

# Evaluation of the survival of sand lobster seeds after transportation at different densities

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**Abstract.** This study aimed to evaluate the effect of density on the survival of sand lobster fry after transportation. The study used a completely randomized design (CRD), consisting of three density treatments: 10 individuals/container, 15 individuals/container, and 20 individuals/container. The lobster juvenile were reared for 15 days and fed twice daily. The parameters observed were total hemocytes, plasma glucose, and lobster fry survival. The results showed that the density treatment of 10 individuals/container produced the highest survival rate, namely  $76.67 \pm 5.77\%$ . The densities of 15 individuals/container and 20 individuals/container each produced survival rates of  $64.44 \pm 3.85\%$  and  $43.33 \pm 5.77\%$ , respectively.

## 1 Introduction

Sand lobster (*Panulirus humarus*) has become one of the leading commodities in Indonesia's aquaculture sector, especially in the southern and eastern coastal regions, which have favorable ecosystem conditions [1]. Global market demand for lobster continues to increase, whether fresh, live, or frozen, opening up significant economic opportunities. However, the development of lobster aquaculture still faces challenges, one of which is the suboptimal technical management of aquaculture, including stocking density used in the grow-out system. Stocking density is a crucial factor that directly affects the survival of farmed organisms.

Determining the ideal stocking density is crucial to avoid competition among individuals, cannibalism, water quality degradation, and physiological stress, which ultimately lead to reduced lobster survival rates. Several studies indicate that excessively high stocking densities can result in growth retardation due to increased competition for space and feed, as well as elevated concentrations of ammonia and other toxic compounds in the culture medium [2]. Conversely, while low stocking densities may improve individual performance, they are economically inefficient due to suboptimal use of space and feed [3]. Therefore, research on variations in stocking density for lobster larvae is important to identify the optimal balance between productivity and cultivation efficiency. This study was conducted to determine the differences in the effects of various stocking densities on the survival of lobster larvae in a controlled cultivation system.

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## 2 Methods

### 1.1 Time and location

This study was conducted in July 2023 at a lobster seed collector in Ujung Genteng village, Sukabumi. Test parameter analysis was carried out at the Aquatic Organism Health Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB, Dramaga, Bogor.

### 1.2 Experimental design

This study was designed using a completely randomized design (CRD) with three treatments and three replicates. The treatments consisted of a density of 10 fish/tank, a density of 15 fish/tank, and a density of 20 fish/tank.

### 1.3 Experimental animal and maintenance management

Sand lobster fry used in the transportation process in the second phase of the research. The seeds weighed between 30 and 50 grams per seed. The container used was a  $60 \times 60 \times 45$  cm<sup>3</sup> fiber tub. Nine aquariums were equipped with aerators and a recirculation system. Each aquarium was stocked with sand lobster fry according to the treatment and replicates applied.

Lobster fry were placed in a rearing container and maintained for 30 days to monitor post-transportation survival. During the rearing period, the lobster fry were fed small fish twice daily, and the water in the container was changed daily by siphoning. Water quality measurements (temperature, DO, pH) were carried out daily throughout the rearing period, while observations (ammonia, nitrite, and nitrate) were conducted every 7 days. Hemolymph and hemolymph glucose were collected and measured at the beginning and end of the rearing period.

### 1.4 Test parameters

Parameters that are survival rate, total hemocytes, and hemolymph glucose levels

#### 1.4.1 Survival rate

The survival rate based on the equation proposed by Zonneveld formula [4] is as follows:

$$SR = \frac{Nt}{No} \times 100 \quad (1)$$

where:  $SR$  = Survival Rate (%),  $Nt$  = Number of lobsters at the end of the cultivation period,  $No$  = Number of lobsters at the beginning of the cultivation period.

#### 1.4.2 Total hemocytes

Hemolysate was collected in 0.1 mL using a 1 mL syringe containing 0.1 mL of sodium citrate anticoagulant, then homogenized for 5 minutes. The first drop of hemolysate from the syringe was discarded, then the hemolysate was dropped onto a hemocytometer and the number of cells per mL was counted using a light microscope with 40x magnification. The

cell count results are calculated using the formula according to Blaxhall and Daishley [4], namely:

$$\text{Total hemocytes} = [(\text{average total cells}) \times (1/\text{large box volume}) \times (\text{dilution factor})] \quad (2)$$

