Original Article

Production, remediation, health management, and economic returns of pond-based multi-trophic aquaculture giant tiger shrimp (*Penaeus monodon*) in varying feeding rates

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Abstract: An analysis and understanding of the proof of concept of Integrated Multi-Trophic Aquaculture (IMTA) technology, with varying feeding rates, in the context of disease and environmental management for better production of pond-based semi-intensive *Penaeus monodon* aquaculture, is evaluated in this study. The grow-out culture experiment was conducted for four months (120 days), utilizing eighteen units of 250 m^2 ponds to hold the experimental trials. Six treatment combinations in triplicate were employed, comprising *P. monodon* as the main cultured species, with *Chanos chanos* and *Oreochromis niloticus* as the co-cultured species. The nutrient extractive species consisted of macroalgae *Ulva fasciata* and *Gracilaria verrucosa*, and mussel *Perna viridis*, at 2.5 and 5% feeding rates. The growth and survival of shrimp and finfish showed no significant difference among all treatments. Water quality levels among the treatments remained within optimal ranges. Shrimp reared within the shrimp-tilapia system and subjected to a 5% feeding rate manifested infection with White Spot Syndrome Virus (WSSV). Net revenue between the two feeding rates was consistently better (129.72-167.69 USD) at 2.5% feeding rates across all treatments. Overall, the shrimp-tilapia and IMTA systems demonstrated superior management and production performances compared to the shrimp polyculture system in terms of shrimp and fish production, plankton diversity, nutrient levels, and disease management. A 2.5% feeding rate is deemed appropriate for a shrimp-milkfish-*Gracilaria*-green mussel IMTA system.

Introduction

Penaeus monodon, commonly known as the giant tiger shrimp, holds paramount economic significance among penaeid shrimp species in the Indo-West Pacific region, encompassing aquaculture and wild capture sectors (Alam and Pálsson, 2016). This breakthrough led to substantial aquacultural expansion and revenue generation in the Philippines during the 1980s (Primavera, 1992; Rosario, 2005). Improper farming practices in the shrimp industry, such as high stocking densities and excessive use of feed, have led to environmental deterioration and disease outbreaks, ultimately resulting in the industry's collapse in the 1990s (Fast, 2000; Macusi, 2022). The industry's transition from milkfish to prawn intensified these adverse effects (Macusi, 2022). To revive the sector, adopting good aquaculture practices, improving environmental management, and prioritizing disease control are crucial (Macusi, 2022). Additionally, innovations in shrimp pond culture, such as the use of specific pathogen-free and specific pathogen-resistant shrimp seed, reduced water exchange, and biosecure systems, have been introduced to address these issues (Fast, 2000). Further, nutrient cycling is equally essential to establishing an ecological balance in an aquaculture system and achieving sustainable production.

Despite the risks and bottlenecks, *P. monodon* remains the Philippines' third most important aquaculture animal species (BFAR, 2019) due to its high market price and demand. Studies addressing deteriorating environments have recently focused on environment-friendly shrimp farming technologies, including green water, bio-floc, immunostimulants,
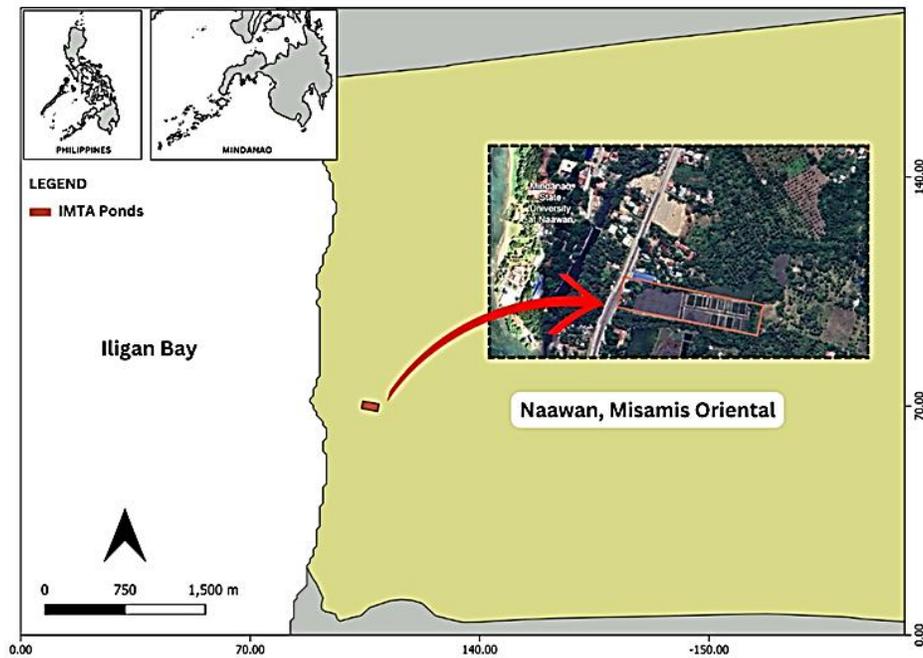
Figure 1. Experimental set-up of Integrated Multi-Trophic Aquaculture (IMTA) subdivided ponds located in Naawan, Misamis Oriental, Philippines (8°25'34"N, 124°17'27"E).

probiotics, and integrated multi-trophic aquaculture (IMTA), as they yield promising and improved production results. Studies have shown the potential of IMTA and algal remediation technologies in balancing ecological conditions and maximizing production. Silkina (2019) and Nardelli (2018) highlight algal biotechnology's effectiveness in waste remediation and nutrient recovery, with the latter specifically focusing on the use of *Ulva lactuca* in an IMTA system. Aubin (2017) further emphasizes the potential of IMTA in increasing system efficiency and reducing waste effluents. These techniques have garnered the attention of aquacultural researchers worldwide as a potential intervention to mitigate environmental degradation. However, documented studies in IMTA showed limited trophic species combinations, contrary to the popular algal green water technology. Moreover, information on the biological composition, particularly the bacterial communities in IMTA, is limited and site-specific.

Understanding feeding strategies in *P. monodon* is crucial for optimizing growth and feed utilization (Arnold et al., 2016). This encompasses factors such as the frequency and ration size of diets and the incorporation of microbial biomass, which can significantly enhance growth (Arnold et al., 2016). The feeding behavior of *P. monodon*, characterized by a preference for animal protein, further highlights the importance of comprehensively understanding their feeding strategies (Hag, 1984). Feeds in *P. monodon* aquaculture constitute the main production expenses. Understanding feeding rates in *P. monodon* aquaculture is vital for maximizing growth and minimizing waste production. Mohanty et al. (2004) emphasize that the aquaculture system's stocking density influences increased feed conversion ratio and sedimentation rate. Is it therefore that the feeding rate in a system warrants careful consideration? In this study, the proof of concept of IMTA technology with varying feeding rates is analyzed and evaluated for *P. monodon*, with a focus on disease and environmental management to enhance production.

