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Integrated Multitrophic Aquaculture of Molobicus Tilapia, White Shrimp (*Penaeus vannamei*) and Seaweed (*Gracilaria* sp.) in Tanks

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Abstract - Integrated Multitrophic Aquaculture System (IMTA) is a polyculture system which grows organisms from different level of the food chain in one environment where wastes from one species serves as the source of food or nutrients for other organisms. In this study, the IMTA system was tried using Molobicus tilapia (main cultured and fed species), white shrimp (unfed species) and *Gracilaria* sp. (as biofilter species). The study aimed to determine the growth and survival of Molobicus Tilapia at different stocking densities (20, 40, and 60 fish per tank) co-cultured with *P. vannamei* and seaweed *(Gracilaria heteroclada)* in round plastic tanks and to monitor the different water parameter in terms of dissolved oxygen, pH, temperature, ammonia, nitrite, and nitrate. Results showed no significant differences in weight gain, length gain, specific growth rates (weight and length), and survival rates of tilapia at the different stocking densities tested. However, in terms of growth rates and survivorship of the white shrimps, there were significant differences among treatments. Treatment III (highest stocking density of tilapia) showed fastest growth in terms of length and weight of the white shrimp, but also resulted to lowest survival rates as compared to Treatments I and II (lower stocking densities of tilapia). Faster growth of white shrimps was observed in Treatment III because of the higher feed ration given to the tilapia as well as higher amount of wastes in the form of uneaten food and fecal matter. However, the higher stocking densities of tilapia in Treatment III also resulted to a density-dependent effect on survival rates of the white shrimps. During the course of the 75-day culture period, there was a decreasing trend in the biomass of the seaweeds (*Gracilaria* sp.), which coincided with increasing trend of nitrogenous compounds such as ammonia, nitrite, and nitrate, which was more pronounced in higher stocking densities of tilapia in Treatment III. High ammonia levels are well known to negatively affect growth and survival rates of aquatic organisms.

Key words*:* Molobicus tilapia; *Penaeus vannamei*; *Gracilaria*; biofilter; polyculture

INTRODUCTION

Globally, aquaculture continues to be the fastest growing animal food-producing sector. The world capture fishery production has been relatively static since the late 1980s, as compared to the growth of aquaculture in providing supply of fish for human consumption [1]. Hence, aquaculture has always been considered as an option to provide the fish supply to the ever-increasing population. However, aquaculture has also been blamed because of the environmental impacts of the effluents on the receiving ecosystems. Therefore, only aquaculture systems that are environmentally sustainable must be encouraged.

The so called Integrated Multitrophic Aquaculture System (IMTA) uses species that are not only commercially valuable, but are also environmentally sustainable. Itco-cultures organisms from different level of the food chain in one environment where wastes from one species serves as the source of food for another and unconsumed feeds and other organic matter serves as fertilizer for the growth of plants like seaweeds. The idea of co-culturing two or more species from different level of food chain in the same environment maximizes production per unit per time, thus increasing production and creating a healthier environment [2].

Recent precautionary approach to responsible aquaculture, developed from traditional extensive polyculture, integrate the culture of fish or shrimp with vegetables, microalgae, shellfish and/or seaweeds. Integrated mariculture can take place in coastal waters or in ponds and can be done intensively. These nutrient-assimilating photoautotrophic plants use solar energy to turn nutrient-rich effluents into profitable resources. Plants counteract the environmental effects of the heterotrophic fed fish and shrimp and restore water quality [3].

In the Philippines, culturing of salt tolerant tilapia (Molobicus tilapia) as the major species with white shrimp (*Penaeus vannamei*) and seaweeds (*Gracilaria sp.*) is new and worth testing as the combination of species fits the criteria for an IMTA system. An earlier study on the culture of Molobicus tilapia, white shrimp and seaweeds in tanks showed promising results. In an earlier study, Molobicus tilapia were stocked at 20, 30, and 50 fish per tank (126-L capacity) with each tank having similar stock of white shrimp and seaweeds, the results showed no significant differences in growth performance in terms of weight and length of Molobicus tilapia and white shrimp [4]. Hence, another study was done to verify the previous results but using higher stocking densities of Molobicus tilapia, the main cultured species.

