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# Growth and survival rate of mud clam larvae (*Geloina* sp.) in relation to rearing densities and diets

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**ABSTRACT**

*Different stocking densities and feed types were evaluated in rearing larvae of mud clam Geloina sp. The experiment 1 included four feed treatments as (i) 100% fresh algae (Nannochloropsis sp and Chaetoceros sp with 1:1 ratio), (ii) 100% dry algae (Spirulina powder), (iii) 75% fresh algae + 25% dry algae, and (iv) 50% fresh algae +50% dry algae. Mud clam larvae were cultured in 5L bottles at the stocking density of 2,000 larvae/L. Experiment period was lasted 17 days from D-larvae to umbo stage (from planktonic to benthic stage). Experiment 2 included three treatments with different nursing densities of clam larvae from (i) 2,000 larvae/L, (ii) 4,000 larvae/L to (iii) 8,000 larvae/L. The best feed type from experiment 1 was applied for feeding in experiment 2. Results from experiment 1 showed that in treatment with 100% fresh algae, the length and width of larvae reached highest values (217.3µm and 230.0 µm) on day 17. In this treatment, metamorphosis rate (31.2%), survival rate (10.5%) and larvae production (300 ind./L) also reached higher values than in other treatments ($p<0.05$). In experiment 2, the best result was obtained when nursing clam larvae at 2,000 ind./L. Length and width of larvae reach highest values (218.3µm and 231.3µm on day 17, respectively), metamorphosis rate (34.9%), survival rate (10.3%) and larvae production (294 ind./L) were also highest in this treatment.*

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## 1 INTRODUCTION

Mud clam (*Geloina* sp.) is one of the edible bivalve species. This genus is one of the largest mangrove bivalves, growing to maximum shell length of 8 cm. The utilization of bivalve species becomes popular due to its high protein content and deliciousness (Ismail, 2015).

Several studies have been conducted on this species and mainly focused on characteristics of reproduction (Morton, 1985) or distribution and morphology (Gimin *et al*., 2005). In Vietnam, several studies have been carried out to culture mud clam in shrimp pond or integrated culture with shrimp and mud crab (Nguyen Van Trai, 2015). With the study on reproductive cycle, Quach Kha Ly and Ngo Thi Thu Thao (2011) reported the spawning activities of mud clam occurred year around with the peaks in May and November in mangrove system of Ca Mau province. Recently,

Ngo Thi Thu Thao *et al*. (2018) also reported the induced methods that could improve the spawning efficiency of mud clam from U Minh Thuong, Kien Giang province.

Nowadays, mud clams have been cultured with different systems; however, mostly seed sources are collected from the wild. Therefore, with limited numbers it can not fulfill the requirements for the culture in large scale. Methods to induce spawning of mud clams in hatchery were also recommended by Quach Kha Ly and Ngo Thi Thu Thao (2011), and Ngo Thi Thu Thao *et al*. (2018). However, the techniques for larval nursing or spat rearing for this species are still very limited. It is necessary to find out suitable techniques in order to improve survival rates and growth performance of mud clam larvae in the hatchery. This study was aimed to find out the suitable density and practical diet for nursing mud clam larvae in hatchery conditions. The results from this study will contribute initial information for artificial seed production process of mud clam in Vietnam, especially in the Mekong Delta.

## 2 MATERIALS AND METHODS

### 2.1 Clam broodstocks

Matured clam broodstocks with shell length ≥40 mm were collected from Kien Giang province and transported to the hatchery in Can Tho University. The gonad development of bloodstocks had to be at stage III or IV of development. At the hatchery, broodstocks were cultured for 2-4 days in composite tanks with aeration and the salinity at 10 ppt to recover their health after transportation. Then the thermal shock method was applied to induce clam spawning and to collect larvae for experiment.

### 2.2 Experimental design

#### *2.2.1 Effects of different feed types in rearing mud clam larvae*

Sea water was filtered by using filter bag, then treated with chlorine at the concentration of 30 ppm in 2-3 days with strong aeration. EDTA (ethylene diamine tetracetic acid) was added at the concentration of 10 mg/L to neutralize heavy metals in rearing sea water. Salinity was maintained at 10 ppt during experimental period. After broodstock spawning, fertilized eggs were collected and incubated for 24 hours until reach D-larvae stage and then transferred into 5L-glass bottles with initial stocking at 2,000 larvae/L.

