Integrated aquaponic food production systems are capable of producing more food on less land using less water than conventional food systems, and marine systems offer the potential of conserving freshwater resources. However, there have been few evaluations of species combinations or operational parameters in marine aquaponics. The goal of this experiment was evaluation of stocking density ratio of Pacific whiteleg shrimp \((\text{Litopenaeus vannamei})\) to three edible halophytes \((\text{Atriplex hortensis, Salsola komarovii, and Plantago coronopus})\) with two C/N ratios in a \(3 \times 2\) factorial design. There were three stocking density ratios (shrimp: plant), 2:1, 3:1, and 5:1; and two C/N ratios, 12 and 15. The results indicated that stocking density ratio exerted a significant impact on shrimp growth. Shrimp reared in 2:1 and 3:1 treatments had better growth performance. In contrast, plants were affected by both stocking density ratio and C/N ratio. Halophytes grown in stocking density ratios of 3:1 and 5:1 with a C/N ratio of 15 had better growth performance and nutrient content. The concentrations of TAN and \(\text{NO}_2^-\) were below 0.2 mg/L throughout the experiment, including the higher stocking density ratio treatments. In conclusion, the stocking density ratio of 3:1 with a C/N ratio of 15 was suggested as the optimal condition for the operation of marine aquaponics in which whiteleg shrimp and the three halophytes are target crops.

**Keywords:** marine aquaponics, shrimp to plant ratio, C/N ratio, \(\text{Litopenaeus vannamei}\), halophytic plants, biofloc, sustainable food production, water pumpless system design

**INTRODUCTION**

Integrated aquaponic food production systems are capable of producing more food, on less land, using less water and a lower environmental impact than conventional food production systems (Somerville et al., 2014; Alshrouf, 2017; Goddek et al., 2019). Adopting a salt-, or brackish water aquaponics system significantly increases the potential animal species that could be raised, and many of the potential species have good name recognition and demand in the marketplace. Further, marine aquaponics reduces the reliance on freshwater resources. Water usage in a marine recirculating aquaculture system can be as low as 16 L per kg of seafood produced, while usage in freshwater recirculating aquaculture system is approximately 50 L per kg of production (Klinger and Naylor, 2012). Marine systems rely on saltwater for initial fill and replacement of evaporative losses, given the system is near a natural source of saltwater (Klinger and Naylor, 2012). Freshwater would only be needed in these situations to adjust salinity to desired levels. Additionally, marine and/or brackish water species tend to have lower FCR (on average) and grow faster than freshwater species (Fry et al., 2018). Of the potential animal species, the Pacific whiteleg shrimp...
(Litopenaeus vannamei) appears to have potential in the short-term as global production was second highest among aquaculture industries in 2018 (FAO, 2020) (4966.2 thousand metric tons) and they display rapid growth (FAO, 2006), high market price (Ross et al., 2017) and strong global demand (FAO, 2020). Whiteleg shrimp are tolerant of a wide range of salinities (Gao et al., 2016; Ray and Lotz, 2017; Pinheiro et al., 2020; Chu and Brown, 2021) and stocking densities (Otoshi et al., 2007; Krummenauer et al., 2011; Araneda et al., 2020) (90 to 600 shrimp/m²) making them strong candidates for marine aquaponics. Further, shrimp might alleviate the “economic drain” of fish raised in freshwater aquaponic systems (Quagrainie et al., 2018). However, the complex interaction between marine aquaponic subsystems and taxa has received little attention in the scientific literature.

Halophytes (salt-tolerant plants) only represent 2% of terrestrial plant species and most of them are not common commodities; however, they have been used for many purposes such as food and forage crops, oils seeds, phytoremediation and medicinal purposes (Glenn et al., 1998, 1999; Ventura and Sagi, 2013; Panta et al., 2014; Panth et al., 2016; Kim et al., 2017).

Red orache (Atriplex hortensis), okahijiki (Salsola komarovi), and minutina (Plantago coronopus) are edible halophytes that possess high nutrient concentrations (protein, amino acids, vitamins, and minerals) and have been successfully raised in marine aquaponics (Chu and Brown, 2021). However, the ratio of subsystem components, which will impact the flow of nutrients and health of subsystem taxa, has not been evaluated in marine aquaponic food production systems.

Several broad generalizations have been developed to help conceptualize the sizing and ratios of aquaponic subsystems. For example, current recommendations include 60 to 100 grams of fish feed/d/m² of plant growing area, a 1:2 ratio of fish tank volume to hydroponic media, a 7.3:1 ratio of plant-bed surface to fish-tank surface area, and a 3:1 ratio of hydroponic tank volume to fish production tank volume (Rakocy, 2012; Somerville et al., 2014; Lam et al., 2015). Generalized ratios fail to acknowledge the biological variability associated with potential species combinations. For example, feed consumption varies between species of fish and crustacean, dietary formulations contain varying concentrations of crude protein and amino acids, nutrient needs of plants vary as a function of growth stage (vegetative vs. fruiting), plant nutrient uptake is influenced by environmental conditions (pH, temperature and the microbiome in the rhizosphere), and the recommendations assume the space and nutritional needs of the system microbiome are adequate. The ratio of specific animals to plants might be a more realistic view of the physiological interactions and flow of nutrients that must occur in an integrated system. Consequently, the ratio of animals to plants must be understood for economical and sustainable operation.

Biofloc technology (BFT) has been used for decades to manage and control the water quality at safe ranges for target organisms in high density aquaculture (Avnimelech, 1999). Biofloc technology is an agglomeration of diverse microbes, which includes heterotrophic and autotrophic bacteria, algae, zooplankton, fungi, and viruses. The major function of BFT is to assimilate and mineralize toxic metabolites such as total ammonia nitrogen (TAN) and nitrite (NO₂⁻) in the water (Panigrahi et al., 2018; Luo et al., 2020). Biofloc technology requires the addition of organic carbon to manipulate the C/N ratio and accelerate the development of the microbial communities. A C/N ratio between 10 and 20 is recommended for aquaculture (Xu et al., 2016, 2018; Panigrahi et al., 2018), while the recommendation for aquaponics systems is lacking. Moreover, inoculating probiotics is a promising approach to ensure the dominant bacteria are beneficial organisms (Crab et al., 2012).

While it is common to amend the growing environment for better plant nutrient uptake and a higher yield, there is little information about the optimal C/N ratio in nutrient solutions for plant cultivation (Rodriguez-Kábana, 1986; Schenck, 2001; White, 2012; Pyakurel et al., 2019; Li et al., 2020).

The goal of this project was to evaluate critical ratios of crop density in aquaculture and hydroponics subsystems and provide operational guidelines for marine aquaponics. Specific objectives were to evaluate stocking densities and the C/N ratio on growth and production of whiteleg shrimp and three halophytes.

