Fisheries Journal, 15 (5), 2633-2645 (2025)
http://doi.org/10.29303/jp.v15i5.1837

RELATIONSHIP OF WATER PHYSICO-CHEMICAL FACTORS WITH TOTAL VIBRIO IN THE HEPATOPANCREAS OF WHITELEG SHRIMP (*Litopenaeus vannamei*) IN TRADITIONAL PONDS RUNGKUT, SURABAYA

Hubungan Faktor Fisika-Kimia Air dengan Total Vibrio pada Hepatopankreas Udang Vaname (*Litopenaeus vannamei*) di Tambak Tradisional Rungkut, Surabaya

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(Received August 29th 2025; Accepted October 25th 2025)

ABSTRACT

Whiteleg shrimp *(Litopenaeus vannamei)* is a valuable commodity in global aquaculture, significantly boosting shrimp farmers' income and local economies. In Indonesia, traditional ponds is the most farming uses with limited pond management, affecting water quality in physical and chemical aspects. Poorly managed water quality can stress shrimp, increasing vulnerability to pathogens like *Vibrio* bacteria, which causes Vibriosis and can lead to significant losses. This study aims to determine water physico-chemical factors that affect *Vibrio* levels in the hepatopancreas of whiteleg shrimp in traditional ponds in Rungkut, Surabaya. Data were collected through direct observation and lab analysis. Simple linear regression with a 5% significance level showed that dissolved oxygen (DO) and ammonia significantly different total *Vibrio* in hepatopancreas of whiteleg shrimp. DO had a P-value of 0,025 with a negative effect, while ammonia had a P-value of 0,020 with a positive effect.

Keywords: Whiteleg Shrimp, Traditional Pond, Physico-Chemical Factors, Total *Vibrio*

ABSTRAK

Udang vaname *(Litopenaeus vannamei)* adalah komoditas penting dalam akuakultur global dengan nilai ekonomi tinggi, berkontribusi pada pendapatan petambak dan ekonomi lokal. Budidaya vaname di Indonesia umumnya masih menggunakan metode tradisional dengan manajemen tambak terbatas. Kondisi fisika-kimia air tambak yang kurang optimal dapat menyebabkan stres pada udang dan meningkatkan resiko infeksi patogen, seperti bakteri *Vibrio* yang memicu penyakit Vibriosis, mengancam kesehatan udang dan menimbulkan kerugian. Penelitian ini bertujuan mengetahui faktor fisika-kimia air yang mempengaruhi total *Vibrio* pada hepatopankreas udang vaname di tambak tradisional Kecamatan Rungkut, Surabaya. Metode yang digunakan adalah survei dengan pengumpulan data melalui pengamatan langsung dan analisis laboratorium. Analisis regresi linear sederhana dengan taraf signifikansi 5%

menunjukkan bahwa oksigen terlarut (DO) dan amonia berpengaruh nyata terhadap total *Vibrio* pada hepatopankreas udang vaname. DO memiliki nilai p 0,025 dengan korelasi negatif, sedangkan amonia memiliki nilai p 0,020 dengan korelasi positif.

Kata Kunci: Udang Vaname, Tambak Tradisional, Fisika-Kimia Air, Total *Vibrio*

INTRODUCTION

Whiteleg shrimp (*Litopenaeus vannamei*) is a crucial commodity in the aquaculture industry due to its high economic value. Global whiteleg shrimp production in 2018 reached approximately 4.5 million tons, valued at $22 billion, demonstrating its significant role in the global economy (Iskandar *et al.,* 2022). In Indonesia, whiteleg shrimp is a leading commodity, contributing significantly to farmer incomes and local economies, particularly in Asian countries, including Indonesia, where it plays a significant role in both domestic and international markets (Yunarty *et al.,* 2022). Traditional pond farming is one of the dominant cultivation methods in Indonesia.

In Rungkut District, Surabaya City, whiteleg shrimp are cultivated traditionally, despite facing various productivity challenges due to environmental, host, and pathogen factors (Ponce-Palafox *et al.,* 2019). Environmental factors, such as water quality, significantly influence the success of whiteleg shrimp cultivation. Pond water quality plays a key role in determining the success of whiteleg shrimp cultivation (Sahrijanna & Septiningsih, 2017). Poor water quality can cause stress in shrimp, increase the risk of diseases such as Vibriosis caused by Vibrio bacteria, and lead to mass mortality which is detrimental to the economy of shrimp farmers (Mandal & Duyari, 2024). Several studies have shown that the accumulation of ammonia and nitrite, as well as increases in temperature and salinity, can affect water quality and increase the risk of pathogen infection (Ariadi *et al.,* 2021; Liu *et al.,* 2024). Furthermore, poor water quality can weaken the shrimp's immune system and facilitate the entry of pathogens such as Vibrio, which causes vibriosis (Oktaviana & Febriani, 2023). Decreased pH levels, low dissolved oxygen levels, and increased TOM (organic matter) are also known to increase the abundance of Vibrio bacteria (Alfiansah *et al.,* 2020).

