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The combined effects of salinity and temperature on the survival and development of zoea, megalopa and crab instar larvae of mud crab, *Scylla tranquebarica* (Fabricius 1798)

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Abstract

An experiment was carried out to determine the combined effects of temperature and salinity on the survival and development of zoea, megalopa and crab instar stages of mud crab *Scylla tranquebarica* (Fabricius 1798). Salinities of 15, 25, 35 and 45 ppt were tested for each of the zoea stages; 5, 15, 25, 35, 45 ppt for the megalopae and 0, 5, 15, 25, 35 and 45 ppt for crab instar 1 (C1), at temperatures of 20, 26 and 32 ^{o}C. Two-way ANOVA showed a significant effect of temperature, salinity and interaction between temperature and salinity on the survival of all stages tested. Larvae at Z1–Z4 stages tolerated 25-35 ppt in 20 ^{o}C and 25–45 ppt in 26 and 32 ^{o}C. There was successful metamorphosis of Z5 larvae to megalopa at 25–35 ppt in 26 and 32 ^{o}C while all larvae died at 15 and 45 ppt, in all temperatures. The megalopae survived and developed to crab instar at 25–35 ppt in 26 and 32 ^{o}C. The first crab instar (C1) survived to the second crab instar (C2) at 5-45 ppt in 26 and 32 ^{o}C and at 15–45 ppt in 20 ^{o}C.

Introduction

In the Philippines, mud crab aquaculture relies heavily on wild-caught juveniles. Recently, supply has become unreliable as a result of over collection and destruction of mangrove habitat. To ensure sustainability of the mud crab aquaculture industry, there is a strong need to establish hatchery techniques for the commercial production of juveniles.

Interest in the worldwide development of the hatchery technology of mud crabs had focused mainly on the species *Scylla serrata* (Forskål 1775) as reported in Africa (Hill 1974; Hill 1979; Davis et al. 2004); Australia (Heasman and Fielder 1983; Mann et al. 1999; Ruscoe et al. 2004; Nurdiani and Zeng 2007); China (Zeng and Li 1992); Japan (Cowan 1984; Hamasaki et al. 2002; Hamasaki 2003); India (Marichamy and Rajapackiam 1991) and the Philippines (Baylon and Failaman 1999; Quinitio et al. 2001; Baylon 2010; Quinitio et al. 2011). Despite significant research efforts in the larval rearing of *S. serrata,* low survival in hatchery operation remains a problem.

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Scylla tranquebarica (Fabricius 1798), based on the classification of Keenan et al. (1998), is one of the three species of mud crabs of the genus *Scylla* found in Philippine waters that are of high commercial value (Camacho and Aypa 2001; Williams and Primavera 2001). It is a popular species for aquaculture and shares many of the attributes of *S. serrata* such as fast growth and appeal to the local and international markets (Camacho and Aypa 2001). To date, only two studies had been published on the larval rearing of *S. tranquebarica* (Maheswarudu et al 2007; Baylon 2009).

To develop seed production technology for crabs, temperature and salinity are two environmental factors that should be studied (Chen and Cheng 1985). The effect of one factor or a combination of both salinity and temperature, on the survival and development of the larvae had been documented in *S. serrata* (Brick 1974; Bhuiyan and Islam 1981; Chen and Cheng 1985; Davenport and Wong 1987; Zeng and Li 1992; Yunus et al. 1994; Hamasaki 2003; Ruscoe et al. 2004; Nurdiani and Zeng 2007; Baylon 2010) and in *Scylla olivacea* (Herbst 1796) (Baylon 2011). This study aimed to determine how varying salinities and temperatures affected the survival and development of larvae of *S. tranquebarica*. The results obtained will be valuable in the improvement of hatchery techniques for the species.

Materials and Methods

Broodstock management and larval rearing

A sexually mature female *S. tranquebarica* weighing 550 g, was collected from the wild and maintained in a 250-L cylindrical concrete tank with 10 cm sand substrate and strong aeration. Salinity of the rearing seawater was 35 ppt and temperature was 26-29 ^{o}C. The crab was fed to satiation, with fresh mussel, *Perna viridis* (Linnaeus 1758) meat. Daily water change was approximately 30% by flow through and cleaning of the tank was done every other day. After spawning, the berried crab was disinfected with 150 ppm formaldehyde for 30 min and transferred to a tank with no sand substrate to prevent bacterial and fungal infestation of the eggs. Eggs hatched to zoeae after 12 days of incubation.

