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The Effect of Feed Composition on the Structure of Zooplankton Communities in Fishponds

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Abstract: With the intensification of aquaculture, the structure and dynamics of aquatic ecosystems are highly affected. At the same time, for a pond fish farmer, one of the most important tasks is to establish and maintain stable and favourable zooplankton populations. In this paper, we assess the effects of different supplementary feed types on zooplankton communities in freshwater fishponds. In an outdoor, experimental fishpond system, carp individuals of 2+ years of age were stocked and fed with either a fishmeal-based diet (FF), a plant meal containing experimental feed (PF) or cereals (CT). To compare the diversity of the zooplankton communities, we used the Shannon diversity index, and to assess the effects of environmental factors and the feed ingredients, we applied canonical correspondence analysis. We described the dynamics of zooplankton communities, where the biodiversity of rotifera and Crustacean communities showed temporal differences. In order to examine the effects of feed ingredients, temporal clustering was applied. The different diets did not consistently alter the composition of zooplankton communities. Consequently, the plant meal containing experimental feed had no negative effect on the planktonic biodiversity, which makes it suitable as an alternative feed source for fishponds at the applied level.

Keywords: cladocera; copepoda; rotifera; fishponds; aquaculture; carp; Shannon diversity; CCA

1. Introduction

The human population is constantly growing, leading to a steady increase in the developments of agriculture and aquaculture. Nowadays, aquaculture is the fastest growing livestock sector in the world [1]. In parallel, our ecosystems worldwide are rapidly losing their functional, genetic and phylogenetic diversity due to habitat changes, human exploitation of natural resources and the spread of pathogens, exotic and domestic animals and plants [2]. In addition to the economic approach to technological development in fisheries, the ecological approach should not be overlooked. The global fish production was over 171 million tonnes, 47% of which (around 80 million tonnes) were accounted for by aquaculture in 2016. In that year, the world aquaculture carp production was 4.5 million tonnes [1]. In recent decades, carp production has intensified in Europe and other parts of the world [3,4].

In Hungary, carp (*Cyprinus carpio* Linnaeus, 1758) production is dominant in the production of fish for consumption. In 2018, it reached 11,400 tonnes, accounting for 79.5% of pond-farmed fish [5].

As aquaculture production increases with feed intake and waste, the amounts of organic matter, nutrients and suspended solids also increase [6]. There is a growing concern about the potential negative environmental impacts of aquaculture [7], in particular, the discharge of nutrients from the effluent of such systems. The impact of feed ingredients on water quality has been widely studied in different systems in as much as they affect the water quality and growth performance of fish in the receiving water [8–11]. Extensive systems are under pressure to intensify due to market demands. The sustainable intensification requires feed types where the highest yields can be achieved with the least nutrient loading on the ecosystem.

A traditional practice to achieve that is supplementary feeding with manuring, which increases both production and natural productivity in the semi-intensive fishpond systems [3,4,12,13]. Replacing cereals with complex feeds makes production more profitable and has a positive impact on fish flesh quality [14–16], however, conventional fish feeds are often made from fishmeal and oil, and the use of such ingredients is unsustainable [17]. The use of fishmeal-based feeds has started to decline in recent years. Compared to the 1990–2000s, the proportion of fishmeal in carp feeds has been reduced by 1–2% in recent years [18–20]. As a result, the aquaculture feed industry has turned to alternative sources [17]. Plant-based ingredients have been used lately. However, the inclusion of alternative plant ingredients has its own challenges in terms of product quality [21–23] and environmental impacts [24].

One of the most important finfish species in global freshwater aquaculture is the common carp. It is the oldest domesticated fish and the most widespread cyprinid species in aquaculture [25,26]. The most common production technology in Europe is carp monoculture grown in earthen ponds [27]. Farming of carp in extensive and semi-intensive pond aquaculture is based on natural production and feeding with cereals. In this case, the most important task of the fish farmer is to establish and maintain a continuously growing zooplankton population using agro-technological tools [28]. Carp adapts well to semi-intensive pond farming conditions and this technology has less environmental impact than other intensive systems [29]. These fishponds are generally shallow, artificially created water bodies designed for fish production purposes. In addition, they make a major contribution to freshwater biodiversity. It is important to monitor the fishponds physically and chemically to understand their impact on aquatic ecosystems [30]. These fishponds are heavily influenced by human activities and weather conditions [31,32].

