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Original Research Articles

Intensive white shrimp (*Litopenaeus vannamei*) culture integrated with green mussel (*Perna viridis*), seaweed (*Gracilariopsis bailiniae*), and tilapia (*Oreochromis mossambicus*): impacts on water quality and growth of the shrimp

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The production of *Litopenaeus vannamei* has significantly intensified, and integrated multi-trophic aquaculture (IMTA) has emerged as an effective farming technique to sustain the shrimp industry. Integrated aquaculture reduces pollution while increasing aquaculture production. This production system could give both ecological and economic benefits. Its contribution to the reduced amount of nutrients from intensive shrimp farming effluents and to the improvement of water quality conditions and growth of white shrimp are significant. This study aimed to evaluate the impacts on water quality and growth performance of shrimp (*L. vannamei*) at the same time assess the viability of rearing shrimps integrated with either green mussel, *Perna viridis* (GM alone), GM+seaweed (*Gracilariopsis bailiniae*), GM+tilapia (*Oreochromis mossambicus*), or GM+seaweed+tilapia. Shrimps were cultured outdoors for 60 days in a recirculating system at an average water exchange rate of 6 L hr^{-1}. Shrimps were stocked at 400 shrimp m^{-3} in shrimp tank while seaweed (2kg m^{-2}), green mussels (50 individuals per meter line), and tilapia (350 grams m^{-3}) were cultivated separately in plastic baskets inside the biofiltration tank. Shrimp effluents were recirculated from the shrimp tank to the biofiltration tank. After 60 days of culture, results showed that shrimps without integration had the lowest average body weight (ABW), survival, weight gain, specific growth rate (SGR), biomass gain, and high feed conversion ratio (FCR) compared to shrimps with integration. This experiment confirmed that shrimp can be cultured intensively with either green mussel (GM alone), GM+ seaweed, GM+tilapia, or GM+seaweed+tilapia without adversely affecting the water quality and its growth performance and could even result in better yield than the shrimp cultured without integration. This research demonstrated the benefits of integrated aquaculture and could be further verified using large-scale culture.

INTRODUCTION

The global demand for seafood has increased as the global population continues to rise. To meet this demand, global aquaculture production in 2020 reached 122.6 million tonnes and is forecasted to be a 15% increase by 2030.[1] There is a pressing need to intensify and expand sustainable aquaculture production to bridge the supply-demand gap. This intensification, however, must be balanced with the need for cost-effective technology and minimal negative impacts on the environment.[2] The solution lies in efficient protein production systems, particularly those founded on intensive and integrated aquatic animal farming. Developing sustainable aquaculture systems, such as integrated aquaculture, will be a crucial player in the industry's future.[3]

Integrated multi-trophic aquaculture (IMTA) is the integration of species with different trophic levels in the culture system to take advantage of the residues from the production of one species for the culture of other species.[4,5] In IMTA systems, nutrients from uneaten feed and the excreted waste of fed species become food for extractive species, reducing nutrient release into the environment while enhancing overall productivity.[1] The integration of

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the inorganic (macroalgae/aquatic weed) and organic extractive species (bivalves) known as the un-fed and the fed/ cultured species (finfish/shrimp) in the same system gives IMTA advantages in terms of mitigation, economic gain, and sustainability than in monocultures.[6] Hence, it has economic and environmental benefits and is a potential solution to reduce the negative environmental impacts of aquaculture activities while increasing aquaculture production.

In recent years, shrimp has become the world's most valuable aquaculture species, and *Litopenaeus vannamei* has become one of the most important aquaculture species for human consumption.[7] In 2020, the total production of white shrimp was 5.8 million tons.[1] This species became well-known for its short culture period, fast growth, advanced immune system, capability to be cultured in high density, high salinity tolerance, and better feed conversion ratio.[8] Production of *L. vannamei* has spread whether cultured alone or in combination with other species.[9] Considering the role of seaweed in improving water quality, it has been cultured together with shrimp, tilapia, and bivalves. Tilapia is an ideal species for culture in an IMTA system.[10] This species has a high tolerance for suspended solids, moderate dissolved oxygen, and high stocking densities.[11] The integration of bivalves as extractive species in IMTA is due to its large biofiltration capacity. Mussels are efficient non-selective filter-feeders, thus providing a rationale for their use in IMTA systems as an eco-friendly water purification process in fish farming.[12] Using bivalves as extractive species creates favorable water quality conditions for better growth performance of shrimps.[13] Integrated farming of fish, shrimp, oysters, and aquatic weeds were better performers in terms of total production and improvement of water quality than in conventional polyculture.[6] White shrimp cultivated by the IMTA system are more effective in improving shrimps' performance productivity than shrimp cultivated by the monoculture system.[14]

Therefore, this study was conducted to evaluate the impacts on water quality parameters and the growth performance of shrimp (*L. vannamei*) integrated with either green mussel, *P. viridis* alone (GM), GM+seaweed (*G. bailiniae*), GM+tilapia (*O. mossambicus*), or GM+seaweed+tilapia. Likewise, this study also assessed the viability of rearing shrimps integrated with GM alone, GM+seaweed, GM+tilapia, or GM+seaweed+tilapia. This will test whether the system can still sustain holding on additional stocks without affecting the growth of the shrimp. If this integrated culture is proven to successfully sustain these species, it can be a viable candidate for large-scale production.

