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Assessment of the Correlation Between *Vibrio* spp. Abundance in Whiteleg Shrimp (*Litopenaeus vannamei*) and Pond Water, Jepara, Central java, Indonesia

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Abstract

Vibrio spp. are key bacterial groups influencing pond health in shrimp aquaculture. This study examined the relationship between Vibrio abundance in Pacific white shrimp (Litopenaeus vannamei) and pond water in intensive grow-out ponds in Jepara, Central Java. Ten ponds were monitored for two months (DOC 7–56) using weekly paired shrimp and water sampling. Ponds were categorized by surface area as small (100–250 m²), medium (560 m²), and large (1,000 m²). Vibrio abundance was quantified using APW enrichment and TCBS agar plating, followed by Spearman's correlation and generalized linear model analyses. Across all ponds, Vibrio levels were consistently higher in shrimp (5.7×10^4–2.6×10^9 CFU/g) than in pond water (10^3–10^5 CFU/mL). In 9 of 10 ponds, correlations between shrimp and water Vibrio were weak or non-significant, indicating that waterborne levels alone do not reliably reflect bacterial loads in shrimp. Only one pond showed a strong and significant correlation. Generalized linear model results indicated no significant differences in Vibrio abundance among pond size categories in either shrimp or water. These findings demonstrate that pond size did not significantly influence Vibrio dynamics and highlight the need for simultaneous monitoring of shrimp and pond water. Sustained high Vibrio levels in shrimp may serve as an early-warning indicator of elevated microbial risk, even when water quality remains within acceptable ranges.

1. Introduction

Shrimp aquaculture is among the fastest-growing food production sectors globally, with Pacific white shrimp (*Litopenaeus vannamei*) accounting for over 80% of farmed shrimp and reaching 6.83 million tonnes in 2022 (Shinn *et al.*, 2025). Its rapid expansion is driven by rising seafood consumption, increasing demand for high-protein foods, and the shift from declining capture fisheries to intensive aquaculture systems (Chen, Kumar, *et al.*, 2024). Asian and Southeast Asian countries play a central role in sustaining global production. Even though shrimp is cultivated in nearly 70 countries, about 80% of output comes from major producers such as China, Thailand, Indonesia, Vietnam, Ecuador, and India (Eun *et al.*, 2020). Given this production concentration, microbiological control is crucial for maintaining farm performance and competitiveness in international markets. Within the aquaculture sector, the FAO estimates that disease outbreaks result in annual economic losses exceeding USD 9 billion, representing roughly 15% of the total global value of farmed fish and shellfish production (Kumar *et al.*, 2021). Within Southeast Asia, Indonesia is a key producer, but its diverse tropical environments continue to pose challenges for consistent production outcomes (Tran *et al.*, 2017).

Among the microbial hazards affecting shrimp aquaculture, Vibrio species grow rapidly under favorable conditions and, as motile heterotrophs capable of associating with various aquatic organisms, occupy diverse ecological niches (Kumarage *et al.*, 2022; Takemura *et al.*, 2014). In tropical pond systems, these characteristics allow Vibrio to associate with both rearing water and shrimp hosts, enabling populations to respond differently to environmental conditions in the water column and within the shrimp host, and potentially amplifying variability in bacterial abundance across pond systems. Their natural presence in marine and brackish waters, along with their ability to proliferate under unstable environmental conditions and adapt to a wide range of environmental stresses, makes them a persistent concern

(Sampaio *et al.*, 2022). Pathogenic species such as *V. parahaemolyticus*, *V. vulnificus*, and *V. harveyi* are recognized as major causes of severe shrimp diseases, including Acute Hepatopancreatic Necrosis Disease (AHPND) and luminous vibriosis (Kumar *et al.*, 2021). Shrimp affected by vibriosis typically show symptoms such as slow growth and skin necrosis (Yudiati *et al.*, 2021), illustrating the extent of physiological and tissue damage caused by these pathogenic Vibrio strains. These infections have led to substantial global production losses and pose additional risks to human health (Brum *et al.*, 2021; Shanmugasundaram *et al.*, 2015).

The expansion and increasing virulence of Vibrio populations have been associated with warming coastal waters and environmental changes (Brumfield *et al.*, 2025), emphasizing the importance of health management in pond systems (Brum *et al.*, 2021). Although Vibrio contamination affects food safety, its most immediate impacts in aquaculture relate to farm biosecurity, pond stability, and the ability of producers to maintain consistent yields (Brauge *et al.*, 2024). Theoretically, differences in pond scale may further shape these processes, as smaller ponds are often more susceptible to rapid bacterial proliferation due to limited water volume and greater environmental fluctuations. In contrast, larger ponds generally exhibit more buffered conditions but may display distinct environmental characteristics, such as relatively lower and more stable temperatures. These differences highligh the need for careful water quality monitoring to understand Vibrio dynamics across pond systems. Therefore, an understanding of how water quality influences Vibrio dynamics in the water and corresponds to bacterial levels in shrimp is required (Sampaio *et al.*, 2022).

The abundance of Vibrio within shrimp is also shaped by host factors such as immune function, microbiome composition, feeding activity, and physiological stress (Chang *et al.*, 2024; Zhang and Sun, 2022). However, the connection between environmental Vibrio levels and their accumulation in shrimp tissues remains poorly defined, particularly in tropical aquaculture systems in Indonesia, where pond sizes and management practices vary widely. In tropical intensive pond systems, key water quality parameters such as light intensity (lux), temperature, salinity, pH, dissolved oxygen (DO), and water transparency are considered important in shaping Vibrio dynamics. However, dissolved oxygen (DO) and salinity are commonly reported as particularly influential factors in shrimp pond environments. To address this gap, the present study investigates the association between Vibrio abundance in pond water and in *L. vannamei* tissues across ponds of different sizes and sampling periods.

2. Material and methods

2.1 Sample and Sampling Method

Ten grow-out ponds used for farming Pacific white shrimp (*L. vannamei*) in Jepara, Central Java, were selected as sampling sites (Figure 1). The ponds were classified into three size categories to represent different production scales: small ponds (100–250 m²; n = 7), medium ponds (560 m²; n = 1), and large ponds (1,000 m²; n = 2). This classification was intended to capture variation in pond area and management intensity across the production system.

