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THE IMPACT OF AQUACULTURE ON THE ORGANISATION OF ZOOPLANKTON COMMUNITIES

PhD Thesis

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1. Introduction and objective

Growing agriculture and aquaculture are trying to exploit nature's values and resources to feed the ever-growing human population. Agricultural research is therefore focused on serving this growing demand in order to be as economical and sustainable as possible. We cannot ignore the fact that, at the same time, it is equally important to avoid and minimise exploitation and damage to nature. Thus, in addition to research aimed at increasing production, it is just as important to analyse the growing impact on nature and to examine ways of reducing the harmful effects. Natural communities, like wetlands, need to be protected in all their components. Each has its place in the multitude of living organisms living together, and no one can be called superfluous or more important than another. They all contribute to the stability of the system, to the proper flow of materials and energy, and to the conservation of biodiversity. By exploiting nature's resources for agriculture, we alter the abiotic, non-living environmental conditions of the system, which can also cause adverse changes in the structure of the biotic communities. In doing so, the stability of the system is at risk. For these reasons, we must also place due emphasis on the importance of agroecological research. Aquaculture production also has an impact on natural ecosystems, and it is therefore important to study it. Zooplankton communities are an important element of this production, as well as of natural systems, as they are both a natural food source for farmed fish and an essential element of the energy pyramid of natural aquatic ecosystems.

In my thesis, I set out to provide a complex, systemic analysis of the relationship between aquaculture and zooplankton communities. I directly examined pond communities of pond fish production under the effects of different feeds, the community formed by the treatment of effluent from an intensive flow through system with an "Artificial wetland water treatment system" from an African catfish (*Clarias gariepinus*) farm, and the communities of the Kákafok oxbow lake as a natural receiving water body. For the latter, I also used functional and phylogenetic approaches that are increasingly coming to the fore in modern ecology. The basic hypothesis of my thesis is that nutrient-rich environments will result in less diverse

communities than nutrient-poor conditions. Along this hypothesis, I seek to answer the following questions:

- Is there a difference between commercial fish feed containing fish meal and fish oil, an experimental vegetable oil-based fish feed, and zooplankton communities that have developed under conventional grain feeds?
- Does this experimental fish feed cause such stress to zooplankton communities that its suitability for fish ponds becomes questionable?
- What kind of zooplankton community develops in ponds of an "artificial wetland water treatment system" treating nutrient-rich effluent from intensive aquaculture?
- How does aquaculture effluent affect the functional, phylogenetic and taxonomic diversity of Rotifera communities in natural receiving waters?

3. Materials and methods

3.1 Testing and sampling sites.

The central site of the Hungarian University of Agriculture and Life Sciences - Research Centre for Fisheries and Aquaculture (MATE - HAKI) (formerly National Agricultural Research and Innovation Centre, Research Institute of Fisheries and Aquaculture) has a net pond area of 90 497 m2. The ponds are filled and drained from the Szarvas - Békésszentandrás (Kákafok) oxbow lake of River Körös, adjacent to the institute, and back to the oxbow lake. Of these ponds, 4 ponds, with a total area of 11 871 m^2, are operated as an experimental "artificial wetland water treatment system", which treats the effluent from an intensive African catfish (*Clarias gariepinus*) farm, which is pumped into the system. The treated water flows continuously by gravity from the treatment system to the said oxbow lake.

Studies on zooplankton communities were carried out in three different nutrient supply units of this complex system. These were:

- Zooplankton communities growing under different carp feeding conditions in the fishpond experiment (hereinafter: fishpond experiment).
- Zooplankton community sampled in a pond of an "artificial wetland water treatment system" treating effluent from an intensive African catfish farm (hereinafter: "artificial wetland water treatment system").
- Investigation of the Rotifera communities of the experimental ponds of MATE-HAKI and the effluent waters of the "artificial wetland water treatment system" of the Szarvas - Békésszentandrás (Kákafok) oxbow lake (hereinafter: Kákafok oxbow lake).

3.1.1 Fishpond experiment

The fishpond experinemt was carried out in a carp feeding experiment. The primary objective was to compare the effects of a commercially available fishmeal-based feed, an experimental vegetable meal and oil-based feed and a conventional grain feed on fish. An important component of any such experiment is an environmental impact assessment, so water chemistry and zooplankton composition studies were also carried out. The experiment was carried out in uniformly sized fish ponds in the MATE HAKI experimental ponds in 2015. The ponds had an average area of 1754 ± 74 m^2 and an average depth of 1.3 m. The feed used for the experiment was adapted to the semi-intensive rearing conditions of the fish. The main difference between the two diets is the fish meal and soy content. The third type of feed was grain, which are traditionally used in Hungary. The experiment was carried out in nine earthen ponds with three replicates per feed. The ponds were stocked from the nearby Kákafok oxbow lake of river Körös. Each pond contained 200 carp (mean weight: 745 ± 80 g), aged 2+ years.