MATERIALS AND METHODS

EXPERIMENTAL TREATMENTS AND CULTURE CONDITIONS

The culture of *Litopenaeus vannamei* was conducted at the Brackishwater Aquaculture Center of the Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Philippines. Treatments tested for this study were: (a) control (shrimp, *L. vannamei* only); (b) shrimp+green mussel (*Perna viridis*) or GM alone; (c) shrimp+green mussel and seaweed (*Gracilariopsis bailiniae*) or GM+seaweed; (d) shrimp+green mussel+tilapia (*Oreochromis mossambicus*) or GM+tilapia; (e) shrimp+green mussel+seaweed+tilapia or GM+seaweed+tilapia. Treatments and the control group were replicated thrice.

WATER QUALITY MONITORING

Water parameters such as salinity, dissolved oxygen, and water temperature were monitored twice daily using a refractometer and dissolved oxygen meter. Water pH was measured by a pH pen twice a week. Alkalinity, unionized ammonia, nitrite, and phosphorus levels were determined weekly and analyzed following the method of Strickland and Parsons.[15] Total suspended solids (TSS) were also measured weekly using the APHA method.[16]

STOCK AND STOCK SAMPLING

Shrimp weighing 3.6-4.0 grams were stocked at a stocking rate of 400 shrimp m^{-3} in a clear glass aquarium. Each aquarium was filled with 50 L seawater. Seaweed and green mussels were washed with running seawater, cleaned from epiphytes, and acclimated with effluent-free seawater for one week. This served as the starvation period for the seaweed and green mussels before the growth trial. Healthy vegetative cuttings of seaweed, green mussels, and tilapia were cultivated separately in plastic baskets (27 cm x 19 cm x 14 cm) inside the biofiltration tank. Seaweed was stocked at 2 kg m^{-2},[17] tilapia at 350 grams m^{-3}, and green mussel (4.3 cm - 4.8 cm) at 50 individuals per meter line.[18] The seaweed stocking rate was maintained at 2 kg m^{-2} for every stock sampling. Shrimp effluents were being recirculated from the shrimp tank to the biofiltration tank. Effluents from the shrimp tank were recirculated to its corresponding biofiltration tank at an average flow rate of 6 L hr^{-1}.[19]

Shrimp weight was monitored weekly. Uneaten feeds and feces were siphoned out daily. The recirculating system's connecting pipes, pumps, and waterlines were checked and monitored to ensure a consistent water flow rate for the entire culture period. These ensure enough water flow for optimal biofiltration. There was no water change during the culture period, and water loss due to water sampling, evaporation, and siphoning out of uneaten feeds and feces during stock sampling were compensated by the addition of seawater to the experimental units from the main reservoir (treated seawater). This was done to maintain the desired water volume of all the treatments. The set-up was also shaded with transparent plastic roofs to ensure that rain would not affect the result of the experiment, and that light and temperature conditions were the same as in the field.

Table 1. Water quality parameters of *L. vannamei* cultured for 60 days.

	Treatments				
	Control	*GM Alone*	*GM+Seaweed*	*GM+Tilapia*	*GM+Seaweed+Tilapia*
Salinity (ppt)	25.00±0.00^{a}	25.33±0.33^{a}	25.67±0.33^{a}	25.00±0.00^{a}	25.33±0.33^{a}
Dissolved Oxygen (mg L^{-1})	4.36±0.11^{a}	4.62±0.09^{a}	4.74±0.04^{a}	4.43±0.21^{a}	4.76±0.30^{a}
Temperature (^{0}C)	25.47±0.03^{a}	25.83±0.07^{a}	25.70±0.06^{a}	25.70±0.06^{a}	25.70±0.15^{a}
pH	7.55±0.06^{a}	7.66±0.03^{a}	7.66±0.01^{a}	7.67±0.02^{a}	7.67±0.04^{a}
Alkalinity (mg L^{-1} CaCO3)	87.39±2.89^{ab}	76.35±2.51^{c}	75.69±4.64^{c}	95.46±3.28^{a}	83.14±2.82^{bc}
TSS (mg L^{-1})	528.67±21.33^{a}	351.00±11.68^{b}	353.67±6.49^{b}	395.33±9.94^{b}	364.33±17.07^{b}
Ammonia (ppm)	1.81±0.07^{c}	0.90±0.08^{d}	1.08±0.09^{d}	2.58±0.04^{a}	2.22±0.11^{b}
Nitrite (ppm)	0.44±.04^{b}	0.57±0.00^{a}	0.54±0.01^{a}	0.54±0.02^{a}	0.46±0.02^{b}
Phosphorus (ppm)	2.05±0.10^{a}	0.95±0.09^{c}	1.06±0.02^{bc}	1.22±0.02^{b}	1.14±0.04^{bc}

Data are presented as Mean±SE (n=3). Values in the same row with different superscripts are significantly different (*p*<0.05).

