


Effects of zebra mussels on cladoceran communities under eutrophic conditions

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Abstract The purpose of this study was to determine how zebra mussels affected cladoceran community structure under eutrophic conditions. We conducted a mesocosm study where we manipulated the presence of zebra mussels and the presence of large-bodied *Daphnia* (*Daphnia magna* and *Daphnia pulex*). We also conducted a complimentary life-table experiment to determine how water from the zebra mussel treatment affected the life history characteristics of the cladoceran species. We anticipated that small- and

large-bodied cladoceran species would respond differently to changes in algal quality and quantity under the effects of zebra mussels. Large-bodied *Daphnia* successfully established in the zebra mussel treatment but failed to grow in the control. We did not observe positive relationships between food concentrations and cladoceran abundances. However, the phosphorus content in the seston indicated that food quality was below the threshold level for large-bodied cladocerans at the beginning of the experiment. We believe that zebra mussels quickly enhanced the phosphorus content in the seston due to the excretion of inorganic

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phosphorus, thus facilitating the development of large-bodied *Daphnia*. In conclusion, our results suggest that zebra mussels can alter the phosphorus content of seston in lakes and this can affect the dynamics of crustacean zooplankton.

Keywords Zooplankton · Chlorophyll · Food quality · Phosphorus limitation · Life-table experiments

Introduction

The invasive zebra mussel, *Dreissena polymorpha* (Pallas, 1771), has negatively impacted aquatic ecosystems in habitats outside of its native range (Higgins & Vander Zanden, 2010). Zebra mussels are efficient filter feeders that consume algae and compete with native crustaceans. Zebra mussels can reduce total algal biomass (Karatayev et al., 1997, 2002; Vanderploeg et al., 2002; Kelly et al., 2010), selectively consume and sort algal particles (Baker et al., 1998, 2000), and promote cyanobacteria especially in systems with low trophic (Raikow et al., 2004). Although less well studied, zebra mussels also have the potential to influence the nutritional quality of algae with respect to elemental composition [mainly studied as carbon (C), nitrogen (N), and phosphorus (P)] and/or polyunsaturated fatty acids [PUFA, mainly studied as eicosapentaenoic acid (EPA)]. For example, zebra mussels excrete nutrients (nitrogen and phosphorus) into the water column (Wilson, 2003; Wojtal-Frankiewicz & Frankiewicz, 2011) that can in turn affect algal elemental quality. In support, zebra mussels increased the phosphorus content of seston in mesotrophic mesocosms thus favoring the development of large *Daphnia* species (Feniova et al., 2015). In contrast, zebra mussels reduced the content of EPA in the mesocosms (Feniova et al., 2015), and they have been shown to selectively consume EPA-rich seston (Makhutova et al., 2013). Therefore, zebra mussels may suppress crustaceans through their indirect effects not only on food quantity, but also on food quality.

If zebra mussels can alter the nutritional quality of algal resources, this could have important implications for crustaceans. For example, crustaceans often exhibit reductions in growth and reproduction if there is a mismatch between algal elemental quality and/or EPA and the body requirements of individual taxa (Urabe & Sterner, 1996; Hessen & Andersen, 2008).

There are also differences in the nutritional requirements of large- and small-bodied zooplankton (Andersen & Hessen, 1991). Large-bodied species are more likely to be successful when carbon is limiting because they are more effective filterers than small-bodied species (Brooks & Dodson, 1965; Gliwicz, 2003; Sikora & Dawidowicz, 2014). However, large-bodied species may be more vulnerable to phosphorus limitation because phosphorus is used for somatic growth (Sterner & Schulz, 1998). Small-bodied species are less vulnerable to decreases in the EPA content of algae than large-bodied species. Sikora et al. (2016) showed that saturation thresholds for EPA-dependent growth increased with increasing species and/or clone body size in *Daphnia*. Combined, these studies suggest that zebra mussels have the potential to modify crustacean communities through bottom-up effects on the nutritional quality (e.g., C:N:P ratio and PUFAs) of algae.

We expect that the effects of zebra mussels on algal abundance and quality will be different under eutrophic conditions compared to those that were observed at lower trophic (Feniova et al., 2015). Raikow et al. (2004) also showed that cyanobacteria such as *Microcystis* were favored by *Dreissena* in low but not high-nutrient lakes. Nevertheless, the mechanisms of zebra mussel effects on zooplankton and phytoplankton under different levels of trophic are still obscure.

We conducted a mesocosm experiment under eutrophic conditions where we manipulated the presence of zebra mussels to determine how they influenced algal food quality and/or quantity and cladoceran community structure. Under eutrophic conditions, carbon is unlikely to be a limiting factor while phosphorus or EPA could be in shortage. Therefore, we hypothesized that zebra mussels would alter algal composition and nutritional quality for crustaceans with respect to cyanobacteria abundance, C:N:P stoichiometry, and/or EPA concentrations. We anticipated that small- and large-bodied cladoceran species would respond differently to changes in algal

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quality or quantity. Therefore, we also added large-bodied *Daphnia* to the mesocosms to see if their establishment was affected by changes in algal quality. Finally, we conducted a concurrent life-table experiment where crustaceans were grown in water from the different mesocosm treatments to determine how zebra mussels affect individual crustacean life history characteristics.

Methods

Mesocosm setup

We conducted our experiments in 12 mesocosms ($0.94 \times 0.64 \times 0.50$ m; 300 l, food safe, high-density polyethylene (HDPE) containers) from June 26 to August 18, 2014 (44 days total). The mesocosms were located on the shore of Lake Mikołajskie (Mazurian Lake District, northeastern Poland, $21^{\circ}35'E$, $53^{\circ}48'N$) at the Hydrobiological Station of the Nencki Institute of Experimental Biology, Polish Academy of Sciences. The mesocosms were filled with unfiltered water from the eutrophic Lake Mikołajskie (Chróst et al., 2009) that contained in situ phytoplankton and zooplankton. The cladoceran community that was added to the mesocosms from Lake Mikołajskie included *Ceriodaphnia pulchella* G.O. Sars, 1862 (a dominant species among cladocerans), *Chydorus sphaericus* (O.F. Müller, 1776), *Bosmina coregoni* Baird, 1857, *Bosmina longirostris* (O. F. Müller, 1776), and *Diaphanosoma brachyurum* (Liévin, 1848). The copepod community included *Thermocyclops oithonoides* (Sars G.O., 1863) (a dominant species among copepods), *Thermocyclops crassus* (Fischer, 1853), *Eudiaptomus gracilis* (Sars G.O., 1863), *Eudiaptomus graciloides* (Lilljeborg, 1888), and *Mesocyclops leuckarti* (Claus, 1857).

Experimental treatments

We established 4 treatments by manipulating the presence of large-bodied zooplankton and zebra mussels in a 2×2 factorial design. Each treatment was replicated in triplicate mesocosms. The treatment with unfiltered lake water only served as the control (C). The large-bodied zooplankton (Z) treatment was established by adding two large-bodied cladoceran species that were reared in laboratory cultures:

Daphnia magna Straus, 1820 (originated from Binnensee, Germany) and *Daphnia pulicaria* Forbes, 1893 (originated from Lake Brome, Canada). We added both *D. magna* and *D. pulicaria* to mesocosms in the Z treatments at densities of 1.0 ind. l^{-1} for each species at the beginning of the experiment on Day 1. The zebra mussel (M) treatment was established by adding zebra mussels at a wet weight of 250 g m^{-2} , or approximately 200 individuals per mesocosm. Similar levels of zebra mussel biomass have been reported in two Polish lakes (lakes Licheńskie and Ślesieńskie) where biomass ranged from 0.02 to 2.79 kg m^{-2} (Sinicyna & Zdanowski, 2007). We collected the zebra mussels from nearby Lake Boczne and transported it to the field station in coolers. We added zebra mussels to the mesocosms within 24 h of collection on Day 1 of the experiment. The size range of mussels used in the experiment was 7–24 mm. Zebra mussel mortality did not exceed 3% by the end of the experiment.

Water quality analysis

We measured temperature and dissolved oxygen concentrations daily from the center of each mesocosm using a WTW multi-parameter probe 3410 with optical sensor FDO925. We collected water samples from the center of each mesocosm after they were gently mixed for analyses of nutrient concentrations 4 times over the course of the experiment (on days 1, 4, 24, and 44) using a Limnos sampler (2.6 l). Phosphates (P- PO_4), nitrate and nitrite nitrogen (N- NO_3 , N- NO_2), and ammonium concentrations (N- NH_4) were measured according to the analytical procedures described in Standard Methods (2005).