Today's intensification in aquaculture is affecting the structure and dynamics of aquatic ecosystems and those of zooplankton communities. This group of organisms also contributes to the growth of economically important fish species. They are main supporters of the energy transfer between phytoplankton and fish [33]. Zooplankton is considered to be a significant source of amino acids, protein, fatty acids, lipids, enzymes and minerals [34,35]. While cereal feed itself is protein-poor and has high carbohydrate content, zooplankton is rich in protein and carbohydrate-poor [36]. However, zooplankton also acts against uneven growing [37] and fish health. The composition and abundance of zooplankton communities can be considered both as indicators of water quality and productivity [38], and useful for managing successful and predictable fish yields [39]. Such bioindicators are often used because they show rapid changes due to environmental factors [40]. Zooplankton communities in fishponds have been studied previously, but these were not related to the effects of different feed ingredients. Pechar et al. [41] noted that over the past 50 years, the dominance of fishpond zooplankton species has shifted towards smaller species. Ruttkay [42,43] described the differences between zooplankton communities in mono- and polyculture of carp. Donászy [44] measured the zooplankton biomass formed in the fishponds with different treatments (fish-cum-duck technology, wheat feeding, enhanced fertilization) with a large sample number. Körmendi [45] described the rotifera fauna of fishponds in the Southern Transdanubia region of Hungary. He showed 63 taxa, of which *Asplachna* and *Brachionus* species were dominant.

The main goal of this paper is to assess the effect of different supplementary feed types on zooplankton communities. In an outdoor, experimental fishpond system stocked with 2-plus-year-old carps, three different feeds were utilised: 1) fishmeal- and fish oil-based feed (FF) as a conventional and commercial feed, 2) plant meal- and plant oil-containing feed (PF) as an experimental, sustainable diet and 3) cereals, the traditional supplementary feed as control (CT). We hypothesised that the impact of experimental feed on community composition does not differ from that of conventional and traditional feeds, and thus experimental feed does not pose a greater environmental burden, and therefore can be recommended as a sustainable alternative to conventional fishmeal-based feeds.

2. Materials and Methods

2.1. Experimental Design

The experiment was conducted in fishponds of uniform size on the site of the National Agricultural Research and Innovation Centre, Research Institute for Fisheries and Aquaculture (NAIK HAKI, Szarvas, Hungary) (Figure 1) in 2015. The average area of ponds was 1754 ± 74 m^2 and the average depth was 1.3 m. The feed used for the experiment was compiled according to the semi-intensive breeding conditions of the fish. The proportion of ingredients in the experimental and conventional feeds is shown in Table 1. The two feed types had practically the same crude protein and crude fat concentrations. The main difference between the two feeds was in the content of fishmeal and soy. The third type of feed was cereal (CT), which is traditionally used in Hungary. The experiment was carried out in nine earthen ponds, three replicates per feed. The ponds were filled from the nearby oxbow lake of River Körös. Each pond contained 200 individuals of 2-plus-year-old carp (average weight: 745 ± 80 g).

Table 1. The ingredients of the experimental and conventional, commercial feeds.

Fishmeal-Based Feed (FF) (conventional and commercial feed)		**Plant Meal-Based Feed (PF) (experimental feed)**	
Ingredient	%	Ingredient	%
Fishmeal 60	14.0	Fishmeal 60	0.0
Winter wheat	20.5	Winter wheat	16.5
Maize	27.5	Maize	27.5
Full-fat soy	6.5	Full-fat soy	9.5
Extruded soy	17.5	Extruded soy	29.5
Blood meal	5.0	Blood meal	8.0
Fish oil	2.0	Linseed oil	2.0
Other	7.0	Other	7.0

Figure 1. The experimental design at the site of the National Agricultural Research and Innovation Centre, Research Institute for Fisheries and Aquaculture (NAIK HAKI); CT—Control; FF—Fishmeal-based feed; PF—Plant meal-based feed.

2.2. Sample Collection

The zooplankton community was sampled three times in 2015 (June, August and September). Each time, 50 litres of surface water were taken and filtered using 50 µm mesh plankton net, then concentrated to 100 ml. Samples were preserved with added formaldehyde (4% final concentration) and stored at 4 °C until identified by light microscope following standard keys [46–53]. Density was measured with a 5 ml counter chamber. The specific dry mass values needed for biomass estimation were based on literature data [54].

Simultaneously with the zooplankton sampling, the whole water column was sampled and water chemistry parameters were analysed. Total nitrogen (TN) [55], ammonium nitrogen (TAN) [56], total phosphorus (TP) [57], total suspended solids (TSS) [58], chlorophyll-a (Cl_a) [59] and conductivity (CON) [60] were measured according to the standards of the Hungarian Standards Institution in the National Agricultural Research and Innovation Centre, Research Institute of Irrigation and Water Management (NAIK ÖVKI, Szarvas, Hungary) Laboratory for Environmental Analytics.

2.3. Diversity Evaluation

To compare the diversity of the zooplankton communities of the experimental ponds, we used the Shannon diversity index in R software environment (© The R Foundation, Vienna, Austrua) [61] with *vegan* package [62].

2.4. Statistics

For statistical evaluation, the effects of environmental factors on the structure of zooplankton communities were analysed by canonical correspondence analysis (CCA) in R with vegan package. Estimated biomass was used for species variables, while environmental variables included concentrations of water chemistry parameters (total ammonium nitrogen, total, nitrogen, total phosphorus, total suspended solids, electrical conductivity, chemical oxygen demand, chlorophyll-a) and total feed components (fishmeal and soy component, wheat feed).

