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Effects of stocking density of tilapia on the performance of a membrane filtration–recirculating aquaponic system

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ABSTRACT

The discharge of water effluents from aquaculture systems pollutes the waters of the ambient environment and is a major concern in the development of sustainable aquaculture. In this study, we examined the effects of fish stocking density on water quality and biomass yields in a recirculating aquaponic–membrane filtration system used for co-culturing tilapia (*Oreochromis niloticus*) and water spinach (*Ipomoea aquatica*). The system was composed of a fish tank, sludge tank with a filtration membrane, and hydroponic tank. Tilapia (average initial weight, 1.8 g) was examined at three densities: 450 (low), 600 (medium), and 750 (high) individuals per tank. The initial density of water spinach was 8.3 g m^{-2}. The experimental period with no water exchange lasted for 12 weeks. Our results revealed that the increasing density of tilapia correlated positively with the increasing weight gain of water spinach but negatively with the growth of tilapia. The average weight gains were 10.8, 8.3, and 4.0 g in the low-, medium-, and high-density groups, respectively. The levels of NH_4^+–N, NO_2^-–N, NO_3^-–N, orthophosphate, biochemical oxygen demand, total bacterial counts, and turbidity in the fish and plant tank effluents were significantly higher in the high-density group than in the low-density group. The total weight gains of plants were 1,204, 1,402, and 1,708 g in the low-, medium-, and high-density groups, respectively. These results indicated that membrane filtration combined with a hydroponic component maintained a suitable water quality in the system for the survival of fish. However, the highest fish biomass gained was observed in the medium-density group, and the highest plant yield was obtained in the high-density group.

Keywords: Aquaponic; Membrane filtration; Stocking density; Tilapia; Water spinach

1. Introduction

Recirculating aquaculture systems (RASs) are being rapidly developed to meet the demands of limited land and water resources worldwide. In RASs, fish are cultured at a high density under controlled environmental conditions; they yield higher biomass under conditions of lower land and water availability than do traditional pond aquaculture systems [1]. RASs were estimated to require 90%–99% less water and land compared with pond aquaculture systems [1]. Discharge water and waste from RASs are minimal because the culture water is efficiently recycled. In addition, RASs facilitate a consistent production of cultured fish by eliminating risks of disease transmission during the rearing period. However, organic loadings (feces and uneaten feed) in RASs cause a high oxygen demand and increase toxic nitrogen-containing nutrient ions such as ammonia and nitrite [2,3]. Therefore, some daily water exchange might still be necessary to reduce the accumulation of dissolved nutrients in the system.

RASs are typically connected to additional treatment components for reducing organic wastes and nitrogenous

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compounds in the culture water. Treatment processes are often designed for physical removal, such as settling tanks and mechanical filtration for removing a large amount of waste produced in RASs, as well as for biochemical removal, such as the transformation of toxic ammonia to nontoxic nitrate ions [1,3]. RASs can be further combined with a hydroponic system (soil-less plant culture system) called a recirculating aquaponic system (RAPS) to reduce nutrients in water [4]. An RAPS is commonly composed of three basic components: fish culture, water treatment, and plant culture components [5–7]. Vegetable crops in an RAPS directly assimilate nutrients that are excreted by fish, dissolved feed, and fish waste, and also microbial degradation of organic products. RAPSs have received considerable attention because they combine fish and plant culture in a single system, thereby reducing the nutrient loading of water, minimizing water exchange, and yielding a plant harvest in addition to fish production [4,8,9]. However, recirculating water in an RAPS must still be connected to a series of water filtration compartments to treat and remove the solids produced in the fish culture compartment [6,7,9–11].

A membrane filtration bioreactor is a treatment system that incorporates components of settleable and suspended solid removal and biofiltration; it performs an outside-in and low-pressure filtration process for yielding clean water permeates [2,12,13]. The high performance of the system is caused by the high activated sludge concentration in a single and compact component, thus eliminating the need for additional facilities and spaces for clarifying the treated water [14]. This type of system has been completely commercialized for treating wastewater and drinking water [12,15].

Studies have focused on the use of membrane filtration with RAS for replacing traditional filtration systems. Their results revealed that, depending on the system design, ammonia and suspended solids in water can be effectively decreased. However, routine exchanges of the culture water are still necessary because of the accumulation of nitrogenous compounds [13,14,16]. Our preliminary study incorporated a hollow-fiber membrane filtration component with an RAPS, with no water exchange in the experimental period [17]. The system effectively retained the solids and microbes in a single sludge tank through membrane filtration, consequently accelerating the nitrification process and reducing the nutrient ion levels in the connected components. The study indicated that this incorporation improved the overall performance of the system. Furthermore, such a design reduced the clogging of plant roots and increased the absorption of nitrogenous compounds by the plants, as well as the yields of fish and plant biomass [17].

The present study is a sequential investigation focusing on the effects of three fish densities on a culture system that combines membrane filtration and an RAPS, with no water exchange for 12 weeks. Water was only supplied for evaporation and mud discharged from the sludge tanks. The study evaluated the effects of fish stocking density on the performance of the system, namely fish biomass, plant yields, and water quality parameters.

2. Materials and methods

2.1. Fish culture tanks

We investigated the effects of three fish culture densities on fish biomass, plant yields, and nutrients in the membrane filtration–RAPS. This study was conducted in a greenhouse of the Lantan Campus of Chiayi University. The system was composed of three major compartments, namely two rectangular tanks (fish culture and hydroponic tanks) and a circular sludge tank (Fig. 1). The tanks were connected using polyvinyl chloride tubes, and each treatment was performed in duplicate.

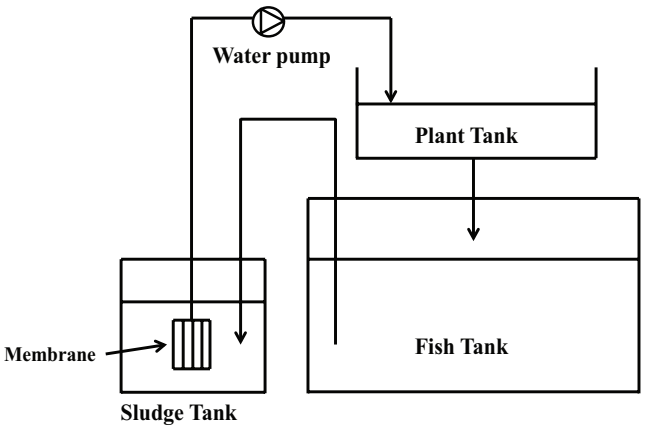

Fig. 1. Scheme of the integrated membrane filtration and recirculating aquaponic system used in this study.

