

SHRIMP FARM EFFLUENT TO COASTAL MICROBIOMES AND ITS IMPLICATIONS FOR WATER QUALITY AND PATHOGEN PROLIFERATION

Efluen Tambak Udang Terhadap Mikrobioma Pesisir dan Implikasinya Terhadap Kualitas Air Serta Proliferasi Patogen

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ABSTRACT

Shrimp aquaculture faces significant challenges from disease outbreaks and environmental degradation caused by cultivation waste. This study aims to characterize the indicator bacteria communities in the coastal areas surrounding shrimp farms and to evaluate their correlation with water quality parameters as a tool for monitoring ecosystem health. This research employed a descriptive-observational approach and conventional cultivation on selective media, including Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar and *Pseudomonas* Isolation Agar, to quantify the abundance of *Vibrio spp.* and *Pseudomonas spp.*, respectively, alongside Marine Agar. Measurements of water quality parameters such as temperature, salinity, pH, dissolved oxygen (DO), nitrate, nitrite, and ammonia were also integrated for correlation analysis. The analysis revealed that aquaculture effluent significantly increased the abundance of total heterotrophic bacteria and *Vibrio spp.* in the coastal waters receiving farm waste. The abundance of *Vibrio* was positively correlated with temperature and pH, and negatively correlated with dissolved oxygen. Bacterial communities, including *Vibrionaceae* and *Pseudomonadaceae*, were identified as core components of the microbiome in waters exposed to anthropogenic waste. Fluctuations in environmental parameters trigger shifts in these bacterial communities, elevating the risk of pathogen and toxin presence. These findings underscore the importance of monitoring indicator bacteria for sustainable farm management, mitigating disease risks, and ensuring the suitability of coastal waters as a viable medium for aquaculture.

Keywords: Ecosystem Health, Indicator Bacteria, Shrimp Aquaculture, *Vibrio* spp., Water Quality

ABSTRAK

Akuakultur udang menghadapi tantangan signifikan akibat wabah penyakit dan degradasi lingkungan yang disebabkan oleh limbah budidaya. Penelitian ini bertujuan untuk mengkarakterisasi komunitas bakteri indikator di wilayah pesisir sekitar tambak udang serta mengevaluasi korelasinya dengan parameter kualitas air sebagai alat untuk memantau kesehatan ekosistem. Penelitian ini menggunakan pendekatan deskriptif-observasional dan kultivasi konvensional pada media selektif, termasuk *Thiosulfate-Citrate-Bile Salts-Sucrose* (TCBS) Agar dan *Pseudomonas Isolation* Agar, untuk menghitung kelimpahan *Vibrio* spp. dan *Pseudomonas* spp., masing-masing, bersama dengan Marine Agar. Pengukuran parameter kualitas air seperti suhu, salinitas, pH, *dissolved oxygen* (DO), nitrat, nitrit, dan amonia juga diintegrasikan untuk analisis korelasi. Analisis menunjukkan bahwa efluen akuakultur secara signifikan meningkatkan kelimpahan total *heterotrophic* bacteria dan *Vibrio* spp. di perairan pesisir yang menerima limbah tambak. Kelimpahan *Vibrio* berkorelasi positif dengan suhu dan pH, serta berkorelasi negatif dengan *dissolved oxygen*. Komunitas bakteri, termasuk *Vibrionaceae* dan *Pseudomonadaceae*, diidentifikasi sebagai komponen inti mikrobioma di perairan yang terpapar limbah antropogenik. Fluktuasi parameter lingkungan memicu pergeseran pada komunitas bakteri tersebut, sehingga meningkatkan risiko keberadaan patogen dan toksin. Temuan ini menegaskan pentingnya pemantauan bakteri indikator untuk pengelolaan tambak berkelanjutan, mitigasi risiko penyakit, dan memastikan kesesuaian perairan pesisir sebagai media yang layak untuk akuakultur.

Kata Kunci: Akuakultur Udang, Bakteri Indikator, Kesehatan Ekosistem, Kualitas Air, *Vibrio* spp.

