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Different Stocking Levels and Giving of *Bacillus* sp. to the Growth and Survival of Post Larvae (PL) of the Vanamei Shrimp (*Litopenaeus vannamei*)

Andi Puspa Sari Idris[1*] and Patang[2]
[1]Department of Aquaculture, Pangkep State Agricultural Polytechnic, Indonesia
[2]Department of Agricultural Technology, Makassar State University, Indonesia

***Corresponding Author: andipuspa@gmail.com**

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ABSTRACT
This research aimed to determine the effect of different stocking densities of vanamei shrimp and the provision of *Bacillus* sp. in the cultivation media on the growth and survival of vanamei shrimp (*Litopenaeus vannamei*). This study used a completely randomized design with four treatments and three replications, namely treatment A: density of vanamei shrimp 22 individuals/liter; treatment B: density of white shrimp 27 individuals/liter; treatment C: density of white shrimp 32 individuals/ liter and treatment; and D: density of white shrimp is 37 individuals/liter. *Bacillus* sp. was added to each treatment at 1ppm. The parameters tested were absolute weight growth, absolute length growth, daily growth, and survival. Meanwhile, the water quality parameters measured are temperature, pH, salinity, dissolved oxygen, nitrite, and ammonia. The data were analyzed for variance using the SPSS application, and the water quality data were descriptively analyzed. Compared to other stocking densities, treatment A was the best based on results. This can be seen from the data of absolute growth, absolute length growth, daily growth, and survival. Water quality data viz. temperature, pH, salinity, dissolved oxygen, nitrite, and ammonia are still in the water quality range suitable for the life of the vanamei shrimp test animals.

INTRODUCTION

According to **Asnawi and Mukhlis (2008)**, one fishery commodity that could displace Indonesia's exports of oil and gas is shrimp (*Litopenaeus vannamei*), a species with a high economic value that is capable of generating foreign exchange for the nation **(Babu *et al*., 2014; Herawati & Hutabarat, 2015; Lama *et al*., 2020; Usman *et al*., 2022)**. Aside from that, vanamei shrimp are among the best fisheries species now farmed in Indonesia and have the benefit of being productive, with a yield of 6–10 tons/ha/year in addition to being resistant to disease **(Yasin, 2013; Ariadi *et al*., 2021; Akmaluddin *et al*., 2023)**. The existence of vanamei shrimp in Indonesia as an introduced shrimp and as an alternative commodity is considered capable of replacing the tiger shrimp (*Penaeus monodon*) as a positive business diversification **(Susianingsih *et al*., 2016)**. This is due to its benefits, which include easier cultivation, faster growth **(Supriyono *et al*., 2007)**. Furthermore, benefits incorporate the increased resistance to disease and environmental factors, particularly the white spot syndrome virus **(Haliman &**

Adijaya, 2005). Two elements, including water quality, management factors, and the quality of shrimp larvae, can lead to the establishment of disease assaults on shrimp **(Illijas *et al*., 2023)**.

Efforts to increase the productivity of vanamei shrimp are also not free from various problems, one of which is the emergence of bacterial diseases such as vibriosis **(Azwansyah, 2022)**. According to **Irma *et al*. (2022)**, the vibriosis disease that struck shrimp larvae in Indonesia in 1991 caused a 70% decrease in larval output. Shrimp deaths resulting from vibriosis attacks in waterways can reach 85% **(Anton *et al*., 2020)**. Giving probiotics is one way to combat this **(Akmaluddin *et al*., 2023)**. The findings of **Mustafa *et al*. (2019)** demonstrated that the growth and survival values of vanamei post larvae given probiotics increased to 8g/ head and 97.33%, respectively. *Bacillus* sp. **(Keysami *et al*., 2007; Rahiman *et al*., 2010; Widanarni *et al*., 2010)** is one of the probiotics that can be used to inhibit vibrio attacks because it produces the AHL-lactonase enzyme, which can inhibit the intercellular communication mechanisms between bacterial cells and can prevent pathogenetic genes from being expressed by pathogenic bacteria **(Permanti *et al*., 2018)**.

Nowadays, the application of probiotics in aquaculture is increasingly popular because it is environmentally friendly and is able to improve the health and growth of cultivated organisms **(Seenivasan *et al*., 2014)**. Probiotics are essential for sustaining the culture environment, decreasing pathogen populations, boosting growth, boosting survival, and influencing production **(Vine *et al*., 2006; Yuhana, 2010; Akter *et al*., 2017)**. Probiotics have been applied extensively in aquaculture activities for the purpose of improving water quality, boosting immune responses, controlling disease **(Gunarto & Hendrajat, 2008; Qi *et al*., 2009; Ekasari *et al*., 2014)**, and providing nutritional and enzymatic contributions to the digestion of aquaculture organisms. One genus of bacteria that has been applied extensively as a source of probiotics in a variety of organisms is *Bacillus* sp. **(Elshaghabee *et al*., 2017)**. Additionally, probiotic bacteria addition was found to boost immunity and lower the prevalence of WSSV infection in vanamei shrimp, according to **Partida-Arangure *et al*. (2013)**. According to **Burhanuddin *et al*. (2016)**, probiotic treatment significantly impacted vanamei shrimp growth and survival. According to **Usman and Rochmady (2017)**, probiotic dosage had a significant impact on relative growth but had no effect on the survival rate of tiger prawn postlarvae. **Suwoyo and Mangampa (2010)** and **Nengsih (2015)** informed that the application of probiotics had a quite good effect on the water quality conditions of the vanamei shrimp rearing media. Giving probiotics can increase the immune response **(Ramezani-Fard *et al*., 2014)**, resistance to disease attacks, growth performance, survival, feed utilization, and digestive enzyme activity **(Gupta *et al*., 2016)**. Non-pathogenic properties are one of the important criteria that determine whether an organism can be used as a good probiotic candidate **(Rahiman *et al*., 2010; Yuhana, 2010)**.

