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# The Relationship Between $NH_3$ And $NO_2$ Content and Bacterial Abundance Vibrio on Media Cultivation Shrimp Vaname (*Litopenaeus vannamei*)

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**Abstract**

*Indonesia, as an archipelagic country, holds great potential in marine and fisheries resources. Whiteleg shrimp (Litopenaeus vannamei) is one of the most economically valuable aquaculture commodities, with production reaching 5,812.2 thousand tons in 2020, an increase of 52.8% compared to 2015. However, disease outbreaks remain a major challenge in shrimp farming. One common disease is vibriosis, caused by Vibrio sp., an opportunistic, highly virulent, Gram-negative and facultative anaerobic bacterium. Therefore, early detection of Vibrio abundance is essential for prevention and control efforts. This Final Internship Project (KPA), titled "Abundance of Vibrio Bacteria in Whiteleg Shrimp Culture Media," aimed to determine the abundance of Vibrio sp. in shrimp ponds and analyze the influence of water quality parameters ($NH_3$, $NO_2$, and plankton). The internship was conducted from February 6 to April 17, 2025, at PT Parigi Aquakultura Prima, Central Sulawesi. Activities included water sampling, $NH_3$ and $NO_2$ testing, plankton density measurement, sterilization, TCBS media preparation, bacterial inoculation, and enumeration. Observations were carried out in six pond plots with weekly sampling from Day of Culture (DOC) 9 to 79. The regression model obtained was $Y = 164.808 + 153.951X_1 + 2092.103X_2 + 0.000X_3$, indicating that increasing $NH_3$ and $NO_2$ levels positively contribute to Vibrio abundance, each raising bacterial numbers by 153,951 and 2,092,103 units per one-unit increase, respectively. Plankton showed no significant effect. Thus, $NH_3$, $NO_2$, and plankton have partial influence on Vibrio abundance, although plankton contributes minimally.*

## Introduction

Indonesia is an archipelagic country that is very rich in natural resource potential. sea And fishery (Jayawibawa, 2024; Astari et al., 2024; Agung & Almubaroq, 2022). Shrimp vaname (Litopenaeus vannamei) is one of the national fisheries and marine sectors that is in high demand by consumers and has high value in international trade. World shrimp production has increased over the past two decades, reaching 9.5 million tons in 2020. In the same year, farmed shrimp production reached 61.5% of total shrimp production. Whiteleg shrimp (Litopenaeus vannamei) is the most economical shrimp species, with production contributing more than half of total global shrimp production. In 2020, total production shrimp vaname reach 5812.2 thousand tons, its value experienced an increase of around 52.8% compared to 2015 production (FAO, 2022). However, shrimp farming is currently under pressure to increase production to meet the growing demand for food as a result of the growing human population. However, in Cultivation activities sometimes encounter obstacles, namely the emergence of disease in shrim (Amelia et al., 2021; Sahidin et al., 2022; Zwetlana et al,. 2023).



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Diseases frequently found in fish and shrimp are caused by pathogenic microorganisms such as parasites, bacteria, and viruses, which are factors that contribute to the failure of shrimp farming, resulting in both social and economic losses (Admasu & Wakjira, 2021; Mondal et al., 2022; Kumar et al., 2021). One disease that frequently attacks cultivated whiteleg shrimp is vibrosis. This disease is opportunistic, highly virulent, and dangerous, and can lead to low survival rates in farmed animals. Most cases of this disease are caused by poor water quality (Sumini & Kusdarwati, 2020; Ariadi & Mujtahidah, 2022). Vibrio sp. bacteria are the cause. disease vibriosis Which Lots found on cultivation shrimp intensive systems (Aulia, 2019; Scabra et al., 2023; Sony et al., 2021). Vibrio sp causes mortality in mass shrimp farming because this bacteria is a type bacteria grams negative that nature facultative anaerobes (Mahulaw et al., 2020; Wahyuni et al., 2025; Widigdo et al., 2021).

The implementation of the shrimp farming system will have an impact on the farming environment (Al Eissa et al., 2022). specifically media Which in a way direct will influence animal Aquaculture includes shrimp. The intensive system for whiteleg shrimp cultivation not only has positive impacts, but also presents several drawbacks, such as the presence of high levels of organic matter due to the use of artificial feed and high stocking densities. Intensive whiteleg shrimp cultivation systems generally have an abundance of Vibrio bacteria (Said et al., 2024; Kurniawinata et al., 2021; Madusari et al., 2022).

By Because That, to minimize, control And prevent farmed shrimp attacked disease need done detection early abundance bacteria vibrio in cultivated waters. The condition of pond water quality will affect the condition and performance of the cultivated shrimp (Gao et al., 2016; Supriatin et al., 2024; Apresia et al., 2024). The water quality that is fluctuating will make shrimp easy experience stress consequence condition abnormal (Ariadi et al., 2019; Bian et al., 2025). Stressed shrimp are very susceptible to disease, one of which is vibriosis, a disease caused by the bacteria Vibrio sp (Babu et al., 2021).

Therefore, in this Final Practical Work (KPA) activity, the author has chosen the title "Abundance of Vibrio Bacteria in Vaname Shrimp Cultivation Media" (Litopenaeus vannamei) In Fishpond Intensive PT. Paris Aquaculture Prima Sulawesi “Center” to find out factors that influence bacterial abundance vibrio as well as relatedness between quality water with abundance bacteria vibrio.

As for objective from activity Work Practice End (KPA) This is as follows : 1) Calculating the abundance of Vibrio sp bacteria in vaname shrimp ponds (Litopenaeus vannamei) in the laboratory PT. Parigi Aquakultura Prima, Central Sulawesi; 2) Analyzing the influence of water quality parameters NH3, NO2 and plankton on the abundance of Vibrio sp. bacteria.

## Methods

### Time And Place


*Figure 1. Map location PT. Paris Aquaculture Prima*



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Work Practice End (KPA) implemented date 06 February sd 17 April 2025 located in PT. Paris Aquaculture Prima Village Lovebirds, Subdistrict Moutong, Parigi Moutong Regency, Central Sulawesi Province.

