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CHARACTERIZATION OF INDICATOR BACTERIAL COMMUNITIES IN SHRIMP PONDS FOR EVALUATING THE HEALTH OF CULTURE MEDIA

Karakterisasi Komunitas Bakteri Indikator Pada Tambak Udang untuk Mengevaluasi Kesehatan Media Budidaya

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ABSTRACT

Shrimp aquaculture is a crucial subsector of Indonesia's marine economy, yet its sustainability is threatened by disease outbreaks. Bacterial infections contribute to 22% of disease cases during the shrimp grow-out phase. This study aims to characterize indicator bacterial communities, particularly *Vibrio* spp., *Pseudomonas* spp., *Bacillus* spp., and total bacteria, as a tool for evaluating the health of culture media in shrimp ponds. The research method employed was descriptive-observational. The analysis revealed that water quality parameters significantly influence the dynamics of the bacterial community. Specifically, a temperature increase above 32°C can enhance the virulence of pathogenic bacteria such as Vibrio parahaemolyticus by 40-60%, whereas temperatures below 26°C can reduce the diversity of beneficial bacteria by 25- 3 5%. Furthermore, daily salinity fluctuations exceeding 5 ppt heighten osmotic stress in shrimp and increase the risk of bacterial infections. A total bacterial abundance exceeding 10^6 CFU/mL is associated with the accumulation of organic matter from residual feed and shrimp feces, indicating a decline in water quality. Conversely, an abundance of *Bacillus* spp. greater than 20% of the total heterotrophic bacteria and a Vibrio/total bacteria ratio below 1% were consistently associated with healthy pond conditions. This study underscores that monitoring indicator bacterial communities is an effective proactive strategy for sustainable pond management and disease risk mitigation.

Key words: Bacterial Community, Indicator Bacteria, Shrimp Pond, *Vibrio* spp., Water Quality

ABSTRAK

Budidaya udang merupakan subsektor penting dalam ekonomi kelautan Indonesia, namun keberlanjutannya terancam oleh wabah penyakit. Infeksi bakteri menyumbang sekitar 22% dari kasus penyakit selama fase pembesaran udang. Penelitian ini bertujuan untuk mengkarakterisasi komunitas bakteri indikator, khususnya *Vibrio* spp., *Pseudomonas* spp., *Bacillus* spp., serta total bakteri, sebagai alat untuk mengevaluasi kesehatan media budidaya di tambak udang. Metode penelitian yang digunakan adalah deskriptif-observasional. Hasil analisis menunjukkan bahwa parameter kualitas air secara signifikan memengaruhi dinamika komunitas bakteri. Secara khusus, peningkatan suhu di atas 32°C dapat meningkatkan virulensi bakteri patogen seperti *Vibrio* parahaemolyticus sebesar 40–60%, sedangkan suhu di bawah 26°C dapat menurunkan keanekaragaman bakteri menguntungkan sebesar 25–35%. Selain itu, fluktuasi salinitas harian yang melebihi 5 ppt meningkatkan stres osmotik pada udang dan memperbesar risiko infeksi bakteri. Kelimpahan total bakteri yang melebihi 10^6 CFU/mL berkaitan dengan akumulasi bahan organik dari sisa pakan dan feses udang, yang menunjukkan penurunan kualitas air. Sebaliknya, kelimpahan *Bacillus* spp. yang lebih dari 20% dari total bakteri heterotrof serta rasio *Vibrio*/total bakteri di bawah 1% secara konsisten berkaitan dengan kondisi tambak yang sehat. Penelitian ini menegaskan bahwa pemantauan komunitas bakteri indikator merupakan strategi proaktif yang efektif untuk pengelolaan tambak berkelanjutan dan mitigasi risiko penyakit.

Kata Kunci: Bakteri Indikator, Komunitas Bakteri, Kualitas Air, Tambak Udang, *Vibrio* spp.

