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## Evaluation of Possible Causes of Non-Predatory Mortality of Crustacean Zooplankton in a Small Siberian Reservoir

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*Non-predatory specific mortality (NPSM) of dominant zooplankton species, Daphnia (longispina group) and Cyclops vicinus, was estimated during 1997-2000 sampling seasons on the basis of a new direct method: live/dead sorting and sediment trap measurements. Simultaneously measured ecological factors, such as water temperature, pH, dissolved oxygen, biomass of cyanobacteria, diatoms, greens and euglens, levels of polyunsaturated fatty acids of  $\omega$ 3 family,  $\alpha$ -linolenic (ALA) and eicosapentaenoic (EPA), were considered as possible causes of the mortality using multivariate canonical correlation analysis. Toxicity was discarded on the basis of another set of study of the reservoir. As found, the best predictor of Daphnia mortality was EPA level, negatively correlated with NPSM value. Nevertheless, large part of variance of Daphnia mortality and practically all variance of Cyclops NPSM remain unexplained and need future investigation.*

*Keywords: non-predatory mortality, Daphnia, Cyclops vicinus, ecological factors, canonical correlation analysis.*

### Introduction

Dynamics of zooplankton populations are known to be of great importance for overall functioning of aquatic ecosystems. Abundance of zooplankton evidently depends on two factors: growth (somatic and generative) and mortality. Meanwhile, data on mortality are very sparse compare to that of growth due to methodical problems (e.g., Polishchuk and Ghilarov, 1981). Consumption by predators, fish (e.g., Luecke et al., 1990; Hulsmann et al., 1999) and invertebrates (e.g., Razlutskiy, 1996; Wojtal et al., 1999) are often the main cause of zooplankton mortality in many locations and seasons. Nevertheless, non-predatory mortality also can play a determinant

role (Luecke et al., 1990; Boersma et al., 1996; Mehner et al., 1998; Dubovskaya et al., 1999; Hulsmann and Weiler, 2000; Hulsmann and Voigt, 2002). In general, causes of non-predatory mortality of zooplankton are believed to be limiting physical and chemical factors, disease, senescence (Velimirov, 1991) and also food (edible phytoplankton) limitation (Luecke et al., 1990; Boersma et al., 1996). However, particular causes of variations of the non-predatory mortality in diverse sites and periods are still unknown. Non-predatory mortality estimations were indirect and included double error, connected with estimations of birth rate and consumption by fish. Finding of any quantitative correlation of such unreliable

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value with potentially determinant ecological factors seemed to be doubtful. Since a direct method for measuring of non-predatory specific mortality (NPSM) has been elaborated (Gladyshev et al., 2003a; Dubovskaya et al., 2003), it gives a chance to study particular causes of variations of this type of mortality.

The aim of present study was to evaluate possible causes of seasonal variations of non-predatory specific mortality of two dominant zooplankton groups in a small Siberian reservoir. Physical (temperature), chemical (pH, dissolved oxygen) factors and biomass of dominant phytoplankton taxa were considered. As found previously on the basis of a set of biotests, including a standard *Daphnia* bioassay (Dubovskaya et al., 2002), there was no toxicity in the reservoir during the studied period. Hence, toxicity was excluded from the consideration. Meanwhile, biochemical quality of seston, namely levels of polyunsaturated fatty acids (PUFA) of  $\omega 3$  family, which are known to be of importance for zooplankton population dynamics (Muller-Navarra, 1995; Gulati and DeMott, 1997; von Elert, 2002; Bec et al., 2003), were also studied as possible factors that influenced the mortality.

### Study site, Materials and Methods

The study was undertaken in a reservoir, locally called as Bugach pond, situated 56°03' N and 92°43' E in the Bugach river (secondary tributary of the Yenisei River). Its surface area is about 0.32 km<sup>2</sup>, and its maximum depth is 7 m. Detailed descriptions of the reservoir ecosystem are given elsewhere (Gladyshev et al., 2001, Sushchik et al., 2003). Reservoir was sampled weekly from May to September in 1997-2000 from a site near the middle (station No.1). Samples were taken with an 8-L Ruttner-like sampler and were pooled from depths 1–2 m.

#### 1. Field measurements and sampling

Water temperature and dissolved oxygen were measured with a meter N5221 (MERA-ELWRO, Poland); pH was measured using meter “pHep-2” (Hanna Instruments, USA).

Phytoplankton samples of 0.15–0.5 L volume were filtered through ‘Vladipor’ filters (Mytitschi, Russia) (pore size, 0.75–0.85  $\mu\text{m}$ ). Microalgae were counted and identified under a microscope. To calculate biovolume and wet biomass of algae, they were equated to appropriate geometrical shapes (or their combinations) and relevant sizes were measured using an ocular micrometer (LOMO, St-Petersburg, Russia).

Zooplankton samples (16–32 L) were filtered through a plankton net (mesh size, 0.08 mm), stained with aniline blue to distinguish between live and dead organisms (Seepersad and Crippen, 1978; Gladyshev, 1993) and fixed with 10% formalin. Zooplankton were counted and identified under a microscope.

