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# Direct and Indirect Impacts of Fish on Zooplankton Communities in Experimental Mesocosms

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**Abstract:** Regulation factors of phytoplankton and zooplankton dynamics is of crucial importance but this topic is not studied well because of complex phytoplankton-zooplankton interactions. Zooplankton, in particular cladocerans, can be regulated bottom-up either via food quantity or food quality (in terms of polyunsaturated fatty acids (PUFA) or phosphorus (P) contents in phytoplankton). Fish can recycle nutrients and in turn change PUFA and P contents in food resources thus modifying the bottom-up regulation. Besides fish can change phytoplankton structure through consumption of crustaceans which selectively graze on phytoplankton. Our goal was to establish the main drivers of crustacean dynamics which can switch in dependence of fish presence/absence with the main focus on cladocerans.. The experiments were carried out in 300-L plastic containers which were filled with water containing natural plankton from the eutrophic Lake Jorzec and mesotrophic Lake Majcz (northeastern Poland). We manipulated trophic levels and fish presence/absence. Small and large cladoceran species responded differently to food quantity and quality. Small *Ceriodaphnia* was regulated mainly by resource concentration while large species were limited by PUFAs. Fish likely increased food quality in terms of PUFA, primarily eicosapentaenoic acid (EPA), thus providing conditions for more successful development of *Daphnia* than in the fish-free treatments. Phosphorus in seston was likely limiting for zooplankton. However, food quality in terms of phosphorus is less important than PUFA because zooplankton can accumulate nutrients in their body.

**Keywords:** zooplankton; phytoplankton; nutrients; population growth rate, small and large cladocerans; fish effects; stoichiometric elemental composition; polyunsaturated fatty acids; mesocosm experiments

## 1. Introduction

Zooplankton are regulated by algal resources not only in terms of carbon (C) concentrations, but also in terms of other essential compounds. In particular, the content of phosphorus (P), nitrogen (N), and also polyunsaturated fatty acids (PUFA, including eicosapentaenoic acids [EPA] and docosahexaenoic acids [DHA]), are important for zooplankton development and reproduction. These substances may limit the growth of zooplankton, especially in mesotrophic and eutrophic conditions where C concentrations exceed threshold food concentrations. Shortages of key dietary elements like C, N, and P for consumer metabolic demands can alter the synthesis of major macromolecules such as lipids, proteins, and nucleic acids [1], However zooplankton can regulate the content of these elements in their body, retaining those that are in shortage, while excreting those that are in excess through homeostasis [2–4].

Sundbom and Vrede [5] suggested that growth limitation in cladoceran zooplankton by PUFAs was a secondary effect of P limitation. They considered that nutrient stress caused changes in the biochemical composition of *Daphnia*, which in turn slowed growth. Therefore, P limitation can determine fatty acid composition, which in turn adversely affects growth. As Gulati showed, with a decrease in the C:P ratios of algal resources from 673 to 59 μmol/μmol, the importance of fatty acids for daphnids increased [6].

The elemental and biochemical composition of crustaceans differs between species resulting in species-specific food quality requirements. Lake crustacean communities are typically dominated by copepods and cladocerans, which differ in their nutrient requirements. Copepods have a high N:P (ca. 30–50) ratio in their tissues, and therefore relatively high N and low P demands for their development [7]. In contrast, cladocerans have a lower tissue N:P (ca.14), and thus high P and low N demands [3]. Cladocerans are also more vulnerable to EPA-limitation in nature [8], while copepods have higher requirements for DHA [9–10]. Thus food quality can help to determine crustacean community structure.

Small and large-bodied species of cladocerans respond differently to environmental factors [11] and they are likely to have different levels of threshold concentrations of C (abundance of food resources), C:P ratios and/or EPA. Large cladocerans have lower threshold food concentrations (i.e. food concentration at which population death rate equals birth rate) than small bodied species [12–15]. However, P requirements are higher in large-bodied species than in small-bodied species, because P is used for somatic growth [16]. Therefore, large-bodied species can be more vulnerable to P limitation. Sikora et al. [17] also showed that small-bodied *Daphnia* species were less vulnerable to EPA shortage [18].

Zooplankton food quality is dependent on phytoplankton composition. For example, total fatty acids of green algae were comprised of 40% PUFA, while cyanobacteria were only comprised of 6% [6,19]. Phytoplankton quality may also depend on its P and N content [20]. Planktivorous fish have the ability to alter phytoplankton quantity and quality indirectly through grazing and nutrient cycling. Fish can change phytoplankton composition indirectly through selective consumption of cladocerans which prefer food items from 1 to 30 mm [21]. Copepods are better able to escape fish predators than cladocerans because they have higher locomotor activity [23-25]. For example, copepods escaped fish attacks 90% of the time while *Daphnia* avoid predator attacks only 15% of the time [22]. The field studies [26] were in accordance with above experimental data demonstrating that cladocerans were selectively consumed by fish despite higher density of the copepods. Furthermore, fish prefer large than small cladocerans [27,14]. Therefore, fish selectively reduce the abundance of the most effective filter feeders, mainly large daphnids, thus leading to increase of edible particulate food items for zooplankton [28].

Fish can also exert bottom-up effects on zooplankton by indirectly altering phytoplankton communities. For example, fish excrete nutrients into the water column that can stimulate phytoplankton growth and alter its species composition [29], and they can stimulate or inhibit the development of individual phytoplankton taxa passing through their guts in “viable gut passage” [30– 31].

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The goal of this experiment was to determine how fish and trophic state affected crustacean community dynamics, with particular emphasis on cladocerans. We conducted a mesocosm experiment where we manipulated the presence of fish in water from a mesotrophic and a eutrophic reservoir, and conducted supplementary life-table experiments. We were interested in how fish affected cladocerans both indirectly through changes in algal food quantity and quality, and directly through predation. We predicted that fish would enhance the quality of algal resources (e.g. PUFA and/or nutrient content), while at the same time structuring cladoceran communities through size-selective predation. We also hypothesized that small and large cladoceran species would respond differently to variations in algal quantity and quality.

## 2. Materials and Methods

The experiment was carried out in a series of 12 plastic mesocosms (300-L total volume, 0.94 × 0.64 × 0.50 m), half of which were filled with water containing natural phytoplankton and zooplankton from the eutrophic Lake Jorzec (northeastern Poland, Mazury Lakes, lake area 41.9 ha, max depth 11.6 and mean depth 5.5 m) and the other half filled with water from the mesotrophic Lake Majcz (area 163.5 ha, max depth 16.4 m, mean depth 6 m, [32]). We manipulated the presence/absence of fish in the mesocosms for a total of 4 treatments: water from the eutrophic lake without fish (E); water from the mesotrophic lake without fish (M); water from the eutrophic lake with fish (EF); water from the mesotrophic lake with fish (MF). Each treatment was replicated in triplicate mesocosms and the experiment was conducted for 31 days. To create the fish treatments, one individual ruff *Gymnocephalus cernuus* (Linnaeus, 1758) between 7.5–11 cm was added to each mesocosm. Fish were kept in 5 L boxes that were suspended in the mesocosms. The boxes had large slots that allowed zooplankton to pass freely, but kept the fish inside. The fish were let out of the cage for only an hour (between 8 to 9 p.m.) each day to feed freely. Previous research has shown that fish can exhibit unrealistically high predation rates in mesocosm studies and the cages were used to limit predation on zooplankton throughout the experiment [33]. The mesocosms were open to the atmosphere, but in the event of rain, they were covered with a polyethylene multilayer film to prevent contamination.

Crustacean zooplankton samples were collected using a 2.6-L Limnos sampler every 10 days from the center of each mesocosm after they were gently mixed and fixed with 4% formaldehyde. The most abundant cladocerans were *Chydorus sphaericus* (O. F. Müller, 1776), *Bosmina longirostris* (O. F. Müller, 1776), *Ceriodaphnia pulchella* Sars, 1862, и *Diaphanosoma brachyurum* (Liévin, 1848). Since large daphnids were absent in the water from the mesotrophic and eutrophic lakes, we added *Daphnia magna* Straus, 1820 (originated from Binnensee, Germany) and *Daphnia pulicaria* Forbes, 1893 (originated from Lake Brome, Canada) into each mesocosm at densities of 1.0 ind. $L^{-1}$ for each species at the beginning of the experiment on Day 1 to study the different responses of large and small cladoceran species. Copepod communities were represented by *Eudiaptomus graciloides* (Lilljeborg, 1888), *Mesocyclops leuckarti* (Claus, 1857), *Thermocyclops oithonoides* (Sars G.O., 1863). Crustaceans were identified to species. The average animal length was used to estimate the wet weight of crustaceans by applying the equations after Błędzki and Rybak [34].

Phytoplankton samples were collected after thoroughly mixing the water in the mesocosms on the same sampling dates as the zooplankton samples. Samples were preserved with Utremel solution and 4% formaldehyde. Phytoplankton samples were concentrated by sedimentation [35] and counted under a light microscope (Nikon Optiphot 2). Cell sizes were measured under a microscope using an ocular micrometer. Algae biomass was calculated based on cell size and their approximations to simple geometric shapes [36,37]. The size structure of phytoplankton was represented by three size classes: < 30 μm; 30 – 50 μm, > 50 μm. Crustaceans mainly consume algal cells < 30 μm [21], while algae > 50 μm are generally too large for consumption.

