

## **Efficacy of various vegetable plants in aquaponic ebb-flow system for Asian redbtail catfish (*Hemibagrus nemurus*) cultivation**

<sup>1</sup>Lies Setijaningsih, <sup>1</sup>Titin Kurniasih, <sup>1</sup>Eri Setiadi, <sup>2</sup>Dudi Lesmana, <sup>3</sup>Moh Mustakim, <sup>2</sup>Yudi Wahyudin, <sup>1</sup>Muhamad Yamin, <sup>1</sup>Novita Panigoro, <sup>4</sup>Armen Zulham, <sup>5</sup>Ulfauza, <sup>6</sup>Safar Dody, <sup>1</sup>Ediwarman, <sup>1</sup>Rahmat

<sup>1</sup> Research Center for Fishery, National Research and Innovation Agency, Cibinong, Bogor, Jawa Barat, Indonesia; <sup>2</sup> Department of Aquaculture, Universitas Djuanda, Bogor, Indonesia; <sup>3</sup> Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Mulawarman University, Samarinda, Kalimantan Timur, Indonesia; <sup>4</sup> Research Center for Cooperative, Corporation, and People's Economy – National Research Innovation Agency, Jakarta, Indonesia; <sup>5</sup> Department of Aquaculture, Jakarta Technical University of Fisheries (Politeknik Ahli Usaha Perikanan), Jakarta, Indonesia; <sup>6</sup> Research Center for Oceanography, National Research and Innovation Agency, Jakarta, Indonesia. Corresponding author: T. Kurniasih, titin.kurniasih@brin.go.id

**Abstract.** Implementing an aquaponic system is an innovative method of cultivating fish that combines hydroponics and aquaculture. Aquaponic has been applied in fish farming systems in Indonesia, although its effectiveness has been limited to certain fish species. In this study, two green veggie plants, water spinach (*Ipomoea aquatica*) and caisin (*Brassica rapa* var. *parachinensis*) were selected to evaluate the effects of different plant species on water quality, fish growth, survival rate (SR), plant growth, and bacterial abundance. This study used an ebb-flow aquaponic system to cultivate an Asian redbtail catfish (*Hemibagrus nemurus*) with an initial weight of  $8.86 \pm 0.14$  g and length of  $10.45 \pm 0.21$  cm for 60 days. The findings indicated that the average amounts of TAN, nitrite, nitrate, and orthophosphate in water spinach were lower than in caisin. Fish cultivation using water spinach treatment showed better growth performance in terms of length and weight compared to caisin, as reflected in both survival growth rate (SGR) and feed conversion ratio (FCR). Furthermore, water spinach treatment yielded the highest SR (91.86%), followed by caisin (71.90%), and control (63.81%). The water spinach treatment exhibited the highest bacterial abundance in both nitrifying and denitrifying bacteria. These results indicate that the ebb-flow aquaponic system produced better outcomes than non-planted, with water spinach outperforming caisin in terms of water quality as well as fish and plant growth.

**Key Words:** *Hemibagrus nemurus*, aquaponics ebb-flow system, water quality, growth performance.

**Introduction.** Aquaponics is an emerging method for increasing fish and vegetable production (Konig et al 2018) that could provide integrated food production by utilizing fish culture waste, such as waste secreted by fish, urine, and uneaten feed, as an organic fertilizer for plants (Stathopoulo et al 2018; Yep & Zheng 2019). According to Dalsgaard et al (2013), aquaponic systems have demonstrated efficacy in utilizing scarce land and water resources, as evidenced by their water reuse efficiency of 95–99%. This efficiency is achieved through microbiological processes that use water from fish cultures as a plant nutrient (Baganz et al 2021). Subsequently, the biofiltered water obtained from the vegetation flows back into the fish tank, producing an environment suitable to both fish and plant growth as compared to conventional methods (Turnsek et al 2020). This system is considered a green technology as it does not release waste into the environment (Joyce et al 2019).

Aquaponic systems have employed a variety of fish species, including tilapia (Setijaningsih & Umar 2015; Kaburagi et al 2020; Ani et al 2022), catfish (Pasch et al 2021; Setijaningsih et al 2022), and common carp (Filep et al 2016; Shete et al 2017).

However, studies on Asian redbtail catfish (*Hemibagrus nemurus*) remain limited. This fish is a freshwater species with high economic value in Southeast Asia, which includes China, the Malay Peninsula, and the Indonesian Archipelago (Gustiano et al 2015). It is prized for its savory, delicious flavour and low-fat content. Currently, the demand for consumable-sized of this fish is largely met by wild catches owing to limited availability from aquaculture. Although this fish grows well in both cages and aquaculture ponds, low survival rates have resulted in reduced fish production (Hasan et al 2022).

The utilization of several fish species in aquaponic systems has been integrated with a variety of plants, primarily green leafy vegetables such as lettuce (Forchino et al 2017; Ani et al 2022), water spinach (Endut et al 2011; Nuwansi et al 2017), and pak choi (Hu et al 2015; Ru et al 2017). The success of these plants varies according to where the aquaponic is placed (Verma et al 2023). The plants that are employed in aquaponic systems play an important part in the biofiltration process because each plant species has specific characteristics in terms of nutrient absorption, root structure, and growth rate, that all of which can influence the system's growth and production (Hu et al 2015; Verma et al 2023). Two leafy green plants, specifically water spinach and caisin have been reported to function effectively as biofiltration agents in aquatic systems (Endut et al 2016; Mahabrur & Zulkanain 2023). These vegetables are also popular in Southeast Asia.