### 1.4.3 Glucose level

Hemolymph samples were centrifuged for 10 minutes at a speed of 1000 rpm to separate the hemolymph plasma. Next, 0.5  $\mu\text{L}$  of hemolymph plasma was added to 3.5 mL of ortho-toluidine color reagent in glacial acetic acid. The mixture was placed in boiling water for 10 minutes. After cooling to room temperature, the hemolysate glucose concentration was measured using a spectrophotometer at a wavelength of 635 nm. The absorbance value was then converted into hemolysate glucose concentration in mg/100 mL. The hemolysate glucose concentration was calculated using the formula from Wedemeyer and Yasutake [5], which is:

$$GD = \frac{AbsSp}{AbsSt} \times GSt \quad (3)$$

where:  $GD$  = Hemolymph glucose concentration (mg/100 mL),  $AbsSp$  = Absorbance of samples,  $AbsSt$  = Absorbance of standart,  $GSt$  = Standard glucose concentration (mg/100 mL).

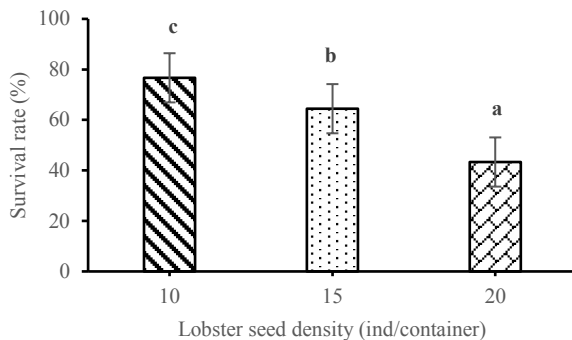
## 1.5 Data analysis

The data on diversity coefficients and physical and chemical properties of the media were tabulated and analyzed descriptively. Other data were processed and analyzed using SPSS Ver.22 software at a 95% confidence level. If there were significant differences between the mean values of the treatment data, a least significant difference test was performed [6].

# 2 Result

## 2.1 Survival rate

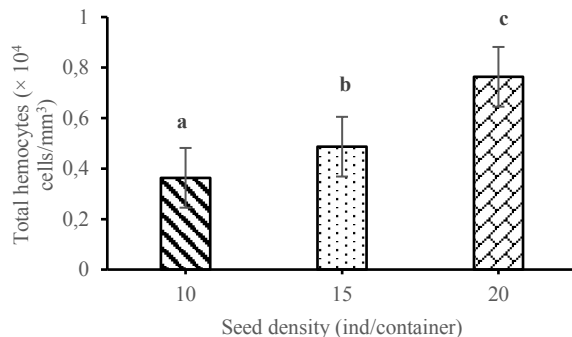
The survival rate for each treatment can be seen in (Figure 1). The treatment with 10 fish per container resulted in the highest survival rate (76.67 $\pm$ 5.77%), which was significantly different from the other treatments ( $p < 0.05$ ). The treatments with densities of 15 and 20 fish per tank had survival rates of 64.44 $\pm$ 3.85% and 43.33 $\pm$ 5.77%, respectively.



**Fig.1.** Survival of sand lobsters at different densities. Different letters in each bar (mean value  $\pm$  standard deviation) indicate statistically significant differences ( $P < 0.05$ ).

## 2.2 Total hemocytes

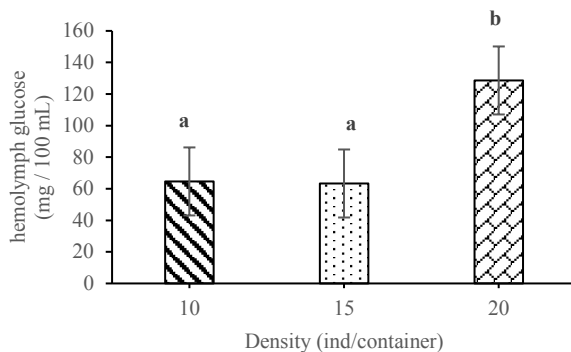
The total hemocytes from each treatment can be seen in (Figure 2). The treatment of 10 individuals/container had the lowest total hemocytes ( $0.36 \pm 0.04 \times 10^4$  cells/ $\text{mm}^3$ ), which was significantly different from the other treatments ( $p < 0.05$ ). The treatments with densities of 15 and 20 fish per container had total hemocytes of  $0.49 \pm 0.03 \times 10^4$  cells/ $\text{mm}^3$  and  $0.76 \pm 0.06 \times 10^4$  cells/ $\text{mm}^3$ , respectively.



