Short Research Article

~~Strategy~~ The strategy of Phytoplankton Administration in Larval Rearing to Improve the Survival Rate of Vannamei Shrimp Larvae of F1 and Free-Pathogen

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ABSTRACT

Aims: Vannamei shrimp (*Litopenaeus vannamei*) larvae F1 is the dream of shrimp cultivators to be cultivated in ponds. This is because F1 larvae have fast growth, especially if the larvae have disease-free certification (specific pathogen-free, SPF). The purpose of this scientific paper is to evaluate strategies for feeding phytoplankton in larval rearing to increase the survival rate of shrimp larvae F1 of vannamei shrimp.
Place and Duration of Study: This research activity was carried out from November to December 2021 at the Shrimp Hatchery Laboratory, Department of Aquaculture Technology, Pankep State Polytechnic Agriculture.
Methodology: The nauplii were obtained from the shrimp hatchery industry and then stocked in 2 units of tanks measuring 4m x 4m x 1.5m. Before stocking, the larvae are acclimatized to temperature and salinity. The larvae were fed live feed (*Skeletonemacostatum*) from the stage of Nauplii-6 to Mysis-2. In addition, the larvae were also fed artificial feeds and *Artemia* nauplii. Observation of larval development was carried out at each stage using a microscope, and observations of the population number of each stage were carried out volumetrically. Parasite analysis of each stage was conducted in the Makassar Fish Quarantine.
Results: The results showed that during the stage shift from Nauplius-6 to Zoea-1, the larvae succeeded in passing the zoea syndrome (empty intestine). The population decline was only reduced by 2% (from 1,200,000 individuals decreased to 1,175,600 individuals). The final population (PL10) was 1,084,000 (SR 90%). The parasite analyses results showed that the larvae were free from any disease. Administration of phytoplankton (*S. costatum*) at the early stage (Nauplius-6) at a dose of 60 x 106cells/mlhas succeeded in reducing the mortality rate (2%) in the critical stage (zoea syndrome) of F1 vannamei shrimp larvae.

Keywords:*Skeletonemacostatum , SPF, Zoea syndrome, Nauplius*

1. INTRODUCTION

Vannamei shrimp (*Litopenaeus vannamei*) cultivation is also not free from the constraints in the cultivation system. Similar to tiger prawns, vannamei shrimp also often experience crop failure due to disease attacks resulting in mass deaths. The emergence of disease

attacks on shrimp can be caused by two factors, namely water quality management and shrimp larvae quality factors. Poor water quality management is a trigger for disease attacks in shrimp farming. However, the development of water management technology has developed rapidly, so that water quality management can be carried out effectively by the farmer. But the quality of larvae as a factor causing disease is very difficult to handle properly by the farmer. This is caused by the provision of shrimp larvae carried out by the shrimp hatchery industry so that the farmer does not have the authority to determine the quality of the larvae provided. Therefore, it is necessary to produce larvae that have high quality to overcome the problem of supply of larvae to the farmer in order to encourage increased shrimp production.

Vannamei shrimp larvae first generation (F1) is the dream of shrimp cultivators to be cultivated in ponds. This is because F1 larvae have faster growth, especially if the larvae have a specific pathogen-free (SPF) certificate from a Certification Berau accredited by the National Accreditation Committee (KomiteAkreditasi Nasional). Increasingly widespread crop failures in vannamei shrimp cultivation, make farmers look for superior shrimp larvae. The superior shrimp larvae are resistant to disease and have fast growth. Crop failure often resulted from disease attacks in the second month of cultivation, which is very detrimental to farmers and the shrimp cultivation industry. Therefore, F1 shrimp larvae that have fast growth (Kusyairi et.al., 2019) will be able to bear these losses, even though they are attacked by disease in the second month, they can still harvest because the size of the shrimp meets consumer standards so that they can cover expenses during cultivation. Disease attacks that cause frequent crop failures in the second month of cultivation are thought to be due to shrimp larvae being carried with disease seeds (carriers). Therefore, the shrimp larvae must have disease-free certification from KAN.