#### 2. Fatty acid analysis

To assay particulate fatty acids (FAs), 100 mL water samples were first filtered through the 0.08-mm nylon mesh. The seston was then collected from these prescreened water samples by vacuum filtration onto membrane filters ‘No.8 Vladipor’ (pore size 0.75–0.85  $\mu\text{m}$ ) precovered with a layer of BaSO<sub>4</sub> to facilitate the separation of residues. The filters were dried at 35 °C for about 30 min, and the residues then separated from filters, placed into methanol and frozen. Further FA analysis is described in detail elsewhere (Gladyshev et al., 2000). Briefly, lipids were extracted by chloroform–methanol (2 : 1, v/v) three times. After evaporation of the solvent, methyl esters of FAs (FAMES) were prepared in a mixture of methanol– sulphuric acid (20 : 1, v/v) at 90°C for 2 h. The FAMES were analyzed by gas chromatography–mass spectrometry (GC-MS) using a model GCD Plus (Hewlett-Packard, Palo

Alto, CA, USA) equipped with a 30 m × 0.25 mm HP-5 capillary column. The conditions for GC - MS analyses are described in Gladyshev et al. (2000). Identification of FAMES was achieved by comparing their mass spectra with those in the Hewlett-Packard database and with those of the authentic standards (Sigma, St. Louis, MO, USA).

### 3. Mortality estimation

Non-predatory specific mortality (NPSM) was measured using the method, described by Gladyshev et al. (2003a). Briefly,

$$m_i = \frac{\Delta y_i}{\Delta t_i \cdot N_i} + G_i \cdot \frac{y_i}{N_i} \quad (1)$$

where  $m_i$  is non-predatory specific mortality (NPSM, day<sup>-1</sup>),  $y_i$  and  $N_i$  is the abundance (ind.·m<sup>-3</sup>) of dead and alive individuals respectively in sample  $i$ ,  $\Delta t_i = t_{i+1} - t_i$ , with  $t_i$  (day) being the instant of taking of sample  $i$ ,  $\Delta y_i = y_{i+1} - y_i$ ,  $G_i$  being the specific rate of elimination (day<sup>-1</sup>) of dead individuals from the sampled part of the column at the instant  $t_i$ . The specific rate of elimination is calculated as

$$G_i = \frac{v_i}{h} \quad (2)$$

where  $v_i$  is sinking velocity (m·day<sup>-1</sup>) of dead individuals,  $h$  is the depth of the layer of sampling of  $N_i$  and  $y_i$ . In this study  $h=2$  m (epilimnion). The sinking velocity, measured by sediment traps, was found to depend on wind speed,  $U_i$ , m·s<sup>-1</sup> (Dubovskaya et al., 2003):

$$v_i = 3.709 \cdot U_i^{-0.984} \quad (3)$$

### 4. Statistical analysis

Canonical correlation analyses (CCA) was carried out by conventional way (Jeffers, 1981), using STATISTICA 6.0 software (StatSoft, Inc.).

## Results

### 1. Physical and chemical factors

Water temperature (Fig. 1a) had more or less regular pattern of seasonal dynamics with summer oscillations around 20 - 22°C, except 1999, when comparatively cold spring and hot summer occurred. Concentration of dissolved oxygen (Fig. 1b) often exceeded 100% of saturation and declined down to about 50% in early June, middle of July and late August. Values of pH (Fig. 1c) were high, except late spring - early summer of 1997-1998.

### 2. Phytoplankton

Cyanobacteria (Fig. 1d) had very high biomass in June-July of 1998 and 2000. During these periods, as well as at the end of August of 1997 and 1999 *Aphanizomenon flos-aquae* (L.) Ralfs dominated and *Anabaena flos-aquae* (Lyngb.) Breb. was subdominant. In turn, *An. flos-aquae* dominated at the beginning of August, 1997 and in June-July of 1999, while *Aph. flos-aquae* was subdominant. At the beginning of August, 2000 *Aph. flos-aquae* was solo cyanobacterial dominant. In August, 1998, at the end of July-beginning of August 1999 *Microcystis aeruginosa* Kutz. emend. Elenk. dominated. In August-September, 2000 *Planktothrix agardhii* (Gom.) Anagn. et Kom. dominated.

Biomass of diatoms (Fig. 1e) was very high in spring-early summer, except 1997 when biomass of these algae was negligibly small during all period of the study. In May-June of 1998 and 2000 *Stephanodiscus* sp. dominated and *Cyclotella meneghiniana* Kütz. was subdominant, while in the same period of 1999 the opposite ratio of biomass of these species occurred. In August, 1998 *C. meneghiniana* occurred with very low biomass. At the end of July, 2000 *Stephanodiscus* sp. appeared and had considerable biomass down to September and *C. meneghiniana* was subdominant.

