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Effect of Salinity on the Growth, Survival and Development of the Commercially Important Portunid Crab Larvae of *Portunus sanguinolentus* (Herbst)

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Abstract: Tolerance of the larvae of *P. sanguinolentus* to salinity stress was studied in the laboratory at different water salinities ranging from 15 to 40‰. Complete larval development from hatching through metamorphosis to first crab instar occurred only in salinities from 25 to 40‰. Even though 1% of the larvae at 25‰ were able to moult up to megalopa, they did not successfully moult to first crab instar stage. The total duration for the successful completion of larval development was 30-33 days, 17-20 days, 14-17 days and 18-21 days, respectively in the salinities of 25, 30, 35 and 40‰. There was variation in the development rate of larvae at different salinities, i.e., 0.029, 0.038, 0.040 and 0.031, respectively, in the salinities of 25, 30, 35 and 40‰. The optimum salinity range for each zoeal and megalopal stages varies. The optimum salinity for the zoea I and megalopa was 35‰, and for zoeae II, III and IV, it was 30-35‰. However, comparatively the larvae in 35‰ salinity showed high development and survival rate and hence the 35‰ salinity was found to be the optimum salinity. Where as 30 - 35‰ was considered as the optimum salinity range to complete the larval development of *P. sanguinolentus*.

Key words: *Portunus sanguinolentus,* salinity, megalopa, instar, first crab, moult

INTRODUCTION

Among the laboratory studies on the effect of salinity on development of crab larvae of a number of species have demonstrated the range of responses of larvae to the environment. The biological effects of variations in salinity are considered to be among the most important factors influencing marine organisms (Ponce-Palafox *et al*., 1997). The salinity is particularly important because it represents ecological master factor for many aquatic organisms and it is easier to measure and control than many other environmental entities. The objective of this work was to study the effect of salinity on larval development of *P. sanguinolentus,* in relation to survival and duration of larval stages. To accomplish this aim, a factorial designed experiment was carried out in the laboratory under controlled conditions.

MATERIALS AND METHODS

The block coloured brooder about to hatch was collected from Parangipettai landing centre. The study was conducted from June 2009 to July 2009. After hatching, the larvae were maintained in different test salinities following the method of Kannupandi *et al.* (2000). The larvae were tested at 6 different salinities, 15, 20, 25, 30, 35 and 40‰ following the method of Costlow *et al*. (1966). Since the three spot crabs are marine in origin, lower salinities such as 5‰ and 10‰ were not included in the experiment. The tests were conducted in small glass finger bowls containing 100ml water with 10 larvae per bowl. Hundred larvae were subjected to test salinity in the range of 15 to 40‰. Hence freshly hatched larvae were separated into 6 groups of 100 individuals each. Three groups of larvae were placed directly in bowls containing seawater of salinities 25, 30 and 35‰. The other groups were gradually acclimated for 2 hours to their final rearing salinities (40, 20 and 15‰). Experimental salinities were obtained by filtering seawater (35‰) and diluting it with glass distilled water. Seawater of 40‰ was obtained by evaporating seawater.

Daily counts were made of exuviae and surviving larvae. The larvae were transferred daily to clean bowls containing freshly filtered seawater of the same salinity. Sample of each larval stage and exuviae were preserved in 10% formalin for further observations. Experiments were terminated when all larvae had either dead or moulted to first crab instar. Triplicate was maintained for each salinity.

Live feed culture:

***Chlorella marina*:** The inoculum of *C. marina* was inoculated into the seawater enriched with ammonium

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Table 1: Survival (%) and development of *P. sanguinolentus* larvae reared at different salinities

	Salinities											
	15‰		20‰		25‰		30‰		35‰		40‰	
Stages	Mean ± SD	Survival (%)	Mean ± SD	Survival (%)	Mean ± SD	Survival (%)	Mean ± SD	Survival (%)	Mean ± SD	Survival (%)	Mean ± SD	Survival (%)
Zoea I-	-	-	7.12±0.25	21	4.75±0.64	32	3.50±0.40	71	3.12±0.25	90	3.87±0.25	55
Zoea II	-	-	-	-	4.50±0.40	28	3.62±0.25	68	3.12±0.25	95	4.00±0.40	49
Zoea III	-	-	-	-	3.75±0.28	20	3.25±0.28	63	2.75±0.28	90	2.87±0.47	46
Zoea IV	-	-	-	-	4.37±0.47	8	3.25±0.28	76	2.87±0.25	95	3.37±0.47	37
Megalopa	-	-	-	-	13.5±0.40	1	6.00±0.40	69	4.25±0.28	87	7.12±0.47	54

sulphate, super phosphate and urea in a ratio of 10:1:1. The green colour developed within 3 to 4 days was the indication of *C. marina* development