Chlorophyll *a* was estimated using a PHYTOPAM fluorimeter (WALZ, Germany) that individually measured the concentrations of green, cyanobacteria and brown algae (diatoms & dinoflagellates) on the same dates as the plankton samples. All the hydrochemical samples were taken also at a 10-day interval and analyzed using standard methods [38]. Concentrations of N-NO3, N-NO2, N-NH4 and P-PO4 were determined by Dionex ICS 1100 ion chromatograph; total phosphorus (TP) and total nitrogen (TN) were measured by the Shimadzu. The total concentration of inorganic nitrogen was determined as the sum of nitrate, nitrite, and ammonium.

Temperature and dissolved oxygen concentrations were measured daily from the center of each mesocosm using a WTW multi-parameter probe 3410 with optical sensor FDO925. Concentrations of dissolved oxygen varied from 8 to 10 mg/L indicating that there was no oxygen limitation in the mesocosms. Electrical conductivity of water which varied from 271–354 μS/cm was measured with a Hatch probe.

We collected seston (all the particles and live organisms that passed through a 100-μm mesh sieve) for elemental (C, P, N) and fatty acid (FA) analyses (EPA, DHA and total FAs) on the first and final days of the experiment. One sample (5-10 L) was filtered onto precombusted glass-fiber GF/F filters (Whatman, USA) until intensive color developed on the filter. The fatty acid subsamples were dried for 30 minutes and then transferred into a chloroform–methanol mixture and frozen. Filters for organic carbon, phosphorus and nitrogen were dried at ambient temperature overnight and stored dry in a desiccator until further analyses.

Samples of zooplankton for elemental (C, P, N) and fatty acid (FA) analyses (EPA, DHA and total FAs) were also taken on the first and final days of the experiment. Preliminarily, 40 – 50 L of water was passed through a 100-μm mesh sieve to remove large items (filamentous algae, sticks etc.) from collected material on the sieve. Then the zooplankton on the sieve were dried with the filter paper and was divided into subsampled for fatty acid and elemental analyses. Subsamples for fatty acids were weighed and placed into a chloroform–methanol mixture and frozen. Subsamples of zooplankton on phosphorus, nitrogen and carbon were weighed and afterwards kept at 75°C overnight and then stored in a desiccator. We did not collect seston and zooplankton samples for nutrient and PUFA analysis during the course of the experiment because we did not want to disturb plankton community dynamics.

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 [39]. Calibration curves for the elemental analyzer were generated using aspartic acid and standard soil reference material. Contents of particulate P were estimated following the conventional photocolorimetric method [40]. The background P content of the filters was preliminarily measured and subtracted from the sample values. The procedure for fatty acid analyses of the seston and zooplankton is described in detail elsewhere [41,42].

We conducted life-table experiments beginning on the 12$^{th}$ day of the mesocosm experiment. Life-tables were performed in 500 mL bottles that were filled with water from each mesocosm after filtration to remove crustaceans and other large items through a 100-μm mesh sieve. Water in the bottles was exchanged at two-day intervals, and the bottles were thoroughly washed. The bottles were hung in the center of each mesocosm in the middle water layer. The openings of the bottles were covered with a sieve (50 μm) through which the crustaceans could not get out while phytoplankton easily penetrated into the bottle. In each of the 12 mesocosms, initially, 7–10 newborn specimens of one of the three dominant species of cladocerans, namely, *D. magna, D. pulicaria* and *C. pulchella* were placed in individual bottles. In total, each mesocosm had three bottles (one with each of the three species) such that the treatments and replicates in the life-table experiment matched the mesocosms experiment. Every other day, living individuals were removed from the bottle, counted and returned. We recorded the duration of development until maturity and the number of eggs in each clutch. 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 ($r$) [43,44]. We measured the concentrations of chlorophyll in each bottle and mesocosm every other day to control for potential discrepancies in food concentrations between bottles and corresponding mesocosms.

We used one-way ANOVA with Fisher's LSD post hoc tests to compare nutrient concentrations, namely, sum of inorganic nitrogen compounds ($N\text{-}NO_3$, $N\text{-}NO_2$ and $N\text{-}NH_4$), $P\text{-}PO_4$ and N:P ratio, concentrations of total chlorophyll, chlorophyll of diatoms & dinoflagellates and green algae, and biomasses of copepods and cladocerans, *D. magna*, *D. pulicaria* and *C. pulchella*. Concentrations of cyanobacteria chlorophyll were compared between E and EF using Mann-Whitney nonparametric U-test. The data on Day 1 of the experiments were not used in the statistical analysis because there was not any effect of fish on plankton community. The figures of nutrient and biological parameters dynamics were made using $\log_{10}$-transformed data. We used one-way ANOVA to compare concentrations (C, N, P) and ratios (C:N, C:P, N:P) of indicators of elements' content in seston and zooplankton, and also concentrations of indicators of biochemical quality (EPA, DNA, Total FA) and their contents per organic carbon (EPA:C, DNA:C, Sum FA:C) in seston and zooplankton. Concentrations of ratios C:P, C:N and EPA:C, DHA:C, Sum FA:C were compared between zooplankton and seston using Mann-Whitney nonparametric U-test. We used one-way ANOVA to compare demographic parameters of *D. magna*, *D. pulicaria* and *C. pulchella* in life-table experiments. Effects of treatments (M, MF, E, EF) and time on phytoplankton biomasses of different size groups were compared by two-way repeated measures ANOVA (RM ANOVA). In the absence of normal distribution (Kolmogorov-Smirnov one-sample test for normality) Kruskal–Wallis (H) test was used. Statistical analyses were performed in PAST, version 3.20.

Canonical correspondence analysis of fatty acid composition of seston was performed using STATISTICA software, version 9.0 (StatSoft, Inc.). Dependence of demographic parameters of D. magna, *D. pulicaria* and *C. pulchella* on total chlorophyll concentration was calculated using regression analysis which was carried out using STATISTICA software, version 9.0 (StatSoft, Inc.).

## 3. Results

The concentrations of N and P and the N:P ratio in the water did not differ between the treatments (Figure 1). The concentration of P remained relatively stable throughout the experiment, likely due to P regeneration. However, the concentration of N decreased throughout the experiment, and as a result, the N: P ratio also decreased.

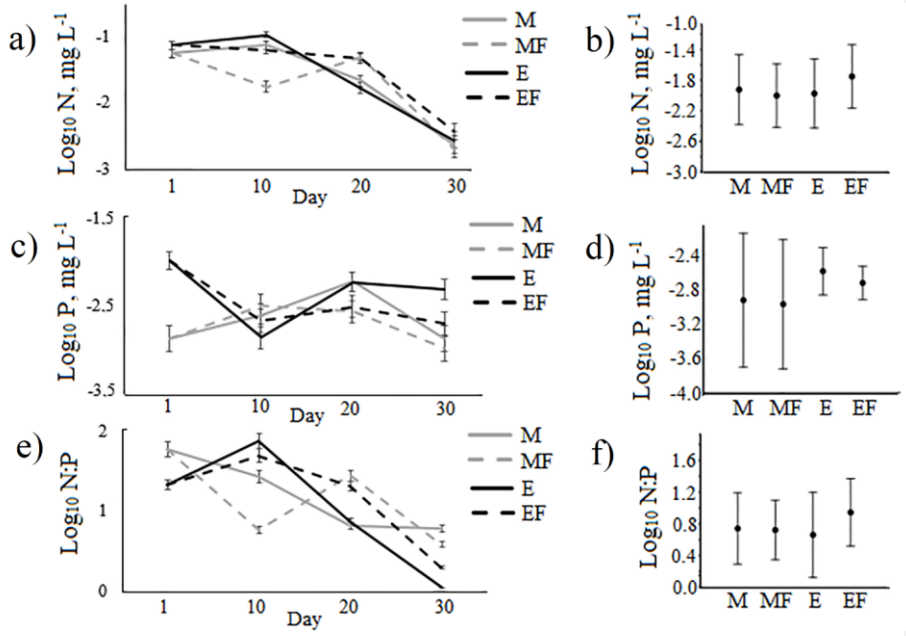

**Figure 1**. Dynamics of nutrient concentrations in the experiments (a, c, e) and treatment means (b, d, f); M – mesotrophic conditions, MF – mesotrophic conditions with introduced fish, E – eutrophic conditions, EF – eutrophic conditions with introduced fish.

The concentrations of total chlorophyll and diatom & dinoflagellate chlorophyll were significantly higher in the treatments with fish than in the corresponding treatments without fish at each trophic level (Figure 2). The highest concentrations of total chlorophyll and diatoms were in the EF treatment, and the lowest was in the M treatment. In the MF and E treatments, the concentrations of chlorophyll were not statistically different but they were higher than in M and lower than in EF. The concentration of green algae chlorophyll was not statistically different between the treatments. Cyanobacteria were either absent or rare in mesotrophic treatments. In the eutrophic treatments, cyanobacteria chlorophyll was higher in the fish treatment than in the eutrophic treatment without fish.