Generally, the study aimed to determine the growth and survival of Molobicus Tilapia at different stocking densities co-cultured with *P. vannamei* and seaweed *(Gracilaria heteroclada)* in round plastic tanks. Specifically, the study aimed to: (1) determine the mean weight and length gain, specific growth rates, and survival rate of the Molobicus tilapia, the main cultured and fed species; (2) to determine the mean weight and length gain, specific growth rates, and survival rate of the *P. vannamei*, the unfed species relying on uneaten feeds and excreta of tilapia; (3) monitor the growth of the seaweed *(Gracilaria heteroclada)*, the biofilter species; and (4) monitor the different water parameter in terms of dissolved oxygen, pH, temperature, ammonia, nitrite, and nitrate.

MATERIALS AND METHODS

Research Design

The study employed the experimental method of research. It consisted of three treatments with three replicates arranged in a completely randomized design (CRD). Molobicus Tilapia was co-cultured with *P. vannamei* and *Gracilaria sp.* in round plastic tanks and the growth performance and survival of the cultured species were monitored within 75 days. The growth parameters that were measured includes weight gain, length gain, specific growth rate and survival rate.

Location of the Study

The study was conducted at Pangasinan State University – Binmaley Campus using nine plastic drums filled with brackishwater. The location map of the Pangasinan State University – Binmaley Campus where the study was conducted is shown in Figure 1.

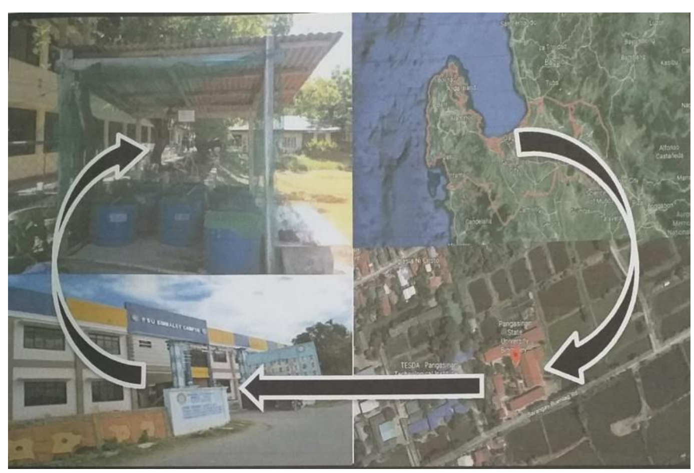

Figure 1. Location map of PSU-Binmaley Campus, Binmaley, Pangasinan.

Experimental Treatments

The study was composed of three (3) Treatments, I, II and III with three replicates (Table 1). Each treatment had 20, 40, and 60 pieces of Molobicus Tilapia stocked per round plastic tank or drum, respectively.

Table 1. Experimental treatments of the study.

Treatment	Molobicus Tilapia (pcs.)	*P. vannamei* (pcs.)	*Gracilaria sp.* (grams)
I	20	20	200
II	40	20	200
III	60	20	200

Experimental Units

Nine plastic round tanks or drums with a measurement of 59 cm in diameter, 46 cm in height and 38.5 cm water depth. The drums were cleaned with soap and water and filled with 125.76 liters of brackish water. They were provided with aeration to ensure sufficient amount of dissolved oxygen. Saltwater was obtained from San Isidro Norte beach. Salinity was maintained at 25 ppt. Drums were covered with nets to avoid loss of stocks.

Figure 3. Culture units used in the study.

Experimental Organisms

Molobicus Tilapia

Three hundred sixty pieces of Molobicus Tilapia with the same sizes ranging from 2-3 cm in length and weight of 0.21-0.48 g were used in the study. Fish were obtained from BFAR-NIFTDC, Bonuan Binloc, Dagupan City. Molobicus Tilapia was the main or fed species stocked in tanks filled brackishwater with a salinity of 25 ppt.

White Shrimp

One hundred eighty pieces of *P. vannamei* with the same sizes (PL20) ranging from 1.5-2.5 cm in length and weight of 0.2-0.15g were used in the study. They were obtained from FSI Shrimp Hatchery in Labrador, Pangasinan. They were co-cultured with the Molobicus Tilapia and *Gracilaria* sp. to utilize the fecal matter and uneaten feeds in the bottom which is their habitat.