GROWTH PARAMETERS AND FEEDING MANAGEMENT

Shrimps were fed commercial shrimp feed with 40% protein content four times daily, and tilapia were fed commercial tilapia feed with 33% protein content twice daily. After stock sampling, the feeding rate was adjusted weekly based on the new body weight and survival rate. Growth parameters such as Average Body Weight, Weight Gain, Specific Growth Rate, Feed Conversion Ratio and Survival Rate were measured weekly.

Weight Gain = Weight $_{final}$ − Weight $_{initial}$

Specific Growth Rate (SGR) = 100 x (ln $_{final\ weight}$ − ln $_{initial\ weight}$)/day

Feed Conversion Ratio (FCR) = feed intake (g)/weight gain (g)

Survival Rate = 100 x (initial number of shrimp − final number of shrimp)/ initial number of shrimp

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS 23.0 version. The data were analyzed using one-way ANOVA to determine the significant differences between treatments. If there was a considerable difference, the Duncan Multiple Range Test (DMRT) was done for post hoc multiple comparisons of means. The significance level was set at 95% significance level (p<0.05). The data were expressed as mean ± standard error (n=3 replicates).

RESULTS

WATER QUALITY

After 60 days of culture, salinity, dissolved oxygen, temperature, and pH were similar in shrimp with integration and control. However, mean alkalinity was highest in shrimp with GM+tilapia and lowest in shrimp with green mussel, *P. viridis* (GM alone), and in shrimps with GM+ seaweed (*G. bailiniae*). Total Suspended Solids (TSS) were significantly higher in the control group than in shrimp with integration (**Table 1**).

For unionized ammonia level, shrimp with GM+tilapia had the highest level and lowest in shrimp with GM alone and with GM+Seaweed. Meanwhile, nitrite concentrations were statistically higher in shrimp with GM alone, GM+Seaweed, and GM+Tilapia compared to shrimp with GM+Seaweed+Tilapia and the control group. Significant phosphorus level differences were found in shrimp with GM alone and the control. The mean phosphorus level was higher in the control than in shrimp with integration of either green mussel, *P. viridis* (GM alone), GM+ seaweed (*G. bailiniae*), GM+tilapia (*O. mossambicus*), or GM+seaweed+tilapia (**Table 1**).

It was observed that the weekly levels of alkalinity, total suspended solids (TSS), nitrite (**Figure 1**), ammonia, and phosphorus (**Figure 2**) vary in the rearing water of shrimps with integration and the control. Ammonia and nitrite started to differ significantly (p<0.05) among treatments at Week 1 while alkalinity and phosphorus at Week 2. It was also observed that TSS levels in the rearing water of shrimps without integration or in the control group at Week 3 were significantly higher (p<0.05) than that of the shrimps with integration.

GROWTH PERFORMANCE

Shrimps showed better performance in shrimp with integration as shown in their average body weight, weight gain, survival rate, biomass gain, and specific growth rate (**Table 2**). Shrimp in the control group or without integration had the lowest average body weight, weight gain, and specific growth rate. They differed significantly (p<0.05) in shrimp with the integration of either green mussel (GM alone), GM+ seaweed, GM+tilapia, or GM+seaweed+tilapia. Moreover, shrimp with integration had the highest weight gain and specific growth rate. (**Table 2**).

Shrimp without integration or the control group had the lowest survival rate and biomass gain. The feed conversion ratio (FCR) was also higher in the control group than in

