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4 Survival Rate and Quality of Phronima Suppa (*Phronima* sp) Zoea with the Cryptobiosis Application

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Abstract. Phronima Suppa (*Phronima* sp) application on hatchery and pond cultivation has succeeded in increasing the survival, growth, and production of black tiger shrimp (*Penaeus monodon*). Phronima has great potential in improving the performance of the national and global shrimp industry while also substituting the use of *Artemia salina*. Phronima suppa lives endemic in Tasiwalie Village and Wiringtasi Village in Pinrang District, South Sulawesi Province. Phronima Suppa distribution to various regions and countries is constrained by storage systems using water media. Phronima dormant's biomass production is an effective solution to the constraints of storage and transportation. The application of cryptobiosis produces low temperature stress to produce Phronima dormant's. This study aims to determine the effective temperature and activation period to produce Phronima biomass dormant's. The study was conducted at the Phronima Suppa Installation of Universitas Muslim Indonesia in Tasiwalie Village. The treatments in this study were three and each with three replications. The treatment of cryptobiosis in the first phase of the study consisted of temperature levels of 8 °C, 4 °C, and 0 °C. The activation period of dormant Phronima biomass with the application of cryptobiosis at a temperature of 8 °C in the second phase of the study was 3 hours, 8 hours, 13 hours and 24 hours activation period. The highest biomass survival (60%) was generated in the 24 hours activation period with cryptobiosis at a temperature of 8 °C.

Keywords : Phronima Suppa, cryptobiosis, dormant, black tiger shrimp.

1. Introduction

Black tiger shrimp (*Penaeus monodon*) has become Indonesia's leading export commodity. The ever-increasing market demand is driving the extensive expansion of shrimp farming areas and the increasing application of intensive tiger shrimp cultivation technology. Massive application of chemicals, drugs, and chemicals for the prevention of pathogens, predators, and improvements in water quality has increased environmental pollution and decreased pond carrying capacity. The intensive application of shrimp farming encourages an increase in the use of artificial feed which contributes to an increase in feed residue and pollution of organic matter in ponds and aquatic environments. Pathogenic infections, especially the White Spot Syndrome Virus (WSSV) and *Vibrio harvey*, affect about 39,022 ha of pond areas in South Sulawesi Province that were unproductive or idle from 1988 to 2007. Harvest failure in South Sulawesi is predicted to cause losses for farmers around 33, 4 million USD per year. Losses due to shrimp disease in Indonesia are estimated to reach more than USD 300 million or more than IDR 3 trillion per year [1][2].

Since 2005 a population of Phronima suppa (*Phronima* sp) micro crustacean species that lived endemic in Wiringtasi Village and Tasiwalie Village, Suppa District, Pinrang Regency. Phronima Suppa was not found on ponds outside the two villages [3]. The presence of Phronima Suppa is an indicator of the rise of tiger prawns in areas that are being attacked by the WSSV and *V. harvey* viruses. The area of the pond that was found living Phronima Suppa and being infected with WSSV succeeded in producing tiger shrimp with a survival rate of around 70 percent. In contrast, the tiger shrimp area without Phronima Suppa is only able to produce shrimp with a survival rate of 10 percent [4]. Phronima Suppa plays an important role in providing the nutrients needed



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by tiger prawns, improving water quality and pond soil as well as enhancing immunity against shrimp pathogen infections [5]. The development of Phronima Suppa natural feed makes Pinrang Regency the biggest tiger shrimp supplier in South Sulawesi. Phronima has great potential in improving the performance of the national and global shrimp industry while also substituting the use of *Artemia salina*. At present Artemia is still imported, thus weakening the competitiveness of the Indonesian shrimp farming industry.

Cryptobiosis application is making low temperature stress to produce dormant organism. Cryptobiosis application is carried out on Phronima Suppa zoea stadia with various temperature levels. The results of previous studies, the activation period of one hour and two hours produced a very significant survival difference ($P < 0.05$). Survival rate was not significantly different in all treatment in the three-hour activation period.^[6] Some types of crustaceans have additional organs such as labirint so they can survive in a humid environment. Young Phronima stadia survival about all that is placed at a high level in the media without air and wet because no statistical differences were found. Application of cryptobiosis and Phronima storage on waterless media is needed because it is compatible with the distribution and trading of Phronima Suppa. Distribution and trading of the Phronima Suppa use of water that is not practical and creates various difficulties in storage and transportation.

The storage and transportation of Phronima Suppa zoea without water media will expand the distribution and using of Phronima in Indonesia or applications globally. This condition can be achieved if Phronima Suppa zoea is in a dormant condition. Dormant is a Phronima Suppa zoea in a condition of temporary loss of insensibility. Phronima Suppa zoea will experience physiological changes in the dormant process. In a dormant state Phronima's metabolic activity is at a low level so that it can be distributed without water media^[6]. Dormant Phronima biomass production is an effective solution to storage and transportation constraints. ³ This study aims to determine the cryptobiosis temperature level and the effective activation period to produce dormant Phronima biomass.

