Original Research Articles

Effects of different feeds and stocking densities on growth and survival rates of mud crab (*Scylla paramamosain*) at the stage from megalopa to crablet-1

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Mud crabs (*Scylla* genus) are luxury foods in high demand internationally. The efficient techniques for mud crab hatcheries are vital for providing breeds for their aquaculture, which is rapidly growing in many countries. This study aims to investigate the effects of different feeds and stocking densities on mud crabs' growth and survival rates (*Scylla paramamosain*) in the stage from megalopa to crablet-1 stage. Two separate experiments were conducted indoors in the 60-liter round plastic tanks (containing 50 liters of water at a 28‰ salinity). Experiment 1 investigated four feeds: frozen Artemia biomass, pureed shrimp meat, Lansy pellet feed (48% protein), and NRD pellet feed (55% protein). Megalopae (mean weight of 5.8 mg) were stocked at a density of 10/L. In experiment 2, the megalopae (mean weight of 5.4 mg) were stocked at densities of 20, 30, and 40/L and were fed the Lansy pellet feed, which was the best one selected from experiment 1. High survival rates were obtained at all four feeds (82.2–87.5%) and three stocking densities (88.4–90.1%). The growth performances in Lansy feed and frozen Artemia biomass were better than those in pureed shrimp meat and NRD pellet feed, which was seen through higher indicators of daily weight gain (DWG) and specific growth rate in weight (SGRw) ($p < 0.05$). Despite the survival rate showing no significant difference among the feeds, their highest value (87.5%) was observed in the Lansy pellet feed. On the other hand, the growth performances showed a significant decrease at stocking densities ⩾ 30/L, as complemented by a significant decrease in DWG and SGRw at these stocking densities ($p < 0.05$). Both survival rate and metamorphosis durations did not show significant differences among the feeds or stocking densities ($p > 0.05$). The investigated feeds and stocking densities suit the nursing mud crab (*S. paramamosain*) megalopa. In contrast, the Lansy pellet feeds had a stocking density of 20/L, resulting in the highest nursing efficiency.

INTRODUCTION

Mud crab is the common name of the crab species of the Scylla genus. Crabs are among the seafood items that are globally distributed, with a consistently growing demand.[1, 2] It has been determined that four species are under the genus Scylla, including *Scylla olivacea*, *S. paramamosain*, *S. serrata*, and *S. tranquebarica*. All these species are known to have great potential for commercial aquaculture because they tolerate environments with poor water quality and are highly resistant to pathogens. Mud crabs are currently cultured extensively in many countries, especially Southeast Asian countries.[1-4] Among them, two species, *S. paramamosain* (locally called Lotus crab) and *S. olivacea* (called Fire crab), are distributed naturally in Vietnam, with over 95% of *S. paramamosain* and only about 5% of *S. olivacea* in their population.[5,6]

S. paramamosain is recently considered one of the most important species for aquaculture in coastal areas of the Mekong Delta region of Vietnam, where the impacts of climate change and saltwater intrusion are more and more evident because it has good adaptability to environmental

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conditions in these areas and has high prices in both domestic and foreign markets.[4] The Mekong Delta region has over 350,000 ha of culturing area for mud crabs, with a total production of about 38,000 tons[4] out of around 53,000 tons produced in the country.[7] Along with the development of commercial crab farming, the breed production industry of mud crabs has achieved snappy progress in the Mekong Delta, with over 600 hatcheries and 1.5 billion crablets produced annually in the region,[8] providing almost all the breed for the farming. However, hatchery activities of mud crab still have limitations, even in Vietnam as well as many other nations, due to low survival rates, hence the lack of crablets for aquaculture on large scales.[2,8,9]

The larval mud crabs often maintained in the hatcheries include three stages: zoea-1 to zoea-5 larvae, zoea-5 to megalopa, and megalopa to the crablet phase.[10] The critical metamorphosis stage from megalopa to crablet-1 in mud crabs has often indicated inconsistent and unreliable results, with low survival rates.[3,11] The mean survival rates of nursing mud crab megalopae often fluctuate from 5.0 to 58.0%.[3] High mortality levels in this stage may be caused by: (i) nutrient factors (both quantity and quality) that result in the occurrence of Molting Death Syndrome[12-14]; (ii) cannibalism at high levels, as crabs in the megalopa stage possess pincers[15-18]; (iii) poor water quality[19-21]; and (iv) bacterial diseases, parasites, and fungi,[22,23] among others. Therefore, the practice of directly stocking megalopa in ponds is not recommended. Instead, the nursery is needed to grow mud crabs of a larger size before pond stocking, and practical nursery techniques still need further development.[24]

