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Evaluation of Pacific Whiteleg Shrimp and Three Halophytic Plants in Marine Aquaponic Systems under Three Salinities

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Abstract: The effect of salinity on the growth performance of whiteleg shrimp (*Litopenaeus vannamei*) and three halophyte plants, red orache (*Atriplex hortensis*), okahijiki (*Salsola komarovii*), and minutina (*Plantago coronopus*), in a marine aquaponic system with biofloc was evaluated in this study. The experiment was conducted for 4 weeks, and the three treatments were 10, 15, or 20 ppt (parts per thousand). The growth performance of the shrimp and the three halophytes were affected by the salinity. Compared to the shrimp reared in 10 ppt, those reared in 15 and 20 ppt had higher final weight, weight gain rate (WGR), and specific growth rate (SGR), and lower feed conversion ratio (FCR). The results from shrimp raised in 15 ppt were 2.0 ± 0.1 g, 89.9 ± 2.2%, 2.3 ± 0.0%, and 1.5 ± 0.0, respectively, and those in 20 ppt were 2.0 ± 0.1 g, 93.9 ± 5.4%, 2.4 ± 0.1%, and 1.4 ± 0.1, respectively. On the other hand, the growth performance and nutrient content in halophyte plants decreased with the increasing salinity. In general, the three halophyte plants had better results in the 10 and 15 ppt treatments than those in 20 ppt. Therefore, the salinity of 15 ppt was suggested as the optimal condition for the integrated cultivation of whiteleg shrimp and the three halophytes in marine aquaponics. Additionally, they are compatible species for the development of marine aquaponics.

Keywords: marine aquaponics; *Litopenaeus vannamei*; halophyte; *Atriplex hortensis*; *Salsola komarovii*; *Plantago coronopus*; biofloc; probiotics; water-pump-less system design; sustainable food production

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1. Introduction

Aquaponics is a new, rapidly emerging, eco-friendly food production system (FPS) that links recirculating aquaculture systems (RASs) with hydroponics (plant production in water, without soil) [1]. This system combines the merits of both aquaculture and hydroponics, such as reduction in water usage, increased food production per unit area, reuse of wastewater and eliminating environmental pollution [2–4]. Given these merits, aquaponics has been viewed as a promising approach for sustainable food production in the future. However, high electricity demand is a drawback of aquaponic systems [4]. To overcome this shortcoming, airlift, a technology that raises liquids by the pressure of air, is a more electrically efficient and alternative approach to promoting water flow and reducing electricity consumption from water pumps [4]. Moreover, this technology can be beneficial to areas with less power supply. Therefore, the technology was applied to the system design of the present study.

On the other hand, most aquaponics systems are freshwater, which is an increasingly limited resource while one of the primary elements for food production. Approximately 70% of the global supply of freshwater is in use in current food production systems [5,6]. Another option for aquaponic FPS would be marine or saline systems. There are thousands of potential fish and invertebrates that could be raised in marine aquaponic systems and numerous high-value plant crops are tolerant of low salinity environments [7].

Currently, marine aquaponics is a relatively new concept compared to freshwater aquaponics and is still in the early stages of development. To date, relatively few species combinations have been evaluated. Many freshwater systems use relatively slow growing and low-value fish and are an "economic drain", whereas the higher value and rapid turnover of plants provides the economic return on investment [8]. In contrast, marine animals, as a group, often display more rapid growth than freshwater fish and shellfish and can have a higher economic value, providing a possible solution to the economic drain issue of freshwater systems. Therefore, exploring different combinations of saltwater organisms and salt-tolerant plants in marine aquaponics could be a significant addition to the global FPS.

Marine shrimp are one of the preferred seafood items globally and the most preferred crustacean, especially Pacific whiteleg shrimp, *Litopenaeus vannamei*, which has become the major species cultured worldwide [9,10]. Several attributes contribute to this preference including faster growth rate, high economic value, demand in the market, high density tolerance, and adaptability to wide ranges of several environmental parameters, such as salinity (3–45 ppt) and temperature (20–35 °C) [11–18]. Given these advantages, especially the high-density tolerance, super-intensive shrimp culture (>300 shrimp/m^2) has become common [17,19,20], but raises the concern of negative environmental impacts from shrimp farm effluents, for instance, spreading outbreaks of disease, inducing eutrophication, etc.

Biofloc technology (BFT) was developed to help manage water quality in intensive aquaculture systems. BFT relies on a diverse microbial community, composed of autotrophic and heterotrophic bacteria, algae, zooplankton, fungi, and viruses, to mineralize and assimilate toxic metabolites, such as ammonia-N and nitrite-N, in the culture water, and it is widely applied to the culture of tilapia (*Oreochromis* sp.), whiteleg shrimp (*Liopenaeus vannamei*), and other omnivorous species which are tolerant to high turbidity [21–24]. However, high concentrations of nitrate-N remain a concern, despite the fact it is less toxic to aquatic animals than other nitrogenous compounds. Nitrate-N removal can be accomplished with the use of plants in an integrated aquaponic culture system [23,25], but there have been a few published reports of BFT use in aquaponics [26,27].