Materials and Methods
Experimental design and species combinations: The experiment was conducted in the fishponds of Mindanao State University, Naawan, Misamis Oriental, Philippines. The culture duration was four months (120 days), and eighteen units of 250 m² ponds were used for the experimental trials. Six treatment

Legend:
-P. monodon
-G. verrucosa
-O. niloticus
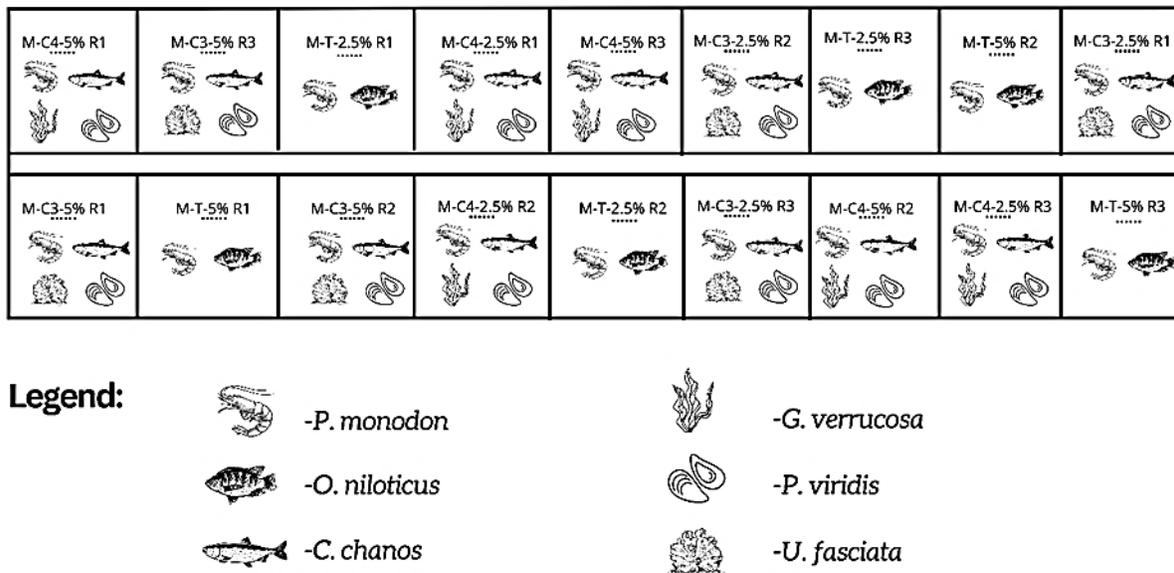-P. viridis
-C. chanos
-U. fasciata

Figure 2. Experimental set-up with treatments randomly assigned in triplicate in ponds.

combinations with varying feeding rates (FR) were designed and randomly assigned in triplicate in ponds comprising polyculture and IMTA (Fig. 1), namely: M-T-5% (control): *P. monodon* with *Oreochromis niloticus* @ 5% FR; M-T-2.5%: *P. monodon* with *O. niloticus* @ 2.5% FR; M-C3-5%: *P. monodon*, *Chanos chanos*, *Ulva fasciata*, and *Perna viridis* at 5% FR; M-C3-2.5%: *P. monodon*, *C. chanos*, *U. fasciata*, and *P. viridis* at 2.5% FR; M-C4-5%: *P. monodon*, *C. chanos*, *Gracilaria verrucosa*, *P. viridis* at 5% FR; M-C4-2.5%: *P. monodon*, *C. chanos*, *G. verrucosa*, and *P. viridis* at 2.5% FR.

   Penaeus monodon is the main cultured species, with *C. chanos* and *O. niloticus* as the co-cultured species. The nutrient-extraction species include macroalgae *U. fasciata* and *Gracilaria*, as well as mussels *P. viridis* (Fig. 2). The choice of *C. chanos* was based on its commercial value, position on the lower trophic level, seed stock availability, and existing aquaculture technologies. The choices of seaweeds and mussels were based on their bioremediation capabilities, as reported in the literature. The selection of species was also based on the salinity requirement optimal for the growth of *P. monodon*, being 20-30 ppt (Ye et al., 2009), closer to its iso-osmotic point, which is 25 ppt, and where osmotic stress is lowest, therefore promoting growth (Cheng and Liao, 1986). A 5% feeding rate of

commercial feed was provided for the main cultured species.

Pond preparation: Standard pond preparation, including draining, pest control, tea seed application, drying, liming, flushing, and water filling, was performed. Tea seed was applied at 100 kg/ha, and lime at 2,000 kg/ha. After three days from liming, the ponds were filled with tidal water up to 30 cm deep. The succeeding water filling was conducted when the water turned green, indicating the growth of natural food, usually done after three to four days. Water is filled up to 100 cm (1 m) in preparation for stocking. These preparations were simultaneously conducted in all the experimental ponds to ensure that the environmental conditions, including sediment and water quality metrics, were standardized.

Sourcing, acclimation, and stocking of culture organisms: Healthy seed stocks of *P. monodon* and finfish were procured from nearby hatcheries and nurseries with known track records within Northern Mindanao, Philippines. Before procurement, the shrimp and finfish were preconditioned to the salinity of the experimental pond. Healthy seaweeds were collected adjacent to Naawan, Misamis Oriental, while mussels were collected from Panguil Bay, Philippines. All collected seed stocks were transported to the experimental sites in Naawan. Upon arrival, they were properly cleaned and acclimated to the pond

water conditions following standard protocols (Apud, 1984; Arriesgado et al., 2025).