**Procedure Work**

***Means And infrastructure***

According to Arikunto & Yuliana (2012), the definition of facilities is anything that can facilitate and expedite the implementation of a business, which can be in the form of objects or money. According to Mulyasa, (2012:49) state means is equipment And equipment which are directly used and support the work process, especially the performance process, such as buildings, rooms, tables and chairs, as well as work tools and media. Meanwhile, infrastructure is all something Which is the main support for the implementation of a process. The facilities and infrastructure used in the laboratory PT. Parigi Pima Aquaculture, can seen on Table 1 and

Table 1. Facilities in the PT. PAP laboratory.

| No. | Facility | Specifications | Use |
|---|---|---|---|
| 1. | Electrical power supply (PLN) | - | Provides electrical power for the laboratory. |
| 2. | Generator | - | Serves as an emergency power source. |
| 3. | WiFi | - | Provides network access for laboratory computer equipment. |
| 4. | Computer | - | Used for inputting specific data in the laboratory. |

Table 2. Infrastructure in laboratory PT. PAP

| No | Infrastructure | Specification | Utility |
|---|---|---|---|
| 1. | Room quality water | | Room special water quality measurement. |
| 2. | Room microbiology | | Room special microbiological testing. |

**Tool And Material**

As for tool And material Which used in activity identification bacteria *Vibrio* sp in laboratory PT. PAP is as following :

Table 3. Tools used in the PT. PAP laboratory

| No. | Equipment | Function |
|---|---|---|
| 1. | Laminar Flow Cabinet | Protects samples from contamination. |
| 2. | Oven | Dry sterilization (specifically for petri dishes). |
| 3. | Incubator | Incubates microbes. |
| 4. | Autoclave | Wet and dry sterilization. |
| 5. | Duran Bottle | Container for media preparation. |
| 6. | Petri Dish | Container for bacterial cultivation. |
| 7. | Sample Bottle | Container for sample storage. |
| 8. | Bunsen Burner | Sterilizes bacterial inoculation tools. |
| 9. | Lighter | Used to ignite flame. |
| 10. | Hot Plate Stirrer | Heats and stirs media. |



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| | | |
|---|---|---|
| 11. | Magnetic Stirrer | Mixes media using a magnetic bar. |
| 12. | Spatula | Used to transfer materials during weighing. |
| 13. | Analytical Balance | Weighs materials accurately. |
| 14. | Micropipette | Transfers small sample volumes accurately. |
| 15. | Yellow/Blue Tips | Tips for micropipette to collect small sample volumes. |
| 16. | Microtube | Container for dilution samples. |
| 17. | Vortex Mixer | Rapidly mixes dilution samples. |
| 18. | Tube Rack | Holder/support for tubes. |
| 19. | Drigalski Spreader | Spreads samples on agar media. |
| 20. | Measuring Cylinder | Measures liquid volume. |
| 21. | Refrigerator | Storage for cooling samples. |
| 22. | Lab Coat | Protects the body from chemicals. |
| 23. | Colony Counter | Counts bacterial colonies. |
| 24. | Hand Counter | Counts bacteria and plankton manually. |
| 25. | Dropper/Pasteur Pipette | Transfers liquid samples drop by drop. |
| 26. | Microscope | Observes types and numbers of plankton. |
| 27. | Haemocytometer | Measures plankton density. |
| 28. | Cover Glass | Creates a thin (even) layer of sample under the microscope. |
| 29. | Test Tube | Holds and reacts chemicals in small scale. |
| 30. | Syringe | Transfers samples accurately into test tubes. |

**Step Work**

Work steps are stages that are carried out sequentially, with objective For finish something work. As for step Work which was carried out to determine the relationship between ammonia (NH 3), nitrite (NO 2) and plankton with The abundance of *Vibrio sp* bacteria can be seen as follows:

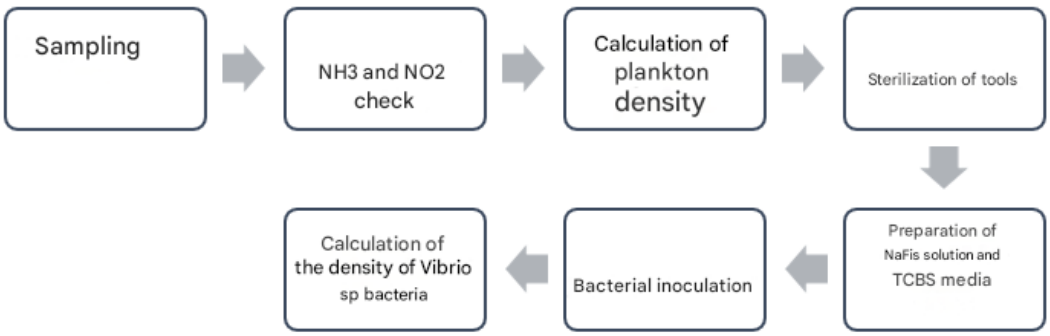


*Figure 2. Steps Work*

**Quality Water**

***Ammonia ($NH_3$)***

To determine the concentration of ammonia ($NH_3$) in the culture water, the test begins with water sampling at PT. Parigi Aquakultura Prima. Sampling is conducted every Sunday at 06.00 WITA at the midpoint of the pond water, after which the collected water is immediately placed into a sterile bottle to prevent contamination. The sample is then transferred into a chemical



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reaction tube using a 5 ml syringe. Two scoops of ammonia test powder are added to the sample, followed by homogenization to ensure the reagent is well mixed. The homogenized sample is then transferred into a reagent bottle, where the resulting color change is compared with the reference chart provided in the test kit. Finally, the ammonia ($NH_3$) level is recorded according to the matching color scale obtained.

***Nitrite ($NO_2$)***

To measure the nitrite ($NO_2$) concentration in the culture water, sampling is conducted once a week at 06.00 WITA at the midpoint of the pond water at PT. Parigi Aquakultura Prima. The collected water is placed into sterile bottles to maintain sample integrity and avoid contamination during handling. The sample is then transferred into a chemical test tube using a 5 ml syringe. Two spoons of nitrite test powder are added to the sample, after which the mixture is homogenized to ensure proper reagent reaction. The homogenized sample is subsequently poured into a reagent bottle, and the resulting color is compared against the color reference chart provided in the nitrite test kit. The nitrite ($NO_2$) concentration is determined based on this color comparison and recorded as the final measurement result.

***Plankton***

To identify the type and calculate the density of plankton present in the culture pond, a specific examination procedure is carried out. Plankton sampling at PT. Parigi Aquakultura Prima is performed once a week at 06.00 WITA by collecting water from the midpoint of the pond and placing it into sterile bottles. Prior to testing, a dropper and distilled water are prepared in a chemical reaction tube. The water sample is then transferred into a test tube using a 5 ml syringe. A small portion of the sample is taken using a dropper and carefully placed on a hemocytometer. The hemocytometer is covered with a cover glass to protect the microscope lens from direct contact with the sample and to create a thin, even layer that facilitates observation.

Microscopic examination is conducted using 10X and 4X magnification to observe the plankton within the sample. The type of plankton observed is identified, and plankton density is counted using a hand counter. The final number representing plankton density is recorded as the observation result.

