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Effects of shrimp-fish polyculture on immune parameters, disease resistance of white shrimp and the prevalence of *Vibrio* spp

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Abstract

We pre-grew shrimp under polyculture (shrimp-tilapia 'ST' and shrimp-mullet 'SM') and monoculture (P-control) conditions until they reached the size of 10–12 g. Then, the shrimps were collected and injected with *Vibrio alginolyticus* (2.1×10^5 CFU shrimp^{-1}). Some shrimp, originating from the shrimp monoculture, was also injected with sterile saline solution, served as N-Control. Over 8 days, mortality in N-control was significantly lower than that in the other treatments ($p = 0.001$). Total haemocyte counts (THC), phenoloxidase activity (PO) and respiratory burst activity (RB) in haemocytes decreased sharply after injection and were significantly different in P-control, ST and SM as compared to N-control ($p < 0.05$). However, there were no significant differences in shrimp survival and immune parameters among P-control, ST and SM ($p > 0.05$). It is concluded that the application of shrimp-fish polyculture systems does not contribute to the robustness of cultured shrimp as verified by challenge through injection. The second experiment was carried out to verify the effect of shrimp-mullet polyculture on the prevalence of *Vibrio* spp. Mullet (3.45 g) were supplemented (either biomass of 500 or 700 g m^{-3}) into six aquaria where shrimp (0.48 g) was stocked at a density of 80 g m^{-3}. The other six aquaria were just stocked with shrimp as P-control and N-control. Aquaria were inoculated with *V. harveyi* at a density of 10^3 CFU ml^{-1}, except for N-control. Results showed that the presence of mullet at either biomass of 500 or 700 g m^{-3} could reduce TCBS counts in water by 20.9% and 24.3%, respectively, as compared to shrimp monoculture.

KEYWORDS
immune response, monoculture, shrimp polyculture, *Vibrio*

1 | INTRODUCTION

The shrimp aquaculture industry worldwide has faced various serious diseases that are mainly caused by pathogenic bacteria and viruses (Amparyup et al., 2013). These diseases contributed heavily to shrimp production losses during the last two decades. One of the serious diseases in shrimp farming related to bacterial species (Lavilla-Pitogo et al., 1998), especially caused by *Vibrio* spp, which are considered to cause severe economic losses in the shrimp farming worldwide (Chatterjee, 2012). Many studies, working with apparently healthy shrimp, have shown that 30% of the bacterial flora was *Vibrio* spp (Soto-Rodriguez et al., 2015). Several species of Vibrio genus such as *V. harveyi*, *V. anguillarum*, *V. splendidus*, *V. parahaemolyticus* and *V. alginolyticus* have been reported as the

causative agents of bacterial disease in shrimp (Austin & Zhang, 2006; Chatterjee, 2012; Lightner, 1996). Furthermore, an emerging bacterial disease, caused by *V. parahaemolyticus* genus (acute hepatopancreatic necrosis disease - AHPND), has occurred in Asian and Mexican shrimp aquaculture, resulting in shrimp mortality up to 70% and an annual loss amounting to USD 1 billion (De Schryver et al., 2014; Soto-Rodriguez et al., 2015).

Vibriosis disease is normally considered to be the consequence of an opportunistic infection, when shrimp are immunologically weak (Moriarty, 1998), eventually in combination with stressful environmental conditions (Srinivas et al., 2019) and high bacterial concentration in the water column. Several studies have indicated that *Vibrio* spp. concentrations in the water at equal to or greater than 10^4 CFU ml^{-1} cause mass mortality for white shrimp *L. vannamei* (Cheng et al., 2005; Liu & Chen, 2004; Wang & Chen, 2005). Moreover, there is also a developing awareness that the prevalence of *Vibrio* spp is ultimately affected by water parameters such as temperature, pH, salinity and nutrients present in the water column (De Souza Valente & Wan, 2021). Barraza-Guardado et al. (2013) documented that *Vibrio* genus grow rapidly when the aquatic environment is enriched by accumulating organic matter.

Different technologies such as greenwater (De Schryver et al., 2014), quorum sensing inhibitors (Defoirdt et al., 2007), antibiotic strategy and integration aquaculture have been tried to prevent or control diseases in shrimp aquaculture (Tendencia et al., 2006). Ekasari et al. (2014) claimed that one should not only focus on implementing biosecurity measures to prevent and control the disease but also consider an integrated approach involving, improving the cultured animal immunity and maintaining good water quality. Among previous recommended technologies for preventing or controlling bacterial disease in shrimp cultivation, integration of shrimp and other aquatic organisms, especially polyculture of shrimp and finfish, is considered to be a feasible biosecurity measure. Paclibare et al. (2002) reported that shrimp-finfish integration has the potential in preventing Vibriosis in shrimp farming. Several studies investigated the effect of the presence of different fish species in polyculture with shrimp on luminescent bacteria prevalence (Hellio et al., 2002). For instance, seabass, milkfish, snapper, grouper and siganid (Tendencia et al., 2006) and tilapia species (Tendencia et al., 2004) have been reported to limit CFU counts of luminescent bacteria. Loc et al. (2016) found that tilapia polyculture pathogenic *Vibrio parahaemolyticus* strain causing the acute hepatopancreatic necrosis disease (AHPND) were reduced in numbers. Mullet (*Mugil liza*) could reduce effectively the population of *Vibrio* spp. in the shrimp and mullet integrated culture (Borges et al., 2020). Based on these previous findings, it is hypothesized that the integration of white shrimp and grey mullet may have an inhibitory effect on the prevalence of pathogenic agents in the water column. In addition, it is also hypothesized that the better water quality and competition between shrimp and fish for food in shrimp-fish polyculture systems might enhance the health condition of the cultured organisms, resulting in improved disease resistance of the shrimps. Thus, the present study was carried out to evaluate the effect of shrimp-fish polyculture systems on immune parameters, disease resistance of white shrimp and its effect on the prevalence of *Vibrio* spp. in shrimp aquaculture.

2 | MATERIALS AND METHODS

The Animal Ethics Committee of HUAF has been established in 2021, so formal ethics approval was not required or obtained at the time this study was conducted. In the absence of a regulatory framework for formal ethical approval, the work was conducted according to the ethical standards of the UK Home Office, based on training received by the co-author at the Institute of Aquaculture, University of Stirling, Stirling, UK.

2.1 | Experiment 1

2.1.1 | Shrimp preparation

White shrimp juveniles were obtained from a local commercial farm in Hue province, Vietnam. After acclimation in the laboratory for 2 weeks, shrimp (0.50 ± 0.16 g) were randomly distributed into three fibreglass tanks (capacity of 5 m^3) where one tank was used to stock only shrimp at a density of 60 shrimp m^{-3} (monoculture tank) and the two other tanks served as polyculture tanks. Shrimp (60 shrimp m^{-3}) and grey mullet (*Mugil cephalus* - 1.51 ± 0.02 g at a stocking density of 10% of shrimp biomass) were co-cultured in one polyculture tank. Whereas in the other polyculture tank, shrimp (60 shrimp m^{-3}) and tilapia (*Oreochromis niloticus*—1.49 ± 0.05 g at a stocking density of 10% of shrimp biomass, with the fish being kept in a cage installed in the tank) were raised together. Shrimp were fed three times a day (06:00, 12:00 and 18:00 h) at a feeding rate of 3 – 10% body weight per day using 38% crude protein commercial pellets (GroBest Co. Ltd. Vietnam). Shrimp were cultured under seawater conditions (15–20 g L^{-1}) until they reached the experimental size of 10–12 g.

