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Integrated Multi-Trophic Recirculating Aquaculture System for Nile Tilapia (*Oreochlomis niloticus*)

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Abstract: Three densities of the sex-reversed male Nile tilapia, *Oreochromis niloticus* (20, 25, 50 fish/m^3) were cultivated in an integrated multi-trophic recirculating aquaculture system (IMRAS) that involves the ecological relationship between several living organisms, i.e., phytoplankton, zooplankton, and aquatic plants. The results indicated that, by providing proper interdependency between various species of living organisms, the concentrations of ammonia, nitrite, nitrate, and phosphate in the system were maintained below dangerous levels for Nile tilapia throughout the cultivation period. The highest wet weight productivity of Nile tilapia of 11 $\pm$ 1 kg was achieved at a fish density of 50 fish/m^3. The aquatic plants in the treatment tank could effectively uptake the unwanted nitrogen (N) and phosphorus (P) compounds with the highest removal efficiencies of 9.52% and 11.4%, respectively. The uptake rates of nitrogen and phosphorus by aquatic plants could be ranked from high to low as: *Egeria densa* > *Ceratophyllum demersum* > *Vallisneria spiralis* and *Vallisneria americana* > *Hygrophila difformis*. The remaining N was further degraded through nitrification process, whereas the remaining P could well precipitate in the soil sediment in the treatment tank.

Keywords: Nile tilapia; phytoplankton; zooplankton; aquatic plant; aquaculture; multi-trophic; recirculating aquaculture system

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1. Introduction

A rapid increase in the world population has accelerated the demand for food, and this leads to challenges in providing an adequate supply of nutrients via intensive agriculture. Typical agricultural systems, particularly aquaculture systems, are mono-cultured where the target aquaculture species such as fish or shrimp is cultivated in a batch mode. The system has to be large enough that the left over feed and wastes from the culture are being naturally treated with several types of microorganisms inhabiting the system. However, it is quite common to have a high-density culture where the system has to be fed with a large quantity of feed and in certain cases with extra aeration. In this case, problems always arise when the waste cannot be adequately treated, resulting in an unsuitable living conditions for the culture. This can lead to stress which negatively affects the growth and the productivity of the system. One attempt to deal with this problem is to have a recirculating system where the unwanted

waste is taken out of the culture tank and treated very effectively elsewhere. Recirculating aquaculture system (RAS) is an integrated closed cultivation system where the circulation between the cultivation and treatment tanks helps maintain the quality of the water. This clean water allows a better control of disease [1] and promotes a better growth of aquatic animals which enhances the productivity of the system. In addition, the treatment tank can also act like a holding basin when the cultivation tank needs to be emptied for maintenance. This ability to collect clean water eliminates the need of water from the external irrigation system and prevents unnecessary contamination which might come from external sources. In RAS, wastewater from an aquatic culture containing major nutrients such as nitrogen and phosphorus compounds is not only treated by typical nitrification and denitrification processes in the biological filters [2–6] or integrated biofloc systems [7–11], but also by the uptake of vegetable/ornamental or aquatic plants [12–16]. By providing a proper balance between these various species, this RAS shares a common important concept with an integrated multi-trophic system, which is the synergistic relationship between the living organisms that helps promote the sustainability and the economics of the whole system. In multi-trophic systems, such waste will be used as feed for other organisms, e.g., aquatic plants, simulating the symbiotic relationship in natural ecosystems. The design of this multi-trophic aquaculture is quite important as this will affect the economics of the aquaculture. Well selected food-chain-like organisms enable the farmers to generate more income from by-products that can be harvested from the system. There is a therefore a clear need to develop the multi-trophic recirculating aquaculture system (MRAS) prototype as an integrated closed loop system for Nile tilapia-plankton-aquatic plant cultivation in Thailand to ensure future success of the system and to help guarantee the security of the food supply for the quickly increasing global population.

In this work, this multi-trophic recirculating aquaculture system was based on Nile tilapia (*Oreochromis niloticus*) as the major species. Due to their rapid growth rate and high resistance to disease, Nile tilapia is one of the most farmed aquatic animals [17–21]. Moreover, they require relatively low oxygen for survival [22–24] and a natural surface oxygen transfer is generally adequate for their effective growth. This excludes the need for surface aeration which is a major electricity cost for the system. However, typical culture practice for Nile tilapia still does not incorporate the concept of RAS which renders the culture system susceptible to several environmental disturbances such as water quality, natural drought, etc. Moreover, Nile tilapia excrete waste in the form of nitrogen and phosphorus compounds, which if not treated properly can exert negative effects on the environment [15,25–28]. Nitrogen and phosphorus excreted from Nile tilapia are used as the feed for microalgae which in this case is *Chlorella* sp. This algal species could grow reasonably well in a tropical climate and therefore could be cultured with minimal maintenance. The biomass of *Chlorella* sp. is fed to *Moina macrocopa* tanks which can be more easily harvested when compared to *Chlorella* sp., whose small size leads to harvesting difficulties. The remaining nitrogen and phosphorus waste are used to grow ornamental aquatic plants (*Egeria densa*, *Ceratophyllum demersum*, *Hygrophila difformis*, *Vallisneria spiralis* and *Vallisneria americana*) which can also be harvested, where the clean water is recirculated back to the fish tank. With this configuration, the system will more effectively utilize the feed and the farmers will benefit from having a variety of products apart from the major aquacultural species (the economics of the culture system were further improved using the combination of phytoplankton or microalgae cultivations and zooplankton). The symbiotic mechanism of the various organisms constitutes the novel concept of the integrated multi-trophic recirculating aquaculture system (IMRAS) which is the main focus of this research.

