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Correlation Between *Phytoplankton* and Bacteria in Pond Water with the Productivity of Vannaamei Shrimp Ponds (*Penaeus vannamei*) Cultivated with an Intensive System

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Abstract: The level of pond productivity is influenced by the environmental conditions of maintenance caused by the physiochemical and biological quality of pond waters. Biological parameters of waters consist of the abundance of *Phytoplankton* and Bacteria. The purpose of this study was to determine the correlation between the abundance and types of *Phytoplankton* and Bacteria in pond water to the productivity level of whiteleg shrimp (*Penaeus vannamei*) ponds in different DOCs cultivated with an intensive system. The research method used observed maintenance parameters, namely the diversity and abundance of *Phytoplankton* including *Green Algae* (GA), *Blue Green Algae* (BGA), *Diatome*, and *Dinoflagellate* groups. Other parameters include *Total Bacteria Count* (TBC), *Total Vibrio Count* (TVC) Yellow, and *Total Vibrio Count* (TVC) Green. These research parameters will be subjected to Multiple Linear Regression analysis on the total harvest, feed conversion value (FCR), and survival rate (SR) of whiteleg shrimp obtained from each pond. The results of the study showed that the abundance of *Phytoplankton* based on the group from the lowest to the highest value was the *Dinoflagellate* group of 0 cells/mL with the GA group of 1.52 x 10^6 cells/mL. Parameters found that were significant to the level of pond productivity were the abundance of TBC and BGA ($P < 0.05$). The conclusion is that the level of pond productivity is influenced by the abundance of *Phytoplankton* and bacteria based on the diversity of their species.

Keywords: Bacteria; Correlation; *Phytoplankton*; Productivity Level

Introduction

Vannamei shrimp (*Penaeus vannamei*) originates from the subtropical areas of the west coast of America (Anggi Nugraha *et al*., 2022); (Asmild *et al*., 2024). Vaname shrimp (*P. vannamei*) is one of the fishery commodities that has economic value with high prospects (Suwoyo & Hendrajat, 2021); (Inayah *et al*., 2023). The productivity of vaname shrimp tends to be better than other shrimp because it has a strong immune system against pathogen attacks, high stocking density, with an optimal feed conversion rate, low feed protein composition of around 20% and 35% and has a wide tolerance for salinity and temperature with a survival rate of 50% to 60% (And Safaa M. Sharaf, 2022); (Siddiqui *et al*., 2022). The success of vaname shrimp cultivation is supported by several factors including water quality conditions, the presence of *Phytoplankton* and *Vibrio* sp. bacteria. The quality of the cultivation water is an important indicator in the success of the maintenance cycle in vaname shrimp ponds.

Phytoplankton has a very important function in vaname shrimp cultivation, namely as natural food and an indicator of ecological parameters (Akbarurrasyid *et al*., 2022a); (. *et al*., 2024).

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Phytoplankton that are abundant in waters have a correlation with the quantity of nutrient concentrations such as nitrate, phosphate, and silicate (Rahmah *et al*., 2022). *Vibrio* sp. bacteria are pathogenic bacteria that can cause losses in the vaname shrimp cultivation cycle because they can cause large-scale deaths (Manchanayake *et al*., 2023a); (V. Kumar *et al*., 2021). The types of bacteria *Vibrio parahaemolyticus*, *Vibrio harveyi*, and *Vibrio alginolyticus* can cause disease in vaname shrimp. This is in accordance with the opinion of (De Souza Valente & Wan, 2021); (Manchanayake *et al*., 2023b) that *Vibrio*sis disease is often found in vaname shrimp, both cultivated and not in the cultivation cycle.

Vaname shrimp infected with *Vibrio parahaemolyticus* bacteria experience body imbalance and tend to swim to the surface of the water looking for an oxygen source where the aerator point is located (Pratiwi *et al*., 2021); (Ulfiani *et al*., 2022). Based on the explanation above with the description of the obstacles and constraints and the relationship between the vaname shrimp cultivation cycle, in-depth research is needed regarding the relationship between the abundance of *Phytoplankton* and *Vibrio* in vaname shrimp pond water.

