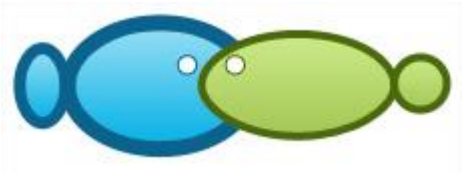

Effects of combined temperature and salinity on growth and digestive enzymes of mud crab *(Scylla paramamosain)* from larvae to juvenile

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Abstract. The present study evaluated the growth performance, survival rate, and digestive enzyme activities of mud crabs, *Scylla paramamosain* from larvae to juvenile stages in high temperatures and at different salinities. The study comprises three experiments that include theree temperature levels (27°C, 30°C, and 33°C) and two salinity levels (25‰ and 30‰). The mud crabs were fed according to their developmental stage. Thus, *Artemia* (umbrella stage) was used for zoeal stages, Lansy PL and *Artemia* for crablet 1 to crablet 5, and *Artemia* biomass for crablet 5 to the juvenile stage. In the nursing of *mud crab*, the survival rate was influenced by the temperature and salinity combinations. The survival rate was higher at the salinity of 25‰ as it follows: from zoea to megalopa (8.50%) and megalopa to crablet 1 (25.5%) in 30°C, crablet 1 to crablet 5 (27.3%) in 33°C, and crablet 5 to the juvenile (31.3%) in 27ºC. As the temperature increased, the survival rate decreased in zoea and crablet 5, however it was the opposite in megalopa and crablet 1 (survival decreased as temperature decreased). The highest growth of mud crab was observed for 33°C temperature and 25‰ salinity in all the developmental stages. Digestive enzyme activities such as trypsin, chymotrypsin, and amylase were highly active in crablet 5 and juvenile in the salinity 30‰ at temperatures 30°C and 33°C, respectively. The findings of this study indicated that the combination of the temperature and salinity does not affect significantly the digestive enzyme activities of crablet 5 and juvenile satges; and the 33°C temperatures and 25‰ salinity are suitable for nursing mud crab from megalopa to juvenile stages.
Key Words: *Scylla paramamosain*, temperature, salinity, growth, enzyme.

Introduction. *Scylla paramamosain* is a large portunid crab found along the coasts of southern China and many countries in the Indo-Pacific. The species is considered an important species for both fisheries and aquaculture in China, due to its abundance, rapid growth rate, and high market acceptance (Ye et al 2011; Dewantara & Sulistiono 2017). In Vietnam, the most prevalent species used in mud crab aquaculture farms is *S. paramamosain* (Christensen et al 2004). Research on mud crab breeding has been extensively carried out in Hawaii, Africa, Australia, Japan, Indian, Malaysia, Philippines, Indonesia, Thailand, and Vietnam (Ong 2018). However, there is currently unmet demand for seed supply (crablets) for mud crab culture on a mass scale (Petersen et al 2013). The majority of mud crab farmers rely on wild seed collection that has declined due to over-exploitation, decreasing mangrove habitats (Le Vay et al 2001), and pollution (Ma et al 2006). Meanwhile, the hatchery-reared seed is limited due to low success rates in first-stage crablet hatchery production (around 10%) (Hamasaki et al 2011). The erratic larvae survival rate and unsatisfactory crablet development can be caused by sub-optimal conditions of any of the following interrelated factors including diets, diseases, and conditions of rearing (Waiho et al 2018).

Temperature is one of the most important environmental factors influencing marine organism physiological behavior, and distribution (Ruscoe et al 2004). Temperature is also known to exert a strong influence on the molting frequency, which is controlled by the ecdysteroid and its receptors (Bortolin et al 2011). The average larval growth period from zoea 1 to zoea 5 at 30ºC in the *S. paramamosain* was reportedly shortened by 7 days compared to 25ºC when the larvae zoea 1 failed to molt at a lower temperature 15ºC (Zeng & Li 992). Shelley & Lovatelli (2011) reported that the

temperature of 25-32°C is preferred for mud crab larviculture reflecting the species in tropical to subtropical distribution, but the survival from zoea to megalopa is affected when larvae are cultured at temperatures over 30°C found in China.

Salinity is one of the most variable water quality factors, and it can have a direct impact on aquatic organisms' osmotic regulation (Wang et al 2019) as well as their immune and digestive enzyme activities (Wang & Chen 2006). In addition, under different salinity conditions, amino acid composition and flavor are also different (Jackson et al 1992). Salinity is another significant environmental factor that influences *S. paramamosain* overwintering (Zhen & Shao 2001). Salinity not only affects the survival rate but also the molt and metamorphosis time of mud crab larvae (Nguyen & Truong 2004). At low salinities, both high and low temperatures resulted in mass mortality of newly hatched larvae, demonstrating the combined impact of temperature and salinity on larval survival. However, a higher temperature range of 28-30°C and a salinity range of 20-30‰ is recommended for aquaculture because it shortens the culture period (Nurdiani & Zeng 2007). All enzymes had identical temperature profiles, with maximum activity at 50°C. The activity of enzymes increased with increasing temperature, peaking at 50°C (Pavasovic 2004). The effect of salinity on the digestive enzyme was also reported by Zhou et al (2020) where the activity was highest at 25‰ and was the lowest at 4‰ (Pavasovic 2004).

The above-cited studies indicated that the effects of single environmental factors such as temperature and salinity on mud crabs have been intensively conducted. However, the combined effects of these two factors, especially at elevated temperatures have not been documents yet. This study, therefore, aimed to determine how elevated temperatures at different salinities affect the growth, survival, and digestive enzyme activities of mud crab (*S. paramamosain*) from larvae to juvenile stages. The results acquired will be an asset to the hatchery techniques of this species.

Material and Method

Broodstock management. A berried female of *S. paramamosain* was collected from a mud crab hatchery in Ca Mau province in Mekong Delta, Viet Nam. The crab was kept in a 100-L tank at the experimental laboratory (wet-lab) of the College of Aquaculture and Fisheries, Can Tho University, Can Tho city, Viet Nam. Saline water was transported from Soc Trang province, a coastal province in the Mekong Delta. Before use, saline water was diluted to 30‰, then treated with chloride (50 mg L^{-1}) and filtered with 5 µm bag net. Heaters were used to rise and control temperatures in 30ºC and 33ºC treatments and coolers (Teco Seachill TR 10) were used in 27ºC treatment to prevent temperature fluctuation. After hatching, zoea was aggressively gathered via siphon and transferred to the rearing tanks.

