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Water quality and microbial interactions in aerofloc systems for whiteleg shrimp aquaculture in round ponds in east Java, Indonesia

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ABSTRACT

Intensive shrimp aquaculture in tropical regions is increasingly challenged by recurrent bacterial disease outbreaks, particularly vibriosis and acute hepatopancreatic necrosis disease (AHPND), alongside growing pressure to reduce antibiotic use while maintaining high productivity. In this context, production systems capable of stabilizing water quality and microbial balance are critical for sustainable intensification. East Java represents a typical tropical intensive shrimp-farming environment characterized by high stocking densities and elevated disease risks. This study investigated microbial and water quality dynamics in an aerofloc-based intensive white shrimp (*Litopenaeus vannamei*) culture system using a round-tank configuration under tropical conditions. The aerofloc system, which integrates continuous aeration and enhanced water circulation, was evaluated as an alternative to conventional biofloc, recirculating aquaculture systems, and traditional pond systems in terms of its capacity to support microbial balance under intensive culture conditions. Shrimp were cultured at different stocking densities (11–44 individuals m^{-2}), and key water quality parameters, organic matter proxies, microbial populations (total bacteria and presumptive Vibrio), and plankton communities were monitored. The results indicated stable water quality conditions and a predominance of non-Vibrio bacterial populations across treatments, with microbial and planktonic patterns closely associated with organic matter dynamics. These findings highlight the relevance of aerofloc-based systems in managing microbial balance under high-intensity tropical shrimp culture, with important implications for disease risk mitigation, reduced reliance on antibiotics, and the sustainable intensification of shrimp aquaculture.

Keywords: aerofloc system, water quality, microbial interaction, plankton dynamics, *Vibrio,* whiteleg shrimp sustainable aquaculture.

INTRODUCTION

Intensive white shrimp (*Litopenaeus vannamei*) aquaculture has undergone rapid intensification to meet increasing global demand, particularly in tropical regions (Jing et al., 2025). However, this intensification has been accompanied by recurrent bacterial disease outbreaks, including vibriosis and acute hepatopancreatic necrosis disease (AHPND), which are strongly associated with unstable water quality and microbial imbalance (Nazarudin, et al., 2025). At the same time, growing regulatory and market pressure to reduce antibiotic use has intensified the need for production systems capable of maintaining microbial stability under high stocking densities. These challenges highlight the scientific urgency of understanding water quality–microbial interactions in intensive shrimp culture systems (Thompson et al., 2022; Kurniawan et al., 2021).

Improvements in technology, especially for water quality and microbial balance, were adopted to reduce the impact caused by such problems (Rizky et al., 2022). One of these was the aerofloc system, which combined principles of biofloc with enhanced aeration using a venturi pump. The aerofloc system represents a modification of the conventional biofloc technology, where the integration of venturi-based aeration enhances water circulation and oxygenation beyond what is typically achieved in standard biofloc systems. Aerofloc-based culture systems have emerged as a modification of biofloc approaches by integrating continuous aeration, enhanced water circulation, and controlled organic matter dynamics within confined production units. Beyond technical differences, aerofloc systems represent a distinct microbial engineering environment, where intensified oxygen transfer and mixing influence organic matter mineralization, microbial competition, and plankton development. These mechanistic features distinguish aerofloc systems from conventional biofloc, recirculating aquaculture systems, and traditional ponds, particularly in their potential to stabilize microbial communities under intensive tropical culture conditions. The way it works allows for the water to stay in motion and oxygenated, aiding in the formation of microbial flocs that help break down organics and recycle nutrients (Ramesh et al., 2024). This directional water flow creates a more homogeneous distribution of dissolved oxygen and suspended particles, while also minimizing the accumulation of organic waste in blind spots. In this study, the aerofloc system was applied in round ponds, which are increasingly favored in intensive aquaculture due to their hydrodynamic advantages. The circular design promotes even water movement, reduces dead zones, and facilitates efficient sludge removal through a centrally located drain.

In intensive shrimp culture, a limited number of water quality parameters play a disproportionate role in shaping microbial dynamics and disease risk. The success of aerofloc system heavily relies on water chemistry interactions with microbial populations. Organic matter accumulation governs heterotrophic bacterial growth, while alkalinity and pH regulate nitrification processes that influence ammonia toxicity and microbial competition (Kurniawan et al., 2021). Optimal plankton and bacteria behaviors depend on pH, ammonia (NH_3), ammonium (NH_4^+), salinity, nitrite (NO_2^-), phosphate (PO_4^{3-}), total hardness, calcium and magnesium levels, bicarbonate, alkalinity, and total organic matter. These processes are closely linked to the proliferation of opportunistic bacteria such as *Vibrio* spp., which respond rapidly to shifts in organic loading and water quality instability. Understanding the interaction among organic matter, key water quality parameters, and microbial populations is therefore essential for evaluating system performance under intensive conditions (Li et al., 2022).

Despite growing adoption of aerofloc systems, most previous studies have focused on production performance or isolated water quality indicators, with limited attention to the integrated dynamics among microbial populations, organic matter, and plankton communities under varying stocking intensities. In particular, empirical field-based evidence linking microbial balance – rather than single-parameter thresholds – to water quality dynamics in aerofloc systems remains scarce. This gap limits the mechanistic understanding required to design environmentally robust intensive shrimp production systems.

This study aimed to analyze the correlations between water quality parameters, microbial populations, and plankton communities in an aerofloc-based intensive white shrimp culture system operated in round tanks under tropical conditions. East Java was selected as a representative tropical intensive farming context characterized by high stocking densities and elevated disease pressure, rather than as a site-specific case study. By adopting a correlation-based analytical approach across multiple stocking densities, this study seeks to generate transferable insights into microbial–water quality coupling that are relevant to intensive shrimp culture systems beyond local monitoring applications. The findings are expected to help refine aerofloc technology and support more sustainable aquaculture practices, in line with global goals such as the sustainable development goals (SDGs).