Biological analysis

We also collected water samples (2.6-l Limnos sampler) from the center of each mesocosm after they were gently mixed for the analysis of chlorophyll concentrations and zooplankton identification and enumeration (on days 1, 4, 14, 24, 34, and 44). Chlorophyll concentration was estimated using a PHYTOPAM fluorometer (Walz, Germany) which estimates chlorophyll concentrations for three groups of algae individually [cyanobacteria, brown (mostly diatoms), and green algae]. Zooplankton samples were preserved in a 4% formaldehyde solution and all

crustaceans were identified to species. We measured the lengths of up to 100 individuals of each taxon for biomass estimates based on length:weight relationships from Balushkina & Vinberg (1978).

Biochemical analyses

We analyzed seston immediately after the mesocosms were filled from Lake Mikołajskie and again at the end of the experiments from the C, M, and MZ treatments. We focused on these three treatments because large-bodied *Daphnia* did not develop in the Z treatments; therefore, the Z treatments were similar to control for all response variables that were measured in this study (see Results below) such that we did not anticipate an effect of *Daphnia* on seston quality. We collected 7–15 l of water from each mesocosm and filtered it onto precombusted glass-fiber GF/F filters (Whatman, USA). The filters for fatty acid analysis were dried at ambient temperature for about 30 min, and then placed into vials containing 3 ml of chloroform–methanol (2:1, v/v) and stored at -20°C until further analysis. We dried filters for organic carbon and nitrogen at 75°C overnight and stored dry in a desiccator until further analyses. The samples for particulate phosphorus were filtered onto membrane filters (Vladipor, Mytischki, Russia, pore size 0.75–0.85 μm).

Live individuals of three dominant species *D. magna* (100–150 ind.), *D. pulicaria* (100–150 ind.), and *C. pulchella* (200–300 ind.) were collected from the zooplankton samples for elemental and fatty acid analyses. We kept the cladocerans in filtered water from their respective mesocosms for several hours before the analyses to allow them to empty their guts. Animals were then collected onto a mesh sieve and placed on filter paper to remove the surface moisture, and then they were subsampled for fatty acid analyses. The sample sizes ranged in 8–20 and 4–10 mg of wet weight for fatty acid and organic carbon analyses, respectively. The fatty acid subsamples were then transferred into a chloroform–methanol mixture and frozen.

We analyzed the fatty acid content of the seston and cladocerans according to the protocol of Gladyshev et al. (2015). We used a common shorthand notation for fatty acids of the form A:Bn-X, where A represents the number of carbon atoms in the molecule, B gives the number of double carbon–carbon bonds, and X

gives the position of the double bond closest to the terminal methyl group. Organic carbon (C) and nitrogen (N) were measured using a Flash EA 1112 NC Soil/MAS 200 elemental analyzer (ThermoQuest, Milan, Italy), as described in Gladyshev et al. (2007). Calibration curves for the elemental analyzer were generated using aspartic acid and standard soil reference material. Contents of particulate total phosphorus (P) were estimated following the conventional photocolometric method (Murphy & Riley, 1962).

Life-table experiments

Life-table experiments were conducted to determine how demographic parameters of the large-bodied species (*D. magna*, and *D. pulicaria*) and a dominant small-bodied cladoceran species from the initial zooplankton community (*C. pulchella*) were affected by zebra mussel changes in algal quantity and/or quality. We did not want to disturb the mesocosms in the experiment described above, therefore, we set up four additional mesocosms to obtain water for the life-table experiments. Two mesocosms were established without zebra mussels (i.e., the same as the control) and two were established with zebra mussels (i.e., as the MZ treatment) exactly as described above. Life-table experiments were performed simultaneously with the mesocosm experiment.

Life-table experiments were conducted in 500 ml bottles using a flow through system. To start the experiment, 20–30 new born individuals (less than 24 h old) of each species were placed into bottles. Each species was grown in monoculture in triplicate bottles with water collected from both mesocosms with and without zebra mussels. The bottles were filled with water that was collected from the mesocosms and first filtered through a mesh sieve (50 μm) to remove crustaceans or other large objects. The flow through system was designed so that the entire volume of each bottle was replenished twice a day (e.g., 1 l flow per day) to ensure that resource abundance was similar to that in the mesocosms. We collected the following parameters every 2 days from the start of the experiment until approximately the third clutch: the total number of individuals of each species, clutch sizes, and the time of maturation. We limited our observations to the third clutch because previous studies on cladoceran life histories have shown that later clutches contribute negligibly to population growth rate

(r) (Porter et al., 1983; Pijanowska et al., 2006). We collected data from the bottles over the course of two generations for each species. The experiments with the first generation were conducted starting on Day 1 and the second generation after Day 24, which was the day when generations of each species reached the third clutch so these two generations did not overlap.

Life-table experiments were used to calculate population growth rate: $r = \ln\{\sum l(x)m(x)\}/T$, where $l(x)$ and $m(x)$ were the survivorship function, or probability that an individual survives to age x , and birth function, or age-specific clutch size, respectively, and T was the mean generation time.

Statistical analyses

Since water quality and biological parameters in the mesocosms were measured 4 times (nutrients) or 6 times (biomass of crustaceans and chlorophyll concentrations), we used factorial repeated measures ANOVA (FRM ANOVA) with treatment and time as the two factors. With respect to treatment, we analyzed four levels including C, M, Z, and MZ.

If the data properties of symmetry according to Mauchly's criterion were violated in the FRM ANOVA, the degrees of freedom of the F test for Time and Time \times Treatment factors were adjusted using epsilon Greenhouse–Geisser corrections. If P in the Mauchly's test was more than 0.05 (i.e., sphericity assumption is not rejected), we did corrections of the degrees of freedom for the F test. If significant treatment effects were detected with FRM ANOVA, we used Fisher's LSD post hoc test ($P < 0.05$) to determine which means differed. We also analyzed the effects of the two-factor combination (Time \times Treatment) to determine whether the Treatment factor was dependent on time.

Since data on nutrient and biological parameters did not meet the requirements of Levene's criterion such as dispersion homogeneity of data and that the combined effects of two factors were not additive, we made log transformation of the data to adjust them for statistical analysis. The figures of nutrient and biological parameters dynamics were also made for log-transformed data to make them correspond to statistical results. FRM ANOVA analysis was conducted using Statgraphic XVII.II software.

We used one-way ANOVA to compare values of the food quality indicators (C:N:P and EPA) of seston

and zooplankton. In the absence of normal distribution (Kolmogorov–Smirnov one-sample test for normality $D_K - s$), Kruskal–Wallis test was used. The number of all variables (3 replicates \times 4 treatments) was equal to 12, since we compare the whole data set rather than pairs of variables. Fatty acid composition of dominant cladoceran species was compared using principal component analysis (PCA). The calculations were carried out using STATISTICA software, version 9.0 (StatSoft, Inc.).

In order to analyze the linear associations between total chlorophyll concentrations and the biomass of small cladocerans, *Daphnia* and copepods, we evaluated Pearson's correlation coefficients, and conducted two-tailed hypothesis test of these coefficients using Fisher's z transformation.

For the life-table experiment, we used parametric one-way ANOVA to compare parameters including clutch sizes and population growth rate between *D. magna*, *D. pulicaria*, and *C. pulchella* in the control and zebra mussel treatments for each species during the first and second generations. If F (Fisher's test) gave significant difference between the means, multiple Tukey's HSD post hoc tests ($P < 0.05$) were used to determine which means were significantly different.

Data on the juvenile development time violated the conditions of randomness of measurements and equality of variances, thus we could not use parametric one-way ANOVA of variance. Therefore, comparison of juvenile development time between control and zebra mussel treatments for two generations was performed using nonparametric one-way ANOVA of variance on the basis of rank values according to the Kruskal–Wallis test (KW). If KW showed significant difference between means, we performed multiple Bonferroni post hoc procedure ($P < 0.05$) to determine which means were significantly different. Statistical analysis of experimental data using one-factor ANOVA was performed using the integrated software Biosystem office (Petrosyan, 2014) and R 3.3 (R Core Team, 2017).