2. Materials and Methods

2.1. System Setup

In this work, a duplicate cultivation of sex-reversed male Nile tilapias (*Oreochromis niloticus*) was carried out in the control and treatment systems. In the control cultivation, fish were cultured in the oval shape opaque fiber (diameter 0.8 m, depth 0.4 m, working volume of 200 L) where the

water was not circulated and not treated (representing typical cultivation practice). On the other hand, the treatment system consisted of a series of tanks connected together as shown in Figure 1. This system, called an integrated multi-trophic recirculating aquaculture system (IMRAS), included a fish tank (Section 2.2), phytoplankton tank (Section 2.3), zooplankton tank (Section 2.4) and aquatic plants tank (Section 2.5). The water in the aquatic plants tank was pumped to the fish tank using a submersible pump. An overflow conduit was installed from the fish tank to the phytoplankton tank and the aquatic plants tank. A valve was provided to allow a partial overflow of the water from the fish tank to the phytoplankton tank. This valve remained open until the phytoplankton tank was filled up, at which point the valve was shut and the tank was then operated in a batch mode for the cultivation of *Chlorella* sp. as described in Section 2.3. Once the stationary growth phase was reached, the phytoplankton culture was transferred to the zooplankton tank as feed for *Moina macrocopa* as described in Section 2.4, and the overflow valve was turned on again. A part of the water from the fish tank continuously overflowed to the aquatic plants tank before being pumped back to the fish tank to finish the cycle. The water pumping rate was set at 700 mL/min which is equivalent to a recirculation with a hydraulic retention time of one day.

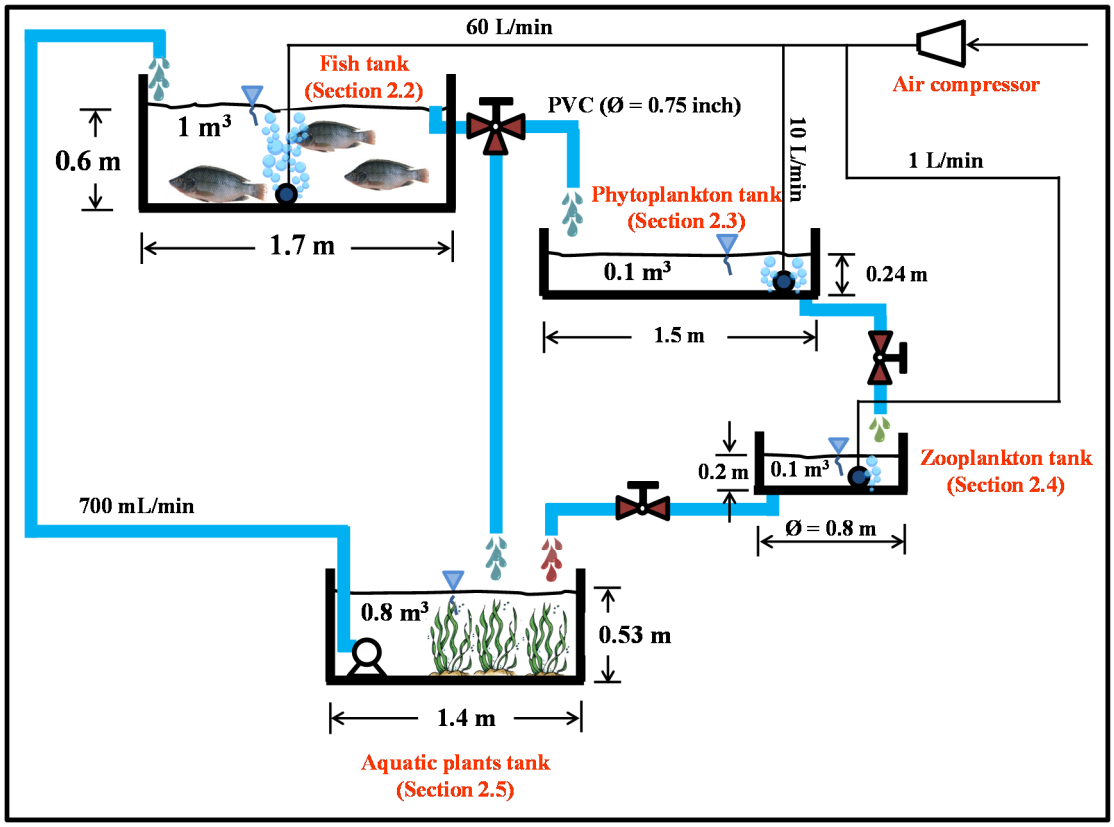

Figure 1. Integrated multi-trophic recirculating aquaculture system (IMRAS) setup.

During the experiment, the growth rates of Nile tilapia, *Chlorella* sp., *Moina macrocopa*, and aquatic plants, along with the water quality such as concentration of ammonia, nitrite, nitrate, phosphate, alkalinity, temperature and dissolved oxygen (DO), were measured following the standard methods for water and wastewater analysis [29].

2.2. Fish Tank (Nile Tilapia)

The fish tank was made from fiber glass with a working volume of 1000 L (dimension: length 1.7 m, width 1 m, depth 0.6 m). An air compressor (LP100, Resun) was used to provide dissolved

oxygen at a level greater than 5 mg/L and also to promote liquid circulation. This level of dissolved oxygen was reported to be enough for growth of this fish [30].

The experiment started with three different fish stockings, i.e., 20, 25 and 50 fish/m^3, with an initial average weight of 2 g/fish. This was meant to examine the effect of fish density on the final productivity, where 50 fish/m^3 represents a typical high density culture whilst 20 and 25 fish/m^3 a low density culture. Feeding was provided twice a day (morning and evening) each at 5% of the total fish weight. The commercial feed composition was 28% crude protein (as provided by Charoen Pokphand Foods PCL, Thailand: nitrogen and phosphorus content were 5.24% and 1.14% of dry weight matter). This amount of protein was recommended as suitable for Nile tilapia by Ribeiro et al. [31]. Note that during the experiment, the weight of all fish was recorded at every 28 days. The experiment was carried out for 112 days before harvest.