**MATERIALS AND METHODS**

**Aquaponic System Design**

Eighteen individual aquaponic systems were constructed at the Aquaculture Research Lab, Purdue University. The following are the components of each system; an aquaculture tank (113.6L), a hydroponic tank (102.2L), and a biofilter tank (18.9 L; Figure 1). To avoid shrimp escape and algal growth, plastic mesh, and lids were placed on the top of aquaculture tanks and hydroponic tanks, respectively. The lids on the top of hydroponic tanks were used to inhibit light into the tank, and also used as the floating rafts to support plants. Every biofilter tank was equipped with a 25-micron filter bag and bio-balls (surface area 98 ft²/ft³; Pentair Aquatic Eco-Systems, Inc., Apopka, FL, United States). Bio-balls in the biofilter tank were used to provide high surface area for attachment and colony expansion of microbes, and the filter bag was used to filter water from shrimp culture to prevent plant roots from clogging by biofloc. Air stones were installed in aquaculture tanks, hydroponic tanks, and biofilter tanks to maintain dissolved oxygen (DO) level above 6 mg/L. To maintain the water temperature within the optimal range 26–28°C for shrimp, submersible heaters (300w; Aqueon, Wisconsin, United States) were used in aquaculture tanks. Light source for plant growth was provided via LED light tubes (40w, 5000 lumens, 4000K daylight white; Kihung LED, Guangdong, China), that were suspended at a height of 16.5 cm over the plant growth bed. Quantum sensor (MQ-500 Full-Spectrum Quantum Meter; Apogee Instruments, Inc., UT, United States) was used to measure light intensity. The photosynthetically active radiation averaged 234 μmolm⁻²s⁻¹. The photoperiod was 14 h light and 10 h dark.

**Biological Material**

**Shrimp**

Pacific whiteleg shrimp were purchased from a commercial shrimp farm (RDM Aquaculture, Fowler, IN, United States), and transported to the Aquaculture Research Lab. Water temperature
during transport was 24°C and salinity was 15 ppt. Shrimp were separated into three 700 L tanks, and quarantined for 1 week before moving into aquaponic systems. During quarantine, shrimp were fed a commercial shrimp feed (Zeigler Brothers, Gardners, PA, United States) twice a day at 8 a.m. and 5 p.m., with a total daily amount of 3.0% of body weight divided into equal aliquots.

Plants
Seeds of red orache, okahijiki, and minutina, were purchased from a commercial source (Johnny’s Selected Seeds, Winslow, ME, United States) and sowed in horticubes, soilless foam medium (OASiS® Grower Solutions, Kent, OH, United States). Fresh water was used for plant irrigation in the first week of germination. To prevent osmotic shock on plants, salinity was increased at a rate of 2–3 ppt every 48 h from the second week until the desired salinity (15 ppt) was reached.

Experimental Design and System Management
A $3 \times 2$ experimental design was established in this study; 3 ratios of shrimp to plants (2:1, 3:1 or 5:1) and 2 C/N ratios (12 or 15). Treatments were designated 2:1ñ12 (stocking density ratio 2:1 with C/N ratio 12), 2:1ñ15, 3:1ñ12, 3:1ñ15, 5:1ñ12, and 5:1ñ15. Treatments were randomly assigned to three replicate experimental systems. The study was conducted for 4 weeks, from July 4 to August 1, 2020. Before the experiment started, all experimental systems were seeded with Bacillus spp. (EZ-Bio; Zeigler Brothers, Gardners, PA, United States), and inoculated water from established systems used in prior research. Sea salt (Instant Ocean®, Blacksburg, VA, United States) was used to adjust the salinity to 15 ppt. One week prior to the experiment, shrimp were weighed and placed in aquaculture tanks to produce nutrients for plants. The stocking density of shrimp was 200 shrimp/ m² (40 shrimp/tank), 300 shrimp/ m² (60 shrimp/tank), or 500 shrimp/ m² (100 shrimp/tank). The average weight of individual shrimp was 1.50 g. The stocking density of plants was 100 plants/ m², which was equivalent to 24 plants (8 plants per species) in each hydroponic tank. Commercial shrimp feed (Zeigler Brothers, Gardners, PA, United States) was provided twice a day at 8 a.m. and 5 p.m., with a total daily amount of 3.0% of body weight divided into equal aliquots. Guaranteed analysis of the feed was 35% protein, 7% fat, and a maximum of 2% fiber.

Probiotics (EZ-Bio; Zeigler Brothers, Gardners, PA, United States) were used to manage water quality and the microbial community within every system. EZ-bio (Bacillus spp.) was inoculated at 10 mg/ L into every system once a week prior to starting the experiment. As soon as shrimp were moved into aquaculture tanks, additional doses of probiotics were added every other day in the first week, twice per week in the second week, and once per week beginning in the third week continuing until the end of the experiment (Crab et al., 2010; Chu, 2014). Molasses (Hawthorne Gardening Co., Vancouver, WA, United States) was added to aquaculture tanks as an organic carbon source to adjust the C/N ratio in the water with the same frequency of the probiotic inoculations. The amount of molasses added was based on the carbon-nitrogen content of shrimp feed and the carbon content of the molasses to adjust the C/N ratio to 12 or 15 (Xu et al., 2016). Potassium bicarbonate was added to maintain the alkalinity above 60 mg/L, and 10% sulfuric acid was applied to keep the pH below 8. Throughout the 4-week experiment, there was no water discharged or exchanged except for replacement due to evaporation.

Measurement of Water Quality
During the experiment, dissolved oxygen, temperature (OxyGuard Handy Polaris DO meter, Farum, Denmark), and pH (pHTestr™ 10 Pocket pH Tester, Vernon Hills, IL, United States) were measured twice per day at 8 a.m. and 5 p.m. before feeding. Salinity (Vital Sine™ Salinity Refractometer,
Pentair Aquatic Ecosystems, Apopka, FL, United States) was measured once per day at 8 a.m. Water samples were collected twice per week from the aquaculture tank before feeding, to determine the concentrations of total ammonia nitrogen (TAN), nitrite-N (NO$_2^-$), nitrate-N (NO$_3^-$), phosphate (PO$_4^{3-}$), and alkalinity using HACH reaction kits (HACH, Loveland, CO, United States). Total suspended solids (TSSs) and volatile suspended solids (VSSs) were measured once a week by United States EPA method 1684.