Results

Mesocosm experiments

Phosphorus (P–PO₄) and nitrogen concentrations (sum N–NO₃, N–NO₂ and N–NH₄) significantly changed

Table 1 Results of factorial repeated measures ANOVA (FRM ANOVA) with factors treatment and time

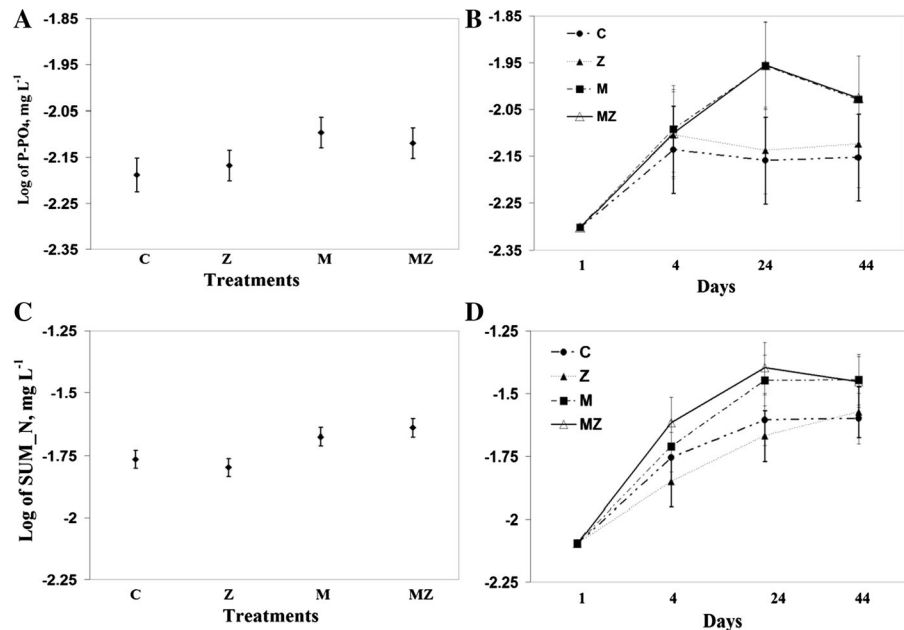
Source	DF ₁ /DF ₂	F ratio	P*	Mauchly's Sphericity test
Nutrient concentrations (mg l ⁻¹)				
Log (<i>P-PO4</i>), FRM ANOVA ($F_{23,24} = 9.45$; $P \ll 0.001$)				
Treatments (C, Z, M, MZ)	3/8	4.7	0.04	W = 0.09
Time, days (1, 4, 24, 44)	2.4/19.5*	50.3	$\ll 0.001$	$X^2 = 16$
Time × Treatments	7.3/19.5*	2.58	0.05	DF = 5 $P^{**} = 0.007$
Log (SUM_N), FRM ANOVA ($F_{23,24} = 35.49$; $P \ll 0.001$)				
Treatments (C, Z, M, MZ)	3/8	11.3	0.003	W = 0.27
Time, days (1, 4, 24, 44)	2/16	240.8	$\ll 0.001$	$X^2 = 8.6$
Time × Treatments	6/16	2.97	0.02	DF = 5 $P^{**} = 0.12$
Chlorophyll concentration (µg l ⁻¹)				
Log (Brown), FRM ANOVA ($F_{31,40} = 5.9$; $P \ll 0.001$)				
Treatments (C, Z, M, MZ)	3/8	3.9	0.05	W = 0.004
Time, days (1, 4, 14, 24, 34, 44)	2.5/20.1*	19.4	$\ll 0.001$	$X^2 = 32.7$
Time × Treatments	7.5/20.1*	3.4	0.01	DF = 14 $P^{**} = 0.003$
Log (Greens), FRM ANOVA ($F_{31,40} = 17.3$; $P \ll 0.001$)				
Treatments (C, Z, M, MZ)	3/8	15.7	0.001	W $\ll 0.001$
Time, days (1, 4, 14, 24, 34, 44)	1.8/14.8*	70.2	$\ll 0.001$	$X^2 = 235$
Time × Treatments	5.5/14.8*	5.6	0.004	DF = 14 $P^{**} \ll 0.001$
Log (Cyanobacteria), FRM ANOVA ($F_{31,40} = 5.5$; $P \ll 0.001$)				
Treatments (C, Z, M, MZ)	3/8	19.2	$\ll 0.001$	W $\ll 0.001$
Time, days (1, 4, 14, 24, 34, 44)	3.7/29.4*	12.7	$\ll 0.001$	$X^2 = 50.6$
Time × Treatments	7.4/29.4*	2.2	0.04	DF = 14 $P^{**} \ll 0.001$
Log (Total biomass), FRM ANOVA ($F_{31,40} = 5.8$; $P \ll 0.001$)				
Treatments (C, Z, M, MZ)	3/8	3.9	0.05	W = 0.05
Time, days (1, 4, 14, 24, 34, 44)	5/40	16.2	$\ll 0.001$	$X^2 = 38.4$
Time × Treatments	15/40	4.6	0.001	DF = 14 $P^{**} = 0.19$
Zooplankton biomass (mg l ⁻¹)				
Log (Copepoda), FRM ANOVA ($F_{31,40} = 3.0$; $P \ll 0.001$)				
Treatments (C, Z, M, MZ)	3/8	0.28	0.8	W = 0.01
Time, days (1, 4, 14, 24, 34, 44)	2.3/18.4*	7.33	0.004	$X^2 = 25.6$
Time × Treatments	6.9/18.4*	2.4	0.06	DF = 14 $P^{**} = 0.03$
Log (Small species), FRM ANOVA ($F_{31,40} = 7.3$; $P \ll 0.001$)				
Treatments (C, Z, M, MZ)	3/8	3.9	0.05	W $\ll 0.001$
Time, days (1, 4, 14, 24, 34, 44)	2.1/16.6*	41.1	$\ll 0.001$	$X^2 = 55.2$
Time × Treatments	6.2/16.6*	2.4	0.08	DF = 14 $P^{**} \ll 0.001$

Table 1 continued

Source	DF ₁ /DF ₂	F ratio	P*	Mauchly's Sphericity test
Log (<i>Daphnia</i>), FRM ANOVA ($F_{15,20} = 5.4$; $P = 0.003$)				
Treatments (Z, MZ)	1/4	10.0	0.03	$W = 0.15$
Time, days (1, 4, 14, 24, 34, 44)	5/20	3.9	0.01	$X^2 = 5.7$
Time \times Treatments	5/20	4.7	0.006	DF = 9
				$P^{**} \leq 0.06$

F Fisher's test and its significance; DF₁, DF₂ degrees of freedom of F ratio; P* P value for the factor effects, W Mauchly's statistical test, X² Chi-square statistical test based on W statistics, DF degrees of freedom for Chi. Sq. test, P** P value for Mauchly's test, P significant values are given in bold; Green House–Geisser corrections of DF₁ and DF₂ were marked with*

Fig. 1 Comparison of log-transformed nutrient concentrations between means (A, C) and on every date (B, D); C control, Z treatments with introduced *Daphnia* species, M treatments with introduced zebra mussels, MZ treatments with introduced *Daphnia* species and zebra mussels. Vertical bars indicate 95% of Fisher's LSD intervals



over time and they were significantly greater in the zebra mussel treatments (M and MZ) than they were in the C and Z treatments (Table 1, Fig. 1). Large-bodied zooplankton did not affect nutrient concentrations as there were no significant differences between the C and Z treatments or between the M and MZ treatments for either nutrient. The greatest differences between treatments without zebra mussels and treatments with zebra mussels were observed during Day 24 of the experiment for both nutrients.

Concentrations of green algae were significantly higher while those of cyanobacteria were significantly lower in the M and MZ treatments compared to the C and Z treatments (Table 1, Fig. 2). Between the M and MZ treatments as well as between the C and Z

treatments, there were no significant differences in chlorophyll concentrations of either cyanobacteria or green algae. Mean concentration of brown algae was significantly higher in the M treatment than in the control. Other treatments did not differ significantly although there were significant time-dependent difference between zebra mussel treatments and treatments without zebra mussels in chlorophyll concentration of brown algae. Total chlorophyll concentrations were not affected either by zebra mussels or large-bodied *Daphnia* (Table 1, Fig. 2G, H).