2.1.2 | Vibrio alginolyticus

A known pathogenic strain of *V. alginolyticus* (AQ-13–91) was collected from the Laboratory of Aquaculture & Artemia Reference Center (ARC), Ghent University, Belgium. Bacteria inocula were cultured on tryptic soy agar (TSA supplemented with 2% NaCl, Hi Media-India) for 24 h at 28°C, and a single colony was transferred to 10 ml tryptic soy broth (TSB supplemented with 2%NaCl, Hi Media-India) for 24 h at 28°C for use as a bacterial stock. Bacterial stock cultures were centrifuged at 7155 *g* for 20 min at 4°C, followed by removing the supernatant fluid. Afterwards, the bacterial pellet was re-suspended in sterile saline solution (0.85% NaCl) at a concentration of 1.1×10^6 CFU ml^{-1} for the challenge test (the concentration of pathogenic bacteria used in this experiment was determined based on a previously established lethal dose—LD_{50} test—unpublished data).

2.1.3 | Experimental setup

The shrimp in the inter-molt stage (the molt stage can be determined by the examination of uropoda according to the method described by Liu and Chen (2004)) ranging from 10 to 12 g, with no significant size difference were chosen for the experimentation. Challenge tests were carried out according to the methods described by Liu and Chen (2004). In brief, the experiment included four treatments with four replicates each, in which four aquaria were used for shrimp originating from shrimp + mullet tank (SM) and four aquaria for shrimp from shrimp + tilapia tank (ST). In addition, the other eight aquaria shrimp originating from the shrimp monoculture tank were challenged (positive; P-control) and non-challenged (negative; N-control). Shrimp were anaesthetized using a sub-lethal dose (100 mg L^{-1}) of tricaine methanesulphonate (MS-222). Shrimp were then challenged with *V. alginolyticus* by injection using needles (25 gauges) into the ventral sinus of the cephalothorax of each shrimp at a dose of 2.1×10^5 CFU $shrimp^{-1}$ (20 μl of bacterial suspension per shrimp) and randomly distributed into sixteen fibreglass aquaria (60 L) at 20 shrimp $aquarium^{-1}$. However, in the N-control aquaria, shrimp were injected with an equal volume of sterile saline solution.

After injection, shrimp were kept in seawater (15–20 g L^{-1}) for 8 days and fed two times a day (8:00 and 17:00 h) at a feeding rate of 3% body weight per day using 38% crude protein challenging pellets. During the experiment, aeration was supplied constantly in experimental aquaria by air stones to maintain dissolved oxygen concentration of 5 mg L^{-1}. The water temperature was maintained at 28–30°C, and pH 7.2–7.5. Experimental aquaria were cleaned daily by siphoning and loss of water was compensated. Immune parameters were measured when mortality appeared and at a time point that mortality terminated.

2.2 | Experiment 2

2.2.1 | Aquatic organisms

Pacific white shrimp (*L. vannamei*) were bought from a certified commercial hatchery located in Thua Thien Hue province, Vietnam, whereas fingerling grey mullet (*M. cephalus*) were collected from a commercial hatchery in Quang Tri province, Vietnam. After reaching the research station, aquatic organisms were acclimated to laboratory conditions for 3–4 weeks prior to obtaining the experimental size (shrimp = 0.48 ± 0.15 g, and fish = 3.45 ± 0.24 g).

2.2.2 | Vibriosis

The known pathogenic species *V. alginolyticus* (AQ-13-91), *V. harveyi* (BB120) and *V. parahaemolyticus* (PV01) were taken from the Laboratory of Aquaculture & Artemia Reference Center (ARC), Ghent University, Belgium. Bacterial stock preparation was done as described for the first experiment. For the antibacterial assay, colonies were chosen directly from TSA plates. However, for an inhibitory test, TSB stock cultures were centrifuged at 7155 *g* for 20 min at 4 °C, followed by removing the supernatant fluid. The bacterial pellet was re-suspended in saline solution to obtain a concentration of 10^3 CFU ml^{-1}.

2.2.3 | Mucus collection and extraction

Grey mullet (80–90 g) were reared (salinity of 15–20 g L^{-1}) in fibreglass tank (5 m^3) with a stocking density of 30 fish per tank. Fish were fed twice a day (8:00 and 17:00 h) at a feeding rate of 2% body weight per day using 30% crude protein commercial pellet (GroBest Co. Ltd. Vietnam). Fish were regularly examined for their physical health condition; only apparent healthy fish were sampled for mucus collection. Mucus was collected from 15 fish as described by Ross et al. (2000) with a slight modification. In short, fish were starved for 24 h and the mucus was collected non-lethally by anaesthetizing the fish with a sub-lethal dose (100 mg L^{-1}) of tricaine methanesulphonate (MS-222). Individual anaesthetized fish was transferred into a polyethylene bag (containing 10 ml of 100 mM NaCl) where fish was gently moved back and forth inside the bag for 2–3 min to slough off the mucus and then returned to the rearing tanks. The mucus samples obtained from individual fish were pooled to yield one mucus sample to be used for the next extraction procedure.

Mucus extraction was implemented by following the method of aqueous solvents as described by Subramanian et al. (2008). The pooled mucus from 15 fish was freeze-dried, re-suspended in 100 mM (w/v) ammonium bicarbonate at 1 mg ml^{-1} and centrifuged at 9500 *g* for 10 min at 4°C. The 5 kDa molecular weight cut-off centrifugal filter devices (Amicon®Ultra-15, Millipore Corporation, USA) were used to desalt the supernatant. The mucus retention was freeze-dried and re-suspended in PCR water (1 g mucus in 20 ml PCR water) for the antimicrobial assay.

2.2.4 | Experimental setup

Antibacterial assay

- **Disk diffusion method**: An antibacterial assay was performed according to the method described by Magarinos et al. (1995) and Tendencia (2007). Briefly, colonies of the *V. parahaemolyticus*, *V. harveyi and V. alginolyticus* on the TSA plates were separately suspended in saline solution (the turbidity compared with #5 MacFarland) and swabbed onto nutrient agar plates. After drying for 15 min, disks impregnated with the fish mucus stock solution (diluted with PCR water at 1 g mucus in 20 ml PCR water) of 30 μl were put on the plates. Plates were incubated for 24 h at 30°C and observed for clearing zones around the disks.
- **Spot inoculation assay**: To check the antibacterial effect of the mullet's mucus, another assay was also conducted following

the method described by Magarinos et al. (1995) and Tendencia (2007). Colonies of *V. parahaemolyticus*, *V. harveyi* and *V. alginolyticus* on the TSA plates were collected and suspended in saline solution (the turbidity compared with #5 MacFarland). The bacterial suspension of the *V. parahaemolyticus*, *V. harveyi* and *V. alginolyticus* were spread onto nutrient agar plates and dried for 15 min. A volume of 10 μl of the fish mucus was spot inoculated separately onto nutrient agar plates. Plates were incubated for 24 h at 30°C and observed for clearing zones around the inoculated spot.

Effect of grey mullet in polyculture with white shrimp on the prevalence of V. harveyi

Twelve fibreglass aquaria (60 L) were filled with disinfected seawater at a level of 50 L and were stocked with *L. vannamei* at a density of 80 g m^{-3}. Six aquaria were stocked with shrimp only (P-control and N-control). The other six aquaria were supplemented with *Mugil cephalus* at a density of either 500 g m^{-3} (SM5) or 700 g m^{-3} (SM7) and considered as experimental treatments. Each treatment was performed in triplicates (dissolved oxygen concentration at 5 mg L^{-1}, temperature at 28–30 °C).

