



28 **Abstract**

29 Interactions between phytoplankton and zooplankton affect the overall functioning of  
30 lakes. Herbivores are habitually confronted with food of inferior quality, usually a result of low  
31 nutrient concentrations in plant material. Large-bodied cladocerans are better competitors for  
32 food than small-bodied species but they are more vulnerable to low food quality. Understanding  
33 the effects of food quality on zooplankton structure and competition between small - large  
34 bodied herbivorous is of considerable interest. We want to find out how differences in C:N:P  
35 ratios between phytoplankton and zooplankton communities affect their abundances in a  
36 freshwater food web. We want also to assess the role of phytoplankton and zooplankton as sinks  
37 of the phosphorus and nitrogen. Therefore, we conducted a 31-day mesocosms experiment with  
38 water from a mesotrophic and a eutrophic lake (with natural plankton communities). To simulate  
39 changes in the plankton communities large-bodied *Daphnia magna* and *Daphnia pulicaria* were  
40 added. Samples for zooplankton, phytoplankton and water chemistry were taken every 10 days.  
41 Samples for elemental analysis (C:N:P) of seston and zooplankton were collected on the first,  
42 and on the final day of the experiment.

43 Our mesocosms experiment showed mismatch in C:P between seston (high) and  
44 zooplankton (low), which suggests that most of the phosphorus is incorporated in zooplankton  
45 biomass. This evidenced that zooplankton is an effective sink of phosphorus, while nitrogen is  
46 accumulated mainly by primary producers. Our results also indicated more stability in  
47 stoichiometry with increasing trophic levels of organisms. However, there were significant  
48 changes in the zooplankton structure. The increasing dominance of large *Daphnia* resulted in  
49 reduction of C:P ratio in zooplankton. Low food quality (C:P) did not limit the growth of large  
50 *Daphnia* in the experimental conditions, which competed effectively with small planktonic  
51 cladocerans and with Rotifera. Over time, inedible algae began to dominate resulting in increase  
52 of relative biomass of periphyton grazers, which suggests that plankton community is  
53 transformed into littoral system in mesocosms for about 30 days.

54 **Keywords:** lake nutrient stoichiometry; elemental and biochemical composition; zooplankton;  
55 phytoplankton; food quality; trophic status.

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## 1. Introduction

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Plankton communities are the key component in lake ecosystems and interactions between primary producers (phytoplankton or photoautotrophs) and primary consumers (zooplankton or heterotrophs) affect the overall functioning of freshwater ecosystems. However, the transfer of energy and matter between these two important trophic levels is complicated by the profound differences in the elemental and biochemical composition between primary producers and consumers (Feniova et al., 2018). Stoichiometric theory predicts how elemental ratios affect consumer growth and nutrient cycling (Sterner and Hessen, 1994; Elser and Urabe, 1999). Phosphorus (P) and nitrogen (N) are key limiting nutrients whereas carbon (C) is rarely in shortage. Since wide variation in the C:N:P ratio of autotrophs contrasts with the narrow range found in herbivores (DeMott and Tessier, 2002), stoichiometric models are especially relevant at the lower levels of food chains. Based on an expanding range of studies in ecological stoichiometry, it has become increasingly clear that there is often a mismatch in the elemental composition between food resources and their consumers (Reiners, 1986; Hessen, 1992; White, 1993), and that this mismatch can have a large impact on the performance of individual consumers and on the transfer efficiency of carbon, nitrogen and phosphorus (Elser et al., 2000; Sterner and Elser, 2002; Anderson et al., 2004). Food quality commonly does not meet consumer requirements because of nutrient deficiency (Plath and Boersma, 2001). As true filter-feeders, daphniids have a limited potential to select for higher quality food that makes them especially susceptible to deterioration of food quality (DeMott, 1986; Butler et al., 1989, Gladyshev et al., 2000; Feniova et al., 2015, 2018; Taipale et al., 2016).

*Daphnia* and phosphorus interactions have attracted much attention, in part because of their important role in lakes and in part because daphniids have higher requirements for P compared with other zooplankton (DeMott and Van Donk, 2013). Threshold elemental ratio models predict the levels of resource C:P and/or C:N at which the grazer's growth ceases as a result of nutrient limitation affecting the grazer's nutrient content, ingestion rate, assimilation efficiencies, respiration costs, and nutrient excretion. C:P thresholds were found for different *Daphnia* species (Sterner, 1997; Frost et al., 2004; Shimizu and Urabe, 2008; Khattak et al., 2018). In microcosm experiments dealing with interactions between grazers and primary producers, the stoichiometric mismatch can be estimated with good accuracy where well-defined single-species phytoplankton diet is offered (Hessen et al., 2013). However, in pelagic systems the food resources for zooplankton consist of a heterogeneous mixture of phytoplankton and dead/live particles collectively labeled "seston" which creates specific problems for researchers, as C:P in seston vary greatly (Hecky et al., 1993; Elser et al., 2000). C:P can change as much as 10 fold, as a consequence of various biotic and abiotic factors across pelagic ecosystems (Hessen et al., 2013). Nevertheless, phosphorus content in phytoplankton is commonly low so seston quality measured as C:P can be a regulating factor for pelagic grazers, nutrient cycle, and community composition.

Large and small cladoceran species can respond differently to the same environmental factors. Their strategies of survival are different (Hart and Bychek, 2011). According to the size-

97 efficiency hypothesis, large-bodied cladocerans are better competitors for food than small-bodied  
98 species (Brooks and Dodson, 1965; Gliwicz, 2003, Sikora and Dawidowicz, 2014). However,  
99 large species are more sensitive to changes in food quality (Sikora et al. 2016). Thus, both food  
100 quantity and quality can be a driver of zooplankton community dynamics and their structure. In  
101 nature environmental factors act simultaneously and it is hard to estimate the role of individual  
102 factor. Therefore, mesocosms could be a valuable tool because they allow researchers to isolate  
103 treatment effects by controlling other abiotic and biotic variables (Dzialowski et al., 2014;  
104 Feniova et al., 2015). Additionally, they offer the potential to rigorously replicate experimental  
105 treatments (Drenner and Mazumder, 1999; Huston, 1999). Because of this, mesocosms can be  
106 used to study populations or communities (Odum, 1984). Compared to the whole ecosystem  
107 studies, mesocosms are also relatively easy and inexpensive to set up and operate. Therefore, we  
108 applied the mesocosms approach which offers more opportunities, flexibility, and experimental  
109 control than, field studies (Dzialowski et al., 2014).

110 Both algal species composition and total phytoplankton biomass alter with changing  
111 nutrient concentrations, thus affecting food webs at all levels. Consumers further regulate and  
112 may even accelerate discrepancies in nutrient stoichiometry by various feedbacks, release, and  
113 recycling pathways (Gilbert, 2012). That is why herbivores are very important in nutrient cycling  
114 in freshwater ecosystems. Via excretory processes, animals can supply nutrients (nitrogen and  
115 phosphorus) at rates comparable with major nutrient sources, and nutrient cycling by animals can  
116 support a substantial proportion of the nutrient demands of primary producers (Ejsmont-Karabin,  
117 1984; Vanni, 2002). On the other hand, zooplankton can also act as nutrient sinks relative to  
118 phytoplankton (Vanni, 2002). For example, Urabe et al. (1995) found that sequestration of P in  
119 *Daphnia* bodies can lead to low rates of P recycling and increased P limitation for  
120 phytoplankton. Finally, zooplankton regulates phytoplankton by grazing and can cause a shift to  
121 the digestion-resistant algae.

122 The importance of zooplankton in nutrient recycling within the water column tend to  
123 increase with lake size (Fee et al., 1994). Especially, in deep lakes, zooplankton contributes  
124 significantly to translocate nutrients from the epilimnion to the deeper layers (Karpowicz and  
125 Ejsmon-Karabin, 2017; Sługocki and Czerniawski, 2018). This dualism in the functional role of  
126 zooplankton, which acts simultaneously as a donor and a sink of nutrients, is very important for  
127 the nutrient cycle in lakes. However, there are still major gaps in our knowledge about elemental  
128 homeostatic regulation. In particular, while some organisms (e.g. *Daphnia*) are quite well studied  
129 in terms of stoichiometry, other representatives, even major taxa groups, have never been dealt  
130 with (Hessen et al., 2013).

131 Therefore, our goal was to determine how differences in food quality in terms of C:N:P  
132 ratios under different trophic conditions affect zooplankton abundance and its elemental  
133 composition. We conducted a mesocosm experiment with water from the mesotrophic and  
134 eutrophic lakes. To simulate changes in the plankton communities large-bodied *Daphnia magna*  
135 and *Daphnia pulex* were added. We predicted that (i) stoichiometric ratios would be more  
136 persistent in zooplankton than in seston and water, (ii) large herbivorous (*Daphnia*) could be a

137 very effective sink of nutrient, especially when food quality is low, (iii) large-bodied cladocerans  
138 would competitively suppress small-bodied zooplankton if competing for food.  
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## 140 2. Materials and methods

141 We conducted a 31-day experiment in a 6 mesocosms (0.94 x 0.64 x 0.50 m; 300 L) from  
142 21 June to 20 July 2017. The mesocosms were located on the shore of Lake Mikołajskie  
143 (Mazurian Lake District, northeastern Poland, 21°35'E, 53°48'N) at the Research Station of the  
144 Nencki Institute of Experimental Biology, Polish Academy of Sciences. The mesocosms were  
145 filled with water containing natural plankton communities which were collected and transported  
146 from the mesotrophic Lake Majcz (Feniova et al., 2015) and the eutrophic Lake Mikołajskie  
147 (Feniova et al., 2018). We had two treatments which included mesotrophic and eutrophic  
148 conditions, each in triplicate mesocosms. The initial zooplankton communities did not contain  
149 typical large Cladocera, so we added 25 individuals of *Daphnia magna* Straus (originated from  
150 Binnensee, Germany) and 25 individuals of *Daphnia pulicaria* Forbes (originated from Lake  
151 Brome, Canada) in each mesocosm, to evaluate the effect of competition between large and  
152 small cladoceran. We covered the mesocosms with polyamide net with 100 µm mesh size to  
153 avoid invertebrate predation (e.g. *Chaoborus* larvae), and when it rained, we covered the  
154 mesocosms with polyethylene film to avoid contamination with organic and other substances that  
155 may have been incorporated in the rainwater.