The density level is another factor that affects vanamei shrimp production in addition to probiotic provision. A shrimp's survival rate can be decreased by cannibalism resulting from an excessively high shrimp seed density according to **Syahid *et al*. (2006)**. In this respect, **Briggs *et al*. (2004)** postulated that vanamei shrimp (*L. vannamei*) can grow well at stocking densities of around 60–150 individuals/m^2. Meanwhile, the stocking density for vanamei shrimp cultivation carried out in various regions in Indonesia, according to **Haliman and Adijaya (2005)**, was around 100-125 individuals/m^2. Because shrimp have limited movement and find it difficult to absorb the minerals needed by the body, shrimp tend to have thin skin, reduced appetite, and are easily stressed **(Taqwa *et al*., 2010)**. Stocking density plays an important role in shrimp cultivation by determining the number of fries to be reared **(Gunarto & Hendrajat, 2008)**. One of the factors that influence the success rate of shelling vanamei shrimp is stocking density **(Usman *et al*., 2022)**. **Effendie (1997)** in this context elucidated that, stocking density is said to be optimal if shrimp are stocked in high numbers, but competition for food and space can still be tolerated by shrimp.

Therefore, the present study aimed to determine the effect of different stocking densities of vanamei shrimp and the provision of *Bacillus* sp. in the cultivation media on the growth and survival of vanamei shrimp (*Litopenaeus vannamei*).

MATERIALS AND METHODS

This research was designed using a randomized design consisting of 4 treatments and 3 replications, so there were 12 experimental units (Fig. 1). The treatments tried were:

Treatment A = Density of vanamei shrimp 22 individuals/liter
Treatment B = Density of vanamei shrimp 27 individuals/liter
Treatment C = Density of vanamei shrimp 32 individuals/liter
Treatment D = Density of vanamei shrimp 37 individuals/liter

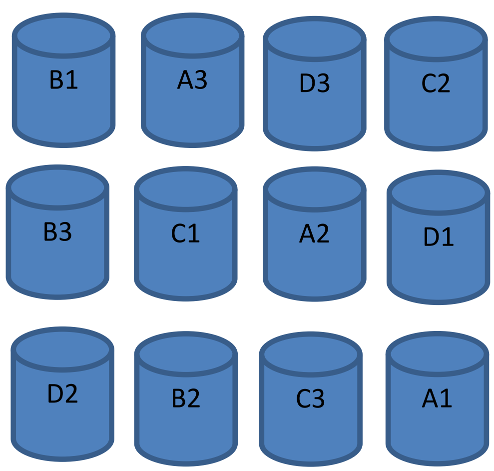

Fig. 1. Experimental unit design plan

The test animals used were vanamei shrimp larvae of uniform size, size PL 7, which were first acclimatized for 30 minutes. After that, the test animals were kept for 16 days. The feed used is commercial feed, namely the Lanzy PL brand with a feed dose of 7ppm. Feeding is carried out 4 times/day, namely at 7.00, 11.00, 15.00, and 19.00. The media used in this research was sea water with a salinity of 25– 30ppt obtained from water channels around the Pangkep State Agricultural Polytechnic water channel research location. Before use, sea water was first sterilized with 30ppm chlorine and was given strong aeration for 24 hours. Next, dolomite was spread, namely 12 g/container (equivalent to 500kg/ ha). The probiotics applied in the research media were *Bacillus* sp., ready to use with a dose of 1ppm **(Risdianto *et al*., 2015)**. The use of molasses as bacterial food in a ratio of 1:1 was cultured for 24 hours **(Izzah *et al*., 2014)**. The distribution of probiotics was carried out six times, namely the 2nd day after spreading the dolomite (2 days before spreading the vanamei shrimp), the 3rd day, the 6th day, the 9th day, the 12th day, and the 15th day during the shrimp rearing period. Twelve sixty-liter plastic buckets were utilized as rearing container, and they were arranged at random with the understanding that the environment at the study site was thought to be uniform. Sterilized water was poured into each container **(Usman *et al*., 2022)**. Thirty liters of water was placed in a container with fixed aeration. The rearing container was checked for damage and cleaned of filth before being used. It was then allowed to dry for three to five days **(Renitasari *et al*., 2023)**. The cleaned vanamei shrimp rearing container was labeled according to the treatment. Next, the container was filled with 30 liters of water/container and aerated. Dolomite was then added in each container. Vanamei shrimp sampling was carried out to determine the absolute weight growth rate, absolute length growth rate, and survival rate of white vanamei shrimp. Measurement or sampling was carried out by taking 10 test shrimp from each treatment. In order to prevent the shrimp from becoming easily stressed out by temperature fluctuations, sampling was done first thing in the morning, utilizing a scoop to collect shrimp samples in a container. The shrimp were weighed on analytical scales following a sample. The shrimp were put back in the growing container after being sampled.

Calculation of absolute weight gain was conducted as stated by **Effendie (1997)** namely:

$$\mathbf{W = W_t - W_0} \quad (1)$$

Where:

W = Weight gain (g)

$\mathbf{W_t}$ = Average weight of fish at the end (g)

$\mathbf{W_0}$ = Average weight of fish at the start (g)

- Calculation of absolute length growth was conducted as stated by **Effendie (1997)**

$$\mathbf{P_m = L_t - L_0} \quad (2)$$

Where:

$\mathbf{P_m}$ = Absolute increase in length (cm)

$\mathbf{L_t}$ = Final average length (cm)

$\mathbf{L_0}$ = Initial average length (cm)

- The daily growth rate of vanamei shrimp during the study was determined using the formula **Zonneveld *et al*. (1991b)**, as follows:

$$\mathbf{SGR = (L_nW_t\text{-}L_nW_o)/\ T_t \times 100\%} \quad (3)$$

Where:

SGR: Daily growth rate (%/day)

$\mathbf{W_t}$: Average weight of shrimp at the end of the study (g)

W_0: Average weight of shrimp at the start of the study (g)

T_t: Length of maintenance (days).