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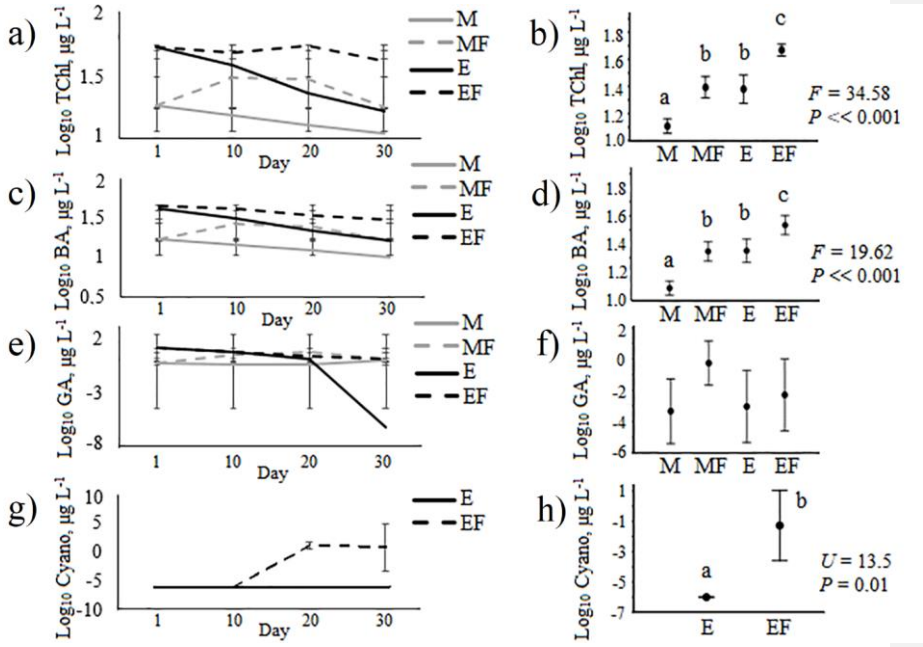


**Figure 2**. Dynamics of chlorophyll concentrations in the experiments (a, c, e, g) and treatment means (b, d, f, h); different letters indicate significant differences at $P < 0.05$ after Fisher's LSD post hoc test (F) and Mann-Whitney nonparametric U-test (U). TChl – concentrations of total chlorophyll, BA – chlorophyll of diatoms & dinoflagellates, GA – green algae and Cyano – cyanobacteria, M – mesotrophic conditions, MF – mesotrophic conditions with introduced fish, E – eutrophic conditions, EF – eutrophic conditions with introduced fish.

The C:N and C:P ratios in the seston did not differ between the treatments or from the start to the end of the experiment (Table 1). C:P mean ratios varied from 176.97 to 743 μmol/μmol (or from 68.5 to 287.6 mg/mg). Therefore, there could be shortage in phosphorus in seston for zooplankton, since there is evidence that 90–100 μmol/μmol can be limiting [45-47]. In contrast, N:P ratios of the seston did differ and were highest in the EF treatment.

**Table 1.** Results of one-way ANOVA comparing means ( ± SE) of ratios of indicators of nutritive quality of seston in experimental mesocosms: E – eutrophic, M – mesotrophic , F – fish, i – initial (June) date, f – final (July) date, 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).

| | **$M_i$** | **$MF_i$** | **$E_i$** | **$EF_i$** | **$M_f$** | **$MF_f$** | **$E_f$** | **$EF_f$** | **F(H)** | ***P*** |
|---|---|---|---|---|---|---|---|---|---|---|
| C:N, (mg/mg) | 7.16±0.25 | 6.71±0.28 | 8.18±0.33 | 7.54±0.19 | 7.39±2.12 | 6.96±0.16 | 8.29±0.52 | 6.79±0.16 | 12.4 | 0.09 |
| C:P (mg/mg) | 136.6±15.2 | 129.0±5.2 | 182.5±10.6 | 150.1±12.9 | 173.9±103.1 | 156.7±36.1 | 68.5±3.5 | 287.6±67.3 | 12 | 0.10 |
| N:P (mg/mg) | 19.0±1.5$^{ab}$ | 19.2±0.5$^{ab}$ | 22.8±1.9$^{b}$ | 19.9±1.3$^{ab}$ | 21.6±7.0$^{ab}$ | 22.4±4.9$^{ab}$ | 8.3±0.8$^{a}$ | 42.0±9.2$^{c}$ | 4.0 | 0.01 |

Results of canonical correspondence analysis of fatty acid (FA) composition (% of total FAs) in seston are given in Figure 3. At the start of experiment, FA composition did not differ considerably between eutrophic (E) and mesotrophic (M) mesocosms (Figure 3). However, there were differences in the M treatment due to a higher levels of docosahexaenoic acid (DHA, 22:6n-3) (Figure 3). At the end of the experiment, higher levels of FAs with odd numbers of carbon atoms and iso-FAs with branched carbon chain, which are the markers of bacteria, were characteristic of seston in all the treatments except EF (Figure 3). EF separated from the other treatments at the end of experiment due to high levels of 16:3n-3, 16:2n-6 and 18:3n-3 which are markers of green algae (Figure 3).

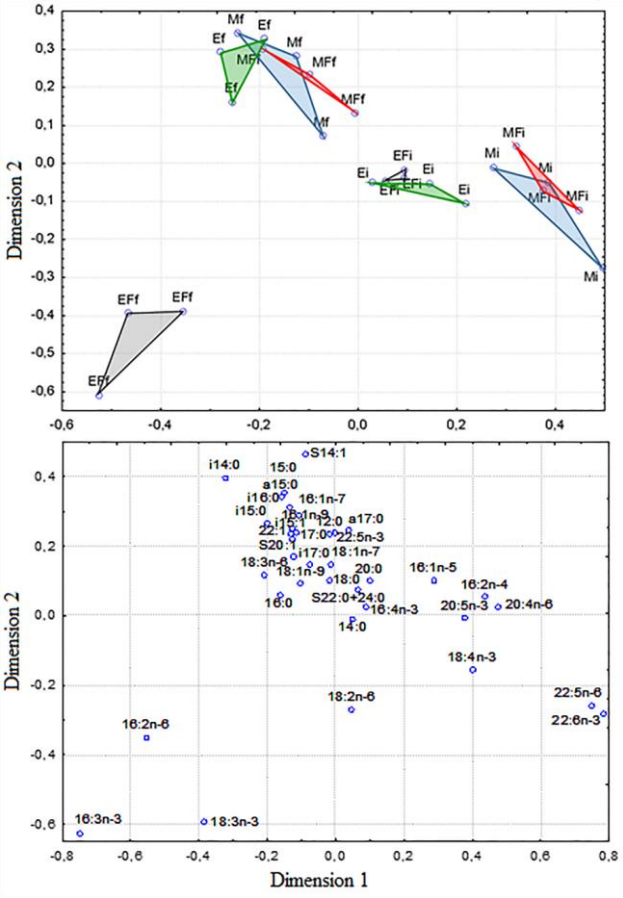


Figure 3. Results of canonical correspondence analysis of fatty acid (FA) composition (% of total FAs) of seston: E – eutrophic, M – mesotrophic conditions, F – fish, i – initial (June) date, f – final (July) date.

Mean concentrations of EPA and DHA in the seston were significantly higher in all the treatments at the start of the experiment than at the end of the experiment, but there were no differences between the treatments (Table 2). At the start, total FA concentrations in eutrophic treatments, regardless of the presence or absence of fish, were higher than those in mesotrophic treatments. In contrast, at the end of experiment, total FA concentrations in EF were significantly

higher than in E. Total FA concentrations in mesotrophic treatments either with fish or without fish did not significantly differ between the start and the end of the experiment.

**Table 2**. Results of one-way ANOVA comparing means (± SE) of concentrations of indicators of nutritive quality of seston in experimental mesocosms: EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid, Total FA – sum of all fatty acids, E – eutrophic, M –mesotrophic , F – fish, i – initial (June) date, f – final (July) date; means labelled with the same letter are not significantly different at $P < 0.05$ after Tukey HSD post hoc test.

| Treatment | EPA, µg/L | DHA, µg/L | Total FA, µg/L |
|---|---|---|---|
| $M_i$ | 3.78 ± 0.34$^{A}$ | 4.02 ± 0.49$^{A}$ | 54.86 ± 6.94$^{AC}$ |
| $MF_i$ | 4.47 ± 0.51$^{A}$ | 3.03 ± 0.90$^{A}$ | 54.55 ± 6.04$^{AC}$ |
| $E_i$ | 4.61 ± 0.24$^{A}$ | 4.12 ± 0.11$^{A}$ | 101.07 ± 11.74$^{BD}$ |
| $EF_i$ | 4.93 ± 0.48$^{A}$ | 4.10 ± 0.26$^{A}$ | 113.06 ± 9.89$^{B}$ |
| $M_f$ | 0.71 ± 0.24$^{B}$ | 0.08 ± 0.05$^{B}$ | 15.45 ± 3.12$^{A}$ |
| $MF_f$ | 1.56 ± 0.48$^{B}$ | 0.52 ± 0.39$^{B}$ | 29.51 ± 4.02$^{AC}$ |
| $E_f$ | 0.52 ± 0.05$^{B}$ | 0.05 ± 0.05$^{B}$ | 16.76 ± 0.94$^{A}$ |
| $EF_f$ | 1.60 ± 0.42$^{B}$ | 0.89 ± 0.65$^{B}$ | 68.85 ± 13.92$^{CD}$ |

Contents of fatty acids per organic carbon (C) in the seston (mg/g) are given in Table 3. EPA:C ratios were similar in all the treatments except for low values observed in EF at the end of the experiment (Table 3), which probably means a significant decrease of the relative abundance of diatoms in the phytoplankton community. DHA:C tended to be higher at the start of the experiment in all the treatments than at the end of the experiment (Table 3) which probably represents a decrease in the relative abundance of chrysophytes over the course of the experiment in all the treatments. Total FA:C ratio did not differ between the treatments or between the start and the end of the experiment (Table 3).

