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How Water Quality In Intensive Shrimp Ponds Fuel *Vibrio* sp. Colonization

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ABSTRACT

The global production of vaname shrimp (*Litopenaeus vannamei*) reaches 6.8 million tons, making it a major species in the aquaculture industry. However, the success of this culture is often threatened by *Vibrio*sis disease caused by *Vibrio* sp. This study aimed to analyze the relationship between water quality parameters and *Vibrio* sp. bacterial abundance in intensive vaname shrimp ponds. Water quality measurements were conducted every seven days including temperature, dissolved oxygen, salinity, and pH, while *Vibrio* sp. density was calculated using selective culture method and PCR confirmation. The results showed that *Vibrio* sp. abundance had a significant correlation with water quality parameters, especially temperature (r = -0.622), dissolved oxygen (r = -0.378), pH (r = 0.377), and salinity (r = 0.597). *Vibrio* sp. density increased with rearing time and showed the highest value at 30ppt salinity and 30°C temperature. In addition, detection of *Vibrio* genes related to Acute Hepatopancreatic Necrosis Disease (AHPND) showed positive results even though *Vibrio parahaemolyticus* was not detected in pond water samples. This study confirms that environmental factors play an important role in *Vibrio* sp. dynamics and need to be properly managed to prevent disease outbreaks in vaname shrimp intensive culture systems.

INTRODUCTION

Vaname shrimp (*Litopenaeus vannamei*) is a major aquaculture commodity in the world. Most of the vaname shrimp producing countries include Ecuador, China, India and Indonesia **(FAO, 2022)**. Increasing market demand encourages production to continue to be increased in various countries **(Halim & Juanri, 2016; Kilawati *et al*., 2025)**. Globally,

vaname shrimp production has reached 6.8 million tons, making it the most widely cultivated species in the aquaculture industry **(FAO, 2024)**. This production is expected to continue to increase as intensive culture practices become more widespread. The high intensification of shrimp culture also has negative impacts including disease outbreaks that cause losses to farmers **(Anderson *et al.*, 2019; Kilawati *et al.*, 2025)**. In the shrimp industry, it is estimated that 60% of shrimp production losses are caused by viral diseases and 20% by most bacterial pathogens, especially Vibriosis **(Flegel, 2012)**.

Vibriosis is a disease that can cause mortality in *L. vannamei* culture and this disease is caused by infection with bacteria of the genus *Vibrio* **(Elias *et al.*, 2023)**. Species of *Vibrio* genus bacteria that can cause Vibriosis in vaname shrimp include *V. harveyi, V. parahaemolyticus, V. alginolyticus, V. anguillarum, V. vulnificus,* and *V. splendidus* **(Jayasree *et al.*, 2006)**. *Vibrio* sp. bacteria are Gram-negative halophilic bacteria that are naturally found in aquatic environments **(Xie *et al.*, 2005)**. Species such as *V. parahaemolyticus* can infect vanname shrimp through wounds on the exoskeleton and will spread through the hemolymph in the vanname shrimp circulation system **(Soto-Rodriguez *et al.*, 2015)**. *Vibrio* sp. bacteria are also associated as causative agents of Acute Hepatopancreatic Necrosis Diseases (AHPND) or Early Mortality Syndrome (EMS), and White Feces Disease (WFD) **(Kumar *et al.*, 2020)**.

AHPND has been widely reported to cause significant losses in vaname shrimp culture with high mortality rates **(Shin *et al.*, 2018; Kilawati *et al.*, 2024)**. The main symptoms are damage to the hepatopancreas until it turns pale and shrinks, shrimp skin becomes soft with the digestive tract appears empty. Shrimp mortality usually occurs 30-35 days, as early as 10 days after shrimp stocking in ponds **(OIE, 2019)**. The results showed that the bacterium *V. parahaemolyticus* is commonly the causative agent of AHPND with the main target organ being the gastrointestinal tract **(Tran *et al.*, 2013; Soto-Rodriguez *et al.*, 2015)**. Hepatopancreas damage is caused by toxins released by the bacteria. The toxin will enter the hepatopancreas through the stomach and cause sloughing of tubular epithelial cells **(Tran *et al.*, 2013; Kilawati *et al.*, 2024)**. Mitigation of significant economic losses caused by AHPND is hampered by knowledge gaps in the pathogenic mechanisms of bacterial infections in shrimp **(Nguyen *et al.*, 2020)**.