MATERIALS AND METHODS

Round pond and aerofloc

The whiteleg shrimp culture pond was circular, with a diameter of 10 meters and a wall height of 1.2 meters. The pond frame was constructed using wire mesh and lined with a high-density polyethylene (HDPE) membrane to ensure water retention and durability (Kurniawan et al., 2025). It was equipped with a central drain and a drainage system located at the middle of the pond, featuring a bottom slope of 2–3% toward the center to facilitate sludge collectionThe stocking density in this study was 350 individuals m^{-3}, with the culture period running from DOC 0 to 44. The observation window for water quality, plankton, and microbial variables was conducted at DOC 11, 19, 25, 30, 37, and 44. This observation range was selected to represent the early to mid-culture phase of biofloc/aerofloc-based farming, during which heterotrophic microbial communities and initial nitrification processes typically begin to develop, and fluctuations in oxygen and inorganic nitrogen are commonly observed.

The seedstock consisted of PL8 shrimp with an initial mean body weight of approximately 0.001 g per individual. Circular pond circulation was maintained using venturi units that combined air injection and induced shear flow to promote floc suspension and direct settleable solids toward the central drain. The performance of this aeration–hydrodynamic regime was evaluated through scheduled observations at the selected DOCs to examine its association with temporal trends in pH, alkalinity, inorganic nitrogen forms (NH_3/NH_4^+), and the ratio of presumptive Vibrio to total heterotrophic bacteria, rather than to establish causal control or system optimization.

A venturi pump was installed along the pond wall with clockwise rotation on both sides to enhance aeration and water circulation. The venturi system works by creating a pressure differential that draws atmospheric air into the water stream, generating fine air bubbles that increase dissolved oxygen and promote continuous mixing (Ramesh et al., 2024). This mechanism also supports the formation and suspension of bioflocs, which consist of aggregated microorganisms, organic matter, and detritus that function as a natural water treatment and supplemental feed source (Khanjani et al. 2022). The pump design and flow pattern were optimized to ensure even distribution of oxygen and minimize dead zones within the pond. This study utilized five separate ponds as replicates, and all measurements were performed independently in triplicate to ensure data reliability and producibility.

Measurement water quality parameters

Water quality parameters, including pH, ammonium (NH_4^+), unionized ammonia (NH_3), phosphate (PO_4^{3-}), alkalinity, calcium hardness, total hardness, bicarbonate (HCO_3^-), and total organic matter (TOM). Total organic matter in this study represents oxidizable organic matter quantified using the potassium dichromate oxidation method, analogous to a COD-based approach, and expressed as mg L^{-1}. While this method does not distinguish specific organic carbon fractions (e.g., TOC), it provides a practical proxy for organic load in biofloc-based aquaculture systems. (Yuan et al., 2025; Zhou et al., 2021)

NH_4^+ and PO_4^{3-} were quantified using modified titrimetric procedures adapted from Standard Methods, as commonly applied in routine pond water assessments.The measurements were conducted immediately after sampling to ensure accuracy, with pH determined using a calibrated digital pH meter, while alkalinity, hardness, and bicarbonate were quantified by acid-base and EDTA complexometric titrations. Ammonium and phosphate concentrations were analyzed using acid-base titration after appropriate sample preparation, and TOM was determined by potassium dichromate oxidation followed by titration with ferrous ammonium sulfate (Habibie, 2022). All analyses were performed in triplicate using analytical grade reagents, and results were expressed in parts per million (ppm).

Microbial analysis

Plankton samples (250 mL) were collected from each pond on the sampling dates and immediately fixed with 1% Lugol's iodine (Pimentel et al., 2025). Plankton identification and enumeration were performed under a compound microscope (Olympus CX21, Tokyo, Japan) using a Sedgewick-Rafter counting chamber (De Paiva-Maia et al., 2013). The number of plankton organisms was counted in multiple random fields of view and averaged to calculate the density All counts were performed in triplicate and expressed as (cell/L).

Water samples for bacteria analysis (250 mL) were aseptically collected from each pond and serially diluted (10^{-1}–10^{-6}) in sterile 0.85% saline solution (Imazumi et al., 2025). To determine total heterotrophic bacterial abundance, 0.1 mL of each dilution was spread onto Marine Agar (Difco), whereas *Vibrio* spp. was enumerated using Thiosulfate Citrate Bile Salts Sucrose (TCBS; Oxoid) agar (Yeoh et al., 2021). Colonies identified on TCBS agar represent presumptive *Vibrio* spp. and do not confirm pathogenic species or virulence. All plates were incubated at 28–30 °C for 24–72 h, depending on the medium. After incubation, plates showing 30–300 colonies were selected for counting. Yellow and green colonies on TCBS agar were recorded as presumptive *Vibrio* species, while colonies on Marine Agar represented total heterotrophic bacteria (Fu et al., 2023). The results were expressed as colony-forming units per milliliter (CFU/mL).

Data processing and analysis

The data were tabulated and analyzed using Microsoft Excel. Descriptive statistics were applied to summarize the mean and standard deviation (SD) of each parameter. Correlation analyses were conducted to evaluate the relationships among water quality parameters, plankton abundance, and bacterial counts using Pearson and Spearman correlation coefficients. Temporal trends are presented descriptively. (Kim et al., 2022). Multivariate analyses, including principal component analysis (PCA) (Imaizumi et al, 2022), were performed to visualize patterns and interactions among abiotic and biotic parameters throughout the culture period. Graphical visualizations were generated using OriginPro 2023 to support data interpretation and ensure reproducibility of analytical outcomes. Correlation and PCA analyses were applied as exploratory tools to describe temporal patterns and co-variation among parameters, rather than for formal hypothesis testing. Given the limited number of sampling points and the repeated-measures structure, correlation coefficients are interpreted cautiously and without strong causal inference.