3. Results

3.1. Abundance

The highest density of zooplankton occurred in pond FF1 during the study period (22 September). The bulk of the zooplankton population was the common crustacean, *Bosmina longirostris*, accounting for 31.7% of the total population. Looking at the averages of total number of individuals per treatment, the highest density of zooplankton was measured for the treatment with fish oil-based feed in June. The lowest density was also in the FF1 pond in August, while the lowest average density was for the wheat feed as control at the same time (Figure 2). The density ratio of zooplankton groups varied throughout the year. The proportion of rotifera in CT1 and PF3 ponds was about 50% in August and 30–40% in June in PF1, PF2, FF2 ponds. Their share in the zooplankton community was low for ponds FF1, CT2, CT3, FF3 and below 20% for the studied period. In terms of treatments, the rotifera proportion was between 10% and 30% with the peak in August, except in the case of feed containing fishmeal, where this was seen in June. Cladocera assemblages were highly variable, but in September they dominated the zooplankton community in all ponds. With each treatment, their proportion was constantly increasing. The density ratio of copepoda organisms greatly varied between ponds and also seasons (20–90%), but in general for all treatments, their dominance in June was reduced by September.

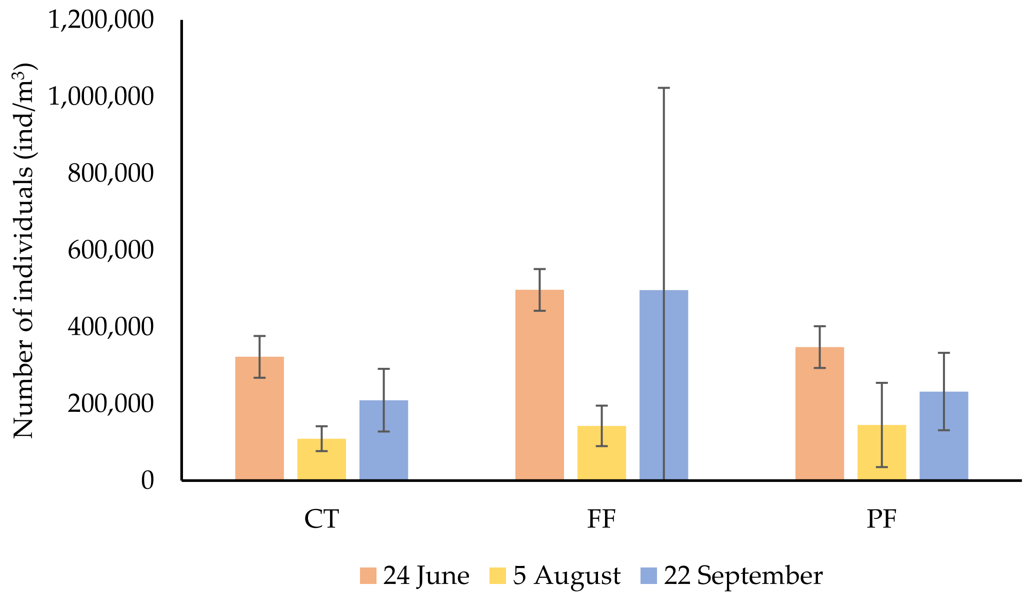


Figure 2. Average number of individuals of the zooplankton species per treatment at each sampling time; CT—Control; FF—Fishmeal-based feed; PF—Plant meal-based feed.

3.2. Biomass

The highest biomass was recorded in pond FF1 (22 September) during the study period, and most was given by *Bosmina longirostris*. Examining per treatment, the average biomass of ponds with fishmeal feed was the highest in June (Figure 3). On average, this treatment produced almost 1.7 times higher biomass than the other two feed types in the season. At the time of sampling in early August, the ponds had low zooplankton masses, with the smallest value occurring in PF1. There were also low values similar to each other in CT1 and CT2 ponds. The difference between the highest and lowest average biomass was greater than 2.9-fold. The lowest average biomass was found in the case of the plant-based feed in August (Figure 3). The proportion of rotifera was negligible in the total biomass of the studied groups, and it was hardly measurable in comparison to crustaceans. In the zooplankton community, the biomasses of cladocera and copepoda were dominant, but their proportions varied from pond to pond. Ponds CT1-FF1, PF1-CT2-FF2 and CT3-FF3-PF3 were the most similar in their tendency, while the annual biomass change was different in PF2.