3.1.2 The „artificial wetland water treatment system"

The established wetland experimental water treatment system was developed from four earthen ponds at the MATE - HAKI central site in 2000. Each unit of the system had a surface area of approximately 2500 m2, totalling 1 ha; the water depth averaged 1.5 m in the first two ponds and 50 cm in the aquatic ponds. The ponds

have been in continuous operation since 2001. Until 2011, the fish farm water was partially treated. In 2012, units W1, W2 were renovated. The effluent from the African catfish farm is pumped directly to the stabilisation pond marked W1, the volume of which is recorded daily. This totalled approximately 333 500 m^3 in 2016 and approximately 177 000 m^3 in the first half of 2017. From here, the water reached the stabilisation pond W2 by gravity, from where it flowed in parallel into the aquatic vegetation units W3 and W4. My samples were taken from pond W2.

Among the ponds planted with aquatic macrophytes, common reed (*Phragmites australis*) dominates in pond W3, while in pond W4, reed dominates (*Typha latifolia* and *Typha angustifolia*). In the year of the study, no fish stocking occurred in the ponds. The treated water from these ponds is combined in a common outlet channel and discharged into the Kákafok oxbow lake via the MATE HAKI L1 channel.

3.1.3 Kákafok oxbow lake

The Kákafok oxbow lake, which connects the settlements of Szarvas and Békésszentandrás, is the largest flood protected oxbow lake of the Tisza catchment area and the fifth largest standing water in Hungary. It is 29 km long, covers 207 hectares, has an average depth of 2.2 m and holds 4.5 million m^3 of water. The lake is semi-paleopotamous, where it can be supplemented or replenished by pumping water from the living river. The oxbow lake is filled at the beginning of the year and the water level is lowered at the end of the year for winter. There is no significant water movement or flow between these two events, so I considered it to be stagnant water. Thus no influx of species is possible during the year. The oxbow lake is used for the storage of inland access water and irrigation water, as well as for various activities (fishing, angling, water sports and recreation).

Five sampling points (K1-K5) were selected at different locations along a 3.5 km part to investigate the effects of the effluent from the African catfish (*Clarias gariepinus*) farm and the effluent from the experimental ponds of MATE HAKI. There are no other known discharges in this section of the oxbow lake. The inflow at site K1 was nutrient rich and partly geothermal in origin. It was treated with an "artificial wetland water treatment system" prior to discharge into the oxbow lake. During the treatment process, the thermal water

from the catfish farm cooled, so no thermal pollution occurred at the point of inflow. Additional sampling points were selected based on the characteristics of the oxbow lake. Since there was no significant water flow, I only considered the distance from the inflow. Point K2 was at 500 m, K3 was at 2.5 km (intensive fish sampling took place between points K1 and K3, so I did not designate sampling points here), K4 was at 3.0 km and K5 was at 3.5 km from the inflow point.

3.2 Sampling

Water samples were taken with a column sampler to investigate the water chemistry parameters, by sampling a two metre high water column. For the fish ponds and the ponds of the „artificial wetland water treatment system", water samples were taken at the outfall structure, while in the oxbow lake, water samples were taken at the midline at the designated points. The volume of the water samples was 1.5 litres. The analysis of the different parameters (total nitrogen, ammonium nitrogen, total phosphorus, total suspended solids, chlorophyll-a and conductivity) was carried out by the accredited Environmental Analytical Testing Laboratory of the Hungarian University of Agricultural and Life Sciences - University Laboratory Centre according to the standards of the International Standards Organisation.

For zooplankton studies, 100 litres of water were filtered through a 50 μm mesh plankton net for each sample. The filtered samples (100 ml) were placed in a 120 ml plastic bottle, preserved by adding formaldehyde (4% final concentration) and taken to the laboratory where they were stored at 4°C until taxon identification. The zooplankton analyses were carried out on the taxa of greatest importance in the group: Cladocera, Copepoda and Rotifera. For species identification, I used a Zeiss microscope and a Nikon ShuttlePix P-400Rv digital microscope, counting the number of individuals in each part of the 5 ml counting chamber in the sample and then expressing the composition in units of individuals/m^3. Depending on the density, I examined the whole or a part of the counting chamber. The aim was to identify at species level, but this is not possible for some groups, so in such cases I assigned a higher taxonomic category.