At the end of the experiment, the weight gain (g), daily weight gain (g/d), feed conversion ratio (FCR) and survival rate (%) are calculated as follows:

$$\text{Weight gain } (g) = \text{Final wet weight } (g) - \text{Initial wet weight } (g) \quad (1)$$

$$\text{Daily weight gain } (\text{g/d}) = \frac{Weight\ gain\ (g)}{Cultivation\ time\ (d)} \quad (2)$$

$$\text{FCR} = \frac{Total\ amount\ of\ fish\ feed\ fed\ (g)}{Total\ wet\ weight\ gain\ (g)} \quad (3)$$

$$\text{Survival rate } (\%) = \frac{Total\ number\ of\ fish\ at\ final\ (fish)}{Total\ number\ of\ fish\ at\ initial\ (fish)} \times 100 \quad (4)$$

In addition, Nile tilapia sample was analyzed for its moisture content, dry weight matter and chemical compositions in order to calculate nitrogen and phosphorus mass balances.

2.3. Phytoplankton Tank (Chlorella sp.*)*

Chlorella sp. was cultivated with the modified M4N medium [32,33] where KNO_3 and K_2HPO_4 were omitted as N and P sources were obtained from the fish excrete. The culture tank was made from transparent glass with a working volume of 100 L (length 1.5 m, width 0.28 m, depth 0.24 m). The initial biomass density was 0.01 g/L (10^6 cell/mL) with a continuous aeration rate of 0.1 vvm (10 L/min). A sample was collected once a day to measure dry weight. *Chlorella* sp. was harvested as it entered the stationary growth phase (generally after 4 days of cultivation) and was used as a feed for *Moina macrocopa*.

2.4. Zooplankton Tank (Moina macrocopa)

The zooplankton tank was made from fiber glass with a working volume of 100 L (diameter 0.8 m, depth 0.2 m). *Chlorella* sp. as harvested in Section 2.3 was used in the cultivation of *Moina macrocopa* with an initial concentration of 0.1 g/L. An aeration rate of 0.01 vvm (1 L/min) was supplied at the center of the tank in order to increase the level of dissolved oxygen in water and also to prevent cell precipitation. *Moina macrocopa* generally spent 4 days to reach its stationary phase in which it was harvested with 150 µm plankton net. The culture water after cell removal was sent to be treated in the aquatic plants tank (Section 2.5).

2.5. Aquatic Plants Tank

The aquatic plants tank was the major component of the treatment because most nitrogen and phosphorus compounds were removed here and used for the growth of the aquatic plants. Moreover, aquatic plants could capture the suspending sediment from the fish tank, which helped maintain not only the level of nitrogen and phosphorus, but also the clarity of the water in the system. The aquatic plants tank was made from fiber glass with a total working volume of 800 L (length 1.4 m; width 1.2 m;

depth 0.53 m). The tank was operated under outdoor condition to utilize sunlight as an energy source for the growth of the aquatic plants.

In this tank, soil was filled at the bottom at the height of 5 cm, where the water depth was 48 cm. Several aquatic plants, i.e., *Hygrophila difformis*, *Vallisneria spiralis*, *and Vallisneria americana*, each with initial fresh weight of 100 g were planted and distributed evenly in the soil, whereas *Egeria densa* and *Ceratophyllum demersum* (100 g each) were floated on the water surface. Aquatic plants were harvested every 14 days such that the remaining weight of each plant was equal to the initial fresh weight (100 g each). The harvested aquatic plants were analyzed for their dry weights, moisture contents, nitrogen and phosphorus balances.

3. Results and Discussion

3.1. Growth of Nile Tilapia

The integrated multi-trophic recirculating aquaculture system (IMRAS) was operated without replacing the water (fresh clean water was regularly added to replenish water lost by evaporation) for 336 days or three fish crops and the growth of the fish was demonstrated in Figure 2. The results indicate that the fish in the low density system (20 fish/m^3) grew at a faster rate when compared with those from the higher fish densities (i.e., 25 and 50 fish/m^3). However, the system with the fish density of 50 fish/m^3 provided the highest total productivity (wet-weight) of 11 $\pm$ 1 kg fish/m^3 whereas the densities of 20 and 25 fish/m^3 could only produce a total wet-weight of 6.8 $\pm$ 0.3 and 5.3 $\pm$ 0.5 kg fish/m^3, respectively. The average wet-weight of Nile tilapia at density of 50 fish/m^3 increased from 2.4 $\pm$ 0.6 g/fish to 240 $\pm$ 16 g/fish within 112 days of growth which corresponds to the average daily weight gain of 2.1 $\pm$ 0.1 g/fish-day. Feed conversion ratio (FCR) and survival rate were 1.5 $\pm$ 0.2 and 91% $\pm$ 1%, respectively. These are reasonably good when compared with the results obtained from the other density condition and therefore the density of 50 fish/m^3 was selected for further investigation. The summary of growth characteristics (weight gain, daily weight gain, FCR, and survival rate) is given in Table 1. Note that the growth characteristics obtained from this system is comparable to those from other reported systems, e.g., the daily weight gain from the "recirculating greenwater system" was 1.75 g/fish-day [34] and from the "cage culture system" was 1.15 $\pm$ 0.02 g/fish-day [35].

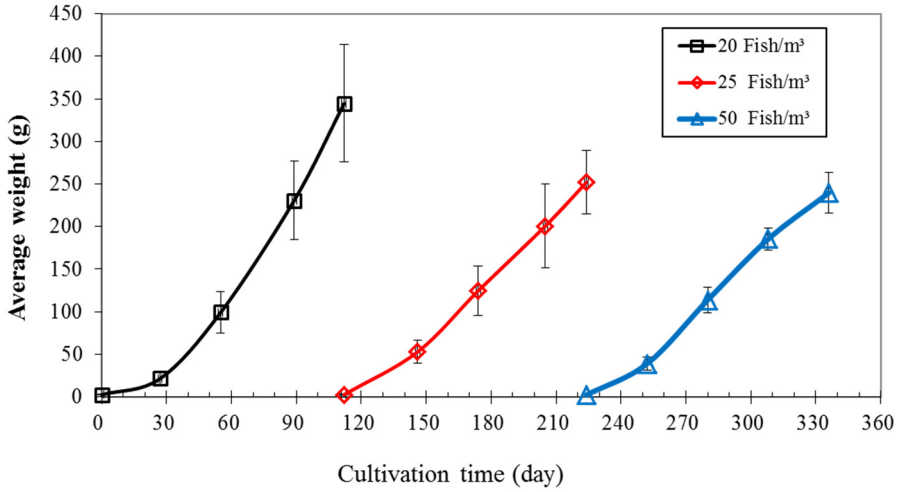

Figure 2. Growth curve of Nile tilapia (note that error bars in this report represent standard deviation of the duplicate results).