Experiment 1 was completely randomized design with three replications of four feeding treatments including i) 100% fresh algae (control, FA); ii) 100% dry algae (DA); iii) 75% fresh algae + 25% dry algae (3FA:1DA); and iv) 50% fresh algae + 50% dry algae (FA: DA). In control group, clam larvae were fed with the mixture of fresh algae species of *Nannochloropsis* sp. and *Chaetoceros* sp. at the cell ratio of 1:1, at the density of 5,000 cells/mL with two feeding times a day at 8:00 and 18:00. Previous study confirmed that the diet with two algal species resulted in better growth and survival rate of white clam, *Meretrix lyrata* (Nguyen Dinh Hung *et al*., 2003). The amount of dried algae in other treatments for daily feeding was calculated based-on the dry cell weight of the live algae in the control diet.

In first five days of culturing, the feces or death algae at the bottom of culture medium were removed daily by siphoning, and new sea water were added to maintain the water volume. Experiment was set up indoor conditions with light intensity around 1,000 lux at day time and darkness at night time. Experiment period was lasted 17 days from D-larvae to umbo stage and transferring into clam spat.

#### *2.2.2 Effects of different densities in rearing mud clam larvae*

Experiment 2 was completely randomized design with three replications of three treatments including i) 2,000 larvae/L; ii) 4,000 larvae/L; and iii) 8,000 larvae/L.

The best diet from Experiment 1 was applied for feeding in Experiment 2. The feeding amount and schedule, experiment period and management were similar to those in Experiment 1.

### 2.3 Data collection

#### *2.3.1 Water quality parameters*

Temperature in each culture bottle was recorded daily at 7:00 and 14:00. pH values were examined by pH meter (HANA), concentrations of $NH_4^+$ and $NO_2^-$ were measured at day 1, 3, 6, 9, 12, 15 and 17 of culture period by SERA test (Made in Germany).

#### *2.3.2 Growth, metamorphosis and survival of larvae*

Larvae were collected at day 1, 3, 6, 9, 12, 15 and 17 to measure the shell length and width (µm). At each sampling time, 10-20 larvae in each glass bottle were collected and stained with lugol solution. Shell length and width of each larvae was measured under microscope with micro-ruler.

Metamorphosis rate (%) of larvae from D-stage to Umbo stage was determined from day 9 to day 17 of rearing period and will be calculated as follows:

Metamorphosis rate (%) = 100 × (Number of Umbo larvae/ Number of observed larvae).

To record the survival rate, larvae in rearing bottle was collected at four points around and one point at the middle of bottle with the volume of 5 mL. Then, they were filtered through suitable mesh size. A number of alive larvae were counted by Sedgwick Rafter counter and calculated the survival rate.

Survival rate (%) = 100 × (alive larvae/initial number of larvae at the beginning of experiment)

*2.3.3 Data analysis*

One-way ANOVA followed by Duncan test was applied to detect the significant difference of collected parameters among treatments at $p<0.05$ using SPSS program version 22.0.

## 3 RESULTS

### 3.1 Effect of different diets on growth and survival rate of mud clam larvae

*3.1.1 Variation of environmental conditions*

Mean temperature was from 26.4 to 27.3°C in the morning and from 28.4 to 28.5°C in the afternoon. pH values also varied slightly in the morning (8.2-8.3) and in the afternoon (8.4-8.5). However, the concentrations of $NH_4^+$ and $NO_2^-$ were changed among treatments (from 0.2 - 0.5 mg/L). There was no significant difference in temperature or pH values among treatments; however, the concentrations of $NH_4^+$ and $NO_2^-$ were high in fully dry algae feeding treatment (Table 1).

**Table 1: Variation of environmental conditions in different treatments**

| Treatment | Temperature (°C) | | pH | | $NH_4^+$ (mg/L) | | $NO_2^-$(mg/L) | |
|---|---|---|---|---|---|---|---|---|
| | 7:00 | 14:00 | 7:00 | 14:00 | 7:00 | 14:00 | 7:00 | 14:00 |
| FA | 26.4±0.2 | 28.5±0.1 | 8.2±0.1 | 8.5±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 |
| DA | 27.3±0.1 | 28.4±0.1 | 8.3±0.1 | 8.4±0.1 | 0.5±0.4 | 0.5±0.4 | 0.5±0.4 | 0.5±0.4 |
| 3FA:1DA | 27.3±0.1 | 28.4±0.2 | 8.3±0.1 | 8.5±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 |
| FA:DA | 27.3±0.1 | 28.4±0.1 | 8.3±0.1 | 8.5±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 |

*3.1.2 Shell length and shell width of clam larvae*

Shell length of larvae in FA (100% fresh algae) was always longer than that in other treatments (Table 2). On day 17, shell length of larvae was highest in FA treatment (217.3 µm), whereas these numbers were lower in 2FA:1DA (199 µm), FA: DA (198.6 µm) and DA (167.9 µm). There was no significant difference in shell length of larvae between 3FA:1DA and FA:DA treatment on day 17 ($p>0.05$).