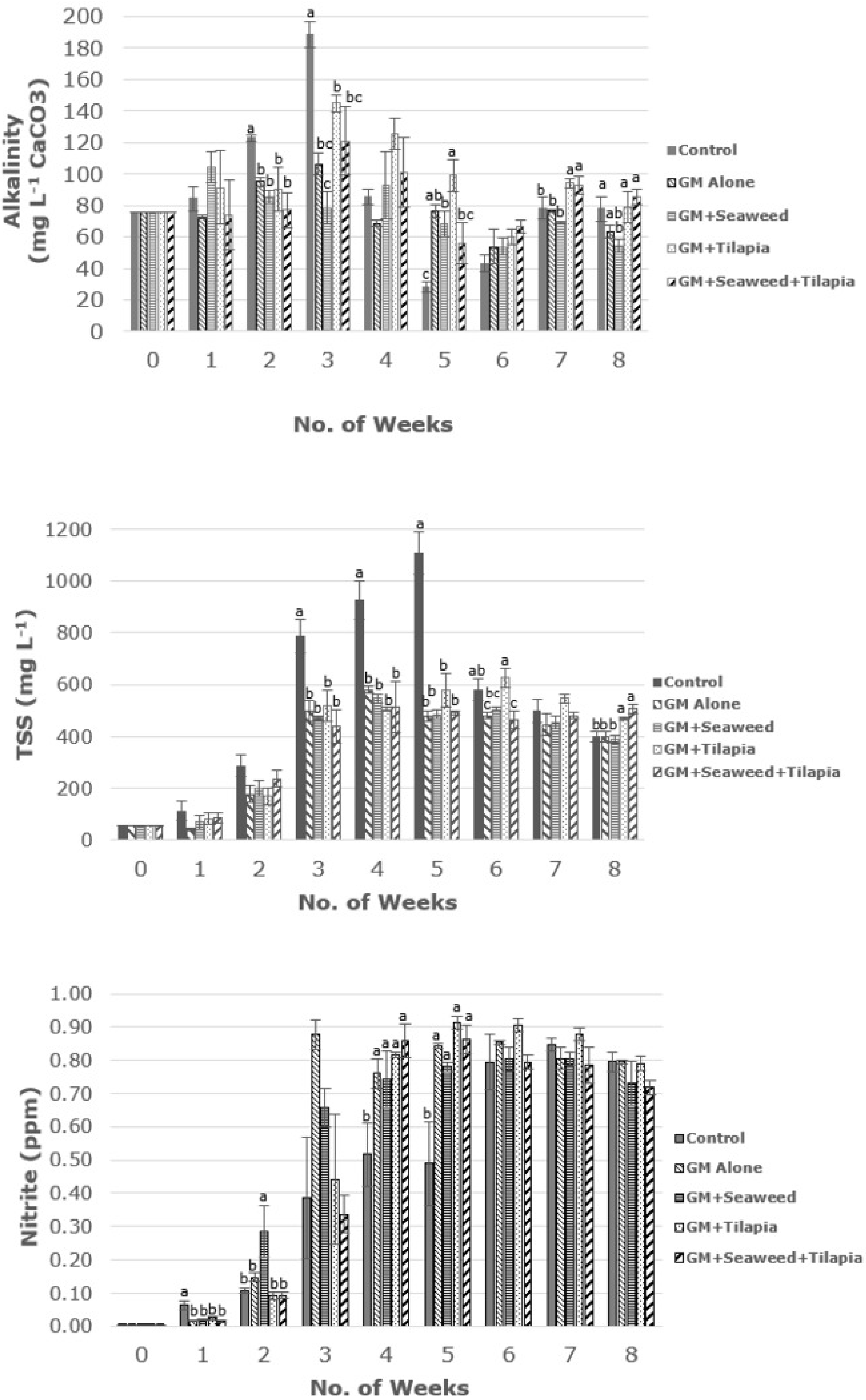

Figure 1. Weekly alkalinity, total suspended solids, and nitrite levels in the experimental units during the 60-day culture period.

Values are presented as Mean±SE (n=3), and different superscripts are significantly different (p<0.05).