Furthermore, the cultivation of *H. nemurus* in an aquaponic system integrated with two different plant species has not been thoroughly investigated. Therefore, the main objective of this research was to assess the efficacy of using different plant species: water spinach and caisin in cultivating *H. nemurus*, focusing on their impact on water quality improvement, growth performance, survival rate, and biomass production generated in an aquaponic ebb-flow system.

## Material and Method

**Materials.** *H. nemurus* fingerlings were obtained from the Sukabumi Freshwater Aquaculture Center, with an initial average weight and length of  $8.91 \pm 0.03$  g and  $10.38 \pm 0.02$  cm, respectively. The fish were stocked in each pond at a uniform density of 1.400 fish specimens by pond (size is specified below). Water spinach and caisin seedlings were germinated first, then moved to aquaponic containers after reaching a height of 7 cm. Each aquaponic container had five planting points, with each point containing a cluster of five plants weighing approximately 10 g per cluster. Both water spinach and caisin were planted in containers seven days after the fingerlings were placed into the aquaponic ebb-flow system.

**Experimental design and unit.** The study lasted for 60 days, from March to April 2023, and used an experimental technique with three treatments and three replicates. It was conducted in an aquaponic ebb-flow system at the Center for Production, Inspection, and Certification of Fishery Products in Jakarta, Indonesia. The treatments applied in this study involved different vegetable plants used as biofiltration media: (a) water spinach, (b) caisin, and (c) control (no plants), with wood charcoal and fern roots as planting media. Wood charcoal and fern roots were selected for their economic, practical, and environmentally friendly. Existing research indicates that charcoal can enhance fish health, improve water quality and nutrient absorption, and remove pollutants from aquaculture systems (Salisu et al 2023; Wong et al 2024) due to its large surface area and wide pores, which provide extensive space for bacterial absorption and adhesion.

The cultivation ponds consisted of nine concrete ponds, with dimensions  $4 \times 2 \times 1$  m (length  $\times$  width  $\times$  height), and hydroponic containers measuring  $28 \times 28 \times 25$  cm<sup>3</sup> (length  $\times$  width  $\times$  height). An aquaponic ebb-flow system was formed by connecting the planting media containers with paralon pipes placed around the entire perimeter of the ponds, as shown in Figure 1. The components of aquaponic ebb-flow system are a reservoir and a water pump that function as the 'ebb-flow system', distributing water to all connected plant containers.

This system works by pumping water into a reservoir, which flows through to all associated plant containers in a flow-through system. When the water reached a certain level, it was transferred to the hydroponic unit through paralon pipe and returned to the fish-rearing pond by gravity to complete the cycle. The biofiltered water entered the reservoir and flowed back into the fish tank after setting up a small waterfall. This system was designed to accomplish both water aeration and a constant water flow to the pond. In this model, plant roots are submerged in water for a set amount of time before being permitted to dry out (not submerged) to enable root respiration (Figure 1). The aquaponic ebb-flow installation was set up outdoors, receiving direct sunlight, and the study was conducted during the dry season, ensuring that the experiment was not exposed to rainwater.

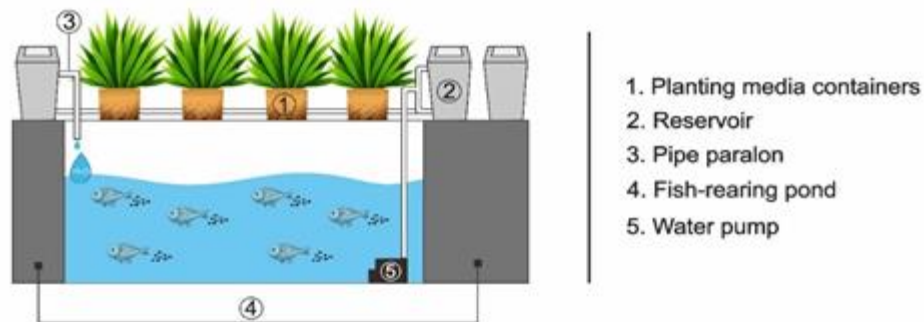


Figure 1. Schematic and circuit of an aquaponic ebb-flow system.

In all treatments, the fish were given artificial pellets twice a day, in the morning and the evening, at a rate of 3% of their body weight. Water quality parameters (temperature, pH, dissolved oxygen (DO), total ammonia nitrogen (TAN), nitrite ( $\text{NO}_2$ ), nitrate ( $\text{NO}_3$ ), and orthophosphate), growth performance, feed conversion ratio (FCR), survival rate (SR), and abundance of nitrifying and denitrifying bacteria were measured every 10 days over a 60 day period. Plant biomass was obtained from harvests conducted on days 25 (first harvest) and 55 (second harvest).

**Determination of water quality.** Temperature, pH, and DO were recorded using a YSI multiparameter model 556, while the levels of TAN, nitrite, and nitrate were analyzed according to the APHA (2012) method, using flame atomic emission spectrophotometry. The measurement of orthophosphate was performed using ion chromatography (Cerozi & Kevin 2016).

**Determination of growth performances of fish and plant biomass.** Biometric data calculations for fish included measurements of weight, length, percentage weight, percentage length, and specific growth rate. Fish weight was measured by weighing each fish using a digital scale (Digital A&D, HT-120) with a precision of 0.01 g, and fish length was determined using a caliper with an accuracy of 0.1 mm.

Fingerlings' growth in each treatment was calculated using the specific growth rate (SGR) and feed conversion ratio (FCR). SGR was determined using Setijaningsih et al (2022) and Ani et al (2022) formulas, while FCR was determined as the ratio between total feed provided (g) and total weight gain (g) during the observation period (Endut et al 2011; Ani et al 2022). The survival rate (SR) of the fingerlings was estimated using the approach of Venkatachalam et al (2018), and the total fish biomass was estimated using the method described by Srivastava et al (2012). Subsequently, plant sampling involved measuring plant height and leaf length as well as calculating plant biomass on days 25 (first harvest) and 55 (second harvest).

