Aquaculture Studies, 25(4), 182-192, AQUAST1917
http://doi.org/10.4194/AQUAST1917

PROOF
RESEARCH PAPER

Application of Multi-trophic Aquaculture to Improve Management of Bacterial Load and Health in Pond Culture of *Penaeus monodon* (Fabricius, 1798)

Elgen M. Arriesgado[1], Jomar F. Besoña[1,*], Victor R. Navarro[1], Rustan C. Eballe[1], Dan M. Arriesgado[1]

[1]School of Marine Fisheries and Technology, Mindanao State University at Naawan, Naawan, Misamis Oriental. Philippines.

How to Cite

Arriesgado, E.M., Besoña, J.F., Navarro, V.R., Eballe, R.C., Arriesgado, D.M. (2025). Application of Multi-trophic Aquaculture to Improve Management of Bacterial Load and Health in Pond Culture of *Penaeus monodon* (Fabricius, 1798). *Aquaculture Studies*, *25(4), AQUAST1917*. http://doi.org/10.4194/AQUAST1917

Article History
Received 04 April 2024
Accepted 09 April 2025
First Online 16 April 2025

Corresponding Author
E-mail: jomar.besona@msunaawan.edu.ph

Keywords
IMTA
Green-water technology
Microbial analysis
Tiger shrimp

Abstract

The integrated multi-trophic aquaculture (IMTA) system, utilizing various organic and inorganic extractors, was evaluated as a sustainable approach to improving shrimp health, managing disease, and optimizing environmental conditions in semi-intensive pond culture of *Penaeus monodon*. The experiment was conducted in four treatments, with triplicates: (T0) monoculture of *P. monodon* (control), (T1) *P. monodon* in green water technology (polyculture) with *Oreochromis niloticus*, (T2) *P. monodon* in IMTA with *Chanos chanos, Ulva fasciata,* and *Perna viridis,* and (T3) *P. monodon* in IMTA with *C. chanos, Gracilaria verrucosa*, and *P. viridis*. The study took place in Naawan, Misamis Oriental, Philippines, using 12 ponds (250 m² each) with a 5% feeding rate based on body weight. Microbial analysis revealed the presence of bacteria such as *Vibrio, Escherichia coli, Pseudomonas,* and *Staphylococcus aureus* in water, soil, and shrimp in some treatments, particularly T0, though all were within normal ranges. Acute Hepatopancreatic necrosis disease was not detected, but white spot syndrome was observed in T0. No significant differences (P>0.05) were found in growth and survival across treatments, except for T0. IMTA treatments (T2, T3) showed higher total production and net income, with T1 yielding the highest ROI. Results highlight IMTA's potential to reduce pathogenic bacteria and improve shrimp production and profitability.

Introduction

The Philippines was the seventh-largest producer of tiger shrimp in 2020, with an annual production of almost 50,000 metric tons, following Vietnam, Indonesia, China, Bangladesh, India, and Myanmar (FAO, 2021). The culture of tiger shrimp (*Penaeus monodon*) has received significant attention due to its unique taste, high nutritional value, and sustained demand in the global market (Primavera, 1997). The commercialization of this species has driven farmers to intensify farming practices to further boost production. However, this intensification led to the collapse of the global *P. monodon* industry, particularly in the Philippines (Cruz et al., 2008), as recent production in 2020 failed to reach the 50,000 metric ton mark, according to a report from the Philippine Statistics Authority (PSA, 2022).

The intensification of shrimp farming has accelerated environmental degradation, leading to poor water and soil quality and creating a favorable environment for pathogenic microbes (Leung et al., 2000). This has resulted in uncontrolled eutrophication and a drastic increase in toxic nitrogenous compounds,

causing stress, weakness, and fatalities among shrimp. Moreover, it has triggered disease outbreaks, such as luminous bacterial infections (caused by *Vibrio harveyii*), white spot syndrome (caused by the white spot syndrome virus [WSSV]), and acute hepatopancreatic necrosis disease (AHPND, caused by *Vibrio parahaemolyticus*), among others (Lavilla-Pitogo et al., 1990; Magbanua et al., 2000; de la Peña et al., 2015).

To address the environmental and production challenges faced by the shrimp farming industry, various studies and technologies have been developed, including Integrated Multi-Trophic Aquaculture (IMTA). IMTA is an engineering model that mimics the energy cycle in intensive aquaculture systems (Chopin, 2006). It involves the farming of species at different trophic levels, enabling the uneaten feed, waste, and by-products of one species to serve as fertilizers, feed, and energy for other species, capitalizing on synergistic interactions among them (Chopin et al., 2001, 2008; Troell et al., 2003; Neori et al., 2004; Besoña et al., 2024).

In the Philippines, the study by Arriesgado et al. (2022) identified suitable IMTA species combinations that enhanced the growth, survival, and profitability of *Penaeus monodon* in pond aquaculture. However, significant research gaps remain, particularly regarding comparisons between *P. monodon* monoculture with green-water technology, identifying the most effective species combinations, and investigating the role of IMTA in reducing microbial loads and improving environmental conditions in semi-extensive pond systems. Addressing these gaps is essential for developing a scientifically grounded and comprehensive protocol for sustainable *P. monodon* culture using IMTA. This study aims to fill these gaps by specifically focusing on microbial analysis and its implications for disease management and environmental sustainability, an area not fully explored in the previous work by Arriesgado et al. (2022).

Therefore, this study seeks to evaluate the effectiveness of IMTA technology in semi-intensive *P. monodon* pond culture for disease and environmental management, with a focus on improving microbial management and health of *P. monodon*. The study also aims to advance IMTA technology from its existing applications for *P. monodon* farming.

Materials and Methods

Experimental Set-up

Culture trials were initiated to evaluate four treatments with three replications following a completely randomized experimental design (CRD). This study utilized 12 ponds, each measuring 250 m², at MSU Naawan, Naawan, Misamis Oriental, Philippines (9023) (Figure 1) for IMTA on microbial, health and environmental management.