**Table 2: Shell length and width of larvae (µm) in different treatments**

| Days | Diets | | | |
|---|---|---|---|---|
| | FA | 3FA:1DA | FA:DA | DA |
| **Shell length (µm)** | | | | |
| 1 | 133.3±0.6$^b$ | 128.1±0.6$^a$ | 127.0±0.7$^a$ | 127.3±0.8$^a$ |
| 3 | 136.2±0.6$^c$ | 134.1±0.8$^b$ | 133.2±0.4$^b$ | 130.1±0.3$^a$ |
| 6 | 141.3±3.2$^c$ | 138.5±0.3$^{ab}$ | 137.9±0.6$^b$ | 134.6±0.6$^a$ |
| 9 | 147.8±2.1$^b$ | 142.1±0.6$^a$ | 141.9±0.8$^a$ | 144.5±5.1$^{ab}$ |
| 12 | 151.0±3.5$^b$ | 146.2±1.0$^a$ | 146.3±1.0$^a$ | 145.7±2.1$^a$ |
| 15 | 165.2±5.7$^b$ | 158.8±0.8$^a$ | 159.0±0.7$^a$ | 155.2±2.1$^a$ |
| 17 | 217.3±0.6$^c$ | 199.0±0.4$^b$ | 198.6±1.0$^b$ | 167.9±0.4$^a$ |
| **Shell width (µm)** | | | | |
| 1 | 142.4±1.4$^c$ | 136.5±0.3$^b$ | 135.9±0.4$^b$ | 132.3±2.7$^a$ |
| 3 | 145.9±1.4$^c$ | 140.3±0.1$^b$ | 139.0±0.9$^b$ | 134.3±0.9$^a$ |
| 6 | 149.2±1.9$^c$ | 145.3±0.5$^b$ | 143.4±1.5$^b$ | 139.8±0.4$^a$ |
| 9 | 153.2±4.8$^b$ | 145.6±0.3$^a$ | 145.5±0.9$^a$ | 144.4±0.8$^a$ |
| 12 | 158.6±1.7$^b$ | 153.3±0.4$^a$ | 153.3±0.4$^a$ | 153.3±2.1$^a$ |
| 15 | 174.3±5.5$^c$ | 168.4±0.1$^b$ | 168.0±0.3$^b$ | 162.1±1.5$^a$ |
| 17 | 230.0±0.1$^c$ | 205.8±0.8$^b$ | 203.3±2.9$^b$ | 174.8±0.4$^a$ |

*Values of the same row with the same letters are not significantly different ($p>0.05$)*

On day 17, shell width of larvae was also highest in FA (230 µm), whereas it was 205.8 µm, 203.3 µm and 174.8 µm in 3FA:DA and FA:DA, DA treatments, respectivively. Shell width of larvae in 3FA:DA and FA treatment was also not significantly different on day 17 ($p>0.05$).

*3.1.3 Metamorphosis and survival rate of mud clam larvae*

Clam larvae began to metamorphose at day 9 (Table 3). Metamorphosis rates of larvae on days 9, 12, 15 and 17 in FA treatment were 7.5, 8.9, 26.6 and 31.2%, respectively and were higher than in other treatments ($p<0.05$).