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Rotifer (*Brachionus plicatilis*): After inoculating the rotifer (30 individuals / mL) into the *Chlorella* tank, the yeast was added daily as supplementary feed to the rotifers. After the microscopical observation on 3rd or 4th day rotifers were harvested and to the tank an equal amount of *Chlorella* with the medium was added for further culture of rotifer. Continuous vigorous aeration was given and the temperature was maintained as 30±2.0ºC throughout the culture period.

***Artemia* nauplii (OSI Brine shrimp eggs, USA):** The *Artemia* nauplii were harvested from the *Artemia* hatching tank and placed in a plastic tub with required quantity of water. The enrichment solution (Culture Selco - INVE, Belgium) was added at a concentration of 0.1%. The nauplii were enriched for 12 h and after washing in seawater the nauplii were fed to the crab larvae.

Feeding Regime: After 3 h of hatching, the feeding was started. The zoea I was fed with the rotifer *B. plicatilis*; zoea II to IV were fed with rotifer dominated *Artemia* nauplii and zoea V and megalopa were fed with *Artemia* nauplii dominated formulated feed. The feed was given twice a day at 8'O clocked in the morning and 5'O clock in the evening *ad libitum*. Food was changed each day with freshly hatched *Artemia* nauplii.

RESULTS

Intermoult period varied among larval stages of *P. sanguinolentus* in different test salinities. The results of survival, mortality and developmental durations were presented in Table 1.

Survival / Mortality:

15‰ Salinity: All the freshly hatched zoeae I survived for 3 days. 54% of the larvae died on the 4th day. Overall 75% of the larvae died before 5th day. Before 11 days, 97% of zoea I died. All larvae died within 12 days from the time of hatching.

20‰ Salinity: 79% of the individuals died in the zoea I stage. Rest of the larvae died in the II zoeal stage. All the larvae died before metamorphosing to the III zoea. No larva survived to complete metamorphosis.

25‰ Salinity: Mortality was lower when compared to other lower salinities. Mortality rate was 68% in zoea I, 72% in zoea II and 80% in zoea III. Overall 92% of the zoea IV died before metamorphosis to the megalopa. Megalopa almost failed to reach the first crab stage. Exceptionally with a prolonged duration, only one megalopa survived to reach the first crab.

30‰ Salinity: Mortality was lower than that observed in other lower salinities. Mortality was 29% in the zoea I, 32% in zoea II, 37% in zoea III, 24% in zoea IV and 31% in megalopa respectively. Few megalopa survived to complete metamorphosis.

35‰ Salinity: Mortality was the least (13%) in this salinity. Mortality was 10% in the zoea I and zoea III stages. In zoea II, zoea IV and megalopa, the mortality was 5, 5 and 13%, respectively.

40‰ Salinity: The total mortality in this salinity was 46%. The percentage mortality of zoea I, zoea II, zoea III, zoea IV and megalopa were 45, 51, 54, 63 and 46%, respectively.

Development:

15‰ Salinity: No development occurred to any later stage in this salinity.

20‰ Salinity: After 7 days, 21% of the individuals moulted to the zoea II. None of the zoea II survived for moulting to zoea III.

25‰ Salinity: Only one larva in this salinity was able to metamorphose to the first crab. Moulting from zoea I to all subsequent zoeal stages, megalopa and first crab was prolonged. The zoeae I, II, III, IV and megalopa required 4.75±0.64, 4.50±0.40, 3.75±0.28, 4.37±0.47 and 13.50±0.40 days, respectively. Total duration for complete larval development was 30-33 days. The development rate was 0.029.

Table 2: Optimum salinity range for different zoeal stages and megalopa of *P. sanguinolentus*

Stage	Salinity range (‰)
Zoea I	35
Zoea II	30 - 35
Zoea III	30 - 35
Zoea IV	30 - 35
Megalopa	35

30‰ Salinity: The zoea I took 3.50±0.40 days to moult to zoea II. Zoeae II, III, IV and megalopa lasted 3.62±0.25, 3.25±0.28, 3.25±0.28 and 6.00±0.40 days, respectively before metamorphosing to first crab instar. Overall duration for the complete metamorphosis was 17-20 days. The rate of development was 0.038 days.