AHPND caused by *Vibrio* sp. bacteria in vaname shrimp ponds is strongly influenced by environmental factors **(Boyd & Phu, 2018)**. In open containers, the environmental conditions of vaname shrimp culture will continue to experience drastic or fluctuating changes, which can cause bacteria that were previously harmless to become pathogenic **(Lafferty *et al.*, 2004)**. Several studies have reported that *V. parahaemolyticus* can't grow at low temperatures (10°C), but the bacteria can grow in high NaCl concentrations (9%) **(Fujikawa *et al.*, 2009)**. The use of low salinity water (<20 ppt) has been shown to reduce AHPND outbreaks in some cases **(OIE, 2019)**. **Kuaphiriyakul and Preeprem (2022)** reported changes in virulence gene expression when the bacteria were cultured at different temperatures and salinity levels. The optimal bacterial density was observed at 30°C with

6% NaCl concentration. The highest virulence conditions were observed at 35 ppt salinity and a temperature of 28°C.

Physiological stress experienced by shrimp due to environmental changes has been widely studied, but the relationship between environmental changes and the presence of *Vibrio* sp. bacteria related to AHPND in vaname shrimp culture containers is rarely evaluated. Based on the description above, it is important to analyze the relationship of water quality to the abundance of *Vibrio* sp. bacteria in intensive shrimp culture.

MATERIALS AND METHODS

Study area

This study was conducted for 3 months from June to August 2024. Shrimp rearing was carried out in the Intensive Vaname Shrimp Pond of Bone Marine and Fisheries Polytechnic, East Tanete Riattang District, Bone Regency, South Sulawesi. Bacterial culture and identification were carried out at the Laboratory of the Takalar Brackish Water Aquaculture Center, South Sulawesi.

Shrimp rearing in ponds

Shrimp culture in HDPE-lined ponds was carried out intensively with a stocking density of 120 shrimp/m³. Pond preparation includes pond cleaning and drying. Water sterilization with chlorine (Tjiwi Kaporit 60, Indonesia) at a dose of 30mg/ L and sodium thiosulfate (AOJIN, China). Programmed feeding of at least 28% protein (Tables 1 and 2). Water exchange was performed daily at a rate of 30%, especially after day 30 (DOC 30). The rearing period was 49 days.

Table 1. Blind feeding program

Day of culture	Shrimp weight (grams)	Shrimp length (cm)	Feed code	Feeding frequency(times/day)	Estimated SR (%)	Initial feeding (Kg)
1 - 10	8 - 1,2	0,6- 1,2	681 V	4	100	6 Kg/
11- 20	1,2- 2,5	1,2- 2,0	681 V-682 V	4	99	185.000 larvae
21- 30	2,5- 3,5	2,0- 3,5	682 V- 683 PV	4	98	

Table 2. Feed program based on feeding rate

Day of culture	Shrimp weight (grams)	Shrimp length (cm)	Feed code	Feeding rate (%)	Feeding frequency (times/day)	Feeding trays (%)	Control time (hours)
30 – 38	3,5 – 5,0	6,0 – 9,0	683PV-683SP	5,6 – 4,6	4	0,6	2,5
39 -60	5,0 – 10,0	9,0 – 12,0	683 SP	4,6 – 3,4	4-5	0,8	2
60 -78	10,0 – 15,0	12,0 – 14,0	683 SP	3,4 – 2,9	5	1,0	1,5

78 -93	15,0 – 20,0	14,0 – 16,0	683 SP	2,9 – 2,5	5	1,2	1,5
93 -105	20,0 – 25,0	16,0 – 17,0	683 SP	2,5 – 2,2	5	1,4	1,0
105 115	25,0 – 30,0	17,0 – 18,0	683 SP	2,2 – 2,0	5	1,6	1,0
>115	>30,0	>18,00	683 SP	2,0	5	1,8	1,0

Water quality measurement

Water quality measurements were conducted *in situ* daily during the afternoon (15:00–16:00). Parameters measured included temperature and dissolved oxygen using a DO meter (YSI Pro20I, USA), salinity using a refractometer (ATAGO CO., LTD, Japan), and pH using a pH meter (WANT Balance Instrument Co., Ltd., China). Meanwhile, nitrite, TAN (Total Ammonia Nitrogen), and TOM (Total Organic Matter) were analyzed following the methods of **Menon (1988)**, **Parsons *et al*. (1989)**, and **APHA (2005)**. Data were compiled collectively and presented every 7 days, specifically on days of culture (DOC) 7, 14, 21, 28, 35, 42, and 49.

Observation of *Vibrio* sp.