The semi-intensive stocking density per square meter was as follows: 20 post larvae (PL 15-45) of 0.25 g average body weight (ABW) for *P. monodon*, 1 to 2 fingerlings of 5 g *C. chanos* (Apud, 1984), *O. niloticus*, 50 juveniles of 2-3 cm shell length (Cranford, 2013), for mussels and the macroalgae at 500 g (modified from Msuya, 2007). The seaweeds were checked every 15 days and partially harvested if the density was increased by their growth or replaced if the quantity was lost (mostly from cultured fish grazing (for *Ulva*) and disintegration (for *Ulva*). Stocking was done early in the morning, and the shrimps, macroalgae, and mussels were the first to be stocked in the ponds. Shrimps were stocked in hapa nets inside the pond at a density of 200 PL/m^2. The macroalgae were placed in towable plastic trays and trays made of B-net and bamboo (1.0x1.5 m) and were submerged 5.08 cm from the water surface. The mussels were placed in platforms made of B-net and bamboo, layered plastic trays, and arranged so that the bottom-most layer was 2.54 cm higher than the pond substrate. Confinements were covered with B-net and were placed in the four corners of the pond, the center, and near the pond gate. After two weeks, the shrimps were released from the hapa net, and the finfish were also stocked one week after the release of the shrimps.
Monitoring and laboratory analyses: Water management involved replenishing water lost to evaporation. Analysis of water and soil was conducted based on APHA (1989) and AOAC (1990). Soil analysis included organic matter, soil phosphorus, and total nitrogen, while water analysis included ammonia-nitrogen (NH$_3$-N), nitrite-nitrogen (NO$_2$–-N), nitrate-nitrogen (NO$_3$–-N), phosphates, transparency, total dissolved solids (TDS), salinity, temperature, and dissolved oxygen. The multi-water parameter meter (Hanna Instruments HI9829) was used to monitor dissolved oxygen, salinity, TDS, temperature, and pH daily. The spectrophotometric method analysis of phosphates, nitrates, nitrites, and ammonia was conducted as initial and final analyses at the MSUN laboratory, while daily analysis of the

parameters was performed on site using test kits.

Microbial analysis of water, soil, and harvested shrimps, as well as differential diagnosis of health and disease in shrimps, was conducted. The preparation of the sample dilutions and bacteriological assays of the water, sediment, and shrimp was performed following the methods described by APHA (2005). Water samples were collected using 500 mL sterile glass bottles at halfway (40 cm) deep from surface water and 0.4 cm above the sediment (bottom water) from five different locations (gate and four corners) in each pond. Sediment samples (10 cm depth in soil) were collected in the same locations and placed in another sterile glass bottle. The samples were homogenized, and a sub-sample of 200 g for soil and 500 mL for water was used for the analysis. The water and soil samples were placed in ice chests and were immediately transported to the accredited microbiological laboratory near MSU at Naawan. The time from collections and analysis was less than four hours.

The health conditions of the shrimps were monitored regularly through daily observations of their behavior and bodies for gross clinical signs. Detection of prevalent *P. monodon* diseases, such as White Spot Syndrome Virus (WSSV) and Acute Hepatopancreatic Necrosis Disease (AHPND), was conducted using appropriate molecular analysis methods, including polymerase chain reaction (PCR) and quantitative PCR (qPCR), to ensure accurate identification and assessment of pathogen prevalence. The required number of *P. monodon* samples was preserved in 95% ethanol and transported to the BFAR XII laboratory in General Santos City, Philippines. Samples of shrimps were also sent to an accredited microbiological laboratory for disease and microbial differential diagnosis and bacterial profiling.

Phytoplankton and zooplankton were monitored every 30 days. Twenty-liter water samples were collected and concentrated in 500-mL sterile plastic bottles. The water was filtered through a cylindrical conical net with a 15 μm mesh, and then concentrated to 10 ml. Concentrated plankton were preserved in 5% buffered formalin for further qualitative and

quantitative analysis. Phytoplankton was analyzed by directly enumerating a 1 mL subsample using a haemacytometer and an electronic compound microscope. Zooplankton analysis involved transferring 1 mL of a subsample to a Sedgewick-Rafter, and then identifying and counting cells within 10 randomly chosen squares under an optical stereomicroscope. Plankton analyses were conducted at MSU's Naawan laboratory, identifying species up to the genus level as described by APHA (1992) and Bellinger (1992). The plankton density was then estimated following Stirling (1985).

Growth, survival, and production of tiger shrimp and finfishes: Growth performance was assessed monthly by measuring average body weight (ABW) and specific growth rate (SGR) from samples of 50 shrimp (*P. monodon*) and 30 finfish (*C. chanos,* and *O. niloticus*). Survival rate (%) was determined at harvest as the proportion of individuals remaining relative to the initial number stocked. Feeding was conducted four times daily using commercial feeds, adjusted to a 5% feeding rate (%FR) of the biomass. Growth parameters, including ABW, %SR, SGR, and feed conversion ratio (FCR), were calculated using standard formulas described by Hasan et al. (2011):

ABW (g) = Total weight of sampled individuals / Number of individuals

SGR (%/day) = [(ln final ABW − ln initial ABW) / culture duration (days)] × 100

%SR = (Final number of individuals / Initial number of individuals) × 100

FCR = Total feed consumed (TFC) / Total yield (TY)

Economic performance was assessed based on key indicators, including the cost of production, gross revenue, net profit, and return on investment (ROI). These were calculated as follows:

Net Profit (NP) = Sales Profit (SP) − Production Cost (PC)

% ROI = (NP / PC) × 100

An ROI greater than 10% was considered indicative of potential economic sustainability (Tietze et al., 2001).

Statistical analysis: Statistical analyses were performed using Microsoft Excel 2010 and R software Version 4.4.2. A t-test was applied to evaluate the growth and survival of fishes (tilapia and milkfish) in two treatments. ANOVA was conducted on the growth and survival results of shrimps and milkfish assessed in more than two treatments, and in bacterial analysis. Data transformation on growth, survival, and bacterial counts results was applied using the method of Gomez and Gomez (1984), and the results were expressed as the mean of triplicate samples for each treatment ± standard deviation. Tukey's test was applied to determine the significant differences between treatment means. The Shannon index of diversity was applied in plankton analysis. In all analyses, a 5% significance level was employed to show the significance of the tested treatments.

Results

Growth and survival: The experiment showed no significant difference in the growth and survival of shrimps and fishes between the treatments (Table 1). Specific growth rates (SGR) were recorded at 4.11-4.23% for shrimp, 3.08-3.09% for tilapia, and 3.01-3.13% for milkfish. Survivorship ranged from 57.67-61.67% for shrimp, 66.17-68.50% for tilapia, and 97.33-98.67% for milkfish (Table 1). All treatments resulted in a >55% survival rate; in an IMTA shrimp-milkfish-Ulva-green mussel treatment, 5 and 2.5% feeding rates were given (59 and 60%, respectively), and in a shrimp-tilapia combination, 5 and 2.5% feeding rates were given (59 and 61.67%, respectively) (Figs. 3, 4).