Production of pathogen-free F1 shrimp larvae requires the provision of phytoplankton at the initial stage (nauplii to zoea stadia) as natural food. This activity of giving phytoplankton often causes problems in shrimp hatcheries which causes a low level of survival rate of larvae. The initial stage of shrimp larvae is the critical stage of the larvae. Especially in the zoea stage known as zoea syndrome (Kumar et. al., 2017; Wiradana et. al., 2022), where the larvae have an empty gut so they will experience mass death. The ability of the larvae to take food from outside for the first time is a critical point for shrimp larvae. Larvae that are able to take food from outside for the first time (commencement of exogenous feeding) will be able to survive, whereas larvae that are unable to take food from outside will not be able to survive or will die. Therefore, the provision of phytoplankton must be timely, in quantity and frequency to pass this critical phase of shrimp larvae. In addition, when preparing or cultivating plankton it is an activity that must be sterile from contamination by other microorganisms, such as protozoa which become parasites for shrimp larvae.

As a solution to overcome problems in providing phytoplankton to the early stages of vannamei shrimp larvae, a special strategy is needed for feeding management, including a strategy for providing phytoplankton. The strategies needed include the type of phytoplankton, density (dose) of phytoplankton, time, and giving of frequency. In addition, the strategy for providing phytoplankton requires sterile media and air.

The purpose of this study was to evaluate the strategy of administering phytoplankton to the survival rate of F1 free-pathogen larvae of the vannamei shrimp.

2. METHOD

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2.1 Place and Duration of Study

This research activity was carried out from November to December 2021 at the Shrimp Hatchery Laboratory, Department of Aquaculture Technology, Pankep State Polytechnic Agriculture.

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2.2 Materials

The medium used in the culture of shrimp larvae is in the form of 2 units of concrete tubs measuring 4 m x 4 m x 1.5 m which are equipped with an aeration system and water intake. Nauplii obtained from the shrimp hatchery industry. The water used comes from the sea, which is pumped and filtered and then stored and deposited at the same time. After that, it is filtered using an ozone machine and a UV filter before being sent to the larval rearing tank and algae mass culture.

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2.3 Stocking of Shrimp Larvae

Stocking of shrimp larvae (nauplii stage) was carried out by first acclimatizing to temperature and salinity, by immersing the nauplii bags in the rearing tank for approximately 30 minutes. After the temperature of the water in the nauplii bag was the same as the temperature of the water in the rearing tank, next, a plastic bag of nauplii was opened and filled ~~it~~ with water ~~of~~ from the rearing tank little by little to acclimatize to salinity. Then, the nauplii ~~was~~ were removed from the plastic bag by carefully pouring them into the rearing tank. A total of 1,200,000 nauplii were stocked in 2 concrete ponds measuring 4 m x 4 m x 1.5 m or 600,000 nauplii were stocked in each pond. The pond is equipped with an aeration system.

2.4 Feeding

The type of feed given to vannamei shrimp larvae during the rearing process is natural feed, phytoplankton, and zooplankton as well as artificial feed. The natural food for phytoplankton that is given is *Skeletonemacostatum* and the natural food for zooplankton that is given is Artemia salina. Algae stocks were obtained from the Siddo Brackish Water Cultivation Center, Barru Regency, South Sulawesi. The algae stocks were mass cultured using a fiber tank (2 tons) filled with seawater and fed with 15 ppm NPK, 2 ppm EDTA (Ethylen Diamine Tetra Acetic Acid), and 0.5 ppm silicate. Algae mass culture is carried out for 2-3 days. As for the artificial feed given, namely feed in powder form such as spirulina flour, vitamin P1, and frippak.

