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Effect of Salinity on Survival and Metamorphosis from Zoea to Megalopa of the Mud Crab *Scylla serrata* Forskal (Crustacea: Portunidae)*

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Abstract

Survival and metamorphosis of each of the five zoeal stages (Z1, Z2, Z3, Z4, Z5) of *Scylla serrata* larvae hatched and reared at 32 ppt and abruptly transferred to salinities of 12, 16, 20, 24, 28 and 32 ppt were compared. These were monitored at 1, 3, 6, 12, 24, 48 and 72 h of exposure. Larvae in all zoeal stages were able to tolerate abrupt transfer to 20, 24, 28 and 32 ppt salinities up to the 72 h of exposure, with consistent low survival at Z3 stage in all salinities. Total mortality at 12 ppt occurred on 12 h at Z1, 48 h at Z2, and 24 h at Z3 and Z4, while very few Z5 larvae survived up to 72 h. There was very low survival at 16 ppt in all zoeal stages. Metamorphosis to the next larval stage occurred at salinities of 20 to 32 ppt. In another experiment, zoeae were continuously reared from Z1 up to megalopa in 5 different water salinity regimens. In treatment 1, a 32 ppt salinity was maintained throughout culture period; in treatment 2, from 32 ppt, there was 1 ppt reduction in salinity every day; in treatment 3, 24 ppt salinity was maintained throughout the culture period, in treatment 4, 32 ppt at Z1, 24 ppt at Z2 and 20 ppt from Z3 to megalopa; and in treatment 5, there was a gradual decrease from 32 ppt in Z1 to 25 ppt in Z5. Highest metamorphosis to megalopa occurred where salinity was constant at 32 ppt. The computed population development indexes of larvae in all salinity regimens showed no significant differences, suggesting that salinity did not affect the duration of development of the surviving larvae.

Introduction

The mud crab classified by Keenan et al. (1998) as *Scylla serrata* is locally known as “king crab” or “alimango” in the Philippines. It spends most of its life cycle in brackish waters such as mangroves and estuaries where

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courtship and mating occur. Berried crabs then migrate 18 to 20 kilometers offshore where their eggs hatch into zoea. Megalops and crablets migrate back to near shore areas of lower salinity, such as rivers and mangroves for feeding and shelter until they grow to maturity (Arriola 1940; Hill 1974; Cowan 1984, Brown 1993).

Ong (1964) was the first to employ gradual lowering of salinity from 32 ppt in zoea 1 to 25 ppt to megalopa in the larval rearing of *S. serrata* in the laboratory. Recent reports on the larval rearing of this crab from other countries used higher salinities. A salinity range of 30 to 33 ppt was reportedly used in Japan (Cowan 1984); with seawater, filtered or unfiltered (Faazaz and Utama 1995) and 32 ppt (Yunus 1994) in Malaysia; 29 to 32 ppt (Zainoddin 1992), and 32 ± 2 ppt (Marichamy 1993) in India; 33 to 34.5 ppt in Hawaii (Brick 1974); 30 to 32 ppt in Darwin Aquaculture Center (Williams and Shelley 1998) 32 ± 2 ppt (Heasman and Fielder 1983) and 33 to 36 ppt at Bribie Island Aquaculture Center (Mann 1998) in Australia.

When Keenan et al. (1998) established the existence of four species of *Scylla* in the Indo-Pacific region, they strongly suggested that each species of *Scylla* evolved from a different habitat and may have different salinity optima for larval growth and survival. It is therefore logical to establish the salinity requirements of the zoeal stages of *S. serrata*. This is of primary importance in the maintenance of the optimum water conditions necessary for the development of a successful hatchery technology for this economically important species.

This study consisted of two phases. The first phase involved abrupt exposure of each zoeal stage to different salinity levels and the second phase involved continuous rearing of the larvae from zoea 1 to megalopa in different salinity regimens.

Materials and Methods

Spawning

A sexually mature female *S. serrata* weighing 780 grams, measuring 16.5 cm and 11 cm in carapace width and length was induced to spawn by ablating an eyestalk. The ablated crab was then maintained in a 300 L circular concrete tank provided with 10 cm sand substrate and strong aeration with approximately 30% daily flow through water change. Salinity of the rearing water was maintained at 32 ppt. Fresh mussel meat was provided to the spawner at 20% body weight. After spawning, the berried crab was transferred to a new tank with no substrate to prevent bacterial and fungal infestation of the eggs. Eggs hatched to zoea stage after 12 days of incubation.