In Rungkut District, two areas, Wonorejo and Medokan Ayu Villages, manage whiteleg shrimp ponds using traditional methods. These ponds suffer from suboptimal management, such as the lack of pond preparation, poor water quality management, infrequent water changes, and relying solely on seawater flow at high tide. This can impact water quality and increase the risk of disease, which impacts pond productivity, such as vibriosis, a common occurrence in whiteleg shrimp cultivation. Research Objectives This study aims to determine the total number of Vibrio in the hepatopancreas of whiteleg shrimp in two traditional shrimp ponds in Rungkut District, Surabaya, and to determine the physicochemical factors of water that influence the total number of Vibrio in the hepatopancreas of whiteleg shrimp in traditional shrimp ponds in Rungkut District, Surabaya. The benefits of this study are to provide information on the total number of Vibrio in the hepatopancreas of whiteleg shrimp and the physicochemical factors of water that influence the total number of Vibrio in the hepatopancreas of whiteleg shrimp in traditional shrimp ponds in Rungkut District, Surabaya. In addition, this study can be a reference for research related to the relationship between physicochemical factors of water and the total number of Vibrio in the hepatopancreas of whiteleg shrimp in traditional shrimp ponds in other regions.

RESEARCH METHODS

Place and Time

This research was conducted in two traditional fish ponds in Rungkut District, Surabaya, at two different stations located in Wonorejo and Medokan Ayu Villages, as well as in a laboratory for further analysis. The study lasted 65 days, from July to September 2024.

Tools and materials

This research was conducted in two traditional fish ponds in Rungkut District, Surabaya, at two different stations located in Wonorejo and Medokan Ayu Villages, as well as in a laboratory for further analysis. The study lasts 65 days, from July to September 2024.

Research Parameters

The main parameters measured in this study were the physicochemical factors of the water, including temperature, water color, pH, DO, salinity, TAN, ammonia, nitrite, nitrate, phosphate, and TOM, as well as the total Vibrio count in the hepatopancreas of whiteleg shrimp. Supporting parameters included the total Vibrio count in the water, survival rate, and histopathology of the hepatopancreas of whiteleg shrimp.

Temperature

Temperature measurements are taken by immersing the thermometer directly at the water sampling point. The thermometer must be fully submerged to a depth of 30 cm below the water surface, ensuring that it does not fall. The temperature displayed on the thermometer is then recorded as pond water temperature data. The same procedure is applied to other water sampling points (Jamal *et al.,* 2022).

Water Color

Water color was measured organoleptically through visual observation, directly assessing the visible color of the pond water. Water samples were also taken using a 50 mL beaker and observed under optimal lighting. The visible color was then recorded as the pond water color. Common water colors found in shrimp ponds range from green, brownish green, brown, to greenish brown (Cahyanurani & Hariri, 2021).

pH

pH measurements were performed by taking water samples from a sampling point at a depth of 50 cm using a 5-liter bucket equipped with a rope. The pH meter was dipped directly into the bucket, and the pH reading on the screen was recorded as the pond water pH data (Jamal *et al.,* 2022).

Dissolved Oxygen (DO)

Dissolved oxygen (DO) measurements are performed using a DO meter. The instrument is then turned on and the DO meter probe is inserted directly into the pond water at the sampling point. The probe is inserted to a depth of 50 cm from the water surface. The DO value read on the meter screen is recorded as the pond water DO data (Jamal *et al.,* 2022).

Salinity

Salinity measurements were performed using a refractometer. Water samples were taken from a sampling point 50 cm below the water surface using a 5-liter bucket equipped with a rope. The water in the bucket was collected using a dropper and then dripped onto the refractometer prism. The salinity reading on the refractometer scale was recorded as the pond water salinity data (Jamal *et al.,* 2022).

Total Ammonia Nitrogen (TAN)

TAN measurements were conducted by the Situbondo Brackish Water Aquaculture Center (BPBAP) laboratory in accordance with the test guidelines listed in IKM/7.2.14.K/BPBAPS using the indophenol blue technique through spectrophotometry. A 25 mL filtered sample was placed in an Erlenmeyer flask, then 1 mL of phenol solution, 1 mL of

Na Nitroprusside solution, 2 mL of alkaline citrate solution, and 0.5 mL of sodium hypochlorite solution were added as an oxidizer. The mixture was incubated for at least 1 hour, then the absorbance was measured using a spectrophotometer at a wavelength of approximately 640 nm, with a maximum measurement time limit of 24 hours after the addition of the reagent. The results of this TAN measurement will be written on a test result sheet by the laboratory and used as data on the TAN content of pond water.

Ammonia (NH_3)

To calculate free ammonia (NH_3) from the Total Ammonia Nitrogen (TAN) concentration, the Boyd conversion method is used. The Boyd table allows the conversion of TAN to free ammonia (NH_3) by taking into account the pH and temperature of the water. The pH and temperature data are entered into this table to calculate the free ammonia content from the measured TAN. The results of these calculations are then recorded on a laboratory test results sheet and used as data on the ammonia content in pond water.