Seaweeds

A total of 1.8 kg of *Gracilaria heteroclada* was used in the study. These were obtained from ponds in Barangay Biec, Binmaley, Pangasinan. This aquatic plant is capable of absorbing excess metabolites of tilapia and white shrimp, thus, avoiding its build-up in the system [5]. It also served as shelter and habitat for the *P. vannamei.*

Stocking and Sampling

Before stocking, the experimental organisms were acclimatized for two to three days before increasing the salinity of the water until it reached the required salinity. The initial weight and length of the stocks were measured as well as the weight of *Gracilaria sp.* using digital weighing scale (OHAUS) while the total length of the fish was measured to the nearest 1 mm using a Vernier caliper. The succeeding samplings were done every day 15 days for a total of 75 days of culture period.

Each plastic drum in each Treatment was stocked with twenty pieces of *P. vannamei*, and two hundred grams of *Gracilaria sp.* whereas Molobicus Tilapia were stocked at 20, 40, and 60 pieces as Treatment I, II, and III respectively.

Stocking of experimental organisms in each plastic drum was done early in the morning (6:00-9:00 A.M) and this was done at random following the experimental design of the study. Scoop net was used to avoid stress to the fish.

Feeds and Feeding

Floating commercial pellet feed (Tateh feeds) was used as feed for the Molobicus Tilapia. The Molobicus Tilapia were fed correspondingly at 5% of their body weight throughout the culture period. The feeding was done thrice a day at 9 am, 12 noon and 3 pm by broadcasting method.

Water Quality Monitoring

The water parameters such as the dissolved oxygen and water temperature were done daily every 6:00 am using a D.O meter (YSI) and a pH meter for the water pH. The salinity, ammonia, nitrite and nitrate were monitored once a week every 6:00 am by using refractometer (ATAGO) and saltwater master kit (API), respectively.

Data Gathering Procedures

Data on growth performance of Molobicus Tilapia, *P. vannamei* and *Gracilaria sp.* were computed using the following formula:

Absolute Growth

Weight

Total growth in weight (W) were computed using the following formula:

Mean Weight gain (g) = Final Weight (g) – Initial Weight (g)

Length

Total growth in Length (TL) were computed using the following formula:

Mean Length gain (cm) = Final Length (cm)- Initial Length (cm)

Specific Growth Rate

SGR= (ln final weight – ln initial weight)/ days X 100

where: ln=natural log

Survival Rate (%)

The survival rate was measured by its percentage of Molobicus Tilapia and *P. vannamei* surviving from the first day until the last of the experiment.

SR = Final Number of Stocks/Initial Number of Stocks x 100

Analysis of Data

Growth performance and survival rate were analyzed by the use of Analysis of Variance (ANOVA) to determine any significant differences among treatment means with the use of Microsoft Excel (2010).

RESULTS AND DISCUSSION

Growth Molobicus Tilapia in Terms of Weight

Table 2 shows the absolute growth in weight of Molobicus Tilapia. In Treatment I, they grew from a mean initial weight of 1.63 g to a mean final weight of 18.98 g after 75 days. On the other hand, tilapia in Treatment II grew from 1.72 g to 16.86 g and in Treatment III, fish grew from 1.54 g to 15.35 g.

In terms of absolute weight gained, tilapia in Treatment I had obtained the highest with 17.35 g followed by Treatment II and Treatment III with 15.14 g and 13.81 g respectively. However, the statistical analysis revealed no significant differences (P>0.05) among the treatments.

Table 2. Growth of Molobicus Tilapia in the 75-day culture period in terms of weight.

