

The use of Common duckweed (*Lemna minor*) as a biological filter in the rearing of hybrid African catfish (*Clarias gariepinus* x *Heterobranchus longifilis*)

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SUMMARY

The African catfish (*Clarias gariepinus* x *Heterobranchus longifilis*) is one of the most important fish species in Hungarian aquaculture. The larvae and juveniles are reared at high feeding rates, which can reach up to 10% of the biomass. Intensive feeding results in large amounts of effluent water, directly proportional to the intensity of fish production. The aim of this experiment was to investigate the potential of Common duckweed (*Lemna minor*) as a biological filter and its nutrient removal capacity during intensive fry rearing of the hybrid African catfish. In a recent study, two experimental recirculation systems, identical in water volume and design, were set up, in one of which duckweed was grown under aquaponic conditions, where the concentrations of nitrogen and phosphorus forms were monitored and the production parameters of the fish were determined. The results obtained from the experiment showed that the use of duckweed as a biological filter improves the water quality of the recirculation system. A significant amount of plant biomass was produced, which mainly reduced the nitrate and orthophosphate concentrations in the system water. As a consequence, the survival of hybrid catfish was improved, which also affected biomass growth, but had no effect on other parameters.

Keywords: aquaculture; aquaponics; African catfish; duckweed

INTRODUCTION

The global economic importance of the African catfish (*Clarias gariepinus*) stems from its high environmental tolerance and easily controllable reproductive biology. It contributes to global food security due to its production-friendly characteristics (Kánainé Sipos et al., 2019; Lisachov et al., 2023). African catfish is one of the most important fish species in Hungarian aquaculture, the second most produced species after common carp (*Cyprinus carpio*). With a fish production for human consumption of over 4,500 tons of African catfish, Hungary is the largest producer in the European Union (MAHAL, 2023). A larger stock of African catfish was first introduced to Hungary in 1987, with a total of 2,000 feeding larvae (Péteri et al., 1989), and has played an important role in Hungarian intensive aquaculture since then.

Clarias species, including *C. gariepinus*, have unique adaptive abilities that make them valuable in aquaculture and as model animals for the study of terrestrial adaptation (Lisachov et al., 2023). In addition to its resilience and technological tolerance, African catfish have rapid growth and favourable feed conversion ratio. At the same time, the growth performance and resistance of hybrids produced by crosses with vundu catfish (*Heterobranchus longifilis*) can be further enhanced (Ekelemu, 2010). To exploit the genetic potential of the hybrid, the larvae and juvenile fish are reared at high feeding rates, which can reach up to 10% of the biomass between 1 and 10 g bodyweight (Hecht, 2013). Intensive feeding results in large amounts of effluent water with high organic matter content, the load of which is directly proportional to the intensity of fish production (Turcios et al., 2014). The quantity and composition of the

effluent water generated during the production of African catfish can have a profound impact not only on the ecological footprint of production but also on its economics, in particular in terms of water tempering in recirculation systems.

Duckweed (*Lemnaceae*) is a fast-growing aquatic plant with a wide ecological tolerance and high nutrient removal capacity. For this reason, it has significant potential in biological water treatment (Gupta and Prakash, 2013; Zhou et al., 2023). Studies have demonstrated its ability to effectively remove nutrients such as nitrogen and phosphorus from wastewater (Al-Qutob and Nashashibi, 2012; Chaudhary and Sharma, 2014). Duckweed has the ability to reduce ammonium levels below recommended limits in both freshwater and brackish water systems (Oron, 1994; Yahaya et al., 2022). It is also effective in removing heavy metals, organic chemicals and reducing the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of wastewater (Körner et al., 2003; Ozengin and Elmaci, 2007).

