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ARTICLE

Removal of nitrate and phosphate by aquatic plants during aquarium-based ornamental fish production

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Abstract

Objective: The rising demand for ornamental fish and plants in aquariums is met through industrial production. However, higher production densities may negatively impact water quality (such as ammonia, nitrate, nitrite, and dissolved oxygen levels), thus impacting production. This can be mitigated by utilizing specific aquatic plants to promote sustainable ornamental fish production. This study aimed to determine how the water quality in ornamental fish tanks can be improved using two floating aquarium plant species: Najas grass *Najas guadalupensis* and Java moss *Taxiphyllum barbieri*.

Methods: The efficiency of nitrate and phosphate filtration by the two plant species was determined in aquariums containing Endler Guppies *Poecilia wingei*. The duration of the study was 4 weeks, and the water quality parameters were measured weekly. The growth rates of the two plants were measured at the beginning and end of the study period.

Result: Najas grass effectively maintained lower nitrate and phosphate levels while showing robust growth. By week 4, nitrate levels in control tanks rose to 33.75 and 35.00 mg/L in the two independent experiments, while nitrate in tanks with Najas grass only reached 8.75 and 11.50 mg/L. Phosphate levels in control tanks increased to 2.42 and 2.40 mg/L compared to 1.075 and 1.05 mg/L in tanks with Najas grass. In single-species tanks, Najas grass showed a 1.6-fold biomass increase, while Java moss showed a 1.2-fold increase. In tanks with both species, Najas grass biomass increased significantly, whereas Java moss biomass decreased.

Conclusion: The superior competitive ability of Najas grass (allelopathy and increased nutrient uptake) underlies the findings of this study and indicates that this species is a better option for maintaining low levels of nitrate and phosphate in aquarium water. This finding can contribute to creating a cleaner and healthier environment for fish species involved in industrial ornamental fish production and trade.

KEYWORDS

aquaristics, aquarium plants, *Najas guadalupensis*, nitrate, phosphate, *Taxiphyllum barbieri*, water quality

INTRODUCTION

Impact statement

The application of appropriately selected aquatic plants holds promise for enhancing the efficiency of ornamental fish production. Further studies can contribute to a more precise understanding of the effects of different plant species and their combinations in this context.

Keeping ornamental fish and aquariums remains a popular hobby today, contributing to an estimated annual value of US\$15–20 $\times 10^9$ in the legal ornamental fish trade globally (Biondo and Burki 2020; Roslan et al. 2021; Jones et al. 2022). However, sustainable satisfaction of this demand can only be achieved through the industrial production of aquarium fish (Evers et al. 2019). The environment of the ornamental fish is maintained under large-scale conditions that are often deficient in terms of water quality parameters, such as pH, dissolved oxygen level, nutrient levels, and water hardness. However, these parameters are critical for fish health, growth, and behavior. Fish actively release catabolic products into the water through feces and via the gills, and nitrogen- and phosphorus-containing substances from the decomposition of unconsumed food can decrease the quality of the water. If the waste is not efficiently managed, the water in the tank becomes turbid (aesthetic problem), and the production of harmful ammonia during decay can prove detrimental to the fish (Endut et al. 2010; Estim et al. 2019). The delicate equilibrium between the water parameters can be disrupted, resulting in impeded reproduction, reduced growth, and increased susceptibility to disease; in severe cases, it can cause mass mortality and reduced production (Roslan et al. 2021; Jones et al. 2022).

The concentration of nitrate and phosphate compounds produced during decay processes in the water is a persistent limiting factor in closed fish production systems, such as aquariums or recirculating aquaculture systems. Aquariums are generally equipped with filtration systems to maintain the water quality at an appropriate level. However, these filters may not provide perfect protection and can be costly; therefore, it is advisable to complement these systems with plants. For example, biological filtration aids in the oxidation of ammonia (NH_3) to nitrite (NO_2^-) and then to nitrate (NO_3^-) in aquarium water, which can be easily absorbed and utilized by aquatic plants (Estim et al. 2019; Roslan et al. 2021; Indriani et al. 2023). The level of phosphate in the aquarium can originate not only from the activities of the organisms in the aquarium, but also from external sources, such as food or chemicals used for buffering the tap water, resulting in excess amounts (Landolt and Kandeler 1987:638–650; Boyd and Tucker 2012).

Aquatic plants maintain the complex balance of water in nature. For instance, they improve water quality; increase dissolved oxygen levels during the day, which can be utilized by the fish (Martin 2013; Hamid et al. 2015); and positively influence fish behavior by providing natural shelters and spawning substrates (Teixeira-de Mello et al. 2016; Tsang and Gerlai 2022). Aquatic plants use dissolved carbon dioxide, nitrogen, and phosphate compounds in water as energy sources, thereby ensuring their own production. Thus, plants can offer a simple, fast, cost-effective, and natural solution for removing carbon dioxide, ammonia, nitrate, and phosphate compounds from aquarium water and keeping them at lower levels.

Najas grass *Najas guadalupensis* is a monocotyledonous plant belonging to the Najadaceae family and is native to Canada, the United States, and Central America to South America. It is widespread in these areas, owing to its asexual reproduction and high tolerance of water quality (water hardness, pH, and organic and inorganic pollutants). The species was recently introduced into Europe and the Near East, where it was mainly recorded in the Mediterranean region (e.g., Greece), Palestine, and Israel (Witztum and Chaouat 1991; Hussner 2012). However, it has been established in the thermal waters of northern European countries due to the aquarium trade (Király 2009; Lukács et al. 2016; Hrivnák et al. 2019). Najas grass has the potential to become invasive because it can cover entire riverbeds and lake beds (Witztum and Chaouat 1991). The extent of its spread and displacement of native species remains unclear (Witztum and Chaouat 1991; Hussner 2012). It grows well even in very hard water with a hardness of up to 358 mg/L (20 degrees of carbonate hardness [dKH]; 2–20 dKH) and develops most intensively in neutral-pH water; however, it can survive in the pH range of 6–9. Najas grass can absorb nutrients from the water and the soil for its growth. For optimal growth, substrate concentrations of 75 mg of nitrogen/kg of sand and 150 mg of phosphorus/kg of sand are suitable (Gosselin et al. 2018). One study reported that the growth of Najas grass is optimal when 2 g of controlled-release fertilizer per kilogram is present in the sand (Hasandras et al. 2018). Najas grass prefers a temperature between 20°C and 30°C, typical of its native habitat (Lowden 1986). The advantages of this aquatic plant include its rapid growth and floating lifestyle because it does not require a substrate, given that substrate is not employed in tanks used for industrial ornamental fish production or trade. However, this plant can be attached to solid objects. Thus, it is a very popular aquarium plant due to these properties.

