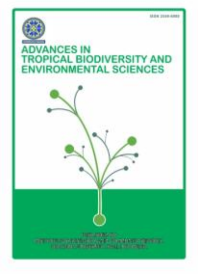

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Analysis of Abundance and Composition of Culturable Bacteria and Their Relationship with Water Quality Parameters in Intensive Shrimp Ponds

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Abstract. Intensive cultivation of Pacific white shrimp (*Penaeus vannamei*) is a crucial pillar of the aquaculture economy, but it faces sustainability challenges due to water-quality degradation driven by microbial activity. This study aims to analyze the total bacterial count (TBC) and total *Vibrio* count (TVC), identify the dominant culturable bacterial genera, and determine their statistical relationship with physicochemical water quality parameters in intensive shrimp ponds. The research methodology included regular sampling of water and sediment over one cultivation cycle. Water quality was analyzed using standard methods, while bacterial enumeration was performed using the spread plate technique on Marine Agar and Thiosulfate Citrate Bile Salts Sucrose Agar media. Pearson's correlation analysis was used to test the relationship between variables. The results show that some water quality parameters, particularly ammonia and TOM, tended to increase and exceed the SNI 01-7246-2006 quality standard as the cultivation cycle progressed. The abundance of TBC and TVC in sediment (average 10^6-10^7CFU/g) was significantly higher than in the water column (average 10^4-10^5 CFU/mL). Correlation analysis revealed a strong and significant negative relationship between DO and TBC ($r=-0.85$, $p<0.01$) and TVC ($r=-0.79$, $p<0.01$). Conversely, a strong and significant positive correlation was found between TOM and TBC ($r=0.91$, $p<0.01$) and TVC ($r=0.88$, $p<0.01$), as well as between ammonia and both TBC and TVC. It is concluded that the abundance of culturable bacteria, especially TBC and TVC, serves as a sensitive biological indicator of organic load and ecological stress in intensive pond systems. The strong predictive relationship between these microbial metrics and key parameters, such as DO and TOM, underscores the importance of microbiological monitoring for proactive management and improved sustainability in aquaculture.

Keywords: *Penaeus vannamei*; intensive culture; water quality; bacterial abundance; correlation

I. INTRODUCTION

The cultivation of Pacific white shrimp (*Penaeus vannamei*) is a leading commodity in the global and national fisheries sector, driven by high market demand and a relatively short production cycle. To meet this demand, cultivation systems have shifted towards intensification, characterized by high stocking density, high-protein feed inputs, and reliance on technologies such as aeration to maintain environmental conditions. While this practice can significantly increase production volume, intensive cultivation brings serious ecological consequences [1]. A major challenge is the large-scale accumulation of organic waste from uneaten feed and shrimp feces. This accumulation directly leads to a decline in water quality, which is the primary limiting factor for the productivity, sustainability, and profitability of intensive shrimp farming enterprises [2].

At the heart of the pond ecosystem's dynamics is a complex community of microorganisms, particularly bacteria, which play a dual role [3], [4], [5]. On the one hand, heterotrophic bacteria are essential to biogeochemical cycles, acting as primary decomposers that break down organic waste and recycle nutrients, such as nitrogen. This process is vital to maintaining the pond system's capacity to assimilate waste. On the other hand, an environment rich in nutrients and organic matter can trigger a shift in the microbial community structure, leading to the proliferation of opportunistic pathogenic bacteria. Among these pathogens, species from the genus *Vibrio* are a major concern because they are the main causative agents of vibriosis, which often results in mass mortality and significant economic losses in shrimp cultivation [6]. Therefore, understanding the balance between beneficial bacteria (e.g., the genus *Bacillus*, often used as probiotics) and potentially harmful bacteria (e.g., *Vibrio* and *Pseudomonas*) is crucial [7], [8], [9].

Physicochemical water quality parameters and the bacterial community in ponds cannot be viewed as two separate entities; they are interconnected in a dynamic feedback loop. An increase in the concentration of organic matter (measured as Total Organic Matter, or TOM) and inorganic nitrogen compounds, such as ammonia (NH3), provides an abundant substrate for the growth of heterotrophic bacteria. The aerobic decomposition process carried out by this dense bacterial population consumes a large amount of dissolved oxygen (DO), leading to hypoxic or anoxic conditions that are harmful to shrimp. Thus, bacterial abundance and activity directly modulate vital water quality parameters, and conversely, water quality conditions determine the types and abundance of bacteria that can thrive [10], [11], [12].