Tilapia (*Oreochromis niloticus*) was maintained in 2,000-L rectangular fiberglass tanks (length, 198 cm; width, 120 cm; and height, 78 cm). The water depth was 63 cm in the culture tanks, with a volume of approximately 1,500 L. Juvenile tilapia (average initial weight, 1.8 ± 0.1 g) was obtained from the Zheng Yi Hainan Tilapia Hatchery, Pudai, Chiayi County, Taiwan. The fish culture densities were as follows: low (450 fish per tank; biomass L^{-1}, approximately 0.54 g), medium (600 fish per tank; biomass L^{-1}, approximately 0.72 g), and high (750 fish per tank; biomass L^{-1}, approximately 0.90 g). The fish tanks were aerated during the experiment to maintain a dissolved oxygen (DO) level of more than 5.4 ± 0.1 mg L^{-1}. Water loss in the fish tanks caused by evaporation was supplemented by fully aerated tap water once weekly. Fish were fed pelleted commercial feed (Sun Victory Feedstuff Co., Ltd., Pingtung, Taiwan), with a composition of 11% moisture, 43% crude protein, 3% crude fat, 3% crude fiber, and 16% ash. Throughout the experiment, fish were fed twice daily with a feed concentration of 5% of their weight; this concentration was adjusted biweekly after 10% of the fish in each tank were weighed as a group. The fish weight gain (%) and specific growth rate (SGR) for each tank were determined at the end of the experiment.

$$\text{Fish weight gain (\%)} = \{[\text{final weight (g)} - \text{initial weight (g)}/\text{initial weight (g)}]\} \times 100 \quad (1)$$

$$\text{SGR (\% d}^{-1}\text{)} = [(\ln \text{final weight (g)} - \ln \text{initial weight) (g)/d}] \times 100 \quad (2)$$

2.2. Sludge and plant tanks

The sludge and plant tanks were setup by modifying the method described by Wang et al. [17]. The 200-L sludge tank contained approximately 120 L of water. Approximately 400 L of water in the fish tanks was siphoned from the bottom to the

sludge tanks daily when the water level of the sludge tanks was reduced because of pumping out to the hydroponic tank. Each sludge tank had one submerged polyvinylidene fluoride hollow-fiber membrane (MOTIMO Membrane Technology, Tianjin, China). This comprised 12 bundles of membranes, with each bundle containing 54 membranes. The length, outer diameter, inner diameter, and pore size of a single membrane were 90 cm, 1.1 mm, 0.6 mm, and 0.1 μm, respectively. The surface filtration area of each membrane setup in the sludge tank was approximately 2 m^2. Additional aeration was applied beneath the membrane module to maintain the DO level at more than 5.0 ± 0.2 mg L^{-1} for supporting microbial nitrification and reducing the fouling on the membrane surface [18]. A reverse osmosis booster pump was used to collect water from the sludge tank that passed through the membrane and flowed into the connecting hydroponic tank. The pump was controlled using a timer set at a suction period of 5 min (20 L h^{-1}), followed by a relaxation period of 1 min. The transmembrane pressure (TMP) was measured at the suction side of the membrane once weekly; the maximum TMP was set at 45 kPa.

The plants were cultured in rectangular hydroponic tanks (inner space, 77 × 77 × 32 cm^3) placed on top of the fish tanks. The tank had a hole in the center to facilitate water inflow into the fish tanks below through gravity; the flow speed was controlled at 20 L h^{-1} by using a 1-inch pipe and a valve. The water depth of the hydroponic tank was 20 cm, and the water surface was covered by a polystyrene plate (68 × 68 × 3 cm^3) with 81 holes. These holes were evenly arranged in 9 × 9 lines; on average, each hole was 2 cm in diameter and 5 cm apart. Each hole contained two seeds of water spinach coated with polyurethane foam, except for the hole in the center that allowed water inflow into the fish tanks. The initial plant density was 8.3 g m^{-2} [19], and the plant biomass was measured every 4 weeks. The total plant yields are reported as wet weight.

2.3. Water quality

Water quality in the system was monitored by measuring the temperature, pH, DO, oxidation–reduction potential (ORP), total ammonia–nitrogen (TAN), nitrite–nitrogen (NIN), nitrate–nitrogen (NAN), and orthophosphate (OP) levels twice weekly. Biochemical oxygen demand (BOD), total bacteria counts (TBC), turbidity, mixed liquid suspended solids (MLSS), mixed liquid volatile suspended solids (MLVSS), and TMP were analyzed once weekly. The levels of pH, DO, and turbidity were measured using corresponding instruments that were calibrated following the manufacturers' instructions. The levels of TAN, NIN, NAN, BOD, COD, TBC, MLSS, and MLVSS were measured by following standard methods [20].

2.4. Statistical analyses

Statistical analyses were conducted using SAS software (SAS Institute, Cary, North Carolina) [21]. The growth rates of tilapia and spinach were calculated by gradual weight gains over time. The effects of the different densities on fish biomass, plant yields, and water quality parameters were evaluated using analysis of variance and Duncan's multiple-range comparison, with the significance set at 0.05.

3. Results and discussion

3.1. Fish biomass

In this study, stocking density significantly affected tilapia growth. In all groups, the average initial weight of tilapia was 1.8 g. In the second week, the average weights of tilapia were 3.9 and 3.7 g in the low- and medium-density groups, respectively; these values were significantly higher than the weight of tilapia in the high-density group (3.3 g). The average weights significantly varied among the groups from the sixth week and were 6.8, 5.1, and 4.1 g in the low-, medium-, and high-density groups, respectively. At the end of this study (12th week), the average weights of tilapia increased to 12.6, 10.1, and 5.8 g in the low-, medium-, and high-density groups, respectively (Table 1 and Fig. 2(a)). The average weight gain of tilapia in the low-density group (10.8 g) showed a significant increase, being 30% and 170% higher than the weight gains of tilapia in the medium-density (8.3 g) and high-density (4.0 g) groups, respectively. Both weight gain and SGR negatively correlated with increasing stocking densities of tilapia. The present study indicated that tilapia growth negatively correlated with increasing stocking density, even in the membrane filtration–RAPS setup. Similar results have typically been recorded in studies on similar stocking densities of fish [22–24]. This was because of less feed and living space as well as worse water conditions for fish in the higher stocking density groups.

In addition, the total increased biomasses of fish (average increased weight × fish number) were 4,680, 4,980, and 3,000 g in the low-, medium-, and high-density groups, respectively. The increased biomass of fish was the highest in the medium-density group, followed by the low-density group, whereas the high-density group yielded the lowest fish biomass. This could be explained by unsuitable conditions in the high-density group that containing higher nitrogenous compounds (Table 2) or the lack of adjustments of the feeding regime to that appropriate for higher growth potential. However, the water conditions were maintained suitable for fish survival in all groups. This was supported by the survival ratios of tilapia among the groups (99%–100%), which showed no significant differences (Table 1).

Table 1
The parameters of fish growth and plant yield in the aquaponic–membrane system with three fish stocking densities

Density treatments	L	M	H
Fish number (tank)	450	600	750
Initial average WT (g)	1.8	1.8	1.8
Final average WT (g)	12.6^a	10.1^b	5.8^c
Average WT gain (g)	10.8^a	8.3^b	4.0^c
SGR (% d^{-1})	2.3^a	2.1^b	1.4^c
Survival rates (%)	100^a	99^a	99^a
Fish production (g $tank^{-1}$)	4,680^b	4,980^a	3,000^c
Initial WT (g $tank^{-1}$)	107	107	107
Plant yield (g $tank^{-1}$)	1,214^c	1,402^b	1,708^a

Note: WT, weight, SGR, Specific growth rate. Significant differences ($p < 0.05$) were indicated by different superscripts (a, b, c).

