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Effects of different temperatures on the growth and survival of mud crab (*Scylla paramamosain)* larvae

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ABSTRACT

This study aimed to evaluate the effects of temperature on the growth and survival of mud crab (Scylla paramamosain) larvae in two stages including zoea-1 to megalopa (the first experiment) and megalopa to crablet-1 (the second experiment). Each experiment was conducted in a completely randomized design with four temperature levels (27, 30, 33, and 36°C) in triplicate. Stocking density of 200 ind./L for the first experiment and 4 ind./L for the second experiment. Fiberglass tanks of 500-L (containing 300-L and 250-L of 25‰ water for the first and second experiment, respectively) were used in the experiments. The results showed that S. paramamosain larvae at zoea-3 and zoea-4 died at 33°C or above, while the highest survival rate of this stage was found at 27°C (11.5%). The growth and larval stage index during the zoea to megalopa stage were significantly higher in 30°C ($p<0.05$) compared to those in the 27°C treatment. In the second experiment, the survival rates of crablet-1 were highest (72.9%) at 27°C and lowest (34,7%) at 36°C. The growth rate of crablets at this stage increased with increased temperature. The highest carapace width and weight (3.41 mm and 0.030 g, respectively) were recorded at 36°C. The findings of this study suggested that mud crab larvae should be reared at the temperature range of 27-30°C to maximize their production.

1. INTRODUCTION

Temperature is considered one of the most important factors influencing marine species **(**Ponce-Palafox et al., 1997). Temperature is determined to be the most important modifier of energy flow and growth of aquatic animals (Brett, 1979). Temperature can affect the survival and development of crustaceans (Ruscoe et al., 2004; Kuhn, 2017). Their intermolt periods have been reported to be influenced by temperature (Leffler, 1972; Wainwright & Armstrong, 1993). Effects of temperature on the development and survival of *Scylla serrata* and *S. paramamosain* were well-documented in numerous published papers (Hill, 1974; Hill, 1980; Heasman & Fielder, 1983; Chen & Cheng, 1985; Zeng & Li, 1992; Nurdiani & Zeng, 2007; Do et al., 2020). Hamasaki (2003) found that the best temperature for survival of *S. serrata* larvae was 29°C, and the duration of larval rearing was significantly shortened with increasing temperature between 23°C and 32°C.

S. paramamosain is a common species found in the lower Mekong delta, Vietnam (Macintosh et al., 2002). It has received increasing attention for its

great taste, abundant nutrients, and medicinal uses (Wang et al., 2005; Ma et al., 2014). In the last decade, *S. paramamosain* showed its potential to contribute to aquaculture development in the future. The Mekong delta had 100 hatcheries, which produced around 200,000 juveniles/hatchery/year in 2009 (Tran & Nguyen, 2009). The number of *S. paramamosain* hatcheries significantly increased, reaching 480 in 2017 with the yearly production per hatchery ranging from 0.5 to 12 million crablets (Tran et al., 2017). *S. paramamosain* farming has been rapidly growing but is being predicted to be affected by climate change under increasing temperatures. According to IPCC (2018), the global temperature could increase from 2°C to 2.8°C in 2100, in which the Mekong delta was predicted to increase to over 32°C by 2050 (Mainuddin et al., 2010). Previously, studies on *S. paramamosain* have mainly focused on nutrition, seed production, rearing system, and digestive enzyme activities (Tran & Le, 2017; Tran, 2017; Tran et al., 2019; Tran, 2018). In an in vitro study, the embryonic development duration increases with the decrease in temperature, which is 9 days at 30°C compared to almost one month at 20°C (Zeng, 2007). Do et al. (2020) suggested that the temperature range of 27 to 30°C is optimal for mud crab juvenile culture. However, knowledge of the impacts of temperature on this species, especially at larval stages is unknown**.** To provide more information and guidelines for *S. paramamosain* production by elucidating optimal water requirements, the present study investigated the effects of different temperature levels on growth performance and survival of *S. paramamosain* at the larval stages.