Water and soil quality: The water quality of all treatments was within optimal levels (Table 2) for most parameters, except for dissolved oxygen, soil organic matter, soil nitrogen, and phosphorus.

Microbial profile: Luminous bacterial populations were undetected in all treatments. The total population of *Vibrio* (Fig. 5) in the pond water was usually below the threshold level of 10^4 CFU mL^{-1} for shrimps (Peeler and Maturin, 1992; Lightner, 1996; Kharisma and Manan, 2012).

Plankton profile: Figure 6 illustrates the plankton profile across various treatments. The highest number of species was recorded at IMTA treatment with

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Table 1. Growth and survival performance of *Penaeus monodon*, *Chanos chanos*, and *Oreochromis niloticus* at 2.5 and 5% feeding rates.

Treatments	ABW (g)	Weight gain (g)	Growth/day (g^{d-1})	Specific Growth Rate (%)	Survival Rate (%)
P. monodon					
M-T-5%	24.8±5.36	24.23±5.36	0.20±0.04	4.23±0.18	59.00±5.29
M-T-2.5%	23.1±3.08	23.76±3.08	0.20±0.03	4.22±0.11	61.67±4.16
M-C3-5%	23.1±3.74	22.86±3.74	0.19±0.03	4.19±0.14	59.00±6.24
M-C3-2.5%	21.0±5.93	21.45±5.93	0.18±0.06	4.11±0.23	60.00±7.55
M-C4-5%	23.9±7.04	23.44±7.04	0.19±0.05	4.19±0.29	57.67±3.51
M-C4-2.5%	22.6±7.04	22.51±7.04	0.19±0.06	4.15±0.29	57.67±16.29
C. chanos					
M-C3-5%	279.58±46.46	272.08±46.46	2.27±0.39	3.01±0.13	97.67±2.52
M-C3-2.5%	276.82±22.67	269.32±22.67	2.24±0.19	3.01±0.07	97.33±2.52
M-C4-5%	319.60±7.84	312.10±7.84	2.60±0.07	3.13±0.02	98.33±1.53
M-C4-2.5%	288.07±38.50	280.57±38.50	2.34±0.32	3.03±0.11	98.67±1.15
O. niloticus					
M-T-5%	202.05±2.88	197.05±2.88	1.64±0.02	3.08±0.01	68.50±3.91
M-T-2.5%	203.67±5.98	198.67±5.98	1.65±0.05	3.09±0.02	66.17±3.01

Note: Different superscripts within treatments indicate a significant difference among means (*P*<0.05)
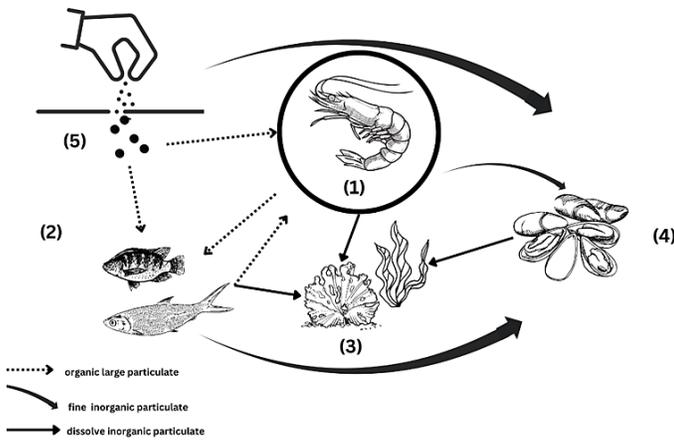

Figure 3. The concept of nutrient cycling using organisms from different trophic levels in an integrated multi-trophic aquaculture system (IMTA) (1) Shrimp, (2) Fish, (3) Seaweeds, (4) Mussel, and (5) Commercial feeds.
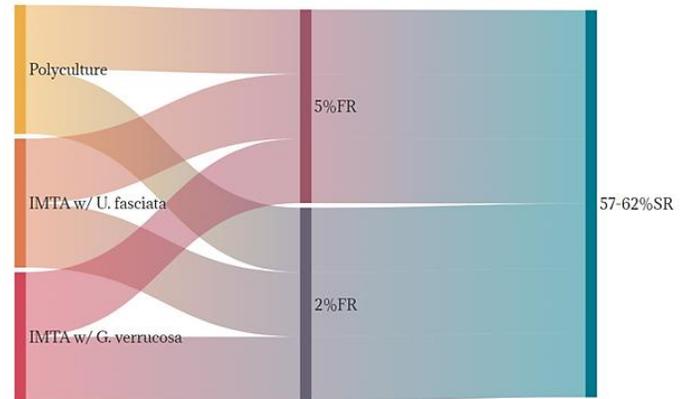

Figure 4. Stankey diagram showing the survival performance of *Penaeus monodon* in different systems at 2.5% and 5% feeding rates, showing no significant difference (*P*>0.05).

G. verrucosa at a 2.5% feeding rate, with 14 species. The highest number of families and genera was recorded at 13 and 14, respectively, observed in IMTA ponds, and the lowest in shrimp polyculture, with nine families and seven genera. A higher diversity index of plankton was also observed in IMTA ponds, with a good Shannon diversity index (≥2) usually observed in IMTA ponds having *G. verrucosa*. However, the densities of the plankton (10^2-10^3) in all treatments were less than the recommended 10^4/ml (Fig. 7).

Health and disease diagnostic: The disease differential diagnosis conducted on all treatments exhibited no discernible signs of infection (Table 3). Subsequent confirmatory molecular analyses, employing polymerase chain reaction techniques (Table 3), revealed negative results for Acute Hepatopancreatic Necrosis Disease (AHPND) across all treatment groups. However, shrimp reared within the shrimp-tilapia system and subjected to a 5% feeding rate manifested infection with White Spot Syndrome Virus (WSSV), whereas all other treatments displayed negative results (Table 3).

Production and profitability: The polyculture of *P. monodon* and *O. niloticus* with a 2.5% feeding rate exhibited the highest ROI among all treatments. The IMTA treatment with *G. verrucosa* at 2.5% has shown

Table 2. Water parameter levels were observed within the range in all treatments.

