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Relationship Between Temperature and Salinity Variables on the Abundance of *Vibrio* sp. and the Growth of *Vannamei* Shrimp (*Litopenaeus vannamei*)

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Abstract

Vannamei shrimp (Litopenaeus vannamei) is one of the most widely cultivated and produced crustacean species worldwide and its production in strongly influenced By water quality. This study was conducted from October 2023 to February 2024 in commercial shrimp pond in Pacitan, East Java. This study aimed to determine the relationship between temperature and salinity and *Vibrio* sp. abundance and *Vannamei* shrimp growth. This study used a quantitative descriptive method involving feed and media probiotic experiments. Data were analyzed using SPSS vr.27 software to examine the relationship between temperature, salinity, *Vibrio* sp. Abundance, and *Vannamei* shrimp growth. The results showed that the average total *Vibrio* value for Pond P was 5.501 x 10^3 CFU/mL and for Pond K was 6.22 x 10^3 CFU/mL.The correlation analysis indicated a significant relationship between temperature and *Vibrio* sp. abundance, while no significant association was observed for salinity. In addition, the combined effects between temperature and salinity were significantly related to *Vannamei* shrimp growth in Pond P(a Sig. F Change value of 0.047. The R value obtained for Pond P is 0.840), whereas no significant relationship was detected in Pond K. This study concludes that temperature was significantly relationship with Vibrio sp. abundance, whereas salinity was more strongly significant relationship with *Vannamei* shrimp growth. Pond P was given probiotic feed and probiotic media treatment, while Pond K was given probiotic feed treatment only.

1. Introduction

Indonesia is a maritime country with abundant natural resources, especially in coastal areas. Coastal natural resources that can be managed and developed include capture fisheries and aquaculture (Sutoyo et al., 2022). One type of coastal aquaculture that can increase natural resources is *Vannamei* shrimp (*Litopenaeus vannamei*) farming. Shrimp farming is an opportunity with high potential. *Vannamei* shrimp is one of the marine fishery commodities that has high economic value. In addition, this shrimp is highly attractive to consumers. *Vannamei* shrimp has several advantages, such as high selling price, easy to cultivate, and resistant to disease (Dahlan et al., 2017). Water quality plays an important role in aquaculture because it directly affects the survival of *Vannamei* shrimp, so it is intensively controlled and maintained to prevent pathogens or diseases that attack shrimp. A decline in *Vannamei* shrimp production can be caused by diseases that attack shrimp health. Several types of *Vibrio* sp. bacteria are disease agents that cause shrimp mortality on a small or large scale. Water quality parameters, including temperature and salinity, can negatively impact aquaculture when they fall outside the normal ranges.

Temperature is a difficult parameter to control in water because it is influenced by location and weather. Areas with high intensity will lower the water temperature. An increase in water temperature will have several effects, such as decreasing dissolved oxygen, and if the temperature exceeds the shrimp's tolerance limit, it will cause the shrimp to die.

Salinity is the total concentration of ions dissolved in water. The optimal salinity range for the growth and survival of *Vannamei* shrimp is 15-30 ppt. The ideal sainity is in correlation with the shrimp's osmoregulation mechanism. High salinity is caused by high evaporation, whereas low salinity is caused by the freshwater input mixing with seawater.

In *Vannamei* shrimp farming, probiotics are used to improve shrimp health while maintaining pond environment quality. Probiotics in *Vannamei* shrimp farming are known to boost the immune system, improve water quality, suppress the population of *Vibrio* sp. bacteria, and increase feed nutrient utilization (Umasugi et al., 2018). Probiotics break down quickly in the food chain, do not accumulate in the body, and do not cause drug resistance. Probiotics in the form of beneficial microorganisms such as *Bacillus* sp., *Lactobacillus* sp., or nitrifying bacteria can be administered through feed or added directly to pond water.

