Effects of salinity and substrates on the growth performance and survival rates of juvenile mud crab (*Scylla paramamosain*)

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Abstract. The purpose of this study was to determine the optimal salinity level and the most appropriate substrate types for the growth and survival rates of juvenile mud crab *Scylla paramamosain*. The present study was conducted with two experiments by the effects of four different salinity levels (5, 10, 15, and 20‰) and four types of substrates (Greenhouse Sunshade Net (GSN); *Meretrix meretrix*'s shell (MS); Sand (SS); and combination of Greenhouse Sunshade Net and *Meretrix meretrix*'s shell (GSN-MS)). Each experiment was performed in triplicates by completely randomized design. The first experiment indicates that the growth rate (% day^{-1}) of body weight of crabs at salinity of 15‰ was the highest, followed by the salinity of 10‰ or 20‰, and the lowest was with the salinity of 5‰. The highest production of juvenile crab and survival rate at the end of the experiment were significantly recorded at a salinity of 15‰ compared to the residual salinities ($p < 0.05$). However, the survival rate and crab production did not show a statistically significant difference ($p > 0.05$) between 5‰ and 10‰ or 20‰ treatments. The results show that the growth performance (carapace width and body weight), survival rate, production, and coefficient of variation were significantly different among the substrate treatments ($p < 0.05$). The combination substrate of GSN-MS showed the highest survival rate and production of crab, followed by GSN, MS, and SS treatments. Besides, the coefficient of variation GSN-MS treatment was significantly better than those of the remaining group ($p < 0.05$). These findings strongly suggest that mud crab *S. paramamosain* should be reared at salinity 15‰ and the combination of Greenhouse Sunshade Net and *Meretrix meretrix*'s shell as subtrate should be used.

Key Words: aquaculture, mud crab, net, production, salinity, substrate.

Introduction. The mud crabs (*Scylla paramamosain*) are widely distributed throughout tropical and subtropical waters of the Pacific Ocean, supplying resources for commercial exploitation, as well as seed for aquaculture, providing a source of income for many coastal communities worldwide (Williams et al 1999). Mud crab farming is considered as an alternative to shrimp farming in coastal areas as shrimp disease outbreaks (Paterson & Mann 2011). Mud crab is also considered an ideal species in poly-culture aquaculture of brackish water ponds with shrimp, fish, and other aquatic species, improving the profit of a water surface area (Shyne Anand et al 2018). However, the source of crab seeds supplied for commercial farming is mainly from natural collection (Mirera 2011; Sujan et al 2021). In Vietnam, mud crab *S. paramamosain* has been raised since 1989 and is the most commonly farmed species, due to its fast-growing, large size, wide food ranges, and high tolerance to changes in environmental factors (Shelley & Lovatelli 2011). Therefore, this species is suitable for farming in saltwater intrusion areas, especially since its product is easy to preserve after harvest.

Salinity is a significant environmental factor for estuarine organisms because it has numerous physiological and ecological effects on them. Because mud crabs are primarily found in estuaries, salinity has an impact on their growth by influencing food intake, feed conversion ratio, and biological energy accumulation (Anger 1991; Minagawa 1992). Fluctuations in salinity are a cause of reduced aquaculture productivity because it could be stressful to aquatic animals, resulting in slowing growth (Gong et al 2015; Rahi et al 2020). Mud crab *S. paramamosain* juvenile can withstand salinity of 5-40‰ and grow normally at salinity of 3-5‰ (Niu et al 2022). Coastal areas with salinity of 10-30‰ are suitable for mud crab farming (Wang et al 2018).

Crab species have cannibalism throughout the nursery and cultural cycle, resulting in reduced productivity and survival rates (Mann & Paterson 2003). Cannibalism continues to occur even when an adequate food supply is available (Pereira Strictar et al 2017). It is recommended that using suitable substrates as shelter in mud crab nursery is an effective solution to reduce cannibalism. Previous studies have demonstrated that the presence of substrates in rearing tank significantly reduces the aggressive behavior and stress of *Scylla* sp. Application of substrates in mud crab rearing may increase survival by minimizing the frequency of encounters among individual crabs. Many studies on different types of substrates have been conducted to reduce cannibalism in mud crab rearing, such as using sheet nets (Syafaat et al 2021), Greenhouse Sunshade Net (GSN) (Quinitio et al 2001; Kwong 2013), coconut leaves, PVC pipes, seaweed *Gracilariopsis* sp. (Quinitio et al 2001).