The newly hatched zoeae were reared to crab instar in a 1000-L cylindrical, flat-bottomed fiberglass larval tank provided with mild aeration. The salinity and temperature range in larval rearing was similar to that in the spawner tank.

The larvae were fed with rotifers *Brachionus sp* from Z1 to Z2. The diet was shifted to newly-hatched *Artemia* nauplii (M & M brand from Great Salt Lake, Utah) from Z3 onwards. *Brachionus* was given at a density of 15-20 rotifers mL^{-1} while *Artemia* nauplii density was increasing with development: 0.5 *Artemia* nauplii mL^{-1} at Z3; 1.0 *Artemia* nauplii per mL at Z4 and 1.5 *Artemia* nauplii mL^{-1} at Z5. *Brachionus* were fed the microalgae*, Nannochloropsis oculata.* When larvae had metamorphosed to megalopae, *Artemia* density was increased to 5 nauplii mL^{-1} while crab instar

were provided with minced mussel as food. The feeding and water management protocols were patterned after the *S. serrata* seed production of juveniles by Quinitio et al (2001).

Experimental design and set up

The tolerance of *S. tranquebarica* at each developmental stage (zoea 1 to 5, megalopa and crab instar) was tested at various salinity levels: 15, 25, 35 and 45 ppt for each of the five zoeal stages; 5, 15, 25, 35, 45 ppt for the megalopae; and 0, 5, 15, 25, 35, 45 ppt for the crab instar. The larvae in each of the salinity level were reared at 20, 26 and 32 ^{o}C. There were a total of 12 temperature-salinity combinations tested for the zoeal stages, 15 for the megalopa and 18 for the crab instar. The salinity was determined using a hand refractometer with automatic temperature compensation (Westover TM Hand Refractometer Model RHS10ATC). The salinity of 45 ppt was prepared by adding sea salt to the seawater (Minagawa 1992; Nagaraj 1992) while lower salinity of 5, 15 and 25 ppt were prepared by mixing filtered and dechlorinated freshwater with seawater.

When the larvae needed for the experiment were of the same developmental stage, which occurred in the morning every 2-3 days for zoeal stages and every 3-5 days for megalopa and crab instar stages, they were collected from the 1000-L source tank in three plastic basins with a diameter of 30.48 cm and a depth of 7.62 cm, and brought to the temperature-controlled room.

The basin with zoeae was allowed to float in the water bath with a depth of 7.5 cm. The process lasted for about 1 h until the water temperature in the basin was similar to that of the 3.5-L cylindrical plastic containers in the water bath where the larvae were reared. Zoeae were stocked at 30, megalopae at 15 and crab instar at 10 individuals per container. The zoeae were transferred to the rearing containers by means of a large bore pipette while megalopae and crab instar were transferred by means of a plastic spoon to avoid injury. The same procedure was done for the megalopae and crab instar. Acclimation of larvae was not done for the different salinity levels. Results of previous work by the author (unpublished) demonstrated no significant difference in larval viability between gradually acclimated and abruptly introduced when transferring zoea to different salinities.

The set-up for 20 ^{o}C was maintained in a water bath in an air-conditioned cubicle. The 26 ^{o}C set-up was maintained in the water bath without heaters while the 32 ^{o}C set-up was maintained with 300-W submersible thermostat-controlled heaters. In the warm set-up, air lifts were positioned at regular intervals for equal distribution of heat. Water temperature was maintained within $\pm$1 ^{o}C. There were three replicates per salinity-temperature treatment. The feeding protocol was similar to that described for the larvae and crab instar in 1000-L rearing tanks. All replicates in one temperature treatment were confined to one water bath.

Survival, onset of development and instar duration were monitored daily during transfer to new containers with the appropriate salinity and temperature. The days when larvae across the three

replicates in each treatment had developed to the next stage was recorded as the days of the onset of development. Instar duration was determined by counting the number of days from the onset of molting to the next stage, to the day when all the larvae had completed the molt. Larvae that had metamorphosed to megalopa and crab instar stages were promptly removed from the containers to prevent them from preying on the remaining larvae. The experiment was terminated when all zoeae, megalopae and crab instar had grown to the next stage of development.