**Table 3.** Results of one-way ANOVA comparing means (± SE) of content per organic carbon (C) of indicators of nutritive quality of seston in experimental mesocosms: EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid, Sum FA – sum of all fatty acids, E – eutrophic, M – mesotrophic, F – fish, i – initial (June) date, f – final (July) date; means labelled with the same letter are not significantly different at $P < 0.05$ after Tukey HSD post hoc test. If ANOVA is insignificant ($P > 0.05$), letter labels are absent.

| Treatment | EPA:C, mg/g | DHA:C, mg/g | Total FA:C, mg/g |
|---|---|---|---|
| $M_i$ | 7.7 ± 0.6$^{A}$ | 8.2 ± 1.0$^{A}$ | 113.0 ± 19.0 |
| $MF_i$ | 7.6 ± 0.6$^{A}$ | 5.2 ± 1.5$^{AC}$ | 93.0 ± 9.5 |
| $E_i$ | 5.6 ± 0.2$^{A}$ | 5.0 ± 0.4$^{AB}$ | 122.1 ± 10.1 |
| $EF_i$ | 5.7 ± 0.3$^{A}$ | 4.8 ± 0.3$^{AB}$ | 130.6 ± 6.5 |
| $M_f$ | 5.1 ± 1.1$^{AB}$ | 1.0 ± 0.6$^{BD}$ | 129.9 ± 41.1 |
| $MF_f$ | 6.1 ± 0.6$^{A}$ | 1.5 ± 1.0$^{BCD}$ | 130.4 ± 32.0 |
| $E_f$ | 5.0 ± 0.9$^{AB}$ | 0.4 ± 0.4$^{D}$ | 161.5 ± 15.2 |
| $EF_f$ | 2.0 ± 0.5$^{B}$ | 1.1 ± 0.7$^{BCD}$ | 85.5 ± 16.5 |

At the beginning of the experiment, chrysophytes had the highest biomass in all the treatments (44–61% of the total algal biomass) (Figure 4) with *Dinobryon* sp. dominating. At the end of the experiment, the green filamentous algae *Oedogonium* sp. and *Mougeotia* sp. contributed 42–59% to the total algae biomass. Cyanobacteria *Limnothrix redekeii* Van Goor and *Oscillatoria* sp. dominated in the

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EF treatment at the end of the experiment (28% of the total algal biomass), while their biomass remained insignificant in the other treatments, including in the mesotrophic treatment with fish (4%). These results were in accordance with DHA:C analysis in seston which showed that chrysophytes declined in all the treatments.

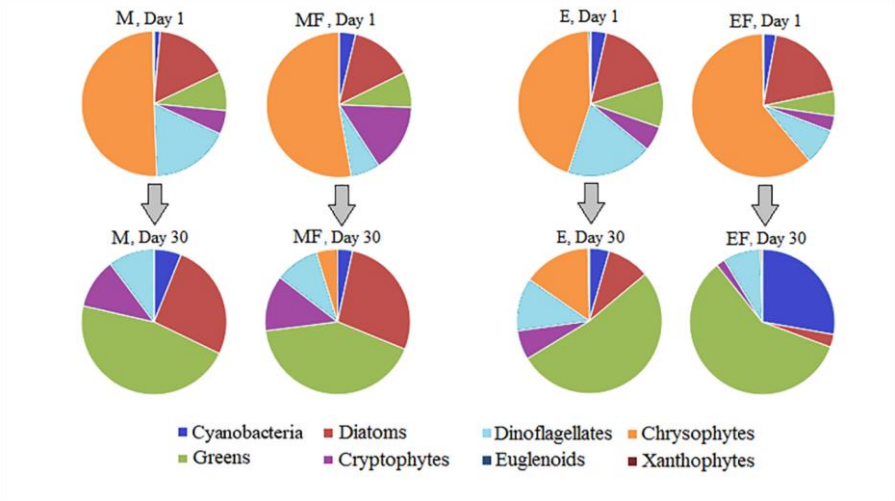


**Figure 4.** Taxonomic structure of phytoplankton community on Day 1 and Day 30 in M – mesotrophic conditions, MF – mesotrophic conditions with introduced fish, E – eutrophic conditions, EF – eutrophic conditions with introduced fish.

The size structure of algae changed with time and in response to fish (Figure 5, Table 4). In all of the treatments, algae between 30–50 µm disappeared by the end of the experiment. In the EF treatment, the biomass of algae < 30 µm decreased, while the biomass of algae > 50 µm increased relative to the other treatments.

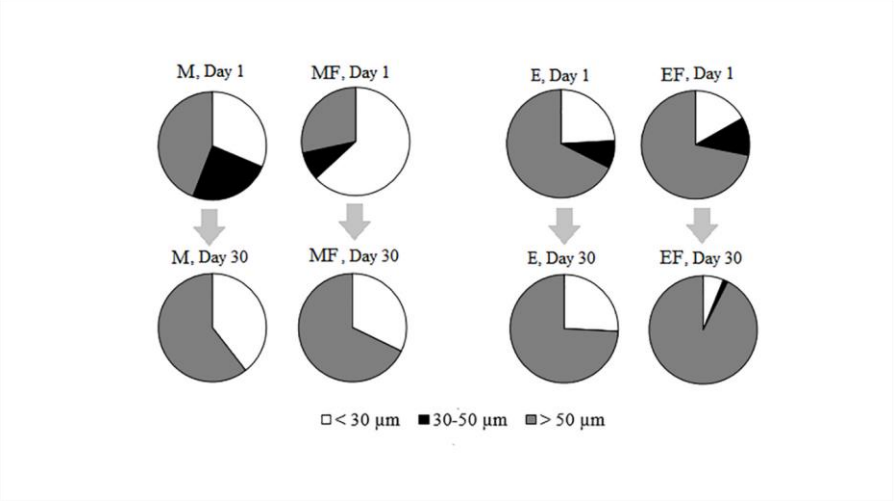


**Figure 5.** Size structure of phytoplankton on Day 1 and Day 30 in M – mesotrophic conditions, MF – mesotrophic conditions with introduced fish, E – eutrophic conditions, EF – eutrophic conditions with introduced fish.

348 **Table 4.** Results of two-way repeated measures ANOVA (RM ANOVA) with factors treatment (M,
349 MF, E, EF) and time. M – mesotrophic conditions, MF – mesotrophic conditions with introduced
350 fish, E – eutrophic conditions, EF – eutrophic conditions with introduced fish. Significant results ($p$
351 < 0.05) are shown in bold.
352

| Source | Sum of sqrs | df | Mean square | *F* | *P* |
|---|---|---|---|---|---|
| | Mesotrophic conditions (M, MF) | | | | |
| *Phytoplankton biomass < 30 µm* | | | | | |
| Fish (presence/absence) | 0.20 | 1 | 0.20 | 5.70 | 0.14 |
| Time, days (1, 10, 20, 30) | 1.85 | 3 | 0.62 | 7.26 | **0.02** |
| Time× Fish | 0.14 | 3 | 0.05 | 0.97 | 0.47 |
| *Phytoplankton biomass 30–50 µm* | | | | | |
| Fish (presence/absence) | 0.005 | 1 | 0.005 | 1.39 | 0.36 |
| Time, days (1, 10, 20, 30) | 4.26 | 3 | 1.42 | 28.26 | **0.001** |
| Time× Fish | 0.69 | 3 | 0.23 | 9.38 | **0.01** |
| *Phytoplankton biomass > 50 µm* | | | | | |
| Fish (presence/absence) | 0.10 | 1 | 0.10 | 0.47 | 0.57 |
| Time, days (1, 10, 20, 30) | 0.77 | 3 | 0.26 | 4.25 | 0.06 |
| Time× Fish | 0.83 | 3 | 0.28 | 4.33 | 0.06 |
| | Eutrophic conditions (E, EF) | | | | |
| *Phytoplankton biomass < 30 µm* | | | | | |
| Fish (presence/absence) | 0.37 | 1 | 0.37 | 20.8 | **0.04** |
| Time, days (1, 10, 20, 30) | 2.98 | 3 | 0.99 | 24.5 | **0.001** |
| Time× Fish | 0.38 | 3 | 0.12 | 2.13 | 0.20 |
| *Phytoplankton biomass 30–50 µm* | | | | | |
| Fish (presence/absence) | 0.02 | 1 | 0.02 | 0.16 | 0.73 |
| Time, days (1, 10, 20, 30) | 8.47 | 3 | 2.82 | 423.1 | **<< 0.001** |
| Time× Fish | 0.45 | 3 | 0.15 | 0.95 | 0.47 |
| *Phytoplankton biomass > 50 µm* | | | | | |
| Fish (presence/absence) | 3.53 | 1 | 3.53 | 43.1 | **0.02** |
| Time, days (1, 10, 20, 30) | 44.97 | 3 | 14.99 | 291.1 | **<< 0.001** |
| Time× Fish | 4.28 | 3 | 1.43 | 13.89 | **0.004** |

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357 The biomass of cladocerans did not differ between eutrophic and mesotrophic conditions in the
358 fish treatments, or in the fish-free treatments (Figure 6). However, their biomass was significantly
359 reduced in fish treatments relative to that in fish-free treatments in the mesotrophic conditions. The
360 biomass of copepods did not differ between the treatments of the experiment.