INTRODUCTION

Shrimp farming is a crucial subsector of Indonesia's marine economy. However, the sustainability of this industry faces serious threats due to disease outbreaks (Delphino et al., 2022). Data shows that bacterial infections, in particular, are responsible for approximately 22% of all disease cases occurring during the shrimp grow-out phase (Bhassu et al., 2024; Kumar et al., 2025). The high frequency of these outbreaks not only causes significant economic losses for farmers but also encourages unsustainable management practices, such as reliance on antibiotic use. Breaking this cycle of losses requires a fundamental paradigm shift, shifting from reactive management to proactive strategies focused on disease prevention and maintaining healthy pond ecosystems (Carlino-Costa & Belo, 2025; Delphino et al., 2022; Macusi et al., 2022; Turlybek et al., 2025; Verdegem et al., 2023).

Traditionally, pond health monitoring has relied heavily on physicochemical parameters such as temperature, salinity, and dissolved oxygen (C. Boyd, 2017; Ramdani et al., 2025; Yohana et al., 2024). While important, these parameters often fail to capture the biological complexity underlying disease dynamics. Pond ecosystems are dynamic microcosms, where the health of the culture medium and, consequently, the health of the shrimp is largely determined by the microbial communities that inhabit them (Cornejo-Granados et al., 2025; Emmanuel et al., 2025; Garcia et al., 2025; Zhang & Sun, 2022; Zheng et al., 2017).

This research focuses on the characterization of the main indicator bacteria. Specifically, this research examines pathogenic genera (Vibrio spp.), opportunistic genera (Pseudomonas spp.), and genera that have potential as probiotics (Bacillus spp.). Understanding the population dynamics of these bacterial groups and, more importantly, the ratios between these groups, such as the Vibrio/Total Bacteria ratio, can serve as a much more sensitive early warning system for disease risk.

Analyzing a complex system requires a comprehensive and stepwise approach, which also validates the methodology used in this study. The series of analyses used included: (1) Descriptive Analysis, to establish baseline values and identify water quality anomalies across multiple locations; (2) Correlation Analysis, to identify linear relationships between environmental triggers and microbial responses; (3) Principal Component Analysis (PCA), to reduce the complexity of multivariate data and visualize the clustering of sampling locations based on their overall health profile; and (4) Community Analysis, to assess the diversity and structure of microbial populations (Betiku et al., 2021; Cassol et al., 2025; Ma et al., 2021; Potiron, 2025; Yang et al., 2023). This combination of analyses enabled this study to move beyond observation and develop quantitative benchmarks applicable to pond management.

The objectives of this study were to (1) characterize water quality parameters and indicator bacterial communities at various locations in an aquaculture system, (2) identify statistical relationships between environmental parameters and microbial dynamics, and (3) establish quantitative thresholds of indicator bacteria that can be used as proxies to evaluate the health of the culture medium.

RESEARCH METHODS

This research uses descriptive-observational methods to characterize environmental and microbiological conditions at key points in the shrimp cultivation system. Sampling was carried out at several locations representing different gradations of conditions, including active cultivation ponds, inlet water reservoirs, and outlet channels from internal wastewater treatment plants.

Water Quality Analysis

Physicochemical parameters were measured both in-situ and in the laboratory. Temperature and salinity are measured directly in the field. Water samples were collected and taken to the laboratory for further analysis to determine dissolved oxygen and nitrate (NO_3^-) concentrations. This measurement aims to characterize basic environmental variations between sampling locations (Do et al., 2025; Lebbie et al., 2025; Setyaningrum et al., 2023; Srinivas & Venkatrayulu, n.d.; Supriatin et al., 2024).

Microbiological Analysis

Bacterial enumeration was carried out using the viable plate count method. Water samples from each location were serially diluted and spread on certain agar media. Total Heterotrophic Bacteria (THB) was counted using non-selective media. Selective enumeration of Vibrio spp. carried out using Thiosulfate-Citrate-Bile-Sucrose (TCBS) Agar media. Other indicator genera, such as Pseudomonas spp. and Bacillus spp., isolated and counted using special selective media. All plates are incubated under appropriate conditions, for example at a temperature of 28–30°C for 24–48 hours, and the colonies formed are calculated as Colony Forming Units (CFU) per mL. This enumeration data was used to calculate the Vibrio/THB ratio and percentage abundance of Bacillus spp., which are the main metrics in this study (Alfiansah et al., 2020; Barbosa et al., 2022; Kemsawasd et al., 2024; Nuri Kamaliah et al., 2025; Nursyirwani et al., 2022).