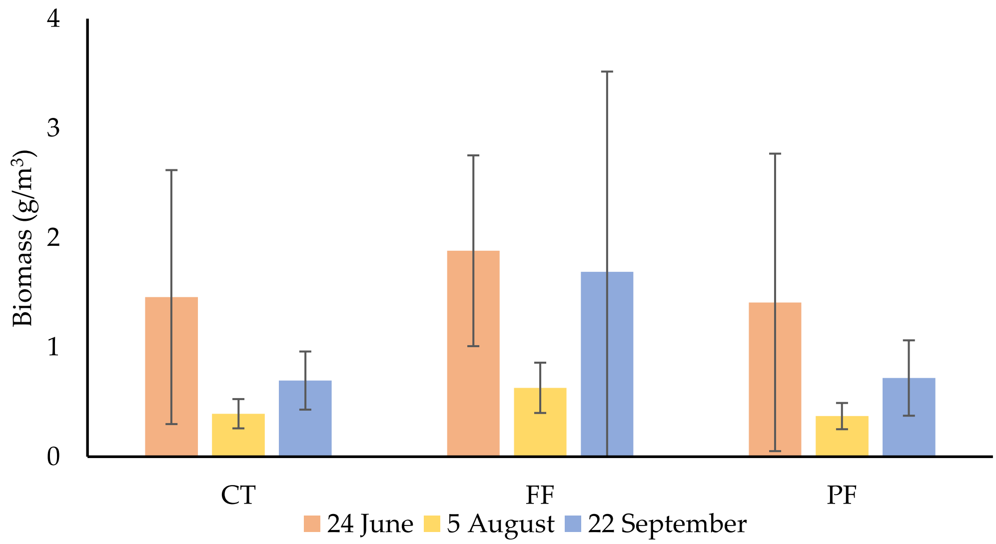


Figure 3. Average biomass of the zooplankton species per treatment at each sampling time; CT—Control; FF—Fishmeal-based feed; PF—Plant meal-based feed.

3.3. Rotifera Assemblage

Twenty-three rotifera taxa were found in the studied fishponds (Table S1), with the smallest number occurring in FF1. The species pool of the other ponds was similar. The dominant elements changed according to the phenological rhythm. The *Asplanchna intermedia* and *Asplanchna pridonta* reached their peak in June, but later only sporadically appeared. The *A. intermedia* did not occur in the ponds treated with fishmeal feed from August on, while *A. pridonta* was absent in the control ponds during the whole experiment. As of June, *Brachionus angularis*, *Brachionus calyciflorus*, *Brachionus falcatus* were dominant elements throughout the study period. From this genus, *Brachionus diversicornis* was not found in FF ponds in August and September, whereas *Brachionus urceolaris* behaved in a contrary way. Beside them, the populations of the warm stenothermic *Filinia opoliensis*, *Keratella tropica* and *Polyarthra euryptera*, also known as the summer plankton [47,48], were significant. In September, in addition to *Keratella cochlearis*, the presence of *Keratella irregularis*, which is very similar to the former species, could be detected in the rotifera assemblage, in some cases outnumbering *K. cochlearis*. In June 2015, the less common *Brachionus variabilis*, which was not previously registered in Hungary and which was present in large numbers in CT1 and FF1 ponds, was found in the rotifera community, but with little abundance in CT2, CT3, FF3 units. Many species appeared only sporadically; *Lecane luna*, *Lepadella rhomboids*, *Testudinella patina*, *Pompholyx sulcate*, *Trichocerca pusilla* were present only in one or two ponds, and their quantity was low. The same applied to *Hexarthra mira*, which only occurred in control ponds.

3.4. Cladocera Assemblage

Altogether, 14 cladocera taxa were found in the studied fishponds (Table S2) and 3 to 9 taxa per pond formed this assemblage. Among the species, *Bosmina longirostris* was a decisive element of the cladocera community throughout the year, and in June, the proportion of *Daphnia cucullata* was significant, which decreased later. *Moina micrura* dominated in August and September. In a preliminary survey, we recorded the presence of *Daphnia ambigua* in one of the ponds for which occurrence has not been published from Hungary previously [63]. This species was also present in the 2015 study period and was a stable member of the zooplankton community during the May and June sampling, except for in pond FF2. Preliminary samplings also revealed the occurrence of *Daphnia parvula*, which was previously known on the American continent. It has appeared in several places in Europe in recent decades [60]. In the 2015 collections, a small number of individuals were present in CT1 and FF2 ponds. In the present study, we recorded the presence of a cladocera species—*Ceriodaphnia rigaudi* (syn: *Ceriodaphnia cornuta f. rigaudi* Sars, 1896)—whose geographical distribution, according to Bledzki and Rybak [64], was found only in Spain on the European continent. According to our investigation, a significant number of reproductive individuals and stable populations of the species could be found in the ponds during this period. In the population, the summer was characterised by the presence of juveniles and females with subitan eggs. Males were present from the end of August, and in September the proportion of females with resting eggs was significant. The species is small; females are 0.4 mm long on average, with a typical rostriform rostrum on the head. In the ephippium, there is one resting egg. The end of the postabdomen is inclined obliquely, with 4–6 spines growing towards the end of the body, and the postabdominal claw is smooth.

3.5. Copepoda Assemblage

From June onwards, in all ponds, *Acanthocyclops robustus* was a typical, dominant organism, with a large proportion of the total zooplankton biomass given by individuals of this species at different life stages. Next to it, the presence of *Cyclops vicinus* was detected in two FF ponds in low abundance and only in September, therefore, despite its large size, it did not have a significant biomass. This species is especially characteristic in the winter plankton communities.

