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Growing Conditions and System Productivity in a Closed-Loop Aquaponic System Under Varying Stocking Density

Jeannie-Rose G. Fabula[1*], Helen F. Gavino[2], Chito F. Sace[2], Marvin M. Cinense[2], Emmanuel V. Sicat[2], Jose S. Abucay[3]

[1] Crops and Resources Research and Development Center, Central Luzon State University, Nueva Ecija, Philippines
[2] Department of Agricultural and Biosystems Engineering, Central Luzon State University, Nueva Ecija, Philippines
[3] College of Fisheries, Central Luzon State University, Nueva Ecija, Philippines
* Corresponding author's e-mail: jgfabula@clsu.edu.ph

ABSTRACT

Aquaponics is an integrated form of a multi-commodity production system that combines a recirculating aquaculture system with the hydroponic cultivation of crops using the same water via recirculation using pumps. However, the ideal density of cultured aquatic species and the suitable fish/plants/fish feed combinations applicable under aquaponics must be established to determine its impact on the system's performance, including the local growing conditions that could affect its productivity. Eighteen aquaponic systems following a closed-loop water recirculation method were established for the production of red tilapia, giant river prawns, lettuce, and duckweed. The study aimed to establish the ambient growing condition, water quality, and productivity of the system subjected to different stocking densities of fish (RT_{24} – 24 fish/m^3, and RT_{48} – 48 fish/m^3) and prawn (P_0 – zero prawn, P_{12} – 12 prawns/m^2, and P_{25} – 25 prawns/m^2. Results show that with an ambient air temperature and humidity ranging from 30–35 °C and 52–71% during the production, the obtained water quality conditions in the system were: water temperature 27–30 °C; dissolved oxygen (DO) 2.8–3.3 mg/L; pH – 8.3; total ammonia nitrogen (TAN) close to 0, Nitrite – 0; Nitrate – 40 to 160 mg/L; total dissolved solids (TDS) – 580 mg/L; and a daily water loss of 1.47% which were within the tolerable growth conditions of the different species. The stocking density of 24 fish/m^3 and 12 prawns/m^2 resulted in better growth and yield performance of the cultured aquatic species. However, the stocking densities had no significant effect on the growth and yield of lettuce and duckweed.

Keywords: aquaponics, red tilapia, giant river prawn, stocking density, water quality, growing condition, productivity.

INTRODUCTION

Aquaponics, or the production of various species in a closed-loop water system (Bosma et al., 2017), is a special form of a recirculating aquaculture system, specifically a polyculture consisting of fish tanks (aquaculture) and plants cultivated in the same water circle (Graber & Junge, 2009). The integration results in the production of various species, which promotes diversity and adds stability to the system (Sace & Fitzsimmons, 2013).

As a technology, aquaponics is a working model of sustainable food production as waste products from its aquaculture component are converted and utilized as a nutrient source for its hydroponic component. Water is re-used through biological filtration and recirculation. In addition, aquaponics promotes local food production providing access to healthy foods as no fertilizer or pesticides are applied, and enhances the local economy by generating higher income due to higher yields (Diver & Rinehart, 2010).

However, various challenges are needed to be addressed in aquaponics. These include the need for more scientific research affecting its successful operation, the proper aquaponics design necessary to attain optimal crop yield, and the suitable fish/plants/fish feed combinations for high profitability

(Kim, 2018). Additional research is needed to evaluate and communicate the best practices for using the technology (Love et al., 2014).

Tilapia is among the popular species cultured in aquaponics. Still, the relative stocking density to maximize its production, profitability, and sustainability must be identified (Balcazar et al., 2006), specifically for its hybrid species, the red tilapia (*Oreochromis* sp.). Freshwater prawns are another excellent option for integration into the system. In particular, the giant river prawn (*Macrobrachium rosenbergii*) can feed on a wide range of aquatic or terrestrial species residuals in an aquaponic system (Marques et al., 2016). The polyculture of freshwater prawns with compatible aquatic species and crops must be explored further (Tambalque et al., 2015).

Another input and the main source of nutrients in aquaponics is fish feed. It significantly affects the quality of water that recirculates in the system (Yildiz et al., 2017). Fish diets must be made of sustainable and locally sourced materials to reduce production costs (Junge et al., 2017). Duckweed (*Lemna* sp.) is an aquatic plant rich in numerous nutrients. Live duckweed used as a fish diet can potentially lower the cost of fish feed (Popa et al., 2017).

Hence, an aquaponic system producing multiple species – red tilapia, freshwater prawn, lettuce, and duckweed, was evaluated to establish the growing conditions resulting from the integration of these species in a closed system that would balance the system's nutrient production versus plant uptake and identify the suitable stocking density that would result into higher efficiency and productivity.

MATERIALS AND METHODS

System design and establishment

A 16 × 8 m protective structure covered with ultraviolet (UV) treated polyethylene (PE) plastic film and 50% shade net was utilized as the growing area. The structure did not have a control system to manage the environmental condition during the experiment. Eighteen aquaponic systems were installed in the growing area. The suggested start-up ratio of 1:1 (Diver & Rinehart, 2010; Underwood & Dunn, 2016) was utilized in the study. The total volume of water in the rearing tanks was equal to the volume of the grow bed media to meet the required ratio. A "system" referred to in the study consisted of two rearing tanks, a media bed, a single submersible pump, and an air pump.

Following the 1:1 start-up ratio, the entire volume of water in each system was 0.78 m^3. Two separate tanks were used in growing the red tilapia and the freshwater prawn to control the negative interaction of the species. The intermediate bulk container (IBC) made from PE material with a dimension of 1.2 × 1 × 1.16 m was used as the rearing tank for the fish. The IBC tank contained 0.50 m^3 of water. It has a steel frame used to carry the fabricated prawn tank on top. The prawn tank's dimension was set to 1 × 0.60 × 0.50 m. It was made from hollow steel frames with PE sheet liner. The overall height of the rearing tanks was 1.56 m. The prawn tank carried 0.28 m^3 of the remaining volume of water for the entire system. An artificial shelter was incorporated into the prawn tank; it was constructed using polyvinyl chloride (PVC) frames with layers of meshed net. It enhanced the tank's surface area and helped improve the survival percentage of prawns.

The media bed technique was used to grow Fanfare lettuce. The grow bed had a large surface area that was beneficial in serving as the biofiltration device of the system. It had a dimension of 2.4 × 1.30 × 0.35 m. It was fabricated using reinforced steel bars with a stand and lined with a PE sheet to hold a media volume equal to 0.78 m^3. The flood and drain technique was used in irrigating the grow beds. A bell siphon was constructed to facilitate the flooding and draining of the system. Pea gravel was selected as the hydroponic media, which was filled up to a depth of 25 cm.

The one-pump system rule was applied to minimize the investment cost of the system (Rakocy, 2012). The 60-watt submersible pump (S3000, Resun, Shenzhen, CN) lifted the water from the fish tank with a total dynamic head of 150 cm. The pump discharge ranged from 15 to 20 liters per minute (lpm). This pump capacity was enough to meet the necessary recirculation requirement for small-scale units (FAO, 2017) to cycle the entire volume of water in the system every hour. An 18-watt air pump (ACO, Resun, Shenzhen, CN) was used to provide the aeration of the system with a rated output of 28 lpm having six outlets for air lines and air stones. The said output was enough to meet the suitable airflow rate of 4–8 lpm for small-scale systems (Somerville et al., 2014). Due to economic reasons, two systems were sharing with a single air pump.