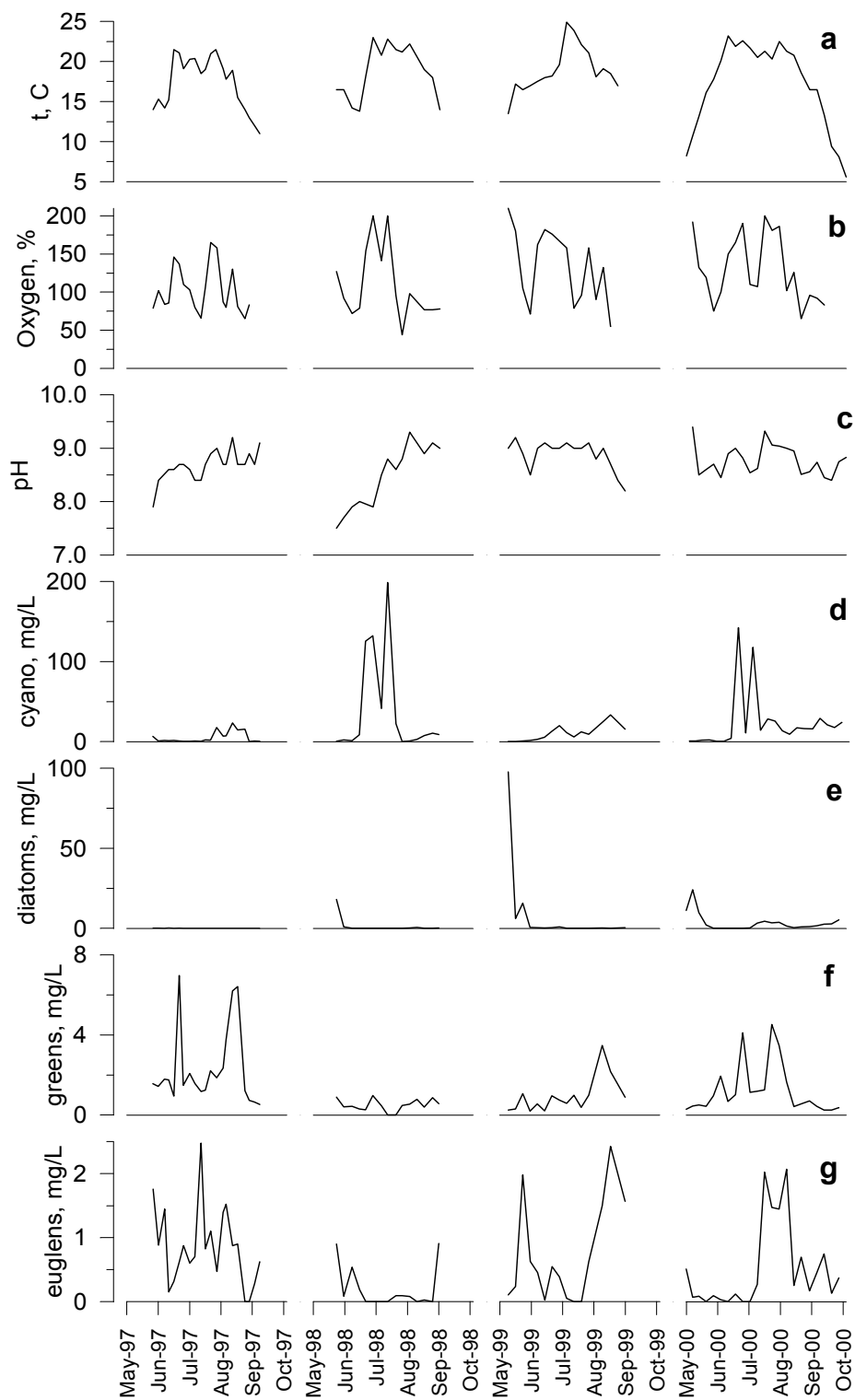


Fig. 1. Ecological factors in the Bugach reservoir (Siberia, Russia), sampling station 1: **a** - water temperature, **b** - dissolved oxygen, **c** - pH, **d** - biomass of cyanobacteria, **e** - biomass of diatoms, **f** - biomass of greens, **g** - biomass of euglens

Green algae (Fig. 1f) had low biomass compare to that of cyanobacteria and diatoms. Two peaks, at the beginning and at the end of summer generally occurred. In June-July of 1997 *Scenedesmus acuminatus* (Lagerh.) Chod. and *Crucigenia tetrapedia* (Kirchn.) W. West, G. West dominated and *Scenedesmus quadricauda* (Turp.) Bréb. was subdominant. At the end of July, in August and in September *Scenedesmus bijugatus* (Turp.) Kütz. and *Chlamydomonas* sp. dominated, *Sc. acuminatus*, *Phacotus lenticularis* Ehren., *C. tetrapedia* were subdominant. In 1998 *Sc. acuminatus* (in June), *Pediastrum boryanum* (Turp.) Menegh. (in July), *Chlamydomonas* sp. and *Pandorina morum* (Müll.) Bory (in August-September) dominated among greens and had comparatively low biomass. In 1999 *Chlamydomonas* sp. dominated, except the beginning of July when *Pandorina morum* (Müll.) Bory, *Oocystis lacustris* Chod. dominated with low biomass. In May, 2000 *Sc. acuminatus* was dominant, in June - *Pediastrum boryanum* and *Coelastrum microporum* Näg., which together with *Chlorococcum infusionum* (Schrank) Menegh. at one date were dominant not only among greens, but among the total phytoplankton. At the beginning of July, 2000 *P. morum* dominated, at the end of July and, in August *Chlamydomonas* sp. was dominant among greens.

Euglens (Fig. 1g) had low biomass compare to the all other taxa, mentioned above. In 1997 *Trachelomonas volvocina* Ehr. and *Phacus pleuronectes* (Ehr.) Duj. dominated during all the sampling period, and in one date of June the former was dominant in the total phytoplankton. In 1998 they also were dominant among euglens, and *Euglena granulata* (Klebs) Schmitz. and *Phacus longicauda* (Ehr.) Duj. sometimes dominated at the beginning and at the end of sampling season. In 1999 *E. granulata* dominated down to the middle of August and *Euglena proxima* Dang. appeared at the end of the season, while *Ph. pleuronectes*

and *T. volvocina* were subdominant. In 2000 *E. proxima* was abundant in spring and especially in August, *T. volvocina* and *Lepocinclis ovum* (Ehr.) Mink. were subdominant.