Treatments	Culture Period (Days)						Weight Gain	SGR (%/d)
	Initial	Day 15	Day 30	Day 45	Day 60	Day 75		
I (20pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)								
R1	1.53	2.65	4.76	7.55	15.60	21.13	19.60	3.50
R2	1.60	3.00	4.27	8.19	13.46	18.25	16.65	3.25
R3	1.77	2.77	4.53	8.50	13.50	17.57	15.80	3.06
Mean	**1.63**	**2.81**	**4.52**	**8.08**	**14.19**	**18.98**	**17.35^{ns}**	**3.27^{ns}**
II (40pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)								
R1	1.69	2.83	4.70	9.41	13.45	18.21	16.52	3.17
R2	1.77	2.93	4.64	9.83	13.02	17.87	16.10	3.08
R3	1.69	2.93	4.86	7.32	10.61	14.50	12.81	2.87
Mean	**1.72**	**2.90**	**4.73**	**8.85**	**12.36**	**16.86**	**15.14^{ns}**	**3.04^{ns}**
III (60pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)								
R1	1.52	3.00	4.47	9.49	12.13	16.51	14.99	3.18
R2	1.52	2.89	5.11	9.17	12.60	15.45	13.93	3.09
R3	1.59	2.80	4.50	7.93	10.10	14.09	12.50	2.91
Mean	**1.54**	**2.90**	**4.69**	**8.86**	**11.61**	**15.35**	**13.81^{ns}**	**3.06^{ns}**

ns = not significant

In terms of specific growth rate in terms of weight, the highest value was attained in Treatment 1 at 3.27%, followed by Treatments 3 and 2 at 3.06% and 3.04%, respectively. However, the ANOVA revealed no significant differences among the treatment means ($P>0.05$).

In a similar experiment by Casing et al. (2019) [4] using the same species combination but different stocking densities of Molobicus tilapia at 20, 30, and 50 fish per drum, they found no significant differences in weight gains (8.10, 7.50, and 8.30 g for Treatments I, II, and III, respectively) among the different treatments after 75 days of culture. However, the present study produced larger fish at the end of the 75-day experiment than the earlier study [4]. No significant differences in SGRs (weight) were also noted by Casing et al. (2019) in their study [4].

Growth of Molobicus Tilapia in Terms of Length

Table 3 shows the growth performance of Molobicus tilapia in terms of length.

Table 3. Growth of Molobicus Tilapia in the 75-day culture period in terms of length.

Treatments	Culture Period (Days)						Length Gain	SGR (%/d)
	Initial	Day 15	Day 30	Day 45	Day 60	Day 75		
I (20pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)								
R1	4.66	5.26	6.36	7.55	9.77	11.37	6.71	1.19
R2	4.62	5.92	6.28	7.70	9.60	10.40	5.78	1.08
R3	4.49	5.70	6.20	7.88	9.40	10.52	6.03	1.14
Mean	**4.59**	**5.63**	**6.28**	**7.71**	**9.59**	**10.76**	**6.17^{ns}**	**1.14^{ns}**
II (40pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)								
R1	4.85	5.92	6.37	8.18	9.13	10.58	5.73	1.04
R2	4.63	5.62	6.27	8.24	8.96	10.14	5.51	1.05
R3	4.55	5.62	6.42	7.60	8.89	10.22	5.67	1.08
Mean	**4.68**	**5.72**	**6.35**	**8.00**	**8.99**	**10.31**	**5.64^{ns}**	**1.05^{ns}**
III (60pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)								
R1	4.52	5.68	6.31	7.95	8.56	10.22	5.70	1.09
R2	4.55	5.94	6.56	8.03	8.67	10.20	5.65	1.08
R3	4.37	5.43	6.33	7.72	8.71	9.90	5.53	1.09
Mean	**4.48**	**5.68**	**6.40**	**7.90**	**8.65**	**10.11**	**5.63^{ns}**	**1.08^{ns}**

ns = not significant

As shown in the table above, Treatment I again obtained the highest mean length gain of 6.17 cm followed by Treatment II with 5.64 cm and Treatment III with 5.63 cm after the 75-day culture period. Highest SGR of Molobicus Tilapia was recorded in Treatment I with 1.14% per day followed by Treatment III with SGR of 1.08% per day and lastly, Treatment II with 1.05% per day. However, ANOVA shows no significant differences (P>0.05) among the treatment means.

Similar results were obtained in an earlier study [4] where there were no significant differences in length gain and SGRs (length) observed among treatment means.

Growth of White Shrimp in Terms of Weight

Table 4 presents the mean weight gain of the White Shrimp (*Penaeus vannamei)* during the 75-day Culture Period. Highest weight gain was achieved in Treatment 3 at 3.61 grams followed by Treatments 2 and 1 at 2.55 g and 2.04 g, respectively. Based on the result of the Analysis of Variance, Treatment III gaining the highest weight gain was statistically different from Treatments I and II. Treatments I and II were not statistically different from each other (P>0.05).