In the P-control, SM5 and SM7 aquaria at 6 h after stocking with shrimp and fish, Vibrio (*V. harveyi*, BB120) was added to a final density of 10^3 CFU ml^{-1} (Leobert et al., 2001; Tendencia et al., 2006). The N-control aquaria were supplemented with an equal volume of sterile saline solution. There was no water exchange, and no external food was given throughout the experimental period of 12 days (Tendencia, 2007). Total ammonium nitrogen, nitrite nitrogen and pH level of the rearing water were measured every 2 days.

2.3 | Water quality analysis

Daily water temperature, dissolved oxygen (DO), pH and salinity in all tanks were measured in situ using a portable DO metre (Hanna Model HI-9146, Rumani), pH metre (pH/temperature Hanna Model-HI98190, Rumani) and a refractometer (Atago Model 2491-master's, Japan) respectively. Water in the experimental tanks was sampled every 2 days at 09:00 h for analyses of total ammonium nitrogen (NH_4^+ - N) and nitrite nitrogen (NO_2) using multi-spectrophotometers (Hanna Model-HI83099, Rumani).

2.4 | Aquatic organisms' survival and immune parameters determination

2.4.1 | Aquatic organism survival

$$\text{Survival (\%)} = (\text{number at harvest}/\text{number at stocking}) \times 100.$$

2.4.2 | Immune parameters determination

Shrimp's immune parameters were measured at the start of the challenge, day 2 (when the first mortality appeared) and day 7 (the mortality almost terminated). Six shrimps per treatment were taken for the measurement of immune parameters. Total haemocyte counts (THC), phenoloxidase activity (PO) and respiratory burst activity (RB) were measured according to the methods described by Liu and Chen (2004) and Ekasari et al. (2014). In short, 0.1 ml of haemolymph was taken out from the ventral sinus of each shrimp with a 1 ml syringe (25 gauge) containing 0.9 ml of precooled anticoagulant solution (30 mM trisodium citrate, 0.34 M sodium chloride, 10 mM EDTA, 0.12 M glucose and pH 7.55). To measure total haemocyte counts, a drop of the diluted haemolymph was placed on a haemocytometer to count the number of haemocyte cells under a light microscope (in triplicates).

Phenoloxidase activity, considered as the formation of dopachrome produced from L-dihydroxyphenylalanine (L-DOPA) (Hernández-López et al., 1996), was measured by centrifuging 500 μl of the diluted haemolymph at 700 *g* for 20 min at 4°C, followed by discarding the supernatant fluid. The pellet was then rinsed, re-suspended gently in cacodylate-citrate buffer (0.01 M sodium cacodylate, 0.45 M sodium chloride, 0.10 M trisodium citrate and pH 7.0) and centrifuged again. The pellet was re-suspended in 200 μl cacodylate buffer (0.01 M sodium cacodylate, 0.45 M sodium chloride, 0.26 M magnesium chloride and pH 7.0), and an aliquot of 100 μl was incubated with 50 μl trypsin (1 mg ml^{-1}) as an activator for 10 min at 25°C. Then, 50 μl of L-dihydroxyphenylalanine (L-DOPA) was added and followed by 800 μl of cacodylate buffer 5 min later. The other aliquot of 100 μl cell suspension, as a no-activation control measurement, was prepared at the same time in 850 μl cacodylate buffer and 50 μl L-DOPA. The optical density at 490 nm was measured using a Hitachi U-2900 Spectrophotometer. The optical density of the shrimp's phenoloxidase activity was expressed as dopachrome formation in 50 μl of haemolymph.

Respiratory burst activity of haemocytes was measured based on the reduction in nitroblue tetrazolium (NBT) to formazan (production of the superoxide anion O_2^-), as described by Song and Hsieh (1994) and Ekasari et al. (2014). Briefly, the diluted haemolymph of 100 μl was incubated for 30 min at room temperature, followed by centrifugation at 1000 *g* for 20 min at 4°C. The supernatant was discarded, and the pellet was stained with 100 μl of nitroblue tetrazolium (0.3% in Hank's balanced salt solution) for 2 h at room temperature. The NBT solution was removed by centrifugation at 1000 *g* for 10 min; haemocytes were fixed with 100 μl of absolute methanol and washed three times with 100 μl 70% methanol and air-dried. 120 μl of KOH (2 M) and 140 μl of dimethylsulfoxide (DMSO) were added to dissolve the formazan. The optical density was measured at 630 nm using a microplate reader (Molecular Devices, USA), and respiratory burst was expressed as NBT-reduction in 10 μl of haemolymph.

2.5 | Microbial analysis

Water samples for bacterial counts were collected before 1 h and after 24 h inoculation of the pathogenic agent into the experimental aquaria and then every 2 days until the termination of 12 days (Tendencia et al., 2006). Total bacteria counts (TBC) were determined using soy agar (TSA, Hi Media-India), and TCBS counts were specified using thiosulphate citrate bile salt agar (Hi Media-India). TCBS is a selective media, at least to a certain degree, for the genus *Vibrio*. Series of 10-fold diluted cultured water were spread plated on TSA or TCBS Agar in triplicates.

2.6 | Statistical analyses

Parametric assumptions (water quality parameters, immune parameters, shrimp survival and bacteria counts) were checked using Levene´s test for homogeneity of variances and Shapiro–Wilk´s test for normality. Survival of shrimp and fish was analysed using arcsin transformed data. The samples with homogenous variances were analysed using the Duncan test, while Dunnett´s T3 test was used for the samples with unequal variances (Zar, 1999). All data were normally distributed, homoscedastic and compared by using one-way ANOVA, followed by the Duncan test. Shrimp survival and bacteria counts were evaluated using one-way ANOVA with repeated measures in which the experimental factors were the analysed factors, and the days of culture were an additional factor (Rao, 1998). All the tests were performed using the SPSS program version 20.0 for Windows. Differences were tested at a level of significance of 5%.

3 | RESULTS

3.1 | Experiment 1

3.1.1 | Water quality parameters

Water quality parameters such as temperature, DO, pH, NH_4^+ - N and NO_2 tended to be similar and not significantly different among treatments (Table 1). These water parameters did vary slightly. The temperature of culture water was 28.0 to 29.8 °C, dissolved oxygen (DO) fluctuated from 4.99 to 5.96 mg L^{-1}, and pH was between 7.13 and 7.59. A low range of NH_4^+ - N and NO_2 concentrations can be seen in all aquaria (0.17–0.63 mg L^{-1} and 0.21–0.47 mg L^{-1}, respectively). Generally, water quality parameters in the experimental aquaria seemed to be stable throughout the challenge period.

3.1.2 | Survival of the white shrimp

Twelve hours after injection, the shrimps showed specific symptoms such as slow swimming and poor eating. The mortality of the shrimp started on day 2 and lasted 7 days (Figure 1). Shrimp survival varied from 47 ± 6% to 90 ± 4%. Shrimp survival in the P-control, ST and SM treatments was significant different from survival in the N-control ($p = 0.001$). However, there were no significant differences among P-control, ST and SM treatments ($p > 0.05$). A significant effect of time on shrimp survival was observed during first 6 days of the challenge test ($p = 0.001$).

3.1.3 | Immune parameters of *L. vannamei*

The immune parameters of the *V. alginolyticus* injected shrimp (P-control, ST and SM) tended to decrease sharply after injection and then recovered slowly towards the end of the experiment. Meanwhile, these parameters in the shrimp injected with sterile saline solution (N-control) seemed to be stable throughout the challenge period of 8 days (Figures 2, 3 and 4). Initially, THC, PO and RB activities in haemocytes of the shrimp, which varied from 11.61 ± 0.86 × 10^6 to 13.13 ± 0.98 × 10^6 Cell ml^{-1}, 0.15 ± 0.03 to 0.19 ± 0.02 (OD_{490} 50 μ L^{-1}) and 0.24 ± 0.01 to 0.26 ± 0.02 (OD_{630} 10 μ L^{-1}), respectively, were not significantly different among treatments ($p > 0.05$).