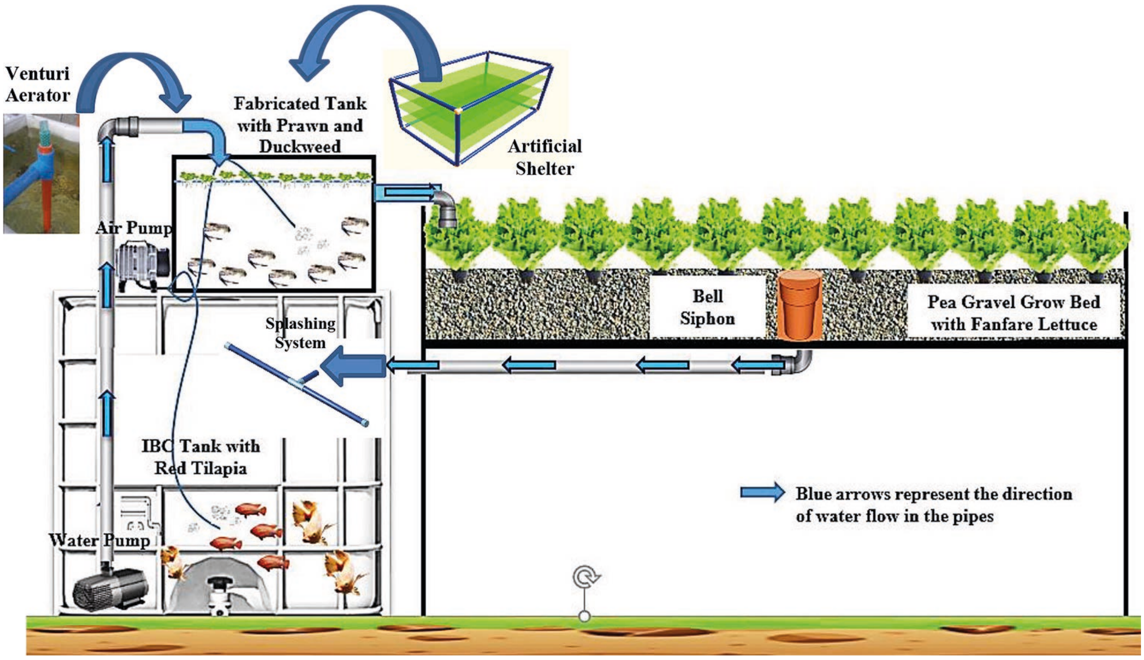

Figure 1. The schematic diagram of the aquaponic system

During the production, additional components were added to improve the DO level of the system. These include the venturi aerator attached at the end of the discharge pipe to the prawn tank, and a splashing system connected at the end of the grow bed drain pipe to the fish tank (Figure 1).

Experimental design and management

All systems were initiated to operate for three days to volatilize the water's chlorine content and check if the systems had leaks. After dechlorination, fish-less cycling was executed. Systems were "fully cycled" when their ammonia and nitrate levels peaked and then declined to below 1 mg/L (Bernstein & Lennard, 2019). It took 15 days to cycle the systems where nitrates were detected on average at about 20 mg/L, nitrite level was zero, and TAN was less than 1 mg/L. After cycling, red tilapia fingerlings (≈3 to 4 cm in length, ≈1.06 grams in weight) and freshwater prawn postlarvae or PL (ranging from 1.5 to 2.0 cm in length, ≈1 to 2 grams in weight) were acclimated and added to the tanks.

The study involved two experimental factors: (1) the stocking density of red tilapia: RT_{24} – minimum stocking density with 24 fish/m^3, and RT_{48} – average recommended stocking density with 48 fish/m^3 based on the aquaponic media-bed sizing model by Lennard (2012), and (2) the stocking density of freshwater prawn: P_0 – zero prawn, P_{12} – recommended stocking density – 12 prawns/m^2, and P_{25} – higher than the recommended, 25 prawns/m^2 following the recommendations of BFAR-NFFTC. Each treatment combination had three replications; 18 aquaponic systems were laid out in the protective structure following a factorial in Completely Randomized Design.

Lettuce seedlings were transplanted in the media bed following a planting density of 25 plants/m^2 (Somerville et al., 2014) five days after adding fish. Two lettuce croppings were performed at 60 days intervals. Data sampling was conducted 30 days after planting. Duckweed was added to the prawn tanks with a plant standing density of 400 g/m^2 (Skillicorn et al., 1993).

The daily feed ratio (DFR), or the amount of feeds given per treatment, was calculated every two weeks to minimize the stress on the fish during sampling for the determination of average body weight (ABW). Commercially available fish feeds (Premium Tilapia Feeds, BMEG, San Miguel Corp., Phils.) were utilized. The following equations were used to determine the daily feed ratio (BFAR, 2000):

$$\mathrm{ABW} = \frac{\text{total weight of fish randomly sampled(g or kg)}}{\text{number of fish sampled}} \tag{1}$$

$$\mathrm{DFR} = \mathrm{ABW} \times \text{Feeding Rate}(\%) \times \times \text{Stocking Density}(\mathrm{pc}) \tag{2}$$

Table 1. The feeding rate, feed type, and feeding frequency which was used in the study

Weight of fish (g)	Feeding rate (% body weight)	Type of fish feed	Feeding frequency (meals per day)
1–5	7	Fry Mash	3
5–20	5	Starter	3
20–100	3	Grower	2
>100	1	Finisher	1

Duckweed was harvested whenever necessary to control its density and used as an additional fish diet. However, it was only given to the fish whenever duckweed's growth exceeded the initial stocking density and clogged the system. Uneaten feeds or scraps from the fish tank conveyed via continuous water pumping and recirculation were used as feeds for the freshwater prawns. The fish and prawns were harvested after 145 days, while lettuce crops were harvested 30 days after transplanting.

The following were the data gathered in the study:

1. Environmental factors such as air temperature and relative humidity were measured daily (8:00 AM and 2:00 PM) using the digital thermometer/ hygrometer (Thermopro TP50 temperature and humidity monitor, Guandong, CN).
2. Daily measurement (8:00 AM and 2:00 PM) of the water quality parameters - pH, TAN, nitrite, nitrate, conductivity, water temperature, and DO using the multiparameter meter (YSI Pro DSS, Xylem Inc, Ohio, USA) and test kits (API Freshwater Master Test Kit, PA, USA).
3. Crop growth and yield parameters during harvest - plant height, root length, number of leaves, fresh and dry biomass of lettuce.
4. Growth and yield of aquatic species - length, weight, height, and number of surviving species for both fish and prawns, measured during harvest. Weight gain, specific growth rate (SGR), survival percentage, and feed conversion ratio (FCR) were computed using the following equations:

$$Weight\ Gain = Final\ Weight - \\ -Initial\ Weight \tag{3}$$

$$Specific\ growth\ rate\ \left(\frac{\%}{day}\right) = \\ = \frac{\ln\left(\begin{matrix} Final\ Weight - \\ -Initial\ Weight \end{matrix}\right)}{Culture\ period\ (day)} \times 100 \tag{4}$$

$$Survival\ Percentage\ (\%) = \\ = \frac{\begin{matrix} Number\ of \\ survival\ fish \end{matrix}}{\begin{matrix} Number\ of\ fish\ at \\ the\ beginning\ of\ the\ study \end{matrix}} \tag{5}$$

$$FCR = \frac{Amount\ of\ feed\ fed\ (kg)}{Weight\ gained (kg)} \tag{6}$$

5. Growth and yield of duckweed - the amount of duckweed harvested from the tanks was weighed and recorded to determine the rate of production based on the initial plant density.
6. Water consumption - after the initial filling-up of the rearing tanks, the total amount of water used/added in each system was recorded for the whole duration of the study.

Statistical analysis

The mixed model procedure using SAS University Edition software (SAS Institute Inc., Cary, North Carolina, U.S.A.) was used in the Analysis of Variance (ANOVA). The Tukey's Least Significant Difference (LSD) test was utilized in comparing treatment means. The effects of the treatments were considered statistically significant at the 0.05 probability level.