Method

Time and Place of Research

The research was conducted for 30 days, from August to September 2024. Whiteleg shrimp (*P. vannamei*) cultivation was carried out in intensive ponds in the Kebumen area, Central Java. *Phytoplankton* analysis, *Total Vibrio Count* (TVC), and water quality tests were carried out at the Kebumen Area-Based Shrimp Cultivation (BUBK) laboratory, Central Java.

Research Method

The research method used was the survey method by determining the location of the pond, the number of pond plots (twelve) and the same shrimp DOC for further direct observation of the maintenance parameters once a week during the research period. The maintenance parameters observed were the diversity and abundance of *Phytoplankton* including *Green Algae* (GA), *Blue Green Algae* (BGA), *Diatome*, and *Dinoflagellate* groups. Other parameters included *Total Bacteria Count* (TBC), *Total Vibrio Count* (TVC) Yellow, and *Total Vibrio Count* (TVC) Green. The research parameters will be subjected to Multiple Linear Regression analysis on the total harvest, feed conversion value (FCR), and survival rate (SR) of vaname shrimp obtained from each pond. Data collection through observation of water quality parameters, pond specifications, and stocking density was carried out once every seven days during the research period, and will then be analyzed statistically (Mramba & Kahindi, 2023). Casual design methodology with the application of ex post-facto analytical methods, is used in research that examines natural events (without treatment). Samples are taken periodically during the maintenance period, with the method used being descriptive analysis (Musa *et al*., 2023). According to (Ariadi & Mujtahidah, 2021) stated, there is a relationship between water quality parameters and the optimal level of vaname shrimp cultivation.

Phytoplankton Analysis

The abundance and diversity of *Phytoplankton* were analyzed in the BUBK Kebumen environmental laboratory using a microscope and a Neubauer hemocytometer. The diversity of *Phytoplankton* species can be identified using a guidebook, namely the Plankton Collection and Analysis Method. *Phytoplankton* identification can be known at each water sampling with a sample bottle volume of 50 ml, then 1 ml of water sample was taken using a micropipette, then analyzed using a 400x magnification microscope. Plankton water samples were taken from sample bottles with a volume of 50 ml and tested for identification and abundance analysis using a Neubauer hemocytometer with the aid of an Olympus CX22 microscope based on the APHA (American Public Health Association) method as follows: Calculation of *Phytoplankton* abundance was carried out using the equation according to APHA-1989.

$$N = \frac{Oi}{Op} x \frac{Vr}{Vo} x \frac{1}{Vs} x \frac{n}{p} \quad (1)$$

Description:
N (Number of samples per liter of individuals)
Oi (Width of sedgewick rafter cover (mm2) 1000 mm2)
Op (Width of observation under microscope (mm2) 1 mm2)
Vr (Volume of water sample (mL) 10 mL)
Vo (Volume of water sample observed (mL) 1 mL)
Vs (Volume of filtered water sample (L) 6 L)
n (Number of plankton observed under microscope)
p (Number of fields observed under microscope)

Total Vibrio Count (TVC) Analysis

TCBS agar media with a dry surface is used for isolation and calculation of the number of *Vibrio* sp. colonies that have previously gone through the incubation stage at a temperature of 35 to 37^{o} C for 24

to 48 hours. The spreader model and the colonies that appear are used as ALT (Total Plate Count) according to the applicable provisions and the colonies that appear are recorded according to their colors, namely green and yellow. The Indonesian National Standards Agency uses the following formula for calculating the number of colonies on a plate:

$$N = (\frac{\sum C}{[(1xn1)+(0,1\ x\ n2)]x(d)}) \quad (3)$$

Description:
N (Number of product colonies (cells/ml))
∑C (Number of colonies on all plates)
n1 (Number of plates in the first dilution)
n2 (Number of plates in the second dilution)
d (First dilution used)