At day 2, a significant difference in THC could be seen in the shrimps from P-control, ST and SM treatments as compared to

TABLE 1 Range and mean value ($n = 4$) of water quality parameters in shrimp culture water in the experiment of the effect of shrimp–fish polyculture on disease resistance and immune parameters of shrimp

	Treatment			
Parameters	**P-control**	**ST**	**SM**	**N-control**
Temperature (°C)	28.0–29.8 29.28 ± 0.13[a]	28.0–29.8 29.29 ± 0.10[a]	28.0–29.7 29.20 ± 0.07[a]	28.0–29.7 29.21 ± 0.08[a]
pH	7.13–7.53 7.29 ± 0.07[a]	7.13–7.53 7.30 ± 0.08[a]	7.15–7.59 7.29 ± 0.09[a]	7.15–7.58 7.26 ± 0.06[a]
DO (mg L^{-1})	5.02–5.96 5.33 ± 0.15[a]	5.08–5.61 5.31 ± 0.14[a]	4.99–5.55 5.32 ± 0.17[a]	5.01–5.61 5.29 ± 0.14[a]
NH_4^+ - N (mg L^{-1})	0.17–0.59 0.36 ± 0.05[a]	0.18–0.56 0.35 ± 0.05[a]	0.19–0.61 0.37 ± 0.02[a]	0.18–0.63 0.34 ± 0.06[a]
NO_2 (mg L^{-1})	0.25–0.46 0.34 ± 0.05[a]	0.24–0.44 0.32 ± 0.04[a]	0.23–0.45 0.33 ± 0.05[a]	0.21–0.47 0.29 ± 0.03[a]

Note: Mean values in the same row with different superscript letters differ significantly ($p \leq 0.05$). The data correspond to the mean of three replicates ± standard deviation.

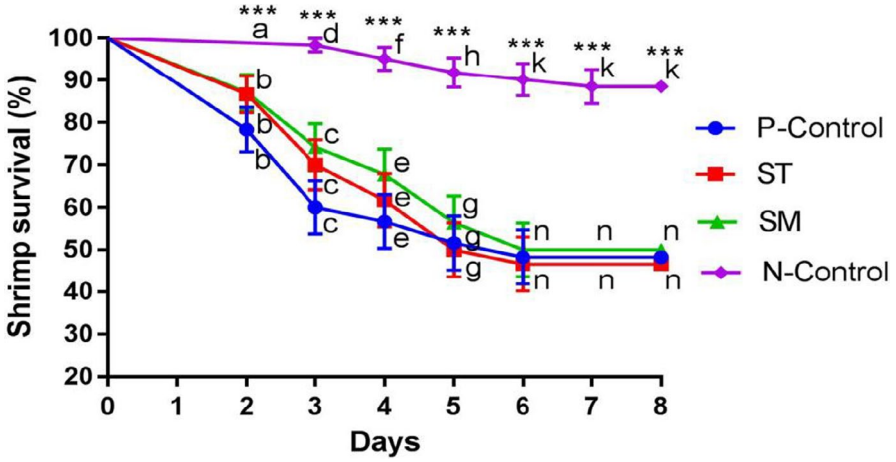

FIGURE 1 Mean (±SD) of survival (%) of white shrimp challenged with *V. alginolyticus* in the different treatments. Values noted with a different letter are significantly different ($p \leq 0.05$)

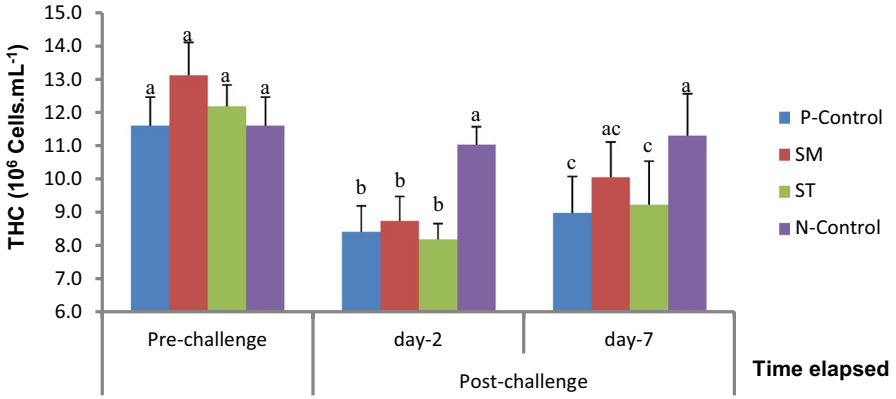

FIGURE 2 Mean (±SD) total haemocyte count (THC) in the haemolymph of *L. vannamei* at the start, day 2, and day 7 after injection with *V. alginolyticus*. Data on the same day with different letters are significantly different ($p \leq 0.05$)

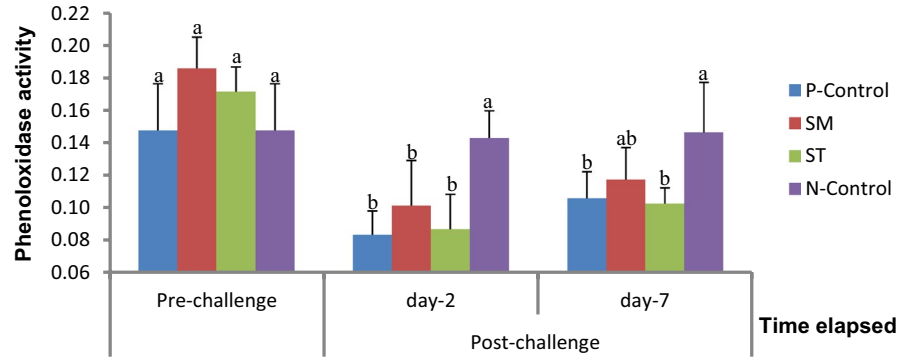

FIGURE 3 Mean (±SD) phenoloxidase activity in the haemocytes of *L. vannamei* at the start, day 2, and day 7 after injection with *V. alginolyticus*. Data on the same day with different letters are significantly different ($p \leq 0.05$)

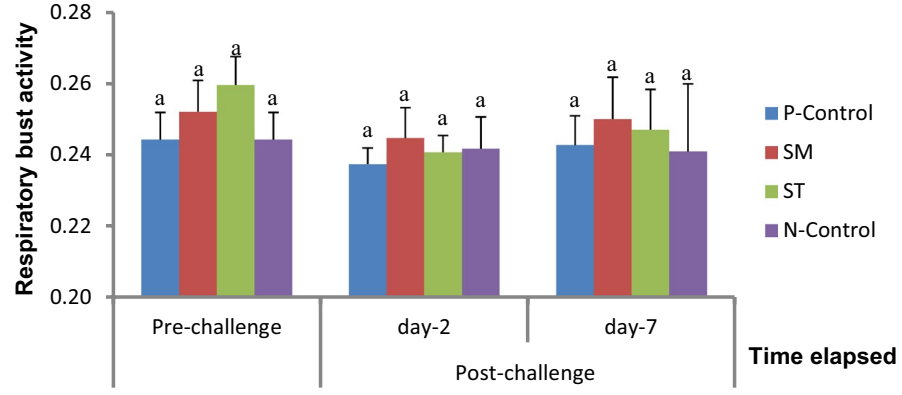

FIGURE 4 Mean (±SD) superoxide anion production in the haemocytes of *L. vannamei* at the start, day 2, and day 7 after injection with *V. alginolyticus*. Data on the same day with different letters are significantly different ($p \leq 0.05$)