RESULTS AND DISCUSSION

System's growing conditions

The recirculating water served as the heart of the integrated aquaponic system. Water quality was maintained for the overall functioning of the systems. Among the factors that affected the system's performance were the observed local growing conditions during study implementation. The measured environmental and water quality parameters were the following:

Ambient air temperature and humidity

The average ambient air temperature inside the study area was 30.69 °C in the morning and 35.63 °C in the afternoon; the average morning humidity was 71% and 52% in the afternoon (Figure 2). The average ambient air temperature outside the protective structure was 36.88 °C in the morning and went as high as 41.04 °C in the afternoon. The average humidity was 51% in the morning and decreased to 42% in the afternoon. The ideal humidity range for optimum plant growth in an aquaponic system was around 50 - 70% (Abdullah & Mazalan, 2022); this condition was attained in the study.

However, due to the high air temperature in the study area, especially in the afternoon, the optimum condition for lettuce production ranging from 16–25 °C (Licamale, 2009) was not maintained. As a result, the crops suffered tip burn, and their yield was significantly reduced. This was similar to the findings of Rogers (2013), wherein the increase in air temperature reduced lettuce yield, increased the risk of bolting, affected its color development, and caused its bitter taste.

The ideal humidity range was achieved by installing shade nets inside and outside the protective structure. It provided a 20% decrease in air temperature in the morning and 13% in the afternoon; however, it was not enough to meet the required air temperature in the growing area. Additional equipment was needed to improve the ventilation in the study area. However, it would require an added cost to the system, which could affect the viability assessment of the technology as the aim was toward low-cost production.

Water temperature

The average water temperature in the fish and prawn tanks was 27.48 °C and 27.28 °C in the morning and 29.46 °C and 28.98 °C in the afternoon. There was an average increase of 5.82% in water temperature during the afternoon. Water temperature was maintained at 27.3 to 29.5 °C, which was favorable to the red tilapia, freshwater prawn, and nitrifying bacteria as they were able to tolerate water temperature as high as 32 °C (BFAR-NFFTC, n.d., FAO, 2017, Romana-Eguia et al., 2020, Somerville et al., 2014). However, the crop component was affected since lettuce favors colder water and grows best at temperatures ranging from 21 to 24 °C (Sallenave, 2016). The water temperature between treatments did not vary significantly (Figure 3).

Among the factors that can influence water temperature were local climatic conditions, ambient air temperature, tank materials, exposed piping and length of pipe runs, placement of tanks, insulation of components in the system, the total water volume of the system, and available backup systems (Sawyer, 2015). The factors that significantly affected the system's water temperature were climate and ambient air temperature since all the system's components were properly insulated. Hence, during crop selection for aquaponic production, the plant growth requirement should also match the prevailing climatic conditions in a particular area where the system will be established and should be considered to ensure adaptability and productivity.

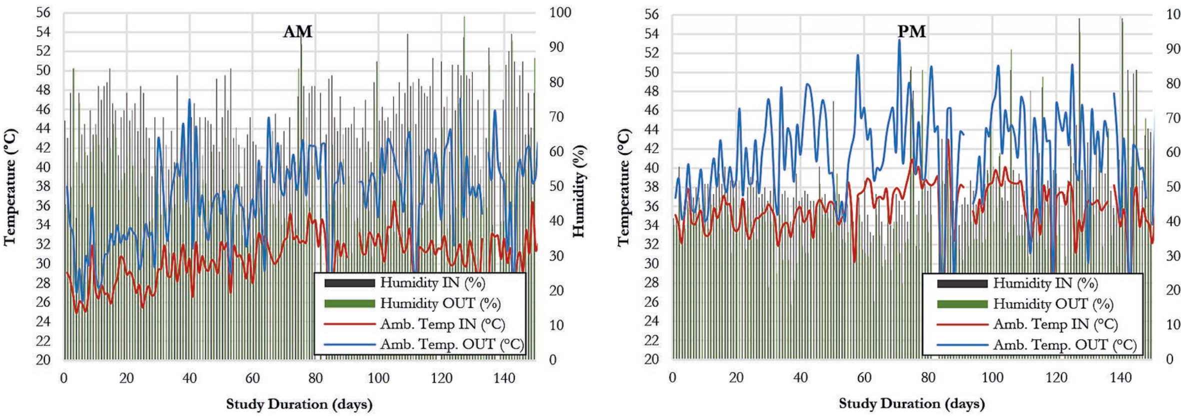

Figure 2. Daily ambient air temperature (°C) and humidity (%) at 8:00 AM and 2:00 PM in the study area

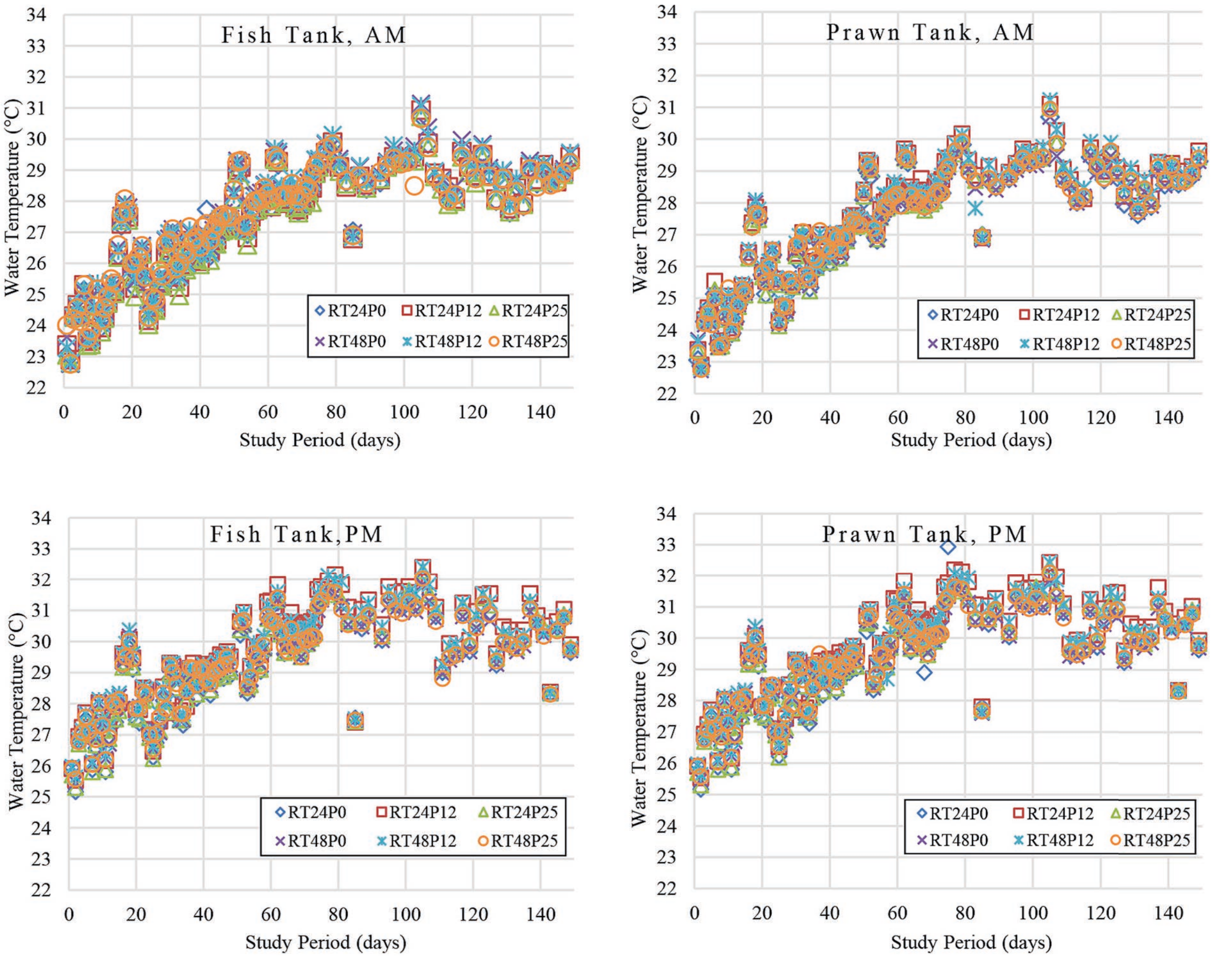

Figure 3. Observed water temperature in the rearing tanks

Dissolved oxygen

The DO level in the fish tank ranged from 1.23 to 5.25 mg/L in the morning and 1.19 to 4.89 mg/L in the afternoon. For the prawn tank, it ranged from 1.21 to 5.48 mg/L in the morning and 1.12 to 4.94 mg/L in the afternoon (Figure 4).