The increasing growth rate of shrimp has negative consequences in terms of increased organic waste load. Aquaculture ponds with higher waste loads will result in an increase in the number of pathogenic bacteria. Pathogenic bacteria such as *Vibrio* spp., *Aeromonas* spp., and *Pseudomonas* spp. can cause serious diseases in shrimp, resulting in unstable growth rates, stress, and even mass mortality. Shrimp growth rate performance can be measured by Average Body Weight (ABW), which represents the average weight per shrimp, and Average Daily Growth (ADG), which indicates the daily weight gain of shrimp (Yunarty et al., 2022).

Temperature and salinity parameters are among the determinants of success in *Vannamei* shrimp farming. Temperature and salinity conditions can determine shrimp growth rates and pathogen abundance in farming ponds. Understanding this relationship can provide a scientific basis for designing more effective water quality management strategies, especially in intensive shrimp ponds. This study aims to determine the relationship between temperature and salinity variables and the abundance of *Vibrio* sp. and *Vannamei* shrimp growth, so as to provide appropriate water quality management solutions.

2. Material and methods

2.1 Research Location and Time

This study was conducted in Ngoyan Pond, Worawari Village, Kebonagung Subdistrict, Pacitan, East Java (Figure 1). The study lasted for one shrimp farming cycle, or three months.

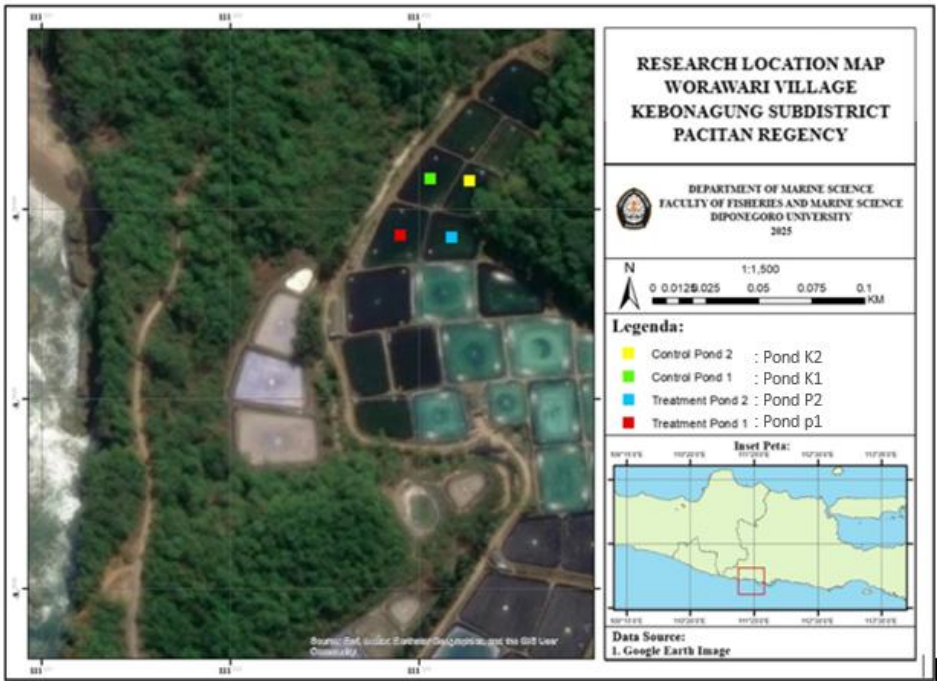

Fig. 1. Research Location at Ngoyan Pond, Worawari Village

2.2 Research Design

This study used a descriptive method by collecting all data from the research location through documentation, observation, and experimentation. The data were analyzed using quantitative descriptive methods in the form of tables and graphs. The experiments were conducted in the field with a four-pond design, namely two treatment ponds that were given probiotic feed and media (Pond P) and two control ponds that were only given probiotic feed (Pond K). The research design used a completely randomized design (CRD) with two replicates per treatment.