Experiment 1: Salinity tolerance of each zoeal stage

Salinity tolerance of each zoeal stage (Z1, Z2, Z3, Z4, Z5) was determined. For each test, a completely randomized design was used. There were

five replicates for every salinity level tested for Z1, and three replicates of each salinity level for Z2 to Z5.

Vigorously swimming zoeae were collected within the first three hours of hatching from the tank with salinity of 32 ppt. Larvae were then distributed to thirty, 4 l circular plastic buckets filled with 3 l of water of different salinity levels. Stocking density was at 10 larvae l^{-1} (30 larvae per container). Larvae were abruptly transferred to the different test salinities. The experimental containers were maintained in a rectangular wooden waterbath to avoid temperature fluctuation. Salinity levels of 12, 16, 20, 24, 28 and 32 ppt were prepared by mixing appropriate volume of filtered seawater and aerated tap water. Water salinity was then adjusted to its required level using a hand refractometer with automatic temperature compensation (WestoverTM Hand Refractometer Model RHS10ATC) calibrated on distilled water at 22°C. Throughout the experiment the larvae were fed *Tetraselmis*-fed *Brachionus* (density = 20 ind· ml^{-1}) and newly-hatched *Artemia* nauplii (M & M brand from Great Salt Lake, Utah; density = 0.5 ind· ml^{-1}). All larvae were transferred to new containers every morning using a plastic spoon.

Physico-chemical parameters such as temperature, pH and dissolved oxygen of the rearing water were monitored daily and were within the optimal tolerable range. Temperature, pH and dissolved oxygen ranged from 26 to 29°C, 8.18 to 8.85 and 4.8 to 7.0 $\text{mg}\cdot\text{l}^{-1}$ respectively. Larval survival was assessed 1, 3, 6, 12, 24, 48 and 72 h after exposure to the new salinity. Metamorphosis of the larvae to the next developmental stage was also determined and the experiment was terminated when more than 50% of the larvae had metamorphosed. Duration of the test was 72 h for Z2, Z3 and Z4 and 96 h for Z1 and Z5 due to the delay in molting.

The remaining zoeae were maintained in 40 l plastic basins at 32 ppt seawater and were fed the same quantity of food. These larvae were used in the salinity tolerance test of subsequent stages.

Experiment 2. Effect of different rearing salinity regimens

A separate setup was prepared using larvae from the same batch used in the first experiment. The larvae were reared continuously at different salinity levels for 15 days until metamorphosis to megalopa. Newly-hatched larvae were stocked in 4 l containers filled with 3 l seawater at a density of 10 ind· l^{-1} . There were five treatments with five replicates each. In treatment 1, larvae were reared at 32 ppt during the entire duration of the test. In treatment 2, salinity started at 32 ppt with a 1 ppt reduction in salinity daily. In treatment 3, the larvae were maintained at a constant salinity of 24 ppt. In treatment 4, salinity was kept at 32 ppt during Z1, 24 ppt at Z2, and 20 ppt at Z3 to Z5 stages. In treatment 5, there was a gradual lowering of salinity from 32 ppt at Z1 to 25 ppt at Z5. The methods for the preparation of the different salinities, feeding and water change were similar to those in the first experiment. The population development indexes (PDI) of the larvae in different salinity regimes were determined using the method described by Villegas and Kanazawa (1980).

Percentage survival and metamorphosis rates were arcsine transformed before applying one way analysis of variance. Duncan's multiple range test was used to compare treatment means (Gomez and Gomez 1984).

Results

Experiment 1. Salinity tolerance of each zoeal stage

Figure 1 shows survival (%) of the different zoeal stages hatched at 32 ppt and abruptly transferred to different salinities. At 12 ppt, Z1 larvae settled at the bottom of the container within 1 h of exposure. Less than 50% survived at 6 h and total mortality occurred at 12 h. At 16 ppt, although larval survival was still 47.3% at 72 h, this was lower than survival at 20 to 32 ppt. A significantly high survival was consistently obtained up to 72 h at salinities of 20 (73.3%), 24 (72.7%), 28 (74.0%) and 32 ppt (67.3%).

All Z2 larvae were dead after 48 h exposure to 12 ppt. At 16 ppt, 36.7% of the larvae survived up to 72 h. Significantly high survival (61.1 to 72.2%) was also obtained at salinities of 20 to 32 ppt.

No Z3 larvae survived 24 h of exposure to 12 ppt. Survival was at 7.8% and 33.3% at 16 and 20 ppt respectively, significantly lower than at higher salinities (50.0 to 61.1% at 24 to 32 ppt).