The local climatic conditions, especially the ambient air temperature, affected water temperature and may have influenced the tanks' DO levels. Water temperature affects oxygen solubility (Patel & Vashi, 2015). This was observed especially in the afternoon, where a 13.76% reduction in the tanks' DO levels was observed.

The DO level in the tanks was more crucial to the aquatic species than the crops. Lettuce roots were oxygenated by the constant flooding and draining of the hydroponic bed facilitated by the bell siphon. Since there was a fluctuating oxygen concentration in the system, aeration was improved by adding a venturi aerator in the prawn tank, installing a splashing unit in the fish tank, and periodic cleaning of the pumps and pipes. The additional system components promoted water movement, surface agitation, and the maintenance of the pumps and pipes increased water cycling, which improved the DO level of the systems.

The density of aquatic species also influenced the oxygen concentration in the system (Ani, et al., 2022). It was observed that the treatments having the minimum recommended stocking density of fish had a higher DO level of more than 2% compared to the treatments with the average stocking density of red tilapia. The treatments without prawns also showed a higher DO level than those with prawns. The result suggests that increasing the stocking densities decreases the DO level in the systems.

In general, the tolerable DO level was maintained, which was crucial for the survival of the aquatic components in the system. Tilapia tolerates DO levels as low as 2–3 mg/L (Somerville et al., 2014). Piping, or the event when fish gasped for air on the surface, was rarely observed, indicating that the DO level required for the fish to survive was maintained. Prawns also sustained their growth to

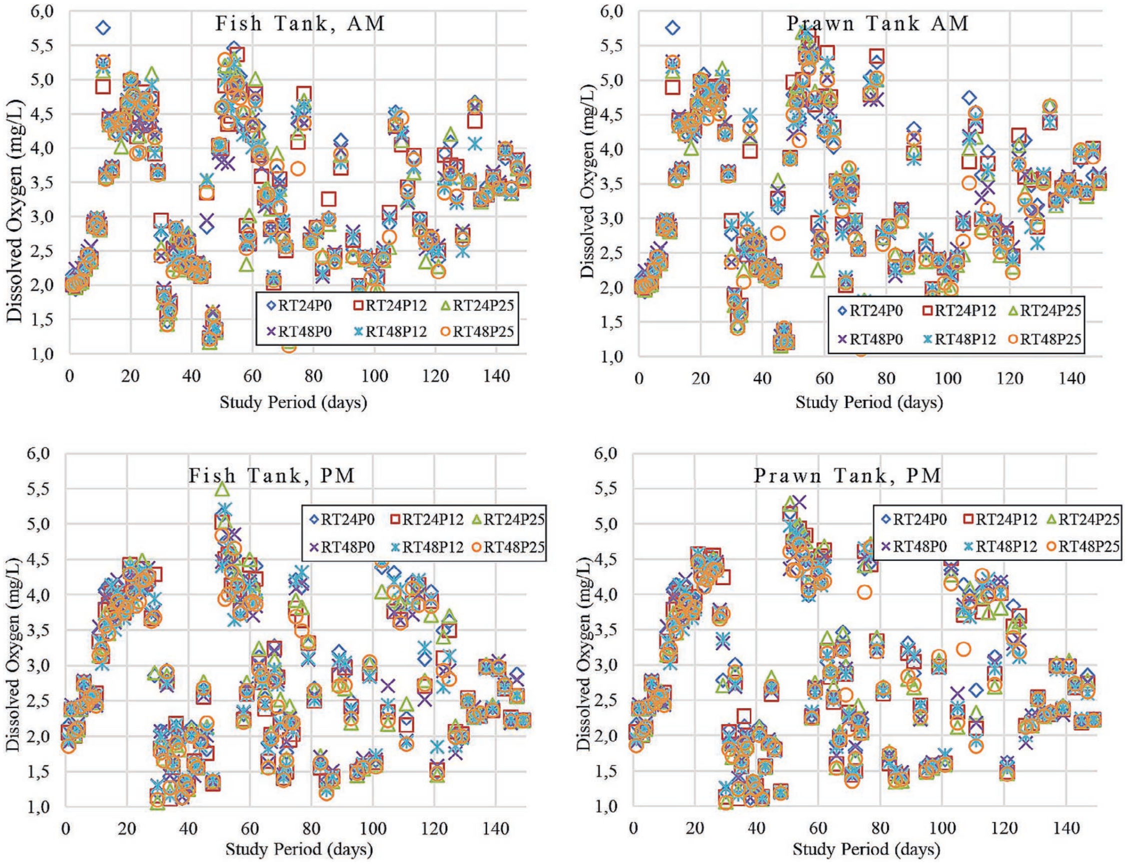

Figure 4. Observed dissolved oxygen (mg/L) in the rearing tanks

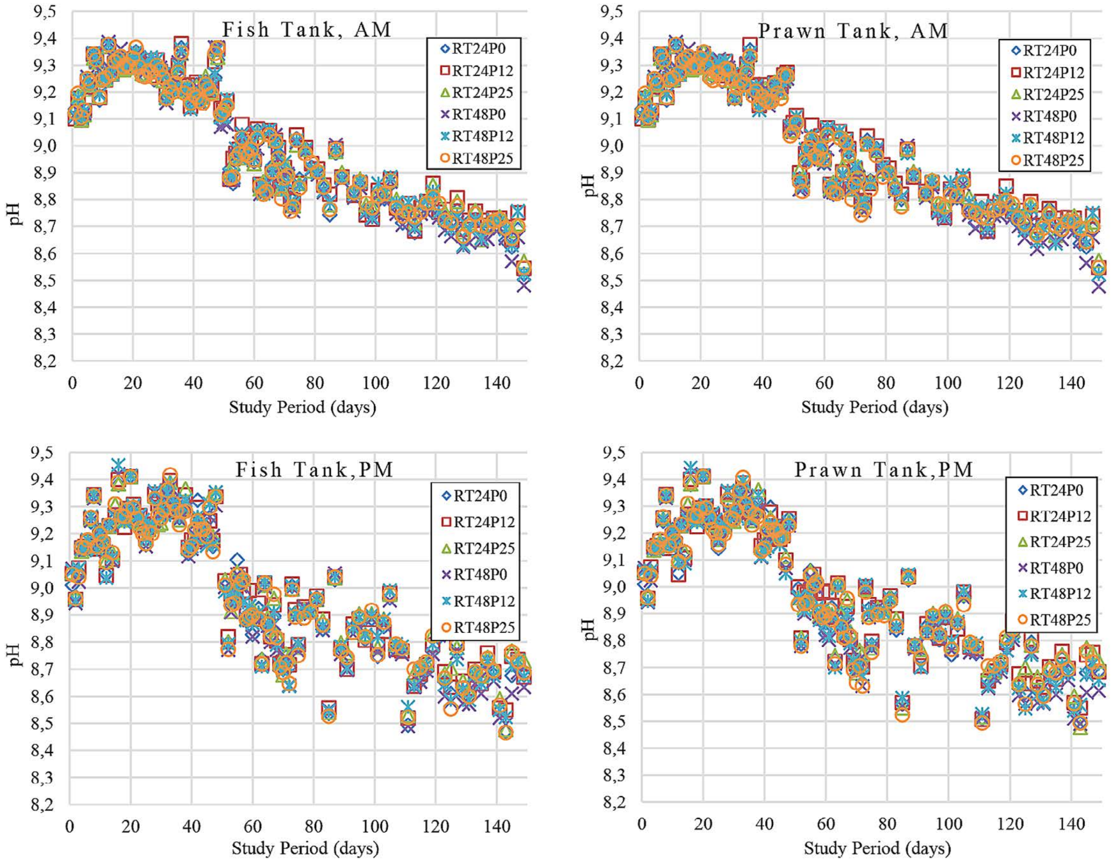

Figure 5. Water pH in the rearing tanks observed at 8:00 AM and 2:00 PM

a DO level of at least 3 mg/L; a value lower than that would still be tolerable as long as aeration is provided (D'Abramo et al., 2006).

