Preface

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It's such a great pleasure for me to welcoming all of you on behalf of Faculty of Fisheries and Marine Universitas Airlangga, for the 2nd international conference on fisheries and marine science. The 1st International Conference on Fisheries and Marine Sciences (InCoFiMS) 2018 has been successfully carried out which facilitated hundreds of publications to the Scopus-indexed proceeding of IOP and connected many researchers. The prior experience encourages to improve the quality of the conference through The 2nd InCoFiMS with the broader topic called "Sustainable Fisheries and Marine Development and Management". This expanded level of this conference with the theme of "Sustainable Fisheries and Marine Development and Management" is expected capable of connecting students, lecturers, researchers, government and professionals from across the world to meet, greet, share and discuss about the potential and best practices in the field of fisheries and marine during the period of focusing on SDG's.

The aims of this conference is to developed and improve the goals of Universitas Airlangga to be of the Top 500 University in the world by improving aquaculture and Fisheries Sustainable sector. For this conference, we also cooperate with Scopus Indexed Publisher. In order to assist students, lecturers and researchers in disseminating their findings, to publish selected papers which are expected helping societies to implement the findings in the focus on developing aquaculture and fisheries sustainable.

I strongly hope that all of the participants from around the world enjoy the conference at the Historical City of Surabaya, the second biggest city in Indonesia with competitive economic activities for the future of Fisheries and Marine development.

Once again, I am most grateful for your participant and your support. Thank you

Dr. Ahmad Shofy Mubarok

Chief of 2ND INCOFIMS

seaweed *Eucheuma cottonii*.

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Provision of bacteria from shrimp pond sediment towards N/P ratio, plankton abundance, and total bacteria in the culture media of white shrimp (*Litopenaeus vannamei*)

W H Satyantini[1,4], M Salsabila[2], D D Nindarwi[1], A M Sahidu[3], and A T Mukti[1]

[1]Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Jl. Mulyorejo, Surabaya60115, East Java, Indonesia
[2]AquacultureProgramme Study, Faculty of Fisheries and Marine, Universitas Airlangga, Jl. Mulyorejo, Surabaya60115, East Java, Indonesia
[3]Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Jl. Mulyorejo, Surabaya60115, East Java, Indonesia

*Corresponding author: worohastuti79@gmail.com/woro_hs@fpk.unair.ac.id

Abstract. This study aims to determine the effect of the administration of bacteria isolated from shrimp pond sediment *Bacillus mycoides, B. subtilis*, and *Pseudomonas diminuta* towards plankton abundance, N/P ratio, and total bacteria in the culture media of white shrimp (*Litopenaeus vannamei*). This experimental research using a Completely Randomized Design (CRD) consisting of four treatments based on the difference in the bacterial density, namely K(0 cell/mL),B1 (10^6 cells/mL), B2 (10^7 cells/mL),and B3 (10^8 cells/mL), with four replications each treatment and the shrimps were carried out for 28 days. The parameters were observed are plankton abundance, N/P ratio, and total bacteria. This research showed that probiotics could increase plankton abundance, N/P ratio, and total bacteria. The results of this research indicate that the different density of bacteria from shrimp pond sediment significantly different ($p<0,05$) towards plankton abundance and total bacteria in the culture media of white shrimp, while the administration of bacteria from shrimp pond sediment could increase the N/P ratio. The B3 gave the best results on plankton abundance, N/P ratio, and total bacteria of13,566.88 cells/mm^3, 7.22, and 2.93 x 10^9 cells/mL respectively.

1. Introduction

White shrimp is a fishery commodity that is widely cultivated in Indonesia as an effort to increase Indonesian shrimp production replacing tiger shrimp that has decreased production[1]. White shrimp production in Indonesia reached 315,000-355,000 tons in 2018 [2]. Increasing market demand must be supported with intensively cultivated which relies on artificial feed during maintenance, thus causing a build-up of leftovers that are not eaten at the bottom of the pond. This can cause a decrease in water quality. One way that can be taken to maintain water quality is to provide probiotics.

