



The Strategy of Phytoplankton Administration in Larval Rearing to Improve the Survival Rate of White Shrimp (*Penaeus vannamei*) Larvae of F1 and Free-Pathogen

Muhammad Ikbal Illijas ^{a*}, Luqman Saleh ^a,
Nur Rahmawaty Arma ^a, Andriani ^a and Rahmi Mulyani ^a

^a Aquaculture Department, Pangkep State Polytechnic of Agriculture, Pangkajene dan Kepulauan, South Sulawesi, 90655, Indonesia.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The white shrimp (*Penaeus 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 white 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.

*Corresponding author: Email: muhammadikbali@yahoo.com;

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 were acclimatized to temperature (28°C) and salinity (30 ppt). The larvae were fed live feed (*Skeletonema costatum*) 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 (40x), 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 10⁶ cells/ml has succeeded in reducing the mortality rate (2%) in the critical stage (zoea syndrome) of F1 white shrimp larvae.

Keywords: *Skeletonema costatum*; SPF; zoea syndrome; nauplius.

1. INTRODUCTION

White shrimp (*Litopenaeus vannamei*) cultivation is also not free from the constraints in the cultivation system. Similar to tiger prawns, white 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 [1]. Poor water quality management is a trigger for disease attacks in shrimp farming [2]. 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 [3,4]. 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.

White 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 (Komite Akreditasi Nasional). Increasingly widespread crop failures in white 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 [5] 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 [6,7], 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 white 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 white shrimp.

2. METHODOLOGY

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, Pangkep State Polytechnic Agriculture, South Sulawesi, Indonesia.

2.2 Materials

The medium used in the culture of shrimp larvae was in the form of 2 units of concrete tanks measuring 4 m x 4 m x 1.5 m which are equipped with an aeration system and water intake. The tanks were placed inside the building. Nauplii obtained from the shrimp hatchery industry. The water used obtained 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.

2.3 Stocking of Shrimp Larvae

Stocking of shrimp larvae (nauplii stage) was carried out by first acclimatizing to temperature (30°C) and salinity (28 ppt), 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 from the rearing tank little by little to acclimatize to salinity. Then, the nauplii 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 white shrimp larvae during the rearing process was live feed, phytoplankton, and zooplankton as well as artificial feed. Live feed for phytoplankton that is given was *Skeletonema costatum* and live feed for zooplankton that is given was *Artemia salina*. Algae stocks were obtained from the Sidde 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 (Ethylene 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.

Feeding of the live feed, *Skeletonema costatum* (Table 1) in the rearing of white 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. Phytoplankton density increased with increasing larvae growth. The post larval stage was no longer given phytoplankton. Feeding *Artemia salina* nauplii in the process of rearing white shrimp larvae began when the larvae reached MPL stage to post larvae 10 (PL10), which were 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 denser 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)

Day of Culture (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:

$$\text{Population} = \frac{\text{Average of Sample Number}}{\text{Sample Water Volume}} \times \text{Rearing Tank Volume}$$

The survival rate of white shrimp larvae is the ratio of the number of larvae that live and the total post larvae of white shrimp that are stocked. Survival rate can be calculated using the formula [8] as follows:

$$SR = \frac{N_t}{N_0} \times 100$$

Description:

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

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, 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 (Fig. 1) was observed using a microscope (40X). 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

Table 2. Feeding program of artificial feed

Day of Culture (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

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 [9].

The zoea stage is known to be the most critical stage in the development of shrimp larvae because it is susceptible to various environmental changes and pathogen infections [10]. Zoea syndrome is characterized by an empty larval intestine and mortality can reach 100% [11]. However, based on the results of observations under a microscope, it showed that the intestines of the zoea larvae looked full (brown in colour).

The mysis stage resembled 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 tends to like zooplankton towards the end of the mysis stage. There are three substadia and that can 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 white shrimp larvae during the rearing process showed that the survival rate of white 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 [12]. However, zoea syndrome was not occurred 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