**Growth Performance**

**Shrimp**

Response parameters for shrimp included survival, weight gain, specific growth rate (SGR), and feed conversion ratio (FCR). The following formulae were used:

\[
\text{Survival} \% = \frac{\text{Final number of shrimp}}{\text{Initial number of shrimp}} \times 100;
\]

\[
\text{Weight gain} \% = \frac{(\text{Final biomass (g)} - \text{Initial biomass (g)})}{\text{Initial biomass (g)}} \times 100;
\]

\[
\text{Specific growth rate}\% = \frac{\ln (\text{Final biomass (g)}) - \ln (\text{Initial biomass (g)})}{\text{day}} \times 100; \text{and,}
\]

\[
\text{Feed conversion ratio} = \frac{\text{Total feed intake (g)}}{(\text{Final biomass (g)} - \text{Initial biomass (g)})}
\]

**Plants**

Only edible parts of individual plants were collected and weighed at the beginning and end of the experiment. Initial plant samples were obtained from the extra plant seedlings and chosen those that were similar to the seedlings transplanted into systems. Initial plant samples were weighed at the same day of the transplantation. Initial and final fresh weights were used to calculate relative growth rate (RGR). Dry weight was measured after plant samples were dried in an oven at 100 °C until constant weight. The water content (WC) in plants was determined through final fresh weights and final dry weights. Formulæ used to calculate plant growth and water content are shown below. In addition, dried plant samples were ground and sieved (with a 10-mesh screen) and kept in 50 ml centrifuge tubes for nutrient analysis. Plant tissue analysis was conducted by the Midwest Laboratory (Omaha, NE, United States).

\[
\text{Relative growth rate}\% = \frac{\ln (\text{Final biomass (g)}) - \ln (\text{Initial biomass (g)})}{\text{day}} \times 100; \text{and,}
\]

\[
\text{Water content}\% = \frac{(\text{Final fresh weight (g)} - \text{Final dry weight (g)})}{\text{Final fresh weight (g)}} \times 100
\]

**Statistical Analysis**

Shrimp and plant growth performance, nutrient content in plants, and water quality parameters were analyzed using JMP v14.0 (SAS Institute Inc., Cary, NC, United States). Treatment means were compared by two-way analysis of variance (ANOVA). Statistical differences between means was determined by Tukey’s honestly significant difference test (HSD) at $p \leq 0.05$.

**RESULTS**

**Shrimp Growth**

The survival of shrimp in all treatments was above 95% and there were no significant ($p > 0.05$) differences among treatments. The growth of shrimp was significantly ($p < 0.05$) impacted by stocking density, but not by the C/N ratio or the interaction of factors (Table 1). Shrimp raised in 2:1–12 treatment had better growth performances (final weight, weight gain, and SGR) and a lower FCR. The mean values of final weight, weight gain, and SGR were significantly greater ($p < 0.05$) in the treatment 2:1–12, averaging 2.68g, 73.9%, and 1.97, respectively, compared to 5:1–12 and 5:1–15 treatments, yet, values were not significantly ($p > 0.05$) different from that of the 2:1–15, 3:1–12, and 3:1–15 treatments. Similarly, FCR was significantly lower ($p < 0.05$) in shrimp raised in 2:1–12 treatment compared to shrimp raised in 5:1–12 and 5:1–15 treatments, whereas it was not significantly ($p > 0.05$) different from the other treatments.

**Plants**

Survival of red orache, okahijiki, and minutina was 100% in all treatments. Stocking density ratio significantly ($p < 0.05$) affected the growth of all plants, and C/N ratio significantly ($p < 0.05$) affected water content (WC) of red orache (Table 2), the final fresh weight (FFW), final dry weight (FDW), RGR and WC of okahijiki (Table 3), and the FFW and RGR of minutina (Table 4). The interaction of the two factors exerted no significant ($p > 0.05$) effect on growth of plants.

**Yield and Mineral Nutrient Content**

According to the result of two-way ANOVA (Table 5), the yield, and the concentration of N, P, K, Mg, Ca, and S were significantly ($p < 0.05$) affected by plant species and stocking density ratio. The C/N ratio significantly ($p < 0.05$) affected the yield and the concentration of Mg. The interactions between plant species and stocking density ratio significantly ($p < 0.05$) affected the results of yield and the concentration of N, P, K, Mg, and Ca.

The 5:1–15 treatment had better plant production among treatments (Table 5). Red orache and okahijiki had significantly higher ($p < 0.05$) yield in 5:1–15 treatment. The yield was about twice that of red orache, while around 1.5 to 2 times higher than 2:1–12 and 3:1–12 treatments in okahijiki. Minutina had a better yield among the three species. Its production was also higher in 5:1–15 treatment, whereas, the production in the 5:1–15 treatment was only higher than that of 2:1–12 treatment.

In general, the concentrations of N, P, K in plants were increased with the increasing stocking density and C/N ratio (Table 5). In contrast, the concentration of Mg and Ca displayed an opposite trend. The treatment with lower stocking density ratio and C/N ratio had a higher concentration of Mg and Ca, while the concentrations of S and Na in plants were not significantly ($p > 0.05$) different among treatments.

**Water Quality**

During the experiment, temperature and dissolved oxygen (DO) were maintained at 28–29°C and 6.1–7.3 mg/L in all treatments, respectively. The salinity was monitored and controlled every day.
TABLE 1 | Response of shrimp in marine aquaponics at three stocking density ratios (SD ratio) of shrimp to plant and two C/N ratios for 4 weeks.

| Treatment | Initial weight (g) | Final weight (g) | Weight gain (%) | SGR | FCR | Survival (%) |
|-----------|--------------------|------------------|-----------------|-----|-----|---------------|
| SD ratio  | C/N ratio          |                  |                 |     |     |               |
| 2:1       | C/N ratio          |                  |                 |     |     |               |
| 12        | 2.68 ± 0.25 a      | 73.9 ± 10.9 a    | 1.97 ± 0.22 a   | 1.50 ± 0.22 c | 100 ± 0.0 |
| 15        | 2.59 ± 0.27 ab     | 71.3 ± 12.0 ab   | 1.92 ± 0.25 ab  | 1.55 ± 0.24 bc | 99.2 ± 1.4 |
| 3:1       | C/N ratio          |                  |                 |     |     |               |
| 12        | 2.38 ± 0.22 b      | 58.0 ± 5.2 abc   | 1.63 ± 0.12 abc | 1.89 ± 0.17 abc | 99.4 ± 1.0 |
| 15        | 2.56 ± 0.14 ab     | 64.8 ± 1.6 abc   | 1.78 ± 0.03 abc | 1.67 ± 0.03 bc | 97.2 ± 2.5 |
| 5:1       | C/N ratio          |                  |                 |     |     |               |
| 12        | 2.37 ± 0.17 b      | 50.2 ± 3.8 c     | 1.45 ± 0.09 c   | 2.14 ± 0.15 a  | 96.7 ± 2.3  |
| 15        | 2.39 ± 0.15 b      | 53.2 ± 3.2 bc    | 1.52 ± 0.07 bc  | 2.01 ± 0.12 ab | 96.3 ± 1.5  |

P values are means ± SD (n = 40, 60, and 100 for stocking density 200, 300, and 500, respectively). Means within column followed by different letters are significantly different based on Tukey’s honestly significant difference (HSD) test (α = 0.05). ns, *** mean no significant or significant at p ≤ 0.01, or 0.001, respectively.