35‰ Salinity: The zoeae I, II, III, IV and megalopa required 3.12±0.25, 3.12±0.25, 2.75±0.28, 2.87±0.25 and 4.25±0.28 days, respectively. The time taken for complete metamorphosis was 14-17 days, which was the lowest among all test salinities. The development rate was 0.040, which was the highest among all the salinities tested.

40‰ Salinity: The intermoult period of zoeae I, II, III, IV and megalopa was 3.87±0.25, 4.00±0.40, 2.87±0.47, 3.37±0.47 and 7.12±0.47, respectively. The total larval development duration was 18-21 days, which was more than that of 30 and 35‰ salinities. The development rate was 0.031, which was very close to the value of 25‰, i.e., 0.029.

Optimum Salinity: The optimum salinity range for each zoeal and megalopal stages varies (Table 2). The optimum salinity for the zoea I and megalopa was 35‰ and for zoeae II, III and IV, it was 30-35‰. However, comparatively the larvae in 35‰ salinity showed high development and survival rate and hence the 35‰ salinity was found to be the optimum salinity and 30-35‰ was considered as the optimum salinity range to complete the larval development of *P. sanguinolentus*.

DISCUSSION

The results of the present study clearly show that the salinity (15-40‰) influences the growth, survival and development to complete the larval development of the three-spot crab, *P. sanguinolentus*. First crab stage appeared only in the salinities of 30, 35 and 40‰ with highest survival rates achieved in 35‰ (87%), followed by 30‰ (69%) and 40‰ (54%). Hence, it is confirmed that the optimum salinity range for culturing the larvae of *P. sanguinolentus* lies in the range of 30-35‰. A similar range of optimum salinities have been reported for the larvae of estuarine crab, *Thalamita crenata* (Kannupandi *et al.*, 1997) and in inshore water crab, *P. corallicola* (Kannupandi *et al.*, 2005).

The survival to the further zoeal stages was nearly meager in salinities below the optimal levels (15-20‰). About 20% of zoea I metamorphosed to zoea II at 20‰ and ultimately they failed to reach zoea III. In connection to the lower survival at suboptimal salinities, Charmantier *et al.* (2002) emphasized that most of the newly-hatched larval stages are regarded as being more sensitive to the low saline waters. High mortality of larvae in the low salinities may be attributed to prolonged moulting as a result of difficulties in casting of old cuticle. The hardening of new cuticle also takes too long time, resulting in larval mortality due to osmotic loss of important ions (Hagerman, 1973). Anger *et al.* (1998) stated that larvae of *C. maenas* respond sensitively against continuous or even short-term transitory exposure to reduced salinities, i.e., $\leq$20‰. Significant decreases were found in the rates of early zoeal survival, development, growth, respiration, and assimilation. Since the rate of oxygen consumption per larva tended to decrease at low salinities, the concurrent depression of larval growth cannot be explained with enhanced metabolic demands under hypoosmotic stress. Rather, this was a consequence of a reduced assimilation capacity; future measurements of larval ingestion rates at different salinities should show whether this was a consequence of decreasing food uptake, decreasing conversion efficiency, or both.

The mortality at the lower salinities is possibly due to imbalance in osmoregulatory mechanism (Foskett, 1977). Gills are the important sites of active ion transport in adult decapods. Larvae lack gills or develop gills in the penultimate or ultimate zoeal stages (Foskett, 1977). Most crabs do not develop gills until the penultimate or ultimate zoeal stage. Megalopal stage of the family Xanthidae, Grapsidae, Ocypodidae and Portunidae possesses well-developed gills (Yang and McLaughlin, 1979; Charmantier, 1998; Anger *et al.*, 2000). The presence of these potential salt-absorbing tissues would be advantageous for the survival of larval and postlarval forms in low salinities, occurring in estuaries or more brackish and freshwater environments (Rabalais and Gore, 1985). The ability for hyperosmotic regulation in very low salinities is in general developed only by later larval or early juvenile stages of decapod crustaceans (Foskett, 1977; Charmantier *et al.*, 1981, 1988). Larvae with advanced morphological features (e.g., Gills) could experience greater survivorship and develop in low salinities (Rabalais and Gore, 1985). In many other crustaceans like *Macrobrachium australiense, M. shokitai, M. rosenbergii, Caridina breverostris, Penaeus aztecus* and *Astacus leniusculus*, the larvae possess salt absorbing epithelia or gills (Talbot *et al.*, 1972; Shokita, 1973; Harrison *et al.*, 1981; Lee and Fielder, 1981).