In the practice of nursing crustaceans, different feeds, like natural feeds, live feeds, and formulated feeds, have often been used in the stage of megalopa metamorphosis to the crablet stage. For the natural and live feeds, Artemia with live or frozen forms has often been practiced with the addition of prawn or shrimp meat, fish meat, and dried shrimp. Besides, Acetes shrimp,[25] dried mud worms, or artificial feed (larva-shrimp feed),[3,9,10,26] minced trash fish, green mussels,[15] brown mussels,[1] or artificial feed[10,27-29] have also been used. Some previous studies have shown that the role of natural feed in the hatchery in the larval stages of mud crabs cannot be completely substituted for artificial feed, but the combination of them is still recommended.[30,31] Still, in the megalopa stage, formulated particle feed could completely replace live prey.[29,32] The development of pellet diets or aquafeed for aquaculture species has gained much interest nowadays, as pellets offer many advantages compared to natural feeds. In terms of nutrient content, artificial feed offers a nutritionally balanced diet with known nutrient content, such as total lipids and protein, that will promote growth and reproduction in the crustacean.[33] In the communal rearing technique, cannibalism is a major problem.[24] Manipulating the levels of protein and lipids is important for growth and reproduction in the crustacean group. The formulated feed could provide sufficient nutrition for them.[34] Our investigation aimed to compare mud crab's growth performance and survival rate (*S. paramamosain*) at the megalopa-to-crablet-1 stage nursed using different feeds (including pellets containing different protein contents, natural and live feeds) and different stocking densities. This is an important link for optimizing nursing techniques in the early stages of the crabs to develop commercial aquaculture of this crab species.

MATERIALS AND METHODS

EXPERIMENTAL MATERIALS

One-day-old megalopa of mud crabs (*S. paramamosain*), with a mean initial body weight of 5.40–5.80 mg and a mean initial carapace width of 0.65 mm, were used in the two experiments that were purchased at a hatchery in Bac Lieu City, Bac Lieu Province, Vietnam, and were transported in plastic bags at a density of 200–300/L.[35] After transport, megalopae were gradually acclimated for one week to the water salinity of 28‰ and temperature conditions of the experiments before they were stocked in experimental tanks.

Four feeds used in experiment 1 were included: (1) Frozen Artemia biomass (containing 54.5% protein, 12.9% total fat, and 11.2% moisture in dried biomass) was transferred from the farming area in Vinh Chau district, Soc Trang province, Vietnam, and stored cold (-21°C) for use throughout the experiment. Before feeding, Artemia was completely thawed and washed with fresh water to remove dirt in Artemia biomass; (2) pureed meat of Stork shrimp (*Metapenaeus tenuipes* Kubo, 1949), a locally common shrimp species that is often used as live feed in aquaculture. The average analyzed nutrient profile of shrimp meat includes protein 19.4±0.56%, lipid 1.15±0.19%, and water 76.3±0.57%[36]; (3) Lansy pellet feed, a feed for shrimp post-larvae (containing contents of 48% protein, 9% fat, and 9% moisture); and (4) NRD pellet feed, a feed for ornamental fish (containing contents of protein: 55%, fat: 9%, and moisture: ⩽ 8%). Both pellet feeds have a particle size of 500 μm and were made by INVE Company in Thailand. In experiment 2, Lansy pellet feed, which observed the highest crab performance in experiment 1, was used.

The water used for the two experiments was sediment-removed saline at 28‰ salinity. The water was treated with 30 mg/L chlorine and continuously strongly aerated for 3 to 4 days, then pumped into the experimental tanks through a filter bag with a mesh size of 5 μm.

The 60-liter round plastic tanks were used for the experiments.

The US National Research Council's guide for the Care and Use of Laboratory Animals was followed.

