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**ORIGINAL RESEARCH ARTICLE**

# Effect of Salinity on Growth and Survival of Portunid Crab, *Charybdis eriata* Larvae

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## ABSTRACT

The salinity is particularly important because it represents ecological master factor for many aquatic organisms. The objective of this work was to study the effect of salinity on larval development of *C. feriata* in relation to survival and duration of larval stages. Survival was very limited for both 15 and 20‰ salinities. In 25‰ salinity, maximum survival was reported (33%) in zoea I and minimum (1%) in megalopa. In 30‰ salinity, survival was reasonable when compared to other salinities. Maximum survival was reported in zoea I (71%) and minimum survival was reported in megalopa stage (54%). In 35‰ salinity, survival was maximum when compared to other test salinities. Maximum survival of 95% was recorded in zoeal stage I. Minimum survival was recorded in megalopa stage (75%). In 40‰ salinity, maximum survival was recorded in zoea I (55%) and minimum was recorded in megalopa stage (32%). Development was very limited for both15 and 20‰ salinities. In 25‰ salinity, the zoeae I, II, III, IV,V, VI and megalopa required 4.70, 3.50, 3.40, 3.51,3.42,3.58 and 6.48 days, respectively. Total duration for complete larval development was around 28-30 days. In 30‰ salinity, almost all zoeal stages took approximately 3-4 days to reach next stage and VI zoea took 6.38 days to moult into megalopa. Overall duration for the complete metamorphosis was 25-27 days. In 35‰ salinity, each zoeal stage required 3-4 days to reach next stage. The time taken for complete metamorphosis was 22-25 days which was the lowest among all test salinities. In 40‰ salinity, each zoeal stage took 3-4 days to reach the next stage. The total larval development duration was 25-30 days, which was considered to be more than that of 30‰ and 35‰ salinities. Based on the study the optimum salinity range lies between 30-40‰ and optimum salinity was considered to be 30‰ for almost all larval stages.

**Key words:** Growth, survival, *C. feriata*, larvae, salinity, effect.

## INTRODUCTION

The salinity is particularly important because it represents an ecological master factor for many aquatic organisms and it is easier to measure and control than many other environmental entities [1,2,4]. Its influence has been noted in larval survival, development, morphology, the moulting cycle, growth, feeding, metabolism, energy partitioning and behavior [3,5,6]. The objective of this work was to study the effect of salinity on larval development of *C. feriata*, in relation to survival and duration of larval stages.

## MATERIALS AND METHODS

The collection of gravid females and their maintenance was followed as earlier. After hatching, the larvae were maintained in different test salinities following the method of [1,2]. The larvae were tested at 6 different salinities, 15%, 20%, 25%, 30%, 35% and 40% following the method of [7]. Since the 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 (55 mm diam. X 50 mm depth) containing 100ml water with 10 larvae per bowl. 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

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filtered seawater of the same salinity. Experiments were terminated when all larvae had either dead or moulted to megalopa. Triplicate was maintained for each test salinity.

**Live Feed Culture**

***Chlorella marina***

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

**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±2.0°C 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 hours and after washing in seawater the nauplii were fed to the crab larvae.

**Feeding Regime**

After 3 hours 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 clock 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 is varied among larval stages of *C.feriata* in different test salinities.The results of survival and developmental durations are presented in (**Table 1**).

**Survival (%)**

**15‰ Salinity**

All the freshly hatched zoeae I survived only for few days and subsequently died before reaching to next stage.

**20% Salinity**

The survival was only 20% up to II stage. No single II zoeae moulted into III zoeal stage.

**25% Salinity**

Survival rate was 33% in zoea I, 28% in zoea II, 20% in zoea III, 8% in zoea IV, 6% in zoea V, 4% in zoea VI and 1% in megalopa.

**30‰ Salinity**

Survival was reasonable when compared to other salinities. Maximum survival was reported in zoea I (71%) and minimum survival was reported in megalopa stage (54%).The survival was reduced considerably from zoea I to megalopa stage.

**35% Salinity**

Survival was maximum when compared to other test salinities. Maximum survival of 95% was recorded in zoeal stage I. Minimum survival was recorded in megalopa stage (75%).

**40‰ Salinity**

Maximum survival was recorded in zoea I (55%) and minimum was recorded in megalopa stage (32%).

**Intermoult duration (days)**

**15% Salinity**

No development occurred to any later stage in this salinity.