156 We collected samples with a 2.6-liter Limnos sampler from the center of each mesocosm  
157 after they were gently mixed, every 10 days for the analysis of water chemistry, zooplankton and  
158 algae identification and enumeration (Days 1, 11, 21 and 31). We also collected zooplankton on  
159 these days by filtering five-liters of water through a 50-µm plankton net and fixed with 4%  
160 formaldehyde. In addition, 6 L of water from each mesocosm were collected to count clutch  
161 sizes of dominant cladoceran species (*Ceriodaphnia pulchella*, *Daphnia magna* and *Daphnia*  
162 *pulicaria*) alive. After recording clutch sizes, all the crustaceans and water were returned back to  
163 corresponding mesocosm. Rotifers and crustaceans were identified to species and counted  
164 totally. Additionally, the size of at least 10 individuals of each species were measured in every  
165 sample. The average animal length was used to estimate the dry weight of crustaceans by  
166 applying the equations after Błędzki and Rybak (2016). The biomass of rotifers was established  
167 using length-weight relations from Ejsmont-Karabin (1998).

168 To estimate effects of food conditions on population growth of dominant cladoceran  
169 species, we calculated population growth rates based on fecundity data and duration of juvenile  
170 development using equation:

$$171 \quad r = \ln \{ \sum l(x)m(x) \} / T,$$

172 where  $l(x)$  and  $m(x)$  are survivorship and fecundity, respectively,  $T$  – average time of generation.  
173 Survivorship and juvenile development were established in cohort experiment which was  
174 conducted over the course of the mesocosm experiment in 500 mL bottles. In flow-through  
175 system with water exchange twice a day from mesotrophic and eutrophic conditions, cohorts of  
176 7-10 individuals of *Ceriodaphnia pulchella*, *Daphnia magna* and *Daphnia pulicaria* in each

177 bottle were kept. Every other day, we recorded their state, first clutch day and survivorship.  
178 Survivorship was 100% both in mesotrophic and eutrophic conditions.

179 Phytoplankton abundance was measured in situ with a submersible spectrofluorometer  
180 (FluoroProbe, bbe-Moldaenke). The Fluoroprobe estimates chlorophyll *a* concentrations of four  
181 phytoplankton groups based on their fluorescence excitation spectra: (GI) Chlorophyta and  
182 Euglenophyta (chlorophyll *a* and *b*); (GII) Heterokontophyta, Haptophyta, and Dinophyta  
183 (chlorophyll *a* and *c*, fucoxanthin–peridinin); (GIII) Cryptophyta (chlorophyll *a* and *c*,  
184 phycobilins); (GIV) Cyanophyta (chl *a*, phycobilins). The default norm spectra for each  
185 Fluoroprobe unit provided by the manufacturer are determined by measuring fluorescence  
186 excitation spectra of laboratory cultures of phytoplankton species representative of the major  
187 pigment groups (Beutler et al., 2002). The bbe FluoroProbe with the additional transmission  
188 sensors provides automatic chromophoric dissolved organic matter (CDOM) and turbidity  
189 correction.

190 Temperature, conductivity (EC), and concentration of dissolved oxygen were measured  
191 using an HQ40D Multi Meter (Hach-Lange GmbH). The analyses of chemical parameters of  
192 water were performed in the laboratory. The concentrations of ions ( $\text{PO}_4^{3-}$ ,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ )  
193 were determined using a Dionex ICS 1100 ion chromatograph. The concentrations of total  
194 nitrogen (TN), dissolved organic carbon (DOC) and total carbon (TC) were analyzed by the  
195 high-temperature catalytic combustion in Shimadzu TOC-L Series analyzers. The analyses of  
196 total phosphorus (TP) and  $\text{SiO}_4^{4-}$  ions were conducted according to the standard methods  
197 (APHA, 2012).

198 The samples for elemental analyses of seston (all the particles and live organisms that  
199 passed through a net of 100  $\mu\text{m}$  mesh size) and elemental composition of zooplankton were  
200 collected on the first, and on the final day of the experiment. For the seston samples, we  
201 collected 5–10 L of water from each mesocosm and filtered them through precombusted glass-  
202 fiber GF/F filters (Whatman, USA). Then zooplankton samples (4–10 mg of wet weight) were  
203 collected on a mesh sieve and placed on filter paper to remove the surface moisture. We kept  
204 filters for seston analyses at room temperature for a day while zooplankton samples were dried  
205 at 75°C overnight and stored dry in a desiccator until further analyses. Organic carbon (C) and  
206 nitrogen (N) were measured using a Flash EA 1112 NC Soil/MAS 200 elemental analyzer  
207 (ThermoQuest, Milan, Italy), as described in Gladyshev et al. (2007). Calibration curves for the  
208 elemental analyzer were generated using aspartic acid and standard soil reference material.  
209 Contents of particulate total phosphorus (P) were estimated following the conventional  
210 photolorimetric method (Murphy and Riley, 1962).

211 We used one-way analysis of variances (ANOVA) with type III sum of squares (SS)  
212 analysis to determine the difference in water chemistry between the trophic conditions and start-  
213 end of the experiment. One way ANOVA with type III SS was also used to compare differences  
214 in stoichiometry ratios (C:P, C:N, N:P) in water, seston and zooplankton. The significant  
215 differences between stoichiometry ratios were visualized with box plots (mean, median, first and  
216 third quartiles, standard deviation, minimum and maximum). Finally, principal component

217 analysis (PCA) was used to analyze the relations between the stoichiometry ratios in water,  
218 seston and zooplankton. Redundancy analysis (RDA) was performed to find combinations of  
219 abiotic (nutrients) and biotic (phytoplankton) factors affecting the community structure of  
220 zooplankton under experimental conditions. Statistical analyses were performed with XLSTAT-  
221 Ecology (Addinsoft).

### 222 3. Results

223 There were differences in water chemistry between the mesotrophic and eutrophic  
224 treatments. The eutrophic conditions were characterized by higher values of EC (F=5.01;  
225 p=0.05), DOC (F=8.6; p=0.017), and lower concentrations of  $\text{SiO}_4^-$  (F=5.41; p=0.045) (Fig. 1).  
226 There were no significant changes between the start and the end of the experiment in total  
227 carbon, total nitrogen and total phosphorus in the water. However, there were large differences in  
228 nutrient forms available for phytoplankton between the dates, i.e.  $\text{PO}_4^{3-}$  (F=18.13; p=0.002),  
229  $\text{NO}_3^-$  (F=5.89; p=0.038),  $\text{NO}_2^-$  (F=5.5; p=0.044),  $\text{SiO}_4^-$  (F=9.41; p=0.013); DOC (F=38.27;  
230 p=0.0002). The concentration of orthophosphates increased significantly on day 11 and then  
231 abruptly declined (Fig. 1A).  $\text{NO}_3^-$  concentration decreased significantly during the experiment  
232 (Fig. 1B). The concentration of silicates decreased during the experiment, however, they were  
233 limiting only in eutrophic conditions since day 21 (Fig. 1D), while the concentration of DOC  
234 increased during the first 10 days of the experiment, afterwards it remained sustainable in  
235 eutrophic and mesotrophic treatments (Fig. 1C).

236 Phytoplankton density decreased since the 21<sup>st</sup> day of the experiment under eutrophic  
237 conditions, and since the 11<sup>th</sup> day under mesotrophic conditions. GII dominated in phytoplankton  
238 during the first 21 days (Fig. 2A,B), yet, they were rare on the 31<sup>st</sup> day under eutrophic  
239 conditions (Fig. 2A). The dominant taxa in GII group were *Stephanodiscus* sp., *Cyclotella* sp.,  
240 *Fragilaria* sp., *Rhizosolenia longiseta*, *Synedra acus*, *Chrysidalis peritaphrena*, and *Chromulina*  
241 sp. At the end of the experiments Cryptophyta (GIII) dominated under eutrophic (Fig. 2A) and  
242 mesotrophic conditions (Fig. 2 B). The dominant taxa in GIII group were *Ceratium*  
243 *hirundinella*, *Cryptomonas* sp., *Rhodomonas* sp. and *Peridinium* sp. GI (*Mougeotia* sp.,  
244 *Schroederia setigera* and *Closterium* sp.). The results of the redundancy analysis (RDA)  
245 indicated that phytoplankton groups GI and GII were strongly related to  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , and also  
246 could be linked to  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{4-}$  (Fig. 3), while GIII and GIV were less affected by the  
247 nutrient concentrations although their abundances may be related to  $\text{PO}_4^{3-}$  at its low level (Fig.  
248 3).

249 The zooplankton biomass increased during the experiment under eutrophic conditions due  
250 to massive development of Rotifera and *Eudiaptomus graciloides* (Fig. 2C). Rotifers were  
251 abundant at the beginning of the experiment and had almost disappeared by the 21<sup>st</sup> day in  
252 mesotrophic (Fig. 2D) and by the 31<sup>st</sup> day in eutrophic conditions (Fig. 2C). Under mesotrophic  
253 conditions zooplankton biomass rapidly increased until the 11<sup>th</sup> day (Fig. 2D) due to the large  
254 development of small cladocerans (represented mainly by *Ceriodaphnia pulchella*), and started  
255 to decline on the 31<sup>st</sup> day (Fig. 2D). Table 1 shows that fecundity of *C. pulchella* significantly  
256 decreased since the 21<sup>st</sup> day in mesotrophic conditions likely due to lower food quality and/or

257 quantity. Besides populations growth rate of *C. pulchella* was negative in mesotrophic conditions  
258 since the 21<sup>st</sup> day (Tab. 1), which could be a result of competition with large Cladocera.  
259 Therefore, biomass of small cladocerans had significantly decreased by 31<sup>st</sup> day in mesotrophic  
260 conditions (Fig. 2D). Large *Daphnia* (*D. magna* and *D. pulicaria*) successfully established under  
261 eutrophic and mesotrophic conditions and became dominant at the end in both treatments of the  
262 experiments (Fig. 2C, D). The population growth rate of *D. pulicaria* was sustainable during the  
263 whole course of the experiment (Tab. 1), however fecundity significantly decreased since 21<sup>st</sup>  
264 day (Tab. 1), which could indicate deterioration of food quality. Furthermore, at the end of the  
265 experiment, there was a shift in small cladoceran composition, i.e. *C. pulchella* was replaced by  
266 *Scapholeberis mucronata* and *Chydorus sphaericus* which were rare at the beginning of the  
267 experiment.

268 The redundancy analysis (RDA) showed that crustaceans (Cladocera, Calanoida and  
269 Cyclopoida) were likely related to the biomass of phytoplankton from groups GI and GII, as well  
270 as to concentrations of NH<sub>4</sub><sup>+</sup> and DOC in the water (Fig. 3). On the other hand, calanoids and  
271 large cladocerans were connected to the share of GIII and GIV in phytoplankton communities  
272 (Fig. 3). The results of RDA analysis also indicated significant differences in environmental  
273 variables affecting large and small-bodied Cladocera. The small cladocerans were highly related  
274 to axis F1, contrary to the large cladocerans, which were strongly connected to axis F2 (Fig. 3).