Zoeae and crab instar used in the experiments were 1 day old while the megalopae were 4 days old. This criterion was based on earlier observation that newly molted megalopae exhibited high mortalities, which could mask the effect of salinity and temperature on survival. Plastic netting, formed into ribbons and tied with a pebble to prevent floating, was provided as shelter to minimise cannibalism during the megalopa and crab instar stages.

Statistical Analysis

The percentage survival at the end of each developmental stage, were arcsine transformed and analysed using two-way ANOVA. This was used to determine if there was a significant effect of temperature, salinity and interaction between temperature and salinity, on survival. One-way ANOVA was employed on the various salinity-temperature combinations as treatments to find out if differences among treatments were significant. Duncan's Multiple Range Test was then applied to identify which salinity-temperature combinations were significantly different from each other. All statistical analyses were computed using SPSS software, version 10 (SPSS Inc., 44 N Michigan Avenue, Chicago, IL, USA).

Results

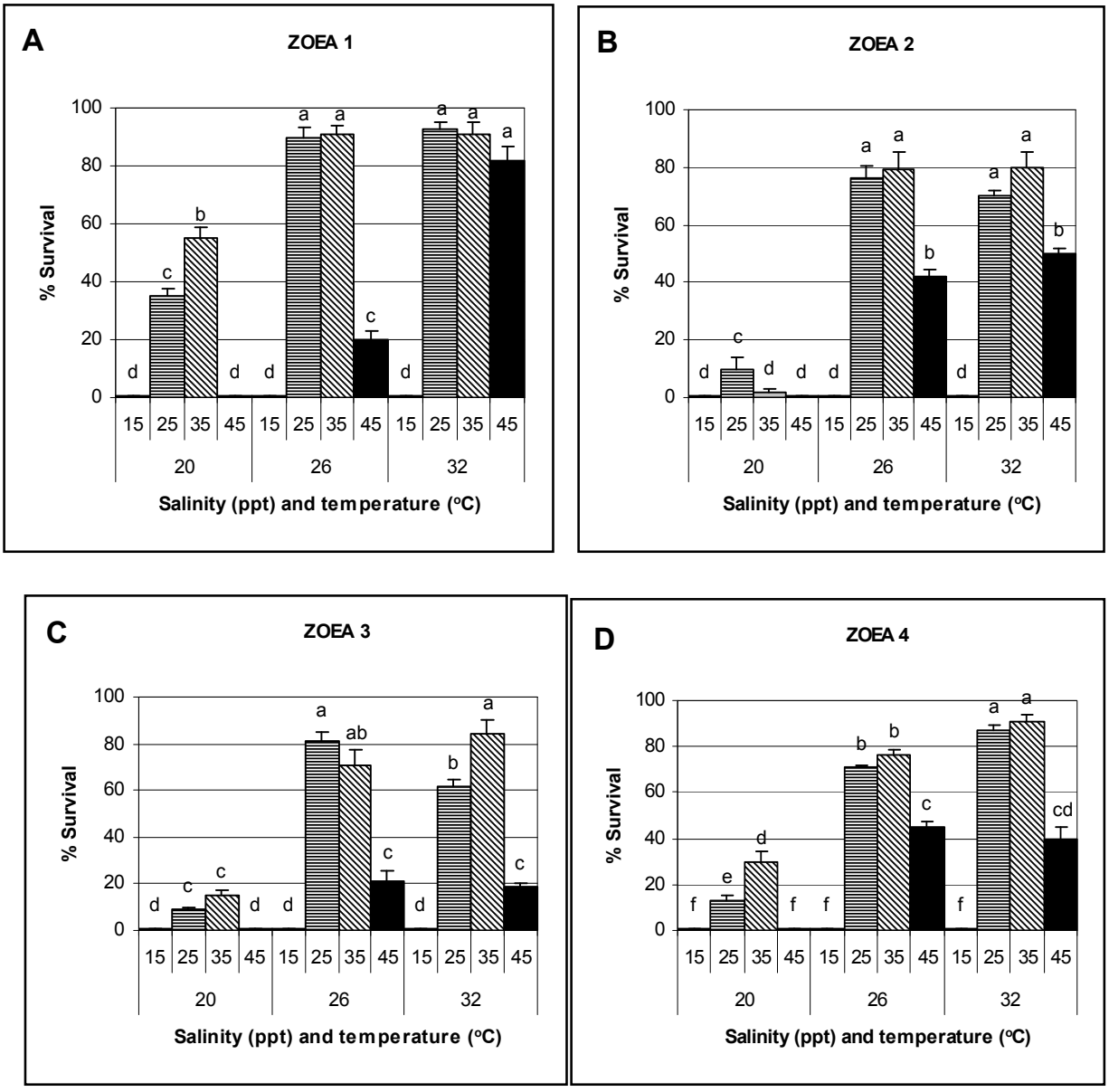
Effects on survival