Experimental design. Three experiments were conducted according to the developmental stages of mud crab including the first experiment from zoeal to megalopa stages then megalopa to crablet 1 stages; the second experiment from crablet 1 to crablet 5 stages, and the third experiment from crablet 5 to juvenile stages. The mud crab (zoea, megalopa, crablet 1, and crablet 5 stages) were distributed randomly to 18 tanks with a capacity of 500-L representing 6 treatments which are combinations of three temperatures (27, 30 and 33ºC) and two salinities (25 and 30‰) with three replicates for each treatment.

For the first experiment, the recommended density for mud crabs from zoea to megalopa was 200 larvae L^{-1} (Shelley and Lovatelli 2011), which means that the total larvae were 60,000 ind./500-L tanks (containing 300-L of water), which means 200 ind. L^{-1}. The experiment was followed for 10 days, which is the time for the transition to the megalopa phase. Then, the stock density was 4 ind. L^{-1} for the stage development from megalopa to crablet. The crabs were observed for 1 week until they entirely metamorphosed to crablet 1. *Artemia* (umbrella stage) was used to feed on zoea 1 to zoea 2, and the larvae were fed 8 times per day at 24:00, 3:00, 6:00, 9:00, 12:00, 15:00, 18:00, and 21:00 with 1-2 g/m^3/time (zoea 1) and 2-3 g/m^3/time (zoae 2). At

zoea stage 3 and 4, larvae were fed with Lansy (feed for marine shrimp postlarvae) (2-3 g/m^3/time) at 9:00 and 15:00, while *Artemia* (3-4 g/m^3/time, incubated 18 hours for zoea 3 and 4-5 g/m^3/time incubated 21 hours for zoea 4) was used at other feeding times. At zoea 5 to megalopa, larvae were fed with Lansy (2-3 g/m^3/time) at 9:00, 15:00, and 21:00 while *Artemia* (5-6 g/m^3/time) was used at other feeding times. From megalopa to crablet 1, crabs were fed with 100% of Lansy (1.5-3 g/m^3/time) at all feeding times. Tanks were provided with 5 mm nets as shelter to prevent crabs from cannibalism in all experiments.

For the second experiment, crablet 1 to crablet 5 stage, the density was 100 ind./200-L tanks, which means 0.1 ind. L^{-1}. Nighty crabs were stocked in the tank and 10 crabs in separate plastic boxes (1 crab/box) for molting observation. Crabs were fed with biomass *Artemia* (3-5 ind./crab/time) four times/day. The duration of this experiment was 20 days. In the third experiment, crablet 5 to juvenile stage, 30 crabs were distributed in 500-L composite tanks (containing 200-L water), which means 0.06 ind. L^{-1}. Also, 10 crabs were reared in separate plastic boxes (1 crab/box) for molting observation. Crabs were fed with biomass *Artemia* (5-8 ind./crab/time) two times per day. The duration of this experiment was 30 days.

Acclimation to variation in water temperature and salinity was done gradually. Temperature levels were increased by 1ºC/8 hours for 30ºC treatment and by 1ºC/4 hours for 33ºC treatment. Salinity in 25‰ treatments was decreased by 1‰/6 hours from 30‰.

Sample analysis. In the first experiment, ten zoeal larvae per tank were collected every 3 days to monitor the growth development and larval stage index (LSI) under a microscope. The length and weight were determined at the end of the experiment, and the number of survived megalopa and crablet 1 were counted for calculating the survival rate. For the second and third experiments, 20 crablets per tank were randomly collected every 10 days for recording growth data (weight and carapace width). Molts of crabs were checked daily in the morning for calculating the molt cycle and molt frequency. The carapace width (CW) was defined as the distance between the two anterior-lateral spines, which was measured using the nearest millimeter caliper; and the weight was measured individually using balances with four decimals for crablet 1 and two decimals for crablet 5 and juvenile.

The larval stage index was calculated by using the following formula:

$$\text{Larval Stage Index (LSI)} = \frac{[(N1 * n1) + (N2 * n2) + (Ni * ni)]}{(n1 + n2 + ni)}$$

Where:
N1, N2, and Ni are the larval stages
n1, n2, and ni are the number of larvae of each stage.

The survival rate of larvae was calculated by using the following formula:

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

At the end of the experiments, to analyze digestive enzyme activity, 3 samples per tank were taken from crablet 5 (removal of a limb, whole-body) and juvenile (intestine and hepatopancreas), were thawed on ice, and homogenized with KH_2PO_4 20 mM and NaCl 6 mM buffer, pH 6.9. The mixture was centrifuged at 4,200 rpm, 4ºC in 30 minutes, and supernatants were extracted and processed at -80ºC for further study. Chymotrypsin activity was measured using the method described by Worthington (1982). Amylase activity was calculated using the method described by Bernfeld (1951) and the method of Tseng et al (1982) was used for trypsin.

Water quality monitoring. Salinity levels in tanks were checked daily using a refractometer (RES-10ATC) and the temperature was measured by using a thermometer at 8:00 AM and 2:00 PM. Nitrite (NO_2) and TAN concentrations were recorded every 3 days

using methods (Griess Ilosvay, Diazonium, and Indophenol blue), using WTW Multi Oxi 3206, which measured dissolved oxygen (DO). WTW Multi 3510 IDS measured the pH. Water was exchanged to keep good water quality at a rate of 30% per tank around two times per week.

Data analysis. The larval stage index was calculated by using the formula below and the zoeal larval survival was presented in percentage (%). The growth and survival of zoea to the juvenile stage under various treatments were analyzed with two-way ANOVA using SPSS 16.0. The DUNCAN test was performed to detect specific significant differences among the treatments at a significance level of $p < 0.05$. Excel 2016 was used for the calculation of the mean and standard deviation.

Results

Water quality parameters. The water quality parameters such as temperature and salinity in the first, second, and third experiments were maintained constant (27°C, 30°C, 33°C and 25‰, 30‰). There was no significant fluctuation observed for pH and dissolved oxygen (DO) in the first and second experiments. The total ammonia nitrogen (TAN) ranged from 0.83±0.00 to 1.22±0.19 among the treatments. In the third experiment, pH and dissolved oxygen (DO) levels did not change significantly. The total ammonia nitrogen (TAN) ranged from 0.40±0.00 to 0.57±0.06 mg L^{-1} among the treatments.

Effect on growth performance. The length of zoea increased gradually with the elevated temperature levels during the first 12 days as indicated in Table 1. Treatment with temperatures of 27°C combined with 25‰ and 30‰ was statistically significantly lower compared to other treatments during the first 9 days ($p < 0.05$). The treatment of 27°C-30‰ (4.39 mm) had the highest growth in length, while the treatment of 30°C-30‰ (4.20 mm) was the lowest on the 15th day, although no statistically significant difference was found among the treatments ($p > 0.05$). There was no observed effect of salinity and combined temperature and salinity on the length of zoea ($p > 0.05$) (Table 1).