When applying BFT in new culture water, accelerating the development of microbial community and ensuring that beneficial bacteria are the dominant species in the biofloc, inoculating probiotics is a promising approach. Crab [28] indicated that inoculating biofloc reactors with probiotic bacteria might exert a biocontrol effect against *Vibrio* spp. Moreover, many studies proved that applying probiotics can improve growth performance and resistance to the adverse environment, as well as increase tolerance to stress on both aquatic animals and plants [29–33].

To develop marine aquaponics, plants capable of growing in saline water are required. Halophytes are a broad category of plant species that tolerate saline conditions. They have been evaluated as food crops, forage crops, oilseed, and energy crops, as well as for phytoremediation (soil desalinization, phytoextraction and phytostabalization), and medicinal purposes [34–36]. Although halophytic plants have been consumed by humans since ancient times [37,38], they are not a common crop species.

Atriplex hortensis is an annual plant and known as red orache or mountain spinach. The plant is native to Europe and Asia, and can also be found in Canada, United States, Australia, and New Zealand. It is grown as a potted herb and colorful salad green for its high nutrient value (protein and amino acids) [39–41].

Salsola komarovii is an annual plant native to China, Korea, Japan and Easter Russia. It is also known as okahijiki or land seaweed. It is rich in protein; leaves and young shoots can be eaten as a vegetable or used as a forage crop [42].

Plantago coronopus is an annual herb rich in vitamins A, C, K and minerals, and it is grown as a leafy vegetable [43]. The common names of this plant are minutina, erba stella and buckshorn plantain. It can be found in Europe, Africa, and Asia. These three halophytes have potential as cash crops and have been proved to grow in high saline conditions [25,38,41,43–47].

Salinity would be a vital factor in the development of marine aquaponics since it can affect the growth performance of both aquatic animal and plants, even though they are able to tolerate salinity at different ranges. Low salinity can reduce immune ability, tolerance to the toxicity of nitrite, and resistance to pathogens and disease [12,48,49]. In contrast, salinity is the main factor and threat for poor growth and productivity of glycophytic plants, even halophytic plants, if the salinity is beyond their threshold [7,50]. Therefore, it is important to find the optimal salinity for the development of marine aquaponics.

The aim of this research was to evaluate the growth performance of potential comparable combination (whiteleg shrimp with three halophytic plants) for the development of marine aquaponics with BFT under different salinities.

2. Materials and Methods

2.1. Aquaponic System Design

Each aquaponic system was equipped with a 113.6 L aquaculture tank, a 102.2 L hydroponic tank, and an 18.9 L biofilter tank (Figure 1). Each aquaculture tank, hydroponic tank, and biofilter tank had an air stone to maintain dissolved oxygen (DO) concentration above 6 mg/L. Aquaculture tanks were covered with plastic mesh to prevent shrimp escape, and hydroponic tanks were covered with lids to prevent algae growth. The lid of each hydroponic tank was also used as the floating raft to support plants. The biofilter tank was filled with bio-balls (surface area 98 ft^2/ft^3) and fitted with a 25-micron filter bag (Pentair Aquatic Eco-Systems, Inc., Apopka, FL, USA). Submersible heaters were added into aquaculture tanks to keep the water temperature within the optimal range 26–28 °C for the shrimp. LED lights (40 w, 5000 lumens, 4000 K daylight white; Kihung LED, Guangdong, China) were suspended at a height of 16.5 cm (6.5 in) over the plant growth bed to provide light for the plants. Light intensity was measured with a quantum sensor (MQ-500 Full-Spectrum Quantum Meter; Apogee Instruments, Inc., Logan, UT, USA). The photosynthetically active radiation averaged 239 µmol m^{-2} s^{-1}. The photoperiod was 14 h light and 10 h dark.

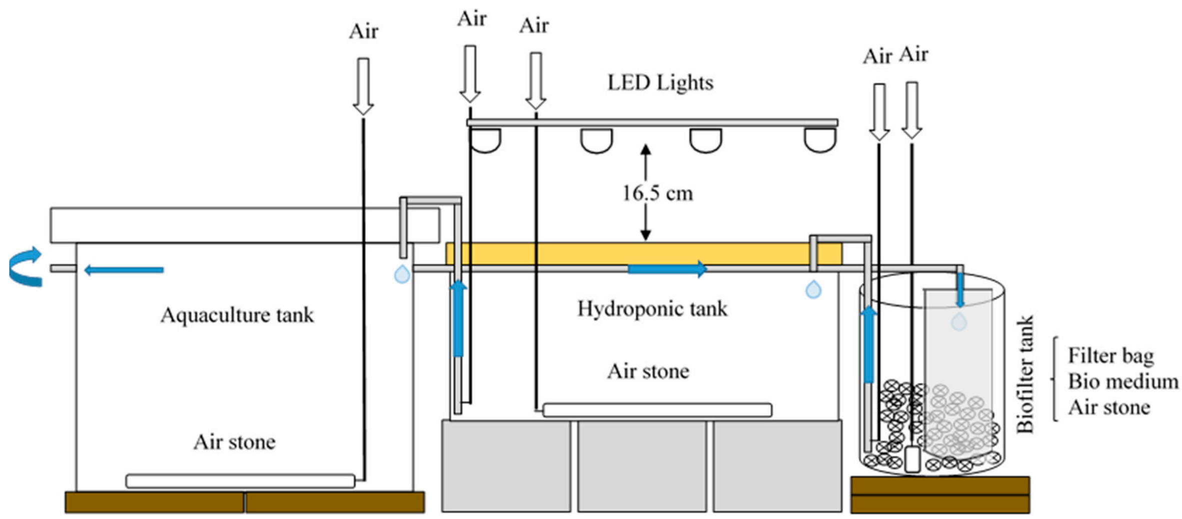

Figure 1. Schematic diagram of an aquaponic system unit.