TABLE 2 | Initial fresh weight (IFW), initial dry weight (IDW), final fresh weight (FFW), final dry weight (FDW), relative growth rate (RGR), and water content (WC) of red orache cultivated in marine aquaponics at three stocking density ratios (SD ratio) of shrimp to plant and two C/N ratios for 4 weeks.

| Treatment | IFW(g/plant) | IDW(g/plant) | FFW(g/plant) | FDW(g/plant) | RGR(%) | WC(%) |
|-----------|--------------|--------------|--------------|--------------|--------|-------|
| SD ratio  | C/N ratio    |              |              |              |        |       |
| 2:1       | C/N ratio    |              |              |              |        |       |
| 12        | 0.19 ± 0.01  | 0.016 ± 0.002| 5.3 ± 1.2 b  | 0.65 ± 0.12 b| 11.8 ± 0.8 b | 87.9 ± 0.9 c|
| 15        | 0.19 ± 0.01  | 0.016 ± 0.002| 6.0 ± 1.5 b  | 0.69 ± 0.18 b| 12.2 ± 0.9 b | 88.3 ± 0.7 bc|
| 3:1       | C/N ratio    |              |              |              |        |       |
| 12        | 0.19 ± 0.01  | 0.016 ± 0.002| 6.0 ± 0.9 b  | 0.69 ± 0.11 b| 12.3 ± 0.5 b | 88.5 ± 0.5 b|
| 15        | 0.19 ± 0.01  | 0.016 ± 0.002| 6.0 ± 1.6 b  | 0.67 ± 0.18 b| 12.2 ± 1.0 b | 88.6 ± 0.6 b|
| 5:1       | C/N ratio    |              |              |              |        |       |
| 12        | 0.19 ± 0.01  | 0.016 ± 0.002| 10.6 ± 2.5 a | 1.06 ± 0.23 a| 14.2 ± 0.9 a | 89.9 ± 0.5 a|
| 15        | 0.19 ± 0.01  | 0.016 ± 0.002| 10.9 ± 1.2 a | 1.06 ± 0.13 a| 14.4 ± 0.4 a | 90.3 ± 0.4 a|

P values are means ± SD (n = 24). Means within column followed by different letters are significantly different based on Tukey’s honestly significant difference (HSD) test (α = 0.05). ns, *** mean no significant or significant at p ≤ 0.01, or 0.001, respectively.

to maintain at the desired level, 15 ppt. The alkalinity in all treatments was not significantly different (p > 0.05) among each other. There were no significant differences (p > 0.05) found in the concentrations of total suspended solids (TSS) or volatile suspended solids (VSS) (Table 6). The pH was affected by the stocking density ratio, the lower stocking density ratio tended to have a higher pH than the higher stocking density ratio. Starting on day 9, the value was significantly higher (p < 0.05) in the stocking ratio of 2:1–12 and 2:1–15 treatment than that of 5:1–12 and 5:1–15 (Figure 2).

The concentrations of TAN, and NO₂−, which are toxic to aquatic animals and plants, remained at safe and low concentrations (both were below 0.2 mg/L) throughout the experiment in all treatments, although the treatments with higher stocking density ratio, 5:1–12 and 5:1–15, had significantly higher (p < 0.05) concentrations than that of lower stocking density ratio, 2:1–12 and 2:1–15 (Figures 3A,B). On the other hand, concentrations of NO₃− and PO₄³− continued increasing throughout the experiment. In general, 5:1–12 and 5:1–15 treatments had significantly higher (p < 0.05) concentrations of NO₃− and PO₄³− than 2:1–12 and 2:1–15 treatments (Figures 3C,D).

DISCUSSION

Shrimp Growth

The results of this study indicate that an increase in stocking density ratio was a negative factor on shrimp growth. In addition, the FCR increased with the increasing stocking density, which increases the cost of production. Similar results were observed in other studies that investigated the effect of stocking density on shrimp production (Mos and Moss, 2004; Ejarza-Leal et al., 2010; Neal et al., 2010; Krummenauer et al., 2011; Sookying et al., 2011; Façanha et al., 2016; Araneda et al., 2020; Fleckenstein et al., 2020). However, survival of shrimp in our experiment was above 95%, while other researchers reported that survival decreased with increasing density (Neal et al., 2010; Krummenauer et al., 2011; Araneda et al., 2020).
The reduced survival and growth of shrimp at higher stocking densities can be related to several factors including the availability of space for growth, competition for feed, cannibalism, and increased waste excretion leading to degraded water quality (Arnold et al., 2006; Wang and Gu, 2010; Ferreira et al., 2020; Luo et al., 2020). The high survival at high stocking density in this study might be attributed to the application of probiotics and molasses, because probiotics improve the resistance to adverse environments and the ability to tolerate stress (Martínez Cruz et al., 2012; Buruiană et al., 2014; Nemutanzhela et al., 2014; Olmos et al., 2020). Moreover, the application of molasses improved the ability of plants to uptake nutrients from the water column (White, 2012). The combined effect from the application of probiotics and molasses maintained the water quality and led to higher survival in the present study, albeit, with reduced growth. It is unclear if the increased density and potential increases in marketable shrimp would offset the slower growth and increased feed costs associated with super-high-density culture, but this possibility needs to be explored.

**Plants**

In general, the growth performance of the three halophytes was better with the increasing stocking ratio and C/N ratio. The nutrient balance is one of the key factors to the success of aquaponics; too few animals (inadequate nitrogen) is limiting to plants, and excessive animal density will result in nitrogen excretion in excess of the plants’ ability to uptake

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**TABLE 3** | Initial fresh weight (IFW), initial dry weight (IDW), final fresh weight (FFW), final dry weight (FDW), relative growth rate (RGR), and water content (WC) of okahijiki cultivated in marine aquaponics at three stocking density ratios (SD ratio) of shrimp to plant and two C/N ratios for 4 weeks.

| Treatment | IFW (g/plant) | IDW (g/plant) | FFW (g/plant) | FDW (g/plant) | RGR (%) | WC (%) |
|-----------|---------------|---------------|---------------|---------------|---------|--------|
| SD ratio  |               |               |               |               |         |        |
| C/N ratio |               |               |               |               |         |        |
| 2:1       | 0.18 ± 0.01   | 0.015 ± 0.002 | 1.6 ± 0.5 c   | 0.17 ± 0.05 c | 7.7 ± 1.1 c | 89.5 ± 1.4 c |
| 15        | 0.18 ± 0.01   | 0.015 ± 0.002 | 2.0 ± 0.8 bc  | 0.21 ± 0.07 bc| 8.4 ± 1.5 bc| 89.9 ± 1.6 bc |
| 3:1       | 0.18 ± 0.01   | 0.015 ± 0.002 | 2.0 ± 0.5 c   | 0.21 ± 0.05 bc| 8.4 ± 0.9 bc| 89.3 ± 1.1 c |
| 15        | 0.18 ± 0.01   | 0.015 ± 0.002 | 3.0 ± 1.4 ab  | 0.28 ± 0.12 ab| 9.7 ± 1.7 ab| 90.8 ± 0.8 ab |
| 5:1       | 0.18 ± 0.01   | 0.015 ± 0.002 | 2.1 ± 1.1 bc  | 0.21 ± 0.09 bc| 8.4 ± 2.0 bc| 89.7 ± 1.9 bc |
| 15        | 0.18 ± 0.01   | 0.015 ± 0.002 | 3.8 ± 1.9 a   | 0.31 ± 0.14 a | 10.2 ± 2.1 a| 91.0 ± 1.0 a  |

P: ns, **, *** mean no significant or significant at p ≤ 0.05, 0.01, or 0.001, respectively.