The density of bacteria observed was derived from water samples of vaname shrimp rearing ponds. Water samples were taken from one pond using a 500mL sampling bottle. A total of 1mL of water samples were inoculated into Thiosulfate Citrate Bile Salt Sucruose Agar (TCBSA) media (HiMedia, India) and incubated for 24 hours at 28-30°C. Bacterial abundance was calculated by the method of **Madigan *et al*. (2003)**:

$$\text{Total Bacterial Count (CFU/mL)} = \Sigma C \times \frac{1}{Fp} \times \frac{1}{S}$$

Where:

ΣC = Total bacterial on the plate

Fp = Dilution factor

S = Inoculation volume (mL)

Bacterial identification

Bacterial isolates grown from water samples at DOC 14 and DOC 49 were analyzed using the Polymerase Chain Reaction (PCR) method to detect *Vibrio parahaemolyticus* and AHPND-associated *Vibrio* species. DNA extraction was performed using the Genomic DNA Mini Kit for Bacteria (Geneaid, Taiwan), including sample preparation, lysis, DNA binding, washing, and elution. Gene detection was carried out using PCR with 2x MyTaq HS Readymix (Meridian Bioscience, UK). PCR amplification results were then electrophoresed on 1% agarose gel (1st BASE, Singapore) with fluorescence RedSafeTm Nucleic Acid Staining Solution (iNtRON, South Korea) (0.3 µL/20 mL agarose). Electrophoresis was performed 40 min at 100 V, 400 mA and visualized with a UV Transilluminator. The genes targeted and the PCR primer sequences used for bacterial gene confirmation are shown in Table (3).

Table 3. Bacterial gene confirmation primers of *V. parahaemolyticus* and *Vibrio* sp. related to AHPND

Gen		Sekuens	Product size (bp)	Reference
AP41	F:	ATGAGTAACAATATAAAAC ATGAAAC	1.269 bp	Dangtip *et al.* (2015)
	R:	ACGATTTCGACGTTCCCCAA		
AP42	F:	TGAGAATACGGGACGTGGG	230 bp	Dangtip *et al.* (2015)
	R:	GTTAGTCATGTGAGCACCTT C		
16SVp	F:	GGTGTAGCGGTGAAATGCGT AG	284 bp	Xiao *et al.* (2021)
	R:	CCACAACCTCCAAGTAGACA TCG		

Data analysis

Data were analyzed by normality test using Shapiro-Wilk method. Correlation test using Sparman Corelation, P=95% to determine the relationship between temperature, pH, salinity and DO with the number of *V. parahaemolyticus* bacteria. Data analysis using SPSS software version 22 (IBM, USA).

RESULTS AND DISCUSSION

Water quality

The research results showed that there were fluctuations in water quality parameters. The measured DO (Dissolved Oxygen) levels remained within the optimal range, thus still supporting the life of the cultured *Litopenaeus vannamei* (vannamei shrimp). The DO range obtained was 6–7 ppm. This is in accordance with the results of research from **Supriatna *et al.* (2017)**, which found that the average concentration of DO in vaname shrimp ponds is 4.84 ± 0.41 ppm with a range of 3.48 ppm to 6.90 ppm. High DO (above 6 mg/L) supports the growth of aerobic microorganisms, including some beneficial bacteria. When DO decreases (e.g. below 5 mg/L), environmental conditions become more favorable for the growth of facultative anaerobic bacteria such as *Vibrio* sp. Pathogenic bacteria grow rapidly in water conditions that are rich in organic matter but have low oxygen levels **(Ariadi *et al.*, 2024)**.

The pH showed a range between 7.5 to 9.00, with the highest pH being 9 which occurred in DOC 42, while the optimal pH is 7 - 8 **(Jelinda *et al.*, 2024)**. pH stability is very important in controlling the density of *Vibrio* sp. bacteria in ponds. pH within the optimal range supports the growth of beneficial microorganisms and maintains the balance of the pond ecosystem, while extreme pH fluctuations can increase stress in shrimp, and trigger high populations of *Vibrio* sp. Therefore, monitoring and management of pH is needed in shrimp culture to prevent health problems and increase pond productivity.

The temperatures showed that DOC 14 reached >33°C and the lowest was DOC 49 with a temperature of 30°C. The highest density of *Vibrio* sp. bacteria in DOC was 10^7 CFU/mL. Fluctuating temperature conditions caused *Vibrio* sp. to be more pathogenic, according to **Hatmati (2003)** water conditions whose water quality fluctuates can cause *Vibrio* sp. more pathogenic or high pathogenicity for shrimp. According to **Arta (2009)**, the optimal temperature for *Vibrio* sp. development was 35–36°C, but drastically decreasing temperatures caused higher virulence **(Kharisma & Manan, 2012)**.