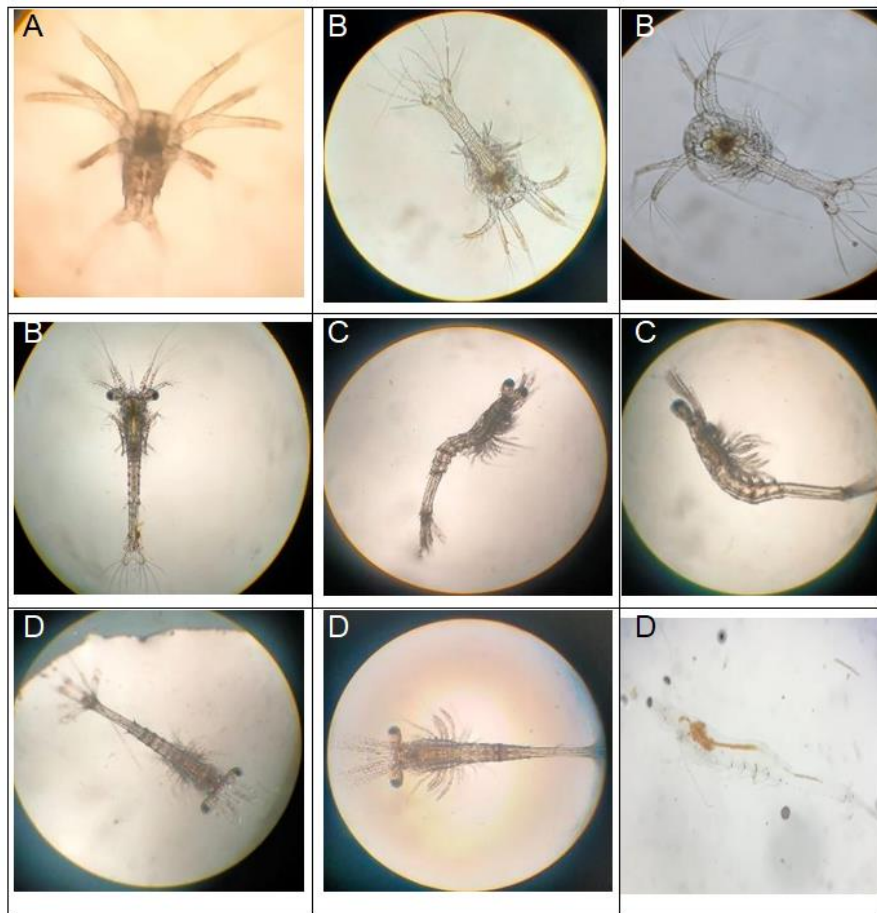


Fig. 1. Larval developments of White shrimp were observed using a microscope with magnitude of 40 X. Nauplius stage (A), Zoea stage (B), Mysis stage (C), and Post larval stage (D)

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 [13,14].

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 parasite

analyses showed negative output (Table 4). It is, according to [15] one superiority white shrimp that is its resilience to disease.

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 [16,17] without leaving harmful residues [18]. The use of ozonation has also been widely used for disinfection of fish and shrimp rearing media [19], especially in the large-scale aquaculture industry.

Table 3. Population number and survival rate (SR)

Day of Culture (DOC)	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

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 Hematopoietic 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	

Table 5. The results of the water quality parameters for white shrimp larvae maintenance

No.	Water Quality Parameters	Measurement Results	Range (PERMEN-KP No. 75, 2016)
1.	Temperature (°C)	27.5-29.5	28-32
2.	Salinity (ppt)	26-34	26-35
3.	pH	7,1-8,57	7-8.5
4.	Oxygen Dissolved (ppm)	4.24-7.22	≥ 4

3.4 Water Quality

The results of measuring the water quality parameters of white 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 measured water quality parameters were in accordance with the standard values of water quality and are in the optimal range to trigger the growth of white 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 [20] where the optimal temperature for growth white shrimp larvae, which range from 28-32°C, this does not significantly affect the growth of the

larvae reared. According to [21], 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 [22].

pH degree in the rearing tanks of white shrimp larvae was found in the range of 7.1-8.57. This pH range is still suitable for white shrimp farming activities and supports the growth and survival of white shrimp. Based on PERMEN-KP No. 75 [20], the optimal pH for the growth of white shrimp larvae is in the range of 7-8.5. Similar to Duan et al. [23], the optimal pH for white 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 [24].

Salinity levels found in white shrimp post larvae rearing have a value in the range of 26-34 ppt. This salinity range is still suitable for white shrimp farming activities. According to (PERMEN-KP No. 75 [20], which states that for white shrimp rearing activities the optimal salinity is 26-35 ppt. This is reinforced by Umami et al. (2018), that white shrimp can live in a range of salinity levels of 0.5-45 ppt. According to Arsad et al. [25], 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 [20], DO levels for rearing shrimp larvae is over than 4 mg/L. This is in line with Arsad et al. [25], DO below 3 mg/l can cause stress and death of shrimp. DO is needed for respiration which is then used for metabolic activities [26]. Oxygen saturation in water is affected by water temperature, because the higher the water temperature, the DO concentration will decrease [27,28].

4. 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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