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Analysis of Water Quality Correlation with the Immune Response of *Litopenaeus vannamei* in Probolinggo, East Java

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***Abstract*— Whiteleg shrimp (*Litopenaeus vannamei*) is a major aquaculture commodity in Indonesia, valued for its resilience, rapid growth, and economic potential. However, water quality remains a critical factor influencing shrimp health, particularly their immune response. This study aimed to examine the correlation between water quality parameters and the immune responses of *L. vannamei* cultivated in Probolinggo, East Java. A descriptive quantitative method was employed, using simple random sampling across three pond sites. Water parameters measured included temperature, pH, dissolved oxygen (DO), salinity, ammonia, nitrate, total suspended solids (TSS), and total dissolved solids (TDS). Immune response indicators such as total hemocyte count (THC), differential hemocyte count (DHC), and phagocytic activity were analyzed. Canonical Correspondence Analysis (CCA) was used to assess correlations. The results revealed significant relationships between water quality and shrimp immune parameters, highlighting the importance of proper environmental management in shrimp farming. These findings provide insights for improving productivity and disease resistance in sustainable aquaculture systems.**

Keywords—*Litopenaeus vannamei*, water quality, immune response, aquaculture, Canonical Correspondence Analysis.

I. Introduction

Vannamei shrimp (*Litopenaeus vannamei*) is a high-value aquaculture commodity with strong development potential in Indonesia. Its key advantages include resilience to disease, adaptability to varying water quality conditions, rapid growth, and suitability for high stocking densities, making it a preferred species in intensive aquaculture systems [1]. However, the success of vannamei farming is closely tied to environmental conditions, particularly water quality. Deviation from optimal water can increase shrimp are more vulnerable to disease outbreaks, experience reduced growth and performance, and may suffer mass mortalities, leading to significant economic losses.

The intensification of shrimp farming often results in fluctuations in water quality, which have a significant impact on shrimp health and immune function. [2]. Parameters such as temperature, pH, salinity, dissolved oxygen (DO), and ammonia are critical for maintaining physiological balance. Ammonia, especially in its unionized form (NH_3), is toxic to shrimp and may cause gill damage and reduced respiration efficiency [3]. Nitrate accumulation, the final product of the nitrification process, can also contribute to physiological stress when exceeding shrimp tolerance thresholds. Other indicators like Total Suspended Solids (TSS) and Total Dissolved Solids (TDS) are important for evaluating water clarity and osmoregulatory balance. Excessive TSS can impair gill function, while TDS reflects the total concentration of dissolved ions, including salts and inorganics, in the culture water [4]. Imbalances in these parameters can result in chronic stress, ultimately weakening the shrimp's immune response.

Shrimp immunity is primarily based on non-specific, innate mechanisms that rapidly respond to foreign substances [5]. As in other crustaceans, *L. vannamei* relies solely on innate immunity, which plays a vital role in recognizing and eliminating pathogens [6]. The shrimp immune system primarily involves hemocytes and phagocytic activity to neutralize microbial threats and other invading agents. Hemocytes serve as the central blood cells responsible for various immune functions such as phagocytosis, clotting, and the secretion of antimicrobial compounds. These cells are classified into granulocytes, hyalinocytes, and semi-granulocytes, each with distinct roles in immune defense [7].

Phagocytosis, the process by which hemocytes ingest and destroy pathogens, is a cornerstone of the shrimp's immune defense [8]. This mechanism is essential for maintaining immune homeostasis, especially in aquaculture environments where shrimp are exposed to varying stress levels and microbial challenges [9].

Despite its importance, limited research has examined the direct correlation between water quality and specific immune responses in *L. vannamei*, particularly under actual pond conditions in Indonesia. Probolinggo, one of the leading shrimp-producing regions, lacks comprehensive studies addressing this

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relationship. This study provides an integrated analysis of the correlation between water quality and immune responses in *L. vannamei*, employing Canonical Correspondence Analysis (CCA) to explore complex interactions between environmental parameters and physiological indicators. By combining environmental monitoring with immunological assessment, this research aims to support sustainable shrimp aquaculture practices and improve health management strategies in East Java.

II. METHOD

A. Research Time and Location

This research was conducted from August 2024 to February 2025 at the Vannamei Shrimp Pond in Probolinggo, East Java. The research was carried out both in-situ and ex-situ. The in-situ research was conducted directly at the research site, while the ex-situ research was performed in the laboratory of the Faculty of Fisheries and Marine Sciences, Brawijaya University. In-situ research involved real time sampling and observation of shrimp health, immune responses, and water quality parameters, whereas ex-situ analysis focused on laboratory-based assays to evaluate various chemical parameters critical to the shrimp's health and environment.

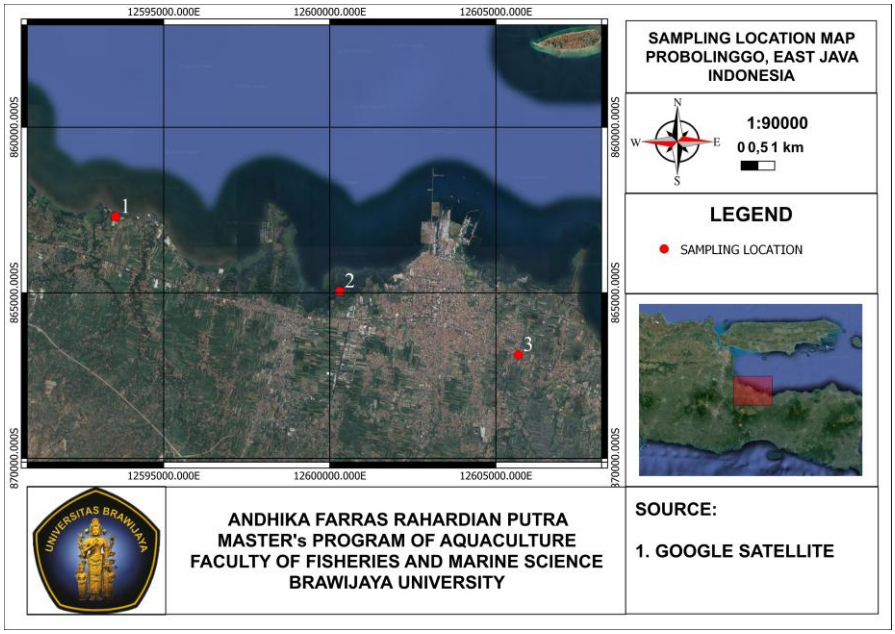


Figure 1. Sampling Location Map in Probolinggo, East Java, Indonesia

B. Method Research

This research employed a quantitative descriptive analysis. Quantitative descriptive research is a methodological approach that integrates descriptive techniques and quantitative analysis to explain phenomena. It aims to portray events, symptoms, or occurrences in a factual, systematic, and precise way. Quantitative data refers to data that can be measured or counted using numerical values, such as age, weight, height, and similar variables [10].