those from the N-control treatment ($p < 0.05$). However, there was no significant difference among P-control, ST and SM treatments ($p > 0.05$) (Figure 2). At day 7, THC of the shrimp increased slightly as compared to day 2. It was significantly lower in P-control and ST treatments as compared to those in N-control treatment ($p < 0.05$). However, there were no significant differences among P-control, ST and SM treatments, and between N-control and SM treatments ($p > 0.05$). A similar trend could be found in PO activity, which was significantly lower for the shrimps in P-control, ST and SM treatments than those in N-control ($p < 0.05$) at day 2. There was no significant difference among P-control, ST and SM treatments ($p > 0.05$) (Figure 2). After 7 days, PO activity, which increased slightly, was significantly lower in P-control and ST treatments than that in N-control ($p < 0.05$). However, there were no significant differences among P-control, ST and SM treatments, and between N-control and SM treatments ($p > 0.05$) (Figure 3). No significant difference in RB activity (superoxide anion O_2^-), which was expressed as the NBT-reduction per 10 μl haemolymph, could be seen for the shrimps in all treatments throughout three sampling points ($p > 0.05$) (Figure 4).

3.2 | Experiment 2

3.2.1 | Water quality parameters

Water quality parameters such as temperature, DO and pH varied slightly as 28.1–29.8 °C, 4.71–5.92 mg L^{-1} and 7.02–7.59 respectively. These water parameters were not significantly different among treatments ($p > 0.05$) and seemed to be stable throughout the experimental period of 12 days (Table 2). However, mean values of total ammonium nitrogen (NH_4^+ - N) and nitrite (NO_2) concentrations in the P-control (0.26 mg L^{-1} and 0.24 mg L^{-1}, respectively) and N-control treatments (0.26 mg L^{-1} and 0.25 mg L^{-1}, respectively) were significantly higher than those in SM5 (0.21 mg L^{-1} and 0.20 mg L^{-1}, respectively) and in SM7 treatments (0.20 mg L^{-1} and 0.20 mg L^{-1}, respectively) ($p < 0.05$).

3.2.2 | Antibacterial activity of mullet's mucus and survival of the experimental organisms

The results of the antibacterial assay showed that mullet's mucus did not have any inhibitory effect on the growth of Vibrio species (*V. alginolyticus*, *V. parahaemolyticus* and *V. harveyi*). After 12 days of starving, survival for white shrimp was 70%–80, and for mullet was 62%–70% (Table 3). The inoculation of *V. harveyi* into the experimental aquaria did not affect the survival of the cultured organisms ($p > 0.05$).

3.2.3 | Effect of the addition of grey mullet in shrimp tanks on the growth of *V. harveyi*

In general, TCBS counts in P-control and shrimp polyculture aquaria tended to decrease steadily from the initial inoculation day (day 1) towards the end of the experiment (day 12). On the contrary, TCBS counts in N-control aquaria (inoculated with sterile saline solution) increased sharply from day 0 to day 6 and levelled off at day 6. The values of TCBS counts then fluctuated slightly around the peak until the termination of the experiment (Figure 5).

TABLE 3 Survival of shrimp and mullet in the experiment inoculated with *V. harveyi* (BB120)

	Treatment			
Survival (%)	SM5	SM7	P-control	N-control
Shrimp[a]	73 ± 6[a]	77 ± 6[a]	70 ± 10[a]	80 ± 10[a]
Grey mullet[b]	70 ± 6[a]	61 ± 10[a]	–	–

Note: Mean values in the same row with different superscript letters differ significantly ($p \leq 0.05$).

The data correspond to the mean of three replicates ± standard error.

[a]One-way ANOVA and Tukey's test.

[b]Student's *t* test ($p \leq 0.05$).

TABLE 2 Range and mean value ($n = 3$) of water quality parameters in shrimp culture water in the experiment of controlling the prevalence of bacteria (Vibrio)

	Treatment			
Parameters	P-control	SM5	SM7	N-control
Temperature (°C)	28.3–29.8 29.34 ± 0.09[a]	28.1–29.8 29.29 ± 0.11[a]	28.2–29.7 29.35 ± 0.05[a]	28.10–29.7 29.33 ± 0.09[a]
pH	7.02–7.55 7.30 ± 0.05[a]	7.03–7.55 7.27 ± 0.08[a]	7.05–7.41 7.21 ± 0.06[a]	7.07–7.59 7.23 ± 0.04[a]
DO (mg L^{-1})	4.73–5.79 5.18 ± 0.13[a]	4.79–5.65 5.23 ± 0.03[a]	4.82–5.92 5.21 ± 0.07[a]	4.71–5.55 5.09 ± 0.10[a]
NH_4^+ - N (mg L^{-1})	0.02–0.43 0.26 ± 0.07[a]	0.01–0.34 0.21 ± 0.02[b]	0.02–0.38 0.20 ± 0.02[a]	0.02–0.42 0.26 ± 0.06[b]
NO_2 (mg L^{-1})	0.02–0.53 0.24 ± 0.01[a]	0.02–0.32 0.20 ± 0.04[b]	0.02–0.31 0.20 ± 0.01[a]	0.02–0.45 0.25 ± 0.01[b]

Note: Mean values in the same row with different superscript letters differ significantly ($p \leq 0.05$).

The data correspond to the mean of three replicates ± standard deviation.

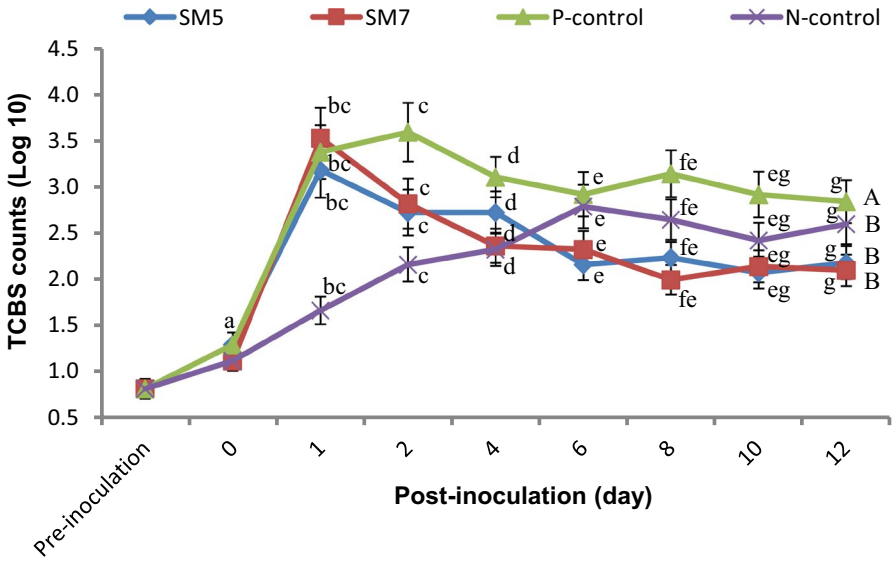

FIGURE 5 Mean (±SD) of TCBS counts in the water column over the experimental period of 12 days. Values noted with different letters (upper-case letters show statistical analysis of treatments 'the whole curves', while lower-case letters present statistical analysis of time series) are significantly different ($p \leq 0.05$)