2. MATERIALS AND METHODS

2.1. Animals and Materials

S. paramamosain gravid females were obtained from Ca Mau province in the Mekong Delta, Vietnam. The animals were held in a 100-L tank in the wet laboratory of the College of Aquaculture and Fisheries, Can Tho University for 12 hours at 25‰ and 27°C (±1°C). Female crabs with full appendices and active movement were selected for spawning in aquaria (one female per aquarium). Active swimming zoeal larvae were collected by siphoning with an aeration tube and transferred into the rearing tanks.

Brine water of 90‰ was obtained from a salt field in Soc Trang province, a coastal province in the Mekong delta. Brine water was orderly diluted to 25‰, treated with chlorine (concentration of 50 mg/L), and filtered through a 5 µm bag before use. The temperature treatments of 30, 33, and 36°C were controlled by the heaters (ATMAN CH 200W), and 27°C was maintained by the chiller (Teco Seachill TR-10).

2.2. Experimental design

The study was conducted over two periods of larval development including (1) from zoea-1 to megalopa stage; and (ii) from megalopa to the crablet-1 stage. All experiments were conducted in a completely randomized design with three replicates per treatment.

Effects of temperature on the development of S. paramamosain larvae from zoea-1 to megalopa stage: Larvae were randomly distributed into 12 tanks (containing 300-L of water), representing four temperature treatments including 27℃ as control; 30; 33 and 36℃ with three replicates. The larval density was 200 ind./L (60,000 larvae/tank).

Effects of temperature on the development of S. paramamosain from megalopa to crablet-1 stage: Megalopa metamorphosed from zoea-5 were immediately distributed into 12 tanks (containing 250-L of water), representing four temperature treatments as described in the first experiment. The density was 4 megalopa/L (1.000 ind./tank).

After stocking, the temperature in every single treatment was increased in different stepwise following experimental temperature treatments (1℃/16 hours in 30℃ treatment, 1℃/8 hours in 33℃ treatment, and 1℃/5.3 hours in 36℃ treatment).

Water was renewed every three days at the ratio of 30% of water in the tanks. The nauplius of *Artemia* (Vinh Chau brand, produced and packaged by the College of Aquaculture and Fisheries, Can Tho University) was used as live food. Umbrella stage *Artemia* was used for larvae from zoea-1 to zoea-3 stage with 8 feedings a day (at 0:00, 3:00, 6:00, 9:00, 12:00, 15:00, 18:00 and 21:00) (equal to 2 g cyst/m^3). At the zoea-4 stage, the larvae were fed with Frippak PL+150 (a commercial shrimp larvae feed of INVE Aquaculture) with 1 g/m^3 at 6:00 and 18:00, while *Artemia* at newly hatched stage was used for the other feeding times (equal to 4 g cyst/m^3). From zoea-5 to early megalopa stage, Lansy-shrimp PL (a commercial shrimp of INVE Aquaculture) was fed 1 g/m^3 at 0:00, 6:00, 12:00, and 18:00. and newly hatched *Artemia* (equal to 6 g/m^3) was used at 3:00, 9:00, 15:00, and 21:00. From the megalopa to the crablet-1 stage, crabs were

completely fed 100% Lansy-shrimp PL (1-2 g/m^3). At this stage, 4 m^2 plastic nets (mesh size of 5 cm) were placed in the tanks to serve as shelters to reduce cannibalism by increasing available surface area and reducing the encounters among crabs.