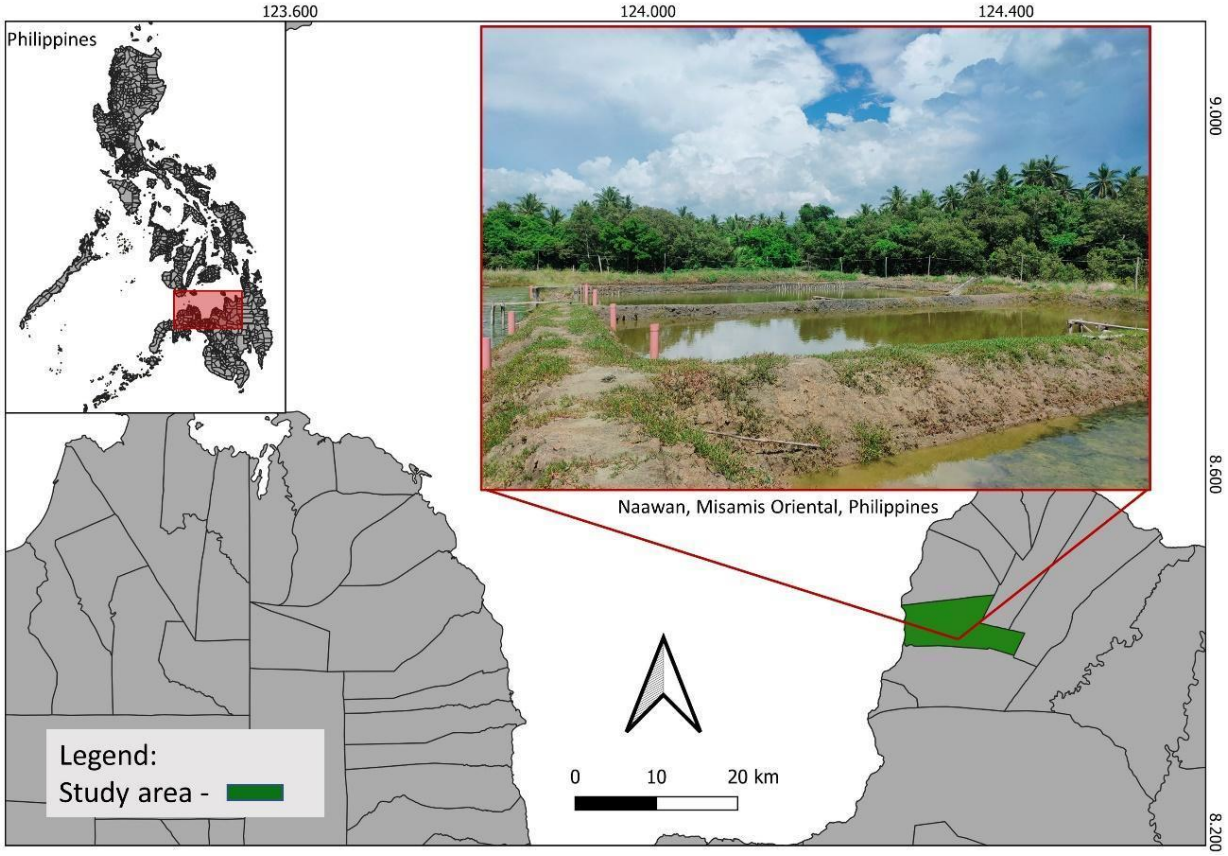

Figure 1. Location of the IMTA ponds in Naawan, Misamis Oriental. Philippines.

Figure 2 illustrates the experimental setup with different treatments, while Table 1 provides a detailed summary of the species composition for each treatment. The species selection was based on commercial value, trophic levels, and the availability of fry and seeds in the area, with the only difference being the macroalgae species. The study was cleared and approved by the institution's ethics committee before its conduct.

Stocking

Size PL_{15} *P. monodon* was stocked and cultured in each treatment pond for four (4) months (120 days) with a stocking density of 20 pcs m^{-2}. *U. fasciata* and *G. verrucosa* (density=0.5 kg m^{-2}) and *P. viridis* (density=30 g m^{-2}) were placed in a separate bamboo-net platform at the bottom of the ponds. The milkfish *C. chanos* (7 cm long, 10 g) was stocked a week later with a density of 0.25 pcs m^{-2}.

Microbial and Molecular Analyses for Disease Diagnosis

Shrimp samples were sent to a laboratory for disease and microbial diagnosis using the methods of morphological pathology, including direct light microscopy and histopathology. Microbial profiling was conducted by aseptically collecting water and soil samples from the ponds and sending them to a nearby microbiological laboratory. Sample collection for diagnostic diseases followed the protocols specified by the laboratory of the Bureau of Fisheries and Aquatic Resources (BFAR) Region 12, General Santos City, Philippines, where the samples were sent for analysis. The prescribed number of *P. monodon* samples were preserved in 90% ethanol and transported to the BFAR Region 12 laboratory for the molecular analysis of the most prevalent diseases in *P. monodon*, namely white spot syndrome virus (WSSV) and acute hepatopancreatic necrosis disease (AHPND).

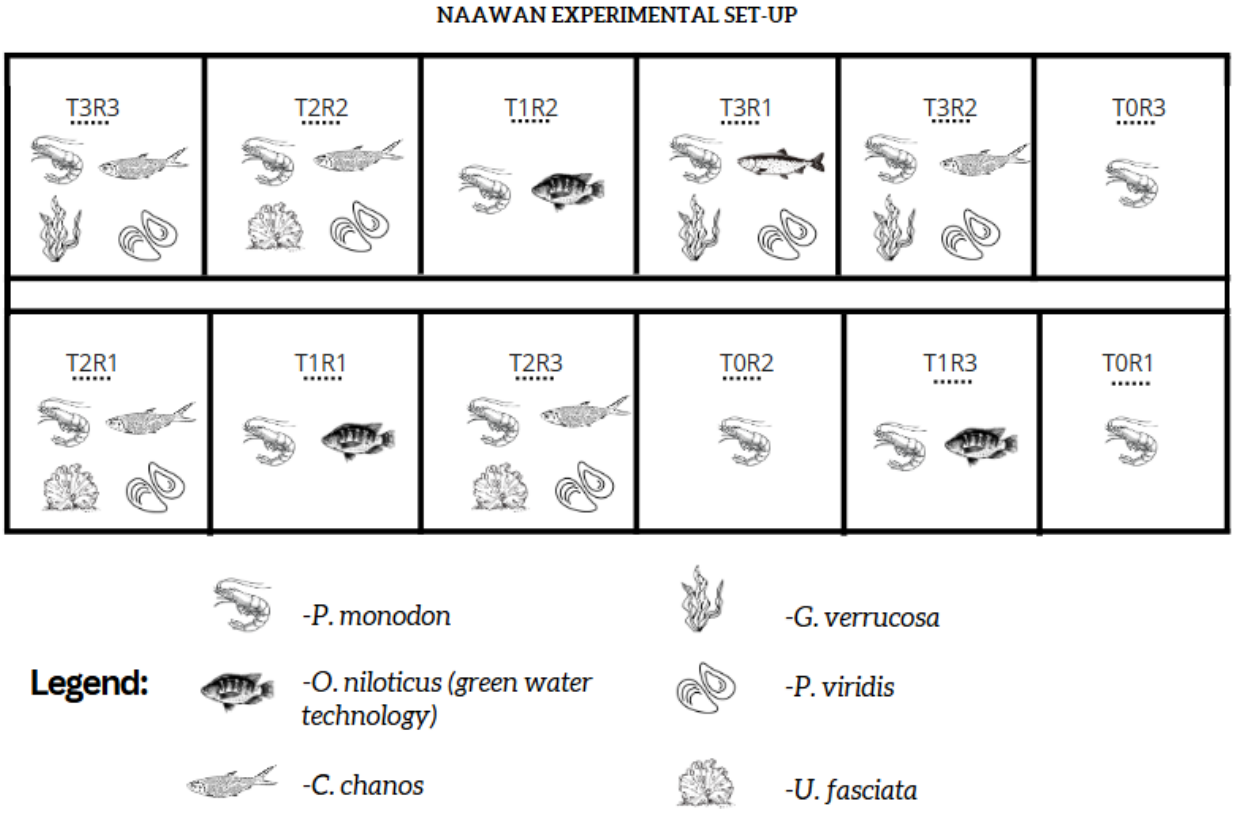

Figure 2. Experimental set-up of various treatments with replicates in the study.

