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Effects of stocking density and diets on survival and growth of lavi-cultured slipper lobster (*Thenus orientalis* Lund, 1793)

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ABSTRACT

A study on the effect of different densities and feed when rearing phyllosoma larvae of slipper lobster (*Thenus Orientalis*) was conducted at Nha Trang Marine Research and Development Center, Institute of Aquaculture Research No. 3 from January to April 2020 to evaluate the effect of density and types of food on survival, growth (phase transition rate, phyllosoma larval size: CL and CW). In general, the impact of feed on phyllosoma larvae was recorded in such parameters as survival rate, stage transition rate, and larval size (CL and CW). The results showed that the survival rate of phyllosoma larvae depends on the density of rearing phyllosoma larvae, and the appropriate density when breeding is 15 fish/L. However, density does not affect the growth of phyllosoma larvae. Besides, the best feed formula for rearing phyllosoma larvae in this study was umbrella *Artemia* + (white clam) fresh meat for the first seven days, and newly hatched *Artemia* + white clam meat continued to be fed from day one, 8th to 24th day.

Keywords: Slipper lobster, *Thenus orientalis*, phyllosoma, diet, density, survival rate, growth rate.

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INTRODUCTION

The *Thenus orientalis* (slipper lobster, also known as shovel lobster, slipper lobster, or flat lobster) is a seafood species with high nutritional and commercial value, capable of being exported and domestic consumption in countries such as India, Australia, Thailand, and Vietnam. According to FAO statistics, in 2010, the total production of lobster caught worldwide was 279,000 tons, of which slipper lobster belonging to the Scyllarid family accounted for about 3.69% of the catch [1]. Among the seven species of shrimps belonging to the Scyllarid family, *Thenus* Leach, 1815 belonging to the order Decapoda and Scyllaridae have the highest commercial value [2, 3]. Among the wild-caught shrimp species in India, *Thenus unimaculatus* (Burton and Davie, 2007) is one of the most critical species contributing to the lobster catch (including the giant *Thenus unimaculatus* shrimp and *Panulirus homarus*) in India annually [4]. In India, shrimp is a source of export seafood with low output compared to other seafood species, but it brings a significant source of foreign currency due to its high commercial value [3]. In Vietnam, the economic value of slipper lobster (*Thenus orientalis*) is second only to cotton lobster (*Panulirus ornatus*), so this shrimp species has the potential to develop into a new species of culture in Vietnam [5]. However, today, the most significant difficulty in developing slipper lobster farming in Vietnam is not yet successfully producing artificial seeds.

In slipper lobster seed production, nutrition plays a crucial role in the survival and development of larvae. The reason is that the phyllosoma larvae of slipper lobster have a characteristic morphology: slab, transparent, fragile, and floating, so the study to determine the suitable food for the larvae is particularly important to researchers. Various feeds have been tested for the rearing of phyllosoma larvae, especially the meat of many mollusks. In the study of Marinovic et al., (1994), fresh green mussel (*Mytilus edulis*) was used as feed when rearing *Ibacus peronii* shrimp larvae from the stage of phyllosoma I to the postlarval nisto stage [6]. In another study, the gonads of green

mussels were minced as food for the larvae of *Scyllarus cultrifer* species. Mikami (1995) used mollusk meat of *Donax deltooides* species to feed the larvae when rearing two species of black cap shrimp *T. orientalis* and *T. indicus* [7]. Besides mollusk meats, as mentioned above, other types of food have also been tested, such as Artemia, fish larvae [8], jellyfish [9], or a combination of many different types of food. Alternatively, changing food according to the stages of larval development was also done to determine the kind of food suitable for rearing phyllosoma larvae of slipper lobster [10].

Larval rearing density is an essential factor affecting the growth and the survival rate of larval rearing of aquatic animals. From an economic point of view, stocking density is a factor that determines the balance between survival rate and quantity of seed produced, which impacts the business's efficiency [11, 12]. Biologically, the stocking density affects the interaction between the larvae reared together; the high stocking density leads to the larvae being easily shocked by the body's energy response, spreading diseases, and competing for food and living space to increase the eating rate [13, 14].

The culture water environment explicitly influences the growth and development of phyllosoma larvae. Larval development can be accelerated when the temperature is increased by increasing the metabolic rate. However, when the temperature exceeds the optimal threshold, the molting process will be delayed, leading to increased larval mortality [15]. Salinity affects the growth and development of phyllosoma larvae of slipper lobster because phyllosoma larvae are commonly distributed in offshore waters, so seawater salinity is high [16]. Ammonia, nitrite, and nitrate are the most abundant nitrogenous inorganic substances in aquatic ecosystems, essential for sustaining the life of various aquatic microbial constituents, including crustaceans [17]. However, when the content of these substances exceeds the threshold, it will adversely affect the larvae and may cause larval death.