The plant's fast growth rate, high protein content (Lévai Jr. et al., 2024) and ease of harvest make it attractive for wastewater treatment (Sun et al., 2020). Duckweed-based systems are cost-effective, environmentally friendly and can provide tertiary treatment performance comparable to conventional wastewater treatment methods (Gupta and Prakash, 2013; Yahaya et al., 2022). These properties make duckweed species a promising option for phytoremediation in various aquatic ecosystems and for use as biological filter plant that can significantly reduce the organic matter content of effluent water from fish production, thereby reducing the environmental load of intensive aquaculture systems (Chaudhary and Sharma, 2014; Zhou et al., 2023).

The aim of our experiment was to investigate the potential of the Common duckweed (*Lemna minor*) as a biological filter and its nutrient removal capacity during intensive fry rearing of hybrid catfish. To this end, two experimental recirculation systems, identical in water volume and design, were set up, in one of which duckweed was grown under aquaponic conditions. During the study, the concentrations of nitrogen and phosphorus forms were monitored and the production parameters of the fish were determined at the end of the experiment.

MATERIALS AND METHODS

Test environment

The 28-day experiment was set up in the Fish Biology Laboratory of the Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen. The study was carried out in two recirculating aquaculture systems (RAS), which were independent of each other, but identical in design, operation and water volume. Both units consisted of 4 plastic circular tanks of 100 litres each, filled with 50 litres of water. The surface area of these tanks for 0.4 m² each. Mechanical and biological filtration of the systems was performed by a pond filter with a Japanese filter and plastic biofilter. Each unit had a 100-litre tank in which a pump was placed to circulate the water.

Treatments

In the case of the Control (C) RAS, the unit was supplemented with a 100-litre buffer tank, while in the case of the Duckweed (DW) system, Common duckweed (*Lemna minor*) were reared in 4 x 25-litre water volumes (100 litres in total), of which 240 g were stocked in total at the start of the experiment. During the experiment, 10 hours of illumination per day was used (4 x 100 W, 6000 K, LED).

Experimental stock

The Common duckweed (*Lemna minor*) used in the experiment came from the Faculty of Agricultural and Food Sciences and Environmental Management's Fish Biology Laboratory's own stock. At the start of the experiment, 60–60 g of duckweed were placed in the plant growing tanks.

The experimental fish stock was a *Clarias gariepinus* x *Heterobranchius longifilis* hybrid catfish with an average weight of 1.52±0.18 g, obtained from artificial propagation. At the beginning of the study, 100–100 catfish were placed in each tank. The fish were fed with commercially available feed with a pellet size of 1 mm. A feeding rates of 5% per day was applied during the experiment. Halfway through the experimental period (after 14 days), the fish population was weighed and feed rations were adjusted. The feed rations were applied every 3 hours, 8 times a day in total, between 08:00 and 17:00 h by hand and between 20:00 and 05:00 h by automatic feeder (JBL AutoFood).

Water quality parameters

During the study, water temperature (T: 24±0.5°C) and dissolved oxygen (DO: 6±0.3 mg/l) were monitored daily using a HACH HQ30d portable meter. Nitrogen forms (NH₃-N, NO₂-N, NO₃-N) and orthophosphate (PO₄-P) concentrations were determined (HACH Lange DR/3900 spectrophotometer) 2 times a week for both systems. The methods used for the determination of the water quality parameters were the following: ammonia – NH₃-N – Method 8038, nitrite – NO₂-N – Method 8507, nitrate – NO₃-N Method 8171, orthophosphate – PO₄-P – Phosphormolybdenum Blue method.

Sampling

At the end of the experimental period, duckweed biomass was measured and quantified, and fish survival (%), individual wet body weight (g), biomass increment (g), growth rate (SGR, %/day), feed conversion ratio (FCR, g/g) and the homogeneity of fish (CV%) were determined. A digital scale with decimal precision (VWR LP-6501, max: 6500 g, 0.1 g accuracy) was used for the measurements.

Statistical analysis

Statistical evaluation of the results of the production parameters and water quality tests was performed using SPSS (version 26) and two-sample t-test (p<0.05).

