




## Article

# Effects of Cyanobacteria on Competitive Interactions Between Different-Sized Cladoceran Species

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**Abstract:** Cyanobacteria negatively affect zooplankton through several mechanisms including mechanical interference, toxicity, and poor food quality due to a shortage of essential lipids. To understand the nature of each of these mechanisms, they should be examined independently. The goal of our study was to assess the influence of cyanobacteria food quality on the competitive outcomes between the small-bodied *Daphnia longispina* and the large-bodied *Daphnia magna*. We conducted life-table experiments to assess  $R^*$  (population threshold food concentration), competition experiments to determine the outcome of competition, and computer simulation experiments at high levels of food supply, which are difficult to realize in laboratory conditions. We used two types of food: the high-quality green algae *Chlamydomonas klinobasis* (GREEN) and the cyanobacterium *Synechococcus elongatus* (CYANO), which contains low levels of essential lipids, but is non-toxic and unicellular. We found that the small-bodied *D. longispina* was a superior competitor in GREEN, while the large-bodied *D. magna* was more abundant in CYANO. We established that the species ratio in GREEN was dependent on competitive interaction, while abundances of daphnids in CYANO were controlled by poor food quality. Since cyanobacteria act as a powerful force for structuring cladoceran communities, the role of competition for food between these two *Daphnia* species greatly declined under their effects.



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**Keywords:** competition; *Daphnia*; food quality; life tables; sterols; modelling; threshold food concentration

## 1. Introduction

It has been known for more than 150 years that cyanobacteria are harmful to zooplankton [1]. However, cyanobacterial blooms have become more frequent in the twenty-first century due to the combined effects of eutrophication and increased temperatures [2]. Blooms of cyanobacteria are increasing in duration, frequency, and scale all over the world. Their expansion poses a great threat to water quality, fisheries, recreational activity, and human health, as has been described in several reviews [3,4]. For this reason, it is important to study the effects of cyanobacteria on various organisms, including zooplankton.

Cyanobacteria are regarded as a poor-quality resource for zooplankton because they produce toxins [4,5]. They are also deficient in essential lipids such as polyunsaturated

fatty acids (PUFA) [6–8] and sterols [9–11] compared to high-quality green algae. Low PUFA contents in algal food constrain cladoceran fitness in nature [12,13]. Sikora et al. [14] showed that small and large cladocerans differed in their responses to deficiencies in eicosapentaenoic acids (EPA). Specifically, small-bodied *Daphnia longispina* O.F. Müller had lower EPA saturation threshold than large-bodied *Daphnia magna* Straus, which is the minimal concentration of EPA above which juvenile growth rate is not constrained. Sterols are precursors of steroid hormones which are responsible for somatic growth of *Daphnia*, while PUFA mainly affect egg production [9]. Hence, the effects of sterol limitation should be more severe for juveniles since they require a greater amount of sterols for growth while PUFA deficiency should have higher impact on matured females. Since shortages of PUFA and/or sterols in cyanobacteria can affect the fitness of daphnids, we suggest that they can also alter competitive interactions. Therefore, it is important to predict the outcomes of competition under different environmental conditions, including algal communities dominated by cyanobacteria.

Competitive interactions between cladoceran species are one of the factors that shape zooplankton community structure [15]. We proposed that cyanobacteria influence the outcome of competition between *Daphnia* species. One approach to study this impact is to use Tilman's resource competition theory and the threshold food concentration ( $R^*$ ), which is the food concentration at which a population stops growing, i.e., when the birth rate equals death rate [16–18]. Superior competitors have lower  $R^*$  values that allow them to grow at lower food concentrations than species with higher  $R^*$  values. It has been shown that environmental factors impact threshold food concentrations in zooplankton, e.g., food quality in terms of phosphorus [19–21] and temperatures [22]. However, it remains unclear how cyanobacteria food quality affects the  $R^*$  of cladoceran species and how these possible alterations in  $R^*$ , in turn, affect competitive interactions.

As mentioned above, small- and large-bodied *Daphnia* species respond differently to deteriorations in food quality. Field studies showed that cyanobacterial blooms did not only cause decreases in zooplankton biomass but also were associated with shifts in abundance from large-bodied to small-bodied species [23]. For example, cyanobacterial blooms led to drastic decreases in the abundance of large *Daphnia* and were associated with rapid increases in small *Bosmina* and *Chydorus* in Lake Vela (Portugal) [24]. Jiang et al. [25] supported the above studies by experimentally showing that the toxic cyanobacterium *Microcystis aeruginosa* caused shifts in competitive advantage from the large-bodied *Daphnia pulex* Leydig to the small-bodied *Bosmina longirostris* (O.F. Müller) in mixed cultures. The authors suggested that toxicity of *M. aeruginosa*, rather than other mechanisms such as physical interference or low food quality in terms of shortage of PUFA and sterols, was responsible for reversing the outcome of competition between large- and small-bodied cladocerans. *Daphnia* was a superior competitor when fed with high-quality algae, but inferior when fed with toxic cyanobacteria as compared to *Bosmina*. Therefore, cyanobacteria can differently affect small- and large-bodied species.

Another reason for the shift in competitive superiority could be the inhibitory effects of filamentous cyanobacteria on food acquisition, which are size-dependent. Gliwicz and Lampert [26] found that the sequence of competitive abilities between three *Daphnia* species was reversed in the presence of the non-toxic filamentous cyanobacterium *Cylindrospermopsis raciborskii*. The smallest *Daphnia cucullata* Sars was a superior competitor when it was fed with the filamentous cyanobacteria, while the largest species *Daphnia pulicaria* Forbes was the best competitor in the treatment with green algae. The intermediate-sized *Daphnia hyalina* Leydig was an intermediate competitor in both cases. However, Kurmayer [27] did not observe a shift in competition between the small-bodied *B. longirostris* and the large-bodied *Daphnia galeata* Sars under the effects of filamentous cyanobacteria relative

to a treatment with high-quality food. Sikora and Dawidowicz [28] also showed that the large-bodied *D. pulicaria* dominated over the small-bodied *D. longispina* in treatments with high-quality food and non-toxic and filamentous cyanobacterium *C. raciborskii*. These contradictory results can be attributed to the multifaceted influence of cyanobacteria on daphnids including negative effects from mechanical interference, toxicity, and/or shortage of essential lipids. Hence, to understand the impact of each of these potential inhibitory mechanisms of cyanobacteria, they should be examined independently.