C. Sampling Method

Simple random sampling technique was used in this research. Simple random sampling is a method used to randomly select samples from a population, where each member of the population has an equal chance of being chosen [11]. The sampling was conducted in three shrimp pond compartments, each with three replications at two week intervals (14 days). Water samples were collected using a plankton net with a mesh size of 25 µm, and shrimp samples were collected using an ancho sampling tool [12]. All collected water and shrimp samples were immediately transferred into sample bottles and stored in a cool box.

D. Sample Analysis

Hemocyte counts were performed by drawing shrimp hemolymph using a 0.1 mL insulin syringe, pre-filled with 10% sodium citrate solution as an anticoagulant. Hemolymph was collected from the ventral side of the haemocoel, specifically between the cuticle of the second abdominal segment [13]. The anticoagulant was added at a 1:1 ratio with the hemocyte sample. Next, 0.1 mL of Trypan Blue was added into an Eppendorf tube, and one drop of the hemocyte suspension was placed on a hemocytometer, then covered with a cover glass. Observations were made under a microscope at 100x or 400x magnification. The Total Hemocyte Count (THC) was calculated using the following formula:

$$THC = Haemocyte\, x\, Dilution\, Factor\, x\, 5\, x 10\text{^}4$$

$$Dilution\, Factor = \frac{Total\ Haemocyte + Diluent}{Total\ Haemocyte}$$

Differential Hemocyte Count (DHC) was also observed with Olympus Microscope under 100x or 400x magnification. The differential Hemocyte Count (DHC) was calculated as follows:

$$Differential\ Harmocyte\ Count = C\%$$

$$Hyalin = \frac{Total\ Hyalin\ Cells}{Total\ Haemocyte}$$

$$Semigranulocyte = \frac{Total\ Semigranulocyte\ Cells}{Total\ Haemocyte}$$

$$Granulocyte = \frac{Total\ Granulocyte\ Cells}{Total\ Haemocyte}$$

The procedure for phagocytic activity testing began with collecting 0.1 mL of hemolymph using a sterile syringe, then mixing it with 10% sodium citrate anticoagulant in a 1:1 ratio in a sterile Eppendorf tube. Then, 0.1 mL of yeast cell suspension was added to assess phagocytic activity. The yeast cell suspension was prepared by dissolving 0.5 grams of dry yeast in 10 mL of NaCl solution, followed by centrifugation for 10 minutes at 1000 rpm to wash the cells. The hemocyte and yeast mixture was homogenized and incubated at room temperature for 20 minutes. After incubation, smear preparations were made on glass slides, air dried, and stained with Giemsa. Observations were made using a light microscope to count cells undergoing phagocytosis [14]. Phagocytic activity was calculated using the following formula:

$$Phagocytic\ Activity = \frac{Phagocytic\ Cells}{Total\ Haemocyte} x100$$

Water quality parameters at the vannamei shrimp pond aquaculture in Probolinggo, East Java, were measured to assess both physical and chemical parameters. Physical parameters included temperature, Total Suspended Solids (TSS), and Total Dissolved Solids (TDS), while chemical parameters included pH, dissolved oxygen (DO), salinity, ammonia, and nitrate levels. Water quality measurements were conducted using both in-situ (on-site) and ex-situ (laboratory-based) methods. The in-situ parameters measured included temperature, dissolved oxygen, salinity, pH, and total dissolved solid. Ex-situ measurements were conducted for ammonia, nitrate, and total suspended solid.

E. Data Analysis

Canonical Correspondence Analysis (CCA) is a multivariate statistical method used to identify and evaluate the relationships between response variables and environmental variables [15]. CCA is widely applied in ecological studies to analyze how environmental factors influence the distribution or biological characteristics of organisms. The method correlates variation in biological data with environmental factors through a canonical correlation-based statistical approach, allowing for the identification of complex patterns both visually and quantitatively [16]. In this research, CCA was used to analyze the relationship between immune parameters (hemocytes and phagocytic activity) and water quality variables. This approach provides a comprehensive framework for understanding the interactions between shrimp health and their environment, facilitating the identification of key environmental drivers influencing immune system function.

III. Results and Discussion

A. Total Hemocyte Count

Based on the observations of the total hemocyte count (THC) at every site and each pond, the following data were obtained:

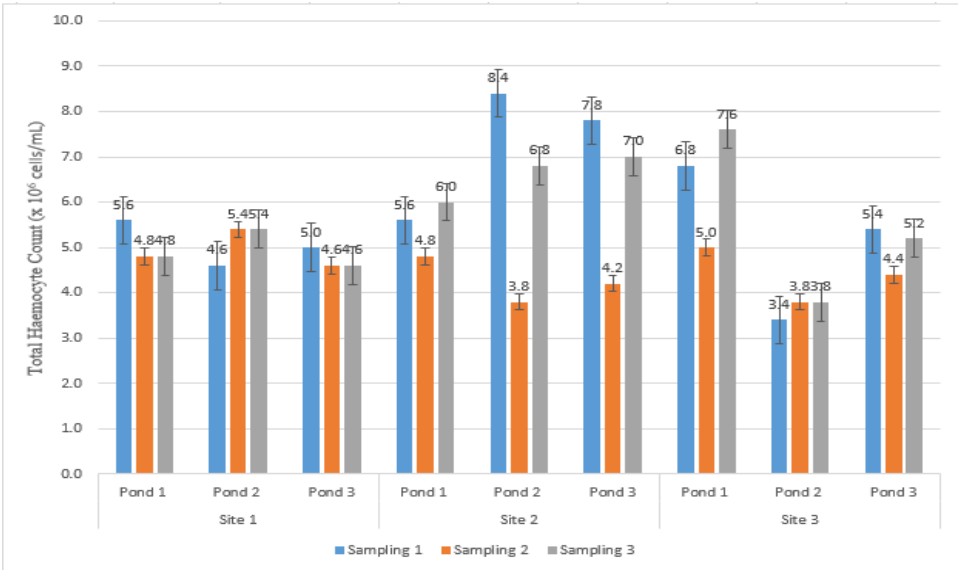


Figure 2. Total Hemocyte Count in *Litopenaeus vannamei*

Total Hemocyte Count (THC) in this research was used to observe the immune response of *Litopenaeus vannamei*. Hemocytes serve as a quantitative parameter in measuring stress responses in organisms. Hemocytes play a critical role in the immune system through phagocytosis, encapsulation, and nodular aggregation [17]. THC consists of three main types of cells: hyalinocytes, semi-granulocytes, and granulocytes. The results of the Total Hemocyte Count measurement are presented in Figure 2.

The data obtained from the total hemocyte count testing ranged from 3.4 × 10^6 to 8.4 × 10^6 cells/mL. The

THC values found at site 1 ranged from 4.6×10^6 cells/mL to 5.6×10^6 cells/mL, at site 2 from 3.8×10^6 cells/mL to 8.4×10^6 cells/mL, and at site 3 from 3.4×10^6 cells/mL to 7.6×10^6 cells/mL. The highest THC was observed at Site 2, Pond 1 in the first sampling period, with a value of 8.4×10^6 cells/mL, and the lowest THC value was at site 3, pond 2 in the first sampling, with a value of 3.4×10^6 cells/mL.

The variations in THC values may be influenced by several factors, such as fluctuations in the aquatic environment or the presence of pathogens [18]. An increase in hemocyte count is associated with environmental factors, where shrimp living in less controlled conditions show higher hemocyte activity, while shrimp in normal environments exhibit normal hemocyte counts. Hemocytes are a key defense mechanism in *Litopenaeus vannamei*, responsible for phagocytosis, nodulation, and encapsulation. A high hemocyte count indicates good shrimp health [19].

The hemocyte count of healthy shrimp is reported to be approximately $1.80 \pm 9.28 \times 10^6$ cells/mL [20]. In this study, the Total Hemocyte Count (THC) of *Litopenaeus vannamei* ranged from 3.4×10^6 to 8.4×10^6 cells/mL. These values are within or above the expected physiological range, suggesting that the shrimp were generally in a healthy condition and that the treatments applied did not induce significant physiological stress. The variation in THC across sampling sites reflects differences in environmental conditions and shrimp immune responses. Notably, the highest THC values were recorded at Site 2, which also had elevated levels of ammonia and temperature. This may indicate an immune activation response triggered by environmental stressors. Similar findings have been reported by Yeh et al. [20] and Sahoo et al. [19], who observed increased hemocyte proliferation in shrimp under stressful or pathogen-exposed conditions. These results underscore the essential role of hemocytes in the non-specific immune defense of shrimp and their sensitivity to changes in water quality.

B. Differential Hemocyte Count

The calculation of the Differential Hemocyte Count (DHC) is divided into three categories: hyalinocytes, semi-granulocytes, and granulocytes. The differences between these categories lie in their function and the stages of pathogen or foreign material entry on the shrimp's body. The following is the Differential Hemocyte Count obtained at the Probolinggo, East Java research site:

TABLE 1.
DIFFERENTIAL HEMOCYTE COUNT IN *LITOPENAEUS VANNAMEI*

Site	Pond	Types of Cells	Differential Hemocyte Count (%)		
			Sampling 1	Sampling 2	Sampling 3
1	1	Hyalinocytes	46.43	45.83	46.34
		Semi-granulocytes	35.71	33.33	34.15
		Granulocytes	17.86	20.83	19.51
	2	Hyalinocytes	47.83	48.15	44.19
		Semi-granulocytes	30.43	33.33	34.88
		Granulocytes	21.74	18.52	20.93
	3	Hyalinocytes	48.00	50.00	48.57
		Semi-granulocytes	32.00	33.33	31.43
		Granulocytes	20.00	16.67	20.00
2	1	Hyalinocytes	53.57	41.67	46.67
		Semi-granulocytes	28.57	33.33	30.00
		Granulocytes	17.86	25.00	23.33
	2	Hyalinocytes	47.62	52.63	44.12
		Semi-granulocytes	33.33	26.32	32.35
		Granulocytes	19.05	21.05	23.53
	3	Hyalinocytes	46.15	47.62	48.57
		Semi-granulocytes	30.77	33.33	31.43
		Granulocytes	23.08	19.05	20.00
3	1	Hyalinocytes	47.06	48.00	44.74
		Semi-granulocytes	32.35	32.00	34.21
		Granulocytes	20.59	20.00	21.05
	2	Hyalinocytes	47.06	47.37	47.37
		Semi-granulocytes	35.29	31.58	31.58
		Granulocytes	17.65	21.05	21.05
	3	Hyalinocytes	44.44	47.83	48.00
		Semi-granulocytes	33.33	30.43	32.00
		Granulocytes	22.22	21.74	20.00

Based on the DHC data from the research conducted in Probolinggo, the hyalinocyte cells ranged from 41.67% to 53.57%, semi-granulocyte cells ranged from 26.32% to 35.71%, and granulocyte cells ranged from 16.67% to 25%. The highest percentage of hyalinocytes was found at site 2, pond 1, in the first sampling, at 53.57%, and the lowest was also at site 2, pond 1 in the first sampling, at 41.67%. The highest percentage of semi-granulocytes was at site 1, pond 1 in the first sampling, at 35.71%, while the lowest was at site 2, pond 2, in the second sampling, at 26.32%. The highest percentage of granulocytes was at site 2, pond 1, in the second sampling, at 25%, and the lowest was at site 1, pond 3, in the second sampling, at 16.67%.