Therefore, to successfully develop the production process of slipper lobster (*Thenus orientalis* Lund, 1793), it is necessary to carry

out studies to determine the appropriate density and feed when rearing phyllosoma larvae of slipper lobster is needed.

MATERIALS AND RESEARCH METHODS

Time, location, and research subjects

The study was carried out from January to April 2020 at Nha Trang Mariculture Research and Development Center, Research Institute for Aquaculture III. Research subjects of slipper lobster larvae (*Thenus Orientalis* Lund, 1793).

The data in this study are part of the research project “Research on exploitation and development of genetic resources of slipper lobster *Thenus orientalis* Lund, 1793”, Code number NVQG 2018/16.

Research materials

Water source: Sea water with a salinity of 30–34 ppt is treated with chlorine (30 ppm), then aerated until chlorine is disappeared and left to settle for 24 h. The water to settle is filtered through a pre-filtration system and fed to the culture tank through an ultrafiltration bag (1 µm).

Shrimp source: Natural broodstock slipper lobster collected, tamed and reared at Nha Trang Mariculture Research and Development Center. The mother shrimp holding the eggs are incubated and hatched in a tank of 0.5–1 m³. Afterward, phyllosoma I larvae were collected, quantified, and arranged for experiments.

Food sources for phyllosoma larvae include *Artemia* (Franciscana strain, USA), blood cockle (*Anadara granosa* Linnaeus, 1758), green mussels (*Perna viridis* Linnaeus, 1758), and white clams (*Meretrix lyrata* Sowerby, 1851).

Research methods

Experimental preparation

Experiment 1: Studying the effect of density on the growth and survival rate of slipper lobster larvae.

The experiment was arranged in 15 plastic buckets ($V = 60$ L), and the volume of water was

50 L/bucket. Food was *Artemia bungee* + green mussel meat for the first seven days, then from day eight until the end of the experiment, feeding newly hatched *Artemia* + green mussel meat. Lighting conditions 12 am/12 pm, light intensity 400 lux. The experiment was repeated five times.

Experimental design: three density treatments (MD):

MD 1: 15 heads/L;

MD 2: 20 fish/L;

MD 3: 25 heads/L;

Experimental time: 25 days

Experiment 2: Effect of food combinations on growth, survival rate, and percentage of molting rate of slipper lobster larvae.

The experiment was arranged in 20 plastic buckets ($V = 60$ L), and the water volume was 50 L/bucket. The density of phyllosoma larvae was 15 individuals/L. Lighting conditions 12 am/12 pm, light intensity 400 lux. The experiment was repeated five times.

Experimental design: including four treatments (TA):

TA 1: Umbrella *Artemia* + blood cockle meat (first seven days)/Newly hatched *Artemia* + blood cockle meat (8th to 24th day);

TA 2: *Artemia* parachute + green mussel meat (first seven days)/Newly hatched *Artemia* + mussel meat green (8th to 24th day);

TA 3: Umbrella *Artemia* + white clam meat (first seven days)/Newly hatched *Artemia* + meat white clams (8th to 24th day);

TA 4: Umbrella *Artemia* + mixed mollusk meat (first seven days)/Newly hatched *Artemia* + mixed mollusk meat (8th to 24th day).

Experimental time: 25 days

Management care

Food preparation and feeding: *Artemia* eggs hatch for 18–20 h and hatch for 24–26 h. Fresh mollusk meat is separated from the shell, gutted, and the mantle removed, then minced and filtered through a 300 µm racket to feed the larvae for the first seven days and filtered through a 400 µm racket for larvae. The larvae were fed from day 8 to day 24. The mixture of mollusk meat in treatment 4 consisted of 3 types: blood cockle + green mussel + white

clam with a ratio of 1:1:1. Daily before feeding, siphon leftovers and dead larvae at the bottom of the bucket. Feed the larvae three times/day.

Water change: Water is changed every three days. Change 100% of the culture water each time. Monitor culture environment factors every three days (salinity, temperature, pH, DO, NO₂⁻ and NH₃).

Data acquisition and interpretation

Data acquisition

Indicators of water environment and larval size: Temperature, pH, and dissolved oxygen (DO) content were determined by Handy Gamma meter, Denmark, with an accuracy of 0.1 °C and 0.1 units, respectively, taste and 0.1 mg O₂/L. Nitrite and ammonia were determined by a colorimetric test kit from Sera, Germany. The length of the cephalothorax (CL - carapace length) and the width of the cephalothorax (CW - carapace width) of larvae were measured by KEYENCE VHX-970F (Japan).

Other indicators:

Ratio of stage transition (*RST*; %): Determine the number of larval stages in all the experimental treatments on the 6th, 12th, 18th, and 24th day of culture:

$$RST = N_i / B * 100\%$$

where: N_i is the total number of phyllosoma larvae stage I, II, III, or IV (child); B is the total number of primary larvae of phyllosoma I (heads).