Nitrite (NO_2^-)

Nitrite levels were measured by the Situbondo BPBAP laboratory using the method listed in IKM/7.2.12.K/BPBAPS, namely testing NO2-N with sulfanilamide spectrophotometrically. The water sample was filtered using filter paper, then measured as much as 50 mL and placed into a 100 mL Erlenmeyer flask. Next, 2 mL of dye solution was added and shaken until homogeneous. After waiting for at least 10 minutes, the absorbance of the sample was measured using a spectrophotometer at a wavelength of approximately 543 nm, with measurements carried out no more than 2 hours after the addition of the reagent. The results obtained will be recorded on a test result sheet by the laboratory and used as data on the nitrite levels of pond water.

Nitrate (NO_3^-)

Nitrate levels are determined using the nitrification stoichiometric relationship. This method is used to determine nitrate concentration in an aquatic system when the concentrations of nitrite and ammonia are known, applying the basic principles of the nitrogen cycle. The nitrification process converts ammonia to nitrite, which is then oxidized to nitrate (Hendrawan *et al.,* 2021). Assuming the nitrification process is complete, nitrate calculations can be performed using the stoichiometric approach.

Phosphate (PO_4^{3-})

Phosphate measurements were carried out by the BPBAP Situbondo laboratory using a spectrophotometric method. A 10 mL sample was taken and placed in a HACH bottle. PhosVer 3 was then added to the sample and shaken until completely dissolved. The sample was then measured using a colorimeter for 2 minutes until the sample turned blue. To read the results, a blank was inserted first and the zero button was pressed. Then, the sample was inserted and the read button was pressed to obtain the phosphate content. The results obtained will be recorded on a test result sheet by the laboratory and used as data for the phosphate content of the pond water.

Total Organic Matter (TOM)

TOM measurements were conducted by the Situbondo BPBAP laboratory using a titrimetric method with potassium permanganate (KMnO4). A 25 mL sample was taken, added to 25 mL of distilled water, and then placed in a beaker. As a blank, 50 mL of distilled water without sample was also placed in the beaker. Then, 10 mL of 0.01 N $KMnO_4$ solution (or 1 mL of 0.1 N $KMnO_4$) and 5 mL of 6 N H_2SO_4 were added to the mixture. The sample was heated on a hot plate for approximately 20 minutes. After heating, the mixture was removed from the hot plate, and 10 mL of 0.01 N oxalic acid (or 1 mL of 0.1 N oxalic acid) was added. Titration was carried out with 0.01 N $KMnO_4$ until a stable color change to pink occurred.

VKMnO4 is the average volume of $KMnO_4$ solution used in the titration. The number 12.64 is a conversion factor obtained from calculations relating the number of moles of $KMnO_4$

reacted with organic matter, where 1 mole of $KMnO_4$ is equivalent to 12.64 mg of organic matter. The results obtained will be recorded on a test results sheet and used as data on the TOM levels of pond water.

Vibrio Total

Total Vibrio counts in pond water samples were measured by the Pasuruan Water Resources and Environmental Management Unit (UPT LKIL) using the total plate count method in accordance with the IKM/7.2.8/UPT-LKIL guidelines. Test tubes were prepared according to the dilution amount, filled with 9 mL of Trisalt solution and the appropriate amount of TCBS agar. The water sample was then diluted sequentially into each test tube. Afterward, 0.1 mL of each dilution was instilled into TCBS agar using the spread plate method. The plates were then placed in an incubator for 24 hours at 35°C.

After 24 hours of incubation on the TCBS agar, Vibrio bacteria were successfully grown and then counted manually, grouping colonies based on similar color. The resulting numbers were then calculated using a formula to determine the number of colonies in the product. The results were recorded on a test result sheet by the laboratory and used as data on the total Vibrio count in pond water.

Research methods

This study uses a survey method by collecting data on main parameters and supporting parameters through direct observation, laboratory analysis, and statements from farmers, as well as comparing the results of parameter measurements from the two traditional ponds.

Research Procedures

The procedure in this study includes determining the research station using two traditional ponds with vaname shrimp commodities in Rungkut District, Surabaya. Traditional pond water sampling was carried out at two points in the pond. Traditional pond water sample testing was carried out in situ every day and ex situ every 10 days for 65 days. Vaname shrimp samples were taken from sampling activities which also carried out SR (survival rate) calculations, clinical symptom observations, total Vibrio testing on vaname shrimp hepatopancreas, preparation and histopathological observation of vaname shrimp hepatopancreas. Water physicochemical data (temperature, color, pH, DO, salinity, TAN, ammonia, nitrite, nitrate, phosphate, and TOM), total Vibrio, and SR were analyzed descriptively quantitatively using Microsoft Excel.

Data Analysis

The histopathological data of vaname shrimp hepatopancreas were tested using Kruskal Wallis, while the relationship between water physicochemical factors and total Vibrio was analyzed using simple linear regression and Pearson correlation coefficient using IBM SPSS.

RESULT

This research was conducted at two traditional whiteleg shrimp ponds in Rungkut District, Surabaya City: Wonorejo and Medokan Ayu Villages. A complete profile of both ponds is presented in Table 1.