Dissolved oxygen (DO) and pH were measured daily using WTW Multi Oxi 3206 and WTW Multi 3510 IDS, with values ranging from 7.57 to 7.73 mg/L and 7.5 to 7.8, respectively. Salinity was also checked daily using a handheld refractometer (RES-10ATC). TAN and nitrite (NO_2^-) concentrations were analyzed weekly using methods of Griess Ilosvay, and Diazonium, and Indophenol blue. Nitrite concentration fluctuated between 0.4 and 0.5 mg/L, while TAN ranged between 0.07 and 0.09 mg/L. Throughout the experiments, the water quality parameters were acceptable for the ideal culture conditions in mud crab (Shelley & Lovatelli, 2011; Dat, 2004; Seneriches-Abiera et al., 2007; FAO, 2007).

2.3. Growth and metamorphosis observation

Larval stage index and growth of zoea were measured every three days by randomly collecting 20 individuals per tank. A microscope with an eyepiece ruler reticle (Olympus SZ51) was used to determine the developmental stages. For the growth of crablet-1, weight and carapace width (at the base of the lateral spines) were individually determined by weighing and measuring 30 crabs per replicate, respectively.

Survival rates (%) were determined as the number of larvae that successfully molted to the megalopa stage (the first experiment) and the crablet-1 (the second experiment) over the total number of stocked larvae in each replicate.

These parameters were calculated by the following formulas:

Larval stage index (LSI)

$$LSI = \frac{(N1x\ n1) + (N2\ x\ n2) + (Ni\ x\ ni)}{(n1 + n2 + ni)}$$

Where: N1, N2, Ni: stage of larvae; n1, n2, ni: the number of larvae of each stage.

Survival rate (SR)

$$SR\ (\%) = \frac{Final\ number\ of\ larvae}{Initial\ numbers\ of\ larvae} \times 100$$

2.4. Statistical analysis

All the data were subjected to statistical analysis involving standard deviation (SD) and mean (M) using Excel 2016. One-way and T-Test analysis of variance (ANOVA) together with DUNCAN's post-hoc tests were used to test for significant differences (at a significant level of 0.05) using the SPSS 16.0.

3. RESULTS

3.1. Effects of temperature on the development of *S. paramamosain* larvae from zoea-1 to megalopa stage.

3.1.1. Metamorphosis of the S. paramamosain larvae

The larval stage index (LSI) varied from 1.0±0.00 to 1.3±0.12 on day 3. LSI was significantly highest at 36°C ($p<0.05$). The impact of temperature on metamorphosis was clearly shown on day 6 with LSI values increasing with temperatures. The same trend was repeated on days 9, 12, 15, and 18. It is noticed that *S. paramamosain* larvae in 36°C only survived to day 6 with zoea-2 and zoea-3 stages, while all the larvae reared at 33°C died after 9 days of exposure when crabs developed from zoea-3 to zoea-4 stage. After 18 days of rearing, the LSI in 30°C treatment (6.18±0.05) was significantly higher than that in 27°C (5.80±0.05) (Table 1).

Table 1. Larval stage index (LSI) of *S. paramamosain* larvae from zoea-1 to megalopa stage under different temperatures

Day	Treatments			
	27°C	30°C	33°C	36°C
3	1.00±0.00^a	1.00±0.00^a	1.1±0.02^a	1.3±0.12^b
6	1.68±0.77^a	2.00±0.00^b	2.65±0.15^c	3.00±0.00^d
9	3.00±0.00^a	3.23±0.76^b	3.83±0.17^c	
12	4.10±0.42^a	4.45±0.32^b		
15	5.06±0.05^a	5.35±0.05^b		
18	5.80±0.05^a	6.18±0.05^b		

Values are expressed as mean±SD. Different letters (a, b, c, d) in the same rows signify a significant difference ($p<0.05$).