The feed conversion value or Feed Conversion Ratio (FCR) is an important indicator in the vaname shrimp maintenance cycle because it is a parameter for successful cultivation. The FCR value of vaname shrimp can be categorized as good if it obtains a value of 1.40 to 1.80. A low FCR value indicates that the condition of natural feed has benefits to support the growth of vaname shrimp so that the use of artificial feed (pellets) can be optimally suppressed. The FCR value can be calculated using the following formula:

$$FCR\ = \frac{F}{(Wt+Wd)-wo} \quad (2)$$

Description:
FCR (Feed Conversion Ratio)
F (Amount of feed given (g))
Wo (Initial weight (g))
Wt (Final weight (g))
Wd (Dead fish weight (g))

Shrimp survival or Survival rite (SR) is a reference for the number of shrimp that survive during the cultivation period. The SR value is a percentage comparison of the number of shrimp that survive at the beginning of the maintenance period with the end of the maintenance period, calculated in the following formula:

$$SR = \frac{Nt}{No}\ x\ 100\% \quad (3)$$

Description:
SR (Survival Rite (%))
Nt (Initial number of stocking (tail))
No (Final number of maintenance (tail))

Data Analysis

Data analysis using the Multiple Linear Regression method. Furthermore, a (t) test is carried out on the hypothesis with a confidence level of 95%.

Result and Discussion

Phytoplankton Abundance

The results of *Phytoplankton* abundance calculations in this study can be seen in Figure 1. Based on the GA group *Phytoplankton* abundance calculation data, the highest density was found in the 12th plot with a value of 1.52 x 10^6 cells/mL and the lowest density was in the 5th plot with a value of 5, 4 x 10^3 cells/mL. The highest BGA group was in plot 5 with a value of 2.26 x 10^6 cells/mL and the lowest density was in plot 7 with a value of 1.74 x 10^2 cells/mL. The highest diatom group was in plot 12 with a value of 6.46 x 10^5 cells/mL and the lowest density was in plot 5 with a value of 1.01 x 10^3 cells/mL. The highest Dinofalagellata group was in plot 7 with a value of 3.5 x 10^3 cells/mL and the lowest density was in plots 10 and 11 with a value of 0 cells/mL. *Phytoplankton* of the GA (Chlorophyta) group, including Nannochloropsis and Chlorella sp. were found in large numbers. which is high in vaname shrimp ponds (Palupi *et al*., 2022). *Chlorella* sp. is a group of GA that can thrive in pond waters which are influenced by environmental carrying capacity, nutrient availability, light intensity, and the level of correlation with the CO2 content of the waters (Budianto *et al*., 2021).

Phytoplankton of the BGA (Cyanobacteria) group in ponds are influenced by water quality conditions and nitrification in the maintenance media (Masithah *et al*., 2019). *Oscillatoria* and *Anabaena* are BGA groups that can produce toxic substances which can harm the cultivation cycle. *Phytoplankton* of the *Diatome* group (*Bacillariophyceae*) can reach high abundance due to the Total Ammonia Nitrogen (TAN) value in the cultivation medium (Ariadi *et al*., 2022). Diatom group, some genera found include *Nitzschia, Chaetoceros*, and *Odontella* (Akbarurrasyid *et al*., 2022b). The *Dinoflagellate* group found in pond waters, some of which *are Choclidinium, Noctiluca* and *Ceratium*. *Phytoplankton* of the *Dinoflagellate* group have a low abundance level so as not to have a negative impact on the vaname shrimp cultivation media (Mahmudi *et al*., 2021).