Table 1

The length of the zoeal stage (mm) during 15 days of the experiment

Treatment	*Experimental time (days)*				
	3	*6*	*9*	*12*	*15*
27°C-25‰	1.54±0.08	1.94±0.11	2.73±0.08	4.17±0.13	4.30±0.01
27°C-30‰	1.54±0.07	1.85±0.15	2.77±0.20	4.15±0.18	4.39±0.12
30°C-25‰	1.70±0.12	2.36±0.12	3.10±0.13	4.13±0.10	4.28±0.09
30°C-30‰	1.87±0.11	2.38±0.10	3.10±0.02	4.16±0.08	4.20±0.10
33°C-25‰	1.70±0.08	2.42±0.15	3.19±0.16	4.34±0.04	4.25±0.05
33°C-30‰	1.72±0.10	2.43±0.22	3.40±0.04	4.30±0.14	4.23±0.05
Temperature					
27°C	1.54±0.07^{a}	1.90±0.13^{a}	2.75±0.14^{a}	4.16±0.14^{a}	4.35±0.09
30°C	1.79±0.13^{b}	2.37±0.10^{b}	3.10±0.08^{b}	4.14±0.08^{a}	4.24±0.10
33°C	1.71±0.08^{b}	2.42±0.17^{b}	3.29±0.15^{c}	4.32±0.10^{b}	4.24±0.05
Salinity					
25‰	1.65±0.11	2.24±0.25	3.01±0.24	4.21±0.13	4.28±0.06
30‰	1.71±0.16	2.22±0.31	3.09±0.29	4.20±0.14	4.27±0.12
P-value Temperature × salinity	0.309	0.777	0.338	0.842	0.258
Salinity	0.213	0.777	0.172	0.847	0.954
Temperature	0.002	< 0.001	< 0.001	0.048	0.064

Note: Values are presented as the mean±SD (standard deviation). Values with different superscript letters (a, b, c) in the same column of temperature indicate a significant difference ($p < 0.05$).

The combined effect of temperature and salinity influenced the metamorphosis index (LSI) of zoea after 3 days of rearing (Table 2). After 12 and 15 days of rearing, the effect of temperature was more defined. The 33°C treatments at 15 days had the highest average LSI 5.97 at 25‰ and 5.93 at 30‰; these values were statistically significantly different ($p < 0.05$) if compared to those of the temperature 27°C and 30°C combined with salinity 25‰ and 30‰.

Table 2

Larval Stage Index (LSI) of the zoeal stage during 15 days of the experiment

Treatment	*Experimental time (days)*				
	3	*6*	*9*	*12*	*15*
27°C-25‰	1.47±0.15^{ab}	2.43±0.06^{a}	3.50±0.10^{a}	4.87±0.06^{a}	5.13±0.15^{a}
27°C-30‰	1.37±0.21^{a}	2.23±0.21^{a}	3.63±0.21^{a}	4.93±0.06^{a}	5.13±0.06^{a}
30°C-25‰	1.67±0.06^{b}	2.97±0.06^{b}	4.00±0.10^{b}	4.87±0.12^{a}	5.73±0.25^{b}
30°C-30‰	1.97±0.06^{c}	2.93±0.06^{b}	4.00±0.00^{b}	4.93±0.06^{a}	5.53±0.06^{b}
33°C-25‰	2.00±0.00^{c}	2.80±0.17^{b}	4.03±0.06^{b}	5.03±0.06^{b}	5.97±0.06^{c}
33°C-30‰	1.97±0.06^{c}	2.83±0.29^{b}	4.10±0.00^{b}	5.00±0.00^{b}	5.93±0.06^{c}
Temperature					
27°C	1.42±0.17	2.33±0.18^{a}	3.57±0.16^{a}	4.90±0.06^{a}	5.13±0.10^{a}
30°C	1.82±0.17	2.95±0.05^{b}	4.00±0.06^{b}	4.90±0.09^{a}	5.63±0.20^{b}
33°C	1.98±0.04	2.82±0.21^{b}	4.07±0.05^{b}	5.02±0.04^{b}	5.95±0.05^{c}
Salinity					
25‰	1.71±0.25	2.73±0.25	3.84±0.27	4.92±0.11	5.61±0.40
30‰	1.77±0.32	2.67±0.37	3.91±0.24	4.96±0.05	5.53±0.35
P-value					
Temperature × salinity	0.021	0.48	0.564	0.357	0.385
Salinity	0.318	0.413	0.205	0.31	0.225
Temperature	< 0.001	< 0.001	< 0.001	0.015	< 0.001

Note: Values are presented as the mean±SD. Values with different superscript letters (a, b, c) in the same column indicate a significant difference ($p < 0.05$).

The temperature has the strongest influence on the width of crablet 1 (Table 3). Crablet 1 had the highest mean width (3.06 mm) in the 33°C-25‰ treatment and the lowest mean width (2.95 mm) in the 27°C-25‰ treatment. The weight of crab in six treatments ranged from 7.28 to 9.86 mg; but the difference among treatments was not statistically significant ($p > 0.05$). The growth of crablet 5 is proportional to temperature and is affected by the combined temperature and salinity (Table 3). After 20 days of rearing, the weight of crablet 5 in the 33°C treatment group achieved the highest average weight (0.76 g). The carapace width of crablet 5 reached a maximum value of 1.41 cm after 20 days of rearing in 30°C-25‰ and minimum (1.12 cm) in 27°C-30‰.

Juvenile mud crabs achieved the highest average weight (4.50 g) in 33°C-25‰ water and the lowest (2.84 g) was in 27°C-25‰ after 20 days of rearing. In terms of carapace width, the interaction of temperature and salinity did not affect the growth rate (Table 3). After 20 days of rearing, the carapace width of juveniles reached the highest value of 2.90 cm and at 33°C-25‰ and lowest value of 2.52 cm at 27°C-25‰. Treatments of temperature groups 30°C and 33°C were not statistically significantly different as compared to the treatments of temperature group 27°C ($p > 0.05$).