ANOVA

SD ratio: ns, **, *** mean no significant or significant at p ≤ 0.05, 0.01, or 0.001, respectively.

C/N ratio: ns, **, *** mean no significant or significant at p ≤ 0.05, 0.01, or 0.001, respectively.

SD ratio*C/N ratio: ns, **, *** mean no significant or significant at p ≤ 0.05, 0.01, or 0.001, respectively.

Values are means ± SD (n = 24). Means within column followed by different letters are significantly different based on Tukey’s honestly significant difference (HSD) test (α = 0.05).

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**TABLE 4** | Initial fresh weight (IFW), initial dry weight (IDW), final fresh weight (FFW), final dry weight (FDW), relative growth rate (RGR), and water content (WC) of minutina cultivated in marine aquaponics at three stocking density ratios (SD ratio) of shrimp to plant and two C/N ratios for 4 weeks.

| Treatment | IFW (g/plant) | IDW (g/plant) | FFW (g/plant) | FDW (g/plant) | RGR (%) | WC (%) |
|-----------|---------------|---------------|---------------|---------------|---------|--------|
| SD ratio  |               |               |               |               |         |        |
| C/N ratio |               |               |               |               |         |        |
| 2:1       | 0.08 ± 0.01   | 0.006 ± 0.001 | 18.3 ± 4.5 b  | 1.38 ± 0.40 | 19.3 ± 0.9 b| 92.5 ± 0.7 b |
| 15        | 0.08 ± 0.01   | 0.006 ± 0.001 | 21.3 ± 5.2 ab | 1.62 ± 0.40 | 19.8 ± 0.9 ab| 92.5 ± 0.4 b |
| 3:1       | 0.08 ± 0.01   | 0.006 ± 0.001 | 18.9 ± 6.1 b  | 1.43 ± 0.49 | 19.3 ± 1.2 b| 92.4 ± 0.3 b |
| 15        | 0.08 ± 0.01   | 0.006 ± 0.001 | 20.6 ± 5.2 ab | 1.52 ± 0.37 | 19.7 ± 0.9 ab| 92.6 ± 0.4 ab |
| 5:1       | 0.08 ± 0.01   | 0.006 ± 0.001 | 21.2 ± 4.6 ab | 1.48 ± 0.31 | 19.8 ± 0.9 ab| 93.0 ± 0.5 a |
| 15        | 0.08 ± 0.01   | 0.006 ± 0.001 | 23.7 ± 4.4 a  | 1.64 ± 0.34 | 20.2 ± 0.6 a| 93.0 ± 0.4 a |

P: ns, **, *** mean no significant or significant at p ≤ 0.05, 0.01, or 0.001, respectively.

ANOVA

SD ratio: ns, **, *** mean no significant or significant at p ≤ 0.05, 0.01, or 0.001, respectively.

C/N ratio: ns, **, *** mean no significant or significant at p ≤ 0.05, 0.01, or 0.001, respectively.

SD ratio*C/N ratio: ns, **, *** mean no significant or significant at p ≤ 0.05, 0.01, or 0.001, respectively.

Values are means ± SD (n = 24). Means within column followed by different letters are significantly different based on Tukey’s honestly significant difference (HSD) test (α = 0.05).
TABLE 5 | Average yield and mineral nutrient concentrations of the three halophytic plants cultivated in marine aquaponics at three stocking density ratios (SD ratio) of shrimp to plant and two C/N ratios for 4 weeks.

| Plant species | Treatment | Yield (kg/m²) | N (%) | P (%) | K (%) | Mg (%) | Ca (%) | S (%) | Na (%) |
|---------------|-----------|---------------|-------|-------|-------|--------|--------|-------|-------|
|               | SD ratio   | C/N ratio     |       |       |       |        |        |       |       |
| Red orache    | 2:1        | 12            | 0.55 c| 3.15 b| 0.36 ab| 1.50 a | 2.64 a | 0.79 a| 0.27  |
|               | 2:1        | 15            | 0.61 bc| 3.12 b| 0.34 b | 1.46 a | 2.39 a | 0.70 ab| 0.27  |
|               | 3:1        | 12            | 0.60 bc| 3.14 b| 0.35 b | 1.47 a | 2.42 a | 0.73 a | 0.24  |
|               | 3:1        | 15            | 0.64 bc| 3.20 b| 0.36 ab| 1.44 a | 2.41 a | 0.71 a | 0.25  |
|               | 5:1        | 12            | 0.98 ab| 3.80 a| 0.43 ab| 1.71 a | 2.32 ab| 0.70 a | 0.27  |
|               | 5:1        | 15            | 1.20 a | 4.02 a| 0.46 a | 1.64 a | 2.04 b | 0.66 b | 0.26  |
| Okahijiki     | 2:1        | 12            | 0.18 c | 3.79 c| 0.99 ab| 3.66 b | 1.79 a | 0.99 ab| 0.31  |
|               | 2:1        | 15            | 0.28 abc| 3.78 c| 0.98 b | 4.28 ab| 1.60 a | 1.04 a | 0.30  |
|               | 3:1        | 12            | 0.22 bc| 3.69 c| 1.01 ab| 3.75 b | 1.90 a | 0.88 ab| 0.30  |
|               | 3:1        | 15            | 0.33 ab| 3.93 bc| 1.00 ab| 4.14 b | 1.68 a | 0.81 b | 0.31  |
|               | 5:1        | 12            | 0.26 abc| 4.32 ab| 1.18 a | 4.99 ab| 1.55 a | 0.94 ab| 0.34  |
|               | 5:1        | 15            | 0.36 a | 4.34 a| 1.17 a | 5.45 a | 1.58 a | 0.89 ab| 0.35  |
| Minutina      | 2:1        | 12            | 1.99 b | 3.89 | 0.64 b | 1.80 a | 1.28 a | 1.29  | 0.54  |
|               | 2:1        | 15            | 2.27 ab| 3.69 | 0.79 ab| 1.83 a | 1.27 a | 1.25  | 0.58  |
|               | 3:1        | 12            | 2.06 ab| 3.65 | 0.79 ab| 2.02 a | 1.24 a | 1.28  | 0.59  |
|               | 3:1        | 15            | 2.17 ab| 3.87 | 0.91 ab| 1.85 a | 1.18 a | 1.27  | 0.55  |
|               | 5:1        | 12            | 2.26 ab| 3.78 | 1.12 a | 1.88 a | 1.25 a | 1.31  | 0.66  |
|               | 5:1        | 15            | 2.52 a | 3.67 | 1.11 a | 1.90 a | 1.14 b | 1.28  | 0.58  |
| P             | **         | ***           | ns    | **    | ns    | ns     | ns     | ns    | ns    |
| ANOVA         | Plant Species | ***           | ***    | ***    | ***    | ***    | ***    | ***    | ***    |
|               | SD ratio    | ***           | ns    | ns    | ns    | *      | ns     | ns    | ns    |
|               | C/N ratio   | ***           | ns    | ns    | ns    | *      | ns     | ns    | ns    |
|               | Plant Species*SD ratio | ns | ns | ns | ns | ns | ns | ns | ns |
|               | Plant Species*C/N ratio | ns | ns | ns | ns | ns | ns | ns | ns |
|               | SD ratio* C/N ratio | ns | ns | ns | ns | ns | ns | ns | ns |
|               | Plant Species’ SD ratio* C/N ratio | ns | ns | ns | ns | ns | ns | ns | ns |