3.6. Diversity Evaluation

In terms of the Shannon diversity indices of the assemblages, the biodiversities of rotifera and Crustaceans (cladocera and copepoda) differed from each other at different sampling dates (Figure 4). With the fishmeal-based treatment, the rotifera community clearly showed a higher degree of diversity than with the other two treatments in June. However, in the case of Crustacean communities' diversity, a clear order of plant-based feed > control > fishmeal-based feed could be identified. In the other samples, the control had the highest diversity in the case of the rotifera communities, followed by plant-based feed and then fishmeal-based feed. In the case of cladocera and copepoda communities, the control showed the highest diversity. In August, this was approached by FF treatment, while PF was much lower. The difference between the latter two disappeared by September and a reversed order was outlined.

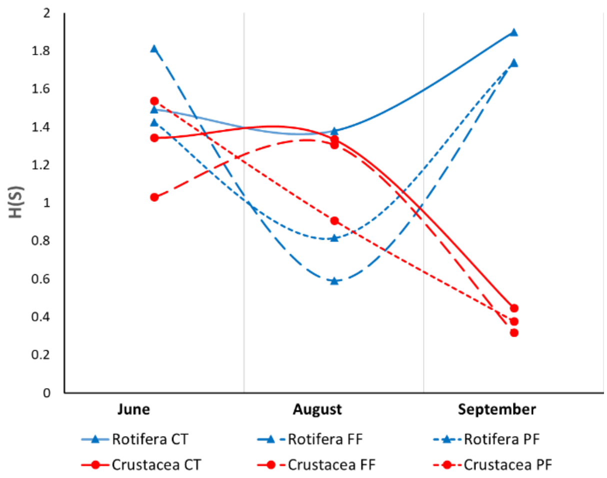


Figure 4. Changes in the Shannon diversity (H(S)) indices of the assemblages of rotifera and Crustacean communities.

3.7. Zooplankton—Environment Relationships

The effects of seven water chemistry and three feed ingredient parameters were assessed on the distribution of rotifera and Crustacea (cladocera and copepoda) using CCA. The results are shown along the first and second axes, where species are represented by dots, and environmental variables by arrows. By placing the communities of Crustaceans (cladocera and copepoda) of each ponds to the ordination space according to their water chemistry parameters, the individual points are grouped according to the sampling times, but the composition of the feed ingredients had no effect on group formation (Figure 5a). The total ammonium nitrogen, total nitrogen, total phosphorus, total suspended solids and the chlorophyll-a had an opposite effect to the electrical conductivity and chemical oxygen demand. The former group positively influenced the community composition in September, while the latter two parameters negatively. Accordingly, water chemistry had little effect on the formation or separation of the community in the other two months. A partial temporal clustering can be observed when examining the effects of treatments (Figure 5b). The values of June and August show a slight overlap. In this case, the plant content, which was typical to experimental feed and control wheat feeding, negatively correlated with the group of August, while the conventional fishmeal feed did the opposite with the community composition of September. Thus, the plant-based diet might be responsible for the separation of the groups of June and August.

There is also a temporal clustering in the ordination space by examining the effect of water chemistry parameters on sets of points representing rotifera communities (Figure 5c). The values for each month are grouped into different quarters of the ordination space, but the confidence ellipses fitted to the values of September and June minimally overlap. The composition of communities of September showed a positive correlation and the communities of August showed a negative correlation with chlorophyll concentration and total suspended solids, while the other parameters showed a negative correlation with the communities of June. Examining the feed components, there is a temporal clustering of rotifera communities, but there is some overlap (Figure 5d). The samples of August form a discrete set, soy being the one to distinguish it from the September samples, and wheat from June samples. In the case of this planktonic group, feed had a smaller effect, but soybean had the largest impact among the ingredients.