**Determination of nitrifying and denitrifying bacteria.** The bacterial abundance of nitrifying and denitrifying bacteria in the water was assessed every 10 days using Most Probable Number (MPN) method (Cappucino et al 2007).

**Data analysis.** Data on fish and plant performance, water quality, and bacterial abundance are presented as a mean±SD. The statistical analysis was performed using SPSS version 25 with a confidence level of 95% (significance at  $P<0.05$ ). The average values of fish production growth, plant growth rate, water quality, and bacterial abundance among the treatments were tested using one-way ANOVA. If there were significant at the 0.05 level, Duncan's multiple range test was employed to compare means and identify significant differences between treatments.

**Results.** The concentration of temperature, pH, DO, nitrite, nitrate, TAN, and orthophosphate in water quality are outlined in Table 1. There were no significant differences ( $P>0.05$ ) in pH and temperature among the treatments, whereas the contents of DO, nitrite, nitrate, TAN, and orthophosphate differed significantly ( $P<0.05$ ) during the experimental periods.

Table 1

Water quality in an aquaponic ebb-flow system

<i>Parameters</i>	<i>Control</i>	<i>Water spinach</i>	<i>Caisin</i>	<i>P-value</i>
Temperature (°C)	27.95±0.32 <sup>a</sup>	27.96±0.37 <sup>a</sup>	27.87±0.32 <sup>a</sup>	0.864
pH	6.91±0.19 <sup>a</sup>	6.93±0.18 <sup>a</sup>	6.90±0.18 <sup>a</sup>	0.969
DO (mg L <sup>-1</sup> )	4.11±0.06 <sup>a</sup>	5.91±1.02 <sup>b</sup>	5.17±0.72 <sup>b</sup>	0.001
TAN (mg L <sup>-1</sup> )	6.21±3.54 <sup>c</sup>	0.92±0.37 <sup>a</sup>	3.52±1.80 <sup>b</sup>	0.002
Nitrite (NO <sub>2</sub> ) (mg L <sup>-1</sup> )	0.09±0.04 <sup>b</sup>	0.02±0.01 <sup>a</sup>	0.04±0.01 <sup>a</sup>	0.001
Nitrate (NO <sub>3</sub> ) (mg L <sup>-1</sup> )	1.62±0.96 <sup>b</sup>	0.52±0.26 <sup>a</sup>	1.00±0.54 <sup>ab</sup>	0.019
Orthophosphate (mg L <sup>-1</sup> )	1.24±0.80 <sup>b</sup>	0.19±0.09 <sup>a</sup>	0.31±0.25 <sup>a</sup>	0.001

Different superscript in the same row shows statistically significant difference ( $P<0.05$ ) among treatments and ± indicates the standard deviation.

The changes in water quality during the experimental periods are presented in Figures 2 and 3. The temperature and pH in the cultivation pond in all treatments during the experimental period ranged from 27.44-28.45°C and 6.62-7.17, respectively (Figure 2), while DO in both aquaponic systems gradually increased (Figure 3).

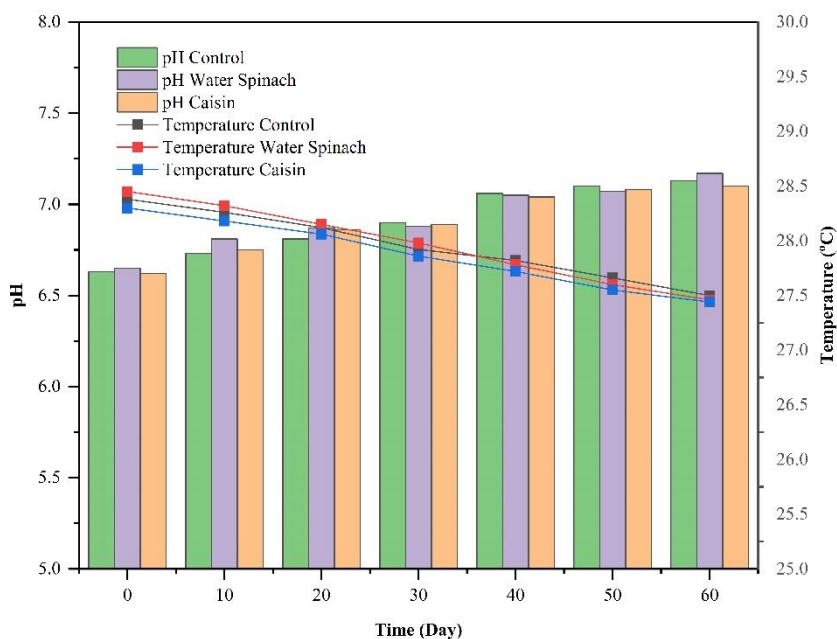


Figure 2. Changes in water quality in temperature and pH.