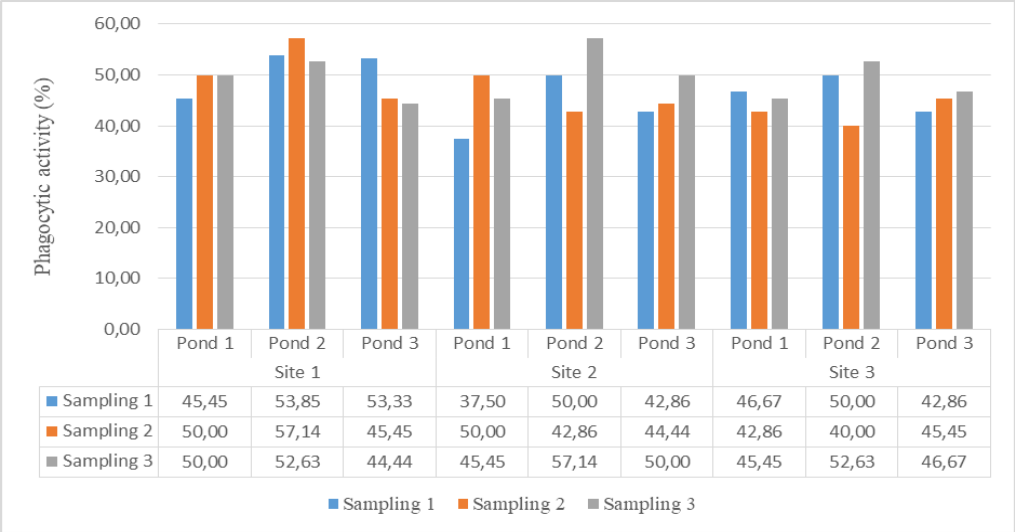
Hyalinocytes cells are phagocytic cells that act early in response to foreign invasion in the body. These cells have a higher nucleus to cytoplasm ratio and fewer granules in the cytoplasm [21]. Semi-granulocytes are

the matured form or next stage of hyalinocytes. These cells are characterized by the presence of granules in their cytoplasm. Semi-granulocytes play a role in encapsulation processes and contribute minimally to phagocytosis, which is primarily carried out by hyalinocytes. Encapsulation is a defense reaction against larger particles that cannot be phagocytosed by hemocytes [22]. Granulocytes are the largest cells, with a smaller nucleus and surrounded by granules. These granular cells, along with semi-granulocytes, are responsible for cytotoxic activity and the production and release of prophenoloxidase [23].

The Differential Hemocyte Count (DHC) in this study revealed hyalinocytes as the most dominant cell type, ranging from 41.67% to 53.57%. Semi-granulocytes ranged from 26.32% to 35.71%, and granulocytes from 16.67% to 25%. The high proportion of hyalinocytes across all ponds reflects their essential role as early responders in the shrimp immune system, particularly through phagocytosis. Semi-granulocytes and granulocytes, which are involved in encapsulation and cytotoxic responses, showed stable but lower proportions. The balance among these cell types suggests that the shrimp were in a steady immune state, with no indications of acute infection. These results align with the findings of Rodriguez and Le Moullac [24], who reported that hemocyte distribution can reflect immunological balance and readiness in crustaceans.

C. Phagocytic Activity

The observation results of phagocytic activity from each sampling location and its repetitions are shown in Figure 3.



	Site 1			Site 2			Site 3		
	Pond 1	Pond 2	Pond 3	Pond 1	Pond 2	Pond 3	Pond 1	Pond 2	Pond 3
Sampling 1	45,45	53,85	53,33	37,50	50,00	42,86	46,67	50,00	42,86
Sampling 2	50,00	57,14	45,45	50,00	42,86	44,44	42,86	40,00	45,45
Sampling 3	50,00	52,63	44,44	45,45	57,14	50,00	45,45	52,63	46,67

Figure 3. Phagocytic Activity in *Litopenaeus vannamei*

Phagocytic activity observed across all sampling sites ranged from 37.50% to 57.14%. Specifically, at Site 1, phagocytic activity ranged from 44.44% to 57.14%, while at Site 2 it ranged from 37.50% to 57.14%, and at Site 3 from 42.86% to 50.00%. The highest activity was recorded at Site 2, followed by Site 1 and Site 3. Although variations were observed, phagocytic activity levels remained relatively stable among the three locations. This suggests that the shrimp maintained a functional non-specific immune system regardless of the environmental differences between ponds.

Phagocytosis is a crucial mechanism in the innate immune response of crustaceans, including *Litopenaeus vannamei*. Hemocytes play an essential role in recognizing, engulfing, and eliminating pathogens through the formation of phagosomes. These vesicles then fuse with lysosomes to create phagolysosomes, where microbial degradation occurs. The immune response is further enhanced through the production of reactive oxygen species (ROS) in a process known as the respiratory burst [24].

The elevated phagocytic activity found at Site 2 may be a physiological reaction to environmental stressors, particularly the higher levels of ammonia and nitrate recorded at that location. These stressors are known to stimulate hemocyte activity as the shrimp attempts to mitigate potential pathogenic threats. According to Musthaq and Kwang [8], exposure to environmental stress, including poor water quality and microbial challenges, can enhance the phagocytic efficiency of shrimp as part of their first-line immune defense.

Therefore, the data support the conclusion that phagocytic activity in *L. vannamei* is closely influenced by environmental conditions, particularly water quality. Monitoring and managing these parameters is essential to maintaining optimal shrimp immune performance in aquaculture systems.

D. Water Quality

Water quality measurements and analyses were conducted both in-situ and ex-situ at the vannamei shrimp ponds in Probolinggo. Physical parameters measured included temperature, TDS, and TSS. Chemical parameters included dissolved oxygen (DO), ammonia, nitrate, salinity, and pH. The results of water quality are presented in Table 2.