Table 1. Growth characteristics of Nile tilapia in the treatment system (mean value).

Growth Parameter	Fish Stocking (Fish/m^3)		
	20	25	50
Initial mean weight (g)	2.6 ± 0.5	2.6 ± 0.6	2.4 ± 0.6
Final mean weight (g)	344 ± 69	252 ± 37	240 ± 16
Weight gain (g)	342 ± 15	249 ± 3	237 ± 16
Daily weight gain (g/d)	3.1 ± 0.1	2.23 ± 0.03	2.1 ± 0.1
Feed Conversion Ratio; FCR	1.36 ± 0.06	1.2 ± 0.1	1.5 ± 0.2
Survival rate (%)	95	86 ± 3	91 ± 1
Productivity (kg/m^3)	6.8 ± 0.3	5.3 ± 0.5	11 ± 1

It should be mentioned here that the results seem to indicate that there is a trade-off between the higher growth obtained from the low density culture and the total weight gain of the fish in the high density culture, and in this case, the high density resulted in greater productivity. However, the systems employed in this work were continuously aerated, which eliminates the effect of night-time oxygen depletion that might occur from the intensive oxygen consumption through respiration. In large scale systems, this aeration might not be practically feasible, and the nocturnal depletion of oxygen might occur and lead to a different conclusion.

3.2. *Growth of Chlorella* sp. *and Moina macrocopa*

Chlorella sp. could grow reasonably well from 0.01 to 0.2 g/L (Figure 3, ◊) within 4 days considering that the system was operated under uncontrolled environmental parameters (light intensity and temperature). Figure 4 illustrates that such growth could be significantly enhanced if the cultivation parameters, e.g., temperature, light intensity and exposure period, could be well controlled [36]. With the outdoor *Chlorella* culture as a feed, *Moina macrocopa* could grow well and the density increased from 0.1 to 0.4 g/L within 4–5 days (Figure 3, Δ) which was slightly better than the value reported in previous literature [37].

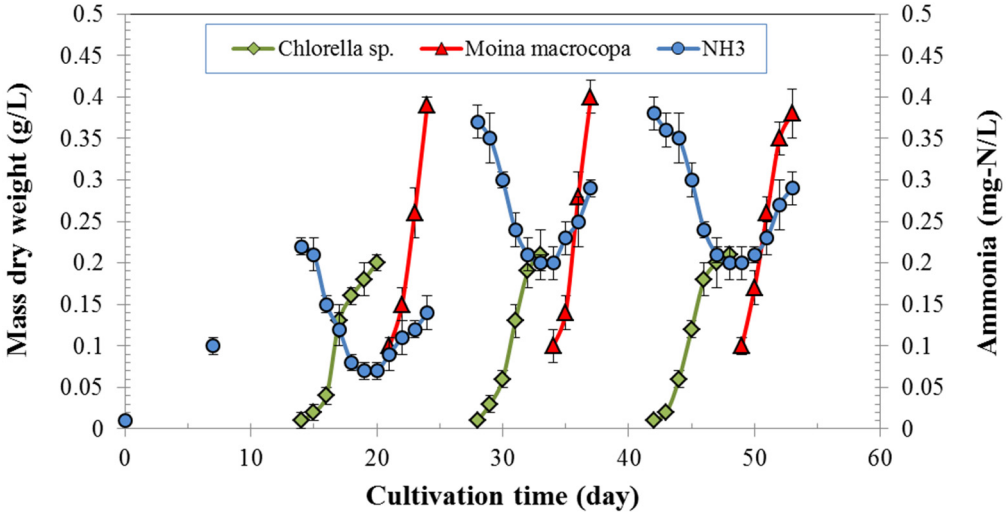

Figure 3. Growth curve of *Chlorella* sp. (◊) and *Moina macrocopa* (Δ) and NH_3 concentration profile (○).

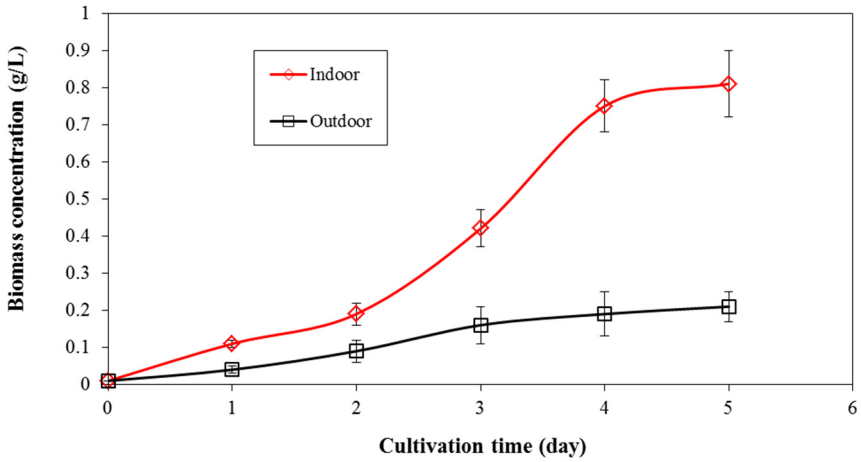

Figure 4. Growth curve of *Chlorella* sp. in Indoor (T = 30 °C, light intensity = 10,000 LUX, light exposure period = 24 h) and Outdoor cultivations (uncontrolled environmental parameters).