RESULTS AND DISCUSSION

Water quality parameters

The average values of water quality parameters were compared to the optimum ranges commonly recommended for whiteleg shrimp aquaculture. Based on the analysis described in Table 1, most parameters were found to be within the acceptable limits, while one parameter exceeded the optimum range. The pH value was 7.88, which is within the recommended range of 7.5 to 8.5. This indicates that the water condition was stable and suitable for shrimp culture. NH_4^+ was measured at 0.57 ppm, and NH_3 at 0.03 ppm. Both values are within safe limits, suggesting that Based on NH_4^+ and NH_3 measurements, nitrogenous compounds remained within observed safety ranges; however, the absence of nitrite and nitrate measurements limits full nitrogen pathway assessment..

PO_4^{3-} was found at 0.37 ppm, which is also within the optimum range. This shows that nutrient accumulation was not excessive. Alkalinity averaged 178.68 ppm, and bicarbonate (HCO_3^-) was 176.63 ppm, both of which are within the ideal range for buffering capacity and pH stability. Calcium hardness was 1118.97 ppm, which is considered good for supporting shrimp molting and shell formation. Total organic matter (TOM) was 85.3 ppm, still below the upper limit of 100 ppm, indicating that organic waste was not yet critical.

However, total hardness was recorded at 6127.2 ppm, which is significantly above the optimum range of 500 to 1500 ppm. This suggests that the water contained a high concentration of dissolved minerals, possibly from calcium, magnesium, or other ions (Boyd and Tucker, 1998). Although high hardness may not be directly harmful, it can affect osmoregulation and may require further monitoring. In conclusion, most water quality parameters were within the recommended levels, showing that the aerofloc system was able to maintain a stable environment (Tong et al., 2025). Only total hardness exceeded the optimum range, which should be observed carefully in future management to avoid possible negative effects on shrimp health.

Although most water quality parameters remained within the recommended range, total hardness consistently exceeded the optimum threshold, reaching values above 6000 ppm. This elevated level of hardness may be attributed to several factors, including the mineral composition of the water source, the use of mineral-rich commercial feeds, or the accumulation of calcium and magnesium ions through the continuous breakdown of organic matter in the biofloc matrix. While high total hardness is not immediately toxic, it can influence the osmoregulatory processes of shrimp, potentially leading to physiological stress, impaired molting, or reduced growth performance if not properly managed. Therefore, monitoring and managing mineral concentrations (Ca and Mg) should be prioritized in future applications of aerofloc systems to ensure optimal shrimp health and system stability.

Table 1. Periodic average values of water quality parameters from day of culture (DOC) 11 to 44

Day of culture	pH	NH_4^+ (ppm)	NH_3 (ppm)	PO_4^{3-} (ppm)	Alkalinity (ppm)	Ca hardness (ppm)	Total hardness (ppm)	HCO_3 (ppm)	TOM (ppm)
Optimum value	7.5–8.3	≤ 0.25	≤ 0.060	0.5–1	120–200	≥ 900	> 4000	-	< 90
11	8.20	0.25	0.020	0.00	187	1125	6039	187	77
19	7.88	0.65	0.030	0.36	184	1105	6204	184	88
25	7.78	0.40	0.014	0.32	173	1155	6019	173	71
30	7.88	0.45	0.019	0.40	175	1138	5989	175	82
37	7.76	0.40	0.022	0.60	174	1068	6253	174	98
44	7.80	1.30	0.048	0.52	166	1123	6258	166	95

Correlation analysis between water quality parameters

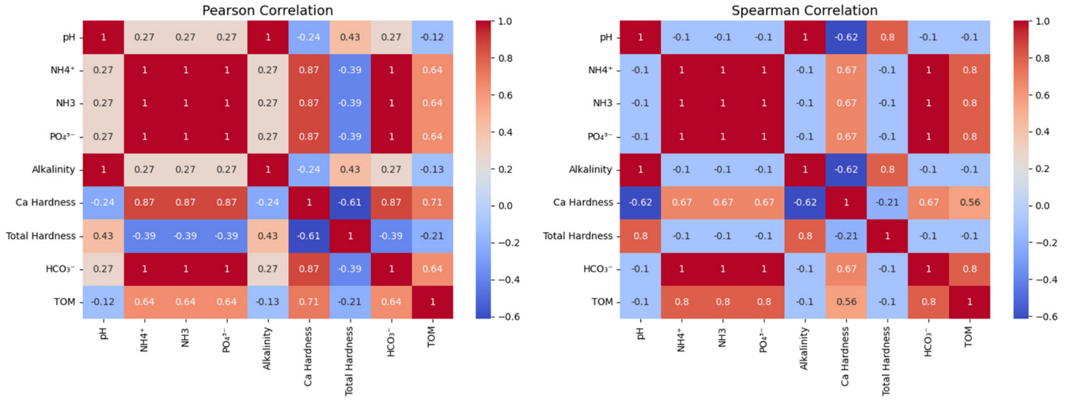
Correlation analysis was conducted to evaluate the relationships between water quality parameters observed during the culture period of whiteleg shrimp in aerofloc systems. Two statistical approaches were applied: Pearson correlation, which measures linear relationships between variables, and Spearman correlation, which assesses the consistency of ranking or order among values. The results whows in the Figure 1.