Statistical Analysis

Statistical analyzes were performed using standard statistical software. Descriptive analysis was used to calculate mean values, standard deviations and extreme values for all water quality and microbiological parameters at each location. Correlation analysis was used to evaluate the strength and direction of linear relationships between environmental parameters and microbiological parameters. Principal Component Analysis (PCA) was performed on standardized data to visualize the clustering patterns of sampling locations and identify the variables that contributed most to the overall variation in the data. Community analysis includes calculation of diversity indices and relative abundance analysis to identify the dominant bacterial genus at each location (Kothari et al., 2021; Longo et al., 2023; Musa et al., 2023; Nazarudin et al., 2025; Siddique et al., 2024; Uddin et al., 2023).

RESULT

Data analysis shows that there is a close and complex relationship between physicochemical environmental conditions, microbial community structure, and the overall health of the cultivation media. This section integrates the obtained statistical results with the identified critical biological thresholds.

Water Quality Variability at Various Sampling Locations

The results of the descriptive analysis showed that there were significant variations in water quality parameters between the different sampling locations, indicating the existence of clear gradations in ecosystem health. As shown in Figure 1, two locations stand out as representing extremes of the spectrum. The Wastewater Treatment Plant site shows signs of heavy eutrophication and high organic load, as indicated by the highest nitrate concentration recorded, namely 51 mg/L. In contrast, the inlet water reservoir location shows much healthier and more stable conditions, which is characterized by the highest dissolved oxygen value, namely 9.66 mg/L. This extreme variability emphasizes that location in the cultivation system is the main determining factor in media quality, so that a uniform management approach for all locations is inadequate (Do et al., 2025; Setyaningrum et al., 2023; Srinivas & Venkatrayulu, n.d.; Supriatin et al., 2024).

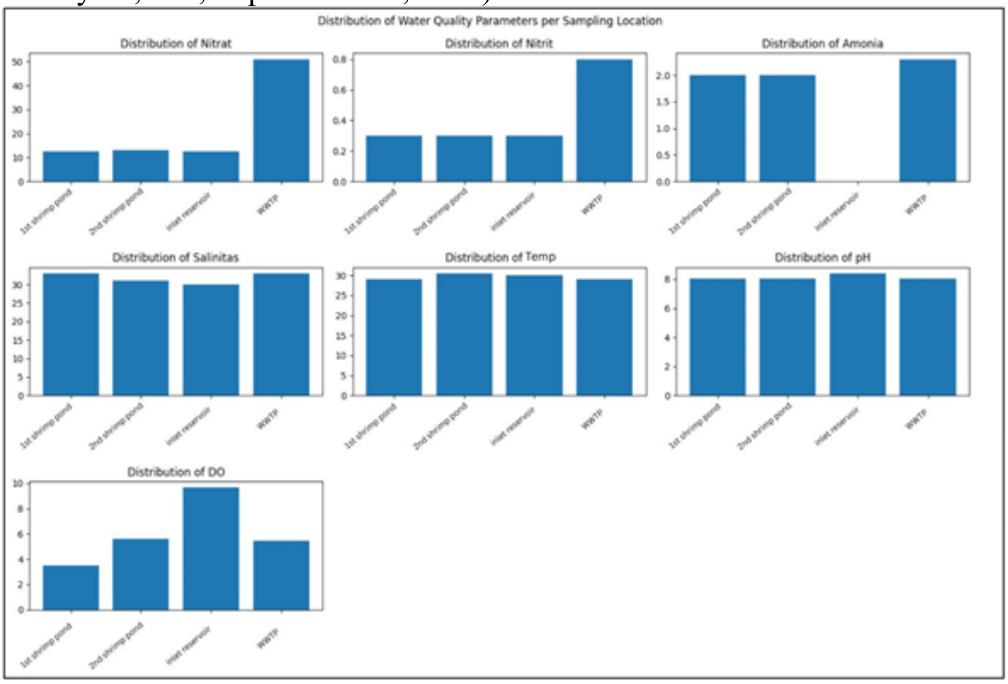

Figure 1. Comparison of main water quality parameters at various sampling locations.