Java moss *Taxiphyllum barbieri* is a member of the Hypnaceae family, belongs to the phylum Bryophyta, and is native to Southeast Asia, India, and the Philippines. It clings to trees and other objects in slow-flowing or stagnant waters. Nowadays, it is frequently used in aquascaping due to its easy maintenance and wide tolerance range. The ideal water temperature ranges from 18°C to 30°C, with a pH preference of 5–8. It is not sensitive to water hardness and thrives in values from 0.0 to 411.7 mg/L (0–23 dKH). Java moss directly absorbs nutrients from the water column through its leaves, making nutrient-rich water or regular fertilization important for its health and growth. The most significant biomass increase was observed at high nitrate levels of 30 mg/L, while vigorous length growth occurred in environments containing ammonium at 30 mg/L. Additionally, lower NH_4^+ concentrations (15 mg/L) combined with NO_3^- resulted in reduced growth (Alghamdi 2003; Cowan et al. 2024). Java moss can improve water quality by absorbing decomposition by-products and toxic substances, like heavy metals (Bačkor et al. 2023). Similar to Najas grass, it is a fast-growing, floating species that can be attached to objects, such as rocks or trees.

The Endler Guppy *Poecilia wingei* is a popular aquarium fish species, owing to its hardiness and numerous colors and forms. Exceptionally eurythermal, it can survive for a limited time in temperatures ranging from 0°C to 41°C. Despite its small size, the Endler Guppy is a prolific eater, and when kept in groups, it produces a significant amount of waste (Hernández-López and Luna-Vivaldo 2021).

The aim of this study was to determine how the water quality in ornamental fish tanks can be improved with two common ornamental aquatic plants. The effects of Naja grass and Java moss, used separately and in combination, on the nitrate and phosphate content of the water were examined.

METHODS

Experimental design

Sixteen glass tanks (width × height × depth = 30 × 35 × 40 cm), each containing 40 L of water, were set up (Figure 1). Substrate was not used in the tanks, and a 4-week pilot system was set up before commencing the live experiments. The test system was structured in precisely the same manner (introducing fish and plants into the tanks) as the subsequent live experiments and was suitable for establishing the beneficial bacterial culture within the filtration system. The tanks were filled with fresh water before setting up the pilot system and each experiment. A 2-week "rest period," during which the tanks were operated empty, was used before starting each live experiment. Chlorine-removing agent (Sera Aquatan; Sera GmbH) and bacterial culture (Panzi SunnyGlobe Bio Enzim Granulatum; Panzi-Pet Kft) were added to the freshly filled tanks.

After the introduction of the fish and plants, 20% of the water was changed every week in addition to using the chlorine-removing agent (Sera Aquatan). Tap water was used to fill and change the water in the tanks; the chemical composition of the tap water is shown in Table 1 (National Center for Public Health and Pharmacy 2024). The tanks were equipped with rear chamber filtration, featuring a sponge and 150 g of ceramic rings (20 × 10 × 10 cm) that provided a large surface area for the culture of beneficial bacteria. Every chamber filtration consisted of four chambers. The first one, which was empty, received water from the tank. The second chamber contained the sponge for physical filtration. The third chamber held the ceramic rings for the biological filtration required by bacteria. The fourth chamber was also empty except for the outflow pipe, into which the tube from the air pump was inserted. The rising air from this tube moved the water through the pipe into the aquarium, thus creating circulation and adequate filtration (Figure 1). A high-powered air pump (JK-HAP8000; JK Fish Company) powered all tanks at 45 W, with a capacity of 75 L/min at a pressure of 0.032 MPa (281.25 L/h per aquarium). A 12/16-mm tube was connected to the air pump, which was routed behind the tanks (length = 1.5 m). Subsequently, it branched into 16 small tubes, each with a mean length of 1.5 m (SD = 10 cm), which passed through an elbow connector into the tanks. The air was injected into the water without aeration (without an air stone). Common lighting was placed above the tanks using an LED tube (one for every three tanks), along with 50-cm LED tubes. An 8-h light : 16-h dark photoperiod was set daily based on the 8–10 h of illumination recommended for freshwater aquaristics. Happet LED 50 lighting (Happet Company Kft) was used as the light source, featuring 6 W, 8000 K, 1000 lm, and a spectrum range of 440–570 nm. The water temperature in the tanks was maintained at 26 ± 2°C by using thermostatic heaters (NHA-50 50W; Aqua Nova).

The aquatic plants and fish were acquired commercially (Trópus Kft). The tanks were divided into four groups based on the treatment: tanks without any plants (control), tanks with only Najas grass, tanks with only Java moss, and tanks with a combination of both plant species. The treatment locations of the tanks were randomly assigned. An equal quantity of plants (100 g) was added to each tank; 50 g of each plant species were added to those with a combination of both plants. The plants were allowed to float freely without attachment. Every week, 7 mL of micronutrient plant fertilizer were administered