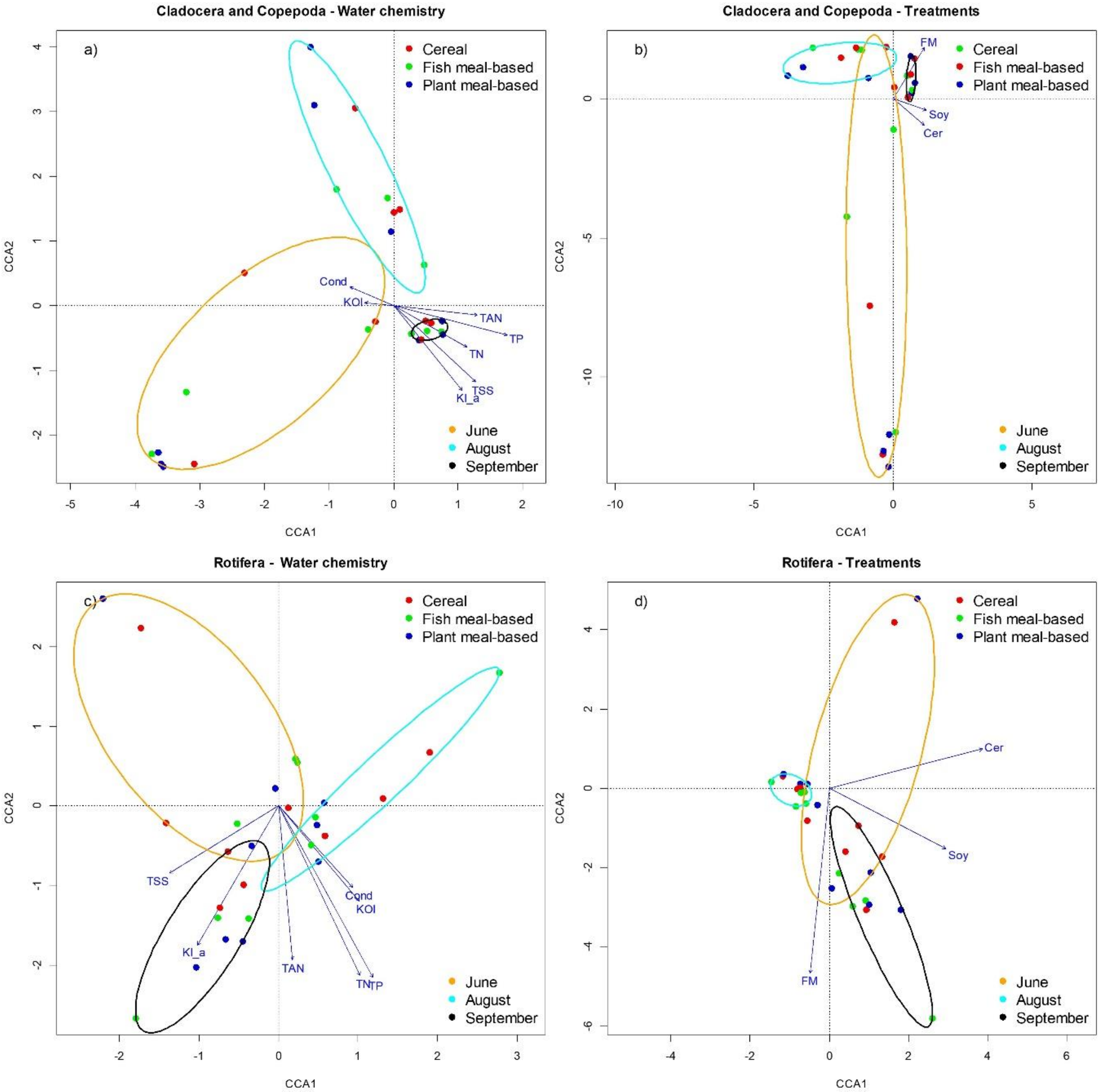
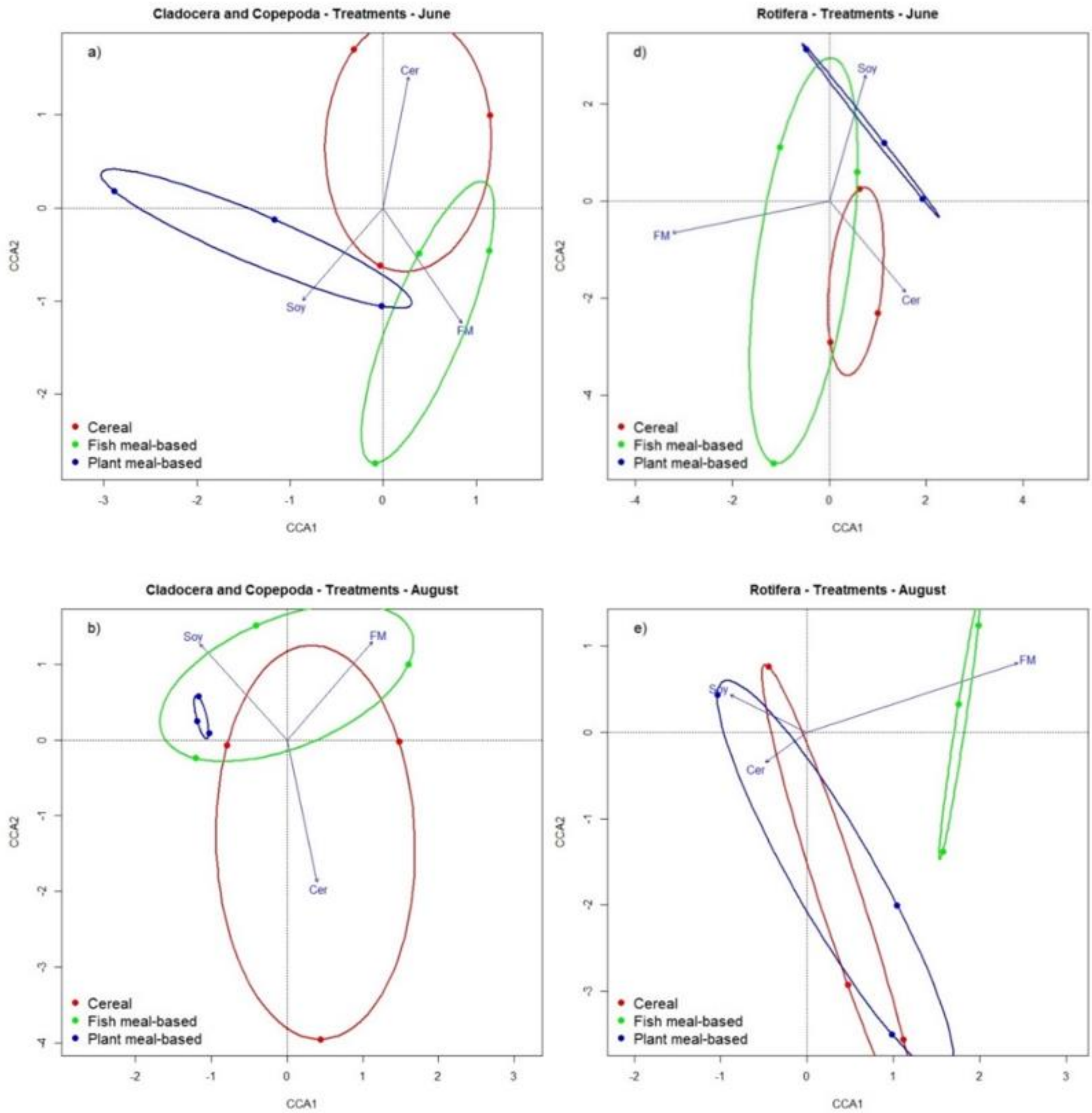