Chlorophyll concentrations were not significantly correlated with the abundance of either copepods or *Daphnia* (Table 2). However, there was a significant

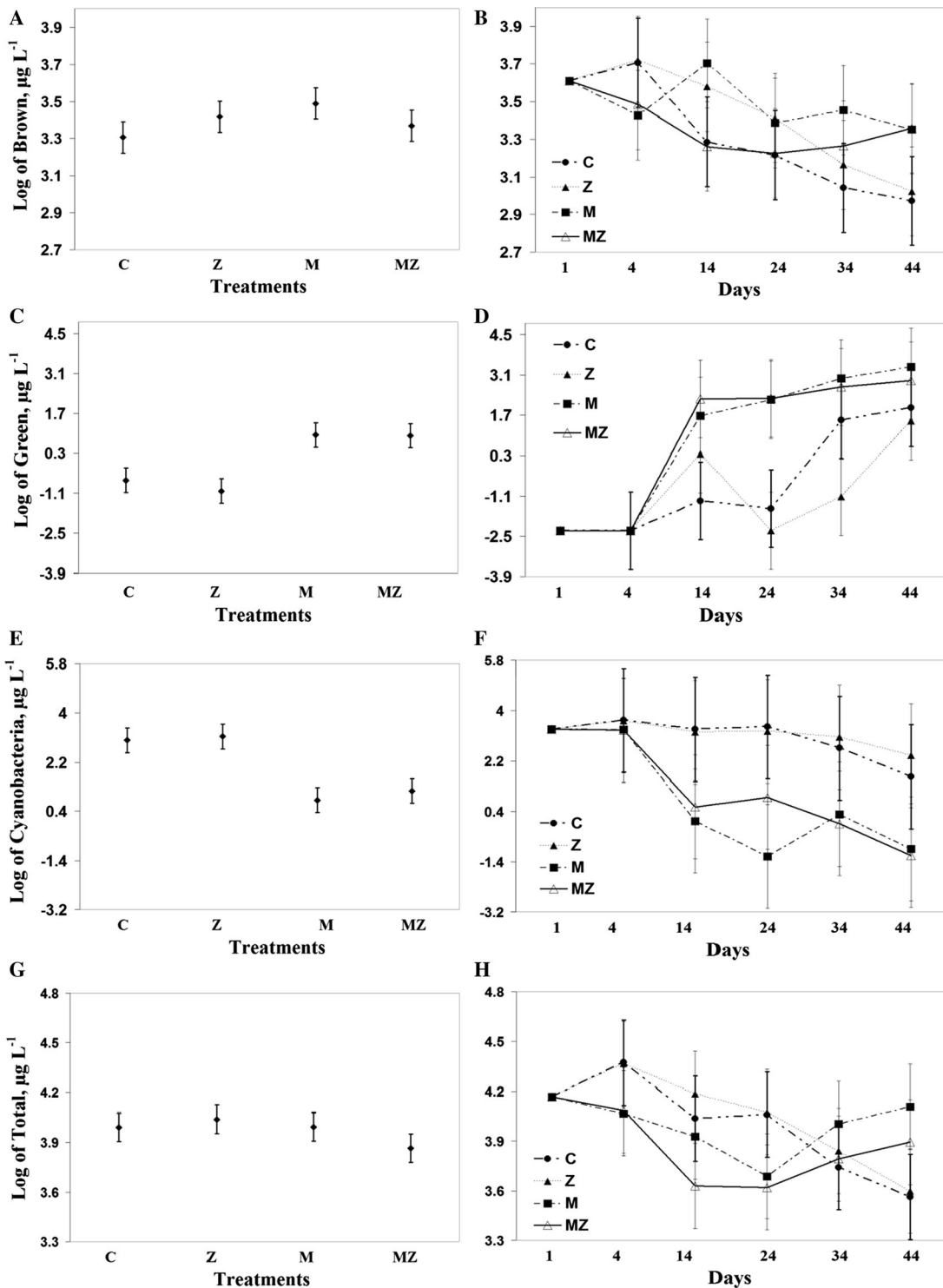


Fig. 2 Comparison of log-transformed chlorophyll concentrations between means (A, C, E, G) and on every date (B, D, F, H); C control, Z treatments with introduced *Daphnia* species,

M treatments with introduced zebra mussels, MZ treatments with introduced *Daphnia* species and zebra mussels. Vertical bars indicate 95% of Fisher's LSD intervals

Table 2 Correlation analysis between biomass of crustaceans and total chlorophyll concentrations in different treatments

	Small cladocerans	<i>Daphnia</i>	Copepods
Control			
Total chlorophyll	– 0.62 (<i>P</i> = 0.006, <i>n</i> = 18)		0.34 (<i>P</i> = 0.17; <i>n</i> = 18)
Z treatment			
Total chlorophyll	– 0.6 (<i>P</i> = 0.01; <i>n</i> = 18)	0.37 (<i>P</i> = 0.14; <i>n</i> = 9)	0.28 (<i>P</i> = 0.26; <i>n</i> = 18)
M treatment			
Total chlorophyll	0.45 (<i>P</i> = 0.06; <i>n</i> = 18)		0.10 (<i>P</i> = 0.69; <i>n</i> = 18)
MZ treatment			
Total chlorophyll	0.31 (<i>P</i> = 0.19; <i>n</i> = 18)	– 0.32 (<i>P</i> = 0.19; <i>n</i> = 18)	– 0.38 (<i>P</i> = 0.12; <i>n</i> = 18)

C control, Z treatments with introduced *Daphnia* species, M treatments with introduced zebra mussels, MZ treatments with introduced *Daphnia* species and zebra mussels

negative correlation between total chlorophyll and the biomass of small-bodied species in the control and Z treatments (Table 2).

The biomasses of copepods and small-bodied cladoceran species did not differ significantly between the treatments (Table 1, Fig. 3A, B, C, D). However, there were significant time effects for these variables (Table 1). Copepod biomass increased until Day 24, afterward it gradually decreased. In contrast, small-bodied cladoceran biomass consistently increased over the course of the experiment regardless of treatment. The biomass of *Daphnia* was significantly greater in the ZM treatment than it was in the Z treatment (Table 1, Fig. 3E, F). *Daphnia* biomass increased in the ZM treatment while it consistently decreased in the Z treatment until Day 24, afterward no *Daphnia* were observed in this treatment. Furthermore, no *Daphnia* neonates were observed in the mesocosms from the Z treatment at any time during the experiment.

Nutritional quality of seston

Concentrations of eicosapentaenoic acid (EPA, 20:5n-3) were significantly higher at the start than at the end of the experiments in all of the treatments (Table 3). When comparing concentrations at the end of the experiments between the treatments, concentrations of EPA were significantly higher in the control than in both of the zebra mussel treatments (Table 3). Concentrations of particulate organic carbon (C) and particulate organic nitrogen (N) in the seston were significantly higher at the start of the experiment

compared to the end of the experiment in all of the treatments (Table 3). In contrast, concentrations of particulate organic phosphorus (P) showed the opposite pattern and were lower at the start than they were at the end of the experiment. C:N values were significantly higher in MZ treatments (Table 3). C:P and N:P values decreased by more than an order of magnitude from the start to the end of the experiments in all the treatments indicating that food quality in terms of phosphorus content improved (Table 3). EPA:C values did not differ between the start and end of the experiments for any treatment (Table 3).

Fatty acid composition of dominant cladoceran species

Using PCA, the dominant cladoceran species were represented in two-dimensional space based on two factors corresponding to the largest eigenvalues from their fatty acid levels (Fig. 4). Factor 1 accounted for 55.0% of the total variance and the highest contributions to Factor 1 were provided by 18:3n-3 and 16:2n-6 on the one hand, and by i17:0 and 15:0 on the other. The second factor accounted for 17.9% of the total variance and the highest contributions to Factor 2 were provided by 20:4n-6 and 20:5n-3 on the one hand, and by ai15:0 and 18:4n-3 on the other. According to the PCA, at the start of experiment, *D. magna* and *D. pulicaria* were close to each other in Factor 1, although they differed moderately in Factor 2. At the end of the experiment in the mesocosms with zebra mussels, *D. magna* shifted significantly upward Factor 2 while *D. pulicaria* moved left along Factor 1 (Fig. 4)