Although molecular techniques such as metagenomics can provide a comprehensive picture of microbial diversity, culture-based methods remain highly relevant, especially for field-level applications. Culture methods, such as counting TBC and *Vibrio* (Total *Vibrio* Count, or TVC), are relatively inexpensive, fast, and can provide quantitative data on indicator bacterial groups whose roles in pond ecosystem health are well understood. This quantitative data is very valuable for day-to-day management decisions. Based on this background, this study was designed to test the hypothesis that there is a strong quantitative relationship between the abundance of culturable bacteria and key water quality parameters in the intensive shrimp pond environment [1], [13].

II. METHODS

Study Location and Schedule

This research was conducted in a commercial intensive *Penaeus vannamei* shrimp pond located on the northern coast of Bali, Indonesia (8°10.648'S. 114° 47.089'E). The pond had an area of 3,000m2, was lined with High-Density Polyethylene (HDPE) plastic, and was operated with an initial stocking density of 120 shrimp/m^2. Sampling was performed weekly for one full cultivation cycle, lasting 110 days, to capture the dynamic changes in parameters as the shrimp grew and waste accumulated.

Sampling

Water and sediment samples were collected according to standard protocols for microbiological and chemical analysis [7], [10]. Water Samples: Composite water samples were taken from five different points in the pond to ensure representativeness. At each point, samples were collected from two depths: the surface (5-10 cm) and the bottom water column (approximately 20 cm above the pond bottom). Water samples for bacteriological analysis were collected using sterile 250 mL bottles, while those for chemical analysis were collected using 1 L polyethylene bottles.

Sediment Samples: Sediment samples were collected from the center of the pond, where organic matter accumulation is highest, using a sterile corer. Only the top sediment layer (0-5 cm) was collected, as this is the zone with the highest microbial activity.

All samples were immediately placed in a cooler with ice (40 °C) and transported to the Microbiology Laboratory at Universitas Pendidikan Ganesha for analysis within 6 hours of collection.

Water Quality Parameter Analysis

Water quality parameters were measured using a combination of in situ and laboratory analysis methods. In Situ Measurements: Temperature, pH, dissolved oxygen (DO), and salinity were measured directly at the site in the morning (07:00 WITA) and afternoon (15:00 WITA) using a calibrated multiparameter water quality probe (YSI ProDSS).

Laboratory Analysis: Water samples brought to the laboratory were analyzed for the following parameters: (1) Total Organic Matter (TOM): Determined by the permanganometric titration method according to APHA (American Public Health Association), (2) Ammonia (NH_3-N): Measured using the phenate method with a spectrophotometer at a wavelength of 640 nm, (3) Nitrite (NO_2-N): Measured using the diazotization method with a spectrophotometer at a wavelength of 543 nm., and (4) Phosphate (PO_4-P): Measured using the ascorbic acid method with a spectrophotometer at a wavelength of 880 nm.

Bacteriological Analysis

Sample Preparation: One gram of wet sediment sample was dissolved in 9 mL of sterile saline solution (0.85% NaCl) and homogenized with a vortex mixer. Water samples and sediment suspensions were then serially diluted (10−1 to 10−6) using the same sterile saline solution.

Bacterial Enumeration: Total Bacterial Count (TBC): 0.1 mL from each appropriate dilution was spread evenly onto the surface of Marine Agar (MA) media using the spread plate method. Petri dishes were incubated at 30 ^{0}C for 48-72 hours. Colonies that grew were counted, and bacterial abundance was expressed as Colony Forming Units per milliliter (CFU/mL) for water samples and CFU per gram (CFU/g) for sediment samples. Total *Vibrio* Count (TVC): The same procedure was performed using the selective medium Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar. Petri dishes were incubated at 30 ^{0}C for 24-48 hours. Yellow (sucrose fermenters) and green (non-sucrose fermenters) colonies that grew were counted as presumptive *Vibrio* spp.

Isolation and Identification: Several colonies with different morphologies from the MA and TCBS plates were randomly selected. These isolates were then purified using the quadrant streak method. Identification to the genus level was performed based on colony morphological characteristics, Gram staining, and a series of standard biochemical tests, including oxidase, catalase, motility, and glucose Oxidation/Fermentation (O/F) tests [7], [8], [9], [14].