This study was conducted to determine the optimal salinity level and the most suitable type of substrate for the growth and survival rate of juvenile mud crab *S. paramamosain*. Our findings provide practical information for seed production and growth-out culture of *S. paramamosain*. These simple techniques can be used by crab rearers to increase the survival and growth of crabs, particularly when low-cost materials are used.

Material and Method. The present study was conducted with two experiments at the Binh Dinh Agricultural Breeding Center, Vietnam. The first and the second experiments were carried out from June to July, and July to August 2023, respectively. The juvenile crabs from two experiments were obtained from the Aquaculture Seed Production Center of Binh Dinh Province, Vietnam. They were artificially produced with the size of the carapace width (CW) and body weight (BW) for experiment 1 (3.40±0.22 mm and 0.013±0.001 g) and experiment 2 (3.42±0.19 mm and 0.014± 0.001 g), respectively. The stocking density of each experiment was 300 individual m^{-2}. Juvenile crabs were reared in circular cement tanks in brown color with a volume of 3 m^3. Saltwater was filtered through a sand filter and treated with chlorine at a concentration of 25 ppm. Crabs were fed with frozen *Artemia* 3 times/day at 6 am, 12 pm, and 6 pm) at 8-12% biomass. Chemical compositions of frozen *Artemia* were (% dry weight basic) crude protein 55.28±0.57 and crude lipid 10.73±0.31 (Nguyen 2011). The water exchange in the rearing tanks was carried out every 5 days at 30% of the volumne by using filtered seawater after adjusting the salinity.

***Experiment 1**: **effects of different salinity levels**.* The experiment included 4 different salinity treatments with a completely randomized design in triplicates: treatment 1: 5‰; treatment 2: 10‰; treatment 3: 15‰; treatment 4: 20‰. Water salinities were obtained by diluting tap water to reach the salinity of the treatments. The water salinity in the experimental tanks was adjusted to decrease by 3‰ day^{-1} until it reached the salinity required by the experimental treatments. After water supply, each tank was arranged with one aerator (air stone) of tank bottom and a Greenhouse Sunshade Net (GSN) (0.25 m^2, 8 pieces m^{-2}).

***Experiment 2**: **effects of different types of substrates**.* This experiment includes 4 randomly designed substrate treatments (Figure 1) with 3 replications for each treatment: treatment 1 - Greenhouse Sunshade Net (GSN); treatment 2 - *Meretrix meretrix*'s shell (MS) with the size of 2-3 cm, covering about 60-70% tank bottom; treatment 3 - sand (SS) layer of 2 cm, with size of 0.2-0.3 mm; treatment 4 - combination of Greenhouse Sunshade Net and *Meretrix meretrix*'s shell, covering about 60-70% tank bottom (GSN-MS). Experimental conditions were similar to experiment 1. Water salinity was adjusted at 15‰.

Greenhouse Sunshade Net (GSN) | *M. meretrix*'s shell (MS) | Sand (SS)

Figure 1. Experimental substrates.

***Environmental conditions of two experiments*.** During the experiment, the basic important water parameters in each tank were monitored twice daily between 06:00 and 07:00 am and between 14:00 and 15:00 pm. Temperature, dissolved oxygen (DO), pH, and alkalinity were 29.1-30.9^{o}C, 5.8-6.0 mg L^{-1}, 7.8-8.0, and 132-140 mg $CaCO_3$ L^{-1}, respectively. Water temperature was measured by using digital thermometer (model: Mi 106) in ^{o}C. DO was measured using digital DO meter (Model: Mi 606) in mg L^{-1} and pH was recorded by digital pH meter (Model: Mi 106). Alkalinity and salinity were measured by Checker MARINE Alkalinity HI755, HANA Instrument Rumani and by handheld refractometer (ATAGO), respectively.

***Crab measurement*.** The BW and CW of crabs were measured with an electronic balance (±0.001 g) and an electronic caliper (±0.01 mm) respectively, according to Fazhan et al (2021). The BW and CW at the beginning and at the end were randomly sampled and measured on 30 crabs or 90 crabs per treatment, respectively. The numbers of alive crabs were recorded to calculate the survival rate (SR) and production.