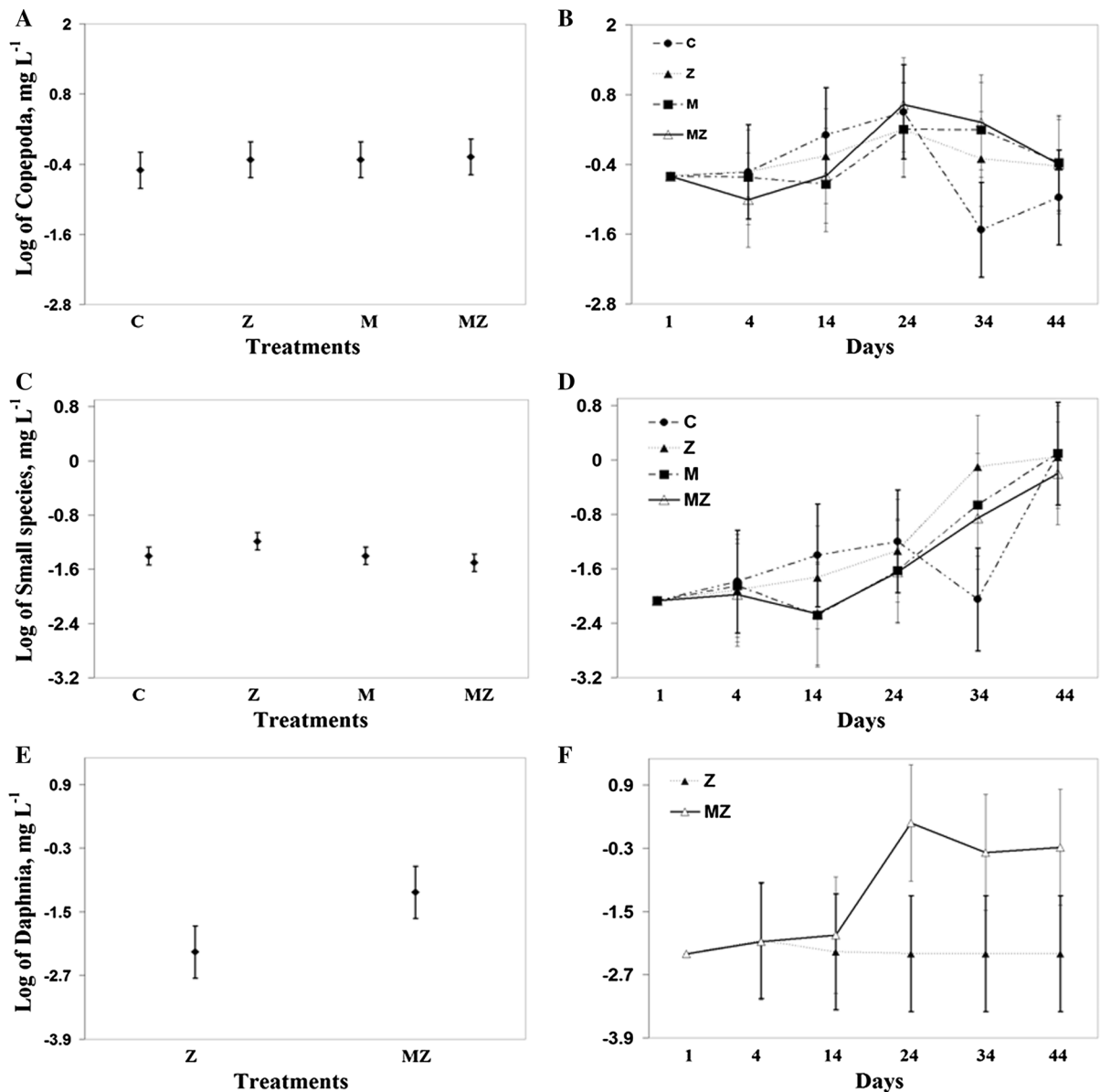


Fig. 3 Comparison of log-transformed biomass of crustaceans between means (**A**, **C**, **E**) and on every date (**B**, **D**, **F**); **C** control, **Z** treatments with introduced *Daphnia* species, **M** treatments

with introduced zebra mussels, **MZ** treatments with introduced *Daphnia* species and zebra mussels. Vertical bars indicate 95% of Fisher's LSD intervals

and became close to *C. pulchella* which was far from both *Daphnia* species at the start of experiment.

Therefore, the above results of the PCA suggest that at the start of the experiments the diets of both *Daphnia* species overlapped but they diverge in their diet by the end of the experiments. Indeed, at the end of the experiment, the percentages of bacterial acids (i15:0, ai15:0, i15:1, 15:0, 17:0) significantly increased in *D. pulicaria* (Table 4). It does not

concern cyanobacteria since they have different fatty acids (FA) composition. Thereby, FA composition of *D. pulicaria* at the end of the experiments was closer to that of *C. pulchella* because percentages of these bacterial acids, which differed significantly at the start of experiment, were similar at the end (Table 4). In contrast, the percentages of some of the bacterial FAs (ai15:0) significantly decreased in *D. magna* (Table 4). In both *Daphnia* species, at the end of the

Table 3 Results of one-way ANOVA comparing mean values (\pm SE) of concentrations and ratios of indicators of nutritive quality of seston in experimental mesocosms: *M* treatments with introduced zebra mussels, *MZ* treatments with introduced *Daphnia* species and zebra mussels, *EPA* eicosapentaenoic

	Start	M	MZ	Control	<i>F</i> (<i>H</i>)	<i>P</i>
EPA, $\mu\text{g/l}$	4.04 ± 0.16^a	0.27 ± 0.07^b	0.44 ± 0.17^b	0.72 ± 0.18^c	136.8	$\ll 0.001$
C, $\mu\text{mol/l}$	142.5 ± 3.2^a	14.9 ± 1.8^b	$17.3 \pm 0.6^{b,c}$	35.3 ± 14.0^c	69.8	$\ll 0.001$
N, $\mu\text{mol/l}$	18.8 ± 0.3^a	2.4 ± 0.1^b	1.9 ± 0.1^b	6.0 ± 2.6^c	35.1	$\ll 0.001$
P, $\mu\text{mol/l}$	0.09 ± 0.01^a	$0.26 \pm 0.04^{b,c}$	$0.17 \pm 0.02^{a,b}$	0.33 ± 0.09^c	4.5	0.038
C:N, $\mu\text{mol}/\mu\text{mol}$	7.6 ± 0.1^a	6.1 ± 0.5^b	9.2 ± 0.4^c	6.1 ± 0.4^b	15.8	0.001
C:P, $\mu\text{mol}/\mu\text{mol}$	1641.3 ± 334.2^a	59.3 ± 5.5^b	106.8 ± 12.6^b	104.6 ± 22.6^b	8.23	0.04
N:P, $\mu\text{mol}/\mu\text{mol}$	216.0 ± 43.7^a	9.8 ± 1.4^b	11.8 ± 1.9^b	17.7 ± 4.5^b	7.82	0.049
EPA:C, $\mu\text{g}/\text{mg}$	2.4 ± 0.2	1.5 ± 0.2	2.1 ± 0.8	2.0 ± 0.7	0.5	0.72

When ANOVA is insignificant ($P > 0.05$), letter labels are absent. Number of variables was equal to 12

acid, *C* organic carbon, *N* nitrogen, *P* phosphorus, *F* Fisher’s test, and its significance, *P* (significant values are given in bold); means labeled with the same letter are not significantly different at $P < 0.05$ after Fisher’s LSD post hoc test (in the absence of normal distribution, Kruskal–Wallis test was used)

