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Effects of salinity on the larval development of a semiterrestrial tropical crab, *Sesarma angustipes* (Decapoda: Grapsidae)

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ABSTRACT: Hatching and larval development of the semiterrestrial tropical brachyuran *Sesarma angustipes* were studied in relation to salinity in laboratory experiments. Adults were found in southern Brazil living in terrestrial habitats and in freshwater creeks. When a free choice was offered in the laboratory, females liberated their larvae indiscriminately in freshwater or seawater Rearing experiments were carried out at 0, 3, 10, 20, and 32‰ S. Unlike in other marine and brackish water species, freshly hatched zoea I larvae remained actively swimming in freshwater, where they survived for almost 2 d. Maximum survival time of zoea I exposed to 3‰ S was ca 3.5 d. The larvae were able to pass through some or all developmental instars (4 zoeal stages and 1 megalopa) only at higher salinities. The zoea I at all salinities ≥ 10‰ S had a high survival rate to the second stage. Its minimum duration of development (with 100% survival), however, was observed at 20‰ S. From the zoea II stage, survival was in general highest and development shortest at 32‰ S, whilst 10‰ S allowed only occasional development to later stages. This indicates a decreasing tolerance to low salinities and a shift in optimum salinity toward seawater in successive zoeal instars. Only 2 megalopae successfully reached metamorphosis to the juvenile crab, both at 20‰ S. These results may reflect the following hypothetical patterns in larval development of *S. angustipes* under field conditions: (1) the zoea I may hatch in freshwater or brackish water; (2) if hatching takes place in freshwater, the larva has 1 to 2 d to be transported by currents to brackish water or seawater; (3) the major part of zoeal development (from the zoea II or III stage) takes place in lower estuaries and coastal oceanic waters with >20‰ S; (4) the megalopa may return to less saline environments. It remains unknown whether the megalopa can successfully metamorphose in freshwater, or if recruitment in rivers and other freshwater biota takes place by upstream migration of juveniles. Our results show that *S. angustipes* must be considered a marine species that has only started its transition to terrestrial and freshwater habitats. Larval morphology of *S. angustipes* was practically identical with that presented in a previous description of *Sesarma ricordi* larvae, confirming the hypothesis that these 2 species are synonyms.

INTRODUCTION

The transition from life in the marine environment to terrestrial and freshwater habitats has been achieved by many brachyuran crab species, in particular members of the families Gecarcinidae, Ocypodidae, Hymenosomatidae, and Grapsidae (Burggren & McMahon 1988). While most of these species need to return to the sea in order to reproduce, others show abbreviated or absent larval development as an adaptation to their life on land or in freshwater (Lucas 1980, Powers & Bliss 1983, Rabalais & Gore 1985). Within the Grapsidae, the mangrove tree crab *Aratus pisoni* Milne Edwards, the Chinese mitten crab *Eriocheir sinensis* Milne Edwards, and a number of *Sesarma* species belong to the best-known examples of such transitions. The latter genus is particularly interesting, as it comprises marine and brackish water species which have 3 or 4 zoeal stages, as well as freshwater and semiterrestrial species with only 2 stages (Rabalais & Gore 1985). Abbreviated larval development is usually associated with lecithotrophy (Hartnoll 1964, Lam 1969).

Sesarma angustipes Dana, 1852 is often referred to by the synonyms *S. miersi* or *S. ricordi* (for taxonomy of this species see Abele 1972a). It is one of those species that can live in marine, terrestrial and freshwater habitats. It occurs on islands in the Carribean Sea (Hartnoll 1965) and along the tropical and subtropical

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coasts of Brazil, south to the state of Santa Catarina (Melo 1984). It is often found in the marginal zones of tropical estuarine mangrove swamps (e.g. Gerlach 1958, Melo 1984). Hartnoll (1965) observed it frequently in Jamaican rivers, up to ca 100 m upstream from their mouths, while some authors also reported it from the freshwater that is collected by bromeliad plants (Sattler & Sattler 1965, McWilliams 1969, Abele 1972b).

The present authors have observed adult *Sesarma angustipes* in Pontal do Sul (state of Paraná, Brazil) occurring regularly in dry terrestrial habitats near a mangrove river (also inside the Centro de Biologia Marinha [CBM] laboratory building), without any tendency to flee toward open water. Moreover, we found them on the nearby island Ilha do Mel (Paraná) and near São Sebastião (state of São Paulo), living on rocks in mountain creeks that merge in mangrove swamps or disappear in sandy beaches on the open Atlantic coast. We never observed *S. angustipes* on a beach or in seawater.