Table 1. Traditional Pond Profile

Information	Wonorejo (A)	Medokan Ayu (B)
Cultivation System	Traditional	Traditional
Area	5.400 m^2	8.300 m^2
Stocking Density	9 ekor/m^2	12 ekor/m^2
DOC (Days of Culture) research	33 days	29 days

The results of measurements of water color, temperature, pH, DO, salinity, TAN, ammonia, nitrite, nitrate, TOM, phosphate, and total Vibrio in each pond are shown below.

Table 2. Pond Water Color

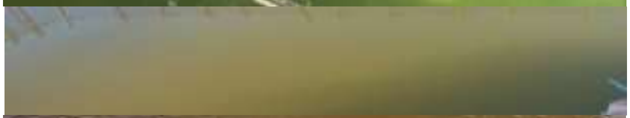
Water Color	**Figure**
Greenery	
Brownish Green	
Brownish	

Table 3. Results of Pond Water Color Measurements

Day th-	**Wonorejo (A)**	**Medokan Ayu (B)**
01-10	Greenery	Brownish
11-20	Greenery	Brownish
21-30	Brownish Green	Brownish Green
31-40	Brownish Green	Brownish Green
41-50	Brownish Green	Brownish
51-60	Brownish Green	Brownish
61-65	Greenery	Brownish