In our study, we focused specifically on the effects of cyanobacteria food quality on the outcome of competition between small- (*D. longispina*) and large-bodied (*D. magna*) species. We used the non-toxic unicellular cyanobacterium *Synechococcus elongatus* to avoid effects of toxicity and mechanical interference, and the green algae *Chlamydomonas klinobasis* as a control. The effects of poor food quality due to deficiencies in PUFA and sterols to our knowledge has not been adequately addressed because it has rarely been studied as an independent factor. The goal of our study therefore was to experimentally determine if the outcome of competition could be altered by the influence of poor-quality cyanobacteria relative to high-quality green algae. We hypothesized that small- and large-bodied daphnids would respond differently to deteriorations in food quality based on the analysis of the published data given above, e.g., due to the higher absolute requirements for essential lipids of large-bodied *Daphnia* compared to small-bodied species. To understand how competitive ability can change under the effects of poor quality cyanobacteria, we determined Tilman's  $R^*$  for both species fed with green algae and cyanobacteria in life-table experiments. We anticipated that if competition is strong, the species with a lower  $R^*$  would be superior. Since the competition experiments are laborious and high levels of food supply (analogue of primary production) can lead to overcrowded and density effects, we also used a computer model to predict species dynamics in mono- and mixed cultures.

## 2. Materials and Methods

### 2.1. Experimental Materials

We used the large-bodied *D. magna* (clone DMN—Novy Vrbensky Rybnik, Czech Republic) and the small-bodied *D. longispina* (clone GB01—Großer Binnensee, Germany) in the competition and life-table experiments. Body size is a crucial ecological trait that influences demographic performance, competitive ability, and survival strategy [15,29–31]. The body length in *D. longispina* was two-fold smaller at first reproduction and its body mass was almost five-fold less than in *D. magna* (Figure 1). The difference in size at first reproduction between the two study species was beyond the range of clonal variation in the corresponding size within each species [32]. Thus, we could study these species as different-sized species.

We used *C. klinobasis* (strain #56 from the Limnological Institute, University of Konstanz) as a high-quality food. It is a unicellular green alga with two anterior flagellae that it uses to move. Since these algae move in the water, the cells do not settle, which helps them to maintain food concentrations even in small volumes of water. *C. klinobasis* is rich in phytosterols and contains alpha-linolenic acid (C18:3  $\omega$ -3),  $\omega$ -3 PUFAs, and  $\omega$ -6 PUFAs [13]. These algae are regarded as a high-quality food for *Daphnia*, providing necessary essential macromolecules [14,33]. *C. klinobasis* was cultivated in batch cultures (0.5 L Erlenmeyer flask filled with 0.2 L of medium) in WC medium and harvested after 4 days. Batch cultures were kept on a shaker (PSU-20i, Biosan, Józefów, Poland) set to 100 rpm, in a thermostatic chamber (ST35, Pol-Eko Aparatura, Wodzisław Śląski, Poland) at  $20 \pm 0.2$  °C and light intensity  $21 \pm 1$   $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ .



**Figure 1.** Adults with the first clutch. Large-bodied *D. magna* (left) and small-bodied *D. longispina* (right).

We used *S. elongatus* strain SAG89.79 as a poor-quality food. *S. elongatus* is a unicellular cyanobacterium [34] which was obtained from SAG collection (University of Göttingen, Germany) and then maintained in the Department of Hydrobiology, University of Warsaw. This *S. elongatus* strain is non-toxic, phosphorus-rich, and well assimilated by crustaceans [6,14,35]. However, it is regarded as a poor-quality food for *Daphnia* because it is deficient in long-chained PUFA [36] and lacks sterols [9,37]. *S. elongatus* was grown in a chemostat in a WC medium [38] at a constant temperature ( $20 \pm 1$  °C) and light intensity of  $56 \pm 1 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ . It was harvested at a stationary phase of growth.

The carbon content of food was measured by the dry combustion method using a Flash 2000 Elemental Analyzer (ThermoFisher Scientific Inc., Waltham, MA, USA). The C content of the algal and cyanobacteria suspensions was determined by reference to photometric light extinction at 800 nm, using previously determined carbon extinction equations.

The medium for experimental zooplankton was prepared by diluting lake water with demineralized water in the proportion 1:2, respectively. Lake water was obtained from the eutrophic Lake Szczęśliwickie, a former clay pit, in Warsaw (Poland). Dilution was applied to reduce the phosphorus content. Lake water was filtered through a Sartoban® capsule filter (pore size 0.2  $\mu\text{m}$ ) to remove bacteria. We then added green algae in the required concentration to create the green algae treatment (GREEN). Since animals fed pure cyanobacterium *S. elongatus* were not able to develop, a mixture consisting of 95% of the required concentration of carbon in the form of cyanobacteria and 5% of green algae was provided to experimental animals (CYANO).

## 2.2. Experimental Design

We conducted life-table experiments with *D. longispina* and *D. magna* in 100 mL glasses to assess demographic parameters, including age at first reproduction, fecundity, and  $R^*$  based on the population growth rate ( $r$ ). We did not determine mortality because we studied food conditions above the threshold food concentration for mortality (we had 100% survivorship).

To avoid the effects of bacterial growth in the water and on the walls, the animals were pipetted daily into sterilized glasses with a fresh medium. Animals were fed with green algae *C. klinobasis* or cyanobacterium *S. elongatus* twice a day at a 12 h interval. One female with eggs was initially put in a 100 mL glass. After hatching neonates, we left only

one individual in the glass. The other neonates and females were removed. Neonates were no more than 12 h old at the start of the experiment. We recorded the age of first reproduction and the number of offspring removed just after hatching. We terminated the experiment after the releasing of neonates from the third clutch because later reproduction has a minor effect on  $r$  [39]. We calculated neonate output which was the sum of neonates born during the first three clutches and the age of first reproduction to compare these parameters between GREEN and CYANO.