Table 1. Species composition per treatment in the integrated multi-trophic aquaculture (IMTA) experiment

Treatment	Species Combination
Treatment 0 (Control)	*Penaeus monodon*
Treatment 1 (T1)	*Penaeus monodon*, *Oreochromis niloticus*
Treatment 2 (T2)	*Penaeus monodon*, *Chanos chanos*, *Ulva fasciata*, *Perna viridis*
Treatment 3 (T3)	*Penaeus monodon*, *Chanos chanos*, *Gracilaria verrucosa*, *Perna viridis*

Water and Soil Analyses

Water, soil, and other laboratory analyses were conducted after the methods of American Public Health Association (APHA, 1989), and the Association of Official Analytical Chemists (AOAC, 1990). Water analyses include ammonia-nitrogen (NH3-N), nitrite-nitrogen (NO2−-N), nitrate-nitrogen (NO3−-N), phosphates, transparency, total dissolved solids (TDS), salinity, temperature, and dissolved oxygen (D.O.). Phosphates, nitrates, nitrites, and ammonia were analyzed following the spectrophotometric method while the remaining parameters used a multiparameter equipment (Hanna Instruments HI9829).

Growth Parameters Analyses

Feeding rates were adjusted based on a 5% feeding rate relative to the body weight of the shrimp, with sampling conducted biweekly. The following growth parameters were determined during sampling and harvest: average body weight (ABW), average daily growth (ADG), specific growth rate (SGR), and survival rate (S.R.). These parameters were calculated using established methods (Steffens, 1989; Hasan et al. 2011), as detailed below:

$$ABW = \frac{W_s}{n} \quad (1)$$

Where, ABW–is the average body weight (g) of the shrimps and fish in a pond, W_s–weight (g) of the samples in a pond, and n–is the number (pcs) of samples in a pond.

$$ADG = \frac{W_f - W_0}{Doc} \quad (2)$$

Where, ADG–is the average daily growth (g day^{-1}) of the shrimps and fish in a pond, W_f–final weight/current of the samples in a pond, W_o–is the initial weight/previous weight of the samples in a pond. DOC – is the age/ days of culture of the shrimps.

$$SGR = \frac{Ln(W_f) - Ln(W_0)}{Doc} \times 100 \quad (3)$$

Where, SGR-is the specific growth rate of the shrimps and fish in a pond, W_f–is the final weight of the samples in a pond, W_o–is the initial weight of the samples, and DOC–is the age/ days of culture of the shrimps.

$$\% SR = \frac{N_f}{N_0} \times 100 \quad (4)$$

Where, % SR is the survival rate of the shrimps and fish in a pond, N_f–final number of shrimps and fish during harvest in a pond, and N_o–initial number of the shrimps and fish during stocking in a pond.

Production and Profitability

The economic return of each treatment was calculated using the following formulae:

$$TP = P_{shrimp} + P_{fish} \quad (5)$$

Where T.P.- is the total production of the shrimp and fish in a pond, P_{shrimp} - is the total shrimp production in a pond, and P_{fish} is the total fish production in a pond.

$$N.P. = G.P. - P.C. \quad (6)$$

Where N.P.-is the net profit of one pond, G.P.-is the gross profit of a one pond, and P.C.-is the production cost of a one pond.

$$\% ROI = \frac{NP}{PC} \times 100 \quad (7)$$

Where ROI- is the return of investment of a pond, N.P. – is the net profit of a pond, and P.C. is the production cost of a pond.

$$TP_{shrimp\ annual}\ (kg\ 250\ m^2) = P_{shrimp} x 2.5\ crops \quad (8)$$

Where $TP_{shrimp\ annual}$- is the total production of shrimp in a year per 250 ^{m2} in a pond, and P_{shrimp} - is the shrimp production in a pond per crop.

$$TP_{shrimp+fish\ annual}\ (kg\ m^2) = TP x 2.5\ crops \quad (9)$$

Where $TP_{shrimp+fish\ annual}$ – is the total shrimp and fish production in a year per 250m^2 pond, and T.P.-is the total production of the shrimp and fish in a pond per crop.

For the economic evaluation, market prices for the species involved in this study were sourced from local market surveys and the price lists provided by the Southeast Asian Fisheries Development Center (SEAFDEC), Philippines. The market prices used to calculate gross profit and return on investment were based on the most current data available at the time of the study.

Statistical Analysis

Prior to statistical testing, all growth, survival, and microbial parameters were assessed for normality and homogeneity of variances using the Shapiro-Wilk test and Levene's test, respectively. A one-way analysis of variance (ANOVA) was performed to determine significant differences among treatments for parametric data, including growth performance (ABW, ADG, SGR, and SR), water quality parameters, and microbial loads in water, soil, and shrimp. Tukey's Honestly Significant Difference (HSD) test was applied for post hoc multiple comparisons to identify specific treatment differences when significant differences ($P<0.05$) were detected. Non-parametric data were analyzed using the Kruskal-Wallis test, followed by Dunn's test for pairwise

comparisons when applicable. All statistical analyses were conducted using IBM SPSS Statistics (v.19). Data are presented as mean±standard deviation (SD) unless otherwise specified.

Results

Microbial Load Analysis for Disease Diagnosis

The bacterial profile of water in the control ponds (T0), where *P. monodon* was cultured in monoculture, exhibited the highest heterotrophic bacterial population (Table 2). *Vibrio harveyii* was absent in all water samples across treatments. However, *V. cholerae*, *V. parahaemolyticus*, and *V. alginolyticus* were detected in the water samples from various treatments. T0 had the highest counts of *V. cholerae* (7,600 cfu/mL), *V. alginolyticus* (2,000 cfu/mL), and *V. parahaemolyticus* (1,880 cfu/mL). In contrast, *V. cholerae* was the only Vibrio species detected in T1, with a lower count of 6,600 cfu/mL, and no other *Vibrio* species were observed. The IMTA treatments (T2 and T3) demonstrated minimal to no presence of *Vibrio* species in the water, especially in T3, which showed only a *V. cholerae* count of 4,000 cfu/mL. Treatment 2 had *V. cholerae* (3,940 cfu/mL), *V. alginolyticus* (1,000 cfu/mL), and *V. parahaemolyticus* (20 cfu/mL). Additionally, an unidentified *Vibrio* species (*Vibrio* sp.1) and *Escherichia coli* were detected in T0, T2, and T3.

In soil samples, several *Vibrio* and *Pseudomonas* species were detected across all treatments. T0 exhibited the highest counts for *V. cholerae* (4,600 cfu/mL), *V. alginolyticus* (5,116.67 cfu/mL), and *V. parahaemolyticus* (500 cfu/mL), followed by T2, which had *V. cholerae* (3,940 cfu/mL), *V. alginolyticus* (1,000 cfu/mL), and *V. parahaemolyticus* (20 cfu/mL). Treatment 1 showed *V. cholerae* (6,600 cfu/mL), while T3 exhibited *V. alginolyticus* (150 cfu/mL). Another unidentified species of *Vibrio* (*Vibrio* sp.2) was also detected. Although *Salmonella* was not detected, *Pseudomonas* species were notably present in the soil samples of T0 and T2.