- Calculation of survival used the formula of **Effendie (1997)** and **Purnamasari *et al*. (2017)**, namely:

$$SR = N_t/No \times 100\% \qquad (4)$$

Where:

SR = Life graduation degree (%)

N_t = Amount at the end of maintenance (tail)

No = Number at the start of stocking (tail)

Monitoring and replacing water are two aspects of the management of water quality that are done. Every morning before feeding, water quality parameters were measured as part of the monitoring process. Temperature, salinity, and pH were the three factors that were measured on-site. Meanwhile, parameters such as dissolved oxygen, nitrite, and ammonia were measured every 3 days. Dissolved oxygen measurements were carried out *in situ*. Measurement of ammonia levels was carried out by taking 200ml of water samples from each container. The water samples were tested for ammonia levels using the phenate spectrophotometer method **(Indonesian National Standards, 2005)** in the range of 0.1mg/ l to 0.6 ppm. Meanwhile, measurements of nitrite levels were carried out by taking 200ml of water samples from each container. Then, the water samples were tested for nitrite levels using the spectrophotometer method **(Indonesian National Standards, 2004)** in the range of 0.01mg/ l to 1.0 ppm. Water changes were carried out 3 times at an interval of 3 days with a percentage of 50-80% **(Usman *et al*., 2022)**.

The data obtained from this research activity were those recorded for the absolute growth, absolute length growth, daily growth, and survival of the vanamei shrimp analyzed using the ANOVA Test, then continued with the Tukey-Test using the SPSS version 22 program. Meanwhile, the data measuring water quality parameters was analyzed descriptively.

RESULTS AND DISCUSSION

1. Absolute weight growth

The results of this study show that the lower the stocking, the higher the average absolute growth of the vanamei shrimp, as seen in Fig. (2), where the treatment with a stocking density of 22 fish/liter showed the highest average weight value. The results of the significance test show a sig value, namely 0.001, which means <0.05, showing that the treatment has an influence on the absolute weight growth of the vanamei shrimp larvae. **Novitasari *et al.* (2017)** reported that *Bacillus* sp. up to 5ppm can be added to technical media molasses to increase the absolute growth of the vanamei shrimp larvae. According to **Cholik *et al*. (1990)**, and supported by the current research, stocking density can affect growth and survival rates by influencing competition for space, feeding requirements, and environmental factors. Stocking density is just one aspect that affects growth **(Purba, 2012)**; other factors that affect growth include internal and external influences **(Febriani *et al*., 2018)**.

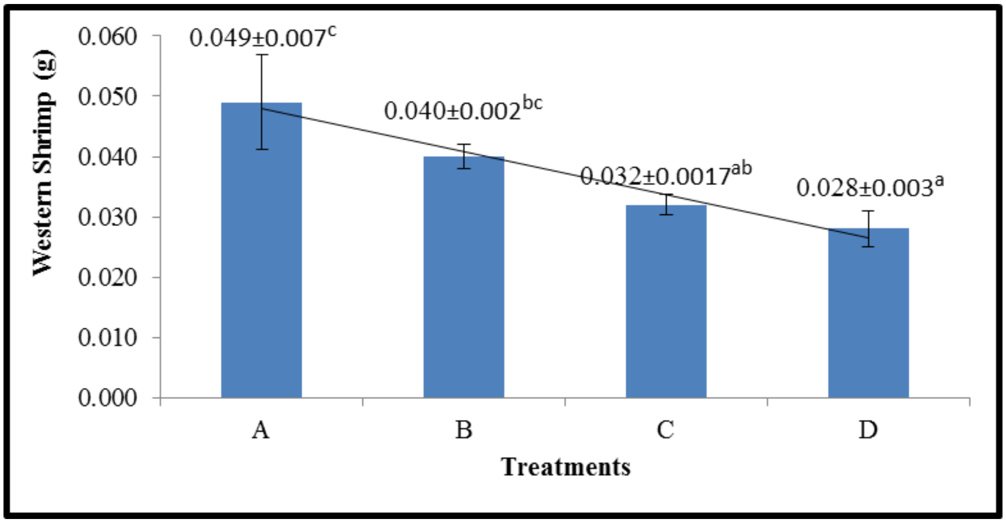

Fig. 2. Average absolute weight growth of vanamei shrimp (g)

The use of probiotics helped in the absorption of feed nutrients into the body and can increase the shrimp's body immunity **(Basir, 2013)**. **Fernando (2016)** stated that vanamei shrimp reared with the addition of probiotics tended to provide higher growth compared to those without. According to **Usman and Rochmady (2017)**, giving shrimp an appropriate amount of probiotics will enable them to correctly digest their meal and absorb the nutrients they require, which will impact their growth. According to **Purba (2012)**, providing maintained shrimp with a diet with a sufficient nutritional content will ensure their survival and activity while also hastening their growth. To clarify this notion, **Fujaya (2008)** stated that fish do not use all of the food they ingest for growth. The majority of the energy obtained from food is utilized for maintenance and basal metabolism; the rest is allocated for growth, reproduction, and activity.