EXPERIMENTAL DESIGN

This study aimed to compare the effects of four feeds and three stocking densities on the growth and survival rates of mud crabs (*S. paramamosain*) at the stage from megalopa to crablet-1. The study included two separate experiments with a completely randomized design in three replications for each placed indoors. The experiments were carried out at the Aquaculture Experimental Hatchery of Bac Lieu Uni-

Figure 1. The nursery tanks were arranged with shelters

versity in South Vietnam from April to May 2020 and are described as follows:

EXPERIMENT 1: COMPARING THE GROWTH AND SURVIVAL RATES OF MUD CRABS (S. PARAMAMOSAIN) AT THE STAGE FROM MEGALOPA TO CRABLET-1 NURSED USING DIFFERENT FEEDS

This experiment aimed to assess the impact of four distinct feeds on mud crabs' growth and survival rates (*S. paramamosain*) during the transition from megalopa to crablet-1 stage. The experiment was conducted in twelve 60-liter round plastic tanks (containing 50 liters of water at a salinity of 28‰). The megalopae, with a mean weight of 5.80 mg and a mean carapace width of 0.65 mm, were stocked into the tanks at a density of 10/L. The crabs were fed four experimental feeds (frozen Artemia biomass, pureed shrimp meat, Lansy pellet feed, and NRD pellet feed) four times a day (6:00, 12:00, 18:00, and 24:00) according to a satisfying diet adjusted based on the situation of the nursery tank, fluctuating between 15-20% body weight per day. Plastic mesh panels with a mesh size of 4 mm were arranged into the bottoms of tanks as shelters for crabs, with a shelter density of 2 m^2 of plastic mesh per m^2 of tank bottom area[37] (**Figure 1**). The water in the tanks was constantly slightly aerated to ensure dissolved oxygen demand for the crabs during the nursing period. It was changed periodically every two days, changing 30% of the volume of the tanks with prepared water in the settling tanks. The fecal matter and uneaten feeds were removed daily from tanks at 21:00 by siphoning. Before siphoning, the aeration systems in the tanks were turned off to settle the sediment at the bottom of the tanks.

EXPERIMENT 2: COMPARING THE GROWTH AND SURVIVAL RATES OF MUD CRABS (S. PARAMAMOSAIN) AT THE STAGE FROM MEGALOPA TO CRABLET-1 NURSED AT DIFFERENT STOCKING DENSITIES

In this experiment, megalopae with a mean body weight of 5.4 mg and carapaces width of 0,65 mm were arranged into nine 60-litter-around plastic tanks (containing 50 liters of water at a salinity of 28‰) at stocking densities of 20, 30, and 40/L. The Lansy pellet feed, the best feed chosen from experiment 1, provided crabs with a daily diet to apparent satiation, equal to 15-20% of the biomass. The feed ratio was divided equally and given four times a day (6:00, 12:00, 18:00, and 24:00). The regimes of management and care in this experiment were done in the same manner as in experiment 1.

DATA COLLECTION

Water quality parameters in the tanks in both experiments were controlled before and during the nursing period, including temperature and pH, which were measured twice a day at 7:00 and 14:00, while TAN ($N\text{-}NH_4^+$) and nitrite ($N\text{-}NO_2^-$) were checked every three days at 14:00. All were tested using Sera GH test kits made in Germany.

Metamorphosis duration was determined from the megalopae stocked in the experiment tanks until they completely metamorphosed into crablet-1 through visual observation. The observation began after five days of stocking, with a frequency of every three hours.

Data for growth and survival rates were collected when the megalopae completely metamorphosed into crablet-1. The crablet-1 weights were weighed using an electrical analytical balance calibrated to 0.01 mg. In addition, ten s of crablet-1 from each tank were randomly collected and measured for the length and width of the carapace using biometric measurements under a microscope at a magnification of 10x with a millimeter unit. The monitored parameters of growth and survival rates from two experiments were determined using the following equations:

DWG (g day^{-1}) = (final body weight – initial body weight)/duration of the experiment;

SGRw (% day^{-1}) = 100 (ln(final weight) – ln(initial weight))/duration of experiment;

Survival rate (%) = 100(final number of crablets-1/initial number of megalopas)

DATA ANALYSIS

The variance homogeneity was assessed using Levene's test. The percentage data were transformed to arcsine before conducting statistics. A one-way analysis of variance (ANOVA) together with Duncan's post-hoc tests were used to identify significant differences between the mean values (significance level of $p < 0.05$) using SPSS 20.0.

RESULTS

PARAMETERS OF NURSING PERFORMANCE USING DIFFERENT FEEDS

WATER QUALITY PARAMETERS

The water quality parameters monitored during the nursing period are presented in **Table 1**. The fluctuating ranges included temperature from 29.08 to 30.11 °C, pH from 7.8 to 8.00, TAN at 0.01 mg/L, and nitrite at 0.001 mg/L. Overall, they remained stable throughout the experiment.