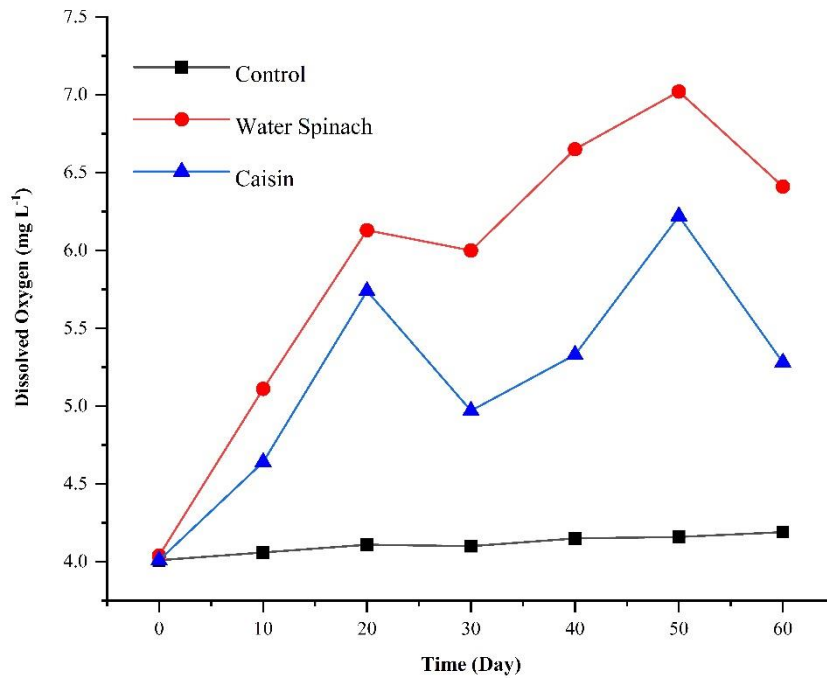


Figure 3. Fluctuation of DO in the cultivation pond in all treatments during the experimental period.

The TAN, nitrate, and orthophosphate levels in all treatments exhibited a similar pattern, with increases occurring throughout the experiment. The lowest increase was noted in the aquaponic system with water spinach, followed by caisin and control, respectively (Figure 4).

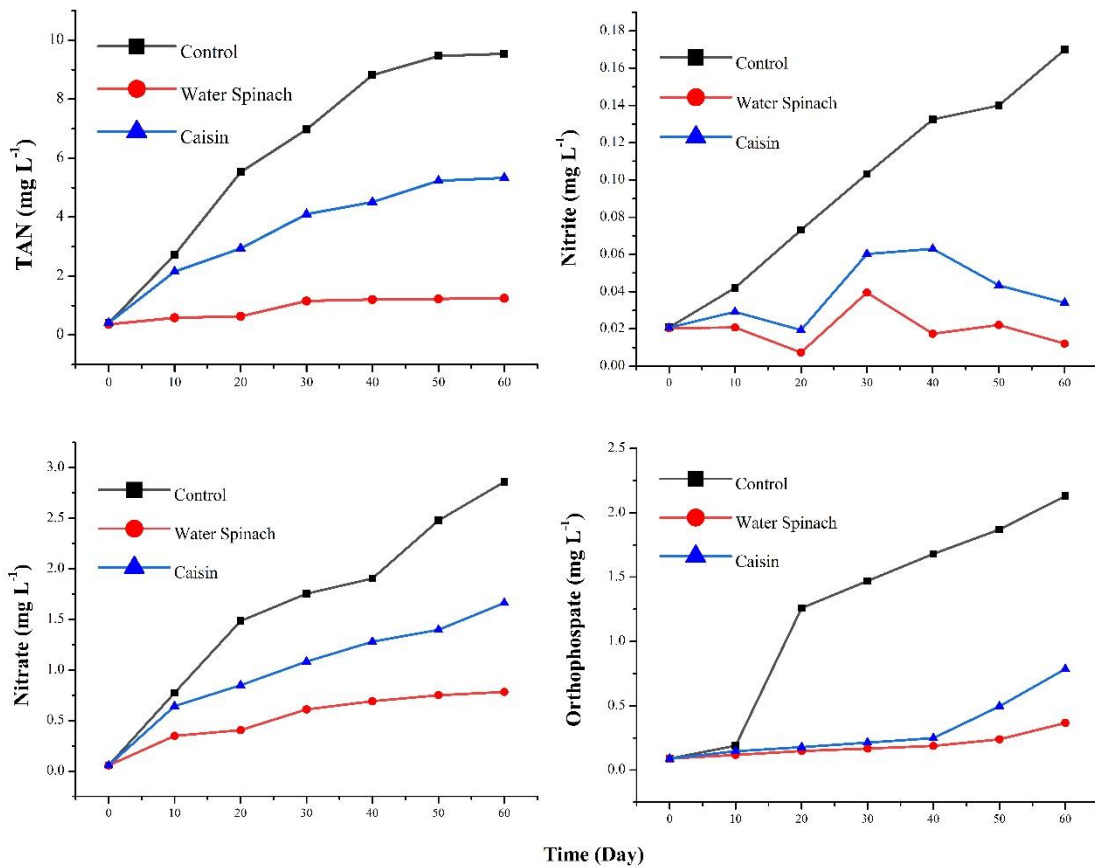


Figure 4. TAN, nitrite, nitrate, and orthophosphate levels in the cultivation pond.

The results indicate that the use of diverse plant species for final length, final weight, SGR, and SR differed significantly ( $P < 0.05$ ) among the treatments, except for FCR ( $P > 0.05$ ) (Table 2). The aquaponic system implementing water spinach exhibited the most favorable growth parameters among the treatments, as evidenced by the highest percentage increase in fish length and weight (100.47% and 819.16%, respectively), as well as the highest SGR in water spinach ( $3.69 \pm 0.03$ ), followed by caisin ( $3.41 \pm 0.02$ ), and the lowest in the control group ( $2.97 \pm 0.02$ ).