In shrimp samples, the highest total heterotrophic bacterial plate count was observed in T1 (32,000 cfu/mL), followed by T2 (16,000 cfu/mL), T3 (5,700 cfu/mL), and T0 (2,500 cfu/mL). *Vibrio* species were not detected in shrimp samples. *E. coli* was present in all treatments at counts of less than 3 cfu/mL, while *Staphylococcus aureus* was observed at 10 cfu/mL. No *Shigella* or *Salmonella* were detected. All bacterial counts remained within normal ranges, indicating no significant pathogenic risk.

Molecular Analysis for Disease Diagnosis

Reverse transcription polymerase chain reaction (RT-PCR) analyses confirmed that all treatments tested negative for Acute Hepatopancreatic Necrosis Disease (AHPND), despite the presence of *V. parahaemolyticus* in water and soil samples (Table 3). However, T0 tested positive for White Spot Syndrome Virus (WSSV), which likely contributed to the significantly lower survival rate of *P. monodon* in this treatment ($P<0.05$). While *V. parahaemolyticus* was present in all treatments, its abundance remained low, suggesting that its role in disease development was minimal in this study.

Table 2. Bacteria profiling for all treatments. Values with different superscripts are significantly different ($P<0.05$). Values are lower than the maximum recommended bacterial limits for good-quality products (ICMSF, 1986)

Bacteria	Treatments			
	T0	T1	T2	T3
Water (CFU/mL):				
Total Heterotrophs	4.76×103	5.03×10^{3}	4.435×10^{3}	5.4×10^{3}
Enterobacter	5.60×10^{2}	4.20×10^{2}	3.80×10^{2}	6.225×10^{2}
Total Coliform	4.30×10^{2cd}	265.00^{d}	930.00^{bc}	$1.01\times10^{3\,b}$
E. coli	7.0×10^{b}	4.0×10^{b}	3.90×10^{2a}	4.0×10^{b}
V. cholerae	7.6×10^{3}	6.6×10^{3}	3.940×10^{3}	ND - 0
V. alginolyticus	2.0×10^{3}	ND - 0	1.0×10^{3}	ND - 0
V. parahaemolyticus	1.88×10^{3a}	ND - 0	2.0×10^{b}	ND - 0
Vibrio sp.1	1.0×10^{3}	ND - 0	1.0×10^{3}	1.0×10^{3}
Soil (CFU/mL):				
Total Heterotrophs	$7.69\times10^{3\,b}$	$2.825\times10^{3\,b}$	$8.5\times10^{3\,b}$	$3.32\times10^{4\,a}$
Vibrio sp.2	$8.133\times10^{3\,a}$	ND - 0	$6.7\times10^{3\,a}$	$3.2\times10^{3\,b}$
V. cholerae	$4.6\times10^{3\,b}$	ND - 0	$2.78\times10^{3\,b}$	ND - 0
V. alginolyticus	$5.117\times10^{3\,a}$	$9.50\times10^{2\,b}$	$6.90\times10^{2\,b}$	$1.50\times10^{2\,b}$
V. parahaemolyticus	$5.0\times10^{2\,a}$	ND - 0	$1.50\times10^{2\,b}$	ND - 0
Pseudomonas sp.1	$6.6\times10^{2\,bc}$	ND - 0	$9.0\times10^{2\,bc}$	$1.6\times10^{3\,bc}$
Pseudomonas sp.2	$2.0\times10^{2\,b}$	$2.120\times10^{3\,a}$	$2.5\times10^{3\,b}$	$5.0\times10^{2\,b}$
Pseudomonas sp.3	$ND - 0^{b}$	$ND - 0^{b}$	$6.25\times10^{2\,b}$	$ND - 0^{b}$
Harvested shrimp (CFU/mL):				
*Aerobic Plate Count	2.5×10^{3}	3.2×10^{4}	1.6×10^{4}	5.7×10^{3}
E. coli	<3.0	<3.0	<3.0	<3.0
Staphylococcus aureus	10	10	10	10
Salmonella	Absent	Absent	Absent	Absent
Shigella	Absent	Absent	Absent	Absent

Note: ND – not detected

Pond Water and Soil Parameters

Water quality parameters in all treatments were within the recommended ranges for *P. monodon* culture as defined by Mohanty et al. (2014), except for salinity, which exceeded optimal levels and may have influenced growth performance (Table 4). Ammonia levels in T0 reached the upper permissible limit, whereas the green-water technology (T1) and IMTA treatments (T2 and T3) maintained ammonia concentrations within acceptable ranges. Statistical analyses showed significant differences in ammonia levels between T0 and the other treatments ($P<0.01$).

Growth and Survival Parameters

Figure 3 presents the growth and survival data of the cultured species. *P. monodon* from T1 (green-water pond) exhibited the highest mean body weight (21.81±1.45 g), followed by IMTA ponds (T2=19.01±1.12 g, T3=16.92±1.08 g), while T0 (monoculture) produced the shrimps with lowest body weight (13.21±1.03 g, $P<0.05$). Specific growth rate (SGR) was significantly higher in T1 (4.15% d^{-1}) compared to T0 (3.72% d^{-1}, $P<0.05$). Survival rates were highest in T3 (58.87%), followed by T2 (55.63%) and T1 (51.12%), while T0 had the lowest survival (16.64%, $P<0.01$). Growth and survival in T1 and IMTA treatments (T2 and T3) were significantly better than in monoculture ($P<0.05$).

For *C. chanos* (milkfish), T3 had the highest average body weight (322±8.71 g), followed by T2 (310±9.02 g), without statistically significant differences between these treatments ($p=0.28$). Survival rates were similar across T2 and T3 (98.13%, $p=0.92$). *O. niloticus* (tilapia) had an average body weight of 125.36±7.15 g, with a survival rate of 31.20% ($P<0.05$).

Production and Profitability

The total biomass production in a 250 m² pond showed that T1 (green-water technology) yielded the highest shrimp biomass (30.53 kg), followed by T3 (27.61 kg) and T2 (27.22 kg), while T0 had the lowest production (6.51 kg, $P<0.01$). Fish biomass was highest in T3 (91 kg, $P<0.05$) (Table 5).

Economic analysis showed that T2 and T3 had the highest gross incomes (USD 258.43 and USD 265.37, respectively). T3 yielded the highest net profit (USD 29.19), followed by T1 (USD 24.98), T2 (USD 22.26), and T0, which recorded a financial loss (USD -41.49). Return on investment (ROI) was highest in T1 (16.39%), followed by T3 (12.36%) and T2 (9.42%), while T0 exhibited the lowest ROI (-53.92%). Annual production estimates indicated that T1 had the highest shrimp production (3.82 tons/ha), whereas T3 produced the highest total biomass of shrimp and fish (11.38 tons/ha, $P<0.05$). T0 exhibited the lowest productivity and economic performance among all treatments.