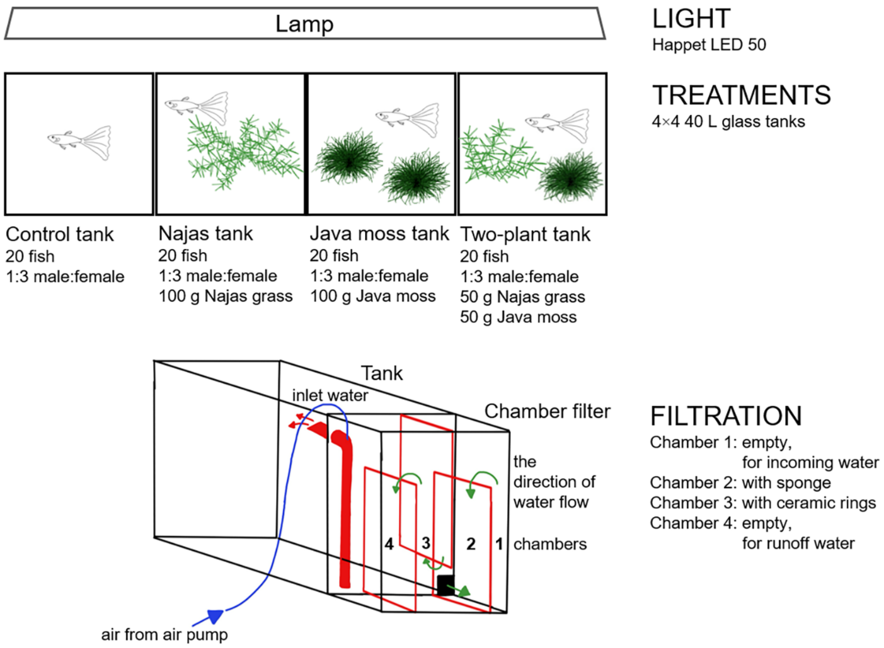

FIGURE 1 Schematic diagram of the experimental setup and chamber filtration. Tanks contained Endler Guppies and Naja grass, Java moss, both plant species, or neither plant species (control).

to all tanks through 1 mL of daily dosing. Sera Florena plant fertilizer (Sera GmbH), which contained potassium chloride (2.58%), magnesium chloride and magnesium oxide (0.65%), calcium oxide (0.31%), sodium hydroxide, and iron–EDTA (0.07%), was used.

Guppy groups (20 individuals measuring 1.5–2.0 cm, with a total starting fish biomass per aquarium of 10.88 ± 0.718 g [mean ± SD]) were introduced into the tanks simultaneously with the plants. The gender ratio of the fish was 1:3 (male : female). Additionally, each tank received one algae-eating catfish (Jumbie Teta *Ancistrus cirrhosus*), approximately 10 cm in size, to combat brown algae. The fish were fed daily with a frozen and dry food diet during the light cycle, 3–4 h before dark, with 1.5 g of fish food used per aquarium. The frozen food included brine shrimp *Artemia salina* and red cyclops *Cyclops* spp. The average nutritional values per 100 g for brine shrimp were as follows: 4.20% raw protein, 0.40% raw fat, 0.70% raw fiber, 93.50% moisture, and 0.60% ash (Léger et al. 1987; Sorgeloos et al. 1998). For the *Cyclops* spp., the average nutritional values per 100 g were 4% raw protein, 0.60% raw fat, 0.60% raw fiber, 93.30% moisture, and 0.50% ash. JBL Novo vert flake fish food (JBL GmbH) with plant content was served as dry food to ensure balanced nutrition for the fish. The composition and nutrient content of the JBL Novo vert flake food were as follows: 32% protein, 5.3% fat, 3% fiber, and 8.5% ash; plus vitamins A, D3, E, and C.

Measuring the plants and fish in the tanks

The weight of the plants in each tank was measured during the first week and last week of the experiments. Before measuring, excess water on the plants was carefully blotted using paper towels. Even though the study did not specifically aim to examine the health status of the fish, the weight and quantity of the fish were measured in the first week and last week of the experiments. Two tanks were randomly selected from each treatment, and the total weight of the 20 fish was measured.

Measuring the aquarium water parameters

Tests commonly used by small- and medium-sized businesses engaged in the production and trade of ornamental aquarium fish were selected for use in this study. The water quality parameters were measured in every tank. Weekly nitrate and phosphate measurements were conducted using Sera tests during the light period before feeding (Sera NO_3^-/Nitrate Test and PO_4^{3-}/Phosphate Test; Sera GmbH). Ammonia levels in the aquarium water were also assessed weekly using a Sera test (Sera Ammonium/Ammonia Test; Sera GmbH). The pH of the water in the tanks was measured using the Panzi pH Test (Panzi-Pet Kft) during the initial and final weeks of the experiments.

TABLE 1 The chemical composition of Szeged, Hungary, tap water, which was used in the experimental tanks. Data are from the National Center for Public Health and Pharmacy (2024).

Variable	Median	Maximum
Arsenic (μg/L)	7	10
Boron (mg/L)	0	0.06
Fluoride (mg/L)	0.42	0.54
Nitrate (mg/L)	0	0.8
Nitrite (mg/L)	0.01	0.12
Nitrite: waterworks outlet (mg/L)	0.02	0.05
Bound active chlorine (mg Cl_2/L)	0	0.44
Ammonium (mg/L)	0.7	1.12
Chloride (mg/L)	2	7
Iron (μg/L)	93	189
Manganese (μg/L)	33	44
Chemical oxygen demand–permanganate (mg O_2/L)	1.29	1.93
Sulfate (mg/L)	5	12
Sodium (mg/L)	39.8	47.9
Total hardness as CaO (mg CaO/L)	118	124

Statistical analysis

Statistical analyses were based on the mean data values from two independent experiments (each with 4×4 parallel aquariums). The statistical analyses were performed using GraphPad Prism for Windows version 8.0.1.244 (GraphPad Software). Normal distribution of the data was assessed using the Shapiro–Wilk test. Statistically significant differences were determined using two-way analysis of variance (ANOVA) and Tukey's post hoc test. Results were considered significant at *p*-values less than or equal to 0.05.

RESULTS

No statistically significant differences in the total mass of the Endler Guppies were observed among the tanks at the beginning and end of the two experiments (Table 2). Each algae-eating catfish weighed 10–12 g, and their weight remained constant throughout the experiment. There were no fish losses during the entire experiment.

TABLE 2 Total biomass of Endler Guppies (g; mean ± SD) in the aquariums ($n = 2$) containing Naja grass, Java moss, both plant species, or neither plant species (control). Statistically significant differences were determined using two-way analysis of variance with Tukey's post hoc test. Results were considered significant at $p \le 0.05$. ns, nonsignificant.