The cephalothorax length (*CL*) and cephalothorax width (*CW*) of larvae were determined at the end of the experiment, with the number of samples per larval stage of each treatment being 30 samples.

Survival rate (*SR*; %): Determine the number of viable larvae in all experimental treatments at the 6th, 12th, 18th, and 24th days of culture. The formula for survival:

$$SR = A/B * 100\%$$

where: A is the total number of live larvae (heads); B is the total number of initial larvae (heads).

Data interpretation

Compare the difference between treatments by Tukey's test through SPSS 22.0 software at the significance level ($p < 0.05$). The collected data are calculated as mean and standard deviation by Excel 2013. The data are presented as mean \pm standard deviation (*SD*).

RESULTS AND DISCUSSION

Effect of density on growth and survival rate of slipper lobster larvae

Water environmental factors monitored during density experiment

The fluctuations of water environmental factors in the treatments during the experimental period are presented in Table 1. The water ecological factors in the treatments were relatively stable because the experiment was arranged in the same area, and the water mode changed and performed synchronously with the same water source. This situation is most evident in the two factors of temperature and salinity (28.5 °C and 33.5 ppt) when the mean values of these two factors are not different in all treatments.

The pH and dissolved oxygen (DO) are important environmental factors in larval shrimp rearing. In this experiment, the pH was stable at 7.8–7.9. DO was stable in the 5.1–5.3 mg/L range and did not differ significantly in these values when comparing treatments.

Several other environmental parameters were also monitored in this experiment, such as nitrite and ammonia content. The values of these two parameters do not fluctuate much and are always in the appropriate range for rearing the larvae of black cap shrimp, respectively, in the range of < 0.2 mg/L (nitrite) and < 0.01 mg/L (nitrite) and < 0.01 mg/L (ammonia).

Different species of slipper lobster will have different appropriate temperature thresholds for rearing phyllosoma larvae, so it is necessary to determine the proper temperature threshold for each species, such as *Scyllarides aequinoctialis*

(Lund, 1793) is 24 °C [18], *Scyllarus americanus* is 25 °C [19]. For shrimp species of *Thenus orientalis*, Kagwade and Kabli (1996) suggested the appropriate temperature for rearing phyllosoma larvae in the range of 26–29 °C; in our study, the average temperature recorded was 28.5 °C, consistent with the

studied temperature range of [20]. Temperature plays a vital role in developing phyllosoma larvae; the increased temperature can accelerate metabolism to help larvae develop faster. Still, when the temperature exceeds the optimal threshold, the development process also slows down as the survival rate of larvae decreases [7].

Table 1. The environmental factors monitored during the density experiment

| Treatment | Salinity (ppt) | Temp. (°C) | pH | DO (mg/L) | NO ₂ ⁻ (mg/L) | NH ₃ (mg/L) |
|-----------|-------------------------|-------------------------|------------------------|------------------------|-------------------------------------|------------------------|
| MD 1 | 33.5 ± 0.2 ^a | 28.5 ± 0.3 ^a | 7.9 ± 0.2 ^a | 5.3 ± 0.4 ^a | < 0.2 | < 0.01 |
| MD 2 | 33.5 ± 0.2 ^a | 28.5 ± 0.3 ^a | 7.8 ± 0.4 ^a | 5.1 ± 0.2 ^a | < 0.2 | < 0.01 |
| MD 3 | 33.5 ± 0.2 ^a | 28.5 ± 0.3 ^a | 7.8 ± 0.3 ^a | 5.3 ± 0.1 ^a | < 0.2 | < 0.01 |

Note: Different letters in the same column represent different values of statistical significance ($p < 0.05$). The data in the table are the mean ± standard deviation ($n = 5$). Density (MD) 1: 15 heads/L; Density 2: 20 heads/L; Density 3: 25 heads/L.

Because the habitat of each shrimp species is different, the appropriate salinity for larval rearing also varies from species to species. When rearing phyllosoma larvae of *Thenus orientalis*, Mikami (1995) found that phyllosoma larvae developed slowly, and the time between molting was prolonged when raised at a salinity of 20 ppt and 25 ppt [7]. Tran Ngoc Hai et al., (2012) suggested that the 30–33 ppt salinity range is suitable for rearing this species of cap shrimp larvae. In our experiment, the salinity for rearing phyllosoma larvae of slipper lobster was 33.5 ppt; this salinity was not significantly different from the salinity range studied by [10]. Research on the saltiness of rearing phyllosoma larvae of *Scyllarus americanus* showed that at two low salinities of 25.8 and 29.3 ppt, the larvae had to go through a second stage before moving to the postlarval stage of nisto, in contrast to rearing at 32.7 ppt salinity [19].

The appropriate oxygen (DO) threshold for rearing phyllosoma larvae of slipper lobster is not well known; however, most studies on larval rearing are usually performed regularly in recirculation or water exchange systems with continuous aeration. In this experiment, we also performed a 100% water change every three days with aeration 24/24, so DO content is always > 5 mg/L; this is also the appropriate oxygen threshold for most aquaculture subjects.