2. Experimental Details

The study was conducted from May to August 2017 at the Phronima Suppa Installation of Universitas Muslim Indonesia in Tasiwalie Village, Suppa District, Pinrang Regency, South Sulawesi Province. Phronima Suppa zoea which is used as a test organism is obtained from nature and selected to obtain a uniform size and prevent contamination.

This study was divided into two stages and each stage consisted of three treatments and each had three replications. Cryptobiosis treatment in the first stage of the study consisted of temperature stress levels of 8 °C, 4 °C, and 0 °C respectively. Zoea was made dormant for 3 hours, 8 hours, 13 hours and 24 hours respectively (Table 1). Each research container contained 50 tail Phronima Suppa zoea and was created in humid conditions. Phronima zoea is activated by inserting it into a container filled with water media at a temperature of 29 - 32 °C, salinity 19-23 ppt, acidity level of 8.6 and dissolved oxygen is controlled at levels > 6.6 ppm. The first phase of the study continued with the second stage of the study to obtain cryptobiosis temperatures and optimal activation periods. The first stage treatment research matrix is presented in Table 1 below.

Table 1. First Stage Research Treatments				
Treatment				
Temperature	Activation Period			
	3 hours (P)	8 hours (Q)	13 hours (R)	24 hours (S)
0 °C (A)	AP	AQ	AR	AS
4 °C (B)	BP	BQ	BR	BS
8 °C (C)	CP	CQ	CR	CS

The research parameters observed during the study were survival rate with the following formula^[7]:

$$S_o = \frac{N_t}{N_o} \times 100\%$$

S_o = Phronima Suppa zoea survival rate after activation (%)

N_t = the number of Phronima Suppa zoea post activation (tail)

N_o = the number of Phronima Suppa zoea before activation (tail)

3. Results And Discussion

Cryptobiosis Temperature Determination

Determination of optimal cryptobiosis temperature is needed to produce Phronima Suppa zoea dormant's but has a high survival rate. Optimal cryptobiosis temperature will support the continuation of physiological processes so that the resulting Phronima zoea dormant's can still be activated and developed into a young and adult Phronima. The results of the research on cryptobiosis temperature are shown in Table 2 and Figure 1.

Table 2. Determination of the Temperature of Cryptobiosis Phronima Suppa Zoea

Activation Period (hours)	Survival Rate		
	Temperature (°C)		
	0 °C	4 °C	8 °C
3	0 %	90 %	90 %
8	0 %	70 %	80 %
13	0 %	70 %	80 %
24	0 %	25 %	25 %
Average	0 %	63.75 %	71.25 %

The highest zoea survival (71.25%) was produced at a stress temperature of 8 °C higher than the treatment temperature of 4 °C and 0 °C. The 4 °C temperature treatment produced 63.75% survival rate and the 0 °C temperature treatment produced the lowest survival rate (0%). The cryptobiosis temperature treatment of 8 °C resulted in the Phronima Suppa zoea being more quickly dormant (1 - 2 minutes) and the highest survival rate (71.25%) compared to others treatments. Application of 4 °C temperature produced the longest dormant zoea (10 - 20 minutes) compared to the other two treatments and lower survival rate (63.75%) compared to 8 °C temperature treatment. The faster Phronima Suppa zoea becomes dormant, it has the opportunity to produce a higher survival. The faster the dormancy occurs the potential for organ damage to be more so that it affects the survival of Phronima zoea. Aquatic organisms are poikilothermic or cannot regulate their body temperature independently so that it depends on the temperature of the waters.^[8]

