The technology of shrimp larvae transportation: ecophysiology and bioeconomy effects on high stocking density shrimp *Litopenaeus vannamei*

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Abstract. Intensification is part of modern shrimp farming technology, feed, and high stocking density. High density could save time and production costs. This research aimed to determine the optimal tolerance of shrimp larvae with high stocking density per bag unit without oxygen liquid in terms of environmental physiology (ecophysiology) and economic feasibility aspects (bioeconomics). The shrimp larvae used were post-larvae 10 with a weight of 0.0026 ± 0.0021 g, which were previously fasted. The method used was a simulation of a three-hour trip with treatments: 200 larvae/packing (control), 400 larvae/packing, and 600 larvae/packing. The treatment for each packing was 450 ml of water and oxygen gas and added ice cubes in each styrofoam during delivery at night. The results showed that the stocking density of 600 larvae/packing had the best value. In terms of ecophysiology, the survival rate was highest when arriving at the location, with 98.20% of total larvae. The lowest oxygen consumption in the metabolic process was 0.76 ± 0.10 mg/L. Reviewing a bioeconomic, the potential profit per packing reaches IDR 995,528. It can be concluded that the use of oxygen gas and the addition of ice cubes in transportation increased the survival of shrimp larvae.

1. Introduction

Modern shrimp culture is characterized by intensification, feed technology, pellets, and high stocking density in one pond area. One of the leading aquaculture products is the white shrimp commodity. One of the leading aquaculture products is the white shrimp

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commodity, *Litopenaeus vannamei*. Many sectors are related to vaname shrimp farming, both in grower and hatchery culture. It is essential to pay attention to the live transportation from the seller to the buyer's location in the hatchery sector. If there are many tourist trips, the tourist is the seller or entrepreneur of shrimp larvae at a loss. Generally, industrial-scale shrimp entrepreneurs are not worried about this because they use aeration technology such as adding liquid oxygen to the larval packing bag or aeration technology in micro/nanobubble.

Industrial-scale entrepreneurs are currently able to apply high-density cultivation. It can be achieved if the need for dissolved oxygen through aeration in shrimp culture ponds is met because it is the absolute main factor needed in creating an atmosphere that supports the growth of this type of biota [1,2]. This has minimal impact on mortality during the journey from the harvest location to the stocking location. Packaging of shrimp larvae to consumers is one of the critical supply chains for shrimp larvae hatchery businesses, especially for conventional farmers. Reducing costs in this process is mandatory to support the increase in profit for producers, especially for small-scale hatcheries such as traditional non-corporate system cultivators.

Especially for conventional shrimp larvae cultivators, one effort to reduce costs or capital costs when sending larvae is to reduce the number of larvae densities per bag even though oxygen gas has been added. Stunning the traditional way with the addition of ice cubes and oxygen gas is expected to increase the number of larvae in the bag. Therefore, this study aimed to develop an optimal tolerance system for shrimp larvae with high stocking densities per bag unit and environmental, physiological aspects (ecophysiology) and economic feasibility aspects of high stocking densities (bioeconomics).

2. Methods

The research was conducted at a company that provides quality larvae around the Province of Banten, and this activity began on August 22-23, 2020. The Covid 19 pandemic caused research to be carried out outside the production site to avoid contamination by cultivation activities. In this study, oxygen gas (liquid oxygen was generally used) was added through an oxygen tube at packing. The addition was to the habits of shrimp larvae farmers. The methods in this study were divided into several series, starting from the preparation of shrimp *Litopenaeus vannamei* larvae, preparation of raw water for packaging, determination of research treatment, and transportation simulation. The first step is by weighing the weight of the larvae, PL10, with a weight of 0.0026 ± 0.0021 g. The second step is preparing raw water by adding an aeration technology such as bubbles or bubbling [3].