Quan et al., (2021) studied the effect of threshold toxicity and resilience of ammonia

content in culture water on stage I of *Ibacus novemdentatus* larvae and revealed that the ammonia concentration suitable for larval rearing should be at 0.05 mg/L or less [21]. The results of ammonia content recorded in our experiment (< 0.01 mg/L) were in the appropriate range compared to the author's assessment in the above study. Similarly, the nitrite content in our investigation <0.2 mg/L is also a suitable threshold for most aquaculture subjects' average growth and development.

Effect of density on the growth of phyllosoma larvae

Rate of larval stage transition:

The results of monitoring density's effect on the larval metamorphosis rate are presented in Table 2. In general, the value of the larval transition rate in the treatments was variable in different behavior at the time of testing (days 6, 12, 18, and 24). However, this value observed no statistically significant difference between experimental treatments. The results showed that the stocking density of phyllosoma larvae did not affect the rate of stage transition after 24 days of the experiment.

According to the study of Sachlikidis (2010), when rearing cotton lobster larvae (*Panulirus ornatus*) with different stocking densities (5, 10, 20, and 40 shrimp/L) [22], there was no significant difference noted in larval development at any of the tested

densities. Similar results were also observed in the study of Smith and Ritar (2006) when rearing phyllosoma larvae of *Jasus edwardsii* showed no effect of stocking density on the

growth rate of phyllosoma larvae [14]. These results are also consistent with the results recorded from our study on phyllosoma larvae of the *Thenus orientalis* species.

Table 2. Effect of density on stage transition rate during phyllosoma larvae rearing

| Treatment | Ratio of stage transition (%) | | | | | | | | | |
|-----------|-------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--------------------------|--------------------------|-------------------------|--------------------------|--------------------------|
| | Day 6 th | | Day 12 th | | | Day 18 th | | | Day 24 th | |
| | Phyl I | Phyl II | Phyl I | Phyl II | Phyl III | Phyl II | Phyl III | Phyl IV | Phyl III | Phyl IV |
| MD 1 | 85.3 ± 3.7 ^a | 14.7 ± 3.7 ^a | 49.5 ± 1.9 ^a | 41.7 ± 2.4 ^a | 8.8 ± 2.0 ^a | 42.5 ± 12.5 ^a | 44.0 ± 8.8 ^a | 13.6 ± 4.3 ^a | 70.4 ± 8.3 ^a | 29.6 ± 8.3 ^a |
| MD 2 | 82.2 ± 2.3 ^a | 17.8 ± 2.3 ^a | 47.6 ± 3.6 ^a | 42.9 ± 4.4 ^a | 9.5 ± 1.4 ^a | 43.4 ± 13.1 ^a | 42.4 ± 9.2 ^a | 14.1 ± 5.0 ^a | 72.7 ± 9.4 ^a | 27.3 ± 9.4 ^a |
| MD 3 | 86.2 ± 2.2 ^a | 13.8 ± 2.2 ^a | 41.4 ± 4.8 ^a | 48.2 ± 6.3 ^a | 10.4 ± 2.9 ^a | 35.9 ± 18.4 ^a | 48.7 ± 16.8 ^a | 15.4 ± 4.9 ^a | 68.4 ± 10.8 ^a | 31.6 ± 10.8 ^a |

Note: Different letters in the same column represent different values of statistical significance ($p < 0.05$). The data in the table are the mean ± standard deviation ($n = 5$). Density (MD) 1: 15 heads/liter; Density 2: 20 heads/liter; Density 3: 25 heads/liter. Phyl I: phyllosoma larval stage I, same as of Phyl II, III, and IV.

In contrast, some other research results showed that too high larval rearing density affects the length of time between molting, metamorphosis time, and the reduction of metamorphosis rate of shrimp species *Lysmata seticaudata* (Risso, 1816) [23] and cap shrimp [24]. This result may be because the densities selected for early larval rearing trials were significantly higher than the appropriate stocking densities of these species.

Effect of density on the survival rate of phyllosoma larvae

The effect of feed on the survival rate of phyllosoma larvae after 24 days of rearing is presented in Table 3. In general, the survival rate of shrimp larvae in treatment 1 (15 fish/L) was the highest compared with that of shrimp larvae in treatment 1 (15 fish/L) with treatments TA 2 (20 animals/L) and TA 3 (25 animals/L) in this experiment. The difference in survival values was recorded in the density experiment starting from day 12; the survival rate of larvae was higher in treatment one compared to TA 3, which was statistically significant. ($p < 0.05$). On the 18th and 24th days, the survival rates between treatments were statistically different, with the survival values from high to low in the order of treatment TA 1, TA 2, and TA 3.