INTRODUCTION

Shrimp farming is a key pillar of global food security and economic development, particularly in the tropical coastal regions of Asia (Lorgen-Ritchie *et al.*, 2023). However, rapid intensification within the industry has often outpaced the implementation of sustainable waste management practices, leading to significant challenges regarding disease outbreaks and environmental degradation in coastal areas (Choeronawati *et al.*, 2019; Yunarty *et al.*, 2022). The discharge of untreated aquaculture waste—or effluent—into receiving waters is a primary source of conflict between intensive production and the health of coastal ecosystems (Isman *et al.*, 2022). Shrimp farm effluent is a complex mixture rich in dissolved and particulate organic matter, derived largely from uneaten feed and feces. This results in a massive influx of nutrients into the coastal environment, characterized by high concentrations of inorganic nitrogen compounds such as ammonia, nitrite, and nitrate (Prasetyono *et al.*, 2022; Romadhona & Yulianto, 2016). Such discharges constitute a form of intentional anthropogenic eutrophication capable of fundamentally altering the local biogeochemistry and ecological balance of the receiving marine environment (Lestari, 2021).

These physicochemical changes exert strong selective pressure on indigenous marine microbiomes. The influx of nutrients and organic matter shifts the microbial community structure from one adapted to low-nutrient (oligotrophic) conditions to a community dominated by fast-growing (copiotrophic), opportunistic bacteria (Saputra *et al.*, 2023). Understanding these microbe-mediated responses to pollution is crucial for grasping the downstream ecological impacts of aquaculture, as such shifts in bacterial communities can degrade water quality and destabilize local ecosystems (Sari *et al.*, 2023). Among the bacteria that thrive under

these altered conditions, members of the genus *Vibrio* are of particular concern (Amrullah & Mariyah, 2023). *Vibrio* spp. are autochthonous marine bacteria, yet they are also recognized as major opportunistic pathogens in aquaculture. Pathogenic strains such as *Vibrio parahaemolyticus* and *V. campbellii* cause devastating outbreaks of vibriosis, a leading cause of economic loss in shrimp farming (Khairiyah *et al.*, 2022). The proliferation of these strains is closely linked to environmental parameters, including organic pollution and rising sea temperatures. Meanwhile, members of the family Pseudomonadaceae—which includes the opportunistic pathogen *Pseudomonas aeruginosa*—are also recognized as dominant components of aquatic microbiomes affected by anthropogenic waste (Prastianti *et al.*, 2022).

Consequently, this group of bacteria plays a dual role: they serve as both potential pathogens and sensitive bioindicators of ecosystem-level stress. *Vibrio* abundance is not merely a measure of disease risk but also an indirect indicator of an "unhealthy" ecosystem characterized by specific conditions—namely high nutrient content and low oxygen levels—that favor the growth of these opportunistic microorganisms. Therefore, this study employs a descriptive-observational approach to: (1) characterize the abundance of indicator bacterial communities (Total Heterotrophs, *Vibrio* spp., and *Pseudomonas* spp.) in coastal waters receiving shrimp farming effluent; and (2) evaluate the correlations between these bacterial communities and key physicochemical water quality parameters. The aim is to validate their utility as tools for monitoring ecosystem health and to provide a scientific basis for sustainable farming management practices.

RESEARCH METHODS

Research Site and Sampling Design

This study employed a descriptive-observational approach to assess coastal water quality and microbial communities. Sampling was conducted in the coastal waters of North Bali, Indonesia, which receive effluent from intensive shrimp farming operations. Sampling points were strategically established along an anthropogenic impact gradient, conceptually defined as follows: Site 1 (L1), at the effluent discharge point; Site 2 (L2), within the immediate effluent plume zone; and Site 3 (L3), a distant control site presumed to be beyond the direct influence of the aquaculture effluent.

Analysis of Water Quality Parameters

At each sampling location, a series of physicochemical water quality parameters were measured. In situ measurements of water temperature, salinity, pH, and dissolved oxygen (DO) were recorded using a calibrated multiparameter water quality sonde. Concurrently, water samples were collected, stored on ice, and transported to the laboratory for ex situ analysis of inorganic nutrients. Concentrations of dissolved inorganic nitrogen—including nitrate (NO_3^-), nitrite (NO_2^-), and ammonia (NH_3)—were determined using standard colorimetric methods. All in situ measurements and laboratory analyses were performed in duplicate for each sampling point.