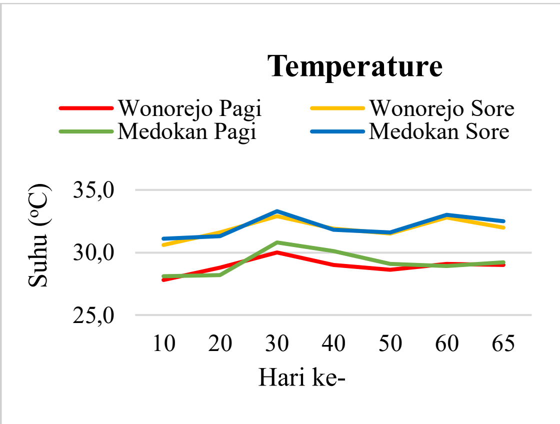


Figure 1. Pond Water Temperature Graph

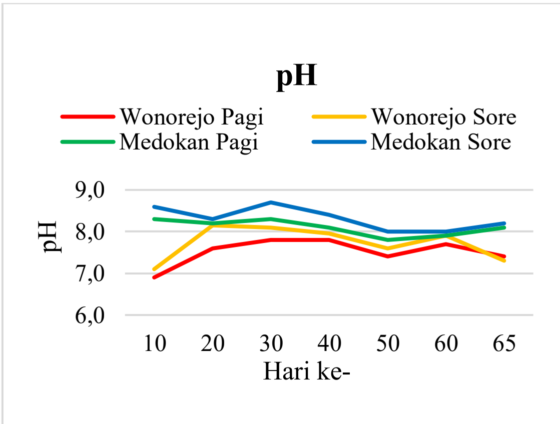


Figure 2. Graph of pH of Pond Water

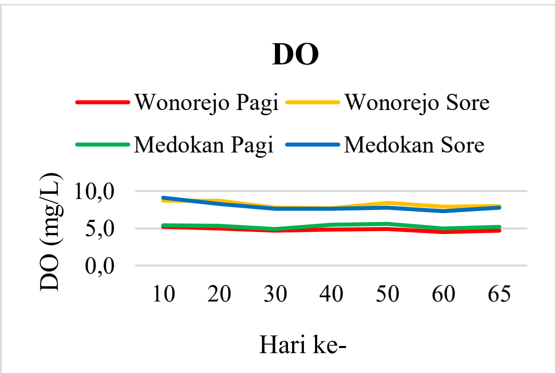


Figure 3. DO Graph of Pond Water

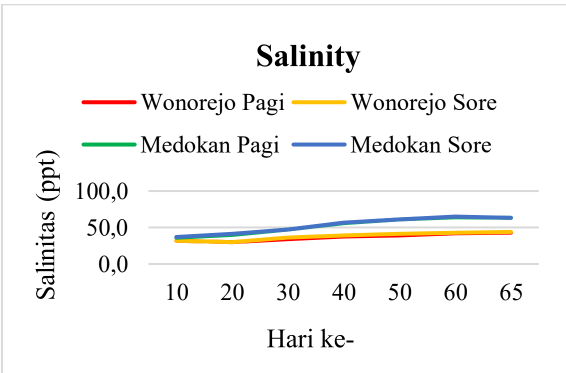


Figure 4. Graph of Pond Water Salinity

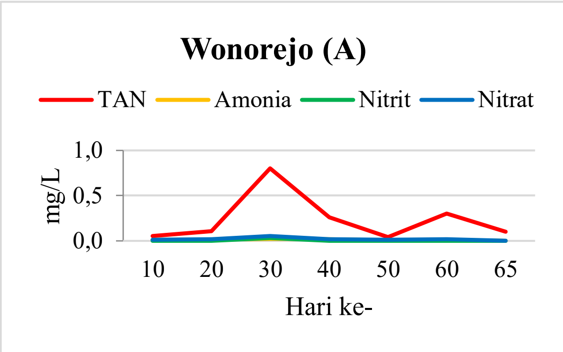


Figure 5. Graph of TAN, Ammonia, Nitrite and Nitrate in Wonorejo Pond

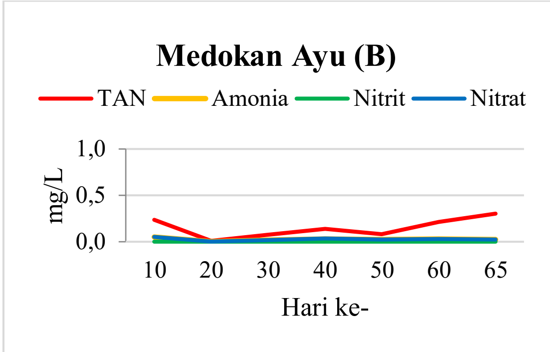


Figure 6. Graph of TAN, Ammonia, Nitrite, and Nitrate in Medokan Ayu Pond

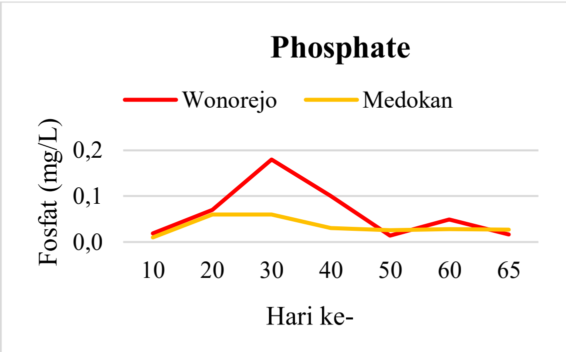


Figure 7. Phosphate Graph of Pond Water

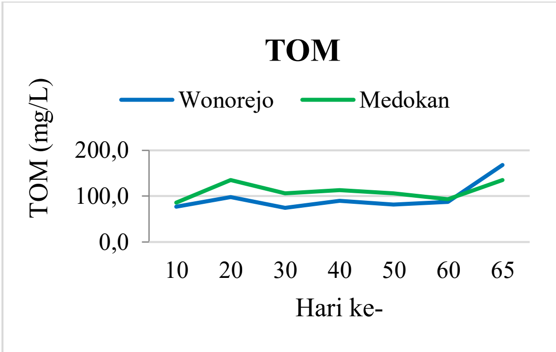


Figure 8. TOM Graph of Pond Water

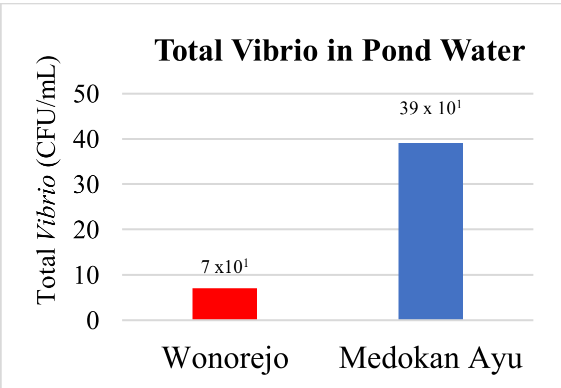


Figure 9. Graph of Total Vibrio Values in Pond Water

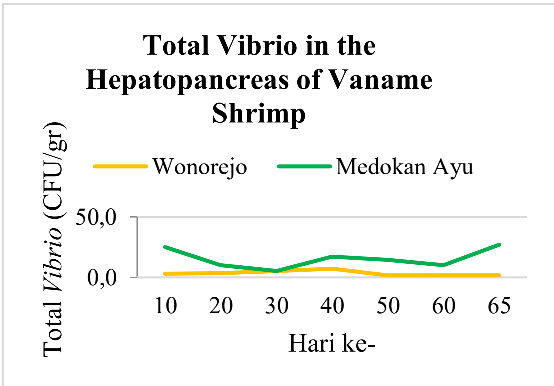


Figure 10. Graph of Total Vibrio in the Hepatopancreas Organ of Vaname Shrimp

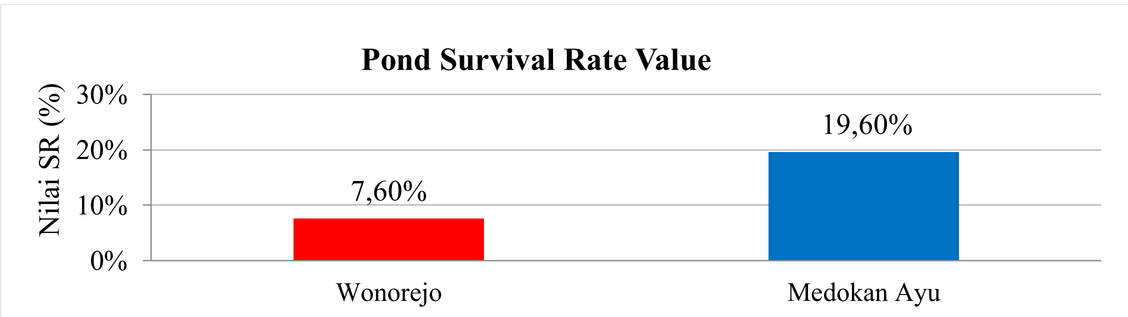


Figure 11. Graph of Pond SR Values

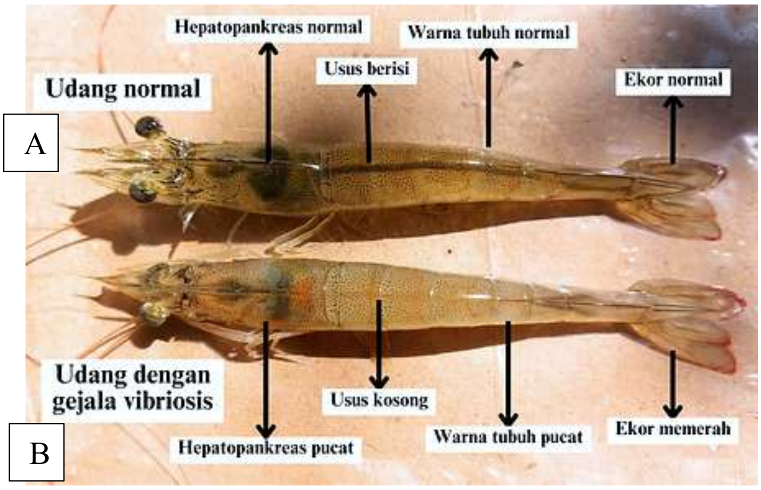


Figure 12. Normal Shrimp and Shrimp with Vibriosis Symptoms
Information: A. Normal shrimp. B. Shrimp with Vibriosis symptoms. In images A and B there are differences in the color of the hepatopancreas, intestinal contents, body color and tail color.

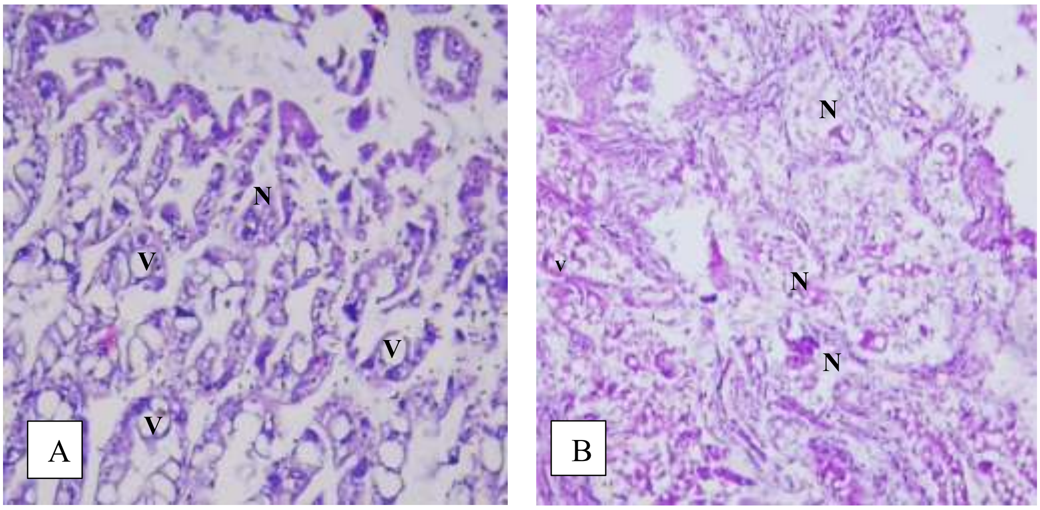


Figure 13. Histopathological Image of Vaname Shrimp Hepatopancreas with Hematoxylin Eosin (HE) Staining at 400x Magnification.
