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Ontogeny of osmoregulation and salinity tolerance in a mangrove crab, *Sesarma curacaoense* (Decapoda: Grapsidae)

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Abstract

The grapsid crab *Sesarma curacaoense* is believed to represent the closest saltwater relative to the ancestor which gave rise to an adaptive radiation of endemic freshwater and terrestrial species on the island of Jamaica. Living in mangrove swamps with variable salinity conditions and showing semiterrestrial behaviour, *S. curacaoense* exhibits ecological adaptations to non-marine conditions. In laboratory experiments, we studied the salinity tolerance during development from hatching to the end of the first juvenile stage. Successful development through metamorphosis occurred in the full salinity range tested (15–32‰), although mortality was significantly enhanced and development delayed at 15‰. In another series of experiments, we studied the ontogeny of the capability for osmoregulation, which is considered as the physiological basis of osmotic stress tolerance. Our results show that *S. curacaoense* is from hatching a fairly strong hyperosmoregulator in dilute media. This capability increased gradually from hatching throughout the larval and juvenile development. In seawater (32‰) and at an enhanced salt concentration (44‰), the zoeal stages remained hyperosmoconformers. The capability for hypoosmoregulation in concentrated media appeared first in the megalopa stage and increased thereafter. Adult crabs were observed to be strong hyper–hypo-osmoregulators in a salinity range from at least 1‰ to 44‰. The unusually early appearance of strong regulatory capabilities, particularly in dilute media, is interpreted as a physiological preadaptation that should have facilitated the evolutionary process of adaptive radiation in non-marine environments on Jamaica. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Grapsidae; *Sesarma*; Larvae; Juveniles; Osmoregulation; Salinity tolerance

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1. Introduction

Grapsid crabs, in particular those belonging to the subfamily Sesarminae, are frequent inhabitants of transitional habitats between the marine intertidal and adjacent freshwater or terrestrial zones. In most of these species, however, reproduction depends on an export of the larval stages into the ancestral environment, the sea, and later reimmigration of megalopae or benthic juveniles into the brackish or semiterrestrial parental habitats (for review see Hartnoll, 1988; Anger, 1995a). Relatively few grapsid species have evolved also the capability to breed and develop in land-locked environments, so that they became entirely independent from the sea. Most of those fully terrestrial or limnic crab species have evolved in two conspicuous centres of adaptive radiation, one in the Indopacific region (Ng, 1988; Ng and Tan, 1995), the other on the Caribbean island of Jamaica (Hartnoll, 1964; Schubart et al., 1998a). The endemic Jamaican crabs have recently been investigated quite extensively, including descriptions of new species (see Schubart et al., 1998b, and earlier papers cited therein), as well as studies of behavioural, physiological, and life-history traits (e.g. Anger and Schuh, 1992; Diesel and Horst, 1995; Schubart and Diesel, 1999).

According to morphological, distributional, and recent molecular genetic evidence, all endemic Jamaican freshwater and terrestrial sesarminids have evolved from the same marine ancestor, which was very closely related to an extant species, *Sesarma curacaoense* (Hartnoll, 1964, 1971; Abele and Means, 1977; Schubart et al., 1998a). This crab lives in coastal mangrove swamps, which are characterized by limited exchange with the sea and thus, highly variable and unpredictable conditions of salinity (ranging from oligohaline to hypersaline) and of plankton production (Atkinson and Taylor, 1988; Macintosh, 1988). The larvae of *S. curacaoense* are not exported to the adjacent sea but released into stagnant, temporary water puddles within the adult habitat (Schuh and Diesel, 1995). It is generally believed that also the ancestor, which gave rise to the adaptive radiation on Jamaica, lived in a similar transitional coastal habitat and hence, was already tolerant of physical, chemical, and nutritional instability. *S. curacaoense* has thus been considered as a suitable model of the initial life-history adaptations to non-marine habitat conditions, which most probably evolved in the transitional zones between the sea, freshwater, and land.

Both field and laboratory observations showed that not only adult *S. curacaoense* are well adapted to the variable and occasionally extreme conditions occurring in this habitat, but also their larvae: (1) they pass through an abbreviated mode of development, with only two zoeal stages and a megalopa (Anger et al., 1995); (2) they show enhanced energy reserves remaining from unusually large eggs; as a consequence, the zoeal stages are largely independent of planktonic food availability (Anger and Schultze, 1995; Schuh and Diesel, 1995; Anger, 1995b); (3) they are euryhaline (Schuh and Diesel, 1995).