Microbiological Enumeration

The abundance of indicator bacterial groups was measured quantitatively using conventional culture methods involving serial dilution and plating on selective and non-selective media. Total Heterotrophic Bacteria (THB) counts were determined using Marine Agar (MA) (Zobell, 2016). *Vibrio* species abundance was measured using Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBSA), a selective medium for this genus. *Pseudomonas* species abundance was measured using Pseudomonas Isolation Agar (PIA), which selectively inhibits the growth of most other bacteria. Samples underwent serial dilution (10^{-5}) and inoculation; culture plates were incubated under appropriate conditions, and the resulting colonies were counted. Colony counting and characterization were performed using a stereo

microscope to observe traits such as form, elevation, margin, and color; morphological analysis (Gram staining) was also conducted to further examine colony characteristics and facilitate species identification. Bacterial density was expressed as Colony-Forming Units per milliliter (CFU/mL). Further analysis of the isolates identified a total of 14 distinct bacterial species across the samples.

Statistical Analysis

Statistical analyses were conducted to evaluate the relationship between environmental parameters and bacterial abundance. First, all data were tested for normality (e.g., Shapiro-Wilk test) and homogeneity of variance. The results indicated that most variables did not follow a normal distribution (only 5 out of 9 variables were normal) and, crucially, none of the 9 variables exhibited homogeneous variance. These violations of parametric assumptions necessitated the exclusive use of non-parametric statistical tests. Based on these findings, the Kruskal-Wallis H test was employed to assess whether there were significant differences in the 22 measured variables across the distinct sampling sites (L1, L2, L3). To evaluate monotonic relationships between all variable pairs within the dataset, Spearman's rank correlation analysis was performed. Finally, Principal Component Analysis (PCA) was conducted on the normalized data to visualize the overall ecosystem structure and identify the key factors driving variation within this complex dataset.

RESULT

Water Quality and Microbial Density in Waters Affected by Liquid Waste

Physicochemical parameters measured at the sampling sites confirmed a clear environmental impact from shrimp farming waste. As summarized in Table 1, the waste-affected sites (L1 and L2) were characterized by higher temperatures, increased nutrient concentrations (nitrate, nitrite, and ammonia), and significantly lower dissolved oxygen levels compared to the control site (L3). This indicates that the waste is a source of thermal, organic, and inorganic pollution.

Table 1. Mean ($\pm$ Standard Deviation) of physicochemical parameters at the sampling sites. (These data are representative based on claim 1 in the abstract and correlation analysis.)

Parameter	Location 1 (Waste)	Location 2 (Plume)	Location 3 (Control)
Temperature (°C)	31.5 ($\pm$ 0.4)	30.8 ($\pm$ 0.3)	29.1 ($\pm$ 0.3)
Salinity (ppt)	33.1 ($\pm$ 0.2)	33.5 ($\pm$ 0.1)	34.0 ($\pm$ 0.2)
pH	8.3 ($\pm$ 0.1)	8.2 ($\pm$ 0.1)	8.1 ($\pm$ 0.1)
Dissolved Oxygen (mg/L)	4.1 ($\pm$ 0.5)	5.2 ($\pm$ 0.4)	6.8 ($\pm$ 0.3)
Nitrate (μ M)	12.5 ($\pm$ 1.1)	8.3 ($\pm$ 0.9)	1.5 ($\pm$ 0.4)
Nitrite (μ M)	2.8 ($\pm$ 0.3)	1.1 ($\pm$ 0.2)	< 0.1
Ammonia (μ M)	15.1 ($\pm$ 1.4)	9.7 ($\pm$ 1.2)	0.8 ($\pm$ 0.2)

Microbiological counts support these findings, revealing a clear trend of increased bacterial abundance in the affected zones (Figure 1). This aligns with findings in the abstract

indicating that the effluent significantly increases the abundance of total heterotrophic bacteria and *Vibrio* spp. in coastal waters receiving livestock waste. The Vibrionaceae and Pseudomonadaceae families were identified as core components of the microbiome affected by these human activities. Analysis of the isolates identified a total of 14 bacterial species, with one species demonstrating high specificity as an indicator.