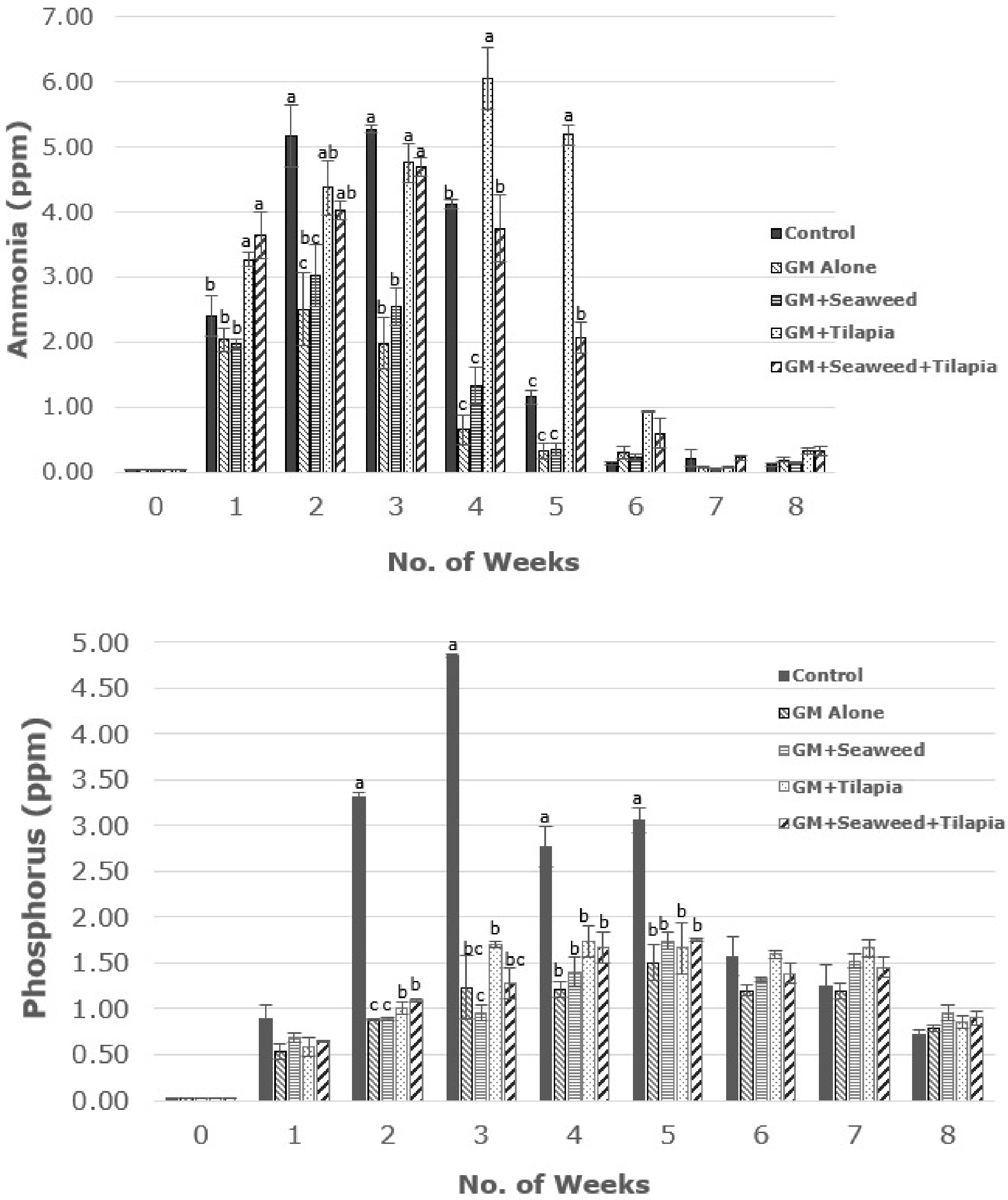

Figure 2. Weekly ammonia and phosphorus levels in the experimental units during the 60-day culture period.

Values are presented as Mean±SE (n=3), and different superscripts are significantly different (p<0.05).

shrimp with integration. The survival rate of shrimp was significantly higher (p<0.05) in the group with integration compared to the control. Further, the shrimp reared with integration had higher production than the control. Biomass gain was significantly higher (p<0.05) in shrimp cultured with GM+tilapia, GM+seaweed+tilapia, GM+seaweed, and GM alone compared to the control. Moreover, with integration, FCR was significantly improved (p<0.05). Overall, growth, survival, biomass gain, and FCR in shrimp cultured with integration did not differ significantly among the groups (**Table 2**).

The mean weekly average body weight of the shrimp increases until 60 days of the culture period. However, the average body weight of the shrimp in the control appeared to be significantly lower than the rest of the treatment starting at Week 4. During harvest at Week 8, the average body weight of shrimp in the control group was the lowest among the treatments (**Figure 3**).

The weekly survival rate of the shrimp was decreasing until harvest. The number of shrimps in the control group significantly (p<0.05) declined starting Week 4 until harvest. Even though it decreased, the survival rate of shrimp with integration still performed better than the control. As shown in the graph at Week 4, up to harvest survival rate was significantly (p<0.05) higher than the control group (**Figure 4**).

Table 2. Growth performance and survival of *L. vannamei* cultured for 60 days.

	Treatments				
	Control	*GM Alone*	*GM+Seaweed*	*GM+Tilapia*	*GM+Seaweed+Tilapia*
ABW (grams)	11.42±0.09[b]	16.10±1.35[a]	16.81±1.48[a]	17.50±0.30[a]	16.11±0.72[a]
Weight Gain (grams)	7.62±0.29[b]	12.34±1.23[a]	12.81±1.48[a]	13.63±0.37[a]	12.27±0.72[a]
Survival (%)	42±1.67[b]	68±4.41[a]	68±6.01[a]	65±5.77[a]	70±2.89[a]
Biomass Gain (kg m^{-3})	1.52±0.23[b]	3.33±0.11[a]	3.43±0.13b[a]	3.56±0.38[a]	3.45±0.34[a]
SGR	1.83±0.10[b]	2.41±0.87[a]	2.37±0.15[a]	2.52±0.06[a]	2.39±0.08[a]
FCR	3.15±0.24[a]	1.67±0.09[b]	1.44±0.17[b]	1.84±0.19[b]	1.55±0.07[b]

Data are presented as Mean±SE (n=3). Values in the same row with different superscripts are significantly different (*p*<0.05).