**Bacteria *Vibrio* sp**

***Sterilization Tool***

Sterilization of laboratory tools is carried out to eliminate harmful microorganisms and ensure that all equipment used during the research process remains free from contamination. The procedure begins with soaking petri dishes in an anti-germ solution for approximately four hours to prevent the growth of pathogenic bacteria during culture activities. After soaking, the disinfectant solution is replaced with clean water mixed with sunlight detergent, and the petri dishes are scrubbed using a sponge. Once cleaned, the dishes are rinsed with fresh water, dried thoroughly, and wrapped in newsprint. The wrapped petri dishes are then placed in an oven for dry sterilization, which is performed for 30 minutes at 175°C. Following sterilization, the petri dishes are allowed to cool before agar media is poured into them.

Sterilization of other laboratory tools, such as microtubes and yellow or blue pipette tips, is conducted using an autoclave. The tips and microtubes are arranged neatly in a single plastic container and placed in the autoclave basket. Prior to operation, it is ensured that the distilled water level in the autoclave is within the recommended range not too little or excessive. The autoclave is then set to tool sterilization mode at 121°C for 15 minutes. After the process is



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complete, the equipment is not removed until the internal pressure has decreased to match room pressure. Once stabilized, the safety valve is opened and the sterilized items are taken out. For Drigalski spreaders, sterilization is performed differently by rinsing them with ethanol and heating them over a Bunsen burner immediately before use.

***Making Nacl PHYSIOLOGICAL 85% (1000 Ml)***

Preparation of the solution begins by weighing 0.85 grams of Sodium Chloride (NaCl) using an analytical balance. The measured NaCl is then dissolved in 100 ml of distilled water inside an Erlenmeyer flask. After the solution is well mixed, the flask is covered with cotton wrapped in aluminum foil and secured with a rubber band to prevent contamination. The solution is then sterilized using an autoclave at 121°C for 15 minutes.

***Making Media TCBS***

The preparation of TCBS media begins by adding 1000 ml of distilled water into a previously sterilized container. Thiosulfate Citrate Bile Salt Agar (TCBS) media and 15.2 grams of NaCl are then measured and added into the container containing the distilled water. A magnetic stirrer bar is placed inside the Duran bottle to ensure that the solution can be stirred and homogenized properly. The container is covered and heated on a hot plate at 200°C until the mixture reaches a boiling point. Once boiling, the solution is removed and allowed to cool inside the laminar flow cabinet until it reaches approximately 50°C, reducing the risk of contamination. After reaching the desired temperature, 15 ml of TCBS media is poured into each petri dish and left undisturbed until the medium solidifies.

When the media has completely hardened, the petri dishes are stored in a cooling cabinet with the plates positioned upside down to maintain quality and prevent condensation from dripping onto the agar surface.

***Inoculation Bacteria***

Before conducting bacterial enumeration, each petri dish is labeled to facilitate identification during the counting process. A 100 µl (0.1 ml) sample is then taken using a micropipette and carefully placed onto the prepared TCBS media. The sample is evenly spread across the surface of the agar using a sterilized Drigalski spreader that has been flamed over a Bunsen burner. Following inoculation, the petri dishes are incubated at 30°C for 24 hours with the plates positioned upside down to prevent condensation from dripping onto the media surface.

***Count Colony Bacteria***

After a 24-hour incubation process, bacterial colonies will form in each petri dish. The number of Vibrio bacteria is calculated using the plate count method or Total Plate Count (TPC), which is counting the number of colonies that grow on TCBS agar media in each petri dish. In accordance with Hartati's opinion (2013), the calculation of the number of Vibrio bacteria is carried out using the plate count method or Total Plate Count (TPC). colony bacteria done on cup Which there is 25 until 250 colony.

**Method Retrieval Data**

According to Windasari (2018), primary data refers to information obtained directly from the original source, either individuals or objects being studied, through activities such as interviews, surveys, and experiments. In this research, primary data collection was carried out through several methods. Observation was conducted by monitoring various activities in the laboratory of PT. Parigi Aquakultura Prima, including the measurement of water quality parameters, especially chemical and biological indicators. Interviews were also held to gather



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additional information related to the relationship between water quality and the abundance of Vibrio sp. bacteria. Hands-on practice was performed by actively following and carrying out laboratory procedures similar to the observation stage, involving direct participation in measuring chemical and biological parameters. In addition, documentation in the form of photographs was collected as supporting evidence during the Final Internship Work (KPA) at PT. Parigi Aquakultura Prima.

**Data Secondary**

Secondary data is data obtained from sources other, such as books and other reading materials related to the problem being researched (Puspita, 2013; Pratikno et al., 2022). This data was obtained from literature studies, the internet and libraries related to practice Which done. Data secondary Which in take covering aspect techniques, financial aspects and general data on the practice location and other data that supports the Final Practical Work Report (KPA).

**Analysis Business**

***Income***

Income is incoming or outgoing costs which in get from sales results. The formula for determining income according to Syahrir (2020):

$$\text{Income} = \text{Total receipts} - \text{Total expenses}$$

***Break event point (BEP)***

Break even point is a break-even point analysis with a calculation formula according to Syahrir (2020):

$$\text{Production BEP} = \frac{\text{Total Cost}}{\text{Sauan price}}$$

$$\text{Production BEP} = \frac{\text{Total Cost}}{\text{Sauan price}}$$

***Benefits Cost Ratio (T/C Ratio)***

B/C Ratio is a comparison between the costs incurred and the expected benefits from one production period.

$$\text{B/Cratio} = \frac{\text{Number of receipts}}{\text{Total Production Cost}}$$

***Flower Capital***

Capital interest is a reward for the owner of the production factor who provides the capital or using the capital in something activity effort. The point is is owner capital as owner factor production, will get Capital interest, due to the use/lending of funds as business capital. The method for calculating capital interest is as follows:



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**Cost Still + Cost No Still + Cost Depreciation x with ethnic group the bank interest rate in effect at that time.**

***Pay Back Period (PP)***

Payback period (PP) is a calculation of the investment return period with profits or referred to as the return of benefit value with cost value with the calculation formula according to Syahrir (2020) as follows:

$$PP = \frac{\text{Total investment}}{\text{Net profit}} \quad \text{x 1 year}$$

**Data Analysis**

The data analysis methods used are divided become two, that is method analysis inferential (regression linear (multiple) is used to determine the extent to which the independent variable influences the dependent variable. The next analytical method is quantitative descriptive analysis, which describes, shows, or summarizes data in a constructive manner. One such quantitative descriptive analysis method is the correlation method, which is used to describe the relationship between variables.