The type of development in *Sesarma angustipes* has not yet been ascertained with certainty. Since this species often lives in isolated freshwater habitats, abbreviated or direct development might have been expected, as well as a regular meroplanktonic development through several larval stages. The latter mode of development is more likely, as Abele (1972a) considered *S. ricordi* a synonym of *S. angustipes*, after Díaz & Ewald (1968) had described 5 zoeal and 1 megalopa stage in *S. ricordi* from Venezuela. Hartnoll (1965) assumed that the zoeae of *S. angustipes* hatch in freshwater and then develop in the sea; however, this assumption remained unconfirmed by experimental evidence. Therefore, we investigated hatching and development of *S. angustipes* larvae under controlled conditions in the laboratory.

MATERIALS AND METHODS

Male and ovigerous female *Sesarma angustipes* were collected in November 1988 in and near to a freshwater mountain creek on the marine island of Ilha do Mel (Paraná, Brazil) and then transferred to the CBM (Pontal do Sul). There they were maintained in a large glass aquarium in which small plastic aquaria (21 × 11 × 3.5 cm) were inserted, with some bottom space left free. The smaller aquaria were filled with either seawater (ca 32 ‰ S) or freshwater. The crabs could move freely on the dry bottom (with stones facilitating climbing), and they could easily enter and leave the small seawater and freshwater aquaria. This experiment was intended to (1) provide information on possible differential habitat preferences in adult males and females, (2) show where the females eventually release their larvae, and (3) yield freshly hatched larvae for rearing experiments.

Freshly hatched zoea larvae were collected with wide-bore pipettes and transferred directly to cultivation vials (30 ml, for individual rearing) and glass bowls (400 ml, for mass rearing); the latter were used for morphological studies. Salinities tested in individual rearing experiments were: 0 (freshwater, from tap), 3, 10, 20 ‰ S, and ambient coastal seawater (ranging between 29 and 33 ‰ S, but mostly near 32 ‰ S). Both freshwater and seawater were filtered prior to use or mixing. In each salinity, 50 sibling larvae (originating from the same female) were used. The experiments were carried out at a constant 25 (± 1) °C and a 12:12 h L:D light regime. Water and food (freshly hatched San Francisco Bay Brand™ *Artemia* sp. nauplii) were changed daily. During the first 4 d, the cultures were checked every 12 h for mortality and moults, thereafter only every 24 h (during a water change).

Morphological studies were carried out with fixed material (both larvae and exuviae stored in 4 % formaldehyde in seawater) that was later dissected under a Wild stereo microscope and examined under a Leitz compound microscope. Thirty individuals were checked in each of the zoeal stages and 10 in the megalopa. Statistical comparison of mean values with equal or unequal variances (after an F-test) was carried out using different t-statistics (Sachs 1984).

RESULTS

Habitat selection experiment

During ca 2 wk of daily observation, no clear habitat selection was exhibited by either males or females. Most individuals remained outside the water, but both freshwater and seawater also were entered indiscriminately.

Hatching of larvae took place in both types of water, with a slight preponderance in freshwater. A few hours after hatching, the larvae released in freshwater or seawater showed no differences in level of activity. Positive phototaxis and shadow response (Forward 1977) were equally strong in both groups.

In no case was moulting, mating, or egg-laying observed after the release of a hatch.

Larval development and morphology

Under optimum conditions, larvae developed through 4 zoeal stages and a megalopa to the first juvenile crab. Salinity had strong effects on survival rate and, to a lesser degree, on duration of development (see below), but not on the number or morphology of larval instars. Larval morphology was examined and compared with the descriptions given by Díaz & Ewald (1968) for *Sesarma ricordi* from Venezuela. A few details in the setation of appendages and the telson were found to differ slightly in some individuals, whereas most larvae corresponded in all details with Díaz & Ewald's descriptions. Variation was observed mainly in the first maxilliped of the zoea III, where setation formulae of 2, 3, 1, 2, 5 and 0, 2, 2, 3, 2 occurred, besides that reported by Díaz & Ewald (2, 2, 2, 2, 6). In the telson of the megalopa, sometimes a central seta occurred on the posterior margin, in addition to 3 short setae on each side and 2 small lateral setae or spines. These deviations were considered taxonomically irrelevant, as they were within the range of individual variability. Our material may thus be considered taxonomically identical with that described by Díaz & Ewald.