The preparation of raw water for packing media in shrimp larvae bags is transported as much as 5 tons of water or approximately 5,000 L. The third step was the provision of larvae with research treatment in plastic bags. The treatments applied were 200 larvae/bag, 400 larvae/bag, and 600 larvae/bag, with the volume of water/bag being 450 mL. The density was based on the minimum standard in white shrimp larvae supplying companies, 2,000 shrimp larvae/45,000 mL of media water in the bag. This study was compressed (empirically) to 200 shrimp larvae/450 mL of media water in the bag. The stocking density of each bag that has been suitable for treatment is then given oxygen gas and then tied with rubber, then put in styrofoam and ice cubes inserted in plastic. The fourth step was a short-distance transportation simulation process with a three hours-trip from 10.00 pm to 01.00 am. The mileage division was divided into three based on the length of time the oxygen gas ran out once oxygen gas was given to the bag. Short distances are from one hour to six hours, medium distances are from seven hours to twelve hours, and finally, long distances are at least twelve hours to twenty-four hours (a day).

In this research, shrimp larvae that have been packaged according to treatment in bags are then shaken in a box container randomly every 60 minutes which are divided into several times. This activity was considered the same as in the field when transporting using a pickup car. Water quality was measured every hour from the beginning to the end of the study for three hours. The parameters measured were water quality parameters in physical and chemical parameters: temperature, TAN (Total Ammonia Nitrogen), and dissolved oxygen (DO). Production performance parameters in the form of OCR (Oxygen Consumption Rate) and survival rate of larvae.

2.1 Water Quality

The following are the water quality parameters and tools used in this study, as well as the measurement times listed in the table below:

Table 1. Water quality parameters and specifications for measuring water quality

Parameter	Unit	Method/Tools	Specification	Measurement Time
Temperature	°C	Thermometer	Lutron 5510	Every hour
TAN	mg/L	Spectrophotometer	Lutron 5510	Every hour
pH	-	pH-meter	ATC pH tester	Every hour
DO	mg/L	DO-meter	Lutron 5510	Every hour

2.2 Oxygen Consumption

The level of oxygen consumption was measured by obtaining the difference between the DO of the medium after and before transportation. The formula used was as follows [4]:

$$OCR = \frac{V \times DO_{t0} - DO_{tn}}{W \times T}$$

Description :
OCR : Oxygen consumption rate (mgO_2/g/hour)
V : Volume of water in tank (L)
DO_{t0} : Dissolved oxygen concentration at the beginning of observation (mg/L)
DO_{tn} : Dissolved oxygen concentration at time in n (mg/L)
W : Biota weight (g)
T : Observation period (hour)

2.3 Survival Rate

The survival rate is calculated using the formula [5]:

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

Description:
SR : Survival rate (%)
N_t : Calculated using the formula t (larvae)
N_0 : Number of live shrimp on day 0 (larvae)

2.4 Data Analysis

The design used in this study was a Completely Randomized Design (CRD) with three replications. Water quality data were analyzed descriptively, while production performance data were analyzed using analysis of variance with a 90% confidence level. If there was a significant difference between treatments, then further tests with *Duncan's Multiple Range* using SPSS 16.

3. Results and Discussion

3.1 Water Quality

Temperature, dissolved oxygen, TAN, and pH are the main water qualities that play an essential role in conditioning a good aquatic environment. These parameters will affect other chemical and physical parameters. Water quality in the form of temperature, TAN content, pH levels, and oxygen consumption rate in shrimp larvae media was presented in Figure 1. The results of observations of water quality show different fluctuations in each treatment and observation time. The results of the water temperature measurement show an increase in the value from the beginning to the end of the observation. The temperature increase was slower in the treatment of the density of 200 larvae, especially during the observation at the first hour. The time of transportation carried out at night causes the temperature to be maintained in the range of 20.37-22.97°C.