Probiotics are microorganisms that produce compounds that can prolong the growth phase of other species and are described as agents that have opposite functions to antibiotics[3]. The use of probiotics in the field of cultivation aims to maintain the balance of microbes in the aquatic environment through the process of biodegradation[4]. The working mechanism of probiotics is that probiotic bacteria will absorb or degrade organic or toxic substances and improve water quality [5]. Probiotics are useful as decomposers of organic matter into minerals and convert toxic compounds into non-toxic substances,

such as ammonia and nitrite compounds, which are poisonously converted into free nitrogen compounds through the process of nitrification and denitrification [6].

Probiotic bacteria generally have a tolerance to salinity, temperature, and pH [5]. Bacteria commonly used as probiotics in the field of cultivation are *Bacillus* and *Pseudomonas*. Application of *Bacillus subtilis* with a density of 10^8 CFU / ml on white shrimp pond culture can improve the water quality of the pond [7]. *Pseudomonas* sp. can produce enzymes such as proteases, amylases, and lipases it can also break down proteins, carbohydrates and other organic compounds into CO_2, ammonia gas, and other simpler compounds[8].

Application of decomposing bacteria or probiotics in shrimp culture media can improve water quality which will have an impact on increasing plankton as well as nitrogen and phosphorus ratios. Probiotics affect the N/P ratio which will affect the abundance of certain phytoplankton[9]. Plankton will grow at high nitrogen and phosphorus levels and low dissolved oxygen, which is an indicator of a good aquatic environment. Plankton which is expected to grow, is Bacillariophyta and Chlorophyta because it is a food source for zooplankton and shrimp[10].

Probiotics will reduce the content of organic matter in the waters and maintain the availability of nutrients from the decomposition of organic matter so that plankton will be maintained stable and harmful gas content for shrimp will decrease[6]. Probiotics such as *Bacillus* sp. can degrade organic nitrogen content into inorganic nitrogen and be able to produce higher nitrate content in water, to increase the nutrients needed by plankton[11].

The amount of probiotic bacteria given can affect the water quality of white shrimp rearing media. Giving probiotics with bacterial density that is too high does not always give the best results[12]. Based on this background, it is necessary to research the addition of *Bacillus* and *Pseudomonas* probiotics to plankton abundance, N/P ratio, and total bacteria in the white shrimp (*Litopenaeus vannamei*) culture to produce an environment in good condition and it is expected that shrimp health will also be good. The purpose of this study is to determine the effect of giving probiotics with different densities to the abundance value of plankton, N/P ratio, and total bacteria in the white shrimp (*Litopenaeus vannamei*) pond.

2. Materials and methods

The research started from January until April 2019 in Faculty of Fisheries and Marine, UniversitasAirlangga, Surabaya. This study using a completely randomized design (CRD), which used 4 treatments, and each treatment has 4 replications. The treatments given included K (0 cells/ml), B1 (10^6cells/ml), B2 (10^7cells/ml), and B3 (10^8 cells/ml).

2.1 Materials

This study used 320 white shrimpsaging 30 Days of Culture (DOC) and weighing 3-5 grams from the Brackishwater Aquaculture Fisheries Center in Tuban, East Java. Probiotic bacteria used came from the isolation of intensive shrimp pond sediments, namely *Bacillus subtilis*, *Bacillus mycoides*, and *Pseudomonas diminuta*.

2.2 Methods

2.2.1 Provision of media inoculants and probiotic bacteria

The probiotic bacterial multiplication was carried out using a media mix. The media mix is a bacterial growth media made from a mixture of several ingredients. In this study, the materials used to make the media were glucose 6%, molasses 2.5%, tofu liquid waste 10%, skim milk 10%, and distilled water added with sodium chloride 1.5%.

B. subtilis, *B. mycoides*, and *P. diminuta*that has been grown in the media Tryptone Soya Broth (TSB) is then harvested by centrifuge at a speed of 3,000 rpm for 15 minutes, then the supernatant is removed until the remaining sediment is left. Furthermore washing with Phosphate Buffer Saline (PBS), then homogenized using vortex. This washing was done twice. Bacteria were taken as much as

500 µl and put into 500 ml mix media, then incubated in an incubator at 30°C and harvested at the 32nd hours.

2.2.2 White shrimp culture

White shrimp are kept for 28 days in an aquarium with a volume of 47 liters containing brackish water with 15 ppt salinity with shrimp density 16 shrimp/aquarium. Feed was given4 times a day and in the form of pellets with 40% protein content in adlibitum. Cleaning water was done once per week. Water that wasted when the siphoning was replaced with new water following the amount of water wasted.