In the present investigation, we studied ontogenetic changes in the capability of osmoregulation in *S. curacaoense*, attempting to understand the physiological basis of salinity tolerance in its early life-history stages. Since the Crustacea show a universal relationship between tolerance of osmotic stress and availability of osmoregulatory functions (Charmantier, 1998), our findings may aid to the understanding of evolutionary adaptations that should have evolved early during the conquest of limnic environments by originally marine crabs.

2. Methods

2.1. Origin of materials, larval rearing, tests of salinity tolerance

Adults of *Sesarma curacaoense* were collected in 1992 and 1993 from coastal mangrove (*Rhizophora mangle, Avicennia germinans*) swamps in Jamaica and transported alive to the Helgoland Marine Biological Station (Germany). The crabs were maintained in aquaria with 25‰ salinity and constant 24°C, a 12:12 h light–dark cycle, and fed with mangrove leaves and frozen isopods (*Idotea* spec.).

Freshly hatched zoeae were mass-reared under the same conditions of temperature and light as the adult crabs, using 500-ml beakers with gentle aeration. Except for the test of salinity tolerance (see below), all rearing experiments were carried out at 25‰. The culture medium was changed daily, and the larvae were fed freshly hatched *Artemia* sp. (San Francisco Bay Brand™) nauplii at a density of ~10/ml. Moults and mortality were recorded daily, and newly moulted larvae were separated (unless moulting was synchronous), so that each rearing beaker contained exclusively individuals with the same age within a given instar. The larval stages were identified according to size and morphological criteria (Anger et al., 1995). The semibenthic megalopae and the benthic juvenile crabs were given pieces of nylon gauze (300 μm mesh size) as a substrate. The average duration of successive developmental stages was 1–2 days (zoea I, II), 8–12 days (megalopa), and 8 days (crab I).

The salinity tolerance of the larval stages of *S. curacaoense* had been studied before only in material obtained from field-caught ovigerous females (Schuh and Diesel, 1995). In order to check for possible effects of differential acclimation, we tested the tolerance of reduced salinities also in larvae that hatched from eggs produced and incubated under constant conditions of temperature (24°C) and salinity (25‰). Freshly hatched larvae were transferred directly to the test conditions (15, 20, 25 and 32‰; 25 individuals per treatment). The desired salinities were obtained by dilution of filtered (1 μm) natural seawater from the North Sea (~32 ‰) with deionized tap water.

2.2. Measurements of osmoregulation

Osmoregulation was measured in larvae, early juveniles (instar I), and adult crabs reared under constant conditions (24°C, 25‰). At least ten individuals in each developmental stage were sampled from the cultures and exposed directly to various experimental test media. Their salinities were expressed as osmotic pressure (in mosm/kg) or salt content of the medium (in ‰); a value of 3.4‰ is equivalent to 100 mosm/kg (29.41 mosm/kg per 1‰). The osmolalities tested in all stages were: 156, 302, 500, 755, 961 and 1297 mosm/kg (or 5.3, 10.3, 17.0, 26.7, 32.7 and 43.7‰ salinity); adult crabs were exposed also to 31 mosm/kg (1.0‰). The media were prepared by adding desalinated freshwater or salt (Tropic Marin®, Wartenberg, Germany), respectively, to natural seawater and then stored for the entire duration of the experiments in 5-l plastic containers. The osmotic pressure of the media was measured with a micro-osmometer Model 3 MO (Advanced Instruments, Needham Heights, MA, USA) requiring 20 μl per sample.

Since the haemolymph osmolality reaches within a few hours (larvae) or in about 1 day (adult crabs) a steady state relative to the ambient water osmolality (Charmantier, 1998; Charmantier et al., 1998), we allowed for an acclimation time of 20–24 h in larvae and early juveniles, but 48 h in adults. The experimental exposure to the test media began approximately in the middle of the moulting cycle (intermoult, stage C; see Drach, 1939). All experiments were conducted at a constant temperature of 24°C.

After the incubation time, larvae and juveniles were separated from the test media, briefly dried on filter paper, quickly immersed in mineral oil to avoid evaporation and desiccation, and the remaining adherent water was aspirated through a glass micropipette. Subsequently, the hemolymph was sampled with a new micropipette inserted in the heart. In adult crabs, the hemolymph was collected via a hypodermic needle after section of the propodite of a fourth or fifth pereiopod previously dried with filter paper. The hemolymph was quickly transferred into mineral oil to avoid evaporation.