According to Miftahuddin et al., (2021), the correlation coefficient value to determine the relationship between variables can be seen in the following table:

Table 4. Coefficient criteria correlation

| No. | r Value Range | Interpretation |
|---|---|---|
| 1. | 0.00 – 0.199 | Very low |
| 2. | 0.20 – 0.399 | Low |
| 3. | 0.40 – 0.599 | Moderate |
| 4. | 0.60 – 0.799 | Strong |
| 5. | 0.80 – 1.000 | Very strong |

## Results and Discussion

**Activity Technical**

***Factor Which Influence Abundance Bacteria Vibrio sp***

**Ammonia ($NH_3$)**

***Retrieval Sample Ammonia ($NH_3$)***

Water sampling at PT. Parigi Aquakultura Prima is carried out once a week at 06.00 WITA at the midpoint of the pond water, using Secchi disk Then entered to in bottle sterile. Next, the sample is taken to the laboratory for water quality analysis.

**Measurement Ammonia ($NH_3$)**

The concentration of ammonia (NH 3) was measured using the Salicylate test kit method. Then use mark phosphate ($PO_4$) as proxy. Matter This based on on the fact that both phosphate and ammonia are the same organic material decomposition products (Hamuna, et al., 2018). The method for measuring NH3 is to separate sample water on tube reaction chemistry use syringe as much as 5 ml, add 2 scoops of nitrate test powder to the sample and mix. Next, transfer the



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mixed water sample to a reagent bottle. Match the color of the water sample in the reagent bottle to the test kit booklet, then record the ammonia (NH3) level measurement results

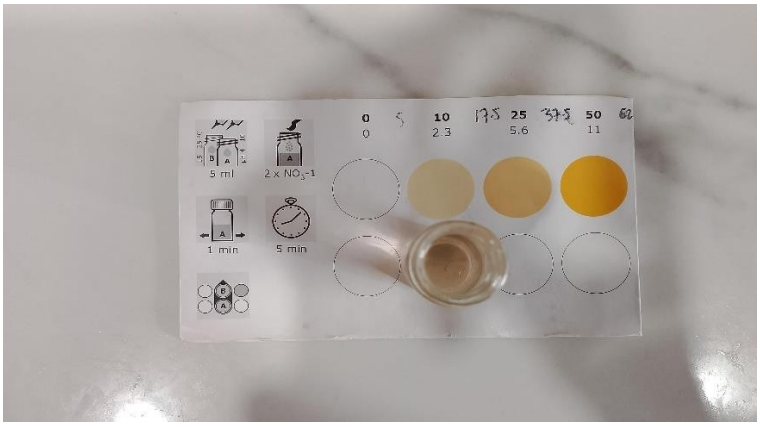


*Figure 3. Checking NH3 use test kit*

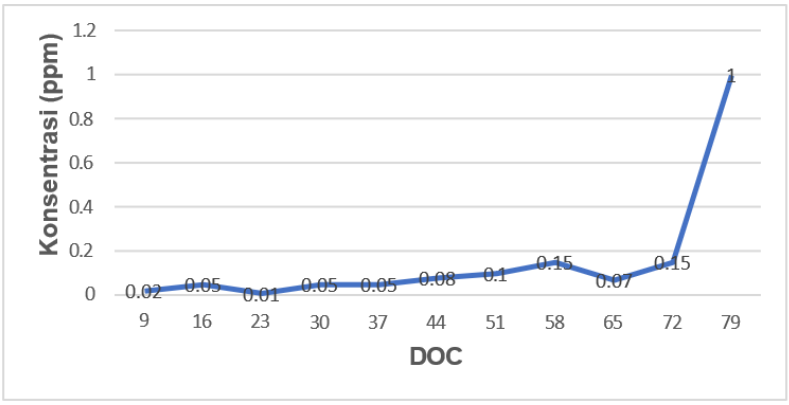


*Figure 4. Graphs total concentration $NH_3$*

Based on the graph presented, there is a fluctuation in ammonia (NO 3) levels during the maintenance period. In DOC 9, the ammonia levels in the media cultivation is at on mark 0.02 ppm, Then on moment enter DOC 16 saw an increase and then decreased again when entering DOC 23. Ammonia levels continued to spike when entering DOC 30 until DOC 44. Then, on DOC 51 its value return experience decline and reach mark highest on DOC 79 with value 1 ppm. Hal This This is caused by the accumulation of excessive, incompletely decomposed organic matter. This condition is a key factor supporting the growth and abundance of Vibrio sp. bacteria. Vibrio bacteria are known to be highly adaptable to environments rich in organic matter and poor water quality, such as high levels of NH3. and low dissolved oxygen.

When the NH 3 level increases, the stress on the shrimp will also increase. This will weaken the shrimp's immune system and become more susceptible to Vibrio bacterial infection. Therefore, the graph not only reflects changes in water chemistry but also signals the potential risk of increased abundance of pathogenic bacteria in the pond. This emphasizes the importance of water quality management, reducing ammonia accumulation, and monitoring population bacteria in a way routine as part from strategy prevention of internal diseases fish farming. (Preena, et al., 2021). Then, on The final DOC ammonia concentration value decreased and was at 17.5 ppm.

Although chemically $NH_3$ can be toxic at high concentrations, in aquaculture ecosystems this compound still plays an important role as the initial substrate in the nitrogen cycle. Ammonia



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(NH 3) is oxidized to nitrite (NO ₂) by bacteria nitrification. Compound nitrite here it is Which used by bacteria heterotrophic like Vibrio (Preena, et al., 2021). As for mark The maximum threshold for ammonia (NH 3) based on the company's SOP is ≤ 1 ppm.

**Nitrite ($NO_2$)**

***Retrieval Sample Nitrite (NO2)***

Water sampling at PT. Parigi Aquakultura Prima is carried out once a week at 06.00 WITA at the midpoint of the pond water, using Secchi disk Then entered to in bottle sterile. The samples were then taken to the laboratory for water quality analysis. Samples for nitrite ($NO_2$) were taken simultaneously with samples for ammonia (NH3). The water samples for Vibrio testing were then transferred into sample bottles, while those for plankton testing were transferred into samples. And parameter quality water chemistry heeded to in tube reaction chemistry.

***Measurement Nitrite ($NO_2$)***

To determine the nitrite (NO2) levels in a cultured water sample, a specific test is required. The steps involved are separating a 5 ml water sample into a chemical test tube using a syringe. Add 1 scoop of nitrite test powder to the sample and mix thoroughly. Next, transfer the sample to a 5 ml container. sample Which has in homogenize to in bottle reagent And Match the color of the sample water in the reagent bottle with the test kit paper, then record the NO 2 level measurement results. NO ₂ data is also represented using phosphate as a proxy.