Temperature is closely related to dissolved oxygen. The higher the temperature, the smaller the dissolved oxygen (DO) in the water and biological and chemical processes will increase, so oxygen consumption will also increase [6]. This increase in oxygen consumption results in a decrease in dissolved oxygen in the water. This can be observed at DO, which decreases from the beginning to the end of the observation. Dissolved oxygen was an essential factor in aquaculture activities. Not only do shrimp consume oxygen for respiration, but oxygen is also used to stabilize water quality. Metabolic waste can be high along with shrimp density. The observations showed that the density treatment of 600 larvae had the lowest DO value compared to other treatments. The highest DO levels were observed at the treatment density of 200 larvae at each observation time. Reduced oxygen in aquaculture is high density and larger shrimp sizes [7]. The leading cause of reduced dissolved oxygen in water is waste materials that consume oxygen [8].

Shrimp was a poikilothermic biota, so any increase in water temperature around the environment will cause the temperature of cold-blooded animals to increase due to increased metabolism in their bodies, and stress plus abnormal stocking density has an impact on increasing oxygen demand. This causes a decrease in the concentration of oxygen in the water medium. Converting oxygen into energy (metabolism) and producing ATP or energy in cells also produces urea or feces rich in ammonia. This was the cause of the increase in ammonia, which was measured using the TAN parameter. The results of the TAN measurement showed an increase from the beginning to the end of the observation. The proportion of unionized ammonia increases as the temperature and pH increase. Un-ionized ammonia dominates at high pH and is toxic, whereas ammonium is relatively non-toxic at low pH [9]. The presence of residual respiration impacts the balance of hydrogen ions in the water medium. This was evident from measuring the pH value periodically, experiencing fluctuations in each treatment every hour of observation.

The treatment with a density of 600 larvae was observed to decrease lower than the other treatments in the second hour and increase in the 3rd hour of observation. Likewise, the 200 and 400 larvae density decreased in the 2nd hour and increased in the 3rd hour. The increase in TAN occurred more slowly at the treatment density of 200 larvae observed in the 1st hour. This indicates that the low density has a lower TAN value than the higher density. TAN increases due to the secretion of shrimp which also uses oxygen, the higher the density, the higher the ammonia secretion produced [10]. TAN is the most toxic form of un-ionized ammonia nitrogen and has a negative impact on shrimp health, so its presence needs to be controlled [11].

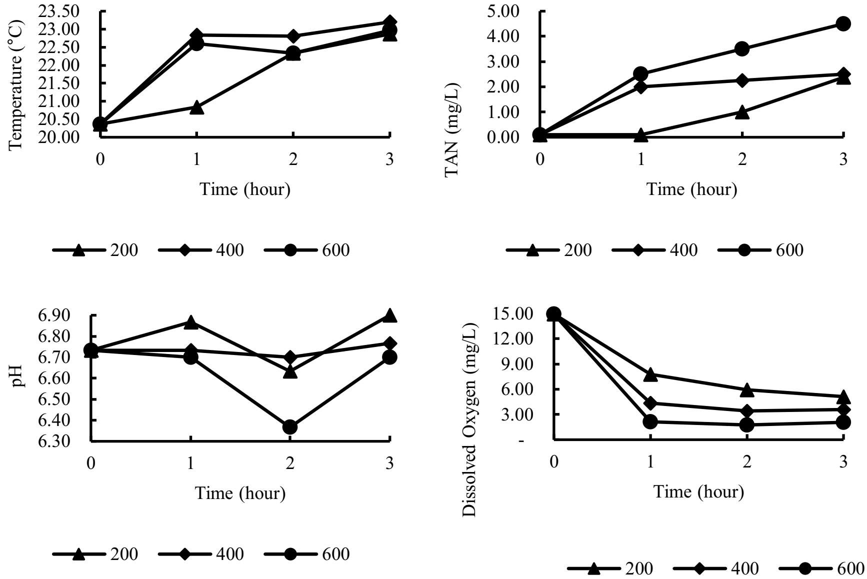

Fig. 1. Fluctuations in Temperature, TAN, pH, and oxygen consumption rate in Vaname Shrimp Larvae Media with Different Densities

3.2 Ecophysiology

Ecophysiology was a study of the physiology of organisms related to the surrounding environment. In this case, two parameters describe the interaction between biota (larvae) and the environment (water media in bags) in this research, the level of oxygen consumption and the survival rate during treatment.