2.2.3 Provision of probiotics

Probiotics are given through maintenance water at a dose of 1 ml/liter. Probiotics are given 2 times per week, with different densities of10^6cells/ml, 10^7cells/ml, and 10^8cells/ml.

2.2.4 Parameter measurement

2.2.4.1 Plankton abundance

Calculation of plankton abundance using Sedgewick-Rafter counting chambers. Plankton abundance parameters were calculated using the formula proposed by [13]is:

$$No./mL = \frac{C \times 1000\, mm^3}{A \times D \times F}$$

Where:
C : the number of organisms counted
A : wide field of view (mm^2)
D : depth of Sedgewick-Rafter Cell (mm)
F : number of calculated visual fields

2.2.4.2 N/P ratio

N/P ratio measurements using the colorimetric method. Nitrogen measurements consist of Total Ammonia Nitrogen (TAN), nitrates, and nitrites using the Merck ammonia test kit, the Merck nitrate test kit, and the Merck nitrite test kit. Total inorganic nitrogen can be calculated by the formula stated by [14], that is:

$$N_i = N_{(TAN)} + N_{(Nitrit)} + N_{(Nitrate)}$$
$$N_{(TAN)} = (14/18).(\text{TAN})$$
$$N_{(Nitrit)} = (14/46).(NO_2^-)$$
$$N_{(Nitrate)} = (14/62).(NO_3^-)$$

Phosphorus measurements using the Merck phosphate test kit. The content of inorganic phosphorus can be calculated using the formula proposed by [14], that is:

$$Pi \text{ in } PO_4^{3-} = (31/95).(PO_4^{3-})$$

The N/P ratio can be measured by the formula stated by [14], that is:

$$N/P \text{ Ratio} = N_i/P_i$$

Where:
N_i : Total nitrogen
P_i : Total fosfor

2.2.4.3 Total bacteria

Water samples from white shrimp rearing media were taken using sterile bottles. Then the water sample is diluted using Phosphate Buffer Saline (PBS). Diluted water samples were taken as much as 0.1 ml using a micropipette and spread evenly on Tryptone Soya Agar (TSA) media using drigalsky. TSA saline media that had been spread with water samples were then incubated for 24 hours in an incubator with a temperature of 30°C.

Calculation of total bacteria in white shrimp rearing media uses the Total Plate Counting (TPC) method. Formula for calculating total bacteria according to [15]is as follows:

$$Bacterialcount(CFU/ml) = numberofcoloniesperplatex\frac{1}{dilutionfactor}$$

2.2.4.4 Survival rate

The survival rate of white shrimp can be calculated using the formula from [16]:

$$Survivalrate(\%) = \frac{Fish\ at\ the\ end\ of\ rearing}{Fish\ at\ the\ beginning\ of\ rearing} \times 100\%$$

3. Results and discussion

3.1 Results

3.1.1 Plankton abundance

Table 1. Plankton abundance in the culture media of white shrimp (*Litopenaeus vannamei*)

Treatments	Plankton Abundance (cells/ml) ± SD			
	Week-1	Week-2	Week-3	Week-4
K (0 cells/ml)	$859.87^{a,b}$ ± 121.966	$1,942.68^{c}$ ± 217.556	$3,757.96^{b}$ ± 337.037	$8,248.41^{b}$ ± 335,026
B1 (10^6 cells/ml)	$1,210.19^{a}$ ± 435.114	$5,208.07^{a}$ ± 1,551.663	$3,025.48^{b}$ ± 394,356	$8,657.87^{b}$ ± 691.917
B2 (10^7 cells/ml)	$1,210.19^{a}$ ± 396.066	$1,910.83^{c}$ ± 428,855	$3,566.88^{b}$ ± 375.023	$5,923.57^{c}$ ± 1,224.081
B3 (10^8 cells/ml)	668.79^{b} ± 217.555	$3,439.49^{b}$ ± 697.736	$5,000^{a}$ ± 787.851	$13,566.88^{a}$ ± 2,044.839

Description: Different superscript in the same column shows significantly different result ($p<0.05$).