Effects of salinity on larval survival and duration of development

Freshly hatched larvae were quite active and appeared viable in any salinity, from 0 to 32‰ S. However, first mortality was found in freshwater after 12 h exposure (Fig. 1). Most larvae in this experimental group died within the following 24 h, with complete mortality within 48 h. Larvae maintained at 3‰ S survived significantly longer, with 100% survival during the first 60 h. Thereafter, mortality increased drastically, and the last larvae died within the following 36 h (Fig. 1).

When first mortality appeared at 3‰ S (during the third day), one mass culture with this salinity was changed to ca 15‰ S to save the larval material of this group. Thereafter, mortality in this culture was low during a major part of zoeal development; the initial stress exerted by very low salinity caused no apparent irreversible damage to the larvae.

At 10‰ S, survival was 100% until the first moult occurred on the third day after hatching (Fig. 1). Of these, 70% (35 individuals) moulted successfully to the second zoeal stage, and 28% reached the zoea III instar. Only 2 individuals at this low salinity reached the zoea IV stage, and none the megalopa (Fig. 2).

Survival patterns at 20 and 32‰ S were very similar (Fig. 1). Survival at 20‰ S decreased only after the zoea III instar. At 32‰ S all larvae reached the zoea IV, while only 76% survived at 20‰ S (cf. Fig. 2). However, in the megalopa stage there was complete mortality in full seawater (mostly during or shortly after moulting to this stage), whereas 2 individuals (4%) survived through metamorphosis at 20‰ S.

Cumulative survival rates from hatching to succes-

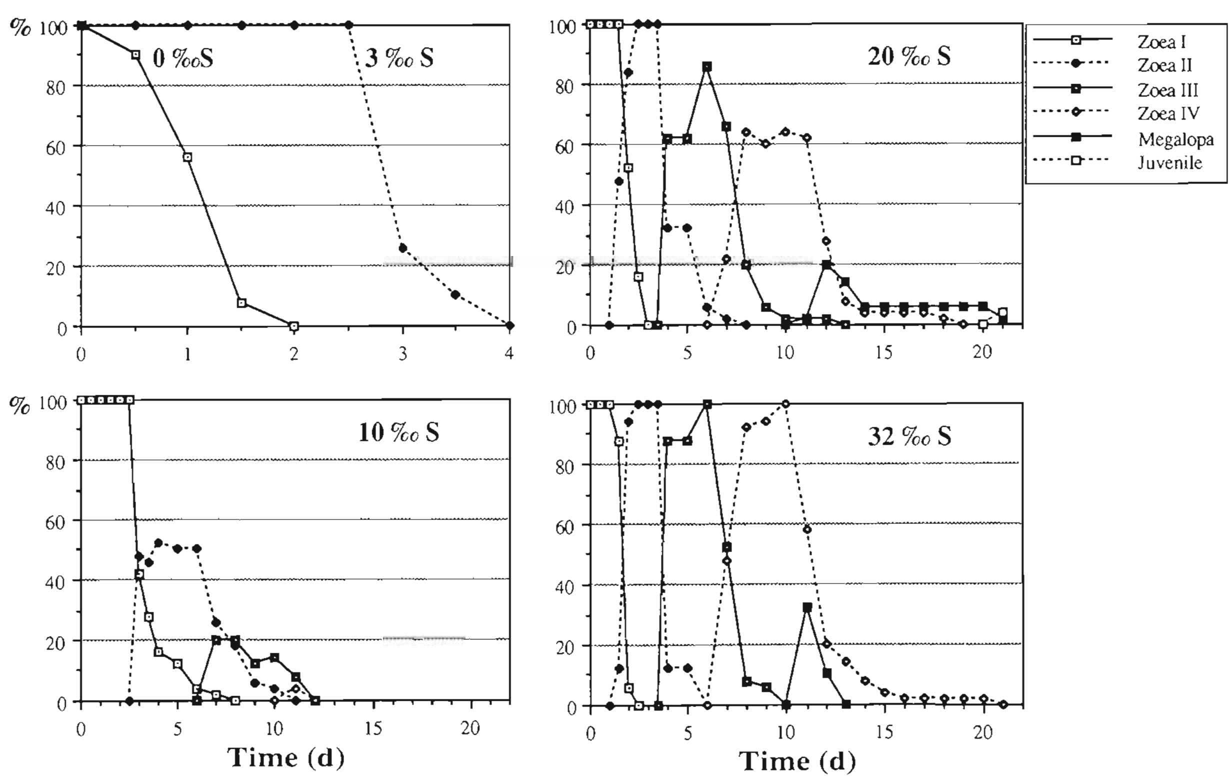

Fig. 1 *Sesarma angustipes*, larval stages. Survival (%) and development through successive instars in relation to salinity (‰ S); initial n = 50