Time point	Treatment	Experiment 1	Experiment 2
Week 0	Control	10.5±0.707	10.5±0.707
	Naja grass	10.5±0.707	11±0
	Java moss	11±1.414	11±0
	Naja grass and Java moss	11.5±0.707	11±1.414
Week 4	Control	12±1.414	11±414
	Naja grass	11.5±0.707	12±0.0
	Java moss	12±0.0	12±1.414
	Naja grass and Java moss	12±1.414	12±0.0
	p	ns	

Based on the two-way ANOVA, there was no difference in nitrate levels between the two independent experiments, whereas there were significant differences between the treatments. At the start, the nitrate level was 5 mg/L in all aquariums and in both experiments. In the control tanks, the nitrate levels increased consistently and significantly from an average of 10.0 and 11.0 mg/L (experiments 1 and 2, respectively) in the first week to 33.75 and 35.0 mg/L by the fourth week (Figure 2). In contrast, in tanks containing only Najas grass, the nitrate levels increased slowly from 7.75 and 7.75 mg/L during the first week to 8.75 and 11.50 mg/L by the fourth week. In tanks containing only Java moss, the nitrate levels increased from 8.25 and 9.50 mg/L during week 1 to 15.0 and 16.0 mg/L by week 4. When both plant species were used together, the nitrate levels increased from 8.25 and 6.75 mg/L in the first week to 12.75 and 11.50 mg/L by the fourth week (Figure 2).

In the first week, the nitrate levels in the control tanks did not significantly differ from those in the plant-containing tanks (Figure 2). From the second week, the nitrate levels were significantly lower in the plant-containing tanks compared to the control tanks. From the second week, significant differences in nitrate levels were observed between the control tanks and those containing Najas grass (both experiments: $p < 0.0001$), Java moss (experiment 1: $p = 0.0286$; experiment 2: $p = 0.0015$), and both plants (experiment 1: $p = 0.0002$; experiment 2: $p < 0.0001$). The nitrate levels measured in the third and fourth weeks showed strong significant differences between the control and plant-containing tanks (experiment 1: $p < 0.0001$; experiment 2: $p < 0.0001$; Figure 2). Interestingly, the nitrate levels in Java moss tanks tended to be higher than those in other plant-containing tanks but were not significantly higher (Figure 2).

Similar to the nitrate levels, there were differences in phosphate between the treatments but not between the two experiments. At the beginning of the experiment, the phosphate level was 0 mg/L in all tanks within both

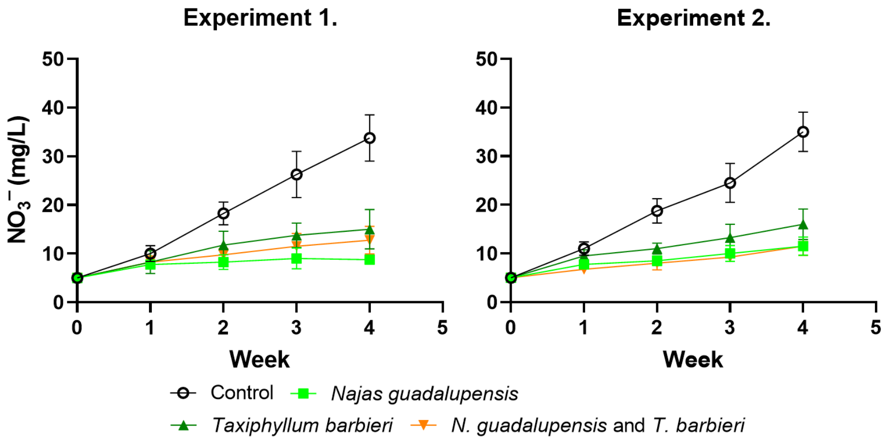

FIGURE 2 Nitrate levels over the 4 weeks in the aquariums containing Endler Guppies and Naja grass, Java moss, both plant species, or neither plant species (control). The data are presented as mean ± SD ($n = 4$). Statistically significant differences were determined using two-way analysis of variance with Tukey's post hoc test. Results were considered significant at $p \leq 0.05$.

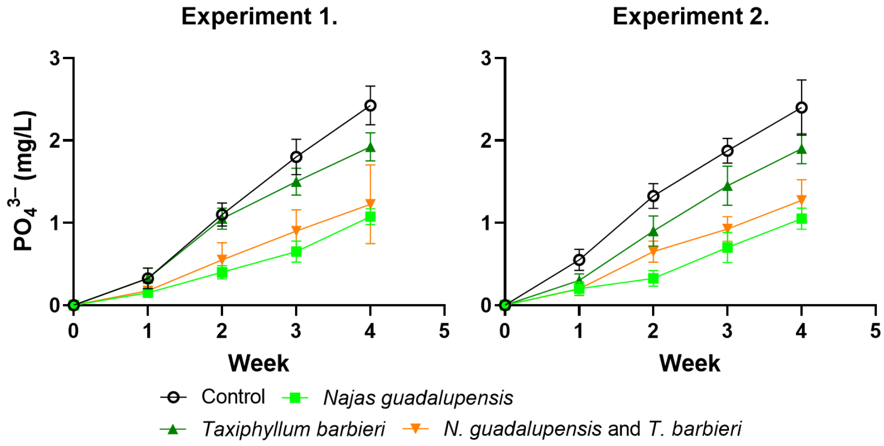

FIGURE 3 Phosphate levels over the 4 weeks in the aquariums containing Endler Guppies and Naja grass, Java moss, both plant species, or neither plant species (control). The data are presented as mean ± SD ($n = 4$). Statistically significant differences were determined using two-way analysis of variance with Tukey's post hoc test. Results were considered significant at $p \leq 0.05$.

experiments (Figure 3). In the control tanks, the phosphate content increased from 0.325 and 0.550 mg/L (experiments 1 and 2, respectively) in the first week to 2.42 and 2.40 mg/L by the fourth week. In contrast, the increase in phosphate levels in tanks with only one plant species occurred much more slowly. In the first week, the phosphates in tanks containing only Najas grass were, on average, 0.15 and 0.20 mg/L, which increased to 1.075 and 1.05 mg/L by the fourth week. In week 1, the phosphate levels in tanks containing only Java moss averaged 0.325 and 0.3 mg/L, which increased to 1.925 and 1.9 mg/L by week 4. Tanks containing both plant species had phosphate levels of up to 0.175 and 0.2 mg/L in the first week and 1.225 and 1.275 mg/L by the fourth week (Figure 3).