2.3 Field Data Collection

The sampling technique used was purposive sampling, which is a technique for obtaining samples by selecting samples from the population as desired by the researcher. This technique was used because all the shrimp samples caught were weighed. Water quality data was collected directly (in situ) from the aquaculture ponds using a Baruno JALA device. Water samples were collected once a week and then tested in a laboratory to calculate the abundance of Vibrio sp.

2.4 Data Analysis

Graphs were created to visualize the data using Microsoft Excel. Data management was performed using SPSS 27.0 software. The initial stage of data processing involved conducting normality tests as a prerequisite for multiple correlation tests and Spearman's correlation tests. Normality tests used the Kolmogorov–Smirnov to determine data distribution. The Spearman correlation test was used to determine the degree of relationship between temperature and salinity and the abundance of *Vibrio* sp. The results were interpreted by looking at the Sig. (2-tailed) value and the correlation coefficient (r) value to determine the direction of the relationship between variables. Multiple correlation tests were used to determine the presence or absence of a simultaneous relationship between temperature and salinity and *Vannamei* shrimp growth. The results were interpreted by looking at the Sig. F Change value and the correlation coefficient (R) value to determine the degree of closeness.

3. Results

3.1 Water Quality Parameters

Measurements of temperature and salinity variables in the cultivation ponds were taken daily at 06:00 and 18:00 WIB. The data obtained from the temperature and salinity measurements are presented in Figure 2 and Figure 3.

The temperature variables during the cultivation period in the four ponds (P1, P2, K1, and K2) are presented in Figure 2. The measured temperature parameters ranged from 28°C to 32°C, with the highest values recorded between DOC 1 and DOC 8 and the lowest values recorded between DOC 77 to DOC 79. During the cultivation period, the salinity variables in the four ponds (P1, P2, K1, and K2) are presented in Figure 3. The results of salinity parameter measurements in the cultivation ponds ranged from 36 to 17 ppt. At the start of cultivation from DOC 1 to DOC 28, the salinity value was 30-36 ppt, then from DOC 29 until harvest, the salinity value ranged from 17-29 ppt.