Sampling was conducted using a time-series design over period two-month, with seven sampling events performed at weekly intervals, spanning from DOC 7 to DOC 56. At each sampling time, water and shrimp samples were collected simultaneously from the same fixed point within each pond to obtain paired and spatially consistent data (Mustafa *et al.*, 2022). This fixed point was located near the pond corner where organic matter tends to accumulate due to aerator-driven water circulation, representing an area of elevated microbial activity. All samples were collected aseptically, stored in ice-cooled containers, and transported to the laboratory on the same day for immediate analysis.

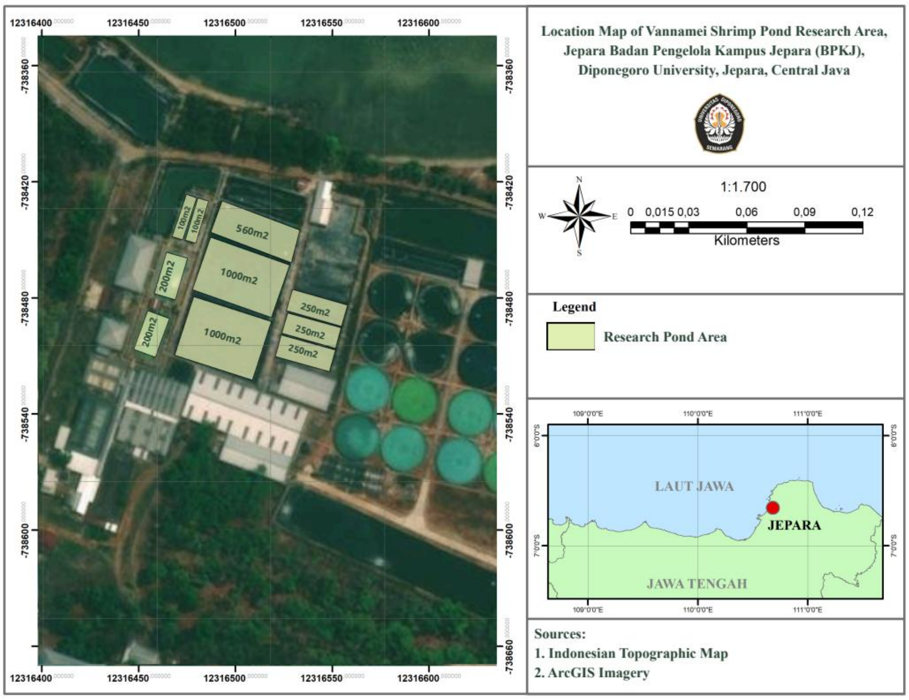

Figure 1. Sampling Location

2.2 Materials and Equipment

Alkaline Peptone Water (APW) was utilized for bacterial enrichment and serial dilution, whereas Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar supplemented with 2% sodium chloride (NaCl) was applied as the selective medium for the isolation and enumeration of *Vibrio* spp. (Scruggs *et al.*, 2024).

2.3 Sample Preparation and Selective Agar

Shrimp samples were prepared by aseptically dissecting the hepatopancreas and muscle tissue, followed by homogenization of 1 g of the combined tissue in 10 mL of Alkaline Peptone Water (APW) to obtain the 10^0 dilution. Serial dilutions (10^{-1}–10^{-3}) were subsequently prepared in 9 mL of APW (Tawhid *et al.*, 2024). Pond water samples were diluted directly to 10^{-1} and 10^{-2} by transferring 1 mL of water into 9 mL of APW. For selective isolation, 0.1 mL of each dilution was spread onto TCBS agar supplemented with 2% NaCl. Each dilution of both shrimp and water samples was plated in duplicate to ensure consistency of bacterial enumeration. Plates were incubated at 34–38 °C for 24–48 h to allow selective recovery of Vibrio colonies (Faulds *et al.*, 2023).

2.4 Colony Enumeration and Correlation Analysis Method

Colony enumeration was conducted using the Total Plate Count (TPC) method. *Vibrio* colonies on TCBS agar were counted after incubation, and viable bacterial concentrations were calculated using the standard plate count formula (Chen *et al.*, 2024):

$$\text{TPC} = \frac{(Number\ of\ Colonies)\ x\ Dilution\ Factor}{Volume\ Plated}$$

Correlation analysis between Vibrio abundance in shrimp and pond water was conducted using Spearman's rank correlation coefficient (ρ). All statistical analyses were performed in SPSS 25, and correlation estimates were generated for each pond size category and sampling period. In addition, a generalized linear model (GLM) was applied to evaluate whether variations in Vibrio abundance among ponds could be explained by measured water quality parameters.

Table 1. Spearman Correlation Range (Selala *et al.*, 2019)

Spearman's ρ (rho)	Strength of Relationship
0.00 – 0.20	Very Weak / Negligible
0.21 – 0.40	Weak
0.41 – 0.60	Moderate
0.61 – 0.80	Strong
0.81 – 1.00	Very Strong

3. Results

3.1. Total Abundance of Bacteria in Shrimp and Pond Water

The total abundance of *Vibrio* spp. in shrimp and pond water across all ponds and sampling days is shown in Table 3 and Table 4. These values summarize the bacterial counts for each pond category and sampling time, providing an overview of *Vibrio* distribution throughout the two-month grow-out period. The measured water quality parameters (Table 2) also showed no notable differences in mean values among the three pond categories, indicating that the environmental conditions were relatively similar across ponds.

Table 2. Water Quality Parameters

Parameter	Small Pond (Mean ± SD)	Mid Pond (Mean ± SD)	Large Pond (Mean ± SD)	Water Quality Standard
Lux (*lux*)	44.118±7699	46.393±5146	46.976±6351	>10.000
Temperature (°C)	27.88±0.95	28.74±1.13	28.62±0.98	26-32^{O}C
pH	8.26±0.24	8.26±0.12	8.28±0.14	7.0-8.5
Dissolved Oxygen /DO (mg/l)	4.58±1.06	4.58±0.98	4.60±0.98	>4 mg/L
Salinity (ppt)	35.02±3.85	35.70±3.56	35.89±3.47	25-35 ppt

Table 3. Total Vibrio Abundance in Shrimp (CFU/g)