Similar to nitrate, the phosphate levels in the control tanks during the first week were not significantly higher than those in plant-containing tanks. From the second week to the end of the study, the phosphate levels were significantly lower in the tanks with only Najas grass and the tanks with both species than in the control tanks. In contrast, for the Java moss tanks, the phosphate levels of the water were significantly lower compared to those in the control tanks only in the fourth week (both experiments: $p = 0.0182$; Figure 3). The tanks with Najas grass had significantly less phosphate than control tanks from the second to the fourth weeks of the two independent experiments (both experiments: $p < 0.0001$). In tanks containing both plants, the phosphate level remained significantly low continuously from the second week (experiment 1: $p = 0.0038$; experiment 2: $p < 0.0001$) to the fourth week (both experiments: $p < 0.0001$). However, the phosphate content in tanks with only Najas grass and tanks with both plant species remained significantly lower than that in the Java moss tanks. By the second week, this differences were significant (experiment 1: $p = 0.0182$; experiment 2: $p = 0.0016$) in the case of the tanks with Najas grass and varied in significance (experiment 1: $p = 0.0182$; experiment 2: $p = 0.9797$) for the tanks containing both plants, whereas these tanks showed strong significant differences by the third week (both experiments: $p<0.0001$) and especially by the fourth week (experiment 1: $p<0.001$; experiment 2: $p<0.0003$; Figure 3).

The two-way ANOVA indicated that plant biomass significantly differed between the treatments only in the fourth week. In the single-species tanks, the mass of the plants showed a significant increase, with an average increase of 1.6-fold for Najas grass (from 100 and 100 g in experiments 1 and 2, respectively, to 162.0 and 166.3 g; both experiments: $p<0.0001$; Figure 4A). Similarly, in tanks containing only Java moss, plant mass also showed a significant 1.3-fold increase (from 100 and 100 g in experiments 1 and 2, respectively, to an average of 131.8 and 131.5 g; experiment 1: $p=0.0171$; experiment 2: $p=0.0185$). Between the two species, Java moss exhibited a more moderate increase in mass compared to Najas grass (experiment 1: $p=0.0276$; experiment 2: $p=0.0063$) in the single-species tanks (Figure 4A). In tanks with both plant species, the mass of Java moss showed an average decrease of 1.2-fold, but it was not a significant decrease tendency with time compared to its initial biomass (Figure 4B).

Despite the weekly ammonia level measurements, ammonia could not be detected in the water of the tanks. No significant differences in pH were detected between the treatments or between measurements taken during the first and last weeks of the two independent experiments (the pH was around 8 in all tanks throughout the time period; Table 3).

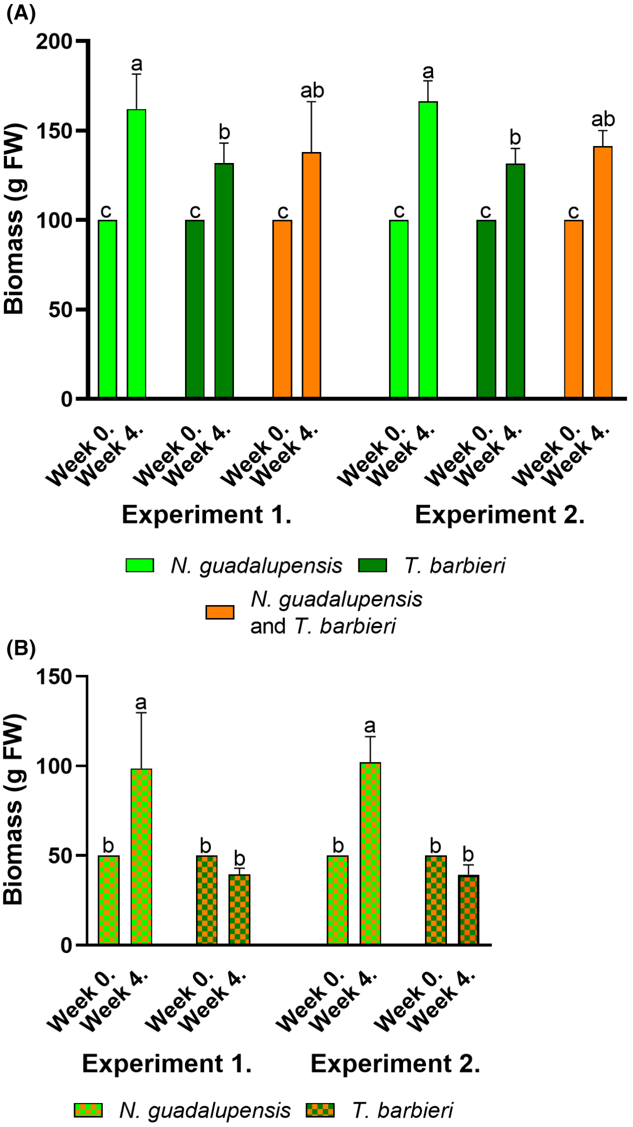

FIGURE 4 Variation in plant mass (g fresh weight [FW]) over the 4 weeks in the aquariums containing Endler Guppies and Naja grass, Java moss, both plant species, or neither plant species (control): (A) mass of plants in single-species aquariums and the total mass of plants in dual-species aquariums; and (B) mass of each plant species within dual-species aquariums. The data are presented as mean ± SD ($n=4$). Statistically significant differences were determined using two-way analysis of variance with Tukey's post hoc test. Results were considered significant at $p \le 0.05$.

DISCUSSION

Although the present study did not focus on the health status of the fish, it is worth mentioning that no differences in the growth and survival of Endler Guppies were observed between the control and treatment groups (Table 2). Therefore, the major impact of plants on fish performance in closed systems would be on the water quality, leading to reduced stress and disease incidence. According to some studies, compounds released by the plants (such as O_2, organic acids, allelochemicals, and certain enzymes) into the water can contribute to other beneficial effects on the fish; for example, they might suppress the growth of pathogens and algae (Norris 1983; Tsang and Gerlai 2022) or influence the color of the fish (Sköld et al. 2016; Indriani et al. 2023). However, these effects may vary for different aquatic plants due to differences in the compositions of their compounds.

Nitrate levels of around 10–30 mg/L can be considered toxic, but this varies depending on the sensitivity of the aquatic organisms, exposure time, and nitrate concentration (Estim 2010; Estim et al. 2019). According to Camargo et al. (2005), freshwater fish are generally more sensitive to nitrate, and even relatively low concentrations can have harmful effects on them, whereas marine fish typically tolerate higher concentrations of nitrate. A nitrate concentration of 10 mg/L can adversely affect Rainbow Trout *Oncorhynchus mykiss*, Chinook Salmon *O. tshawytscha*, and Cutthroat Trout *O. clarkii* during long-term (30-day) exposures. Tilak et al. (2007) showed that nitrate significantly affected the hemoglobin and oxygen uptake in Common Carp *Cyprinus carpio*, especially at high concentrations (967.63 mg/L).