Parameters	M-T-5%	M-T-2.5%	M-C3-5%	M-C3-2.5%	M-C4-5%	M-C4-2.5%	Ideal
Water							
Ammonia (NH_3)	0.01-0.05	0.01-0.03	0.01-0.04	0.01-0.07	0.01-0.02	0.01-0.03	<0.1
Nitrite (NO_3)	0.03-0.04	0.02-0.04	0.03-0.05	0.02-0.04	0.02-0.05	0.02-0.04	<0.1
Nitrate (NO_2)	0.02-0.04	0.02-0.04	0.02-0.04	0.04-0.05	0.03-0.04	0.02-0.04	<0.5
Phosphate (PO_4)	0.03-0.04	0.03-0.07	0.04-0.07	0.03-0.06	0.01-0.04	0.04-0.09	<0.1
Dissolved oxygen (ppm)	2.10-5.20	2.00-5.40	2.50-5.70	2.40-5.50	2.10-5.30	2.10-5.20	>2
TDS (ppm)	18.10-28.10	18.00-27.40	18.50-28.60	17.80-26.70	17.90-18.60	21.20-28.80	<100
pH	7.10- 8.60	7.20-8.50	7.10-8.60	7.20-8.50	7.20-8.50	7.10-8.50	7.5-8.5
Temperature (°C)	28.10-32.70	28.10-32.80	28.20-32.60	28.10-32.70	28.10-32.60	28.10-32.60	28-33
Salinity (ppt)	20.50-22.70	20.50-22.70	20.40-22.80	20.40-22.80	20.40-22.80	20.50-22.70	15-25
Transparency (cm)	35.1045.00	35.60-44.50	35.50-40.50	30.50-41.50	35.50-40.30	34.90-42.50	35-45
Soil							
OM (%)	2.01-2.60	1.82-1.82	1.98-2.22	1.58-1.79	1.46-2.13	1.22-1.82	>0.1
Nitrogen (%)	NS-0.26	NS-0.12	NS-0.17	NS-0.12	NS-0.16	NS-0.11	0.1
Phosphorus (ppm)	0.41-0.49	0.43-0.44	0.277-0.61	0.28-0.58	0.55-0.66	0.32-0.52	0.1

*Mohanty et al. (2014)

Table 3. Disease differential diagnosis and confirmatory molecular analysis on harvested shrimps in different treatments.

Site	Treatments	Diagnosis
Naawan, Misamis Oriental, Philippines	M-T-5%	• Normal pigmentation; Clear hepatopancreas; No erosion on exoskeleton • Heterotrophs load is high and beyond the level of normal flora
	M-T-2.5%	• Normal pigmentation; Clear hepatopancreas; No erosion on exoskeleton • Heterotrophs load is high and beyond the level of normal flora
	M-C3-5%	• Normal pigmentation; Clear hepatopancreas; No erosion on exoskeleton
	M-C3-2.5%	• Normal pigmentation; Clear hepatopancreas; No erosion on exoskeleton • Heterotrophs load is high and beyond the level of normal flora
	M-C4-5%	• Normal pigmentation; Clear hepatopancreas; No erosion on exoskeleton
	M-C4-2.5%	• Normal pigmentation; Clear hepatopancreas; No erosion on exoskeleton

Confirmatory molecular analysis on AHPND and WSSV of *P. monodon* in different treatments

	M-T-5%	M-T-2.5%	M-C3-5%	M-C3-2.5%	M-C4-5%	M-C4-2.5%
AHPND	-	-	-	-	-	-
WSSV	+	-	-	-	-	-

+ indicates a positive detection, - indicates a negative detection

the lowest Feed Conversion Ratio (FCR), while the control showed the highest. The annual viable shrimp yield of 3.31 tons, breakeven production of a one-hectare farm based on Hatch et al. (1996), was surpassed by an IMTA shrimp-milkfish-Gracilaria-green mussel (Table 4) in this study (3.75-4.05 T) and by the shrimp-tilapia combination (4.29-4.43 T).

Discussions
Growth and survival: The promising result of the 2.5% feeding rate in this study will reduce feed costs and, at the same time, avoid intensified feeding and

waste accumulation. The growth and survival performances between the two feeding rates did not significantly differ in shrimp and the co-cultured fishes, implying that the 2.5% feeding rate is adequate for an IMTA pond system. Hasan et al. (2013) asserted that intensified feeding generally results in a large amount of sedimentation with high levels of nutrient accumulation, causing stress and diseases in farmed fish (Hasan et al., 2013), thus lower production and economic returns (Edwards et al., 1996). A study by Janakiram et al. (2011) presented a growth of 1.55 to 2.4 g per week (0.22-0.34 g/day) for a semi-
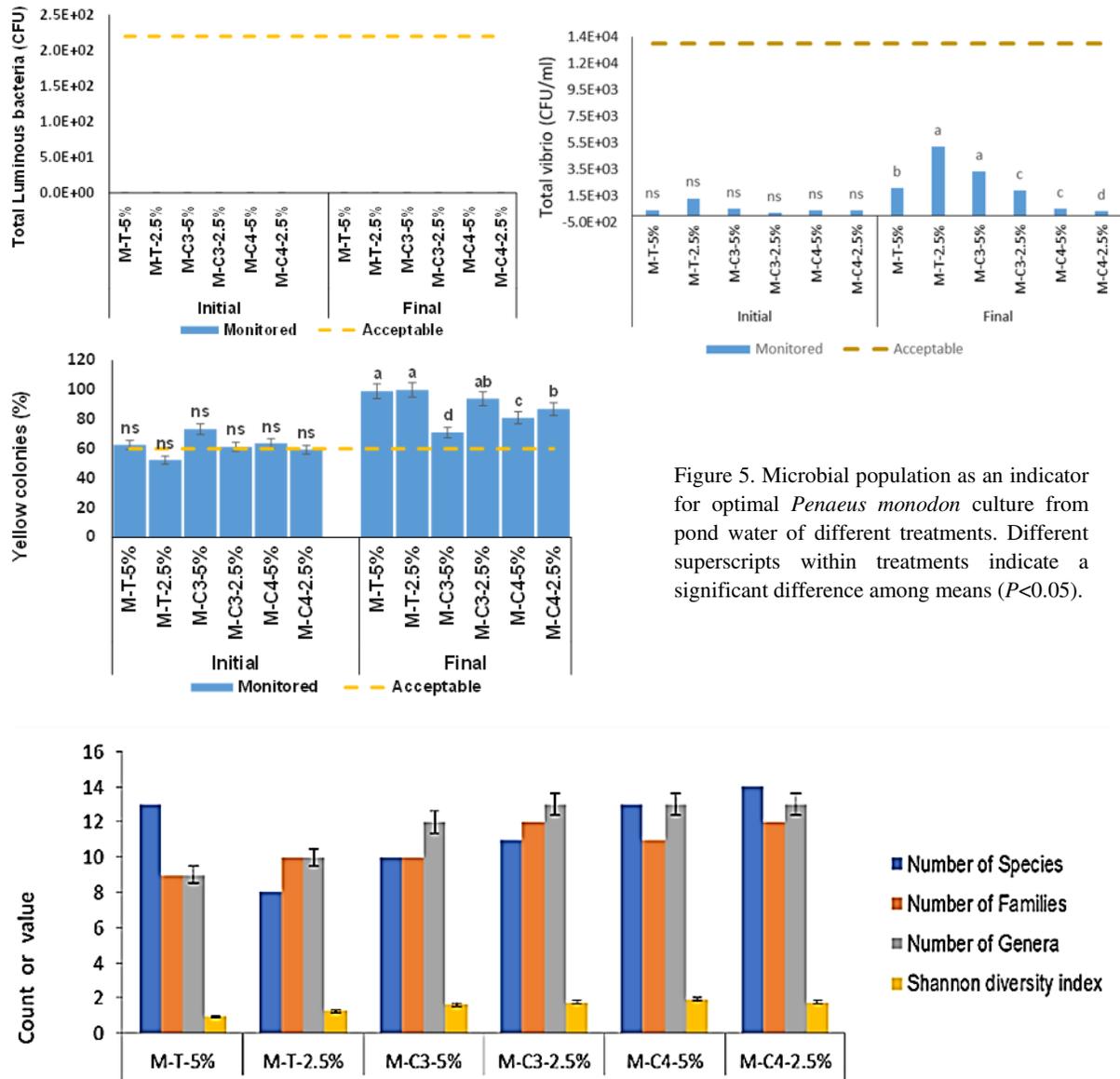
Figure 5. Microbial population as an indicator for optimal *Penaeus monodon* culture from pond water of different treatments. Different superscripts within treatments indicate a significant difference among means (*P*<0.05).