TABLE 2.
WATER QUALITY PARAMETERS IN VANNAMEI SHRIMP CULTURE PONDS

Water Quality Parameters	Site 1			Site 2			Site 3		
	Pond 1	**Pond 2**	**Pond 3**	**Pond 1**	**Pond 2**	**Pond 3**	**Pond 1**	**Pond 2**	**Pond 3**
Temperature (°C)	27-28.2	27-28.4	26.9-28	27.2-28.1	27.9-28.6	27.7-27.9	27.2-28.1	28.3-28.8	27.7-27.9
Salinity (ppt)	29-31	29-31	29-31	5-6	5-6	5-6	10	8-10	10
DO (mg/L)	4.1-5.9	3.9-6.2	4-6.1	3.5-5.1	3-4.8	3.8-4.7	4.3-4.7	4.8-5.1	4.4-4.9
pH	7.73-8.12	7.67-8.24	7.7-8.19	6.7-7.15	6.75-7.49	6.82-7.32	6.85-7.12	6.82-7.26	6.74-7.2
Nitrate (mg/L)	0.62-0.92	0.5-0.83	0.5-0.88	0.1-0.14	0.09-0.17	0.13-0.21	0.1-0.14	0.11-0.17	0.13-0.25
Ammonia (mg/L)	0.11-0.16	0.18-0.27	0.09-0.25	0.57-0.62	0.24-0.38	0.41-0.51	0.3-0.62	0.28-0.45	0.32-0.5
TSS (mg/L)	231-281	207-296	218-284	189-271	206-264	197-258	155-268	187-247	211-233
TDS (mg/L)	2824-3239	2872-3456	3037-3815	1997-2113	1817-2085	2008-2156	6624-6856	6523-6795	6674-6752

Based on the research result presented in Table 2, water temperature across the three sampling sites ranged from 27–28.4°C at Site 1, 27.2–28.6°C at Site 2, and 27.2–28.8°C at Site 3. These temperature values remain within the acceptable range for the survival and growth of *Litopenaeus vannamei*. Considering the metabolic rate observed in this research [25], maintaining a temperature between 25 °C and 30 °C is recommended for the effective cultivation of *Litopenaeus vannamei*. *Litopenaeus vannamei* is generally cultured at a temperature range of 20-33 °C [26]. As a result, the temperature conditions observed during the research are still considered suitable for shrimp aquaculture. Temperatures above the optimal range may accelerate shrimp metabolism, increasing energy demands and physiological stress. Conversely, temperatures below the optimal range tend to reduce feeding activity and suppress growth performance [27].

Salinity levels also showed considerable variation between sites, with values ranging from 29–31 ppt at Site 1, 5–6 ppt at Site 2, and 8–10 ppt at Site 3. While vannamei shrimp are known to tolerate a broad salinity range of 0–31 ppt, optimal growth occurs at salinity levels between 15–25 ppt, with the preferred range being 25–30 ppt [27]. Low salinity conditions, such as those observed at Sites 2 and 3, can impair osmoregulatory function and increase physiological stress, which may compromise the immune system and reduce survival [28]. Only Site 1 exhibited salinity levels that are within the optimal range, suggesting a more favorable environment for shrimp development at that location.

The dissolved oxygen (DO) levels recorded during the research were within the range of 3.9–6.1 mg/L at Site 1, 3–5.1 mg/L at Site 2, and 4.3–4.9 mg/L at Site 3. These values meet the minimum DO requirement for shrimp survival, which is no less than 4 mg/L, with the optimal range being 4–10 mg/L [4]. According to [29], a dissolved oxygen range of 4-8 mg/L is considered suitable for shrimp's life. Adequate DO is crucial for supporting metabolic activities, especially in intensive systems where high biomass leads to increased oxygen demand [30]. Higher metabolic rates due to growth or stress require more oxygen, and insufficient oxygen can negatively affect health and performance [31].

Based on the pH measurement results obtained in this research, the values ranged from 7.67 to 8.24 at site 1, from 6.70 to 7.49 at site 2, and from 6.74 to 7.26 at site 3. pH of approximately 7.0-9.5 affect hemocyte count, phenoloxidase activity, bacteriolytic activity and antibacterial activity [32]. Within this range, shrimp can grow optimally and tolerate pH levels between 6.5 and 9.0. The ideal pH value for aquatic organisms ranges from 6.8 to 8.5. The pH concentration of water influences the feeding behavior of shrimp and the chemical reactions that occur in the water. Moreover, pH levels below the tolerance range can cause molting difficulties in shrimp, resulting in soft shells and lower survival rates [33]. From the research results, only site 1 exhibited pH values within the optimal range for the growth of *L. vannamei*, while site 2 and site 3 had values below the optimal range. This condition could negatively affect the growth and overall health of the shrimp.

Based on the nitrate presented at each sampling location, the concentrations ranged from 0.50 to 0.92 mg/L at site 1, from 0.09 to 0.21 mg/L at site 2, and from 0.10 to 0.25 mg/L at site 3. The optimal nitrate concentration for the growth and survival of *L. vannamei* is between 0 and 1.5 mg/L [34]. Nitrate is an essential nutrient involved in protein synthesis for both plants and animals [35]. However, nitrate accumulation in aquaculture systems may lead to reduced water quality, triggering eutrophication and plankton blooms that may cause anoxic conditions during nighttime due to decreased dissolved oxygen levels. The results of this research indicate that nitrate concentrations at all sampling sites were within the optimal range, suggesting that nitrate levels are not expected to negatively impact the performance or environment of *L. vannamei* aquaculture.

The ammonia concentrations obtained in this research ranged from 0.11 to 0.27 mg/L at site 1, from 0.24 to 0.62 mg/L at site 2, and from 0.20 to 0.50 mg/L at site 3. Ammonia is a nitrogenous compound that is toxic to cultured organisms, even at low concentrations. Fundamentally, the acceptable range of ammonia should not exceed 0.1 ppm [36], and the safe level of free ammonia in aquaculture systems should be below 0.02 mg/L [37]. The results of this research indicate that ammonia concentrations at all sampling locations ranged from 0.11 to 0.62 mg/L, which are still within the tolerance threshold for the survival and growth of *L. vannamei*.