Figure 5. Canonical Correspondence Analysis (CCA) results of the effect of water chemistry and feed ingredient parameters on the distribution of rotifera and Crustacean (cladocera and copepoda) communities. Abbreviations of water chemistry parameters (a–c): TN—Total nitrogen; TAN—ammonium nitrogen; TP—total phosphorus; TSS—total suspended solids; Cl_a—Chlorophyll-a; CON—conductivity. Abbreviations of feed ingredients: Cer—Cereals; FM—Fishmeal. Confidence ellipses are drawn according to months.

Looking at the community-scale effect of feed ingredients at a monthly level (Figure 6a–f), the crustacean and rotifera communities responded differently. In the case of the former, the ellipses fitted in the ordination space do not separate without overlap in any month (Figure 6a–c). There is no clear effect of feed ingredients on community composition. A complete clustering cannot be said for rotifera under the influence of feed ingredients (Figure 6d–f), but in two months, characteristic communities of certain feed components have emerged. In August, the effect of fishmeal-based feed is separated from the other two treatments (Figure 6e), while in September, grain-fed ponds developed a rotifera community that sharply separated from the other two treatments (Figure 6f). However, in view of all this, no general claims can be made during the growing season about the difference in the effects of feed on zooplankton communities.



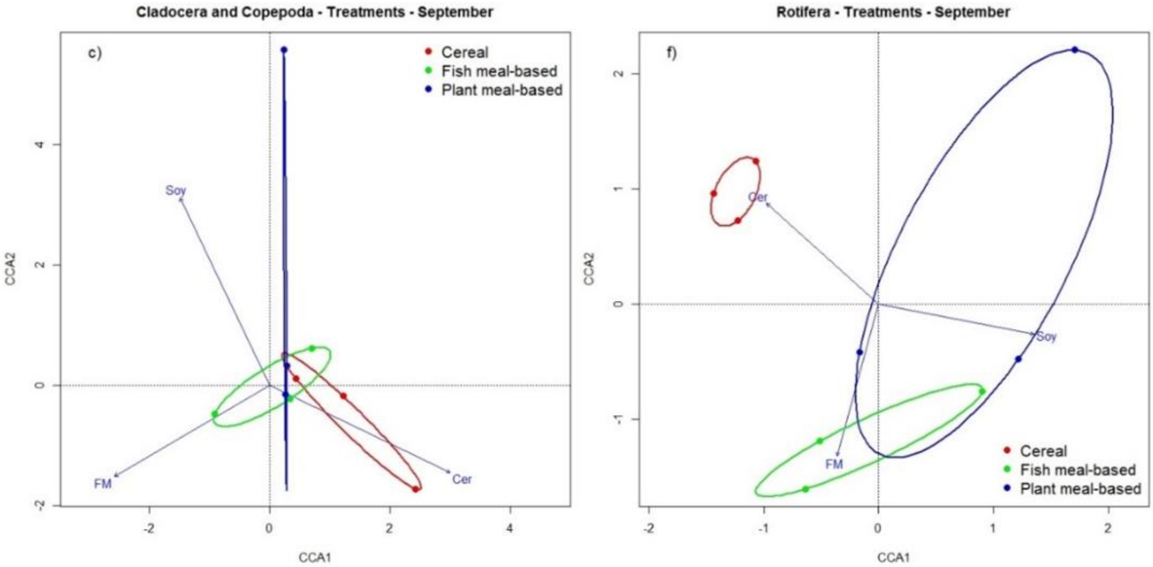


Figure 6. Canonical Correspondence Analysis (CCA) results of the community-scale effect of feed ingredient parameters on the distribution of rotifera and Crustacean (cladocera and copepoda) communities by months. Abbreviations of water chemistry parameters (a–c): TN—Total nitrogen; TAN—ammonium nitrogen; TP—total phosphorus; TSS—total suspended solids; Cl_a—Chlorophyll-a; CON—conductivity. Abbreviations of feed ingredients: Cer—Cereals; FM—Fishmeal.