2.2. Biological Material

2.2.1. Shrimp

Pacific white shrimp (*Litopenaeus vannamei*) were acquired from a private producer (RDM Aquaculture, Fowler, IN, USA) and transported to the Aquaculture Research Laboratory, Purdue University. Transportation conditions were temperature 24 °C and salinity 16 ppt. Shrimp were quarantined for 1 week before moving into aquaponic systems. During the quarantine, commercial shrimp feed (Zeigler Brothers, Gardners, PA, USA) was provided two times daily at 8 a.m. and 5 p.m., with a total daily amount of 3.5% of body weight divided into equal aliquots.

2.2.2. Plants

The seeds of three halophytic plants, red orache (*Atriplex hortensis*), okahijiki (*Salsola komarovii*), and minutina (*Plantago coronopus*), were obtained from a commercial source (Johnny's Selected Seeds, Winslow, ME, USA) and sowed in horticubes, soilless foam medium (OASIS® Grower Solutions, Kent, OH, USA). During germination, plants were irrigated with fresh water for the first week. To reduce osmotic shock on plants, salinity of irrigation water was increased 2–3 ppt every 48 h from the second week until the desired salinity was obtained.

2.3. Experimental Design and System Management

Shrimp and three halophytic plants were evaluated in 3 salinity treatments; 10, 15, or 20 ppt. The study was conducted in Aquaculture Research Lab at Purdue University from 4 April to 2 May 2020. During the week of quarantine, shrimp were cultured in three 700 L tanks, and slowly acclimated to the desired salinity at a rate of 2 ppt every 24 h. One week before the experiment, shrimp were weighed and placed into systems to produce nutrients for plants. The stocking density of shrimp was 200 shrimp/m^2 (40 shrimp/tank), and the average weight of individual shrimp was 0.96 g. There were 24 plants (8 plants per species) in each hydroponic tank, which is equivalent to a density of 100 plants/m^2. Commercial shrimp feed (Zeigler Brothers, Gardners, PA, USA) was provided two times daily at 8 a.m. and 5 p.m., with a total daily amount of 3.5% of body weight divided into equal aliquots. The guaranteed analysis of the feed was 35% protein, 7% fat, 1.1% phosphorus, and 4% fiber.

Water flow within each system was promoted via airlift with a flow rate of 3 L/min. To manage water quality and the microbial community within each system, probiotics (EZ-Bio; Zeigler Brothers, Gardners, PA, USA) were used. EZ-bio (*Bacillus* spp.) was inoculated at 10 ppm into each of the 9 systems three days before the shrimp were placed in aquaculture tanks. As soon as shrimp were moved into aquaculture tanks, additional doses of probiotics were added every day in the first week, every other day in the second week, twice per week in the third week, and once per week beginning in the fourth week continuing until the end of the experiment [23,51]. Molasses (Hawthorne Gardening Co., Vancouver, WA, USA) was added as an organic carbon source to adjust the C/N ratio in the water once per day after the first feeding. 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 15 [52]. Sodium bicarbonate and potassium bicarbonate were added to maintain the alkalinity above 60 mg/L; 10% sulfuric acid was applied to keep the pH below 8. To protect plant roots from clogging by biofloc from shrimp culture, the water passed through the 25-micron filter bag before irrigation.

The experimental design was a completely randomized design (CRD) with salinity as the main factor. Each treatment had three replicates for a total of nine experimental systems. Throughout the 4-week experiment, there was no water discharged or exchanged except for replacement due to evaporation.

2.4. Measurement of Water Quality

During the experiments, dissolved oxygen, temperature (OxyGuard Handy Polaris DO meter, Farum, Denmark), and pH (pHTestr™ 10 Pocket pH Tester, Vernon Hills, IL, USA) were measured twice per day at 8 a.m. and 5 p.m. before feeding. Temperature, dissolved oxygen, and pH were maintained at 25–27 °C, 6.1–7.4 mg/L, and 7.3–8.0, respectively. Salinity (Vital Sine™ Salinity Refractometer, Pentair Aquatic Eosystems, Apopka, FL, USA) 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-N (TAN), nitrite-N (NO_2^-), nitrate-N (NO_3^-), phosphate (PO_4^{3-}), and alkalinity using a HACH kit (HACH, Loveland, CO, USA). Total suspended solids (TSSs) and volatile suspended solids (VSSs) were measured once per week following US EPA method 1684.