275 Comparison of stoichiometry ratios indicated that zooplankton had the highest stability of  
276 elemental composition (Fig. 4). The largest differences were observed in water (C:P and C:N  
277 ratio) and in the seston (C:P and N:P ratio). The seston was characterized by high C:P and N:P  
278 ratios, and low C:N ratio (Fig. 4), which indicates that seston contained little phosphorus and  
279 much nitrogen. Low C:P (2-3 times lower than in water and seston) and C:N ratios (Fig. 4) in  
280 zooplankton suggest the high content of phosphorus and nitrogen. The results of PCA analysis  
281 also confirmed that stoichiometric ratios of zooplankton are highly correlated, in contrast to  
282 seston and water (Fig. 5). The significant differences between seston and zooplankton  
283 stoichiometry was shown in Fig. 6. The zooplankton stoichiometry changed in dependence of  
284 zooplankton size structure, while N:P ratios of zooplankton differ significantly between the  
285 beginning and end of the experiment ( $F = 5.34, p = 0.05$ ) and between trophic conditions ( $F =$   
286  $7.25, p = 0.027$ ). N:P of zooplankton was lower at the end of the experiment relative to the start  
287 of the experiments (Fig. 6A) that could be explained by the domination of large *Daphnia* in the  
288 zooplankton (Fig. 2 C,D). C:P and N:P ratios of zooplankton were higher in mesotrophic than in  
289 eutrophic conditions (Fig. 6B,C) which could be associated with a higher share of small  
290 cladoceran (*Ceriodaphnia pulchella*). The trophic conditions did not significantly affect the  
291 seston stoichiometry except C:N which differed between the start and the end of the experiment  
292 ( $F=26.02; p=0.001$ ). Nitrogen content in seston at the end of the experiment decreased (Fig. 6D).  
293 The diatoms which dominated at the beginning were replaced by cryptophytes (Fig. 2A,B).  
294 Water stoichiometry did not differ between the treatments.  
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#### 296 4. Discussion

297 The results of our mesocosm experiments provided strong evidences that stoichiometric  
298 ratios are more sustainable at higher trophic levels. We have found much wider range of  
299 variation in C:P and N:P ratios in water and autotrophs which contrasted with the narrow range  
300 of variation in zooplankton. The elemental composition of zooplankton in our experiments was  
301 similar to the theoretical Redfield ratio (Redfield, 1934). The contents of carbon, nitrogen and  
302 phosphorus in zooplankton were  $45.5 \pm 3.6 \%$ ,  $9.7 \pm 1.3\%$  and  $1.0 \pm 0.3 \%$ , respectively. These  
303 are typical values, which are found in zooplankton in temperate lakes (Hessen, 2008; Hessen et  
304 al., 2013) as well as in brackish waters of the Baltic Sea (Walve and Larsson, 1999). The  
305 relatively persistent elemental composition of zooplankton means that they can keep elemental  
306 content at a particular level (Hessen, 1990; Andersen and Hessen, 1991). To maintain  
307 stoichiometric balance of body with that of food, zooplankton, besides selective feeding, have to  
308 adjust their growth efficiency so that the elements in short supply have to be accumulated while  
309 elements in surplus are excreted (Sterner, 1990). In this way, zooplankton may overcome the  
310 nutrient shortage of phytoplankton (Hessen and Andersen, 1992; Daufresne and Loreau, 2001).

311 We have found significant differences in the phosphorus content between phytoplankton  
312 and zooplankton. The C:P ratio of zooplankton was 2-3 times lower than in seston. According to  
313 the threshold elemental ratio model, the element in the least supply is assimilated with maximum  
314 efficiency. In the case of P, this is often set to 1 (100%), while for C it may be set to 0.6, linearly  
315 declining toward zero with increasing C:P (Hessen et al., 2013). *Daphnia* also excretes nutrients  
316 at a high N:P ratio, when there is low P content in algae (Sterner et al., 1992). Olsen et al. (1986)  
317 showed that the release rate of P by *Daphnia* became zero when C:P ratio in the food exceeds a  
318 critical value in the range 320-430. The results of our studies also suggest that most of the  
319 phosphorus is incorporated in *Daphnia* body, which leads to depletion of orthophosphate in  
320 water. This indicates that zooplankton is a very effective sink of phosphorus and can retain  
321 phosphorus in the body if its content is insufficient in seston. Hudson et al. (1999) suggested that  
322 small animals such as zooplankton are much more important than fish as nutrient sources. Our  
323 results are in full accordance with this statement.

324 High nitrogen content in seston and zooplankton in our mesocosms indicates that nitrogen  
325 was effectively accumulated by primary producers. Photosynthesis launch the transfer of matter  
326 and energy through the trophic web by converting inorganic nutrients into new organic  
327 compounds. Phytoplankton contains intracellular nitrogen in the form of nitrate, ammonium,  
328 amino acids, protein, RNA, and pigments (e.g. Conover, 1979; Rhee, 1978; Dortch, 1982). The  
329 accumulation of nutrients by phytoplankton and zooplankton likely caused the decline of  
330 accessible forms of nitrogen ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ) and orthophosphates in water since the 21<sup>st</sup> day of our  
331 experiments. Thus, nutrient shortage in combination with the growing zooplankton pressure  
332 could result in reduction of phytoplankton biomass. Under the eutrophic conditions, the silicon  
333 could be an additional factor limiting the growth of diatoms, a dominant component of  
334 phytoplankton. Silicon is a major limiting nutrient for diatoms growth and hence controls  
335 primary production (e.g. Martin-Jezequel et al., 2000; Gilpin et al., 2004). Finally, nutrient  
336 deficiency together with high pressure of herbivores at the end of the experiments in all the

337 mesocosms resulted in the domination of the digestion-resistant algae as *Ceratium hirundinella*  
338 and *Peridinium* sp. These species are large and commonly have horns which protect them from  
339 consumption by zooplankton (Margalef, 1983; Pollinger, 1988; Bustamante Gil et al., 2012).  
340 Both defense theory and aquatic trophic cascade theory suggest that resistant to consumption  
341 algae should be favored under heavy grazing pressure (Agrawal, 1998).

342 Ecological stoichiometry predicts that consumers selectively retain the limiting element  
343 while excreting excess elements in order to maintain their C:N:P stoichiometry (Sterner and  
344 Hessen, 1994; Sterner and Elser, 2002). For example, lake crustacean communities are typically  
345 dominated by copepods and cladocerans, which differ in their nutrient requirements. Copepods  
346 have a high N:P (ca. 30–50) ratio in their tissues, and therefore a relatively high N and low P  
347 demand for their development (Elser and Urabe, 1999). In contrast, cladocerans have a high P  
348 and low N demand, and thus have a lower N:P (ca. 14) (Sterner and Elser, 2002). Based on these  
349 broad taxonomic differences in stoichiometric requirements, it has been predicted that copepods  
350 would dominate in P-limited environments while cladocerans would be more abundant in N-  
351 limited environments. Nevertheless, the results of our experiment in P-limited environments did  
352 not cause the shift to copepods domination in eutrophic and mesotrophic conditions. To explain  
353 it, it is necessary to analyze threshold elemental C:P ratios which for larger *Daphnia magna*  
354 equaled 150-170 (Sterner, 1997; Frost et al., 2004; Shimizu and Urabe, 2008; Khattak et al.,  
355 2018), and for smaller *Daphnia galeata*, it was 385 (Urabe and Watanabe, 1992), while the  
356 growth rate of *Daphnia* was strongly reduced at seston C:P ratios >500 (Plath and Boersma,  
357 2001). The lowest C:P ratios in seston in our experiment were 260-330, i.e. much lower limiting  
358 values for the growth of large *D. magna* and *D. pulex*, which for this reason successfully  
359 developed and dominated in zooplankton biomass under the experimental conditions. In support,  
360 recent results from Mediterranean lakes with seston C:P ratio similar to that in our treatments  
361 (<350) indicated that such values of C:P did not influence the daphniid growth (Villar-Argaiz et  
362 al., 2018). However, the fecundity of large *D. pulex* and small *C. pulchella* significantly  
363 decreased in the middle of the experiment, which could be associated with decrease of food  
364 concentration.

365 The shift of size structure of zooplankton from small species to large species can be  
366 explained in view of stoichiometric data. The increase of large *Daphnia* abundance and decrease  
367 of smaller cladocerans resulted in the decline of C:P and N:P ratios. It is well-known that  
368 daphniids maintain a lower and more constant C:P ratio than other zooplankters (Hessen and  
369 Lyche, 1991) and have high requirements for P compared with other crustacean zooplankton  
370 (DeMott and Tessier, 2002; Khattak et al., 2018). *Daphnia magna* and *D. pulex* effectively  
371 competed with smaller cladoceran (*Ceriodaphnia pulchella*) and with Rotifera. As a result,  
372 rotifers were almost eliminated by the end of the experiment. The large *Daphnia* suppressed both  
373 small and large rotifers likely due to the combined effects of interference (small rotifers) and  
374 exploitative competition (large ones) (Gilbert, 1988; Karpowicz and Ejsmont-Karabin, 2018). It  
375 is often assumed that large-bodied cladocerans can outcompete small-bodied cladocerans  
376 because they are more efficient filter feeders and can filter a broader range of particle sizes (e.g.

377 size-efficiency hypothesis, Brooks and Dodson, 1965). Similar mesocosm experiments revealed  
378 that large *Daphnia* as a result of the exploitative competition with smaller cladocerans,  
379 significantly reduced the fecundity of *Ceriodaphnia pulchella* (Feniova et al., 2015). This fact  
380 confirmed that there may be a strong competition between large *Daphnia* and *Ceriodaphnia*  
381 *pulchella*, which share the same resources.

382 Nevertheless, in all the mesocosms, *Scapholeberis mucronata* and *Chydorus sphaericus*  
383 reached high densities, despite their very low abundance at the beginning of the experiment. The  
384 increase in the population of those species could be due to the fact that they use other resources  
385 than *Daphnia*. Euneustonic *Scapholeberis mucronata* can attach to the surface tension film with  
386 the ventral parts of their carapace and move along it and graze (Gladyshev, 2002; Zieliński et al.  
387 2016). *S. mucronata* is also ideally adapted for grazing on periphyton (Karpowicz et al., 2016),  
388 which covered the mesocosm walls. Similarly, small-bodied *Ch. sphaericus* which belong to the  
389 Chydoridae adapted to creeping along submerged surfaces, either macrophytes or bottom  
390 substrates, but they are poor swimmers (Fryer, 1968), although some species occasionally leave  
391 their substrate (Whiteside, 1974). As most Chydoridae remain in close contact with a substrate,  
392 they belong to microbenthos rather than to zooplankton. *Ch. sphaericus* is an exception, as it has  
393 two alternative ways of life. It can be found in the littoral zones of lakes among macrophyte  
394 vegetation and on bottom substrates that are rich in organic material (Goulden, 1971; Keen,  
395 1973; Whiteside, 1974; Williams, 1982), as well as in the water column in the open water zone  
396 of eutrophic lakes and ponds (Vijverberg et al., 1990; Ewald, 1991), especially during extensive  
397 Cyanobacteria blooms (Gannon, 1972; Vijverberg and Boersma, 1997; Górnjak and Karpowicz,  
398 2014). Therefore, at the end of the experiment there was a shift in small cladoceran group from  
399 pelagic *Ceriodaphnia pulchella* to periphyton grazers (*Scapholeberis mucronata* and  
400 Chydoridae). The shift happened as a result of “wall” effect of mesocosm (high surface area to  
401 volume ratios) (Carpenter, 1996, 1999; Schindler, 1998; Petersen et al., 1999). For this reason,  
402 we suggest that mesocosms experiments should not exceed 30 days to properly simulate relations  
403 in plankton communities.