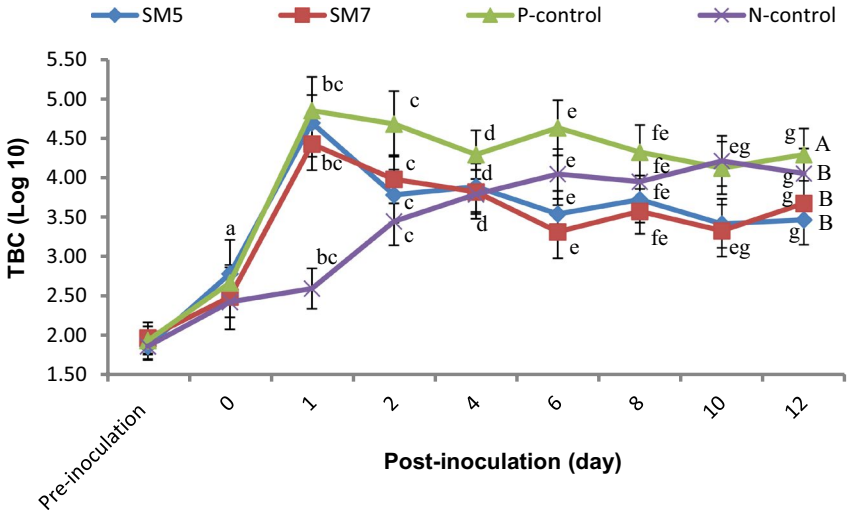

FIGURE 6 Mean (±SD) of total bacteria counts in the water column over the experimental period of 12 days. Values noted with different letters (upper-case letters show statistical analysis of treatments 'the whole curves', while lower-case letters present statistical analysis of time series) are significantly different ($p \leq 0.05$)

A significant difference in TCBS counts (for the whole curves) was observed in P-control as compared to other treatments ($p \leq 0.05$). However, there was no significant difference among N-control, SM5 and SM7 ($p > 0.05$). TCBS counts within each treatment were significantly different at day 0 as compared to days 1, 2, 4, 6, 8, 10 and 12 ($p < 0.05$) at day 1 compared to days 4, 6, 8, 10 and 12 ($p < 0.05$), at day 2 compared to days 4, 6, 8, 10 and 12 ($p < 0.05$), at day 4 compared to days 6, 8, 10 and 12 ($p < 0.05$), between days 6 and 12, and between days 8 and 12 ($p < 0.05$). However, there were no significant differences between days 1 and 2; and between days 6, 8 and 10 ($p > 0.05$) on one hand, and between days 10 and 12 ($p > 0.05$) on the other hand (Figure 5).

A similar trend can be seen in total bacteria counts (TBC) which tended to decrease steadily from the initial inoculation day (day 1) towards the end of the experiment (day 12) in P-control, SM5 and SM7 treatments. On the contrary, TBC in N-control increased sharply from day 0 to day 6, levelled off at day 10. The values of TBC were significantly lower in N-control, SM5 and SM7 treatments than those in P-control ($p < 0.05$). There were no significant differences among N-control, SM5 and SM7 treatments ($p > 0.05$). The fluctuation of TBC within each treatment seemed to be identical to TCBS counts (Figure 6).

4 | DISCUSSION

4.1 | Effect of shrimp-fish polyculture systems on immune parameters, disease resistance of white shrimp

In the present study, after *V. alginolyticus* injection, a reduction in the levels of THC, PO and RB activities for the shrimps in P-control, ST and SM was observed. However, it was not the case for the shrimps in the N-control (injected with sterile saline solution). Several studies have reported a decrease in both THC and PO when white shrimp were injected with a pathogen (Cheng et al., 2005; Ekasari et al., 2014; Liu & Chen, 2004; Wang & Chen, 2005). The amount of transcript encoding prophenoloxidase decreased

by 50% when blue shrimp (*L. stylirostris*) were injected with *V. alginolyticus* (Le Moullac & Haffner, 2000). A reduction in the concentration of THC, PO and RB activities is a normal physiological response in case of infection (Ekasari et al., 2014; Le Moullac & Haffner, 2000; Liu et al., 2010). If not mortal, it takes approximately 9–12 days for shrimp to recover their immune system from pathogen infections (Chang et al., 2003). On the contrary, there was no significant difference in the level of THC, PO and RB for the shrimp in P-control and shrimp pre-grown in polyculture systems, and no significant difference in shrimp survival was found among P-control, ST and SM treatments ($p > 0.05$). This implies that the application of shrimp-finfish polyculture systems does not contribute to the robustness of cultured shrimp as verified by challenge through injection with this pathogen.

The present work suggests that the shrimp-finfish polyculture may not enhance the health condition of the cultured shrimp, resulting in improved disease resistance of the shrimp. It is different from biofloc systems. Phenoloxidase activity (PO) and respiratory burst activity (RB) of the white shrimp grown in the biofloc systems were higher than those of the shrimp from the conventional system (Ekasari et al., 2014). The survival of *L. vannamei* challenged with *V. parahaemolyticus* was enhanced when shrimp were reared in the biofloc system (Kumar et al., 2020). Furthermore, Shinn et al. (2018) also documented that the biofloc can protect white shrimp from *V. parahaemolyticus* and assist in controlling bacterial infections.

4.2 | Effect of integration of white shrimp and grey mullet on the prevalence of *Vibrio* spp

Feeding can result in an increase in the TBC in the water column (Tendencia et al., 2006). Hence in experiment 2, feed was not given to the cultured organisms (shrimp and mullet) throughout the experimental period of 12 days to avoid the influence of this potential confounding factor. Under these experimental conditions, there was a significant reduction in the concentration of *Vibrio* spp. in the water. Borges et al. (2020) observed a similar effect when comparing shrimp monoculture (SM) with shrimp integrated cultures (SIC). Not onlyTCBS counts were significantly higher in SM as compared to SIC, but also here, this difference did not affect the survival of shrimp monoculture.

A lower in TCBS counts (expressed as CFU ml^{-1}) in SM5 and SM7 than those in P-control probably related to the accumulation of organic matter (OM) released in aquaria by the cultured organisms throughout the experimental period. It has been proven that the total suspended solid (TSS) concentrations (unpublished data) in the control were higher than those in shrimp integrated aquaria. Also, in daily observation of the experimental aquaria, it appeared that the aquaria bottoms in shrimp-fish polyculture treatments were cleaner than those in the control. Furthermore, some water quality parameters such as the total ammonium nitrogen (NH_4^+ - N) and nitrite (NO_2) in the control were also higher than those in shrimp integrated aquaria. This might be caused by organic matter recycling in shrimp integrated system. Cruz et al. (2008) reported that the presence of consumers (e.g. tilapia, mullet, rabbitfish and milkfish) in shrimp culture help to clean up the particulate organic waste. Thus, it could be concluded that the presence of mullet in shrimp integrated aquaria, in the present study, might be conducive to a reduction in organic matter (OM) in the integrated culture, although it is not clear how the feeding behaviour of mullet brings about this effect. A higher amount of OM, a favourable nutrient source for the growth of bacteria, in the control as compared to in shrimp integrated aquaria might be conducive to the proliferation of *Vibrio* spp. bacteria as detected by TCBS and TBC plating.