**Fig. 2.** Total sand lobster hemocytes at different densities. Different letters in each bar (mean value  $\pm$  standard deviation) indicate statistically significant differences ( $P < 0.05$ ).

## 2.3 Glucose level

Hemolymph glucose levels for each treatment can be seen in (Figure 3). Treatment 10 fish/container had a hemolymph glucose level of  $64.63 \pm 2.88$  mg/100 mL, which was not significantly different from treatment 15 fish/container ( $63.33 \pm 5.84$  mg/100 mL). The treatment with 20 individuals per container resulted in the highest hemolymph glucose level of  $128.61 \pm 3.06$  mg/100 mL.



**Fig. 3.** Hemolymph glucose of sand lobsters at different densities. Different letters in each bar (mean value ± standard deviation) indicate statistically significant differences ( $P < 0.05$ ).

### 2.4 Water quality

The water quality range during lobster fry maintenance is still within the range tolerated by lobster fry (Table 1).

**Tabel. 1.** Range of water quality for lobster seed maintenance.

Parameter	Unit	Range
Dissolve oxygen	mg L <sup>-1</sup>	5,00 – 5,13
pH	-	8,51 – 8,61
Salinity	g L <sup>-1</sup>	28,0 – 30,0
Temprature	°C	27,0 – 29,0
Ammonia total (TAN)	mg L <sup>-1</sup>	0,17 – 2,77
Nitrite (NO <sub>2</sub> )	mg L <sup>-1</sup>	0,03 – 1,48
Nitrate (NO <sub>3</sub> )	mg L <sup>-1</sup>	0,01 – 1,67

## 3 Discussion

Stress is defined as a state in which homeostasis is disturbed beyond normal limits and the recovery process continues. through metabolic pathways and affects the fish immune system [7]. In the crustacean immune system, hemocytes act against pathogens or foreign particles through phagocytosis, encapsulation, degranulation, and synodular aggregation [8].

Lobster fry housed in confined spaces at high densities can cause physical and environmental stress. Physical stress can include limited space for movement, while environmental stress can include increased ammonia concentrations and decreased water quality [9]. This stress can affect the lobster's immune system, which is directly related to the total number of hemocytes in the hemolymph.

High stocking dens can cause an increase in lobster metabolism in response to stress. When lobsters are stressed by high stocking dens, their bodies respond by increasing their metabolism, which impacts their energy needs. This energy demand can affect glucose levels in the hemolymph [10]. The stress caused by high stocking densities causes the release of stress hormones such as adrenaline and cortisol, which stimulate the breakdown of glycogen into glucose (glycogenolysis) to provide rapid energy. Glucose levels in the hemolymph increase in response to the greater energy needs, adapting to the stress [11].

A dissolved oxygen value range of 5.00 – 5.13 mg L<sup>-1</sup> indicates adequate oxygen levels in the water to support normal lobster seed metabolism. In general, higher oxygen levels reduce oxidative stress in lobsters. A pH range of 8.51 – 8.61 indicates slightly alkaline water.

This pH range is considered ideal for many lobster species, including sand lobsters, which typically prefer a slightly alkaline pH to support their physiological balance [12].

A salinity between 28.0 – 30.0 g L<sup>-1</sup> indicates adequate water conditions for the needs of sand lobsters. Salinities higher or lower than this range can cause osmotic stress and affect the lobster's ion balance. A temperature range of 27.00 – 29.00 °C is within the ideal temperature range for the growth of sand lobster fry. Temperatures that are too high or too low can cause a decrease in metabolic rate, weaken the immune system, or increase the likelihood of thermal stress, which can damage the lobster's body tissue [13].

A total ammonia value between 0.17 – 2.77 mg L<sup>-1</sup> reflects ammonia levels that can vary and are potentially harmful at higher concentrations. Ammonia is a waste product produced by lobsters and other organisms in aquaculture systems, and its accumulation in water can cause toxicity, disrupt their respiratory system, and damage body tissues [14]. A nitrite value range of 0.03 – 1.48 mg L<sup>-1</sup> indicates relatively low nitrite concentrations. Nitrite is an intermediate product in the nitrogen cycle and can be highly toxic to lobsters at high concentrations. A nitrate value range of 0.01 – 1.67 mg L<sup>-1</sup> ppm indicates relatively low nitrate concentrations, which are generally harmless [15].

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