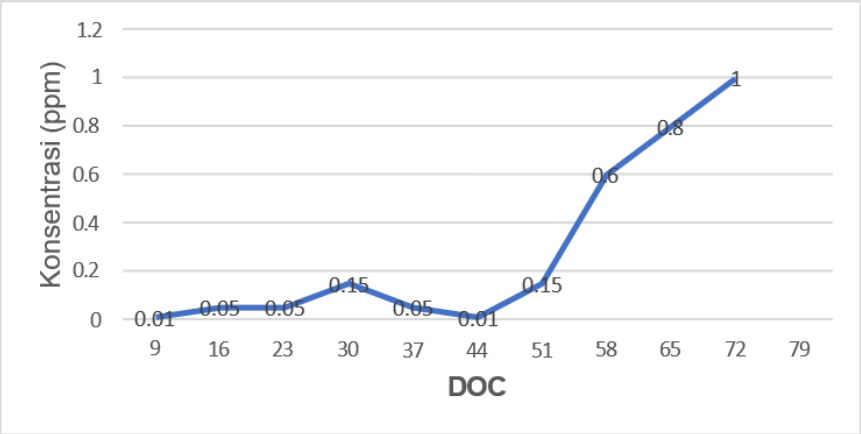


*Figure 5. Graphs total concentration $NO_2$*

Chart in on describe improvement level nitrite (NO 2) in The increasing age of the shrimp culture ponds directly affects the quality of the aquatic environment. The increase in NO2 levels in DOC 9 was 0.01 and continued to increase. on moment enter DOC 16 until DOC 30. Then, Upon entering DOC 37, nitrite levels decreased to 0.01. This occurred because water quality management had been carried out through water changes and siphoning of the bottom of the cultivation pond.

Furthermore, at the end of the cultivation period, namely from DOC 51 to DOC 79, nitrite levels continued to increase, this was caused by the accumulation of leftover feed, feces and carcass plankton shrimp as well as bacteria Which There is on base pool. As for The maximum threshold value of nitrite (NO 2) concentration based on the company's SOP is ≤ 1 ppm.



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**Plankton**

***Retrieval Sample***

Samples for checking plankton are taken the same as water quality samples. chemistry, every Morning day One time a week that is on o'clock 06.00 WITA. Samples were taken using a Secchi disk and placed into sterile bottles. sample water for plankton moved to in tube reaction chemistry.

***Checking Type And Plankton Density***

Plankton types were identified using a microscope based on their morphological characteristics, while plankton density was calculated in units of cells/ml using a hemocytometer under a microscope.

***Calculation Amount Density Plankton***

Calculation of the density of plankton is done by taking a small sample of water in a chemical test tube using a dropper and then placing it on a hemocytometer. Then, cover the hemocytometer using a cover glass to protect the microscope lens from direct contact with the sample. Observe and calculate the number of plankton. density plankton use microscope with magnification 10X and 4×. Then, the number of individuals of each type visible under the microscope was converted to the volume of the sample water.

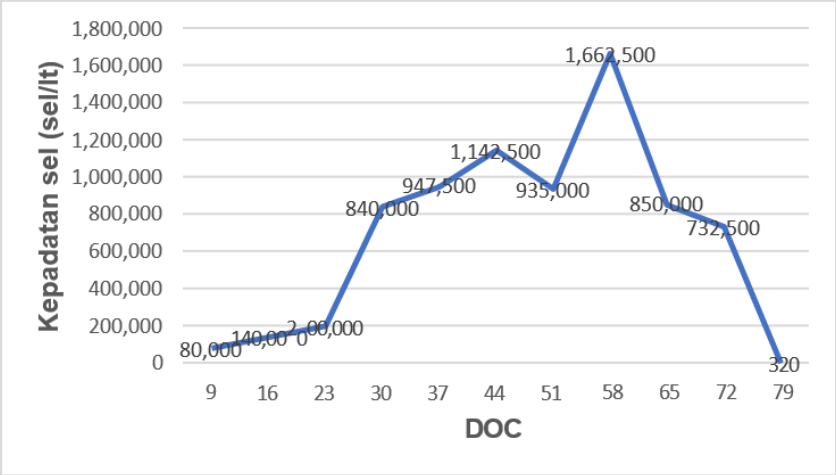


*Figure 6. Total density plankton*

Based on the graph presented, there is a fluctuation in the total number of plankton. in shrimp cultivation media. On DOC 9 to DOC 23 Plankton abundance is relatively low and is still relatively stable, but is starting to increase on moment enter DOC 30 until DOC 37. Furthermore, on upon entering DOC 44 the amount increase And return experience decline in DOC 51. Then, it increased sharply when entering DOC 58 with a total plankton count of 1,663,500 cells/liter. The increase in plankton numbers in the cultivation pond was caused by the daily addition of dolomite lime. This phenomenon indicates that plankton abundance is greatly influenced by environmental factors, nutrition, and possibly other biotic activities occurring in the pond. In addition, That, plankton can increase level oxygen dissolved (dissolved oxygen) through photosynthesis, creating optimal conditions for microbial metabolism. However, from DOC 65 to DOC 79, there was a drastic decrease, meaning plankton death occurred.



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In relation to the abundance of Vibrio bacteria, these fluctuations in plankton can be an important indicator. Plankton are part of the aquatic food chain and can act as hosts or competitors for bacteria. When plankton abundance is high, competition for nutrients can increase, suppressing Vibrio populations. Conversely, when plankton abundance is low, space and nutrients are available. available can utilized by Vibrio For develop breed more fast. Moreover, mass plankton death can increase dissolved organic matter in the water which is an ideal growth substrate for Vibrio p. Therefore That, monitoring abundance plankton in a way periodically very important in the management of water quality in aquaculture ponds to control the population of pathogenic bacteria such as Vibrio. (Azam et al., 1983; Kirchman, 2010; Stabili et al., 2022)

**Isolation Bacteria *Vibrio* sp**

Before isolation activities, it is necessary to prepare the equipment that will be used to support the success of bacterial isolation activities. All equipment to be used must be washed and cleaned first using a soapy solution and scrubbed with a sponge. The inside of the equipment Sample bottles and drums are cleaned using a bottle cleaner, a small, elongated brush. The soaped equipment is then rinsed under running water until clean and free of residue.

Meanwhile, the equipment in the form of a petri dish is soaked using anti-german for ±4 hours with the aim of preventing the emergence of pathogens from bacteria on cup used planting bacteria. Cup petri which has been washed and then dried in a draining basket lined with cloth in a room equipped with air conditioning and UV lights, then the sterilization stage is carried out.

**Sterilization Tool**

Sterilization is the process of cleaning all equipment to kill all organisms. According to Heliyana and Rismayani (2022), sterilization is a process using specific methods that results in a state where no living microorganisms can be detected. The sterilization process for equipment is as follows:

***Sterilization Dry (Dry sterilization method)***

Dry sterilization is a sterilization method using an oven. The dry sterilization method is used on laboratory equipment that cannot be wet and equipment that will not melt, burn, or change shape when exposed to high temperatures. Ovens can be used to sterilize petri dishes and volumetric pipettes (Kharisma and Manan, 2012).