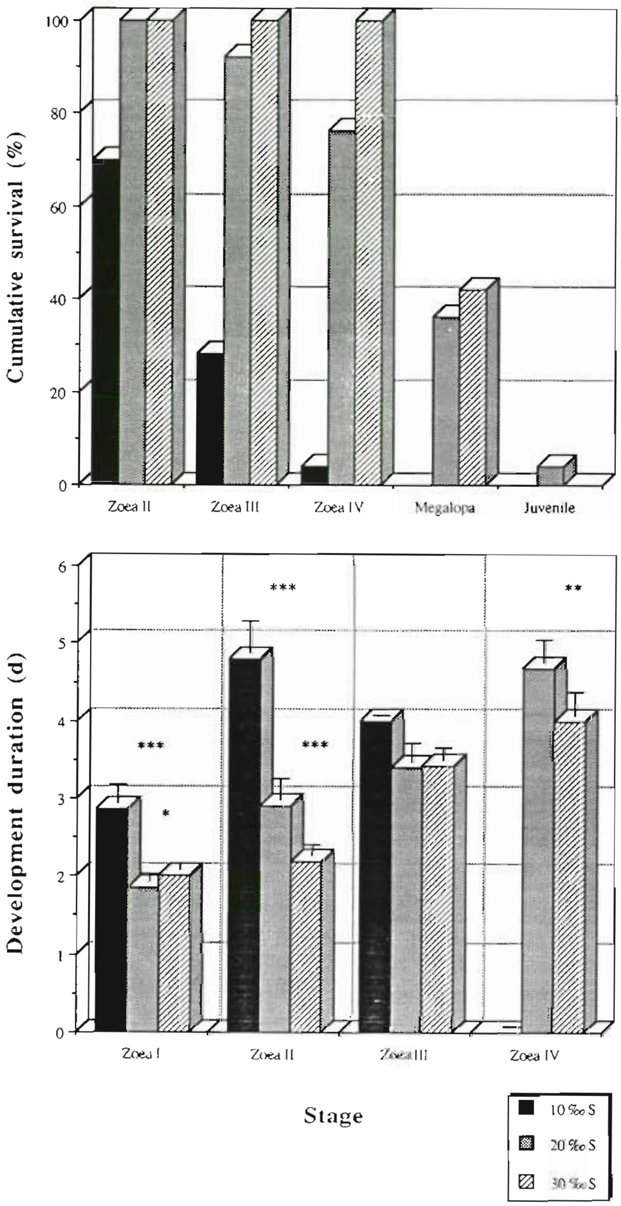

Fig. 2. *Sesarma angustipes*, larval stages. Cumulative survival rate (%) from hatching to subsequent instars (upper graph; initial n = 50); duration of development (d; x ± 95 % confidence intervals) at 10, 20, and ca 32 ‰ S (lower graph), asteriks mark statistically significant differences between mean development times: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

sive developmental stages are compared in Fig. 2 (upper graph) for the 3 highest salinities. This comparison shows again that salinities ≤ 10 ‰ S become detremental as soon as the first moult is approached. On the other hand, 20 ‰ S resulted in increased mortality (compared with 32 ‰ S) in later zoeal instars, but allowed at least some survival of megalopa larvae through metamorphosis.

Since the larvae were reared individually, duration of development may be calculated separately for each stage. Fig. 2 (lower graph) shows that a strongly reduced salinity (10 ‰ S) increased duration of development significantly. Water of 20 ‰ S in the zoea I instar had a slight but statistically significant ($p < 0.05$) accelerating effect on duration of development as compared to seawater, whereas later it had no effect (zoea III) or even caused a delay (zoea II, IV).

The patterns shown by these developmental data agree in general with the survival data described above. They may be summarized as follows. (1) Freshly hatched zoea I larvae of *Sesarma angustipes* may survive in freshwater for 24 to 36 h. (2) At 3 ‰ S the zoeae may survive for more than 3 d, but there is complete mortality when the first moult is approached. (3) 10 ‰ S has a significant delaying effect on development, but most zoea I larvae moult successfully to the second stage, a few may reach the third or fourth instar. Later zoeal stages cannot develop in such a low salinity. (4) 20 ‰ S represents a very suitable condition for early larval stages and possibly, again for the ultimate larval instar, the megalopa. (5) Seawater (32 ‰ S) appears optimum for the zoeal stages II to IV, whereas the megalopa did not survive in this condition.