3.2. Plant yields

In contrast to the biomass of fish, the yields of water spinach significantly increased with increasing stocking density of tilapia. The initial weights of individual spinach seedlings were approximately 1.3 g, and the average weight was 107.0 g in each plant tank (Table 1 and Fig. 2(b)). At 12 weeks, the weights of water spinach per tank were 1,321, 1,509, and 1,815 g in the low-, medium-, and high-density groups, respectively; the corresponding weight gains were 1,214, 1,402, and 1,708 g. These results revealed that the high-density group supplied the highest nutrient levels to the plants and resulted in higher plant growth than did the low- and medium-density groups. The nutrients produced in the aquaculture process, namely nitrogen and phosphorus compounds, accumulate in water and contribute to eutrophication. The plants integrated into the RAPS grow by assimilating these nitrogen and phosphorus nutrients from the water. The plants grew well and maintained favorable water quality, despite no water being exchanged in the present study. This result indicates that the present design can markedly reduce the amount of aquaculture discharge. Similar studies have revealed that favorable water quality was maintained by plants in an RAPS that was also tested without water renewal [17,25].

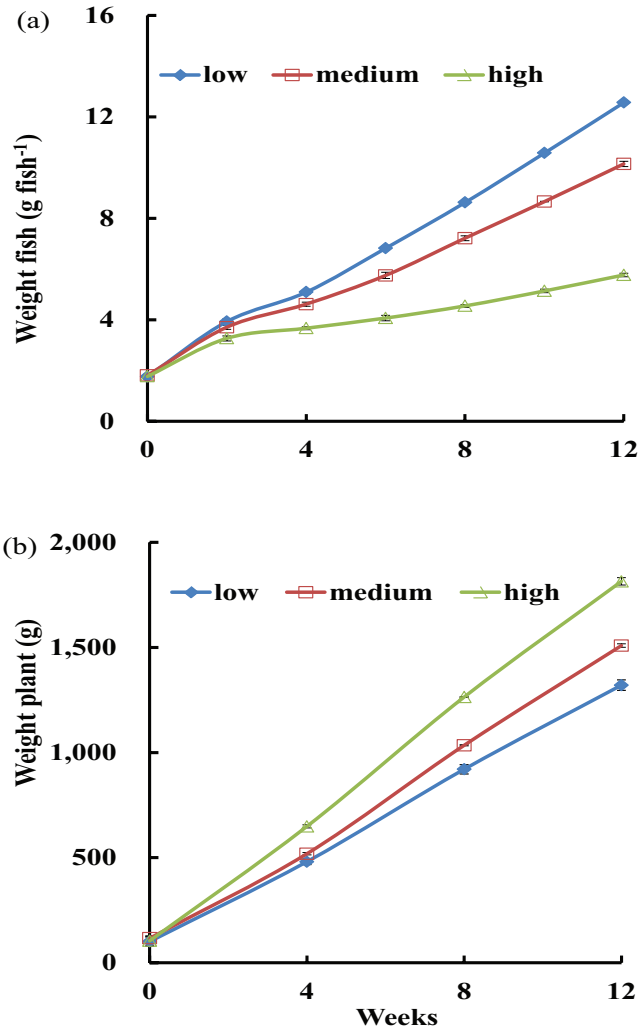

Fig. 2. (a) Average biomass yield of tilapia (g fish^{-1}) and (b) total yield of water spinach (g) in the recirculating aquaponic–membrane filtration system at three fish densities.

3.3. Water quality

3.3.1. Water temperature, pH, DO, and ORP

In this study, water temperature, DO, and pH were maintained at optimal conditions for the growth of fish and plants. The average of these parameters revealed no significant differences among the three groups and among the sampling sites: fish tank, sludge tank, membrane permeate, and plant tank effluents (Table 2). The water temperatures varied daily between 25.8°C and 29.5°C, with an average of 28.4°C–28.6°C, and were affected by the ambient temperature changes in the system. The water temperature in the system was adequate for the growth of both tilapia and plants [26,27].

During the study, the pH of the water samples was 7.8–8.5, with an average of 8.0–8.3 in the tanks; these levels showed no significant fluctuations. The levels were stable and showed no significant differences among the groups and sampling sites (Table 2). The concentrations of nutrient ions (e.g., Fe^{2+}, Mn^{2+}, PO_4^{3-}, Ca^{2+}, and Mg^{2+}) were reported to be decreased in the hydroponic culture at pH > 7 [28], resulting in nutrient deficiencies in the cultured plants. However, in this study, the plants showed no significant nutrient deficiencies.

The higher fish density resulted in lower DO levels in both fish and plant tanks. The average DO levels in the fish tanks were significantly lower in the high-density group (5.7 mg L^{-1}) than in the medium- and low-density groups (both, 5.9 mg L^{-1}; Table 2). Similar results were observed in the plant tanks, where the average DO levels were lower (5.9 mg L^{-1}) in the high-density group than in the medium- and low-density groups (6.0 and 6.1 mg L^{-1}, respectively). However, the DO level in the sludge tank was lower (4.5 mg L^{-1}) in the medium-density group than in both low- and high-density groups (4.8 and 4.9 mg L^{-1}, respectively). Contrastingly, the DO levels were 5.7 mg L^{-1} in the membrane permeate for all groups, with no differences. Boyd [29] suggested that the DO level of an aquaculture system should be maintained at more than 5 mg L^{-1} for normal activities of the cultured organisms. Moreover, the minimum DO level for a constant nitrification activity is recommended to be 0.5–4.0 mg L^{-1} [30]. Therefore, the present results indicate that the DO levels were correspondingly sufficient for maintaining fish biomass and for microbial activities (nitrification and mineralization) in the tanks of the studied system.

The ORP levels rapidly decreased in the initial week and were thereafter maintained at 131.9–139.6 mV at all sampling sites during the study; no significant differences were observed among the groups (Table 2).

3.3.2. Nitrogenous compounds

The highest fish stocking density significantly increased the TAN concentration in most water samples. The average TAN levels in the fish, sludge, and plant tanks of the high-density group were 0.54, 0.28, and 0.24 mg L^{-1}, respectively; these values were significantly higher than the corresponding levels in

Table 2
The measured water quality of four sampling sites in the aquaponic–membrane system with three fish stocking densities