Figure 6. Plankton profile (number of species, genera, families, and diversity index).

intensive *P. monodon* farm. These growths were comparable in the IMTA system (0.18 to 0.28 g/day) and in the shrimp-tilapia system (0.20 g/day) in this study.

The survival rates of shrimp in the current study were higher than the general survival trend of *P. monodon* experienced by farmers in Northern Mindanao at present (<15%, pers. com). A survival rate of 12.3-46.5% was reported by Janakiram et al. (2011) for a semi-intensive *P. monodon* farm. The survival rates in this study were higher, ranging from 57.67 to 62%, in both the IMTA system and the shrimp-tilapia system. This is comparable to shrimp survival at the peak of production in the 1980s (70%

in Apud, 1984) and in shrimp culture using the biofloc technique (68-81%, AftabUddin et al., 2020). However, the cost of biofloc in relation to its affordability and adoptability by shrimp farmers in Northern Mindanao requires evaluation. The stocking density of co-cultured fishes may have also influenced the survival of shrimp. Pudadera and Lim (1982) reported that a polyculture of *P. monodon* and finfish will yield a better result if the finfish has a lower stocking density. Simão et al. (2013) also emphasized that increasing the density of shrimp would not affect the finfish; however, increasing the density of the finfish decreases the final weight and survival rate of the shrimp.

Table 4. Pond production of IMTA and other systems in Naawan.

| Treatments	M-T-5%	M-T-2.5%	M-C3-5%	M-C3-2.5%	M-C4-5%	M-C4-2.5%
A. Production (kg)						
Shrimp	42.90	44.30	40.90	38	40.5	37.5
Tilapia	27.69	26.97				
Milkfish			54.74	53.85	62.86	56.83
Total	70.59	71.27	95.64	91.85	103.36	94.33
B. Operating costs (USD)						
Feeds	106.52	63.29	171.41	108.44	171.41	108.44
Seeds	16.79	16.79	33.44	33.44	33.44	33.44
Others	25.19	25.19	25.19	25.19	25.19	25.19
Total operating costs	148.50	105.27	230.04	167.08	230.04	167.08
C. Revenue estimates* (USD)						
Shrimp @ USD 5.30/kg	227.25	234.83	216.71	201.61	214.59	198.96
Tilapia @ USD 1.41/kg	39.16	38.13				
Milkfish @ USD 1.77/kg			96.76	95.19	111.10	100.46
Total revenue	266.41	272.96	313.48	296.80	325.68	299.42
Net revenue	117.92	167.69	83.44	129.72	95.65	132.34
D. ROI	79.41	159.30	36.27	77.64	41.58	79.21
E. FCR	2.36	1.40	2.08	1.36	2.25	1.07
F. Annual production estimates per hectare						
Cycle of production	2.50	2.50	2.50	2.50	2.50	2.50
Shrimp (tons)	4.29	4.43	4.09	3.80	4.05	3.75
Shrimp and fish	7.06	7.13	9.56	9.19	10.34	9.43
% shrimp production increased from 2.7-ton semi-intensive baseline (Hatch et al., 1996)	58.89	64.07	51.48	40.74	50.00	38.89
% shrimp production increase from 1 the baseline present production in Northern Mindanao	329.00	343.00	309.00	280.00	305.00	275.00

1 USD = 56.57
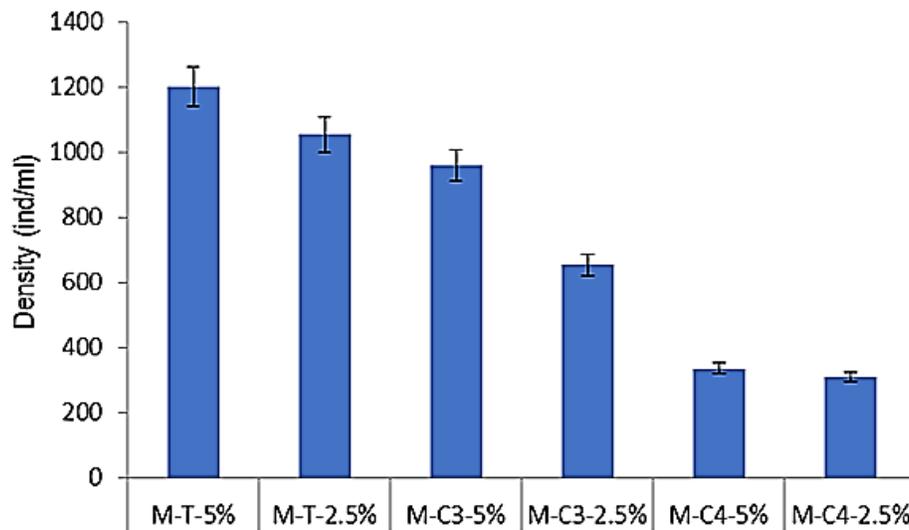
Figure 7. Plankton profile (density: counts/ml).

Our results further demonstrate a pronounced growth and survival performance of milkfish over tilapia. The better growth and survival of milkfish combined with shrimp is supported by Eldani and Primavera (1981), indicating that this combination has an advantageous influence on the growth and survival of *C. chanos*. Milkfish are known to feed on organic detritus, uneaten feeds, and even feces of other animals in the pond (Fang, 2018). Milkfish are active, opportunistic, and continuous feeders (Chiu et al., 1987); therefore, they have the advantage of serving as a co-culture fish for shrimp in an IMTA pond system. The growth and survival performance of shrimp in the current study suggest that an IMTA