The study on the rearing density of lobster larvae of *Panulirus japonicus* species showed that the survival rate decreased as the stocking density increased. At the stocking density of 40 shrimp/L, the larvae survival rate of the experiment was 54%, the highest in the treatments of Matsuda and Takenouchi (2005) [25]. According to Sachlikidis (2010), when rearing larvae of cotton lobster (*Panulirus ornatus*) with different stocking densities (5, 10, 20, and 40 shrimp/L) [22]. The study showed that the survival rate of larvae was significantly lower when comparing the stocking density of 20 and 40 fish/L compared with the stocking density of 5 and 10 fish/L. Similar results were also observed in Smith and Ritar’s study, where high stocking density affects survival when rearing phyllosoma larvae of *Jasus edwardsii* species [14]. Matsuda and Takenouchi (2005) concluded that the stocking density of early *Panulirus japonicus* larvae should not exceed 40 larvae/L [25]. Meanwhile, according to Sachlikidis (2010), to maximize the survival of the *P. ornatus* lobster larvae during rearing, the appropriate stocking density in the early stage is less than 20 shrimp/L [22]. The suitable density for rearing slipper lobster (*Thenus orientalis*) larvae in their experiment was 1/2 lower than *Panulirus japonicus* in the above study; this may be due

to the larger size of the phyllosoma stage I larvae of *Thenus orientalis* compared to *Panulirus japonicus*. Most research results suggest that larval rearing density negatively correlates with survival and growth rates, such as shrimps [24] and sea crabs for crustaceans

with mutual feeding properties. [26]. Thus, the appropriate density for rearing phyllosoma larvae of capuchin shrimp and lobster species will depend on the size of phyllosoma larvae, catching habits, time of transition, metamorphosis, etc.

Table 3. Effect of density on the survival rate of phyllosoma larvae

| Treatment | Survival rate (%) | | | |
|-----------|-------------------------|--------------------------|-------------------------|-------------------------|
| | Day 6 th | Day 12 th | Day 18 th | Day 24 th |
| MD 1 | 91.2 ± 4.7 ^a | 51.5 ± 3.2 ^a | 34.5 ± 3.0 ^a | 27.3 ± 2.4 ^a |
| MD 2 | 83.0 ± 6.7 ^a | 49.4 ± 2.8 ^{ab} | 26.5 ± 3.1 ^b | 19.5 ± 1.5 ^b |
| MD 3 | 85.2 ± 6.2 ^a | 44.1 ± 2.1 ^b | 19.2 ± 2.4 ^c | 12.1 ± 1.6 ^c |

Note: Different letters in the same column represent different values of statistical significance ($p < 0.05$). The data in the table are the mean ± standard deviation ($n = 3$). Density (MD) 1: 15 heads/L; Density 2: 20 heads/L; Density 3: 25 heads/L.

Effect of feed on growth and survival rate when rearing slipper lobster larvae

Water environment factors monitored during feed experiment

The fluctuations of water environmental factors in the treatments during the experiment are presented in Table 4. The waters environmental factors monitored in this

experiment were within the appropriate range for experimental slipper lobster larvae growth and development. The values of environmental factors in the culture water recorded in this experiment did not have significant differences between treatments and were similar to the results of monitoring ecological factors in the culture water in the density experiment. The discussion of these environmental factors was done in experiment 1.

Table 4. Water environment factors monitor during the feed experiment

| Treatment | Salinity (ppt) | Temp. (°C) | pH | DO (mg/L) | NO ₂ ⁻ (mg/L) | NH ₃ (mg/L) |
|-----------|-------------------------|-------------------------|------------------------|------------------------|-------------------------------------|------------------------|
| TA 1 | 33.0 ± 0.3 ^a | 28.5 ± 0.3 ^a | 8.1 ± 0.3 ^a | 5.3 ± 0.3 ^a | < 0.2 | < 0.01 |
| TA 2 | 33.0 ± 0.3 ^a | 28.5 ± 0.2 ^a | 8.0 ± 0.4 ^a | 5.0 ± 0.1 ^a | < 0.2 | < 0.01 |
| TA 3 | 33.0 ± 0.3 ^a | 28.5 ± 0.2 ^a | 7.9 ± 0.3 ^a | 5.1 ± 0.2 ^a | < 0.2 | < 0.01 |
| TA 4 | 33.0 ± 0.3 ^a | 28.5 ± 0.1 ^a | 7.8 ± 0.2 ^a | 5.1 ± 0.3 ^a | < 0.2 | < 0.01 |

Note: Different letters in the same column represent different values of statistical significance ($p < 0.05$). The data in the table are the mean ± standard deviation ($n = 3$). TA 1: umbrella *Artemia* + blood cockle meat (first seven days)/newly hatched *Artemia* + blood cockle meat (day 8th to 24th); TA 2: umbrella *Artemia* + green mussel meat (first seven days)/newly hatched *Artemia* + green mussel meat (day 8th to day 24th); TA 3: umbrella *Artemia* + white clam meat (first seven days)/newly hatched *Artemia* + white clam meat (day 8th to day 24th); TA 4: umbrella *Artemia* + mixed mollusk meat (first seven days)/newly hatched *Artemia* + mixed mollusk meat (day 8th to day 24th).