Table 2  
Growth performances and survival rate of *Hemibagrus nemurus*

Parameters	Control	Water spinach	Caisin	P-value
Initial body length (cm)	10.45±0.21 <sup>a</sup>	10.47±0.23 <sup>a</sup>	10.38±0.03 <sup>a</sup>	0.217
Final body length (cm)	14.33±0.34 <sup>a</sup>	20.98±0.65 <sup>c</sup>	18.30±0.76 <sup>b</sup>	0.000
Percentage length gain (%)	37.13±3.26 <sup>a</sup>	100.47±7.95 <sup>c</sup>	76.35±7.04 <sup>b</sup>	0.000
Initial body weight (g)	8.86±0.27 <sup>a</sup>	8.86±0.14 <sup>a</sup>	8.82±0.21 <sup>a</sup>	0.792
Final body weight (g)	52.77±0.60 <sup>a</sup>	81.39±0.94 <sup>c</sup>	68.66±0.76 <sup>b</sup>	0.000
Percentage weight gain (%)	496.16±19.56 <sup>a</sup>	819.16±16.36 <sup>c</sup>	678.95±23.36 <sup>b</sup>	0.000
Specific growth rate (%)	2.97±0.02 <sup>a</sup>	3.69±0.03 <sup>c</sup>	3.41±0.02 <sup>b</sup>	0.000
FCR	1.10±0.07 <sup>b</sup>	0.94±0.03 <sup>a</sup>	0.99±0.03 <sup>a</sup>	0.017
Survival rate (%)	63.81±0.25 <sup>a</sup>	91.86±0.07 <sup>c</sup>	71.90±0.72 <sup>b</sup>	0.000

Different superscript in the same row shows statistically significant difference ( $P < 0.05$ ) among treatments and  $\pm$  indicates the standard deviation.

The utilization of water spinach and caisin in an aquaponic ebb flow system with *H. nemurus* led to significant differences ( $P < 0.05$ ) in average plant weight between two harvesting periods during the experimental phase as seen in Table 3.

Table 3  
Biomass production

Parameters	Water spinach	Caisin	P-value
Weight of plant (kg) / Crop yield			
First harvest			
Total average per unit	12.73±1.22	9.61±0.31	0.040
Total Biomass	41.18 <sup>b</sup>	28.85 <sup>a</sup>	-
Second harvest			
Total average per unit	14.44±1.88 <sup>b</sup>	8.38±1.90 <sup>a</sup>	0.017
Total Biomass	38.33	25.13	-
Total yield/biomass (first and second harvest)	79.50	53.98	-
Plant height (cm)			
Min	30	29	-
Max	45	40	-
Root length (cm)			
Min	3	1	-
Max	5	3	-

Different superscript in the same row shows statistically significant difference ( $P < 0.05$ ) among treatments and  $\pm$  indicates the standard deviation.

In this study, the average of the first and second harvest, along with the total plant biomass produced by water spinach (12.73, 14.44, and 79.50 kg, respectively), were higher than those produced by caisin (9.61, 8.38, and 53.98, respectively). Water spinach had plant heights ranging from 30-45 cm and root lengths between 3-5 cm, but caisin had slightly lower results, with plant heights of 29-40 cm and root lengths ranging from 1-3 cm. In this study, both water spinach and caisin were harvested at growth and weight levels adequate for commercial consumption.

The most significant microorganisms in aquaponics are nitrifying and denitrifying bacteria, which decompose waste into molecules that can be utilized by plants. The abundance of nitrifying and denitrifying bacteria is shown in Figures 5 and 6. Both nitrifying and denitrifying bacteria proliferated during the experiment, with significant differences ( $P < 0.05$ ) observed at each interval (except on day 0) across all treatments. The aquaponic system with water spinach showed the highest increase, followed by caisin, and control. On day 60, the number of nitrifying bacteria in the control, water spinach, and caisin treatments reached  $58.93$ ,  $156.50$ , and  $106.60 \times 10^7 \text{ cell mL}^{-1}$ , respectively, while the number of denitrifying bacteria reached  $21.95$ ,  $93.30$ , and  $52.70 \times 10^7 \text{ cell mL}^{-1}$ , respectively. This is consistent with the TAN levels seen in this study, where the water spinach treatment had the lowest TAN concentration, followed by caisin and control, indicating that a greater number of nitrifying bacteria is associated with the nitrification process.

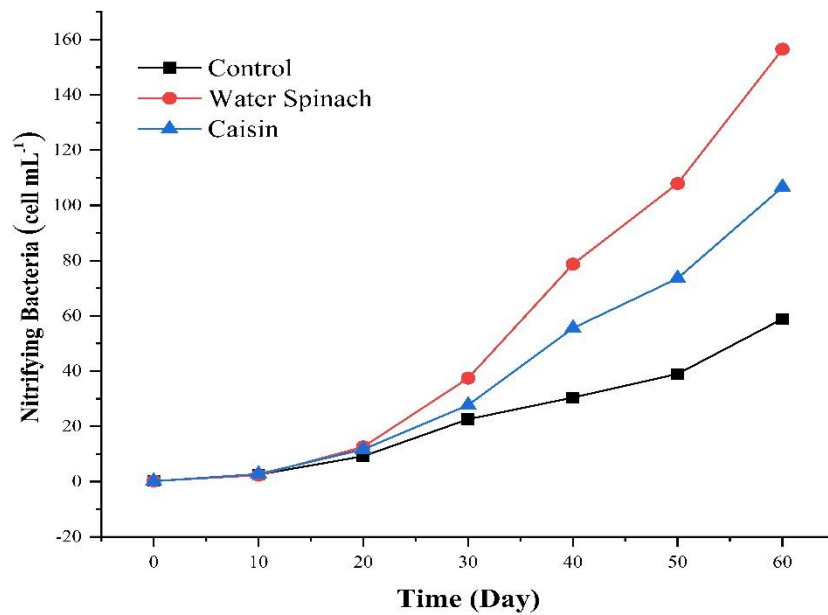


Figure 5. Abundance of nitrifying bacteria during cultivation period.