Table 4. Growth of White Shrimp *(Penaeus vannamei)* in 75 days in terms of weight gain and SGR.

Treatments	Culture Period (Days)						Weight Gain (g)	SGR (%/d)
	Initial	Day 15	Day 30	Day 45	Day 60	Day 75		
1 (20pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)								
R1	0.09	0.31	0.43	0.51	0.98	1.96	1.87	4.11
R2	0.08	0.28	0.36	0.45	1.01	2.12	2.04	4.37
R3	0.11	0.45	0.63	0.72	1.15	2.31	2.20	4.06
Mean	**0.09**	**0.35**	**0.47**	**0.56**	**1.05**	**2.13**	**2.04[b]**	**4.18[b]**
2 (40pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)								
R1	0.08	0.39	0.53	0.66	1.76	2.89	2.81	4.78
R2	0.09	0.55	0.64	0.70	1.78	2.14	2.05	4.23
R3	0.12	0.40	0.51	0.63	1.08	2.92	2.80	4.26
Mean	**0.10**	**0.45**	**0.56**	**0.66**	**1.54**	**2.65**	**2.55[b]**	**4.42[b]**
3 (60pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)								
R1	0.10	0.38	0.55	0.76	1.89	3.56	3.46	4.76
R2	0.07	0.40	0.49	0.65	1.78	3.76	3.69	5.31
R3	0.13	0.50	0.61	0.75	1.97	3.80	3.67	4.50
Mean	**0.10**	**0.43**	**0.55**	**0.72**	**1.88**	**3.71**	**3.61[a]**	**4.86[a]**

Significant at 0.05 level
**having the same letter shows no significant different from each other*

In terms of specific growth rate in weight of white shrimp, Treatment III attained the highest SGR with a mean of 4.86% per day followed by Treatments II and I with mean values of 4.42% and 4.18%, respectively. The results of the study showed that the higher stocking density of Molobicus Tilapia, the higher the feeding ration given to them resulting to higher release of excreta and uneaten feeds that served as food for the white shrimp *(P. vannamei)*, thus contributing to their growth. Analysis of Variance revealed that Treatment III was significantly different from Treatments I and II ($P<0.05$).

The above results are in stark contrast to that observed by Casing et al. (2019) in their earlier study [4]. They observed no significant differences in weight gains of white shrimp among the different treatments.

Growth of White Shrimp in Length

Length gain by the White Shrimp *(P. vannamei)* throughout the 75-day Culture Period is shown in Table 5. Treatment III obtained the highest increment having a mean length gain of 5.45 cm followed by Treatment II with a mean value of 4.57 cm.

Table 5. Growth of White Shrimp *(Penaeus vannamei)* in 75-day culture period in terms of length.

Treatments	Culture Period (Days)						Length Gain	SGR (%/d)
	Initial	Day 15	Day 30	Day 45	Day 60	Day 75		
I (20pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)								
R1	2.42	3.67	4.61	4.87	5.12	6.02	3.60	1.22
R2	2.45	3.70	4.31	4.42	4.94	6.50	4.05	1.30
R3	2.49	4.04	4.68	4.81	5.09	6.45	3.96	1.27
Mean	**2.45**	**3.80**	**4.53**	**4.70**	**5.05**	**6.32**	**3.87**[b]	**1.26**[b]
II (40pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)								
R1	2.43	4.49	4.42	4.52	5.25	7.12	4.69	1.43
R2	2.51	4.37	4.89	4.94	5.76	6.95	4.44	1.36
R3	2.58	3.92	4.66	4.79	5.68	7.15	4.57	1.36
Mean	**2.51**	**4.26**	**4.66**	**4.75**	**5.56**	**7.07**	**4.57**[b]	**1.38**[b]
III (60pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)								
R1	2.26	3.87	5.11	5.24	6.01	7.80	5.54	1.61
R2	2.39	4.4	4.22	4.45	5.78	7.40	5.01	1.51
R3	2.70	4.07	4.92	5.19	7.46	8.50	5.80	1.53
Mean	**2.45**	**4.11**	**4.75**	**4.96**	**6.42**	**7.90**	**5.45**[a]	**1.56**[a]

Significant at 0.05 level
**having the same letter shows no significant different from each other*

On the other hand, Treatment I got the lowest increment with 3.87 cm after the 75-day culture period. ANOVA revealed that there were significant differences (P>0.05) among the treatments. Treatment III which has the highest length gain was significant from Treatments I and II.