**Table 3: Metamorphosis and survival rate of mud clam larvae**

| Treatment | Rearing day | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 9 | 12 | 15 | 17 |
| **Metamorphosis rate (%)** | | | | | | | |
| FA | 0.0 | 0.0 | 0.0 | 7.5±0.1$^{d}$ | 8.9±0.6$^{d}$ | 26.6±1.2$^{d}$ | 31.2±0.6$^{d}$ |
| 3FA:1DA | 0.0 | 0.0 | 0.0 | 4.3±0.2$^{c}$ | 7.4±0.3$^{c}$ | 21.9±0.2$^{c}$ | 26.2±0.4$^{c}$ |
| FA:DA | 0.0 | 0.0 | 0.0 | 3.4±0.1$^{b}$ | 6.6±0.3$^{b}$ | 18.9±0.6$^{b}$ | 22.5±0.1$^{b}$ |
| DA | 0.0 | 0.0 | 0.0 | 2.3±0.1$^{a}$ | 5.8±0.3$^{a}$ | 15.8±0.7$^{a}$ | 18.8±0.6$^{a}$ |
| **Survival rate (%)** | | | | | | | |
| FA | 100$^{a}$ | 56.7±0.8$^{d}$ | 31.0±0.6$^{d}$ | 21.8±0.7$^{d}$ | 16.8±0.7$^{d}$ | 15.1±0.4$^{d}$ | 10.5±0.6$^{d}$ |
| 3FA:1DA | 100$^{a}$ | 42.7±0.6$^{c}$ | 24.1±0.3$^{c}$ | 18.2±0.7$^{c}$ | 12.8±0.4$^{c}$ | 11.0±0.6$^{c}$ | 5.8±0.6$^{c}$ |
| FA:DA | 100a | 34.9±0.9$^{b}$ | 19.4±0.5$^{b}$ | 14.3±0.3$^{b}$ | 10.9±0.4$^{b}$ | 8.4±0.2$^{b}$ | 4.5±0.6$^{b}$ |
| DA | 100$^{a}$ | 29.4±0.8$^{a}$ | 14.9±1.0$^{a}$ | 10.3±0.6$^{a}$ | 9.0±0.4$^{a}$ | 6.4±0.3$^{a}$ | 3.9±0.6$^{a}$ |

*Values of the same column with the same letters are not significantly different ($p>0.05$)*

Survival rate of larvae in all treatments decreased abruptly from day 3 to 17 (Table 3). At day 3, 6, 9, 12, 15 and 17, the survival rate of larvae in FA treatment was highest and significantly different from others ($p<0.05$). Especially, at day 17, survival rate was 10.5% in FA treatment and double times higher than in 3FA: DA (5.8%) and FA: DA (4.5%). In treatment fed 100% dry algae, the survival rate was lowest at day 17 (3.9%).

### 3.2 Effects of different rearing densities on growth and survival rate of clam larvae

*3.2.1 Environmental conditions*

Mean temperature in rearing medium varied from 26.4-26.5°C in the morning and 28.5°C in the afternoon. pH values varied from 8.2- 8.5, and mean concentrations of $NH_4^+$ (0.2 mg/L) or $NO_2^-$ (0.2 mg/L) were stable and not significantly different among treatments (Table 5).

**Table 5: Variation of environmental conditions in different stocking densities**

| Density | Temperature (°C) | | pH | | $NH_4^+$ (mg/L) | | $NO_2^-$ (mg/L) | |
|---|---|---|---|---|---|---|---|---|
| (larvae/L) | 7:00 | 14:00 | 7:00 | 14:00 | 7:00 | 14:00 | 7:00 | 14:00 |
| 2000 | 26.4±0.2 | 28.5±0.1 | 8.2±0.1 | 8.5±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 |
| 4000 | 26.5±0.2 | 28.5±0.1 | 8.2±0.1 | 8.5±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 |
| 8000 | 26.4±0.2 | 28.5±0.1 | 8.3±0.1 | 8.5±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 | 0.2±0.1 |

*3.2.2 Shell length and shell width of clam larvae at different rearing densities*

Shell length of larvae has no significant differences among treatments ($p>0.05$) on day 1, 3, 6 and 9. However, the significant differences were detected at day 12, 15 and 17 of rearing period ($p<0.05$), showing the effects of density on shell growth of clam larvae (Table 6). On day 17, shell length of larvae was highest in lowest rearing density (218.3 µm) and significantly higher than in the remaining treatments ($p<0.05$). As shown in Table 6, shell width of clam larvae was affected by rearing density earlier than shell length. From day 6 to 17, the highest number was always presented in rearing density of 2,000 larvae/L, meanwhile it was similar between middle or highest density.