Table 3

Weight and carapace width of mud crab at different stages compiled from three experiments

Treatment	Crablet 1		Crablet 5		Juvenile	
	Weight (mg)	Width (mm)	Weight (g)	Width (cm)	Weight (g)	Width (cm)
27°C-25‰	7.28±0.45	2.95±0.08^{a}	0.58±0.06^{ab}	1.12±0.06^{a}	2.84±0.19^{a}	2.52±0.09^{a}
27°C-30‰	7.95±0.63	2.96±0.02^{a}	0.57±0.05^{bc}	1.12±0.05^{c}	2.97±0.48^{a}	2.55±0.20^{a}
30°C-25‰	9.86±3.88	3.02±0.03^{ab}	0.73±0.08^{c}	1.41±0.06^{c}	3.69±0.44^{b}	2.80±0.10^{b}
30°C-30‰	8.74±0.69	3.04±0.06^{ab}	0.69±0.16^{a}	1.22±0.11^{a}	3.56±0.18^{b}	2.76±0.07^{b}
33°C-25‰	9.50±2.17	3.06±0.04^{b}	0.76±0.04^{abc}	1.35±0.02^{ab}	4.50±0.11^{c}	2.90±0.05^{b}
33°C-30‰	9.10±2.02	3.05±0.05^{b}	0.72±0.04^{bc}	1.30±0.03^{bc}	4.03±0.32^{c}	2.84±0.04^{b}
Temperature						
27°C	7.62±0.55	2.97±0.57^{a}	0.57±0.05^{a}	1.12±0.05	2.91±0.33^{a}	2.54±0.14^{a}
30°C	9.30±2.56	3.03±0.44^{ab}	0.71±0.11^{b}	1.31±0.13	3.62±0.31^{b}	2.78±0.08^{b}
33°C	9.30±1.83	3.05±0.42^{b}	0.74±0.04^{b}	1.33±0.03	4.27±0.34^{c}	2.87±0.05^{b}
Salinity						
25‰	8.88±2.49	3.01±0.07	0.69±0.10	1.29±0.14	3.68±0.76	2.74±0.18
30‰	8.60±1.24	3.02±0.05	0.66±0.11	1.21±0.10	3.52±0.55	2.72±0.17
P-value						
Temperature × salinity	0.852	0.770	0.938	< 0.044	0.282	0.72
Salinity	0.733	0.576	0.423	0.016	0.319	0.679
Temperature	0.296	0.043	0.008	< 0.001	< 0.001	< 0.001

Note: Note: Values are presented as the mean±SD. Values with different superscript letters (a, b, c) in the same column indicate a significant difference ($p < 0.05$).

Effect on molting. The average number of molting times among treatments changed between 2.93 and 3.27 times after 20 days of rearing the crablet 5 (Table 4). Crablet 5 molted the most in the 30°C-25‰ treatment (3.27 times), and the least in the 33°C-25‰ treatment (2.93 times). The average molting cycle ranged from 6.08 to 6.36 days. Treatment 33°C-30‰ had the shortest molting cycle (6.08 days), while treatment 27°C-25‰ had the longest (6.36 days).

The average maximum molting (2.78 times) for juvenile mud crabs was observed in 33°C-30‰ while minimum molting (2.02 times) was in 27°C-30‰ (Table 4). The average number of molting times between treatments varied between 2.02 and 2.78 times. Treatment with temperature 27°C was statistically significantly different from the treatments with temperature groups 30°C and 33°C ($p < 0.05$). The average molting cycle lasted 7.54 to 8.73 days on average. The molting cycle was the shortest (7.54 days) in 30°C-25‰ and the longest (8.73 days) was in 27°C-30‰. There was no significant difference between the treatments of 30°C and 33°C ($p > 0.05$) compared to treatments of 27°C ($p < 0.05$).

Table 4

Molting frequency and molting cycle during crablet 1 to 5 and crablet 5 to juvenile stage

Treatment	Crablet 1 to 5 (2^{st} experiment)		Crablet 5 to Juvenile (3^{rd} experiment)	
	Molting frequency (time)	Molting cyle (day)	Molting frequency (time)	Molting cyle (day)
27°C-25‰	3.11±0.10	6.36±0.36	2.10±0.13^{a}	8.35±0.32^{b}
27°C-30‰	2.97±0.15	6.34±0.37	2.02±0.14^{a}	8.73±0.18^{b}
30°C-25‰	3.27±0.12	6.15±0.16	2.53±0.24^{b}	7.54±0.41^{a}
30°C-30‰	3.23±0.20	6.26±0.35	2.57±0.15^{b}	7.75±0.33^{a}
33°C-25‰	2.93±0.25	6.31±0.16	2.74±0.21^{b}	7.99±0.27^{a}
33°C-30‰	3.23±0.15	6.08±0.21	2.78±0.13^{b}	7.73±0.38^{a}

Temperature				
27°C	3.04±0.14	6.35±0.33	2.06±0.13^{a}	8.54±0.31^{b}
30°C	3.25±0.15	6.21±0.25	2.55±0.18^{b}	7.65±0.35^{a}
33°C	3.08±0.15	6.19±0.22	2.76±0.16^{b}	7.86±0.33^{a}
Salinity				
25‰	3.10±0.21	6.27±0.23	2.46±0.33	7.96±0.45
30‰	3.14±0.20	6.23±0.30	2.46±0.36	8.07±0.56
P-value				
Temperature × Salinity	0.097	0.584	0.761	0.256
Salinity	0.626	0.741	0.979	0.484
Temperature	0.122	0.575	<0.001	<0.001

Note: Values are presented as the mean±SD. Values with different superscript letters (a, b, c) in the same column indicate a significant difference ($p < 0.05$).

Effect on survival. The survival rates of mud crab from larvae to juveniles reared in six different treatments with elevated temperatures at different salinities are shown in the Figures 1, 2, 3, and 4. The survival of zoea to megalopa was highest in 30°C-25‰ (8.50%) whereas the lowest was in 33°C-30‰ (0.52%) (Figure 1). Crablet 1 survived the most in 30°C-25‰ (25.5%) and the least in 27°C-30‰ (0.85%) (Figure 2). Temperature and salinity have a highly significant effect on the survival of crablet 1 ($p < 0.05$). After 20 days of raising, the survival rate of crablet 5 did not differ among the treatments, ranging from 21.3 to 27.3% (Figure 3). The highest survival rate (27.3%) was in 33°C-25‰ and the lowest (21.3%) in 27°C-25‰, but the difference was not statistically significant ($p > 0.05$). The survival rate of juvenile mud crabs ranged from 27.7 to 31.3% after 20 days of rearing (Figure 4). High survival of juveniles was observed when reared at 27°C-25‰ (31.3%) while very low survival was in 33°C-25‰ (27.7%). The survival rates of crablet 5 and juveniles were not significantly affected by temperature or salinity ($p > 0.05$).