### 3. Polyunsaturated fatty acids

Level of 18:3 $\omega$ 3,  $\alpha$ -linoleic acid (ALA), had a mid-summer maximum in 1999 and an August-September maximum in 2000 (Fig. 2a). Level of 20:5 $\omega$ 3, eicosapentaenoic acid (EPA), in both years of sampling peaked at the beginning and at the end of growing seasons (Fig. 2b).

### 4. Zooplankton

Seasonal dynamics of abundance of dominant zooplankton species - *Daphnia cucullata* Sars in 1997 and *Daphnia longispina* O.F.Muller in the other years had irregular patterns (Fig. 2c). NPSM of *Daphnia* in general was high during periods of low population abundance and low during population increases (Fig. 2d).

The dynamics of abundance of subdominant zooplankton species - adult females of *Cyclops vicinus* Uljanine, like that of *Daphnia*, was irregular (Fig. 2e) and NPSM also was high during population declines and low abundance (Fig. 2f).

### 5. Canonical correlation analysis

To reveal possible interactions between studied components of the ecosystem, the multi-variant statistical technique, canonical correlation analysis, was used. Firstly, correlations of non-predatory specific mortality of *Daphnia* and *Cyclops* as dependent variables (right set) with water temperature, pH, concentration of dissolved oxygen, biomass of cyanobacteria, diatoms, greens and euglens as independent variables (left set) for the sampling periods of 1997-2000 (correlation matrix No. 1) were analyzed using CCA. Low percentage of total variance extracted indicated comparatively low relationship between these two sets of variables. Moreover, canonical correlation coefficient,  $R_1$ , pertained to the first canonical root,