2.5. Growth Performance

2.5.1. Shrimp

The initial weights, final weights and the number of shrimp at the beginning and end of the experiment were collected to calculate growth indices such as survival rate, weight gain rate (WGR), specific growth rate (SGR), and feed conversion ratio (FCR). The following formulae were used:

$$\text{Survival rate (\%)} = (\text{Final number of shrimp}/\text{Initial number of shrimp}) \times 100 \quad (1)$$

$$\text{WGR (\%)} = (\text{Final biomass (g)} - \text{Initial biomass (g)})/\text{Initial biomass} \times 100 \quad (2)$$

$$\text{SGR (\%/day)} = [\text{Ln(Final biomass (g))} - \text{Ln(Initial biomass (g))}]/\text{days} \times 100 \quad (3)$$

$$\text{FCR} = \text{Total feed intake (g)}/(\text{Final biomass (g)} - \text{Initial biomass (g)} \quad (4)$$

2.5.2. Plants

The portions of all plants above the raft system were weighed individually at the beginning and end of the experiment. Plant fresh weights were used to calculate relative growth rate (RGR). Plant samples were dried in an oven for 72 h at 100 °C and measured for dry weight. Plant fresh weights and dry weights were used to calculate water content (WC) in plants. Dried plant samples were ground and filtered through a 10-mesh sieve and kept in plastic vials for nutrient analysis. Plant tissue analysis was performed by the Midwest Laboratory (Omaha, NE). The following formulae were used to calculate plant growth:

$$\text{RGR (\%/day)} = [\text{Ln(Final biomass (g))} - \text{Ln(Initial biomass (g))}]/\text{days} \times 100 \quad (5)$$

$$\text{WC (\%)} = (\text{Final fresh weight (g)} - \text{Final dry weight (g)})/\text{Final fresh weight} \times 100 \quad (6)$$

2.6. Statistical Analysis

All data were statistically analyzed via JMP v14.0 (SAS Institute Inc., Cary, NC, USA). Statistical difference will be determined using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference test (HSD) at $p \leq 0.05$.

3. Results

3.1. Shrimp Growth

The survival rate in the three treatments was above 90% and there were no significant differences ($p > 0.05$) among treatments. In the present study, the salinity showed a significant impact ($p < 0.05$) on the results of final weight, WGR, SGR, FCR, and productivity. Generally, values of final weight, WGR, SGR, and productivity increased with the increasing salinity. Mean final weight, WGR, SGR, and productivity of the shrimp were 11%, 19%, 14%, and 5%, respectively, higher when cultured at higher salinity (20 ppt) than that at lower salinity (10 ppt). Yet, there was no significant difference between shrimp raised in 15 and 20 ppt salinity. In contrast, FCR was significantly higher ($p < 0.05$) by 21% in shrimp raised in 10 ppt compared to shrimp raised in 20 ppt; values for shrimp raised in 15 ppt were not significantly different ($p > 0.05$) in terms of FCR from those raised at either 10 or 20 ppt (Table 1).

3.2. Plants

During the experiment, the survival rate of red orache, okahijiki, and minutina in all three treatments were 100%. Salinity has significant impacts on the growth performance of all three halophytes. Final fresh weight, RGR, WC, and yield of all three plants were inversely related to salinity (Table 2). In addition, concentrations of nutrient elements

(N, P, K, Mg, and Ca) in plant tissue were also inversely related to salinity; however, the percentage of sodium in plant tissue was increased with the increasing salinity (Figure 2).

Table 1. Growth performance of shrimp cultured in aquaponic systems in three salinities (10, 15, or 20 ppt).

Salinity (ppt)	10	15	20
Initial Weight (g)	0.96 ± 0.12	0.96 ± 0.12	0.96 ± 0.11
Final Weight (g)	1.82 ± 0.16 b	2.00 ± 0.10 a	1.99 ± 0.07 a
WGR (%)	79.24 ± 6.09 b	89.88 ± 2.18 ab	93.93 ± 5.39 a
SGR (%)	2.08 ± 0.12 b	2.29 ± 0.04 ab	2.40 ± 0.10 a
FCR	1.67 ± 0.13 a	1.47 ± 0.04 ab	1.38 ± 0.08 b
Survival Rate (%)	93.3 ± 2.9	95.0 ± 2.5	95.8 ± 1.4
Productivity (kg/m^2)	0.35 ± 0.01 b	0.37 ± 0.01 ab	0.37 ± 0.01 a

Values are means ± SD. Values within rows followed by a common letter do not differ significantly based on Tukey's honestly significant difference (HSD) test (α = 0.05).

Table 2. Initial fresh weight (IFW), initial dry weight (IDW), final fresh weight (FFW), final dry weight (FDW), relative growth rate (RGR), water content (WC), and yield of red orache, okahijiki, and minutina cultivated in aquaponic systems with different salinities (10, 15, or 20 ppt).