TABLE 3 The pH in the aquariums ($n = 4$; mean ± SD) containing Endler Guppies and Naja grass, Java moss, both plant species, or neither plant species (control). Statistically significant differences were determined using two-way analysis of variance with Tukey's post hoc test. Results were considered significant at $p \leq 0.05$. ns, nonsignificant.

Time point	Treatment	Experiment 1	Experiment 2
Week 0	Control	7.25 ± 0.5	7.25 ± 0.5
	Naja grass	7.5 ± 0.577	7.75 ± 0.5
	Java moss	7.25 ± 0.5	7.5 ± 0.577
	Naja grass and Java moss	7.25 ± 0.5	7.5 ± 0.577
Week 4	Control	7.75 ± 0.5	7.25 ± 0.5
	Naja grass	8.00 ± 0.0	7.75 ± 0.5
	Java moss	7.75 ± 0.5	7.5 ± 0.577
	Naja grass and Java moss	7.75 ± 0.5	7.75 ± 0.5
	p	ns	

While this study did not directly assess the health status of Endler Guppies, the absence of mortality and the lack of noticeable behavioral differences between the control and treatment tanks suggest that the fish tolerated the observed nitrate concentration of 30 mg/L in this study (Table 2; Figure 2). However, these observations were limited to a 4-week period, and longer term studies are needed to draw definitive conclusions about the impact on fish performance. Smallbone et al. (2016) used a closely related species, the Trinidadian Guppy *Poecilia reticulata*, to investigate the impact of excessive fertilization on disease development and biodiversity. Although their results showed that chronic nitrate poisoning mitigated the severity of infection, it also resulted in significant fish mortality. Fish mortality was 10% at a nitrate concentration of 50 mg/L compared to 43% at 250 mg/L. Considering this, the experimental system in the current study would likely have reached the toxic nitrate concentration of 50 mg/L during the fifth week in the absence of plants (Figure 2). This further emphasizes the beneficial effect of plants on the water quality of the aquarium.

According to our results, the nitrate levels in the control tanks significantly increased over time, while the increase was slower in the plant-containing tanks, reaching around 10 mg/L (Figure 2). Roslan et al. (2021) examined the effects of duckweed *Lemna* spp. and mosquito ferns *Azolla* spp. on the water nutrient content in an aquaculture system containing Goldfish *Carassius auratus*. Similar to our findings, those authors discovered that the plants consumed significant amounts of nutrients, particularly ammonia and nitrate. Although we did not directly measure the filtration efficiency of each plant species, the observed reductions in nitrate levels indicate that both Najas grass and Java moss effectively uptake these nutrients. The combined application of the two plant species further enhanced this effect, suggesting a cumulative impact on nitrate reduction (Figure 2).

The phosphate levels in the plant-free tanks increased significantly over time; however, this increase was slower in tanks containing only Najas grass and in tanks with both plant species (Figure 3). The combined application of the two plant species significantly reduced the phosphate levels, especially from the second week to the fourth week (Figure 3). Our findings suggest that certain plant species can bind nitrates and phosphates. Submerged aquatic plant communities exhibit a phosphorus uptake mechanism that is not found in water surface-dominating macrophytes because submerged floating plants can absorb phosphorus compounds throughout their entire surfaces (e.g., stems and leaves; Granéli and Solander 1988). However, further research is needed to determine the more efficient nitrate- and phosphate-fixing aquatic plant species, for which the mesocosm study of Dierberg et al. (2002) can provide a good basis. They examined the phosphorus uptake of some submerged floating plant species for 8 months and found that Najas grass absorbed the most phosphorus, followed by hornwort *Ceratophyllum demersum*, whereas muskgrass *Chara* spp. showed significant uptake only 7 days after treatment and Illinois pondweed *Potamogeton illinoensis* had the lowest uptake.

The mass of plants in tanks with only one plant species was significantly increased in this study (Figure 4A), possibly due to the utilization of nitrate and phosphate from the water. Similar findings were observed by Dierberg et al. (2002), who reported that Najas grass produced the most biomass compared to the other species. In contrast, Najas grass biomass increased significantly, while Java moss biomass showed a nonsignificant decrease in tanks containing both species (Figure 4B). These results indicate that Najas grass may have rapid nutrient uptake capabilities and, with its potential allelopathic substances, may significantly limit the abundance and growth of other species. Norris (1983) recommended using Najas grass to reduce the quantity of cyanobacteria in natural waters. The invasive characteristics of Najas grass in many countries support this notion (Witztum and Chaouat 1991). Therefore, its introduction or escape into natural waters should be minimized. The current study also illustrates the importance of carefully selecting the combination of aquatic plant species for use in aquariums, as the species may compete with each other. Thus, the joint application of Najas grass and Java moss is not advisable.

Ammonia could not be detected in the water, possibly because the fish were placed in tanks equipped with proper physical and biological filtration, where bacteria *Nitrosomonas* spp. quickly oxidized the produced ammonia into nitrite; alternatively, the conversion of nitrite to nitrate is carried out by the bacteria *Nitrobacter* spp. The pH did not change significantly in the tanks (pH remained at around 8; Table 3). This is similar to the results of Roslan et al. (2021), who reported that the pH of the tanks did not differ significantly and ranged from 7.81 ± 0.39 (mean ± SD) to 7.89 ± 0.46. In our study, the sodium hydroxide present in the plant nutrient that counteracts the acidification of the tanks could be a possible explanation for this.

Owing to their floating nature, the plants that we applied can easily get caught in the filtration system, causing blockages and operational disruptions; in particular, the Java moss stuck to the inlet in the present study. Therefore, the compatibility of Najas grass and Java moss for aquariums that employ hang-on-back filters, canister filters, or sponge filters must be taken into consideration. The floating nature can limit the efficiency of the filter and increase maintenance needs. Additionally, the plants can hinder the collection of fish. Structural modifications, such as using a mesh or filter insert at the intake pipe of the filter or choosing the correct filtration speed, can help to minimize the risk of plant entanglement. In addition, proper securing or containment of the plants can also reduce interference. A separate tank can be operated with proper water flow through which the water is passed, and the plant can be stored there. If it is important to provide hiding places for the fish and to make them feel comfortable, the drain should be designed in such a manner that it does not cause any trouble (for example, a prefilter grid in front of the inlet). It is also essential to perform regular maintenance, such as periodic filter cleaning and pruning of the plants, to minimize the risk of blockages and operational disruptions. Plants that float on the surface of the water (e.g., duckweed and mosquito ferns; Roslan et al. 2021) or species that require a substrate (e.g., *Anubias* spp., *Microsorum* spp., and *Alternanthera* spp.; Tsang and Gerlai 2022; Indriani et al. 2023) can also be used.