We used three food concentrations to assess  $R^*$  at both food types: 0.54, 0.36 and 0.18 mg C L<sup>-1</sup>. In total, we had 12 treatments (2 species × 3 food concentrations × 2 food types). We started with 10 replicates for each treatment; however, the number of replicates in some treatments decreased due to the loss of animals during experiment manipulations, but by no more than by three replicates (at least 7 individuals).

The population growth rate ( $r$ ) was calculated using the Euler–Lotka equation [40,41]:

$$1 = \sum (l_x m_x e^{-rx})$$

where  $l_x$  is age specific survival,  $m_x$  is age specific fecundity and  $x$  is age in days. The sum was taken over the duration of the experiment or from  $x = 1$  until the day of the third clutch.

Competition experiments were conducted in 400 mL glass jars. The medium and the jars were exchanged every day to prevent development of bacteria in the water and on the walls. Experiments with monocultures and mixed culture of both species lasted for 60 days in the treatments fed with *C. klinobasis*. Algae were added twice a day at a 12 h interval in the amount 0.09 mg C L<sup>-1</sup> (totaling 0.18 mg C L<sup>-1</sup> per day). All the treatments (2 monocultures and mixed culture) were conducted in triplicate replicates. There was no population growth in cyanobacteria treatments under such mode of feeding because of the low food supply. For this reason, we could not perform competition experiments with cyanobacteria. However, we used a model approach, which is described below.

We started the competition experiment with 5 neonates ( $\leq 12$  h old) of *D. magna*, and 20 neonates of *D. longispina*. The initial number of newborn individuals of *D. longispina* was greater than *D. magna* in order to balance the differences in biomass. Females with eggs prior to experiment were kept in the similar conditions for several days, but before that, they were reared under favourable conditions in the media and fed with *C. klinobasis* at a concentration 1 mg C L<sup>-1</sup> to obtain equally healthy individuals. We counted animals under a microscope (Nikon SMZ1000, Nikon Corporation, Tokyo, Japan) every 6th day.

Competition and life-table experiments were performed during a summer photoperiod (16L:8D) with 10.3  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity (Li-Cor 189 quantum sensor, Li-Cor Biosciences®, Lincoln, NE, USA). The experimental jars were kept in a water bath at 24 °C with  $\pm 0.1$  °C accuracy using Zefir (Adarex, Poland) submersible thermostats.

### 2.3. Computer Modelling

We used the same computer modelling approach as previous research on phosphorus limitation of cladocerans [21]. This approach allowed us to simulate dynamics of species with complex age structures in which age classes were distinguished by individual traits (somatic growth rate, fecundity, birth and death rates, and ingestion rates) [21]. The algorithm was written in C++ programming language. We used this model to predict the population dynamics of two cladoceran species in monocultures and mixed cultures. The species in the model were distinguished by demographic parameters, including individual growth rate, fecundity, mortality, ingestion rate. The parameters differed between three stages of development including neonates, young individuals, and matured females. In the model, we changed the food supply, which was analogue of primary production. This modelling approach was required to predict the outcome of competition between species



which differ considerably in competitive ability, measured as threshold food concentration ( $R^*$ ). In this case, at low food supply, the species with higher  $R^*$  could not increase in abundance. However, at high food supply, the species with lower  $R^*$  can be overcrowded, which creates additional factors, such as density effects. Modelling simulations allow us to avoid overcrowding effects, which are common in laboratory experiments. The equations of the model and details of its description are given in Feniova et al. [21], in which we simulated the dynamics of large- and small-bodied *Daphnia* under the influence of algal phosphorus content. Here, we used models to simulate the dynamics of modelled *D. longispina* and *D. magna* at high food supply (maximum possible food concentration  $0.54 \text{ mg C L}^{-1}$ ) which was three-fold greater than the low food supply used in the laboratory experiments.

Functions of relationships between individual demographic parameters, including fecundity, mortality, and age at first reproduction, and the food concentration were determined based on results from the life-table experiments and from [21]. These functions were approximated by linear relationships. The ingestion rates were taken from the literature [42–44]. We presented the parameters for the models in Supplementary Material 1 which included the demographic parameters for each of the three stages for each species and for each food type and equation for calculating each parameter under a particular food concentration.

#### 2.4. Statistical Analysis

We used a two-way fixed general linear model ANOVA (GLM-ANOVA) to assess the effects of food quality (FQ) (two types of FQ: cyanobacteria, green algae) and food concentration (FC) (three levels of FC: 0.18, 0.36,  $0.54 \text{ mg C L}^{-1}$ ) and their interaction (FQ  $\times$  FC) on the age of first reproduction (AFR) and neonate output in *D. magna* and *D. longispina*. Multiple comparisons of Tukey post hoc tests ( $p < 0.05$ ) were used for all analyses when significant treatment effects were detected by GLM-ANOVA. All data were log-transformed prior the analyses to achieve normality of residuals and improve homoscedasticity of variance. Although the Tukey test can typically withstand some deviations from normality, it is less resistant to heterogeneity of variance. Therefore, we used the Tukey test with Welch's approximation for multiple comparisons when samples differed in homogeneity of variance [45]. Statistical analyses and figure construction were performed in R v. 4.3.3. [46] and in NCSS v. 7.

Linear regression analysis was performed to identify the relationship between the population growth rate ( $r$ ) ( $y$ -axis) and food concentration ( $x$ -axis) for real and simulated species. The intersection of the regression line with the  $x$ -axis was designated as  $R^*$ , representing the food concentrations at which  $r$  was zero. We determined  $R^*$  for each species grown in GREEN and CYANO. In accordance with Tilman's resource competition theory [16–18], we regarded species with the lowest  $R^*$  as the superior competitor for a given food quality.

We used GLM-ANOVA to assess the effects of competition on the abundances of *D. longispina* and *D. magna* in the mono- and mixed cultures. In this analysis, we used a one-way GLM-ANOVA with four levels to detect differences in the means during stationary period of the experiment from Day 17 until the end of the experiment.