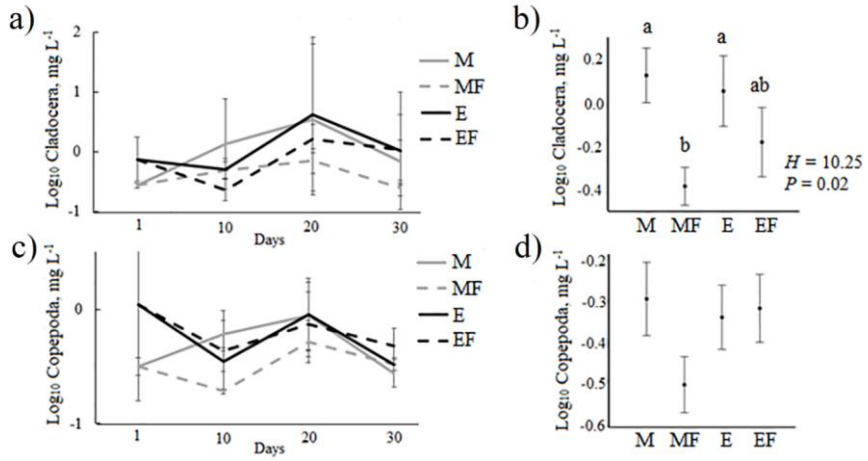


**Figure 6.** Dynamics of biomass of crustaceans in the experiments (a, c) and treatment means (b, d); different letters indicate significant differences at $P < 0.05$ after Kruskal – Wallis test (H); M – mesotrophic conditions, MF – mesotrophic conditions with introduced fish, E – eutrophic conditions, EF – eutrophic conditions with introduced fish.

Biomasses of *D. pulicaria* and *D. magna* did not differ between mesotrophic and eutrophic conditions in the free-fish treatments and between mesotrophic and eutrophic conditions in the fish treatments (Figure 7). However, the biomasses of these species in the treatments without fish were significantly higher than in the treatments with fish where they were very rare. Biomasses of *C. pulchella* did not differ between the treatments with fish and the treatments without fish either in mesotrophic or eutrophic conditions (Figure 7). Initially *C. pulchella* was more abundant in the M treatment and this species peaked earlier in M than in E treatments.

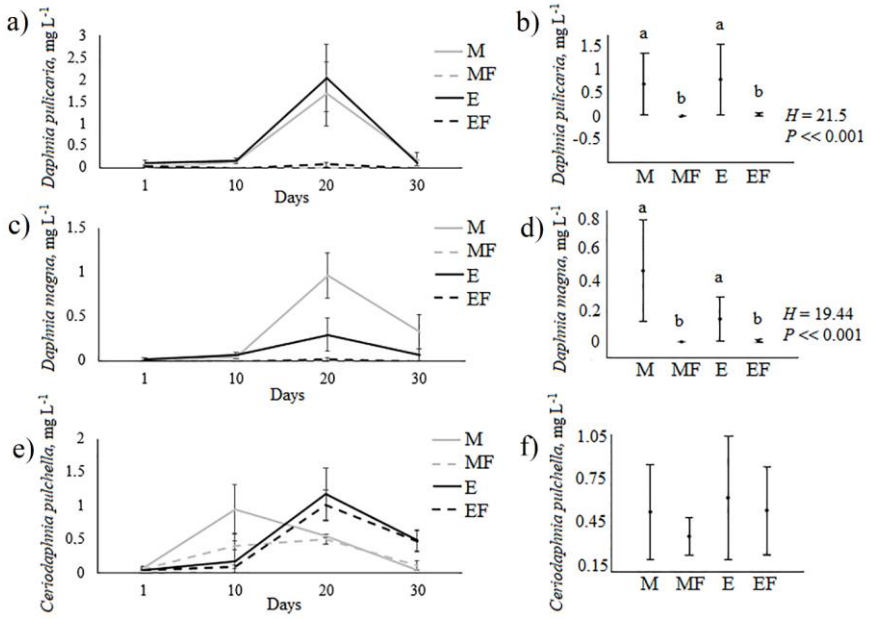


**Figure 7.** Dynamics of biomasses of *D. magna*, *D. pulicaria* and *C. pulchella* in the experiments (a, c, e) and treatment means (b, d, f); different letters indicate significant differences at P < 0.05 after Kruskal – Wallis test (H); M – mesotrophic conditions, MF – mesotrophic conditions with introduced fish, E – eutrophic conditions, EF – eutrophic conditions with introduced fish.

C: P and C: N in zooplankton did not differ between the treatments and between the beginning and end of the experiment (Table 5). However, C: P and C: N in zooplankton was significantly lower than in the seston in all of the treatments except one – C:N was not significantly greater in the seston in the M treatment at the end of the experiment (Table 6).

385 **Table 5.** Results of one-way ANOVA comparing mean values ( ± SE) of ratios of indicators of nutritive quality of zooplankton in experimental mesocosms: E –
386 eutrophic, LE – low-eutrophic , F – fish, i – initial (June) date, f – final (July) date, C - organic carbon, N - nitrogen, *P* - phosphorus, F Fisher's test, and P
387 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
388 normal distribution, Kruskal–Wallis test was used).
389

390

| | **$M_i$** | **$MF_i$** | **$E_i$** | **$EF_i$** | **$M_f$** | **$MF_f$** | **$E_f$** | **$EF_f$** | **F(H)** | ***P*-value** |
|---|---|---|---|---|---|---|---|---|---|---|
| C:N (mg/mg) | 4.65±0.52 | 4.09±0.07 | 4.46±0.26 | 3.82±0.42 | 4.50±0.09 | 5.18±0.28 | 4.26±0.39 | 4.10±0.12 | 1.8 | 0.15 |
| C:P (mg/mg) | 37.0±0.6 | 59.7±0.6 | 50.0±10.5 | 45.1±5.1 | 42.6±0.4 | 35.6±4.0 | 44.3±1.0 | 44.2±2.4 | 13.7 | 0.06 |

**Table. 6.** Results of Mann-Whitney comparing mean values (± SE) of ratios C:P and C:N of zooplankton and seston in experimental mesocosms: E – eutrophic, M – mesotrophic , F – fish, i – initial (June) date, f – final (July) date; means labelled with the same letter are not significantly different at $P < 0.05$. Significant results ($P < 0.05$) are shown in bold.

| Treatment | C:P (mg/mg) | | *P*-value | C:N (mg/mg) | | *P*-value |
|---|---|---|---|---|---|---|
| | zooplankton | seston | | zooplankton | seston | |
| $M_i$ | 37.0±0.6$^A$ | 136.6±15.2$^B$ | ***P* = 0.03** | 4.65±0.52$^A$ | 7.16±0.25$^B$ | ***P* = 0.03** |
| $MF_i$ | 59.7±0.6$^A$ | 129.0±5.2$^B$ | ***P* = 0.03** | 4.09±0.07$^A$ | 6.71±0.28$^B$ | ***P* = 0.03** |
| $E_i$ | 50.0±10.5$^A$ | 182.5±10.6$^B$ | ***P* = 0.03** | 4.46±0.26$^A$ | 8.18±0.33$^B$ | ***P* = 0.03** |
| $EF_i$ | 45.1±5.1$^A$ | 150.1±12.9$^B$ | ***P* = 0.03** | 3.82±0.42$^A$ | 7.54±0.19$^B$ | ***P* = 0.03** |
| $M_f$ | 42.6±0.4$^A$ | 173.9±103.1$^B$ | ***P* = 0.03** | 4.50±0.09$^A$ | 7.39±2.12$^A$ | $P = 0.31$ |
| $MF_f$ | 35.6±4.0$^A$ | 156.7±36.1$^B$ | ***P* = 0.03** | 5.18±0.28$^A$ | 6.96±0.16$^B$ | ***P* = 0.03** |
| $E_f$ | 44.3±1.0$^A$ | 68.5±3.5$^B$ | ***P* = 0.03** | 4.26±0.39$^A$ | 8.29±0.52$^B$ | ***P* = 0.03** |
| $EF_f$ | 44.2±2.4$^A$ | 287.6±67.3$^B$ | ***P* = 0.03** | 4.10±0.12$^A$ | 6.79±0.16$^B$ | ***P* = 0.03** |

In zooplankton, EPA:C and total FAs did not differ between the treatments at the beginning of the experiment (Table 7). By the final day, however, both of these indicators of zooplankton quality had decreased in fish-free treatments (M and E) relative to the initial date while in the fish treatments these indicators remained at the initial level. Therefore, zooplankton quality in terms of EPA:C and/or FA:C in the fish treatments were higher than in the corresponding treatments without fish. The content of DHA in zooplankton was initially higher in eutrophic (E and EF) than in mesotrophic treatments (M and MF). By the end of the experiment, DHA content had decreased in the fish-free treatments (M and E) relative to initial values, while in the fish treatments (MF and EF) DHA content did not significantly change. The changes in contents of PUFAs and FAs in zooplankton did not follow corresponding changes in the seston. We suggest that assimilation and consumption of phytoplankton taxa can be different and zooplankton content of quality indicators better characterized food conditions for zooplankton than seston quality indicators.