Dry sterilization is performed using a special oven for sterilizing petri dishes. These are dried and wrapped in newspaper, then placed in the oven and heated to 170°C for 18 minutes. This is consistent with the opinion expressed by Ikenganyia. et al., 2017 that recommendation temperature And duration time Laboratory drying oven for sterilization of laboratory equipment is 170°C for 18 minutes, 180°C for 7.5 minutes and 190°C for 1.5 minutes.

This method uses very high temperatures and a long time to kill or eliminate pathogens that cause contamination in tissue cultures, such as bacteria and fungal spores. The oven works using heat conduction, first... formerly heat surface part outside equipment, Then absorb hot And move it to part middle tool (Alkhadim, 2018).

***Sterilization Wet (Steam sterilization method)***

The wet sterilization method is performed using an autoclave operated with steam under pressure (Misra, 2012). The standard temperature and pressure required for the autoclave sterilization process are carried out at a high temperature for a short period of time, which is



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preferred over a lower temperature for a longer period. Some standard temperatures or pressures used are 115°C/10 psi, 121°C/15 psi, and 132°C/27 psi (psi = pounds per square inch).

Sterilization using an autoclave is divided into two types: liquid and soil sterilization. For sterilization of yellow tip, blue tip, and media TCBS and NaFis liquid were carried out using an autoclave for 15 minutes at a temperature of 121°C. This is in accordance with the opinion of Gupta and Shukshith, 2016, who stated that However, the temperature and pressure generally used are 121°C/15 psi. The time setting commonly used with this wet sterilization method is 10-15 minutes. These conditions are very effective in killing bacteria and fungal spores (Ikenganyia et al., 2017). The important thing to note in this sterilization process is that the sterilization time is calculated after the autoclave reaches condition normal that is on temperature 121°C And pressure 15 psi, No starts when pressing the "on" button (Gupta and KS, 2016).

***Use Ethanol 96%***

Ethanol 96% is used specifically for Driglaski sterilization. Ethanol is highly volatile and flammable, requiring careful and concentrated combustion to minimize risks (Misra and Misra, 2012). Before inoculating bacteria, Driglaski is dipped in 96% ethanol and then heated over a Bunsen burner to kill microorganisms. The Driglaski sterilization process is usually carried out in a laminar air flow (LAF). The use of ethanol with a concentration of 70-96% is effective against pathogens (Auliya et al., 2019).

**Making Media TCBS**

According to Ihsan & Retnaningrum, 2017, Thiosulfate citrate bile salt (TCBS) media is a selective medium that is able to inhibit unwanted bacteria, media This differential for growth bacteria vibrio so that Bacterial colonies growing on colored media (yellow, orange, green, and bluish green), in the form of circular colonies, and entire colony edges. TCBS media is made by adding 1000 ml of distilled water, then weighing 88 ml of TCBS. gr and 15.2 gr NaCl is then added to

in sterilized durian. The magnetic stirrer rod is inserted into the durian, then covered with cotton and aluminum foil and secured with a rubber band. The durian is then heated on a hot plate by pressing the on/off button on the hot plate and then adjusting the temperature and speed of the magnetic stirrer by turning the control. The medium is heated until it boils. After boiling, the hot plate is turned off and the durian is removed. and left alone to be cooled until temperature 50 o C then poured in a sterile petri dish as much as 15ml/cup. Leave the petri dish until the medium is dry There is in inside become hard And congested, Then insert to in the refrigerator in an upside down condition.

**Inoculation Bacteria**

Bacterial inoculation was carried out in the laboratory of PT. Parigi Aquakultura Prima using the spread plate method. The spread plate method is a method for inoculating bacteria by spreading them on the surface of solid media using a glass rod to spread the bacterial cells. on surface medium so that (Rosmania et al., 2023). Inoculation Bacteria were inoculated in a laminar to reduce the risk of contamination, inoculation was carried out without dilution (100), where 100 µl (0.1 ml) of water sample was taken using a micropipette and inserted into a petri dish containing TCBS agar media, then spread or leveled using driglaski. Incubation done with position inverted cup at 30°C for 24 hours.



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**Calculation colony bacteria**

Bacteria Which grow after process incubation during 24 O'clock will counted using a colony counter by marking and counting all bacterial colonies on the plate, then calculated using the Total Plate Count (TPC) formula to determine the total number of Vibrio sp. Bacterial colony counting is a method used to determine the number of bacterial colonies found in a medium (Rosmania & Yanti, 2020).

Based on results isolation bacteria vibrio sp Which has done on the media TCBS, seen existence bacteria with two type characteristics colony different that is colored yellow (yellow) And green (green). In accordance with explanation Ambat et al., (2022) That colony bacteria Vibrio colored green is caused by characteristic bacteria Which No capable ferment sucrose, whereas colony Vibrio yellow Which capable ferment sucrose on TCBS agar media and is also able to lower the pH on TCBS agar media. The results of the total calculation of Vibrio sp bacteria can be seen in the table:

Table 5. Results of *Total Plate Count* (TPC) calculations

| No. | Total Vibrio | Yellow | Green | Total Count (≤3.100 / ≤3.000 / ≤100) |
|---|---|---|---|---|
| 1 | 9 | 20 | 0 | 20 |
| 2 | 16 | 40 | 0 | 40 |
| 3 | 23 | 80 | 20 | 100 |
| 4 | 30 | 300 | 0 | 300 |
| 5 | 37 | 440 | 0 | 440 |
| 6 | 44 | 560 | 0 | 560 |
| 7 | 51 | 1080 | 0 | 560* |
| 8 | 58 | 830 | 5 | 835 |
| 9 | 65 | 750 | 40 | 790 |
| 10 | 72 | 678 | 11 | 689 |
| 11 | 79 | 490 | 70 | 1080 |

Based on table on can seen that, on sample water colony composition bacteria vibrio colored yellow (yellow) more dominate compared to green vibrio. According to Sarinda (2010), the presence of green bacteria colony colored yellow Which more tall compared to colony green not harmful for shrimp Because group bacteria colony green Which is a characteristic from bacteria vibrio reason disease vibriosis like Vibrio Harvey