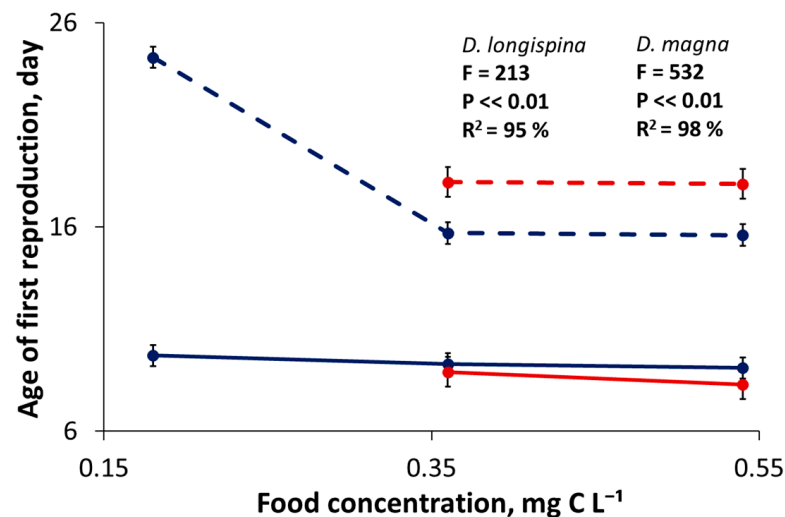
We used a residual analysis to evaluate the deviation of the model data from the experimental data. To assess the normality of the distribution of residuals, we utilized the Shapiro–Wilk test (SW-test), which involves comparison of the quintiles of the fitted normal distribution to the quintiles of the residuals. The basis of this test is a comparison of the quintiles of the fitted normal distribution to the quintiles of the residuals. If the  $p$  value of the SW test exceeds 0.05, it indicates that the hypothesis of normal distribution of residuals

with 95% confidence was not rejected. Furthermore, the t-criterion was employed to assess the null hypothesis concerning the mean of the residuals. The statistical parameters of the model verification are presented in Supplementary Material 2. The parameters and methods of residual analysis allowed us to conclude that the model adequately predict the dynamics of both species (Table S5, Figure S1, Supplementary Material 2).

### 3. Results

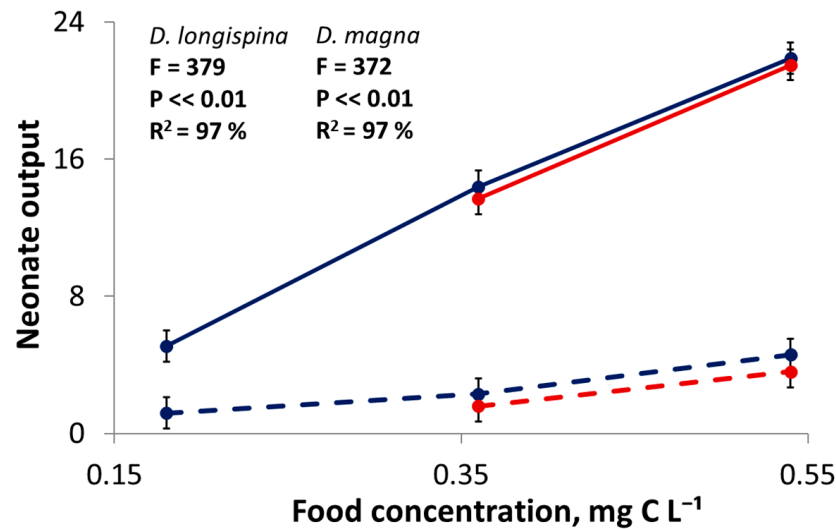
#### 3.1. Life-Table Experiments

Age at first reproduction (AFR) was significantly earlier in GREEN compared to CYANO for both species of *Daphnia* at each food concentrations (Figure 2). The AFR of *D. longispina* in GREEN was  $13.5 \pm 0.7$  days. We did not include this value in Figure 2, which shows the results of the GLM-ANOVA pairwise comparison between the parameters. *D. longispina* did not reach maturity at  $0.18 \text{ mg C L}^{-1}$  in CYANO, and for this reason, we did not have a corresponding paired value. The individuals in all the replicates at the lowest food concentration died on days 14–15. Cyanobacteria had significant negative effects on the AFR of both species (Tables S1 and S2). Food concentration and interaction effects of food quality and food concentration only had significant impacts on AFR for *D. magna*. The AFR of *D. longispina* was not dependent on food concentration and interaction effects of food quality and food concentration (Table S2).



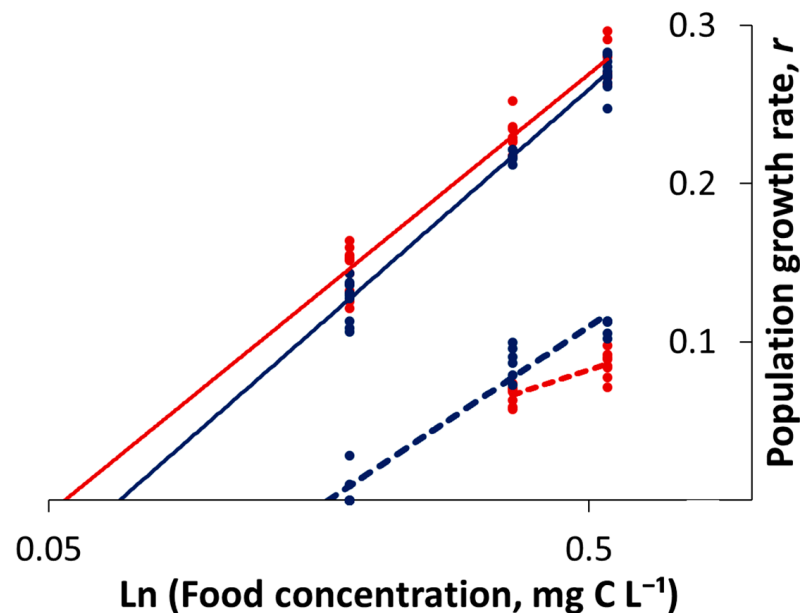
**Figure 2.** Multiple comparisons of AFR of *D. longispina* (red) and *D. magna* (blue) at three food concentrations between GREEN (solid lines) and CYANO (dashed lines) for each species separately. Error bars denote 95% of Tukey's HSD intervals.