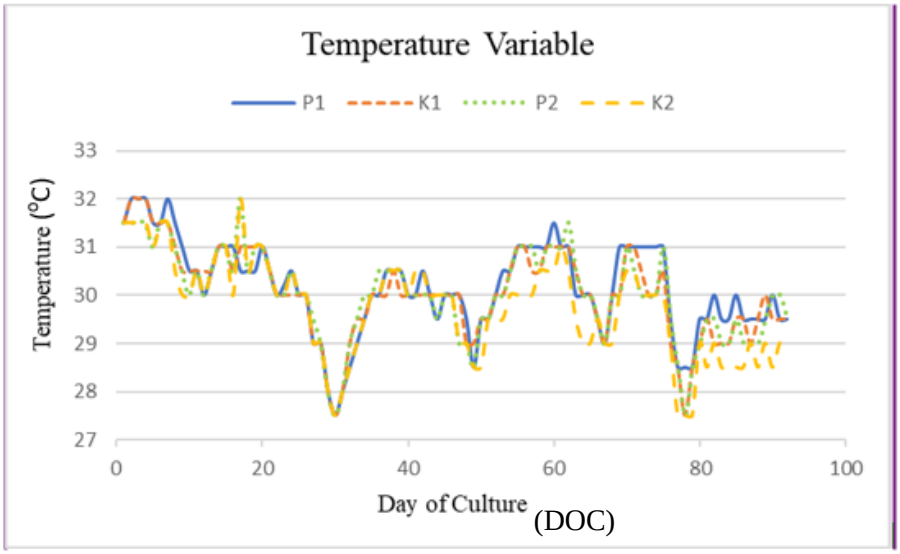

Fig. 2. Temperature Variable Graph

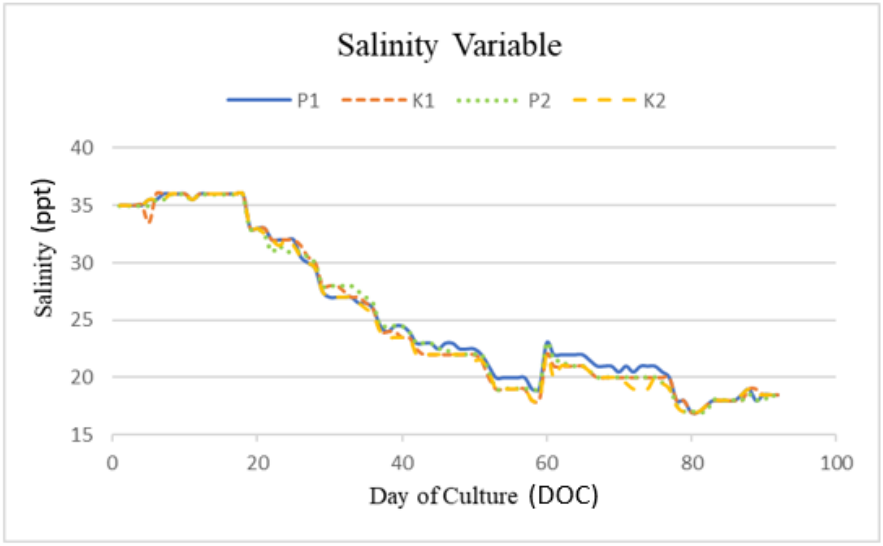

Fig. 3. Salinity Variable Graph

3.2 Abundance of *Vibrio* sp.

The abundance of *Vibrio* sp. obtained from 14 samples taken from the treatment pond and control pond is presented in Table 1.

The results obtained from 14 samples taken from the treatment pond showed an average total *Vibrio* value of 5.501 x 10^3 CFU/mL, while the control pond showed a value of 6.22 x 10^3 CFU/mL. The highest abundance of *Vibrio* sp. was found in DOC 49, with 2.26 x 10^4 CFU/mL in the treatment pond and 2.17 x 10^4 CFU/mL in the control pond. The average value in the treatment pond was lower than that in the control pond because the treatment pond was given feed probiotics and media probiotics, while the control pond was only given feed probiotics.

3.3 Shrimp Growth Rate

The growth rate of *Vannamei* shrimp consists of Average Body Weight (ABW) and Average Daily Growth (ADG), which are presented in Figure 4 and Figure 5. Data for ABW and ADG were obtained from eight growth sampling events. Sampling was conducted from DOC 41 through DOC 91, at one-week intervals.

The final growth value in the treatment pond was 18.60 g/individual and in the control pond was 18.46 g/individual. In the treatment pond, *Vannamei* shrimp growth was superior to that in the control pond. The ABW trend line showed an increasing growth rate in both *Vannamei* shrimp culture ponds.

Shrimp weight per day fluctuated over the one week. The lowest ADG value was 0.18 g/day at DOC 48 in the treatment pond and 0.19 g/day at DOC 55 in the control pond. The largest ADG value in the treatment pond was 0.44 g/day at DOC 91, while the largest ADG value in the control pond was 0.41 g/day at DOC 69 and DOC 84.