3.2.1 Oxygen Consumption Rate

The level of oxygen consumption was a parameter to determine the metabolic rate closely related to growth [12]. The level of oxygen consumption was an illustration of the amount of oxygen consumed by biota from the oxygen consumption rate available in aquaculture media. Based on Figure 2, it was known that the highest value in the treatment of 200 larvae/bag was 3.66 ± 0.44 mgO_2/g/hour and the lowest value in the treatment of 600 larvae/bag was 0.76 ± 0.14 mgO_2/g/hour. The low level of oxygen consumption in the treatment of 600 larvae/bag indicated that the larvae on high stocking density media could not consume much oxygen 0.0022 mgO_2/g/hour for each PL 10 shrimp larvae. The lowest temperature in the treatment supported this fact, which tends to be colder in the range of 22.97±0.31°C compared to other treatments, 23.20±0.20°C.

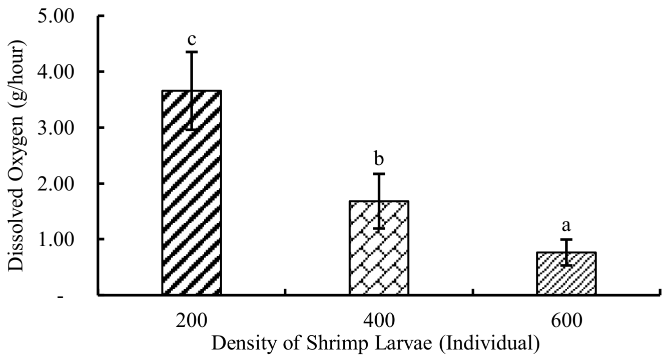

Fig. 2. The level of oxygen consumption of vaname shrimp larvae on short-distance transportation with different densities

Low-temperature levels can affect the metabolic rate in the body of vaname shrimp larvae, including being able to inhibit the movement of larvae. As stated, temperature affects the level of oxygen consumed by shrimp and the salinity and activity level of biota [13]. Shrimp that are slow to move affect the lack of oxygen consumed, converted into energy [14]. If the shrimp larvae are slow to move, the potential for ammonia or waste was also low, so the chance of environmental stress/pressure and death due to poisoning was low. The energy produced will be converted to growth, not to metabolism.

3.2.2 Survival Rate

All treatments were not statistically significantly different. Based on the results of the research, it was found that the highest survival rate or survival rate was found in the treatment with a stocking density of 600 larvae/bag (98.20±0.67%), followed by a stocking density of 400 larvae/bag, 97.12±1.76%, and the lowest was at 200 larvae/bag (97.08 ± 0.72%). From a numerical point of view, the best choice in treating 600 shrimp larvae/bag, but according to [15], statistical significance was a direct result of mathematical equations and not necessarily relevant to describe all influencing factors in the field. A possible approach for making decisions regarding statistically non-significant outcomes was to draw relationships with key parameters closer to the parameters being analyzed (Figure 3) oxygen consumption level. Judging from the level of oxygen consumption parameters, the best value was the treatment of 600 larvae/bag. The value of the frequency of media shaking, the volume of media water, the health status of the test biota, and other parameters are some of the factors that affect significantly or are not related to the statistical test.

The survival rate of shrimp larvae was calculated based on the number of shrimp larvae. Live at the end of the treatment so that the statistically significant difference was more towards the label and the potential impact in the study itself was not an external impact, for example, economic impact or other impacts that are not the purpose of the research. Based on the above, the recommended survival rate in this study was the treatment of 600 shrimp larvae/bag with a survival rate of 98.20±0.67%.