CONCLUSION

This study determined the efficiency of nitrogen and phosphate filtration by Najas grass and Java moss in single-species and combined-species tanks during ornamental fish farming. While the study did not directly measure filtration efficiency, the significant reductions in nitrate and phosphate levels indicate the nutrient uptake capabilities of the plants. Additionally, although no adverse impacts on fish performance were observed over the 4-week period, longer term studies are necessary to draw definitive conclusions regarding fish health. In conclusion, appropriately selected aquatic plants can significantly contribute to maintaining water quality in artificial aquatic systems, such as those used for ornamental fish production and trade. However, the impact of aquatic plants on water quality may vary by species, and the plant species may significantly affect each other's growth—an aspect that has received limited attention in such systems. Therefore, further research may be necessary to better understand the effects of different plant species and their combinations. Practical implications can be drawn from the results of this study, contributing to the efficiency of ornamental fish farming, as appropriately selected aquatic plants and combinations of plants will enable effective reduction of nitrate and phosphate levels in tank water.

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CONFLICT OF INTEREST STATEMENT

The authors have no competing interests or conflict of interest.

DATA AVAILABILITY STATEMENT

Data will be made available upon request.

ETHICS STATEMENT

Hereby, we declare that throughout the research, all experimental procedures and protocols were strictly conducted in accordance with ethical guidelines. The treatment of animals employed in the study consistently respected the well-being and rights of the animals. The handling and evaluation of data adhered to the principles of objectivity and transparency. We acknowledge the societal significance of the research findings and commit to making the results publicly available. There are no conflicts or influencing factors, and the research was independently funded. Every member of the research team is dedicated to ethical research practices and scientific integrity.

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REFERENCES

Alghamdi, A. A. (2003). *The effect of inorganic and organic nitrogen sources and their combination on growth and metabolism of* Vesicularia dubyana [Doctoral dissertation, Michigan Technological University]. Saudi Digital Library. https://drepo.sdl.edu.sa/handle/20.500.14154/59580

Bačkor, M., Goga, M., Singh, P., & Tuptová, V. (2023). Mechanisms of copper toxicity and tolerance in the aquatic moss *Taxiphyllum barbieri*. *Plants*, *12*(20), Article 3607. https://doi.org/10.3390/plants12203607

Biondo, M. V., & Burki, R. P. (2020). A systematic review of the ornamental fish trade with emphasis on coral reef fishes—An impossible task. *Animals*, *10*(11), Article 2014. https://doi.org/10.3390/ani10112014

Boyd, C. E., & Tucker, C. S. (2012). *Pond aquaculture water quality management*. Springer Science & Business Media.

Camargo, J. A., Alonso, A., & Salamanca, A. (2005). Nitrate toxicity to aquatic animals: A review with new data for freshwater invertebrates. *Chemosphere*, *58*, 1255–1267. https://doi.org/10.1016/j.chemosphere.2004.10.044

Cowan, N., Ashwood, D., Drewer, J., Toteva, G., & Heal, M. R. (2024). A low-tech, low-cost method to capture point-source ammonia emissions and their potential use as a nitrogen fertiliser. *PLOS ONE*, *19*, Article e0296679. https://doi.org/10.1371/journal.pone.0296679

Dierberg, F. E., DeBusk, T. A., Jackson, S. D., Chimney, M. J., & Pietro, K. (2002). Submerged aquatic vegetation-based treatment wetlands for removing phosphorus from agricultural runoff: Response to hydraulic and nutrient loading. *Water Research*, *36*(6), 1409–1422. https://doi.org/10.1016/S0043-1354(01)00354-2

Endut, A., Jusoh, A., Ali, N., Nik, W. W., & Hassan, A. (2010). A study on the optimal hydraulic loading rate and plant ratios in recirculation aquaponic system. *Bioresource Technology*, *101*(5), 1511–1517. https://doi.org/10.1016/j.biortech.2009.09.040

Estim, A. (2010). *Water quality management in a marine fish hatchery system* [Doctoral dissertation, Universiti Malaysia Sabah]. Universiti Malaysia Sabah Institutional Repository. https://eprints.ums.edu.my/id/eprint/38187

Estim, A., Saufie, S., & Mustafa, S. (2019). Water quality remediation using aquaponics sub-systems as biological and mechanical filters in aquaculture. *Journal of Water Process Engineering*, *30*, Article 100566. https://doi.org/10.1016/j.jwpe.2018.02.001

Evers, H. G., Pinnegar, J. K., & Taylor, M. I. (2019). Where are they all from? – Sources and sustainability in the ornamental freshwater fish trade. *Journal of Fish Biology*, *94*(6), 909–916. https://doi.org/10.1111/jfb.13930

Gosselin, J. R., Haller, W. T., Gettys, L. A., Griffin, T., & Crawford, E. S. (2018). Effects of substrate nutrients on growth of three submersed aquatic plants. *Journal of Aquatic Plant Management*, *56*, 39–46.

Granéli, W., & Solander, D. (1988). Influence of aquatic macrophytes on phosphorus cycling in lakes. In G. Persson & M. Jansson (Eds), *Phosphorus in freshwater ecosystems: Proceedings of a symposium held in Uppsala, Sweden, 25–28 September 1985* (Developments in Hydrobiology 48, pp. 245–266). Springer. https://doi.org/10.1007/978-94-009-3109-1_15

Hamid, N. B. B., Masiri, B., Kaamin, M. B. M., Abd Kadir, A. B., Bakar, S. K. B. A., & Ibrahim, N. H. B. (2015). Research on the effectiveness of an aquatic plant (*Utricularia aurea*) for fish preservation. *Journal of Civil Engineering Research*, *5(4A)*, 1–5. https://doi.org/10.5923/c.jce.201501

Hasandras, H., Moore, K. A., & Gettys, L. A. (2018). Growth of the aquatic plant southern naiad in varying percentages of sand and controlled-release fertilizer. *HortTechnology*, *28*, 252–256. https://doi.org/10.21273/HORTTECH03987-18

Hernández-López, M., & Luna-Vivaldo, I. (2021). Life history traits of Endler's fish (*Poecilia wingei*). *AACL Bioflux*, *14*, 1727–1733.