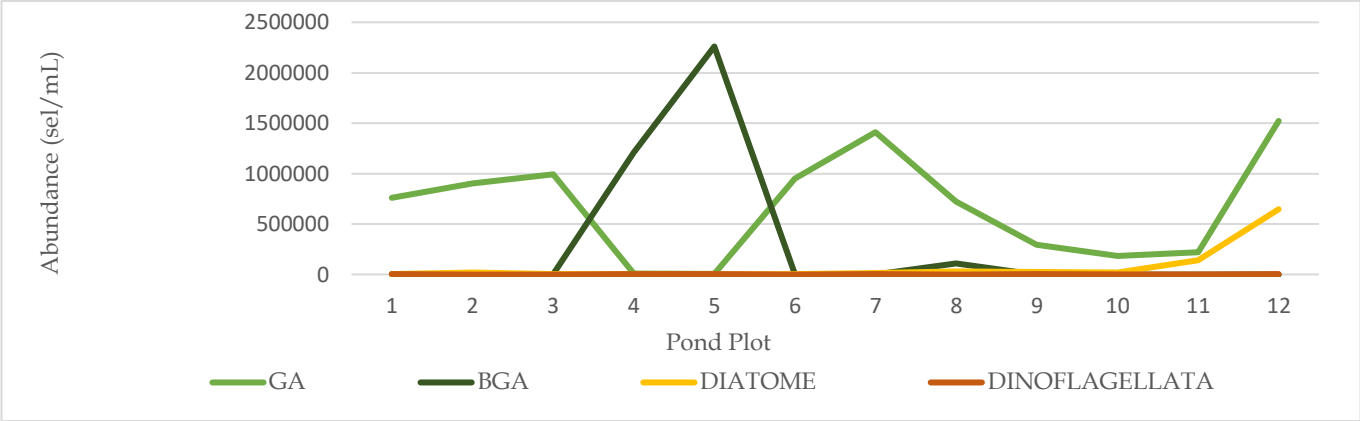

Figure 1. Abundance and Diversity of *Phytoplankton* in All Vaname Shrimp Pond Plots

Abundance of Bacteria

The results of the calculation of the abundance of Bacteria in this study can be seen in Figure 2. Based on the calculation data of the abundance of TBC, the highest density was found in plot 1 with a value of 2.60 x 10^5 CFU/mL and the lowest density in plot 5 with a value of 1 x 10^3 CFU/mL. The abundance of TVC Yellow was found to be the highest in plot 5 with a value of 1.67 x 10^4 CFU/mL and the lowest density in plot 3 with a value of 2.50 x 10^3 CFU/mL. While TVC Green had the highest density in plot 5 with a value of 8 x 10^3 CFU/mL and the lowest density in plots 7.90 and 10 with a value of 0 CFU/mL. Probiotic bacteria can suppress the presence of *Vibrio* sp. in the cultivation media, indicated by a higher abundance of TBC compared to the abundance of TVC. The abundance of TBC in the culture media tends to decrease compared to TVC as the shrimp DOC increases, indicating that *Vibrio* sp. bacteria grow well in conditions of decreasing water quality (Widigdo *et al*., 2020). Fluctuations of *Vibrio* sp. in cultivation ponds follow the biological metabolic response and physiological adaptation to the maintenance media (Garibay-Valdez *et al*., 2020).

Vibrio sp. bacteria have a yellow or green color on the TCBS plate due to the presence of different types of bacteria growing in the agar medium. TVC Green colonies growing on TCBSA plates have a structural similarity of approximately >80% with *V. fishery* and *V. Mimicus* (Asni *et al*., 2023). The bacteria that produced green colonies on TCBS media were identified as *Vibrio campbellii* (S. Kumar *et al*., 2021). TVC Yellow was identified using microbiological and biochemical methods, thus finding the types *V. alginolyticus* and *V. Fluvialis*. The *V. Harveyi* bacteria in the identification of its biochemical and physiological properties showed sucrose fermentation activity and produced a yellow color on TCBS media (Pavlinec *et al*., 2022).

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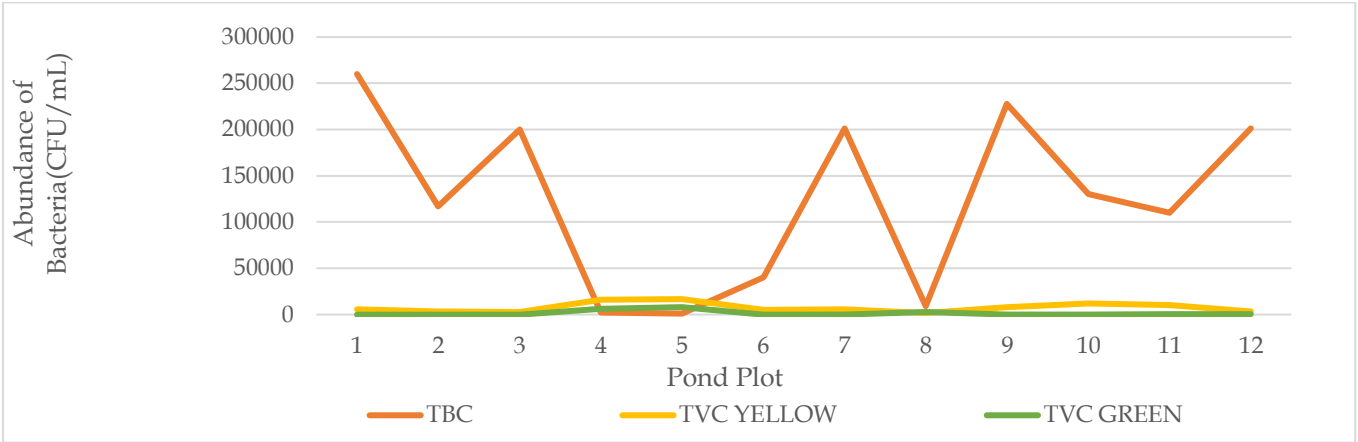