All Z4 larvae were dead by 24 h after exposure to 12 ppt. Survival was significantly higher at 20 (80.0%), 24 (84.4%), 28 (85.6%), and 32 ppt (91.1%) compared with 16 ppt (25.5%).

Very few Z5 larvae (3.3%) survived at 12 ppt but 28.9% survived at 16 ppt. up to 72 h. Significantly higher survival was obtained at salinities of 24 (87.8%), 28 (94.4%), and 32 (91.1%) than at 20 ppt (75.6%).

Table 1 shows the rate of metamorphosis to the next larval stage for each zoeal stage abruptly exposed to different salinities and reared until larvae metamorphosed to the next larval stage (72 to 96 h).

The highest rate of metamorphosis at Z1 to Z2 was obtained at 28 ppt (58%) followed by larvae exposed to 20, 24 and 32 ppt (34.6%, 48.7%, and 24.7% respectively) (P < 0.05). There was no metamorphosis at 12 and 16 ppt.

Table 1. Percentage metamorphosis to next larval stage of each zoeal stage of mud crab *S. serrata* larvae reared in different salinity levels

Treatment	Larval Stages				
	Z1 - Z2	Z2 - Z3	Z3 - Z4	Z4 - Z5	Z5 - M
12 ppt	0 ^c	0 ^d	0 ^c	0 ^d	0 ^d
16 ppt	0 ^c	23.3 ^c	0 ^c	6.7 ^c	0 ^d
20 ppt	34.6 ^c	37.8 ^b	6.7 ^b	60.0 ^b	22.2 ^c
24 ppt	48.7 ^b	45.6 ^{ab}	17.8 ^a	67.8 ^{ab}	34.4 ^{bc}
28 ppt	58.0 ^a	48.9 ^{ab}	26.7 ^a	67.8 ^{ab}	47.7 ^{ab}
32 ppt	24.7 ^d	57.8 ^a	31.1 ^a	77.8 ^a	55.6 ^a

No metamorphosis of Z2 to Z3 took place at 12 ppt and only 23.3% metamorphosed at 16 ppt. Highest rate of metamorphosis was obtained at 32 ppt (57.8%) followed by 28 ppt (48.9%) and 24 ppt (45.6%). At 20 ppt, Z3 larvae were produced (37.8%) but this was significantly lower compared with the rest of the higher salinities.

At 12 and 16 ppt Z3 larvae did not metamorphose to Z4. A low percentage of metamorphosis occurred at 20, 24, 28 and 32 ppt (6.7%, 17.8%, 26.7%, 31.1% respectively).