I calculated the necessary biomass estimates using specific data. For species encountered during the study but not reported in the literature, the specific weights of similar species were used.

3.3 Statistical analyses

The effects of environmental factors on zooplankton community structure were analysed by canonical correspondence analysis (CCA) in the R software environment using the *vegan* software package. I used the counted number of individuals of each taxon in the analysis, 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 (fish meal and soybean meal, and forage wheat).

To investigate the impact of effluent water on Rotifera communities in the oxbow lake, statistical analysis of rank-abundance curves was performed using the vegan software package in R. The curves were generated in EXCEL.

To compare Rotifera biodiversity between sample sites, I used the Rényi diversity ordering.

I used eight ecological and life-history traits to analyse the functional traits of the Rotifera species: body size, trophy type, feeding type, physical protection, body wall type, corona type, habitat preference, tolerance. Distance between species based on functional traits was determined by Gower distance measurement using the *StatMatch* software package. These values varied between 0 and 1 based on the specificity of the method.

To determine phylogenetic distances, I measured the length of branches on phylogenetic trees in the literature, which indicates the distance from a common ancestor. These were first standardised with each other and then the distances were converted to a range of 0 to 1. This was necessary because of the common scaling of functional and phylogenetic distances.

The relationship between the functional and phylogenetic distance matrices was tested using Mantel's test with *ade4* package in 9999 random replicates. The evolution of functional traits and how they have been conserved over time can then be assessed.

Because functional and phylogenetic traits carry complementary information about the differences between species, I have used these and their combination in my thesis.

I quantified functional and phylogenetic diversity using Rao quadratic entropy. This was done using the *SYNCSA* software package.

4. Results

1. The following results were obtained from the study of zooplankton communities growing under different carp feeding conditions in the fishpond experiment:

- From fish producting point of view, the quantity and quality of zooplankton communities were adequate in the pond studied, and there were no significant differences in community structure between treatments.
- In the ponds, "unconsumed" zooplankton density and biomass indicated that the communities were established in the abundance (0.06-70 g m-3) and composition (dominance of Bosmina-Cyclopidae) typical of carp monoculture.
- There were 23 Rotifera, 14 Cladocera and 2 Copepoda species.
- Several non native species were present in the community. Among the Rotifera, *Brachionus variabilis* may be epizoidal (clinging to the surface of Daphnia, Ceriodaphnia) or free-living. Of the Cladocerans, *Daphnia ambigua* and *Daphnia parvula* are widespread in North America, but have only recently appeared in Europe. Of the zooplankton taxon, the most striking Cladocera occurrence was *Ceriodaphnia rigaudi*, which is typical of the warmest, tropical and subtropical zone.
- Looking at environmental background factors and feed components, the different treatments had little effect on community composition, as expected. Community composition was determined by season rather than treatment. In general, communities were not related to different feed components.

- The results showed that the plant-based experimental feed had no negative effects on plankton assemblages, making it suitable as a sustainable fish feed for pond aquaculture.

The results have been published in the Water journal:

Tóth, F., Zsuga, K., Kerepeczki, É., Berzi-Nagy, L., Sándor, Z., Körmöczi, L. 2020 The Effect of Feed Composition on the Structure of Zooplankton Communities in Fishponds. Water, 12, 1338. IF.: 3.103 https://doi.org/10.3390/w12051338

Further Hungarian publications on the topic:

Tóth F., Zsuga K., Kerepeczki É., Berzi-Nagy L., Jakabné Sándor Zs., Körmöczi L. 2020. Halastavi zooplankton közösségi összetételben rejlő különbségek eltérő takarmányösszetevők használata mellett, XLIV. Halászati Tudományos Tanácskozás, Szarvas, 2020. 09. 23-24. Halászatfejlesztés 37. 93-96.

Zsuga K., Tóth F., Kerepeczki É., Berzi-Nagy L. 2018. *Ceriodaphnia rigaudi* (Richard 1894), Új Cladocera faj megjelenése a hazai faunában. Hidrológiai Közlöny 98. évfolyam (különszám), 102-105

2. While studying the zooplankton community growing in a pond of an "artificial wetland water treatment system" managing the effluent water of an intensive African catfish farm, I came to the following conclusions:

- Of the naturally growing zooplankton communities in the W2 unit of the water treatment system, three Cladocera, nine Rotatoria species and the Copepoda taxon were detected.
- Of the Cladocerans, two large species, *Daphnia magna* and *Moina macrocopa*, emerged as the main recyclers of nutrients in the system with high densities, with alternating dominance over the study period.