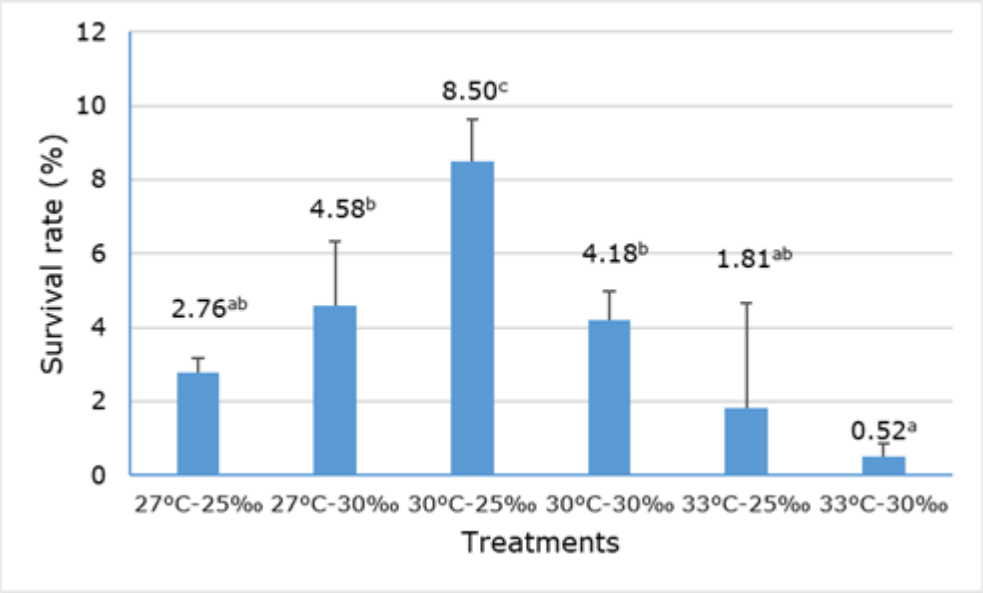

Figure 1. The survival rate of zoea to megalopa (Note: Values are presented as the mean±SD. Different letters (a, b, c) above bars indicate significant differences among treatments, p-value temperature × salinity < 0.05).

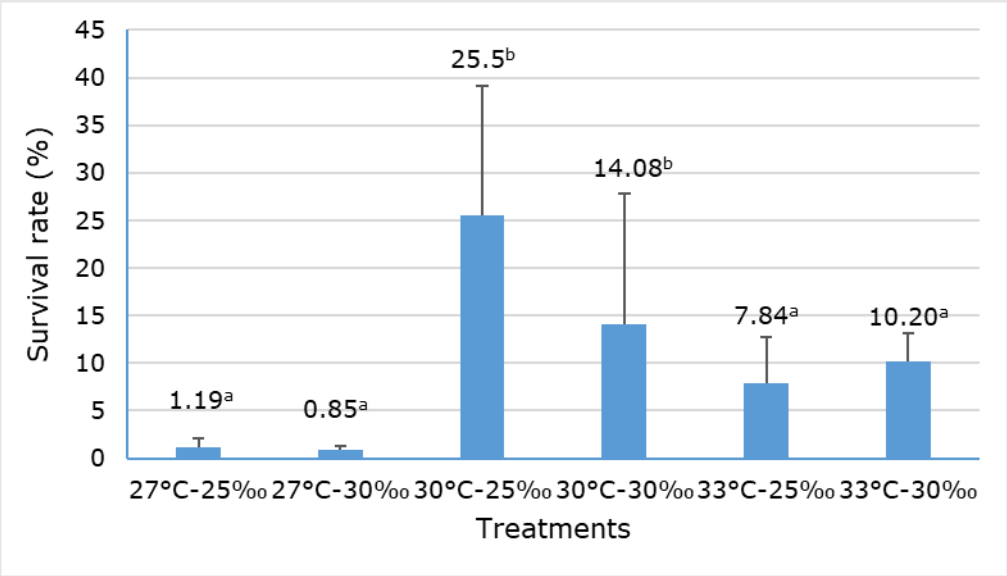

Figure 2. The survival rate of crablet 1 (Note: values are presented as the mean±SD. Different letters (a, b) above bars indicate significant differences among treatments, p-value temperature × salinity < 0.05).

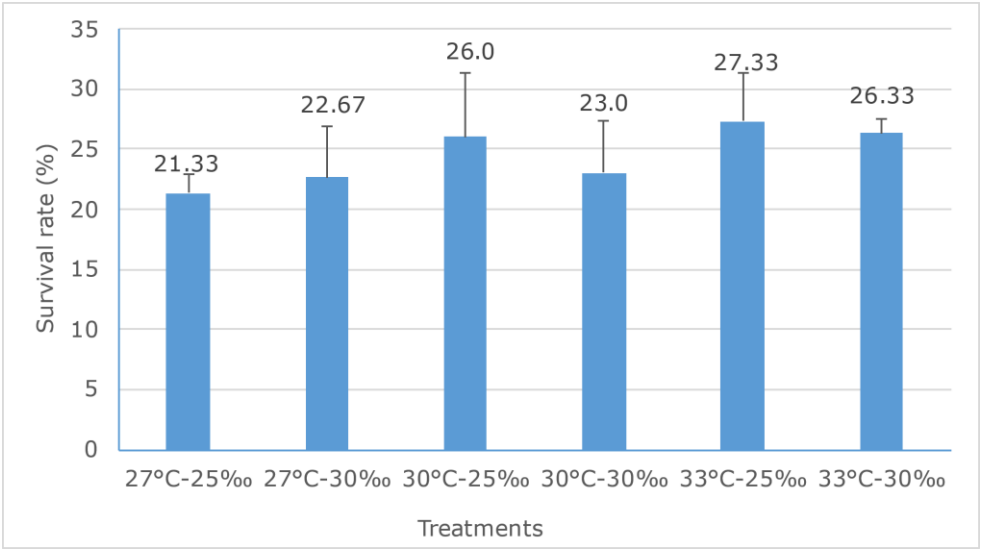

Figure 3. The survival rate of crablet 5 (Note: values are presented as the mean±SD; no significant difference among treatments, p-value temperature × salinity > 0.05).