DOC	**S1-S**	**S2-S**	**S3-S**	**S4-S**	**S5-S**	**S6-S**	**S7-S**	**M1-S**	**L1-S**	**L2-S**
7	4,1 x 10^8	3,8 x 10^8	2,8 x 10^8	4,3 x 10^8	-	-	-	-	6,8 x 10^8	-
14	-	-	-	-	4,7 x 10^7	1,2 x 10^7	3,2 x 10^7	1,4 x 10^7	-	2,6 x 10^9
21	1,7 x 10^7	1,4 x 10^7	3,8 x 10^5	7,1 x 10^6	3,8 x 10^5	1,5 x 10^6	3,5 x 10^5	1,9 x 10^5	1,9 x 10^7	3,8 x 10^6
28	1,7 x 10^6	1,4 x 10^6	9,6 x 10^5	3,8 x 10^5	4,3 x 1^6	1,3 x 10^6	2,1 x 10^6	1,1 x 10^6	3,2 x 10^6	7,0 x 10^6
35	5,2 x 10^6	4,1 x 10^6	2,3 x 10^6	6,3 x 10^6	5,9 x 10^6	3,0 x 10^6	1,8 x 10^6	2,8 x 10^5	1,0 x 10^6	5,4 x 10^6
42	4,1 x 10^5	9,4 x 10^5	3,2 x 10^5	1,5 x 10^6	1,6 x 10^6	2,1 x 10^6	1,4 x 10^7	5,1 x 10^6	3,1 x 10^5	1,5 x 10^6
49	9,2 x 10^6	5,7 x 10^4	8,3 x 10^4	4,5 x 10^5	1,0 x 10^7	4,1 x 10^5	1,2 x 10^6	1,8 x 10^6	3,5 x 10^4	3,2 x 10^6
56	9,7 x 10^5	1,0 x 10^6	7,6 x 10^6	2,4 x 10^6	7,8 x 10^5	2,8 x 10^6	4,2 x 10^6	1,9 x 10^6	1,7 x 10^6	2,4 x 10^6
Total Abundance CFU/g	6,4 x 10^7	5,8 x 10^7	4,3 x 10^7	6,4 x 10^7	1,0 x 10^7	3,4 x 10^6	8,0 x 10^6	3,5 x 10^6	1,0 x 10^8	3,7 x 10^8

The abundance of *Vibrio* spp. in shrimp samples (Table 3) showed considerable variation among ponds and across sampling times. *Vibrio* abundance in the small ponds ranged from 5.7 × 10^4 to 4.3 × 10^8 CFU/g, with the highest values (S1-S to S7-S) observed early in the monitoring period (DOC 7 and 21). In the medium-pond category (M1-S), peak abundance also occurred early in the monitoring period, reaching 10^7 CFU/g, before declining to an average of 10^5–10^6 CFU/g from DOC 21 to 56. In the large-pond category (L1-S to L2-S), the highest values were similarly recorded at the beginning of the culture period, at 6.8 × 10^8 and 2.6 × 10^9 CFU/g, while subsequent abundances fluctuated within the range of 10^4–10^7 CFU/g from DOC 21 to 56.

Pond water samples (Table 4) showed lower total Vibrio abundance across all ponds compared to the levels observed in water. In the small-pond category (S1-W to S7-W), values ranged between 10^3 and 10^5 CFU/ml, with no consistent peak observed among ponds. In the medium-pond category (M1-W), abundances remained relatively stable at around 10^4 CFU/ml, falling within the range of 2.0 × 10^4 to 5.2 × 10^4 CFU/ml. Meanwhile, in the large-pond category (L1-W to L2-W), initial values ranged from 4.8 × 10^3 to 1.2 × 10^5 CFU/ml, and no substantial increase in bacterial abundance was observed across DOC intervals.

The maximum limit of Vibrio bacteria commonly referenced for shrimp pond environments is approximately 10^4 CFU/ml. However, the total abundance detected in shrimp tissues frequently exceeded this level, ranging from 10^4 to 10^8 CFU/g and in some cases reaching up to 10^9 CFU/g. In pond water, Vibrio concentrations were also relatively high, with most values falling within 10^4 to 10^5 CFU/ml.

Table 4. Total Vibrio Abundance in Water (CFU/ml)

DOC	S1-W	S2-W	S3-W	S4-W	S5-W	S6-W	S7-W	M1-W	L1-W	L2-W
7	$4{,}3 \times 10^4$	$7{,}6 \times 10^4$	$4{,}7 \times 10^4$	$1{,}3 \times 10^4$	-	-	-	-	$9{,}3 \times 10^4$	-
14	-	-	-	-	$1{,}5 \times 10^4$	$2{,}8 \times 10^3$	$7{,}2 \times 10^3$	$2{,}0 \times 10^4$	-	$2{,}5 \times 10^4$
21	$1{,}5 \times 10^5$	$1{,}7 \times 10^4$	$2{,}5 \times 10^4$	$6{,}8 \times 10^3$	$2{,}7 \times 10^3$	$3{,}4 \times 10^4$	$7{,}2 \times 10^3$	$6{,}5 \times 10^4$	$2{,}2 \times 10^4$	$7{,}8 \times 10^3$
28	$1{,}5 \times 10^4$	$4{,}0 \times 10^3$	$1{,}3 \times 10^4$	$9{,}1 \times 10^3$	$2{,}4 \times 10^4$	$4{,}0 \times 10^4$	$1{,}8 \times 10^4$	$3{,}5 \times 10^4$	$2{,}3 \times 10^4$	$5{,}1 \times 10^3$
35	$1{,}0 \times 10^5$	$2{,}8 \times 10^5$	$1{,}8 \times 10^4$	$1{,}3 \times 10^5$	$7{,}0 \times 10^4$	$6{,}9 \times 10^4$	$8{,}7 \times 10^4$	$3{,}2 \times 10^4$	$1{,}2 \times 10^5$	$8{,}7 \times 10^4$
42	$1{,}0 \times 10^4$	$3{,}4 \times 10^3$	$3{,}8 \times 10^3$	$7{,}2 \times 10^3$	$8{,}7 \times 10^3$	$4{,}3 \times 10^4$	$9{,}9 \times 10^4$	$5{,}2 \times 10^4$	$4{,}8 \times 10^3$	$2{,}6 \times 10^4$
49	$6{,}2 \times 10^5$	$2{,}9 \times 10^3$	$1{,}5 \times 10^3$	$6{,}9 \times 10^3$	$5{,}5 \times 10^5$	$2{,}4 \times 10^4$	$7{,}4 \times 10^4$	$1{,}0 \times 10^4$	$2{,}6 \times 10^4$	$6{,}7 \times 10^4$
56	$1{,}1 \times 10^3$	$5{,}6 \times 10^3$	$9{,}1 \times 10^3$	$4{,}4 \times 10^3$	$1{,}2 \times 10^5$	$7{,}2 \times 10^4$	$1{,}7 \times 10^5$	$2{,}4 \times 10^4$	$2{,}0 \times 10^4$	$4{,}2 \times 10^4$
Total Abundance CFU/ml	$1{,}3 \times 10^5$	$5{,}5 \times 10^4$	$4{,}1 \times 10^4$	$2{,}5 \times 10^4$	$1{,}1 \times 10^5$	$4{,}1 \times 10^4$	$6{,}6 \times 10^4$	$4{,}8 \times 10^4$	$4{,}5 \times 10^4$	$3{,}7 \times 10^4$