Means within a column of each plant species followed by different letters are significantly different based on Tukey’s honestly significant difference (HSD) test (α = 0.05). ns, *, **, *** mean no significant or significant at p ≤ 0.05, 0.01, or 0.001, respectively.

TABLE 6 | Mean water quality values (range) for marine aquaponics at three stocking density ratios (SD ratio) of shrimp to plant and two C/N ratios for 4 weeks.

| Treatment | Temperature (°C) | DO (mg/ L) | Alkalinity (mg/ L) | TSS (mg/ L) | VSS (mg/ L) |
|-----------|-----------------|------------|-------------------|-------------|-------------|
|           | SD ratio        | C/N ratio  |       |       |        |        |        |        |        |
|           | 2:1             | 12         | 28.5 ± 0.3 (28.0–28.9) | 6.8 ± 0.2 (6.2–7.3) | 71.1 ± 11.5 (60–100) | 40.9 ± 5.9 (32.0–51.0) | 26.1 ± 6.9 (12.5–37.0) |
|           | 2:1             | 15         | 28.6 ± 0.29 (28.1–28.9) | 6.8 ± 0.2 (6.2–7.3) | 74.8 ± 11.9 (60–100) | 41.2 ± 8.4 (29.0–56.0) | 25.6 ± 6.6 (16.5–35.0) |
|           | 3:1             | 12         | 28.5 ± 0.3 (28.0–28.9) | 6.8 ± 0.2 (6.2–7.3) | 72.6 ± 12.6 (60–100) | 40.8 ± 7.5 (28.5–52.0) | 24.9 ± 6.2 (14.0–33.5) |
|           | 3:1             | 15         | 28.5 ± 0.3 (28.0–28.9) | 6.8 ± 0.3 (6.1–7.3) | 71.1 ± 11.5 (60–100) | 41.4 ± 6.3 (29.5–51.5) | 27.0 ± 5.6 (16.5–34.0) |
|           | 5:1             | 12         | 28.7 ± 0.2 (28.1–29.0) | 6.8 ± 0.2 (6.2–7.3) | 66.7 ± 9.6 (60–80) | 43.9 ± 5.9 (34.5–55.5) | 28.8 ± 6.2 (18.5–42.0) |
|           | 5:1             | 15         | 28.5 ± 0.2 (28.0–28.8) | 6.7 ± 0.2 (6.1–7.2) | 79.3 ± 10.4 (60–100) | 40.3 ± 5.8 (33.5–52.0) | 25.6 ± 5.7 (17.0–33.0) |

Values are means ± SD.

In our study, plants were benefiting from a higher shrimp stocking density, in which more nutrients are provided for plants. All plants had greater yield in treatment 5:1 (Table 5). A similar trend was also reported by Shete et al. (2015); the plant production was higher in fish to plant ratio of 1:1, followed by 1:2 and then 1:3. Furthermore, the concentration of macronutrients (N, P, and K) in plant tissues were also higher in both 5:1 treatments than the other stocking density ratio treatments. The higher concentrations of TAN, NO$_3^-$, and PO$_4^{3-}$ found in the 5:1 treatment was likely the result of the higher feed inputs into those treatments. The steady increase in NO$_3^-$ and PO$_4^{3-}$...
in all treatments may indicate the saturation of plant's assimilation limits. Further research is needed to evaluate a longer culture duration or a different strategy on plant harvest (sequential stocking) since the concentration of all N- and P-compounds in the water will likely increase after every harvest (Yang and Kim, 2020b), which might be a concern for shrimp culture.

While not as pronounced as the effect of shrimp stocking density, the C/N ratio also exerted an impact on plant production characteristics. Additional carbon for amending the growing environment improves nutrient uptake by plants, increases crop yield, and alleviates phytotoxicity, caused by trace metals, salinity, pesticides, phytotoxins, or allelochemicals (Rodriguez-Kabana, 1986; Schenck, 2001; White, 2012; Pyakurel et al., 2019; Li et al., 2020). Overall, the effect of C/N ratio on plant growth, yield, and mineral nutrient concentrations in plants was relatively minor compared to stocking density.

**Water Quality**

To manage the water quality well, a robust microbial community is indispensable in aquaponics. Promoting the establishment of microbial flora by inoculation of probiotics or inoculations from mature water, used-biome, or biofloc from stabilized systems to new systems are efficient practices (Otoshi et al., 2011; Xu and Pan, 2012; Pinheiro et al., 2020; Chu and Brown, 2021). Xu and Pan (2012) inoculated bioflocs, characterized by *Bacillus* sp. as the predominant bacteria, into experimental tanks before their study. The concentrations of toxic nitrogenous waste, TAN and NO$_2^-$, in their study were maintained below 0.51 mg/ L and 1.25 mg/ L, respectively. In the present study, the concentration of TAN and NO$_2^-$ remained lower than 0.2 mg/ L throughout the experiment, even in the high stocking density treatments. Additional research needs to be conducted examining the frequency of probiotic application and varying harvest scenarios, as after every plant harvest, the TAN and NO$_2^-$ will likely increase (Yang and Kim, 2020b).

Concentrations of TAN and NO$_2^-$ were higher in the higher C/N treatments while the NO$_3^-$ concentrations were lower. Similar results were also reported in other studies (Xu et al., 2016, 2018). The additional organic carbon facilitates growth of heterotrophic bacteria, which compete for nutrients and space inside the biofilm or biome, with the nitrifying bacteria (NB), which are composed of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria.
FIGURE 3 | Concentrations of TAN (A), nitrite (B), nitrate (C), and phosphate (D) measured during the 4-week experiment. Each point represents the means of 3 replicates ± standard error. Lower-case alphabet letters represent significant differences, followed by one-way ANOVA and Tukey's HSD test ($\alpha = 0.05$).