The highest salinity measurement was 35 ppt in DOC 28 and the lowest was 25 ppt in DOC 7. Based on the bacterial density graph, the highest bacterial density was observed at 30 ppt salinity on DOC 49, reaching 10^7 CFU/mL. Meanwhile, at salinity levels of 34–35 ppt on DOC 28 and 35, bacterial abundance remained relatively high at 10^6 CFU/mL. The abundance of *Vibrio* sp. has a strong correlation with alkalinity, pH, salinity, and phytoplankton density **(Rizaldi *et al.*, 2023)**. Salinity affects not only *Vibrio* sp. but also other microbial communities in the pond, such as probiotic (non-pathogenic) bacteria, which can influence the microbial balance. If salinity is stable within the optimal range, probiotic bacteria can compete with *Vibrio* sp. for nutrients and living space, suppressing pathogen density. However, if salinity is too low or high, probiotics may not survive, potentially allowing *Vibrio* sp. to become more dominant and cause infections in shrimp.

The results of nitrite, TAN and TOM measurements are presented in Figs. (2, 3). Nitrite concentrations were relatively low and fluctuated. There was a slight increase at DOC 14-21, but after that it remained low until DOC 49. The fluctuation of nitrite indicated the presence of a nitrification process, where ammonia is converted to nitrite by nitrifying bacteria. However, the low nitrite levels may be due to rapid conversion into nitrate or the presence of an effective water quality management system **(Ciji & Akhtar, 2020)**. In this study, water quality management included regular water exchange (10-20% daily), continuous aeration to maintain dissolved oxygen above 5 mg/L, and the application of physical filters before water was released into the ponds. In addition, probiotics containing *Bacillus* spp. are administered twice a week to support microbial balance and suppress opportunistic pathogens, including *Vibrio* species. This strategy is known to reduce nitrogenous waste accumulation and maintain microbial stability in intensive shrimp farming systems **(Chen *et al.*, 2024; Huang & Li, 2024)** Meanwhile, TAN concentrations tended to increase over time. On DOC 7, the levels were low, but they significantly increased from DOC 14 to DOC 49, reaching approximately 1.8 mg/L at the end of the observation period. The increase in TAN indicates the accumulation of ammonia compounds in the pond, originating from shrimp excretion and the decomposition of organic matter **(Zhao *et al.*, 2020; Islamy *et al.*, 2024)**.