RESULTS AND DISCUSSION

Nitrification is an important biological process in the nitrogen cycle, whereby ammonia (NH₃) in soil and water is converted into nitrates (NO₃⁻) by nitrifying bacteria through a two-step oxidation process (van Kessel, 2015). This process takes place in an aerobic environment, as oxygen is required for the oxidation reactions. Recirculating aquaculture systems (RAS) utilize the nitrification process to convert toxic ammonia and nitrite produced during fish metabolism and accumulating in the system into less toxic nitrate (Kuhn et al., 2010).

Figure 1 shows a comparison of averages of water quality parameters. No statistical difference is shown for ammonia and nitrite (Control ammonia 0.30±0.28 mg/l; nitrite 0.20±0.17 mg/l, Duckweed ammonia 0.25±0.17 mg/l, nitrite 0.12±0.11 mg/l). There is already a significant difference between the means for nitrate and orthophosphate (Control nitrate 6.01±1.71 mg/l, orthophosphate 0.31±0.1 mg/l, Duckweed nitrate 3.49±1.13 mg/l, orthophosphate 0.13±0.07 mg/l) indicating that the duckweed treatment resulted in better water quality parameters. This is supported by Sims and Hu's (2013) study, which investigated the organic matter removal capacity of ponds planted with duckweed and algae. Their results showed that duckweed ponds were more efficient at removing organic matter (ammonia-N to nitrate-N and orthophosphate-P) than algae ponds.

Figure 1. Comparison of water quality parameters. Results are means \pm SE of eight water quality measurements within a treatment. Asterisk indicates significant difference compared to the control ($P < 0.05$)

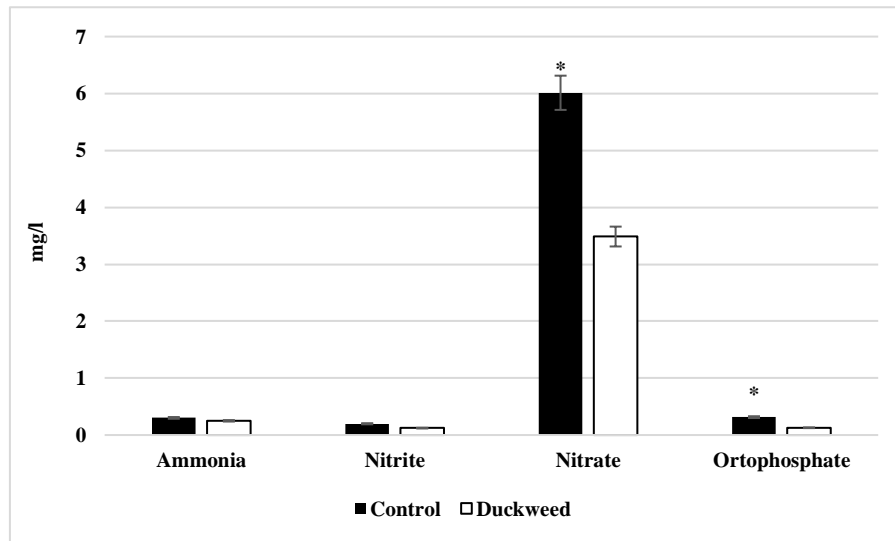
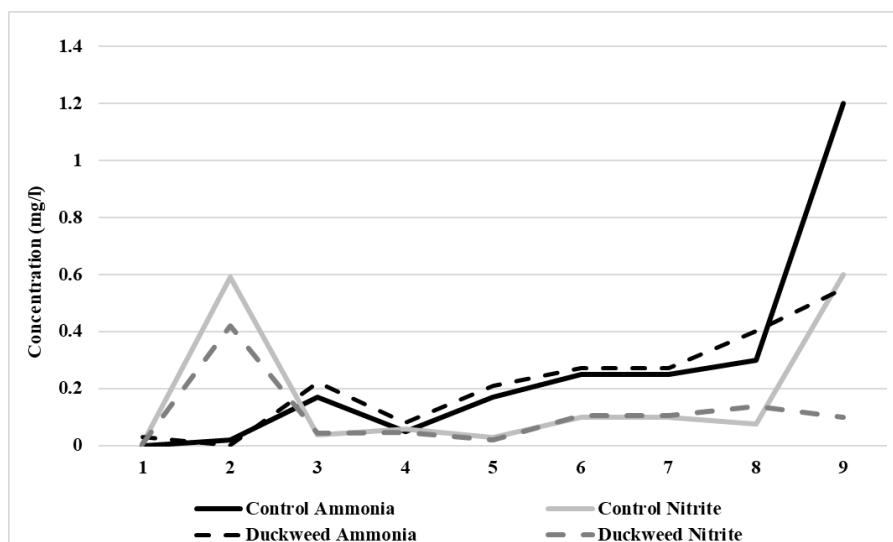