Discussion

Microbial Load Parameters for Disease Diagnosis

Several studies have identified *Vibrio parahaemolyticus*, *V. alginolyticus*, and other *Vibrio* species as pathogenic bacteria commonly found in shrimp hatcheries and ponds (Nonwachai et al., 2010). Although *V. cholerae* is less virulent than *V. parahaemolyticus* (Haldar et al., 2007), its infection can still result in fatality for marine organisms like *P. monodon* (Joseph et al., 2015). Our study observed the absence of *V. cholerae* in both soil and water samples from IMTA systems reared with green mussel and

Table 3. RT-PCR results for AHNP and WSSV using the shrimp samples

Diseases	T0	T1	T2	T3
AHPND	Negative	Negative	Negative	Negative
WSSV	Positive	Negative	Negative	Negative

Table 4. Water parameter level range observed in all treatments

Treatments	T0	T1	T2	T3	Ideal Values (Mohanty et al., 2014)
NH3 (ppm)	0.01 - 0.1	0.01 - 0.05	0.01 - 0.04	0.01 - 0.02	<0.1
PO4 (ppm)	0.03 - 0.04	0.03 - 0.04	0.04 - 0.07	0.01 - 0.04	<0.1
NO3 (ppm)	0.03 - 0.05	0.03 - 0.04	0.03 - 0.05	0.02 - 0.05	<0.1
NO2 (ppm)	0.02 - 0.04	0.02 - 0.04	0.02 - 0.04	0.03 - 0.04	>0.5
D.O (ppm)	3.3 – 5.8	2.1 - 6.7	2.5 - 4.9	2.1 - 5.0	>2
TDS (ppm)	20.0 - 26.5	20.0 - 28.1	20.2 - 28.6	20.2 - 32.2	<100
pH	7.19 - 8.3	7.1 - 8.6	7.1 - 8.6	7.2 - 8.5	7.5-8.5
Transparency (cm)	30.5 – 45	35.1 – 45	35.5 - 40.5	35.5 - 40.3	35-45
Salinity (ppt)	29 – 32	28.6 – 32	28.2 - 32	28.4 – 32	15-25
Temperature (°C)	29 – 33	29.1 - 32.7	29.8 - 32.2	28.5 - 32.6	28-33

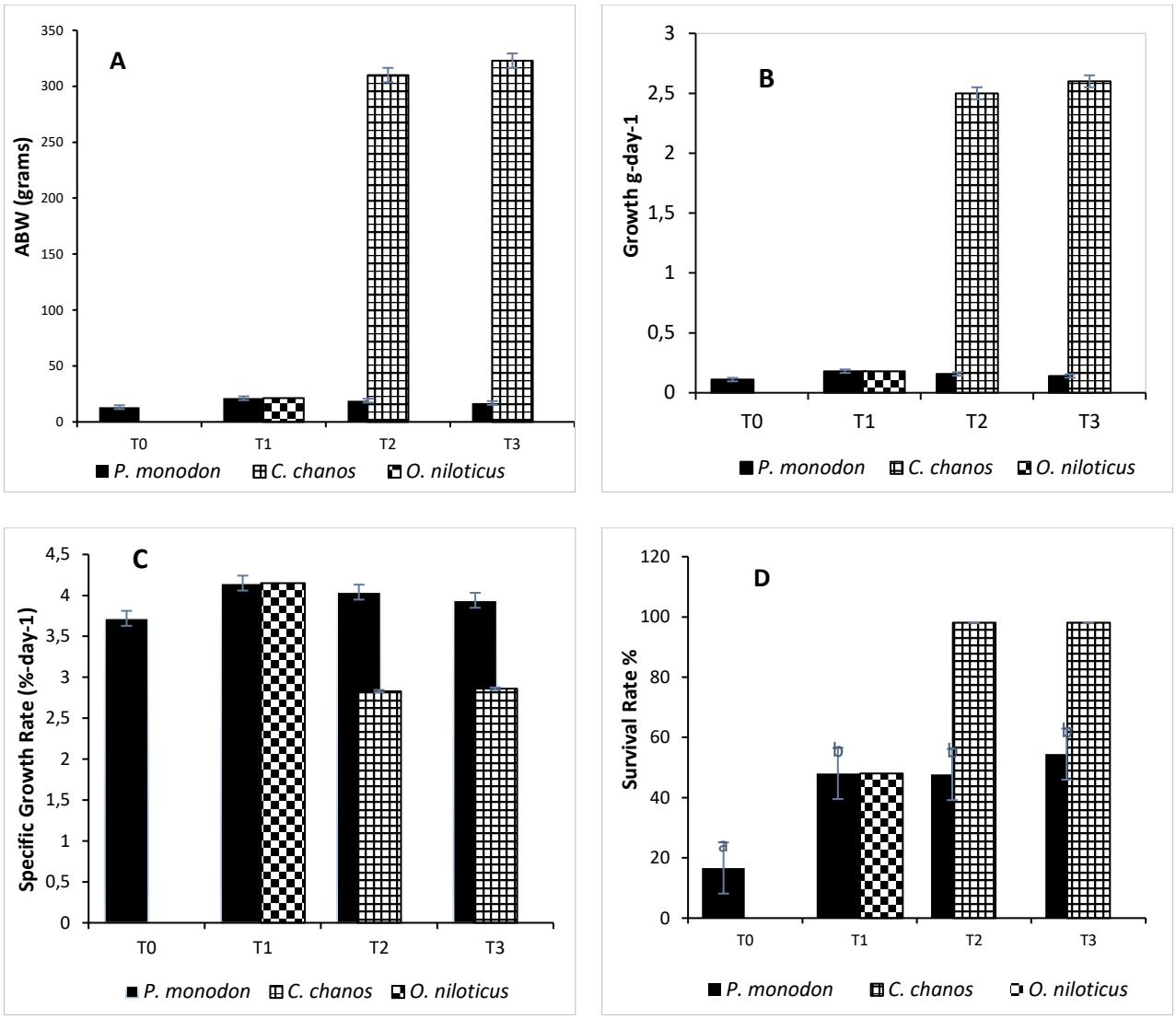

Figure 3. Growth and survival of *P. monodon, C. chanos and O.niloticus* in IMTA pond.

G. verrucose (P<0.05). This finding supports the notion that these organisms inhibit *V. cholerae*, potentially improving disease resistance in shrimp. Notably, statistical analysis (ANOVA, P<0.05) confirmed that treatment groups T1 and T2 exhibited lower *V. cholerae* counts compared to monoculture control (T0).

Additionally, *V. parahaemolyticus* and *V. alginolyticus* are associated with diseases like AHPND (Lai et al., 2015) and WSSV infection. Our results showed that *V. parahaemolyticus* was absent in both water and soil samples of T1 and T3 (P<0.05), suggesting that the IMTA treatments, with the green mussel and *G. verrucosa* combinations, helped mitigate the growth of Vibrio species. The reduction of *V. alginolyticus* was similarly significant in T1 and T3 (P<0.01), demonstrating the effectiveness of these treatments in controlling opportunistic pathogens. Our study corroborates the findings by Peeler and Maturin (1992) and Kharisma and Manan (2012), who noted that *Vibrio* populations above 10^4 CFU/mL increase disease susceptibility.

The microbial analysis conducted in this study further validated the benefits of IMTA systems in preventing pathogen growth, with significant reductions in *Vibrio*, *E. coli*, *Pseudomonas*, and *Staphylococcus aureus* counts (P<0.05) in T1 and T3 compared to T0.