The values of Total Suspended Solids (TSS) obtained in this research ranged from 207 to 284 mg/L at site 1, from 189 to 271 mg/L at site 2, and from 155 to 268 mg/L at site 3. TSS is a critical component influencing

biofloc production in intensive and super intensive shrimp farming. High concentrations of TSS (greater than 500 mg/L) may have negative effects on shrimp health and performance, particularly for individuals larger than 15 grams [38]. Excess particulate matter may lead to gill clogging, reducing oxygen exchange and potentially increasing shrimp mortality. The results from this research show that TSS concentrations remained within normal levels, ranging from 155 to 284 mg/L, and therefore are not expected to adversely affect shrimp health and performance.

Based on the data in Table 2, Total Dissolved Solids (TDS) values at the shrimp farming sites showed significant variation among ponds and locations. The lowest TDS value was recorded at site 2, pond 2, ranging from 1,817 to 2,085 mg/L, while the highest value was recorded at site 3, pond 1, ranging from 6,624 to 6,856 mg/L. Overall, the TDS values across all ponds ranged from 1,817 to 6,856 mg/L, indicating farming systems with low to moderate salinity. Although *L. vannamei* is known to tolerate a wide range of salinity conditions, the optimal TDS range for good growth is between 10,000 and 35,000 mg/L, which corresponds to a salinity of 10 to 35 ppt [39]. Low TDS levels may disrupt the osmoregulatory processes in shrimp, increase stress levels, and reduce growth efficiency, while excessively high TDS values can impair metabolic function and increase the energy demand for osmotic adaptation [40]. Therefore, most ponds at site 1 and site 2 were below the optimal threshold, which may affect aquaculture performance if not managed properly through appropriate feeding strategies and water quality control.

Water quality parameters varied across sites. Site 1 had optimal salinity (29–31 ppt) and pH (7.7–8.2), while Site 2 exhibited low salinity (5–6 ppt) and suboptimal pH (6.7–7.4), along with higher ammonia concentrations (up to 0.62 mg/L). Site 3 had intermediate conditions but still fell below the optimal range for several parameters. According to Boyd [4] and Kordi and Tanjung [25], fluctuations in water quality can induce stress and suppress shrimp immunity. The stability of water quality at Site 1 may explain the moderate immune responses observed there, while Site 2's harsher environment likely contributed to increased THC and phagocytic activity as adaptive immune mechanisms.

E. Canonical Correspondence Analysis

The Canonical Correspondence Analysis (CCA) method was applied in this research to comprehensively examine the relationships between independent and dependent variables. In this context, the analysis was conducted to determine the correlation between water quality parameters in shrimp culture ponds and immune response variables, which include total hemocyte count, differential hemocyte count, and phagocytic activity. The results of the CCA analysis are presented in Figure 4.

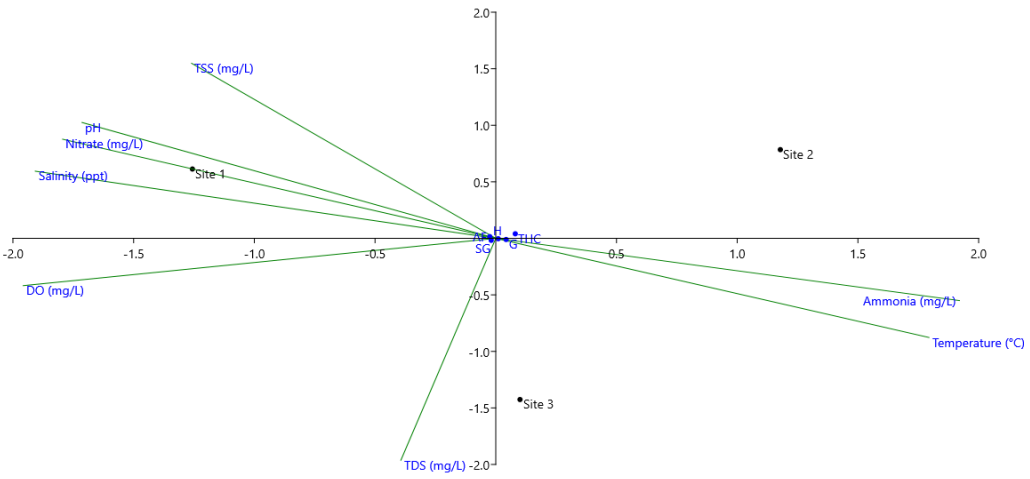


Figure 4. Analysis of the Relationship between Water Quality and the Immune Response of Vannamei Shrimp

The results of the Canonical Correspondence Analysis (CCA) indicate varying relationships between water quality parameters and the immune responses of *Litopenaeus vannamei* across the three sampling sites. Site 2 was positioned close to temperature and ammonia vectors, along with immune indicators such as Total Hemocyte Count (THC) and granulocytes, suggesting that higher temperatures and ammonia concentrations at this site may have triggered heightened immune activity. In contrast, Site 1 clustered near pH, salinity, and nitrate vectors, and was associated with hyalinocytes, indicating a more stable and favorable environment for shrimp immunity. Meanwhile, Site 3 appeared opposite to dissolved oxygen (DO), total suspended solids (TSS), and total dissolved solids (TDS), implying suboptimal water quality that may negatively impact shrimp immune performance. Overall, temperature and ammonia emerged as the most influential factors affecting immune cell variation. The use of CCA provided a multidimensional understanding of how environmental stressors shape immune responses, supporting its application as a robust analytical tool for integrated water quality and shrimp health monitoring in aquaculture systems [15], [16].