Experimental indices were calculated as follow:

Crab growth rates: Specific growth rate (SGR) by carapace width (SGR_{CW}) or by body weight (SGR_{BW}) was calculated according to the formulas:

$$\mathrm{SGR_{CW}\ or\ SGR_{BW}}\ (\%\ day^{-1}) = \left[\frac{\log(\mathrm{X_2}) - \log(\mathrm{X_1})}{\mathrm{t_2} - \mathrm{t_1}}\right] \times 100$$

where: X_1 is the carapace width or body weight at measurement time t_1 and X_2 is the carapace width or body weight at measurement time t_2.

Survival rate (SR) (%):

$$\text{SR (\%)} = \frac{\text{The number of crab alive at the end of the experiment}}{\text{The number of crab at the beginning of the experiment}} \times 100$$

Production (ind m^{-2}):

$$\text{Production (ind m}^{-2}\text{)} = \frac{\text{Final crab number}}{\text{Floor area of the tank (m}^2\text{)}}$$

Coefficient of variation (%):

$$\text{CV (\%)} = \frac{\text{Standard deviation of CW or BW}}{\text{Mean of CW or BW}}$$

***Data analysis*.** We compared the statistical significance of differences between treatments in terms of BW, CW, SGR_{BW}, SGR_{CW}, and SR using one-way ANOVA and Tukey's post-hoc test. Computational analyses were run by SPSS 20.0 software at a significance level of $p < 0.05$.

Results

***Experiment 1**: **effects of different salinity levels**.* The crab's growth rate (SGR_{CW} and SGR_{BW}) was affected by the salinity of the water environment. Crab rearing at 5‰ salinity had a statistically significant slower growth rate in both SGR_{CW} and SGR_{BW} compared to the remaining salinity treatments ($p < 0.05$). At a salinity of 15‰, the SGR_{BW} of crabs was the highest, followed by the salinity of 10‰ or 20‰, and the lowest was the salinity of 5‰.

The highest production of crab juvenile and SR at the end of the experiment were significantly recorded at a salinity of 15‰ compared to the remaining salinities ($p < 0.05$). However, the SR and crab production did not show a statistically significant difference ($p > 0.05$) between 5‰ and 10‰ or 20‰ treatments. Salinity also affects the flocking rate of nursery crabs. The highest coefficient of variation in terms of weight (CV_{BW}) and carapace width (CV_{CW}) was recorded at treatment 5‰, followed by 10‰ or 20‰ treatments, and the lowest was treatment 15‰. The experimental indices of *S. paramamosain* juvenile reared at various salinities are described in Table 1.

Table 1

The growth performance, survival rates, and production of *S. paramamosain* juvenile at different salinities

Experimental indices	*Salinity treatments*			
	5‰	*10‰*	*15‰*	*20‰*
CW_i (mm)	3.40±0.22	3.40±0.22	3.40±0.22	3.40±0.22
CW_f (mm)	17.84±2.55^a	18.35± 2.37^b	18.59±2.18^b	18.40±2.42^b
SGR_{cw} (% day^{-1})	5.53±0.03^a	5.62±0.02^b	5.67±0.01^b	5.63±0.00^b
BW_i (g)	0.013±0.001	0.013±0.001	0.013±0.001	0.013±0.001
BW_f (g)	1.02±0.35^a	1.09±0.34^b	1.13±0.32^b	1.09±0.33^b
SGR_{BW} (% day^{-1})	14.45±0.09^a	14.64±0.01^b	14.78±0.01^c	14.65±0.01^b
SR (%)	36.81±1.18^a	37.19±1.34^a	41.26±1.01^b	37.52±0.84^a
Production (ind m^{-2})	110.44±3.53^a	111.56±4.03^a	123.78±3.02^b	112.56±2.52^a
CV_{CW} (%)	14.43±0.57^c	13.04±0.06^b	11.84±0.07^a	13.31±0.60^b
CV_{BW} (%)	34.07±1.31^c	31.07±0.76^b	28.22±0.69^a	30.82±0.77^b

Different superscript letters on the same row indicate significant differences among the treatments ($p < 0.05$). CW_i and BW_i = the carapace width and body weight of crab at the beginning of the experiment; CW_f and BW_f = the carapace width and body weight of crab at the end of the experiment; SGR_{cw} (% day-1) and SGR_{BW} (% day^{-1}) are the specific growth rate of crab in carapace width and body weight respectively; SR (%) = survival rate; CV = coefficient of variation.