The results demonstrate that it was possible to enhance the economics of the system by introducing proper bio-components in the food chain. In this case, a high value animal feed *Moina macrocopa* (2–3 $/kg) was introduced to convert and upgrade the low value *Chlorella* biomass which was again fed on NH_3 excreted from the fish culture. It is interesting to observe that, during the growth of *Chlorella* sp., NH_3 was being used for growth and the concentration of NH_3 dropped significantly. The level of NH_3 bounced back again (Figure 3, ○) as *Moina macrocopa* grew and it also excreted NH_3 during its growth stage.

3.3. Growth of Aquatic Plants

Figure 5 illustrates the wet-weights of the five aquatic plants which were harvested every 14 days. The different plants grew at different rates but the productivities of all aquatic plants followed the same pattern. Most plants grew at a relatively slow rate at the beginning which was due to the limited nitrogen source. In other words, the initial concentrations of nitrogen and phosphorus levels from the fish tank that flew into the aquatic plants tank were inadequate for growth (as the fish was still small). The growth rate increased considerably particularly for *Egeria densa* and *Ceratophyllum demersum* during the first 42 days, implying that there were more abundant nitrogen/phosphorus compounds not only due to the accumulation of the uneaten or remaining feed, but also from the acquisition of the plants to the environment of the tank. The other plants, i.e., *Hygrophila difformis, Vallisneria spiralis, and Vallisneria americana*, also grew at a faster rate but the changes in the growth rate were not as obvious when compared to the two species mentioned above. The total fresh weight of all aquatic plants could be ordered from high to low as follows: *Egeria densa* (14.9 $\pm$ 0.7 kg), *Ceratophyllum demersum* (13.2 $\pm$ 0.5 kg), *Vallisneria americana* (3.87 $\pm$ 0.09 kg), *Vallisneria spiralis* (3.67 $\pm$ 0.03 kg), and *Hygrophila difformis* (1.74 $\pm$ 0.06 kg).

It is noted that aquatic plants directly assimilated nitrogen and phosphorus into the biomass. However, the analysis of N balance in the following section shows that this nitrogen assimilation only accounted for a small fraction of nitrogen input (in animal feed), and most of the nitrogen was lost from another unknown mechanism which, in this case, was believed to be the conversion of NH_3 and NH_4^+ to NO_3^- via nitrifying bacteria and perhaps also via the denitrifying activities as the level of oxygen under the water level in the tank could well exhibit anaerobic condition. These groups of bacteria were generally found in the sediment of the culture tank [38,39].

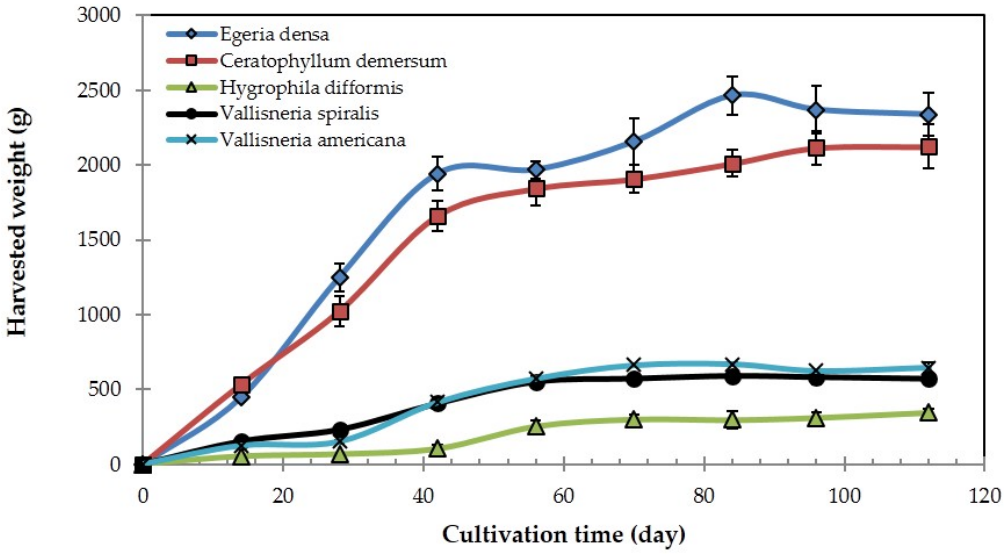

Figure 5. Average harvested weight of aquatic plants (14-day harvesting interval).

Aquatic plants were also reported to have beneficial effect as they acted like a filter for the suspended sediment [40]. This helped enhance the level of dissolved oxygen (DO) in the fish tank, as this sediment is usually organic matters which could undergo aerobic decomposition in the fish tank. Preventing this organic decomposition therefore eliminated the unnecessary oxygen uptake in the fish tank resulting in a better control of DO level in the cultivating system [41,42].

The findings from this section suggest that the selection of the aquatic plant species used in the treatment tank should be carefully considered to ensure high treatment efficiency and also a reasonably level of economic benefit. The rapid growth plants must be used to provide a reliable water treatment/filtering capacity whereas the slow growth plants should also be provided as they are usually of high value and could enhance the feasibility of the system.

3.4. Water Quality

Figure 6 illustrates nitrogen and phosphorous compounds profiles in the fish and aquatic plant tanks where nitrogen (NH_3, NH_4^+, NO_2^- and NO_3^-) and phosphorous (PO_4^{3-}) levels in both tanks continuously increased during the first 50 days and remained constant until the end of experiment. Maximum nitrogen and phosphorous concentrations in the fish tank were 0.38 $\pm$ 0.02, 0.57 $\pm$ 0.02, 55 $\pm$ 2 mgN/L and 0.32 $\pm$ 0.03 mgP/L, for ammonia, nitrite, nitrate and phosphate, respectively. These levels of nitrogen compounds were still lower than the dangerous level for Nile tilapia (dangerous level indicated by the dash line in Figure 6 as suggested by Hart et al. [43]; Liao and Mayo [44]; Masser et al. [45]), but still higher than those in the aquatic plants tank where the corresponding concentrations were reduced to 0.28 $\pm$ 0.02, 0.33 $\pm$ 0.02, 38 $\pm$ 2 mgN/L and 0.20 $\pm$ 0.02 mgP/L, respectively. This indicates that the water in the treatment system could be self-cleaned by the provided concocted ecosystem. It is noted that the levels of ammonia, nitrite, nitrate and phosphate at the end of the control system were 0.52 $\pm$ 0.04, 1.20 $\pm$ 0.04, 135 $\pm$ 11 mgN/L and 2.45 $\pm$ 0.04 mgP/L which were relatively high, indicating inadequate treatment capacity in such a system.