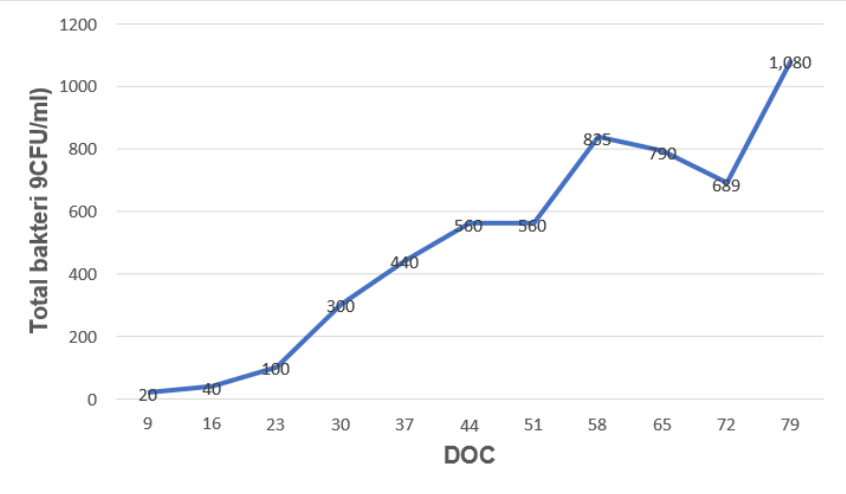


*Figure 7. Total bacteria Vibrio sp*



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It can be seen that there is a fluctuation in the total abundance of Vibrio sp bacteria during the cultivation of whiteleg shrimp (Litopenaeus vannamei). This is influenced by the water conditions of the culture media during maintenance, fluctuations in the abundance of Vibrio sp bacteria in pond waters fluctuate due to the dynamics of the dynamic pond aquatic ecosystem (Madusari et al., 2022).

Vibrio sp bacteria in shrimp cultivation media is not affected by the age of the shrimp being cultivated, this is in accordance with the statement (Ariadi & Mutjahidah, 2022) that the abundance of Vibrio sp bacteria fluctuates dynamically according to physiological adaptation factors to the surrounding environmental conditions and the level of biological metabolism of the bacteria themselves. However, in The graph above shows that the abundance of Vibrio sp bacteria increase Enough rapidly on time end cycle cultivation. Matter This caused by level burden waste in waters cultivation Keep going increase along with the increase in the cultivation inputs provided. According to Aisyah et al., (2022) the increase in the number of V. alginolitycus, V. iimicus, V. fluvialis bacteria in the cultivation media shrimp vannamei is impact from material organic And high nutrient levels in the pond. Efforts to manage water quality are carried out during process cultivation that is with method do siphoning, water changes and probiotic administration are carried out with the aim of reducing the amount of organic material and nutrients in the cultivation media and suppressing the growth of Vibrio sp. bacteria.

Vibrio bacterial counts fluctuate significantly during shrimp culture at various stages of life. Early in the culture period, from DOC 16 to DOC 58, bacterial counts increase steadily as leftover feed and waste accumulate, providing a nutrient source for the bacteria. Then, from DOC 65 to DOC 72, the total bacterial count shows a gradual downward trend, indicating improvements in management. pool, Which impact positive towards control number of bacteria. Furthermore, when entering DOC 79 there was the highest spike in the overall chart Which signify that indicator condition pool Which worsening again and increasing stress on shrimp.

Thus, fluctuations in the number of Vibrio bacteria are closely related to change condition environment pool And management maintenance. Critical points such as those in DOC 79 are of significant concern because they indicate a drastic increase in the bacterial population and have the potential to harm shrimp health.

Factors Which influence abundance This between other is water quality like content material organic, temperature, salinity, And level nutrients. Besides In addition, interactions between organisms in the pond ecosystem also affect population dynamics. bacteria Vibrio sp. And in support by factor external, like weather and the provision of probiotics also plays a role in influencing the number of Vibrio sp bacteria during the cultivation process.

***Influence $NH_3$, $NO_2$ And Plankton to Abundance Bacteria Vibrio sp***

Analysis of the relationship between water quality parameters such as ammonia (NH 3), nitrite (NO 2) And plankton with abundance bacteria Vibrio done For to determine the extent of each parameter's contribution in influencing the growth of these bacteria in the maintenance media for whiteleg shrimp (Litopenaeus vannamei). This type of parameter was chosen because it is ecologically closely related to the dynamics of bacterial populations in cultivation waters, particularly in whiteleg shrimp ponds.

$NH_3$ and $NO_2$ are nitrogen compounds resulting from the decomposition process of organic materials. organic And remainder metabolism shrimp. On level certain, second the compound can become poison for shrimp Which cultivated, but Also is an indicator of high nutrient loads



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in water. This condition can indirectly increase the growth of Vibrio sp., a heterotrophic bacterium that utilizes organic matter and nutrients for its reproduction.

Meanwhile, plankton, particularly phytoplankton, serve as the most crucial component in maintaining the balance of the pond ecosystem. However, excessive plankton can trigger blooms, ultimately leading to a decrease in dissolved oxygen and an increase in organic matter when large numbers of plankton die. This creates highly favorable conditions for the massive growth of Vibrio sp. bacteria.

To determine the relationship between these parameters and abundance bacteria Vibrio sp, so done analysis correlation And regression

using the application IBM SPSS25. Correlation used For see to what extent connection between variables without notice connection causality directly, whereas regression used For predict influence each variable ($NH_3$, $NO_2$, and plankton) on the abundance of Vibrio sp. bacteria. The results of the correlation test will provide an overview of the strength and direction of the relationship. between parameter quality water with amount bacteria. With Thus, the graph correlation Which will served furthermore become base important in determining strategy management quality water use pressing growth bacteria Vibrio sp in system cultivation intensive.

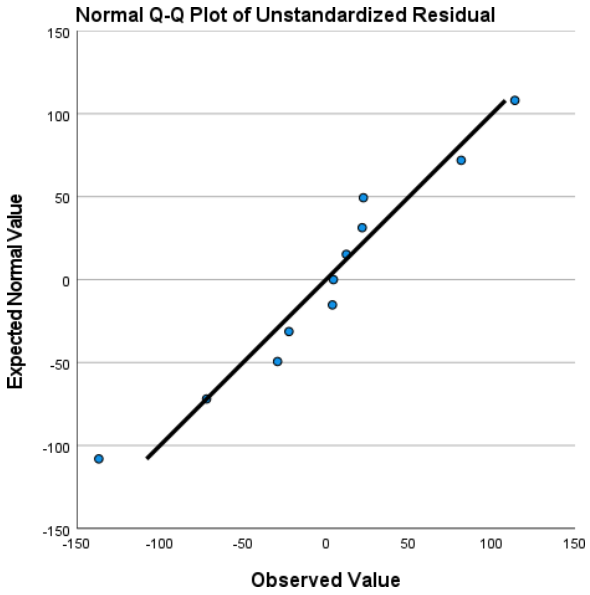


*Figure 8. Line correlation $NH_3$, $NO^2$, Plankton to Vibrio Sp*

In Figure X, it can be seen that the points mostly follow the diagonal line, indicating that the residual distribution of the regression model approaches a normal distribution. This graph is the result of a normality test using the QQ Plot (Quantile-Quantile Plot) method, which compares the cumulative distribution of observed residual data (X-axis) with the expected cumulative distribution of a normal distribution (Y-axis).