Figure 2 highlights the changes in ammonia and nitrite concentrations during the experimental period. The figure shows that the ammonia and nitrite concentrations were almost equal until day 26 of the experiment, when the ammonia and nitrite concentrations started to increase significantly in the control treatment. During this period, ammonia

concentration doubled and nitrite concentration increased more than sixfold compared to the duckweed treatment. This suggests that if the experiment had been continued, there would have been a significant difference between the ammonia and nitrate concentrations of the two treatments, as was observed for nitrate and orthophosphate content.

Figure 2. Changes in ammonia and nitrite concentrations during the experimental period



In Table 1, the duckweed treatment was found to be more beneficial for the survival of the fish, as the water was less loaded with organic matter, which presumably provided more favourable conditions for the fish, and thus the duckweed treatment resulted in higher survival (Control $54 \pm 12.36\%$ Duckweed $75 \pm 12.14\%$). This is

also supported by the results of Pamula et al. (2019), who found that when rearing African catfish, better survival is achieved when rearing in aquaponic conditions and the more plants in the system, the higher the survival rate of the fish.

Table 1. Production parameters of fish. Results are means \pm SE of four tanks within a treatment. Asterisk indicates significant difference compared to the control ($P < 0.05$)

	Treatments	
	Control (C)	Duckweed (DW)
Survival (%)	54 \pm 12.36	75\pm12.14*
Individual weight (g)	3.74 \pm 0.78	3.51 \pm 0.25
Fish biomass (g)	149,60 \pm 71,67	264,22 \pm 59,9
Biomass growth (g)	134,89 \pm 73,30	247,89\pm59,95*
SGR (%/day)	3.39 \pm 1.21	2.98 \pm 0.60
FCR (g/g)	1.59 \pm 0.37	1.28 \pm 0.15
Homogeneity (CV %)	48,30 \pm 9,38	37,57 \pm 7,49

Nindum et al. (2023), in their study, cite the ammonia and dissolved oxygen content of water as two of the main factors that can affect the growth and survival of African catfish. In the recent experiment, there was no difference between the ammonia levels of the treatments, and those were showing low values while the oxygen content of the water was around 6 mg/l throughout the experiment. This also confirmed the assessment of individual body weights shown in Table 1, which showed that there was no difference in the values reached at the end of the experiment between treatments (Control 3.74 \pm 0.78 grams, Duckweed 3.51 \pm 0.25 grams). The individual body weight of fish is influenced by population density, the higher the population density the lower the individual body weight (Hengsawat et al., 1997). This is presumably the reason why at the end of our experiment the control treatment, which had a lower survival rate and thus biomass density, had a higher individual body weight compared to the duckweed treatment, although the difference did not prove to be significant.

Regarding fish biomass, there was no statistical difference between the two experimental groups, though the tendency shows the Duckweed treatments' biomass was higher. The Control group's biomass was 149.60 \pm 71.67 grams at the end of the experiment, and for the Duckweed treatment it was 264.22 \pm 59.9 grams.

There was a significant difference in fish biomass growth in favour of the duckweed-treated group (Control 134.89 \pm 73.30 grams, Duckweed 247.89 \pm 59.95 grams). It is important to note here that the higher increment could be due to better survival, as more individuals survived to the end of the experimental period in the duckweed groups.

