

# Effects of different temperatures on the growth, survival and digestive enzyme activities of mud crab *Scylla paramamosain* at juvenile stage

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**Abstract.** The effects of temperature on the growth, survival, and digestive enzymatic activities of mud crab *Scylla paramamosain* were investigated in laboratory conditions. Crab juveniles (0.13 g) were exposed to four temperatures (27-28°C, 30-31°C, 33-34°C, and 36-37°C), the salinity of 25‰, and three replicates for 30 days. The density was 30 individuals per 500-L tank. The carapace width and weight in 27-28°C treatment ( $2.41 \pm 0.02$  cm and  $2.62 \pm 0.05$  g) were significantly lower than those in other treatments, while the highest values were in the 36-37°C treatment ( $3.99 \pm 0.10$  cm and  $10.3 \pm 0.68$  g). The digestive enzymatic activities gradually decreased with temperature rise except for amylase activity which increased up to 33°C and then decreased at the higher temperature. The molting cycle was shortest at 36-37°C ( $10.6 \pm 0.16$  days), which was significantly lower than other treatments. Crabs reared at 36-37°C showed the highest molting frequency ( $2.05 \pm 0.05$  times). The survival rate of crabs after 30 days was significantly reduced at the 36-37°C treatment ( $28.9 \pm 2.94\%$ ), compared to lower temperature treatments with the highest survival rate being  $51.1 \pm 4.01\%$  at 27-28°C. The temperature of 27-28°C was the optimal temperature for rearing this species at the juvenile stage.

**Key Words:** digestive enzyme, growth performance, molting cycle, *Scylla paramamosain*, survival rate.

**Introduction.** Mud crab *Scylla paramamosain* has been considered as a viable candidate for aquaculture with key characteristics such as high market price, rapid growth, simple feed, and abiotic requirements (Wickins & Lee 2002). Information on larval rearing of mud crab was first reported by Ong (1964). In 1983, mud crab juveniles reared in tanks in Taiwan showed a survival rate of 50 to 70% (Cowan 1984). In the Mekong Delta region of Vietnam, there were around 100 mud crab seed production hatcheries, which accounted for nearly 200,000 juveniles per hatchery per year (Hai & Phuong 2009). The number of mud crab hatcheries increased, reaching 480 hatcheries in 2017 with yearly productivity from 0.5 to 12 million juveniles per hatchery (Hai et al 2017). However, it has been recognized that temperature is one of the important factors that should be studied in the seed production technology of mud crabs (Chen & Cheng 1985) besides vibriosis (Jithendran et al 2010; Wu et al 2016), nutrition (Holme 2008; Holme et al 2009) and water quality (Li et al 2008; Li et al 2012). According to IPCC (2018), atmospheric temperature could increase from 2 to 2.8°C in 2100, while the Mekong river Delta, Vietnam was predicted to increase to over 32°C by 2050 (Mainuddin et al 2010).

Among environmental abiotic factors, the temperature was emphasized as a major confounding parameter influencing marine species (Ponce-Palafox et al 1997). Thus, it is determined to be the most important modifier of energy flow and hence the growth of aquatic animals (Brett 1979). Higher feeding activity in summer than in winter was found in *Gammarus fossarum* (Pockl 1992; Maltby et al 2002; Coulaud et al 2011; Charron et al 2013). Consequently, it could be suggested that temperature modulates digestive enzymatic activities (Trellu & Ceccaldi 1980; Charron et al 2013).

The effects of temperature on the growth and survival of *Scylla serrata* were reported in numerous studies (Hill 1974, 1980; Heasman & Fielder 1983; Chen & Cheng 1985; Zeng & Li 1992; Nurdiani & Zeng 2007). Hamasaki (2003) found that the optimal temperature for survival of *S. serrata* larvae was 29°C in which the larval rearing period

being significantly shortened with temperatures from 23 to 32°C. In *S. parammosain*, Khoa et al (2019) discovered that there are variations in the activities of digestive enzymes (trypsin, chymotrypsin, pepsin, and amylase) during larval development. Additionally, several studies were carried out on this species over the last decade (Hai & Viet 2017a, b; Hai et al 2017; Khoa 2018). However, these studies mainly focused on determining nutrition, seed production and rearing systems. But the effects of temperature have attracted less attention of researchers. Hence, there is a distinct lack of information on its impacts on this species as compared to commercially cultured crustacean species.