IV. CONCLUSION

This study demonstrated that water quality plays a critical role in influencing the immune response of *Litopenaeus vannamei* cultivated in Probolinggo, East Java. Parameters such as temperature, pH, dissolved oxygen, salinity, ammonia, nitrate, total suspended solids (TSS), and total dissolved solids (TDS) were found to correlate significantly with key immune indicators, including total hemocyte count (THC), differential hemocyte count (DHC), and phagocytic

activity. Canonical Correspondence Analysis (CCA) revealed that temperature and ammonia were the most influential environmental factors affecting shrimp immune function, particularly THC and granulocyte levels, suggesting that environmental stressors can trigger adaptive immune responses. These findings offer a scientific basis for enhancing pond management practices through improved water quality control to support shrimp health, reduce disease risks, and promote sustainable aquaculture. Based on these results, it is recommended that future research explore real-time water quality monitoring systems integrated with immunological biomarkers to optimize shrimp health management. Additionally, local policymakers and shrimp farmers should collaborate to implement adaptive water treatment protocols tailored to the specific environmental conditions of each farming region.

REFERENCES

[1] S. Anwar and A. Abdurrohman, "Utilization of Internet of Things Technology for Monitoring Vannamei Shrimp Ponds Based on Android Smartphones Using Nodemcu Wemos D1 Mini," *Infotronik: Journal of Information and Electronics Technology*, vol. 5, no. 2, pp. 77–83, 2020.

[2] M. G. Emerenciano, A. N. Rombenso, F. D. N. Vieira, M. A. Martins, G. J. Coman, H. H. Truong, *et al*., "Intensification of penaeid shrimp culture: an applied review of advances in production systems, nutrition and breeding," *Animals*, vol. 12, no. 3, p. 236, 2022.

[3] M. F. Haqqi, Y. P. Hastuti, and K. Nirmala, "Toxicity of Total Ammonia Nitrogen (TAN) Exposure on the Productivity and Physiological Response of Vannamei Shrimp *Litopenaeus vannamei* on Day of Culture 30," *Indonesian Journal of Aquaculture*, vol. 19, no. 1, pp. 1–12, 2021.

[4] C. E. Boyd, Water Quality in Ponds for Aquaculture. Birmingham, AL: Birmingham Publishing Co., 1990.

[5] A. Ridlo and R. Pramesti, "Application of Seaweed Extract as an Immunostimulant Agent for the Non-Specific Defense System in Shrimp (*Litopenaeus vannamei*)," *Indonesian Journal of Marine Science*, vol. 14, no. 3, pp. 133–137, 2009.

[6] H. Manoppo and E. F. Kolopita, "Immune Response of Crustaceans," *Aquaculture Journal*, vol. 2, no. 2, pp. 22–26, 2014.

[7] M. Shen, Y. Hu, K. Zhao, C. Li, B. Liu, M. Li, *et al*., "Occurrence, bioaccumulation, metabolism and ecotoxicity of fluoroquinolones in the aquatic environment: a review," *Toxics*, vol. 11, no. 12, p. 966, 2023.

[8] S. K. S. Musthaq and J. Kwang, "Reprint of 'Evolution of specific immunity in shrimp–A vaccination perspective against white spot syndrome virus'," *Developmental & Comparative Immunology*, vol. 48, no. 2, pp. 342–353, 2015.

[9] F. Impellitteri, A. S. Curpăn, G. Plăvan, A. Ciobica, and C. Faggio, "Hemocytes: a useful tool for assessing the toxicity of microplastics, heavy metals, and pesticides on aquatic invertebrates," *International Journal of Environmental Research and Public Health*, vol. 19, no. 24, p. 16830, 2022.

[10] A. Ghanad, "An overview of quantitative research methods," *International Journal of Multidisciplinary Research and Analysis*, vol. 6, no. 08, pp. 3794–3803, 2023.

[11] S. Noor, O. Tajik, and J. Golzar, "Simple random sampling," *International Journal of Education & Language Studies*, vol. 1, no. 2, pp. 78–82, 2022.

[12] A. Hariri, "Intensive Cultivation of Vannamei Shrimp (*Litopenaeus vannamei*) in Circular Ponds at CV. Tirta Makmur Abadi, Lombang Village, Batang-Batang District, Sumenep, East Java," *Grouper: Scientific Journal of Fisheries*, vol. 12, no. 2, pp. 35–46, 2021.

[13] G. Maharani, J. T. Sunarti, and T. Juniastuti, "Hemocyte damage and count in tiger shrimp (*Penaeus monodon* Fab.) affected by zoothamniosis," *Scientific Journal of Fisheries and Marine*, vol. 1, no. 1, 2009.

[14] A. P. Yanuar and H. Manoppo, "Non-Specific Immune Response of Common Carp Fed Orally with Baker's Yeast as Immunostimulant," *e-Journal of Aquaculture*, vol. 5, no. 2, 2017.

[15] C. J. Ter Braak, "Canonical Correspondence Analysis: A New Eigenvector Technique for Multivariate Direct Gradient Analysis," *in Proc. of Ecology*, vol. 67, no. 5, pp. 1167–1179, 1986.

[16] P. Legendre and L. Legendre, Numerical Ecology, 2nd ed., 1998.

[17] I. Ismawati, R. A. Destryana, and N. Huzaimah, "Immunity of vannamei shrimp (*Litopenaeus vannamei*) fed with kasembukan leaf (*Paederia foetida* Linn.) as a dietary supplement," *Jurnal Kelautan: Indonesian Journal of Marine Science and Technology*, vol. 12, no. 2, pp. 201–206, 2019.

[18] B. Raza, Z. Zheng, J. Zhu, and W. Yang, "A review: Microbes and their effect on growth performance of *Litopenaeus vannamei* (white leg shrimps) during culture in biofloc technology system," *Microorganisms*, vol. 12, no. 5, p. 1013, 2024.

[19] P. K. Sahoo, B. R. Pillai, J. Mohanty, J. Kumari, S. Mohanty, and B. K. Mishra, "In vivo humoral and cellular reactions, and fate of injected bacteria *Aeromonas hydrophila* in freshwater prawn *Macrobrachium rosenbergii*," *Fish & Shellfish Immunology*, vol. 23, no. 2, pp. 327–340, 2007.

[20] S. P. Yeh, Y. N. Chen, S. L. Hsieh, W. Cheng, and C. H. Liu, "Immune response of white shrimp, *Litopenaeus vannamei*, after a concurrent infection with white spot syndrome virus and infectious hypodermal and hematopoietic necrosis virus," *Fish & Shellfish Immunology*, vol. 26, no. 4, pp. 582–588, 2009.