**Table 7**. Results of one-way ANOVA comparing means (± SE) of content of indicators of nutritive quality of zooplankton in experimental mesocosms at the beginning and end of the experiment: EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid, Total FA – sum of all fatty acids, E – eutrophic, M – mesotrophic , F – fish, i – initial (June) date, f – final (July) date; means labelled with the same letter are not significantly different at $P < 0.05$ after Tukey HSD post hoc test. If ANOVA is insignificant ($P > 0.05$), letter labels are absent.

| Treatment | EPA:C, mg/g | DHA:C, mg/g | Total FA:C, mg/g |
|---|---|---|---|
| $M_i$ | 1.05 ± 0.04$^A$ | 0.75 ± 0.10$^{ABD}$ | 12.88 ± 0.73$^A$ |
| $MF_i$ | 0.97 ± 0.06$^A$ | 0.56 ± 0.09$^{AC}$ | 10.28 ± 0.68$^{AC}$ |
| $E_i$ | 0.91 ± 0.03$^A$ | 0.99 ± 0.04$^B$ | 11.57 ± 0.25$^{AD}$ |
| $EF_i$ | 0.85 ± 0.02$^{AC}$ | 1.06 ± 0.01$^B$ | 10.55 ± 0.08$^{ADC}$ |
| $M_f$ | 0.47 ± 0.09$^B$ | 0.25 ± 0.03$^C$ | 3.66 ± 0.34$^E$ |
| $MF_f$ | 0.91 ± 0.13$^A$ | 0.67 ± 0.14$^{ABC}$ | 7.60 ± 1.18$^{BC}$ |
| $E_f$ | 0.54 ± 0.03$^{BC}$ | 0.36 ± 0.09$^{CD}$ | 5.29 ± 0.38$^{BE}$ |
| $EF_f$ | 0.82 ± 0.05$^A$ | 0.89 ± 0.11$^{AB}$ | 9.27 ± 0.78$^{CD}$ |

419 The contents of EPA and total FAs was significantly lower in zooplankton than in phytoplankton
420 (Table 8). DHA was lower in seston at the beginning of the experiments, however, it was reduced in
421 seston by the end of the experiment and did not differ significantly between seston and zooplankton.
422 In general, we can conclude that zooplankton poorly accumulated PUFA and FAs in contrast to P
423 and N.
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**Table. 8.** Results of Mann-Whitney (*U*) comparing means (± SE) of contents per organic carbon (C) of indicators of nutritive quality of zooplankton and seston in experimental mesocosms: EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid, Total FA – sum of all fatty acids, E – eutrophic, M – mesotrophic , F – fish, i – initial (June) date, f – final (July) date; means labelled with the same letter are not significantly different at $P < 0.05$. Significant results ($P < 0.05$) are shown in bold.

| Treatment | EPA:C, mg/g | | *P*-value | DHA:C, mg/g | | *P*-value | Total FA:C, mg/g | | *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| | zooplankton | seston | | zooplankton | seston | | zooplankton | seston | |
| $M_i$ | 1.07±0.03$^A$ | 7.7±0.6$^B$ | **$P = 0.03$** | 0.77±0.07$^A$ | 8.2±1.0$^B$ | **$P = 0.03$** | 12.87±0.73$^A$ | 113±19.0$^B$ | **$P = 0.03$** |
| $MF_i$ | 1.00±0.06$^A$ | 7.6±0.6$^B$ | **$P = 0.03$** | 0.57±0.09$^A$ | 5.2±1.5$^B$ | **$P = 0.03$** | 10.30±0.71$^A$ | 93.0±9.5$^B$ | **$P = 0.03$** |
| $E_i$ | 0.93±0.03$^A$ | 5.6±0.2$^B$ | **$P = 0.03$** | 0.97±0.03$^A$ | 5.0±0.4$^B$ | **$P = 0.03$** | 11.60±0.25$^A$ | 122.1±10.1$^B$ | **$P = 0.03$** |
| $EF_i$ | 0.87±0.03$^A$ | 5.7±0.3$^B$ | **$P = 0.03$** | 1.10±0.00$^A$ | 4.8±0.3$^B$ | **$P = 0.02$** | 10.53±0.09$^A$ | 130.6±6.5$^B$ | **$P = 0.03$** |
| $M_f$ | 0.47±0.09$^A$ | 5.1±1.1$^B$ | **$P = 0.03$** | 0.23±0.03$^A$ | 1.0±0.6$^A$ | $P = 0.30$ | 3.67±0.33$^A$ | 129.9±41.1$^B$ | **$P = 0.03$** |
| $MF_f$ | 0.93±0.13$^A$ | 6.1±0.6$^B$ | **$P = 0.03$** | 0.63±0.15$^A$ | 1.5±1.0$^A$ | $P = 0.31$ | 7.60±1.15$^A$ | 130.4±32.0$^B$ | **$P = 0.03$** |
| $E_f$ | 0.53±0.03$^A$ | 5.0±0.9$^B$ | **$P = 0.03$** | 0.37±0.09$^A$ | 0.4±0.4$^A$ | $P = 0.66$ | 5.30±0.36$^A$ | 161.5±15.2$^B$ | **$P = 0.03$** |
| $EF_f$ | 0.83±0.07$^A$ | 2.0±0.5$^B$ | **$P = 0.03$** | 0.90±0.12$^A$ | 1.1±0.7$^A$ | $P = 1$ | 9.27±0.79$^A$ | 85.5±16.5$^B$ | **$P = 0.03$** |

Table 9 indicates that during the life-table experiments, the average concentrations of total chlorophyll, diatoms & dinophytes and greens in the fish treatments were higher than in the corresponding treatments without fish. In the eutrophic conditions, cyanobacteria concentrations were higher in the fish treatments than in the treatment without fish. In the mesotrophic conditions, cyanobacteria were either absent or rare. Table 10 shows that there were no differences in resource concentrations between the mesocosm and life-table experiments except MF bottles with *Daphnia* species where food concentrations were lower in life table experiments than in the corresponding mesocosms.

**Table 9.** Results of one-way ANOVA comparing means ( ± SE) of chlorophyll concentrations for the period of life-table experiments in E – eutrophic, LE – low-eutrophic , F – fish treatments, F Fisher's test; *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 (H) was used).

| Chlorophyll | M | MF | E | EF | *F (H)* | *P* |
|---|---|---|---|---|---|---|
| Total, μg $L^{-1}$ | 13.26±0.48$^{a}$ | 29.60±1.21$^{b}$ | 22.95±1.21$^{c}$ | 42.28±1.26$^{d}$ | 125.4 | **<<0.01** |
| Diatoms&Dinoflagellates, μg $L^{-1}$ | 12.61±0.37$^{a}$ | 25.23±0.95$^{b}$ | 20.71±0.66$^{c}$ | 28.98±0.69$^{d}$ | 102.3 | **<<0.01** |
| Greens algae, μg $L^{-1}$ | 0.62±0.14$^{a}$ | 4.33±0.46$^{b}$ | 2.23±0.60$^{c}$ | 5.74±0.79$^{b}$ | 16.66 | **<<0.01** |
| Cyanobacteria, μg $L^{-1}$ | 0$^{a}$ | 0.04±0.04$^{a}$ | 0$^{a}$ | 7.56±1.13$^{b}$ | 77.63 | **<<0.01** |

**Table 10.** Results of one-way ANOVA comparing means (± SE) of total chlorophyll concentrations (μg $L^{-1}$) for the period of life-table experiments in mesocosms and bottles with *D. pulicaria, D. magna* and *C. pulchella*. E – eutrophic, M – mesorophic, F – fish treatments, F Fisher's test, and *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 (H) was used).

| Treatments | Mesocosms | *D. pulicaria* | *D. magna* | *C. pulchella* | *F (H)* | *P* |
|---|---|---|---|---|---|---|
| M | 13.3±0.5 | 12.4±0.5 | 14.2±1.6 | 15.4±1.4 | 4.1 | 0.25 |
| MF | 29.6±1.2$^{A}$ | 20.9±0.8$^{B}$ | 19.8±0.8$^{B}$ | 31.5±1.5$^{A}$ | 53.8 | **<<0.01** |
| E | 22.9±1.2 | 25.7±1.5 | 23.8±1.3 | 27.0±1.5 | 1.2 | 0.33 |
| EF | 42.3±1.3 | 41.7±1.9 | 46.4±4.5 | 45.9±1.3 | 4.3 | 0.23 |

In the life-table experiments, the rate of population growth (*r*) differed both between the treatments and between species within individual treatments (Table 11). The population growth rate in *C. pulch*ella was higher than that in the two species of *Daphnia* in all the treatments and was always positive. The minimum *r* in *C. pulchella* was observed in mesotrophic conditions while its maximum population growth rate was recorded in eutrophic conditions with fish. Strongly negative *r* values were observed in both *Daphni*a species in the M and E treatments, i.e. these species were gradually dying out over the course of the experiment. In the two *Daphni*a species, r was positive in MF (although the resource concentration in this treatment was lower than in the mesocosms, see Table 10) and slightly negative in EF but it was higher than in M and E. The survival rate of all individuals in the life-table was 100%. Therefore, *r* mainly depended on the clutch sizes and the duration of juvenile development until maturity. Table 11 shows that *C. pulche*lla always had eggs after reaching maturity. Moreover, fecundity of *C. pulchella* was significantly dependent on the concentration of the total chlorophyll (Figure 8). In *Daphnia*, there was no relationship between the fecundity and

concentration of the food resource. Both *Daphnia* laid eggs in the treatment with fish, but eggs were extremely rare in the mesocosms without fish. In the MF treatment, in both *Daphnia* species, there were more than 2 eggs per female whereas in EF, fecundity was less than one egg per female (Table 11). Therefore, fish positively affected the population growth rate of *Daphnia* via changing food quality and/or quantity. The juvenile developmental time until maturity (first clutch) in both *Daphnia* species was lower in MF and EF than in fish free treatments, M and E. *C. pulchella* reached maturity for 4 days, i.e. faster than daphnids, in all the treatments except M where the first clutch was laid in 10 days after birth. In this treatment, the average concentration of food was the lowest compared to the other treatments.