GROWTH PERFORMANCE

Growth performance was determined based on parameters such as the mean weight, DWG, and SGRw, and the mean width and length of carapaces. Data in **Table 2** shows that

Table 1. Water quality parameters during nursing mud crab (*S. paramamosai*n) from megalopa to crablet-1 using different feeds

Feeds	Temperature (°C)		pH		TAN (mg/L)	NO_2^- (mg/L)
	7:00	14:00	7:00	14:00		
Frozen Artemia biomass	29.1±0.2	30.1±0.2	7.8±0.1	7.8±0.1	0.01±0.00	0.001±0.000
Pureed shrimp meat	29.1±0.2	30.1±0.2	8.0±0.0	8.0±0.1	0.01±0.00	0.001±0.000
Lansy pellet feed	29.1±0.2	30.1±0.2	8.0±0.0	8.0±0.0	0.01±0.00	0.00±0.0000
NRD pellet feed	29.1±0.2	30.1±0.2	8.0±0.0	8.0±0.0	0.01±0.00	0.001±0.000

Table 2. Mean weight, width and length of carapace, DWG, and SGRw of mud crab (*S. paramamosain*) from megalopa to crablet-1 nursed using different feeds

Feeds	Frozen Artemia biomass	Pureed shrimp meat	Lansy pellet feed	NRD pellet feed
Initial weight (mg)	5.8 ±0.1	5.8 ±0.1	5.8 ±0.1	5.8 ±0.1
Final weight (mg)	9.3±0.2^{a}	8.8±0.1^{b}	9.4±0.1^{a}	9.0±0.2^{ab}
Initial carapace widtd (mm)	0.65±0.02	0.65±0.02	0.65±0.02	0.65±0.02
Final carapace widtd (mm)	2.96±0.16^{a}	2.94±0.17^{a}	2.97±0.10^{a}	2.94±0.10^{a}
Initial carapace lengtd (mm)	3.80±0.22	3.80±0.22	3.80±0.22	3.80±0.22
Final carapace lengtd (mm)	2.84±0.18^{a}	2.89±0.18^{a}	2.89±0.15^{a}	2.88±0.15^{a}
DWG (mg day^{-1})	0.61±0.02^{a}	0.52±0.01^{b}	0.62±0.01^{a}	0.53±0.01^{b}
SGRw(% day^{-1})	7.8±0.2^{a}	7.0±0.1^{b}	8.0±0.1^{a}	7.2±0.10^{b}

Values are presented as mean±SD. Values with different superscript letters (a, b) in the same rows show a significant difference (p < 0.05).

the final carapace width ranged from 2.94 to 2.97 mm and the final carapace length ranged from 2.84 to 2.89 mm; both showed no significant differences among the feeds used (p > 0.05). Meanwhile, the highest final weight was observed in Lansy shrimp pellet feed (9.4 mg), which did not show a significant difference from that in frozen Artemia biomass and NRD pellet feed (9.3 and 9.0 mg, respectively) (p > 0.05). The lowest final mean body weight was found in pureed shrimp meat (8.8 mg) and showed a significant difference from that in both Lansy pellet feed and frozen Artemia biomass (p < 0.05) but did not significantly differ from that in NRD pellet feed.

DWG and SGRw in Lansy feed and frozen Artemia biomass were significantly higher than those in pureed shrimp meat and NRD pellet feed (p < 0.05). Both did not show significant differences between frozen Artemia biomass (0.61 g day^{-1} and 7.8% day^{-1}) and Lansy pellet feed (0.62 g day^{-1} and 8.0% day$^{-1)}$ or between pureed shrimp meat (0.52 g day^{-1} and 7.0% day^{-1}) and NRD pellet feed (0.53 g day^{-1} and 7.2% day^{-1}) (**Table 2**).

SURVIVAL RATE

The survival rates were observed to be rather high (82.2–87.5%) and did not show significant differences among the investigated feeds (p > 0.05). The highest survival rate was at Lansy pellet feed (87.5%), followed by frozen Artemia biomass (85.3%), NDR pellet feed (84.9%), and pureed shrimp meat (82.2%) (**Figure 2**).