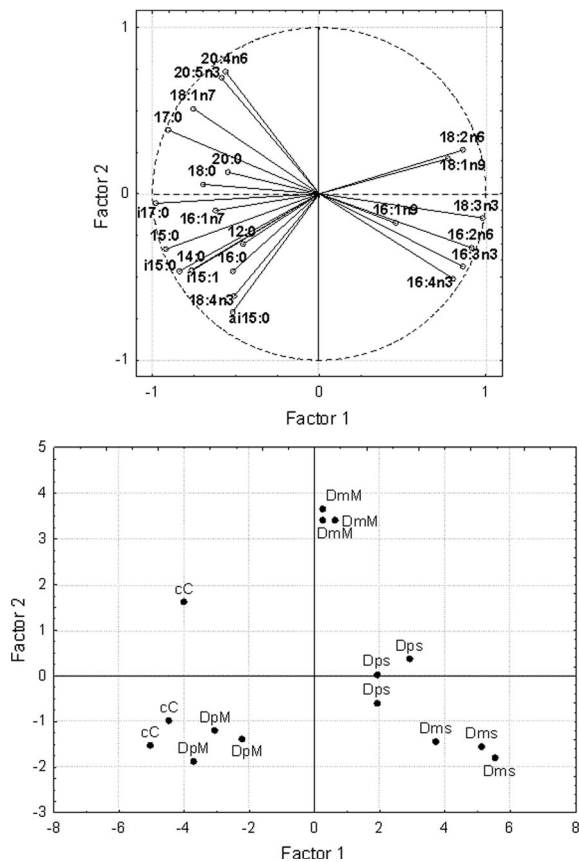


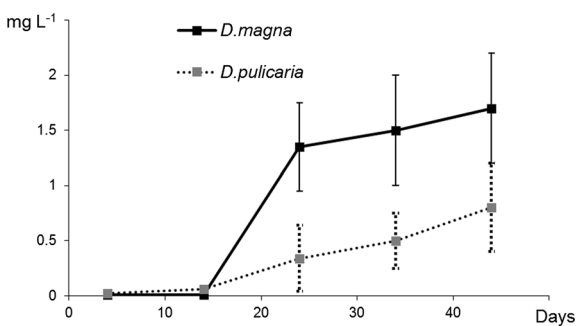
Fig. 4 Principal component analysis of FA levels in zooplankton from mesocosms: *Dms* *Daphnia magna*, start; *Dps* *Daphnia pulicaria*; start, *DmM* *D. magna* + mollusks, end; *DpM* *D. pulicaria* + mollusks, end; *cC* *Ceriodaphnia pulchella* control

experiments, percentages of 16:2n-6, 16:3n-3, 18:3n-3 significantly decreased (Table 4) indicating a decrease in the contribution of green algae in their diet. Besides in *D. pulicaria*, at the end of experiment, percentages of 18:2n-6 decreased indicating a stronger decrease in algal diet compared to that of *D. magna* (Table 4). Percentages of 20:4n-6 increased significantly at the end of the experiments in both *Daphnia* species indicating an increase of proportion of allochthonous organic matter in their diet (Table 4). In *D. magna*, percentages of 20:5n-3 at the end of experiments increased significantly by about an order of magnitude indicating an abrupt increase in the proportion of diatoms in their diet (Table 4). In contrast, in *D. pulicaria*, the percentage of 20:5n-3 increased by only 1.4 times (Table 4). In *D. magna*, percentages of 18:0 and 18:1n-7 increased significantly (Table 4), providing a moderate left moving along Factor 1 (Fig. 4). These data are in accordance with the fact that when reared in the culture, both *Daphnia* were fed with green algae and showed that in the experiments, *D. magna* added diatoms to their diet, and *D. pulicaria* dramatically switched to bacteria. This divergence in their diet was observed in the MZ treatment only because in the other treatments at the end of the experiments both *Daphnia* were absent (Fig. 3G, H). Figure 5 demonstrated that in the MZ treatment, *D. magna* and *D. pulicaria* did not displace one another and coexisted throughout the experiment.

Table 4 Mean values (\pm SE) of percentages (% of total FA) of fatty acids in *Daphnia magna* and *Daphnia pulex* at the start and at the end of experiment with introduced mollusks, and in *Ceriodaphnia pulchella* (control)

	<i>D. magna</i> start	<i>D. magna</i> end	<i>D. pulex</i> start	<i>D. pulex</i> end	<i>C. pulchella</i>
12:0	0.4 \pm 0.1 ^a	0.6 \pm 0.1 ^a	1.1 \pm 0.2 ^b	1.8 \pm 0.1 ^c	0.7 \pm 0.1 ^{a,b}
14:0	1.9 \pm 0.0 ^{a,b}	1.6 \pm 0.0 ^a	1.8 \pm 0.1 ^{a,b}	3.0 \pm 0.2 ^b	4.3 \pm 0.9 ^c
i15:0	0.6 \pm 0.1 ^a	0.6 \pm 0.0 ^a	0.7 \pm 0.1 ^a	2.0 \pm 0.0 ^b	2.2 \pm 0.5 ^b
ai15:0	0.6 \pm 0.0 ^a	0.2 \pm 0.0 ^b	0.3 \pm 0.0 ^b	0.7 \pm 0.0 ^a	0.9 \pm 0.2 ^a
i15:1	0.2 \pm 0.1 ^a	0.2 \pm 0.0 ^a	0.1 \pm 0.0 ^a	1.0 \pm 0.0 ^b	0.7 \pm 0.2 ^b
15:0	0.8 \pm 0.0 ^a	0.9 \pm 0.0 ^{a,b}	1.0 \pm 0.0 ^b	1.4 \pm 0.0 ^c	1.4 \pm 0.1 ^c
16:0	18.7 \pm 1.2 ^{a,b}	17.4 \pm 0.3 ^b	22.4 \pm 0.8 ^{a,c}	25.9 \pm 1.7 ^c	22.2 \pm 2.0 ^{a,c}
16:1n-9	3.0 \pm 1.4	1.8 \pm 0.0	3.2 \pm 0.1	1.9 \pm 0.3	1.8 \pm 0.3
16:1n-7	2.9 \pm 1.4 ^a	2.7 \pm 0.1 ^a	2.1 \pm 0.2 ^a	4.4 \pm 0.4 ^{a,b}	6.4 \pm 1.3 ^b
i17:0	0.5 \pm 0.0 ^a	0.8 \pm 0.0 ^c	0.6 \pm 0.0 ^b	1.2 \pm 0.0 ^d	1.3 \pm 0.0
16:2n-6	0.9 \pm 0.1 ^a	0.3 \pm 0.0 ^c	0.7 \pm 0.0 ^b	0.3 \pm 0.1 ^c	0.2 \pm 0.1 ^c
17:0	0.7 \pm 0.1 ^a	1.7 \pm 0.0 ^c	1.3 \pm 0.0 ^b	1.7 \pm 0.1 ^{c,d}	1.9 \pm 0.0 ^d
16:3n-3	3.6 \pm 0.5 ^a	0.6 \pm 0.0 ^c	1.8 \pm 0.1 ^b	0.7 \pm 0.1 ^c	0.3 \pm 0.1 ^c
16:4n-3	5.6 \pm 0.7 ^a	0.6 \pm 0.1 ^c	2.2 \pm 0.2 ^b	1.4 \pm 0.1 ^{b,c}	0.3 \pm 0.1 ^c
18:0	4.4 \pm 1.0 ^a	7.5 \pm 0.3 ^b	9.6 \pm 0.5 ^b	10.1 \pm 1.0 ^b	9.9 \pm 1.3 ^b
18:1n-9	17.4 \pm 0.4 ^a	16.1 \pm 0.4 ^a	12.5 \pm 0.4 ^b	13.2 \pm 0.8 ^b	8.2 \pm 0.7 ^c
18:1n-7	1.2 \pm 0.1 ^a	7.2 \pm 0.1 ^b	2.9 \pm 0.2 ^{a,c}	4.5 \pm 0.0 ^c	9.2 \pm 1.4 ^b
18:2n-6	7.6 \pm 0.2 ^{a,b}	7.1 \pm 0.3 ^b	8.4 \pm 0.7 ^a	3.3 \pm 0.3 ^c	3.9 \pm 0.4 ^c
18:3n-3	22.7 \pm 0.7 ^a	11.9 \pm 0.3 ^c	17.6 \pm 0.7 ^b	8.2 \pm 0.7 ^d	3.4 \pm 0.4 ^e
18:4n-3	1.7 \pm 0.0	1.2 \pm 0.0 ^a	1.4 \pm 0.0	2.0 \pm 0.1	2.4 \pm 0.6
20:0	0.3 \pm 0.1	0.5 \pm 0.1	0.3 \pm 0.1	0.6 \pm 0.1	0.4 \pm 0.1
20:4n-6	0.1 \pm 0.1 ^a	7.8 \pm 0.1 ^b	0.7 \pm 0.4 ^a	3.2 \pm 0.3 ^c	5.3 \pm 0.4 ^d
20:5n-3	0.7 \pm 0.1 ^a	6.8 \pm 0.2 ^c	1.8 \pm 0.2 ^b	2.5 \pm 0.3 ^d	6.1 \pm 0.2 ^e

Means along the lines (i.e., between species) labeled with the same letter are not significantly different at $P < 0.05$ after Fisher's LSD post hoc test for ANOVA. When ANOVA is insignificant, letter labels are absent

**Fig. 5** Biomass dynamics of *D. magna* and *D. pulex* in zebra mussel mesocosms

Life-table experiments

Clutch sizes of the three study species responded differently to the presence of zebra mussels (Table 5).