***Experiment 2**: **effects of different types of substrates**.* The experimental indices of juvenile *S. paramamosain* reared at different types of substrates are described in Table 2. The results show that the growth performance (SGR_{cw} and SGR_{BW}), SR, production, and CV were significantly different among the substrate treatments ($p < 0.05$). Based on the data analysis, it is evident that the CW and BW were significantly higher in substrates treated with GSN and GSN-MS when compared to MS and SS ($p < 0.05$). In addition, there was a significant difference in the BW of the crab between MS and SS substrates ($p < 0.05$). The SGR_{cw} and SGR_{BW} at day 30 had a similar trend to be significantly greater in the treatments of GSN and GSN-MS than those of MS and SS ($p < 0.05$). Furthermore, there was a significant difference in the SR and production of crabs at different types of substrates ($p < 0.05$). The GSN-MS substrate showed the highest SR and production of crab, followed by GSN, MS, and SS treatments. Besides, the CV in GSN-MS treatment was significantly better than those of the remaining groups ($p <0.05$). These findings strongly suggest that the use of GSN and GSN-MS as substrates for crab juveniles has a positive impact on crab rearing.

Table 2
The growth performance, survival rates, and production of *S. paramamosain* juvenile at different types of substrates

Experimental indices	Substrate treatments			
	GSN	*MS*	*SS*	*GSN-MS*
CW_i (mm)	3.42±0.19	3.42± 0.19	3.42±0.19	3.42±0.19
CW_f (mm)	18.21±2.51^b	18.14±2.51^{ab}	17.98±2.56^a	18.22±2.33^b
SGR_{cw} (% day^{-1})	5.57±0.01^b	5.56±0.01^{ab}	5.53±0.02^a	5.58±0.01^b
BW_i (g)	0.014±0.001	0.014±0.001	0.014±0.001	0.014±0.001
BW_f (g)	1.09±0.36^{bc}	1.07±0.35^{ab}	1.05±0.35^a	1.09±0.33^c
SGR_{BW} (% day^{-1})	14.62±0.02^b	14.55±0.04^{ab}	14.49±0.03^a	14.63±0.02^b
SR (%)	38.89±1.90^c	32.63±2.00^b	26.93±2.28^a	43.78±0.89^d
Production (ind m^{-2})	116.67±5.69^c	97.89±6.00^b	80.78±6.84^a	131.33±2.67^d
CV_{CW} (%)	13.92±0.43^b	13.97±0.43^b	14.41±0.40^b	12.94±0.06^a
CV_{BW} (%)	32.91±0.27^b	32.88±0.86^b	33.47±0.44^b	30.71±0.47^a

Different superscript letters on the same row indicate significant differences among the treatments ($p < 0.05$). CW_i and BW_i = the carapace width and body weight of crab at the beginning of the experiment; CW_f and BW_f = the carapace width and body weight of crab at the end of the experiment; SGR_{cw} (% day-1) and SGR_{BW} (% day^{-1}) are the specific growth rate of crab in carapace width and body weight respectively; SR (%) = survival rate; CV = coefficient of variation.

Discussion. Salinity is one of the most important variables that directly affect the osmotic regulation of aquatic organisms and the molting process of mud crabs (Liu et al 2021; Zhang et al 2021). Parado-Estepa & Quinitio (2011) have reported that the optimal salinity for C1 stages of *S. olivacea*, *S. serrata*, and *S. tranquebarica* was 12-16‰, 8-24‰, and 8-24‰, respectively. On the other hand, Rahi et al (2020) suggested that mud crab *S. olivacea* requires from 3 to 5 days to adapt to the sudden change of salinity and requires up to 30 days for normal growth activity. The present study revealed that the salinity impact on the growth rates in CW and BW during the period of 30-day rearing. Our observations show that the duration of salinity assimilation can stimulate the molting of the crab. Our different experiments for 30 days indicate that salinity of 5‰ is unfavorable for juvenile crabs, resulting in lower growth and SRs. This result is consistent with the report by Pedapoli & Ramudu (2014), who suggested that lower salinity impacted the SR, and feed conversion ratio of juvenile crabs. Moreover, salinity affected the molting frequency of juvenile crabs, impacting the growth performance (Fatihah et al 2017) because molting frequency strongly correlated with the growth rate of crabs (Ruscoe et al 2004). Huang et al (2019) reported that low salinity caused stress to juvenile crabs more than high salinity. The osmoregulation of crabs under stress conditions of salinity resulted in loss of energy for growth (Pourmozaffar et al 2020; Rahi et al 2020). The mud crab *S. paramamosain* is the dominant species in estuarine habitats with marked seasonal salinity changes and can tolerate long periods of low salinity 5-7‰ (Walton et al 2006). This shows a strong match with the present research with no significant differences in SR and production among the salinity treatments. Similarly, according to Mia & Shah (2010), the SR of juvenile crabs between salinity levels of 5, 10 and 15‰ did not have a statistically significant difference ($p > 0.05$), however, higher salinity conditions had a positive relationship with growth performance. Based on the results of this study, the best salinity for rearing juvenile crabs was 15‰ for growth, survival, production, and variability.