The following study was designed to gain additional information on the impacts of temperature on the survival, growth, and digestive enzymatic activities of *S. parammosain* at the juvenile stage. Besides, it was expected to suggest an optimal range of temperature for the hatchery operation and contribute to the sustainable development of mud crab hatchery and farming under the climate change context.

## Material and Method

**Experimental materials.** This study was conducted from April to December 2020 at the College of Aquaculture and Fisheries, Can Tho University, Viet Nam. Mud crab juveniles were obtained from a hatchery in Ca Mau province, the Mekong Delta, Vietnam. After arriving in the wet-lab of the College of Aquaculture and Fisheries, Can Tho University, crabs were disinfected in a formalin (20 mg L<sup>-1</sup>) bath for 15 minutes and then transferred to a 2 m<sup>3</sup> tank at 25‰ water and 27±1°C for three days for acclimation to the new condition. Crablets were fed with live *Artemia* biomass (4-6 ind crab<sup>-1</sup> day<sup>-1</sup>) twice a day (8:00 and 16:00 hour). Prior to experimentation, 40 crabs that were not then used in the experiment, were randomly collected and weighed to obtain a mean initial weight (0.13±0.03 g) using a Sartorius CP2245 electronic balance (accuracy to 0.0001 g). The initial width (0.71±0.21 cm) was determined by using a Vernier Caliper (0-150×0.02 mm).

Brine water was obtained from a salt farm in Soc Trang province, a coastal province in the Mekong Delta. Brine water was diluted to 25‰ with tap water, disinfected with chloride (50 mg L<sup>-1</sup>), and filtered by a bag-net with 5 µm mesh size. The temperature in tanks was increased and maintained according to target levels using heaters (EHEIM Professionel 4+ 350 T, Germany) in 30-31°C; 33-34°C and 36-37°C treatments and coolers (Teco Seachill TR 10, Germany) in the 27-28°C treatment.

**Experimental design.** The experiment was conducted in round fiberglass 500-L tanks (diameter: 100 cm and height: 70 cm), containing 200-L water, which were arranged in three parallel rows of four. A total of 360 individuals with uniform size were randomly distributed to 12 tanks representing four temperature treatments (27-28°C, control); 30-31°C; 33-34°C and 36-37°C) with three replicates for 30 days. For each replicate, 20 juveniles were directly placed in tanks, and 10 crabs individually cultured in round plastic boxes (diameter: 10 cm; height: 10 cm;) for molting observation. On the boxes, small holes were carved to ensure water exchange with water outside. The molts were recorded daily in the morning before feeding to determine the molting cycle and frequency. After distributing crabs into the tanks, the temperature in every single treatment was increased in different stepwise by different temperature levels (1°C per 16 hours for 30-31°C treatment; 1°C per 8 h for 33-34°C treatment and 1°C per 5.3 h for 36-37°C treatment), and experimental temperatures in all treatments reached the target levels at the same time (48 h). Water in tanks was refreshed every three days in a ratio of 30% prepared water which was the same temperature levels. Feeding was performed twice a day as that in the acclimation period. Besides, a total of 4 m<sup>2</sup> nylon nets (mesh size of 5 cm) were placed in the tanks, which play a role as shelters to reduce cannibalism (increasing available surface area and reducing the encounters among crabs). In addition, the experiments were conducted following the laws and regulations controlling experiments with live animals in Vietnam where the study was conducted (Law of Animal Health 2015).

Water quality was checked daily using WTW Multi Oxi 3206 meter that measured dissolved oxygen (DO) and water temperature, while pH was measured by WTW Multi 3510 IDS meter. Salinity levels in the experimental tanks were checked daily using a Handheld Refractometer (RES-10ATC). Nitrite (NO<sub>2</sub><sup>-</sup>) concentrations were weekly recorded using Griess Ilosvay, Diazonium. Alkalinity was checked using Sera test kits (Germany).

**Sample collection.** Ten crab-lets per tank which were then continued to be reared were randomly collected and carefully weighed and measured every 10 days. Growth parameters were computed as mean weight and carapace width as well as the specific growth rate (SGR). The survival rate (%) was determined at the end of the experiment by counting all survived crabs. The digestive enzymatic activities were determined at the end of the experiment. Three crablets from each replicate were collected and immediately dissected on-ice. The hepatopancreas, intestine, and stomach were extracted. Samples were thawed on ice and homogenated with the buffer KH<sub>2</sub>PO<sub>4</sub> 20 mM and NaCl 6 mM, pH 6.9. The mixture was centrifuged at 4.200 rpm for 30 min at 4°C and then the supernatant was collected and stored at -80°C until analysis. Chymotrypsin activity was measured by the method described by Worthington (1982); amylase activity was determined using the method described by Bernfeld (1951), while trypsin activity was analyzed following the methods of Tseng et al (1982). Protein was determined using the Biorad protein assay. Specific activities are expressed as U min<sup>-1</sup> mg<sup>-1</sup> protein.