Two-way ANOVA showed a significant effect ($P<.01$) of temperature, salinity and interaction between temperature and salinity on the survival of zoea, megalopa and crab instar stages.

The survival of each larval stage reared in various combinations of salinity and temperature is shown in Figure 1A-G. Survival of Z1-Z4 larvae was high (62–90%) at 25–35 ppt in 26 and 32 ^{o}C but low (2–55%) at 25–35 ppt in 20 ^{o}C (Fig 1A-D). The larvae survived (18–50%) at 45 ppt at 26 and 32 ^{o}C while none survived at 15 ppt, regardless of the temperature.

There was successful metamorphosis (22-55%) of Z5 to megalopa at 25–35 ppt in 26 and 32 ^{o}C (Fig 1E). The Z5 larvae died in all other salinity-temperature combinations. High survival and development of megalopae to crab instar (60–78%) were observed when reared at 25-35 ppt in 26 and 32 ^{o}C while survival was very low (12–26%) in all other salinity temperature combinations (Fig 1F). As larvae grew to crab instar, there was greater tolerance to low salinity and to low temperature

(Fig 1G). Crab instar 1 survived and developed to crab instar 2 at a salinity range of 15–45 ppt in 20 °C which widened to 5–45 ppt in 26 and 32 °C. There was 100% survival and development to the next stage at 15–35 ppt in 26 and 32°C and at 35 ppt in 20 °C. None of the crab instar survived at 0 ppt salinity, regardless of the temperature.