404

## 405 5. Conclusions

406 The mismatch in C:P ratios between seston (high) and zooplankton (low) suggest that  
407 phosphorus content in zooplankton biomass is higher than in food resources. This indicates that  
408 zooplankton serve as a sink of phosphorus while primary producers accumulate more nitrogen.  
409 Our results supported the idea that stoichiometric ratios at higher trophic levels exert less  
410 variability than lower levels of trophic chain. However, stoichiometric ratios appeared to depend  
411 on size structure of zooplankton. The increasing dominance of large *Daphnia* resulted in  
412 decrease of C:P ratio of zooplankton. The low food quality in our experimental conditions did  
413 not limit the growth of large *Daphnia*, which effectively competed with small planktonic  
414 cladocerans and rotifers. Inedible periphyton algae was gradually replacing plankton algae that in  
415 turn facilitate the growth of periphyton grazers. Therefore, mesocosms experiments should be  
416 short term, about 30 days, to properly simulate relations in plankton communities.

417

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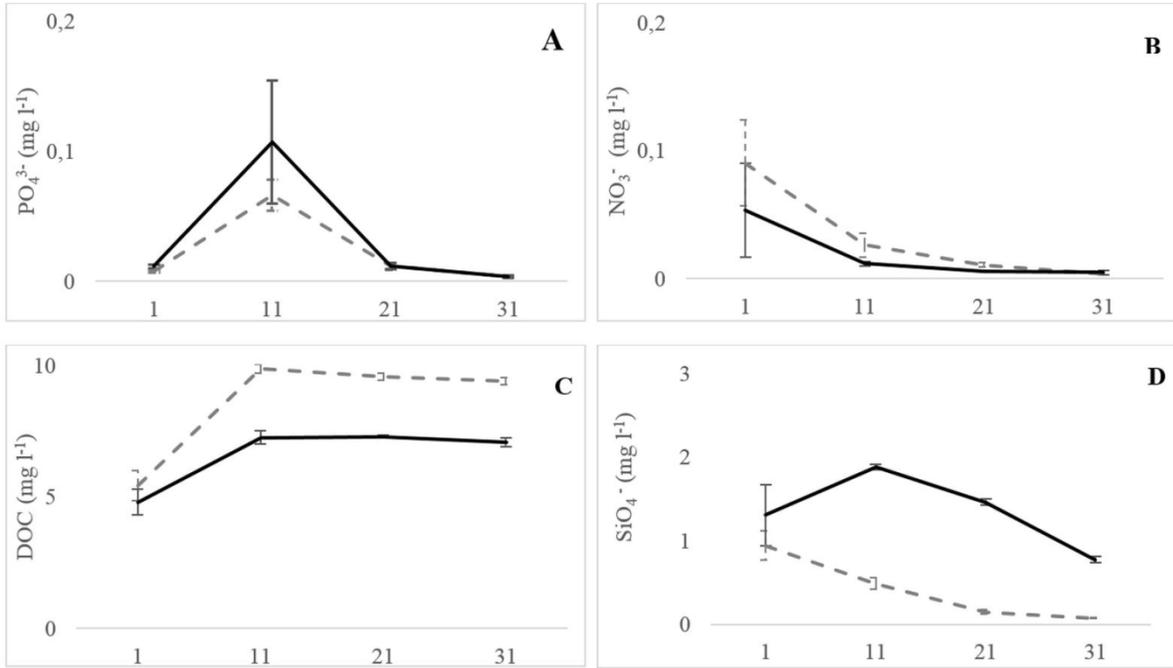
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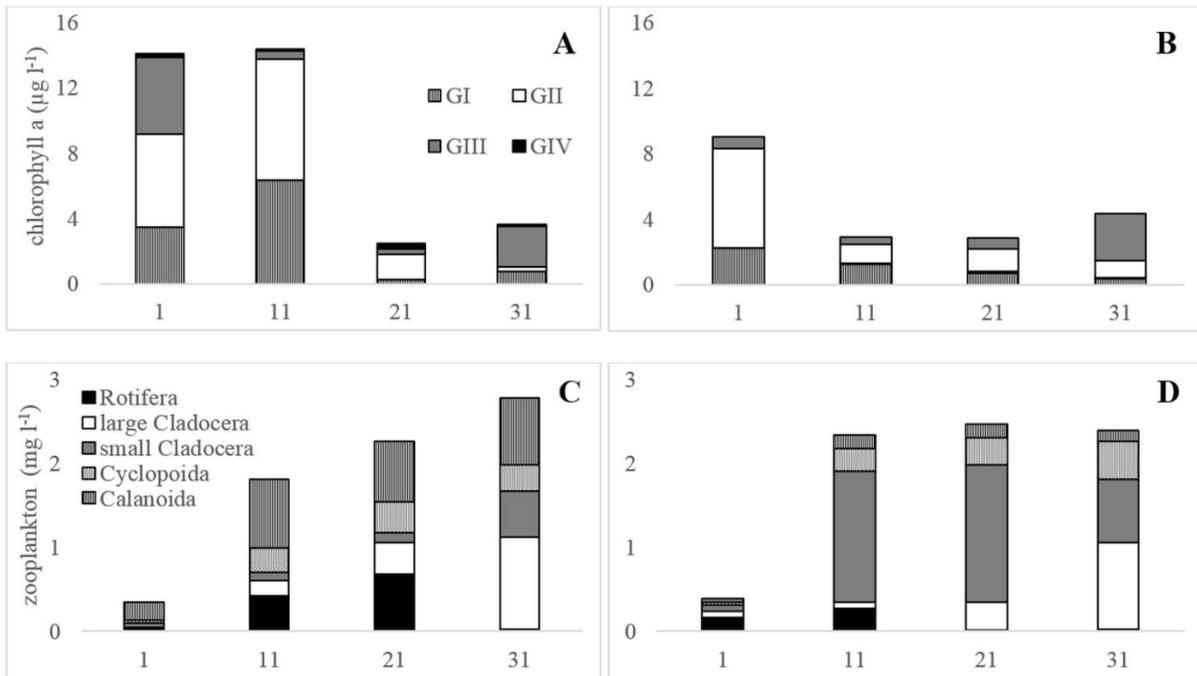
649 Tab. 1. Demographic parameters of *Ceriodaphnia pulchella*, *Daphnia magna*, and *Daphnia*  
 650 *pulicaria* (average  $\pm$  stadard deviation). Abbreviations: E - eutrophic; M – mesotrophic; nd – no  
 651 data available.  
 652

	Day 1		Day 11		Day 21		Day 31	
	E	M	E	M	E	M	E	M
<b>Fecundity</b>								
<i>C. pulchella</i>	nd	3.5 $\pm$ 1.2	2.4 $\pm$ 2.2	3.4 $\pm$ 1.2	2.3 $\pm$ 0.3	0.1 $\pm$ 0.3	2.4 $\pm$ 0.5	0
<i>D. magna</i>	nd	nd	nd	nd	1.7 $\pm$ 1.1	6.4 $\pm$ 2.6	4 $\pm$ 0.8	4.7 $\pm$ 0.6
<i>D. pulicaria</i>	nd	5.7 $\pm$ 0.6	14.7 $\pm$ 5.5	12 $\pm$ 3	2.7 $\pm$ 1.5	2	0.7 $\pm$ 0.6	1.7 $\pm$ 0.5
<b>Population growth rate</b>								
<i>C. pulchella</i>	nd	0.34 $\pm$ 0.05	0.01 $\pm$ 0.9	0.31 $\pm$ 0.29	0.5 $\pm$ 0.03	-2.2 $\pm$ 0.8	0.3 $\pm$ 0.03	-2.44
<i>D. magna</i>	nd	nd	nd	nd	-0.26 $\pm$ 1	0.34 $\pm$ 0.04	0.3 $\pm$ 0.03	0.31 $\pm$ 0.01
<i>D. pulicaria</i>	nd	0.33 $\pm$ 0.01	0.44 $\pm$ 0.05	0.42 $\pm$ 0.03	0.24 $\pm$ 0.07	0.22	nd	0.2 $\pm$ 0.04