DISCUSSION

The very close correspondence in larval morphology of *Sesarma angustipes* from Brazil and *S. ricordi* from Venezuela (Díaz & Ewald 1968) provides strong additional support to Abele (1972a) who concluded from comparison of adult specimens that these 2 species were synonyms. The same correspondence should also be expected for *S. roberti* from Africa, since Hartnoll (1965) synonymized it with *S. angustipes.*

The results of the present study confirm Hartnoll's (1965) speculations on the life cycle of *Sesarma angustipes*. They conform with the view that this species has only begun its transition from marine to freshwater and terrestrial habitats. The adults are very well adapted to tolerate extremely wide variations in salinity and water temperature and to survive long exposure to freshwater or air. The larval stages, in contrast, depend on the existence of aquatic habitats with a higher salinity and enough food organisms to allow planktotrophic development through 5 free-swimming instars. The wide geographical distribution of *S. angustipes* in the South Atlantic likely is due to this regular 'marine' type of larval development.

The first zoeal stage reveals clear adaptations to the species' life in freshwater environments. Even in extremely euryhaline brackish-water species, freshly hatched larvae exposed to freshwater will immediately cease swimming and die within a few hours. In contrast, most *Sesarma angustipes* zoeae remain active and unaffected throughout 1 d, and many individuals may survive even for a second day. This unusual degree of tolerance to osmotic stress would give the zoea I sufficient time to swim actively near the surface and be transported by currents out of their river of origin, into brackish or marine waters.

When the larvae have reached the oligohaline waters of an upper estuary, they would have even more time for their further transport: in a salinity as low as 3‰ S they can survive about 3 d. This should normally be sufficient for them to reach waters with higher salinities, where longer survival times and some further development become possible. However, it is interesting to note that brackish water conditions with 10‰ S still have obvious negative effects on larval survival and development rates in *Sesarma angustipes*, whereas other brackish water species that never live in freshwater (e.g. *Rhithropanopeus harrisii*) may develop even at 2.5‰ S (Costlow et al. 1966).

The tolerance of larval *Sesarma angustipes* to low salinity decreases in successive stages. The zoea I had a quite high survival rate at 10‰ S, and developed equally well at 20 or 32‰ S, with a slightly shorter moult cycle at 20‰ S. Although this difference in duration of development is very small (1.8 vs 2.0 d), it is statistically significant ($p < 0.05$; see Fig. 2), and it may reflect an adaptation of the zoea I to regular release in freshwater and early development in brackish water habitats.

Later zoeal stages of *Sesarma angustipes* exhibit a slight preference for fully marine conditions, where they mostly showed shortest development and highest survival rates. At 10‰ S, they revealed clearly delayed development and increased mortality. This shows that *S. angustipes* in its development is still a marine species.

There appears to be a gradual shift from freshwater to seawater in the salinity optimum of successive larval stages, associated with an increasingly stenohaline response. This pattern suggests a possible genetic adaptation to a regularly occurring ontogenetic migration in the life cycle of *Sesarma angustipes*. A major part of its larval development appears to take place in lower estuaries and in coastal oceanic waters, where salinities between ca 20 and 32‰ S are found. This salinity range also was found to be optimal for larval development in other brackish water *Sesarma* species (e.g. Costlow et al. 1960, Fransozo & Negreiros-Fransozo 1986).

The ability for hyperosmotic regulation in very low salinities is in general developed only by late larval or early juvenile stages of decapod crustaceans (Foskett 1977, Charmantier et al. 1981, 1988). Osmoregulation depends on eyestalk factors (Charmantier et al. 1981) that become functional in *Sesarma* spp. during late zoeal or megalopa development (Costlow 1966). However, the great tolerance to low salinity that is exhibited by the zoea I suggests that this ability may occur much earlier in *S. angustipes*.

The data of the present investigation suggest that the tendency of decreasing tolerance to low salinity with progressive development may be stopped or reversed in the megalopa stage. The only individuals that reached metamorphosis to the first juvenile crab instar were observed at 20‰ S, not in fully marine conditions.

In future experimental studies, larvae of *Sesarma angustipes* should be reared under optimum (high) salinity conditions to the megalopa stage and then transferred to different conditions of gradually decreasing salinity. Those experimental tests, combined with field collections, should show when developmental stages return from the sea to brackish water and eventually, to freshwater habitats, and where their metamorphosis takes place. Once the complete life cycle of *S. angustipes* is known, this species could become an interesting and suitable model for studies of the ontogeny of osmoregulation in semiterrestrial crabs and hence, a model of the development of physiological adaptations in Crustacea during the transition from life in the sea to freshwater and terrestrial environments.

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