ANOVA test results of plankton abundance in the first week showed significantly different ($p <0.05$). The B3 was significantly different ($p <0.05$) to the B1 and B2, but not significantly different ($p> 0.05$) towards K. There was an increase in plankton abundance from week 2 to week 4. ANOVA test results of plankton abundance at the 4th week showed significantly different ($p <0.05$). The highest plankton abundance at week 4 was found in B3 (13,566.88 cells/ml) and was significantly different ($p <0.05$) with K, B1, and B2.

3.1.2 N/P ratio

Table 2.N/P ratio in the culture media of white shrimp (*Litopenaeus vannamei*)

Treatments	N/P Ratio ± SD			
	Week-1	Week-2	Week-3	Week-4
K (0 cells/ml)	0.97^{a} ± 0.125	3.50^{b} ± 0.746	5.39^{a} ± 0.999	6.02^{a} ± 1.667
B1 (10^6 cells/ml)	0.95^{a} ± 0.145	5.06^{ab} ± 1.236	6.97^{a} ± 0.602	5.62^{a} ± 1.230
B2 (10^7 cells/ml)	0.85^{ab} ± 0.036	5.72^{a} ± 1.597	6.92^{a} ± 1.247	6.90^{a} ± 1.226
B3 (10^8 cells/ml)	0.78^{b} ± 0.028	5.02^{ab} ± 1.229	6.89^{a} ± 1.226	7.22^{a} ± 1.279

Description: Different superscript in the same column shows significantly different result ($p<0.05$)

ANOVA test results of the N/P ratio at week 1 showed significantly different ($p < 0.05$).B3 was significantly different ($p < 0.05$) to K, and B1, but not significantly different ($p > 0.05$) to B2. An increase in the value of the N/P ratio from week 2 to week 4. In the 4th week, there were no significant differences ($p > 0.05$) in each treatment. The highest N/P ratio was obtained at the 4th week in B3 (7.22).

3.1.3 Total bacteria

Table 3. Total bacteria in the culture media of white shrimp (*Litopenaeus vannamei*)

Treatments	Total Bacteria (CFU/ml) ± SD		
	Week-0	**Week-2**	**Week-4**
K (0 cells/ml)	$4.23 \times 10^{7\,a} \pm 0.000$	$1.03 \times 10^{5\,d} \pm 0.012$	$1.50 \times 10^{9\,c} \pm 0.014$
B1 (10^6 cells/ml)	$4.23 \times 10^{7\,a} \pm 0.000$	$1.63 \times 10^{5\,c} \pm 0.023$	$1.86 \times 10^{9\,b} \pm 0.019$
B2 (10^7 cells/ml)	$4.23 \times 10^{7\,a} \pm 0.000$	$1.23 \times 10^{6\,b} \pm 0.012$	$2.80 \times 10^{9\,a} \pm 0.013$
B3 (10^8 cells/ml)	$4.23 \times 10^{7\,a} \pm 0.000$	$4.75 \times 10^{6\,a} \pm 0.006$	$2.93 \times 10^{9\,a} \pm 0.003$

Description: Different superscript in the same column shows a significantly different result ($p<0.05$).

ANOVA test results of total bacteria at week 1 showed that they were not significantly different ($p > 0.05$). Duncan test results at week 1 showed that B3 was not significantly different ($p > 0.05$) to K, B1, and B2. A decrease in the total of bacteria in the second week, and an increase in total bacteria in the 4th week. ANOVA test results at week 2 and week 4 showed significantly different results ($p < 0.05$). At the 4th week, the B3 was significantly different ($p < 0.05$) from K and B1, but not significantly different ($p > 0.05$) from B2. The highest of total bacterial was obtained at 4thweeks in the B3 (2.93×10^9 CFU / ml).

3.1.4 Survival rate

Table 4. Survival rate of white shrimp (*Litopenaeus vannamei*)

Treatments	Survival Rate (%) ± SD
K (0 cells/ml)	$49.27^{b} \pm 27.582$
B1 (10^6 cells/ml)	$81.41^{a} \pm 16.058$
B2 (10^7 cells/ml)	$87.98^{a} \pm 13.899$
B3 (10^8 cells/ml)	$89.58^{a} \pm 12.156$

Description: Different superscript in the same column shows significantly different result ($p<0.05$)

ANOVA test results the survival rate of white shrimp culture showed significantly different ($p < 0.05$). The B3 was significantly different ($p < 0.05$) to the K, but not significantly different ($p > 0.05$) to the B1 and B2 treatments. The highest survival rate of white shrimp was obtained in the B3 (89.58%).