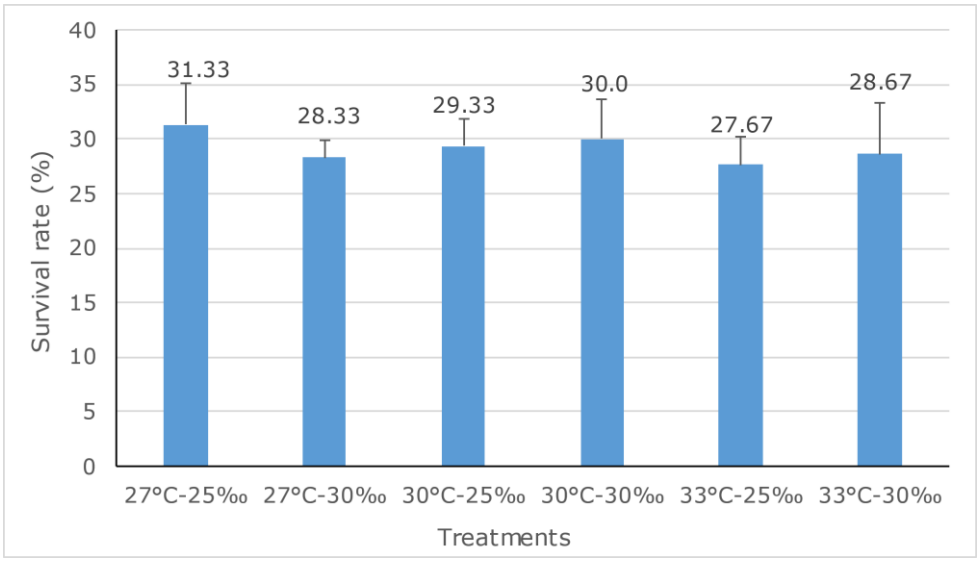

Figure 4. The survival rate of juvenile (Note: values are presented as the mean±SD; no significant difference among treatments, p-value temperature × salinity > 0.05).

Effect on digestive enzyme activity. The results show that trypsin, chymotrypsin, and amylase activities of crablet 5 and juveniles are not affected by temperature and salinity. At 30°C-30‰, all the digestive enzymes were highly active with average values of 0.10; 322 and 53.0 U/min/mg protein for crablet 5, respectively (Table 5). However, trypsin, chymotrypsin, and amylase were all very active in 27°C-25‰ (0.05 U/min/mg protein), 30°C-25‰ (423 U/min/mg protein) and 33°C-30‰ (67.0 U/min/mg protein) respectively for juveniles (Table 6).

For the crablet 5, the enzyme trypsin activity was low (0.07 U/min/mg protein) at 33°C-30‰ whereas in juvenile it was low (0.03 U/min/mg protein) at 30°C-25‰. The chymotrypsin activity for crablet 5 was low in 27°C-30‰ (260 U/min/mg protein) but for juvenile in 33°C-25‰ (326 U/min/mg protein). For juvenile, the amylase activity was low in 30°C-30‰ (43.0 U/min/mg protein) and 27°C-30‰ for crablet 5 (47.7 U/min/mg protein). There was no statistically significant variation in digestive enzyme activity across treatments for crablet 5 as well as juveniles ($p > 0.05$) (Table 5 and 6).

Table 5

Activities of trypsin, chymotrypsin, and amylase of crablet 5

Treatment	*Trypsin (U/min/mg protein)*	*Chymotrypsin (U/min/mg protein)*	*Amylase (U/min/mg protein)*
27℃-25‰	0.09±0.028	307±42.3	52.8±4.3
27℃-30‰	0.09±0.013	260±122	47.7±4.1
30℃-25‰	0.08±0.019	310±65.2	52.8±5.8
30℃-30‰	0.10±0.012	323±25.6	53.0±7.9
33℃-25‰	0.08±0.014	310±41.7	50.3±5.0
33℃-30‰	0.07±0.017	313±45.5	48.0±8.2
P-value			
Temperature × Salinity	0.602	0.703	0.759
Salinity	0.682	0.745	0.419
Temperature	0.286	0.652	0.564

Note: Values are presented as the mean±SD. No significant differences were found among treatments, $p > 0.05$.

Table 6

Activities of trypsin, chymotrypsin, and amylase of juveniles

Treatment	*Trypsin U/min/mg protein*	*Chymotrypsin U/min/mg protein*	*Amylase U/min/mg protein*
27℃-25‰	0.05±0.030	399±139	56.0±10.5
27℃-30‰	0.03±0.010	374±76.5	45.0±17.5
30℃-25‰	0.03±0.000	423±60.2	50.7±6.51
30℃-30‰	0.03±0.015	338±85.9	43.0±5.20
33℃-25‰	0.04±0.021	326±121	56.0±14.9
33℃-30‰	0.04±0.023	358±124	67.0±21.9
P-value			
Temperature × Salinity	0.451	0.641	0.376
Salinity	0.478	0.603	0.707
Temperature	0.677	0.732	0.214

Note: Values are presented as the mean±SD. No significant differences were found among treatments, $p > 0.05$.

Discussion

Water quality parameters. Temperature and salinity were kept constant (27, 30 and 33°C; 25 and 30‰) as required by the treatments. The pH and dissolved oxygen (DO) levels did not change significantly. The fluctuation was 8.19-8.40 for pH; 7.01-8.12 mg L^{-1} for DO and 0.4-1.22 for total ammonia nitrogen (TAN). The ranges of pond water parameters are temperature 24-32°C, salinity 15-30‰, dissolved oxygen 3.9-9.8 mg L^{-1}, and pH 7.6-8.7 (Rodriguez et al 2007). Similarly, Fatihah et al (2017) reported the optimum level of temperature as 27-29°C, DO 5.5-6.8 mg L^{-1}, pH 8.2-8.9, and salinity 25-26‰ for *Scylla tranquebarica*. Shelley & Lovatelli (2011) defined water quality requirements for mud crab production, with an optimal DO of 5 mg L^{-1}, the temperature of 25-35°C, water pH of 7.0 to 9.0, TAN of 3 mg L^{-1}, alkalinity of 80 mg$CaCO_3$ L^{-1}, and turbidity of 30 mg L^{-1}. According to Pedapoli (2014), in a study carried out on the effect of water quality parameters on growth and survivability of *S. tranquebarica* in the grow-out phase, with salinity between 10.4 to 33.2‰, the temperature ranged from 28.6 to 30.4°C, dissolved oxygen level varied between 5.5 to 6 mg L^{-1} and ammonia level ranged between 0.02 to 0.03 mg L^{-1}. The water quality parameters for the larval development and growth of mangrove crab *S. tranquebarica* crablet were salinity 35-48‰, pH 8-8.5, and water temperature 28-31°C (Gunarto et al 2019). The optimum water quality parameters for crablets of *Scylla olivacea* ranged between 5 to 20‰, pH 8-8.9, and dissolved oxygen 4.5-5.7 mg L^{-1} (Gunarto & Parenrengi 2014). Dissolved oxygen concentration greater than 4 mg L^{-1} is not a limiting factor to crablet growth. According to Shelley & Lovatelli (2011), the optimal pH value has the strongest impact on the osmoregulation process of mud crabs, and the enzymes that function on the gills are affected by the pH of the water.