The Pearson correlation analysis showed strong relationships between several parameters. The concentration of ammonium (NH_4^+) had a perfect correlation with free ammonia (NH_3), indicating that changes in NH_4^+ levels directly affect NH_3 levels. This result is consistent with the chemical equilibrium between these two nitrogen forms. Ammonium also showed high positive correlations with phosphate (PO_4^{3-}) and bicarbonate (HCO_3^-), with values of 0.87 and 1.00, respectively. These findings suggest that the accumulation of dissolved compounds tends to occur simultaneously, possibly due to chemical processes in the water such as ion interactions or solubilization. The correlation between pH and NH_4^+ ($r = 0.87$) indicates that higher pH levels are associated with increased ammonium concentrations, which is important because pH influences the chemical form of nitrogen in water.

On the other hand, a moderate negative correlation was found between calcium (Ca) hardness and total hardness, with a value of -0.61. This result is unexpected because calcium is usually a major contributor to total hardness. The negative value may suggest that total hardness is more influenced by other ions, such as magnesium, or that there are variations in ionic composition among ponds. A similar negative correlation was observed between Ca hardness and HCO_3^- ($r = -0.39$), indicating that increases in calcium do not always correspond with increases in bicarbonate, possibly due to precipitation or interactions with alkalinity.

The Spearman correlation analysis revealed similar patterns, but focused more on the consistency of value rankings over time. Ammonium again showed perfect correlations with NH_3, PO_4^{3-}, alkalinity, HCO_3^-, and total organic matter (Pramudia et al, 2022a), all with values of 1.00. This means that although the absolute values may differ, the order of increase or decrease among these parameters remained consistent throughout the culture period. A strong negative correlation was found between pH and Ca hardness ($r = -0.62$), suggesting that higher pH levels are associated with lower calcium hardness. This may be related to the possibility of calcium precipitation at higher pH. Moderate negative correlations were also observed between Ca hardness and both total hardness and HCO_3^-, indicating that fluctuations in calcium levels do not always align with changes in total hardness or bicarbonate content (Pramudia et al., 2022b).

Overall, both correlation methods show that water quality parameters in aerofloc systems interact in complex ways. Positive correlations among dissolved compounds such as NH_4^+, NH_3, PO_4^{3-}, and HCO_3^- suggest that these substances tend to accumulate together (Moehammad et al., 2025). Meanwhile, negative correlations involving Ca hardness highlight the need for further monitoring of ionic composition in the system. Understanding these relationships is important for decision-making in water quality management, including pH control, monitoring of dissolved compounds, and hardness regulation. With a data-based approach,



Pearson Correlation

	pH	NH4⁺	NH3	PO₄³⁻	Alkalinity	Ca Hardness	Total Hardness	HCO₃⁻	TOM
pH	1	0.27	0.27	0.27	1	-0.24	0.43	0.27	-0.12
NH4⁺	0.27	1	1	1	0.27	0.87	-0.39	1	0.64
NH3	0.27	1	1	1	0.27	0.87	-0.39	1	0.64
PO₄³⁻	0.27	1	1	1	0.27	0.87	-0.39	1	0.64
Alkalinity	1	0.27	0.27	0.27	1	-0.24	0.43	0.27	-0.13
Ca Hardness	-0.24	0.87	0.87	0.87	-0.24	1	-0.61	0.87	0.71
Total Hardness	0.43	-0.39	-0.39	-0.39	0.43	-0.61	1	-0.39	-0.21
HCO₃⁻	0.27	1	1	1	0.27	0.87	-0.39	1	0.64
TOM	-0.12	0.64	0.64	0.64	-0.13	0.71	-0.21	0.64	1

Spearman Correlation

	pH	NH4⁺	NH3	PO₄³⁻	Alkalinity	Ca Hardness	Total Hardness	HCO₃⁻	TOM
pH	1	-0.1	-0.1	-0.1	1	-0.62	0.8	-0.1	-0.1
NH4⁺	-0.1	1	1	1	-0.1	0.67	-0.1	1	0.8
NH3	-0.1	1	1	1	-0.1	0.67	-0.1	1	0.8
PO₄³⁻	-0.1	1	1	1	-0.1	0.67	-0.1	1	0.8
Alkalinity	1	-0.1	-0.1	-0.1	1	-0.62	0.8	-0.1	-0.1
Ca Hardness	-0.62	0.67	0.67	0.67	-0.62	1	-0.21	0.67	0.56
Total Hardness	0.8	-0.1	-0.1	-0.1	0.8	-0.21	1	-0.1	-0.1
HCO₃⁻	-0.1	1	1	1	-0.1	0.67	-0.1	1	0.8
TOM	-0.1	0.8	0.8	0.8	-0.1	0.56	-0.1	0.8	1

Figure 1. Analysis of the Pearson correlation and Spearman correlation between water quality parameters

environmental stability in shrimp culture systems can be maintained more effectively, supporting sustainable production outcomes.

The Pearson and Spearman correlation analyses not only revealed statistical relationships among water quality parameters but also offer practical insights for system management. For instance, strong positive correlations between ammonium, phosphate, bicarbonate, and total organic matter suggest that these parameters tend to accumulate simultaneously, likely due to shared sources such as feed input or microbial decomposition. Recognizing these patterns can help farmers anticipate and manage nutrient build-up by adjusting feeding regimes, aeration intensity, or water exchange rates. Similarly, the observed negative correlation between calcium hardness and total hardness is noteworthy. While calcium is a major contributor to total hardness, the inverse relationship observed in this study may indicate a higher proportion of other divalent ions (likely Mg) dominating the hardness profile. However, since magnesium was not directly measured in this study, further investigation is needed to confirm this hypothesis.

Total plankton density and plankton composition

The total plankton density was monitored from day 11 to day 44 in five ponds. The results in the Figure 2, show that plankton populations changed over time, with different patterns in each pond. At the beginning of the culture period (day 11), the plankton density was relatively low, around or below 700,000 cells per liter (Ce/L) in all ponds. This level is considered acceptable and within the optimum range for early-stage shrimp culture.