The specific growth rate (SGR) is strongly influenced by feed intensity and the population density (Edet Afia et al., 2020; Oyedaju, 2016), while the survival rate does not affect this parameter. In our experiment, the feed rates and stocking density were the same within treatments and groups, so there should be no difference based on the literature. According to this and our experiment, the growth rate was not affected by the treatment (Control 3.39 \pm 1.21 %/day, Duckweed 2.98 \pm 0.60 %/day).

The feed conversion ratio (FCR) was not affected by the treatment (Control 1.59 \pm 0.37 g/g, Duckweed 1.28 \pm 0.15 g/g). According to studies, the feeding coefficient is mainly influenced by the protein content

of the feed and the intensity of feeding (Farhat and Khan, 2011; Al-Hafedh and Ali, 2004). In the present experiment, all groups were fed the same feed and the same intensity of feeding was applied.

No difference in homogeneity of the groups was observed between treatments during the experiment. The homogeneity of fish is particularly important in the rearing of African catfish, as the potential for cannibalism is higher in heterogeneous stocks (Baras and Dalmeida, 2001). Cannibalism is a major problem in aquaculture, as it can be as high as 90% in intensive systems for some species (Hecht and Appelbaum, 1988; Pienaar, 1990).

The initial duckweed biomass was 60 grams in each tank (240 grams in total) and the amount of duckweed produced at the end of the experimental period was 313,76 \pm 17.23 g per tank (1255 grams in total). Over the 28-day period, the duckweed increased fivefold over the initial biomass. This amount of biomass is below the yields that duckweed could potentially produce. Some studies have shown that under suitable conditions it can double its own biomass in 2–4 days and reach yields of 10–106 t/ha per year in dry matter under regular harvesting (Stejskal et al., 2022; Leng et al., 1995; Sun et al., 2020).

CONCLUSIONS

Intensive aquaculture in Hungary has experienced significant growth in recent decades, mainly due to the rise of hybrid African catfish. The hybrid African catfish (*Clarias gariepinus* \times *Heterobranchius longifilis*), which is a preferred species hybrid due to its fast growth rate, high environmental tolerance and resilience. The feeding of the hybrid African catfish requires special attention, as it needs high-protein feeds for rapid growth and healthy development in intensive farming systems. Feeding intensity can be as high as 10% at the beginning of the rearing period. Population density is also an important factor for hybrid African catfish. It is tolerant of high population densities (up to 500 kg/m³), but this, coupled with intensive feeding requirements, results in high levels of organic matter (mainly ammonia) in the system and run-off water, which must be managed in some way.

The various species of duckweed can help to promote the development of freshwater aquaculture by removing a significant proportion of the nitrogen forms

in the water (Paolacci et al., 2022) This study has shown that duckweed is an effective biological water purifier under intensive conditions, which increases the sustainability of the system by reducing the number of water exchanges, and also the amount of energy required to heat the water when rearing African catfish, so that not only is production more economical by reducing water exchanges, but less energy is required to maintain the water at optimal temperature. Our studies suggest that the integration of duckweed could be a promising technical element to increase the efficiency of aquaculture systems and reduce their environmental impact. The plant biomass produced can be used as a supplementary feed for fish, crustaceans, poultry and pigs (Leng et al., 1995; Iqbal, 1999) due to its high protein content. It can be seen, therefore, that duckweed has great potential for increasing the

economic and environmental sustainability of intensive fish farming.

The results of the experiment showed that the use of duckweed as a biological filter improves the water quality of the recirculation system. During the 28-day trial, a significant amount of plant biomass was produced, which mainly reduced the nitrate and orthophosphate concentrations in the system water. As a consequence of the improved water quality, the survival of hybrid catfish was improved, which also affected biomass growth. For other production parameters, there were no differences between the two treatments. The results suggest that hybrid catfish and Common duckweed can be effectively integrated into aquaponic fish-plant rearing systems and that Common duckweed can be incorporated into hybrid African catfish fry rearing technology.

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