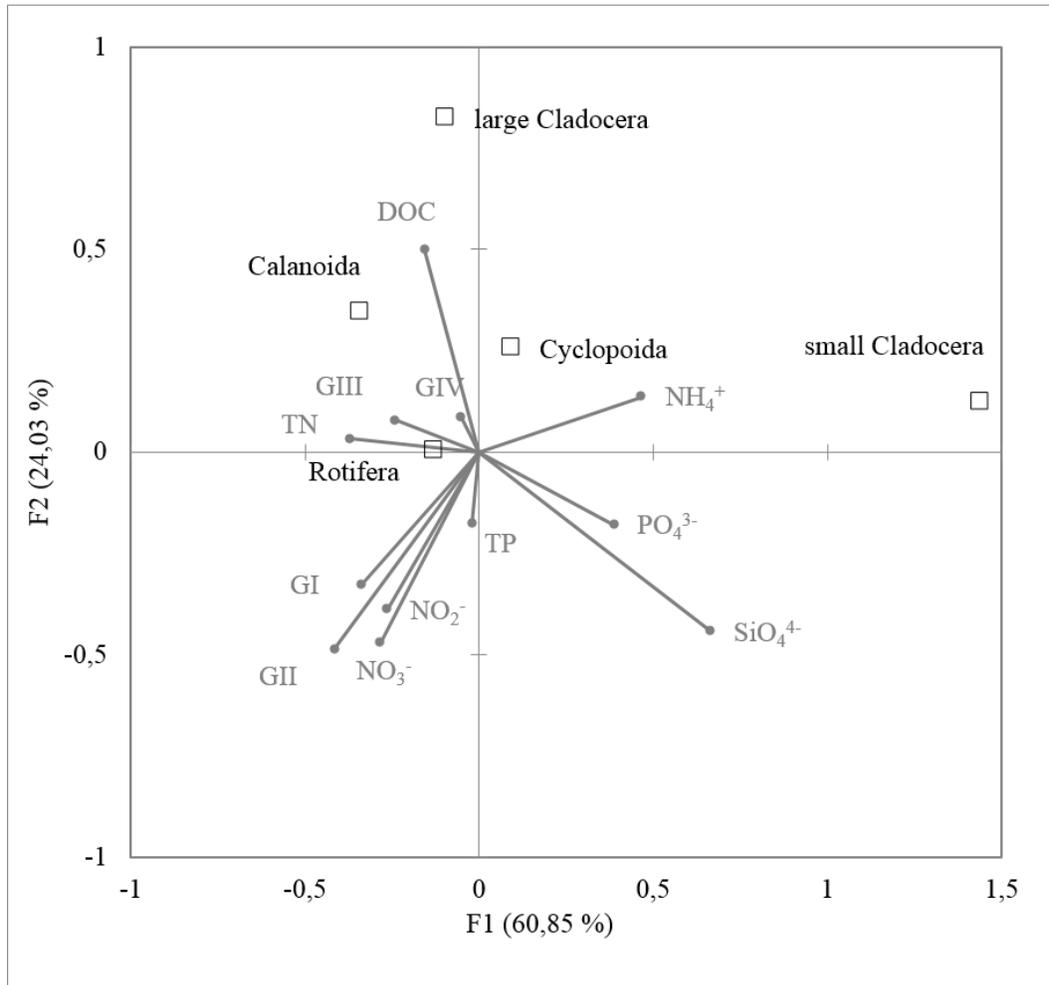
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 656 Fig. 1. Comparison of mean ( $\pm$ SD) nutrient concentration ( $PO_4^{3-}$ ,  $NO_3^-$ , DOC) and  $SiO_4^{2-}$  on every  
 657 date. Dashed grey lines are eutrophic conditions, and solid black lines are mesotrophic  
 658 conditions.  
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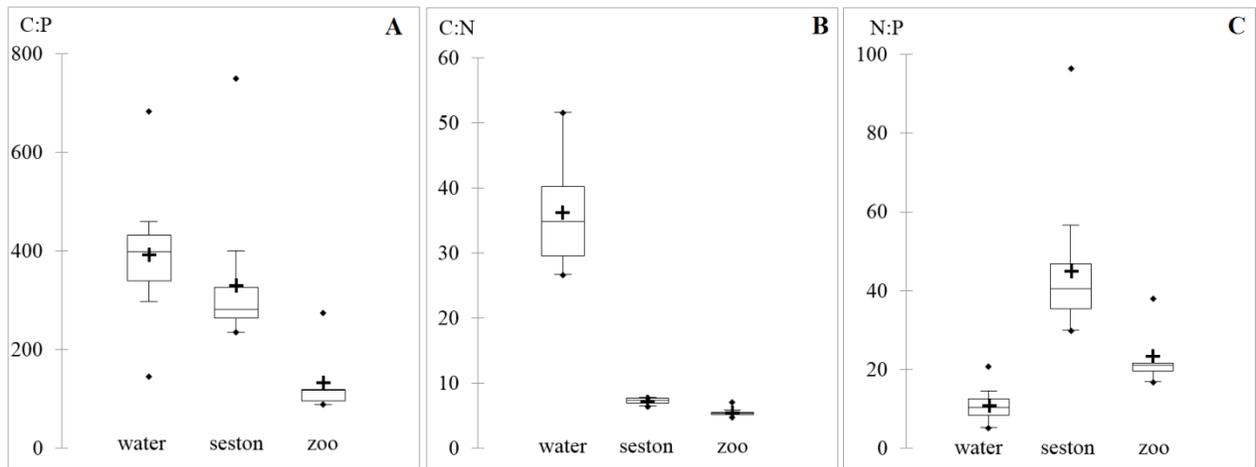


660  
 661 Fig. 2. Comparison of phytoplankton communities (A, B) and zooplankton wet weight biomass  
 662 (C, D) on every date under eutrophic (A, C) and mesotrophic (B, D) condition. Abbreviations  
 663 phytoplankton: GI - Chlorophyta and Euglenophyta; GII – Heterokontophyta, Haptophyta and  
 664 Dinophyta; GIII – Cryptophyta; GIV – Cyanophyta.

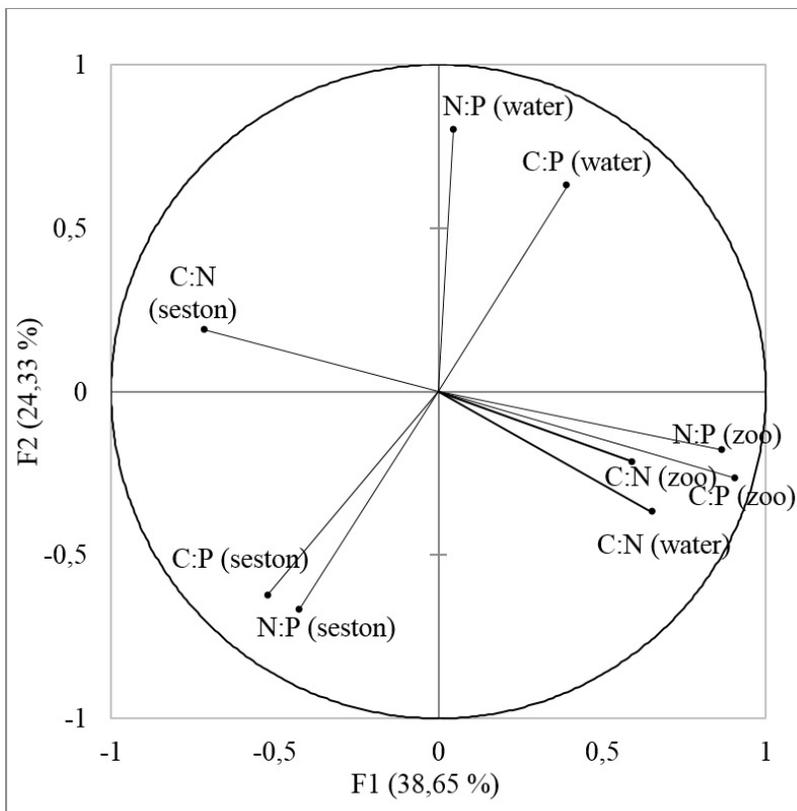


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 666 Fig. 3. The redundancy analysis (RDA) map of major group of freshwater zooplankton and  
 667 environmental variables (nutrients and phytoplankton). Labels of phytoplankton taxa: GI -  
 668 Chlorophyta and Euglenophyta; GII – Heterokontophyta, Haptophyta and Dinophyta; GIII –  
 669 Cryptophyta; GIV – Cyanophyta.

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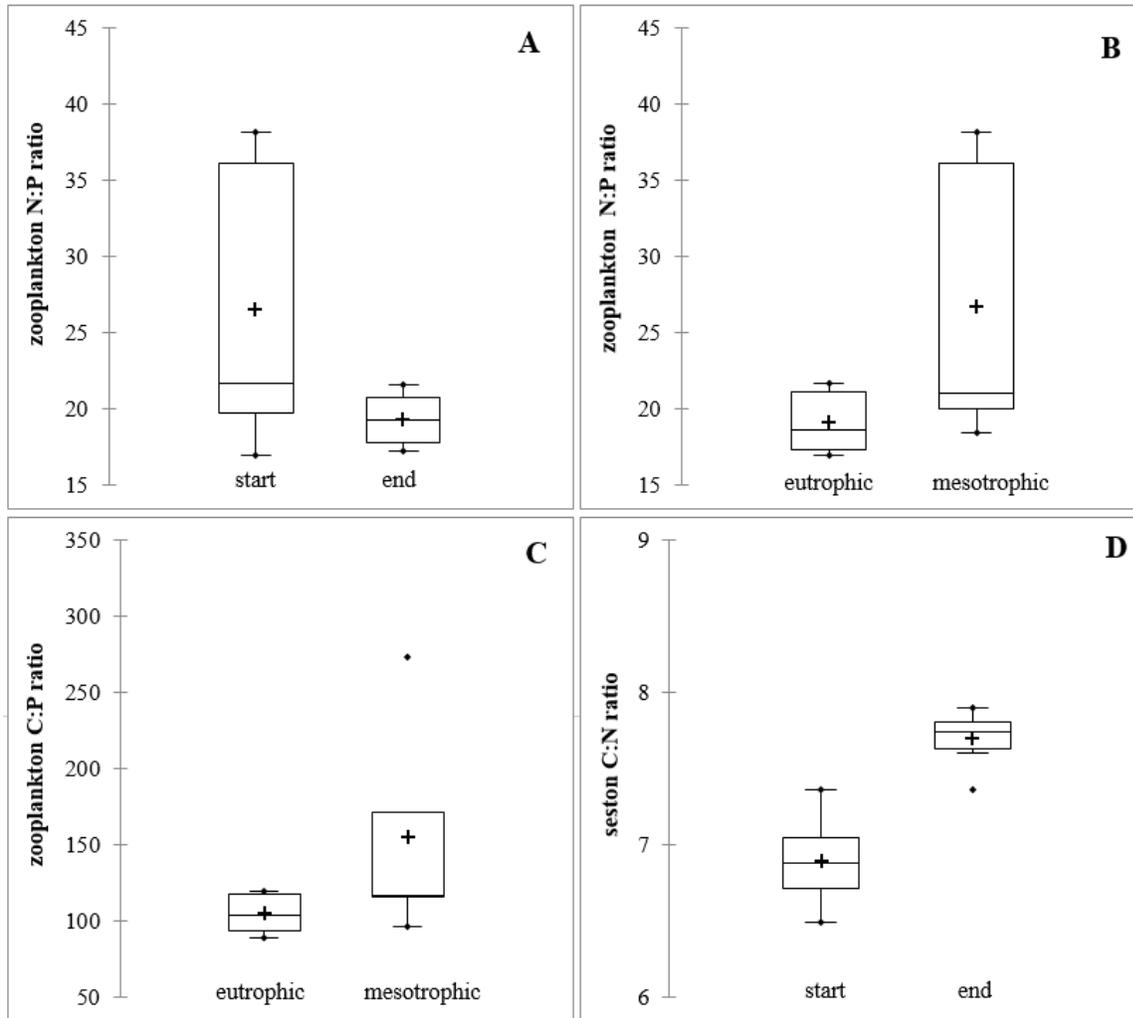