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Feeding of the live feed, *Skeletonemacostatum* (Table 1) in the rearing of vannamei shrimp larvae started from the nauplii-6 stage to the mysis-3 stage larvae. Algae administration was carried out at the zoea stage as much as 60,000 cells/ml. The density of the given phytoplankton will increase with the growth of the shrimp stage, but will decrease when entering the mysis stage. The post larval stage is no longer given phytoplankton. Feeding Artemia salina in the process of rearing vannamei shrimp larvae began when the larvae are MPL stage to post larvae 10 (PL10), which are given four times a day, namely at 09.00, 15.00, 21.00 and 03.00. The density is adjusted according to the growth of shrimp stadia. The higher the shrimp stadia, the more dense the artemia will be. Artificial feed was given when entering the zoea phase (Table 2). The type of artificial feed used was (spirulina flour, vitamin P1, and frippak) as much as 2 ppm. The dose of feed every day has increased, according to the contents of the stomach. In the mysis stage given vitamins (0.33 ppm). The frequency of artificial feeding was six times at 09.00, 13.00, 17.00, 21.00, 01.00 and 05.00.

2.5 Observation of Larval Development

Data collection was carried out by sampling at each stage by observing the development of the larvae at each stage. Each stage of development was observed by observing the completeness of the organs owned by using a microscope.

Table 1. Feeding Program of Live Feed (algae and Artemia)

DOC	Stadia	Algae (cells /mLx1000)	Artemia (g/ day)
1	N_6	60,000	-
2	Z_1	60,000	-
3	Z_2	65,000	-
4	Z_3	70,000	-
5	ZM	70,000	-
6	M_1	65,000	-
7	M_2	60,000	-
8	M_3	-	60
9	MPL	-	150
10	PL_1	-	180
11	PL_2	-	180
12	PL_3	-	180
13	PL_4	-	180
14	PL_5	-	150
15	PL_6	-	150
16	PL_7	-	120
17	PL_8	-	120
18	PL_9	-	60

2.6 Observation of Population Number and Survival Rate

To determine the number of larvae in the rearing tanks using the sampling method by taking samples at 5 points in the tank. Samples were taken using a 100 ml volume biker glass. The number of nauplii collected was counted. The population of larvae in the tank was determined using the formula:

$$\text{Population} = \frac{\text{Rata} - \text{rata jumlah sampel}}{\text{Volume air sampel}} \text{ x volume air bak}$$

The survival rate of vannamei shrimp larvae is the ratio of the number of larvae that live and the total post larvae of vannamei shrimp that are stocked. Survival rate can be calculated using the formula (Effendie, 2002) as follows:

$$SR = \frac{Nt}{No} \times 100$$

Description:

SR : Survival Rate (%)
Nt: Number of larvae at the end of rearing (individual)
N0 : Number of larvae at beginning of rearing (individual)

Table 2. Feeding Program of Artificial Feed

DOC	Stadia	Program (g/ million fry / day)			
		Zoea	Mysis	OT $_{1-6}$	OT $_{7-12}$
1	N $_{6}$	30			
2	Z $_{1}$	47			
3	Z $_{2}$	52			
4	Z $_{3}$	52			
5	M $_{1}$		46		
6	M $_{2}$		55		
7	M $_{3}$		55		
8	MPL		51		
9	PL $_{1}$			73	
10	PL $_{2}$			87	
11	PL $_{3}$			120	
12	PL $_{4}$			144	
13	PL $_{5}$			120	
14	PL $_{6}$			103	
15	PL $_{7}$				126
16	PL $_{8}$				126
17	PL $_{9}$				129
18	PL $_{10}$				126
19	PL $_{11}$				90
20	PL $_{12}$				150

2.7 Parasite Analyses

Parasite tests for shrimp larvae were carried out using the Polymerase Chain Reaction (PCR) system at the Makassar Quarantine Center and tests for parasite content in water were carried out at the Brackish Water Aquaculture Research and Fisheries Research Center, Maros, South Sulawesi.