Hrivnák, R., Medvecká, J., Baláži, P., Bubíková, K., Oťaheľová, H., & Svitok, M. (2019). Alien aquatic plants in Slovakia over 130 years: Historical overview, current distribution and future perspectives. *NeoBiota*, *49*, 37–57. https://doi.org/10.3897/neobiota.49.34318

Hussner, A. (2012). Alien aquatic plant species in European countries. *Weed Research*, *52*, 297–306. https://doi.org/10.1111/j.1365-3180.2012.00926.x

Indriani, R., Hadiroseyani, Y., & Diatin, I. (2023). The growth performance and physiological status of Comet Goldfish *Carassius auratus* in aquascape with different aquatic plant species. *Jurnal Akuakultur Indonesia*, *22*, 36–46. https://doi.org/10.19027/jai.22.1.36-46

Jones, M., Alexander, M. E., Snellgrove, D., Smith, P., Bramhall, S., Carey, P., Henriquez, F. L., McLellan, I., & Sloman, K. A. (2022). How should we monitor welfare in the ornamental fish trade? *Reviews in Aquaculture*, *14*(2), 770–790. https://doi.org/10.1111/raq.12624

Király, G. (2009). *New Hungarian herbal. The vascular plants of Hungary. Identification key* [In Hungarian]. Aggteleki Nemzeti Park Igazgatóság.

Landolt, E., & Kandeler, R. (1987). *The family of Lemnaceae: A monographic study*. Stiftung Ruebel.

Léger, P., Bengtson, D. A., Sorgeloos, P., Simpson, K. L., & Beck, A. D. (1987). The nutritional value of *Artemia*: A review. In P. Sorgeloos, D. A. Bengtson, W. Decleir, & E. Jaspers (Eds.), *Artemia research and its applications: Ecology, culturing, use in aquaculture* (Vol. *3*, pp. 357–372). Universa Press.

Lowden, R. M. (1986). Taxonomy of the genus *Najas* L. (Najadaceae) in the Neotropics. *Aquatic Botany*, *24*(2), 147–184. https://doi.org/10.1016/0304-3770(86)90094-X

Lukács, B. A., Mesterházy, A., Vidéki, R., & Király, G. (2016). Alien aquatic vascular plants in Hungary (Pannonian ecoregion): Historical aspects, data set and trends. *Plant Biosystems*, *150*, 388–395. https://doi.org/10.1080/11263504.2014.987846

Martin, M. (2013). *Aquascaping: Aquarium landscaping like a pro: Aquarist's guide to planted tank aesthetics and design*. Ubiquitous Publishing.

National Center for Public Health and Pharmacy. (2024). *Magyarországi települések ivóvízminősége*. Országos ivóvízminőségi adatbázis (Humán Vízhasználatok Informatikai Rendszere, HUMVI) 2022. https://www.nnk.gov.hu/index.php/kozegeszsegugyi-laboratoriumi-foosztaly/kornyezetegeszsegugyi-laboratoriumi-osztaly/vizhigienes-laboratorium/ivoviz/magyarorszagi-telepulesek-ivovizminosege.html?view=placemark&load_bootstrap=1&thumbnail=1&imagegalery=0&hidedescriptionhtml=0&showdescriptionfullhtml=0&id=27398

Norris, M. K. (1983). *Potential biological control of nuisance blue green algal species by* Najas guadalupensis *and* Hydrodictyon reticulatum [Master's thesis, South Dakota State University]. Open PRAIRIE. https://openprairie.sdstate.edu/etd/4378

Roslan, M. N. A. M., Estim, A., Venmathi Maran, B. A., & Mustafa, S. (2021). Effects of aquatic plants on nutrient concentration in water and growth performance of Fantail Goldfish in an aquaculture system. *Sustainability*, *13*(20), Article 11236. https://doi.org/10.3390/su132011236

Sköld, H. N., Aspengren, S., Cheney, K. L., & Wallin, M. (2016). Fish chromatophores—From molecular motors to animal behavior. *International Review of Cell and Molecular Biology*, *321*, 171–219. https://doi.org/10.1016/bs.ircmb.2015.09.005

Smallbone, W., Cable, J., & Maceda-Veiga, A. (2016). Chronic nitrate enrichment decreases severity and induces protection against an infectious disease. *Environment International*, *91*, 265–270. https://doi.org/10.1016/j.envint.2016.03.008

Sorgeloos, P., Coutteau, P., Dhert, P., Merchie, G., & Lavens, P. (1998). Use of brine shrimp, *Artemia* spp., in larval crustacean nutrition: A review. *Reviews in Fisheries Science*, *6*(1–2), 55–68. https://doi.org/10.1080/10641269891314195

Teixeira-de Mello, F., de Oliveira, V. A., Loverde-Oliveira, S. M., Huszar, V. L. M., Barquín, J., Iglesias, C., Silva, T. S. F., Duque-Estrada, C. H., Silió-Calzada, A., & Mazzeo, N. (2016). The structuring role of free-floating plants on the fish community in a tropical shallow lake: An experimental approach with natural and artificial plants. *Hydrobiologia*, *778*, 167–178. https://doi.org/10.1007/s10750-015-2447-2

Tilak, K. S., Veeraiah, K., & Raju, J. M. P. (2007). Effects of ammonia, nitrite and nitrate on hemoglobin content and oxygen consumption of freshwater fish, *Cyprinus carpio* (Linnaeus). *Journal of Environmental Biology*, *28*, 45–47.

Tsang, B., & Gerlai, R. T. (2022). Common aquarium plants as an enrichment strategy in Zebrafish facilities. *Zebrafish*, *19*(6), 218–223. https://doi.org/10.1089/zeb.2022.0036

Witztum, A., & Chaouat, M. (1991). Contributions to the flora of Israel and Sinai. VI. *Najas guadalupensis* (Sprengel) Magnus in Israel. *Israel Journal of Botany*, *40*(1), 65–66. https://doi.org/10.1080/0021213X.1991.10677178