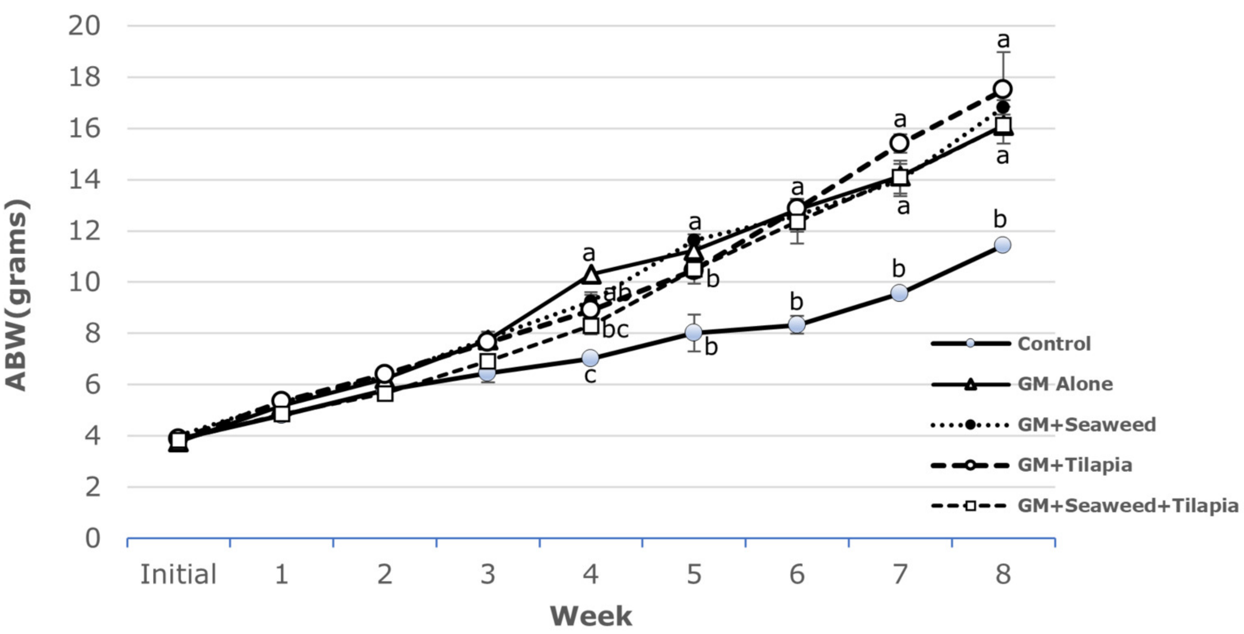

Figure 3. Weekly average body weight of *L. vannamei* cultured for 60 days.

Values are presented as Mean±SE (n=3), and different superscripts are significantly different ($p<0.05$).

DISCUSSION

WATER QUALITY

The shrimp aquaculture industry has proliferated and is continuously growing and developing,[20] and *Litopenaeus vannamei*[21] is the most important penaeid shrimp species farmed worldwide.[22] Along with this development and intensification in white shrimp production is the challenge of water quality deterioration, stress conditions, low feed efficiency, disease outbreaks, and waste disposal.[8,23]

It is important to have cost-effective technologies to sustain the industry and the environment. One efficient and inexpensive way of treating culture effluents is through IMTA.[17,24] It also aims to cultivate various species with different trophic levels that can increase the added value of economic products. Integrated multi-trophic aquaculture both offers ecological and economic benefits.

It is essential to have good water quality throughout the culture period for optimum growth, survival, and shrimp production. In this study, salinity, dissolved oxygen, temperature, and pH have similar values in all treatments. These water quality parameters were within the optimum ranges for growing white shrimp.[25] Even if the culture was done outdoors, the experimental conditions remained the same for all treatments because the experimental unit was shaded with a transparent roof. Moreover, the desired water level was maintained by regularly adding treated seawater from the main reservoir to the experimental units.

Mean values for alkalinity in all treatments with and without integration were lower than the required optimum range of 140-160 mg L^{-1} for growing white shrimp.[25] However, these values were within the suggested value of >75 mg L^{-1} by another study.[26] If the water's alkalinity is below standard, it can be improved by using lime.[27] During the culture period, lime was regularly applied in the experi-