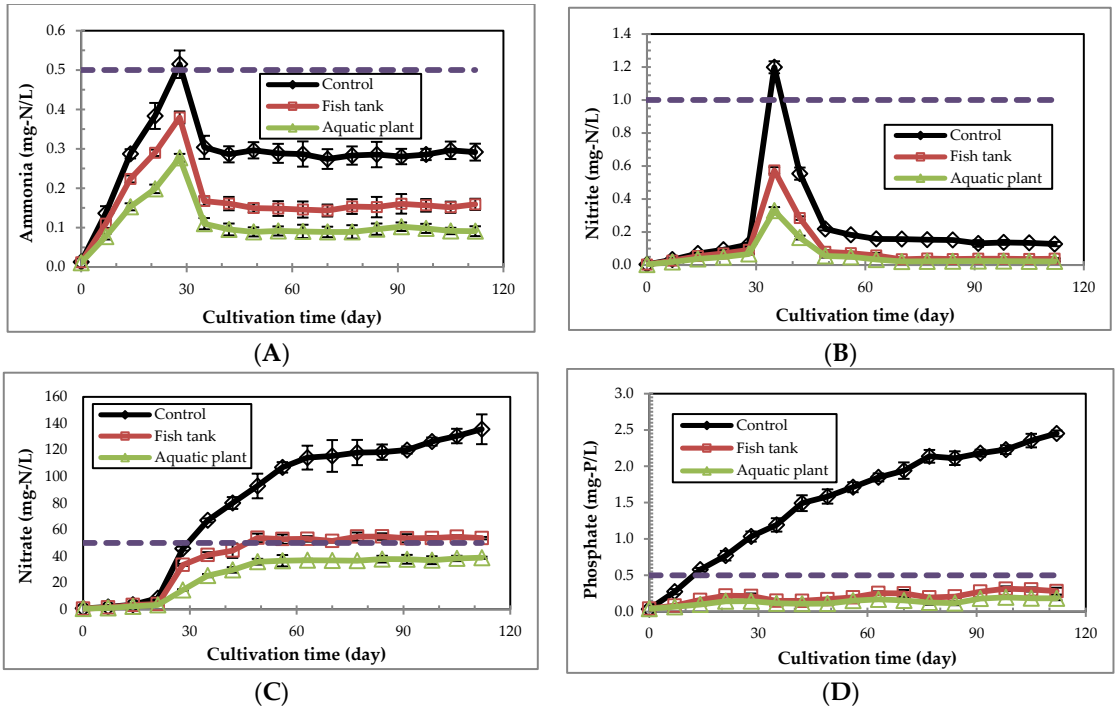

Figure 6. Concentration of ammonia, nitrite, nitrate, and phosphate in fish and aquatic plants tank (mg/L). (**A**) Total ammonia nitrogen (NH_3 and NH_4^+); (**B**) Nitrite nitrogen (NO_2^-); (**C**) Nitrate nitrogen (NO_3^-); (**D**) Phosphate phosphorous (PO_4^{3-}).

Dissolved oxygen (DO) gently decreased both in the fish and aquatic plant tanks. The initial DO concentrations in both tanks were 6.45 and 5.97 mg/L and the final concentrations were 5.55 and 4.65 mg/L, respectively (Figure 7A). In the fish tank, the reduction in DO would be due to a greater need for oxygen from the larger fish [46]. On the other hand, despite oxygen generated from photosynthesis, more oxygen was also required in the aquatic plant tank due primarily to the decomposition of uneaten feed and fish feces and nitrogen compounds through nitrification reaction. DO in the *Chlorella* sp. and *Moina macrocopa* tanks remained mostly unchanged (data not shown) indicating that the activities of the tank could be maintained regardless of the conditions in the other tanks.