pH

The systems' pH gradually decreased over time as the production progressed. The maximum pH in the rearing tanks in the morning was 9.38 until it eventually dropped to 8.36 and 8.27 for the fish and prawn tanks. Afternoon pH peaked at 9.41 for both tanks and went down to 8.40 for the fish and 8.39 for the prawn tanks. A similar trend for pH was observed for all treatments (Figure 5).

The fish and aerobic bacteria used for nitrification have an optimum pH of ~7 to 9, whereas most hydroponic plant species usually prefer pH levels between 5.8 and 6.2 (Rakocy et al., 2006). The recorded high pH condition of recirculated water in the system reduced the productivity of lettuce and duckweed that grows best in aquaponics systems with a pH of 5.8 to 6.2 (FAO, 2019) and 6.5 to 7.5 (Popa et al., 2017) respectively.

A high pH for start-up systems was typical (Storey, 2017). Additionally, the water used during the initial stocking of the rearing tanks was naturally alkaline, with a pH of 9.1. Performing procedures that may lower the system's pH was not applied since adjusting it to more than 0.2 units per day would be drastic to the overall functioning of the units; lowering was also not necessary as the natural process of nitrification would biologically reduce the water's pH (Deer et al., 2021). Moreover, fish respiration also decreases the water pH (FAO, 2017); however, the stocking densities of aquatic species did not produce a significant variation in the system's pH, as the same trend was observed across all treatments.

Total ammonia nitrogen

Ammonia, often referred to as TAN, had two forms existing in equilibrium represented by ionized ammonia (NH_4^+) and un-ionized ammonia (NH_3), which was considered toxic to fish (Wurts, 2003). The average ammonium nitrogen (NH_4-N) in the systems was 0.24 mg/L; it ranged from 0.51

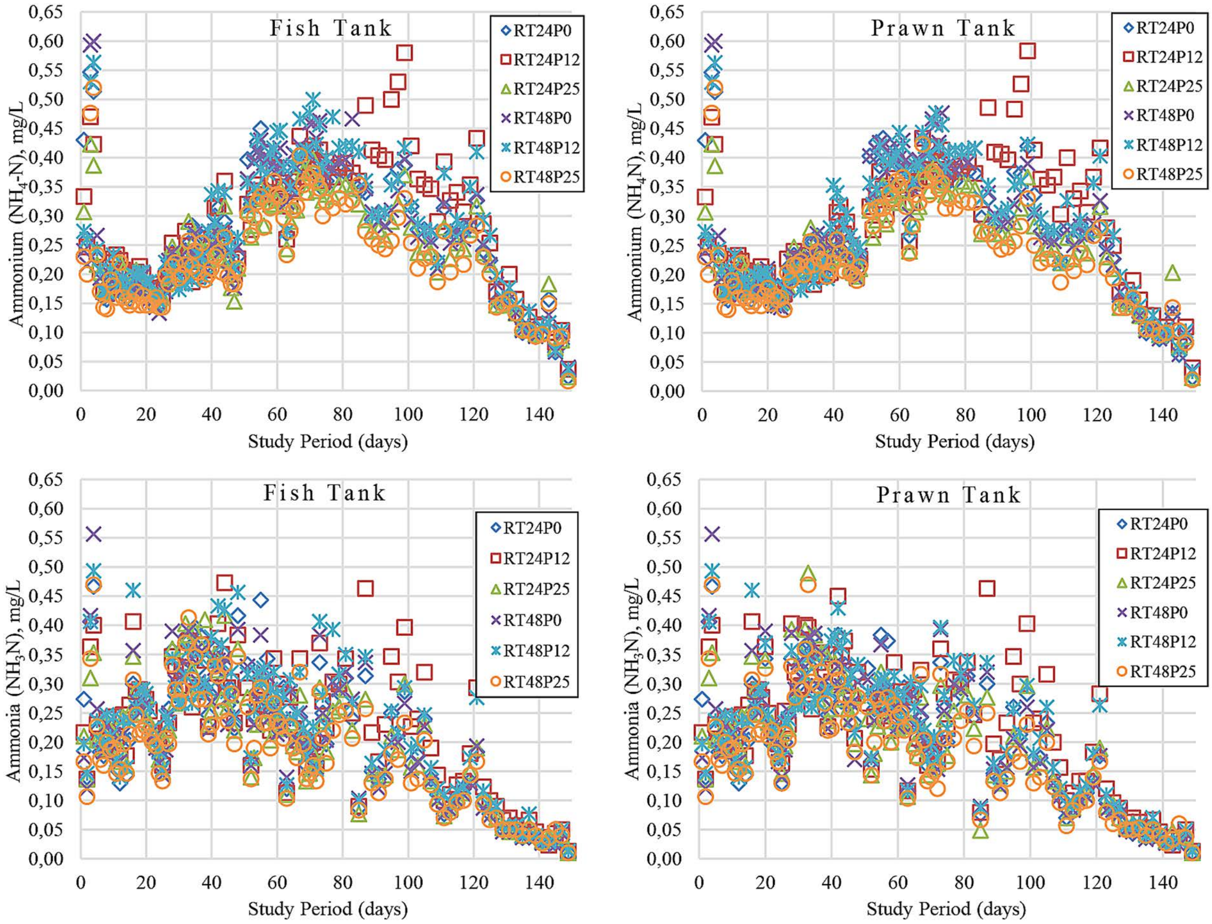

Figure 6. The system's total ammonia nitrogen (NH4-N and NH3-N) as affected by the different stocking densities of fish and prawns

mg/L to 0.01 mg/L (Figure 6). The NH_4-N content of the minimum recommended density (RT_{24}) was 1.49% higher than the average recommended stocking density (RT_{48}).

It may be related to the feed input and FCR of the two treatments. The average recommended stocking density (RT_{48}) had a higher feed input and FCR than the other treatment. Robaina et al. (2019) cited that 95% of the feed inputs were either ingested or digested by the fish, 30 – 40% was converted as its biomass, and 60 – 70% was generated as waste. The RT_{48} treatments converted most of its feed input into its biomass. In contrast, the other treatment generated more waste at a lesser biomass conversion ratio, which resulted in the difference in NH_4-N content.

The NH_4-N content of treatments having the recommended stocking density of 12 prawns/m^2 was 4.37% higher than the zero prawn treatment. Increasing the stocking density of prawns to 25/m^2 translated into a 14.21% decrease in NH_4-N content as wastes generated by the fish component were fed to the prawns.

The average ammonia nitrogen (NH_3-N) was 0.20 mg/L per day; it went from 0.46 mg/L to zero. Similarly, treatments with 24 fish/m^3 had a higher NH_3-N content than treatments with 48 fish/m^3 by 2.89%. The addition of 12 prawns/m^2 increased the NH_3-N content in the systems by 7.89% but increasing it to 25 prawns/m^2 decreased it to 13.84%. Wastes generated wastes in the systems, plus the influence of red tilapia and prawn stocking densities affected the production of NH_4-N and NH_3-N.