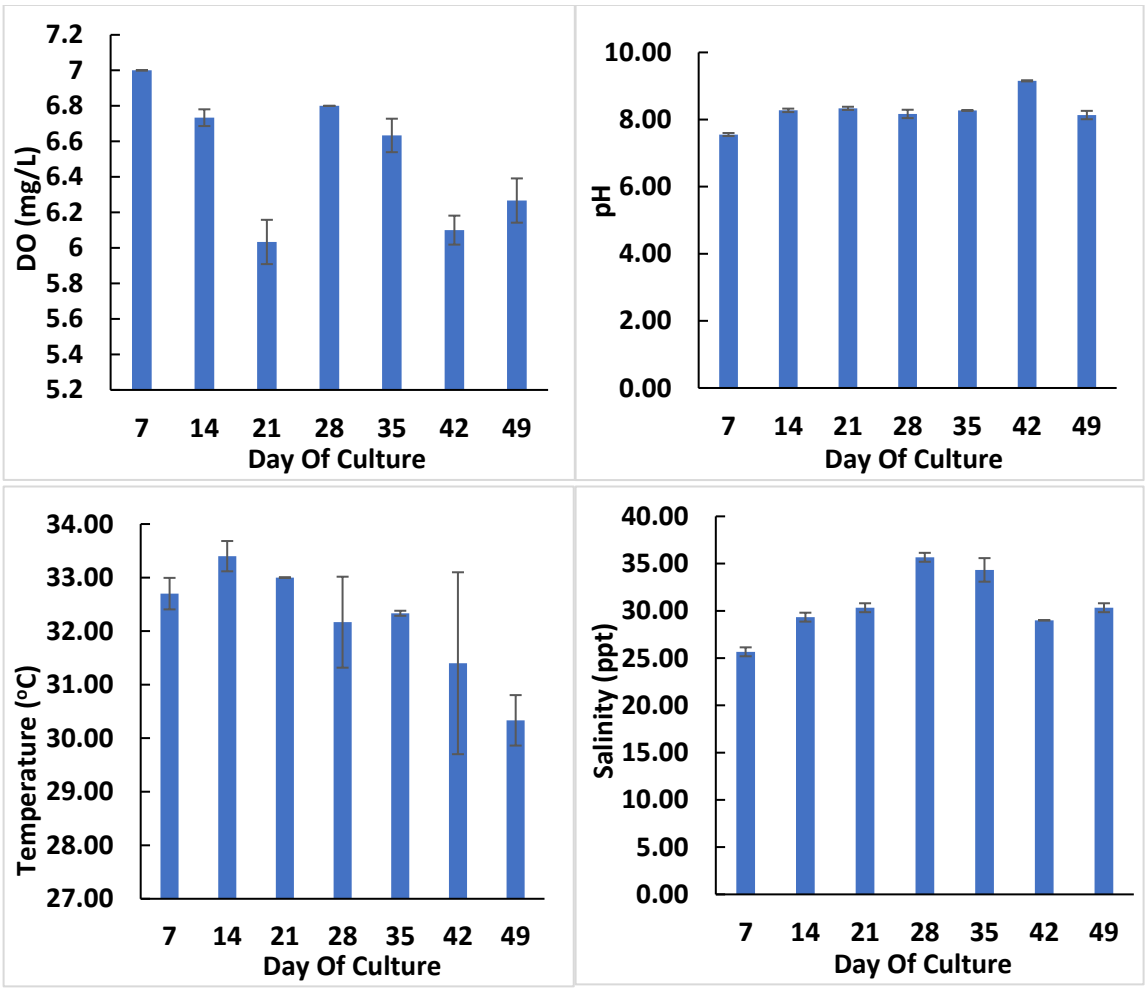

Fig. 1. Water quality measurement results

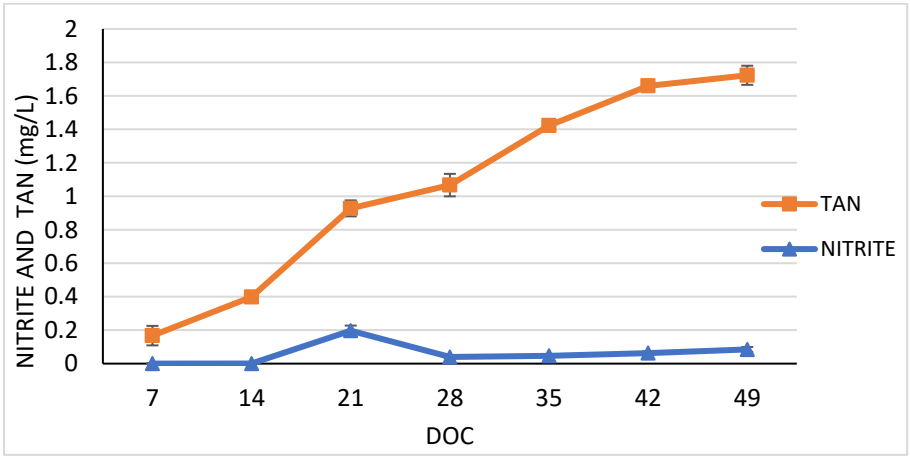

Fig. 2. Nitrite and TAN measurement results in ponds

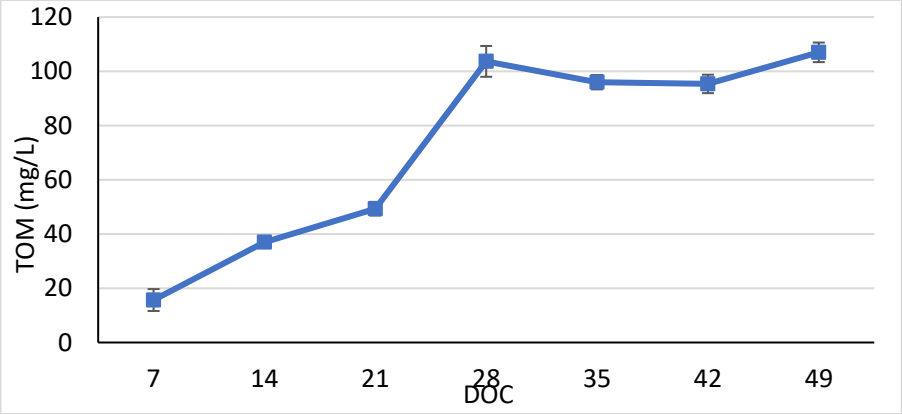

Fig. 3. TOM measurement results in ponds

The concentration of TOM increased from DOC 7 to 28 up to 100 mg/L. This graph shows a gradual increase in TOM with increasing shrimp age. TOM is an indicator of the amount of organic matter in the water, including feed residues, shrimp feces, and dead microorganisms. High TOM values in intensively culture ponds can be caused by factors such as overfeeding, shrimp excretion and fecal accumulation, and decomposition of dead microorganisms and plankton **(Yang *et al.*, 2017)**. In our study, the increase in TOM was primarily associated with overfeeding and the high organic waste load from uneaten feed and shrimp feces, which accumulated at the pond bottom. This was particularly evident in ponds with limited water exchange and suboptimal bottom management practices. Elevated TOM contributes to a reduction in dissolved oxygen levels and promotes anaerobic conditions in the sediment-water interface **(Ariadi *et al.*, 2019)**. These conditions create a favorable environment for *Vibrio* spp., including AHPND-causing strains (such as *V. parahaemolyticus*), to proliferate. The organic rich environment not only serves as a nutrient source but also reduces microbial competition by suppressing beneficial, aerobic bacteria **(Wang *et al.*, 2023)**. As a result, high TOM acts as an indirect driver for the colonization and potential virulence expression of AHPND-related *Vibrio* through enhanced survival and dominance in the pond ecosystem.