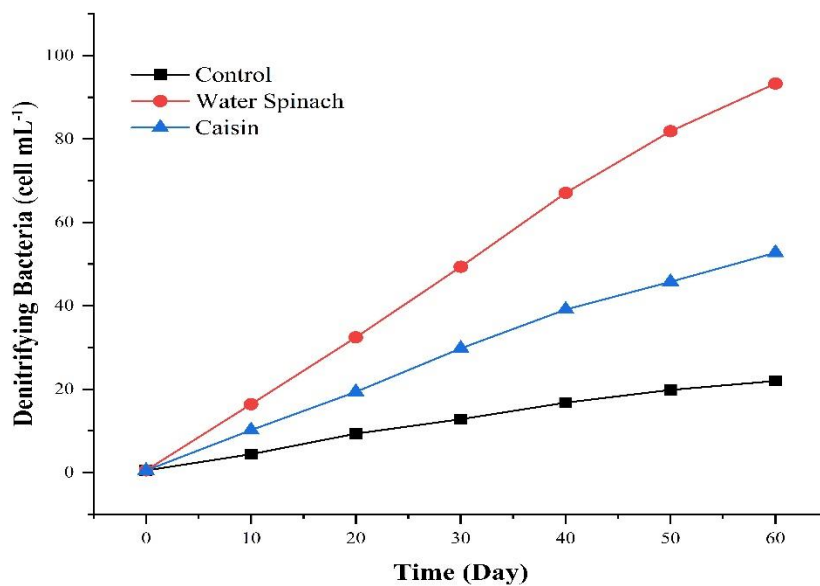


Figure 6. Abundance of denitrifying bacteria during cultivation period.

**Discussion.** The results revealed that there were no significant differences ( $P > 0.05$ ) in temperature and pH between the treatments, while DO, nitrite, nitrate, TAN, and orthophosphate differed significantly ( $P < 0.05$ ) during the experimental periods (Table 1).



However, the use of different plant species had a significant effect on TAN levels. Cultivation of *H. nemurus* without aquaponic system (control) resulted in the lowest DO levels and the highest concentrations of nitrite, nitrate, TAN, and orthophosphate compared to the aquaponic treatments.

The temperature and pH in the cultivation pond in all treatments during the experimental period ranged from 27.44-28.45°C and 6.62-7.17, respectively (Figure 2a). Over the experimental period (days 0 through 60), water temperature showed a decreasing trend, while pH exhibited an increasing one. Kurniawan et al (2022) reported that *H. nemurus* can grow well at pH 5-7.5 and at temperatures of 25-32.5°C, therefore the temperature and pH levels observed in this study are still within the range for cultivating this fish. This is consistent with the findings of Cerozi & Kevin (2016), which stated that the pH in an aquaponic system should be maintained between 5.5-7.2 to ensure optimal availability and absorption by plants. The pH level is an important factor in the performance of aquaponic systems (Kloas et al 2015).

The nitrification process converts ammonia from fish excretions and uneaten fish food into nitrite and then into nitrate, keeping the tolerable limits for cultivation (Preena et al 2021). Nitrogen transformation, which is dependent on oxygen supply, can create suitable environmental conditions for various microbial populations in the aquaponic system (Schmautz et al 2021). During the experimental period, DO levels in both aquaponic systems gradually increased, although DO levels were reduced during certain observation periods. This drop was attributed to rapid plant growth, which resulted in increased root respiration and reduced oxygen diffusion into water from the atmosphere (Moorhead & Reddy 1988). In comparison, DO levels in the control group remained relatively stable (Figure 3). In order to achieve optimal growth rates for both fish and plants, a one-loop aquaponics system relies on maintaining environmental parameters such as temperature, pH, DO and other chemical compositions in the water (Gichana et al 2018).

The rise in TAN concentration in water is associated with increased fish weight and feed intake (Rahmatullah et al 2010). The average nitrate and orthophosphate concentrations during the experimental period did not differ significantly ( $P>0.05$ ) between the treatment of water spinach and caisin (Figure 3). Additionally, the increasing nitrate levels observed during the study period are attributed to the nitrification process by microorganisms through biogeochemical processes driven by changes in dissolved oxygen and pH concentration (Goddek et al 2015). Nitrate ( $\text{NO}_3$ ) is non-toxic to fish and serves as a major nutrient for plant growth (Wongkiew et al 2018), as plants can directly consume nitrate.

In contrast, nitrite levels displayed a different pattern, remaining relatively stable throughout the experimental period, although there was an increase on day 30 in the aquaponic treatments (Figure 3). Conversely, nitrite levels in the control treatment gradually increased during the experimental period. This increase is most likely due to ammonia decomposition, which also occurs through microbial assimilation in the water column (Shete et al 2017). However, in this study, primary ammonia decomposition occurred in the substrate media and plant roots. Baganz et al (2021) found that, in a one-water-loop aquaponic system (permanently connected), nitrification primarily occurs in the hydroponic component due to its substantial specific surface area for bacterial colony growth. Nitrification involves ammonia-oxidizing and nitrite-oxidizing bacteria that require a sufficient biological surface area; porous, lightweight, and inert media in aquaponics (Deer et al 2021). In this study, fern roots and charcoal were also used as substrates for bacterial adhesion, with porous substrates being preferred to optimize aquaponic system performance (Maucieri et al 2018).

Furthermore, microorganisms in aquaponic systems convert phosphorus from fish feed waste and excretions into orthophosphate ions, which can be absorbed by plants (Verma et al 2023). Endut et al (2016) reported that orthophosphate absorption increases as the plants grow. As a result, the decomposition of ammonia and phosphate by microorganisms in aquaponic systems can promote plant growth by providing nutrients while reducing fish stress by improving water quality (Chandramenon et al 2024). However, Van Bussel et al (2013) found that orthophosphate accumulation in RAS



did not negatively impact the health of Turbot fish, with phosphate ( $\text{PO}_4\text{-P}$ ) concentrations ranging from 1.31-0.74  $\text{mg L}^{-1}$  across all treatments during the experiment. Moreover, it has been found that pH can influence orthophosphate concentrations in aquaponic systems, with an increase in pH leading to a drop in total orthophosphate levels (Cerozi & Kevin 2016).