The WWTP location shows signs of severe eutrophication with the highest nitrate concentration, while the incoming water tank shows the healthiest conditions with the highest dissolved oxygen levels.

Bacterial Community Structure and Genus Dominance

Interesting findings emerged when water quality data were linked to microbial community analysis. The Tandon location, which has the best physicochemical conditions, was also recorded as having the highest bacterial diversity, as shown in Figure 2.1. This is in line with basic ecological theory: environments that are stable and have good dissolved oxygen tend to support more diverse, complex and resilient biological communities (Choudhary et al., 2025; Linnaeous Rabadon et al., 2022; Nuri Kamaliah et al., 2025; Nursyirwani et al., 2022; Yang et al., 2023).

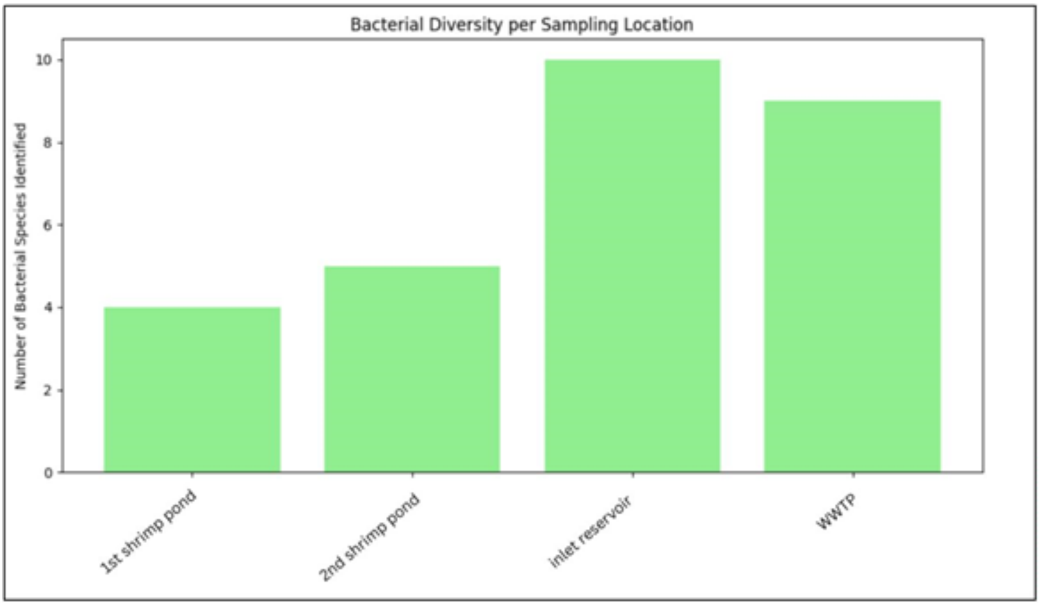

Figure 2 Bacterial diversity index at various sampling locations.

The Tandon site, characterized by the most stable and oxygen-rich conditions, supported the highest bacterial diversity, in line with the ecological theory that stable environments encourage the formation of more complex and resilient communities.

However, despite differences in diversity levels, compositional analysis revealed an important finding: all sites, both healthy and disturbed, were dominated by the same three genera of bacteria, namely Vibrio, Pseudomonas and Bacillus. This finding is very important because it shows that pond health is not determined by the presence or absence of pathogen genera such as Vibrio, but rather by the balance and ratio between these competing groups of bacteria. Figure 3 depicts the distribution of microbes. This supports the hypothesis of a "microbial battle" in the cultivation media, where Bacillus spp. directly compete with Vibrio spp. in fighting for resources and space. Pond health appears to be highly dependent on environmental and biological factors that favor the growth of Bacillus over Vibrio (Ali et al., 2022; Dou et al., 2021; Harpeni et al., 2024; Khanjani et al., 2024; Raza et al., 2024; Soto-Marfileno et al., 2024; Truong et al., 2021; Widiyanto et al., 2020; Wu et al., 2024).