(NO3). The rate of reproduction of heterotrophic bacteria is much faster than NB, while the reproduction rate of AOB is faster than NOB (Hu et al., 2015). Due to the competition with heterotrophic bacteria, and the slower growth rate of AOB and NOB, the efficiency of nitrification decreases with the increasing C/N ratio, resulting in a decrease in TAN removal rate and NO3− productivity (Zhu and Chen, 2001; Michaud et al., 2006; Luo et al., 2020). However, in our study, all nitrogenous waste products remained below concentrations considered toxic. The concentrations of PO4 3− continued to accumulate throughout the experiment, similar to previous results (Boxman et al., 2018; Yang and Kim, 2019, 2020a,b; Chu and Brown, 2021; Huang et al., 2021), and was higher in the treatments with the higher stocking densities and C/N ratio, likely due to higher feed inputs. Another explanation could be the higher C/N ratios resulted in bioflocs dominated by heterotrophic bacteria and less algae (Xu et al., 2016). Moreover, the accumulation of PO4 3− suggests the saturation of plant’s assimilation ability. PO4 3−, one of the compounds that causes eutrophication, is an issue for aquaponics; hence, more research is required to improve the management of PO4 3− in aquaponics and determine how to improve plant’s ability to assimilate PO4 3−.

In aquaponics, pH is another vital parameter that can be affected by nitrification, nutrient assimilation by plants and heterotrophic bacteria, and CO2 excretion by aquatic animals, as well as other factors (Yang and Kim, 2019; Li et al., 2020). The process of nitrification and nitrogenous waste assimilation by bacteria, and CO2 released through the respiration of aquatic animals and microorganisms tends to decrease the pH. Conversely, CO2 removal and nutrient assimilation by plant tends to raise the pH (Ebeling et al., 2006; White, 2012; Somerville et al., 2014). This may explain why the pH level was lowest at the stocking density of 3:1, followed by 2:1, then 2:1. Also, the lower pH can be another possible reason for a better plant growth in higher stocking density treatments, because nutrient availability increases with the decreasing pH (Somerville et al., 2014).

CONCLUSION

The stocking density ratio and C/N ratio exerted significant impacts on the performance of shrimp and plants in marine aquaponics. Shrimp performed better in the stocking density of 2:1 and 3:1, with no impact from the C/N ratio. Conversely, plants performed better in the stocking density of 3:1 and 5:1 with the C/N ratio at 15. Therefore, a stocking density ratio of 3:1 with a C/N ratio at 15 is suggested as the optimal condition for shrimp and the three halophytes in an indoor marine aquaponic food production system. Inoculating the water with biofloc and applying probiotics regularly can enhance the management of water quality and the health of shrimp and plants in aquaponics. Although water quality was maintained at safe levels for shrimp and halophytes during the experiment, more studies with a longer period of cultivation are needed for a better understanding of marine aquaponics using these species.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Y-TC: conceptualization, methodology, investigation, validation, formal analysis, data curation, and writing – original draft
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references

Alshrouf, A. (2017). Hydroponics, aeroponic and aquaponic as compared with conventional farming. Am. Sci. Res. J. Eng. Technol. Sci. 27, 247–255.

Araneda, M., Gasca-Leyva, E., Vela, M. A., and Dominguez-May, R. (2020). Effects of temperature and stocking density on intensive culture of Pacific white shrimp in freshwater. J. Therm. Biol. 94:102756. doi: 10.1016/j.jtherbio.2020.102756

Arnold, S. J., Sellers, M. J., Crocos, P. J., and Coman, G. J. (2006). Intensive Production of juvenile tiger shrimp *Peneaus monodon*: an evaluation of stocking density and artificial substrates. *Aquaculture* 261, 890–896. doi: 10.1016/j.aquaculture.2006.07.036

Avnimelech, Y. (1999). Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture* 176, 227–235. doi: 10.1016/S0044-8486(99)00085-X

Boxman, S. E., Nystrom, M., Ergas, S. J., Main, K. L., and Trotz, M. A. (2018). Evaluation of water treatment capacity, nutrient cycling, and biomass production in a marine aquaponic system. *Ecol. Eng.* 120, 299–310. doi: 10.1016/j.ecoleng.2018.06.003

Browdy, C. L., Ray, A. J., Leffler, J. W., and Avnimelech, Y. (2012). “Biofloc-based aquaculture systems,” in *Aquaculture Production Systems*, ed. J. Tidwell (Oxford: John Wiley & Sons, Inc). 278–307. doi: 10.1007/9781118250105.ch12

Buruiană, C. T., Profră, A. G., and Vizirianu, C. (2014). Effects of probiotic Bacillus species in aquaculture – an overview. *Ann. Univ. Dunarea Jos Galati Fascicule VI Food Technol.* 38, 9–17. doi: 10.16598/rsf.2021.07.007

Chu, Y.-T. (2014). Effects of Different Probiotics on Water Qualities and Growth of Hybrid Culture System of *Litopenaeus vannamei*. Master’s Thesis. Keelung: Brusseis: Ghen University.

Chu, Y.-T., and Brown, P. B. (2021). Evaluation of Pacific whiteleg shrimp and three halophytic plants in marine aquaponic systems under three salinities. *Sustainability* 13:269. doi: 10.3390/su13010269

Crab, R. (2010). *Bioflocs Technology: An Integrated System for the Removal of Nutrients and Simultaneous Production of Feed in Aquaculture*. Ph.D. Thesis. Brusseis: Ghen University.

Crab, R., Chielens, B., Wille, M., Bossier, P., and Verstraete, W. (2010). The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. *Aquac. Res.* 41, 599–567. doi: 10.1111/j.1365-2109.2009.02353.x

Crab, R., Deforêt, T., Bossier, P., and Verstraete, W. (2012). Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture* 355, 351–356. doi: 10.1016/j.aquaculture.2012.04.046

Ebeling, J. M., Timmons, M. B., and Bisogni, J. I. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture* 257, 346–358. doi: 10.1016/j.aquaculture.2006.03.019

Esparza-Leal, H. M., Ponce-Palafax, J. T., Aragón-Noriega, E. A., Arredondo-Figueroa, J. L., García-Ulloa Gómez, M., and Valenzuela-Quiñonez, W. (2010). Growth and performance of the whiteleg shrimp *Peneaus vannamei* (boone) cultured in low-salinity water with different stocking densities and acclimation times. *Sustain. Res.* 41, 878–883. doi: 10.1111/j.1365-2109.2009.02367.x

Facanha, F. N., Oliveira-Neto, A. R., Figueriedo-Silva, C., and Nunes, A. J. P. (2016). Effect of shrimp stocking density and graded levels of dietary methionine over the growth performance of *Litopenaeus vannamei* reared in a green-water system. *Aquaculture* 463, 16–21. doi: 10.1016/j.aquaculture.2016.05.024

FAO (2006). *Cultured Aquatic Species Information Programme Peneaus vannamei*. Rome: FAO.
Schenck, S. (2001). *Fisheries of the United States*, Ross, W., Gallaudet, R. T., and Oliver, C. (2017). *Aquaponics: Integrating fish and plant culture*, in *Frontiers in Marine Science* | www.frontiersin.org