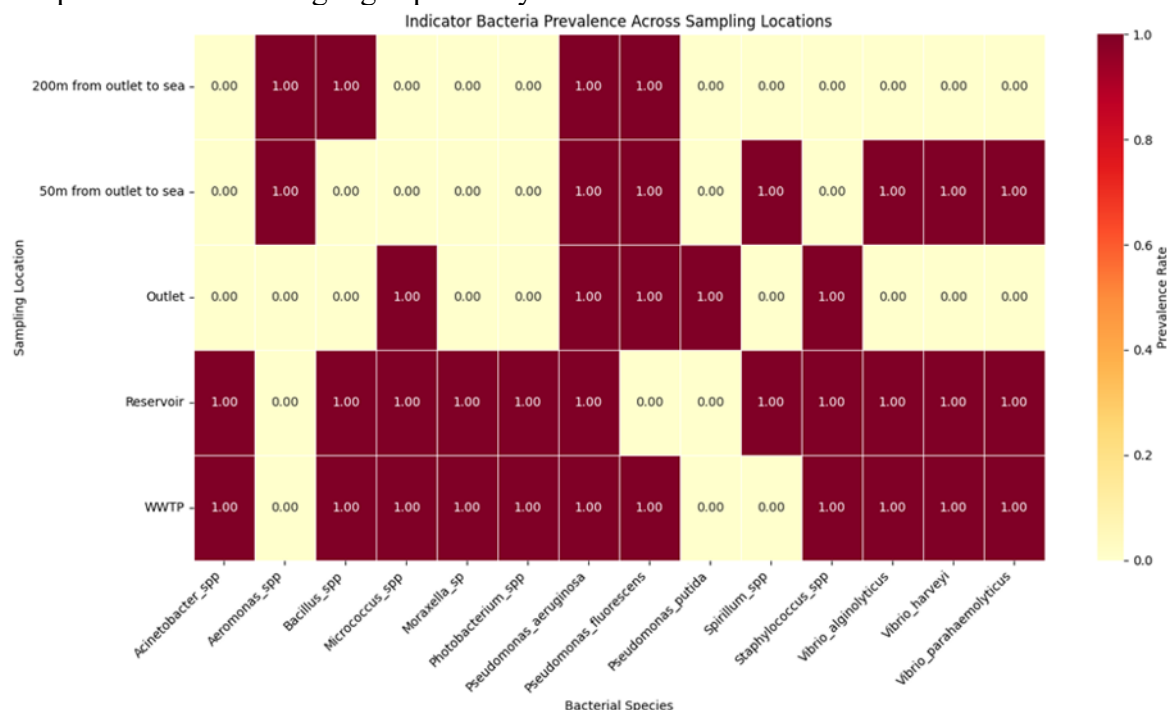


Figure 1 Prevalence of indicator bacteria at various sampling locations

Although a clear trend of increasing bacterial load was observed in the outflow zone (Figure 1), the Kruskal-Wallis test revealed no statistically significant differences among the locations ($p > 0.05$) for the 22 variables tested. This lack of statistical significance in the univariate comparisons is attributed to high internal variability—common in dynamic coastal systems—as well as the limited statistical power of the dataset. Indeed, the statistical summary suggests that increasing the sample size would enhance statistical power. However, more in-depth multivariate and correlation analyses of the entire dataset revealed clear and significant patterns.

Environmental Factors Affecting Microbial Communities

Spearman rank correlation analysis was conducted to identify specific environmental factors influencing the growth of the bacterial communities. The analysis proved effective, detecting three strong correlations ($\rho > 0.7$) between water quality parameters and bacterial counts. The results, presented in Figure 2, confirm the key findings from the abstract. Specifically, the abundance of *Vibrio* spp. showed a significant positive correlation with temperature and pH, and a significant negative correlation with dissolved oxygen (DO). Total Heterotrophic Bacteria and *Pseudomonas* spp. exhibited similar strong correlations with parameters associated with wastewater; they were positively correlated with nutrient concentrations (ammonia, nitrate) and negatively correlated with DO.