2.8 Water Quality

Water quality measurements were carried out every day with a frequency of twice a day, ~~namely~~ in the morning and evening. Water quality parameters measured were temperature, dissolved oxygen, salinity, and pH in situ using Jala Tech Baruno. Data analysis was carried out descriptively by comparing published results in accredited journals, both national and international.

3. RESULTS AND DISCUSSION

3.1 Larval Development

Larval development during larval rearing (Figure 1) was observed using a microscope. In the observations found that the stage change from nauplii to zoea generally takes about 36-40 hours from the time the eggs hatch. At this stage, the development of larval size is very fast and the feeding appendages begin to function and actively feed on phytoplankton. The zoea stage was the weakest stage and was sensitive to strong light and has three substadia. The body can be divided into three parts: carapace, thorax and abdomen. Zoea 1 was characterized by the difference between the cephalothorax and abdomen which was visible when observed with the naked eye and the eyes were present but not on the carapace, zoea 2 was characterized by the eyes starting to be on the eye stalks and is located on the carapace and the rostrum was visible between the two eyes, zoea 3 was characterized by the formation of biramous uropods and spines appear on the abdominal somites (Nuntung et. al., 2018).

The zoea stage is known to be the most critical stage in the development ofvannameivaname shrimp larvae because it is susceptible to various environmental changes and pathogen infections (Pérez-Morales et. al., 2017). Zoea syndrome is characterized by an empty larval intestine and mortality can reach 100% (Haliman et.al., 2006). However, based on the results of observations under a microscope, it showeds that the intestines of the zoea larvae looked full (brown in colorcolour).

The mysis stage resembleds the mature shrimp compared to the previous two stages and it is physically stronger. The type of food that can be eaten is phytoplankton and zooplankton and tends to like zooplankton towards the end of the mysis stage. There are three substadiaand thatcan be distinguished from the development of the thoracic appendages and pleopods (swimming legs). Mysis 1 stage is marked by the formation of a primitive uropod and pleopod appears, mysis 2 is marked by pleopod growth and mysis 3 pleopod parts are getting longer and segmented.

The change in shape from the mysis stage to the post larval stage took place on the ninth day after hatching. The larvae in this stage are similar to adult shrimp and have higher stamina, so they are stronger. This stage was marked by the perfect formation of the pleopod and the presence of hairs on the pleopod that will help the shrimp swim. The ability to swim changes, because the pleopod has developed and started to function. Early post larvae are still pelagic in particular until they enter the benthic PL stage.

3.2 Population Number and Survival Rate of Larvae

The results of calculating the population number and survival rate of vannamei shrimp larvae during the rearing process showed that the survival rate of vannamei shrimp larvae decreased with the change in larval stages (Table 3). The initial population of nauplii larvae at the start of rearing was 1,200,000 (SR 100%). Entering the zoea-1 stage, the larval survival rate (SR) decreased to 98%. In the zoea stage, cases of zoea syndrome are known, where intestinal emptiness occurs in the larvae which will cause 100% mass death (Haliman et. al., 2006). However, zoea syndrome was not occured in the observed zoea stage due to the provision of phytoplankton at the beginning of the stage (Nauplii-6) so that the zoea could take phytoplankton (S. costatum) as a source of energy to survive into the next stages. The population of post larval-10 stage at the end of rearing was 1,084,000 individuals or with a survival rate of 90%. The achievement of the survival rate of shrimp larvae is very high when compared to several research reports (Anita et. al., 2018; Putri et. al., 2020).