The correlation between the system's pH and TAN revealed a strong positive relationship, especially with NH_3-N, suggesting that an increase in the system's pH will result in higher TAN and NH_3-N contents, especially during the early stage of the study when the biofilter of the system was not yet established. The predominant form of ammonia was affected by water temperature and pH at any given time in the aquaponic systems (Francis-Floyd et al., 2009; Yildiz et al., 2017). Overall, TAN was maintained below 1 ppm, which was suitable in aquaponics (Sallenave, 2016).

Nitrate

The nitrate content in the systems started to fluctuate from 0 to 40 mg/L during the cycling period. Conversely, when the aquatic species, especially the feeds, were introduced into the systems, the nitrate content increased inconsistently from 40 mg/L to as high as 160 mg/L.

The average nitrate contents for the different treatments were: $RT_{24}P_0$ – 65.26; $RT_{24}P_{12}$ – 49.49; $RT_{24}P_{25}$ – 48.37; $RT_{48}P_0$ – 67.15; $RT_{48}P_{12}$ – 53.27; and $RT_{48}P_{25}$ – 48.88 mg/L. Increasing the stocking density of the red tilapia from 24 to 48 fish/m^3 increased the nitrate content of the system to 3.79%. However, increasing the prawns' stocking density resulted in lower nitrate content in the systems. The nitrate contents in the P_{12} and P_{25} treatments were 22.39% and 26.56% lower than the zero prawn treatment. The result was again related to the feeding management of species as prawns depended on fish scraps (Figure 7).

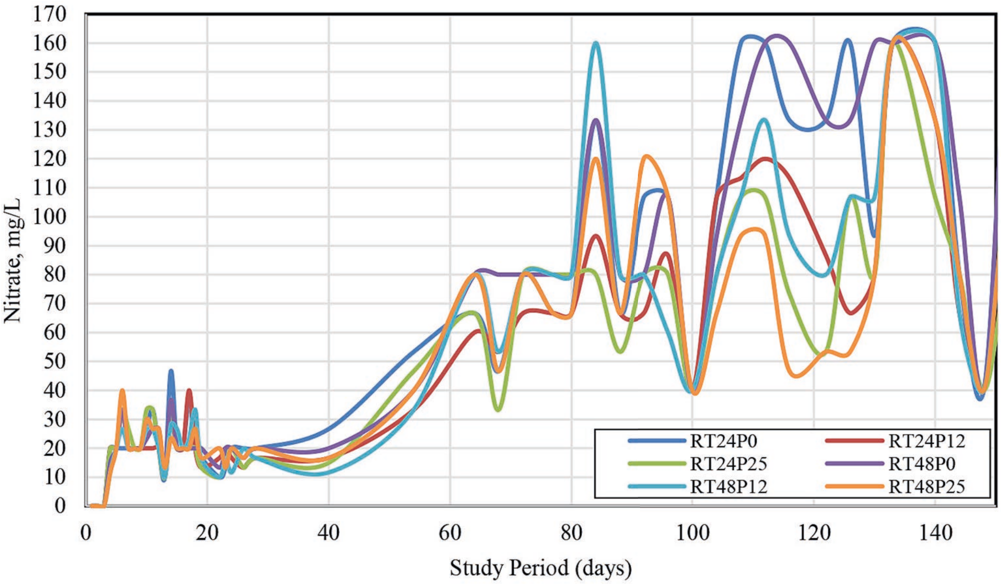

Figure 7. Average nitrate content (mg/L) in the aquaponic systems

Toward the end of the study, both the ammonium nitrogen and ammonia nitrogen levels were nearly zero (Figure 6); this served as an indication that the systems' biofilters were becoming fully established due to the conversion of ammonia into nitrates caused by the nitrifying bacteria (FAO, 2017). Thus, the high nitrate content of water revealed that nutrients were already building up in the system and that the biofilters were effectively working.

Total dissolved solids

The average TDS when the systems were established started at 254.05 mg/L; by the end of the growing period, it reached 580.85 mg/L in the fish tank and 580.03 mg/L in the prawn tank (Figure 8). However, there was no notable difference in the TDS between treatments having varying red tilapia stocking densities. The TDS in the prawn treatments decreased as stocking density intensified; the TDS in the zero prawn treatment was 3.42% higher than the P_{12} treatment and 5.96% higher than the P_{25} treatment.

The result revealed the impact of not feeding the prawns in the study. The amount of dissolved nutrients decreased due to prawns' dependence on the wastes generated in the system to support their growth. Feeding of prawns under integrated production with red tilapia could be considered in future research to improve their growth performance.

Water consumption

After the initial filling-up of water in the rearing tanks, the average amount of water consumed per system was 1920.67 liters, which translates into a daily depletion of 11.43 L/day or 1.47%. The amount was within the expected range of daily water loss for aquaponic systems, as Rakocy et al. (2006) reported, or 0.5 to 10% per day. The result confirmed that the established integrated

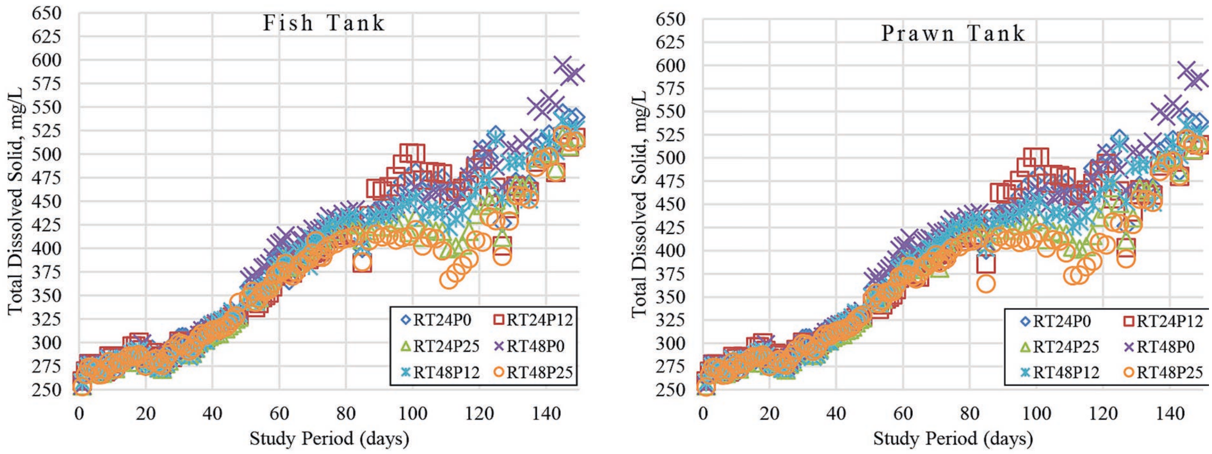

Figure 8. The amount of total dissolved solids (mg/L) in the rearing tanks

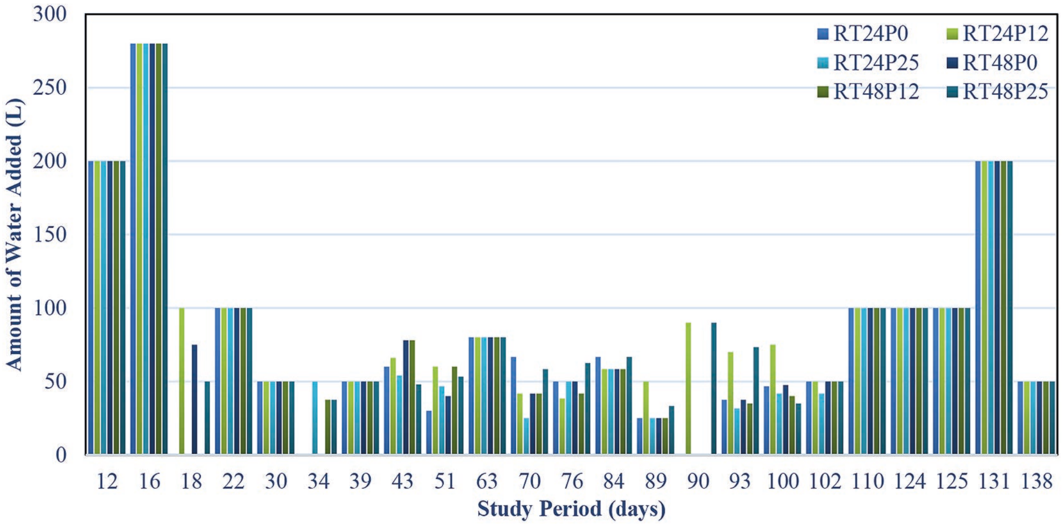

Figure 9. The amount of water used in each treatment during the entire duration of the study

food system utilizes a conservative amount of water to support the growth of multiple species, thus an effective water-saving technology.