The results were published in the proceedings of the National Agricultural Research and Innovation Centre for Conference of Young Researchers:

Tóth F., Kerepeczki É., Berzi-Nagy L. 2018. Megújuló energiára alapozott létesített vizes élőhelyi vízkezelő rendszer hatékonysága, és

a benne rejlő zooplankton termelési lehetőségek, Kutatói utánpótlást elősegítő program II. szakmai konferenciája, 35-44, ISBN 978-615-5748-09-7

3. Results obtained from the study of Rotifera communities of the Kákafok oxbow lake effected by the experimental ponds of MATE-HAKI and the receiving waters of the "artificial wetland water treatment system":

- The survey revealed a species-rich Rotifera fauna with 26 taxa. In spring, the biodiversity increased with increasing distance from the inflow site, but after a summer transition period, the situation partially reversed in autumn.
- The trait conservatism I have found is that taxa that are more closely related have more similar characters.
- In the spring, environmental influence and functional and phylogenetic clustering can be detected in the community at the point of influence. At this point in spring, the emergence and massing of mesosaprobial indicator species, mainly Brachionus species, in the Rotifera community is due to the inflowing effluent water, which reduces the biodiversity of the community.
- In summer, neither biotic interactions nor environmental conditions play a role in the development of community structure. Influent water in summer has no detectable influence on environmental conditions based on community.
- In the autumn, the driving force of community organisation shifts towards biotic interactions, but already at this time, species-poor communities have developed further away from the point of influence. At the point of influence, however, interspecific interactions clearly contribute to organisation.

The results concerning taxon diversity have been published in the Water journal:

Tóth, F., Zsuga, K., Kerepeczki, É., Berzi-Nagy, L., Körmöczi, L., Lövei, G.L. 2020 Seasonal Differences in Taxonomic Diversity of Rotifer Communities in a Hungarian Lowland Oxbow Lake Exposed to Aquaculture Effluent. Water, 12, 1300. IF.: 3.103 https://doi.org/10.3390/w12051300

Section of functional and phylogenetic diversity is submitted to the Ecology and Evolution journal.

Further Hungarian publications on the topic:

Tóth F., Zsuga K., Kerepeczki É., Berzi-Nagy L., Körmöczi L., Lövei G. 2021. A Kákafoki Holt-Körös kerekesféreg közösségének diverzitásváltozásai akvakultúrából származó elfolyóvíz hatására, XLV. Halászati Tudományos Tanácskozás, Szarvas, 2021. 09. 8-9. Halászatfejlesztés 38. 48-52.

5. New scientific results

1. In a zooplankton community surveyed under semi-intensive pond fish production conditions, 23 Rotifera, 14 Cladocera and 2 Copoda were detected. Of these, Rotifera *Brachionus variabilis*, Cladocera *Daphnia ambigua*, *Daphnia parvula* and *Ceriodaphnia rigaudi* are new alien species from Hungary.
2. I found that the plant-based experimental feed had no negative effects on zooplankton assemblages, suggesting its potential use as a sustainable fish feed in pond aquaculture.
3. I have determined the impact of nutrient-rich effluent water from intensive aquaculture on zooplankton communities growing in the "artificial wetland water treatment system" that manages it, a species-poor community of 12 species in total, with variable dominance. The high abundance of *Moina macrocopa* in May was replaced by the dominance of *Daphnia magna* in September after an appearance in August.
4. I made a detailed survey of the Rotifera community of the Kákafok oxbow lake, which maintains a distinct community with 26 taxa, differing in several respects from those described in neighbouring and similar oxbow lakes. According to the functional and phylogenetic relationships of the Rotifera taxa occurring in the oxbow lake, I have established trait conservatism, i.e. taxa that are more closely related have more similar characters. The impact of nutrient loading on the oxbow lake varies seasonally: while in spring environmental effects are most important for community organisation, biotic interactions become dominant in autumn.

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7. Full publication list (highlighting the publications used in the dissertation)

MTMT ID of Flórián Tóth: 10052967

https://m2.mtmt.hu/gui2/?type=authors&mode=browse&sel=10052967

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XXI. századi vízgazdálkodás a tudományok metszéspontjában, II. Víztudományi Nemzetközi konferencia, Szarvas, 2019 03. 22. Angol nyelvű előadás

6. Fresh Blood for Fresh Water Meeting, Tihany, Hungary, 2019. 04. 23-27. Angol nyelvű poszter

LX. Hidrobiológus Napok, Tihany, 2018. 10. 3-5. Előadás

11. Magyar Ökológus Kongresszus, Nyíregyháza, 2018. 08. 28-30. Poszter

XLI. Halászati Tudományos Tanácskozás, Szarvas, 2017. 06. 14-15. Poszter

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7.5. Scientific informative article

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