In this study, the usage of water spinach improved water quality more than caisin. During the experimental period, water spinach had lower average concentrations of TAN, nitrite, nitrate, and orthophosphate (0.92, 0.02, 0.52, and 0.19  $\text{mg L}^{-1}$ , respectively) than caisin (3.52, 0.04, 1.00, and 0.31  $\text{mg L}^{-1}$ ). This could be related to the water spinach plant, which is known for its long fibrous roots (Andriani et al 2019). Water spinach has longer roots than lettuce, pak choi, and choy sum when cultivated in half-flooded and fully flooded conditions, making it ideal for aquaponic systems (Trang et al 2010). Endut et al (2016) found that water spinach generated better water quality than mustard greens in the cultivation of African catfish. Furthermore, the use of fern roots and charcoal as growing media is believed to improve nutrient absorption and pollutant removal in water spinach treatments.

The study found no significant difference ( $P>0.05$ ) in FCR between water spinach and caisin. However, the aquaponic system had a significantly lower ( $P<0.05$ ) FCR value compared to the control (Table 2). Endut et al (2016) reported that in an aquaponic system for African catfish (*Clarias gariepinus*), water spinach showed a lower FCR (1.13) compared to mustard green (1.29). Lower FCR is influenced by good water quality, which ultimately results in higher fish production (Maucieri et al 2018). The highest survival rate was found in the water spinach, followed by caisin and control ( $91.86\pm 0.07$ ,  $71.90\pm 0.72$ , and  $63.81\pm 0.25$ , respectively). This outcome was partly influenced by increased DO levels during the study period, which had a positive effect on the growth of this fish. *H. nemurus* requires a high concentration of DO to maintain homeostasis. This is in line with water spinach treatment, that had the highest DO content ( $5.91\pm 1.02$ ), resulting in the highest survival rate. Essentially, fish growth performance and survival rates depend on various external factors, including water environment, feeding techniques (Supriyono et al 2022), and light intensity, whether direct or indirect (Heltonika & Okta 2017).

Furthermore, water quality is a crucial factor influencing fish growth in aquaponic systems, where better water quality leads to improved fish performance (Yildiz et al 2017). Similar outcomes have been reported in other studies, where the use of water spinach in aquaponic systems resulted in better growth performance than aquatic monoculture systems in species such as grass carp, African catfish, and red tilapia (Zhang et al 2019; Putri et al 2019; Luo et al 2023). Mahabrur & Zulkanain (2023) reported a different outcome, stating that the use of *Brassica rapa* var. *parachinensis* had no effect on tilapia weight, length, or SGR over the 30-day observation period. These differences in research findings can be attributed to variations in the methods employed, duration of treatment, location, and the genetics of the fish species involved (Barbosa et al 2022; Mugo-Bundi et al 2024).

These findings indicate that water spinach has a higher capacity for nutrient absorption than caisin due to its long and widespread root structure. Longer roots allow more particles to be collected or attached to, increasing nutrient absorption, which eventually meets the nutritional needs of water spinach. Bailey & Ferrarezi (2017) observed that most leafy green vegetables have short growth cycles and thrive in nitrogen-rich water with low nutrient requirements and can grow well in media that are fully saturated with water (Trang et al 2010). In the present study, water spinach grew actively and showed no evidence of nutritional imbalance or deficiency during the study period, indicating a positive response to the application of aquaculture wastewater in aquaponic systems in terms of biomass production and growth (Nuwansi et al 2017; Trang et al 2017). Constant oxygen contact with the roots improves nitrification, oxygen supply, and root activity, which leads to higher plant biomass production (Pasch et al 2021). The roots of water spinach play a crucial role in the accumulation of nitrogen (N) and phosphorus (P), with removal rates ranging from 64.1 to 67.3% (Liu et al 2022). Nuwansi et al (2017) reported a greater range of water spinach height in Koi and Goldfish

aquaponics, ranging from 21.94 to 62.98 cm. Furthermore, Trang et al (2010) found that water spinach exhibited greater plant height and root length than caisin. The pH level of the aquaponic system is a crucial factor that affects nutrient absorption by plants. A pH value close to 7 is ideal as it balances the nitrification process and optimal plant nutrient uptake (Wortman 2015).

A category of bacteria known as nitrifiers, specifically autotrophic nitrifying bacteria such as *Nitrosomonas* and *Nitrobacter*, convert toxic ammonia into nitrite and subsequently to nitrate through biochemical oxidation, which can then be absorbed by plants as fertilizer (Bowen et al 2011; Goddek et al 2015). Waste products excreted by fish, such as urine and ammonia, are transformed by nitrifying bacteria within the plant growth area into forms that are readily absorbed by plants for energy and growth (Diver 2006). Hu et al (2015) reported that longer and larger plant roots offer more surface area for oxidizing bacteria to flourish. This phenomenon is observable in water spinach, which has long fibrous roots with numerous rootlets, allowing bacteria to stick to or be captured by the roots, resulting an ideal environment for bacterial proliferation. The growth of bacterial colonies within this system is crucial for converting waste ammonia into nitrate, which is required for plant growth (Rashmi et al 2013).