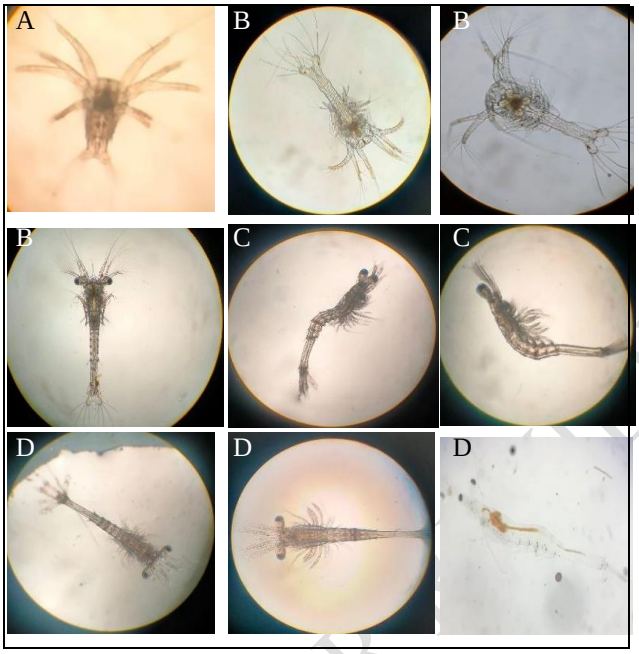

Figure 1. Larval development of Vannamei Shrimp.Nauplius stage (A), Zoea stage (B), Mysis stage (C), and Post larval stage (D).

The Nauplius stage (N6) is marked by the elongation of the posterior part of the body, and the furcal spines increase by one pair to 7+7 with spinulus on the longer spines (Kitani, 1986). Furthermore, the zoea stage of the body shape has changed remarkably. The anterior portion of the body is covered by the carapace, and the posterior is divided into a six-segmented chest and an unsegmented abdomen.

3.3 Parasite analyses

Analysis parasite used Polymerase Chain Reaction (PCR), which was carried out at the Makassar Quarantine Center. Results of parasite analyses showed negative output ~~results~~ (Table 4). It is , according to(Amoah *et . al* ., 2019) , one superiority shrimp vaname that is its resilience to disease.

Table 3. Population Number and Survival Rate (SR)

No	Stadia	Est, Jmlh Seed (tail)	SR(%)
1.	N6	1 . 200 . 000	100
2.	Z1	1 . 175 . 600	98
3.	Z2	1 . 178 . 000	98
4.	Z3	1 . 160 . 500	97
5.	ZM	1 . 138 . 000	95
6.	M1	1 . 136 . 400	95
7.	M2	1 . 116 . 500	93
8.	M3	1 . 119 . 200	93
9.	MPL	1 . 107 . 000	92
10.	PL $_1$	1 . 104 . 300	92
...	....	.....	...
11	PL 10	1 . 084 . 000	90

The results of PCR analysis of disease-causing parasites in shrimp larvae (Table 4) showed that in the mysis and PL10 stages of the shrimp larvae no parasites were detected (negative), as was the case in the water medium which also did not find any parasites. This shows that water management techniques which are a potential source of disease infection can successfully prevent or sterilize water from parasites that can cause disease in shrimp larvae. The use of ultraviolet (UV) radiation is very effective in inactivating various microorganisms ((Bae et. al., 2016; Skowron et. al., 2014) without leaving harmful residues (Shannon et al., 2008). The use of ozonation has also been widely used for disinfection of fish and shrimp rearing media (Novriadi, 2016), especially in the large-scale aquaculture industry.

Table 4. The types of parasites that often infect shrimp larvae

No.	Parasite Type	Nauplii				Air Media
		Nauplii	Zoea	Mysis	PL10	
1.	White Spot Syndrome Virus (WSSV)	nd	nd	Negative	Negative	Negative
2.	Infectious Hypodermal and Hematopoetic Necrosis	nd	nd	Negative	Negative	
3.	Acute Hepato Pancreatic Necrosis Disease/Early Mortality Syndrome (AHPND/EMS)	nd	nd	Negative	Negative	
4.	Taura Syndrome Virus (TSV)	nd	nd	Negative	Negative	