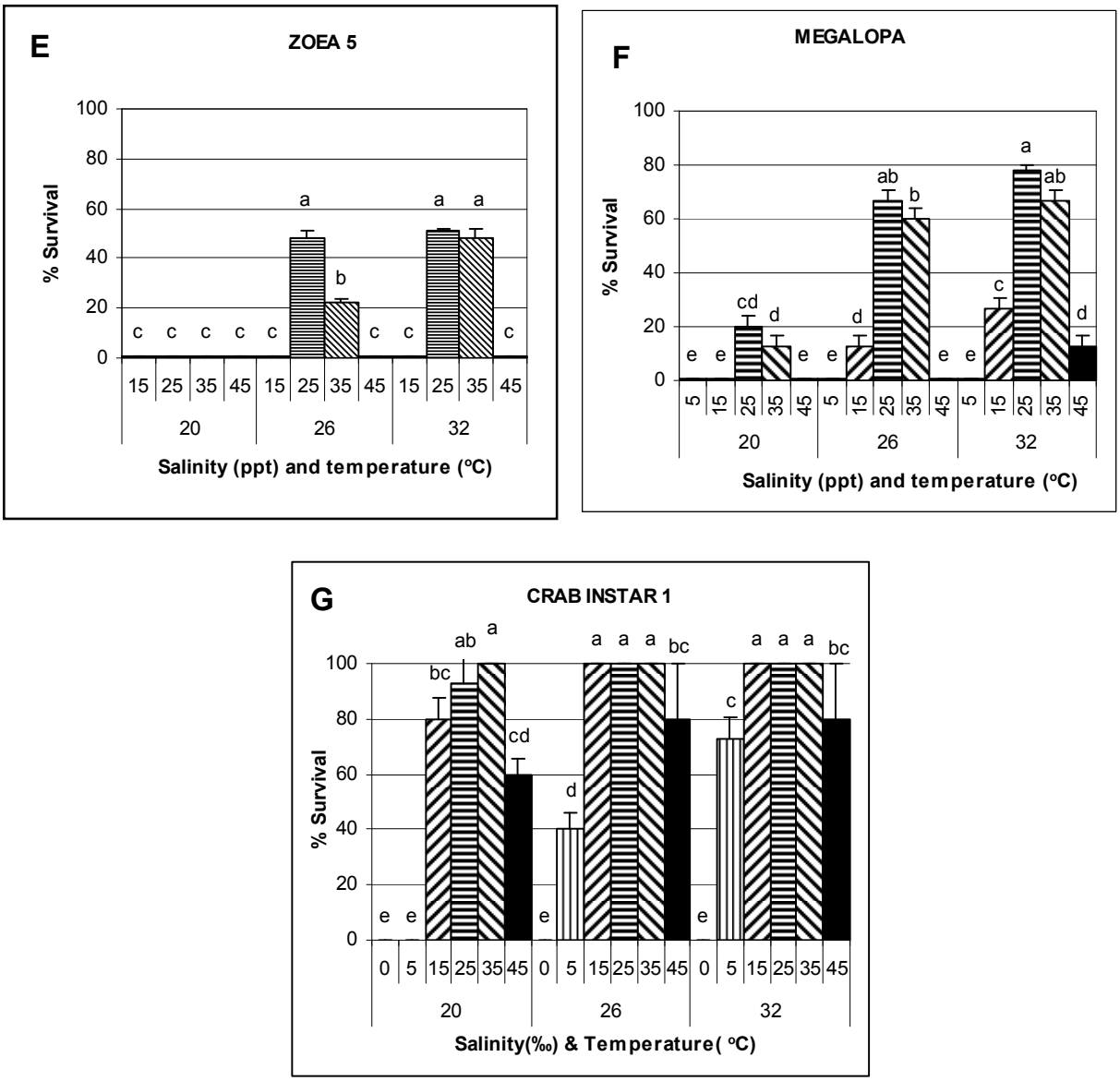


Fig. 1. Percent survival (±S.E.) of each stage of *S. tranquebarica* zoea (A-E), megalopa (F) and crab instar (G) abruptly transferred and reared at various combinations of salinity (0, 5, 15, 25, 35, 45 ppt) and temperature (20, 26, 32 °C). Bars with the same letters are not significantly different (P <.01).

Effects on development

Salinity and temperature affected the onset of development and instar duration (Table 1). In all stages tested across treatments, onset of development was delayed up to 9 days when in colder temperature of 20 °C compared to 26 and 32 °C. For example, at 25 and 35 ppt, Z1 larvae developed to Z2 on the 8th and 7th day in 20 °C while development to Z2 occurred on the 3rd and 2nd day in 26 and 32 °C, respectively. In every temperature level tested, onset of development of larvae at Z1 to megalopa stages occurred on the same day at 25 and 35 ppt. except in Z1 where there was a delay of 1 day at 25 ppt/20 °C and in Z5 at 35 ppt/26 °C. At crab instar, onset of development in every

temperature tested occurred on the same day at 15-35 ppt but at 5 and 45 ppt it was delayed up to 12 days in 20 ^{o}C; 3 days in 26 ^{o}C and 1 day in 32 ^{o}C. In all stages, instar duration was longer at lower temperature of 20 ^{o}C except in Z3 at 25-35 ppt/20 ^{o}C and in megalopa at 25-35 ppt/ 20 and 26 ^{o}C where only few larvae survived, hence there was shorter instar duration. In every temperature level tested, instar duration of larvae at Z1 to megalopa stages was the same for both 25 and 35 ppt except in Z5 where it was longer by 1 day in 25 ppt/ 26 ^{o}C. At crab instar stage, duration of molt was the same at 15-45 in 20 ^{o}C and in 5-45 ppt in 26 and 32 ^{o}C.

Table 1. Onset of development and instar duration (in parentheses) of *S. tranquebarica* zoea, megalopa and crab instar larvae reared at various salinity and temperature levels.
* All larvae died after 24 h of rearing