Figure 2. Abundance and Diversity of Bacteria in All Vaname Shrimp Pond Plots

Harvest Results

The harvest results in this study can be seen in Figure 4. Based on the harvest data in all pond plots, the highest weight was found in plot 3 with a value of 1,213.62 Kg and the lowest in plot 5 with a value of 409

Kg. The last stage of the vaname shrimp cultivation cycle is carried out at the time of harvest with various main standards, namely DOC around >50 days and shrimp size around 99 tails/kg (Mauladani *et al.*, 2020). The harvest is influenced by the quality of the cultivation media in preventing the emergence of diseases that can attack vaname shrimp such as *White Spot Syndrome Virus* (WSSV) (Suryadi *et al.*, 2021)

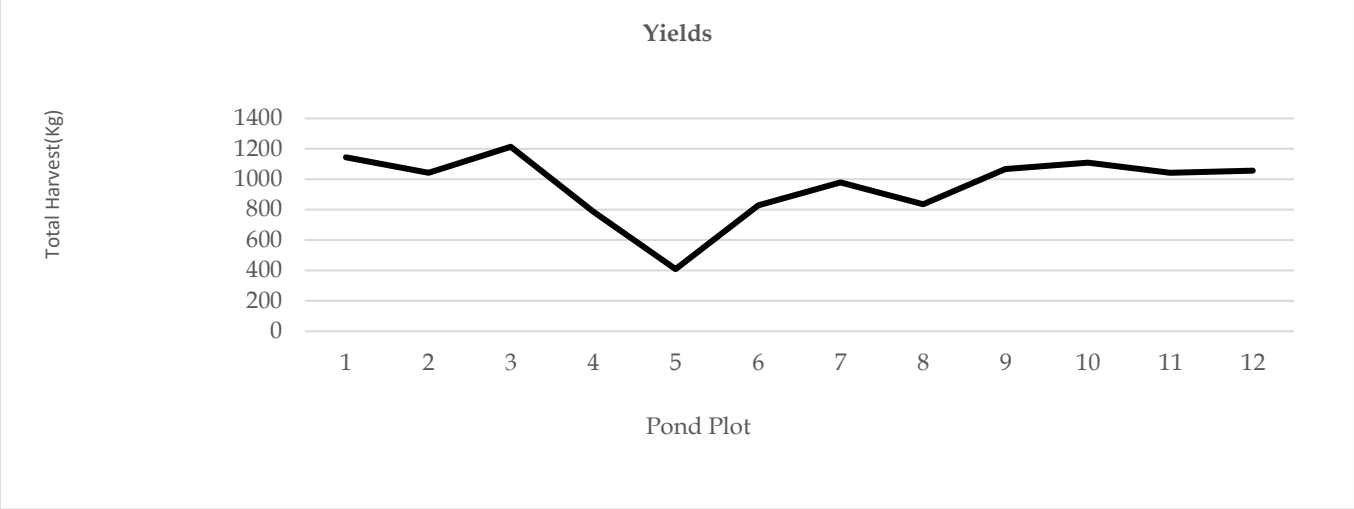

Figure 4. Vaname Shrimp Harvest Results in All Pond Plots

Survival Rate (SR)

The survival rate (SR) of vaname shrimp during the research period can be seen in Figure 5. Based on the SR value data in all pond plots, the highest percentage was found in plot 3 with a value of 87.24% and the lowest percentage in plot 5 with a value of 26.06%. This can be caused by the abundance of the BGA group and *Vibrio* sp. bacteria which are high in plot 5 and the lower density shown in plot 3. *Phytoplankton* of the BGA group produce toxic substances that can cause the death of vaname shrimp (Thawabteh *et al.*, 2023). High TVC values cause the survival percentage of vaname shrimp to decrease (Medina Félix *et al.*, 2017).