Bacteria abundance

The results of the Total *Vibrio* Count (TVC) calculation or bacterial density, from DOC 7 to DOC 49 ranged from 10^4 to 10^7 CFU/mL (Fig. 4). This indicated an increase over time during the rearing period, which was caused by the rising input in the culture system. Consequently, the waste load also increased periodically **(Martinez-Durazo *et al.*, 2019)**.

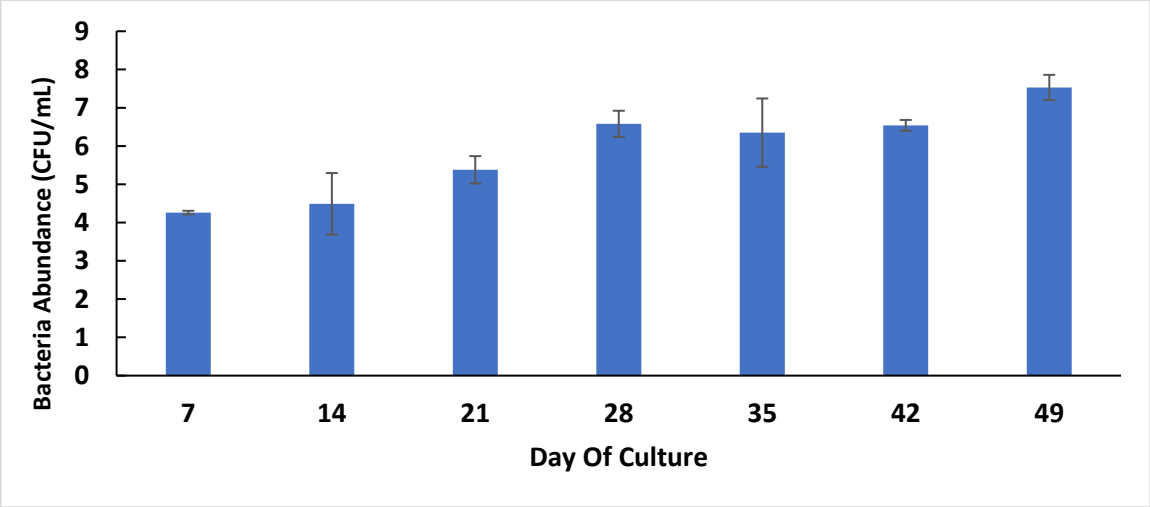

Fig. 4. Population abundance of *Vibrio* sp. bacteria in ponds

The increasing amount of aquaculture inputs such as feed and shrimp metabolic waste in the culture system lead to the accumulation of organic matter in the pond **(Ariadi *et al*., 2019)**. *Vibrio* spp., which are known pathogens in shrimp ponds, can adapt well to aquatic environmental conditions **(Pariakan & Rahim, 2021)**. The population of these bacteria fluctuates in response to environmental changes. Some *Vibrio* sp. species are very sensitive and can be opportunistic in their hosts **(Nitimulyo *et al*., 2005)**. Infection from these bacteria can be very harmful to cultured shrimp because *Vibrio* sp. is able to combine infection with other pathogens **(Ariadi, 2020)**.

Bacterial presence of *V. parahemolyticus* and *Vibrio* sp. strain AHPND

Detection of *V. parahemolyticus* bacteria in pond water samples showed negative results (Table 4). Samples showed positive results for AHPND detection starting from DOC 14 and DOC 49 (Fig. 5). This suggested that although *V. parahaemolyticus* bacteria were not present in pond waters, AHPND-related bacterial genes were still detected in other *Vibrio* sp. bacteria types. The density of *Vibrio* sp. bacteria increased to 10^7 at DOC 49.