Items	Fish tank			Sludge tank			Membrane permeate			Plant tank effluent		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
Temperature	$_{x}28.4^{a}$	$_{x}28.5^{a}$	$_{x}28.4^{a}$	$_{x}28.6^{a}$	$_{x}28.6^{a}$	$_{x}28.6^{a}$	$_{x}28.4^{a}$	$_{x}28.5^{a}$	$_{x}28.5^{a}$	$_{x}28.5^{a}$	$_{x}28.6^{a}$	$_{x}28.5^{a}$
(°C)	(1.0)	(1.0)	(1.0)	(1.1)	(1.1)	(1.1)	(1.0)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)
pH	$_{x}8.1^{a}$	$_{x}8.2^{a}$	$_{x}8.1^{a}$	$_{x}8.0^{a}$	$_{x}8.1^{a}$	$_{x}8.0^{a}$	$_{x}8.3^{a}$	$_{x}8.2^{a}$	$_{x}8.2^{a}$	$_{x}8.3^{a}$	$_{x}8.3^{a}$	$_{x}8.3^{a}$
	(0.2)	(0.1)	(0.2)	(0.2)	(0.1)	(0.2)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
DO	$_{x}5.9^{a}$	$_{x}5.9^{a}$	$_{y}5.7^{b}$	$_{z}4.9^{a}$	$_{z}4.5^{b}$	$_{z}4.8^{a}$	$_{y}5.7^{a}$	$_{y}5.7^{a}$	$_{y}5.7^{a}$	$_{w}6.1^{a}$	$_{w}6.0^{a}$	$_{x}5.9^{b}$
(mg L^{-1})	(0.1)	(0.1)	(0.2)	(0.3)	(0.4)	(0.5)	(0.2)	(0.1)	(0.3)	(0.2)	(0.2)	(0.2)
ORP	$_{x}139.6^{a}$	$_{x}137.0^{a}$	$_{x}136.4^{a}$	$_{x}134.0^{a}$	$_{x}131.9^{a}$	$_{x}133.1^{a}$	$_{x}135.8^{a}$	$_{x}133.3^{a}$	$_{x}132.7^{a}$	$_{x}134.6^{a}$	$_{x}133.4^{a}$	$_{x}133.2^{a}$
(mV)	(19.4)	(13.8)	(15.3)	(15.4)	(17.6)	(16.1)	(18.0)	(13.1)	(14.5)	(17.0)	(14.4)	(14.3)
TAN	$_{x}0.34^{b}$	$_{x}0.4^{b}$	$_{x}0.54^{a}$	$_{y}0.20^{b}$	$_{y}0.25^{ab}$	$_{y}0.28^{a}$	$_{y}0.23^{a}$	$_{y}0.24^{a}$	$_{y}0.25^{a}$	$_{y}0.18^{b}$	$_{y}0.22^{ab}$	$_{y}0.24^{a}$
(mg L^{-1})	(0.16)	(0.22)	(0.28)	(0.16)	(0.17)	(0.18)	(0.17)	(0.17)	(0.11)	(0.17)	(0.15)	(0.11)
NIN	$_{x}166.8^{b}$	$_{x}188.0^{b}$	$_{x}324.2^{a}$	$_{y}7.8^{c}$	$_{y}17.6^{b}$	$_{y}27.2^{a}$	$_{y}7.4^{b}$	$_{y}13.4^{b}$	$_{y}37.4^{a}$	$_{y}5.3^{b}$	$_{y}11.0^{b}$	$_{y}26.8^{a}$
(μg L^{-1})	(99.2)	(104.4)	(61.6)	(4.2)	(15.7)	(14.4)	(17.2)	(9.6)	(49.4)	(14.6)	(3.0)	(42.4)
NAN	$_{x}12.6^{b}$	$_{x}14.7^{a}$	$_{x}15.6^{a}$	$_{y}10.0^{a}$	$_{y}11.1^{a}$	$_{y}11.8^{a}$	$_{y}10.2^{b}$	$_{y}11.0^{a}$	$_{y}12.1^{a}$	$_{z}6.4^{a}$	$_{z}7.2^{a}$	$_{z}7.5^{a}$
(mg L^{-1})	(5.8)	(6.6)	(6.9)	(4.4)	(4.4)	(5.2)	(4.2)	(4.1)	(4.3)	(2.3)	(2.6)	(2.8)
OP	$_{z}0.4^{c}$	$_{y}0.5^{b}$	$_{z}0.8^{a}$	$_{w}1.0^{b}$	$_{x}0.5^{c}$	$_{x}1.1^{a}$	$_{x}0.6^{b}$	$_{x}0.6^{b}$	$_{y}1.0^{a}$	$_{y}0.5^{c}$	$_{x}0.6^{b}$	$_{y}1.0^{a}$
(mg L^{-1})	(0.1)	(0.2)	(0.3)	(0.1)	(0.1)	(0.3)	(0.1)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)
BOD	$_{x}2.3^{b}$	$_{x}3.3^{a}$	$_{x}4.5^{a}$	$_{y}2.0^{b}$	$_{y}2.4^{ab}$	$_{y}2.9^{a}$	$_{z}0.3^{b}$	$_{z}0.4^{b}$	$_{z}0.7^{a}$	$_{z}0.1^{c}$	$_{z}0.2^{b}$	$_{z}0.4^{a}$
(mg L^{-1})	(1.6)	(2.2)	(3.0)	(0.7)	(0.9)	(1.2)	(0.1)	(0.2)	(0.5)	(0.0)	(0.1)	(0.2)
TBC	$_{y}12.1^{c}$	$_{y}19.7^{b}$	$_{y}25.6^{a}$	$_{x}46.7^{c}$	$_{x}51.9^{b}$	$_{x}57.3^{a}$	$_{z}0.1^{c}$	$_{z}0.2^{b}$	$_{z}0.3^{a}$	$_{z}0.1^{c}$	$_{z}0.1^{b}$	$_{z}0.2^{a}$
(×10^{3} mL^{-1})	(2.4)	(4.2)	(3.4)	(2.7)	(3.9)	(8.8)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Turbidity	$_{y}2.1^{c}$	$_{y}4.4^{b}$	$_{y}7.6^{a}$	$_{x}16.2^{b}$	$_{x}17.6^{a}$	$_{x}17.7^{a}$	$_{z}0.2^{b}$	$_{z}0.2^{b}$	$_{z}0.3^{a}$	$_{z}0.1^{b}$	$_{z}0.1^{b}$	$_{z}0.2^{a}$
(NTU)	(0.3)	(1.2)	(3.1)	(0.9)	(0.6)	(0.5)	(0.0)	(0.1)	(0.0)	(0.0)	(0.0)	(0.0)
MLSS				1,306^{c}	1,389^{b}	1,492^{a}						
(mg L^{-1})				(61)	(56)	(56)						
MLVSS				771^{c}	944^{b}	1,061^{a}						
(mg L^{-1})				(56)	(65)	(94)						

DO: dissolve oxygen, ORP: oxidation–reduction potential, TAN: NH_4^+–N, NIN: NO_2^-–N, NAN: NO_3^-–N, BOD: biological oxygen demand, OP: orthophosphate, MLSS: mixed liquid suspended solid, MLVSS: mixed liquid volatile suspended solid, TBC: total bacterial count; CFU: colony forming unit.
Significant differences ($p < 0.05$) among densities and among sampling sites in system are indicated by different superscripts (a, b, c) and subscripts (w, x, y, z), respectively.

the low-density group (Table 2). In addition, the TAN levels in the fish tanks were 0.87–1.42 mg L^{-1} at the beginning of the experiment; they decreased rapidly until day 11, increased to 0.34–0.43 mg L^{-1} on day 15, and plateaued at 0.29–0.48 mg L^{-1} toward the end of the experiment (Fig. 3). The average TAN levels in the fish tanks of the three groups were higher than the corresponding levels in the connected sludge and plant tanks in the systems. The results indicated that the TAN levels increased with the fish density and were 32%, 40%, and 54% lower in the sludge tanks of the low-, medium-, and high-density groups, respectively. No further significant decreases were observed in the TAN levels in the water samples of the following membrane permeate and plant tank. The levels increased with increasing fish stocking density and indicated efficient nitrification processes in the sludge tank with the membrane filtration treatments in all groups, whereas no significant further decrease was observed in the connecting plant tanks. However, Wang et al. [17] reported a sequential decrease in TAN levels in the sludge tank and its connecting plant tank for their RAPS. This might be caused by 2–8 times higher levels of TAN in the present study and the consequent lower microbial transformations and plant absorptions.