Effect on growth and survival from larvae to crablet 1. The development of zoea gradually increased with the temperature levels after 3 days. Treatments with temperature of 33°C and salinities of 25‰ and 30‰ had the maximum development compared to other temperature and salinity groups. However, on the 15th day, the development of zoea was observed to be higher in the treatment with a temperature of 27°C and salinity 30‰ (4.39 mm) whereas the development was slower in 33°C and 30°C treatment. According to Baylon et al (2001), there was no significant difference in the rate of development of larvae in all zoeal stages among different salinity regimes. Nurdiani & Zeng (2007) found that temperature, salinity, and their interaction also had a significant effect on the larval development of *Scylla serrata*. The mean larval development time to megalopa ranged from 13.5 to 18.5 days at 34°C and 20.6 to 22.6

days at 25°C at different salinities. At 25‰ for all temperatures, the fastest mean development of larvae was observed.

The temperature and salinity combinations that influenced all the zoeal stages and metamorphosis of zoea 1 to zoea 5 were 33°C-25‰ and 33°C-30‰, which is similar in *Scylla tranquebarcia* according to Baylon (2013). There was a successful metamorphosis of zoea to megalopa at 25–35‰ in 26°C and 32°C. Salinity did not have any statistically significant effect on the metamorphosis of larvae as compared to temperature. The larval development to megalopa started from day 12 and day 15 which was mainly affected by the temperature. At 33°C, the LSI was higher regardless of the salinity in the present study, which is similar to Nurdiani & Zeng (2007) where the average larval development time to megalopa ranged from 13.5 to 18.5 days at various salinities in *S. serrata.* The LSI was lower in the temperature of 27°C combined with salinities of 25 and 30‰ in all the zoeal stages up to 15 days. This is similar according to Baylon (2013), where there was a delay in the development of zoea at 20°C-25‰ and 26°C-35‰.

The survival rate of the mud crab from larvae to zoea was the highest in the temperature at 30°C (8.50%) and the lowest at 33°C (0.52%). This data was different in *S. tranquebarica* in which the survival of larvae was higher (62-90%) in 26°C and 32°C but lowest (2-55%) in 20°C at 25‰ and 30‰ (Baylon 2013). The survival rate was lower at 27°C and 33°C which are similar to Baylon (2013). Salinity did not affect the survival of zoeal larvae within the range of 20-35‰ significantly; the temperature and salinity combinations affected the larval survival at low salinities and both high as well as low temperature (34°C-15‰, 34°C-20‰, and 25°C-15‰) (Nurdiani & Zeng 2007).

The temperature has a higher influence on the width of crablet 1. The highest mean width was in the temperature 33°C (3.06 and 3.05 mm) and lowest in 27°C (2.95 and 2.99 mm) combined with salinities 25 and 30‰. Salinity did not have a significant effect on the width of the crablet 1. Higher temperatures 30 and 35°C are better than the lower temperature (20 and 25°C), in which the mean carapace width can reach up to 13.7 and 11.6 mm, respectively (Ruscoe et al 2004). In the present study, the mean weight of the crablet ranged from 7.28 to 9.86 mg in different temperature and salinity combinations. Temperature and salinity did not have a significant effect on the weight of the crablet 1. According to Ruscoe et al (2004), both temperature and salinity have highly significant differences in the mean weight of crablets but with no interaction effect. The highest mean weight was at 30°C in salinities 5-20‰.

The survival rate of mud crab from megalopa to crablet 1 reared in six different treatments with the elevated temperature at different salinities ranged from 0.85 to 25.5% with the lowest being 27°C and highest in 30°C. Crab instar 1 (crablet 1) has greater tolerance to low salinity and low temperature as it grows from the larvae; the survival of crab instar 1 to 2 was 100% at 15-35‰ in 26 and 32°C and at 35‰ in 20°C; regardless of the temperature, all the crab instar died at the salinity of 0‰ (Baylon 2013). According to Ruscoe et al (2004), the temperature has a significant effect on the survival of crab instar 2 but no effect due to salinity of 5-40‰; at the temperatures 20, 25, 30, and 35°C, the mean survival rates were 36, 98, 96 and 94%, respectively.

Effect on growth, survival, and digestive enzyme activities from crablet 1 to crablet 5*.* After 20 days of rearing the weight of crablet 5 in the 33°C treatment group achieved the highest average weight (0.76 g), which was statistically significant if compared to the 27°C treatment group ($p < 0.05$). The carapace width of crablet 5 reached a maximum value of 1.41 and 1.35 cm at 30°C-25‰ and 33°C-25‰, respectively. In both the treatments with temperatures 27°C combined with 25 and 30‰, the carapace width was just 1.12 cm. Gunarto et al (2019) concluded in a study conducted in *S. tranquebarica,* that the fastest growth of crablet was 0.55 g and the slowest growth of crablet was 0.14 g, whereas the highest and lowest mean carapace width was 15.2 and 8.88 mm, respectively. According to Syafaat et al (2021), the mean carapace width at 28°C (12.3 mm) was higher than in 24°C (8.81 mm) and 32°C (not significantly different) in *S. paramamosain*. The mean final body weight was also higher at 28°C with the value of 0.29 g and lowest at 24°C with 0.11 g.

The survival rate of crablet 5 did not differ amongst the treatments, ranging from 21.3 to 27.3% in this study. Treatment 33°C-25‰ had the highest survival rate (27.3%) while treatment 27°C-25‰ had the lowest (21.3%), but the difference was not statistically significant ($p < 0.05$). The survival rate of crablet 5 was not affected by temperature and salinity significantly. According to Baylon (2013), the first crab instar (crablet 1) to the second crab instar (crablet 2) survived at 5-45‰ in 26°C and 32°C and 15–45‰ in 20°C in *S. tranquebarica*. Similarly, in *S. paramamosain* survival at 28°C (97.0%) was significantly higher than 32°C (80.0%), but it was not significantly different from either 24°C (87.0%) or ambient temperature (Syafaat 2021). These results are similar to those of Gong et al (2015), and Ruscoe et al (2004), who studied the effects of water temperature at crablet 1 to 2 on *S. paramamosain*, and at crablet 2 to 3 of *S. Serrata*, respectively.