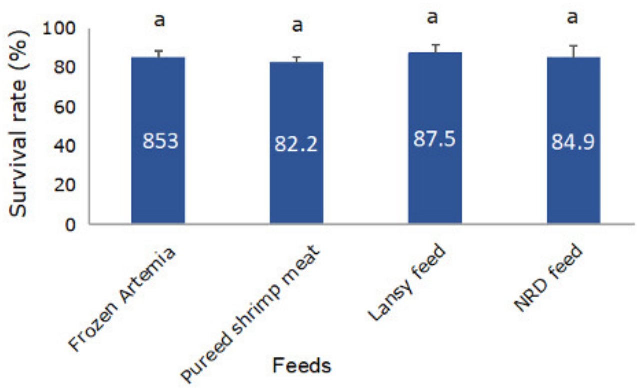

Figure 2. Survival rate of mud crab (*S. paramamosain*) from megalopa to crablet-1 nursed using different feeds

[Values are presented as mean±SD. The bars with different letters (a, b) show a significant difference (p < 0.05)]

METAMORPHOSIS DURATION

Metamorphosis duration was shown to be relatively equivalent at feeds of frozen Artemia biomass, Lansy pellet feed, and NRD pellet feed (10.03 days). Meanwhile, a longer metamorphosis duration was observed in pureed shrimp meat (11.67 days). Here, there were no significant differences in metamorphosis durations among feeds used (p > 0.05) (**Figure 3**).

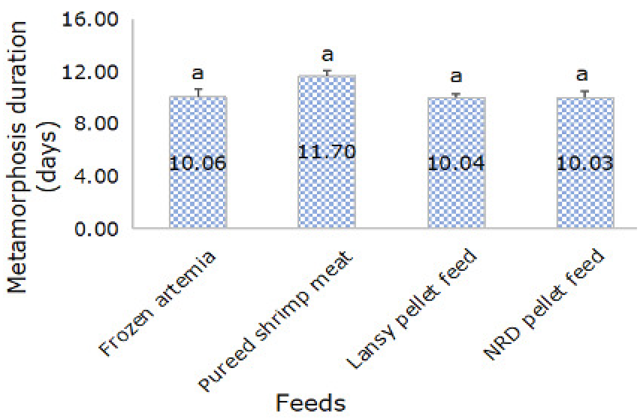

Figure 3. Metamorphosis duration of mud crab (*S. paramamosain*) from megalopa to crablet-1 nursed using different feeds

[Values are presented as mean±SD. The bars with different letters (a, b) show a significant difference
($p < 0.05$)]

PARAMETERS OF NURSING PERFORMANCE AT DIFFERENT STOCKING DENSITIES

WATER QUALITY PARAMETERS

In this experiment, water quality parameters were observed: temperature fluctuated between 27.7 and 29.6 °C; pH 7.7 and 7.9; TAN 0.26 and 0.31 mg/L; and nitrite at a concentration of 0.01 mg/L (**Table 3**).

GROWTH PERFORMANCE

Despite final carapace width (ranging from 2.99–3.33 mm) showing no difference among stocking densities, final weight (9.3–9.9 mg), the final carapace lengths (2.90–2.95 mm), DWG (0.66-0.76 mg day^{-1}), and SGRw (9.1–10.2% day^{-1}) were shown to decrease with increases in the stocking densities, and significant decreases were at a stocking density of 40/L ($p < 0.05$). For DWG and SGRw, significant reductions were shown at stocking densities ⩾ 30/L (**Table 4**).

SURVIVAL RATE

The survival rates observed were pretty high and relatively equal among stocking densities, with values of 89.7%, 90.1%, and 88.4% at stocking densities of 20, 30, and 40/L, respectively (**Figure 4**).

METAMORPHOSIS DURATION

Figure 5 shows that metamorphosis durations from megalopa to crablet-1 had little variation among stocking densities of 20–40/L. They were observed ranging from 12.01 to 12.03 days, and the difference was not found among stocking densities.

DISCUSSION

WATER QUALITY PARAMETERS

Both experiments were conducted indoors, so the water quality parameters showed little fluctuation. The temperature ranged from 29.08 to 30.11 °C, pH from 7.8 to 8.00, TAN at 0.01 mg/L, and nitrite at 0.001 mg/L in experiment 1 (**Table 1**), and they were 27.7–29.6°C, 7.7–7.9; 0.26–0.31 mg/L; and 0.001 mg/L, respectively, in experiment 2 (**Table 3**). Overall, all of them sustained the proper conditions for the life of Scylla crabs during the nursing period. These water quality parameters suggested for mud crab rearing in larval and adult stages were a temperature of 28–30 °C (Ye et al., 2011; Omn[3]), a pH of 7.5–8.5 (0.5 daily variations),[38] TAN < 3 mg/L,[39] and NO_2^- < 10 mg/L at salinity >15‰.[39] Furthermore, it was concluded that the water quality parameters for a shrimp culture can be used as a guide in mud crab farming activities.[24,40] Probably because the water in the tanks was changed periodically and the tanks were siphoned daily, the nitrite concentration was low during larval rearing in all experiment tanks.