Acute Hepatopancreatic Necrosis Disease (AHPND) in *Litopenaeus vannamei* (Pacific white shrimp) is commonly caused by *Vibrio parahaemolyticus* that produces toxins. However, AHPND cases are not always directly caused by *V. parahaemolyticus*. It can be caused by several factors, such as the presence of other bacteria that also carry a similar AP4 gene. The cause of AHPND is not only caused by *V. parahaemolyticus* but also by *V. punensis, V. harveyi, V. owensii, V. campbelli*, and *Shewanella* sp. containing the pVA1 plasmid encoding the binary toxins PirAVP and PirBVP **(Srikanth *et al*., 2008; Almagro-Moreno *et al*., 2015; Phiwsaiya *et al*., 2017; Xiao *et al*., 2017; Osei-Adjei *et al*., 2018; Perez-Acosta *et al*., 2018; Quintana-Hayashi *et al*., 2018; Gomez *et al*, 2019)**. In addition, *V. parahaemolyticus* is capable of communicating and transferring AHPND-associated genes to other *Vibrio* species, particularly through quorum sensing (QS) **(Ming Xue *et al*., 2023; Shuang Liu *et al*., 2023).**

Table 4. Detection results of *V. parahaemolyticus* and *Vibrio* sp. bacteria associated with AHPND

DOC	*V. parahaemolyticus*	*Vibrio* sp. AHPND	*Vibrio* Count
14	Negative	Positive	10^4 CFU/mL
49	Negative	Positive	10^7 CFU/mL

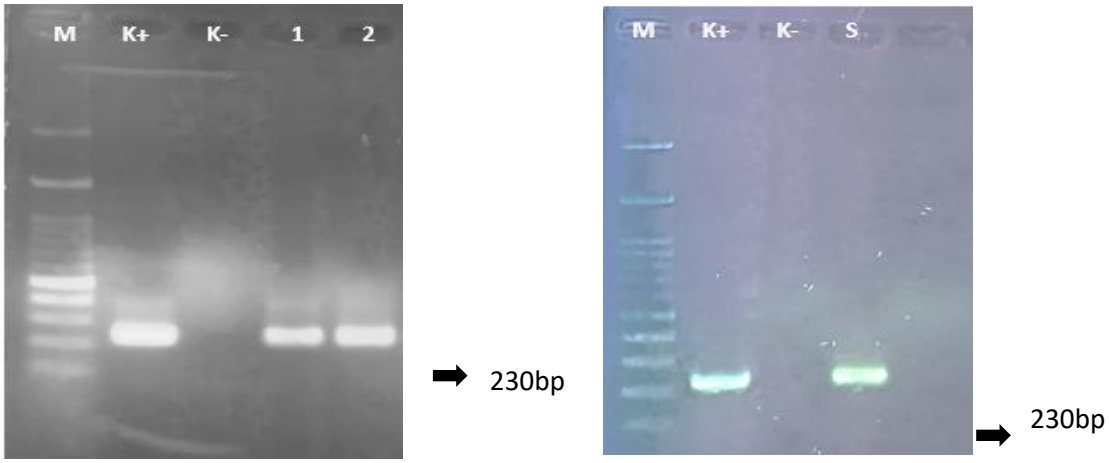

Fig. 5. Electrophoregram of AHPND detection results in pond water samples DOC 14 and 49; M: Marker DNA Ladder 100 bp; K+: AHPND Positive Control (230 bp); K-: Negative Control; 1: AHPND Positive First Sample DOC 14; 2: AHPND Positive Second Sample DOC 14; S: AHPND Positive Sample DOC 49

Corelation between water quality and bacteria abundance

The results of water quality parameter testing in relation to the abundance of *Vibrio* sp. are presented in Table (5). All tested water quality parameters showed a significant correlation with the abundance of *Vibrio* sp. ($P < 0.05$).

Table 5. Spearman correlation analysis of water quality parameters on *Vibrio* bacteria abundance

Spearman correlation test	Correlation of parameters to the abundance of Vibrio sp.			Correlation to relationship rate	
	Sig. Value	Criteria	Conclusion	Correlation Coeficient (r)	Relationship rate
DO with *Vibrio* abundance	0,007	< 0,05	Correlation	-0,378	Strong
Temperature with *Vibrio* abundance	0,000	< 0,05	Correlation	-0,622	Strong
pH with *Vibrio* abundance	0,008	< 0,05	Correlation	0,377	Strong
Salinity with *Vibrio* abundance	0,000	< 0,05	Correlation	0,597	Strong

Based on the analysis results (Table 5), the dissolved oxygen (DO) parameter has a significance value (sig.) of 0.007 and a correlation coefficient (r) of -0.378. This indicates that the DO parameter is correlated with the density of *Vibrio* sp. bacteria, with a strong level of association (Spearman correlation value between 0.334–0.666). The negative correlation value suggests that an increase in *Vibrio* sp. density is associated with a decrease in oxygen concentration, and vice versa. Low oxygen conditions (hypoxia) allow *Vibrio* sp. to proliferate more rapidly, as anaerobic environments better support its metabolic activity and virulence **(Bueno *et al.*, 2020)**. *Vibrio* is capable of utilizing organic compounds that decompose under low-oxygen conditions, which often occur due to the accumulation of organic matter such as feed residues and shrimp feces **(Farizky *et al.*, 2020)**. Additionally, hypoxic conditions can cause physiological stress in shrimp, reduce immune responses, and increase susceptibility to pathogenic bacterial infections. Conversely, sufficient dissolved oxygen levels enhance the activity of aerobic microorganisms that compete with *Vibrio* sp. in the pond ecosystem. These microorganisms can decompose organic matter more efficiently, thereby reducing the nutrient sources available to *Vibrio* sp.