#### **Growth and survival parameters**

$$\text{Survival rate (SR, \%)} = \frac{\text{Number of crab at the end of the experiment}}{\text{Number of initial crabs}} \times 100$$

$$[\text{Specific growth rate (SGR, \% \cdot \text{day})}^{-1}] = \frac{\text{Ln(Wf)} - \text{Ln(Wi)}}{t} \times 100$$

where: Wi is the initial weight (g); Wf is the final weight (g); and t is the experimental time (day).

**Statistical analysis.** All the data were subjected to statistical treatment involving standard error (SE) and mean using Excel 2016. One-way analysis of variance (ANOVA) together with DUNCAN tests were used to test for significant differences (at a significant level of 0.05) by using SPSS 16.0.

#### **Results**

**Water quality parameters of the rearing tanks.** During the experimental period, the temperatures were controlled according to desirable temperature treatments, while the other environmental parameters of all tanks were in the suitable range for *S. paramamosain* rearing such as 7.5-7.7 for pH; 7.60-7.71 mg L<sup>-1</sup> for DO; 0.51-0.53 mg L<sup>-1</sup> for nitrite and 97.5-107 mg L<sup>-1</sup> for alkalinity (Table 1).

The growth performance of *S. paramamosain* juveniles increased with temperature levels on the 30<sup>th</sup> day. The carapace width and weight in 27-28°C treatment (2.41±0.02 cm and 2.62±0.05 g) was significantly lower than those in other treatments, whereas the highest values were found in the 36-37°C (3.99±0.10 cm and 10.3±0.68 g, respectively) from the initial weight and width of 0.13±0.03 g and 0.71±0.21 cm, respectively. The same pattern was found in specific growth rate in weight with 10.1±0.06% in 27-28°C and 14.7±0.23% in 36-37°C (Table 2).

Table 1

The average of water quality parameters in crab rearing tanks within 30 days

| Parameter | Treatments/Temperatures |           |           |           |           |
|-----------|-------------------------|-----------|-----------|-----------|-----------|
|           |                         | 27–28°C   | 30–31°C   | 33–34°C   | 36–37°C   |
| T (°C)    | Morning                 | 27.3±0.05 | 30.5±0.04 | 33.1±0.01 | 36.3±0.07 |
|           | Afternoon               | 28.0±0.01 | 31.4±0.06 | 34.0±0.03 | 37.1±0.03 |
| DO (ppm)  | Morning                 | 7.60±0.01 | 7.61±0.01 | 7.61±0.01 | 7.64±0.01 |
|           | Afternoon               | 7.69±0.01 | 7.71±0.02 | 7.68±0.01 | 7.68±0.02 |
| pH        | Morning                 | 7.50±0.01 | 7.60±0.01 | 7.60±0.01 | 7.60±0.01 |
|           | Afternoon               | 7.70±0.01 | 7.70±0.02 | 7.70±0.01 | 7.70±0.01 |
| Alk (ppm) |                         | 107±0.81  | 100±0.00  | 97.5±1.44 | 104±1.44  |
| Nit (ppm) |                         | 0.52±0.01 | 0.53±0.00 | 0.51±0.01 | 0.52±0.00 |

Note: DO - dissolved oxygen; T – temperature; Alk – alkalinity; Nit – nitrite. Temperature, dissolved oxygen, and pH were recorded daily in morning and afternoon, while alkalinity and nitrite were analyzed in weekly intervals; values are expressed as mean±SE (n = 3).