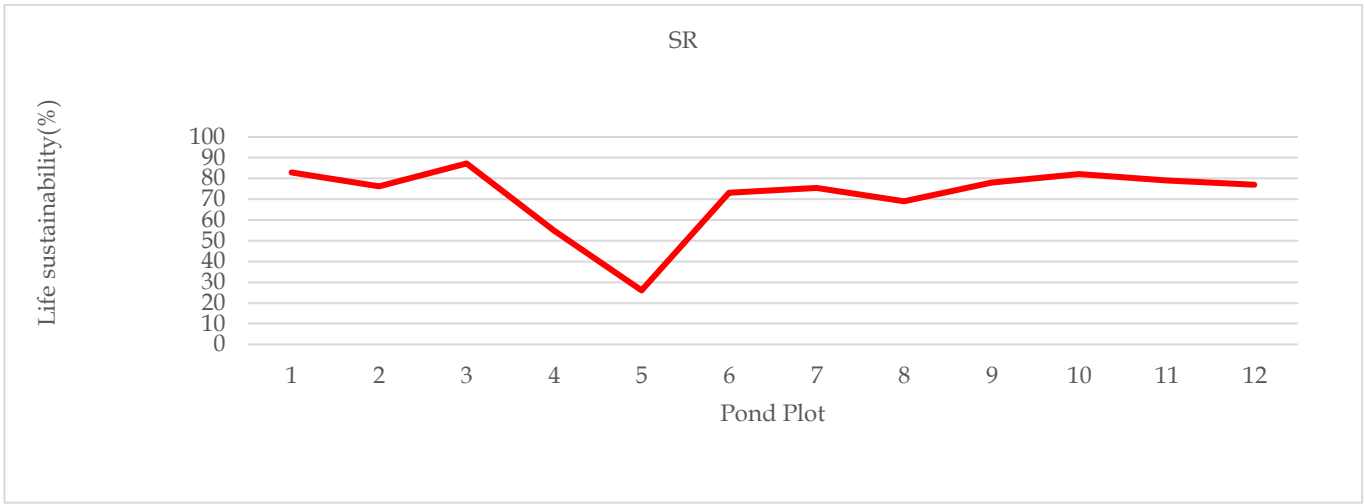

Figure 5. Survival Rate of Vaname Shrimp in All Pond Plots

Feed Conversion Rate (FCR)

The feed conversion rate (FCR) of whiteleg shrimp during the research period can be seen in Figure 6. Based on the FCR value data in all pond plots, the lowest value was found in plot 3 with a value of 1.18 and the highest value in plot 5 with a value of 2.4. Whiteleg shrimp that get optimal nutrition from feed have a low FCR value, which is around <1.5 (Imron & Samara, 2021). The low FCR value of vaname shrimp indicates the environmental carrying capacity in providing *Phytoplankton* as natural food, thereby helping the efficiency of artificial feed (pellets) (Rizky *et al.*, 2022).