In the first generation, clutch sizes of *D. magna* were significantly higher in water from the M mesocosms than from the control. In contrast, clutch sizes were lower for *C. pulchella* grown in the M treatment. In *D. pulex*, clutch sizes did not differ between the treatments. During the second generation, clutch sizes of *C. pulchella* and *D. pulex* did not significantly differ between control and zebra mussel treatment while *D. magna* clutch sizes were significantly greater in zebra mussel treatment than in the control (Table 5). In comparing clutch sizes between the first and second generations in control, clutch sizes of *D. magna* and *D. pulex* were significantly greater during the second generation, while in *C. pulchella* the difference in clutch size between generations was not significant. Clutch sizes of all three species did not differ between generations in the M treatments.

Table 5 Demographic parameters of *D. magna*, *D. pulicaria*, and *C. pulchella* in the control and zebra mussel treatments (mean ± SE); *F* Fisher’s test and its significance; Tukey’s HSD post hoc test (*P* < 0.05) was used for pairwise comparisons of mean clutch sizes along the lines and along the columns; *n* sample size (number of analyzed individuals), *KW* Kruskal–Wallis test, *rv* average rank values along the lines, Bonferroni post hoc procedure (*P* < 0.05) was used for

pairwise comparisons of rank values of duration of juvenile development along the lines and along the columns; *P** values obtained by the parametric one-way ANOVA; *P*** values obtained by nonparametric one-way ANOVA with Kruskal–Wallis procedure along the lines and along the columns; *P** and *P*** significant values are given in bold; means labeled with the same letter are not significantly different along the same line

Treatments	<i>D. magna</i>	<i>D. pulicaria</i>	<i>C. pulchella</i>
Mean clutch size, eggs/female			
First generation starting since day 1			
Control	0.44 ± 0.09 ^a (<i>n</i> = 12)	0.74 ± 0.18 ^a (<i>n</i> = 12)	1.68 ± 0.17 ^b (<i>n</i> = 11)
Zebra mussel	2.52 ± 0.55 ^a (<i>n</i> = 12)	1.64 ± 0.47 ^{a,b} (<i>n</i> = 11)	0.95 ^b ± 0.1 (<i>n</i> = 12)
Control/Zebra mussel	<i>F</i> = 14.17, <i>P</i>* = 0.001	<i>F</i> = 3.42, <i>P</i> * = 0.08	<i>F</i> = 14.36, <i>P</i>* = 0.001
Second generation starting since day 24			
Control	1.02 ± 0.22 ^a (<i>n</i> = 12)	2.17 ± 0.43 ^b (<i>n</i> = 12)	1.68 ± 0.24 ^{a,b} (<i>n</i> = 11)
Zebra mussel	3.05 ± 0.46 ^a (<i>n</i> = 11)	2.58 ± 0.56 ^a (<i>n</i> = 11)	1.09 ± 0.2 ^b (<i>n</i> = 11)
Control/Zebra mussel	<i>F</i> = 13.12, <i>P</i>* = 0.001	<i>F</i> = 0.32, <i>P</i> * = 0.56	<i>F</i> = 3.6, <i>P</i> * = 0.07
Control1/Control2	<i>F</i> = 6.14, <i>P</i>* = 0.02	<i>F</i> = 9.27, <i>P</i>* = 0.006	<i>F</i> = 0.0; <i>P</i> * = 1.0
M1/M2	<i>F</i> = 0.57, <i>P</i> * = 0.46	<i>F</i> = 1.55, <i>P</i> * = 0.23	<i>F</i> = 0.37, <i>P</i> * = 0.55
Duration of juvenile development until maturity, days			
First generation starting since day 1			
Control	17.8 ± 0.07 ^a (<i>n</i> = 35, <i>rv</i> = 114)	6.7 ± 0.23 ^b (<i>n</i> = 29, <i>rv</i> = 82)	5.0 ± 0 ^c (<i>n</i> = 67, <i>rv</i> = 34)
Zebra mussel	16.9 ± 0.05 ^a (<i>n</i> = 40, <i>rv</i> = 141.5)	9.96 ± 0.15 ^b (<i>n</i> = 48, <i>rv</i> = 97.5)	5.0 ± 0 ^c (<i>n</i> = 73, <i>rv</i> = 37)
Control/zebra mussel	<i>KW</i> = 48.7, <i>P</i>** << 0.001	<i>KW</i> = 58.2, <i>P</i>** << 0.001	No significance
Second generation starting since day 24			
Control	9.4 ± 0.18 ^a (<i>n</i> = 23, <i>rv</i> = 63)	10.1 ± 0.29 ^a (<i>n</i> = 13, <i>rv</i> = 69)	5.0 ± 0 ^b (<i>n</i> = 47, <i>rv</i> = 24)
Zebra mussel	8.3 ± 0.21 ^a (<i>n</i> = 22, <i>rv</i> = 51)	7.0 ± 0.32 ^b (<i>n</i> = 30, <i>rv</i> = 34.7)	6.8 ± 0.29 ^b (<i>n</i> = 24, <i>rv</i> = 31.8)
Control/zebra mussel	<i>KW</i> = 13.1; <i>P</i>** = 0.0002	<i>KW</i> = 20.1; <i>P</i>** << 0.001	<i>KW</i> = 42.5; <i>P</i>** << 0.001
Control1/Control2	<i>KW</i> = 47.85, <i>P</i>** << 0.001	<i>KW</i> = 30.1, <i>P</i>** << 0.001	no significance
M1/M2	<i>KW</i> = 51.98, <i>P</i>** << 0.001	<i>KW</i> = 41.0; <i>P</i>** << 0.001	<i>KW</i> = 61.57. <i>P</i>** << 0.001
Population growth rate (day⁻¹)			
First generation starting since day 1			

Table 5 continued

Treatments	<i>D. magna</i>	<i>D. pulicaria</i>	<i>C. pulchella</i>
Control	-0.013 ± 0.017^a ($n = 3$)	0.04 ± 0.025^a ($n = 3$)	0.22 ± 0.009^b ($n = 3$)
Zebra mussel	0.1 ± 0.021 ($n = 3$)	0.13 ± 0.009 ($n = 3$)	0.15 ± 0.015 ($n = 3$)
Control/Zebra mussel	$F = 18.06,$ $P^* = 0.01$	$F = 10.56,$ $P^* = 0.03$	$F = 17.29$ $P^* = 0.01$
Second generation starting since day 24			
Control	0.09 ± 0.017^a ($n = 3$)	0.15 ± 0.019^a ($n = 3$)	0.25 ± 0.006^b ($n = 3$)
Zebra mussel	$0.18 \pm 0.012^{a,b}$ ($n = 3$)	$0.22^b \pm 0.023$ ($n = 3$)	0.13 ± 0.003^a ($n = 3$)
Control/Zebra mussel	$F = 21.19,$ $P^* = 0.01$	$F = 5.51,$ $P^* = 0.08$	$F = 342.25,$ $P^* \ll 0.0001$
Control1/Control2	$F = 18.0,$ $P^* = 0.01$	$F = 11.64,$ $P^* = 0.03$	$F = 6.40,$ $P^* = 0.06$
M1/M2	$F = 11.29,$ $P^* = 0.03$	$F = 13.02,$ $P^* = 0.02$	$F = 2.23,$ $P^* = 0.21$

Control/zebra mussel is comparisons between control and zebra mussel mesocosms, Control1/Control2 is comparisons between the first and second generations in the control treatment, and M1/M2 is comparisons between the first and second generations in zebra mussel mesocosms

The juvenile development time in *D. magna* in the first generation was significantly longer than in the second generation in the C and M treatments. However, in the M treatment, *D. magna* developed faster until maturity than in the control (Table 5). *Daphnia pulicaria* also developed faster in the M treatment in the second generation than in the first generation but in the control it developed faster in the first generation. *Ceriodaphnia pulchella* developed equally fast in the first generation in the zebra mussel and the control treatments while in the second generation, it developed a little bit longer in the zebra mussel treatment.

Mortality was low and did not exceed 0.04–0.08 per capita per day which was in the limits of minimal physiological mortality for cladoceran species (Romanovsky & Feniova, 1985) in either treatment for any study species.

The population growth rate in both *Daphnia* species was lower in the control than in the M treatment in the first and second generations (Table 5). *Ceriodaphnia pulchella* showed the opposite trend as its population growth rate was greater in the control than in the M treatment in both generations. In the second generation, population growth rates of both *Daphnia* species

were greater in both the control and M treatment compared to the corresponding treatments in the first generation. *Ceriodaphnia pulchella* did not show significant differences in growth rates in the M and C treatments between generations.