Plant Species	Salinity (ppt)	IFW (g/plant)	IDW (g/plant)	FFW (g/plant)	FDW (g/plant)	RGR (%)	WC (%)	Yield (kg/m^2)
Red orache	10 ppt	0.05 ± 0.01 a	0.004 ± 0.001 a	3.67 ± 1.28 a	0.34 ± 1.28 a	15.1 ± 1.3 a	90.7 ± 0.2 a	0.37 ± 0.02 a
	15 ppt	0.05 ± 0.01 a	0.004 ± 0.001 a	3.31 ± 0.90 ab	0.34 ± 0.10 a	14.8 ± 1.0 ab	89.7 ± 0.2 b	0.36 ± 0.03 a
	20 ppt	0.05 ± 0.01 a	0.004 ± 0.001 a	2.80 ± 0.99 b	0.29 ± 0.12 a	14.2 ± 1.2 b	89.8 ± 0.2 b	0.31 ± 0.05 a
	p	ns	ns	*	ns	*	***	ns
Okahijiki	10 ppt	0.13 ± 0.01 a	0.011 ± 0.001 a	6.05 ± 2.40 a	0.42 ± 0.17 a	13.3 ± 1.5 a	93.0 ± 0.4 a	0.63 ± 0.10 a
	15 ppt	0.13 ± 0.01 a	0.011 ± 0.001 a	4.78 ± 1.50 ab	0.36 ± 0.11 a	12.6 ± 1.2 a	92.4 ± 0.6 b	0.49 ± 0.09 ab
	20 ppt	0.13 ± 0.01 a	0.011 ± 0.001 a	3.64 ± 1.39 b	0.34 ± 0.12 a	11.5 ± 1.4 b	90.7 ± 1.0 c	0.39 ± 0.02 b
	p	ns	ns	***	ns	***	***	*
Minutina	10 ppt	0.05 ± 0.01 a	0.003 ± 0.001 a	20.28 ± 9.25 a	1.50 ± 0.74 a	20.9 ± 1.8 a	92.7 ± 0.4 a	2.03 ± 0.47 a
	15 ppt	0.05 ± 0.01 a	0.003 ± 0.001 a	8.79 ± 3.45 b	0.68 ± 0.27 b	18.0 ± 1.5 b	92.3 ± 0.4 b	0.88 ± 0.14 b
	20 ppt	0.05 ± 0.01 a	0.003 ± 0.001 a	5.50 ± 2.79 b	0.44 ± 0.23 b	16.0 ± 2.4 c	91.9 ± 0.3 c	0.55 ± 0.11 b
	p	ns	ns	***	***	***	***	**

Values are means ± SD (*n* = 12 and 24 for initial weight and final weight, respectively). Values within columns of each plant species followed by a common letter do not differ significantly based on Tukey's honestly significant difference (HSD) test (α = 0.05). ns, *, **, *** mean not significant or significant at $p \leq 0.05$, 0.01, or 0.001, respectively.

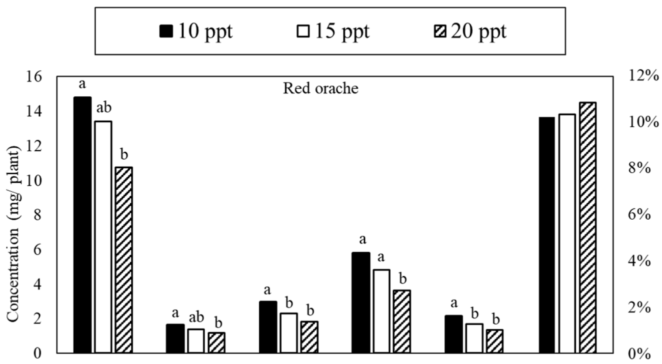

Figure 2. *Cont.*

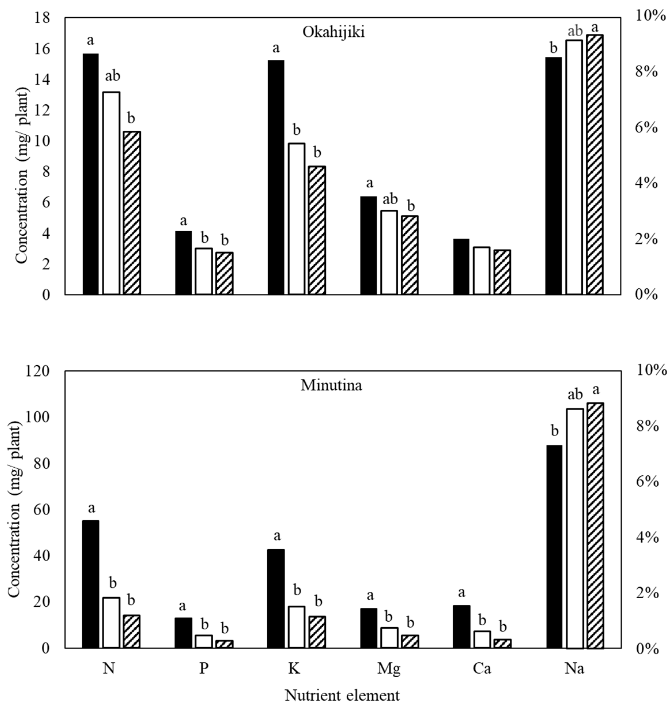

Figure 2. The concentrations of N, P, K, Mg, and Ca, and the percentage of Na in plant tissue. Within each nutrient element, different letters above each bar indicate significant difference between treatments based on Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$).