Table 1. Results of *Vibrio* sp. Abundance Calculations

Day of Culture	Number of *Vibrio* sp. Colonies (CFU/mL)					
	Treatment Pond		Average	Control Pond		Average
	1	2		1	2	
0	5.2×10^{2}	3.6×10^{2}	4.4×10^{2}	1.9×10^{3}	1.3×10^{3}	1.0×10^{3}
7	1.0×10^{1}	1.5×10^{2}	8.0×10^{1}	6.0×10^{1}	2.2×10^{2}	1.4×10^{2}
14	1.7×10^{3}	2.9×10^{3}	2.3×10^{3}	4.5×10^{2}	$3,6\times10^{2}$	4.1×10^{2}
21	2.1×10^{3}	3.1×10^{3}	2.6×10^{3}	1.8×10^{3}	5.9×10^{3}	3.9×10^{3}
28	8.7×10^{3}	7.3×10^{3}	7.9×10^{3}	8.8×10^{3}	8.5×10^{3}	8.6×10^{3}
35	4.5×10^{3}	1.0×10^{4}	7.3×10^{3}	1.8×10^{4}	5.4×10^{3}	1.2×10^{4}
42	6.8×10^{3}	5.6×10^{3}	6.2×10^{3}	1.9×10^{4}	8.8×10^{3}	1.4×10^{4}
49	1.8×10^{4}	2.7×10^{4}	2.3×10^{4}	2.1×10^{4}	2.3×10^{4}	2.2×10^{4}
56	2.4×10^{3}	-	1.2×10^{3}	3.0×10^{2}	2.8×10^{3}	1.6×10^{3}
63	4.7×10^{3}	1.1×10^{3}	2.9×10^{3}	8.9×10^{3}	1.3×10^{3}	5.1×10^{3}
71	6.4×10^{3}	5.0×10^{2}	3.5×10^{3}	1.6×10^{3}	1.5×10^{3}	1.6×10^{3}
77	1.6×10^{3}	1.2×10^{3}	1.4×10^{3}	9.0×10^{2}	6.2×10^{3}	3.6×10^{3}
85	1.3×10^{4}	1.8×10^{4}	1.6×10^{4}	8.1×10^{3}	4.8×10^{3}	6.5×10^{3}
92	5×10^{2}	$5,2\times10^{3}$	2.9×10^{3}	9.2×10^{3}	5.7×10^{3}	7.5×10^{3}
Total *Vibrio*			5.5×10^{3}			6.2×10^{3}
Standard Vibrio level			$\leq 1\times10^{3}$ CFU/mL			