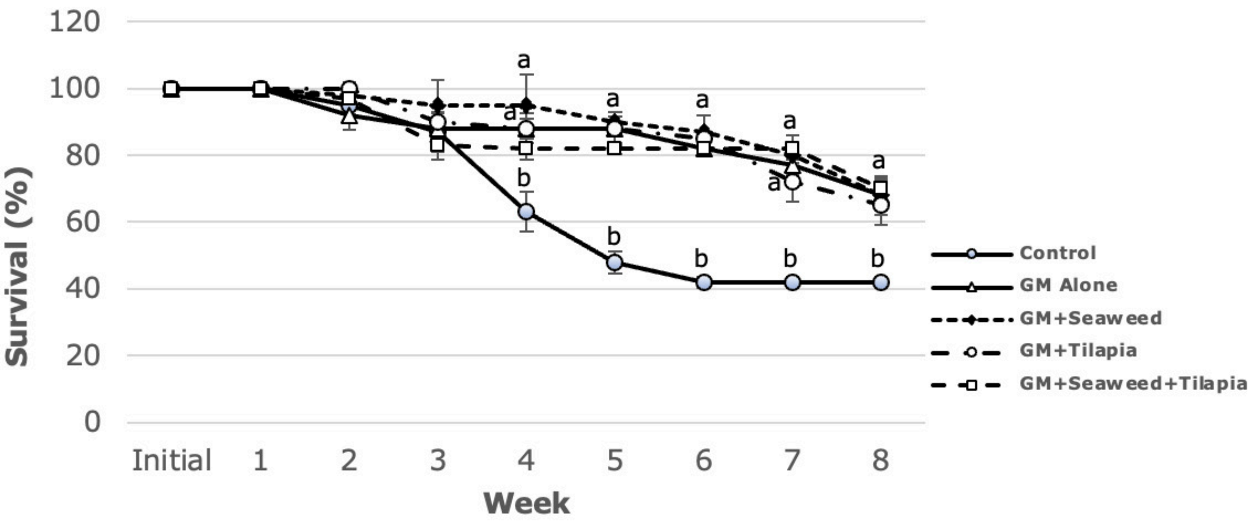

Figure 4. Weekly survival rate of *L. vannamei* cultured for 60 days.

Values are presented as Mean±SE (n=3), and different superscripts are significantly different (p<0.05).

mental units. This contributed to maintaining the pH value. The water pH is also affected by water alkalinity. Alkalinity plays a major role in shrimp culture because of its involvement in the shrimp molting process. Low alkalinity leads to broad pH variations, resulting in reduced growth and even mortality in shrimp.[28]

Total suspended solids (TSS) levels in shrimp with integration of either green mussel, seaweed, or tilapia were within the recommended level of ⩽460 mg L^{-1}.[29] However, TSS mean values for the control exceeded the recommended level, and its mean weekly TSS even reached 1107±81 mg L^{-1}. High TSS levels in the control were probably a stress factor that affected the survival of shrimp. Excess solids in the water can cause rapid oxygen depletion and growth performance problems for the shrimp.[30] The drastic change in the TSS level of the control group might be due to feed inputs because the feed ration was adjusted weekly and was dependent on the body weight and survival rate of the shrimps during stock sampling. It is known that large amounts of particulate organic carbon present as uneaten feed particles, feces, detritus, and microorganisms (bacteria, algae, and zooplankton) compose the total suspended solids in the system.[29] TSS can be controlled by IMTA. The integrated culture of Pacific white shrimp and fish proved efficient in consuming and maintaining TSS levels in the integrated shrimp system.[31] In addition, filter feeders like bivalves[13] and tilapia[32] reduce TSS. Meanwhile, the co-culture of Pacific white shrimp with green seaweed also reduced TSS.[32] These explain why TSS levels in shrimp with integration were more stable throughout the culture period than in the control.

Mean ammonia and nitrite levels for all treatments in this study were within the recommended levels for the culture of white shrimp. The recommended ammonia concentration was <3mg L^{-1}, while nitrite was <10 mg L^{-1} but shrimp could survive in higher concentrations.[25] Tilapia in this study was given tilapia feeds regularly. Additional nitrogen from uneaten tilapia feeds, feces, and urine of tilapia contributed to the high ammonia level of shrimp in GM+Tilapia. Knowing that the water is recirculating from the shrimp tank to the biofiltration tank where additional species were cultured. The amount of ammonia excreted by shrimp and fish depends on the feed, the feeding rates, and the survival rate. However, it was observed that shrimp in the control group had already been exposed to high ammonia levels since the second week of the culture period. In the fourth week, the survival rate of shrimp in the control group started to decline significantly compared to shrimp integrated with either green mussels, seaweed, or tilapia. Ammonia increases oxygen consumption by tissues and gills and reduces the ability of blood to transport oxygen.[28] Thus, longer exposure to high ammonia levels is toxic for shrimp.

The integration system is successful in reducing phosphate-phosphorus concentrations. The phosphorus level obtained in shrimp in the control was significantly higher. Perhaps the presence of green mussels might be the reason why phosphorus levels were lowest in shrimp with green mussels. It is said that bivalves can remove phosphorus through deposition and recycle silicate by transporting it from the water column into the sediment.[13]