2. Shrimp length growth

The average absolute length growth of shrimp was the highest in treatment A at 0.93 ± 0.065, and the lowest in treatment D, with 0.74 ± 0.020 (Fig. 3). The results of the ANOVA test showed a significance value of 0.001, which means that the implementation has an influence on the absolute growth in length because it is <0.05. **Effendie (1997)** stated that growth occurs due to tissue increase and mitotic cell division, which occurs due to an excess input of energy and protein from feed. **Fujaya (2008)** illustrated that, compared to adult fish, growing juvenile fish consumed more energy per unit of body weight. **Purba (2012)** asserts that sufficient nutritional content in the feed and appropriate feed consumption can have a significant impact on the growth of post-larvae of white shrimp larvae. This is also in accordance with the study of **Gunarto and Hendrajat (2008)**, who stated that feed consumption greatly influenced the increase in shrimp body weight because feed consumption determines the entry of nutrients into the body, which will then be used for growth and other needs. The main external factors influencing growth are food and water temperature **(Effendie, 1997)**. Low temperatures will cause the fish to lack appetite; the fish will become slow to develop and have small bodies, resulting in the fish often swimming alone **(Wangni *et al*., 2019)**.

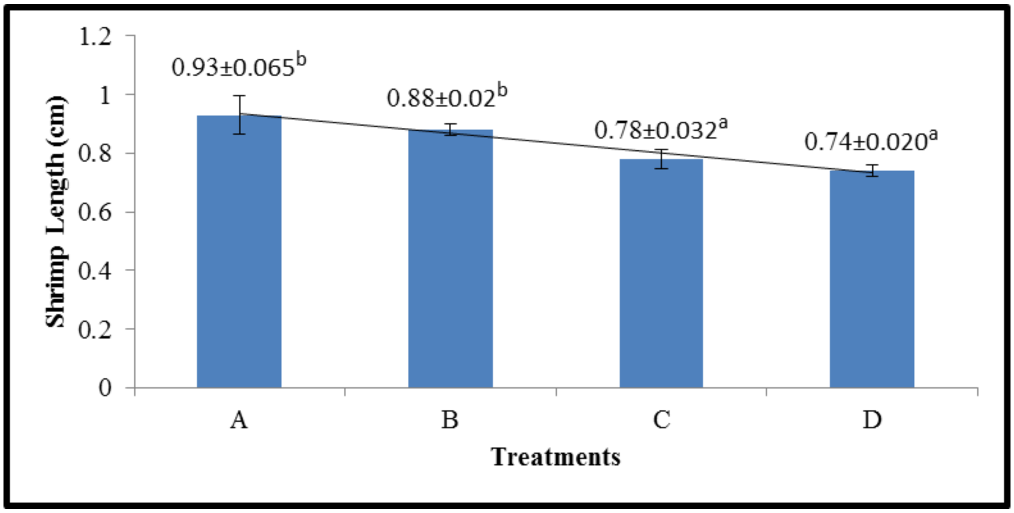

Fig. 3. Average growth of shrimp length (cm)

3. Shrimp daily growth

The average value of daily growth rate during the study was in treatment A, namely 10.74 ±0.25, and the lowest was in treatment D (8.33 ± 0.49%) (Fig. 4). The results of the ANOVA test showed that the treatment had an influence because it has a value of 0.001 and is smaller than 0.05 (< 0.05). The daily growth rate of the vanamei shrimp in this study is greater than the results of research conducted by **Gunarto *et al*. (2008)**, with ranges of 0.12- 0.17g/ day. Physically, growth is manifested throughout time by variations in the quantity or size of cells that comprise body tissue **(Effendie, 1997)**. **Purba (2012)** highlighted that a variety of factors affect growth, which is a complicated biological process. Fish have a sluggish digestive system hence temperature has an indirect impact on fish growth **(Wangni *et al*., 2019)**.

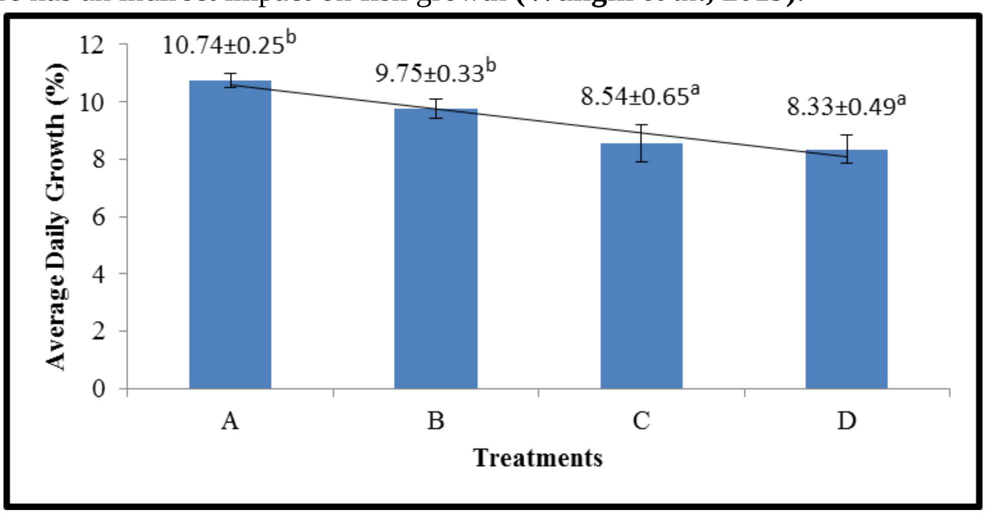

Fig. 4. Average daily growth of shrimp (%)

4. Survival

The highest survival rate of the vanamei shrimp in this study (90.13 ± 5.32) was in treatment A, with the lowest stocking density level, 22 fish/liter, while the lowest survival rate, was in treatment D, recording values of 73.17 ± 5.57% (Fig. 5). The results of the ANOVA test showed a significance value of 0.02, which means that the treatment has an influence on survival because it is less than <0.05. The present results are in line with the opinion of **Rakhfid *et al.* (2017)** considering that the survival value of vanamei shrimp tends to decrease with increasing stocking density. *Bacillus* sp. produces antimicrobial substances, namely bacteriocins, which can increase post-larval survival of vanamei shrimp **(Suriani, 2016)**. The application of probiotics has significantly increased the survival of giant prawns **(Habib *et al.*, 2014; Seenivasan *et al.*, 2014)** and the vanamei shrimp **(Hamsah *et al.* 2017a, b)**. **Nainggolan (2008)** stated that during the moulting process, the mortality rate for shrimp can reach 30%, one of which is caused by cannibalism.