Temp (^{o}C)	Salinity (ppt)	Z1	Z2	Z3	Z4	Z5	Megalopa	Crab instar
20	0							____****
	5							____*
	15	____*	____ **	____ **	____ **	____**	____ **	13–16(4)
	25	8–11(4)	10–13(4)	11–12(2)	9–12(4)	____**	9–12(4)	13–16(4)
	35	7–10(4)	10–13(4)	11–12(2)	9–12(4)	____**	9–12(4)	13–16(4)
	45	11–16(6)	____**	____**	____**	____**	____**	16–19(4)
26	0							____****
	5							7–9(3)
	15	____**	____**	____**	____**	____*	11–13(3)	5–7(3)
	25	3–5(3)	4–6(3)	3–6(4)	4–6(3)	5–11(7)	9–12(4)	5–7(3)
	35	3–5(3)	4–6(3)	3–6(4)	4–6(3)	6–11(6)	9–12(4)	5–7(3)
	45	5–7(3)	6–8(3)	5–7(3)	6–8(3)	____***	____*	7–9(3)
32	0							____****
	5							5–7(3)
	15	____**	____**	____**	____**	____*	8–12(5)	4–6(3)
	25	2–3(2)	3–4(2)	2–3(2)	2–4(3)	4–8(5)	8–12(5)	4–6(3)
	35	2–3(2)	3–4(2)	2–3(2)	2–4(3)	4–8(5)	8–12(5)	4–6(3)
	45	3–5(3)	5–7(3)	4–5(2)	4–6(3)	____***	8–12(5)	5–7(3)

** All larvae died after several days of rearing
*** All larvae died due to failure to metamorphose to megalopa
**** All larvae died after 10 min of exposure
Onset of development – the range in days, when larvae across the 3 replicate containers in each treatment had developed to the next stage.
Instar duration - the number of days from the onset of development to the day when all the larvae in 3 replicates had completed the molt.

Discussion

Successful metamorphosis to megalopa and crab instar at 25-35 ppt in 26 and 32 ^{o}C

The salinity-temperature combinations that supported survival of all zoeal stages, metamorphosis of Z5 larvae to megalopae and megalopae to crab instar were 25–35 ppt/ 26 and 32^{o}C. These results for *S. tranquebarica* are similar for *S. serrata,* where high survival and metamorphosis to megalopae and crab instar occurred at salinities 25–35 ppt in moderate and warm

temperatures of 26 and 32 ^{o}C (Baylon 2010). In *S. olivacea*, the early stage larvae (Z1–Z3) also survived at 25 and 35 ppt in 26 and 32 ^{o}C but unlike the two other *Scylla* species, up to 80% of *S. olivacea* zoea larvae survived in low temperature of 20 ^{o}C (Baylon 2011). *Scylla olivacea* at later larval stages (Z4–Z5) survived a wider salinity range of 15-35 ppt in all temperatures tested, although survival was low at 15 ppt/20 ^{o}C. The same salinity-temperature combinations favoured the survival and development by the megalopae to crab instar.

Among the three *Scylla* species, *S. olivacea* appears to be more tolerant at low salinity (15 ppt) as survival to megalopae of up to 95% occurred when reared at 32 ^{o}C (Baylon 2011). Apparently, *S. olivacea* has better osmoregulatory capabilities compared with *S. serrata* and *S. tranquebarica*. Although it has been reported by Keenan et al. (1998) that both *S. olivacea* and *S. tranquebarica* are usually found in mangroves and coastlines inundated with reduced salinity, the results of the present study could explain the common observation that fewer number of *S. tranquebarica* are caught in mangrove swamps compared to *S. olivacea*.

The optimum salinity-temperature combinations that supported development to juvenile crabs have been reported in other crustacean larvae. In the stone crab, *Menippe mercenaria* (Say 1818), the optimum salinity was 30–35 ppt in 30 ^{o}C (Ong and Costlow 1970). For the hermit crab, *Clibanarius vittatus* (Bosc 1802), it was 25–30 ppt in 25-30 ^{o}C (Young and Hazlett 1978). In the red frog crab, *Ranina ranina* (Linnaeus 1758), successful metamorphosis to megalopa was at 24–37 ppt in 28 ^{o}C (Minagawa 1990) while in the crucifix crab, *Charybdis feriatus* (Linnaeus 1758), it was 25-35 ppt in 26-32 ^{o}C (Baylon and Suzuki 2007). Salinities and temperatures favourable for the survival and development of decapod crustacean larvae and juveniles in laboratory conditions were found to be similar to the salinities and temperatures of their natural environment (Anger 2001).