Increasing fish stocking density resulted in higher NIN concentrations in water. The NIN levels were higher in the high-density group (324 μg L^{-1}) than in the medium- and low-density groups (188 and 167 μg L^{-1}, respectively; Table 2). The NIN levels in the water samples of the fish tank, membrane permeate, and plant tank effluents showed no significant differences between the medium- and low-density groups. NIN, a potentially toxic chemical, disrupts the physiological functions of aquatic animals and causes growth reduction and death at high concentrations [3,31]. Similar to TAN, NIN decreased the most in the sludge tanks of the system of all groups. The NIN levels were the highest in the fish tanks; however, they decreased by 91%–95% in the connecting sludge tanks and showed no further decreases in the connecting plant tanks (Table 2). The high levels of both TAN and NIN in the fish tanks of the high-density group might explain the lower increased biomasses of fish in this group compared with those in the remaining groups.

The NAN levels increased with the stocking density and showed a stepwise decrease from the fish tank to the connecting tanks (Fig. 4). The average NAN levels in the fish tank were 15.6 and 14.7 mg L^{-1} in the high- and medium-density

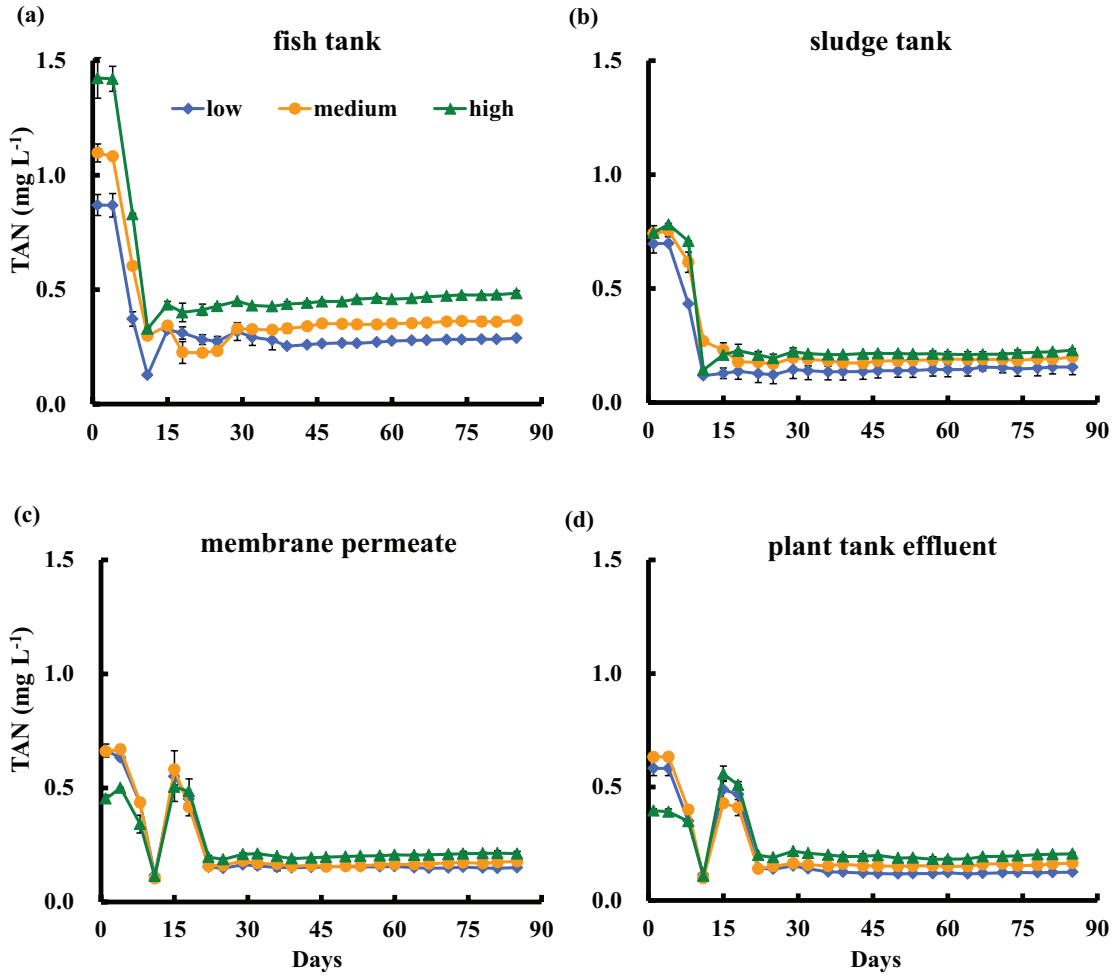

Fig. 3. Comparison of NH_4^+–N concentrations at different fish stocking densities in the recirculating aquaponic–membrane filtration systems at four sampling sites: fish tank (a), sludge tank (b), membrane permeates (c), and plant tank effluents (d).

groups, respectively; the levels were higher than the corresponding level in the low-density group (12.6 mg L^{-1}; Table 2). The NAN levels decreased by 24%–25% in the sludge tanks of the high- and medium-density groups and by 21% in the sludge tank of the low-density group; no significant differences were observed among the groups. The NAN levels did not significantly decrease in the membrane permeates; however, the levels were higher in the medium- and high-density groups than in the low-density group. The NAN values decreased by 35%–38% from the permeates to the plant tank effluents, yielding final levels of 6.4–7.5 mg L^{-1} that did not significantly vary among the groups. The results indicate that both microbial assimilation in the sludge tank and nutrient absorption in the plant tank played important roles in the decreased NAN levels in all density groups. NAN, a major nitrogenous nutrient in water, is produced by nitrification activities and is necessary for aquatic plants [32]. A comparison of the NAN levels in the fish and plant tanks revealed that the overall removal ratios of NAN were 49%–52%, which were lower than >70% and 79%–88%, as reported in an RAPS with marble goby [7] and catfish [19,33], respectively, in a freshwater system. Furthermore, the removal ratios were lower than 25%–172% reported in a marine system [9]. The lower NAN removal ratios in the present study might be because of the lower plant densities compared with those analyzed in previous studies. The effects of a higher plant biomass will be investigated in future studies.

3.3.3. Orthophosphate

The OP levels in this study increased with increasing fish stocking density. The average OP levels were the highest in the fish, sludge, and plant tanks of the high-density group than in those of the medium- and low-density groups. For example, the average OP levels were the highest in the fish tank of the high-density group (0.8 mg L^{-1}), followed by the corresponding tank of the medium-density (0.5 mg L^{-1}) and low-density group (0.4 mg L^{-1}; Table 2). The OP levels increased in the sludge tanks of the high- and low-density groups (0.5–1.1 mg L^{-1}) and decreased slightly in the following membrane permeate (0.6–1.0 mg L^{-1}). The OP levels decreased from 0.6 to 0.5 mg L^{-1} in the plant tank of the low-density group, whereas no significant differences were observed in the corresponding tank of the medium- and high-density groups (Table 2). Typically, the overall OP levels in the plant tank effluents of the three groups were 20%–25% higher than those in their fish tanks.