With and without integration, most of the water quality parameters in this study were almost similar. Seaweed is an excellent nutrient scrubber, and shellfish sequesters carbon, reducing eutrophication from the culture system.[33] Seaweeds serve as an extractive component for dissolved nutrients in culture and stabilize the cultural environments. Studies on integrated systems of seaweed cultured with other economic aquatic animals have developed rapidly in the past several decades. The genus *Gracilaria* (Rhodophyta) offers high bioremediation efficiency. *Gracilaria* can effectively remove nutrients by using excessive nutrients (e.g. N and P) in the IMTA system of fish, scallops, or shrimp co-cultured with algae.[34] *Gracilariop-*

sis bailiniae can remove nutrients by utilizing both NH_4^+ and NO_3^- levels in an indoor-integrated fish-seaweed culture experiment, and the average outflow concentration of NH_4^+ was reduced from half of its pretreated level.[17] *L. vannamei* with seaweed reduced TAN by 25.9%; NO_2-N by 72.8%, phosphate-24.6%, and TSS 12.9%.[32] In contrast, tilapia has filtering structures that can remove the excess of total suspended solids and be used as a bioremediation agent in IMTA systems.[10] Tilapia in shrimp ponds maintain pH, avoid the toxicity of any unionized ammonia nitrogen, and maintain DO level.[35] In addition, it was found that bacteria isolated from the production of green water from tilapia culture can nitrify ammonia aerobically.[36] *P. viridis* is reported to remove up to 50% ammonia and up to 10% of nitrite from shrimp farms.[37] Combining seaweed and green mussel resulted in a more stable level of ammonia and nitrite favorable for shrimp cultivation.[37] For these reasons, integrations of green mussels, seaweed, and tilapia with shrimp greatly affected most of the water quality parameters in this study.

GROWTH, SURVIVAL, AND FEED EFFICIENCY

Shrimps cultivated by the IMTA system were better than those grown without integration (control). The growth, survival rate, and biomass gain of *L. vannamei* in all treatments were significantly higher in the IMTA system. The survival rate for shrimp with integration was 65%-70%. Water parameters in shrimp with integration were within the suitable levels for the culture of *L. vannamei*, contributing to the higher survival rates of shrimps integrated with either green mussel, seaweed, or tilapia compared to the very low survival rate of 42% in the control. This was almost comparable to the significantly high survival rate (60-71%) by another study in IMTA,[14] wherein the presence of extractive species such as green mussels, seaweed, and tilapia proved to provide better water quality for the shrimp.

The productivity of shrimp was higher in the group with integration than in the control group because of higher survival and weight gain obtained in these treatments. Shrimp with integration resulted in the productivity of 3.33-3.56 kg m^{-3} biomass gain compared to the control with only 1.52 kg m^{-3}. A similar final yield, 3.05 kg m^{-3} of the Pacific white shrimp with the same stocking density of 400 shrimp m^{-2}, was obtained in a zero-water change culture.[38] Total production was higher in IMTA due to better water quality, resulting in better growth performances of shrimp.[13] Moreover, FCR was significantly improved, 1.44-1.84 in shrimp with integration compared to 3.15 in the control. This result was comparable to the 1.6-1.7 FCR of the shrimp integrated with tilapia.[5] Almost the same FCR was observed when cultured intensively in a controlled tank at 300 shrimp m^{-3} and 450 shrimp m^{-3} with 1.40 and 1.53-1.60 FCR, respectively.[27] Lower FCR indicates better feed utilization by shrimps in the integrated system.

The presence of tilapia in the production of Pacific white shrimp enhanced shrimp growth, survival, biomass, health, and production.[36] It can improve feed utilization and increase economic returns.[35] Aside from absorbing reactive nitrogen and improving water quality, seaweed can become a shelter and substrate where natural food is gathered.[37] Shrimp survival rate can increase with the addition of substrate, a place of natural food gathered by the shrimp.[14]

The present findings demonstrated that shrimp can be cultured intensively with either green mussels, seaweed, tilapia, or their combination. The addition of additional species to the shrimp culture system did not negatively affect the water quality. This resulted in better growth performance and improved shrimp production due to the better quality created by integrating tilapia, seaweed, and green mussels.

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AUTHORS' CONTRIBUTION

Conceptualization: BESSIE JOY G. ELLE (Lead). Methodology: BESSIE JOY G. ELLE (Lead), MARY JANE APINES-AMAR (Supporting). Formal Analysis: BESSIE JOY G. ELLE (Lead), ROSY L. JANEO (Supporting), MELANIE P. GEN-ODEPA (Supporting). Investigation: BESSIE JOY G. ELLE (Lead), ROSY L. JANEO (Supporting), MELANIE P. GEN-ODEPA (Supporting). Writing – original draft: BESSIE JOY G. ELLE (Lead). Funding acquisition: BESSIE JOY G. ELLE (Equal), MARY JANE APINES-AMAR (Equal). Supervision: BESSIE JOY G. ELLE (Lead). Writing – review & editing: MARY JANE APINES-AMAR (Lead).

COMPETING OF INTEREST — COPE

No competing interests were disclosed with respect to the research, authorship, and/or publication of this article.

ETHICAL CONDUCT APPROVAL — IACUC

Experimental procedures involving plants and animals were carried out within the ethical guidelines. All efforts were made to ameliorate any sufferings of the experimental animals.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

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