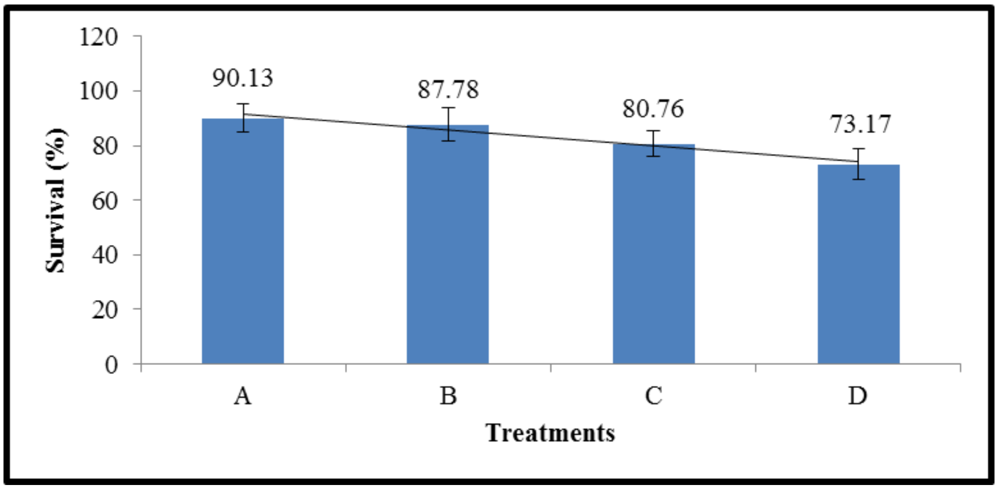

Fig. 5. Average vanamei shrimp survival (%)

Stocking density affected survival and growth rates. High and inappropriate stocking density will also have an impact on water quality **(Renitasari *et al.*, 2023)**. According to research by **Rakhfid *et al.* (2017)**, survival rates are influenced by competition between individuals in utilizing space and obtaining food. The use of high stocking densities will have an impact on water quality and survival rates **(Renitasari *et al.*, 2023)**. Apart from that, **Fuady (2013)** stated that the factors that influence the level of survival in cultivation are abiotic and biotic factors. The moulting process, which does not coincide between one shrimp and another, tends to cause cannibalism of the shrimp that are moulting and subsequently results in death **(Anggoro, 1992)**. The addition of *Bacillus* sp. also has an effect on increasing the survival and absolute growth of the white shrimp post larvae infected with vibrio **(Akmaluddin *et al.*, 2023)**. Mass fatalities from high vibrio populations can result in large financial losses **(Kaligis, 2015)**. If probiotics are taken three days apart at a concentration of 0.5ppm in 0.1ml of water, the population of *Vibrio* sp. can be reduced by 88.49% **(Budi *et al.*, 2016; Mustafa *et al.*, 2019)**.

According to **Permanti *et al*. (2018)**, there was only a 2.7% difference in survival between post-latrial rearing infected with *Vibrio* and larval rearing without *Vibrio* (85.3% survival).

5. Water quality

Poor water quality management is one of the triggers for disease attacks in shrimp farming **(Racotta *et al*., 2004)**. During the research, the water quality of the media was still within the tolerance limits of the vanamei shrimp, showing no effect on the treatment (Table 1). The management of water quality as a medium for cultivating the vanamei shrimp in ponds is one of the decisive elements that affect the effectiveness of shrimp farming **(Mas & Wahyudi, 2018)**. **Suwoyo and Mangampa (2010)** stated that the use of intensive technology in cultivation operations creates the issue of ponds' carrying capacity being reduced for the life of farmed shrimp. **Wiranto and Hermida (2010)** concluded that water quality management is a very important thing to pay attention to in the process of rearing vanamei shrimp larvae. Water quality is acceptable as long as it does not have a negative influence on biota **(Zonneveld & Fadholi, 1991a)**. In addition, according to **Qi *et al*. (2009)**, the application of probiotics in cultivation activities is used to control disease, increase immune responses, provide nutritional contributions and enzymatic effects on the digestion of cultivated organisms, as well as improving water quality. This is in accordance with the statement by **Nengsih (2015)** that the application of probiotics in aquaculture activities can improve the range of water quality in terms of temperature, pH, salinity, oxygen, nitrite, and ammonia (Table 1).