PERFORMANCE OF THE CRABS NURSED USING DIFFERENT FEEDS

The gastrointestinal tracts and articulated appendages of larval crustaceans show remarkable morphological ranges, enabling them to capture and process different feed sources. Therefore, the diet of larval crustaceans frequently changes markedly over developmental stages, depending on their size and feeding abilities.[41] Crab larvae, as well as homarids and carideans, do not have the anterior midgut diverticula and alternative sources of digestive enzymes as those of Penaeid shrimp larvae. Therefore, the choice of appropriate feed types for the rearing of mud crab larvae, especially at the stages of zoea-5 and megalopa, is very important for the yields.[42]

Experiment 1 showed the highest growth performance with Lansy pellet feed and frozen Artemia biomass. This was evident as the highest values of final weight, DWG,

Table 3. Water quality parameters during nursing mud crab (*S. paramamosain*) from megalopa to crablet-1 at different stocking densities

Densities	Temperature (°C)		pH		TAN (mg/L)	NO_2^- (mg/L)
	7:00	14:00	7:00	14:00		
20	27.8±0.1	29.4±1.1	7.9±0.2	7.9±0.2	0.26±0.05	0.001±0.000
30	27.8±0.1	29.3±1.2	7.7±0.2	7.7±0.2	0.31±0.09	0.00±0.0000
40	27.7±0.6	29.6±1.2	7.8±0.2	7.8±0.2	0.29±0.08	0.001±0.000

Table 4. Mean body weight, width and length of carapace, DWG, and SGRw of mud crab (*S. paramamosain*) from megalopa to crablet-1 nursed at different stocking densities

Densities (/L)	20	30	40
Initial weight (mg)	5.4 ±0.7	5.4 ±0.7	5.4 ±0.7
Final weight (mg)	9.9±0.1^{a}	9.7±0.1^{ab}	9.3±0.1^{b}
Initial carapace width (mm)	0.65±0.02	0.65±0.02	0.65±0.02
Final carapace width (mm)	3.00±0.08^{a}	3.33±3.16^{a}	2.99±0.07^{a}
Initial carapace length (mm)	3.80±0.22	3.80±0.22	3.80±0.22
Final carapace length (mm)	2.95±0.11^{a}	2.94±0.11^{ab}	2.90±0.17^{b}
DWG (mg day^{-1})	0.76±0.01^{a}	0.70±0.01^{b}	0.66±0.01^{c}
SGRw (%) day^{-1})	10.2±0.1^{a}	9.6±0.2^{b}	9.1±0.6^{c}

Values are presented as mean±SD. Values with different superscript letters (a, b) in the same rows show a significant difference ($p < 0.05$).

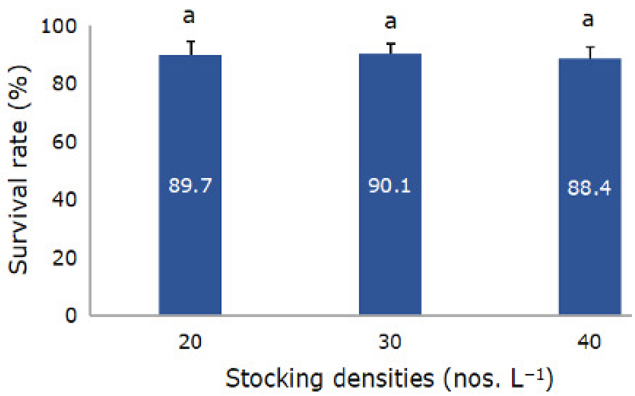

Figure 4. Survival rate of mud crab (*S. paramamosain*) from megalopa to crablet-1 nursed at different stocking densities

[Values are presented as mean±SD. The bars with different letters (a, b) show a significant difference
($p < 0.05$)]

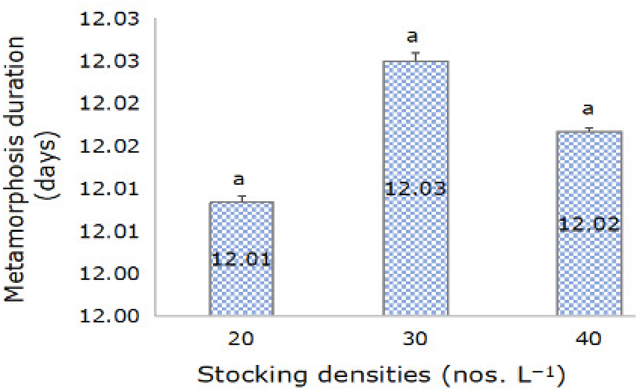

Figure 5. Metamorphosis duration of mud crab (*S. paramamosain*) from megalopa to crablet-1 nursed at different stocking densities