3.1.2. Growth of S. paramamosain larvae at different sampling times

The results reveal that the length of the larvae was improved with increased temperature. After 3 and 6 days, the 36°C treatment showed the highest length (1.75±0.01 mm and 2.58±0.13 mm, respectively) which was significantly higher than those of other treatments, while the lowest length was found in the control treatment (27°C) (1.59±0.02 mm and 1.84±0.01 mm, respectively). No larvae survived in the 36°C treatment on day 9th, leaving the 33°C treatment had the highest length (3.95±0.12 mm) on day 9th, compared to other treatments. On day 12th, only crabs at 27°C and 30°C survived and the higher temperatures showed a better growth rate. It indicates that *S. paramamosain* larvae could not survive at 33°C or above, and the length values of larvae in the 30°C treatment were lower than the control treatment on day 15th and 18th (Table 2).

Table 2. Length of larvae (mm) from zoea-1 stage to megalopa stage

Day	Treatments			
	27°C	**30°C**	**33°C**	**36°C**
3	1.59±0.02[a]	1.67±0.02[b]	1.68±0.01[c]	1.75±0.01[d]
6	1.84±0.01[a]	1.94±0.02[a]	2.18±0.02[b]	2.58±0.13[c]
9	2.92±0.03[a]	3.50±0.03[b]	3.95±0.12[c]	
12	4.01±0.04[a]	4.59±0.04[b]		
15	4.61±0.01[b]	4.51±0.04[a]		
18	4.22±0.03[b]	4.13±0.02[a]		

Values are expressed as mean±SD. Values with different letters (a, b, c, d) in the rows signify a significant difference ($p<0.05$).

3.1.3. Survival rate

The survival rate of larvae in the 27°C treatment (11.5±1.08%) was significantly higher than that of the 30°C treatment (7.5±0.04%). In this study, all the larvae in the 36 and 33°C treatments died after 6 and 9 days, respectively (Fig. 1).

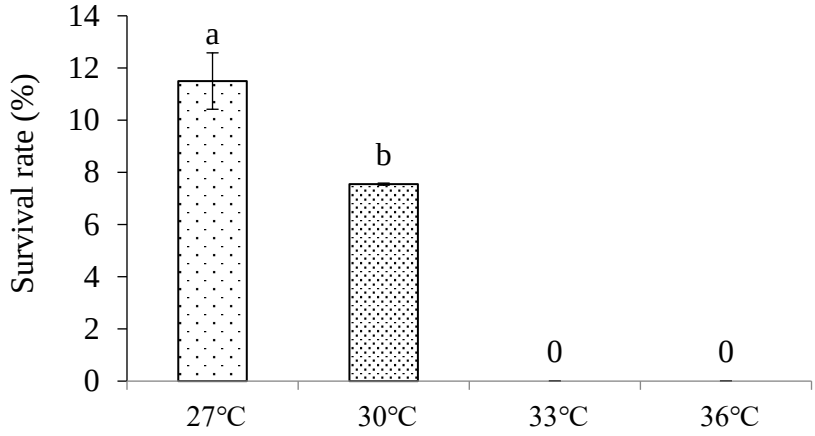

Fig. 1. The survival rate of *S. paramamosain* larvae was reared at different temperatures

(Values are expressed as mean±SD; Bars with different letters (a, b) signify a significant difference, $p<0.05$)

3.2. Effects of temperature on the development of mud crab larvae from megalopa to crablet-1 stage.

3.2.1. Growth of S. paramamosain reared in different temperatures

The temperature had shown influences on the growth performance of the megalopa stage. The growth in carapace width as well as in weight increased with increased temperature levels (Table 3). The highest values were in 36°C treatment (3.41±012 mm, 0.030±0.12 g, respectively) which were significantly higher than the three lower temperature treatments, with the lowest being recorded in the treatment of 27°C (2.13±0.086 mm, 0.020±0.04 g, respectively). It is noticeable that the megalopa reared at 27°C and 30°C showed insignificant differences in both growth rate indicators ($p>0.05$).