Larval respiration was in general reduced at lower salinities (Anger *et al.*, 1998). Salinity tolerance of larvae

without necessary osmoregulatory tissues and/or regulatory mechanisms is more restrictive and survivorship lowers outside of a limited range (Rabalais and Gore, 1985). Larval susceptibility to lower salinity can be a major limiting factor in the distribution of a species (Vernberg, 1983; Krishnan and Kannupandi, 1987; Vijayakumar and Kannupandi, 1987; Balagurunathan and Kannupandi, 1993; Kannupandi *et al*., 1997; Kannupandi *et al*., 2000; Kannupandi *et al*., 2005).

Similarly, mortality at higher salinity (40‰) is likely being due to the inability of the larvae to osmoregulate (Foskett, 1977). Foskett (1977) suggested that since larvae lack a heavy exoskeleton, hyperosmoticity in all salinities might be necessary to provide turgor pressure to insure integrity of the thin larval cuticle. Mortality at high and low salinities may perhaps be attributed to osmotic stress, *i.e*., rupture of cells at low salinity due to hyperosmosis and shrinkage of cells at higher salinities (Perumal and Subramanian, 1985).

Salinity strongly influences the growth and development in tropical crab larvae. In the present study, the growth and development was slow at low salinities (15-25‰). Development and hatching of eggs takes place under a relatively wide range of salinity and water temperature, but this range narrows in successive zoeal instars (Lin *et al*., 1994; Iin *et al*., 2003). In *S. serrata*, salinity along with temperature strongly influences the growth and developmental duration of zoea to reach megalopa (Ong, 1964; Brick, 1974; Haesman and Fielder, 1983; Marichamy and Rajapackiam, 1984, 1992; Jamari, 1992). Similar kind of influence of salinity on development duration was reported for *S. oceanica* by Anil and Suseelan (1999). Larval growth was particularly affected by hypo-osmotic stress during the initial phase (postmoult, intermoult) of the zoea I moult cycle in *C. maenas* (Anger *et al*., 1998). They also reported that, there was a shift in energy partitioning: net growth efficiency decreased under the influence of reduced salinities.

The growth and development at low salinities (15-25‰) is slow, which is probably due to increased rate of excretion (Emerson, 1969; Pandian, 1975; Kinne, 1976; Johns, 1981; Kannupandi *et al.,* 1997). In coastal waters, osmotic stress due to low and variable salinities may reduce growth rates and then fitness of larvae. Low salinities lead to a decrement in growth rates or even loss of weight in larval instars of several marine and estuarine crustaceans (Johns, 1982; Pfaff, 1997; Anger *et al*., 1998; 2000). At stressful salinity (both highest and lowest salinities), protein is used as a source of energy (Johns, 1981). The crustacean larvae have protein as the major portion of their biochemical composition (Frank *et al*., 1975; Capuzzo and Lancaster, 1979; Kannupandi *et al*., 1997). Utilization of dietary and body protein as energy diverts this resource from growth to maintenance needs (Johns, 1981; Kannupandi *et al*., 1997). Laughlin and French (1989) found that crab larvae from low prehatching salinities had a lower dry weight, and suggested compensatory modifications of the nitrogen metabolism, reducing the concentration of osmotically active organic molecules (Schoffeniels and Gilles, 1970). Thus at stressful salinities development is delayed (Krishnan and Kannupandi, 1987; Vijayakumar and Kannupandi, 1987; Balagurunathan and Kannupandi, 1993; Kannupandi *et al*., 1997; Kannupandi *et al*., 2000; Kannupandi *et al*., 2005).

Development at higher salinity (40‰) is quicker because high salinity may accelerate decomposition of the cuticle, which is little quicker in the tropics owing to high temperature (Heegaard, 1971). Anger *et al.* (2000) while working on the *Armases miersii*, growth within individual moulting cycles and the overall level of larval biomass increase per molting cycle appears to be exceptionally low in the normal salinities (from 15 to 45‰). Similar kind of results were obtained by Anger and Schultze (1995) in a preliminary study on elemental composition, growth and exuvial loss in the larval stages of two semi terrestrial crabs, *S. curacaoense* and *A. meirsii*. They also interpreted the possible reasons as a consequence of partial utilization of enhanced internal energy reserves remaining from the egg yolk. These reserves enable the early larvae of this species to survive and develop, when necessary, also with limited or lacking planktonic food sources (Anger, 1995a). A relationship between particularly low zoeal growth and a high endotrophic potential was observed also in other decapod species that have partially emancipated from the sea (Anger, 1995b).