From day 19 to day 25, the density remained low or slightly decreased. However, starting from day 30, there was a noticeable increase in plankton density. The highest peak was observed between day 30 and day 37, where some ponds reached values close to or above 2,000,000 Ce/L. This level is considered high and may indicate excessive plankton growth, which can affect water quality and oxygen levels if not managed properly. After day 37, the plankton density started to decrease again in all ponds. By day 44, the values dropped to a more moderate level, although still higher than the initial stage.

In general, the plankton density was within the optimum range during the early and late stages of culture. The peak between day 30 and 37 was above the recommended level and should be monitored carefully. High plankton density can lead to problems such as oxygen depletion, especially during the night, and may also increase organic matter in the water (Pimentel et al., 2025). Therefore, it is important to control plankton growth through proper aeration, water exchange, and feeding management. Keeping plankton density within the optimum range helps maintain water stability and supports healthy shrimp growth.

Plankton composition was analyzed from day 11 to day 44 of the culture period (Table 2). The percentage of each plankton group was compared to the optimum values commonly recommended for shrimp aquaculture (Chen et al., 2019; Ma et al., 2024). Based on the results, most plankton groups were within the recommended range, except for one group. Chlorophyta showed a high average percentage of 88.74%, which is well above the minimum optimum value of 50%. This result is considered good because Chlorophyta is known to support water quality and is beneficial

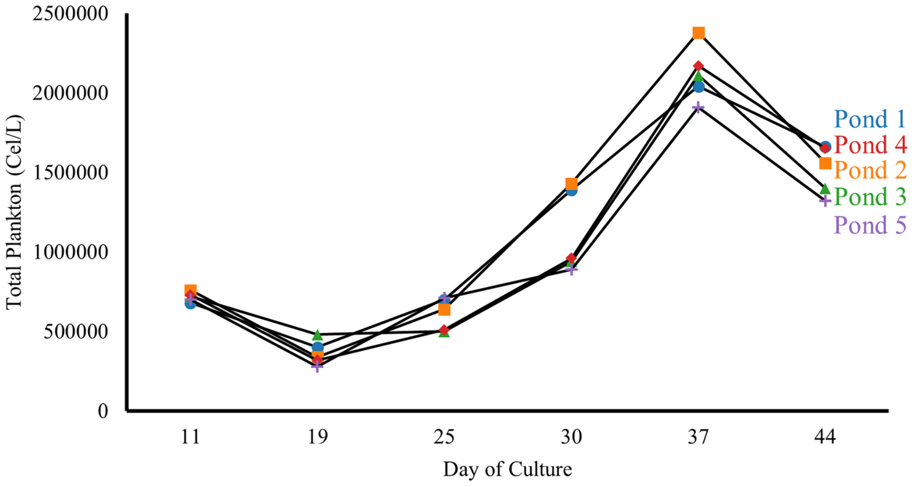


Figure 2. Graphics total plankton density

in shrimp ponds. Cyanophyta had an average of 3.9%, which is below the maximum limit of 5%. This means that the presence of Cyanophyta was still under control and did not reach harmful levels.

Cryptophyta, Eugienophyta, Pyrrophyta, and Protozoa all showed very low average percentages, ranging from 0.04% to 1.04%. These values are all within the optimum limits, indicating that these groups were present in small amounts and did not dominate the plankton community. However, Chrysophyta (Diatoms) had an average percentage of 5.69%, which is far below the optimum value of more than 50%. This result suggests that diatoms were not dominant in the system, even though they are usually considered beneficial for shrimp culture due to their nutritional value and role in stabilizing water quality. In summary, the plankton community was mostly dominated by Chlorophyta, which is favorable. Most other groups were present in low and acceptable amounts. The only group that did not meet the optimum condition was Chrysophyta, which may indicate a need to improve conditions that support diatom growth, such as light availability or nutrient balance.

The dominance of Chlorophyta throughout the culture period is a positive indicator of water quality stability and ecological balance in the aerofloc system. Chlorophyta are known to contribute to oxygen production, nutrient uptake, and serve as a natural food source for shrimp, especially in early life stages. Their prevalence suggests that the system supported favorable conditions for beneficial phytoplankton growth. In contrast, the consistently low proportion of Chrysophyta (diatoms), which are also considered beneficial due to their high nutritional value and role in stabilizing water quality, may indicate suboptimal conditions for their proliferation. This could be related to limited light penetration, insufficient silicate availability, or competition with other phytoplankton groups.

The sharp increase in total plankton density observed between DOC 30 and 37 is likely

Table 2. Plankton composition from day 11 to day 44 of the culture period

Day of culture	% Chlorophyta	% Cyanophyta	% Cryptophyta	% Chrysophyta (diatom)	% Eugienophyta	% Pryrrophyta	% Protozoa
Optimum value	> 50%	< 5%	< 10%	> 50%	< 5%	< 5%	< 5%
11	92.188	0.294	0	6.656	0.000	0.862	0
19	93.304	2.978	0	1.416	0.000	0.500	0
25	85.782	0.884	0	10.054	0.000	2.606	0.674
30	88.002	1.39	0	8.498	0.000	1.482	0.632
37	79.426	16.572	0.406	3.444	0.105	0.280	0.186
44	93.716	1.312	0.286	4.068	0.153	0.496	0

associated with increased feed input and subsequent nutrient accumulation in the system. As organic matter and dissolved nutrients build up, they can stimulate phytoplankton blooms, particularly in systems with high microbial activity like aerofloc. While moderate plankton growth is beneficial, excessive blooms may lead to oxygen fluctuations and increased organic loading, highlighting the need for careful monitoring and adaptive management during mid to late culture phases.