**Table 6: Shell length and width of larvae (µm) in different rearing densities**

| Day | Stocking densities (larvae/L) | | |
|---|---|---|---|
| | 1) 2,000 | 2) 4,000 | 3) 8,000 |
| **Shell length of larvae (µm)** | | | |
| 1 | 133.3±0.6[a] | 133.3±0.6[a] | 133.3±0.6[a] |
| 3 | 136.2±0.6[a] | 136.2±0.6[a] | 136.2±0.6[a] |
| 6 | 141.3±2.8[a] | 141.3±3.2[a] | 140.1±0.7[a] |
| 9 | 147.5±2,0[a] | 143.8±5.4[a] | 141,0±0.9[a] |
| 12 | 151.5±3.6[b] | 145.8±4.5[a] | 143.7±0.8[a] |
| 15 | 168.8±0.6[b] | 164.9±5.4[b] | 158.2±0.6[a] |
| 17 | 218.3±0.3[b] | 208.3±2.9[a] | 206.7±2.9[a] |
| **Shell width of larvae (µm)** | | | |
| 1 | 143.5±0.4[a] | 143.5±0.4[a] | 143.5±0.4[a] |
| 3 | 146.5±1.0[a] | 145.1±0.7[a] | 141.8±1.5[a] |
| 6 | 149.7±1.6[b] | 147.9±0.8[ab] | 146.1±0.7[a] |
| 9 | 155.9±2.6[b] | 153.0±0.4[ab] | 150.7±0.1[a] |
| 12 | 158.1±2.5[b] | 153.8±2.0[a] | 151.4±0.6[a] |
| 15 | 175.6±1.1[b] | 171.2±3.2[a] | 169.1±1.0[a] |
| 17 | 231.3±1.1[b] | 206.8±2.8[a] | 206.7±2.9[a] |

*Values of the same row with the same letters are not significantly different (p>0.05)*

*3.2.3 Metamorphosis and survival rate of mud clam larvae*

In Experiment 2, metamorphosis rate of larvae also occurred at day 9 of rearing. Metamorphosis rate at day 9, 12, 15 and especially day 17 (34.9%) was highest in treatment 1 and significantly different from treatment 2 and 3 ($p<0.05$).

Survival rate of larvae strongly decreased from day 3 to day 17 (Table 9) in all treatments. On day 3, 6, 9, 12, 15 and 17, survival rate was highest in treatment 1, and there were statistically significant differences between treatment 1 and 2 ($p<0.05$). At the end of rearing period, clam larvae in lowest stocking density showed the best survival rate (10.3%), meanwhile, corresponding numbers were low in middle (6.6%) and highest stocking density (6.4%). Regardless of double times rearing density, there were no significant differences in survival rate of larvae between density of 4,000 and 8,000 larvae/L ($p>0.05$).

**Table 9: Metamorphosis and survival rate of mud clam larvae in different rearing densities**

| Density (larvae/L) | Rearing days | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 9 | 12 | 15 | 17 |
| **Metamorphosis rate (%)** | | | | | | | |
| 1) 2000 | 0 | 0 | 0 | 4.4±0.1[b] | 6.6±0.1[b] | 21.5±0.3[b] | 34.9±0.6[b] |
| 2) 4000 | 0 | 0 | 0 | 2.3±0.1[a] | 4.2±0.1[a] | 14.2±0.1[a] | 22.9±0.3[a] |
| 3) 8000 | 0 | 0 | 0 | 2.3±0.1[a] | 4.2±0.2[a] | 13.9±0.2[a] | 22.4±0.2[a] |
| **Survival rate (%)** | | | | | | | |
| 1) 2000 | 100[a] | 59.4±0.5[b] | 35.1±0.4[b] | 29.5±0.6[b] | 18.9±0.4[b] | 17.0±0.4[b] | 10.3±0.2[b] |
| 2) 4000 | 100[a] | 55.0±0.8[a] | 33.1±0.5[a] | 19.2±0.7[a] | 13.4±0.2[a] | 10.6±0.4[a] | 6.6±0.1[a] |
| 3) 8000 | 100[a] | 55.0±0.6[a] | 32.9±0.5[a] | 19.5±1.3[a] | 13.5±0.3[a] | 10.5±0.2[a] | 6.4±0.2[a] |

*Values of the same column with the same letters are not significantly different (p>0.05)*

## 4 DISCUSSIONS

### 4.1 Effects of different diets on growth and survival rate of mud clam larvae

Richard *et al*. (2015) reported that temperature has a strong influence on Manila clam immune activity. However, in both experiments from this study the temperature was not high variation among treatments, and there was probably no effect on the growth of clam larvae. On the other hand, in experiment 1, the concentration of nitrite was highest in treatment fed dry algae, and it might be harmful for larvae during nursing period.