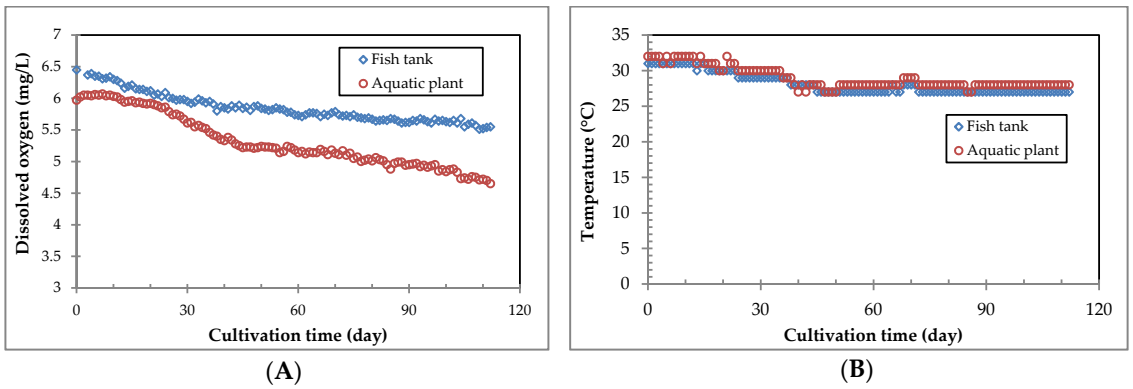

Figure 7. *Cont.*

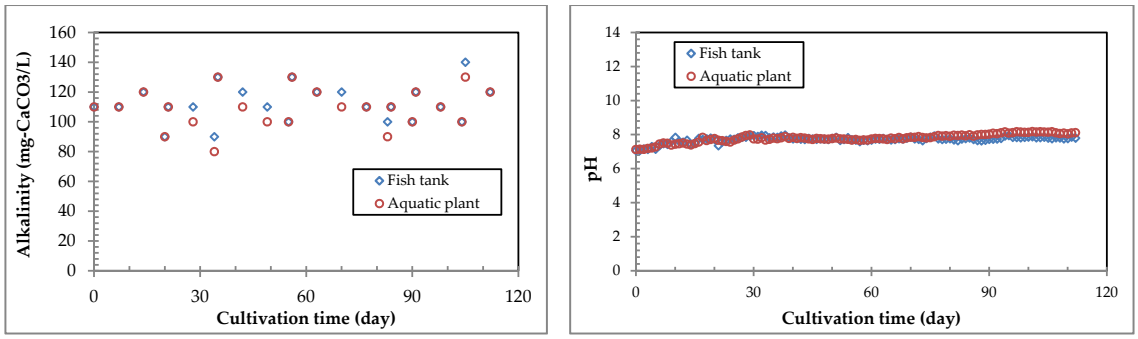

Figure 7. Water qualities in fish and aquatic plant tanks: Dissolved oxygen (**A**); temperature (**B**); alkalinity (**C**) and pH (**D**).

Figure 7 also demonstrates the variation in temperature, alkalinity and pH in the system. Due to a large quantity of water, the uncontrolled system temperature was in the range of 27–32 °C with an average of 28–29 °C which was considered within the optimum range (25–30 °C) for Nile tilapia (Figure 7B) [47–49]. Figure 7C demonstrates that alkalinity dropped with time which was potentially due to the activity of nitrifying bacteria and some other algae that might grow in the system. However, Hart, O'Sullivan, Teaching and Aquaculture [43] suggested that the alkalinity for aquatic animals should be maintained above 100 mg-$CaCO_3$/L, therefore, $NaHCO_3$ was added to stabilize the level of alkalinity above this level. The addition of $NaHCO_3$ could also stabilize the pH value in the system, and Figure 7D illustrates that pH (at 2 p.m.) could be naturally controlled within the range of 6–8.5 which was safe for the living organisms involved in the ecology of this system.

3.5. Nitrogen and Phosphorus Mass Balances

Figure 8 is the summary of the flow of nitrogen compounds within IMRAS. In this experiment, total nitrogen input (790.63 g) came from the use of fish feed throughout the 112 days of each crop. A large quantity of nitrogen (301.13 g·N or 38.09% of the total nitrogen input) could be converted to Nile tilapia. This level is more or less within the range reported elsewhere [16,50]. The remaining nitrogen was converted to: *Chlorella* sp. (7.64 g or 0.97%), *Moina macrocopa* (1.88 g or 0.24%), and aquatic plants (75.29 g or 9.52%). Some nitrogen, e.g., ammonia, nitrite, nitrate (about 91.10 g or 11.52%) was still dissolved in the water at the end of the experiment. Some of nitrogen (48.31 g or 6.11%) might still be adsorbed in the soil while some of nitrogen was not measured directly but calculated as the unaccounted nitrogen. As much as 265.30 g (33.56%) could undergo the decomposition reaction carried out by denitrifying bacteria residing within the eco-system such as in the soil sediment in the aquatic plants tank.

Similarly, Figure 9 displays the flow of phosphorus within IMRAS where the total phosphorus entering the system was 172.01 g (mostly in the fish feed). The amounts of phosphorus converted to Nile tilapia, *Chlorella* sp., *Moina macrocopa* and aquatic plants were 52.75 g (30.67%), 1.20 g (0.70%), 0.22 g (0.13%), and 19.61 g (11.40%), respectively (Figure 9). Again, some phosphorus was still soluble in the water at the end of the experiment and this accounted for about 2.65% (or 4.55 g) of the total phosphorus input. Some of phosphorus (7.07 g or 4.11%) might still be adsorbed in the soil while some of phosphorus was not measured directly but calculated as unaccounted phosphorus. As much as 86.60 g or 50.35% of phosphorus could not be accounted for by the measurement employed in this work. This phosphorus was anticipated to remain partially in the excretion matrix and some could be assimilated to the microorganisms cultivated within the system. It is noted here that the remaining phosphorus in the sediment could pose some concerns on the long-term operation of this system and will need to be extracted at some point.

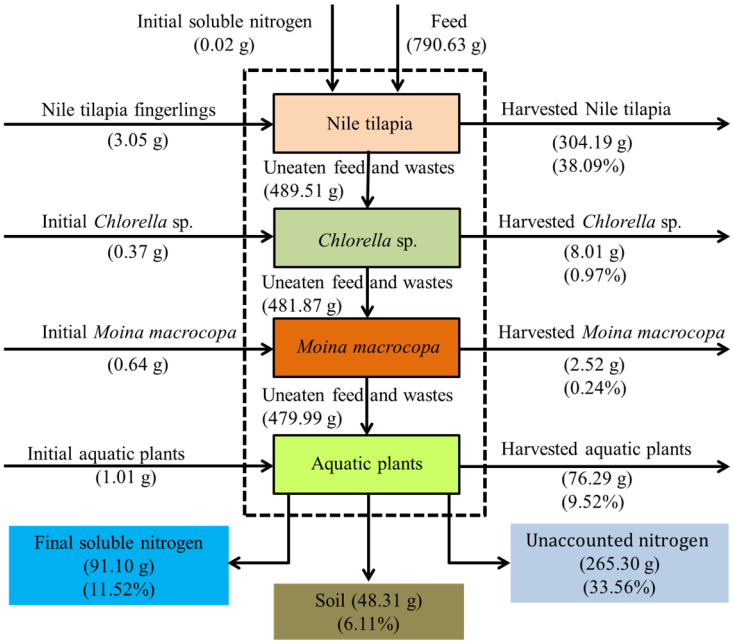

Figure 8. Nitrogen balance of IMRAS.