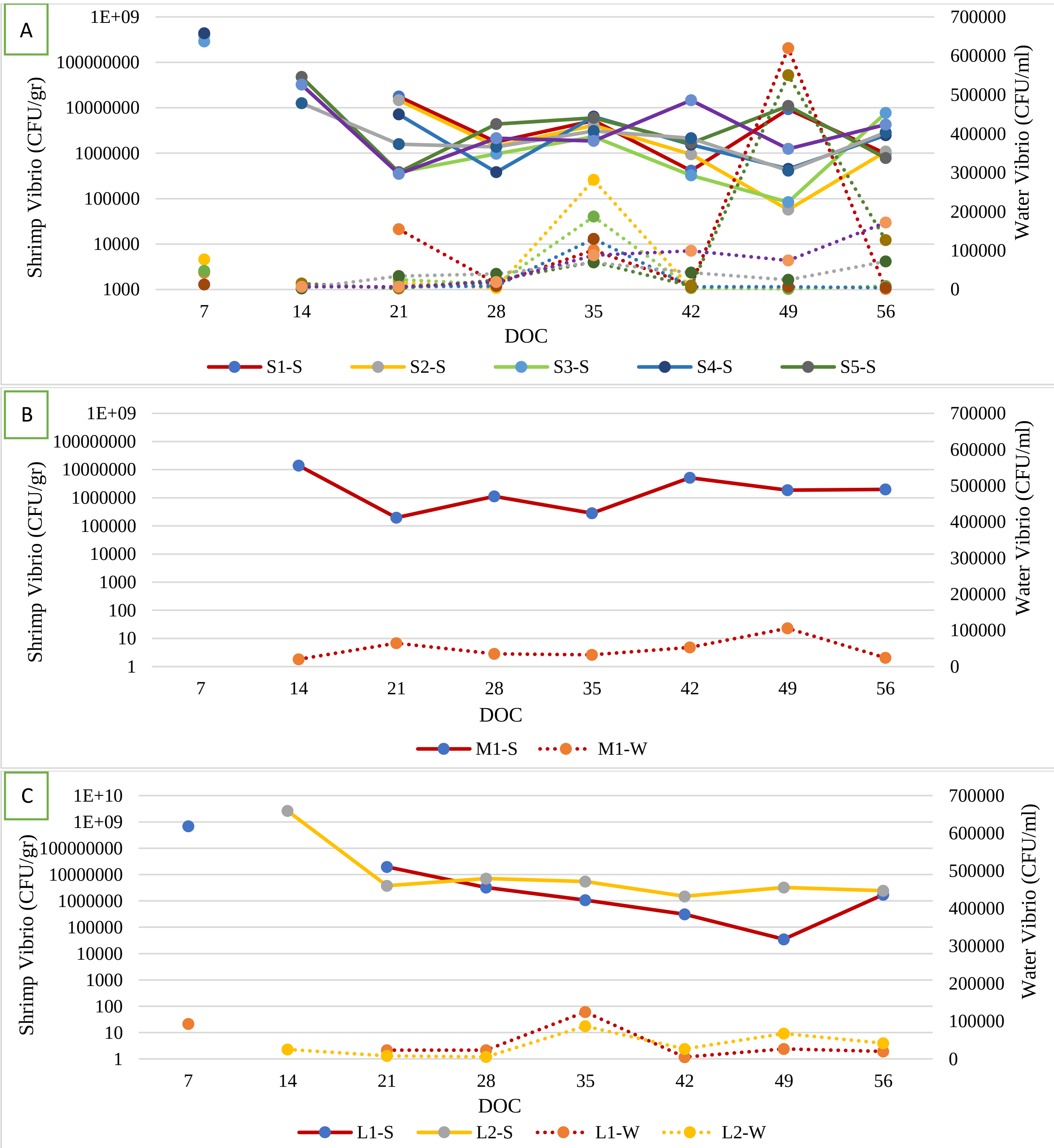

Figure 2. Dynamics of Bacterial Abundance in Shrimp and Pond Water in Small Ponds (A); Medium Ponds (B); and Large Ponds (C)

The maximum limit of Vibrio bacteria commonly referenced for shrimp pond environments is approximately 10^4 CFU/ml. However, the total abundance detected in shrimp tissues frequently exceeded this level, ranging from 10^4 to 10^8 CFU/g and in some cases reaching up to 10^9 CFU/g. In pond water, Vibrio concentrations were also relatively high, with most values falling within 10^4 to 10^5 CFU/ml.

Across all pond categories, shrimp consistently exhibited higher Vibrio abundance than pond water throughout the sampling period. In the small-pond group (Figure 2.A), shrimp showed marked fluctuations with several peaks and declines, while water samples remained lower and relatively stable. A similar pattern was observed in the medium-pond group (Figure 2.B), where shrimp abundance remained at higher levels with moderate variation, and water samples stayed low and steady. In the large-pond group (Figure 2.C), shrimp abundance showed a gradual decline over time, whereas water samples displayed only minor variations with consistently lower values. Overall, the visual trends across all pond sizes indicate that shrimp maintained substantially higher Vibrio loads than pond water, with no major differences in the general pattern among the three pond categories.

The correlation analysis showed wide variation in the relationship between Vibrio levels in shrimp and pond water (Table 5). In small ponds, S1 and S3 had strong but non-significant positive correlations, while S2 showed a very strong and statistically significant one. Ponds S4 and S5 exhibited weak positive correlations, and S6 and S7 showed very weak correlations. The medium pond (M1) displayed a moderate negative correlation. In large ponds, L1 showed a very weak positive correlation and L2 a weak negative correlation. Aside from S2, none of the correlation values were statistically significant.

The association between water quality parameters and Vibrio abundance across ponds was evaluated using a generalized linear model (GLM). The analysis showed no significant effects of the measured water quality parameters on Vibrio abundance in either pond water or shrimp (Table 6). No significant relationship was observed between environmental parameters and Vibrio levels in water ($F = 1.033$; $p = 0.423$) or in shrimp ($F = 0.264$; $p = 0.981$).