3.4 Water Quality

The results of measuring the water quality parameters of vannamei shrimp larvae rearing media during the rearing process are presented in (Table 5). Water quality parameters measured were temperature, salinity, pH and dissolved oxygen. Based on Table 5, the results of measureding water quality parameters wereare in accordance with the standard values of water quality and are in the optimal range to trigger the growth of vannamei shrimp larvae. The water quality parameter value for larval rearing media is temperature in the range of 27.5-29.5

°C, the minimum temperature measurement results obtained are below the optimum range (PERMEN-KP No. 75, 2016) where the optimal temperature for growth vannamei shrimp larvae, which range from 28-32˚C, this does not significantly affect the growth of the larvae reared. According to (Zainuddin et. al., 2014), temperature has a direct effect on shrimp metabolism, at high temperatures the shrimp metabolism is accelerated, while at lower temperatures the metabolism processes are slowed down. Temperature is a limiting factor, where if the temperature drops or rises it will affect dissolved oxygen (DO) levels and several other parameters will be affected indirectly (Sari and Ikbal, 2020).

The degree of acidity (pH) in the rearing tanks of vannamei shrimp larvae was in the range of 7.1-8.57. This pH range is still suitable for vannamei shrimp farming activities and supports the growth and survival of vannamei shrimp. Based on PERMEN-KP No. 75 (2016), the optimal pH for the growth of vannamei shrimp larvae is in the range of 7-8.5. Similar to Duan et al. (2019), the optimal pH for vannamei shrimp growth is around 8.3, while pH values below 6.9 and above 9.7 can cause stress for shrimp. Low pH values can affect the ability of shrimp to digest or absorb proteins and carbohydrates (Yu et. al., 2020).

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Salinity levels found in vannamei shrimp post larvae rearing have a value in the range of 26-34 ppt. This salinity range is still suitable for vannamei shrimp farming activities. According to (PERMEN-KP No. 75 (2016), which states that for vannamei shrimp rearing activities the optimal salinity is 26-35 ppt. This is reinforced by Umami et al. (2018), that vannamei shrimp can live in a range of salinity levels of 0.5-45 ppt. According to Arsad et. al. (2017), salinity plays a role in the process of shrimp osmoregulation and also molting. At too high a salinity level, shrimp growth is decreases because the osmoregulation process is disrupted.

Dissolved oxygen (DO) levels during larval rearing have a range of 4.24–7.22 mg/l. This value indicates that the DO levels are still within the optimal range for shrimp larvae. According to PERMEN-KP No. 75 (2016), DO levels for rearing shrimp larvae is over than 4 mg/L. This is in line with Arsad et. Al. (2017), DO below 3 mg/l can cause stress and death of shrimp. DO is needed for respiration which is then used for metabolic activities (Hidayat et. al., 2014). Oxygen saturation in water is affected by water temperature, because the higher the water temperature, the DO concentration will decrease (Nur et. al., 2017).

Table 5 . The results of ~~measuring the~~ water quality parameters for vannamei shrimp larvae maintenance

No	Parameter Type	Measurement Results	Range (PERMEN-KP No. 75, 2016)
1.	Temperature (°C)	27.5-29.5 °C	28-32oC ⁻_
2.	Salinity (ppt)	26-34 ppt	26-35 ppt
3.	pH	7,1-8,57	7-8.5
4.	Oxygen Dissolved (ppm)	4.24-7.22 ppm	≥ 4 mg/L

3.5 Conclusion

Production of F1 pathogen-free vaname shrimp larvae by applying the strategy of giving phytoplankton at the early stage (Nauplii-6 to zoea stage) which is the critical point of larvae can produce larvae with a survival rate of 90%. This means that the strategy of

providing phytoplankton has successfully passed the critical point where the larvae take food from outside for the first time (commencement of exogenous feeding). This is of course inseparable from other environmental factors that support this success, such as artificial feeding, control of water quality, and other factors.

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