Increased tolerance to wider salinity range with development

The *S. tranquebarica* zoea successfully metamorphosed to megalopa at a narrow salinity range of 25–35 ppt. This range widened to 15–45 ppt at megalopa stage and further expanded to 5–45 ppt at crab instar. Similarly for *S. serrata*, zoea successfully metamorphosed to megalopa at a salinity range of 25–35 ppt which widened to 15–45 ppt at megalopa stage and further expanded to 5–45 ppt at crab instar (Baylon 2010). In *S. olivacea*, the zoea tolerated a much wider salinity range of 15–35 ppt which further increased to 15–45 ppt at megalopae and expanded to 5–45 ppt for crab instar stage (Baylon 2011). Increased salinity tolerance to a wider salinity range with growth has also been reported in other portunid crabs. For *C. feriatus* a narrow salinity range of 25–35 ppt promoted high survival and development of zoea and megalopae, but widened to 25–45 ppt for crab instar (Baylon and Suzuki 2007). Tolerance to salinity is frequently variable during ontogeny, where embryos and larvae of crustaceans are often less tolerant than juveniles and adults (Charmantier 1998).

Increased tolerance to low temperature with development

Scylla tranquebarica zoeae and megalopae exibited high sensitivity to a low temperature of 20 ^{o}C with no successful metamorphosis. It was observed that *S. tranquebarica* zoea reared in cold temperature of 20 ^{o}C had reduced "tumbling" and swimming activities and their abdomens were in flexed position most of the time, unlike those of larvae in 26 and 32 ^{o}C where abdomen were extended. Abdominal flexure was necessary for the crab larvae to capture food prey. Hence, the mortalities could have been caused by starvation due to decline in feeding (Heasman and Fielder 1983). At crab instar stage however, a high survival of 80–100% was obtained at 20 ^{o}C. The crab instar larvae reared in cold temperature were observed to be sedentary and could be mistaken to be dead, but they exhibited movement when disturbed with a plastic spoon. When the crab instar that were produced from the cold setup were harvested and transferred in water temperature of 26 ^{o}C, they immediately became vigorous, similar to those collected from the warm temperature set-up of 32 ^{o}C. The same observation has been reported on *S. serrata* (Baylon 2010) and *S. olivacea* (Baylon 2011).

Faster development at warmer temperatures

With increasing temperature, the onset of development was quicker and development period was shorter. In this present study, it took 21 days for the S. *tranquebarica* zoea larvae reared at 25–35 ppt / 32 ^{o}C to reach the first crab instar and 28 days in 26 ^{o}C . At 20 ^{o}C, none of the zoea survived to reach the megalopa stage. For *S. serrata*, it took 16 days for the zoea to reach the first crab instar at 32 ^{o}C; 21 days in 26 ^{o}C and 50 days at 20 ^{o}C (Baylon 2010). For *S. olivacea* larvae, it took 16 days to reach the first crab instar in 32 ^{o}C; 20 days in 26 ^{o}C, and 45 days in 20 ^{o}C (Baylon 2011). The shorter development period at higher temperatures is likely to be a result of increasing metabolic rate, enzyme activity and hormone levels involved in the molting process (Skinner 1985).

Comparing the three *Scylla* species, both *S. olivacea* and *S. serrata* demonstrated the shortest number of days to reach the first crab instar stage (16 days) while *S. tranquebarica* exhibited the longest duration (21 days) when incubated at 32 ^{o}C. In all the three species, there was a delay in the development in 20 and 26 ^{o}C. In hatchery operation, prolonged molting, especially at later zoeal stages, would be detrimental as this may result in higher cannibalism of megalopae on zoeae, as observed in the mass seed production of *S. serrata* (Quinitio et al. 2001).

Conclusion

Both salinity and temperature affected the survival and development of zoea, megalopa and crab instar of *S. tranquebarica*. Zoeae and megalopae preferred salinity levels of 25 and 35 ppt in warmer temperatures of 26 and 32 ^{o}C. The crab instar demonstrated high tolerance to low temperature of 20 ^{o}C at a salinity range of 15–45 ppt and a wider salinity range of 5–45 ppt at warmer temperatures of 26 and 32 ^{o}C. Compared with *S. serrata* and *S. olivacea*, the larvae of *S.*

tranquebarica appear to be the most sensitive to fluctuations in salinity and temperature and most likely to suffer heavy mortalities if optimum environmental conditions are not established during hatchery operation.

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