Table 1. Range of water quality for vanamei shrimp rearing media during the research

Water quality	Treatment	Range
Temperature (^{0}C)	A	26-29
	B	26-30
	C	26-31
	D	26-32
pH	A	8.1-8.6
	B	8-8.7
	C	8.1-8.7
	D	8.1-8.6
Salinity (ppt)	A	30-31
	B	30-31
	C	30-31
	D	30-31
Dissolved Oxygen (ppm)	A	3.97-4.83
	B	4.06-4.8
	C	4.00-4.89
	D	4.00-4.67
Nitrite (ppm)	A	0-0.1
	B	0-0.1
	C	0-0.2
	D	0-0.3
Ammonia (ppm)	A	0-1.5
	B	0-1.5
	C	0-1.5
	D	0.25-1.5

6. Temperature

Water temperature during the study is illustrated in Fig. (6). One of the physical characteristics of water that is crucial to the existence of organisms is temperature **(Taqwa *et al*., 2008)**. **Huet (1971)** noted that another external factor influencing fish growth is water temperature. Because oxygen is soluble in water, water temperature has an indirect impact on aquatic biota life **(Boyd, 1991)**. Metabolic activity as well as the life and growth of aquatic biota are influenced by temperature **(Wihardi *et al*., 2014; Putri *et al*., 2019)**. The optimal temperature to support the growth of vanamei shrimp is between 26 and 32°C **(Haliman & Adijaya, 2005; Supriatna *et al*., 2020)**. The optimum temperature for vanamei shrimp is between 23 and 30^{o}C **(Wyban & Sweeny, 1991)**. Meanwhile, the water temperature for fish rearing media in this study is in the range of 27.89 to 28.06°C (Fig. 6). Every 100°C increase in temperature will cause a two-time increase in the biochemical rate **(Boyd, 1982)**. Water temperature is related to fish health and is closely related to survival; since fish are cold-blooded animals, the metabolism in the body depends on the temperature of the environment including body immunity **(Haris *et al*., 2017)**. At low temperatures, fish become inactive, often gathering or in groups, not wanting to swim, resulting in reduced immunity, making them susceptible to fungal or parasitic diseases, while high temperatures can accelerate the occurrence of bacterial infections **(Haris *et al*., 2017; Wangni *et al*., 2019)**.

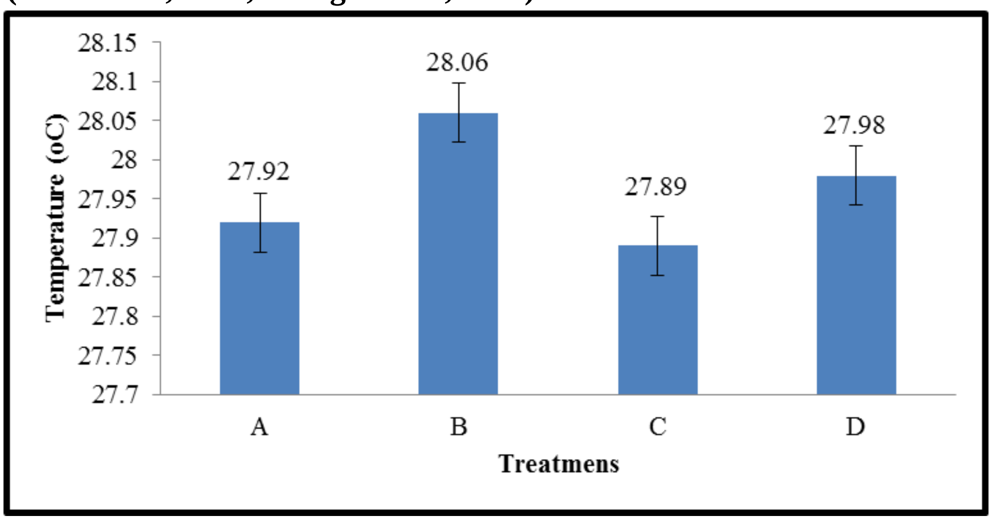

Fig. 6. Average water temperature for fish rearing media (^{o}C)

7. pH

The pH value determines whether or not an aquatic environment is suitable for shrimp **(Ratnawati, 2008; Nengsih, 2015)**. The pH of the media water during the research was in a range that suited the needs of the vanamei shrimp because it was above 8 (8.22 to 8.36) (Fig. 7). This is in line with the opinion of **Boyd (1991)** and **Ariadi *et al*. (2020)** demonstrating that the degree of acidity (pH) that is good for shrimp survival is around 6-9. When the pH falls between 5.4 and 6.4, the process of larvae turning into postlarvae is disrupted, opposed to the case when the pH rises over 7 **(Liew *et al*., 2022)**. Vanamei shrimp are cultivated in water with a pH of 7.8–

8.0, or 7.0–8.5 **(Elovaara, 2003; Supriatna *et al*., 2020)**. Excessive mucus may leak out due to gradual pH changes; the skin will turn white and become more susceptible to infection **(Yunias, 2010)**. **Haris and Yusanti (2019)** stated that the dissolved oxygen content will drop at low pH levels (high acidity). Fish will grow more slowly, become more vulnerable to bacteria and parasites, and possibly even perish as a result of decreased oxygen intake, increased respiratory activity, and decreased hunger **(Wangni *et al*., 2019)**.