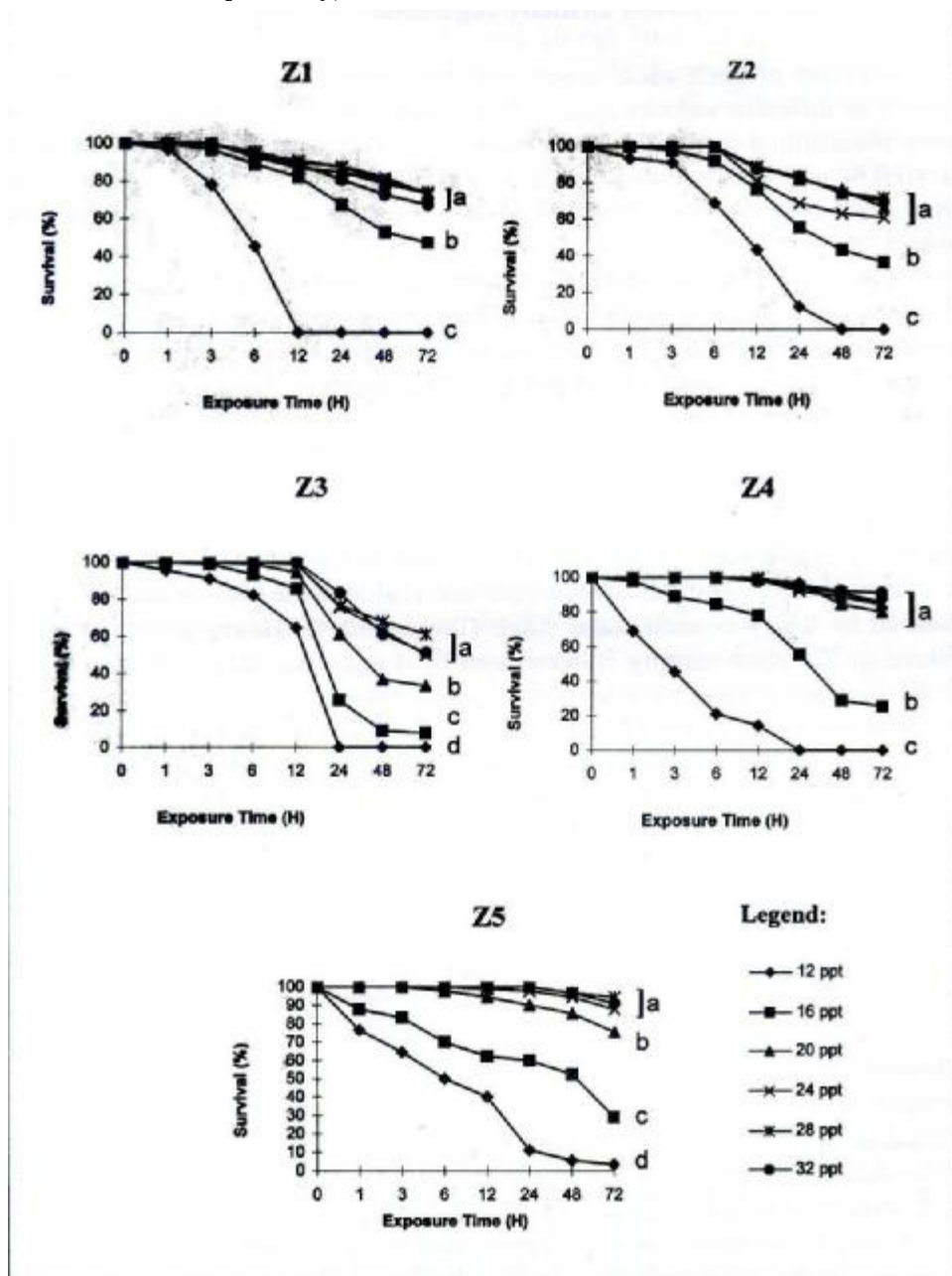


Fig. 1. Percentage survival of five zoeal stages of mud crab *S. serrata* larvae hatched and reared at 32 ppt and abruptly transferred to 12, 16, 20, 24, 28 and 32 ppt salinity

Metamorphosis of Z4 to Z5 was highest when larvae were reared at 32 ppt (77.8%) followed by 28 (67.8%), 24 (62.2%) and 20 ppt (60%). Metamorphosis was very low at 16 ppt (6.7%) with none at 12 ppt.

Molting of Z5 to megalopa was highest at 32 ppt (55.6%), followed by 28 (47.7%), 24 (34.4%) and 20 ppt (22.2%) respectively. No metamorphosis took place at 12 and 16 ppt.

Experiment 2. Survival of zoea and metamorphosis to megalopa when reared at different salinity regimens

Survival of each zoeal stage and metamorphosis to megalopa when reared in different salinity regimens is shown in table 2. Where Z1 larvae were maintained at 32 ppt up to megalopa (Treatment 1), a high survival of 80.6 % was obtained at Z1 but this dropped to 42.7 % at Z2. Survival then gradually declined from Z3 to Z5. Metamorphosis to megalopa was only 6.7%.

When a 1 ppt reduction in salinity was made daily (Treatment 2), there was a sudden drop in survival from 81.3% in Z1 to 40.7% at Z2 that further declined to 15.3 % at Z3. Although Z4 and Z5 stages tolerated the lowering of salinity (22 to 20 ppt for Z4 and 19 to 17 ppt for Z5), none metamorphosed to megalopa.

When reared at a constant salinity of 24 ppt (Treatment 3), there were significantly higher survival at Z1, Z2 and Z3 stages when compared with other treatments. However, there was a decline in survival in subsequent stages so that few (0.7%) Z5 metamorphosed to megalopa.

There was a gradual decrease in survival as salinity was abruptly decreased by 4 ppt in each zoeal stage (Treatment 4). Heavy mortalities occurred at Z3 when salinity was reduced by 4 ppt (from 24 to 20 ppt). None of the surviving zoea metamorphosed to megalopa.

Salinity was gradually reduced from 32 ppt in Z1 to 25 ppt in Z5 (Treatment 5) which required a 1 ppt lowering of salinity every two days. Z3 again turned out to be the critical stage since more than 50% of the surviving Z2 larvae died. Although survival improved at Z4, heavy mortality occurred again at Z5. In this treatment, only 1.3% of the zoea molted to megalopa.