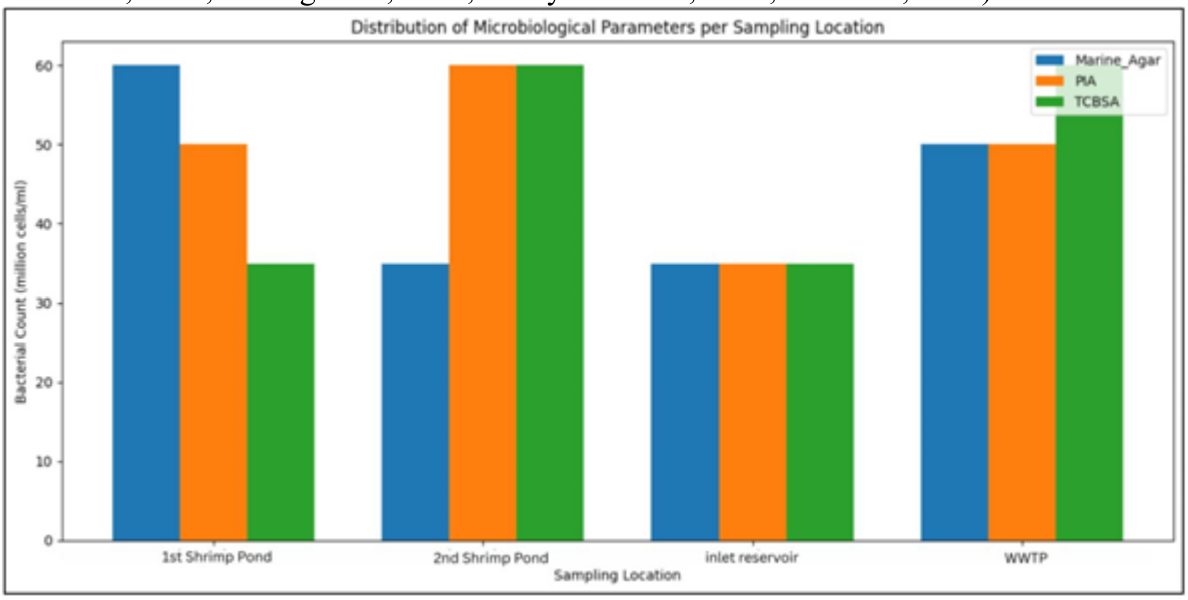

Figure 3 Relative abundance of the main indicator bacterial genera (Vibrio, Pseudomonas, and Bacillus) at various sampling locations.

This graph shows that pond health is not determined by the presence or absence of certain genera, but rather by the balance and ratio between these genera, which confirms the concept of "microbial battle" in the cultivation medium.

Multivariate Relationship between Environment and Microbes

Correlation analysis confirms the existence of a strong relationship pattern between environmental parameters and microbiological parameters (Longo et al., 2023; Nazarudin et al., 2025). In particular, temperature showed significant correlations with several bacterial parameters, confirming its role as a major environmental factor, as shown in the correlation matrix (Figure 4). To disentangle the complex relationship between these various variables, PCA analysis was carried out. PCA succeeded in reducing the complexity of the data, with the first two principal components (PC1 and PC2) explaining 80.6% of the total data variance (PC1 of 58.1% + PC2 of 22.5%).