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 675 Fig. 4. Stoichiometric ratios: C:P (A), C:N (B) and N:P (C) in the water, seston and zooplankton.  
 676 The means are denoted by crosses. The central horizontal bars are the medians. The lower and  
 677 upper limits of the box are the first and third quartiles, respectively. Points above and below the  
 678 means are minimum and maximum, respectively.  
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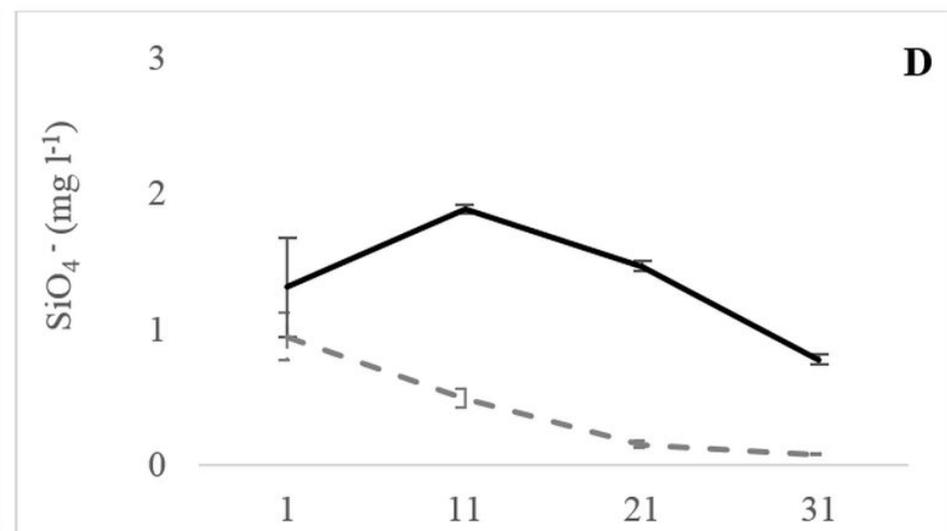
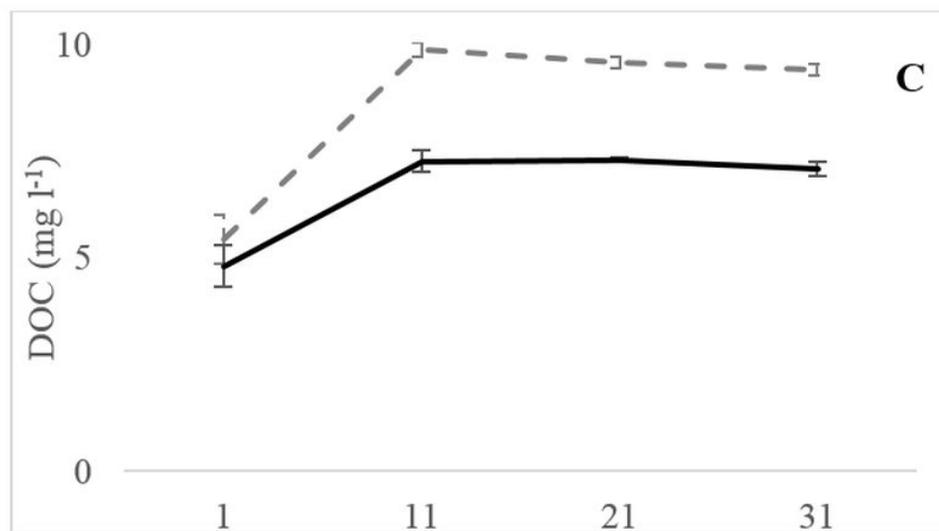
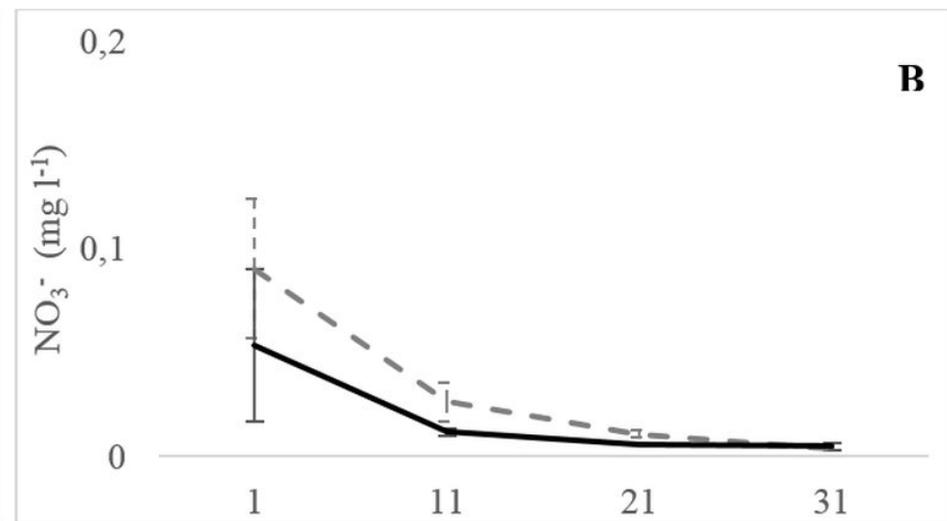
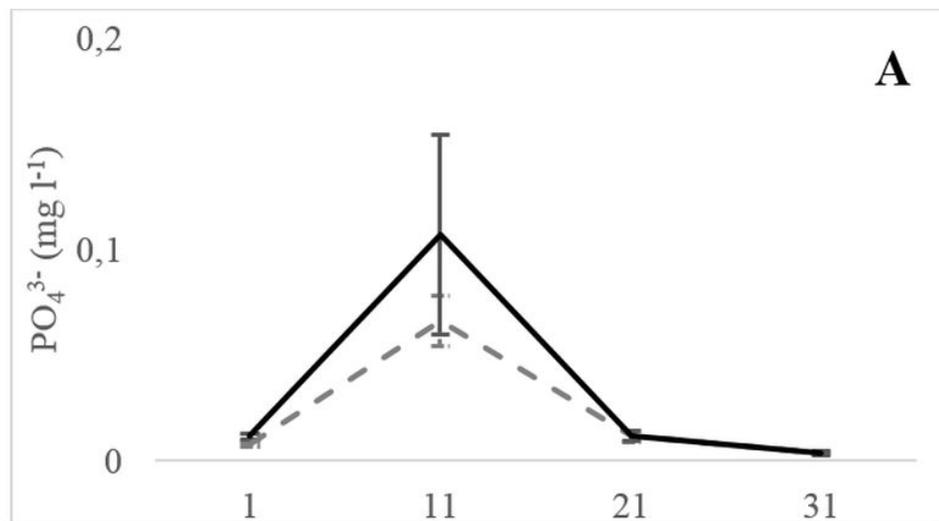
680  
 681 Fig. 5. Comparison of stoichiometry ratios between water, seston and zooplankton visualised by  
 682 the correlation circle of the PCA analysis.  
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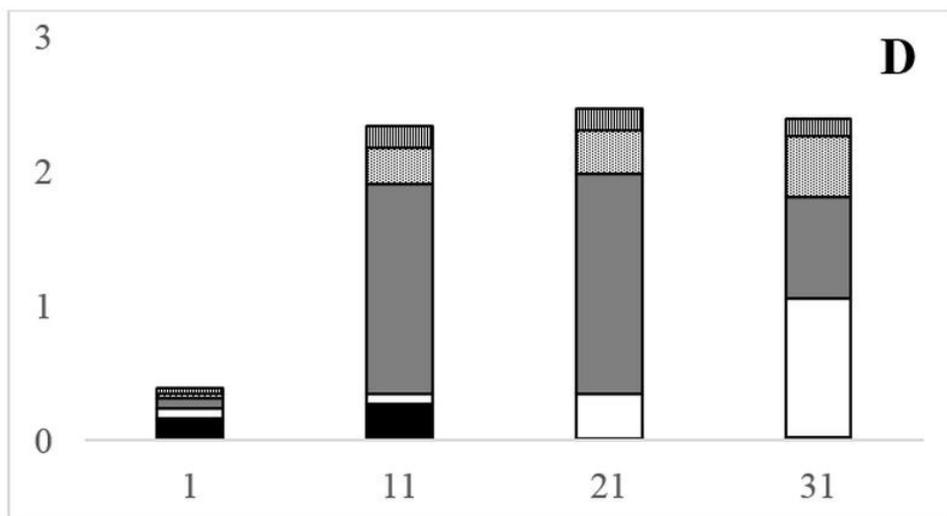
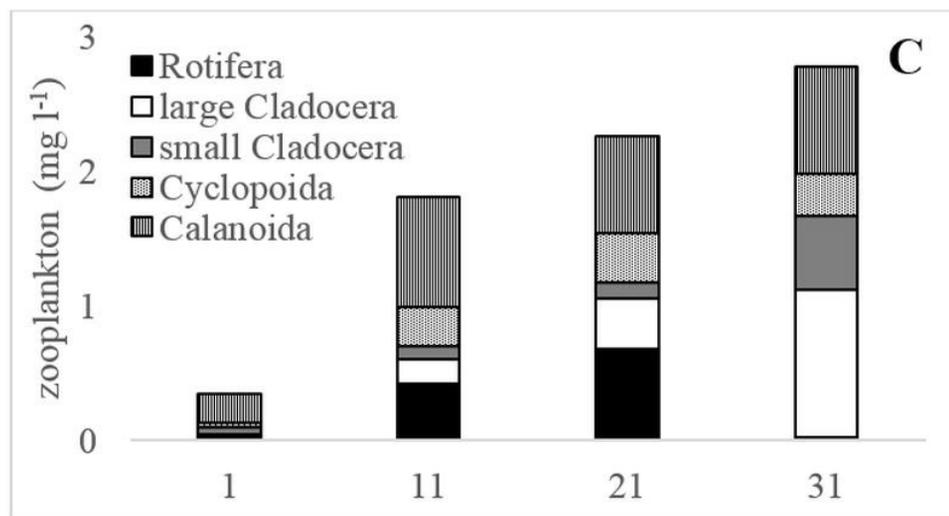
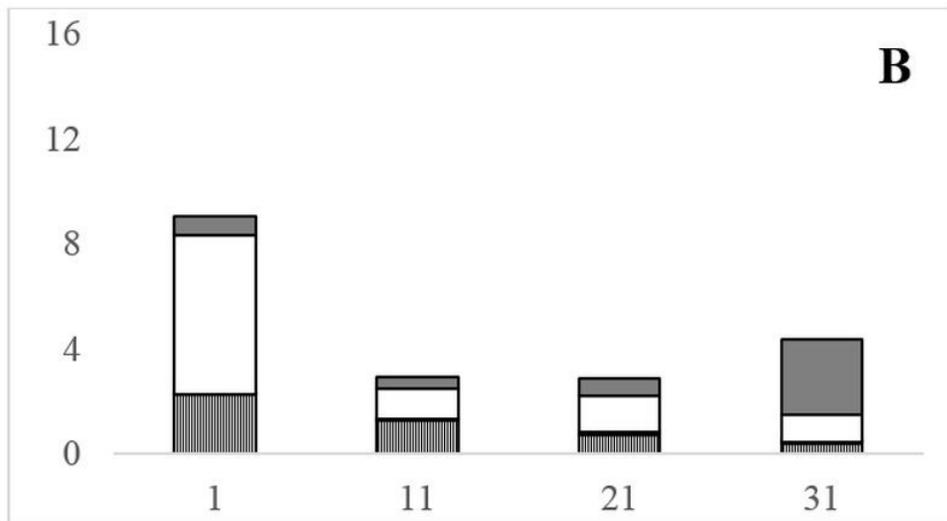
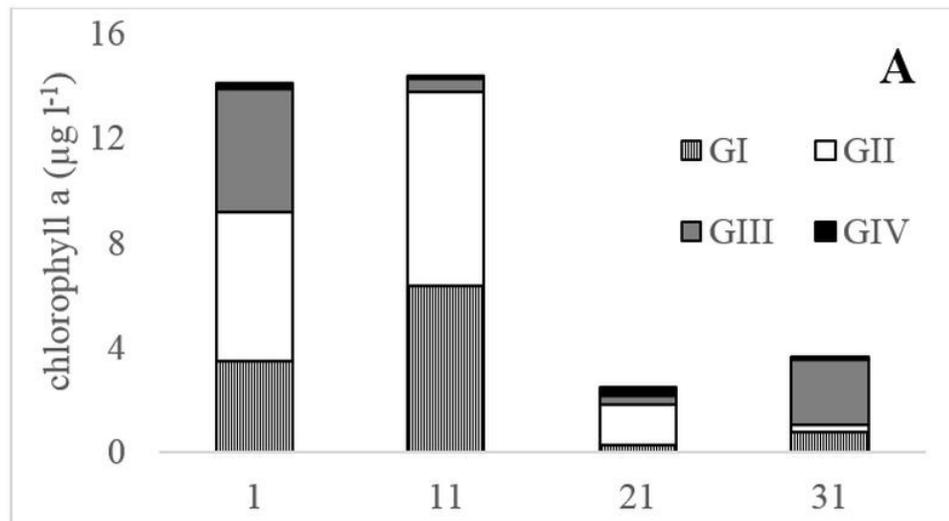
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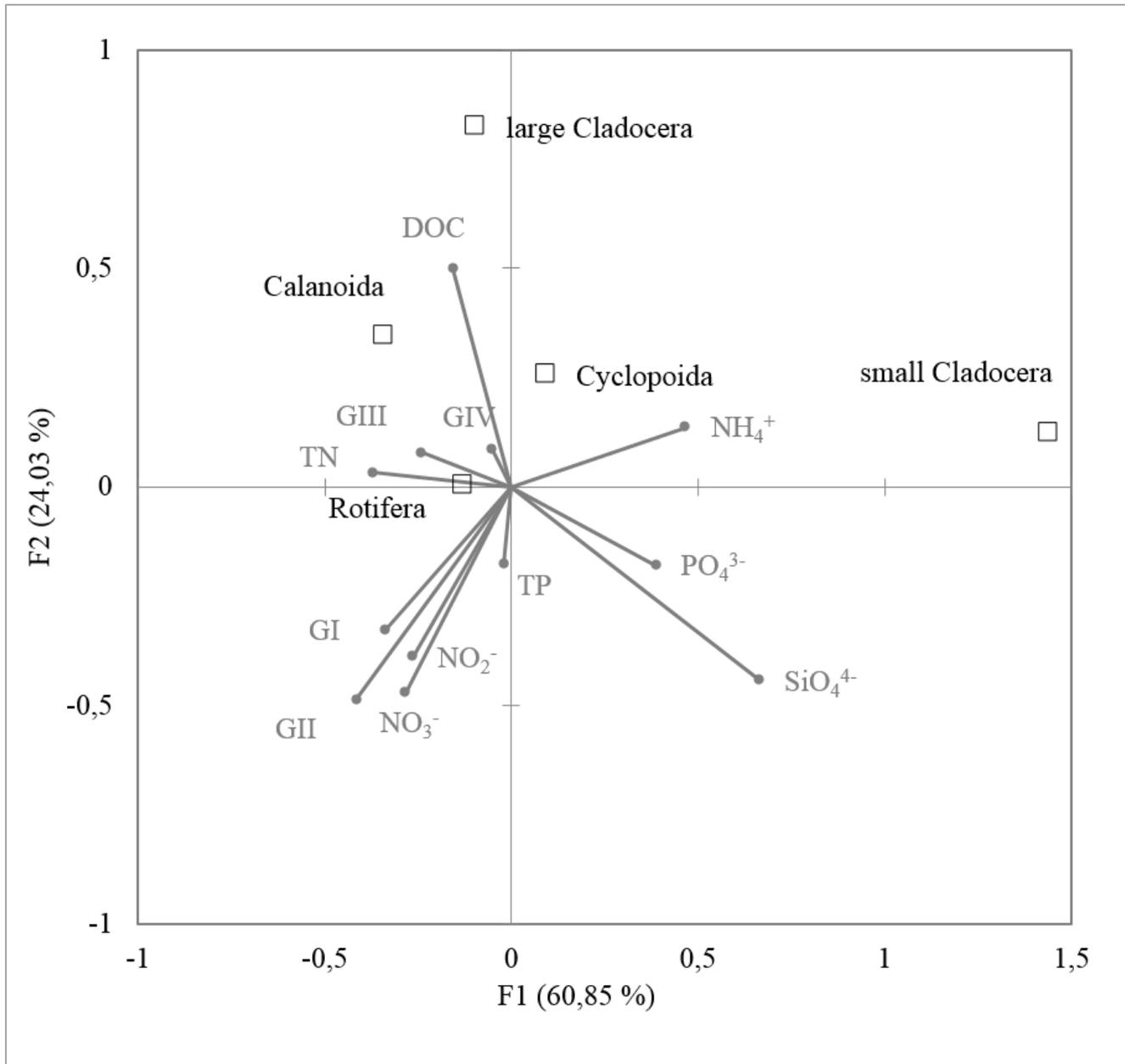