3.2.1. Red Orache (*Atriplex hortensis*)

Values of final fresh weight, RGR, and water content of red orache cultivated in 10 ppt treatment were significantly higher (31%, 6%, and 1%, respectively; $p < 0.05$) than in 20 ppt, yet the results were not significantly different ($p > 0.05$) from 15 ppt regarding final fresh weight and RGR. In the aspect of water content, the highest value (90.7%) was shown in 10 ppt treatment and was significantly higher ($p < 0.05$) than the other two treatments. Although there was no significant difference ($p > 0.05$) among all treatments in dry weight and yield, the results followed the same trend as other parameters as well (Table 2). The concentrations of K and Ca were significantly higher ($p < 0.05$) in plants cultivated in 10 ppt treatment than 15 and 20 ppt treatments. N, P, and Mg were more concentrated in the 10 ppt treatment and were significantly higher than the 20 ppt treatment, yet were not significantly different from the 15 ppt treatment (Figure 2).

3.2.2. Okahijiki (*Salsola komarovii*)

The results of the final fresh weight, RGR, water content, and yield of okahijiki had a similar trend as red orache, but they were more pronounced Values of those indices were 66%, 15%, 2.5%, and 62% higher at the 10 ppt treatment than at the 20 ppt treatment and showed significant difference ($p < 0.05$). Yet, the results were not significantly different from the 15 ppt treatment in general. Similarly, there was no significant difference among treatments for final fresh weight, but the results indicated that salinity was inversely related (Table 2). The concentrations of N and Mg were significantly higher in plants grown at 10 ppt than those grown at 20 ppt, but with no significant difference to the 15 ppt treatment. In addition, the highest concentrations of P and K were in the 10 ppt treatment and showed a significant difference to both 15 and 20 ppt treatments (Figure 2).

3.2.3. Minutina (*Plantago coronopus*)

Compared to previous plants, the results of growth performance displayed an even more obvious trend on the salinity effect. The mean final fresh weight, final dry weight, RGR, and yield were 269%, 275%, 30%, and 269%, respectively, higher at the 10 ppt treatment than at the 20 ppt treatment. Unlike the other two halophytes, 10 ppt treatment had the highest result of all parameters, even in final dry weight, among treatments and showed significant difference ($p < 0.05$). This situation can be found in the result of plant tissue analysis as well. The concentration of N, P, K, Mg, and Ca in plants cultivated in 10 ppt treatment was significantly higher ($p < 0.05$) than those cultivated in 15 and 20 ppt.

3.3. Water Quality

The daily loss of water through evaporation and transpiration was roughly 1–2% of the total volume of water in each system (data not shown). The concentrations of total suspended solids (TSSs) and volatile suspended solids (VSSs) were not significantly different among the three treatments. On the other hand, alkalinity in the 10 ppt treatment was significantly lower ($p < 0.05$) than that of 15 and 20 ppt treatments (Table 3).

Table 3. Mean water quality values (range) for marine aquaponics operated at three salinities for 4 weeks.

Salinity (ppt)	Temperature (°C)	DO (mg/L)	pH	Alkalinity (mg/L)	TSS (mg/L)	VSS (mg/L)
10	26.5 ± 0.4 (25.9–26.9)	7.0 ± 0.5 (6.1–7.4)	7.6 ± 0.2 (7.3–8.0)	65.2 ± 13.1 b (47–80)	28.4 ± 14.7 (9.8–41.5)	12.1 ± 6.7 (1.7–21.8)
15	26.6 ± 0.2 (26.3–27.0)	6.8 ± 0.5 (6.1–7.4)	7.6 ± 0.2 (7.4–7.9)	70.4 ± 11.6 ab (53–80)	28.4 ± 16.3 (10.2–49.7)	11.0 ± 5.3 (2.0–25.0)
20	26.5 ± 0.2 (26.2–26.8)	6.9 ± 0.5 (6.1–7.4)	7.6 ± 0.2 (7.3–7.9)	76.3 ± 14.7 a (53–100)	30.1 ± 22.1 (7.2–64.5)	10.4 ± 5.1 (2.2–23.8)

Values are means ± SD (range). Values followed by different letters are significantly different based on Tukey's honestly significant difference (HSD) test (α = 0.05).

The weekly changes in TAN, nitrite-N, nitrate-N, and phosphate concentrations are shown in Figure 3. TAN, NO_2^-, NO_3^-, and PO_4^{3-} displayed similar trends among the three treatments. The concentration of TAN in every treatment continued to increase in the first two weeks, then started to decrease and remained below 1 mg/L until the end of the experiment (Figure 3A). There were no significant differences ($p > 0.05$) among treatments. On the contrary, NO_2^- and NO_3^- stayed at low concentrations in the first two weeks and started to increase, yet the concentration remained at acceptable levels for shrimp and plants. At the end of the experiment, the concentrations of nitrite and nitrate in the 10 ppt treatment were significant lower ($p < 0.05$) than the other treatments (Figure 3B,C). The concentration of phosphate continued increasing throughout the experiment. Although the concentration in the 10 ppt treatment was slightly lower than the other treatments, there were no significant differences ($p > 0.05$) among treatments (Figure 3D).