[21] B. W. Prastowo, R. Lareu, R. Caccetta, and R. Fotedar, "Determination of cell type and haemocyte morphometric characteristics of Western Australia freshwater crayfish (*Cherax cainii*) at different temperatures *in vitro*," *Aquaculture Engineering and Technology Journal*, vol. 8, no. 2, pp. 1009–1028, 2020.

[22] S. A. S. Wangi, I. Nur, and M. Idris, "Differential hemocyte analysis in vannamei shrimp (*Litopenaeus vannamei*) cultured around mining areas," *Journal of Aquaculture Medium*, vol. 4, no. 2, pp. 77–81, 2019.

[23] B. A. Takwin, D. P. Perdana, M. Muktiniati, and F. Azhar, "Utilization of Jatropha leaf (*Jatropha curcas*) extract as immune system enhancer of vannamei shrimp (*Litopenaeus vannamei*) infected with *Vibrio parahaemolyticus*," *Journal of Aquaculture and Fish Health*, vol. 12, no. 1, pp. 12–20, 2023.

[24] L. Rodriguez and G. Le Moullac, "State of the Art of Immunological Tools and Health Control of Penaeid Shrimp," *Aquaculture*, vol. 191, pp. 109–119, 2000.

[25] M. Kır, M. C. Sunar, M. Topuz, and M. Sarıipek, "Thermal acclimation capacity and standard metabolism of the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) at different temperature and salinity combinations," *Journal of Thermal Biology*, vol. 112, p. 103429, 2023.

[26] C. Bett and L. Vinatea, "Combined effect of body weight, temperature and salinity on shrimp *Litopenaeus vannamei* oxygen consumption rate," *Brazilian Journal of Oceanography*, vol. 57, pp. 305–314, 2009.

[27] M. Supriatna, M. Mahmudi, and M. Musa, "pH model and its relationship with water quality parameters in intensive vannamei shrimp (*Litopenaeus vannamei*) ponds in Banyuwangi, East Java," *JFMR (Journal of Fisheries and Marine Research)*, vol. 4, no. 3, pp. 368–374, 2020.

[28] W. Utami, "Effect of Salinity on Infection by Vibrio harveyi in Vannamei Shrimp (*Litopenaeus vannamei*)," *J. Aquac. Manage. Technol*., vol. 5, no. 1, pp. 82–90, 2016.

[29] S. Bahri, D. Mardhia, and O. Saputra, "Growth and graduation of vannamei shell life (*Litopenaeus vannamei*) with feeding tray (anco) system in AV 8 Lim Shrimp Organization (LSO) in Sumbawa District," *Biologi Tropis*, vol. 20, no. 2, pp. 279–289, 2020.

[30] R. Syah, M. Makmur, and M. Fahrur, "Vannamei shrimp farming with high stocking density," *Journal of Aquaculture Medium*, vol. 12, no. 1, p. 19, 2017.

[31] A. Wafi, H. Ariadi, A. Muqsith, M. Mahmudi, and M. Fadjar, "Oxygen consumption of *Litopenaeus vannamei* in intensive ponds based on the dynamic modeling system," *Journal of Aquaculture and Fish Health*, vol. 10, no. 1, pp. 17–24, 2021.

[32] P. Lu-Qing, J. Ling-Xu, and M. Jing-Jing, "Effects of salinity and pH on immune parameters of the white shrimp *Litopenaeus vannamei*," *Journal of Shellfish Research*, vol. 24, no. 4, pp. 1223–1227, 2005.

[33] M. S. Chakravarty, P. R. C. Ganesh, D. Amarnath, B. S. Sudha, and T. S. Babu, "Spatial variation of water quality parameters of shrimp (*Litopenaeus vannamei*) culture ponds at Narsapurapupeta, Kajuluru and Kaikavolu villages of East Godavari district, Andhra Pradesh," *International Journal of Fisheries and Aquatic Studies*, vol. 4, no. 4, pp. 390–395, 2016.

[34] F. Muaddama, "Comparative Study of Phosphate and N-Nitrogen (Ammonia, Nitrate and Nitrite) Contents in Ponds in Polluted and Unpolluted Coastal Areas," *Journal of Fisheries Science and Technology*, vol. 1, no. 1, pp. 37–48, 2021.

[35] D. J. Schuler, et al., "Acute Toxicity of Ammonia and Nitrite to Pacific White Shrimp, Litopenaeus vannamei, at Low Salinities," J. World Aquac. Soc., vol. 41, no. 3, pp. 438–446, 2010.

[36] S. Arsad, A. Afandy, A. P. Purwadhi, M. V. Betrina, D. K. Saputra, and N. R. Buwono, "Study on vannamei shrimp (*Litopenaeus vannamei*) grow-out cultivation activities using different rearing systems," *Scientific Journal of Fisheries and Marine*, vol. 9, no. 1, p. 1, 2017.

[37] A. Putra, A. S. Yumna, A. T. Alfiazh, B. A. Nugraha, D. Sartika, F. Ramadiansyah, M. Novela, N. J. D. Chairani, S. Samsuardi, S. Ramadhan, Y. D. Wake, I. Ilham, and S. Suharyadi, "Water quality analysis in intensive vannamei shrimp (*Litopenaeus vannamei*) aquaculture systems," *Unram: Fisheries Journal*, vol. 13, no. 3, pp. 871–878, 2023.

[38] M. Emerenciano, E. L. Ballester, R. O. Cavalli, and W. Wasielesky, "Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817)," *Aquaculture Research*, vol. 43, no. 3, pp. 447–457, 2012.

[39] C. E. Boyd, J. F. Queiroz, J. H. Lee, O. Green, A. Rowan, and T. McNevin, *Responsible Aquaculture Practices*. Washington, DC: World Bank Group, 2018.

[40] T. M. Samocha, A. L. Lawrence, D. A. Pooser, and K. Masuda, "The effect of salinity on growth and survival of juvenile *Litopenaeus vannamei*," *Journal of the World Aquaculture Society*, vol. 32, no. 3, pp. 207–217, 2001.