Rakocy, J. E. (2012). "Aquaponics: Integrating fish and plant culture," in *Chu and Brown Marine Aquaponics: Shrimp With Halophytes*, Quagrainie, K. K., Flores, R. M. V., Kim, H. J., and McClain, V. (2018). Economic analysis of aquaponics and hydroponics production in the U.S. Midwest. *J. Aquar. Sci.*, 7, 49–53. doi: 10.3126/jsastr.v7i1.23301

Panigrahi, A., Saranya, C., Sundaram, M., Vinoth Kannan, S. R., Das, R. R., Satish Kumar, R., et al. (2018). Carbon: nitrogen (C:N) ratio level variation influences microbial community of the system and growth as well as immunity of shrimp (*Litopenaeus vannamei*) in biofloc based culture system. *Fish Shellfish Immunol.* 81, 329–337. doi: 10.1016/j.fsi.2018.07.035

Panta, S., Flowers, T., Lane, P., Doyle, R., Haros, G., and Shabala, S. (2014). Halophyte agriculture: success stories. *Environ. Exp. Bot.* 107, 71–83. doi: 10.1016/j.envexpbot.2014.05.006

Panth, N., Park, S. H., Kim, H. J., Kim, D. H., and Oak, M. H. (2016). Protective effect of *Salicornia europaea* extracts on high salt intake-induced vascular dysfunction and hypertension. *Int. J. Mol. Sci.* 17:1176. doi: 10.3390/ijms17071176

Pinheiro, I., Carneiro, R. F. S., Vieira, F., do, N., Gonzaga, L. V., Fett, R., et al. (2020). Aquaponic production of *Sarcocornia ambigu* and Pacific white shrimp in biofloc system at different salinities. *Aquaculture* 519, 1–9. doi: 10.1016/j.aquaculture.2020.10.014

Pyakurel, A., Dahal, B. R., and Rijal, S. (2019). Effect of molasses and organic fertilizer in soil fertility and yield of Spinach in Khotang. *Nepal. Int. J. Appl. Sci. Biotechnol.* 7, 49–53. doi: 10.3126/ijasbi.v7i1.23301

Quagrainie, K. K., Flores, R. M. V., Kim, H. J., and McClain, V. (2018). Economic analysis of aquaponics and hydroponics production in the U.S. Midwest. *J. Aquar. Sci.*, 7, 49–53. doi: 10.3126/ijasbi.v7i1.23301

Rakocy, J. E. (2012). "Aquaponics — Integrating fish and plant culture," in *Aquaculture Production Systems*, ed. J. Til Epid (Oxford): John Wiley & Sons, Inc., 343–386. doi: 10.1002/9781118250165.ch14

Ray, A. J., and Lotz, J. M. (2017). Comparing salinities of 10, 20, and 30% in intensive, commercial-scale biofloc shrimp (*Litopenaeus vannamei*) production systems. *Aquaculture* 476, 29–36. doi: 10.1016/j.aquaculture.2017.03.047

Rodriguez-Kabana, R. (1986). Organic and inorganic nitrogen amendments to soil as nematode suppressants. *J. Nematol.* 18, 129–134.

Ross, W., Gallaudet, R. T., and Oliver, C. (2017). *Fisheries of the United States*, 2017. U.S. Department of Commerce, NOAA Current Fishery Statistics No. 2017. Washington, DC: NOAA.

Schenck, S. (2001). Molasses Soil Amendment for Crop Improvement and Nematode Management. Honolulu, HI: Hawai’i Agriculture Research. Center, 1–7.

Shete, A. P., Verma, A. K., Chadha, N. K., Prakash, C., and Chandrakant, M. H. (2015). A comparative study on fish to plant component ratio in recirculating aquaponic system with common carp and mint. *J. Environ. Sci.*, 29, 323–329.

Somerville, C., Cohen, M., Pantanelli, E., Stankus, A., and Lovatelli, A. (2014). *Small-Scale Aquaponic Food Production. Integrated fish and Plant Farming.* Fisheries and Aquaculture Technical Paper, No 589. Rome: FAO, 262.

Sookiyong, D., Silva, F. S. D., Davis, D. A., and Hanson, T. R. (2011). Effects of stocking density on the performance of Pacific white shrimp *Litopenaeus vannamei* cultured under pond and outdoor tank conditions using a high soybean meal diet. *Aquaculture* 319, 232–239. doi: 10.1016/j.aquaculture.2011.06.014

Ventrica, Y., and Sagi, M. (2013). Halophyte crop cultivation: the case for salicornia and sarcocornia. *Environ. Exp. Bot.* 92, 144–153. doi: 10.1016/j.envexpbot.2012.07.010

Wang, Y., and Gu, Q. (2010). Effect of probiotics on white shrimp (*Penaeus vannamei*) growth performance and immune response. *Mar. Biol. Res.* 6, 327–332. doi: 10.1080/17451009003300893

White, P. J. (2012). "Ion uptake mechanisms of individual cells and roots: short-distance transport," in *Marschner’s Mineral Nutrition of Higher Plants*, ed. P. Marschner (Cambridge, MA: Academic Press), 7–47. doi: 10.1016/B978-0-12-384905-2.00002-9

Xu, W. J., Morris, T. C., and Samocha, T. M. (2016). Effects of C:N ratio on bioclor development, water quality, and performance of *Litopenaeus vannamei* juveniles in a biofloc-based, high-density, zero-exchange, outdoor tank system. *Aquaculture* 453, 169–175. doi: 10.1016/j.aquaculture.2015.1.021

Xu, W. J., Morris, T. C., and Samocha, T. M. (2018). Effects of two commercial feeds for semi-intensive and hyper-intensive culture and four C:N ratios on water quality and performance of *Litopenaeus vannamei* juveniles at high density in biofloc-based, zero-exchange outdoor tanks. *Aquaculture* 490, 194–202. doi: 10.1016/j.aquaculture.2018.02.028

Xu, W. J., and Pan, L. Q. (2012). Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C:N ratio in feed. *Aquaculture* 356–357, 147–152. doi: 10.1016/j.aquaculture.2012.05.022

Yang, T., and Kim, H. J. (2019). Nutrient management regime affects water quality, crop growth, and nitrogen use efficiency of aquaponic systems. *Sci. Hortic.* 256,108619. doi: 10.1016/scientia.2019.108619

Yang, T., and Kim, H. J. (2020b). Comparisons of nitrogen and phosphorus mass balance for tomato-, basil-, and lettuce-based aquaponic and hydroponic systems. *J. Clean. Prod.* 274:122619. doi: 10.1016/j.jclepro.2020.122619

Yang, T., and Kim, H. J. (2020a). Characterizing nutrient composition and concentration in tomato-, basil-, and lettuce-based aquaponic and hydroponic systems. *Water* 12:1259. doi: 10.3390/W12051259

Zhu, S., and Chen, S. (2001). Effects of organic carbon on nitrification rate in fixed film biofilters. *Aquac. Eng.* 25, 1–11. doi: 10.1016/S0144-8690(01)00071-1

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