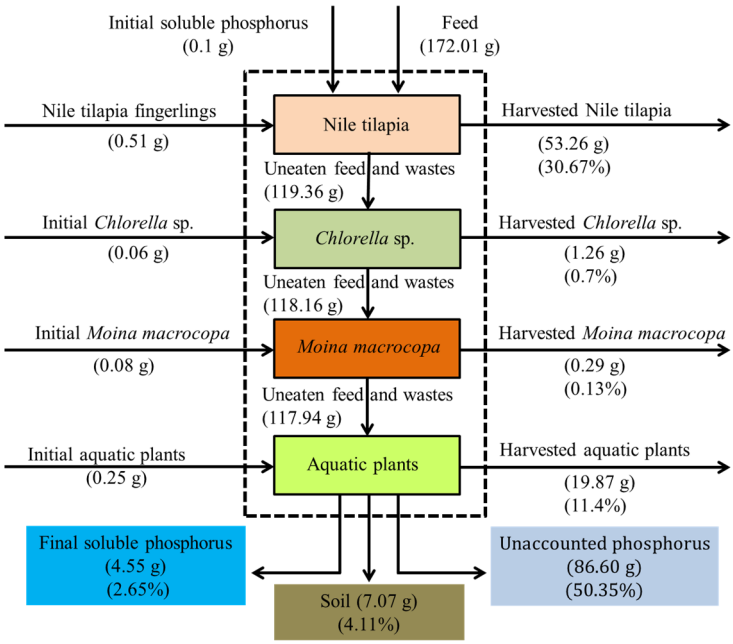

Figure 9. Phosphorous balance of IMRAS.

Figure 10 summarizes that nitrogen and phosphorous of 1311 $\pm$ 26 mgN/m^3/d and 230 $\pm$ 21 mgP/m^3/d were converted to Nile tilapia mass with an initial fresh weight of 2.4 $\pm$ 0.6 g/fish and with a density of 50 fish/m^3. This was from the cultivation period of 112 days where the final fresh weight was 240 $\pm$ 16 g/fish. Phytoplankton and zooplankton could only convert a small fraction of nitrogen and phosphorous to biomass, i.e., at about 41 $\pm$ 7 mgN/m^3/d and 6 $\pm$ 1 mgP/m^3/d. Nitrogen and phosphorous of 328 $\pm$ 80 mgN/m^3/d and 85 $\pm$ 16 mgP/m^3/d, respectively, were converted into all aquatic plants (*Egeria densa*, *Ceratophyllum demersum*, *Vallisneria americana*, *Vallisneria spiralis*, and *Hygrophila difformis*). Nitrogen and phosphorous amounts of 397 $\pm$ 108 mgN/m^3/d and 20 $\pm$ 3 mgP/m^3/d, respectively, were dissolved in the water. Nitrogen and phosphorous amounts of

210 ± 31 mgN/m^3/d and 31 ± 6 mgP/m^3/d, respectively, were adsorbed in the soil, while nitrogen of 1155 ± 114 mgN/m^3/d and phosphorous of 377 ± 17 mgP/m^3/d could not be utilized. This finding suggested that the remaining nitrogen and phosphorous could still be utilized by aquatic plants provided that there is enough area for the plants to grow. A rough linear estimate recommended that the area for the aquatic plants should increase 4–5 times to accommodate the amount of the remaining nitrogen and phosphorus.

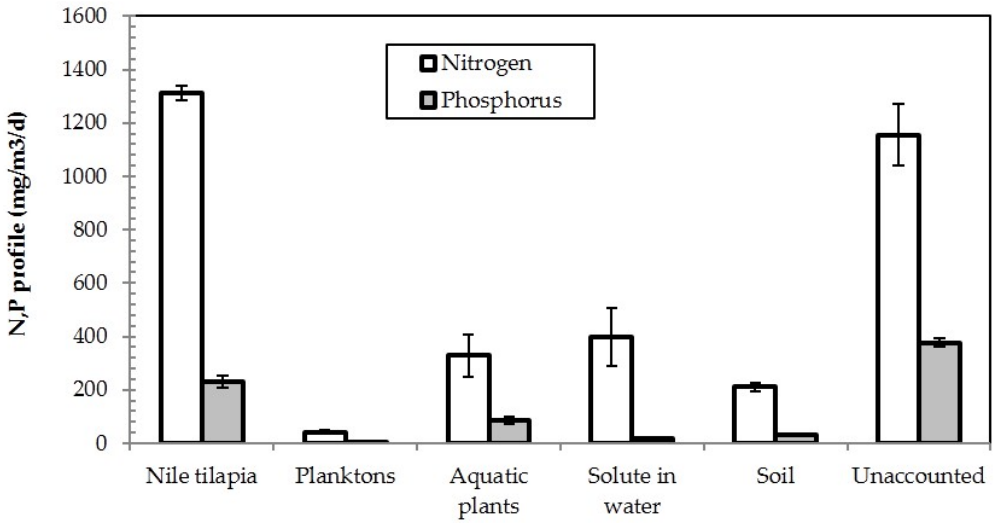

Figure 10. Nitrogen and phosphorous final profile.

4. Conclusions

This work demonstrates the success of the implementation of a closed-loop aquacultural system where a treatment tank is introduced. Although the results revealed that most of the nitrogen and phosphorus were unaccounted for by the fish, planktons and aquatic plants as they were taken up by other metabolisms in the sediment, the introduction of the treatment tank helps to complete the ecology of the system by providing proper conversion of the waste generated by the fish, as some of this waste was converted into valuable products, in the case of phytoplankton, zooplankton, and aquatic plants. Not only does this system benefit from these added-value by-products, but it also enables the recirculation of the culture water, enhancing the reliability of the water management within the system. With the treatment tank, the water quality, ammonia, nitrite nitrate, phosphate, pH and DO could be effectively controlled at safe levels for the cultivation duration of 112 days, and the observed fish productivity was reasonably high.

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