Neonate output (the total number of neonates from each female born during the first three clutches) was significantly higher in GREEN compared to CYANO for both species at each food concentration (Figure 3; Tables S1 and S2). The significant differences in neonate output between the two food quality treatments at the same food concentration indicated the negative influence of cyanobacteria on reproduction. Neonate output was similar in both species, yet the mean tended to be higher for *D. magna* than for *D. longispina* at the two higher food concentrations, while neonate output was higher in *D. longispina* ( $8.9 \pm 1.6$ ) than in *D. magna* ( $5.1 \pm 0.9$ ) at  $0.18 \text{ mg C L}^{-1}$ . The effects of food concentration and the interaction between food quality and food concentration on neonate output were significant for both species (Tables S1 and S2).



**Figure 3.** Multiple comparisons of neonate output of *D. longispina* (red) and *D. magna* (blue) at three food concentrations between GREEN (solid lines) and CYANO (dashed lines) for each species separately. Error bars denote 95% of Tukey's HSD intervals.

The daphnids demonstrated significant relationships between  $r$  and food concentration (Figure 4). The  $R^*$  of *D. magna* in GREEN (0.07 mg C L<sup>-1</sup>) was much lower than in CYANO (0.17 mg C L<sup>-1</sup>) (Figure 4, Table S3). Since *D. longispina* did not reach maturity at the lowest food concentration (0.18 mg C L<sup>-1</sup>), we can only estimate that the  $R^*$  in *D. longispina* was in the range 0.18–0.36 mg C L<sup>-1</sup>, i.e., higher than in *D. magna* (0.17 mg C L<sup>-1</sup>). The  $R^*$  in GREEN was lower for *D. longispina* (0.05 mg C L<sup>-1</sup>) than for *D. magna* (0.07 mg C L<sup>-1</sup>) (Figure 4, Table S3).



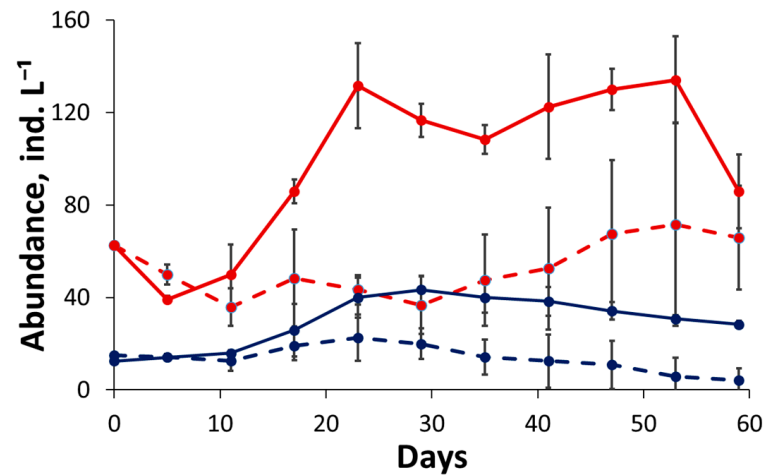
**Figure 4.** Linear regression between population growth rate ( $r$  day<sup>-1</sup>) and food concentration (log scale) in GREEN (solid lines) and CYANO (dashed lines). *D. longispina*—red, *D. magna*—blue (laboratory experiment).  $R^*$  was calculated from the point where the regression line crossed the  $x$ -axis.

### 3.2. Competition Experiment

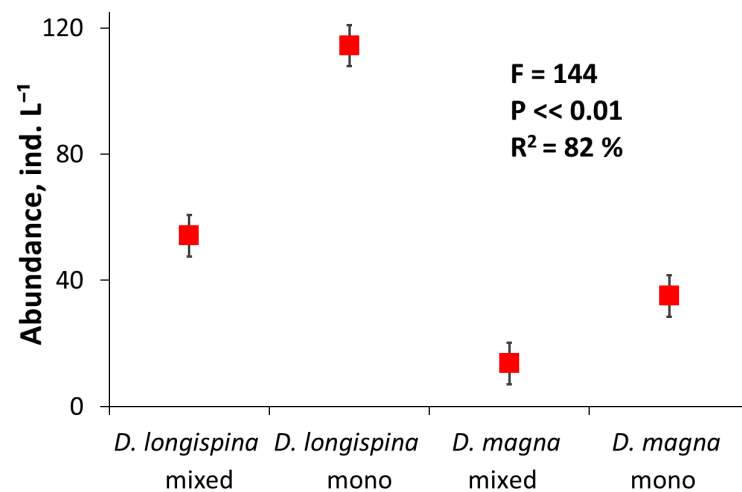
Both *Daphnia* successfully developed in monocultures in GREEN at 0.18 mg C L<sup>-1</sup> food supply (Figure 5). In the mixed cultures, the abundances of both species were lower



than in the monocultures, indicating that there were competitive interactions between species (Figures 5 and 6). *D. magna* was more strongly suppressed by *D. longispina* than vice versa in GREEN which is indicated by the differences between the means in monocultures and mixed culture (Figure 6). At the end of the experiment, only a few adult *D. magna* without eggs remained in the mixed cultures. The competitive dominance of *D. longispina* over *D. magna* in GREEN was consistent with their  $R^*$  values determined in the life-table experiments above, which also suggested that *D. longispina* was a superior competitor relative to its larger counterpart in GREEN. In CYANO with the same food supply, *D. longispina* did not develop. Therefore, there was no similar competition experiment in CYANO.