In terms of SGR of white shrimp *(P. vannamei)* in the 75-day culture period in terms of length, Treatment III achieved the highest increment with a mean value of 1.56% per day followed by Treatment II with a mean value of 1.38% per day and Treatment I with the lowest increment of 1.26% per day. ANOVA revealed that Treatment III which has the highest length obtain had significant difference (*P*>0.05) from the other two treatments.

The above results are different from the earlier study of Casing et al (2019) [4] because they observed no significant differences in length gains and SGRs (length) of white shrimp in the different treatments used. They used similar species combinations of Molobicus tilapia, white shrimp and seaweeds.

Growth Rate of Seaweed *(Gracilaria Heteroclada)*

Table 6 shows the growth performance of seaweed *(Gracilaria heteroclada)* in terms of weight gain throughout the 75-day culture period.

At stocking, all treatments had similar initial weights of 200 grams seaweed *(Gracilaria heteroclada).* During the first 30 days, all treatments gained weight. Treatment I attained the highest increment of 224.17 grams followed by Treatments II and Treatment III with an increment of 220.58 grams and 219.31 grams, respectively. On the 45th day, weight of seaweeds in all treatment decreased constantly until the end of the experiment. Only seaweeds in Treatment 1 showed positive growth at the end of the experiment.

The present results revealed a difference from the data gathered in an earlier similar study [4]. That study revealed that seaweeds *(Gracilaria sp.)* in all treatments attained an increase on the final weight gain after the 75-day culture period. Another probable reason for the low growth of seaweeds in the present study was that the present study was conducted from 20 December 2019 to 5 March 2020 at a time when thallus of *Gracilaria* were not yet fully developed whereas the earlier study [4] was conducted from January 24, 2019 to April 8, 2019 which was already the start of the dry season when *Gracilaria* abounds in nearby brackishwater ponds in Binmaley, Pangasinan.

Tilapia is an omnivorous fish. Young tilapia feeds by grazing algal and bacterial films on hard surface as well as phytoplankton and zooplankton in extensive culture systems, making them a very good filter feeder in its young stage of life but as it grows, it becomes less effective as filter feeders. On the other hand, adult tilapia feeds on macroscopic plants and algae [6]. Hence, it is possible that the larger-sized tilapia produced in the present study nibbled on the seaweeds hindering its growth. On the other hand, shrimp in its larval stage of life feeds on phytoplankton initially and afterwards on zooplankton. Shrimp can be both omnivore and detritivore in its feeding habit. It feeds on decaying matter of plants and animals and may scrape algal and bacterial films on pond sediments [6].

In the aspect of polyculture of tilapia and shrimp, both organisms occupy different niches in the pond. Shrimp occupies the bottom of the pond and tilapia occupies the water column. Tilapia mainly filter feed the plankton in the water column. The fecal matter of tilapia which acts as a detrital rain supports feed for the shrimps at the bottom. Shrimp feeds on the detrital matter at the bottom consisting of algae, bacteria and fecal matter of tilapia [7].

As a matter of fact, many farmers in Asia have adopted tilapia and shrimp polyculture method to counteract disease problems associated with intensive culture of shrimps alone [6, 8]. In this process, tilapia is considered as a bioremediator species [8], specifically that it was believed that the addition of tilapia helps in the suppression of luminous bacteria and helps improve sediment and water quality.

Table 6. Growth of seaweed *(Gracilaria heteroclada)* in terms of weight gain after the 75-day culture period.