Crabs are aggressive and cannibalistic; the mortality rate due to cannibalism in crabs can reach 60% within the first few days (Hussan et al 2011). According to Hamasaki (2003), the molting process is necessary for growth, however, the molting process is also known to cause mud crabs to become more vulnerable to cannibalism or predation. The substrate plays a vital role in creating shelter for crabs to reduce the frequency of encounters between individuals (Mirera & Moksnes 2015). Similarly, the experimental results of Zhou et al (2023) showed a significant difference in the molting rate of *S. paramamosain* with substrate compared to without substrate. This proves that using a substrate to create more shelter for crabs promoted the molting, resulting in faster growth. On the other hand, the shelter serves to reduce the stress levels of the biota and minimize the mortality of crabs caused by cannibalism (Hastuti et al 2020). This study has proven that combination of GSN-MS substrates achieves better crab nursery results in terms of growth, SR, production, and CV compared to the remaining treatments (Table 2). This result is strongly consistent with Ut et al (2007), who reported that the combination of sand with bricks or mollusk shells resulted better than sand only in *S. paramamosain* crab nursery. Li et al (2022) reported that juvenile mud crabs preferred vertical rather than horizontal substrates at the bottom of the rearing tank. The combination of horizontal MS and vertical GSN is believed to contribute to better growth and SR of crab seeds. The SR results of this study are consistent with the report by Islam et al (2017), who documented that low SR during the nursery period of *S. serrata* and *S. paramamosain* can be 60% or greater. In our opinion, the treatment using sand as substrate caused the crabs to quickly bury themselves under the sand during the first few days of the experiment; however, before and after molting, the crabs were unable to bury in the sand bottom, but instead concentrated on the sandy surface and stuck around the tank's walls. Crabs tend to move away from the sandy surface to reduce pressure during the molting process, and because the body is soft after molting, it cannot be immediately buried in the sand to hide, increasing the likelihood of cannibalism by crabs with hard shells. With the GSN substrate, crabs can shelter vertically, creating more shelter space, thus reducing competition for shelter space and food, so the possibility of meeting between individuals is also reduced compared to mollusk shell substrate. Combining GSN-MS substrates, resulting in a combined horizontal and vertical structure, provides more shelter and living space (Figure 2A, B), potentially reducing encounters between individuals and competing for food and shelter, which in turn significantly reduces stress and mortality and, ultimately, increases growth rates. Previous studies have also shown that the arrangement of substrates in the nursery tank is complex, increasing the surface area and hiding space, reducing the encounter rate, and reducing cannibalism (Zmora et al 2005; Daly et al 2009).

(A) Juvenile crabs attached on the GSN substrate | (B) Juvenile crabs attached on the MS substrate | (C) Juvenile crabs attached on the SS substrate

Figure 2. The juvenile crabs attached on different substrates.

Conclusions. Nursery of mud crab *Scylla paramamosain* at a salinity level of 15‰ was the best condition in terms of growth and survival rates, and coefficient of variation compared to other salinity levels. The combination of GSN-MS substrates achieved a better crab nursery result in terms of growth, survival rate, production, and CV compared to the remaining treatments. Our findings recommended that mud crab *Scylla paramamosain* C1 stage should be reared at salinity of 15‰ and the combination of Greenhouse Sunshade Net and *Meretrix meretrix*'s shell as subtrate should be used during rearing.

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Conflict of interest. The authors declare that there is no conflict of interest.

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