The findings from Experiment 1 was similar to the results of Albentosa *et al*. (1997) that the growth rates of *Ruditapes decussatus* larvae fed dried microalgae were significantly lower than those from fresh algae diets. The cell size of *Nannochloropsis*

sp. and *Chaetoceros* sp. is suitable for D-larvae. The content of lipids, proteins (amino acids), carbohydrates and vitamins of various micro algae species is one of the main reasons for considering these organisms as food source for aquaculture animals (Southgate, 2003). Furthermore, their content of highly unsaturated fatty acids, especially eicosapentaenoic acid, arachidonic acid, docosahexaenoic acid and linolenic acid provides the most prominent determinant of the nutritional composition of microalgae (Lavens and Sorgeloos, 1996; Richmond, 2004; Martínez-Fernández *et al*., 2006). Becker (2004) suggested that the larval stage needs a small amount but requires a high quality of microalgae.

The partial replacement of a diet of fresh microalgae has been reported in several studies using dried diets. Doroudi *et al*. (2002) reported that black-lip pearl oyster *Pinctada margaritifera* with the substitution of 25–50% of a live microalgae diet with dried micro-algae (*Tetraselmis* sp) did not significantly reduce survival or growth of D-stage larvae. However, results from the present study showed that dried diet substitution negatively affected growth of larvae, with an increased substitution level resulting in growth decline in general. The slowest growth occurred at complete replacement of fresh algae. Dry powder of *Spirulina* algae easily made pollution in cultured environment and triggered bacterial contamination. Furthermore, with the high protein concentration (~50%), the decomposition of *Spirulina* powder will increase nitrite concentrations in nursing water, and it will be harmful for clam larvae. Laramore (2015) recommended that the concentration of nitrite during clam nursing stage should be lower than 0.2 mg/L. The results from this study showed that, dry algae should be limited utilization when fresh algae are in shortage production for rearing D-larvae of mud clam.

Survival rate of clam larvae decreased gradually along the experiment period, at the late development stages, the survival rate of larvae decreased abruptly because they completed the development and started to transfer from planktonic to benthic stage. Quayle and Newkirk (1989) recommended that supplying food sources with micro algal diets at enough quantity and quality played important role in maintaining the good growth performance, high and stable survival rate of larvae in the hatchery.

### 4.2 Effects of different rearing densities on growth and survival rate of mud clam larvae

Siphoning waste materials from the bottom of cultured vessels and renewing water were conducted daily during experiment, resulting in that $NH_4^+$ and $NO_2$ concentrations were not different among treatments of 4,000 and 8,000 larvae/L. Therefore, those toxic nitrogen compounds could be limited effects on the growth of mud clam larvae in this study. The results indicated that growth rate, metamorphose and survival rate were highest at the density of 2,000 larvae/L. Similarly, according to La Xuan Thao *et al*. (2004), cultured blood cockle larvae at 25 ppt and the density of 2,000 individuals/L were the optimum conditions for nursing blood cockle in planktonic stage. The authors also observed that the growth rate and survival rate of blood cockle larvae were decreased when nursing densities increased from 2,000 to 4,000 larvae/L.

The growth of larvae in high rearing density remained relatively low, supporting the notion that factors other than access to food limit growth at high stocking density (Liu *et al*., 2006). Elevated density triggers a corresponding increase in the collision rate between swimming larvae causing retraction of the velum and cessation of feeding, as well as longer term energy loss associated with shell repair (Cragg, 1980; Sprung, 1984; Liu *et al*., 2006). Higher larval density may also result in rapid accumulation of toxic metabolites that can be detrimental to both immune function and growth (Sprung, 1984; Yan *et al*., 2006; Raghavan and Gopinathan, 2008). Furthermore, Orensanz *et al*. (1991) recommended that decreased survival rate at high densities was possibly due to food and oxygen depletion, predation or other environmental stresses.

Lower stocking densities can shorten the larval swimming period and reduce the risk of mortality. Therefore, rearing larvae with stocking density at 2,000 individuals/L has been recommended; however, in this study, the food limiting factor might be the cause for low results in growth performance, metamorphose and survival rate of clam larvae. It is necessary to conduct more studies on how to increase feeding parallel together rearing densities to optimize the rearing efficiency in the hatchery.

## 5 CONCLUSIONS AND RECOMMENDATION

Using 100% fresh algae (*Nannochloropsis sp* and *Chaetoceros sp*) as a food source for mud clam larvae increased shell length, shell width, survival rate, metamorphosis rate, and larvae production.

Rearing mud clam larvae at the density of 2,000 individuals/L showed best results in shell length, shell width increase, survival rate, metamorphose rate, and larvae production.

Further study is needed to increase rearing densities in the range of 2,000 to 8,000 larvae/L with suitable

amount and schedule of feeding to optimize the rearing efficiency in clam hatchery.

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