system for *P. monodon*-milkfish-*Gracilaria* or *Ulva*-green mussel is profitable. The results also support the role of seaweeds and mussels as extractive organisms, as further discussed in the succeeding subsections. As asserted by Biswas et al. (2020), *P. monodon* co-cultured with finfishes has the highest growth in the treatment added with organic extractor (*Crassostrea cuttackensis*) and inorganic extractor (*Ipomoea aquatica*) compared to the performance of a traditional polyculture.

Water and soil quality: The water quality from the experimental site was within the optimal levels for most parameters. Low dissolved oxygen levels were typically observed after heavy rainfall and when the fish were larger. Low DO levels did not show any adverse effect on the cultured organisms. The water quality monitored for different parameters in IMTA treatment combinations suggests their potential to control and recycle wastes, as evidenced by the ideal range levels of NO_2, NO_3, NH_3, and PO_4 in all treatments. The co-culture of finfishes and the introduction of extractive species are expected to increase the organic load in ponds from faeces. However, the N and P, as well as the organic matter in IMTA ponds, remain optimal, indicating the synergistic effect of organisms and possibly the action of extractive seaweeds and mussels in the pond. As pointed out by several studies (Huo et al., 2012; Skriptsova and Miroshnikova, 2011; Samocha et al., 2015), the application of macroalgae is one of the most effective approaches to utilize inorganic nitrogen and phosphorus. Filter-feeding bivalves provide an efficient method for using organic and particulate wastes (Irisarri et al., 2015; Biswas et al., 2020).

The salinity levels in the ponds ranged within the iso-osmotic point (25 ppt) for *P. monodon*, which helps explain the optimal sizes and survival of the shrimp. Ye et al. (2009) elaborated that a salinity higher than 25 ppt has a substantial impact on shrimp's growth as less energy is budgeted for its growth and metabolism in favor of the more energy spent on osmotic regulation and respiration. Additionally, the higher salinity may have subjected the shrimp to osmotic stress, leading to bacterial infection and the

occurrence of WSSV. Given the WSSV occurrence, the dry season becomes a limiting factor in relation to areas with high salinity. The varying salinity levels in the sampling ponds have minimal impact on the survival of the commodities under experiment. The limitation of water physicochemical parameters in an IMTA system can be overcome by carefully selecting a species combination in relation to the existing conditions of the area and the requirements of the chosen species.

Microbial profile: Total heterotrophs were generally higher in shrimp-tilapia treatments and in IMTA treatments with a 5% feeding rate. Luminous bacterial populations were undetected in all treatments. The total population of *Vibrio* in the pond water was usually below the threshold level of 10^4 CFU mL^{-1} for shrimps (Peeler and Maturin, 1992; Lightner, 1996; Kharisma and Manan, 2012). *Vibrio* counts exceeding 10^4 CFU encourage susceptibility of shrimps to infection and diseases, including vibriosis (Kharisma and Manan, 2012). In this study, the *Vibrio* population usually formed as part of the normal flora. *Vibrio* species, which are often found in green bacterial colonies, pose a threat to shrimp aquaculture. Shanmugasundaram et al. (2015) reported that among the most active pathogenic bacteria involved in the vibrio crisis and responsible for up to 100% trouble in shrimp aquaculture are *V. harveyi, V. anguillarum*, *V. parahaemolyticus*, *V. vulnificus*, and *V. alginoliticus*. Limsuwan (2010) tagged *V. parahaemolyticus* and *V. alginolyticus* as the pathogenic bacteria commonly found in shrimp hatcheries and ponds. The most active pathogenic *Vibrio* species monitored were *V. parahaemolyticus,* and *V. alginoliticus*. *Vibrio parahaemoliticus* was occasionally present in IMTA waters reared with *Ulva*. Paena et al. (2018) asserted that the occurrence of high *Vibrio* counts is caused by high dissolved organic matter from feeds and faeces. Although *Vibrio* is generally a dominant microbial species, the organic load from feed and faeces encourages them to become opportunistic pathogens (Otta and Karunasagar, 2001).

A phytochemical study by Widowati et al. (2014)

supported these results, indicating that the antibacterial activity of *G. verrucosa* extracts is effective against the pathogenic marine bacteria, *V. parahaemoliticus*. *Gracilaria verrucosa* has also antibacterial activity against *V. alginoliticus,* although reported to be weak (Maftuch et al., 2016). Kurniaji et al. (2020) emphasized that a polyculture of *G. verrucosa* and the white leg shrimp could suppress the population of another *Vibrio* species, *V. harveyi*. Moreover, a study has shown that just the presence of algae in the rearing water of shrimp and fish would enhance the survival, growth, and transformation index of the cultivated species (Muller-Feuga, 2000), improve shrimp growth and its immune response (Moss et al., 1992; Primavera et al., 2000) compared to those reared in clear water. Mahrinho-Soriano et al. (2009) suggested co-culturing *Gracilaria* in shrimp farming for water treatment, while the culture of *Ulva* (*U. lactuca*) in shrimp has also been reported by Elizondo-González et al. (2018) to remove 80% of the inorganic nitrogenous compounds in ponds. The green water technology of the shrimp-tilapia system is a proven remediation method that provides the beneficial effect of antibacterial substances as well as production of compounds that inhibit virulence gene regulation (Natrah et al., 2011; Kokou et al., 2012). The above-mentioned existing studies support the results of achieving significantly better growth and survival of *P. monodon* reared in IMTA and the shrimp-tilapia system, despite the presence of the pathogenic *Vibrio* species in almost all treatments.