4. Discussion and Conclusion

In an attempt to replace fishmeal- and fish oil-containing aquafeed with a plant-based diet, we investigated the dynamics of zooplankton communities, which represent the natural food source of fishponds. In the ponds, based on "uneaten" zooplankton density and biomass, the communities were formed in quantity (0.06–70 g/m^3) [65] and quality (dominance of *Bosmina spp.* and *Cyclopidae* [42]) typical to common carp monoculture. *Bosmina* is too small in size for carp, Cyclopidae's movement is too intensive. The density of larger species of cladocera (*Ceriodaphnia*, *Daphnia*), which ensure a primary food source for carp, was initially higher, but later became sporadic. Due to the faster growth of *Moina micrura*, it can be subdominant throughout the year [43]. After the appearance of the alien *Ceriodaphnia rigaudi*, its significant stocks survived in all fishponds. From a fish production point of view, the quantity and quality of zooplankton communities were adequate in the studied ponds, and there was no significant difference in structure between treatments. The number of 23 rotifera, 14 cladocera and 2 copepoda species were similar to the fishpond zooplankton study of Körmendi and Hancz [66] (25, 12 and 1 species, respectively), but 12 common rotifera species and only 4 common cladocera species were found in both studies. This distribution of the species number in a nearby natural ecosystem, the River Hármas-Körös, was 70, 11 and 2 species, respectively [67]. This river creates the base of the oxbow lakes that are the source of the experimental fishponds.

Several alien species were present in the community composition. Among the Rotifers, *Brachionus* variabilis was determined to be episodic (adhering to the surface of *Daphnia*, *Ceriodaphnia*) or has a free-living lifestyle [46,48]. At the time of its appearance, *Daphnia cucullata* and *Daphnia longispina* were found in large numbers in the cladocera assemblages, not adhering to them, but rather free-living. In the later period, it was not recorded and by that time the abundance of *Daphnia* species was not considerable. We did not find *Brachionus variabilis* in ponds treated with plant-based feeds, while in two of these ponds, *Daphnia* species were present. Based on this, the connection between the two species cannot be excluded. Among the cladocera, *Daphnia ambigua* and *Daphnia parvula* are widespread on the American continent [63] but they had only recently appeared in Europe. It can be concluded from the results that establishment, survival and reproduction of *Daphnia* species were successful in this area. According to a new classification, both organisms are considered invasive species [68,69]. The most surprising occurrence of this zooplankton taxon was *Ceriodaphnia rigaudi*, which, according to the literature, is typical to the warmest, tropical and subtropical zones [70–74]. It has not yet been detected from the filling oxbow lake, but its monitoring is recommended in any case as it may be related to climate change.

Based on the diversity indices, the temporal states showed that rotifera and Crustacean diversities are different, as was expected. The diversity of each group was quite variable over time, but the treatments had no significant effect, except in August, when the rotifera diversity in the treated sites was substantially lower than in the controls. In the case of Crustacea, diversity was reduced to a lesser extent in PF treatment compared to the other two feeds. Examining the environmental background factors and the feed components, the different treatments had little effect on the community composition, corresponding to our hypothesis. The structure of the community was determined by season rather than by the treatment. In general, communities are not associated with different diets.

All in all, the different treatments did not represent special conditions for the zooplankton communities, which would cause a change in their composition in both positive and negative directions. Similarly to fishmeal-based commercial feed, the typical zooplankton communities for carp monoculture were formed, which correspond to the fish's natural diet, and have been developed using experimental feed. Based on these results, the plant-based experimental feed had no negative effect on planktonic assemblages, which makes it suitable as a sustainable fish feed in pond aquaculture.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4441/12/5/1338/s1, Table S1: The list of rotifera species of the fishponds according to the treatments, and the calculated number of individuals; CT—Control; FF—Fishmeal-based diet; PF—Plant meal-based diet (PF); SD—Standard deviation; Table S2: The list of Crustacea species of the fishponds according to the treatments, and the calculated number of individuals; CT—Control; FF—Fishmeal-based diet; PF—Plant meal-based diet (PF); SD—Standard deviation

Author Contributions: Conceptualization, É.K. and Zs.J.S.; methodology, L.K..; software, L.K.; validation, L.K.; formal analysis, F.T.; writing—original draft preparation, F.T.; writing—review and editing, L.K., K.Zs., É.K., L.B.-N. and Zs.J.S.; visualization, L.K., F.T.; supervision, K.Zs., Zs.J.S,; project administration, Zs.J.S; data analysis, F.T., L.B.-N.; sampling, F.T., L.B.-N.; zooplankton identification, K.Zs. All authors have read and agreed to the published version of the manuscript

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Conflicts of Interest: The authors declare no conflict of interest.

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