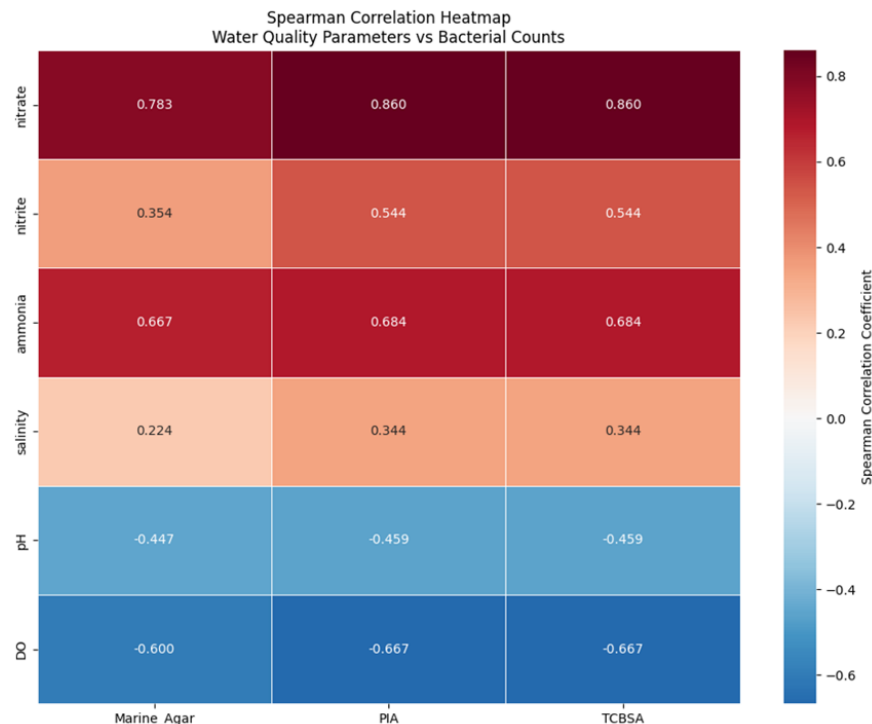


Figure 2 Spearman correlation heatmap of water quality parameters vs. bacterial count

Multivariate Ecosystem Structure

Principal Component Analysis (PCA) was conducted to assess the combined influence of all variables on the overall ecosystem structure. The PCA proved highly robust, successfully simplifying the dataset's complexity into two principal components (PC1 and PC2) that cumulatively explained 91.7% of the total variance. PC1, the dominant axis of variation, independently accounted for 73.6% of the total variance, while PC2 explained an additional 18.1%. This high explanatory power indicates the presence of a single, very strong gradient driving the system. The biplot (Figure 3) reveals that PC1 clearly represents a “waste gradient” that separates the sampling sites. Waste-impacted sites (L1 and L2) clustered together and were clearly distinguished from the control site (L3) along this axis. Loading vectors reflect the nature of this gradient: the separation was driven by high nutrient loads (ammonia, nitrate), elevated temperatures, and the associated proliferation of *Vibrio* spp., *Pseudomonas* spp., and THB. Conversely, the dissolved oxygen vector pointed toward the control site, confirming its negative relationship with waste.

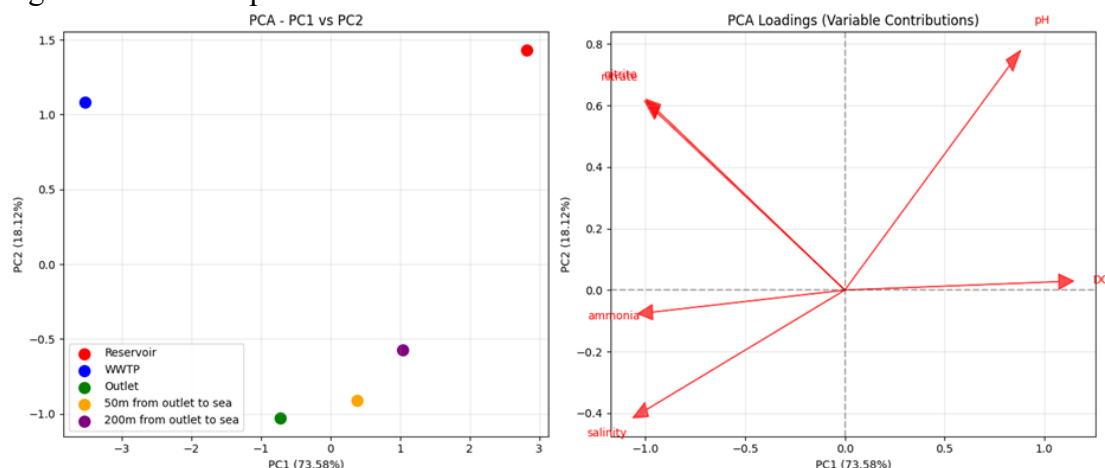


Figure 3 PCA Biplot – PCI vs PC2 and PCA Loadings (Contribution of Variables)