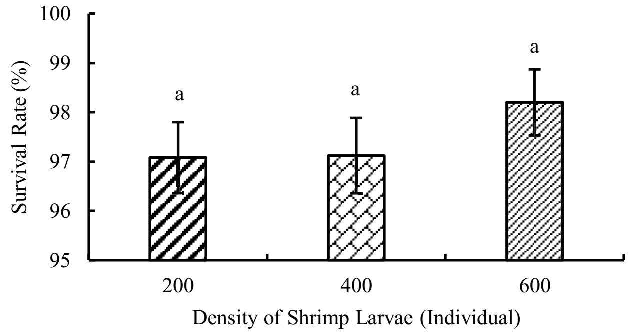

Fig. 3. The survival rate of vaname shrimp larvae at different density transport

3.3 Bioeconomics

Bioeconomics is an activity with economic value based on data on the production and growth of living organisms that can adapt to a fluctuating environment in the context of applied science to achieve prosperity. One of the activities that included bioeconomics was aquaculture activities. Each cultivation activity related to stocking density or density can be compared between the amount of stocking density and the constant output [16]. This activity was inseparable from production, including production costs and investment costs to make profits from the business. It was impossible for good cultivation results, but it was still a loss from the economy of scale. It includes businesses related to vaname shrimp larvae PL 10. In larvae cultivation business activities on a traditional business scale by prioritizing the use of oxygen gas.

Based on table 2, the production cost was the same in all treatments (200 larvae/bag, 400 larvae/bag, and 600 larvae/bag) during the process of transporting larvae from the farmer to the buyer, which was IDR 19,497,000. This was because the supporting material for the transportation of shrimp larvae and the employee costs incurred were the same because the weight of the tail was not the primary consideration but the number of larvae. The unit observed was per unit of transport capacity, one pickup car with a travel time of three hours or a short distance. The capacity of the pickup car was 1,305 litres which could be filled with 58 boxes, and each box contained 50 bags with a volume of 450 mL of water. At the beginning of the departure conditions, it was assumed that all larvae were 100% alive, and during the trip, there were no obstacles such as accidents or traffic tickets on the way or other events.

Table 2. Production costs of vaname shrimp larvae with different densities

Stocking Density (Larvae/Bag)	Total Gas Cost / Bag (IDR)	Packaging Fee / Bag (IDR)	Cost of Ice Cubes and Styrofoam / Bag (IDR)	Pick Up Rental L 300 (IDR)	Total Number of Fry (Larvae)	Total Cost (IDR)
200	400	5,500	600	300,000	580,000	19,150,000
400	400	5,500	600	300,000	1,160,000	19,150,000
600	400	5,500	600	300,000	1,740,000	19,150,000

There was the most considerable total profit value among them in the treatment of 600 larvae/bag (Table 3). The profit value obtained from this treatment was IDR 995,528 or ten times the value with a stocking density of 200 larvae/bag, which was IDR 106,688.

Although not statistically significant, the number of survivors living in the treatment was 98.20% or the lowest compared to the other treatments. The cause of the considerable profit value was the larvae's selling price and the bag's capacity to accommodate the larvae stocking density. On the other hand, this was an indication that the density of 600 larvae/bag was still categorized into the optimal tolerance for the number of biota/bag in secure packing with oxygen gas and ice cubes in styrofoam. The survival rate of larvae was still above 85%, which means it was still good in terms of pressure resistance during the trip.

Table 3. Acceptance of results on different density vaname shrimp larvae

Stocking Density (Larvae/Bag)	Survival Rate of Larvae (%)	Selling Price Per Larva (IDR)	Total Number of Live (Larva)	Revenue (IDR)	Total Profit (IDR)	Profit / Bag (IDR)
200	97.08	45	563,064	25,337,880	6,187,880	106,688
400	97.12	45	1,126,592	50,696,640	31,546,640	543,908
600	98.20	45	1,708,680	76,890,600	57,740,600	995,528

4. Conclusion

The best treatment that can be used at short distances was a density of 600 larvae/bag with a survival rate of 98.20%. The results support this that the level of oxygen consumed by each larva was more efficient at 0.0022 mgO_2/g/hour, which is lower than other treatments and in terms of economic potential, the most significant net profit is IDR 57,740,600 compared to other treatments.

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