The environmental factors recorded in the experiment of rearing the larvae of phyllosoma shrimp *Thenus Orientalis* were not different from the density experiment due to the same experimental culture system. The discussion is similar to the density experiment.

Effect of diet on larval growth

Ratio of larval stage transition:

The effect of feed on the transition rate of shrimp larvae stage is presented in Table 5. On the sixth day of nursing, the larvae showed

phyllosoma stage II in all experimental treatments, with rates ranging from 5.8% to 7.6%; however, no difference was observed in the rate of larvae transitioning to stage II between treatments when compared with each other. Larvae transition to the phyllosoma III stage with a rate ranging from 4.5 to 6.5% on day 12, and the value of this transition rate was not significantly different between treatments in the experimental stages of phyllosoma II and III. On the 18th day of nursing, larvae in all treatments were recorded in all three phyllosoma stages II, III, and IV, with the highest rate being phyllosoma II (70.6–75.9%).

Meanwhile, the percentage of larvae transitioning to phyllosoma stage IV was the lowest (3.8–7.0%), and this rate did not show a significant difference when comparing the treatments. The statistically significant difference ($p < 0.05$) in the rate of phyllosoma III and IV larval transition occurred on the 24th day of rearing, treatment TA 3 (*Artemia* poplar + white clam meat) first seven days/newly hatched *Artemia* + white clam meat (day 8 to 24)) compared with the rest of the treatments. Treatment TA 3 had a higher larval transition to phyllosoma IV (38.3%) than the other treatments.

Table 5. Effect of feed on transition stage during rearing the phyllosoma larvae

| Treatment | Rate of stage transition (%) | | | | | | | | | |
|-----------|------------------------------|------------------------|-------------------------|-------------------------|------------------------|-------------------------|-------------------------|------------------------|-------------------------|-------------------------|
| | Day 6 th | | Day 12 th | | | day 18 th | | | Day 24 th | |
| | Phyl I | Phyl II | Phyl I | Phyl II | Phyl III | Phyl II | Phyl III | Phyl IV | Phyl III | Phyl IV |
| TA 1 | 92.4 ± 2.7 ^a | 7.6 ± 2.7 ^a | 17.6 ± 3.8 ^a | 77.9 ± 5.4 ^a | 6.5 ± 3.7 ^a | 20.4 ± 4.5 ^a | 75.9 ± 5.0 ^a | 3.8 ± 2.0 ^a | 69.0 ± 2.3 ^a | 31.0 ± 2.3 ^a |
| TA 2 | 94.2 ± 1.2 ^a | 5.8 ± 1.2 ^a | 22.2 ± 7.0 ^a | 73.3 ± 8.0 ^a | 4.5 ± 1.2 ^a | 23.2 ± 4.9 ^a | 70.6 ± 6.3 ^a | 6.2 ± 2.1 ^a | 68.3 ± 3.5 ^a | 31.5 ± 1.9 ^a |
| TA 3 | 93.2 ± 0.6 ^a | 6.8 ± 0.6 ^a | 18.6 ± 8.6 ^a | 75.0 ± 7.0 ^a | 6.4 ± 2.2 ^a | 21.3 ± 7.4 ^a | 71.8 ± 9.4 ^a | 7.0 ± 2.2 ^a | 61.7 ± 2.3 ^b | 38.3 ± 2.3 ^b |
| TA 4 | 92.9 ± 2.1 ^a | 7.1 ± 2.1 ^a | 21.1 ± 3.5 ^a | 72.8 ± 5.0 ^a | 6.1 ± 1.9 ^a | 22.5 ± 7.6 ^a | 70.8 ± 7.0 ^a | 6.8 ± 1.2 ^a | 67.0 ± 2.8 ^a | 33.0 ± 2.8 ^a |

Note: Different letters in the same column represent different values of statistical significance ($p < 0.05$). The data in the table are the mean ± standard deviation ($n = 3$). TA 1: umbrella *Artemia* + blood cockle meat (first seven days)/newly hatched *Artemia* + blood cockle meat (day 8th to 24th); TA 2: umbrella *Artemia* + green mussel meat (first seven days)/newly hatched *Artemia* + green mussel meat (day 8th to day 24th); TA 3: umbrella *Artemia* + white clam meat (first seven days)/newly hatched *Artemia* + white clam meat (day 8th to day 24th); TA 4: umbrella *Artemia* + mixed mollusk meat (first seven days)/newly hatched *Artemia* + mixed mollusk meat (day 8th to day 24th). Phyl I: phyllosoma larvae stage I, same as Phyl II, III, and IV.