**201% Salinity**

After 6 days very few individuals moulted to zoea II. None of the zoea II moulted to zoea III.

**25% Salinity**

Moulting from zoea I to all subsequent zoeal stages and megalopa was prolonged. The zoeae I, II, III, IV,V, VI and megalopa required 4.70, 3.50, 3.40, 3.51,3.42,3.58 and 6.48 days, respectively. Total duration for complete larval development was around 28-30 days.

**30% Salinity**

Almost all zoeal stages took approximately 3-4days to reach next stage and VI zoea took 6.38 days to moult into megalopa. Overall duration for the complete metamorphosis was 25-27 days.

**35% Salinity**

Each zoeal stage required 3-4 days to reach next stage. The time taken for complete metamorphosis was 22-25 days which was the lowest among all test salinities.

**40% Salinity**

Each zoeal stage took 3-4 days to reach the next stage. The total larval development duration was 25-30 days, which was considered to be more than that of 30‰ and 35% salinities.

**Optimum Salinity**

The optimum salinity range lies between 30-40% and optimum salinity was considered to be 30% for almost all larval stages.

**Table 1: Survival (%) and intermoult duration (days) of *C.feriata* larvae reared at different salinities**

| Stages | Salinities | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 20‰ | | 25‰ | | 30‰ | | 35‰ | | 40‰ | |
| | Mean ± SD | % of Survival | Mean ± SD | % of Survival | Mean ± SD | % of Survival | Mean ± SD | % of Survival | Mean ± SD | % of Survival |
| Zoea I | 6.14±0.25 | 20 | 4.70±0.65 | 33 | 3.12±0.25 | 71 | 3.50±0.40 | 90 | 3.87±0.25 | 55 |
| Zoea II | - | - | 3.50±0.24 | 28 | 3.12±0.25 | 68 | 3.62±0.25 | 95 | 4.00±0.40 | 49 |
| Zoea III | - | - | 3.40±0.28 | 20 | 3.25±0.28 | 63 | 3.25±0.28 | 90 | 2.87±0.47 | 46 |
| Zoea IV | - | - | 3.51±0.20 | 8 | 3.18±0.25 | 76 | 3.25±0.28 | 95 | 3.37±0.47 | 42 |
| Zoea V | - | - | 3.42±0.41 | 6 | 3.25±0.28 | 69 | 3.32±0.40 | 87 | 3.72±0.47 | 38 |
| Zoea VI | - | - | 3.58±0.22 | 4 | 3.30±0.28 | 62 | 3.41±0.28 | 80 | 4.02±0.33 | 35 |
| Megalopa | | | 6.48±1.21 | 1 | 6.38±0.19 | 54 | 5.42±0.30 | 75 | 6.78±0.47 | 32 |
| Total Days | 6-7 | - | 28-30 | - | 25-27 | - | 22-27 | - | 28-30 | - |

## DISCUSSION

The result of the present study clearly shows that the salinity influences the growth and survival of *C.feriata larvae*. Megalopa stage appeared only in the salinities of 30%, 35% and 40% with the highest survival rates achieved in 35% (75%), followed by 30% (54%) and 40% (32%). Hence, it is confirmed that the optimum salinity range for culturing the larvae of *C.feriata* lies in the range of 30-35%. A similar range of optimum salinities have been reported for the larvae of estuarine crab, *T. crenata* [3] in inshore water crab, *P. corallicola* [4] and three spot crab *P.sanguinolentus* [2].

The survival of the zoeal stages was nearly meager in salinities below the optimal levels (15% to 20%). [5,6] emphasized that most of the newly-hatched larval stages are regarded as being more sensitive to the lower saline waters.High mortality of larvae in the lower 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 [8]. [9] stated that larvae of *C. maenas* respond sensitively against continuous or even short-term transitory exposure to reduced salinities, *i.e* 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 lower 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 [10]. 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 [10]. 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 [6,11,12]. The presence of these potential salt-absorbing tissues would be advantageous for the survival of larval and postlarval forms in lower salinities, occurring in estuaries or more brackish and freshwater environments [13]. The ability for hyperosmotic regulation in very lower salinities is in general developed only by later larval or early juvenile stages of decapod crustaceans [6,10]. Larvae with advanced morphological features (e.g., Gills) could experience greater survivorship and develop in lower salinities [13]. 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 [14,15, 16].