In aquatic systems, various bacterial species build biofilms and play essential roles in nitrification and denitrification processes (Preena et al 2021). Although aquaponic systems differ in terms of fish nutrition and species, they generally have a similar bacterial base (Eck et al 2019). The nitrification process primarily involves autotrophic bacteria, heterotrophic ammonia oxidation, and nitrite oxidation, whereas denitrification processes are driven by heterotrophic and autotrophic bacterial denitrification (Preena et al 2021). The structure, population dynamics, and abundance of nitrifying and denitrifying bacteria are influenced by environmental variables including dissolved oxygen, total nitrogen (TN), pH, conductivity, and humic acid content (Wongkiew et al 2018; Gao et al 2022; Schmutz et al 2021). Therefore, the environmental conditions in which bacteria reside must be carefully maintained, as the nitrification process inherently leads to increased acid production, resulting in a decrease in pH levels because of the constant release of hydrogen ions (Elia et al 2014; Yang & Kim 2020). It has been reported that nitrifying bacteria operate optimally at pH levels between 6.5-8.0 (Goddek & Korner 2019). Furthermore, a pH level of approximately 7 is recommended for successful nitrification in aquaponic units, because a pH lower than 6.5 can interfere with this process and potentially leading to system failure (Yildiz & Bekcan 2017). Additionally, dissolved oxygen levels in the water have a significant impact on the activity of nitrifying bacteria, which become ineffective at DO levels lower than 2 mg L<sup>-1</sup> (Shete et al 2017). During the research period, DO levels tended to increase across all treatments, and the rise in aerobic conditions inhibited the denitrification process due to the shorter contact time between nitrate and denitrifying bacteria (Endut et al 2009).

**Conclusions.** The results of this study indicate that the addition of various plants to ebb and flow aquaponic systems significantly affects the water quality, particularly in regard to DO, nitrite, nitrate, TAN, and orthophosphate levels. The growth performance of *H. nemurus* in terms of weight, length, SGR, and survival rate was significantly influenced by variations in plant usage, except for FCR. Furthermore, the treatment with water spinach produced the best water quality, with average levels of TAN, nitrite, nitrate, and orthophosphate during the study being lower compared to caisin. The water spinach treatment also had the highest fish growth performance, plant biomass, and bacterial abundance throughout the study when compared to caisin and non-planted plants (control). In this study, water spinach proved to be an effective biofilter, as the aquaponic system operated without water exchange and still achieved optimal fish production. Therefore, the cultivation of *H. nemurus* integrated with water spinach offers a feasible method for producing commercial fish on a pond scale.

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Authors:

Lies Setijaningsih, Research Center for Fishery, National Research and Innovation Agency Gedung Biologi, Jl. Raya Bogor KM 47, Cibinong, Nanggung Mekar, Bogor, West Java, 16912, Indonesia, e-mail: lies.setijaningsih@brin.go.id

Titin Kurniasih, Research Center for Fishery, National Research and Innovation Agency Gedung Biologi, Jl. Raya Bogor KM 47, Cibinong, Nanggung Mekar, Bogor, West Java, 16912, Indonesia, e-mail: titin.kurniasih@brin.go.id

Eri Setiadi, Research Center for Fishery, National Research and Innovation Agency Gedung Biologi, Jl. Raya Bogor KM 47, Cibinong, Nanggung Mekar, Bogor, West Java, 16912, Indonesia, e-mail: eris005@brin.go.id  
Dudi Lesmana, Department of Aquaculture, Universitas Djuanda. Jl. Tol Ciawi No.1 Bogor West Java 16720, Indonesia, e-mail: dlesmana20@gmail.com

Moh Mustakim, Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Mulawarman University, Jalan Gunung Tabur No. 1, Gunung Kelua Campus, Samarinda City, East Kalimantan Indonesia 75129 e-mail: mustakim.unmul@gmail.com

Yudi Wahyudin, Department of Aquaculture, Universitas Djuanda. Jl. Tol Ciawi No.1 Bogor West Java 16720, Indonesia, e-mail: yudi.wahyudin@unida.ac.id

Muhamad Yamin, Research Center for Fishery, National Research and Innovation Agency Gedung Biologi, Jl. Raya Bogor KM 47, Cibinong, Nanggung Mekar, Bogor, West Java, 16912, Indonesia, e-mail: muha321@brin.go.id

Novita Panigoro, Research Center for Fishery, National Research and Innovation Agency Gedung Biologi, Jl. Raya Bogor KM 47, Cibinong, Nanggung Mekar, Bogor, West Java, 16912, Indonesia, e-mail: novita.panigoro@brin.go.id

Armen Zulham, Research Center for Cooperative, Corporation, and People's Economy, National Research and Innovation Agency – Jakarta- 12710, Indonesia. E-mail: armenzulham@gmail.com

Ulfauza, Department of Aquaculture, Jakarta Technical University of Fisheries (Politeknik Ahli Usaha Perikanan), Ministry of Marine Affairs and Fisheries, Jl. Pasar Minggu, Jati Padang, Jakarta 12520, Indonesia, e-mail: ulfauzapaul@gmail.com

Safar Dody, Research Center for Oceanography, National Research and Innovation Agency, Jl. Pasir Putih I, Ancol Timur, Pademangan, North Jakarta - Jakarta 14430 e-mail: safar.dody00@gmail.com

Ediwarman, Center Research for Fishery, National Research and Innovation Agency Gedung Biologi, Jl. Raya Bogor KM 47, Cibinong, Nanggung Mekar, Bogor, West Java, 16912, Indonesia, e-mail: ediwarman@brin.go.id

Rahmat, Research Center for Fishery, National Research and Innovation Agency Gedung Biologi, Jl. Raya Bogor KM 47, Cibinong, Nanggung Mekar, Bogor, West Java, 16912, Indonesia, e-mail: bebenharden@gmail.com

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