Table 3. Weight and carapace width (CW) of *S. paramamosain*

Parameters	Treatments			
	27°C	**30°C**	**33°C**	**36°C**
Carapace width (mm)	2.13±0.086[a]	2.5±0.40[ab]	2.90±0.11[b]	3.41±012[c]
Weight (g)	0.020±0.04[a]	0.023±0.01[ab]	0.024±0.00[b]	0.030±0.12[c]

Values are expressed as mean±SD. Different letters (a, b, c) in the rows signify a significant difference ($p<0.05$).

3.2.2. Survival rate

After 7 days of rearing, there were significant differences among temperature treatments. Survival rates decreased when temperature increased. The highest rate (72.9%) was recorded in the 27°C treatment which was significantly higher than those of the other three treatments. It is clearly shown that the survival rate of the megalopa reared at 36°C was reduced dramatically, just 34.7% (Fig.2). In general, the temperature had shown its effects on the survival rate of mud crab from megalopa to crablet-1 stage.

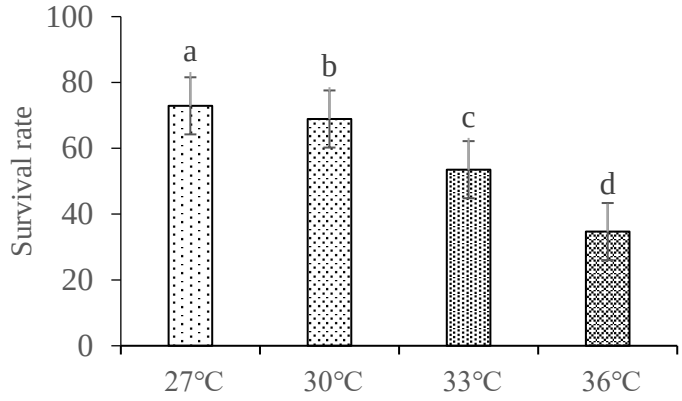

Fig. 2. The survival rate of *S. paramamosain* crablet-1 under different temperatures

(Values are expressed as mean±SD; Bars with different letters (a, b, c, d) signify a significant difference, $p < 0.05$).

4. DISCUSSION

Van-Wormhoudt and Bellon-Humbert (1994) reported that in crustaceans, raised temperatures and proper light intensity increase their molt frequency, while lower temperature prolongs metamorphosis time. Metamorphosis was recorded to be faster with increased temperature (Marichamy & Rapackiam, 1991). According to Smith's crustacean molt-process model, the accumulation of sufficient degree-day exposure is one of the necessary conditions for molting (Smith, 1997). Zeng and Li (1992) stated that the metamorphosis of *S. serrata* larvae was faster in higher temperatures. The results are in agreement with the previous studies carried out on the same species that the length of larvae increased from zoea-1 to zoea-5 stage then decreased at megalopa and crablet-1 stages with values of 1.65; 2.18; 2.70; 3.54; 4.50, 4.01, and 2-3 mm (Nguyen & Tran, 2009; Tran & Truong, 2004). In this study, under the impact of temperature, the LSI value in the 30°C treatment was higher than in the control (27°C) treatment. It is quite different from other species in that growth is continuous (von Bertalanffy, 1938). For crustaceans, growth is discrete and biphasic, and their rigid exoskeleton limits external growth to the brief molting events (Hartnoll, 1982; Brylawski & Miller, 2006). In the current study, on the same monitoring day the higher the LSI values were, the longer the length of the zoea was observed. The present study also indicates that metamorphosis might appear sooner in the higher temperature treatment resulting in higher growth of the larvae. The zoea nursed at 33°C and 36°C metamorphosed faster, but they completely died after 6 days and 9 days, respectively. This suggests that the suitable temperature condition for mud crab from the zoea-1 stage to the megalopa stage was in the range of 27-30°C. The rising temperature leads to increases in metabolic rate and digestive enzyme activities; fish effectively consume more feed to meet energy demand Do & Nguyen, 2010). This could partially explain the higher growth rate of mud crabs. These results are also in agreement with the study carried out by Do et al. (2020) that the growth rate of

crablet-1 increases with temperature levels. In addition, α-amylase and trypsin activities are impacted by elevated temperatures, which were significantly higher than those of lower temperatures (Do et al., 2020).