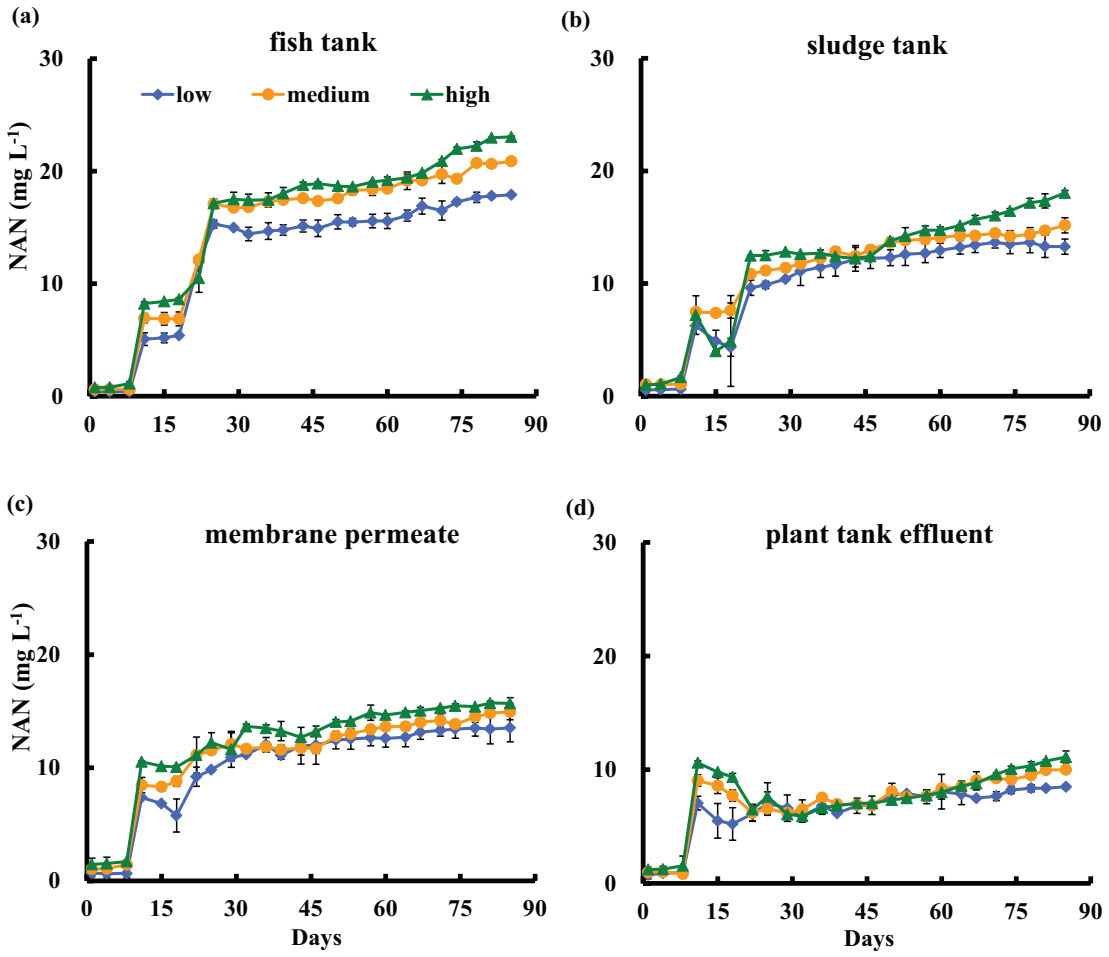

Fig. 4. Comparison of NO_3^-–N concentrations at different fish stocking densities in the recirculating aquaponic–membrane filtration systems at four sampling sites: fish tank (a), sludge tank (b), membrane permeates (c), and plant tank effluents (d).

The results indicated that OP slightly accumulated in the system during the culture period and its levels were higher at the optimal concentrations (0.15–0.2 mg L^{-1}) for the assimilation in plants [34]. Hussain et al. [35] reported similar OP changes in an aquaponic study. The OP levels increased with fish stocking densities in the range of 1.2–1.7 mg L^{-1}. However, the levels decreased in the plant tanks (0.6–1.0 mg L^{-1}). The limited removal of OP in the present study was possibly because of the zero water exchange and accumulation of organic solids in the sludge tank. The OP levels in the fish and plant tanks were constant after an initial increase in the first 2 weeks (Fig. 5), indicating constant removal rates of OP after the 2-week period. A study reported the slight accumulation of phosphate in an RAPS with no water renewal [25].

3.3.4. Biochemical oxygen demand

Increased fish density increased the BOD levels in the water samples of the RAPS. The average BOD levels in the fish tanks of the low-, medium-, and high-density groups were 2.3, 3.3, and 4.5 mg L^{-1}, respectively (Table 2). Compared with the BOD levels in the fish tanks, those in the sludge tanks were 13%–36% lower (2.0–2.9 mg L^{-1}) and were markedly decreased in the membrane permeates (84%–88%; 0.3–0.7 mg L^{-1}) and plant tank effluents (91%–96%; 0.1–0.4 mg L^{-1}; Fig. 6). Comparing the levels in the fish tank to the plant tank, the overall removal ratios of BOD were 91%–96% and were higher than the ratio of 63% reported in an RAPS with marble goby [7]. BOD indicates biodegradable organic loadings and oxygen consumption rates in waters that must be maintained at lower than 30 mg L^{-1} during the culture period for good aquaculture practices [29,31]. A study on RAS recommended an optimal level of BOD of less than 20 mg L^{-1} for the optimal performance of biofilters [36]. Wang et al. [17] reported a BOD removal rate of 89% in another RAPS with a higher fish stocking density. In the present study, the membrane filtration component efficiently reduced the BOD levels in the culture water and maintained the BOD levels in the three groups at substantially lower than the recommended levels. This indicates low organic loadings even in the high-density group in the present study.

3.3.5. Total bacterial counts

The TBC of all water samples in this study changed consistently with fish density. For example, the average TBC in the