Plankton profile: Information on the quality and quantity of plankton in shrimp ponds is fundamental to successful aquaculture management and production. According to Prasad and Singh (2003), the phytoplankton population represents the biological wealth of a water body, whereas the zooplankton forms the principal source of food for shrimp within the water body. In this study, plankton densities were observed to be higher in shrimp-tilapia ponds than in IMTA ponds. The presence of mussels and seaweeds in IMTA ponds may limit plankton population growth, leading to lower plankton densities compared to shrimp-tilapia ponds (Juárez-Rosales, 2020). This is indicative of controlled nutrient accumulation in IMTA ponds, as evidenced by optimal levels of nitrogen, phosphorus, and organic matter (Juárez-Rosales, 2020). The availability of nutrients and top-down control in shrimp ponds significantly influence the size structure and composition of phytoplankton (Cardozo, 2011). IMTA ponds also outperform conventional polyculture in terms of environmental remediation, productivity, and economic return (Biswas, 2020). Despite the addition of co-cultured finfish and mussels, nutrient accumulation is being controlled in IMTA ponds.

The food provisioning function of plankton in this study may not be that critical because a commercial feed was provided to the cultured shrimp and finfishes. However, a lower plankton population could probably bring adverse effects on the water quality of the pond due to an imbalanced ecosystem. Microalgae are known to play a vital role in regulating water quality, and several studies have proven their antimicrobial, antioxidant, and anti-inflammatory properties. For instance, the microalgae *Nitschia* have been assessed to inhibit the pathogenic *Vibrio* (Lio-Po et al., 2002). *Nitzchia* is one of the commonly occurring diatoms in IMTA ponds. Dash et al. (2017) asserted that the proper balance and maintenance of phytoplankton and bacterial communities throughout the production cycle have been claimed to increase productivity, with enhanced water and soil quality, in an intensive culture system. A better production performance had been observed in ponds with a higher plankton diversity index, mainly from IMTA ponds that included *G. verrocusa*. This may suggest the importance of having *G. verrucosa* in an IMTA shrimp pond to help improve plankton diversity.

Health and disease diagnostics: The confirmatory molecular analysis using PCR yielded a negative result for AHPND in all treatments, suggesting that this disease may not have been present at the time of the experiment. Disease differential diagnosis indicated that shrimps reared in IMTA ponds had normal pigmentation, clear hepatopancreas, and absence of erosion. Takahashi et al. (1998) elaborated that stress factors could make shrimp more susceptible

to WSSV infection. Kharisma and Manan (2012) associated the susceptibility of shrimp to infection and diseases with a *Vibrio* population that exceeds 10^4 CFU. The presence of seaweeds and mussels in IMTA and green water in the shrimp-tilapia system may have helped manage the *V. parahaemoliticus* population. In IMTA, this was probably facilitated via nutrient recycling by seaweeds and mussels in ponds.

Production and profitability: The production of shrimp for a semi-intensive farm varied from 0.5 to 5 T/ha/year (Apud, 1984; Rosenberry, 1995; Hatch et al., 1996; Janakiram, 2011), with an average of 2.7 tons (Hatch et al., 1996) and 2 tons (Rosenberry, 1995). Sensitivity analysis by Hatch et al. (1996) indicated a minimum of 55 to 60% survival and 3.31 T/ha/yr production of *P. monodon* is needed to cover the total cost of a semi-intensive system. Among the treatments evaluated in this study, a survival of >55% was observed in an IMTA shrimp-milkfish-*Gracilaria*-green mussel treatment given a 2.5% feeding rate (57.67%) and of the same species combination given a 5% feeding rate (57.67%). The annual viable shrimp yield of 3.31 tons, breakeven production of a one-hectare farm based on Hatch et al. (1996), was surpassed by an IMTA shrimp-milkfish-*Gracilaria*-green mussel (3.75-4.05 T) and by a shrimp-tilapia combination (4.29-4.43 T). These results suggest the viability of an IMTA shrimp-milkfish-*Gracilaria*-green mussel system and shrimp-tilapia combination for both the survival and production sensitivity analysis.

The profitability of an IMTA system is secured by the production of both shrimps and the co-cultured fishes. Total pond production was generally higher in the IMTA system co-cultured with larger ABW fish species, such as milkfish. The highest production performance was observed in the shrimp-milkfish-*Gracilaria*-green mussel combination, achieved with a 2.5% feeding rate, resulting in shrimp production of 68.40 kg, total shrimp and fish production of 126.80 kg, gross revenue of USD 476.27, and a net income of USD 282.15, using a 250 m² pond. The low inputs on feeds resulted in a reduced total operating cost of USD 194.12. An annual net income from this treatment

combination is estimated at around USD 26,514.30.

This study showed that IMTA and shrimp-tilapia systems are economically viable. Low survival, slow growth, high inputs, and WSSV occurrence are some of the combined factors that negatively affected the production performance of a shrimp-tilapia polyculture with 5% feeding rate. A profitable IMTA pond is generally demonstrated when *P. monodon* is co-cultured with milkfish, which yields an overall better production and profitability than with shrimps co-cultured with tilapia. The output from seaweeds and mussels was not evaluated since *Ulva* and *Gracilaria* are not commercially exploited in Northern Mindanao, Philippines, at present. Moreover, the grazing of fish could result in a negative growth of the seaweeds. The application of seaweeds to an IMTA system could lead to opportunities for their aquaculture and for livelihood. *Ulva* and *Gracilaria* are consumed by milkfish. Moreover, the use of *Ulva* and *Gracilaria* helped realize the feed reduction in an IMTA pond system. Cruz-Suárez et al. (2010) reported that when shrimp are fed fresh *U. clathrata,* around 50% of pelleted feed could be saved without negative effects on shrimp growth. Although the utilization of *U. fasciata* needs further evaluation, it was observed to disintegrate more easily than *G. verrucosa* in pond water. Best production and profitability were achieved when the feeding rate was reduced to 2.5%. Both shrimp-tilapia and IMTA systems resulted in better shrimp and overall production and net income, with the highest production observed in shrimp co-cultured with milkfish. The results also showed an improved production by more than 20% from a baseline of 0.5-1.0 ton/kg/ha/year in Northern Mindanao, Philippines.

Conclusion
The study concluded that Integrated Multi-Trophic Aquaculture (IMTA) technology, with a reduced feeding rate of 2.5%, is recommended for sustainable *P. monodon* pond aquaculture. This recommendation is based on its ability to support *P. monodon* growth and survival, ensure high profitability, maintain optimal water quality, and aid in disease prevention.

Further studies are needed to enhance the growth and survival of P. monodon in IMTA ponds with *C. chanos*. In addition, the production and profitability of the mussels and seaweeds must also be considered for the total IMTA production.

Acknowledgement

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