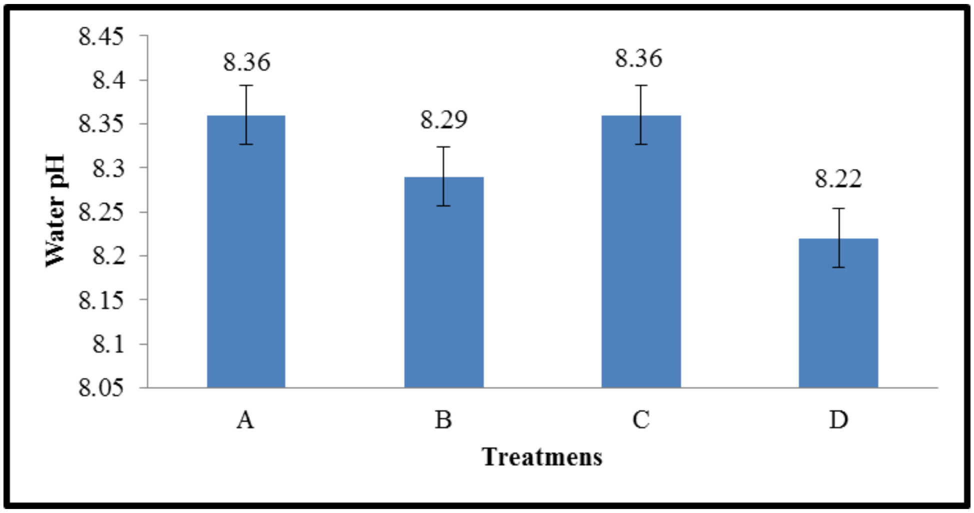

Fig. 7. Average pH of water in fish rearing media

8. Salinity

According to **Anita *et al*. (2017)**, salinity affects or is related to the osmoregulation process, which affects shrimp development and larvae survival. The study's media water had a salinity between 30.52 & 30.92ppt (Fig. 8). According to **Renitasari *et al*. (2023)**, the vanamei shrimp are euryhaline, which allows them to grow in a broad salinity range of 0- 45ppt. Optimal salinity for the vanamei shrimp ranges from 5- 35ppt **(Xincai & Yongquan, 2001)**. Meanwhile, **Saoud *et al*. (2003)** argumented that the vanamei shrimp can grow in waters with salinity ranging from 0.5 to 38.3 ppt. In this context, **Syukri and Ilham (2016)** added that the best salinity for the life of vanamei larvae is 15– 30ppt. Vanamei shrimp can be cultivated in a salinity of 0.5ppt and a stocking density of 170 to 175 individuals/m^2 **(Purnamasari *et al*., 2017)**.

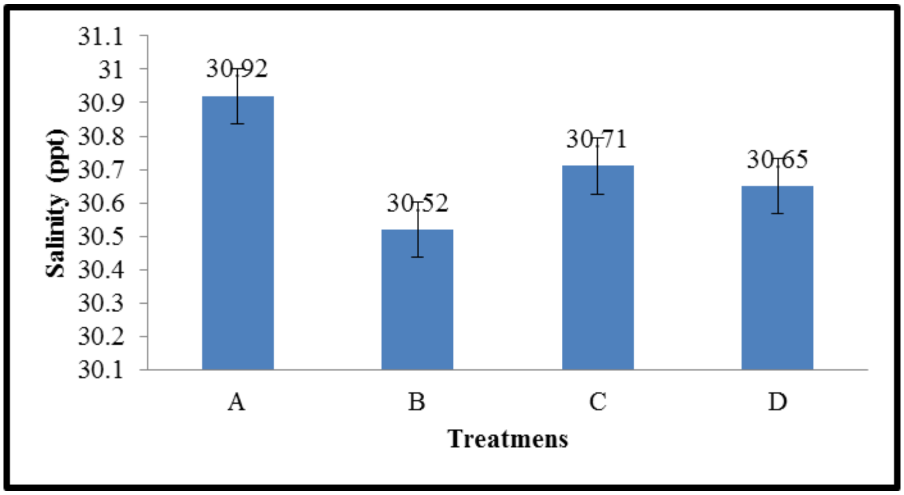

Fig. 8. Average salinity of fish rearing media water (ppt)

9. Dissolved oxygen

The need for oxygen in shrimp cultivation is important in two aspects, namely environmental needs for certain species and consumptive needs, which depend on shrimp metabolism **(Zonneveld *et al*., 1991b)**. Dissolved oxygen content greatly influences shrimp body metabolism **(Haliman & Adijaya, 2005)**. The oxygen content in this study was in the range of 4.27-4.36 (Fig. 9). Thus, the dissolved oxygen content in this study is relatively low compared to **SNI 7311:2009**, ranging from >5 ppm **(Usman *et al*., 2022)**. However, it is still in accordance with the opinion of **Adiwijaya *et al*. (2003)**, illustrating that the optimal range of dissolved oxygen during the rearing period for the vanamei shrimp is around 3.5– 7.5ppm, with dissolved oxygen of 3– 8ppm **(Fegan, 2003; Syukri & Ilham, 2016)**.

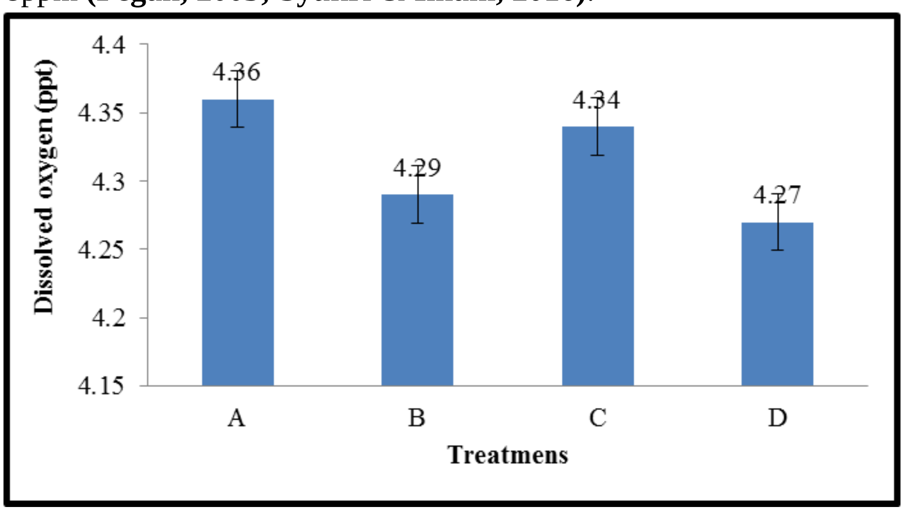

Fig. 9. Average dissolved oxygen in fish rearing media water (ppm)

10. Nitrite

The presence of nitrite (NO_2) describes ongoing biological processes from organic materials with low dissolved oxygen levels **(Effendi, 2003)**. Nitrite in aquaculture waters comes from shrimp waste, which is then mineralized to form ammonia, which is converted into nitrite.