[Values are presented as mean±SD. The bars with different letters (a, b) show a significant difference
($p < 0.05$)]

and SGRw were observed in these feeds. Meanwhile, the lowest growth performance was with the pureed shrimp meat (Table 2). Differences in the nutritional composition of the investigated feeds could cause the differences in crab performance observed. Three of the four investigated feeds—the Lansy pellet feed, frozen Artemia biomass, and NRD pellet feed—contained between 48% and 55% protein and 9% and 12.9% lipid, which are within levels suggested for the growth of mud crabs.[43-45] Namely, the best growth performance and nutrient turnover of mud crab (*S. serrata*) juveniles were found in a 45% crude protein diet.[45] Mud crabs (*S. serrata*) grew well when they were fed with 32–40% dietary protein and 6–12% lipids.[44] Dietary lipid levels ranging from 5.3% to 13.8% appeared to meet the lipid requirement of juvenile mud crab *S. serrata*.[43] Meanwhile, the pureed meat of Stork shrimp contains a lower protein and lipid content, with only 19.4±0.56% and 1.15±0.19%, respectively. This might be the cause of the lower weight gain of crabs consuming this feed. The growth performance of crablet-1 of *S. paramamosain* crabs fed fresh shrimp meat was lower than that of frozen Artemia biomass, which was also found by Anh et al.[46] In addition, the growth performance of *S. paramamosain* megalopa reached its highest level with Lansy pellet feed (containing 48% protein) and decreased with NRD pellet feed (containing 55% protein), which was consistent with the previous study on *S. serrata* juvenile.[45] The researchers highlighted that higher protein levels in the diets of animals compared to their protein nutrient requirements could lead to an imbalance in the dietary nutrient profile and result in excess protein catabolism. This could generate higher ammonia contents in their hemolymph as a product of excretion.[47] Accumulation of ammoniac nitrogen in the hemolymph could negatively affect metabolic processes such as transporting oxygen and balancing osmotic pressure.[48,49] It can increase the metabolic cost of nitrogen excretion.[50,51] In addition, at excessively high dietary protein levels, free amino acids accumulated in the body fluids may become toxic, affecting normal metabolism and compromising normal growth in crustaceans.[52] The information mentioned above may explain the low growth performance of experimental crabs fed NRD pellet feed compared to Lansy pellet feed. Besides, it recommended that in the practice of nursing crustaceans in the megalopa stage, formulated particle feed could completely replace live prey,[29,32] as pellets offer many advantages compared to natural or live feeds. In terms of nutrient content, artificial feed offers a nutritionally balanced diet with known nutrient content, such as total lipids and protein, that will promote growth and reproduction in the crustacean.[33,34] Results of our study also showed that, although the difference was not significant, Lansy pellets resulted in crab performance slightly higher than frozen Artemia biomass feed.

Despite the survival rates and metamorphosis durations showing no significant difference among the investigated feeds, the highest survival rate was observed in the Lansy pellet feed (**Figure 2**). Meanwhile, the lowest survival rate (**Figure 2**) and the longest metamorphosis duration (**Figure 3**) were observed for pureed shrimp meat. It is suggested that feed availability (quantity and quality) is one of the major factors influencing survival in reared crabs.[53,54] The high mortality during the molting phases is often attributed to the poor nutritional status of the larvae.[9] Besides, the shortage of suitable feed is one of the factors leading to cannibalism and decreasing survival in crabs.[55-58] Survival rates (82.2–87.5%) in *S. paramamosain megalopa* in the current study were higher as compared to some other species of the Sylla genus recorded in previous studies. *S. serrata* had survival rates of 38, 46, and 48% with feeds of nauplii Artemia, enriched nauplii Artemia, and frozen crab larvae, respectively,[9] 32.8±4.8% with feeds of mussels or small shrimps (*Acetes sp.*),[55] and 48.3–53.3% with feeds of macerated brown mussel meat (*Modiolus metcalfei*) or fish.[15] *Sylla sp*. obtained a 30–50% survival rate with feeds of newly hatched and adult Artemia.[59] *S. paramamosain* had the highest survival rate of 72.9% when fed 100% Lansy-shrimp feed (called Lansy pellet feed in the present study).[29]