Table 5. Spearman’s Correlation Result

POND	Water and Shrimp		Description
	Correlation Coefficient	Sig. (2-tailed)	
S1	0.714	0.071	Strong (Not Significant)
S2	0.857	0.014	Very Strong (Significant)
S3	0.679	0.094	Strong (Not Significant)
S4	0.357	0.432	Low (Not Significant)
S5	0.393	0.393	Low (Not Significant)
S6	0.179	0.702	Very Low (Not Significant)
S7	0.107	0.819	Very Low (Not Significant)
M1	-0.464	0.294	Mid-Negative (Not Significant)
L1	0.143	0.760	Very Low (Not Significant)
L2	-0.375	0.432	Low-Negative (Not Significant)

Table 6. Generalized Linear Model (GLM) analysis of the relationship between water quality parameters and Vibrio abundance in water and shrimp

Sample	**GLM analysis**	
	F	Sig.
Water Vibrio	1.033	0.423
Shrimp Vibrio	0.264	0.981

3.3. Shrimp Growth

Shrimp growth performance across the culture period is presented in Table 7. Both average body weight and body length exhibited a consistent increase with advancing days of culture (DOC). Mean body weight increased from 0.43 ± 0.32 g at DOC 7 to 12.09 ± 2.21 g at DOC 56, while mean body length increased from 3.50 ± 0.75 cm to 12.80 ± 0.84 cm over the same period. Average daily growth (ADG) showed a generally increasing pattern throughout the culture period, with lower values observed during the early stages and higher growth rates recorded at later DOC.

Table 7. Growth Parameters of *L. vannamei* at Different Days of Culture (DOC)

DOC	Average Body Weight (gr)	Average Daily Growth (gr)	Length (cm)
7	0.43 ±0.32		3.50 ±0.75
14	0.89 ±0.24	0.07	5.24 ±0.41
21	1.50 ±0.22	0.09	6.35 ±0.41
28	2.66 ±0.57	0.17	7.24 ±0.48
35	4.72 ±0.98	0.29	8.97 ±0.95
42	6.21 ±0.86	1.49	10.72 ±1.46
49	9.27 ±2.28	0.44	11.24 ±0.74
56	12.09 ±2.21	0.40	12.80 ±0.84

4. Discussion

The results show that *Vibrio* spp. consistently exhibited higher abundance in shrimp than in pond water across all pond-size categories. This suggests that shrimp tissues such as the intestine, gills, and hepatopancreas provide stable, nutrient-rich microhabitats that support bacterial colonization and growth (Salama and Chennaoui, 2024; Xian-wei and Petersen, 2025). Furthermore, evidence shows that the health status of shrimp is closely interrelated with gut microbiota through the maintenance and modulation of the immune system (Yudiati and Azhar, 2024), indicating that microbial communities within shrimp tissues play an important role in host defense and overall physiological stability. The surrounding water column is characterized by more variable and competitive conditions, where fluctuations in nutrient availability, particle attachment, and interactions with competing bacteria and other microorganisms create a less stable environment for *Vibrio* spp. (Geisser *et al.,* 2025; Thorstenson and Ullrich, 2021). Shrimp-associated microhabitats allow bacterial populations to persist and accumulate more consistently.

The lower vibrio abundance observed in pond water relative to shrimp tissues does not indicate negligible microbial risk. Despite vibrio concentrations in pond water reaching 10^4–10^5 CFU, these levels were recorded under physicochemical conditions that remained stable and within acceptable ranges throughout the study period. These findings indicate that Vibrio levels in both shrimp and pond water were elevated and surpassed the commonly cited environmental threshold (Amalisa *et al.,* 2021). Here, stability refers to the temporal consistency of water quality parameters rather than to reduced microbial abundance (Doren *et al.,* 2013). The low abundance of Vibrio in the pond water was likely related to the stability of water quality parameters (Asni *et al.,* 2023), which remained within normal ranges throughout the study. Stable environmental conditions can suppress the growth of free-living Vibrio, even when temperature, salinity, and pH fall within favorable ranges for bacterial development (Sheikh *et al.,* 2022). Increases in Vibrio generally occur when water quality fluctuates, such as during sudden temperature changes, reductions in dissolved oxygen, higher turbidity, or the buildup of organic matter. These fluctuations create conditions that favor opportunistic bacteria (Brumfield *et al.,* 2023). However, because water quality remained stable during the study, abrupt increases in Vibrio populations in the water column were likely limited.

Consistent with the observed stability of water quality, the generalized linear model (GLM) analysis demonstrated that the measured environmental parameters did not have a significant effect on Vibrio abundance in either pond water or shrimp. The lack of significant relationships suggests that, under relatively stable physicochemical conditions, variation in water quality parameters alone was insufficient to explain differences in Vibrio levels among ponds (Xiong *et al.,* 2014).

Differences among pond sizes further illustrate distinct microbial dynamics. Small ponds exhibited sharper fluctuations in shrimp-associated Vibrio, likely due to limited water volume that makes organic loading and microbial conditions more sensitive to change (Schryver and Vadstein, 2014). Medium ponds displayed more stable patterns, whereas large ponds showed a gradual decline toward the end of the culture period, reflecting more homogeneous environments that buffer rapid microbial shifts.

Temporal changes in shrimp growth during the culture period provide additional context for interpreting Vibrio dynamics (Quigg *et al.,* 2025). As shrimp body weight and length increased progressively with advancing days of culture, the available surface area and internal tissue volume for microbial colonization also increased. Furthermore, the increase in average daily growth (ADG) at later stages of culture indicates elevated metabolic activity and feed intake, which may enhance nutrient availability within the gut environment and support bacterial persistence within shrimp tissues (Rivera et al., 2023; Goh *et al.,* 2023).

Correlation analysis revealed substantial variation in the relationship between Vibrio abundance in shrimp and pond water across the different pond categories. Positive correlations in several ponds indicate that increases in waterborne Vibrio were accompanied by corresponding increases in shrimp, suggesting that the dynamics of the host and surrounding environment moved in parallel. This pattern may occur when environmental fluctuations, microbial interactions, and host-related factors align in a way that allows changes in the water column to be reflected within shrimp tissues (Deris *et al.,* 2022; Makwarela and Seoraj-pillai, 2025). In contrast, negative correlations observed in multiple ponds imply that higher concentrations of Vibrio in water did not necessarily result in greater colonization of the host. Such patterns may arise when management interventions temporarily reduce waterborne Vibrio levels without immediately affecting bacterial populations already established within shrimp tissues. Practices such as partial water exchange, organic matter removal, or probiotic application may alter microbial conditions in the water column while host-associated Vibrio populations respond more slowly (Alfiansyah *et al.,* 2018). Such outcomes may be influenced by physiological defense mechanisms within the shrimp (Yildirim-aksoy *et al.,* 2022), immune responsiveness (Shakweer *et al.,* 2023), or environmental shifts that do not directly affect bacterial attachment on or within the host.