Table 2

Weight, carapace width (CW), and specific growth rate (SGR) of crabs reared at different temperatures

| Sampling time | Treatment | CW (cm)                 | Weight (g)              | SGR (% day <sup>-1</sup> ) |
|---------------|-----------|-------------------------|-------------------------|----------------------------|
| Day 10        | 27-28°C   | 1.53±0.03 <sup>a</sup>  | 0.73±0.04 <sup>a</sup>  | 5.85±0.19 <sup>a</sup>     |
|               | 30-31°C   | 1.67±0.08 <sup>ab</sup> | 0.97±0.14 <sup>ab</sup> | 6.72±0.50 <sup>ab</sup>    |
|               | 33-34°C   | 1.91±0.12 <sup>b</sup>  | 1.24±0.19 <sup>ab</sup> | 7.54±0.54 <sup>b</sup>     |
|               | 36-37°C   | 1.93±0.09 <sup>b</sup>  | 1.35±0.18 <sup>b</sup>  | 7.85±0.48 <sup>b</sup>     |
| Day 20        | 27-28°C   | 1.93±0.04 <sup>a</sup>  | 1.31±0.12 <sup>a</sup>  | 7.78±0.32 <sup>a</sup>     |
|               | 30-31°C   | 2.33±0.09 <sup>b</sup>  | 1.79±0.05 <sup>a</sup>  | 8.84±0.09 <sup>b</sup>     |
|               | 33-34°C   | 2.47±0.18 <sup>b</sup>  | 3.00±0.29 <sup>b</sup>  | 10.54±0.32 <sup>c</sup>    |
|               | 36-37°C   | 2.96±0.09 <sup>c</sup>  | 5.06±0.30 <sup>c</sup>  | 12.29±0.20 <sup>d</sup>    |
| Day 30        | 27-28°C   | 2.41±0.02 <sup>a</sup>  | 2.62±0.05 <sup>a</sup>  | 10.11±0.06 <sup>a</sup>    |
|               | 30-31°C   | 2.88±0.10 <sup>b</sup>  | 4.07±0.17 <sup>a</sup>  | 11.58±0.14 <sup>b</sup>    |
|               | 33-34°C   | 3.23±0.20 <sup>b</sup>  | 5.12±0.60 <sup>b</sup>  | 12.30±0.42 <sup>b</sup>    |
|               | 36-37°C   | 3.99±0.10 <sup>c</sup>  | 10.26±0.68 <sup>c</sup> | 14.65±0.23 <sup>c</sup>    |

Note: Values presented as mean±SE (n = 3). Different letters (a, b, c, d) in the columns of the same sampling time signify a significant difference (p < 0.05).

**Digestive enzymatic activities of mud crab under different temperatures.** There were no significant differences in digestive enzymatic activities among the four temperature treatments. However, the results of the enzymatic analysis showed that the increase in temperature had an impact on all these digestive enzymes. Amylase activity tended to increase with temperature from 27-28 to 33-34°C and then decreased at 36-37°C, while trypsin and chymotrypsin activities were highest at 27-28°C (0.06±0.01 and 761±166 U min<sup>-1</sup> mg<sup>-1</sup> protein, respectively) and gradually decreased by temperature rises to 36-37°C (0.03±0.02 and 404±88.3 U min<sup>-1</sup> mg<sup>-1</sup> protein, respectively) (Table 3).

Table 3

Activities of amylase, trypsin, and chymotrypsin of crabs reared at different temperatures

| Parameter    | Treatment |           |           |            |
|--------------|-----------|-----------|-----------|------------|
|              | 27-28°C   | 30-31°C   | 33-34°C   | 36-37°C    |
| Amylase      | 35.342.85 | 42.2±0.06 | 44.9±2.29 | 30.9±1.58  |
| Trypsin      | 0.06±0.01 | 0.06±0.01 | 0.04±0.01 | 0.03±0.02  |
| Chymotrypsin | 761±166   | 776±225   | 442±59.1  | 404.4±88.3 |

Note: The amylase, trypsin, and chymotrypsin concentration are presented as U.min<sup>-1</sup>.mg<sup>-1</sup> protein. Values are presented as mean±SE (n = 3).

**Molting cycle and molting frequency of crabs under different temperatures.** The results showed that the molting cycle and molting frequency of crabs were affected by raised temperatures (Figure 1). The molting cycle was reduced with increased temperatures, which was significantly shorter at 36-37°C ( $10.6 \pm 0.16$  days) as compared to other treatments. The results indicated that juveniles reared at normal temperature (27-28°C) had the longest molting duration ( $13.2 \pm 0.05$  days). On the other hand, the molting frequency increased in crabs reared under higher temperatures with the highest frequency being in 36-37°C ( $2.05 \pm 0.05$  times) and significantly higher than the other three treatments.