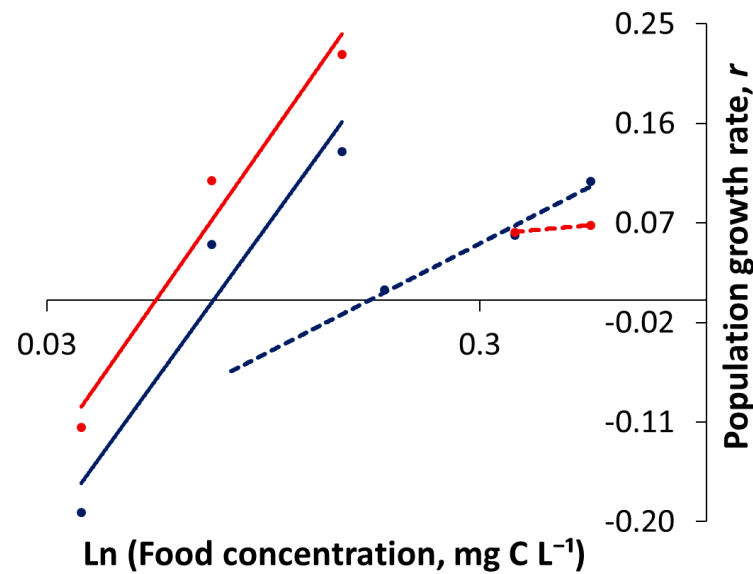
**Figure 5.** Abundance (mean  $\pm$  SD) dynamics of *D. magna* (blue) and *D. longispina* (red) in mono (solid line) and mixed (dashed line) cultures in GREEN at 0.18 mg C L<sup>-1</sup> food supply (laboratory experiment).



**Figure 6.** Results of one-factor ANOVA showing the differences in abundances of *D. longispina* and *D. magna*. Mean values are given with 95% Tukey confidence intervals in mono- and mixed cultures for the stationary period from Day 17 to Day 60.

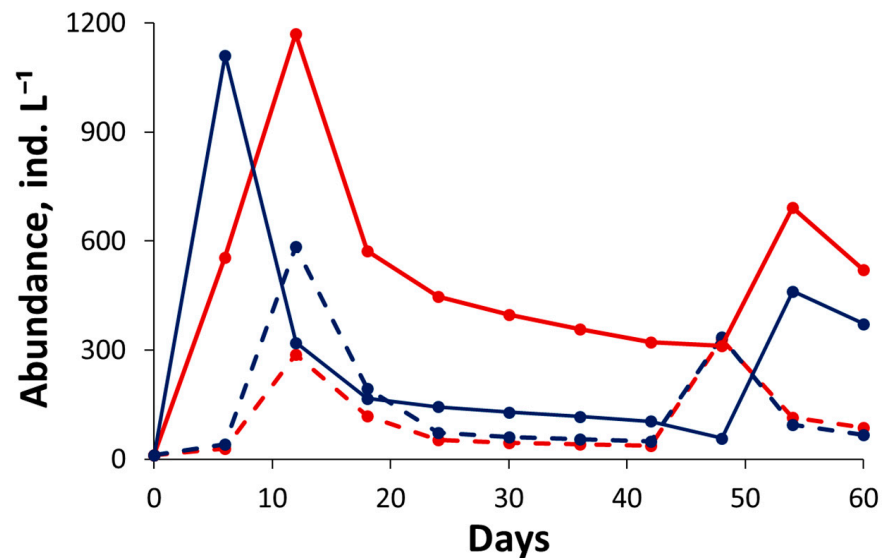
### 3.3. Computer Competition Experiment

$R^*$  for the modelled species calculated based on regression relationships between population growth rate ( $r$  day<sup>-1</sup>) and concentrations of green algae and cyanobacteria were similar to those for real species (Figure 7, Table S4), because they were found using data of life-table experiments.



**Figure 7.** Linear regression between population growth rate ( $r$  day<sup>-1</sup>) and food concentration (log scale) in GREEN (solid lines) and CYANO (dashed lines). Modelled *D. longispina*—red, modelled *D. magna*—blue (computer experiment).  $R^*$  was calculated from the point where the regression line crossed the  $x$ -axis.

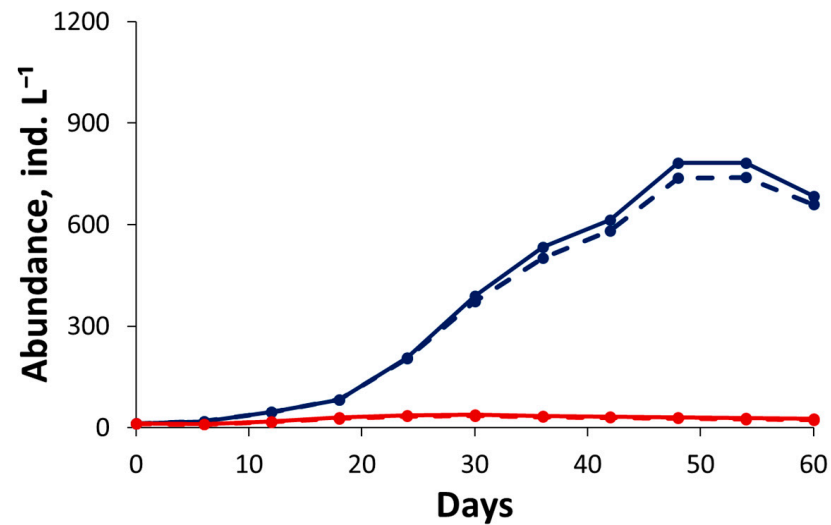
Dynamics of the modelled *D. magna* and *D. longispina* in GREEN at high (max possible food concentration  $0.54$  mg C L<sup>-1</sup>) food supply had two pronounced peaks (Figure 8). Abundances of both species in monocultures were higher than in mixed cultures indicating competitive suppression. In mixed culture both species coexisted with dominance of *D. magna* during the first peak.



**Figure 8.** Abundance dynamics of modelled *D. magna* (blue) and *D. longispina* (red) in mono (solid line)- and mixed (dashed line) cultures in GREEN at high food supply (computer experiment).