Discussion

Although zebra mussels can affect cladocerans by altering either the quantity or the quality of algal resources, we hypothesized that changes in food quality would regulate cladocerans dynamics under eutrophic conditions. Zebra mussels can outcompete crustaceans if they reduce algal concentrations below crustacean threshold levels ($0.5\text{--}2.0 \mu\text{g l}^{-1}$) (Semchenko et al., 2007). However, in our experiments, concentrations of chlorophyll were always above these threshold levels. Moreover, no significant relationships were detected between chlorophyll and crustacean abundances. Therefore, we suggest that competition for food was not an important factor for crustacean dynamics. We believe that our results are consistent with research showing that zooplankton

biomass is not always related to food concentrations, but instead related to algal nutritional quality (McCauley et al., 1990; Müller-Navarra & Lampert, 1996).

When comparing population growth rates of *Daphnia* species between two generations in the life-table experiments, we concluded that by the time of the second generation (day 24), food conditions improved for *Daphnia*. For small *C. pulchella*, population growth rates did not differ between generations. Therefore, it is likely that food quality changed over time and that large- and small-bodied species responded differently to these changes.

Food quality for crustaceans is commonly measured either as elemental composition (e.g., C, N, P) (Sterner & Elser, 2002; Johnson & Luecke, 2012) and/or polyunsaturated fatty acids (e.g., EPA) (Müller-Navarra, 1995; Müller-Navarra et al., 2000; Wacker & von Elert, 2001; Gladyshev et al., 2008; Hartwich et al., 2012). Sikora et al. (2016) showed that EPA-saturation thresholds varied as a function of body size in cladocerans. For the small-bodied *D. longispina* complex, the content of EPA resulting in 75% of the asymptotic growth rate varied between 0.74 and 1.80 ($\mu\text{g EPA mg C}^{-1}$), for the medium-bodied *D. pulicaria* it varied between 2.21 and 3.49, and for the large-bodied *D. magna*, it varied between 5.83 and 7.33 (Sikora et al., 2016). According to other published data for the medium-bodied *Daphnia pulex* Leydig, 1860 (similar in size as *D. pulicaria*) a lower EPA threshold for 90% saturation was 1.3 $\mu\text{g EPA mg C}^{-1}$ (Ravet et al., 2012), and for the large-bodied *D. magna* it was 2.0–4.9 $\mu\text{g EPA mg C}^{-1}$ (Sperfeld & Wacker, 2011). Based on these thresholds, EPA ($\mu\text{g EPA mg C}^{-1}$) was unlikely limiting for small-bodied species at the start of our experiment, but was close to the limiting threshold for large-bodied cladocerans. While EPA content ($\mu\text{g EPA mg C}^{-1}$) did not vary over the course of the experiments nor did it differ between the treatments, population growth rates of *Daphnia* species differed distinctly between generations. Therefore, we believe that these data suggest that EPA content per organic carbon was not the driver of population growth rates of cladocerans in our experiments.

The N:P ratio in the tissues of cladocerans also depends on body size. Small-bodied species have a higher percentage of nitrogen and a lower percentage of phosphorus content in their dry weight than larger

daphnids (Bergström et al., 2015). Therefore, the phosphorus demand is higher in large-bodied species due to their higher somatic growth rate than in small-bodied species that grow more slowly (Sterner & Schulz, 1998). Because daphnids have a relatively higher phosphorus content in their body tissues than most other freshwater zooplankton (Sterner & Hessen, 1994), stoichiometric theory predicts that daphnids should be more sensitive to phosphorus limitation than other taxa (DeMott & Gulati, 1999).

The most likely critical molar C:P ratio in seston above which daphnid production will be limited by seston phosphorus content is ~ 300 (Urabe et al., 1997; Sterner, 1997, 1998; Brett et al., 2000). At the start of our experiment, the C:P ratio was more than fivefold higher than the threshold C:P ratio while at the end of the experiment, this ratio declined below the threshold level. This ratio varied strongly due to changes in phosphorus content in the seston, so phosphorus was the most variable factor. Over the course of the current experiment, C:P ratio decreased over 15-fold. We can presume that C:P ratios gradually decreased in both treatments, i.e., it hardly fluctuated over time. However, zebra mussels likely enhanced inorganic phosphorus concentration in the water faster than it rose in the control. In support, Wilson (2003) demonstrated in his experimental studies that the highest difference in phosphorus concentration in the water between control and zebra mussel treatments was observed between the 8th and 10th days, afterward this difference started to decrease. We suggest that after the phosphorus content of the seston peaked in the zebra mussel treatment, it then started to decline. This peak in phosphorus likely stimulates the growth of *Daphnia*, which accumulate more phosphorus in their body than small-bodied species. Thus, dynamics of phosphorus content in the seston may represent a bell shape in mesocosms with zebra mussels in contrast to the control. Phosphorus content also increased with time in the control but likely more slowly than in zebra mussel mesocosms. We suggest that such increases in phosphorus content in the control were provided by regeneration of phosphorus by microzooplankton groups such as ciliates, nanoflagellates, rotifers which commonly excrete nutrients although not as intensively as zebra mussels (Vanni, 2002). Since zebra mussels may quickly enhance the phosphorus content in food resources, large *Daphnia* species introduced at the

beginning of the experiments could start to successfully develop with zebra mussels during the first generation but they failed in the treatments without zebra mussels. Similar effects were observed in mesotrophic conditions (Feniova et al., 2015) where P-PO₄ enrichment by zebra mussels was found to facilitate the successful development of large-bodied *Daphnia* at the beginning of the experiments. However, by the start of the second generation (day 24), the phosphorus content also increased in the Z treatment but by day 24, all the *Daphnia* had become extinct. Thereby, large-bodied species could have successfully developed in the treatment without zebra mussels if they had been introduced within the time of the second generation as evidenced by the life-table experiments.

Based on the PCA analysis, we found that two closely related species of *Daphnia* (*D. pulex* and *D. magna*) exhibited differences in resource use and they successfully coexisted throughout the experiment in the MZ treatment. Prior to the start of the experiment, these species were reared in culture and fed with *Scenedesmus quadricauda* Chodat, 1926; however, in the experiment, *D. magna* mainly grazed on diatoms while *D. pulex* switched to bacteria as indicated by the fatty acid composition. Such a divergence in their diet likely weakens potential competition between *Daphnia* species. Similar diet patterns were also observed in mesotrophic conditions (Feniova et al., 2015), where *D. pulex* preferred bacteria while *D. magna* preferred diatoms. Cladocerans are known as nonselective filter feeders whose diet is constrained by food particle size (DeMott, 1986). However, they can differently retain or assimilate particulate food items. In support, Taipale et al. (2016) found that cladoceran $\delta^{13}\text{C}$ values did not correlate with seston $\delta^{13}\text{C}$ values and instead correlated with the $\delta^{13}\text{C}$ values of the different phytoplankton taxa, indicating that *Daphnia* selectively assimilated phytoplankton. Selective feeding of *Daphnia* on natural microalgal assemblages was also demonstrated experimentally by Gladyshev et al. (2000).

Life-table experiments supported that these two *Daphnia* species can coexist and based on the population growth rates, they could have equal chances of developing in the mesocosms. However, the relative abundance of these two species, which was 1:2 (*D. pulex*: *D. magna*) in the mesocosms could also be affected by copepod predation and/or other

factors. These findings contradict the niche theory stating that closely related species experience severe competition (Chesson, 2000; Shea & Chesson, 2002; Tilman, 2004). However, it gives a potential explanation for the ‘plankton paradox’ phenomenon for zooplankton (Ghilarov, 1981) where more than one potentially competitive species coexist in the plankton community.

In conclusion, food quality was the main driver of cladoceran dynamics under eutrophic conditions. The introduction of zebra mussels enhanced phosphorus in the seston due to the excretion of inorganic phosphorus, thus favoring the development of large-bodied *Daphnia*. These results were confirmed by the life-table experiment as *D. magna* exhibited larger clutches when grown in water from the zebra mussel treatment. C:P and N:P ratios appeared to be more variable indicators of food quality than PUFA, so seston stoichiometry can operate as a driver of crustacean community dynamics. Therefore, our results suggest that zebra mussels can alter the phosphorus content of seston and, as a result, dynamics of crustacean zooplankton in lakes.

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