During development, phyllosoma larvae will accumulate energy from external food sources in the form of lipids in the body, and this energy will be used in metamorphosis and larval stage transition [28–31]. In this study, treatment TA 3 (feeding with *Artemia* in combination with white clam meat) showed that larvae developed and transitioned faster than the other treatments, indicating the possibility that white clam meat is more suitable for feeding phyllosoma *Thenus Orientalis* larvae than other mollusks.

As reported by Oksana et al., the nutritional composition of bivalve mollusks showed that the mollusk meat has a high

protein content. It also has a relatively high concentration of amino acids such as glycine, glutamate, aspartic acid, alanine, leucine, lysine, arginine, and many inorganic elements such as sodium, potassium, magnesium, calcium, iron, zinc, nickel, and other, present in most parts of the mollusk [27]. With a relatively high and diverse amino acid composition in mollusk meat, it will be an essential source of nutrients for the growth and development of slipper lobster (*Thenus orientalis*) larvae when rearing.

Size of larvae:

The effects of feed on phyllosoma III and IV larval size after 24 days of rearing are

presented in Table 6. Overall, phyllosoma III and IV larval sizes (CL and CW) in the two treatments fed umbrella *Artemia* + green mussel meat (first seven days)/newly hatched *Artemia* + green mussel meat (8th to 24th day) – TA 2 and newly hatched *Artemia* + white clam meat (first seven days)/newly hatched *Artemia* + white clam meat (day 8 to day 24) – TA 3 was more significant than the other two

treatments. However, there was no statistically significant difference ($p > 0.05$) in size (CL and CW) of phyllosoma III larvae noted between the different diets. Statistically significant differences in size (CL and CW) were only observed at the phyllosoma IV stages between TA 3 and TA 1; there was no significant difference when comparing values between 3 treatments TA 2, TA 3, and TA 4 together.

Table 6. Effect of food on the size of phyllosoma larvae

| Treatment | Larvae sizes | | | |
|-----------|------------------------|------------------------|-------------------------|--------------------------|
| | Phyl III | | Phyl IV | |
| | CL (mm) | CW (mm) | CL (mm) | CW (mm) |
| TA 1 | 5.8 ± 0.3 ^a | 6.5 ± 0.3 ^a | 8.6 ± 0.4 ^a | 10.4 ± 0.6 ^a |
| TA 2 | 6.0 ± 0.4 ^a | 6.8 ± 0.5 ^a | 9.3 ± 0.5 ^{ab} | 10.9 ± 0.6 ^{ab} |
| TA 3 | 6.0 ± 0.4 ^a | 6.8 ± 0.4 ^a | 9.5 ± 0.3 ^b | 11.5 ± 0.3 ^b |
| TA 4 | 5.8 ± 0.3 ^a | 6.6 ± 0.4 ^a | 9.0 ± 0.5 ^{ab} | 10.5 ± 0.6 ^{ab} |

Note: Different letters in the same column represent different values of statistical significance ($p < 0.05$). The data in the table are the mean ± standard deviation ($n = 5$). TA 1: umbrella *Artemia* + blood cockle meat (first seven days)/newly hatched *Artemia* + blood cockle meat (day 8th to 24th); TA 2: umbrella *Artemia* + green mussel meat (first seven days)/newly hatched *Artemia* + green mussel meat (day 8th to day 24th); TA 3: umbrella *Artemia* + white clam meat (first seven days)/newly hatched *Artemia* + white clam meat (day 8th to day 24th); TA 4: umbrella *Artemia* + mixed mollusk meat (first seven days)/ newly hatched *Artemia* + mixed mollusk meat (day 8th to day 24th). Phyl III: phyllosoma larvae stage III; Phyl IV: phyllosoma stage IV.

Monitoring the change of size of phyllosoma larvae of slipper lobster through each developmental stage, Mikami & Greenwood (1997) recorded that at phyllosoma stage IV *Thenus orientalis* had a cephalothorax length (mean CL) is 11 mm, and the mean cephalothorax width (CW) is 13.16 mm [15]. This larval size is larger than our results, 9.5 mm (CL) and 11.5 mm (CW). Meanwhile, phyllosoma III larval size in our experiment and Mikami & Greenwood (1997) did not have a big difference, corresponding to CL: 6.0 mm and CW: 6.8 mm compared with CL: 5.20 mm and CW: 7.46 mm [15]. In this study, the difference in larval size between treatments suggested that nutrient content from different food sources might have influenced the size of phyllosoma larvae. So far, information on the larval size of *Thenus orientalis* shrimp, including total body length (TL), cephalothorax length (CL), cephalothorax width (CW), and the developmental stages of larvae of slipper lobster (*Thenus orientalis*), is still limited. Therefore, providing more research data on the

size of slipper lobster larvae is vital for assessing the quantity and quality of shrimp larvae feed used when rearing phyllosoma larvae.