Relationship between abiotic parameters and plankton abundance

The correlation analysis (Figure 3) shows that several water quality parameters have significant relationships with total plankton abundance in the aerofloc system. Among them, TOM has the strongest positive correlation (r = 0.80), followed by Total Hardness (r = 0.75), Calcium Hardness (r = 0.60), and Alkalinity (r = 0.55). These results suggest that the availability of organic matter and minerals plays an important role in supporting plankton growth, both as nutrient sources and as stabilizers of the water environment. Higher levels of TOM may provide essential carbon and nutrients for heterotrophic and mixotrophic plankton, thereby stimulating microbial decomposition and nutrient regeneration processes (De Morais et al., 2020). Similarly, hardness and alkalinity reflect the buffering capacity and mineral content of the water, which are crucial for maintaining stable pH and ionic balance that favor plankton proliferation (Farid et al., 2022). Adequate calcium and carbonate availability can also enhance algal cell wall formation and photosynthetic efficiency, contributing to a more productive plankton community

In contrast, nitrogen-related parameters such as NH_4^+ and NH_3 show moderate negative correlations (r = -0.45 and r = -0.40). This may indicate that high concentrations of nitrogen compounds can have toxic effects on plankton. A negative correlation with pH (r = -0.25) also suggests that higher acidity levels may not be favorable for plankton development.

The strong positive correlation between TOM and plankton density indicates that the aerofloc system effectively functions as a nutrient source for phytoplankton. In this system, bioflocs formed from the accumulation of organic matter and microbial activity can release nutrients such as nitrogen and phosphorus, which are readily available for plankton growth. This reinforces the role of biofloc not only as a waste treatment agent but also as a secondary nutrient source that supports primary productivity in the pond.

Conversely, the negative correlation between plankton density and the concentrations of NH_4^+ and unionized NH_3 suggests the potential toxic effects of nitrogen compounds on phytoplankton. High levels of inorganic nitrogen, particularly in the form of toxic NH_3, can inhibit photosynthesis, damage cell structures, and reduce the abundance and diversity of plankton communities. Therefore, controlling nitrogen accumulation is essential to maintain microbial ecosystem balance and support the sustainability of the aerofloc system.

Multivariate patterns of plankton dynamics based on abiotic parameters

The PCA (Figure 4) provides a more complete view of how abiotic parameters influence

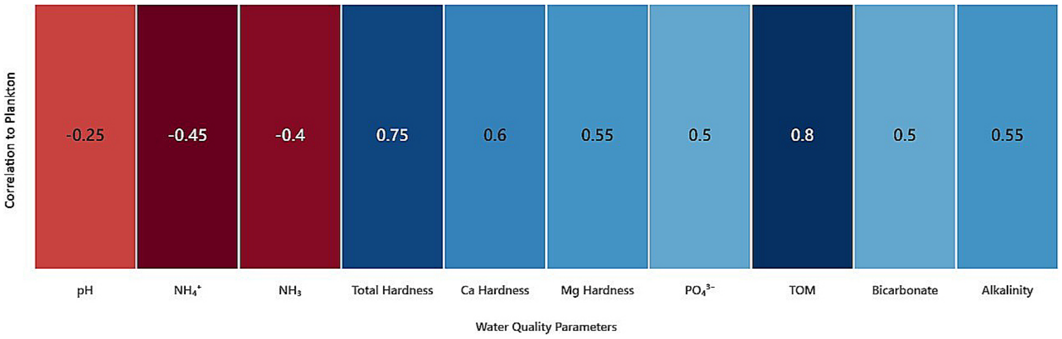


Figure 3. Heatmap of the correlation between abiotic water quality parameters and total plankton abundance in the aerofloc system for whiteleg shrimp aquaculture. This visualization uses the RdBu (Red-Blue) color scale, where red indicates a positive correlation and blue indicates a negative correlation. Pearson correlation coefficients are displayed within each cell, with the color scale ranging from -1 (perfect negative correlation) to +1 (perfect positive correlation)

plankton abundance during the culture period (DOC 11–44). Two main components (PC1 and PC2) explain 62% of the total data variation. The distribution of DOC points shows how environmental conditions change over time.

The direction and length of vectors such as TOM, Total Hardness, and PO_4^{3-} on PC1 indicate that these parameters contribute strongly to early system variability, likely related to the initial phase of plankton growth. During this phase, the system experiences rapid microbial activity and nutrient mineralization, where organic matter decomposition and phosphorus availability play key roles in supporting the initial bloom of phytoplankton and heterotrophic microorganisms (Borges et al., 2024). High concentrations of TOM provide an abundant carbon source, while adequate calcium and hardness levels stabilize floc formation and facilitate nutrient adsorption, enhancing the growth environment for plankton. PO_4^{3-} described by Lemonnier et al., (2016), as a limiting nutrient in aquatic systems, is critical for energy transfer and nucleic acid synthesis, which supports cell division and biomass accumulation during the early exponential phase of plankton growth (Wetzel, 2001).

Meanwhile, parameters such as NH_4^+, NH_3, and pH are more dominant on PC2, reflecting their influence during the later phase of culture, when nitrogen waste tends to accumulate. As feeding rates and organic loading increase, microbial decomposition and ammonification processes lead to elevated concentrations of ammonia and ammonium (Moehammad et al., 2025). Excessive nitrogen compounds, particularly unionized NH_3, can become toxic to plankton and disrupt photosynthetic processes, leading to shifts in species composition and reduced primary productivity (Häder et al., 2020). The higher loading of pH on PC2 may also indicate that nitrogen accumulation and CO_2 fluctuations from microbial respiration influence water acid base balance in the later phase of the system.