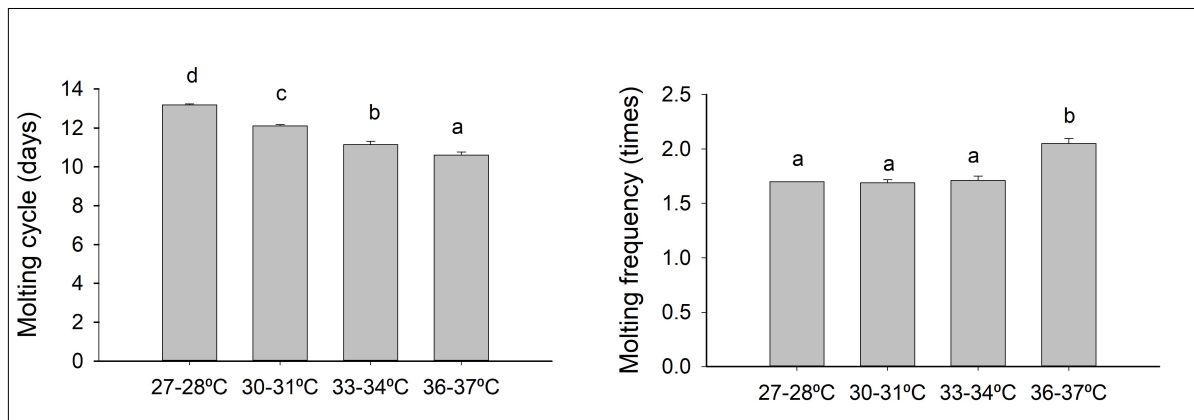


Figure 1. Average molting cycle (left) and molting frequency (right) of crab reared in different temperatures in 30 days. Data are presented as means  $\pm$  SE ( $n = 3$ ). Values with different letters (a, b, c, d) signify a significant difference ( $p < 0.05$ ).

**Survival rate.** The survival rate of crabs after 30 days of rearing was significantly reduced at 36-37°C treatment ( $28.9 \pm 2.94\%$ ), compared to lower temperature treatments with the highest survival rate being  $51.2 \pm 4.01\%$  at 27-28°C. The survival rates were decreased with elevated temperature but did not show a significant difference in the range from 27-28 to 33-34°C (Figure 2).

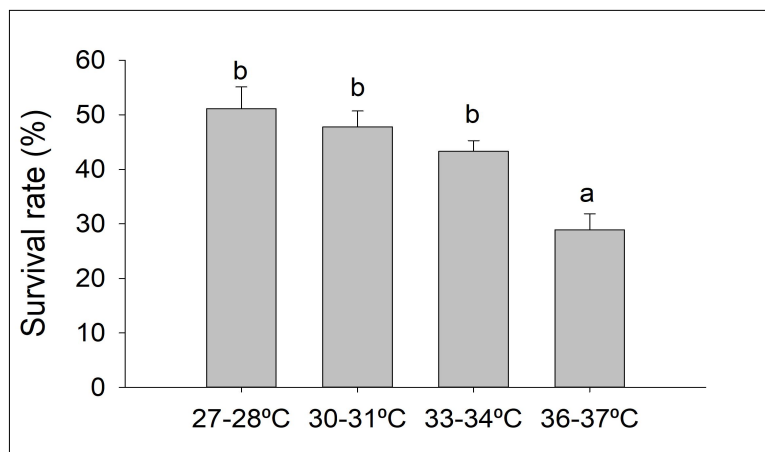


Figure 2. The survival rate of crab reared different temperatures for 30 days. Data are presented as mean  $\pm$  SE ( $n = 3$ ). Values with different letters (a, b) signify a significant difference ( $p < 0.05$ ).

**Discussion.** Throughout the experimental period, the water quality parameters were in an accepted range for mud crab rearing (Shelley & Lovatelli 2011). The result of the current study indicates that the growth in weight and width significantly correlate with temperature rises, which is highest at 36-37°C. Additionally, rising temperatures resulted in increases in molting frequency and shortening of the molting cycle of crabs. It has been demonstrated that the weights of juveniles and sub-adult were almost doubled at

each molt by inflating a greatly expanded new cuticle through imbibing a large amount of seawater (Neufeld & Cameron 1994). This seems consistent with the molting frequency data of the present study, in which the more frequently the crab molt, the more the weight of crabs increases. The results in the current study are in agreement with Huong et al (2020) that *S. paramamosain* at crablet 1 stage showed a higher growth rate at 36-37°C and the lowest value at 27-28°C. The elevated temperature leads to increases in metabolic rate; animals effectively consume more feed to meet energy demand. In the same salinity, the study on *S. serrata* conducted by Heasman & Fielder (1983) showed that larval development and frequency of prey capture increased in a temperature range from 22 to 28°C, compared to lower temperatures. Similar results were shown by Hill (1978) where swimming activities and prey capture frequency at 25 and 20°C were significantly higher than temperatures below 12°C. Baylon (2013) reported that the onset of development *Scylla tranquebarica* larvae was delayed up to 9 days in colder temperature of 20°C compared to 26 and 32°C.