If the data points consistently follow a straight line, it can be concluded that the residuals are normally distributed, which is an important assumption in linear regression analysis. Although there are slight deviations in the middle to upper part of the graph, the overall pattern of the points indicates that the assumption of normality residual is still acceptable. this supports the validity model regression Which displayed on table summary model, in where mark Adjusted R Square as big as 0.962 show that model



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capable explain variation variables dependent as big as 96.2%, And the remaining 3.8% is explained by other factors outside the model.

As for analysis regression Which done on influence $NH_3$, $NO_2$ and plankton on the abundance of Vibrio sp bacteria using the SPSS25 application which produces a multiple linear regression equation model as follows:

Table 6. Results regression analysis multiple linear $NH_3$, $NO_2$, Plankton to Vibrio

| Model | Unstandardized Coefficients | | Standardized Coefficients | | |
|---|---|---|---|---|---|
| | B | Std. Error | Beta | t | Sig. |
| 1 (Constant) | -43,041 | 50,621 | | -,850 | ,423 |
| $NH_3$ | 473,142 | 135,807 | ,384 | 3,484 | ,010 |
| $NO_2$ | 500,838 | 108,251 | ,505 | 4,627 | ,002 |
| Plankton | ,000 | ,000 | ,632 | 8,509 | ,000 |
| a. Dependent Variable: *Vibrio* Sp | | | | | |

Based on the results of the multiple linear regression analysis shown in the table, known that mark constant as big as -43,041 And coefficient regression for each variables free is as following : $NH_3$ (X1) as big as 473.142, $NO_2$ (X2) of 500.838 and Plankton (X3) of 0.000. Thus, the linear regression equation model obtained is:

$Y = -43,041 + 473,142X_1 + 500,838X_2 + 0.000X_3$.

This equation shows that increasing levels of $NH_3$ and $NO_2$ have a positive impact on increasing the abundance of Vibrio sp. bacteria. This means that every one-unit increase in $NH_3$ will increase the abundance of Vibrio Sp by 473.142 and every one-unit increase in $NO_2$ will increase abundance as big as 500,838. Temporary That, variables plankton does not contribute to the increase because the coefficient value is 0.000.

Table 7. Results Test t NH 3, NO 2, Plankton to Vibrio

| Model | Variable | t | Sig. |
|---|---|---|---|
| 1 | (Constant) | -,850 | ,423 |
| | $NH_3$ | 3,484 | ,010 |
| | $NO_2$ | 4,627 | ,002 |
| | Plankton | 8,509 | ,000 |

The significant value (Sig.) for the three variables is below the significance threshold of 0.05. Thus, it can be concluded that $NH_3$, $NO_2$ and plankton are partial influential significant to abundance Vibrio sp. Matter This

reinforced by the calculated t value > t table for each variable, namely 3.484 for $NH_3$, 4,627 For $NO_2$ And 8,509 For plankton > t table that is 1.89458. Therefore, these results support that $NH_3$ and $NO_2$ are important factors influencing the abundance of Vibrio sp bacteria, with NO 2 as the most dominant variable based on the largest beta value, namely 500.838

Table 8. Results coefficient determination $NH_3$, $NO_2$ And plankton to Vibrio

| Model Summary |
|---|



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| Model | R | R Square | Adjusted R Square | Std. Error of the Estimate |
|---|---|---|---|---|
| 1 | ,981 [a] | ,962 | ,946 | 81.06127 |
| a. Predictors: (Constant), Plankton, $NH_3$,$NO_2$ | | | | |

From the results of the determination coefficient above, it explains that the value of the relationship (R) is 0.981. From the output, the determination coefficient (R Square) is 0.962, which means that the influence of $NH_3$, $NO_2$ and Plankton on the abundance of *Vibrio* sp is 96.2%, while the remaining 3.8% is influenced by other factors.

Table 9. Results Test F $NH_3$, $NO_2$ And plankton to *Vibrio*

| ANOVA[a] | | | | | | |
|---|---|---|---|---|---|---|
| **Model** | | **Sum of Squares** | **df** | **Mean Square** | **F** | **Sig.** |
| 1 | Regression | 1174577,133 | 3 | 391525,711 | 59,585 | ,000 [b] |
| | Residual | 45996,503 | 7 | 6570,929 | | |
| | Total | 1220573,636 | 10 | | | |
| a. Dependent Variable: *Vibrio* Sp | | | | | | |
| b. Predictors: (Constant), Plankton, $NO_2$, $NH_3$ | | | | | | |

Based on the results of the F test in the table above, it can be seen that the variables $NH_3$, $NO_2$ and Plankton simultaneously influence the abundance *of Vibrio* Sp. This is reinforced by the calculated F value > F table, namely 59.585 > 4.35 and the significance value below the 0.05 threshold, namely 0.000 < 0.05. Therefore, it can be concluded that that in a way simultaneous or in a way together variables $NH_2$,$NO_2$, and Plankton have a significant positive effect on the abundance of *Vibrio Sp. Bacteria*

## Conclusion

Based on the results of the Final Practical Work (KPA) conducted at PT. Parigi Aquakultura Prima, Central Sulawesi, it can be concluded that the total abundance of Vibrio sp. bacteria (CFU/ml) found in water samples from whiteleg shrimp (Litopenaeus vannamei) ponds sequentially reached 20, 40, 100, 300, 440, 560, 560, 835, 790, 689, and 1,080. The analysis of the influence of $NH_3$, $NO_2$, and plankton on the abundance of Vibrio sp. bacteria using IBM SPSS 25 showed that the Adjusted R Square value obtained was 0.962, with a significance value (Sig.) for all three variables below the threshold of 0.05. This indicates that $NH_3$, $NO_2$, and plankton each have a significant effect on the abundance of Vibrio sp. bacteria. Furthermore, the results of the business analysis demonstrate that whiteleg shrimp cultivation in the intensive ponds of PT. Parigi Aquakultura Prima is financially feasible, as it yields a profit of Rp. 809,849,141 with a Payback Period (PP) of 4.4 years for capital return.

## Suggustion

Suggestions that the author can provide in Practical Work This end (KPA) is for further practice in order to further expand the relationship between quality parameters. water with Vibrio sp, due to bacteria vibrio own range There is a fairly broad association with water quality.



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Therefore, it is possible that the increased abundance of Vibrio sp. bacteria could be related to other water quality conditions.

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