**Table 11.** Demographic parameters (± SE) of *D. magna, D. pulicaria* and *C. pulchella* in E – eutrophic, M – mesotrophic, F – fish treatments; Kruskal–Wallis (H) test, Dunn's post hoc test ($P < 0.05$) was used for pairwise comparisons of population growth rate, fecundity, time of first clutch along the columns and along the lines. *P* significant values are given in bold; means labeled with the same letter/asterics are not significantly different along the same columns/line. Letters are given for comparisons along the columns while asterics show discrepancies along the lines.

| **Treatments** | ***D. pulicaria*** | ***D. magna*** | ***C. pulchella*** | |
|---|---|---|---|---|
| | | Population growth rate | | |
| M | -2±0$^{a,}$ * | -2±0$^{a,}$ * | 0.02±0.01$^{a,}$ ** | $H^*$ = 7.6, $\mathbf{P^*}$ **= 0.02** |
| MF | 0.17±0.03$^{b,}$ * | 0.14±0.03$^{b,}$ * | 0.29±0.02$^{bc,}$ ** | $H^*$ = 6.0, $\mathbf{P^*}$ **= 0.05** |
| E | -2±0$^{a,}$ * | -2±0$^{a,}$ * | 0.27±0.04$^{b,}$ ** | $H^*$ = 7.6, $\mathbf{P^*}$ **= 0.02** |
| EF | -0.013±0.04$^{c,}$ * | -0.15±0.10$^{c,}$ * | 0.37±0.01$^{c,}$ ** | $H^*$ = 6.0, $\mathbf{P^*}$ **= 0.05** |
| | $H$ = 10.6 | $H$ = 10.6 | $H$ = 9.4 | |
| | ***P* = 0.01** | ***P* = 0.01** | ***P* = 0.02** | |
| | | Fecundity | | |
| M | 0$^{a,}$ * | 0$^{a,}$ * | 0.25±0.01$^{a,}$ ** | $H^*$ = 7.6, $\mathbf{P^*}$ **= 0.02** |
| MF | 2.72±1.52$^{b,}$ * | 2.13±0.35$^{b,}$ * | 2.39±0.43$^{b,}$ * | $H^*$ = 0.3, $P^*$ = 0.88 |
| E | 0$^{a,}$ * | 0$^{a.,}$ * | 1.69±0.53$^{b,}$ ** | $H^*$ = 7.6, $\mathbf{P^*}$ **= 0.02** |
| EF | 0.73±0.34$^{ab,}$ * | 0.17±0.03$^{a,}$ * | 3.77±0.32$^{c,}$ ** | $H^*$ = 7.3, $\mathbf{P^*}$ **= 0.03** |
| | $H$ = 10.2 | $H$ = 10.7 | $H$ = 9.5 | |
| | ***P* = 0.02** | ***P* = 0.01** | ***P* = 0.02** | |
| | | Time of first clutch | | |
| M | 30±0$^{a,}$ * | 30±0$^{a,}$ * | 10±1.15$^{a,}$ ** | $H^*$ = 7.6, $\mathbf{P^*}$ **= 0.02** |
| MF | 6.67±0.67$^{b,}$ * | 7.33±0.67$^{b,}$ * | 4±0$^{b,}$ ** | $H^*$ = 6.2, $\mathbf{P^*}$ **= 0.04** |
| E | 30±0$^{a,}$ * | 30±0$^{a,}$ * | 4±0$^{b,}$ ** | $H^*$ = 8.0, $\mathbf{P^*}$ **= 0.02** |
| EF | 9.33±0.67$^{c,}$ * | 10±0$^{c,}$ * | 4±0$^{b,}$ ** | $H^*$ = 7.0, $\mathbf{P^*}$ **= 0.03** |
| | $H$ = 10.5 | $H$ = 10.9 | $H$ = 10.7 | |
| | ***P* = 0.01** | ***P* = 0.01** | ***P* = 0.01** | |

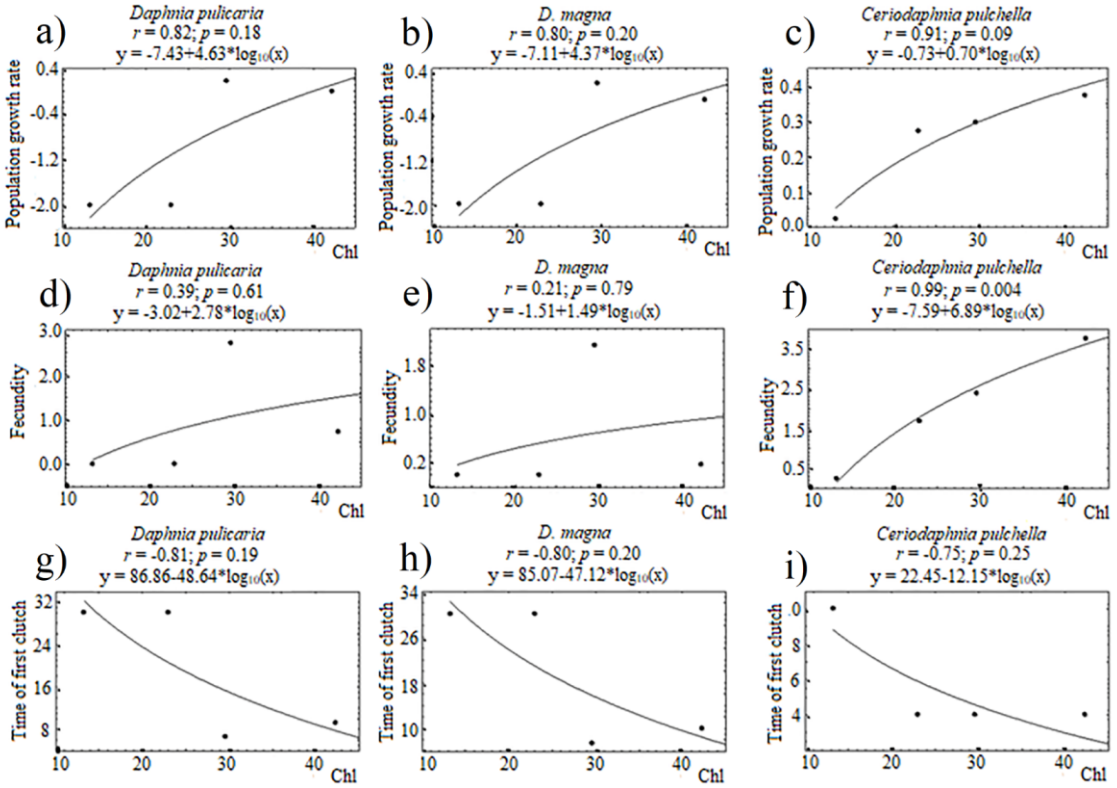


**Figure 8.** Relationships between total chlorophyll (Chl) and population growth rate of *D. pulicaria* (a), *D. magna* (b) and *C. pulchella* (c); between total chlorophyll and fecundity of *D. pulicaria* (d), *D. magna* (e) and *C. pulchella* (f); between total chlorophyll and time of first clutch of *D. pulicaria* (g), *D. magna* (h) and *C. pulchella* (i) in the mesocosms. Above the graphs is the correlation coefficient, the significance level of the regression equation and the regression equation.

Contents of EPA+DHA in *D. magna* and *D. pulicaria* in the experiment, 0.42 ± 0.03 mg/g and 0.36 ± 0.07 mg/g, respectively, did not differ significantly: $P > 0.05$ after Mann-Whitney's U-test. It means that diets of both species of *Daphnia* were similar.

## 4. Discussion

It has long been known that fish can structure zooplankton communities directly through predation. Less understood is how fish indirectly affect zooplankton through changes in algal food quantity and quality. While fish did not directly affect nutrient concentrations in the mesocosms, they did affect algal quality in terms of species composition and seston nutrient and PUFA contents. Fish recycle nutrients either via fish excretion or by changing phytoplankton composition indirectly through consumption of crustaceans. In mesocosms, zooplankton biomass was regulated by fish grazing on zooplankton. Especially it was concerned large daphnids. *C. pulchella* and copepods did not show differences in biomass in the treatments with fish and without fish.

There continues to be debate as to whether food quality or food quantity is a main driver of zooplankton dynamics. Based on theory of the threshold food concentration (TFC), the species with higher TFC should be suppressed by species with lower TFC [48,14]. If it is so, food concentration can be a limiting factor for inferior competitors because superior competitors would decrease it up to its TFC. However, there is evidence indicating that food quality can also affect species dynamics. For example, phosphorus [45,16], polyunsaturated fatty acids (PUFA) or fatty acids (FAs) [49,6] can also be limiting factors that constrain the development of crustaceans even if carbon concentrations are high. In our experiment we had four treatments that were distinguished by trophic state (mesotrophic and eutrophic) and fish (presence/absence) both of which can potentially change phytoplankton structure and/or food quantity and quality. Only the small bodied species *C. pulchella* showed a significant relationship between its fecundity and resource concentration. Population growth rate in *C. pulchella* was the highest at the greatest food concentration (EF treatment) and lowest at the smallest resource concentration (M treatment). In contrast, both species of *Daphnia* had low fecundity at the highest resource concentration (EF) and the highest fecundity at the intermediate resource concentration (MF). Therefore, $r$ for daphnids was the highest in MF and it was strongly negative in M and E. In EF, $r$ was negative but higher than in M and E. Thus, we can conclude that small and large cladoceran species responded differently to the food concentrations. If we can assume that *C. pulchella* abundance was regulated by resource concentration, *Daphnia* abundance was rather dependent on food quality.