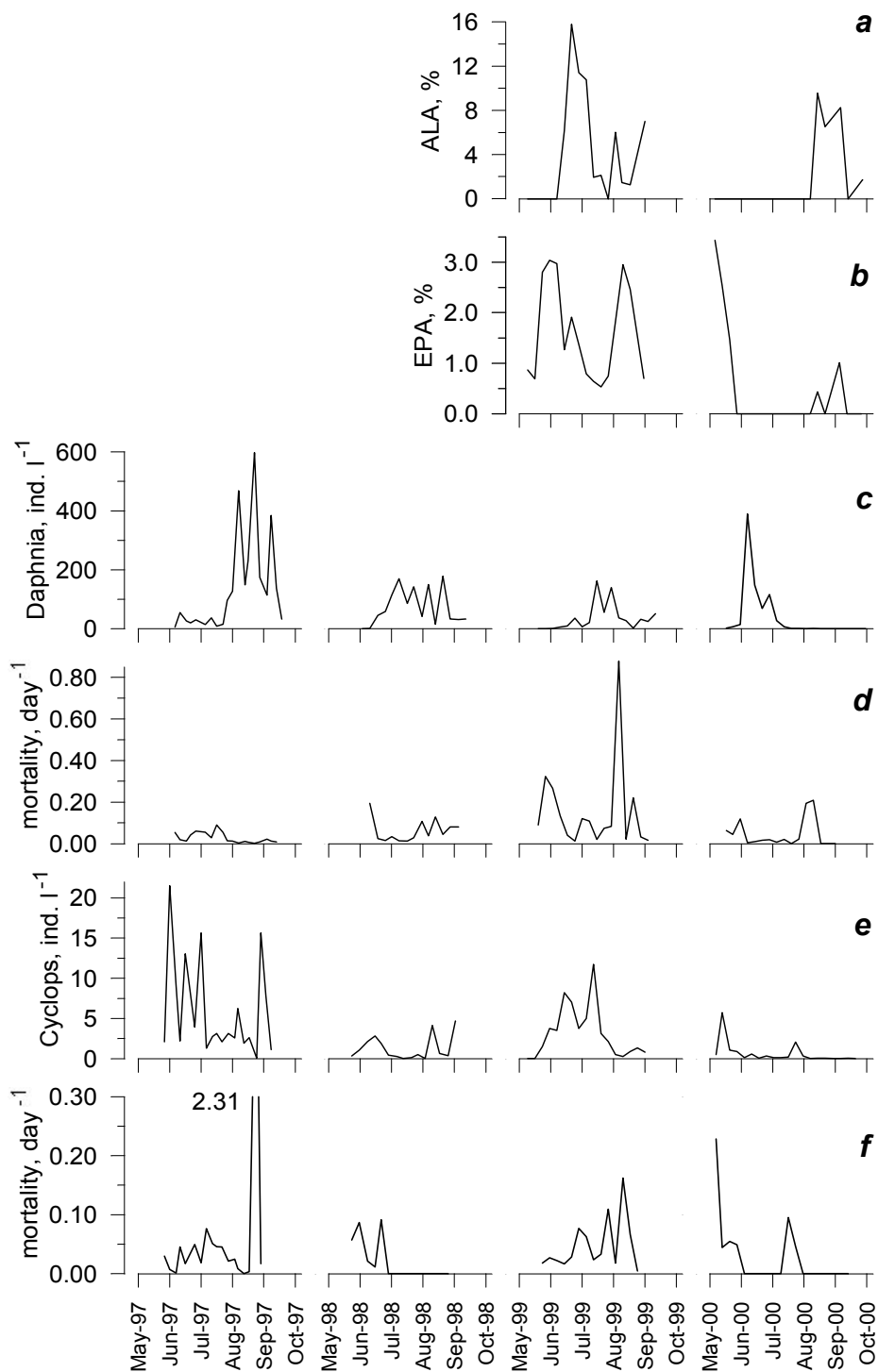


Fig. 2. Dynamics of levels of polyunsaturated fatty acids (as % of the total fatty acids) in seston and characteristics of dominant zooplankton species in the Bugach reservoir (Siberia, Russia), sampling station 1: **a** –  $\alpha$ -linolenic acid, **b** – eicosapentaenoic acid, **c** - abundance of alive individuals of *Daphnia*, **d** – non-predatory specific mortality of *Daphnia*, **e** – abundance of alive individuals of adult females of *Cyclops vicinus*, **f** – non-predatory specific mortality of adult females of *Cyclops vicinus*. 1997 - *D. cucullata*, 1998-2000 - *D. longispina*

was small and statistically insignificant ( $p > 0.05$ ), not to mention  $R_2$  (thereby, factor loading values were omitted in Table 1). Visual analysis of scatter plots of the correlation matrix did not reveal any non-linearity as a possible cause of the low correlations.

While such independent variables as temperature, pH, concentration of dissolved oxygen, could be regarded as factors of potentially instant effect, factors of food quantity and quality (biomass of different algae taxa) were believed to affect the mortality with a delay. For instance, Luecke and coauthors (1990) have demonstrated that another parameter of zooplankton population dynamics, egg ratios, correlated with edible phytoplankton if egg ratio was lagged by one sampling date. Hence, in the second CCA complex (correlation matrix No. 2) the mortality shifted at week interval relative to biomass of the phytoplankton was used. Results of CCA of the second matrix are given in Table 2. Variance extracted and total redundancy values were low, but higher, than those for correlation matrix No. 1. Canonical correlation, pertained to the first root,  $R_1$ , was high and statistically

significant. Factor loadings and canonical weights of variables for the first canonical root indicated that positive interaction of biomass of green algae with *Cyclops* NPSM contributed significantly to the overall canonical correlation. Nevertheless, scatter plot of the canonical correlation pertained to the first root (Fig. 3) demonstrated that the high  $R_1$  value was provided by only one extreme data point.

Additional set of independent variables, potentially related with the mortality (PUFA level), was available for shorter sampling period, 1999-2000. Since the reduction of number of cases (observations) demanded an appropriate reducing of number of variables, the unimportant independent variables, revealed by CCA of the first and second matrixes, were excluded. Thereby, third correlation matrix included the same dependent variables (NPSM of *Daphnia* and *Cyclops*) as right set and new independent variables, levels (per cent of total FAs) of 20:5 $\omega$ 3 (EPA) and 18:3 $\omega$ 3 (ALA) together with biomass of green algae as left set. Cases of the sampling period of 1999-2000 were used. The depended

Table 1. Results of canonical analysis of correlation matrix No. 1 of water temperature, pH, concentration of dissolved oxygen, biomass of cyanobacteria, diatoms, greens and euglens as independent variables (left set) and non-predatory specific mortality of *Daphnia* and *Cyclops* as dependent variables (right set) in Bugach reservoir for the sampling periods of 1997-2000

	Left set	Right set
No. of variables	7	2
Variance extracted (%)	28.7	100.0
Total redundancy (%)	3.8	16.7
Number of valid cases	34	
	Root 1	Root 2
Eigenvalues	0.234	0.086
Canonical R	0.484	0.293
Chi-sqr.	9.0	2.5
df	14	6
p	>0.05	>0.05

Table 2. Results of canonical analysis of correlation matrix No. 2 of biomass of cyanobacteria, diatoms, greens and euglens as independent variables (left set) and non-predatory specific mortality of *Daphnia* and *Cyclops* as dependent variables (right set) in Bugach reservoir for the sampling periods of 1997-2000. The mortality shifted at week interval relative to biomass of the phytoplankton

	Left set	Right set
No. of variables	4	2
Variance extracted (%)	39.7	100.0
Total redundancy (%)	11.5	25.2
Number of valid cases	37	
	Root 1	Root 2
Eigenvalues	0.438	0.062
Canonical R	0.661	0.248
Chi-sqr.	20.8	2.1
df	8	3
p	<0.01	>0.05
Factor loadings:		
	Left set	
cyanobacteria	0.167	-0.381
diatoms	-0.141	-0.425
greens	0.949	0.005
euglens	0.116	0.549
	Right set	
<i>Daphnia</i> NPSM	-0.336	-0.942
<i>Cyclops</i> NPSM	0.950	-0.313
Canonical weights:		
	Left set	
cyanobacteria	-0.263	-0.753
diatoms	0.029	-0.620
greens	1.125	-0.059
euglens	-0.171	0.819
	Right set	
<i>Daphnia</i> NPSM	-0.313	-0.950
<i>Cyclops</i> NPSM	0.942	-0.336