3.2 Discussion

Based on the calculation of plankton abundance, the administration of probiotics can affect plankton abundance every week. Plankton abundance at first week showed significantly different results. This is because in the first week of the study the number of probiotic bacteria that are in the medium of shrimp culture is still small in number. The B3 is different from B1 and B2, and the plankton abundance in B3 is lower than the K, B1, and B2. This can be due to the number of B3 bacteria more than B1 and B2 so that in the first week there is a competition of nutrients in the form of nitrogen and phosphorus. Bacteria need carbon and nitrogen to metabolize[17], while phytoplankton require nitrogen and phosphorus[18].In the second week, there was an increase in plankton abundance because there was an increase in nutrients in the water. The longer the

maintenance period, the higher the organic matter in the water. In B3, bacteria can degrade organic matter into carbon, nitrogen, and phosphorus.

The abundance value of plankton in each treatment increased every week starting from the second week to the fourth week. But in the third week, the value of plankton abundance in B1 decreased. The decrease in plankton abundance can be caused by competition of nutrients in the form of nitrogen consumed by both phytoplankton and probiotic bacteria. Competition between phytoplankton and bacteria can affect the composition of phytoplankton and the bacterial community and can affect the basic function of microbial ecosystems by shifting the balance between phytoplankton and bacteria[19]. Whereas the treatment of B2 and B3 continued to experience an increase in plankton abundance. This is because the number of probiotic bacteria given to the white shrimp culture media is sufficient to degrade organic material so that the nitrogen and phosphorus available in the shrimp rearing media can be utilized by phytoplankton for optimal metabolism so that phytoplankton growth can increase.

In the N/P ratio has a value ranging between 0.78 - 7.22. The value of N/P ratio affects the composition of the phytoplankton class in white shrimp culture media. The dynamics of the composition of nitrate, nitrite, ammonia, and phosphate affect the dynamics of the composition of plankton in the water[20]. The lowest N/P ratio value was obtained in the first week at B3, while the highest N/P value at the fourth week was obtained at B3. The N/P ratio of B1 in the fourth week has decreased. A decrease in the N/P ratio can be caused by the increasing number of bacteria in the shrimp rearing media so that nitrogen is consumed by phytoplankton and bacteria. At the fourth week, the lowest N/P ratio value was obtained at K, while the highest value at B3. This indicates that the addition of probiotics affects the value of the N/P ratio. In this study, the results show that the more N/P ratio increases, the abundance of plankton will also increase. This can be seen in the B3, where the N/P ratio increases, the number of plankton also increases.

In Table 3, the total bacteria in the second week in each treatment decreased. This is because probiotic bacteria are still in the stage of adapting. The decrease in total bacteria is also supported by nitrogen levels in waters that are still low and the number of plankton has increased, resulting in nutritional competition between phytoplankton and bacteria. Whereas in the 4^{th}week, the total bacteria in each treatment has increased. This is supported by nitrogen levels in the waters that increase so that bacteria can utilize nitrogen and carbon to carry out metabolism. The work of extracellular enzymes produced by probiotic bacteria in the form of carbon and nitrogen can affect the growth and metabolic yield of bacteria[21]. The highest total bacteria were obtained in the B3 (2.93×10^9 CFU / ml). The total bacteria in the white shrimp rearing media was influenced by the number of probiotics given.

Probiotics given in this study can affect the survival rate of white shrimp. In Table 4, it can be seen that the highest survival rate of white shrimp is obtained in the B3 (89.58%). The high survival rate of white shrimp is suspected because probiotic bacteria can compete with pathogenic bacteria[22]. Probiotic bacteria-derived fromwhiteshrimp culture media have the potential and can suppress the growth of pathogenic bacteria, including *vibrio* sp.[23].While the lowest survival rate of white shrimp occurred in K (49.27%). The treatment without administration probiotics has the lowest survival rate, this is presumably because there are no additional probiotic bacteria that can suppress pathogens. Factors that affect survival rates include water quality, pathogens, and stress levels[22].

4. Conclusion

Based on the results of the study, probiotic *Bacillus subtilis*, *Bacillus mycoides*, and *Pseudomonas diminuta* with different density can affect the abundance of plankton, the ratio of N/P, and total bacteria on white shrimp(*Litopenaeus vannamei*) culture media.

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