This pattern shows that plankton abundance in the aerofloc system is highly influenced by organic and mineral dynamics in the early phase, and by nitrogen accumulation in the later phase. Therefore, adaptive water quality management based on the culture stage is essential to maintain optimal plankton productivity and ecological stability. In practice, this may involve regulating organic inputs and aeration intensity during the early phase to promote beneficial biofloc formation, and enhancing nitrification or water exchange during the later phase to mitigate nitrogen toxicity (Liu et al., 2025). A stage-based management approach can thus sustain balanced microbial-plankton interactions, which are crucial for maintaining water quality and supporting in intensive aquaculture systems.

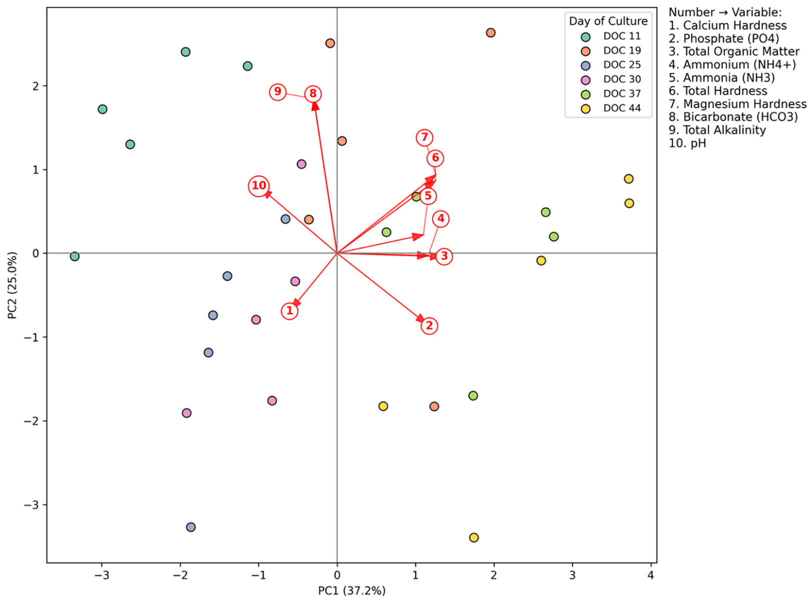


Figure 4. Principal component analysis (PCA) biplot of water quality parameters in aerofloc systems for whiteleg shrimp culture. PC1 (37.2%) and PC2 (25.0%) explain 62.2% of total variance. Arrows represent variable loadings; numbers correspond to variables listed in the legend panel. Colors indicate day of culture (DOC)

The findings from the PCA provide valuable practical insights for phase-based management in aerofloc shrimp culture systems. During the early culture phase, when parameters such as TOM, PO_4^{3-}, and water hardness contribute most to system variability, management efforts should focus on optimizing organic inputs and aeration intensity to promote stable biofloc formation and plankton development. In contrast, during the later culture phase management strategies should shift toward controlling nitrogenous waste through enhanced nitrification, reduced feeding rates, or increased water exchange. This phase-specific management approach can help maintain microbial-plankton balance, prevent water quality deterioration, and support shrimp health and growth.

Dynamics of vibrio and total bacteria in the aerofloc system

Table 3 shows the results of bacterial density measurements in the aerofloc system. The TVC was found to be higher than the recommended optimum level (<1.1 × 10^3 CFU/mL) at all observation points, ranging from 1.274 to 2.178 CFU/mL. However, the ratio of TVC to total bacterial count (TBC) remained below the critical threshold of 10%, with values between 4.73% and 8.70%. This means that *Vibrio* did not dominate the bacterial community.

Specifically, yellow *Vibrio* showed fluctuations, reaching its highest level at DOC 25 (2.140 CFU/mL), then decreasing to 468 CFU/mL at DOC 44. In contrast, green *Vibrio* increased sharply at DOC 44 (1.668 CFU/mL), replacing the previous dominant group. Black *Vibrio* was almost undetectable throughout the cycle, appearing only slightly at DOC 19.

The linear regression analysis between total *Vibrio* and total bacteria (Figure 5) shows a very strong relationship, with the equation $y = 13.45x$ and $R^2 = 0.9285$. This means that when *Vibrio* increases, the total bacterial population also increases. For every 1 CFU/mL increase in *Vibrio*, there is an estimated increase of 13.45 CFU/mL in total bacteria.

It should be noted that *Vibrio* counts reported in this study are based on presumptive identification using TCBS agar and do not distinguish pathogenic from non-pathogenic strains.

Although the total *Vibrio* count exceeded the optimum level, the TVC/TBC ratio stayed below the danger limit. This suggests that the heterotrophic microbial community in the biofloc system was able to suppress *Vibrio* growth through nutrient competition, antibacterial compound production, and complex microbial interactions. According to (Samsuri et al., 2025), high bacterial density in biofloc systems acts as a biological buffer, preventing opportunistic pathogens from dominating.

The shift in *Vibrio* dominance from yellow to green *Vibrio* at the end of the culture period (DOC 44) is an important ecological event. This change may be caused by water quality changes, organic matter accumulation, and microbial strain interactions. explained that changes in carbon and nitrogen availability in biofloc systems can alter microbial balance, giving competitive advantage to certain strains. Green *Vibrio* dominance should be monitored carefully, as this group is often linked to latent pathogens that may cause subclinical disease when shrimp are under stress (Zhang et al., 2024).The strong positive correlation between total *Vibrio* and total bacteria ($R^2 = 0.9285$) shows that *Vibrio* growth is not independent, but influenced by the overall microbial dynamics. This supports the idea that *Vibrio* grows along with total bacteria when organic substrates

Table 3. Bacterial density measurements in the aerofloc system

Day of culture	Yellow *Vibrio*	Green *Vibrio*	Black *Vibrio*	Total *Vibrio* (TVC)	Total bacteria (TBC)	Ratio TVC/TBC
Optimum value	< 1 × 10^3 CFU/ml	< 1 × 10^2 CFU/ml	-	< 1.1 × 10^3 CFU/ml	> 10 × TVC	≤10%
11	1476	70	0	1546	24820	6.48%
19	858	414	2	1274	31740	4.73%
25	2140	38	0	2178	27620	7.97%
30	1638	168	0	1806	20780	8.03%
37	1652	136	0	1788	19320	8.70%
44	468	1668	0	2140	25800	8.10%