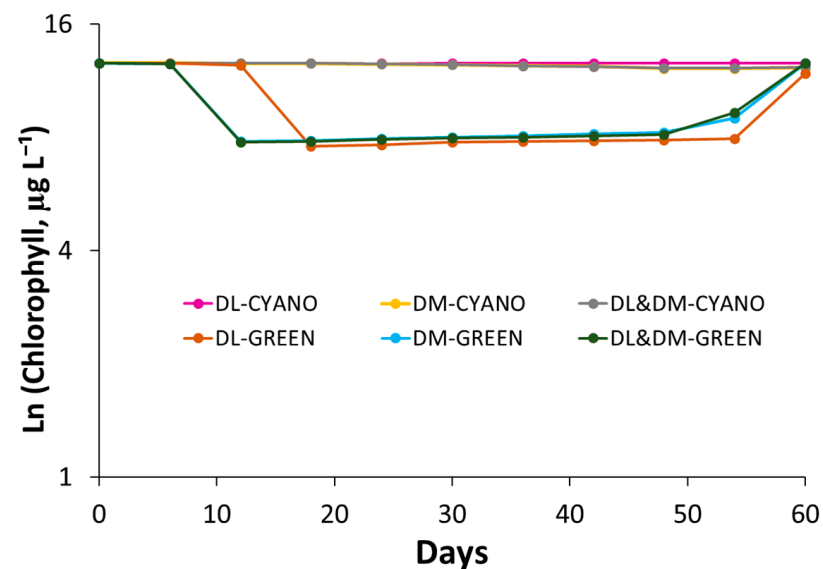
Dynamics of abundances predicted by the computer model in CYANO at high food supply showed that the abundances of both species in monocultures were much lower than in GREEN and there were no fluctuations in abundances over two months because the daphnids grew slowly (Figure 9). In the mixed culture in CYANO at  $0.54$  mg C L<sup>-1</sup> food supply, the modelled *D. magna* dominated over *D. longispina*. The abundance of *D. magna* exceeded that of *D. longispina* by over 20-fold. It is noteworthy that the abundances of both

species in monocultures and mixed cultures were similar, which suggests that competition was weak, if there was any.



**Figure 9.** Abundance dynamics of modelled *D. magna* (blue) and *D. longispina* (red) in mono (solid line)- and mixed (dashed line) cultures in CYANO at high food supply (computer experiment).

Dynamics of food concentration in the cultures with the modelled species was different in CYANO and GREEN (Figure 10). In GREEN, *Daphnia* decreased food concentration to the low level, which was associated with a decline in *Daphnia* abundance in mono- and mixed cultures (Figure 8). However, both *Daphnia* species grew so slowly in CYANO that they were not able to decrease the food concentration as much as in GREEN. The maintenance of a high food concentration in the mixed culture allowed the *Daphnia* species to coexist and keep the abundance at the same level as in monocultures (Figure 9).



**Figure 10.** Dynamics of food concentration (log scale) in mono- and mixed cultures (DL&DM) of modelled *D. magna* (DM) and *D. longispina* (DL) in GREEN and CYANO at high food supply (computer experiment).

#### 4. Discussion

Mechanical interference, toxicity, and/or lack of PUFA and sterols are important factors that contribute to the low quality of cyanobacteria [9,47]. In the current work, we

isolated the influence of food quality on daphnids because we used a non-filamentous (unicellular) and non-toxic cyanobacterium. We proposed that poor-quality cyanobacteria would have a stronger effect on the larger-bodied *D. magna* compared to the smaller-bodied *D. longispina* based on data in the literature [14]. We proceeded from the fact that if sterols are limiting, juveniles would suffer more than adults because sterols are mainly responsible for somatic growth while PUFA more strongly control reproduction [9].

We found that cyanobacteria greatly affected AFR and neonate output. PUFA limitation mainly affects egg production in *Daphnia* [48,49]. Our data showed that neonate output of both species was higher in *D. longispina* than in *D. magna* even at the lowest food concentration, suggesting that *D. longispina* was less constrained by PUFA than *D. magna*. In crustaceans, sterols serve as a precursor for ecdysteroids, which are responsible for moulting [50,51]. Martin-Creuzburg et al. [35] indicated that sterol requirements differ between cladoceran species. In particular, *D. galeata*, which belongs to the *D. longispina* species complex [52], had a two-fold higher threshold sterol level than *D. magna*. These results suggest that the lack of sterols in cyanobacteria could cause a greater response of *D. longispina* relative to *D. magna* when fed with cyanobacteria. Indeed, juvenile *D. longispina* in CYANO did not reach maturity, while juvenile *D. magna* survived until the first reproduction, although juvenile development was prolonged up to 25 days at  $0.18 \text{ mg C L}^{-1}$ . A slowdown of growth by *D. magna* can reduce the expenditure of essential lipids including sterols, thus giving *D. magna* a survival advantage.

Life-table experiments showed that the  $R^*$  values of both species were higher in CYANO compared to GREEN. That means that the  $r$  (population growth rate) of both species was negatively affected by cyanobacteria. According to the linear regression, when *Daphnia* were fed with green algae, their  $r$  was dependent not only on food quality, but also on food concentration. However, there was no clear effect of cyanobacteria concentration on  $r$  in *D. longispina*. In contrast,  $r$  in *D. magna* in CYANO was positively dependent on food concentration, although  $R^*$  was 2.4-fold higher than in GREEN. According to the lower  $R^*$ , *D. longispina* was competitively stronger in GREEN, while it failed in CYANO. Hence, contrary to our expectation, *D. longispina* was more sensitive to cyanobacteria than *D. magna*. We suspect that juveniles of *D. longispina* did not reach maturity because sterols deficiency restricted somatic growth. Based on field data, it was shown that sterols constrain the growth of zooplankton when cyanobacteria comprise  $\geq 40\%$  of total phytoplankton biomass [53]. Moreover, Laine et al. [10] found that the growth of *Daphnia* can be limited by sterols, even when feeding on eukaryotic algae. In our experiment, cyanobacteria constituted 95% of the total food biomass.