The growth performance of *S. paramamosain* in the current work showed that larvae reared in higher temperature conditions presented a better growth rate with shortened larval development time. Although at the 30℃ treatment, the length of larval from zoea-1 stage to megalopa stage was longer than that in 27℃, the survival rate was lower. This was explained that there can be significant cannibalism of mud crab larvae when metamorphosing from zoea-5 to megalopa stage (Shelley & Lovatelli, 2011). In addition, Azra et al. (2018) documented that active swimming and mobility create greater chances of fighting and cannibalism resulting in higher mortality in blue swimmer crab (*Portunus pelagicus*) at high temperatures. The higher mortality could be attributed to incidents of molt death syndrome (MDS), describing as larval mortality because they are not able to completely shed their old exoskeleton during molting. MDS was observed to contribute significantly to high zoea-5 of *S. serrata* mortalities at 34℃ (Nurdiani and Zeng, 2007). Besides, Zeng and Li (1992) reported that high temperature could also trigger high incidents of MDS and the rate of successful metamorphosis from zoea-5 to megalopa of *S. serrata* decreased as temperatures exceeded 30°C. A similar result was also found in *Penaeus monodon* at the juvenile stage, the lowest survival rate was reared at 36℃ treatment (0%), while the highest rate was in the 27℃ treatment (65%) (Do et al. 2018).

The temperature was by far the most significant factor affecting the survival and growth performance of mud crab in this study. Similar results were found by Ruscoe et al. (2004) that *S. serrata* larvae (crablet-2 stage) reared at 30℃ show the highest growth rate and shortest intermolt duration in comparison with those of lower (20 and 25°C) or higher (35°C) temperatures. In the current study, the elevated temperature resulted in decreases in the survival rate of larvae from megalopa to crablet-1 stage, whereby the survival rate at 33 and 36℃ were 53.5% and 34.7% as compared to 72.9% and 68.9% at 27℃ and 30℃ treatments, respectively. Although the *S. paramamosain* larvae in the 33 and 36℃ treatments displayed high carapace width and weight values, their survival rates were lower than those in the 27 and 30℃ treatments. This result showed that the best temperature range for rearing megalopa to crablet-1 stage is 27 to 30℃. This can be explained that the low temperature condition is likely more favorable for the process of molting from megalopa to crablet-1 than high temperature. The aquatic species spend less energy for metabolism at low temperatures, and the saved energy could be used for other physiological processes including the molting process (Syafaat et al., 2020). Syafaat et al. (2020) also documented that the highest survival rate from megalopa to crablet-1 at 24 and 28℃ was 100%, but all crablet-1 died 1 day after molting at 36℃. In the present study, the survival rate of megalopa to crablet-1 was only 34% which was similar to the results of Do et al. (2020) that the survival rate of crabs rearing from crablet-1 to crablet-5 at 36°C was also low (12%). These results verified that the temperature of 36℃ is not suitable for *S. paramamosain* larval rearing. The study found that the optimal temperature for rearing zoea to megalopa and megalopa to crablet-1 was similar to that published by Syafaat et al. (2021), in which the growth and survival rate of *S. paramamosain* were highest at the temperature between 28 and 30°C. The temperature in the range of 27℃ to 30℃ is recommended for *S. paramamosain* larval rearing from zoea to megalopa and megalopa to crablet-1 as it provides a high larval survival rate.

5. CONCLUSIONS

S. paramamosain larvae grows better at 27-30℃ and the highest survival rate is at 27℃. Zoeal larvae die at 33℃ and above. Megalopa stage has a high survival rate at 27℃ and 30℃, but it decreases significantly at the higher temperatures. The growth rate of mud crab larvae increases with increased temperature.

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