The presence of mullet in shrimp integrated aquaria could also release antibacterial substances against bacteria, resulting in a reduction in *Vibrio* spp. concentration in for other reasons. This could be attributed to the microflora associated with shrimp-mullet polyculture or chemical substance released by fish's mucus or fish's intestine (Hellio et al., 2002; Tendencia et al., 2006). Moriarty (1999) called the mechanism competitive exclusion in which one species out-competes another in a natural quest for food and habitat. Several studies proved that the bacteria associated with tilapia culture have antimicrobial activity against *V. harveyi* (Lio-Po et al., 2002). The bacteria, originating from bacterial microbiota of the jewel tilapia mucus, gut and rearing water, can antagonize luminescent vibrio thus effectively inhibiting the population of the pathogen (Lio-Po et al., 2005). Tendencia et al. (2006) reported that hybrid tilapia (*Oreochromis niloticus*) and grouper (*Epinephelus coioides*) at stocking density 500 g m^3 efficiently inhibited the growth of luminescent bacteria in shrimp (biomass = 80 g m^3) rearing water and positively affected shrimp survival. Moreover, some other studies also discovered the antibacterial activity of different fish species in controlling pathogens (Austin & McIntosh, 1988; Magarinos et al., 1995). Dash et al. (2017) suggested that integrated fish and shrimp culture can be an effective alternative to reduce the *Vibrio* spp. bacteria in water.

Also, it is well known that the mucus layer on the surface of fish harbours several antimicrobial agents that provide the first line of defence against invading pathogens (Subramanian et al., 2008). A variety of biologically active substances such as lysozyme, lectins, proteolytic enzymes, flavoenzymes, immunoglobulins, C-reactive protein, apolipoprotein A–I and antimicrobial peptides were found in fish mucus (Ellis, 1999; Kitani et al., 2008; Villarroel et al., 2007). Nevertheless, in the present study, the results obtained from the in-vitro assay showed that mullet mucus had no inhibitory effect on the growth of *V. parahaemolyticus*, *V. harveyi* and *V. alginolyticus*. It could be that the mullet mucus has weak antibacterial activity against pathogenic bacteria or the essay may not have tested at suitable concentrations of mullet's mucus sufficient to detect an inhibitory effect on the growth of *V. harveyi*. Thus, further studies are necessary to confirm the antibacterial activity of mullet mucus, especially, searching for the inhibitory concentration and the inhibitory mechanisms.

5 | CONCLUSION

The present study indicates that the shrimp were pre-grown under the described fish-shrimp polyculture conditions did not either

enhance disease resistance or improve immune parameters. The presence of grey mullet at both biomass of 500 g m^{-3} and 700 g m^{-3} could reduce TCBS counts and TBC in shrimp (biomass = 80 g m^{-3}) rearing water.

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DATA AVAILABILITY STATEMENT

Data available on request from the authors

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REFERENCES

Amparyup, P., Charoensapsri, W., & Tassanakajon, A. (2013). Prophenoloxidase system and its role in shrimp immune responses against major pathogens. *Fish & Shellfish Immunology*, *34*(4), 990–1001.

Austin, B., & McIntosh, D. (1988). Natural antibacterial compounds on the surface of rainbow trout, Salmo gairdneri Richardson. *Journal of Fish Diseases*, *11*(3), 275–277.

Austin, B., & Zhang, X.-H. (2006). Vibrio harveyi: A significant pathogen of marine vertebrates and invertebrates. *Letters in Applied Microbiology*, *43*(2), 119–124. https://doi.org/10.1111/j.1472-765X.2006.01989.x

Barraza-Guardado, R. H., Arreola-Lizárraga, J. A., López-Torres, M. A., Casillas-Hernández, R., Miranda-Baeza, A., Magallón-Barrajas, F., & Ibarra-Gámez, C. (2013). Effluents of shrimp farms and its influence on the coastal ecosystems of Bahía de Kino, Mexico. *TheScientificWorldJournal*, *2013*, 306370.

Borges, B. A. A., Rocha, J. L., Pinto, P. H. O., Zacheu, T., Chede, A. C., Magnotti, C. C. F., Cerqueira, V. R., & Arana, L. A. V. (2020). Integrated culture of white shrimp *Litopenaeus vannamei* and mullet Mugil liza on biofloc technology: Zootechnical performance, sludge generation, and *Vibrio* spp. reduction. *Aquaculture*, *524*, 735234. https://doi.org/10.1016/j.aquaculture.2020.735234

Chang, C.-F., Su, M.-S., Chen, H.-Y., & Liao, I.-C. (2003). Dietary β-1, 3-glucan effectively improves immunity and survival of Penaeus monodon challenged with white spot syndrome virus. *Fish & Shellfish Immunology*, *15*(4), 297–310.

Chatterjee, S. (2012). Vibrio related diseases in aquaculture and development of rapid and accurate identification methods. *Journal of Marine Science: Research & Development*, *s1*. https://doi.org/10.4172/2155-9910.S1-002

Cheng, W., Wang, L.-U., & Chen, J.-C. (2005). Effect of water temperature on the immune response of white shrimp *Litopenaeus vannamei* to Vibrio alginolyticus. *Aquaculture*, *250*(3–4), 592–601.

Cruz, P. S., Andalecio, M. N., Bolivar, R. B., & Fitzsimmons, K. (2008). Tilapia–shrimp polyculture in Negros Island, Philippines: A review. *Journal of the World Aquaculture Society*, *39*(6), 713–725.

Dash, P., Avunje, S., Tandel, R. S., Sandeep, K. P., & Panigrahi, A. (2017). Biocontrol of luminous vibriosis in shrimp aquaculture: A review of current approaches and future perspectives. *Reviews in Fisheries Science & Aquaculture*, *25*(3), 245–255.

de la Leobert, D. P., Lavilla-Pitogo, C. R., & Paner, M. G. (2001). Luminescent vibrios associated with mortality in pond-cultured shrimp Penaeus monodon in the Philippines: Species composition. *Fish Pathology*, *36*(3), 133–138.

De Schryver, P., Defoirdt, T., & Sorgeloos, P. (2014). Early mortality syndrome outbreaks: A microbial management issue in shrimp farming? *PLoS Path*, *10*(4), e1003919.

De Souza Valente, C., & Wan, A. H. L. (2021). Vibrio and major commercially important vibriosis diseases in decapod crustaceans. *Journal of Invertebrate Pathology*, *181*, 107527.

Defoirdt, T., Boon, N., Sorgeloos, P., Verstraete, W., & Bossier, P. (2007). Alternatives to antibiotics to control bacterial infections: Luminescent vibriosis in aquaculture as an example. *Trends in Biotechnology*, *25*(10), 472–479.

Ekasari, J., Azhar, M. H., Surawidjaja, E. H., Nuryati, S., De Schryver, P., & Bossier, P. (2014). Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. *Fish & Shellfish Immunology*, *41*(2), 332–339.

Ellis, A. (1999). Immunity to bacteria in fish. *Fish & Shellfish Immunology*, *9*(4), 291–308.

Hellio, C., Pons, A. M., Beaupoil, C., Bourgougnon, N., & Le Gal, Y. (2002). Antibacterial, antifungal and cytotoxic activities of extracts from fish epidermis and epidermal mucus. *International Journal of Antimicrobial Agents*, *20*(3), 214–219.

Hernández-López, J., Gollas-Galván, T., & Vargas-Albores, F. (1996). Activation of the prophenoloxidase system of the brown shrimp (Penaeus californiensis Holmes). *Comparative Biochemistry and Physiology-C-Pharmacology Toxicology Endocrinology*, *113*(1), 61–66.

Kitani, Y., Kikuchi, N., Zhang, G., Ishizaki, S., Shimakura, K., Shiomi, K., & Nagashima, Y. (2008). Antibacterial action of L-amino acid oxidase from the skin mucus of rockfish Sebastes schlegelii. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *149*(2), 394–400.