Molecular Parameters for Disease Diagnosis

Biotic and abiotic stressors influence the vulnerability of shrimp to diseases like WSSV. Among these, *Vibrio* species play a critical role in disease development (Tendencia et al., 2010). While all water parameters in the treatments were similar, statistical comparisons (P<0.05) revealed that higher *Vibrio* counts in T0 correlated with increased susceptibility to WSSV. This suggests that high organic load in Control treatment could exacerbate disease risks unless properly managed. On the other hand, the absence of pathogenic *Vibrio* species in T3 (P<0.01) highlights the role of IMTA systems in disease prevention.

Table 5. Production and profitability of the different treatments in one pond.

Treatments	T0	T1	T2	T3
Total shrimp production (kg 250m^2)	6.51	30.53	27.22	27.61
Total fish production (kg 250m^2)	6.51	38.26	87.97	91.00
Gross income (USD/pond)	35.45	177.29	280.51	288.39
Operating Cost (USD/pond)	76.95	152.46	236.18	236.18
Net profit (USD/pond)	-41.49	24.83	44.33	52.21
Production Cycle	2.5	2.5	2.5	2.5
Estimated annual net profit (USD)/pond	-103.73	62.07	110.82	130.52
ROI (%)	-53.92	14.53	9.26	12.45
Annual shrimp production per pond (kg 250m^2)	16.275	76.32	68.05	69.025
Annual total production per pond (kg 250m^2)	16.275	95.65	219.92	227.5

P. monodon selling price at USD 5.44, **O. niloticus* selling price at USD 1.45, **C. chanos* selling price at USD 2.18

Pond Water and Soil Parameters

Wang and Chen (2006) emphasized that 25 ppt is the optimum salinity for shrimp immunity against pathogens. In this study, all treatments had similar salinity ranges but were beyond optimal and could potentially hamper their growth and optimal immune function towards disease susceptibility. Statistical analyses (ANOVA, $P<0.05$) indicated that IMTA treatments, particularly those utilizing macroalgae like *G. verrucosa*, resulted in significantly lower levels of ammonia, nitrites, and phosphates compared to monoculture treatments. These findings suggest that IMTA systems efficiently absorb excess nutrients, contributing to improved water quality and disease resistance.

The reduction of suspended solids observed in the IMTA ponds was also statistically significant ($P<0.01$), with *P. viridis* potentially effective in filtering phytoplankton, zooplankton, and organic detritus, preventing nutrient accumulation and potential eutrophication. This highlights better waste management capabilities of IMTA systems. Our results align with previous studies by Pan and Wang (2004) and Gao et al. (2006), which reported similar filtration benefits from bivalves in integrated aquaculture systems.

Growth and Survival of Cultured Species

The integration of *C. chanos* with *P. monodon* at low density (0.25 pcs/m^2) resulted in significantly better shrimp growth compared to monoculture ($P<0.05$), aligning with previous studies by Pudadera and Lim (1982). Additionally, our results demonstrated that IMTA treatments with *G. verrucosa* provided optimal growth conditions for *P. monodon*, as evidenced by the significantly higher growth rates ($P<0.01$) compared to control treatments. These results support the recommendation of incorporating *G. verrucosa* into IMTA systems to enhance shrimp production and profitability.

Production and Profitability

The economic evaluation showed that monoculture (T0) resulted in the lowest and negative profit from all other treatments. Treatment 3, despite having higher costs, yielded the highest biomass and net profit. The combined shrimp and fish production, alongside the pricing of the three species, directly influenced the overall profitability of the treatments. A regression analysis ($R^2=0.89$) confirmed that the IMTA systems' higher biomass production contributed significantly to the increased profitability compared to monoculture treatments.

In conclusion, this study provides compelling evidence that IMTA systems, particularly those incorporating *G. verrucosa*, not only improve water quality and reduce disease susceptibility but also enhance shrimp growth and profitability. The statistically significant reductions in pathogenic *Vibrio* species and improved survival and growth of *P. monodon* in IMTA treatments underscore the system's potential for sustainable shrimp farming. Further studies focusing on the optimization of IMTA pond designs and feeding rates are recommended to enhance the economic feasibility and ecological benefits of this technology.

Ethical Statement

This study was granted and permitted to proceed and was provided with an ethic certification by the Institutional Ethics Review Committee. This study conforms to the ethical and scientific rigor and ensures the welfare of the invertebrate animals involved in this study.

Funding Information

The study was funded by the Department of Science- Philippine Council for Agriculture, Aquaculture and Natural Resources Research and Development (DOST-PCAARRD) through IARRD and implemented by MSU at Naawan.

Author Contribution

Elgen Arriesgado: Conceptualization, Investigation, Methodology, Writing -review and editing;

Jomar Besoña: Data Curation, Formal Analysis, Investigation, Methodology, Visualization and Writing;

Victor Navarro: Data Curation, Formal Analysis, Investigation, Methodology, Writing

Rustan Eballe: Data Curation, Formal Analysis, Investigation, Methodology, Writing

Dan Arriesgado: Resources, Investigation, Methodology, Writing -review and editing, Supervision

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Acknowledgements

The study is part of the project "Evaluating IMTA as an Approach to Disease and Environmental Management for Sustainable Culture of *Penaeus monodon* in Northern Mindanao" funded by the Department of Science- Philippine Council for Agriculture, Aquaculture and Natural Resources Research and Development (DOST-PCAARRD) through IARRD and implemented by MSU at Naawan. The study sites were hosted by Mindanao State University (MSU) at Naawan. Special thanks to Jay D. Tering, Romeo D. Pinaso, Mark dela Peña and Ms. Marnelle Sornito, for the technical assistance.

References

[AOAC] Association of Official Analytical Chemists. (1990). *AOAC Official Methods of Analysis*. 15th Edition. Arlington, VA.

[APHA] American Public Health Association. (1989). *Standard methods for the examination of water and wastewater*. 17th edition. Washington DC.

Arriesgado, E. M., dela Peña, M. A., Pinaso Jr, R. D., Tering, J. D., Navarro, V. R., Eballe, R. C., Besoña, J. F., & Arriesgado, D. M. (2022). Identifying the Suitable Integrated Multi-trophic Aquaculture (IMTA) Species Combination for *Penaeus monodon* (Fabricius, 1798) for a Sustainable Pond Aquaculture. *Philippine Journal of Science*, 151(6B), 2497-2507. https://doi.org/10.56899/151.6b.14

Besoña, J. F., Arriesgado, E. M., Capangpangan R. Y., Arriesgado, D. M., & Pedroso, F. L. (2024). Bioremediation efficiency of sea cucumber *Holothuria scabra* (Jaeger, 1833) on the quality of water and sediment of shrimp *Penaeus monodon* (Fabricius, 1798) pond culture. *International Journal of Aquatic Biology*, 12(1), 57-67. https://doi.org/10.22034/ijab.v12i1.2124

Biswas, G., Kumar, P., Ghoshal, al. T. K., Kailasam, M., De, D., Bera, A., ... Vijayan, K. K. (2020). Integrated multi-trophic aquaculture (IMTA) outperforms conventional polyculture with respect to environmental remediation, productivity and economic return in brackishwater ponds. *Aquaculture*, 516, 734626. https://doi.org/10.1016/j.aquaculture.2019.734626