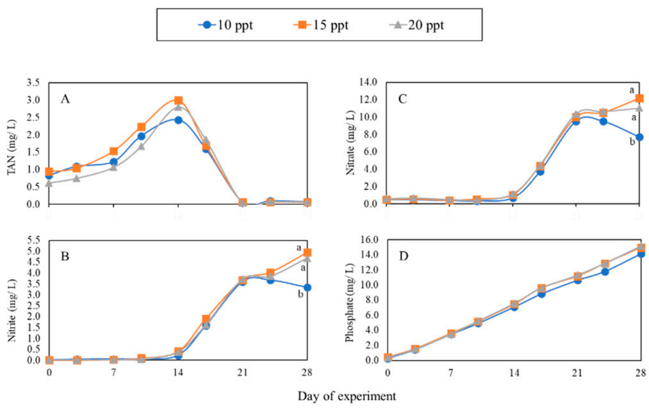

Figure 3. The change of TAN (**A**), nitrite (**B**), nitrate (**C**), and phosphate (**D**) concentrations in marine aquaponics with three salinities for 4 weeks. Lowercase alphabet letters represent significant differences, followed by one-way ANOVA and Tukey's HSD test (α = 0.05).

4. Discussion

4.1. Shrimp Growth

The experiment in the present study took place in lab conditions and was not close to production parameters. Shrimp raised in salinity of 15 and 20 ppt had better growth performance (FCR, WGR, and SGR) than those reared in the 10 ppt treatment. This result was also observed by other researchers [13,27,49,53,54]. Therefore, based on those studies and the results of the present study, the optimal salinity for the growout of whiteleg shrimp would be 15–20 ppt.

In this research, shrimp in the three salinities had a survival rate above 90% with no significant difference among treatments; however, Ray and Lotz [53] reported that mortality in lower salinity was higher. This may be because shrimp reared in lower salinity have poor resistance to nitrite toxicity [48]. In the present study, shrimp cultured in low salinity (10 ppt) had a better survival rate which may be attributed to the application of probiotics, since probiotics can improve the resistance to the adverse environment and increase tolerance to stress [29–32].

4.2. Plants

In the present study, the three plant species successfully grew in marine aquaponics, which indicates that these plant species are capable of growing in soilless conditions at varying salinities. This is in contrast to certain previous experiments. For example, saltwort (*Batis maritima*) had a 30% survival rate [55], and *Sarcocornia ambigua* had a 63% survival [27]. According to Radhakrishnan et al. [33], the growth performance and health of plants can be improved during salt stress when probiotics (*Bacillus* spp.) are used, which may have contributed to our results.

In general, the growth performance of the three halophytic plants in this research declined with the increasing salt concentration. The reduction might be due to the negative water potential, caused by external salinity, which can lead to the diminution of water flow into the plant, reducing nutrient (NO_3^- and PO_4^{3-}) availability. Sodium and chloride will compete with nutrients during nutrient uptake and transportation [25,56]. Figure 3C and D show that the concentrations of NO_3^- and PO_4^{3-} were more concentrated in the 15 and 20 ppt treatments. Furthermore, from the result of plant tissue analysis (Figure 2), nutrient concentrations also were higher in the lower salinity treatment, which can be evidence that nutrient absorption is hindered in a higher salinity environment. This circumstance not only occurred in glycophytic plants but also in halophytic plants. However, compared to glycophytic plants, halophytes are able to minimize salt toxicity and alleviate the effect via comparting and accumulating Na and Cl into vacuoles, located in salt bladders, where halophytes store excess salt [37,56,57]. In addition, higher nutrient concentrations in plants that grow in lower salinity indicated that they have higher nutritional value and are beneficial to human health [58].

Growth of red orache, okahijiki, and minutina, can be stimulated by salinity [43,45,47]. Plants grown in freshwater had a relatively poor growth performance compared to those grown within the salinity tolerances of the chosen species. The thresholds suggested by previous studies of red orache, okahijiki, and minutina were 10, 17.6, and 7.3 ppt, respectively [43,45,47]. Based on the growth performance reported in this study, the minimum thresholds are 15, 15, and 10 ppt, respectively. The reason for the different thresholds between Sai Kachout et al. [45] and the present study may be the media in which the tests were conducted. Salinity in irrigation water can lead to the accumulation of salt in the soil [37,59]. On the other hand, red orache in the present study was grown in hydroponics and the salinity level in the water changed little. Therefore, the tolerance of salinity in hydro- or aquaponics applications needs to be assessed for potential crops.

4.3. Water Quality

With well-established microbial flora, the concentration of TAN and NO_2^- can be maintained at low levels in aquaponics; however, it takes time if no probiotics, mature water or used-biomedia are used [20]. Yang and Kim [60] reported that TAN concentration remained high (above 5 mg/L) in the first 60 days and then stabilized below 0.5 mg/L; the concentration of NO_2^- maintained at a high level between 10 and 12 mg/L from day 16 to the end of the study. Nozzi et al. [56] spent a month acclimating a European sea bass system before their experiment started. In the present study, the bio media in the filter tank were new and clean, which means there was no established microbial flora in the system. Compared to nitrifying bacteria, the reproduction of heterotrophic bacteria is faster, requiring hours rather than days [4]. Moreover, Schmautz et al. [61] indicated that nitrifying bacteria only account for a relatively small fraction in the microorganism community in biofilter (less than 10%), and there are little or no nitrifying bacteria in other areas such as plant roots, periphyton, and fish feces. Therefore, adding probiotics (heterotrophic bacteria) can be an effective and practical approach to manage water quality, and can also assure the microorganisms dominated in the culture water are beneficial to our target organisms, shrimp and plants [29,33,51,62,63]. It only took 3 weeks for TAN concentration to stabilize and remain below 0.1 mg/L in this study, which was similar to [62].