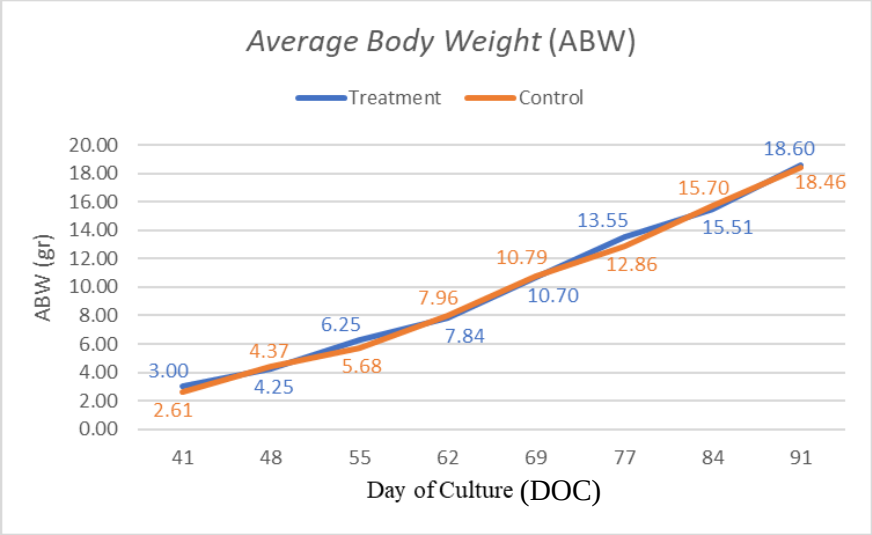

Fig. 4. *Vannamei* Shrimp Growth Rate Weekly

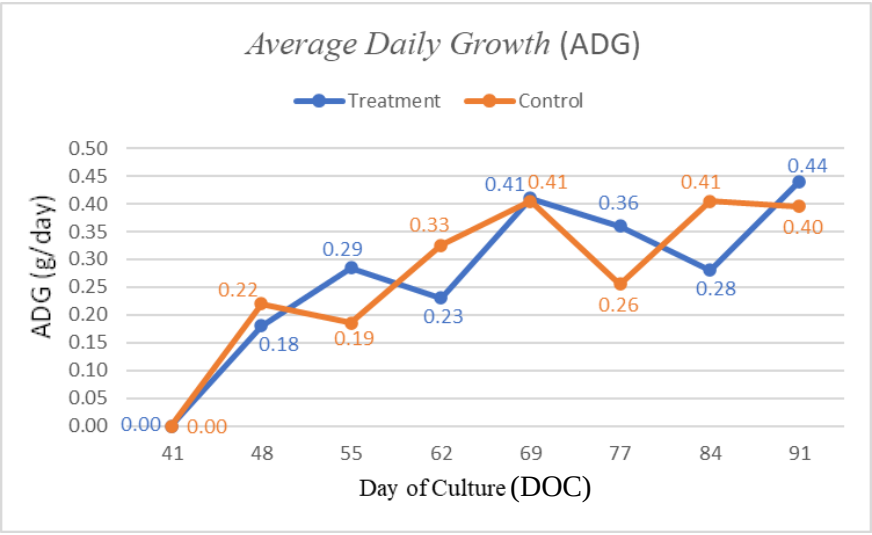

Fig. 5. *Vannamei* Shrimp Growth Rate per Day

3.4 Biomass Calculation

The results of the biomass value calculations for the treatment and control ponds are presented in Figure 6. Biomass calculations were performed from DOC 41 to DOC 91 at one-week intervals.

Biomass calculations were performed after sampling the shrimp, where the Average Body Weight (ABW) results were essential for determining the Food Ratio (FR) (%) value. The biomass calculation results showed that the treatment

pond had a higher value than the control pond. From DOC 69 to DOC 77, the biomass value of both ponds decreased, which was proportional to the ADG value, which also decreased at the same DOC.

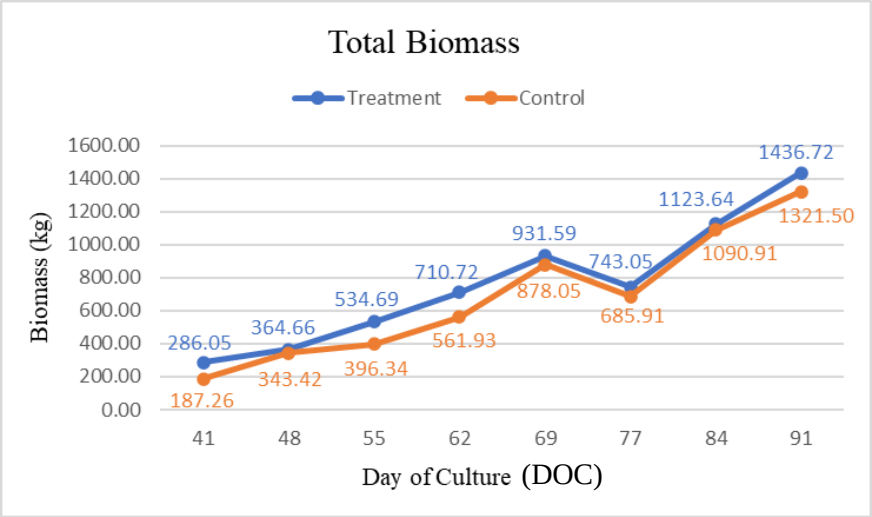

Fig. 6. Total Biomass Weekly

3.5 Relationship between Temperature and Salinity on the Abundance of *Vibrio* sp.

The statistical test used was Spearman's correlation test. Spearman's correlation test was used when the normality test results obtained a Sig. (P Value) < 0.05 (not normal). The results of the relationship between temperature and salinity on the abundance of *Vibrio* sp. are shown in Table 2.