Caro, L. F., Mai, H. N., Cruz-Florez, R., Marcos, F. L., Alenton, R. R., & Dhar, A. K. (2021). Experimental reproduction of White Feces Syndrome in whiteleg shrimp*, Penaeus vannamei*. *Plos one*, 16(12), e0261289. https://doi.org/10.1371/journal.pone.0261289

Chang Z. Q., Neori, A., He, Y. Y., Li, J. T., Qiao, L., Preston S. I. Preston, Liu, P., & Li, J. (2020). Development and current state of seawater shrimp farming, with an emphasis on integrated mutli-trophic aquaculture farms, in China- a review. *Reviews in Aquaculture*, 12(4), 2544-2558. https://doi.org/10.1111/raq.12457

Chary, K., Aubin, J., Sadoul, B., Fiandrino, A., Covès, D., & Callier, M.D. (2020). Integrated multi-trophic aquaculture of red drum (*Sciaenops ocellatus*) and sea cucumber (*Holothuria scabra*): assessing bioremediation and life-cycle impacts. *Aquaculture*, 516, 734621. https://doi.org/10.1016/j.aquaculture.2019.734621

Chiu, Y. N., Sumagaysay, N. S., & Sastrillo, M. A. S. (1987). Effect of feeding frequency and feeding rate on the growth and feed efficiency of milkfish, *Chanos chanos*, Forsskal, al. juveniles. *Asian Fisheries Science*. https://doi.org/10.33997/j.afs.1987.1.1.003

Chopin, T. (2008). *Integrated multi-trophic aquaculture (IMTA) will also have its place when aquaculture moves to the open ocean*. Fish Farmer, 31(2), 40-41.

Chopin, T., & Robinson, S. (2006). *Rationale for developing integrated multi-trophic aquaculture (IMTA): an example from Canada.* Fish Farmer, 29(1), 20-21.

Chopin, T., Buschmann, A. H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G. P., Zertuche-Gonzalez, J. A., Yarish, C., & Neefus, C. (2001). Integrating seaweeds into marine aquaculture systems: a key towards sustainability. *Journal of Phycology*, 37, 975–986. https://doi.org/10.1046/j.1529-8817.2001.01137.x

Cruz, P., Andalecio, M., Bolivar, R., & Fitzsimmons, K. (2008). Tilapia-Shrimp Polyculture in Negros Island, Philippines: A Review. *Journal of The World Aquaculture Society*, 39. 713-725. https://doi.org/10.1111/j.17497345.2008.00-207.x.

Cubillo, A. M., Ferreira, J. G., Robinson, S. M., Pearce, C. M., Corner, R. A., & Johansen, J. (2016). Role of deposit feeders in integrated multi-trophic aquaculture—a model analysis. *Aquaculture*, 453, 54-66. https://doi.org/10.1016/j.aquaculture.2015.11.031

de La Peña L., Cabillon, N. A., Catedral, D., Amar, E., Usero, R., Calpe, A. T., Monotilla, W. D., Fernandez, D., & Saloma, C. (2015). Acute hepatopancreatic necrosis disease (AHPND) outbreaks in *Penaeus vannamei* and *Penaeus monodon* cultured in the Philippines. *Diseases of aquatic organisms,* 166, 251-254. https://doi.org/10.3354/dao02919

Eldani, A., & Primavera, J. H. (1981). Effect of different stocking combinations on growth, production and survival of milkfish *(Chanos chanos* Forskal) and prawn (*Penaeus monodon* Fabricius) in polyculture in brackishwater ponds. *Aquaculture*, 23(1-4), 59-72. https://doi.org/10.1016/0044-8486(81)90007-7

Evania, C., Rejeki, S., & Aryati, R. W. (2018). PERFORMA PERTUMBUHAN UDANG WINDU (*Penaeus monodon*) YANG DIBUDIDAYAKAN BERSAMA KERANG HIJAU (*Perna viridis*) DENGAN SISTEM IMTA. *Indonesian Journal of*

Tropical Aquaculture, 2(2), 44-52. https://doi.org/10.14710/sat.v2i2.2984

FAO. (2020). *The State of World Fisheries and Aquaculture 2020. Sustainability in action.* Rome. https://doi.org/10.4060/ca9229en

Gao, Q. F., Shin, P. K., Lin, G. H., Chen, S. P., & Cheung, S. G. (2006). Stable isotope and fatty acid evidence for uptake of organic waste by green-lipped mussels *Perna viridis* in a polyculture fish farm system. *Marine Ecology Progress Series*, 317, 273-283. https://doi.org/10.3354/meps317273

Gonzales-Corre, K. (1988). *Polyculture of the tiger shrimp (Penaeus monodon) with the Nile tilapia (Oreochromis niloticus) in brackish water fish ponds*. In Proceedings of the Second International Symposium on Tilapia in Aquaculture, Manila, 15-20.

Haldar, S., Chatterjee, S., Asakura, M., Vijayakumaran, M., & Yamasaki, S. (2007). Isolation of *Vibrio parahaemolyticus and Vibrio cholerae* (Non-O1 and O139) from moribund shrimp (*Penaeus monodon*) and experimental challenge study against post larvae and juveniles. *Annals of microbiology*, 57, 55-60. https://doi.org/10.1007/bf03175050

Hasan B.M.A., Guha B., & Dutta S. 2011. Salinity mediated growth responses of Penaeus monodon cultured in two different coastal-saline zones of India. Environment and Ecology 29: 2023–2027.

Hawkins, A. J. , Smith, R. F. , Tan, S. H., & Yasin, Z. B. (1998). Suspension-feeding behaviour in tropical bivalve molluscs: *Perna viridis*, *Crassostrea belcheri, Crassostrea iradelei*, *Saccostrea cucculata* and *Pinctada margarifera*. *Marine Ecology Progress Series*, 166, 173-185. https://doi.org/10.3354/meps166173

Izzati, M. (2011). The role of seaweeds *Sargassum polycistum* and *Gracilaria verrucosa* on growth performance and biomass production of tiger shrimp (*Penaeaus monodon* Fabr). *Journal of Coastal Development*, 14(3), 235-241.