Moreover, most of the water consumed in the study was usually applied to correct/maintain the desired quality in the system. For example, more water was applied during the early stage or when cycling of the systems was being executed (Figure 9). During this phase, the systems were still establishing their biofilters, as reflected in the fluctuating ammonia levels. It corresponds to water management via partial water change to minimize the toxicity in the system. Leaks were another factor contributing to the water losses in the system. Hence, as the systems stabilized and leaks were addressed, the addition of water during the grow-out phase was minimized.

Growth and yield of the red tilapia

The red tilapia's highest weight and weight gain and SGR were obtained from the $RT_{24}P_{12}$ treatment, while the lowest was from the $RT_{48}P_{25}$ treatment (Table 2). The different stocking densities significantly affected the fish's weight, weight gain, and SGR. Treatments stocked with the minimum recommended stocking density of 24 fish/m^3 and the recommended stocking density of 12 prawns/m^2 were significantly heavier and gained more weight than the other five treatments.

However, increasing the stocking density of the red tilapia to 48 fish/m^3 and freshwater prawn to 25/m^2 showed a significant reduction in the fish' body mass, weight gain, and SGR. The said effect might be attributed to balancing the synergy between the two species coexisting under polyculture. Further, in a tilapia-prawn polyculture, prawns were usually stocked at a lower density (Marquez et al., 2016). The intensive stocking densities decreased the beneficial effect of the synergy, as reflected in the reduction of the red tilapia's body mass, weight gain, and SGR.

No significant difference was obtained for the red tilapia's survival percentage, total length, height, and FCR due to the different stocking densities.

Growth and yield of the freshwater prawn

The stocking densities had no significant effect on the freshwater prawns' weight, total length, and survival percentage. The highest weight and total length were obtained from the $RT_{48}P_{12}$ treatment, while the highest survival percentage was computed from the $RT_{24}P_{12}$ treatment (Table 3). The weight, total length, and survival percentage of freshwater prawns with a stocking density of 12 pcs/m^2 was 30%, 10%, and 32% higher than treatments stocked with 25 prawns/m^2. The prawns suffered poor growth in the study due to the limited feed input, as they relied only on the leftovers from the fish tanks. It was further observed that their weight, total length, and survival rate decreased as the stocking density intensified.

Similar to prawn's weight and length, the highest specific growth rate was computed from the $RT_{48}P_{12}$ treatment. The stocking densities had a significant effect on prawn's SGR. The weight of prawns from $RT_{48}P_{12}$ treatment was significantly higher by 8.88% compared to $RT_{24}P_{12}$ treatment and 31.58% higher than $RT_{48}P_{25}$.

The growth rate and survival of prawns were influenced by stocking density, predation, feed management, and temperature (New, 2002). Stocking density and predation contributed to the poor weight of prawns during production. Water temperature is another factor since prawns' optimum water temperature requirement was 26 to 30 °C; they could survive from 22 to 32 °C but

Table 2. The growth and yield of the red tilapia under the integrated recirculating aquaponic system as affected by the different stocking densities

Treatments (stocking density)		Average weight (g/pc)	Weight gain (g)	Specific growth rate, SGR (%/day)	Survival percentage (%)	Total length (cm)	Height (cm)	Feed conversion ratio (FCR)
Fish per m^3	Prawn per m^2							
RT_{24}	P_0	161.72 ± 9.28^{b}	160.66 ± 9.28^{b}	3.50 ± 0.04^{b}	88.89±5.32	20.10±0.52	5.70±0.30	2.31±0.21
	P_{12}	170.28 ± 9.28^{a}	169.22 ± 9.28^{a}	3.53 ± 0.04^{a}	94.44±5.32	20.37±0.52	5.95±0.30	1.97±0.21
	P_{25}	126.36 ± 9.28^{b}	125.30 ± 9.28^{b}	3.33 ± 0.04^{b}	80.56±5.32	18.10±0.52	4.95±0.30	2.61±0.21
RT_{48}	P_0	132.81 ± 9.28^{b}	131.75 ± 9.28^{b}	3.36 ± 0.04^{b}	91.67±5.32	19.70±0.52	5.47±0.30	1.96±0.21
	P_{12}	142.71 ± 9.28^{b}	141.6 ± 9.28^{b}	3.42 ± 0.04^{b}	94.44±5.32	20.20±0.52	6.00±0.30	1.82±0.21
	P_{25}	120.69 ± 9.28^{c}	119.63 ± 9.28^{c}	3.30 ± 0.04^{c}	91.67±5.32	19.05±0.52	5.23±0.30	2.13±0.21

Note: means sharing the same letter are not statistically different at α=0.05.

Table 3. The growth and yield of the freshwater prawn under the integrated recirculating aquaponic system as affected by the different stocking densities

Treatments (Stocking density)		Average weight (g/pc)	Specific growth rate, SGR (%/day)	Total length (cm)	Survival percentage (%)
Fish per m^3	Prawn per m^2				
RT_{24}	P_0	-	-	-	-
	P_{12}	9.27±1.03	1.44±0.09^b	9.38±0.41	87.50±14.45
	P_{25}	5.61±1.03	1.04±0.09^c	8.26±0.41	46.67±14.45
RT_{48}	P_0	-	-	-	-
	P_{12}	10.14±1.03	1.52±0.09^a	9.77±0.41	75.00±14.45
	P_{25}	7.93±1.03	1.33±0.09^b	8.93±0.41	64.44±14.45

Note: Means sharing the same letter are not statistically different at α = 0.05.

will suffer poor growth and activity at the end of those ranges (Costa-Pierce et al., 1984). During the production, the ambient air temperature in the growing area was at its extreme, which affected the temperature of the recirculated water in the system. Moreover, the limited food supply in the systems further influenced the growth of prawns that also resulted in predation, as indicated in the derived survival rate in the different treatments.

Growth and yield of lettuce

Lettuce crops were planted in two cropping periods (Table 4). The highest plant height was recorded during both trials from the RT_{48} treatment and the zero prawn treatment. Also, on average, the plant height of lettuce increased to 21.96% during the second trial compared to the initial trial.

The P_{25} treatment had the most number of leaves regardless of the number of red tilapia stocked for the first trial. However, during the second trial, all treatments have almost the same number of leaves. A 22.22% average increase in the number of leaves was recorded in the second trial compared to the first.

During two planting events, treatments with a stocking density of 24 fish/m^3 produced the longest root length. The highest root length for the first trial was obtained from the $RT_{24}P_0$ treatment (13.60 cm) and $RT_{48}P_{12}$ treatment (14.73 cm) for the second trial. Comparing the two trials showed that lettuce root length increased by 11.55% during the second trial compared to the previous trial.