Information: A. Shrimp hepatopancreas from Wonorejo ponds. B. Shrimp hepatopancreas from Medokan Ayu pond. x In images A and B, vacuolization (V) and necrosis (N) were found in the cells.

Table 4. Results of Vaname Shrimp Hepatopancreas Vacuolization Score

Damage	Shrimp from the Pond	Average ± SD	Percentage	Information
Vacuolation	Wonorejo (A)	2,56 ± 0,50	63,9%	Lots
	Medokan Ayu (B)	2,67 ± 0,47	66,7%	Lots

Superscript (*) in the same column indicates a significant difference (p<0.05)

Table 5. Results of Hepatopancras Necrosis Score of Vaname Shrimp

Damage	Shrimp from the Pond	Average ± SD	Persentage	Information
Nekrosis	Wonorejo (A)	2,11 ± 0,57*	52,8%	Lots
	Medokan Ayu (B)	3,56 ± 0,50*	88,9%	A huge amount

Superscript (*) in the same column indicates a significant difference (p<0.05)

Table 6. Results of Linear Regression Test of Water Physico-Chemical Factors on Total Vibrio in the Hepatopancreas of Vaname Shrimp

No.	Parameter	Linear Regression P Value	Pearson Correlation Coefficient
1	Temperature	0,827	-0,064
2	pH	0,219	0,219
3	DO	0,025*	-0,595
4	Salinity	0,054	0,525
5	TAN	0,560	0,171
6	Ammonia	0,020*	0,612
7	Nitrite	0,609	-0,150
8	Nitrate	0,054	0,525
9	Phosphate	0,275	-0,313
10	TOM	0,428	0,230

Description: Superscript (*) in the Linear Regression P Value column indicates a significant effect ($p<0.05$) on the total Vibrio in the hepatopancras of vaname shrimp.