Although all ponds were managed under similar operational conditions, pond S2 still exhibited a markedly stronger correlation between waterborne and shrimp-associated Vibrio compared to the other ponds. Such differences may arise from unmeasured environmental factors or natural variability within pond ecosystems (Fries *et al.,* 2022), as subtle differences in organic matter distribution, microbial community structure, sediment conditions, or animal behavior can influence microbe–host interactions even under uniform management practices (Zhou *et al.,* 2025). This type of unexplained variation is common in aquaculture systems, where complex biological and environmental processes can produce divergent outcomes among ponds despite identical inputs. Therefore, the strong correlation observed in S2 may reflect pond-specific characteristics that were not captured in the measured parameters (biotic and abiotic factors) (Wang *et al.,* 2022), rather than differences in applied treatments (Wan *et al.,* 2025).

From a practical perspective, these findings emphasize the importance of comprehensive biosecurity monitoring strategies in shrimp aquaculture. Because Vibrio populations can persist and accumulate within shrimp tissues even when water quality parameters remain stable, reliance on water-based monitoring alone may underestimate microbial risks. Routine assessment of shrimp tissues, alongside conventional water quality measurements, is therefore recommended to provide a more accurate evaluation of Vibrio dynamics and potential disease risk in shrimp farming systems (Xiong *et al.*, 2016; Farag *et al.*, 2023).

5. Conclusions

Vibrio spp. were consistently more abundant in shrimp than in pond water across all pond-size categories, indicating that shrimp tissues function as primary reservoirs for bacterial persistence in tropical aquaculture systems. The predominance of weak or negative correlations between waterborne and shrimp-associated Vibrio, together with the GLM results showing that measured water quality parameters did not significantly explain variations in Vibrio abundance, suggests that differences in Vibrio levels among ponds were not driven by the physicochemical parameters assessed in this study. Furthermore, progressive shrimp growth during the culture period likely provided increasing opportunities for bacterial persistence within host tissues, highlighting the importance of host-associated factors in shaping Vibrio dynamics. These findings emphasize that biosecurity monitoring should prioritize shrimp tissue analysis alongside water quality assessment, while acknowledging study limitations related to the short monitoring period and the absence of concurrent environmental variables, such as organic matter levels, in the correlation analysis.

Ethics approval

The study complied with Indonesian animal welfare regulations, and no specific permits were required for the sampling of marine biota. All procedures were conducted in accordance with the relevant institutional and national guidelines governing the use of aquatic organisms in research

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Author contributions

Salsabila Sambhara Putri: writing-original draft, investigation, formal analysis, data curation, methodology; Nuril Azhar and Ervia Yudiati: resources, supervision, writing original draft preparation, writing-review and editing, validation, methodology, funding acquisition, project administration; Agus Trianto: supervision, validation, resources, funding acquisition, writing-review and editing, project administration; Rizki Ahmad Fachreza: Investigation, data curation, formal analysis, methodology.

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Declaration of competing Interest

The authors declare no competing interests.

References

Amalisa, Mahasri, G., and Kismiyati., 2021. The correlation between ectoparasite infestation and total *Vibrio parahaemolyticus* bacteria in pacific white shrimp (*Litopenaeus vannamei*) in super intensive ponds. *Earth and Environmental Science*, (888): 1–7. https://doi.org/10.1088/1755-1315/888/1/012003

Alfiansah, Y. R., Hassenrück, C., Kunzmann, A., Taslihan, A., Harder, J., and Gärdes, A., 2018. Bacterial abundance and community composition in pond water from shrimp aquaculture systems with different stocking densities. *Frontiers in microbiology*, 9: 2457. https://doi.org/10.3389/fmicb.2018.02457

Asni, Rahim, Saleh, R., Landu, A., and Muliadi., 2023. Correlation between water quality parameters and Vibrio sp . bacteria content in traditional vannamei shrimp (*Lithopenaeus Vannamei*). *Journal of Agriculture (JoA)*. 2(2): 121–131. https://doi.org/https://doi.org/10.47709/joa.v2i02.2577

Brauge, T., Mougin, J., Ells, T., and Midelet, G., 2024. Sources and contamination routes of seafood with human pathogenic *Vibrio* spp. : a farm-to-fork approach. *WILEY: Comprehensive Reviews in Food Science and Food Safety*. 33(1): 1–25. https://doi.org/10.1111/1541-4337.13283

Brum, K. D., Usmani, M., Chen, K. M., Gangwar, M., Jutla, A. S., Huq, A., and Colwell, R. R., 2021. Environmental parameters associated with incidence and transmission of pathogenic *Vibrio* spp . *Environmental Microbioogy*. 23: 7314–7340. https://doi.org/10.1111/1462-2920.15716

Brumfield, K. D., Chen, A. J., Gangwar, M., Usmani, M., Hasan, N. A., Jutla, A. S., and Huq, A., 2023. Environmental factors in fluencing occurrence of *vibrio parahaemolyticus* and *Vibrio vulnificus*. *Applied and Enviromental Microbiology*. 89(6): 1–19. https://doi.org/https://doi.org/10.1128/aem.00307-23

Brumfield, K. D., Usmani, M., Long, D. M., Lupari, H. A., Pope, R. K., Jutla, A. S., and Huq, A., 2025. Climate change and vibrio : Environmental determinants for predictive risk assessment. *PNAS*. 122(33): 1–10. https://doi.org/10.1073/pnas.

Chang, Y., Huang, W., Wu, P., Kumar, R., Wang, H., and Lu, H., 2024. Low salinity stress increases the risk of *Vibrio parahaemolyticus* infection and gut microbiota dysbiosis in pacific white shrimp. *BMC Microbiology*. 24(275): 1–16. https://doi.org/10.1186/s12866-024-03407-0.