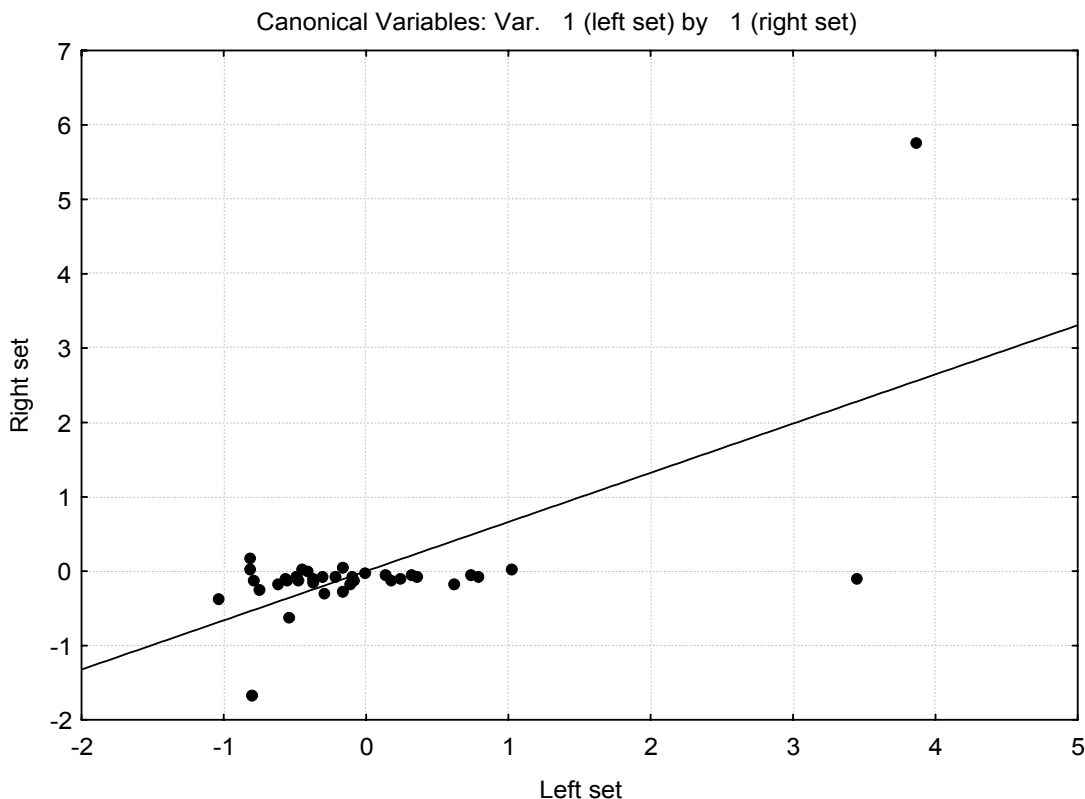


Fig. 3. Plot of correlation between two canonical variables pertained to the first canonical root of correlation matrix No. 2. Matrix No. 2 includes biomass of cyanobacteria, diatoms, greens and euglens as independent variables (left set) and non-predatory specific mortality of *Daphnia* and *Cyclops* as dependent variables (right set) in Bugach reservoir for the sampling periods of 1997-2000.

variables also were shifted, like those in correlation matrix No.2.

Percentage of total variance extracted in left set was very high.  $R_1$  value was also high, although statistically insignificant (Table 3). These values in general indicate comparatively high interactions between the two sets of variables, but such conclusion needs a following statistical verification. The low statistical significance could be due to the low number of valid cases, and also due to non-linearity of relations between the variables. Indeed, a visual analysis of the scatter plot of the canonical correlation, pertained to the first canonical root, indicates that the relationship

seems to be non-linear (Fig. 4). The possible non-linearity needs the analysis of ln-transformed data, but such transformation would inevitably diminish the number of valid cases because of elimination of zero points, i.e., increase the statistical insignificance. Thus, only paired correlations of appropriate data were available. Since NPSM of *Daphnia* and EPA level demonstrated the highest factor loadings and canonical weights (Table 3), product-moment correlation was calculated for their ln-transformed values. Scatter plot of these data is given in Fig. 5. The correlation coefficient,  $r = -0.525$ , was statistically significant ( $p < 0.05$ ) at number of pairs 16.

Table 3. Results of canonical analysis of correlation matrix No. 3 of two  $\omega$ 3 PUFAs (percent of total fatty acids) in seston, biomass of greens as independent variables (left set) and non-predatory specific mortality of *Daphnia* and *Cyclops* as dependent variables (right set) in Bugach reservoir for the sampling periods of 1999-2000. The mortality shifted at week interval relative to independent variables

	Left set	Right set
No. of variables	3	2
Variance extracted (%)	73.6	100.0
Total redundancy (%)	15.4	15.2
Number of valid cases	16	
	Root 1	Root 2
Eigenvalues	0.417	0.070
Canonical R	0.646	0.265
Chi-sqr.	7.3	0.9
df	6	2
p	>0.05	>0.05
Factor loadings:	Left set	
20:5 $\omega$ 3	0.631	0.754
18:3 $\omega$ 3	0.515	-0.846
greens	0.473	0.195
	Right set	
<i>Daphnia</i> NPSM	-0.649	-0.761
<i>Cyclops</i> NPSM	0.229	-0.973
Canonical weights:	Left set	
20:5 $\omega$ 3	0.762	0.555
18:3 $\omega$ 3	0.746	-0.686
greens	0.287	0.007
	Right set	
<i>Daphnia</i> NPSM	-1.208	-0.284
<i>Cyclops</i> NPSM	0.945	-0.805