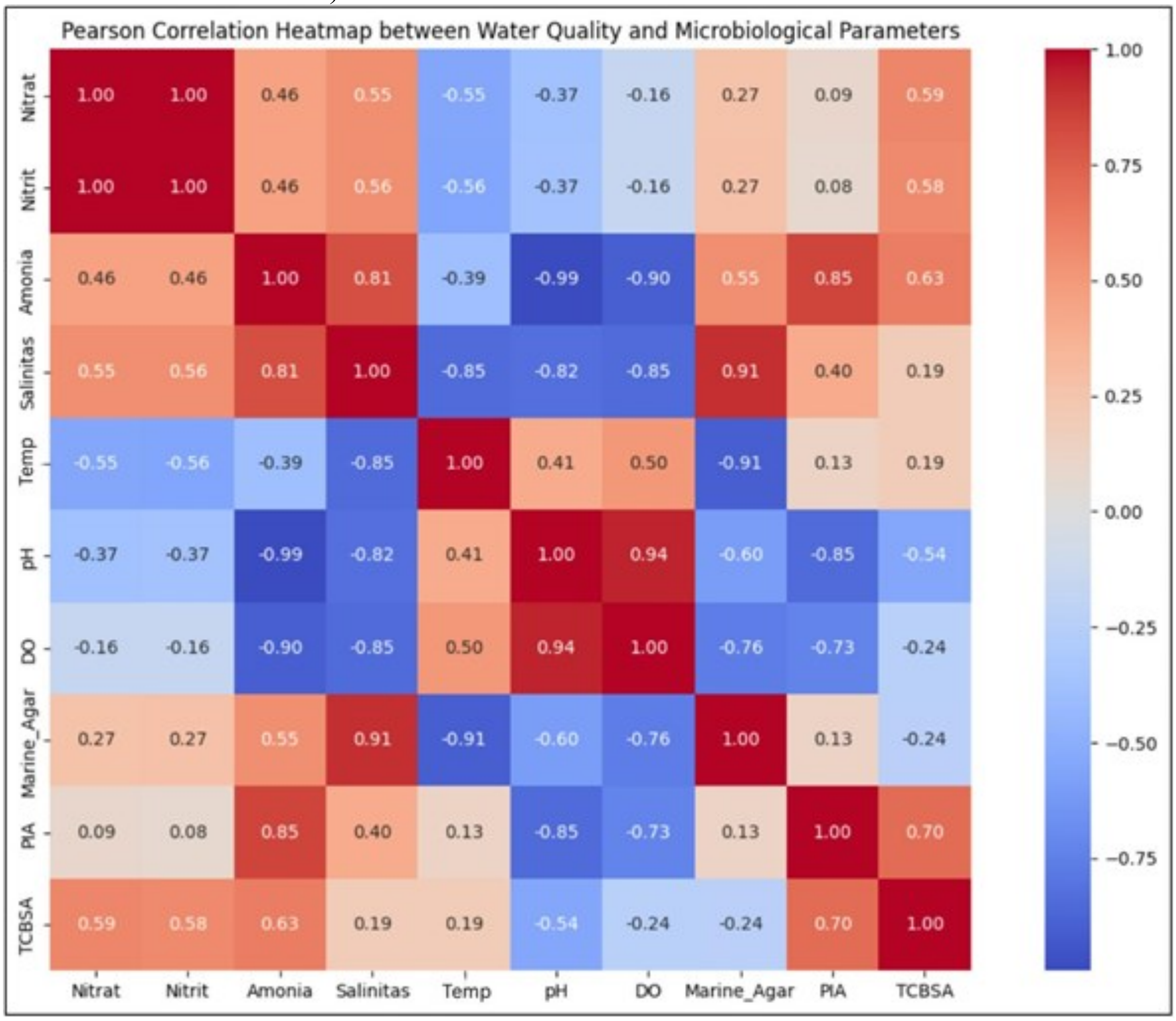

Figure 4. Correlation matrix heatmap showing the strength and direction of linear relationships, both positive and negative, between environmental parameters and microbiological parameters. Temperature showed significant correlations with several bacterial groups, confirming its role as a major environmental factor.