Chen, Y., Kumar, V., Mitra, A., Rahimnejad, S., Tan, B., Niu, J., and Xie, S., 2024. Retrospect of fish meal substitution in pacific white shrimp (*Litopenaeus vannamei*) feed : alternatives, limitations and future prospects. *Review in Aquaculture*. 16(1): 382–409. https://doi.org/10.1111/raq.12843

Chen, Y., Wang, T., Cao, Z., Haochun, K., Li, K., and Cheng, R., 2024. Reducing total plate count of bacteria through dark sealed storage for plant factory nutrient solution sterilization. *HortTechnology*. 34(6): 738–746. https://doi.org/10.21273/HORTTECH05538-24

Deris, Z. M., Iehata, S., Gan, H. M., Ikhwanuddin, M., Najiah, M., Sung, Y. Y., and Wong, L. L., 2022.

Understanding the effects of salinity and *Vibrio harveyi* on the gut microbiota profiles of *Litopenaeus vannamei*. *Frontiers in Marine Science*. 11(230): 1–16. https://doi.org/10.3389/fmars.2022.974217

Eun, J., Chan, S., Chan, S., Jin, H., Yeon, K., Seo, Y., Park, S., Han, S., Hyung, J., and Choi, S., 2020. Molecular detection of Enterocytozoon hepatopenaei and *Vibrio parahaemolyticus*- associated acute hepatopancreatic necrosis disease in Southeast asian *Litopenaeus vannamei* shrimp imported into Korea. *Aquaculture*. 517: 1-4. https://doi.org/10.1016/j.aquaculture.2019.734812

Faulds, N., Williams, J., Evans, K., Hughes, A., Leak, D., Crabtree, D., Sohier, D., Heikkinen, P., Hurskainen, E., Mcmahon, W., Cuthbert, N., Matthews, B., Ruben, L., Sturghill, L., and Godawski, F., 2023. Microbiological methods validation of the thermo scientific tm suretect tm *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* pcr assay for the detection of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* in Seafood Matrix. *Journal of AOAC International*. 106(5): 1254–1277. https://doi.org/10.1093/jaoacint/qsad061

Farag, M. A., Mansour, S. T., Nouh, R. A., and Khattab, A. R., 2023. Crustaceans (shrimp, crab, and lobster): A comprehensive review of their potential health hazards and detection methods to assure their biosafety. *Journal of Food Safety,* 43(1), https://doi.org/10.1111/jfs.13026

Fries, B., Davis, B. J. K., Corrigan, A. E., Depaola, A., Curriero, F. C., and Fries, B., 2022. Nested spatial and temporal modeling of environmental conditions associated with genetic markers of *Vibrio parahaemolyticus* in washington state pacific oysters. *Frontiers in Microbiology*. 13: 1-14. https://doi.org/10.3389/fmicb.2022.849336

Geisser, A. H., Scro, A. K., Smolowitz, R., and Fulweiler, R. W., 2025. Macroalgae host pathogenic *Vibrio* spp. in a temperate estuary. *Frontiers in Marine Biology*. 12(4): 1–14. https://doi.org/10.3389/fmars.2025.1549732

Goh, J. X. H., Tan, L. T. H., Law, J. W. F., Khaw, K. Y., Zengin, G., Chan, K. G., and Goh, B. H. (2023). Probiotics: comprehensive exploration of the growth promotion mechanisms in shrimps. *Progress In Microbes & Molecular Biology,* 6(1). https://doi.org/10.36877/pmmb.a0000324

Kumar, V., Roy, S., Behera, B. K., Bossier, P., and Das, B. K., 2021. Acute hepatopancreatic necrosis disease (ahpnd): virulence, pathogenesis and mitigation strategies in shrimp aquaculture. *Toxins*. 13(8): 1–28. https://doi.org/https://doi.org/10.3390/toxins13080524

Kumarage, P. M., Silva, L. A. D. S. De, and Heo, G.-J., 2022. Aquatic environments: a potential source of antimicrobial- resistant *Vibrio* spp. *Journal of Applied Microbiology*. 33(6): 2267–2279. https://doi.org/10.1111/jam.15702

Makwarela, T. G., and Seoraj-pillai, N., 2025. Exploring the molluscan microbiome : diversity, functio, and ecological implications. *Biology*. 14(8): 1–30. https://doi.org/10.3390/biology14081086

Mustafa, A., Paena, M., Athirah, A., Ratnawati, E., Asaf, R., Suwoyo, H. S., Sahabuddin, S., Hendrajat, E. A., Kamaruddin, K., Septiningsih, E., Sahrijanna, A., Marzuki, I., and Nisaa, K., 2022. Temporal and spatial analysis of coastal water quality to support application of whiteleg shrimp *Litopenaeus vannamei* intensive pond technology. *Suistainability*. 14: 1–25. https://doi.org/10.3390/su14052659

Quigg, A., Gaona-Hernández, A., Hochman, M. S., Ray, S. M., and Schwarz, J. R., 2025. Applied Time Series Analyses (2000–2017) of Vibrio vulnificus and Vibrio parahaemolyticus (Pathogenic and Non-Pathogenic Strains) in the Eastern Oyster, Crassostrea virginica. *Bacteria*, 4(2): 17. https://doi.org/10.3390/bacteria4020017

Quintino-Rivera, J. G., Elizondo-González, R., Gamboa-Delgado, J., Guzmán-Villanueva, L. T., and Peña-Rodriguez, A., 2023. Metabolic turnover rate, digestive enzyme activities, and bacterial communities in the white shrimp Litopenaeus vannamei under compensatory growth. PeerJ, 11, e14747. https://doi.org/10.7717/peerj.14747

Salama, Y., and Chennaoui, M., 2024. Microbial spoilage organisms in seafood products : pathogens and quality control. *European Journal of Microbiology and Infectious Diseases*. 1(2): 66–89. 10.5455/EJMID.20240518114533

Sampaio, A., Silva, V., Poeta, P., and Aonofriesei, F., 2022. *Vibrio* spp.: life strategies, ecology, and risks in a changing environment. *Diversity*. *14*(2): 1–26. https://doi.org/10.3390/d14020097

Schryver, P. De, and Vadstein, O., 2014. Ecological theory as a foundation to control pathogenic invasion in aquaculture. *The ISME Journal*. 8(4): 2360–2368. https://doi.org/10.1038/ismej.2014.84

Scruggs, E. F., Gulley, Z., Steele, G., Alahmadi, M., Barnawi, A., Majrshi, H., and Tiong, H. K., 2024. Recovery of pasteurization-resistant vagococcus lutrae from raw seafoods using a two-step enrichment, its presumptive prevalence, and novel classification phenotypes. *Applied Microbiology*. 4(4): 1434–1452. https://doi.org/10.3390/applmicrobiol4040099