In addition, the metamorphosis duration in the megalopa-to-crablet-1 stage of *S. paramamosain* (10.03–11.70 days) (**Figure 3**) was within the ranges recorded in species of the Sylla genus in other studies. The megalopa mud crab stage often lasts 7–10 days before molting to the first crab stage.[2] Mud crab megalopae took 11–12 days to reach the first crab stage at a salinity of 31‰, while it took only 7–8 days at a lower salinity of 24–31‰.[60] The megalopa duration was recorded as unusually long (15–16 days) at 29°C and a salinity of 34–35‰.[61] These findings indicated that the four feeds were highly effective for nursing the mud crab *S. paramamosain* at the megalopa to crablet-1 stage. The best feeds were Lansy pellet, frozen Artemia biomass, NRD pellet feed, and pureed shrimp meat. Besides, the results also show that NRD pellet feed (55%) decreased the performance of the experiment crabs.

PERFORMANCE OF THE CRABS NURSED AT DIFFERENT DENSITIES

The increases in stocking density may significantly improve production yield per unit area. However, too high a stocking density of crab megalopa can also lead to competition for space and feed utility[62] and crowd physiological stress.[63] Also, the cannibalistic nature of megalopa with claws and free swimming causes sub-lethal injuries (e.g., limb autotomy),[64] reducing the energy available for growth,[64] and affecting the health, growth, and survival of farmed animals.[65-67] The present study indicated the change in stocking densities from 20 to 40/L had no clear effect on the survival rate and metamorphosis duration of *S. paramamosain* from megalopa to crablet-1 (**Figures 4 and 5**). Meanwhile, growth performance significantly decreased with a stocking density ⩾ 30/L, with daily weight gain and SGR in weight significantly lower at these stocking densities ($p < 0.05$) (**Table 4**).

The survival rates (88.4–90.1%) in *S. paramamosain* in this experiment were higher than those found in several previous studies on mud crabs in the same stage under different conditions of nursing.[9,10,15,29,35,55,59,68-70] It has been endorsed that in the communal rearing technique of crabs, cannibalism is a critical problem.[24,29] The adequate availability of feed and shelter could reduce the rate of cannibalism.[34] In this experiment, we used Lansy pellet feed, which resulted in the best growth and survival rates for the crabs from experiment 1, while simultaneously putting plastic mesh panels in the tanks, providing sufficient shelter to the crabs. Also, the experimental tanks were placed indoors, changed periodically, and siphoned waste every day, so the water in the tanks was maintained at good quality with few fluctuations, perhaps helping improve the crabs' survival rate. Furthermore, Lansy pellet feed with stocking densities of 20–40/L has not had a remarkable variation in the metamorphosis duration of mud crab *S. paramamosain* in the reared stage from megalopa to crablet-1. The metamorphosis duration (12.01–12.03 days) in this experiment was within the range found in previous studies in some species of mud crabs.[2,60,61] However, stocking densities ⩾ 30/L significantly reduced the growth performance in DWG and SGRw (**Table 4**). It is recommended that Lansy feed be commonly used for rearing crabs from megalopa to crablet-1, and stocking density should be applied at 20/L to achieve the highest rearing efficiency in our conditions.

CONCLUSION

Four investigated feed types all showed great potential in nursing mud crab (*S. paramamosain*) at the stage from megalopa to crablet-1, with high survival rates in crablet-1 (82.2–90.1%). Among these the Lansy pellet feed (containing 48% protein) and stocking density of 20 /L offered the highest nursing efficiency within the indoor nursing system in the tanks. Also, NRD pellet feed (containing 55% protein) showed a decrease in crab performance. The findings have provided valuable information for choosing appropriate feeds and stocking densities to reduce costs and enhance nursing performance in *S. paramamosain* megalopa. Further studies need to be conducted, including the use of Lansy pellet feed for nursing *S. paramamosain* megalopa at higher stocking densities with nursing systems of hapas and ponds or applying biofloc nursing systems to improve the efficiency of the nursing processes increasingly.

AUTHORS' CONTRIBUTION USING CREDIT

Conceptualization: Tien Hai Ly (Equal), Le Hoang Vu (Equal), Doan Xuan Diep (Equal). Methodology: Tien Hai Ly (Equal), Doan Xuan Diep (Equal). Writing – original draft: Tien Hai Ly (Lead). Writing – review & editing: Le Hoang Vu (Equal), Doan Xuan Diep (Equal). Formal Analysis: Doan Xuan Diep (Lead). Investigation: Doan Xuan Diep (Lead).

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