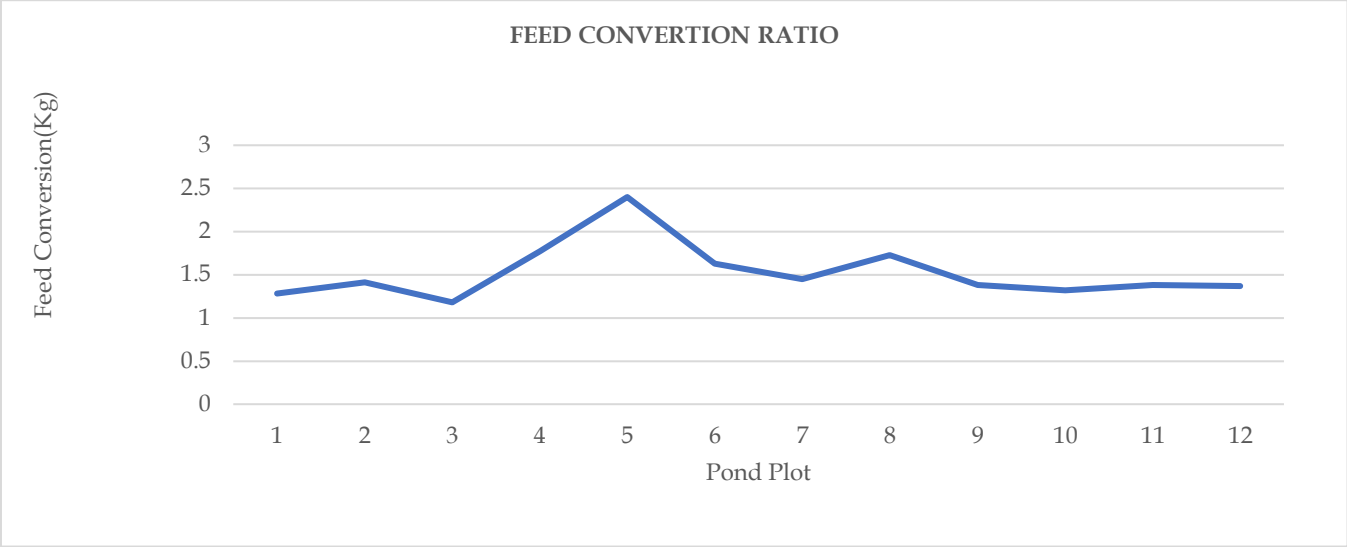

Figure 6. Conversion Value of Vaname Shrimp Feed in All Pond Plots

Based on the regression analysis and partial hypothesis test results (t-test) in table 1, it shows that the TBC variable has a significant difference in the Harvest Results (P<0.05). While variables such as TVC Yellow, TVC Green, GA, BGA, *Diatome*, and Dinoflagellata are not significantly different in the Harvest Results (P>0.05). Total bacteria such as *Clostridium* sp., *Flavobacterium* sp., *Vibrio* sp., *Pseudomonas* sp., and *Bacillus* sp. can grow in different quantities influenced by the cultivation system applied so that it has an impact on the maintenance environment [40]. The growth of gram-positive bacteria such as *Verrucomicrobiaceae* and *Lactococcus* can suppress the presence of pathogenic bacteria that can reduce the productivity of vaname shrimp (Gainza & Romero, 2020). The presence of probiotic bacteria in the maintenance media has been proven to increase the harvest of vaname shrimp, because it can minimize *Vibrio parahaemolyticus Acute Hepatopancreatic Necrosis Disease* (VPAHPND) (Kewcharoen & Srisapoome, 2019).

Table 1. Multiple Linear Regression Coefficient of Abundance and Diversity of *Phytoplankton* and Bacteria with Vaname Shrimp Harvest Yield

Para-Meter	Coef	SE Coef	T	Sig.	VIF
Cons	503	320	1.57	0.191	
TBC	0.001855	0.000651	2.85	0.046	4.74
TVC Y	0.02	0.0208	1.25	0.28	14.48
TVC G	0.07	0.0606	1.29	0.267	34.76
GA	0.000243	0.000241	1.01	0.37	19.84
BGA	-0.000449	0.000211	-2.13	0.10	28.14
Diatom	0.00009	0.000186	0.49	0.65	1.48
Dinofla	-0.1062	0.06	-1.53	0.20	7.84

Based on the regression analysis and partial hypothesis test results (t-test) in table 2, it shows that the TBC and BGA variables have a significant difference in SR (P<0.05). While variables such as TVC Yellow, TVC Green, GA, *Diatome*, and Dinoflagellata are not significantly different in SR (P>0.05). The composition of bacteria in the cultivation media can support the quality of the maintenance environment and increase the SR of vaname shrimp. (Panigrahi *et al*., 2018). The density of TBC (non-pathogenic bacteria) can play an active role in recycling nutrients, thereby helping to maintain the stability of water quality and supporting the survival rate (SR) of cultivated biota. The application of bioremediation bacteria such as *Thiobacillus* spp., *Bacillus* sp., and *Lactobacillus* spp. appropriately can increase the shrimp survival rate (SR) during the maintenance period (Widiyanto *et al*., 2020).