The temperature parameter has a significance value (sig.) of 0.000 (<0.05) and a correlation coefficient (r) of -0.622. In aquatic ecosystems, temperature not only affects the metabolic rate of bacteria but also influences shrimp resistance to infection. Based on the correlation analysis, temperature has a negative relationship with *Vibrio* density, meaning that as temperature increases, the density of *Vibrio* sp. tends to decrease. At higher temperatures, bacterial metabolic rates generally increase; however, overly warm environmental conditions can inhibit the growth of certain pathogenic bacteria, including *Vibrio* sp. Some species of *Vibrio* sp., such as *V. harveyi* and *V. parahaemolyticus*, have an optimal temperature range for growth, typically between 25–30°C **(Sheikh *et al.*, 2022)**. If the temperature exceeds this optimal limit, these bacteria can experience physiological stress that inhibits metabolic activities, including replication. Furthermore, high temperatures can enhance the competitiveness of non-pathogenic microorganisms that are antagonistic to *Vibrio* sp., such as probiotic bacteria or microalgae that produce antibacterial compounds. On the other hand, lower temperatures can inhibit shrimp metabolism, reduce appetite, and weaken their immune system **(Islamy *et al.*, 2025; Islamy *et al.*, 2024; Jiang *et al.*, 2019)**. When the shrimp's immune system is weakened, they become more susceptible to *Vibrio* sp. infection, giving the bacteria a greater opportunity to replicate and cause disease.

The pH parameter has a significance value (sig.) of 0.008 (<0.05) and a correlation coefficient (r) of 0.377. Similarly, the salinity parameter has a significance value (sig.) of 0.000 (<0.05) and a correlation coefficient (r) of 0.597. *Vibrio* sp. generally grows optimally at neutral to slightly alkaline pH levels (7.5–8.5) **(Sampaio *et al.*, 2022)**. When the pH drops below the optimal range (becoming acidic), enzymatic activity in *Vibrio* sp.

is disrupted, leading to decreased nutrient utilization efficiency and inhibited growth. Acidic conditions can also damage the bacterial cell membrane, inhibit cell division, and increase bacterial mortality. In contrast, excessively high pH (alkaline) may induce osmotic stress and ionic imbalance within bacterial cells, potentially damaging protein structures and impairing cellular respiration **(Karagulyan *et al.*, 2022)**. Furthermore, unstable pH can affect shrimp health, weaken their immune systems, and indirectly increase the chances of *Vibrio* sp. colonization and infection. Therefore, maintaining pH stability within the optimal range is essential to control *Vibrio* sp. populations in ponds and mitigate their negative impact on aquaculture species.

The optimal salinity for the growth of *Vibrio* spp. generally ranges between 10–30 ppt, depending on the species **(Schofield *et al.*, 2020)**. At appropriate salinity levels, *Vibrio* can grow rapidly due to osmotic conditions that support cellular metabolism and proliferation. Conversely, when salinity is too low (< 5 ppt), osmotic pressure within *Vibrio* cells may be disrupted, leading to cell lysis or a drastic reduction in metabolic activity, thus decreasing the bacterial population. On the other hand, excessively high salinity (> 35 ppt) can cause dehydration of bacterial cells due to excessive osmotic pressure, thereby inhibiting growth or even causing bacterial death **(Larsen *et al.*, 2004; Gwendolyn *et al.*, 2021).** Moreover, extreme changes in salinity can disrupt microbial community balance in aquaculture ponds, potentially giving *Vibrio* spp. a competitive advantage. This underscores the importance of maintaining salinity within the optimal range to prevent excessive growth of *Vibrio* sp., which can negatively affect shrimp health.

CONCLUSION

This study confirms a strong relationship between water quality parameters and the abundance of *Vibrio* sp. in intensive shrimp ponds. Poor or imbalanced conditions such as elevated temperature, low dissolved oxygen, or high organic matter can promote *Vibrio* proliferation, highlighting the need for proper water quality management to control bacterial colonization and maintain shrimp health.

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