The shoot weight of lettuce during the first trial was comparably lower than its weight during the second trial. The average fresh and dry shoot weight during the second trial was 73.10% and 101.74% higher than the obtained lettuce shoots during the first trial. It was also observed that lettuce shoot yield decreased as the number of prawn species in the system increased. There

Table 4. The growth and yield of lettuce under the integrated recirculating aquaponic system as affected by the different stocking densities

Treatments (stocking density)		Plant height[1] (cm)	Plant height[2] (cm)	No. of leaves[1] (pcs)	No. of leaves[2] (pcs)	Root length[1] (cm)	Root length[2] (cm)	Shoot FW[1] (g)	Shoot FW[2] (g)	Shoot DW[1] (g)	Shoot DW[2] (g)	Root FW[1] (g)	Root FW[2] (g)	Root DW[1] (g)	Root DW[2] (g)
Fish/m^3	Prawn/m^2														
RT_{24}	P_0	25.87±3.00	32.50±3.02	9±0.46	10±0.57	13.60±1.36	14.50±1.59	18.83±1.46	40.33±5.21	1.83±0.19	4.83±0.86	2.97±0.40	3.93±0.70	0.87±0.07	0.97±0.08
	P_{12}	23.70±3.00	31.97±3.02	9±0.46	11±0.57	12.23±1.36	13.03±1.59	19.17±1.46	31.50±5.21	2.00±0.19	4.00±0.86	2.77±0.40	3.53±0.70	0.83±0.07	0.93±0.08
	P_{25}	23.07±3.00	28.40±3.02	10±0.46	11±0.57	12.77±1.36	14.63±1.59	17.00±1.46	31.17±5.21	1.33±0.19	3.00±0.86	2.10±0.40	3.37±0.70	0.73±0.07	0.83±0.08
RT_{48}	P_0	28.23±3.00	33.73±3.02	9±0.46	11±0.57	11.00±1.36	12.80±1.59	21.33±1.46	38.83±5.21	1.67±0.19	3.50±0.86	3.03±0.40	4.13±0.70	0.77±0.07	0.97±0.08
	P_{12}	26.03±3.00	31.03±3.02	9±0.46	11±0.57	12.40±1.36	14.73±1.59	19.17±1.46	32.17±5.21	1.83±0.19	3.17±0.86	2.37±0.40	3.50±0.70	0.87±0.07	0.97±0.08
	P_{25}	26.97±3.00	30.00±3.02	10±0.46	10±0.57	11.77±1.36	12.57±1.59	18.00±1.46	22.50±5.21	1.67±0.19	2.33±0.86	2.80±0.40	3.57±0.70	0.87±0.07	0.93±0.08

Note: FW – fresh weight, DW – dry weight, 1 – 1st cropping, 2 – 2nd cropping.

was a decreasing trend in the crop's shoot yield in the first and second trials.

The highest fresh root weight of lettuce in two cropping periods was obtained from the $RT_{48}P_0$ treatment. For dry root weight, treatments having 48 fish/m^3 recorded the highest dry weight for two trials. Likewise, lettuce's average fresh and dry root weight in the second trial was 37.45% and 13.41% greater than its root weight during the first trial. Lettuce's fresh and dry root weight increased as the stocking density of fish was increased from 24 to 48 fish/m^3, especially without prawns. But, as the stocking density of prawns increased, their root weight decreased.

Overall, the different stocking densities of the red tilapia and freshwater prawns had no significant effect on the lettuce's height, the number of leaves, root length, and the fresh and dry weight of its shoot and roots for two planting events.

The observed increase in the plant height, number of leaves, root length, and shoot and roots fresh and dry weights between the two trials might be attributed to the difference in feed inputs when the planting trials were executed. The quality and quantity of food provided to the fish should also match the nutrient needs of the crops (Mullins et al., 2015). The initial trial of lettuce planting happened right after the systems were newly stocked with red tilapia fingerlings and prawn juveniles. Earlier, the amount of feeds being supplied to the systems matched the weight of the young aquatic species, barely weighing a few grams. The starting feed input then ranged from 4–10 grams per day which was not enough to provide the required nutrients for the crops. The nutrient level in the system was low during the early stage or right after stocking the aquatic species. The system then required more time for nutrients to build up to sustain the growing needs of the crops (Rakocy, 2007).

On the contrary, during the second trial of lettuce production, the systems attained the full potential of their biofilters, providing more nutrients for the crops. Also, the reared fish and prawns have increased their biomass requiring a higher feed input. The amount of feeds supplied into the systems was about 60–80 grams per day. Nutrients in the system had already build-up, thus providing the required nutrition for the crops. The result of the growth and yield performance of lettuce in the study further showed that aside from identifying the ideal stocking density suitable in aquaponics polyculture, the amount of feed given daily to the aquatic species must equate to the crop requirement per growing area. Therefore, this should be considered to obtain the proper functioning of the integrated food system (FAO, 2017).

Integrating prawns into the system also had a noticeable effect on lettuce yield. There was a decreasing trend in its shoot and root weight as prawns' stocking density increased. It may be due to not feeding the prawn since only the red tilapia was being fed in the system. The waste or scraps from the fish tanks that could be converted into nutrients were used as food for the freshwater prawns. Prawn's feeding habits affected and limited the crops' yield.

Moreover, another factor that significantly affected the crop's performance was the growing conditions in the system. The impact of water quality and the prevailing conditions in the growing area were discussed in the previous sections.

Growth and yield of duckweed

The highest recorded duckweed yield of 4.37 g/day was obtained from the $RT_{24}P_{12}$ treatment. There was an observed declining growth rate of duckweed as the stocking density of the red tilapia increased, while its production rate performed better under 12 prawns/m^2 stocking density. However, statistically, the obtained yield per treatment did not result in a significant difference; hence, the influence of the stocking densities of the red tilapia and freshwater prawns may not be the factor that affected the observed trend. Instead, it may be attributed to water quality and the constant water recirculation in the system (Table 5).

Adding duckweed to the systems had notable advantages and disadvantages. Duckweed served as a filtering mechanism in the system since it relied on the waste contained in the water to sustain its growth, thus helping purify the water. It also served as a shade for the prawns and a supplemental diet for the fish.

Table 5. The growth and yield of duckweed under the integrated recirculating aquaponic system as affected by the different stocking densities

Treatments (stocking density)		Duckweed yield (g/day)
Fish/m^3	Prawn/m^2	
RT_{24}	P_0	4.18±0.79
	P_{12}	4.37±0.79
	P_{25}	2.25±0.79
RT_{48}	P_0	3.68±0.79
	P_{12}	4.25±0.79
	P_{25}	3.18±0.79

However, its production rate was affected by the constant movement of the water surface in the prawn tanks as duckweed grows best in a pH ranging from 6.5 to 7.5 and ideally requires slow-moving or still bodies of water (Popa et al., 2017). The suitable pH for duckweed's optimum production was not maintained since the system's pH was too high. Also, water in the tanks was constantly agitated due to constant aeration, further slowing down duckweed's growth. Duckweeds required intensive management and maintenance in the system as they often got clogged in the outlet, which sometimes restricted water recirculation. Hence, the addition of duckweed in the aquaponic system must be carefully analyzed, considering its growth requirement to maximize its yield. Still, its integration must be established without hampering the system's performance.

CONCLUSIONS

A properly assembled closed-loop aquaponic system could be employed to produce red tilapia, freshwater prawns, lettuce, and duckweed. The study established their growing conditions under aquaponic production. In designing the system, priority must be given to attaining the optimum growing environment of the species and the economics of its establishment. Also, the growing conditions in the aquaponic system must be maintained within the safe threshold to increase system efficiency and profitability.

The minimum recommended stocking density of fish and the recommended stocking density of prawns provided better growth and yield of the red tilapia and giant river prawns. However, the growth and yield of lettuce and duckweed were not affected by the different stocking densities.

Lettuce is a commonly grown crop in aquaponics. Therefore, other potential species that could be produced and integrated into the technology must be explored. The species' adaptability to the prevailing conditions in the growing area, their market potential, value-adding mechanism, and the timing and production duration should be considered.

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