DISCUSSION

Geographically, both stations receive their water from the same source: the sea, which flows into the same area. The ponds in Medokan Ayu are closer to settlements than the traditional ponds in Wonorejo and obtain water from smaller tributaries. In Wonorejo, water flows from a larger river and is located closer to the coastline. In the Medokan Ayu ponds, the water color ranges from brownish to greenish brown, with brownish predominating. This water color is influenced by the concentration of organic matter and plankton. In general, the water color does not indicate hazardous conditions and varies between green, brownish green, brownish, and greenish brown. Green indicates a high concentration of phytoplankton, which can support aquaculture productivity (Masithah, 2023). Meanwhile, brownish green indicates a balance between phytoplankton and zooplankton, which typically consist of Chlorophyta and Diatoms (Farabi & Latuconsina, 2023). Light brown water indicates a dominance of plankton from the genus Navicula sp. and Nitzschia sp., which are good for shrimp cultivation (Rauf *et al.,* 2024). The lowest temperature in the Wonorejo pond was recorded on day 10 at 27.8°C, and the highest temperature on day 30 at 32.9°C. In the Medokan Ayu pond, the lowest temperature was recorded on day 10 at 28.1°C, and the highest temperature on day 30 at 33.3°C. These temperatures are influenced by temperature fluctuations due to the dry season and the movement of monsoon winds from the east-southeast. These temperature fluctuations can disrupt the ecosystem balance and negatively impact shrimp production, as well as affect other water quality parameters, such as dissolved oxygen and pH (Putra *et al.,* 2024). The pH results showed that the lowest pH in the Wonorejo pond was recorded on day 10 at 6.9, and the highest pH on day 20 at 8.2.

In the Medokan Ayu pond, the lowest pH was recorded on days 40 and 65 at 8.1, and the highest pH was recorded on day 30 at 8.7. The pH in both ponds was outside the optimal range several times. The increase in pH often coincided with an increase in temperature. In the Medokan pond, on day 60, the pH did not keep pace with the increase in temperature, possibly due to the addition of water. A pH that is too low or too high can negatively impact the shrimp gut microbiota, reducing the growth and health of whiteleg shrimp (Yu *et al.,* 2020). For DO parameters, in the Wonorejo pond, the lowest was recorded on day 60 at 4.5 mg/L, and the highest was recorded on day 10 at 8.3 mg/L. In the Medokan Ayu pond, the lowest DO was recorded on days 30 and 65 at 3.2 mg/L, and the highest was recorded on day 10 at 9.2 mg/L. Based on the data, DO levels in both ponds were outside the optimal range several times. This is in line with the statement that increasing water temperature can reduce dissolved oxygen levels (Ariadi *et al.,* 2021; Rugebregt *et al.,* 2023). Low DO levels can cause stress in shrimp, inhibit their growth, and increase the risk of diseases such as bacterial and viral infections (Atthariq *et al.,* 2024). The lowest salinity in Wonorejo pond was recorded on day 20 at 30 ppt, and the highest salinity on day 65 at 44 ppt. In Medokan Ayu pond, the lowest salinity was recorded on day 10 at 36 ppt, and the highest salinity on day 60 at 65 ppt. Salinity in both ponds exceeded the optimal limit, even exceeding the shrimp's tolerance threshold in Medokan Ayu pond. This is likely due to the long dry season that causes seawater evaporation and the low frequency of water additions. This increase in salinity disrupts homeostasis, slows growth, and reduces survival rates (Long *et al.,* 2021).

TAN in Wonorejo ponds ranged from 0.042 to 0.802 mg/L, with the lowest value on day 50 and the highest on day 30. In Medokan Ayu ponds, TAN ranged from 0.010 to 0.303 mg/L, with the lowest value on day 20 and the highest on day 65. High TAN levels can be caused by the accumulation of organic matter due to lack of water recirculation. Most TAN levels in both ponds were within safe limits, except in Wonorejo ponds on day 30, which exceeded the threshold. This high TAN was likely caused by a decrease in water quality after partial harvesting and the influx of new water. In terms of ammonia parameters, Wonorejo ponds ranged from 0.001 to 0.022 mg/L, with the lowest value on day 65 and the highest on day 30. In the Medokan Ayu pond, ammonia levels ranged from 0.001 to 0.051 mg/L, with the lowest value on day 20 and the highest on day 10. This excess ammonia level is caused by high temperatures during the summer, which increase bacterial activity and nitrification. High ammonia levels can cause oxidative stress, decreased immunity, and susceptibility to pathogens, which can lead to shrimp mortality. Regarding nitrite parameters, nitrite levels in the Wonorejo pond were stable at 0.001 mg/L, except on day 30, when it reached 0.032 mg/L. In the Medokan Ayu pond, nitrite levels remained at 0.001 mg/L throughout the study. The increase in nitrite levels in the Wonorejo pond was likely caused by the addition of partial post-harvest water containing organic matter. Accumulated nitrite can negatively impact the growth and survival of whiteleg shrimp, as well as damage the shrimp's hepatopancreas. For nitrate parameters, levels in the Wonorejo pond ranged from 0.002 to 0.054 mg/L, with the lowest value on day 65 and the highest on day 30. In the Medokan Ayu pond, nitrate levels ranged from 0.002 to 0.052 mg/L, with the lowest value on day 20 and the highest on day 10. Nitrate levels in both ponds remained within safe limits. Nitrate is formed through the nitrification process and acts as a nutrient for plants and algae, but excessive levels can cause eutrophication, potentially harmful to shrimp (Patty, 2019).