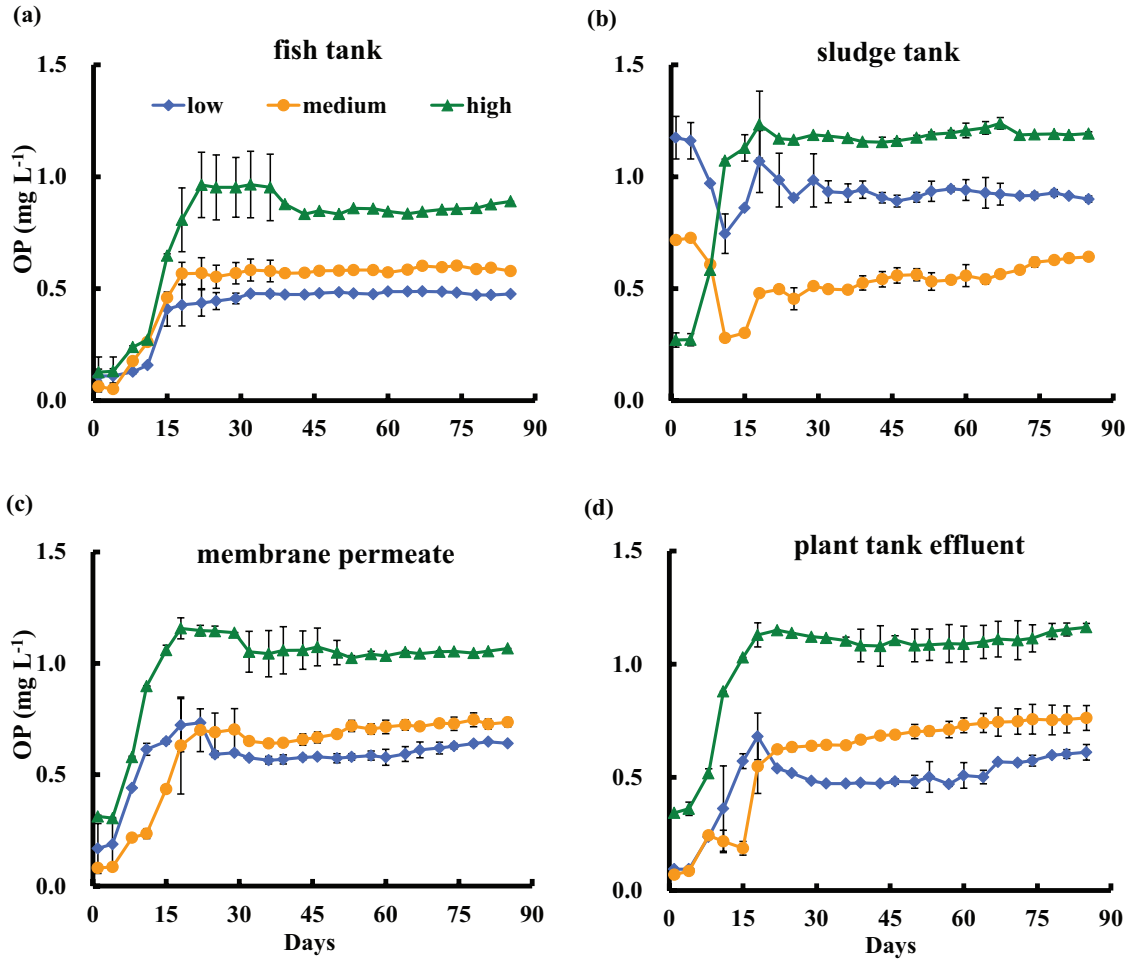

Fig. 5. Comparison of orthophosphate concentrations at different fish stocking densities in the recirculating aquaponic–membrane filtration systems at four sampling sites: fish tank (a), sludge tank (b), membrane permeates (c), and plant tank effluents (d).

fish tanks significantly increased with fish density and were 12.1×10^3, 19.7×10^3, 25.6×10^3 colony forming units (CFU) mL^{-1} in the low-, medium-, and high-density groups, respectively (Table 2). The TBC in the sludge tank rapidly increased to 46.7–57.3 × 10^3 CFU mL^{-1} (224%–386%) and showed significant increases with fish density. The TBC subsequently decreased to 0.1–0.3 × 10^3 and 0.1–0.2 × 10^3 CFU mL^{-1} in the membrane permeate and plant tank effluents, respectively. The present findings reveal that increasing fish density resulted in higher TBC in the culture water. However, membrane filtration effectively retained the microbes in the sludge tank and maintained low TBC in the connecting plant tank. The fish and biofiltration tanks in RAS often had high bacterial counts [2]. Studies have indicated decreased bacterial counts in RAS installed with a membrane filtration component [2,14]. Furthermore, the membrane treatment in an RAPS can effectively reduce the microbial levels and plant root clogging [17]. The present results reveal effectively reduced TBC even in the high-density group.

3.3.6. Turbidity, MLSS, and MLVSS

Turbidity, MLSS, and MLVSS positively correlated with fish stocking density. The average turbidites were 2.1, 4.4, and 7.6 NTU in the low-, medium-, and high-density groups, respectively (Table 2). The turbidites significantly increased to 16.2–17.7 NTU in the following sludge tank and rapidly decreased to 0.2–0.3 and 0.1–0.2 NTU in the membrane permeates and plant tank effluents, respectively (Fig. 7). The turbidites were higher in all components of the RAPS of the high-density group than those of the low- and medium-density groups.

Similar to the turbidity levels, the MLSS and MLVSS levels in the sludge tanks consistently increased with fish density. The average MLSS levels in the high-, medium-, and low-density groups were 1,306, 1,389, and 1,492 mg L^{-1}, respectively, whereas the corresponding MLVSS levels were 771, 944, and 1,061 mg L^{-1} (Table 2).

One of the critical concerns for maintaining optimal processes in an RAPS is to remove solids and reduce turbidites in water through membrane filtration. The turbidites in the permeates and plant tanks were similar to those reported previously [17]. However, the turbidites in the present study were 3.5–12.7-fold and 9–9.8-fold higher in the fish and sludge tanks, respectively, compared with those reported by Wang et al. [17]. This indicates that the hollow-fiber membrane filtration treatment in the present study efficiently retained