The results from the competition experiments were in accordance with the estimated  $R^*$  values. In particular, *D. longispina* dominated in GREEN due to its lower  $R^*$  but only at low food supply. However, *D. magna* at high food supply exceeded the abundances of *D. longispina* during the first peak after which they then coexisted. Dominance of *D. magna* over *D. longispina* in GREEN at high food supply during the first peak of abundance dynamics was attributed to high maximum reproduction potential of *D. magna* which can be manifested only at high food concentrations, but not when food concentration declines [21]. Decreases in food concentration below  $R^*$  by both *Daphnia* species caused declines in abundances due to exploitative competition. Competitive suppression of one species by the other was also confirmed by the differences in abundances of both *Daphnia* species between mono- and mixed cultures in GREEN. In CYANO, *D. magna* coexisted with *D. longispina* at high food supply with a great dominance of *D. magna*. Based on comparison of species abundances and food dynamics in mono- versus mixed cultures, we established that mainly cyanobacteria controlled the dynamics of daphnids in mixed culture and competition was very weak, if present at all. This statement was also supported

by the fact that the food concentration in CYANO was kept at a high level without any fluctuations which allowed both species to grow as in the monocultures, i.e., without the suppression of one species by the other. It is noteworthy that there was no development of *D. longispina* in CYANO at low food supply because its high  $R^*$  exceeded even the maximum food concentrations that were possible in the experiment with low food supply. Thus, there were different ratios in the abundances of the two species at different food types and/or food supply. In general, both competing species coexisted in GREEN at high food supply due to a trade-off between the competitive superiority (low  $R^*$ ) of *D. longispina* and advantage of the high maximum reproduction potential of *D. magna*. Therefore, the potentially competing species can be different in response to food type and food supply. In addition, we suggest that exploitative competition does not always shape the community structure. As our experiments demonstrated, the poor quality of cyanobacteria as a food resource can regulate the population dynamics more strongly than competitive interactions between species.

Jiang et al. [25] also experimentally demonstrated different competitive outcomes between the large *D. pulex* and the small *B. longirostris* when fed with different food types. When the green alga *Chlorella pyrenoidosa* was in their diets, *D. pulex* significantly suppressed *B. longirostris* and vice versa in the cyanobacteria treatment. However, the competitive advantage was reversed in the cyanobacteria treatment due to the toxicity of the non-filamentous cyanobacteria strain of *Microcystis aeruginosa*. In our case, the shift in dominance in CYANO compared to GREEN was attributed to the poor food quality of cyanobacteria (i.e., the lack of PUFA and/or sterols).

The other possible reason for an advantage of *D. magna* over *D. longispina* is its ability to consume bacteria. *D. magna* has one of the smallest mesh sizes of filtering appendages among cladocerans [54]. As a result, its efficiency in consuming bacteria is much higher than in the *D. longispina* species complex [55]. In our experiment, *D. magna* may have gained an advantage due to its ability to feed on cyanobacteria or bacteria that may have developed in the medium. The advantage of *D. magna* for more efficient grazing on bacteria has also been shown by others [28,56,57]. We filtered lake water prior to its usage in the experiment every day to remove bacteria. For this reason, the effect of cyanobacteria on the growth of the daphnids was pronounced. But in cases of contamination of the media by bacteria, the results of the experiments may be different because bacteria can serve not only as a source of carbon, but they can produce essential nutrients, including sterols [58]. There may also be many other factors that can cause shifts in dominance from one species to the other, such as temperature and hypoxia [31], food quality, food resource production, different reproduction strategies, and feeding mode [59]. However, we would like to emphasize that in laboratory conditions, bacteria can strongly influence species abundances which can mask the effects of the investigated factors (e.g., food quality, [60]).

Our study suggests that increases in the proportion of cyanobacteria in the seston give an advantage to large-bodied species with respect to food quality. In this case, exploitative competition is not as important in the shaping of cladoceran communities because poor food quality strongly hinders the development of crustaceans, thus attenuating the effects of competition. However, the other potential mechanisms of cyanobacteria that cause negative effects including toxicity and/or filamentous forms can, on the contrary, have larger negative effects on large-bodied species. Therefore, to predict the outcome of competition between different-sized cladoceran species, we should consider all of the potential mechanisms by which cyanobacteria negatively affect daphnids.

Eutrophication and climate change are causing shifts in phytoplankton community composition towards cyanobacteria, which negatively affect zooplankton production [53]. Decreases in zooplankton biomass and increases in primary production due to the intensive



development of cyanobacteria lead to a reduction in the efficiency of transfer of carbon and essential substances, including sterols and PUFA [53,61] from phytoplankton up the food webs. Such effects can have a great impact on aquatic trophic webs, which can be reflected further in planktivorous fish and higher predators, causing deterioration in the quality of biological resources at each trophic level [62]. Thus, it is important to establish all the mechanisms by which cyanobacteria impact zooplankton communities for the development of approaches to attenuate cyanobacteria impact.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w17071014/s1>, Supplementary Materials 1: Parameters and formulas used in computer experiments. Supplementary Materials 2: Figure S1: Normal probability plots of residuals and Shapiro-Wilk test to verify the assumption that the residuals are normally distributed; Table S1: Results of two way ANOVA of the effects of food quality (FQ) (Cyanobacteria, Green algae) and food concentration (FC) (0.18, 0.36, 0.54 mg C L<sup>-1</sup>) and their interaction (FQ × FC) on the age of first reproduction and neonate output in *D. magna*; Table S2: Results of two way ANOVA of the effects of food quality (FQ) (Cyanobacteria, Green algae) and food concentration (FC) (0.36, 0.54 mg C L<sup>-1</sup>) and their interaction (FQ × FC) on the age of first reproduction and neonate output in *D. longispina*; Table S3: Thresholds food concentrations ( $R^*$ ) and regression parameters for *D. longispina* and *D. magna* in GREEN and CYANO (laboratory experiment); Table S4: Thresholds food concentrations ( $R^*$ ) and regression parameters for *D. longispina* and *D. magna* in GREEN and CYANO (computer experiment); Table S5: Results of comparative analysis of residual for comparison of abundance dynamics of *D. magna* and *D. longispina* in laboratory and computer experiments.

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## Abbreviations

The following abbreviations are used in this manuscript:

GREEN	Green algae treatment
CYANO	Cyanobacteria treatment
PUFA	Polyunsaturated fatty acids
EPA	Eicosapentaenoic acid
$R^*$	Population threshold food concentration

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