Data Analysis

All data obtained were analyzed using SPSS statistical software version 25. Descriptive statistics (mean, standard deviation, and range) were calculated for all water quality and bacteriological parameters. To test the relationship between bacterial abundance (TBC and TVC) and water quality parameters, Pearson's correlation analysis was used. The statistical significance level was set at $p<0.05$.

III. RESULTS AND DISCUSSION

The physicochemical water quality parameters measured during one cultivation cycle showed dynamic fluctuations, reflecting the biological processes and management inputs in the pond. A summary of the data is presented in Table 1.

Table 1.
Average Water Quality Parameters During the Culture Period and Their Comparison with Quality Standards

Parameters	Unit	Average Value ± SD	Range	01-7246-2006 SNI Standard
Temperature	°C	29.8±1.2	28.1−31.5	28.5−31.5
Salinity	g/L	18.5±2.5	15.2−23.8	15−25
pH	-	8.0±0.4	7.5−8.6	7.5−8.5
Dissolved Oxygen (DO)	mg/L	4.8±0.9	3.6−6.5	≥3.5
Total Ammonia (NH^3−N)	mg/L	0.08±0.05	0.01−0.25	≤0.01
Nitrite (NO_2−N)	mg/L	0.05±0.03	0.01−0.15	≤0.01
Phosphate (PO_4−P)	mg/L	0.22±0.10	0.11−0.45	≥0.1
Total Organic Matter (TOM)	mg/L	75.6±20.1	45.2−110.5	≤55

In general, the parameters of temperature, salinity, pH, and DO were within the recommended ranges specified in SNI 01-7246-2006 for intensive *Penaeus vannamei* cultivation. The lowest DO value (3.6 mg/L) was recorded in the morning before sunrise, which is the result of respiration by all pond organisms (shrimp, plankton, bacteria) throughout the night without oxygen production from photosynthesis [3], [7], [10], [11].

In contrast, parameters related to organic waste and nutrients showed deviation from the. The average concentrations of total ammonia (0.08 mg/L), nitrite (0.05 mg/L), and TOM (75.6 mg/L) exceeded the established thresholds. The increase in ammonia and TOM concentrations, especially in the second half of the cultivation cycle, is a direct indication of the accumulation of uneaten feed and shrimp metabolic products that exceed the pond system's natural assimilation capacity. Ammonia, even at low concentrations, is known to be toxic to shrimp, can inhibit growth, and increase susceptibility to disease. The high TOM value also indicates a large organic load, which has the potential to become a substrate for intense microbial decomposition [5], [15], [16].

Abundance and Composition of Cultivated Bacteria

Bacteriological analysis showed a significant difference in abundance between the water and sediment compartments (Table 2). The abundance of TBC and TVC in the sediment was consistently one to two orders of magnitude (10 to 100 times) higher than in the water column. This result confirms the role of sediment as a "microbial engine room" or a major biogeochemical hotspot in the pond ecosystem. High feed input in intensive systems results in the deposition of uneaten feed, feces, and dead organisms (necromass) at the bottom of the pond. This concentrated accumulation of organic matter provides an abundant source of carbon and nutrients, which directly triggers the growth and metabolic activity of heterotrophic bacteria. Conversely, the water column is a relatively more diluted environment. This finding is consistent with other studies showing that sediment is a more dominant contributor of bacteria to the shrimp gut than water. Therefore, the condition of the sediment and its microbial community play a central role in determining the overall ecological health of the pond [13], [17-19].

From the isolation and identification process, several dominant bacterial genera were successfully identified. From the MA medium, the commonly found genera included *Bacillus*, *Pseudomonas*, *Flavobacterium*, and *Micrococcus*. From the selective TCBS medium, the majority of isolates were identified as members of the genus *Vibrio*. The presence of these genera is consistent with previous reports on the microbiome in aquaculture environments.

Correlation between Bacterial Abundance and Water Quality Parameters

Pearson's correlation analysis (Table 3) revealed a strong, significant relationship between bacterial abundance and several key water quality parameters, providing quantitative evidence of dynamic interactions within the pond ecosystem.