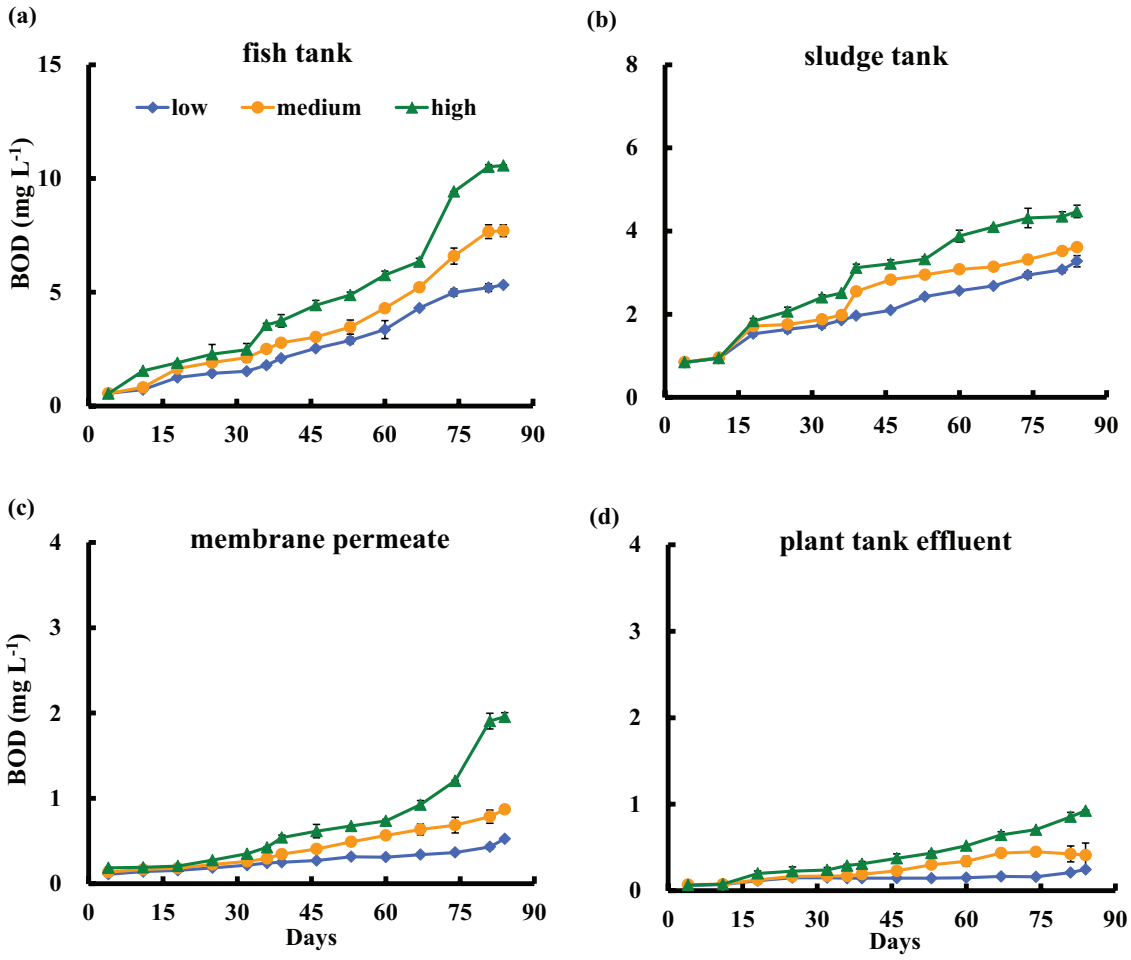

Fig. 6. Comparison of biochemical oxygen demand at different fish stocking densities in recirculating aquaponic–membrane filtration systems at four sampling sites: fish tank (a), sludge tank (b), membrane permeates (c), and plant tank effluents (d).

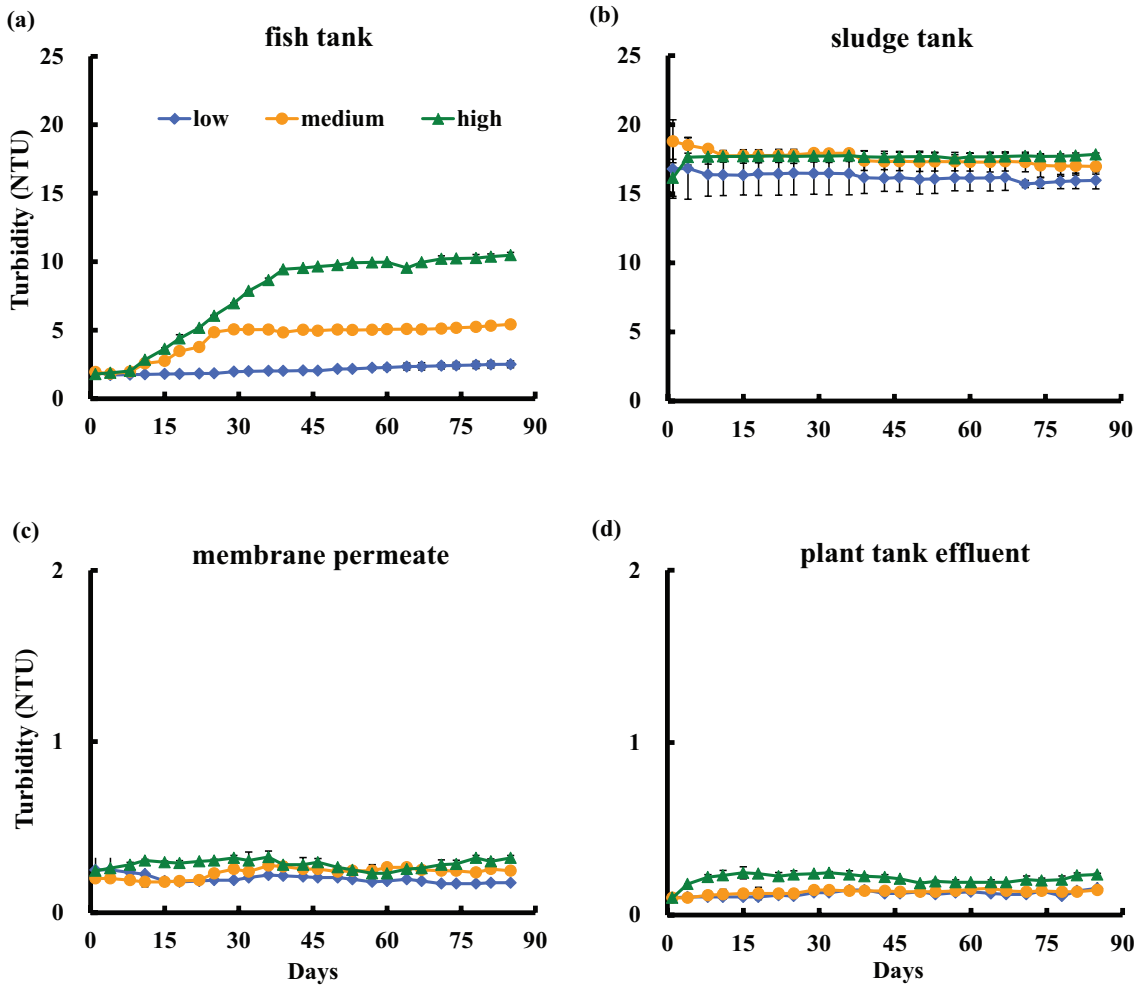

Fig. 7. Comparison of turbidites at different fish stocking densities in recirculating aquaponic–membrane filtration systems at four sampling sites: fish tank (a), sludge tank (b), membrane permeates (c), and plant tank effluents (d).

the suspended solids in the sludge tanks and removed the turbidites in the system even at the highest fish density. The turbidites in an RAPS system are caused by the residual feed, fecal matter, microorganisms, and sloughed biofilm masses. High turbidity in water can clog gills and attach to the body surface of culture organisms, thereby resulting in low growth rates [4]. A reason for the lower fish biomass in the high-density group might be the higher turbidity of the culture water. The present study reveals that membrane filtration can efficiently improve the culture conditions by reducing the water turbidity.

3.3.7. *Transmembrane pressure*

The average TMP of the hollow-fiber membranes in the membrane filtration treatment gradually increased during the study. The average TMP was initially 9 kPa and gradually increased to 36, 42, and 47 kPa in the low-, medium-, and high-density groups, respectively, at the end of the study. The membranes were not cleaned for the treatments, and filtration was performed at a constant average flow volume of permeate of 20 L h^{-1} in all groups during the study. An increase in the TMP in the membrane filtration treatment often resulted in a lower filtration flux and low filtration efficiency. In previous studies, the membrane was cleaned at a TMP of 14–30 kPa [13,18]. The TMP in the low-density group at the end of the present study was similar to the final TMP reported by Wang et al. [17]. In contrast to previous findings, the present results indicate that the setup of the membrane filtration treatment can tolerate higher TMPs and maintain a stable filtration performance.

4. Conclusions

Our pilot study reveals that the integration of an RAPS with hollow-fiber membranes embedded in a compact water filtration unit yields a favorable performance with respect to fish biomass, plant yields, and recirculating water quality. In the present study, the membrane filtration–RAPS maintained suitable water quality and organism growth. The result was valid even under the condition of no water exchange for 12 weeks. The medium-density group yielded the highest fish biomass, 4,980 g, whereas the high-density group provided the highest plant yield, 1,708 g. An ongoing study is

examining the effects of higher plant densities and at a commercially applicable scale with larger aquaponic systems.

Acknowledgments

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