Treatments	Culture Period						Weight Gain
	Initial	Day 15	Day 30	Day 45	Day 60	Day 75	
I (20pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)							
R1	200	215.23	221.72	218.21	215.68	208.31	8.31
R2	200	214.68	222.65	219.13	214.97	209.12	9.12
R3	200	220.94	228.12	223.41	217.88	211.65	11.65
Mean	**200**	**216.95**	**224.16**	**220.25**	**216.18**	**209.69**	**9.69**
I (40pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)							
R1	200	211.13	218.26	210.13	197.35	189.2	-
R2	200	214.52	219.34	211.45	198.12	190.43	-
R3	200	218.34	224.14	213.67	201.23	191.9	-
Mean	**200**	**214.66**	**220.58**	**211.75**	**198.9**	**190.51**	**-**
I (60pcs Tilapia, 20 pcs *P. vannamei* and 200g *Gracilaria sp.*)							
R1	200	210.67	215.67	203.13	194.78	178.92	-
R2	200	209.81	216.12	207.9	185.19	170.13	-
R3	200	221.02	226.14	207.56	182.9	169.19	-
Mean	**200**	**213.83**	**219.31**	**206.20**	**187.62**	**172.75**	**-**

Survival Rate of Molobicus Tilapia and White Shrimp

Tables 7 and 8 showcased the survival rates of the Molobicus Tilapia and White Shrimp *(Penaeus vannamei)* after the 75-day culture period.

Treatment I attained the highest survival rate of 100% followed by Treatment II with 88.33% and Treatment III with the lowest survival rate of 83.33%. These observed mortalities may be attributed to mishandling during sampling. After the 75-day culture Period, 14 pieces of Molobicus tilapia were found dead in Treatment II whereas 30 pieces were found dead in Treatment III. Tilapia stocked at higher densities are more prone to stress. This could also be related to high ammonia levels in the higher stocking densities of tilapia.

Survival rates observed in the study is lower compared to that observed in a similar study [4]. In that study, they observed 98.33%, 96.67% and 88% survivorship for Treatments 1, II, and III, respectively.

Table 7. Survival rate of Molobicus Tilapia in the 75-day culture period.

Treatments	R1	R2	R3	Mean
I	100.00	100.00	100.00	100.00[a]
II	87.50	85.00	92.50	88.33[b]
III	81.67	83.33	85.00	83.33[b]

Table 8. Survival rate of white shrimp *(Penaeus vannamei)* in the 75-day culture period.

Treatments	R1	R2	R3	Mean
I	90	85	85	86.67[a]
II	75	90	85	83.33[a]
III	55	65	55	58.33[b]

As shown on the Table above, Treatment I attained the highest survival rate of 86.67% followed by Treatment II with 83.33% and Treatment III got the lowest survival rate of 58.33%.

The survival rate of white shrimp in the present study was much lower compared to the survival rate of White Shrimp *(Penaeus vannamei)* in the study conducted by Casing et al. (2018) who observed 88.33-98.33% survivorship of white shrimps in their study [4].

Water Quality Parameters

Figure 4 shows the graphical presentation of the increase of ammonia concentration in the study caused by excretal matter from Molobicus Tilapia and uneaten feeds throughout the 75-day culture period. Ammonia concentration was highlighted in this study as it is known to affect the growth and survival of the experimental organisms.

The build-up of ammonia concentration started on the fifth week where there was a decrease in weights of seaweeds. As the seaweed biomass decreased, ammonia concentration also increased. Treatment III with 60 pieces of Molobicus Tilapia had the highest concentration of ammonia followed by Treatment II with 40 pieces of Molobicus Tilapia and Treatment I with 20 pieces of Molobicus Tilapia has the lowest concentration of ammonia during the 75-day culture period. It was observed that the higher the stocking density, the higher the production of excretal matter resulting to rapid increase of ammonia concentrations.

Result of this study was different from the result stated on Casing et al. (2018) [4]. Their study revealed that water quality parameters were optimal due to the increase of the seaweed *(Gracilaria heteroclada)* which served as the biofilter.