The average number of molting times between treatments changed after 20 days of rearing the crablet, between 2.93 and 3.27 times in this study. Crablet 5 molted the most in the 30°C-25‰ treatment (3.27 times), and the least in the 33°C-25‰ treatment (2.93 times). The average molting cycle ranged from 6.08 to 6.36 days. Treatment 33°C-30‰ had the shortest molting cycle (6.08 days), while treatment 27°C-25‰ had the longest (6.36 days). According to Ruscoe et al (2004), there were also significant differences in the instar 3 intermoult duration among treatments due to both temperature and salinity but no interaction effect in *S. serrata*. Generally, crabs held at 30 and 35°C inter-moulted for a shorter time than those held at 25°C. During the molting phase from crablet 1 to crablet 2, the 32°C treatment had the shortest molting interval (2.96±1.22 days) compared to other treatments in *S. paramamosain* (Syafaat et al 2021), though its value was not significantly different from either the 28°C (3.92 days) or ambient temperature treatments. Furthermore, the 32°C treatment (7.66 days) had the shortest molting interval from crablet 5 to crablet 6, and its value was significantly different from both the 28°C (10.7 days) and ambient temperature treatments. In crustaceans, shorter molting interval times at high temperatures are frequent (Yuan et al 2017; Kuhn & Darnell 2019). Molting duration for the crablets of *S. serrata* at 26°C and 32°C were not significantly different from the duration at 20°C which had the longest molting duration period (7 days at 35 and 45‰). The shortest molting duration was at 32°C from 2 days at 5‰ to 4 days at 45‰ (Baylon 2010). According to Parado-Estepa & Quinitio (2011), *S. olivacea* has a shorter molt interval than other species. For *S. olivacea*, the molt interval was shorter in 12-20‰ than in 24-32‰, did not differ between test salinities for *S. serrata,* and was shortest in 20 and 24‰ for *S. tranquebarica*, with fewer individuals achieving the fourth molt in 32‰ than in 8-20‰; so that the molt interval increment in *S. olivacea* was influenced by salinity, not in other species.

The larvae of *S. serrata* do not have anterior midgut diverticula (AMD) which the penaeid shrimp possess. *S. serrata* larvae have very limited digestive capabilities (Serrano 2012). The present study shows that trypsin, chymotrypsin, and amylase activities of crablet 5 are not affected by temperature and salinity. The difference in digestive enzyme activity between treatments was not statistically significant ($p > 0.05$). At 30°C-30‰, the trypsin, chymotrypsin, and amylase were highly active. The enzyme trypsin activity was low at 33°C-30‰, while the chymotrypsin enzyme activity and amylase activity were low. According to Khoa et al (2019) in *S. paramamosain*, after the metamorphosis of megalopa, the trypsin activity increased and reached the highest level in crablet 1 at the temperature range of 26.5-29.5°C and salinity of 30‰ (3.94 U/mg protein). However, the chymotrypsin was low in crablet 1 (0.76 U/mg protein). In contrast, then amylase activity was higher in the zoea 5 (9.36 U/mg protein) but sharply declined in crablet 1 (4.21 U/ mg protein).

Effect on growth, survival, and digestive enzyme activities from crablet 5 to juvenile. The weight of juveniles in the 33°C achieved the highest weight (4.50 g and 4.03 g) after 20 days of rearing, which was statistically significant when compared to the 27°C and 30°C ($p < 0.05$). The carapace width of juveniles reached a highest at 33°C-25‰ and 33°C-30‰, respectively. Romano and Zeng (2006) study in *Portunus*

pelagicus has shown that when compared to the 20, 30, and 35‰ treatments, the carapace width growth at 45‰ was significantly less at each molt. At any crab stage, no significant variations in carapace length, carapace width, or wet weight increases were found ($p > 0.05$) among the 20, 30, and 35‰ treatments. Brown et al (1992) found that high temperatures promoted rapid growth in the stone crab, *Menippe mercenaria*.

Based on this study, the highest survival rate was found in temperature 27°C at 25‰, while the lowest was in 33°C at 25‰ but the difference was not statistically significant ($p > 0.05$) after 20 days of raising. The survival rate of juveniles was not significantly affected by temperature or salinity. According to Romano & Zeng (2006), salinity significantly affected the survival of early *P. pelagicus* juveniles. The average survival rate at 25‰ was the highest (93.3%) followed by 40‰ (86.6%), 30‰ (63.3%), 35‰ (63.3%), 15‰ (50.0%) 45‰ (43.3%), 10‰ (10.0%) and 5‰ (0%). As reported by Heasman & Fielder (1983) on *S. serrata* and observed by Baylon & Suzuki (2007) on *C. feriatus*, reduced survival at low temperatures of 20°C could be due to a decline in feeding.

The present study shows that the 33°C-30‰ treatment resulted in the maximum molting while the 27°C-30‰ treatment resulted in the least. Treatment with temperature 27°C was significantly different from the treatments with temperature groups of 30 and 33°C ($p < 0.05$). The molting cycle for treatment 30°C-25‰ was the shortest (7.54 days), while treatment 27°C-30‰ was the longest (8.73 days). According to Romano and Zeng (2006) in *P. pelagicus*, the mean intermolt duration at 45‰ was substantially longer for each crab stage than at 20, 30, and 35‰. There was no significant difference among the 20, 30, and 35‰ treatments ($p > 0.05$). At 5‰, there was full mortality and no successful molts. According to Baylon (2010), when juveniles of *S. serrata* were reared at lower temperatures, development was delayed and extended.

In this study, temperature and salinity do not affect the activities of trypsin, chymotrypsin, and amylase in juvenile crabs. There was no statistically significant variation in digestive enzyme activity across treatments ($p > 0.05$). In the treatments 27°C-25‰, 30°C-25‰ and 33°C-30‰, the digestive enzymes were all very active. The enzyme trypsin activity was low at 30°C-25‰, chymotrypsin activity at 33°C-25‰, and amylase activity in 30°C-30‰. The optimum temperature for all the digestive enzyme activity was similar in the mud crab at 50°C and relatively low levels of amylase were observed at 4°C; in *S. serrata*, the enzyme activity gradually increases with the rising temperature to a peak of 50°C but rapidly declines as the temperature increases (Pavasovic et al 2004). In a study done by Zhou et al (2020), the activity of the digestive enzyme was highest at 25‰ and lowest at 4‰. *S. paramamosain* had lower digestion at 4 and 12‰ compared to 25‰.

Conclusions. The study on the nursing of *S. paramamosain* from zoeal to juvenile stages with elevated temperature at different salinity indicates that the survival was influenced by the temperature and salinity combinations. The survival rate was highest at the salinity of 25‰ from zoea to crablet 1 in 30°C, crablet 1 to crablet 5 in 33°C, and crablet 5 to juvenile in 27ºC. The temperature increased the survival rate decreased in zoeal stages and crablet 5, however it was the opposite in megalopa and crablet 1 (survival decreased as temperature decreased). The maximum growth of mud crab was observed in the temperature 33°C and salinity 25‰ in all the developmental stages. Digestive enzymes such as like trypsin, chymotrypsin, and amylase were highly active in crablet 1, crablet 5 and juvenile in the salinity 30‰ in temperatures 30°C and 33°C, respectively.

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Conflict of interest. The authors declare no conflict of interest.

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