For phosphate parameters, levels in the Wonorejo pond ranged from 0.014 to 0.180 mg/L, with the lowest value on day 50 and the highest on day 30. In the Medokan Ayu pond, phosphate levels ranged from 0.010 to 0.060 mg/L, with the lowest value on day 10 and the highest on days 20 and 30. Phosphate levels in both ponds were generally within safe limits, except on day 30 in the Wonorejo pond, which was likely caused by partial post-harvest water addition. Excessive phosphate levels can cause eutrophication, which is harmful to aquatic biota (Wulandari *et al.,* 2021). For the TOM parameter, levels in the Wonorejo pond ranged from 77.10 to 168.11 mg/L, with the lowest value on day 10 and the highest on day 65. In the Medokan Ayu pond, TOM levels ranged from 85.95 to 135.25 mg/L, with the lowest value on day 10 and the highest on days 20 and 65. TOM levels in both ponds exceeded safe limits. TOM fluctuations are caused by inadequate water management and irregular pond draining. High TOM levels can compromise shrimp health and support the growth of Vibrio bacteria, as seen in the TOM and total Vibrio graph trends in the Medokan Ayu pond.

Total Vibrio in the Wonorejo pond was 7.0 x 10^1 CFU/mL, while in the Medokan Ayu pond it reached 3.9 x 10^2 CFU/mL. These figures are still within safe limits but require caution. Measurements of total Vibrio in the hepatopancreas of whiteleg shrimp showed that in the Wonorejo pond, the total Vibrio range was 1.69 x 10^5 - 5.1 x 10^5 CFU/g, with the highest value on day 30. In the Medokan Ayu pond, the range was 1.0 x 10^6 - 2.7 x 10^6 CFU/g, with the highest value on day 65. This value exceeds the safe threshold, which can increase the risk of mass shrimp mortality. Poor water quality and weak shrimp immunity can contribute to high Vibrio counts. The clinical symptoms observed in whiteleg shrimp are consistent with previous studies, showing pale body color, empty intestines, and a pale hepatopancreas, along with a reddish telson. Histopathological observations of the whiteleg shrimp hepatopancreas showed organ damage indicative of bacterial infection, with signs of vacuolation and necrosis in the hepatopancreatic tissue from both ponds. This damage aligns with findings that Vibrio bacterial infection can cause severe necrosis and vacuolation due to enzymes and toxins that damage host tissue. If continued, necrosis can trigger mass shrimp mortality, as the hepatopancreas plays a crucial role in shrimp growth, digestion, and the immune system. The Kruskal-Wallis test showed a significant difference in necrosis conditions between the two ponds (P-value = 0.006).

The *Survival Rate* (SR) results showed a very low figure, indicating production failure, possibly influenced by the gradual daily increase in salinity (Nurhasanah *et al.,* 2021). Furthermore, SR can also be influenced by several factors, including fry quality, water quality factors, the presence of harmful pathogens, and poor pond management. The results of linear regression tests and Pearson's correlation coefficients showed that DO and ammonia significantly affected the total Vibrio in the hepatopancreas of whiteleg shrimp. The DO parameter had a P value of 0.025 and a Pearson correlation coefficient of -0.595, indicating a moderate negative correlation, while ammonia had a P value of 0.020 and a Pearson correlation coefficient of 0.612, indicating a strong positive correlation. These results support the finding that DO and ammonia have a strong correlation with the abundance of Vibrio bacteria, which thrive in environments with high nutrients and organic matter, and increase oxygen depletion in ponds (Ariadi *et al.,* 2021; Widigdo *et al.,* 2020).

CONCLUSION

Jumlah total *Vibrio* pada hepatopankreas udang vaname di tambak Wonorejo berkisar antara 1,69 x 10^5 – 7,20 x 10^5 CFU/gram, sedangkan di tambak Medokan Ayu berkisar antara 5,3 x 10^5 – 2,7 x 10^6 CFU/gram. Parameter oksigen terlarut (DO) dan amonia berpengaruh nyata terhadap total *Vibrio* pada hepatopankreas udang, dengan nilai p-value masing-masing 0,025 dan 0,020. DO menunjukkan korelasi negatif, sementara amonia menunjukkan korelasi positif.

ACKNOWLEDGEMENT

The author would like to express his gratitude to the Surabaya City Food Security and Agriculture Service (DKPP), the Wonorejo Tambak Makmur Surabaya Fish Farmers Group, the Podojoyo Surabaya Fish Farmers Group, and all parties who have helped in making this research a success.

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