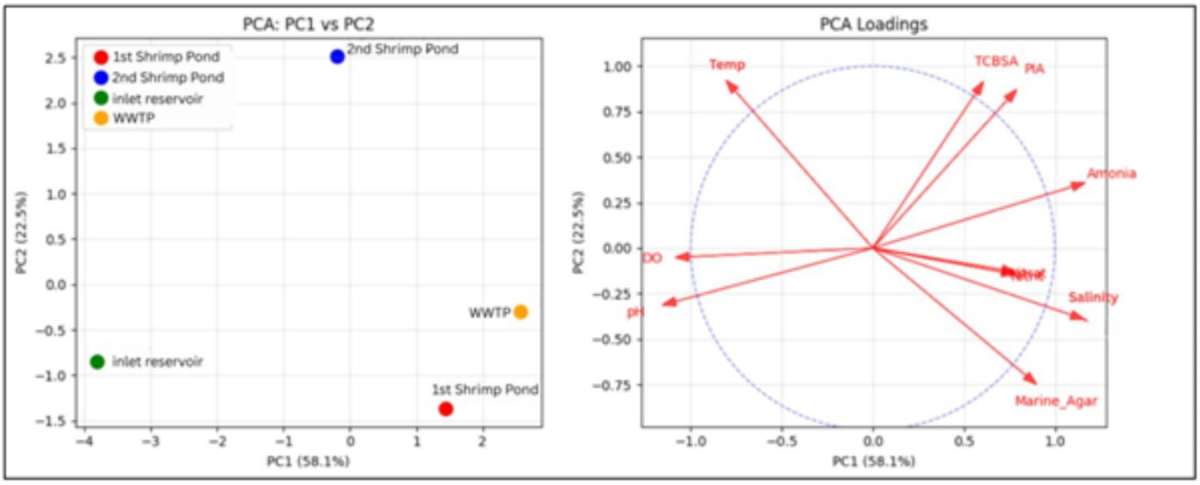

Figure 5 PCA biplot visualizing the clustering of sampling locations based on all measured environmental and microbiological variables. PC1 and PC2 explained 80.6% of the total variance.

The PCA biplot (Figure 5) visually shows a clear clustering pattern at the sampling locations. These locations are separated along the axes, forming a "health map" of the system. As expected, the locations of the Tandon and Waste Water Treatment Plant are far apart, occupy opposite quadrants, and represent two ends of the spectrum, namely healthy conditions and disturbed conditions. The variable vectors in the biplot confirm the relationship: the nitrate vector and Vibrio abundance are strongly correlated towards the quadrant where the WWTP is located, while the dissolved oxygen vector, bacterial diversity and Bacillus abundance are correlated towards the Tandon quadrant (Ali et al., 2022; Soto-Marfileno et al., 2024). This PCA (Figure 5) visually confirms that physicochemical conditions and microbiological health are intrinsically linked and together determine the condition of the cultivation medium.

DISCUSSION

The most significant finding of this study is the quantification of specific environmental and biological thresholds. The ability to move from general correlations to applicable quantitative risk indicators is at the heart of a proactive management strategy. These findings are summarized in Table 1.

The analysis identified three main categories of stress factors:

1. Environmental stress factors

Daily salinity fluctuations exceeding 5 ppt were identified as a significant osmotic stress factor for shrimp, which directly increases the susceptibility of shrimp to bacterial infections. Additionally, temperature was found to have a paradoxical role: temperatures above 32°C drastically increased the virulence of pathogens such as Vibrio parahaemolyticus by 40–60%. In contrast, temperatures below 26°C suppress the beneficial microbiome by reducing its diversity by 25–35% (Dou et al., 2021; Widiyanto et al., 2020). These findings effectively establish a narrow safe operating zone for temperature management (Ali et al., 2022; Khanjani et al., 2024; Raza et al., 2024).

2. Biological stress factors

Accumulation of organic matter from food waste and feces, which can be measured as a proxy for total bacterial abundance, is consistently associated with decreased water quality. A risk threshold was identified when the total bacterial abundance exceeded 10^6 CFU/mL. This condition is a direct indicator of poor feed management or failure of the waste disposal system (Longo et al., 2023; Nazarudin et al., 2025).

3. Microbial health indicators

Most importantly, this research identified two ratio-based benchmarks for defining healthy pond conditions, namely: (a) abundance of Bacillus spp. more than 20% of the total population of heterotrophic bacteria, and (b) the Vibrio/Total Bacteria ratio is maintained below 1% (Khanjani et al., 2024; Musa et al., 2023; Nazarudin et al., 2025; Raza et al., 2024).