Larval respiration was in general reduced at lower salinities [9]. Salinity tolerance of larvae without necessary osmoregulatory tissues and/or regulatory mechanisms is more restrictive and survivorship lowers outside of a limited range [13]. Larval susceptibility to lower salinity can be a major limiting factor in the distribution of a species [1,3,4,17,18].

Similarly, mortality at higher salinity (40%) is likely being due to the inability of the larvae to osmoregulate [10]. [10] 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 higher and lowerer salinities may perhaps be attributed to osmotic stress, *i.e*., rupture of cells at lower salinity due to hyperosmosis and shrinkage of cells at higher salinities [19].

Salinity strongly influences the growth and development in tropical crab larvae. In the present study, the growth and development was slow at lower salinities (15-25ppt). 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 [20]. In *S. serrata*, salinity along with temperature strongly influences the growth and developmental duration of zoea to reach megalopa [21-23]. Similar kind of influence of salinity on development duration was reported for *S. oceanica* by [24] and *P.sanguinolentus* [2]. Larval growth was particularly affected by hypo-osmotic stress during the initial phase (postmoult, intermoult) of the zoea I moult cycle in *C. maenas* [9]. 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 lower salinities (15-25%) is slow, which is probably due to increased rate of excretion [1,2]. In coastal waters, osmotic stress due to lower and variable salinities may reduce growth rates and then fitness of larvae. Lower salinities lead to a decrement in growth rates or even loss of weight in larval instars of several marine and estuarine crustaceans [9,25]. At stressful salinity (both highest and lowest salinities), protein is used as a source of energy [25]. The crustacean larvae have protein as the major portion of their biochemical composition [3]. Utilization of dietary and body protein as energy diverts this resource from growth to maintenance needs [3,25]. [26] 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 [27]. Thus at stressful salinities development is delayed [1-4].

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 [28]. [12] while working on the *Armases miersii*, growth within individual moulting cycles and the overall level of larval biomass increase per moulting cycle appears to be exceptionally low in the normal salinities (from 15 to 45ppt. Similar kind of results were obtained by [29] 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 [30]. 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 [31].

During the course of current study, while maintaining the brooders of *C. feriata* at different salinities, it was observed that the hatching of eggs and release of larvae occurs in the waters of salinity ranging between 30-35ppt. Thus it can be confirmed that, though the adults occupy the estuarine and mangrove niches [32], larvae of this species cannot survive in the waters of lower salinities (15% to 25%). [33] opined that, in the larvae of *C.feriata*, the occurrence of prezoeae increased when eggs were hatched at salinities below the oceanic salinities that this species normally encounters in nature. [34] noted that about 300 species of portunid crabs, very few are euryhaline as adults and none of these spawn in lower 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 [35] and affected the osmoregulatory capacity and starvation tolerance of the first zoea [36]. 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 [37]. The biomass of freshly hatched zoea I depends on initial egg biomass and salinity experienced during embryogenesis [35].

[12] 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 %). Also the timing of exposure was not a very important factor in the salinity tolerance. As an overall tendency, later (acute) exposure to unfavorable salinities appeared to affect growth to slightly lesser degree than long-term exposure. In the megalopa 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. 1. The efficiency of hyperosmoregulation in dilute media (25%) should significantly increase, due to the appearance of functional gills and an enlargement of other transport epithelia [5]. As a consequence of reduction in the energy costs for hyper-osmoregulation, more energy should become available for growth in brackish water. 2. 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 ‰) 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 [38].

In the natural environment, the larvae may escape from unfavorable dilute media by sinking to deeper water layers with higher salinities as this kind of avoidance behaviour is suggested by [39,40]. Further it appears to be a general response of decapod larvae to lower salinity stress [39]. The megalopa has a range of salinity tolerance, i.e., 30-35‰, may be due to the well-developed gills as suggested by [10]. Similar pattern of tolerance to lower salinity was found in the later stages of development, viz., megalopa has been reported in *C. sapidus* [7] and *Neoepisesarma (Neoepisesarma) mederi* [41].

Thus from the current study, it can be confirmed that salinity greatly influences the growth and survival of *C.feriata* larvae. Metamorphosis to the further stages did not happen in the lower salinities (15 – 20%). Even though megalopa was obtained 30, 35 and 40%. The survival and growth is maximum in 35‰. So it is suggest that 35% is optimum salinity for *C.feriata* and 30-40 ppt considered to be optimum range.

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