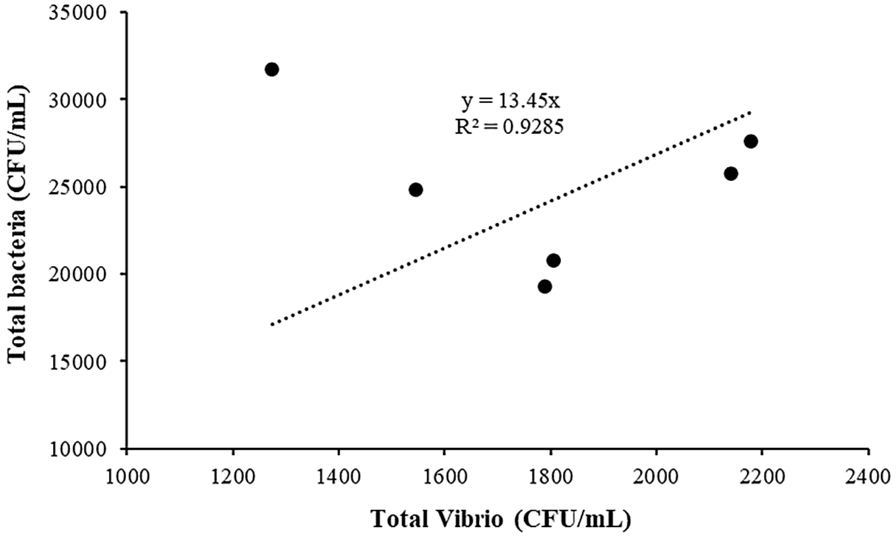


Figure 5. Linear regression analysis between total Vibrio and total bacteria

increase (Soto-Marfelino et al., 2024). However, this also brings a risk: if non-pathogenic bacteria suddenly decrease (for example, due to aeration failure or water quality changes), *Vibrio* may dominate and increase disease risk.

From a management perspective, these findings highlight the importance of microbial community control strategies. These include adjusting the C:N ratio to support non-pathogenic heterotrophic bacteria, applying probiotics (such as *Bacillus* spp. and Actinobacteria) to compete with Vibrio, and maintaining dissolved oxygen to keep flocs suspended and active (Kurniawan et al., 2018). Regular monitoring of the TVC/TBC ratio can also serve as an early warning indicator to detect potential disease risks before clinical symptoms appear. Therefore, the aerofloc system not only improves nutrient efficiency and natural feed production, but also acts as an ecological biocontrol mechanism that strengthens shrimp resistance against pathogens.

The finding that the ratio of TVC to total bacterial count (TBC) remained below the critical threshold of 10%, despite elevated TVC levels, is a significant indicator of the biological control function provided by the heterotrophic microbial community in the aerofloc system. This suggests that the microbial consortium formed within the biofloc effectively suppresses *Vibrio* proliferation through nutrient competition, production of antimicrobial compounds, and complex microbial interactions.

Moreover, the observed shift in dominance from yellow *Vibrio* during the early culture phase to green *Vibrio* in the later phase reflects microbial community dynamics influenced by changes in water quality and shrimp growth stages. The late culture phase is typically characterized by increased accumulation of organic matter and nitrogenous compounds, which may create environmental conditions that favor the proliferation of certain *Vibrio* groups, including potentially pathogenic strains.

To maintain the dominance of non-pathogenic microbes and prevent *Vibrio* outbreaks, adaptive management strategies are essential. Recommended approaches include adjusting the carbon-to-nitrogen (C:N) ratio to support the growth of competitive heterotrophic bacteria, applying probiotics such as Bacillus spp. or Actinobacteria to inhibit pathogenic strains, and enhancing aeration to keep bioflocs suspended and maintain optimal dissolved oxygen levels. Regular monitoring of the TVC/TBC ratio can also serve as an early warning indicator for potential disease risks, allowing for timely intervention before clinical symptoms appear.

This study did not include several routine pond diagnostic parameters such as temperature, salinity, dissolved oxygen, nitrite, nitrate, total suspended solids, floc volume, or chlorophyll-a. Therefore, the interpretation of water quality is limited to selected chemical indicators and their relationships with microbial and plankton dynamics. Nevertheless, these parameters are sufficient to support the correlative analysis presented in this study. Future studies integrating physical, chemical, and biological parameters are required to provide a comprehensive evaluation of aerofloc system performance.

CONCLUSIONS

This study confirms that aerofloc systems implemented in round ponds under tropical conditions can effectively maintain water quality and support beneficial microbial interactions for sustainable whiteleg shrimp aquaculture. Most water parameters remained within optimal ranges, except for total hardness, which exceeded recommended levels and may affect shrimp osmoregulation, highlighting the need for mineral monitoring. The dominance of Chlorophyta indicated favorable ecological conditions, while the low abundance of diatoms suggested potential limitations in light or silicate availability. Correlation and PCA analyses revealed that organic matter and mineral content positively influenced plankton growth in early culture phases, whereas nitrogen accumulation negatively affected plankton in later stages, emphasizing the importance of phase-specific water quality management. Despite elevated *Vibrio* counts, the TVC/TBC ratio remained below the critical threshold, demonstrating effective microbial control by heterotrophic communities. The observed shift from yellow to green *Vibrio* further underscores the dynamic nature of microbial succession during culture. These findings highlight the need for adaptive management strategies, including C:N ratio adjustment, probiotic application, and optimized aeration, to maintain microbial balance and prevent disease outbreaks.

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