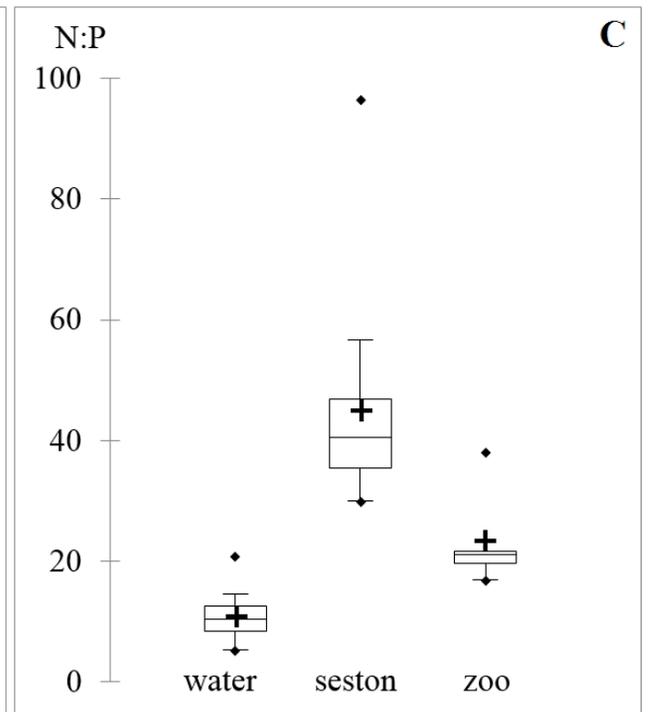
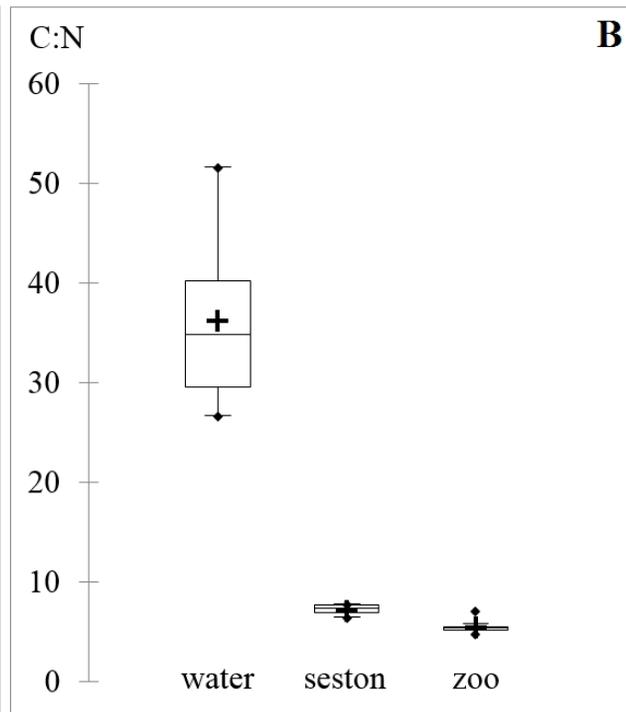
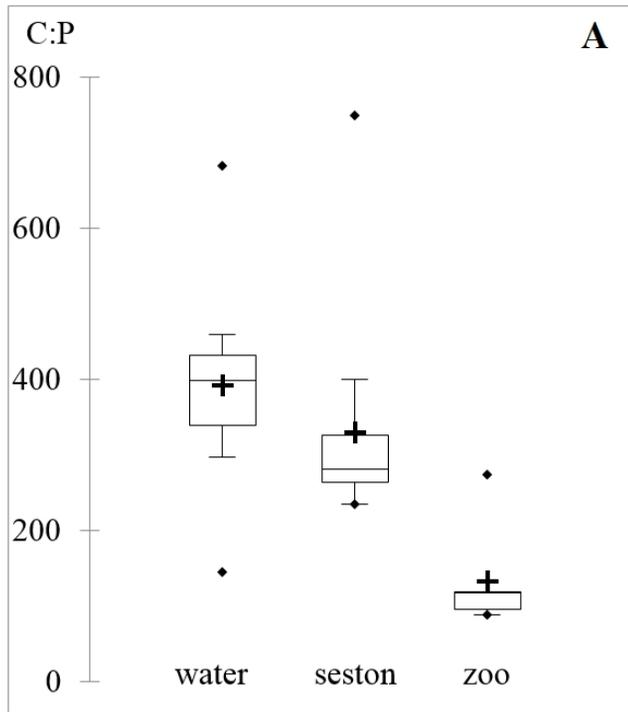
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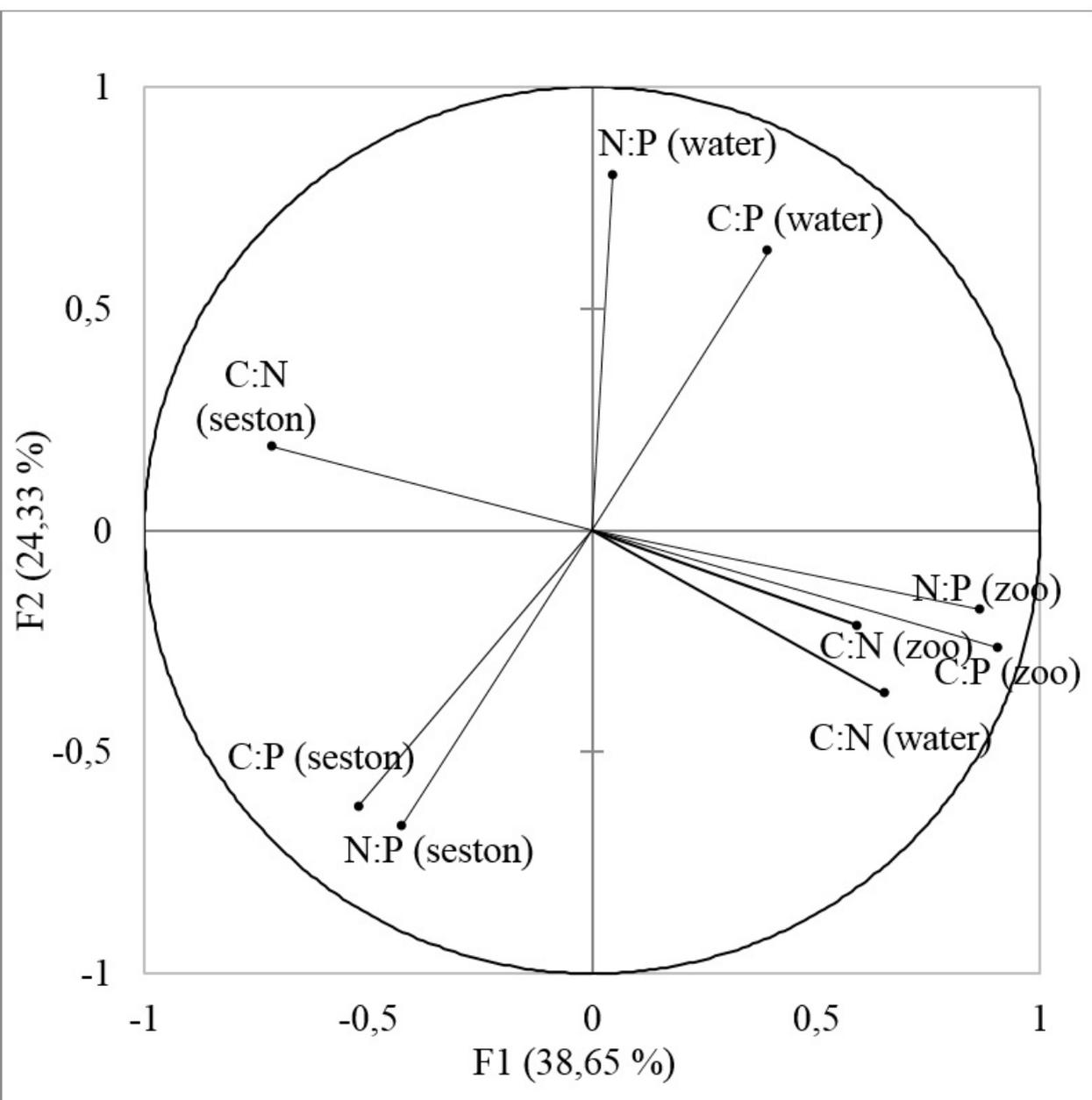
Fig. 6. Significant differences in zooplankton and seston stoichiometric ratios between trophic conditions (B, C) and between the beginning and end of the experiment (A, D).