Under normal conditions, nitrite will be converted into nitrate by bacteria **(Durborow *et al*., 1997)**. The nitrite content of rearing media water in this study was in the range of 0.04-0.08 ppm, with the highest value (0.08 ppm) of media water in treatment C (Fig. 10). The NO_2 level in the water that can be tolerated by shrimp is 0.1–1.0 ppm **(Suprapto, 2005)**. For nitrate, **Adiwijaya *et al*. (2003)** in their study assessed that the optimal range of nitrite for vanamei shrimp cultivation is 0.01–0.05 ppm.

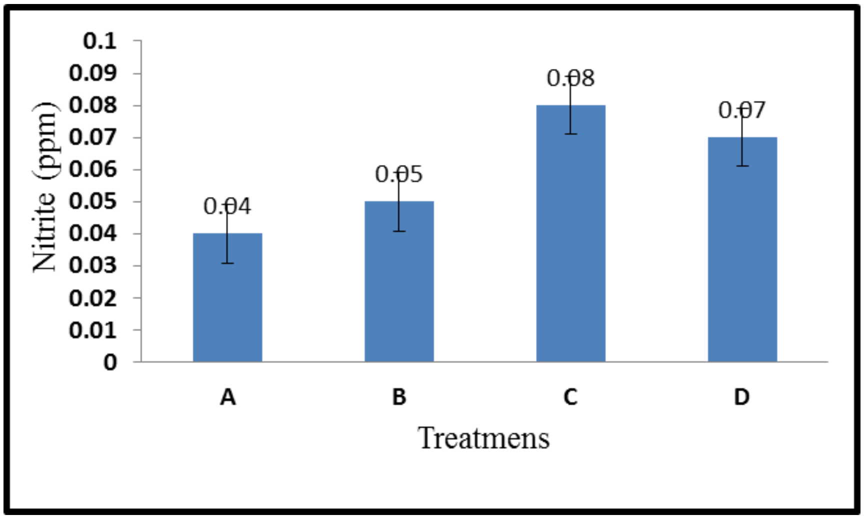

Fig. 10. Average dissolved nitrite in fish rearing media water (ppm)

11. Ammonia

High stocking density will increase the organic material content due to the buildup of food waste and metabolic waste so that the shrimp will be stressed and easily infested with ectoparasites and can end in death **(Usman *et al*., 2022; Renitasari *et al*., 2023)**. **Yudiati *et al*. (2010)** concluded that the use of probiotic bacteria was able to increase the removal of ammonia and organic matter into simple compounds that were actually needed by primary producers for the growth of vanamei shrimp. The ammonia value in this study was in the range of 0.6667 to 0.9333 ppm, with the highest ammonia value found in treatment D, namely 0.9333 ppm (Fig. 11); nevertheless, the ammonia value in this study is still much lower than the opinion of **Boyd and Clay (2002)** who argumented that the ammonia concentration above 4 and 5 ppm will be toxic to shrimp. Eminently, an increase in stocking density is associated with the high levels of ammonia in the water, resulting from the leftover feed and uneaten feed, as well as the increased waste **(Suhendar *et al*., 2020)**.

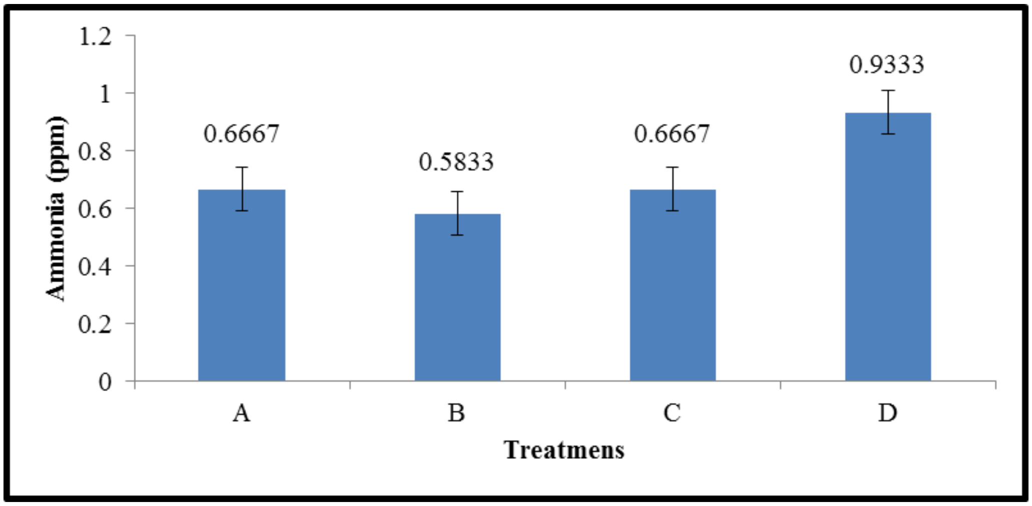

Fig. 11. Average dissolved ammonia in fish rearing media (ppm)

CONCLUSION

Based on the current results, it can be deduced that the lower the stocking, the higher the average absolute growth, absolute length growth, daily growth, and survival of the vanamei shrimp. The highest average absolute growth of the vanamei shrimp was in treatment A (0.049±0.007); the highest absolute length growth of shrimp was in treatment A, with 0.93±0.065; the highest average daily growth rate in treatment A was 10.74±0.25; and the highest average survival in treatment A, namely 22 fish/liter, was 90.13±5.32. Water quality parameters under study are still in the water quality range being suitable for the life of the vanamei shrimp test animals.

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