Joseph, A., & Philip, R. (2007). Acute salinity stress alters the haemolymph metabolic profile of *Penaeus monodon* and reduces immunocompetence to white spot syndrome virus infection. *Aquaculture*, 272(1-4), 87-97. https://doi.org/10.1016/j.aquaculture.2007.08.047

Joseph, T. C., Murugadas, V., Reghunathan, D., Shaheer, P., Akhilnath, P. G., & Lalitha, K. V. (2015). Isolation and characterization of *Vibrio cholerae* O139 associated with mass mortality in *Penaeus monodon* and experimental challenge in postlarvae of three species of shrimp. *Aquaculture*, 442, 44-47. https://doi.org/10.1016/j.aquaculture.2015.02.020

Kharisma, A., & Manan, A. (2012). The abundance of *Vibrio* sp. bacteria on enlargement water of L*itopenaeus vannamei* as the early detection of Vibriosis. *Jurnal Ilmiah Perikanan dan Kelautan*, 4(2). https://doi.org/10.20473/jipk.v4i2.11563

Lai, H. C., Ng, T. H., Ando, M., Lee, C. T., Chen, I. T., Chuang, J. C., ... & Wang, H. C. (2015). Pathogenesis of acute hepatopancreatic necrosis disease (AHPND) in shrimp. *Fish & shellfish immunology*, 47(2), 1006-1014. https://doi.org/10.1016/j.fsi.2015.11.008

Lavilla-Pitogo, C. R., Baticados, M. L., Cruz-Lacierda, E. R., & de la Peña, L. D. (1990). Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines, *Aquaculture*, 91(12), 1990, 1-13. https://doi.org/10.1016/0044-8486(90)90173-k

Leung P., Tran, L., Fast, A. (2000). A logistic regression of risk factors for disease occurrence on Asian shrimp farms. *Diseases of aquatic organisms*, 41, 65-76. https://doi.org/10.3354/dao041065

Lightner, D. V. (1996). Epizootiology, distribution and the impact on international trade of two penaeid shrimp viruses in the Americas. *Revue scientifique et technique (International Office of Epizootics)*, 15(2), 579-601. https://doi.org/10.20506/rst.15.2.944

Magbanua, F., Natividad, K., Migo, V., Alfafara, C., de la Peña, F., Miranda, R., Albaladejo, J., Nadala, E., Loh, P., & Mahilum-Tapay, L. (2000). White spot syndrome virus (WSSV) in cultured Penaeus monodon in the Philippines. *Diseases of Aquatic Organisms*, 42, 77–82. https://doi.org/10.3354/dao042077

Mamauag, R. E., Baliao, D., & Cruz, J. M. d. (2021). Black tiger shrimp culture rejuvenation: the OPLAN Balik Sugpo of SEAFDEC/AQD. *Fish for the People*, *19*(2), 2-7.

Mohanty, R. K., Mishra, A., & Patil, D. U. (2014). Water budgeting in black tiger shrimp *Penaeus monodon* culture using different water and feed management systems. *Turkish Journal of Fisheries and Aquatic Sciences*, 14(2), 487-496. https://doi.org/10.4194/1303-2712-v14_2_20

Neori, A., Chopin, T., Troell, M., Buschmann, A. H., Kraemer, G. P., Halling, C., ... & Yarish, C. (2004). Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. *Aquaculture*, 231(1-4), 361-391. https://doi.org/10.1016/j.aquaculture.2003.11.015

Nonwachai, T., Purivirojkul, W., Limsuwan, C., Chuchird, N., Velasco, M., & Dhar, A. K. (2010). Growth, nonspecific immune characteristics, and survival upon challenge with *Vibrio harveyi* in Pacific white shrimp (*Litopenaeus vannamei*) raised on diets containing algal meal. *Fish & Shellfish Immunology*, 29(2), 298-304. https://doi.org/10.1016/j.fsi.2010.04.009

Otta, Karunasagar, & Karunasagar. (2001). Bacteriological study of shrimp, *Penaeus monodon* Fabricius, hatcheries in India. *Journal of Applied Ichthyology*, 17(2), 59–63. https://doi.org/10.1046/j.1439-0426.2001.00249.x

Paena, M., Syamsuddin, R., Rani, C., & Tandipayuk, H. (2018) The distribution of organic waste discharged from super-intensive vaname shrimp (*Litopenaeus vannamei*) ponds monitored using stable isotopes. *AACL Bioflux*, 11(4), 1089-1097.

Palanca-Tan, R. (2017). Aquaculture, Poverty and Environment in the Philippines. *The Journal of Social, al. Political, al. and Economic Studies*, 43(3/4), 294-315.

Pan, J. F., & Wang, W. X. (2004). Influences of dissolved and colloidal organic carbon on the uptake of Ag, Cd, and Cr by the marine mussel Perna viridis. *Environmental Pollution*, 129(3), 467-477. https://doi.org/10.1016/j.envpol.2003.11.015

Peeler, J. T. & Maturin, L. J. (1992). Aerobic plate count. *In L.A. Tomlinson (ed.)*

Philippine Statistics Authority (2022). *Value production in Philippine Agriculture and Fisheries*.

Primavera, J. H. (1997). Socioeconomic impacts of shrimp culture. *Aquaculture Research*, 28, 815-827. https://doi.org/10.1111/j.1365-2109.1997.tb01006.x

Pudadera Jr, B. J., & Lim, C. (1982). Evaluation of milkfish (*Chanos chanos* Forskal) and prawn (*Penaeus monodon* Fabricus) in polyculture system. *Fisheries Research Journal of the Philippines*, 7(1), 51-59.

Ramos, R., Vinatea, L., Andreatta, E. R., & da Costa, R. H. (2008). Treatment of efluent from ponds of *Litopenaeus vannamei* shrimp rearing through nutrients sedimentation and absortion with the macroalga *Ulva fasciata*. *Boletim do Instituto de Pesca*, 34, 345-353.

Shahar, B., & Guttman, L. (2021). Integrated biofilters with Ulva and periphyton to improve nitrogen removal from mariculture effluent. *Aquaculture*, 532, 736011. https://doi.org/10.1016/j.aquaculture.2020.736011

Sharma, S. K., Shankar, K. M., Sathyanarayana, M. L., Sahoo, A. K., Patil, R., Narayanaswamy, H. D., & Rao, S. (2010). Evaluation of immune response and resistance to diseases in tiger shrimp, *Penaeus monodon* fed with biofilm of *Vibrio alginolyticus*. *Fish & shellfish immunology*, 29(5), 724-732. https://doi.org/10.1016/j.fsi.2010.07.016

Steffens, W. (1989). *Principles of fish nutrition*. Ellis Horwood Limited.

Tendencia, E. A., Bosma, R. H., & Verreth, A. J. (2010). WSSV risk factors related to water physico-chemical properties and microflora in semi-intensive *Penaeus monodon* culture ponds in the Philippines. *Aquaculture,* 302(3–4), 164-168. https://doi.org/10.1016/j.aquaculture.2010.03.008

Trianti, C. M., & Adharini, R. I. (2020). The Utilization of Gracilaria verrucosa as shrimp ponds wastewater biofilter. *E3S Web of Conferences*, 147, 02023. https://doi.org/10.1051/e3sconf/202014702023

Troell, M., Halling, C., Neori, A., Chopin, T., Buschmann, A. H., Kautsky, N., & Yarish, C. (2003). Integrated mariculture: asking the right questions. *Aquaculture*, 226(1-4), 69-90. https://doi.org/10.1016/s0044-8486(03)00469-1

Wang, F. I., & Chen, J. C. (2006). Effect of salinity on the immune response of tiger shrimp *Penaeus monodon* and its susceptibility to *Photobacterium damselae* subsp. damselae. *Fish & Shellfish Immunology*, 20(5), 671-681. https://doi.org/10.1016/j.fsi.2005.08.003

Ye, L., Jiang, S., Zhu, X., Yang, Q., Wen, W., & Wu, K. (2009). Effects of salinity on growth and energy budget of juvenile Penaeus monodon. Aquaculture, 290(1–2), 140–144. https://doi.org/10.1016/j.aquaculture.2009.01.028