly from each other regarding indicators of nutrition quality, such as C:N, N:P, PUFA:P and partly C:P. Thus, no single species should be considered as absolutely valuable or poor in terms of nutritive value. All of them, or at least detritus originating form them, may meet specific elemental and biochemical requirements of particular groups of zooplankton. Here a concern caution is needed because we are talking about stoichiometry and fatty acids, but not other food quality aspects, as morphology and toxins. Consequently, biochemical and elemental composition of dominant phytoplankton species, rather than more coarse taxa, should be regarded for purposes of nutrition ecology of plankton. Even in lakes in which algal cells comprise a modest part of total seston, they may nevertheless be the major source of the detritus, with their stoichiometric or biochemical footprints still being apparent (Hessen et al. 2003).

It should be noted, that while initially the core role of EPA in nutrition of some zooplankton species (population) has been emphasized, we consider also the sum of ω3-PUFA, like in some other studies (Park et al. 2002; Von Elert 2004), because recently there is strong evidences has been obtained concerning the importance of diverse ω3 species for zooplankton growth, such as α-linolenic, 18:3ω3 (Wacker and Von Elert 2001; Sushchik et al. 2003a), stearidonic, 18:4ω3, (Ravet et al., 2003) and eicosatetraenoic, 20:4ω3 (Becker et al. 2004) acids. We found $\Sigma\omega$3-PUFA better related with the whole elemental composition (stoichiometry) of seston, than any single PUFA species. Evidently, phytoplankton taxa are known to differ in PUFA composition, for instance, in Bugach Reservoir *Anabaena* has only ALA, *Stephanodiscus* comprised primarily EPA, and *Peridinium* especially rich in DHA (Sushchik et al. 2004). These differences be important for specific requirements of particular zooplankton species, but this aspect is out of scope of our present study. Besides PUFA, also sterols may be the biochemical determinants of the food quality of phytoplankton for zooplankton (e.g., Von Elert et al. 2003; Martin-Creuzburg et al. 2005). Thus, field data on sterol contents in diverse phytoplankton taxa are very desirable.

Conclusions

In general, in the seston of the eutrophic Reservoir there were no straightforward simple correlations between single element, e.g., phosphorus, and single ω3-PUFA species, but complex significant interaction between the elemental composition (stoichiometry) of seston and the sum of the particulate essential fatty acids as a whole. Different dominant phytoplankton species had specific and relatively constant elemental and PUFA composition. The dominant species of phytoplankton were very diverse with regard to single indicators of nutritive quality (C:N, N:P, PUFA:P, PUFA:C, C:P), thus there were no species of absolutely high or low nutritive value. Subdividing of phytoplankton concerning elemental and biochemical composition, i.e., nutrition quality, into large taxa (cyanobacteria, diatoms, etc.) appeared to be too coarse for purposes of nutrition ecology of plankton.

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