Discussion of this threshold is very important. Vibrio/Total Bacteria ratio <1% is the most powerful Key Performance Indicator (KPI) for disease prevention. Vibrio will always be present in marine environments; the goal is not eradication which is impossible, but rather the suppression of the relative population so that it remains below the 1% threshold. In this context, recommendations for the use of probiotic Bacillus spp. be a very logical and directed strategy. This is not just a random addition of "good" bacteria, but rather a biocontrol strategy designed to actively manage that ratio. By encouraging the abundance of Bacillus spp. up to >20%, natural competition for resources and space can suppress Vibrio populations to remain below the critical threshold of 1% (Ali et al., 2022; Khanjani et al., 2024; Truong et al., 2021).

This series of findings allows us to visualize the Catastrophic Causal Chain in bacterial disease outbreaks. (1) Poor management causes → (2) accumulation of organic matter, which is detected as Total Bacteria >10^6 CFU/mL → (3) decrease in water quality (X. Wang et al., 2024). Simultaneously, (4) uncontrolled environmental stress factors cause → (5) osmotic stress in shrimp and an increase in Vibrio virulence by 40–60%. The end result is: (6) shrimp experiencing physiological stress in poor quality media encounter a more virulent and numerically dominant Vibrio population. It was this combination that likely drove 22% of the disease cases observed.

Table 1. Principal Component Analysis (PCA) biplot which visualizes the grouping of sampling locations based on all measured environmental and microbiological variables.

Category	Parameters	Risk Threshold	Health Threshold	Biological Implications
Environment	Temperature	< 26°C or > 32°C	26 – 32°C	< 26°C reduces biodiversity; > 32°C increases Vibrio virulence.
Environment	Salinity Fluctuations	> 5 ppt/day	< 5 ppt/day	High fluctuations cause osmotic stress in shrimp.
Organic Load	Total Bacteria	> 10^6 CFU/mL	< 10^6 CFU/mL	Indicates accumulation of food waste & feces, as well as poor water quality.
Microbes	Abundance	< 20% from total	> 20% From total	Indicates a healthy probiotic/biocontrol population.
Microbes	Bacillus spp.	> 1%	< 1%	The most powerful indicator of Vibrio disease risk threat.

A clear separation of locations, such as Tandon which is associated with high levels of dissolved oxygen (DO) and Bacillus, and WWTP which is associated with high levels of nitrate and Vibrio, forms a "health map" of the aquaculture system. This indicates an intrinsic relationship between physicochemical conditions and microbiological health.

CONCLUSION

This study successfully characterized the indicator bacterial community and associated environmental parameters to evaluate the health of the culture medium in shrimp ponds. Our findings confirm the key conclusion that monitoring the indicator bacterial community, in addition to standard physicochemical parameters, is a highly effective proactive strategy for sustainable pond management and disease risk mitigation (Deep et al., 2021).

We have established clear quantitative benchmarks that directly link environmental triggers and microbial responses to pond health. Healthy conditions are consistently characterized by a Vibrio/Total Bacteria ratio below 1% and a Bacillus spp. abundance of more than 20% of the total heterotrophic bacteria (Cui et al., 2022). Conversely, disease risk levels increase significantly when total bacterial abundance exceeds 10^6 CFU/mL, indicating a high organic load, operational temperatures outside the safe range of 26–32°C, or daily salinity fluctuations exceed 5 ppt.

Based on these findings, we provide specific recommendations applicable to pond management:

1. Monitoring

Institutions and farmers need to implement routine monitoring of the Vibrio/Total Bacteria ratio. This ratio should be considered a biological Key Performance Indicator (KPI) and an early warning system for potential disease outbreaks.

2. Environmental Control

Pond management should prioritize strict control of temperature and salinity parameters, ensuring they remain within identified safe thresholds to avoid triggering physiological stress in shrimp and increasing pathogen virulence.

3. Bioremediation/Biocontrol

The use of probiotic Bacillus spp. should be strongly considered (Lorgen-Ritchie et al., 2023). This is no longer just a common practice, but rather a targeted biocontrol strategy, specifically to competitively suppress Vibrio populations and proactively manage microbial ratios to maintain a healthy level.

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