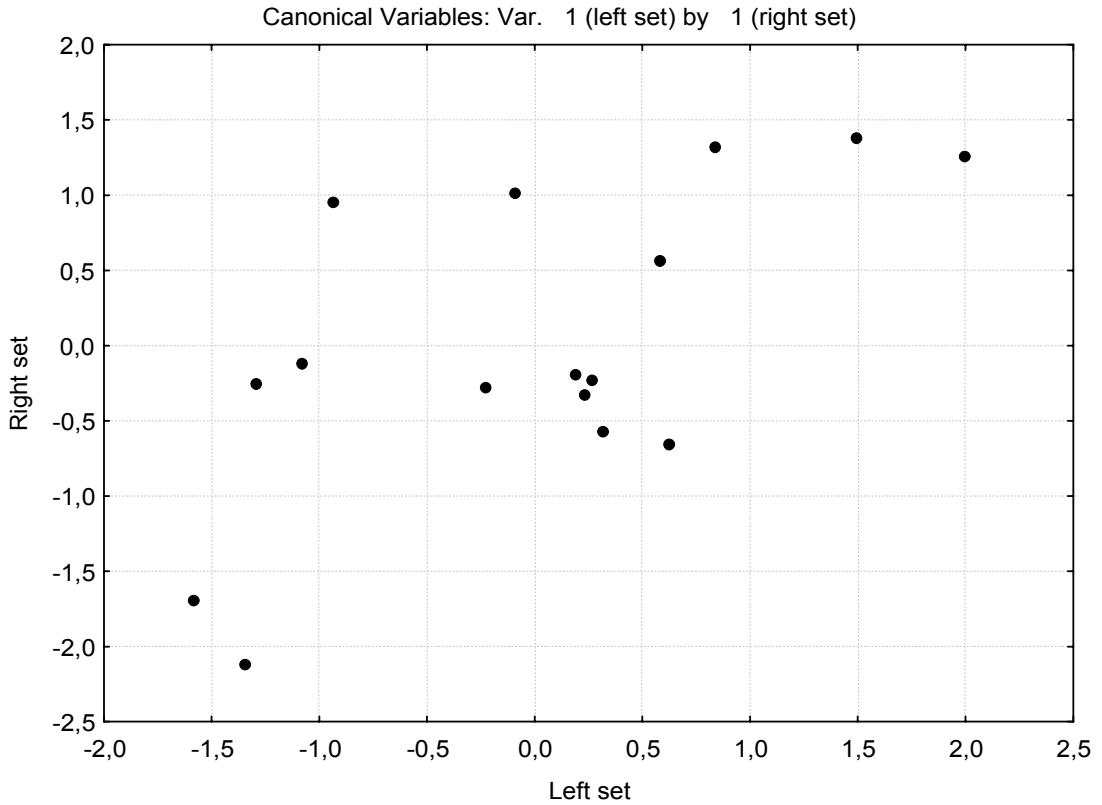


Fig. 4. Plot of correlation between two canonical variables pertained to the first canonical root of correlation matrix No. 3. Matrix No.3 includes percentages of two  $\omega$ 3 PUFAs (of the total fatty acids) in seston, biomass of greens as independent variables (left set) and non-predatory specific mortality of *Daphnia* and *Cyclops* as dependent variables (right set) in Bugach reservoir for the sampling periods of 1999-2000

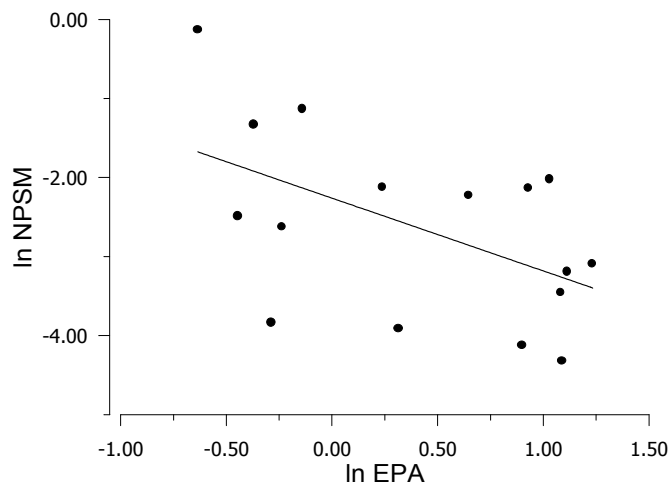


Fig. 5. Correlation between ln of non-predatory specific mortality (NPSM) of *Daphnia* and ln of level of eicosapentaenoic acid (EPA) in seston in Bugach reservoir (Siberia, Russia). May-October, 1999-2000

## Discussion

According to detailed estimation of Dorazio (1984) values of mortality due to longevity, i.e. death at some finite age due to physiological senescence are  $0.003-0.015 \text{ day}^{-1}$ . The non-predatory mortality of *Daphnia* in all the seasons was substantial. Its values in many cases (Fig. 2) were higher than values of above-mentioned physiological mortality. Consequently, the obtained NPSM included influence of other different factors of mortality. Besides, our data are in a good agreement with findings of the other authors, who demonstrated a low contribution of fish predation to total *Daphnia* mortality (Boersma et al., 1996; Mehner et al., 1998; Romare et al., 1999; Hulsmann and Weiler, 2000). Such ecological factors, as water temperature, pH, concentration of dissolved oxygen in the studied site did not influence NPSM value. Evidently, levels of these factors (and their combinations) during the studied period were within limits of tolerance. Biomass of diatoms, cyanobacteria and euglens also did not affect the mortality. Probably, biomass of euglens was on the average too low to influence population of *Daphnia*. Cyanobacteria, which may exude toxins (e.g., Rohrlack et al., 1999) seem to be harmless in the studied period. Indeed, during toxicological studies in the reservoir (Dubovskaya et al., 2002), besides *Daphnia* test, a multi-enzyme test, particularly sensitive to cyanobacterial exudations (Kratasyuk et al., 2001) was applied and revealed no toxicity. As to diatoms, it was found earlier (Sushchik et al., 2003), that at least some species had comparatively low nutritive value, i.e., their biomass did not correlate with EPA level in seston. This early observation may explain the absence of correlation between biomass of diatoms and NPSM of *Daphnia*, revealed in present study.