During the course of current study, while maintaining the brooders of *P. sanguinolentus* at different salinities, it was observed that the hatching of eggs and release of larvae occurs in the waters of salinity ranging between 30-35‰. Thus it can be confirmed that, though the adults occupy the estuarine and mangrove niches (John *et al*., 2004), larvae of this species cannot survive in the waters of low salinities (15 to 25‰). Campbell and Fielder (1987) opined that, in the larvae of *P. sanguinolentus*, the occurrence of prezoeae increased when eggs were hatched at salinities below the oceanic salinities that this species normally encounters in nature. Norse (1977) noted that of the ≈ 300 species of portunid crabs, very few are euryhaline as adults and none of these spawn in low salinities. He hypothesized that although portunids have been ecologically expansive, radiating from a shallow marine origin into a variety of habitats (including freshwater), they are reproductively conservative. Even species adapted to freshwater and estuarine life migrate to sea to release their larvae in waters of suitable salinity.

Prehatching salinity regulated the amount of reserves invested per egg and lost during embryogenesis (Gimenez

and Anger, 2001) and affected the osmoregulatory capacity and starvation tolerance of the first zoea (Charmantier *et al*., 2002; Gimenez, 2002). Differences in salinity experienced as embryos and initial larval biomass acted on a set of physiological and developmental processes taking part in the propagation or reversion of initial variability in larval biomass. Variability in larval biomass may have consequences for survival and growth at advanced larval or juvenile stages (Gimenez and Torres, 2002). The biomass of freshly hatched zoea I depends on initial egg biomass and salinity experienced during embryogenesis (Gimenez and Anger, 2001).

Anger *et al*. (2000) studied the effects of salinity on growth of larval and early juvenile of an extremely euryhaline crab, *A. miersii*. They reported that the biomass increments in successive stages of larvae were, in general, relatively little influenced by salinity, showing conspicuous depression only at the most extreme conditions (5 and 55 ppt). Also the timing of exposure was not a very important factor in the salinity tolerance. As an overall tendency, later (acute) exposure to unfavourable salinities appeared to affect growth to slightly lesser degree than long-term exposure. In the megalopa and crab I, the range of salinities allowing for maximum carbon accumulation appears to widen again, shifting back towards lower salt concentrations. This was explained with two major ontogenic changes:

- The efficiency of hyperosmoregulation in dilute media ($\leq$25 PSU) should significantly increase, due to the appearance of functional gills and an enlargement of other transport epithelia (Charmantier, 1998). As a consequence of reduction in the energy costs for hyper-osmoregulation, more energy should become available for growth in brackish water.
- In the megalopa stage, *A. miersii* attains also the capability of hypo-regulation in concentrated media. Reduced growth in the megalopa and crab I at high salinities ($\geq$ 35 PSU) may thus reflect an increasing energy demand for hypoosmoregulation. When these late stages were exposed, without prior acclimatization, to enhanced salinities, also their mortality and development rates indicated particularly strong osmotic stress (Anger, 1996).

In the natural environment, the larvae may escape from unfavourable dilute media by sinking to deeper water layers with higher salinities as this kind of avoidance behavior is suggested by Roberts (1971) and Anger (1985). Further it appears to be a general response of decapod larvae to lower salinity stress (Roberts, 1971). The megalopa has a range of salinity tolerance, i.e., 30-35‰, may be due to the well-developed gills as suggested by Foskett (1977). Similar pattern of tolerance to low salinity was found in the later stages of development, viz., megalopa has been reported in *C. sapidus* (Costlow, 1967) and *Neoepisesarma (Neoepisesarma) mederi* (Selvakumar *et al*., 1987). In the present study, *P. sanguinolentus* has different optimum salinities for different stages: 35‰ for zoea I, and megalopa, 30-35‰ for zoea II, III and IV, respectively. The salinity requirements of a species may change with the stage in its life history (Vernberg and Vernberg, 1971).

Thus from the current study, it can be confirmed that salinity greatly influences the growth, survival and development of *P. sanguinolentus* larvae. Metamorphosis to the further stages did not happen in the lower salinities (15-25‰). Megalopa metamorphosed to first crab stage in all other salinities, i.e., 30, 35 and 40‰. Among these three, the optimal salinity for the larval development is ranged between 30-35‰.

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