Table 2. Spearman's correlation test of temperature and salinity against the abundance of Vibrio sp.

Pond Code	Variable Y		Temperature	Salinity	Vibrio
Pond P	*Vibrio* sp.	Correlation Coefficient	-699	-336	1.000
		Sig. (2-tailed)	.005	.240	
		N	14	14	14
Pond K	*Vibrio* sp.	Correlation Coefficient	-690	-322	1.000
		Sig. (2-tailed)	.006	.262	
		N	14	14	14

The results of calculations for the treatment and control ponds showed that temperature is significantly related to *Vibrio* sp. abundance, whereas salinity is not. The Sig. (2-tailed) value for temperature with *Vibrio* sp. in the treatment pond was 0.005 and in the control pond was 0.006, where the results were < 0.05, which can be concluded to have a significant relationship.

3.6 The Relationship Between Temperature and Salinity and Growth Rate

The results of statistical tests of the relationship between temperature and salinity variables on *Vannamei* shrimp growth are presented in Table 3.

Table 3. Multiple Correlation Test of temperature and salinity on *Vannamei* shrimp growth

Pond	R	R Square	Adjusted R Square	Std. Error of the Estimet	R Square Change	F Change	Sig. F Change
P	.840	.706	.588	3.58396	.706	5.993	.047
K	.805	.647	.506	3.95468	.647	4.588	.074

In pool P, the Sig. F Change value obtained was 0.047 (< 0.05), indicating statistically significanteffect. The R value obtained was 0.840, which can be classified as a "very strong" correlation coefficient. The calculation results for pool K show that the Sig. F Change value obtained was0.074 (>0.05), indicating no statistically significant relationship.

4. Discussion

Temperature variables in aquaculture activities will affect oxygen solubility, shrimp metabolism, survival, reproduction, morphological growth, and molting rate. Increased temperature will cause salinity to increase along with a decrease in pond oxygen levels (Pan-Lu-Qing et al., 2007; Ariadi et al., 2021; Wafi et al., 2021). Elevated temperatures enhance evaporation, leading to increased salinity, while shrimp respond by enhancing osmoregulatory and respiration processes, which increase oxygen consumption rates to achieve isoosmotic conditions. The average temperature in the four ponds was good for the life and growth of *Vannamei* shrimp, ranging from 28°C to 32°C. The average salinity in the four ponds ranged from 17 to 36 ppt. At the beginning of cultivation in DOC 1 to DOC 28, the variable ranged from 30 to 36 ppt, because the cultivation period took place during the summer. Salinity is an important environmental factor affecting the survival, stress tolerance, and growth performance of *L. vannamei* (Adella et al., 2023). The hypersaline salinity values were caused by high water

evaporation rates due to high temperatures, coupled with low mixing rates of new water.

Shrimp infected with *Vibrio* sp. bacteria show symptoms such as reduced appetite, swimming at an angle, moving closer to air bubbles, redness on the swimming legs and uropods, and necrosis and melanization in the body segments. The administration of probiotics in *Vannamei* shrimp farming is one solution to maintain survival rates during the rearing period and maintain pond water quality. In addition to administering probiotics, efforts can be made to manage water quality, such as siphoning. The average total *Vibrio* in the treatment pond was 5.501×10^3 CFU/ml and in the control pond was 6.22×10^3. The number of *Vibrio* sp. bacteria that grew in this study was still very low compared to the study reported by Fatmala et al. (2019), which found that the average number of *Vibrio* sp. bacterial colonies in *Vannamei* shrimp ponds was 3.96 x 10^6 CFU/ml - 1.46 x 10^6 CFU/ml. The abundance of *Vibrio* sp. in the culture medium in this study is still within the normal range for *Vannamei* shrimp maintenance.