In addition to growth performance, the digestive enzymes of mud crabs in this study showed to be affected by different temperatures. The results of enzymatic analyses are consistent with those reported on the fish (*Clarias batrachus*) with digestive enzyme activities at 30 and 35°C being reduced as compared to that in 25°C (Ahmad et al 2014), although this species possibly grows well up to 32°C (Dehadrai et al 1985). In the current study, the main weight of crabs reared at 36-37°C after 30 days approximately doubled compared to those at 33-34°C treatment and was almost five times higher than that at 27-28°C. A significant correlation between rising growth and falling digestive enzyme activities were reported on large yellow croaker (*Pseudosciaena crocea*) by Ma et al (2005); the author argued that decreases in digestive enzymatic activities were due to rises in body protein (as specific activity is the ratio activity per mg protein), and this does not reflect a lowering in digestive capacity. The increase in protein concentration at higher temperatures was also found in the present study (Table 3). Serrano (2015) reported that the chymotrypsin-like enzyme activity of the mud crab was maximal at 30°C and decreased at higher temperatures. Furthermore, a study in freshwater zooplankton, *Heliodiaptomus viduus* (Gurney) revealed that the optimum amylase activity was at 30°C (Dutta et al 2006).

The molt cycle in mud crabs (and all decapod crustaceans) could result from complex interactions between many endogenous (genetic) and exogenous factors, with the temperature being reported as one of the important factors (Heasman 1980). The molt cycles were between 10.6 and 13.2 days in this study, which are longer than those reported by Huong et al (2020) at the same temperature levels with the duration of molting of crablet-1 fluctuating from 3.55 to 6.77 days. As crabs grow, the molts become less frequent (Kuhn 2017). The rates of physiological processes increase in raised temperature, which includes those associated with molting, thus shortening the period between molts (Gillooly et al 2001). The shortened inter-molt periods when temperature increases were also found in some previous studies on *Callinectes sapidus* (Tagatz 1968; Leffler 1972; Brylawski & Miller 2006), brachyuran crabs (Anger 1984; Kondzela & Shirley 1993), *Callinectes similis* and *C. sapidus* (Kuhn 2017). In the present study, the molt cycle was shortened and molts appeared more frequent in 36-37°C treatment in which crabs showed greater growth performance. However, the increase in molt frequency could lead to a lower survival rate in higher temperature treatments due to cannibalism after molting. A study conducted by Wall et al (2009) revealed that a small proportion of crabs were responding to the molt odors and that this still has the potential to cause a dramatic cumulative reduction in grow-out survival. Thus, the higher mortalities could be attributed to incidents of molt death syndrome (MDS), which is described as larval mortality because they are not able to completely shed their old exoskeleton during molting. In addition to nutritional deficiencies, temperatures could also result in high incidents of MDS (Zeng & Li 1992) that the rate of successful metamorphosis from zoea-5 to megalopa decreased as temperatures exceeded 30°C. The considerably higher incidents of MDS at *S. serrata* were also reported by Nurdiani & Zeng (2007) at 34°C and Hamasaki (2003) at 35°C that the survival in the megalopa stage was extremely low. These phenomena can explain the markedly low survival rate in 36-

37°C treatment where crabs reported a significantly higher molting frequency. Previous temperature studies have shown the optimal temperature range for larval rearing of both *S. paramamosain* (Zeng & Li 1992) and *S. serrata* (Hamasaki 2003) were between 25 and 30°C. A study by Nurdiani & Zeng (2007) on *S. serrata* showed that the highest survival was recorded at both 25 and 28°C.

**Conclusions.** Mud crab juveniles cultured under rising temperatures show a better growth rate, with the molting cycle being shortened. The survival of crabs decreases with temperature elevation but there is no significant difference in survival in a range from 27-28 to 33-34°C. The survival rate is an important factor determining the success of rearing. In this study, the survival rate is highest at 27-28°C and this temperature could be considered as the optimal condition for rearing this species at the juvenile stage although the growth rate of crab is slightly lower than those at higher temperatures.

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