To promote the growth of heterotrophic bacteria, which utilize organic carbon as energy to assimilate nitrogen waste to grow, addition of carbohydrates to increase the C/N ration is the easiest approach [22,64]. Additional carbohydrates also promote the formation of the biofloc, which can serve as supplemental nutrition to the cultured animals [22], and the concentration of that can be determined by measuring total suspended solids. However, filter bags are commonly used to prevent excess biofloc which can clog plants root or irritate the gills of aquatic animals [26]. That may be the reason why the concentrations of TSS and VSS in the present study were lower than other published research [26,52,53,64,65]. In addition, the short cultivation time of the present study may be another reason for the low concentrations of TSS and VSS. Moreover, some authors suggested that management of solids to prevent excess TSS is required [66,67]. Ray and Lotz [68] reported that lower biofloc concentrations improved shrimp production. Although the concentration of TSS in this study was low and might not be enough to serve as supplemental nutrients for shrimp, the biofloc filtered and accumulated in filter bags might be beneficial to plants, since essential micronutrients for plants (e.g., K, Ca S, P, Mg Cu, Mo, Zn, etc.) can be released via mineralization [1,4].

At the end of the present study, the concentrations of NO_2^- and NO_3^- were significantly lower in the 10 ppt treatment than the other treatments (Figure 3B,C), which is similar to values reported by Maicá et al. [54]. In that study, the predominant microorganisms in microbial flora were chlorophytes in the low salinity treatments. While not quantified, watercolor in the 10 ppt treatment was yellow-brownish, while brown in 15 and 20 ppt treatments. Thus, the microbial flora in the 10 ppt treatment may have been dominated by algae rather than heterotrophic bacteria.

Salinity is another factor that affects the relative proportions of NH_3 and NH_4^+ in the water [69]. The concentration of NH_3 increases with higher temperature and pH, and lower salinity. In the present study, the amount of NH_3 in the water only accounts for 2 to 3% of total ammonia nitrogen, which means NH_4^+ was the main source of nitrogen in the water. The lower alkalinity in the 10 ppt treatment may be because NH_4^+ serves as the main source of nitrogen for algae, and the biosynthesis of algae results in the consumption of alkalinity (as $CaCO_3$) [66]. In addition, 3.57 g of alkalinity is consumed when heterotrophic bacteria convert a gram of NH_4^+ into microbial biomass. Heterotrophic bacteria were added into systems regularly in the present study; therefore, the alkalinity in the 10 ppt treatment was not only affected by the assimilation of heterotrophic bacteria, but also the biosynthesis of algae. This explains why the concentration of alkalinity in the 10 ppt treatment remained lower than that in 15 and 20 ppt treatment (Figure 4).

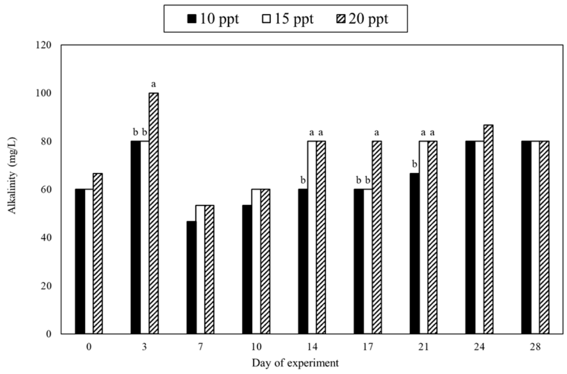

Figure 4. The dynamics of alkalinity in marine aquaponic systems for 4 weeks. Lowercase alphabet letters above bars represent significant differences among treatments, followed by one-way ANOVA and Tukey's HSD test (α = 0.05).

Although there was no significant difference in the concentration of PO_4^{3-} among the three treatments, PO_4^{3-} was slightly higher in the 20 ppt treatment followed by 15 ppt, then 10 ppt treatment. This result was similar to previous research that investigated *Dicentrarchus labrax* and *Beta vulgaris* var. *cicla* produced in freshwater and saltwater aquaponic systems, see Nozzi et al. [56], suggesting that salinity may affect plants to assimilate this critical nutrient. However, Pinheiro et al. [27] reported that the concentration of PO_4^{3-} was significantly lower in high salinity treatment than in low salinity treatment. Therefore, more research is needed to determine how salinity affects plants' ability to assimilate PO_4^{3-} in aquaponics.

5. Conclusions

Regarding marine aquaponics, whitelet shrimp and the three halophytes (*Atriplex hortensis*, *Salsola komarovii*, and *Plantago coronopus*) are suitable combinations for the future development. According to the results of this research, shrimp performed better in the salinity of 15 and 20 ppt; yet, plants performed better in the salinity of 10 and 15 ppt. Therefore, a salinity of 15 ppt is suggested as the optimal saline condition for shrimp and the three halophytes in an indoor marine aquaponics system. In addition, inoculating probiotics do have the efficiency of stabilizing water quality, cultivating microbial community, and enhancing the health of shrimp and plants in the operation of aquaponics. More research on searching suitable species combinations with longer culture time and higher stocking densities is needed for the development of commercial marine aquaponics.

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