Table 2. Multiple Linear Regression Coefficient of Abundance and Diversity of *Phytoplankton* and Bacteria with SR Value of Vaname Shrimp

Para-Meter	Coef	SE Coef	T	Sig.	VIF
Cons	50.10	12.60	3.97	0.01	
TBC	0.000078	0.000026	3.02	0.03	4.74
TVC Y	0.001605	0.000822	1.95	0.12	14.48
TVC G	0.00397	0.00239	1.66	0.17	34.76
GA	0.000017	0.00001	1.74	0.15	19.84
BGA	-0.000033	0.000008	-3.99	0.01	28.14
Diatom	0.000001	0.000007	0.10	0.92	1.48
Dinofla	-0.00622	0.00273	-2.28	0.08	7.84

Based on the regression analysis and partial hypothesis test results (t-test) in table 3, all variables such as TBC, TVC Yellow, TVC Green, GA, BGA, *Diatome*, and Dinoflagellata are not significantly different from the FCR value (P>0.05). This shows that the provision of pellet feed to vaname shrimp can be caused by other factors, not depending on the biological indicators of the cultivation media alone. The FCR value can experience an insignificant difference due to management in feeding (Awaludin *et al*., 2020).

Table 3. Multiple Linear Regression Coefficient of Abundance and Diversity of *Phytoplankton* and Bacteria with FCR Value of Vaname Shrimp

Para-Meter	Coef	SE Coef	T	Sig.	VIF
Cons	2.19	0.44	4.91	0.008	
TBC	-0.000002	0.000001	-2.52	0.065	4.74
TVC Y	-0.000044	0.000029	-1.51	0.204	14.48
TVC G	-0.0001	0.000085	-1.18	0.302	34.76
GA	0	0	-1.16	0.31	19.84
BGA	0.000001	0	2.33	0.08	28.14
Diatom	0	0	-0.37	0.72	1.48
Dinofla	0.000139	0.000097	1.44	0.22	7.84

Conclusion

Phytoplankton abundance based on groups from the lowest to the highest values are GA group 5.40 x 10^3 cells/mL to 1.52 x 10^6 cells/m, BGA group 1.74 x 10^2 cells/mL to 2.26 x 10^6 cells/mL, *Diatome* group 1.01 x 10^3 cells/mL to 6.46 x 10^5 cells/mL, *Dinoflagellate* group 0 cells/mL to 3.50 x 10^3 cells/mL. TBC abundance based on the lowest to the highest values is 1 x 10^3 CFU/mL to 2.60 x 10^5 CFU/mL. *Vibrio* sp. bacteria abundance based on color characteristics from the lowest to the highest values is TVC Yellow 2.50 x 10^3 CFU/mL to 1.67 x 10^4 CFU/mL. While TVC Green 0 CFU/mL to 8 x 10^3 CFU/mL. The level of pond productivity based on the value of Harvest Yield, SR, and FCR respectively from the lowest to the highest value is 409 Kg to 1,213.62 Kg, 26.06% to 87.24%, and 1.18 to 2.4. Parameters found that are significant to the level of pond productivity are the TBC value to Harvest Yield (P <0.05), TBC and BGA values to SR (P <0.05). While the statistical values of all parameters to the FCR value were found to be insignificant (P>0.05). Based on the results of the study, the level of pond productivity is influenced by the abundance of *Phytoplankton* and bacteria based on the diversity of their species.

Author Contributions

Conceptualization, M. M. M.; methodology, M. A.; validation, G. M.; formal analysis, M. M. M.; investigation, M. M. M.; resources, M. A.; data curation, G. M.: writing—original draft preparation, M. M. M.; writing—review and editing, M. A.: visualization, G. M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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