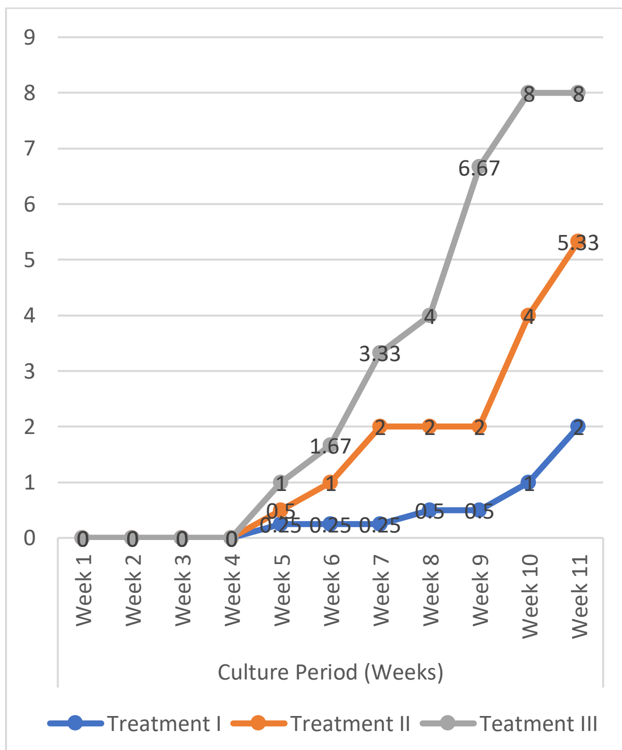

Figure 4. Graphical presentation of the weekly increase of ammonia concentration (ppm) on the study throughout the 75-day culture period.

Table 9 presents the mean water temperature, pH, and dissolved oxygen which were monitored daily at 6:00 – 7:00 am.

Water Parameters in this study are lower than that observed by Casing et al. (2019) specifically temperature, because of the season when the study was conducted [4]. The study was conducted on the month of December to February, which are the coldest months. Lower temperature will result to lower production of dissolved oxygen which leads to the growth development reduction of the cultured species.

Table 9. Mean temperature, dissolved oxygen, pH, salinity, nitrite, and nitrate monitoring in the 75-day culture period.

Treat-ments	Temp (˚C)	pH	DO	Salinity (ppt)	Nitrite (ppm)	Nitrate (ppm)
I	23.8	6.34	4.13	25.18	0.43	8.64
II	24.5	6.39	3.56	25.27	1.59	28.18
III	24.9	6.49	3.24	25.36	16.55	61.81

As shown on the Table above, salinity in all of the Treatments ranged from 25.18-25.36 ppt. According to Westly et al. 2016, good growth of Molobicus Tilapia is achieved at salinity ranging from 15 to 35 ppt. Nitrite (NO_2) and Nitrate (NO_3) concentration also increased with increases in the stocking densities. Based on the results, nitrate and nitrite concentrations increased as ammonia increased as these water parameters are related with each other. Furthermore, the exposure to a high concentration of these parameters, leads to poor growth development and lower survival rate of the experimental organisms.

In analyzing the absorption efficiency and kinetic parameters of the seaweed *Gracilaria cervicornis* for nutrients NH_4^+, NO_3^- and PO_4^{3-}, results showed a reduction of as much as 85.3, 97.5 and 81.2% for NH_4^+, NO_3^- and PO_4^{3-}, respectively [9]. These results suggest that this algal species has good absorption capacity for the nutrients tested and may be a promising candidate as a bioremediator of eutrophized environments. As in certain traditional polyculture schemes, seaweeds can significantly reduce feed use and environmental impact of intensive mariculture and at the same time provide additional income. These nutrient-assimilating seaweeds use solar energy to turn nutrient-rich effluents into profitable resources. Plants mitigate the environmental effects of the heterotrophic fed fish and shrimp and restore water quality [3].

CONCLUSIONS

The different stocking densities of tilapia used in the present study did not affect the growth performances and survival rates of Molobicus tilapia, the main cultured species. However, the different stocking densities positively affected the growth rates of the white shrimp as a result of higher food ration given to tilapia which resulted to higher wastes such as uneaten feed and fecal matter whereas it also negatively affected the survival rates of white shrimp. The decrease in biomass of seaweeds as the experiment progressed resulted to high levels of nitrogenous compounds such as ammonia, nitrite, and nitrates, which may also negatively affect survival rates of shrimps. It is recommended to practice partial harvesting of tilapia at higher stocking densities as they grow and to ensure the presence of seaweeds to serve its role as biofilter under this polyculture system. This IMTA system may also be tried using higher value finfish such as rabbitfishes alongside the white shrimp and seaweeds.

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