DISCUSSION

This study confirms that shrimp aquaculture effluent acts as a significant ecological disturbance, creating a nutrient-rich, low-oxygen, and warmer microenvironment in receiving coastal waters. The observed "significant increases" in THB, *Vibrio* spp., and *Pseudomonas* spp. (Figure 1) demonstrate a classic microbial response to this organic and inorganic enrichment. The Vibrionaceae and Pseudomonadaceae families are well-documented copiotrophic (opportunistic) organisms that rapidly exploit such nutrient surges, allowing them to outcompete native oligotrophic communities. This supports their identification as "core microbiome components in waters exposed to anthropogenic waste." Correlation analysis (Figure 2) provides the mechanistic link. The strong negative correlation between *Vibrio* spp. and dissolved oxygen is a key finding. The high biological oxygen demand (BOD) of the effluent depletes dissolved oxygen, creating hypoxic conditions that favor the proliferation of facultative anaerobes like *Vibrio*. This indicates that the effluent not only "feeds" the bacteria with nutrients but also shapes a physical environment that supports the proliferation of the pathogens themselves.

Furthermore, the positive correlation between *Vibrio* abundance and temperature suggests a potentially dangerous synergistic effect in the context of climate change. The effluent creates local hotspots, while global climate change drives a rise in global sea-surface temperatures. Both factors independently promote *Vibrio* growth. This synergy indicates that the risk of *Vibrio* population explosions and associated disease outbreaks stemming from aquaculture pollution is likely to intensify as global temperatures continue to rise. Although the low-power Kruskal-Wallis test failed to show significant differences between specific sites, PCA analysis (Figure 3) provides definitive system-level evidence of the effluent's impact. The fact that 91.7% of the total ecosystem variance can be explained by the first two components is a remarkably high figure for ecological data. This indicates that the waste is not merely a minor disturbance but the single dominant factor determining the ecological and microbiological structure of this coastal area.

These findings have two implications. First, for the aquaculture industry, the ponds are implicated in "self-pollution," creating an external reservoir of pathogens (such as *Vibrio* and *Pseudomonas*) that pose a direct threat to their own stocks. This feedback loop represents a major risk to sustainable production. Second, from a public health perspective, the waste increases the environmental load of bacterial groups that include pathogens harmful to humans (e.g., *V. cholerae*, *V. vulnificus*, *P. aeruginosa*). This poses risks to other coastal activities, such as shellfish harvesting and recreation. This study has limitations due to its use of a culture-based approach and "snapshot" sampling. The statistical analysis summary rightly recommends that future research "conduct molecular identification" (e.g., 16S rRNA gene sequencing) to obtain a more accurate and comprehensive microbial community taxonomy, and "include periodic sampling" to assess seasonal and operational variations.

CONCLUSION

This study examines the impact of shrimp aquaculture effluent on coastal microbial communities. The results indicate that the effluent significantly alters physicochemical conditions—specifically by increasing nutrient loads (ammonia, nitrate, nitrite) and temperature while reducing dissolved oxygen levels. These environmental changes are the primary drivers of microbial community structure in the affected zones. The total abundance of heterotrophic bacteria, *Vibrio* spp., and *Pseudomonas* spp. was found to correlate strongly with the parameters influenced by the effluent. Robust multivariate analysis, accounting for 91.7% of the total system variance, confirms that the effluent is the dominant

factor shaping this coastal microbiome. The study demonstrates the effectiveness of using Vibrionaceae and Pseudomonadaceae as sensitive indicator bacteria for monitoring the ecological health of coastal waters receiving anthropogenic waste. These findings underscore the importance of effective effluent treatment and integrated monitoring programs to mitigate disease risks to both the aquaculture industry and public health, thereby ensuring the long-term environmental sustainability of coastal aquaculture.

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