Kumar, V., Wille, M., Lourenço, T. M., & Bossier, P. (2020). Biofloc-based enhanced survival of *Litopenaeus vannamei* upon AHPND-causing vibrio parahaemolyticus challenge is partially mediated by reduced expression of its virulence. *Genes*, *11*(1270). https://doi.org/10.3389/fmicb.2020.01270

Lavilla-Pitogo, C. R., Leaño, E. M., & Paner, M. G. (1998). Mortalities of pond-cultured juvenile shrimp, Penaeus monodon, associated with dominance of luminescent vibrios in the rearing environment. *Aquaculture*, *164*(1–4), 337–349.

Le Moullac, G., & Haffner, P. (2000). Environmental factors affecting immune responses in Crustacea. *Aquaculture*, *191*(1), 121–131.

Lightner, D. V. (1996). *A handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp*. World Aquaculture Society.

Lio-Po, G. D., Leaño, E. M., Peñaranda, M. M. D., Villa-Franco, A. U., Sombito, C. D., & Guanzon, N. G. Jr (2005). Anti-luminous Vibrio factors associated with the 'green water'grow-out culture of the tiger shrimp Penaeus monodon. *Aquaculture*, *250*(1–2), 1–7.

Lio-Po, G. D., Leaño, E. M., Usero, R. C., & Guanzon, N. G. Jr. (2002). Vibrio harveyi and the'green water culture'of Penaeus monodon. *Disease Control in Fish and Shrimp Aquaculture in Southeast Asia–Diagnosis and Husbandry Techniques: Proceedings of the SEAFDEC-OIE Seminar-Workshop on Disease Control in Fish and Shrimp Aquaculture in Southeast Asia–Diagnosis and Husbandry Techniques, 4-6 December 2001, Iloilo City, Philippines*. Aquaculture Department, Southeast Asian Fisheries Development Center (pp. 172–180).

Liu, C.-H., & Chen, J.-C. (2004). Effect of ammonia on the immune response of white shrimpLitopenaeus vannamei and its susceptibility to Vibrio alginolyticus. *Fish & Shellfish Immunology*, *16*(3), 321–334.

Liu, K.-F., Chiu, C.-H., Shiu, Y.-L., Cheng, W., & Liu, C.-H. (2010). Effects of the probiotic, Bacillus subtilis E20, on the survival, development,

stress tolerance, and immune status of white shrimp, *Litopenaeus vannamei* larvae. *Fish & Shellfish Immunology*, *28*(5–6), 837–844.

Loc, H., Tran, K., & Lightner, D. (2016). *Effects of tilapia in controlling the acute hepatopancreatic necrosis disease (AHPND)*. School of Animals and Comparative Biomedical Sciences, The University of Arizona.

Magarinos, B., Pazos, F., Santos, Y., Romalde, J. L., & Toranzo, A. E. (1995). Response of Pasteurella piscicida and Flexibacter maritimus to skin mucus of marine fish. *Diseases of Aquatic Organisms*, *21*(2), 103–108.

Moriarty, D. J. A. (1998). Control of luminous vibrio species in penaeid aquaculture ponds. *Aquaculture*, *164*, 351–358.

Moriarty, D. J. (1999). Disease control in shrimp aquaculture with probiotic bacteria. In *Proceedings of the 8th international symposium on microbial ecology* (pp. 237–243). Atlantic Canada Society for Microbial Ecology Halifax.

Paclibare, J. O., Usero, R. C., Somga, J. R., & Visitacion, R. N. (2002). Integration of finfish in shrimp (Penaeus monodon) culture: An effective disease prevention strategy. In *Disease Control in Fish and Shrimp Aquaculture in Southeast Asia-Diagnosis and Husbandry Techniques: Proceedings of the SEAFDEC-OIE Seminar-Workshop on Disease Control in Fish and Shrimp Aquaculture in Southeast Asia-Diagnosis and Husbandry Techniques*, *4-6 December 2001*, *Iloilo City*, *Philippines* (pp. 151–171). Aquaculture Department, Southeast Asian Fisheries Development Center.

Rao, P. V. (1998). *Statistical research methods in the life sciences*. Duxbury Press.

Ross, N. W., Firth, K. J., Wang, A., Burka, J. F., & Johnson, S. C. (2000). Changes in hydrolytic enzyme activities of naive Atlantic salmon Salmo salar skin mucus due to infection with the salmon louse *Lepeophtheirus salmonis* and cortisol implantation. *Diseases of Aquatic Organisms*, *41*(1), 43–51.

Shinn, A., Jiravanichpaisal, J., Griffiths, D., Pokharatsiri, A., Burana, P., Sumon, T., Tongmee, C., Decamp, O., & Galli, L. (2018). Effect of biofloc on the survival of whiteleg shrimp, *Penaeus vannamei* boone 1931, when challenged with a pathogenic strain of vibrio parahaemolyticus, the causative agent of acute hepatopancreatic necrosis disease (AHPND). *Asian Fisheries Science*, *31S*, 210–225. https://doi.org/10.33997/j.afs.2018.31.S1.015

Song, Y.-L., & Hsieh, Y.-T. (1994). Immunostimulation of tiger shrimp (Penaeus monodon) hemocytes for generation of microbicidal substances: Analysis of reactive oxygen species. *Developmental & Comparative Immunology*, *18*(3), 201–209.

Soto-Rodriguez, S. A., Gomez-Gil, B., Lozano-Olvera, R., Betancourt-Lozano, M., Morales-Covarrubias, M. S., & Griffiths, M. W. (2015). Field and experimental evidence of vibrio parahaemolyticus as the causative agent of acute hepatopancreatic necrosis disease of cultured shrimp (*Litopenaeus vannamei*). *Northwestern Mexico*, *81*(5), 1689–1699. https://doi.org/10.1128/AEM.03610-14

Srinivas, D., & Venkatrayulu, C. C. (2019). *International Journal of Advanced Research in Biological Sciences Prevalence of Vibriosis in Penaeus (Litopenaeus) vannamei in three different locations of Nellore district of Coastal Andhra Pradesh*.

Subramanian, S., Ross, N. W., & MacKinnon, S. L. (2008). Comparison of antimicrobial activity in the epidermal mucus extracts of fish. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *150*(1), 85–92.

Tendencia, E. A. (2007). Polyculture of green mussels, brown mussels and oysters with shrimp control luminous bacterial disease in a simulated culture system. *Aquaculture*, *272*(1–4), 188–191.

Tendencia, E. A., dela Peña, M. R., Fermin, A. C., Lio-Po, G., Choresca, C. H., & Inui, Y. (2004). Antibacterial activity of tilapia Tilapia hornorum against Vibrio harveyi. *Aquaculture*, *232*(1), 145–152.

Tendencia, E. A., Fermin, A. C., dela Peña, M. R., & Choresca, C. H. (2006). Effect of *Epinephelus coioides*, *Chanos chanos*, and GIFT tilapia in polyculture with *Penaeus monodon* on the growth of the luminous bacteria *Vibrio harveyi*. *Aquaculture*, *253*(1–4), 48–56. https://doi.org/10.1016/j.aquaculture.2005.06.028

Villarroel, F., Bastías, A., Casado, A., Amthauer, R., & Concha, M. I. (2007). Apolipoprotein AI, an antimicrobial protein in Oncorhynchus mykiss: Evaluation of its expression in primary defence barriers and plasma levels in sick and healthy fish. *Fish & Shellfish Immunology*, *23*(1), 197–209.

Wang, L.-U., & Chen, J.-C. (2005). The immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to Vibrio alginolyticus at different salinity levels. *Fish & Shellfish Immunology*, *18*(4), 269–278.

Zar, J. H. (1999). *Biostatistical analysis*. Pearson Education India.

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