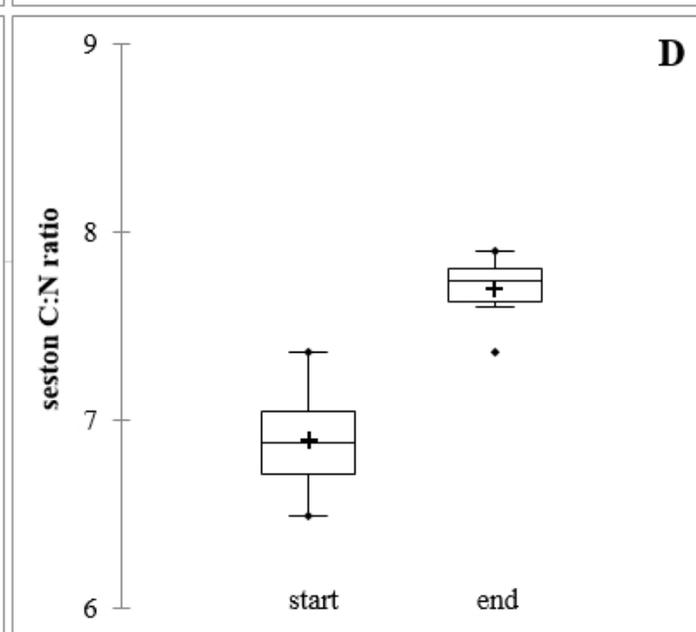
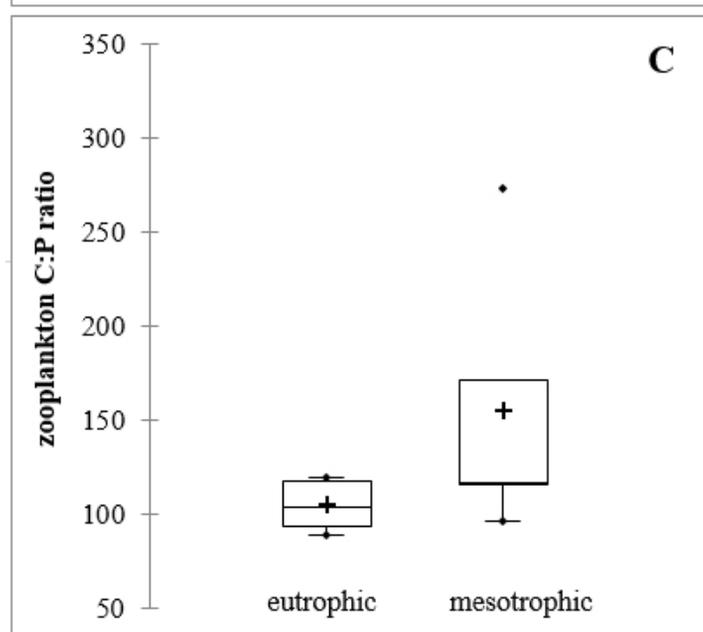
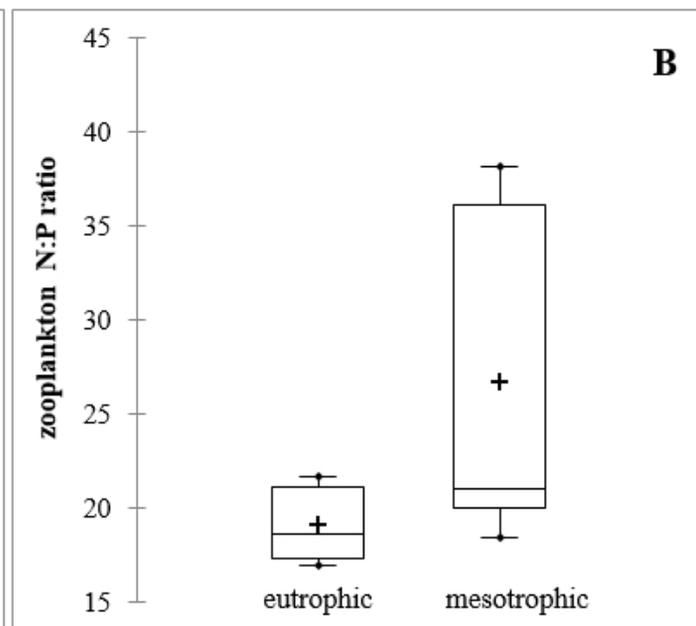
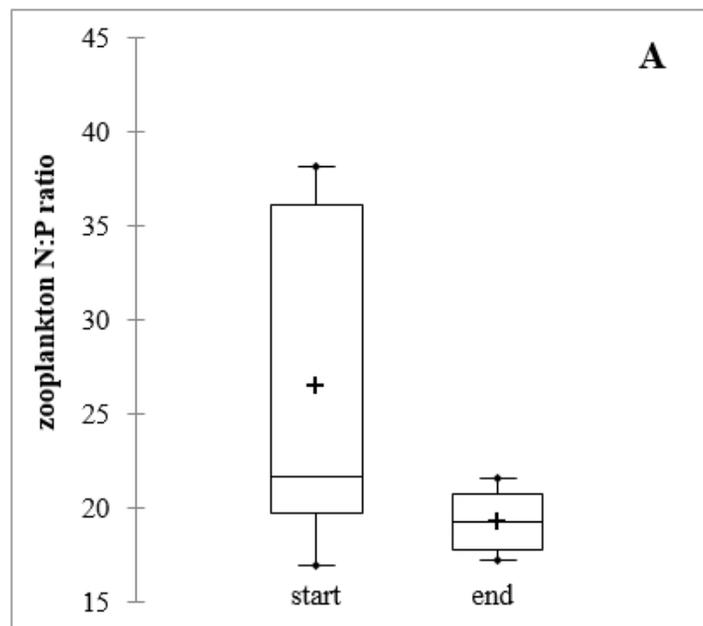












Tab. 1. Demographic parameters of *Ceriodaphnia pulchella*, *Daphnia magna*, and *Daphnia pulicaria* (average  $\pm$  standard deviation). Abbreviations: E - eutrophic; M – mesotrophic; nd – no data available.

	Day 1		Day 11		Day 21		Day 31	
	E	M	E	M	E	M	E	M
<b>Fecundity</b>								
<i>C. pulchella</i>	nd	3.5 $\pm$ 1.2	2.4 $\pm$ 2.2	3.4 $\pm$ 1.2	2.3 $\pm$ 0.3	0.1 $\pm$ 0.3	2.4 $\pm$ 0.5	0
<i>D. magna</i>	nd	nd	nd	nd	1.7 $\pm$ 1.1	6.4 $\pm$ 2.6	4 $\pm$ 0.8	4.7 $\pm$ 0.6
<i>D. pulicaria</i>	nd	5.7 $\pm$ 0.6	14.7 $\pm$ 5.5	12 $\pm$ 3	2.7 $\pm$ 1.5	2	0.7 $\pm$ 0.6	1.7 $\pm$ 0.5
<b>Population growth rate</b>								
<i>C. pulchella</i>	nd	0.34 $\pm$ 0.05	0.01 $\pm$ 0.9	0.31 $\pm$ 0.29	0.5 $\pm$ 0.03	-2.2 $\pm$ 0.8	0.3 $\pm$ 0.03	-2.44
<i>D. magna</i>	nd	nd	nd	nd	-0.26 $\pm$ 1	0.34 $\pm$ 0.04	0.3 $\pm$ 0.03	0.31 $\pm$ 0.01
<i>D. pulicaria</i>	nd	0.33 $\pm$ 0.01	0.44 $\pm$ 0.05	0.42 $\pm$ 0.03	0.24 $\pm$ 0.07	0.22	nd	0.2 $\pm$ 0.04