The most notable relationship was the very strong negative correlation between DO and both TBC and TVC, in both water and sediment. This directly illustrates a fundamental biological process: the higher the bacterial abundance, the higher the rate of oxygen consumption for respiration and the decomposition of organic matter. Conversely, strong positive correlations were observed between TOM and ammonia, and between TBC and TVC. This relationship shows that organic matter and ammonia act as primary substrates that drive bacterial proliferation [1], [20]

Correlations of TOM and bacteria paint a picture of a "system stress feedback loop". The accumulation of waste (an increase in TOM and ammonia) provides "fuel" for the bacterial population explosion (an increase in TBC and TVC). The metabolic activity of this dense bacterial population then depletes the dissolved oxygen supply (a decrease in DO), creating increasingly stressful conditions for the shrimp. A negative correlation between DO and TBC, TVC, and TOM in intensive shrimp ponds, which reinforces these findings [21-22]

Furthermore, this quantitative relationship suggests that the TBC/DO axis can function as a "bio-indicator axis" for the pond's metabolic condition. A high TBC value accompanied by a low DO value is a quantitative marker of a system experiencing an excessive organic load and high decomposition stress, approaching critical hypoxic conditions [1], [12], [18], [20]. Thus, TBC is no longer just a bacterial count but an integrative biological indicator that reflects the health of the ecosystem and the effectiveness of feed and waste management. A significant negative correlation was also found between pH and TVC, indicating that *Vibrio* abundance tends to be higher at slightly lower pH conditions, although still within the alkaline range [23]. Some studies suggest that higher pH (approaching 8.5) can suppress the growth of some *Vibrio* species. Thus, TBC is no longer just a bacterial count but an integrative biological indicator that reflects the health of the ecosystem and the effectiveness of feed and waste management [24-25].

The results of this study also provide strong scientific justification for management practices that focus on controlling the organic load. Practices such as efficient feed management to minimize uneaten feed, regular siphoning of bottom sludge to remove accumulated waste, and the application of probiotics based on *Bacillus* spp. are highly relevant. *Bacillus* probiotics not only accelerate the efficient decomposition of organic matter but can also compete with pathogenic bacteria, such as Vibrio, for space and nutrients, thereby helping maintain a healthy microbial balance. By demonstrating the quantitative relationship between organic waste, potential pathogenic bacterial proliferation, and oxygen depletion, this research confirms that the key to successful intensive cultivation lies in microbial ecosystem management.

Table 2.
Average Abundance of Total Bacteria and Total Vibrio in Water and Sediment Samples

Sample Type	Bacteriological Parameter	Average Abundance ± SD	Units
Water	TBC	$5.2\times10^4\pm2.1\times10^4$	CFU/mL
	TVC	$1.8\times10^3\pm0.9\times10^3$	CFU/mL
Sediment	TBC	$8.9\times10^6\pm3.5\times10^6$	CFU/g
	TVC	$4.5\times10^4\pm1.7\times10^4$	CFU/g

Note: TBC = Total Bacteria count; TBC = Total Vibrio count.

Table 3.
Correlation between Bacterial Abundance and Water Quality Parameters

Parameter	TBC (Water)	TVC (Water)	TBC (Sediment)	TVC (Sediment)
Temperature	0.21	0.18	0.25	0.22
pH	−0.45*	−0.58**	−0.41*	−0.52**
DO	−0.85**	−0.79**	−0.89**	−0.82**
TOM	0.91**	0.88**	0.94**	0.90**
Ammonia (NH^3−N)	0.76**	0.72**	0.80**	0.75**
Nitrite (NO_2−N)	0.65**	0.61**	0.69**	0.66**
Phosphate (PO_4−P)	0.59**	0.55**	0.63**	0.58**

Note: TBC = Total Bacteria count; TBC = Total Vibrio count; *Significant at $p<0.05$; **Significant at $p<0.01$.

CONCLUSION

This study demonstrated a strong, predictable statistical relationship between culturable bacterial abundance and water quality parameters in intensive *Penaeus vannamei* pond systems. The total bacterial count and total *Vibrio* count showed a very significant positive correlation with total organic matter and ammonia concentrations, and a very significant negative correlation with dissolved oxygen levels. These findings confirm that the abundance of heterotrophic bacteria and *Vibrio* can serve as sensitive and integrative biological indicators of the pond's ecological condition. The increase in these bacterial populations directly reflects a high organic waste load and is the primary driver of the decline in dissolved oxygen levels.

The broader implication of this research is that routine monitoring of these bacteriological indicators, when integrated with standard physicochemical parameter measurements, can significantly enhance proactive management capabilities, improve disease-prevention strategies, and ultimately contribute to greater sustainability of the intensive shrimp farming industry.

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CONFLICT OF INTEREST

All authors state no conflict of interest for this publication.

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