Shakweer, M. S., Elshopakey, G. E., Abdelwarith, A. A., Younis, E. M., Davies, S. J., and Elbahnaswy, S., 2023. Comparison of immune response of *Litopenaeus vannamei* shrimp naturally infected with vibrio species , and after being fed with florfenicol. *Fishes*, 8(3): 1–22. https://doi.org/10.3390/fishes8030148

Shanmugasundaram, S., Mayavu, P., Manikandarajan, T., Suriya, M., Eswar, A., and Anbarasu, R., 2015. Isolation and identification of *Vibrio* sp . in the hepatopancreas of cultured white pacific shrimp (*Litopenaeus vannamei*). *International Letter of*

Natural Science. 46: 52–59. https://doi.org/10.56431/p-1dioy2

Sheikh, H. I., Najiah, M., Fadhlina, A., Laith, A. A., Nor, M. M., and Jalal, K. C. A. 2022. Temperature upshift mostly but not always enhances the growth of vibrio species : a systematic review. *Frontiers in Marine Science*. 9: 1–17. https://doi.org/10.3389/fmars.2022.959830

Shinn, A. P., Wongwaradechkul, R., Coates, C. J., and Limakom, T., 2025. Confirmed spread of *Vibrio parahaemolyticus* by aerosols is a risk factor in the infection of shrimp ponds. *Aquaculture*. 598: 1–13. https://doi.org/10.1016/j.aquaculture.2024.741923

Takemura, A. F., Chien, D. M., and Polz, M. F., 2014. Associations and dynamics of vibrionaceae in the environment, from the genus to the population level. *Frontiers in Microbiology*. 5(2): 1–26. https://doi.org/10.3389/fmicb.2014.00038

Tawhid, M., Islam, M. M., and Tandon, S., 2024. Biochemical assay for detection of pathogenic and probiotic bacteria at shrimp and prawn from wild and different culture conditions in bangladesh. *Suranaree Journal of Science & Technology*. 31(1): 1–9. https://doi.org/10.3390/applmicrobiol4040099

Thorstenson, C. A., and Ullrich, M. S., 2021. Ecological fitness of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* in a small-scale population dynamics study. *Frontiers in Marine Science*. 8: 1–16. https://doi.org/10.3389/fmars.2021.623988

Tran, N., Rodriguez, U., Yee, C., John, M., Mohan, V., John, P., Henriksson, G., Koeshendrajana, S., Suri, S., and Hall, S., 2017. Indonesian aquaculture futures : an analysis of fish supply and demand in indonesia to 2030 and role of aquaculture using the asiafish model. *Marine Policy*. 79: 25–32. https://doi.org/10.1016/j.marpol.2017.02.002

Van Doren, J. M., Kleinmeier, D., Hammack, T. S., and Westerman, A., 2013. Prevalence, serotype diversity, and antimicrobial resistance of Salmonella in imported shipments of spice offered for entry to the United States, FY2007–FY2009. *Food Microbiology*, 34(2): 239-251. https://doi.org/10.1016/j.fm.2012.10.002

Wan, S. H., Xu, W., Yu, E. Y. N., and Yung, C. C. M., 2025. Differentiation in vibrio populations across subtropical marine habitats. *Environmental Microbiology*. 27(5): 1–18. https://doi.org/10.1111/1462-2920.70107

Wang, X., Liu, J., Zhao, W., Liu, J., Liang, J., Thompson, F., and Zhang, X., 2022. Fine-scale structuring of planktonic *Vibrio* spp. in the chinese marginal seas. *Applied and Enviromental Microbiology*. 88(23): 1–21. https://doi.org/10.1128/aem.01262-22%0A

Xian-wei, W., and Petersen, J. 2025. Hemolymph microbiota and host immunity of crustaceans and mollusks. *The ISME Journal*. 19(1): 1–16. https://doi.org/10.1093/ismejo/wraf133

Xiong, J., Dai, W., and Li, C., 2016. Advances, challenges, and directions in shrimp disease control: the guidelines from an ecological perspective. *Applied microbiology and biotechnology*, 100(16): 6947-6954. https://doi.org/10.1007/s00253-016-7679-1

Xiong, J., Zhu, J., Wang, K., Wang, X., Ye, X., Liu, L., and Zhang, D., 2014. The temporal scaling of bacterioplankton composition: high turnover and predictability during shrimp cultivation. *Microbial ecology*, 67(2): 256-264. https://doi.org/10.1007/s00248-013-0336-7

Yildirim-aksoy, M., Eljack, R., Peatman, E., and Beck, B. H. 2022. Microbial pathogenesis immunological and biochemical changes in pacific white shrimp, *Litopenaeus vannamei*, challenged with *Vibrio parahaemolyticus*. *Microbial Pathogenesis*. 172: 1–7. https://doi.org/10.1016/j.micpath.2022.105787

Yudiati, E., and Azhar, N., 2024. Growth performance, survival rate, and resistance against ahpnd of *Litopenaeus vannamei* juveniles fed with synbiotic bio-encapsulated artemia. *Jurnal Riset Akuaqulture*. 19(3): 191–204. https://doi.org/http://doi.org/10.15578/jra.19.3.2024.191-204

Yudiati, E., Sedjati, S., Azhar, N., Oktarima, W., and Arifin, Z., 2021. Spirulina water extract and *Lactobacillus bulgaricus* FNCC – 0041 , *Streptococcus thermophilus* FNCC – 0040 secretion as immunostimulants in gnotobiotic Artemia challenge tests against pathogenic *Vibrio parahaemolyticus*, Spirulina water extract and Lactoba. *2nd International Conference on Fisheries and Marine: Earth and Environmental Science*. 1–8. https://doi.org/10.1088/1755-1315/890/1/012018

Zhang, S., and Sun, X., 2022. Core gut microbiota of shrimp function as a regulator to maintain immune homeostasis in response to wssv infection. *American Society for Microbiology: Microbiology Spectrum*. 10(2): 1–11. https://doi.org/10.1128/spectrum.02465-21%0A

Zhou, Z., Lu, J., Zhan, P., and Xiong, J., 2025. Postlarval shrimp-associated microbiota and underlying ecological processes over ahpnd progression. *Microorganisms*. 13(4): 1–20. https://doi.org/10.3390/microorganisms13040720