The most substantial effect on NPSM of *Daphnia* was produced by EPA level in seston.

At high EPA level the mortality was very small, while at low level of EPA values of NPSM were maximal. The principal importance of EPA for growth of *Daphnia* is well known (Muller-Navarra, 1995; Gulati and DeMott, 1997; Muller-Navarra et al., 2000). Our finding is the first evidence that EPA level also influences the non-predatory mortality.

Besides the negative interaction between EPA level and NPSM of *Daphnia*, on the basis of CCA of matrix No. 3 it may be concluded, that ALA level and biomass of green algae also tended to contribute negatively to mortality of this zooplankton genus (Table 2, 3). Green algae  $<30 \mu\text{m}$  are well known to be suitable food for *Daphnia*. Thus, it is clear, why a high biomass of green algae, which species were primarily of edible sizes in the studied reservoir, tended to coincide with low mortality. According to some recent data, namely level of  $\alpha$ -linolenic acid, which is absolutely essential for animals, rather than EPA, can determine growth of some populations of *Daphnia* (Wacker and von Elert, 2001; von Elert, 2002; Sushchik et al., 2003). The tendency of negative correlation between NPSM and ALA level, revealed in present study, is in a good agreement with the data, mentioned above. Thus, in the studied reservoir main causes of the mortality of *Daphnia* were food factors.

Nevertheless, the studied causes explained about a half of variance of non-predatory specific mortality of *Daphnia*. Among the analyzed factors the principal role in this explanation played the food quality, namely EPA level in seston. Meanwhile, a considerable part of variance of NPSM of *Daphnia* population remained undiscovered. Evidently, some other factors, omitted in our study, e.g.,  $\text{POC}_{<30\mu\text{m}}$  concentrations (Hulsmann and Voigt, 2002) or primary production of edible phytoplankton  $<30 \mu\text{m}$  (Kamjunke et al., 1999), may likely explain a substantial residual part of the non-predatory mortality variance. This

assumption may be supported by our finding, that green algae, primarily of edible sizes <30  $\mu\text{m}$ , provided significant negative influence on *Daphnia* mortality value (Table 2), although less substantial than those of EPA and ALA (Table 3).

Besides the food supply, other ecological factors, such as diseases and parasites (Stirnadel and Ebert, 1997; Bittner et al., 2002), also could be the causes of non-predatory mortality. It is interesting to remark, that a study of epibiotic microalgae of *Daphnia* in the Bugach reservoir (Dubovskaya et al., 2005) revealed, that the epibionts did not increase non-predatory mortality of the zooplankton, but could increase namely predatory mortality, since individuals covered with colored microalgae, were more conspicuous and vulnerable for planktivorous fish (Willey et al., 1990; 1993). The Bugach reservoir is inhabited by several species of planktivorous fish, among them *Carassius auratus* Linnaeus, *Leucaspis delineatus* Heckel are rather numerous (Gladyshev et al., 2003b). Nevertheless, bacterial or viral species-specific infection also may be one of causes of non-predatory mortality (Gries and Gude, 1999).

Concerning *Cyclops* we suppose that causes of mortality of this species remain unrevealed, since results of CCA of matrix No. 2 were ambiguous. If assume, that the extreme point (Fig. 3), provided the high value of the correlation coefficient, was valid rather than accidental, this would mean positive direct influence of an increase of biomass of green algae on an increase of mortality of *Cyclops* (Table 2). Such influence looks like doubtful. Although cyclopoids may not utilize some hard-walled green algae due to lack of cellulase in their guts, *C. vicinus* was proven to survive and develop on a pure algal diet, included

greens (Hansen and Santer, 1995). Moreover, results of CCA of matrix No. 3 demonstrated that in absence of the extreme point there was no significant influence of biomass of greens on NPSM of *Cyclops*, neither on the basis of factor loadings nor canonical weights (Table 3). It should be remarked, that the mortality of *Cyclops* in the reservoir on the average was substantially higher (Fig. 2f), than that in laboratory conditions, 0.001–0.014  $\text{day}^{-1}$ , obtained by Hansen and Santer (1995). We believe that some peaks of adult *Cyclops* NPSM may be the result of physiological aging after reproductive period, but the others may be caused by different factors, particularly by diseases and parasites, omitted in our study.

### Conclusion

In summary, a decrease of food quality, namely EPA level in seston, resulted in increase of non-predatory mortality of *Daphnia*, and about 50% of variance of NPSM could be attributed to this factor. At the same time, considerable part of NPSM variance for *Daphnia* and all the variance of NPSM of *C. vicinus* stay to be revealed in a future on the basis of other ecological factors, rather than those considered in present study.

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