The final Average Body Weight (ABW) of the treatment pond was 18.6 gindividual^{-1} and that of the control pond was 18.46 g individual^{-1}. Both ponds experienced an upward trend in shrimp weight each week, but the final ABW in the treatment pond was higher than the control pond. At DOC 72, partial harvesting was carried out in both ponds, thereby reducing feed competition and increasing growth rates. The highest Average Daily Growth (ADG) was in the treatment pond with a value of 0.44 g/day, while the highest ADG in the control pond was 0.41 g/day. Both ponds experienced a simultaneous decline in growth at DOC 77 due to poor water quality and poor shrimp appetite. The protein in the feed was not consumed properly, but the shrimp's metabolism was too high. Additionally, partial harvesting was conducted at DOC 72, which could have caused stress to the shrimp due to this partial activity.

Vibrio sp. bacteria are the cause of vibriosis, a disease commonly found in intensive shrimp farming systems. Various prevention and treatment efforts for *Vibrio* sp. bacteria have been carried out by *Vannamei* shrimp farmers, one of which is the administration of probiotics. One probiotic that reduces the abundance of *Vibrio* sp. in shrimp farming media is the ANTI V-PRO probiotic. ANTI V-PRO probiotic is a probiotic developed using Trichoderma reesei fungi. T. reesei fungi are capable of producing extracellular enzymes that attack *Vibrio* bacteria. The results of testing the relationship between temperature and *Vibrio* abundance in the treatment ponds obtained a Sig. (2-tailed) value of 0.005 and in the control ponds a value of 0.006. The Sig. (2-tailed) values for both ponds were < 0.05, indicating a significant relationship between the temperature variable and *Vibrio* abundance. The relationship between the salinity variable and Vibrio abundance in both ponds showed insignificant results, with Sig. (2-tailed) values >0.05 and a weak correlation level.

The statistical test of correlation between temperature and salinity variables and growth has a significant relationship, as evidenced by the correlation table in the Pearson correlation value. In this study, the most influential variable was salinity, followed by temperature. The results obtained from the Multiple Correlation test in the treatment pond showed a Sig. F Change value of 0.047. This result is < 0.05, which can be concluded that there is a significant simultaneous relationship between temperature and salinity on the growth of *Vannamei* shrimp in the treatment pond. The correlation coefficient (R) was 0.804, which is classified as a very strong correlation. Furthermore, the results obtained from the Multiple Correlation test in the control pond showed a Sig. F Change value of 0.074. The result is > 0.05, which can be concluded that there is no significant simultaneous relationship between temperature and salinity on the growth of *Vannamei* shrimp in the control pond. Continuous management of temperature and salinity variables and maintaining them within the optimal range is very important to maximize growth in *Vannamei* shrimp farming.

5. Conclusions

This study shows that the abundance of *Vibrio* sp. is more influenced by temperature than salinity. The growth rate of *Vannamei* shrimp in the treatment ponds was significantly influenced by both temperature and salinity. Increased temperature will cause salinity to increase, along with a decrease in pond oxygen levels. High temperatures increase evaporation, leading to elevated salinity. In response, shrimp intensify their osmoregulation and respiration processes, which increase the rate of oxygen consumption to achieve isoosmotic conditions. These conditions can have an impact on their growth rate.

Ethics approval

The study complied with Indonesian animal welfare regulations, and no specific permits were required for the sampling of marine biota. All procedures were conducted in accordance with the relevant institutional and national guidelines governing the use of aquatic organisms in research.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Author contributions

Joko Sumarwan: writing-review and editing, validation, methodology. Andika Dinar Saputra: writing-original draft, investigation, formal analysis, data curation, methodology; Agus Trianto: supervision, validation, resources, funding acquisition, writing-review and editing, project administration; Ervia Yudiati: resources, supervision, writing original draft preparation, writing-review and editing, validation, methodology, funding acquisition, project administration.

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Declaration of competing Interest

None

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