Effect of food on the survival rate of phyllosoma larvae

The effect of diet on phyllosoma larvae survival after 24 days of rearing is presented in Table 7. The survival value of lobster larvae decreased by about 50% after 12 days of rearing in all treatments. By the 24th day of nursing, this value is in the range of 16.3–26.5%. On the 24th day of culture, the highest survival rate was recorded in the treatment that fed the larvae *Artemia* bung parachute + white clam meat (first seven days)/newly hatched *Artemia* + white clam meat (8th day to 24th day). (TA 3; 26.5 ± 2.9%), this survival value was statistically significant compared with the rest of the diets at the same testing time ($p < 0.05$). In contrast, the survival rates from the feed treatments were not

significantly different ($p > 0.05$) at all three test times, days 6, 12, and 18.

Moreover, combining foods is also one of the solutions to meet the nutritional needs and provide food with the right size for the larvae of interest to researchers. In the research and trial production of slipper lobster, many diets were used as feed for larvae, such as rotifers, *Artemia* larvae, fish larvae, mollusks, and jellyfish. Mikami and Greenwood (1997) used a combination of mollusk feed (fresh and frozen) combined with *Artemia* larvae as feed for rearing larvae of *Thenus orientalis* and *Thenus* sp.; the results showed the phyllosoma larvae of the two lobster species above developed best in the combination of fresh clam meat (*Donax brazieri*) and *Artemia* larvae [15]. The variety of feeding umbrella *Artemia* and blood cockle meat in the first two days and from the third day onward, newly hatched *Artemia* and blood cockle meat when rearing

white cap shrimp (*Thenus orientalis*) larvae showed a higher survival rate and larval growth was best after 26 days of culture [10]. In our study, the survival rate of larvae in treatment TA 3 (feeding the larvae with umbrella *Artemia* + white clam meat in the first seven days and newly hatched *Artemia* + white clam meat from day 8 to 24) was the highest recorded after 25 days of culture.

During the experiment, the nutritional composition of the mollusks used as larval food and the nutritional requirements of the phyllosoma larvae at each stage was not determined. However, initial experimental results showed differences in the survival and growth of larvae when fed different mollusk species. The difference may be due to mollusks' nutritional composition, larvae' ability to absorb and digest these nutrients, or physical, structural differences in the mollusk meat.

Table 7. Effect of diet on the survival rate of phyllosoma larvae

| Treatment | Survival rate (%) | | | |
|-----------|-------------------------|-------------------------|-------------------------|-------------------------|
| | Day 6 th | Day 12 th | Day 18 th | Day 24 th |
| TA 1 | 86.2 ± 5.7 ^a | 48.6 ± 4.8 ^a | 34.2 ± 2.7 ^a | 18.5 ± 4.3 ^a |
| TA 2 | 87.6 ± 7.2 ^a | 49.3 ± 4.6 ^a | 34.7 ± 4.4 ^a | 16.3 ± 3.9 ^a |
| TA 3 | 85.4 ± 6.1 ^a | 52.4 ± 5.4 ^a | 39.3 ± 5.4 ^a | 26.5 ± 2.9 ^b |
| TA 4 | 84.1 ± 7.9 ^a | 51.7 ± 3.8 ^a | 36.8 ± 5.8 ^a | 18.2 ± 3.0 ^a |

Note: Different characters in the same row represent a difference of statistical significance ($p < 0.05$). The data in the table are the mean ± standard deviation ($n = 5$). TA 1: umbrella *Artemia* + blood cockle meat (first seven days)/newly hatched *Artemia* + blood cockle meat (day 8 to 24); TA 2: umbrella *Artemia* + green mussel meat (first seven days)/newly hatched *Artemia* + green mussel meat (day 8 to 24); TA 3: umbrella *Artemia* + white clam meat (first seven days) / newly hatched *Artemia* + white clam meat (8th day to 24th day); TA 4: umbrella *Artemia* + mixed mollusk meat (first seven days)/newly hatched *Artemia* + mixed mollusk meat (day 8 to 24).

CONCLUSIONS AND SUGGESTIONS

Conclusions

The density of rearing phyllosoma larvae of slipper lobster (*Thenus orientalis*) affected their survival rate after 25 days of rearing but did not affect the development of larvae. The appropriate density for the slipper lobster larval stage is less than 15 heads/L.

Different feed formulations in this experiment affected the survival and growth of white cap shrimp larvae, especially phyllosoma stage IV. The suitable feed formula for white

cap shrimp larvae in this study was umbrella *Artemia* + white clam meat fed to the larvae for the first seven days, and newly hatched *Artemia* + white clam meat continued to be provided from day 8th to 24th.

Suggestions

It is necessary to continue to study the nutritional composition of mollusk feeds and to determine the appropriate amount and size of feed for each stage of larval development and the frequency of feeding during rearing slipper lobster larvae.

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