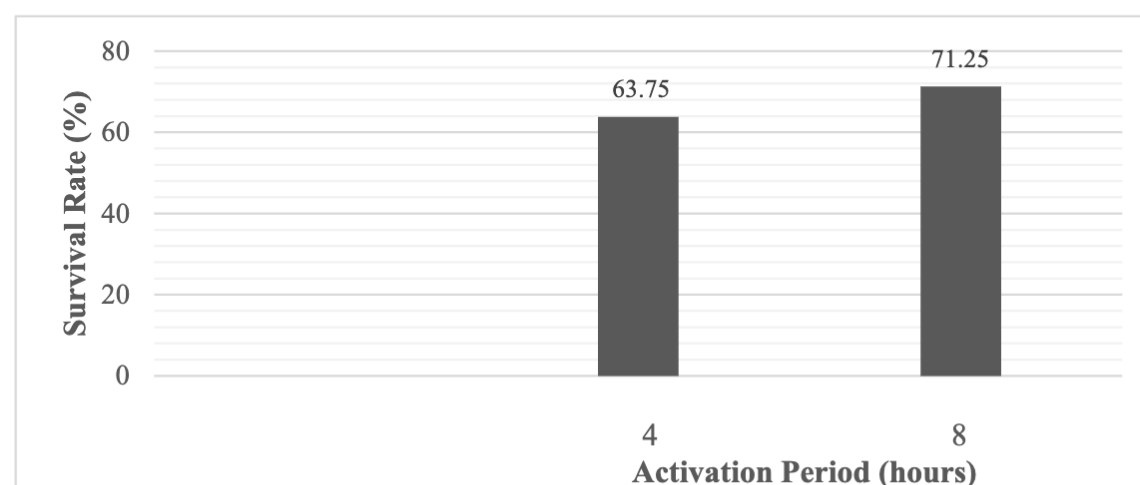


Figure 1. Determination of the Temperature of Cryptobiosis Phronima Suppa Zoea

Phronima Suppa zoea dehydrated in the cryptobiosis process due to the temperature applied not the normal temperature. The dehydration process takes place before the emergency process occurs. The 8 °C temperature treatment resulted in the fastest Phronima zoea dormancy process (1-2 minutes) so that the dehydration process was lower compared to other treatments. The 8°C treatment was determined to be the cryptobiosis temperature treatment in producing Phronima Suppa zoea dormant and became a reference in determining the activation period.

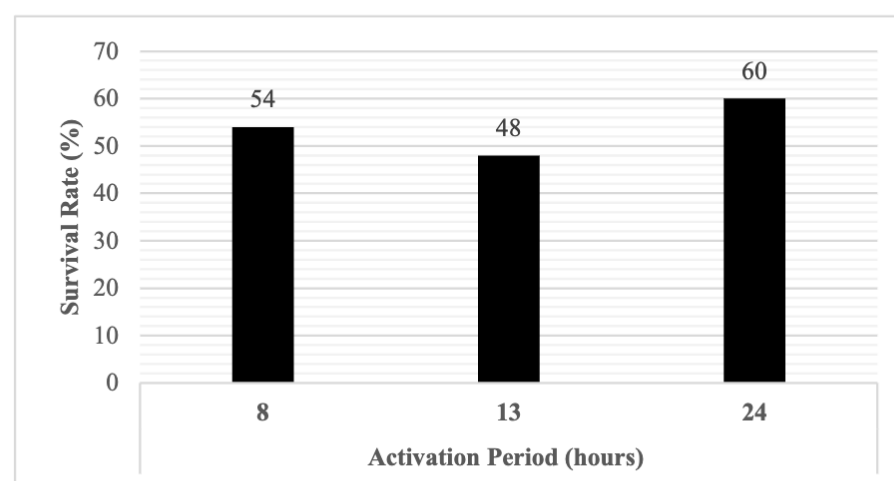
Activation Period

Phronima Suppa zoea survival at different activation periods can be explained in Table 3 and Figure 2. Phronima Suppa zoea survival at the 24 hours activation period results in higher (60%) and lowest (48%) survival at the 13 hours activation period.

Table 3. Phronima Suppa Zoea Survival Rate at Different Activation Period in the 8 °C Cryptobiosis Temperature

Temperature 8 ⁰ C	Survival Rate Activation Period (hours)		
	8 (A)	13 (B)	24 (C)
Average	54 %	48 %	60 %

The highest survival rate for 24 hours activation treatment is influenced by the storage system in humid conditions so that it does not expend great energy to adapt to the given temperature. The storage process of Phronima Suppa zoea for activation period 24 hours every two hours is kept under control in humid conditions. The development of Artemia zoea under optimal conditions between 8 to 16 hours after the dehydration process.^[9] This is highly influenced by external environmental conditions. Low temperature stress will cause changes in the concentration of cortisol and catecholamine in the body of shrimp. Catecholamine can affect the cardiovascular system, metabolic energy, and immunity.^[10]

**Figure 2.** Phronima Suppa Zoea Survival Rate at Different Activation Period

Phronima Suppa zoea becomes dehydrated after going through a dormant process due to osmotic pressure, thus depleting the body's fluid ions. One way Phronima zoea adapts to dehydration is by limiting water permeability so that it can passively limit the release of body fluids.

Water quality

Water quality parameters during the study were still within the limits of growth tolerance and survival of Phronima Suppa zoea. The results of dissolved oxygen (DO) measurements obtained during the study were between 6.6 - 10.8 ppm. The DO range is the optimum range for Phronima Suppa zoea. The results of temperature measurements obtained during the study are 29 - 32 °C. The temperature range is still in the optimum range for Phronima Suppa zoea is 20 - 30 °C.³ Salinity obtained during the study was in the range of 19 - 35 ppt.

4. Summary

Cryptobiosis temperature stress at 8 °C produces the highest survival rate (71.25%) compared to 4 °C and 0 °C. The highest survival rate of Phronima zoea (60%) was produced at a temperature stress cryptobiosis at 8 °C with 24 hours activation period. Phronima Suppa zoea dormant develops well into the stage of young Phronima and adult Phronima after activation. It is recommended that the dormancy process be carried out in a room with temperature controlled and further research to increase the Phronima Suppa zoea survival in waterless media.

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