Table 2. Percentage survival in each zoeal stage and metamorphosis to megalopa of mud crab *S. serrata* larvae reared in different salinity regimens

Treatment	Survival (%)					Metamorphosis to megalopa(%)
	Z1	Z2	Z3	Z4	Z5	
1	80.6 ^a	42.7 ^b	33.3 ^{ab}	29.3 ^a	22.7 ^a	6.7 ^a
2	81.3 ^a	40.7 ^b	15.3 ^c	12.7 ^c	9.3 ^b	0 ^b
3	84.7 ^a	60.7 ^a	42.0 ^a	26.7 ^{ab}	14.7 ^{ab}	0.7 ^b
4	66.0 ^b	38.7 ^b	18.0 ^c	11.3 ^c	0.7 ^c	0 ^b
5	62.7 ^b	45.3 ^{ab}	21.3 ^{bc}	16.0 ^{bc}	6.7 ^{bc}	1.3 ^b

Means in the same column with common superscript are not significantly different (P > 0.05).

Larval growth through metamorphosis was compared using their computed population development indexes (PDI). No significant differences ($P < 0.05$) were found in the mean values of rate of development of larvae in all zoeal stages among different salinity regimens.

Discussion

The present study shows high mortality of zoea larvae when abruptly transferred to lower salinities of 12 and 16 ppt from 32 ppt. This may be due to the inward movement of water across the permeable body surfaces that subjected the larvae to osmotic and ionic stress (Robertson 1960; Gilles and Pequeux 1983). In this present experiment, dead larvae were observed to have swollen bodies probably because of water retention. Gilles and Pequeux (1983) reported that the ability of crustaceans to adapt to a habitat with fluctuating salinity is governed by their capability to either regulate or conform the osmolality inside its body with that of the medium. It is apparent that larvae at these stages have limited capability to osmoregulate. The organism's response to the osmotic and ionic concentration of the medium could also vary with the molt stage and at different periods after ecdysis (Ferraris et al. 1986).

High survival of Z1, Z2, and Z4 was in a salinity range of 20 to 32 ppt; however at Z3 and Z5, a narrower range of salinity (24-32 ppt) was tolerated. Also, massive mortalities always took place at these stages. This suggests that they are more sensitive than the other stages. Ong (1964) reported that Z1, Z2, & Z4 stages undergo an increase in size after molting to the next stage, however, it is at the Z3 stage where the larvae undergo major morphological changes such as the development of an additional abdominal segment from five segments in Z2 to six segments in Z3. The gastric mill of the digestive system also starts to develop during this stage as well as an increase in the number and size of the hepatopancreas (Zeng and Wang 1986). At Z5, the larvae probably require more nutrition to build up tissues and more energy from food to be able to molt to megalopa that involves drastic morphological changes such as the considerable increase in size from Z5 to megalopa and the development of thoracic appendages such as the big pincers and four pairs of legs. Since these are already tremendous demands during these stages, further subjecting the larvae to lower salinities (20 ppt and below) stressed and weakened them and this eventually resulted to death.

High survival and metamorphosis in 32 ppt at Z2, Z3, Z4, Z5 stages reflect the life cycle pattern of this species, where berried crab migrate from less saline water in the rivers and mangroves to water of higher salinity offshore to hatch their eggs in the sea. The result of this study confirms that survival and development of these zoeal stages require high salinity water in the ocean. So far, there has been no report whatsoever on the exact location where metamorphosis to megalopa takes place. The high salinity preference shown by Z5 larvae to develop to megalopa in this

present experiment strongly suggests that molting to the megalopa stage also takes place offshore where water salinity is high. Megalops then move near shore, molt to crablet stage and recruit in the mangroves to feed. Keenan et al. (1998) reported that mature *S. serrata* are dominant in oceans where surface salinity is greater than 34 ppt. In the Red Sea where salinities may reach 40 ppt, only *S. serrata* species occur. The main distributions of the three other species (*S. paramamosain*, *S. tranquebarica* and *S. olivacea*) are concentrated on the South China Sea and Bay of Bengal. These are areas with salinities below 31 ppt.

For commercial hatchery operation of *S. serrata*, it is recommended that salinity be maintained at 32 ppt from Z1 up to megalopa. This method is less tedious and at the same time lessens the cost of hatchery operations since pumping of freshwater from scarce underground sources is no longer required.

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