In general, daphnids are known as nonselective filter feeders that do not selectively consume individual food particles [50]. Their diet spectrum is restricted by the size of food items and varies from 1 to 20–30 mm [21]. Indeed, according to the contents of EPA and DHA, diets of *D. magna* and *D. pulicaria* were similar. Besides, they are both large bodied species and they equally needed to allocate a great portion of consumed energy to their growth. For this reason, their demographic parameters changed similarly in response to the treatment effects. However, in contrast to large daphnids, *C. pulchella* do not need to allocate so much energy to growth and can spend more energy on reproduction. As our experiments showed, *C. pulchella* reached maturity earlier and clutch sizes were always larger than in large *Daphnia* under the same conditions. Population growth rates of *C. pulchella* were always positive and higher than those of the large *Daphnia*. Only in MF, $r$ of *C. pulchella* was similar to that of both *Daphnia* species. It is noteworthy that despite negative $r$ in *Daphnia* in some treatments, survival was 100% everywhere, however, fecundity was close to zero in the treatments without fish. This result is in accordance with contribution theory [51]. According this theory, daphnids prioritize energy allocation. When the concentration of resources is limiting, allocation priority of an individual is to stay alive at the expense of reproduction. When trophic conditions improve, resource partitioning is directed to provide body growth and increase clutch size. Therefore, we suggest that there was strong limitation of *Daphnia* population growth in M and E treatments while $r$ increased in EF treatment although it was still negative and in MF where it was the highest.

For *C. pulchella*, the best trophic conditions were in EF and the worst in M. Since its fecundity was linearly related to food concentration, we can conclude that limiting factor for *C. pulchella*, was resource abundance. But for *Daphnia* species, which had the highest rate in MF, i.e. at intermediate food concentration and negative in EF at the highest food concentration, we suggested that they were rather subject to limitation of resource quality.

The threshold concentration of sestonic EPA for the growth of *Daphnia* is equal to 13 mg $L^{-1}$ [52]. In our experiments, seston EPA concentrations were much lower and therefore EPA may have constrained *Daphnia* growth rates. EPA and DHA concentrations were higher at the beginning of the experiment than at the end while FA did not differ significantly between the start and the end of the experiments in all the treatments. These data indicated that in terms of PUFAs, food quality gradually deteriorated for *Daphnia*. For this reason, abundance of *Daphnia* increased in the first half of the experiment and decreased in the second half. Content of EPA and DHA in phytoplankton, i.e. ratios EPA:C and DHA:C less explicitly differed between the start and the end of the experiment. EPA:C was the least in EF at the end. FA:C did not differ between the treatments and between the start and the end of the experiment.

If comparing PUFA:C and/or FAs:C between zooplankton and phytoplankton, we can see that this ratio is much lower in zooplankton indicating that zooplankton can accumulate only approximately 10% of PUFA and/or FAs relative to phytoplankton. The other part of seston EPA is likely lost, maybe because it is inaccessible for zooplankton. Despite deterioration of food quality in term of PUFAs concentrations by the end of the experiment, EPA:C decreased in zooplankton only in treatments without fish. If fish were present, EPA content in zooplankton at the end was similar to that at the start of the experiment. In addition, this ratio in zooplankton at the end of the experiments was higher in the treatment with fish than in the corresponding treatments without fish. Similarly, DHA:C in zooplankton was higher in EF than in E at the end. FA:C at the end was higher in MF than in M and in EF was greater than in E. Therefore, contents of PUFA and FAs in zooplankton was higher in treatments with fish than in the corresponding treatments without fish. Based on these data, we can conclude that food conditions were worse for zooplankton in the treatments without fish. However, estimations of seston quality in terms of PUFA concentrations did not show differences between treatments with fish and without fish at the end of the experiment.

We think that since the fish significantly reduced the abundance of cladoceran species in the MF and EF treatment, the share of preferred resources by zooplankton species increased in these treatments relative to the M and E treatments, respectively. We suggest that phytoplankton species are not equally accessible for zooplankton due to their sizes or assimilation ability. Therefore, seston quality can be similar in the treatments with fish and without fish, but the edible fraction for cladoceran species can be different. Diet of cladocerans are known to be constrained by food particle size [50]. Besides, they can differently retain or assimilate particulate food items. In support, cladocerans were shown to selectively accumulate EPA from food [53-56]. Additionally, Taipale et al. [57] found that cladoceran $\delta^{13}C$ values did not correlate with seston $\delta^{13}C$ values and instead correlated with the $\delta^{13}C$ 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. [58]. Therefore, we argue that zooplankton contents of PUFA and FAs is a better indicator of food conditions.

If comparing C:P or C:N in seston or zooplankton between the treatments and between the start and the end of the experiments, there were no significant differences between the treatments in both for phytoplankton and zooplankton. C:P ratios in seston were quite high and could constrain the growth of daphnids because they were higher than threshold for *Daphnia* development equaled to 225 – 375 μmol/μmol [46]. However zooplankton C:P was significantly higher than that in seston. There are evidences in literature that *Daphnia* can increase P-retention if P content of resources is in shortage [21,45,59]. Feniova et al. [33] suggest that there was selective accumulation of food particles that are rich in P by *Daphnia*. Such mechanisms of phosphorus retention enabled crustaceans to increase phosphorus in their body. Despite phosphorus content of seston in EF treatment was significantly lower than in the other treatments, phosphorus content in zooplankton did not differ

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significantly. In regard to C:N there was not any mismatch between seston nitrogen content and that of zooplankton. Therefore, we do not think that there was nitrogen limitation in the experiment.

Thus, higher population growth rates of *Daphnia* in the MF and EF treatments relative to corresponding treatments without fish were not related to food concentrations. We assumed that it could be associated with higher share of edible size range (<30 μm) in the treatments with fish but the data on phytoplankton size structure did not confirm this assumption. Taxonomic composition was also similar. Contents of phosphorus and nitrogen in zooplankton in the MF were not higher than in the other treatments. Therefore, we believe that PUFA and FAs, especially EPA, which is the most important component for *Daphnia*, can be the main limiting factors which did not allow *Daphnia* to reproduce in the treatments without fish. Unfortunately, we do not know explicitly why population growth rate in *Daphnia* was higher in MF than in EF. However, results of canonical correspondence analysis of fatty acid (FA) composition (% of total FAs) of seston showed that in EF treatment at the end of the experiment inedible green algae were most abundant. In addition, data on taxonomic composition indicated that in eutrophic conditions the share of attached green algae and cyanobacteria were much higher than in mesotrophic treatments. Since these algae are not accessible for zooplankton, the portion of PUFA and FAs in edible seston particles could be less in eutrophic than in mesotrophic conditions.

Small *Ceriodaphnia* and large *Daphnia* species demonstrated that they differently respond to food conditions. While *Ceriodaphnia* population growth was positively related to food concentrations, *Daphnia* population growth was restricted by food quality likely in terms of PUFAs and FAs. Our findings are in accordance with Sikora et al. [17] which showed that large bodied species were more sensitive to low food quality than small-bodied ones in terms of PUFA and/or C:P ratio. Sikora et al. [18] showed that EPA-saturation thresholds, which are defined as the minimal concentration of EPA above which the juvenile growth rate becomes saturated, increased significantly with increasing body size of the tested species.

In this aspect, small *Ceriodaphnia* have a strategy of "patients" [60] or "stress-tolerators" [61] in the plankton, i.e. species that inhabit degraded environment, while *Daphnia* are typical of "violents" strategy [60] or 'competitors [61] that suppress "patients" when conditions recovered. The shift from "patients" to "violents" in zooplankton communities can be caused by gradual alteration in food quality. In our case, food quality in the middle of the experiments was not sufficient for *Daphnia* to reproduce in the treatments without fish while in the treatment with fish, *Daphnia* were selectively suppressed by fish. For this reason, *Daphnia* were not successful in any treatment in the second half of the experiments. Although during the first 10 days, when food quality was better, large daphnids reproduced and increased in biomass.

To conclude, we suggest that small *Ceriodaphnia* was regulated mainly by resource concentration while large species of cladocerans were limited by food quality and differences in sestion PUFA and FAs. Fish likely increased food quality in terms of PUFA, primarily EPA, thus providing conditions for more successful development of *Daphnia* than in the fish-free treatments. Seston food quality in terms of PUFA and FA and nutrient concentrations appeared not to be good indicators of food conditions due to different constraints for consumption by zooplankton including inappropriate sizes or shapes of resources, low assimilation or ingestion rates. Phosphorus in seston was likely limiting for zooplankton. However, food quality in terms of phosphorus is less important than PUFA because zooplankton can accumulate nutrients in their body.

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**Author Contributions:** I.F. participated in the experiments, wrote the manuscript; E.S. made statistical analyses; M.K. participated at all stages of the studies, processed and analyze crustacean samples; M.G. and N.S. processed PUFA and C, N and P data and analyzed data on fatty acids and elemental compositions in phyto and zooplankton; P.D. was responsible for the methodological part of the experiments and provided crustacean and fish material for the studies; Z.G. processed and analyzed phytoplankton samples; A.G. processed and analyzed nutrient samples; Y.S. participated in the experiments; A.D. supervised and coordinated the research studies and revised and edited manuscript.

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