For all developmental stages, hemolymph osmolality was measured with a Kalber-Clifton nanolitre osmometer (Clifton Technical Physics, Hartford, NY, USA) requiring about 30 nl. The results were expressed either as hemolymph osmolality or osmoregulatory capacity (OC), defined as the difference between the osmolalities of hemolymph and the medium.

2.3. Statistical analyses

Statistical analysis of survival and development data was done with non-parametric tests, as goodness-of-fit *G*-tests showed that they sometimes deviated significantly from a normal distribution (Sokal and Rohlf, 1995). The Kruskal–Wallis *H*-test was employed in multiple comparisons, the Mann–Whitney *U*-test for subsequent pairwise comparisons of mean values. Analysis of variance (ANOVA) and Student's *t*-test were used for multiple and pairwise comparisons of OC data, after appropriate checks for equality of variance.

3. Results

3.1. Salinity tolerance

Larval survival and development from hatching through the first juvenile instar occurred in the entire range of tested salinities (15–32‰), with a slight tendency of higher survival and shorter development at 25‰ (Fig. 1). At the time of metamorphosis from the megalopa to the first crab stage, cumulative survival was significantly higher at the intermediate salinities (20–25‰) than at 15‰ or in seawater (32‰). The lowest salt

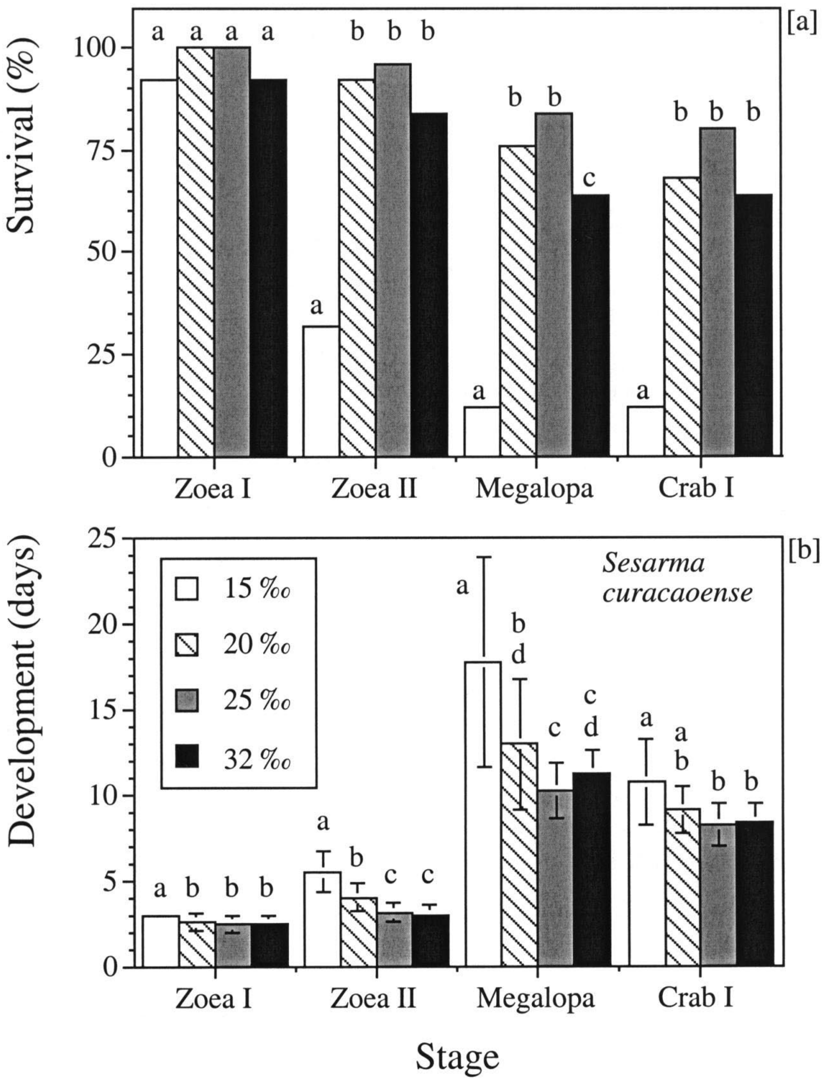

Fig. 1. Survival (a) and duration of development (b), mean±S.D.; initial $n = 25$ per treatment) of the larval stages and first-stage juveniles of *Sesarma curacaoense* reared under four different salinity conditions; different letters above error bars indicate statistically significant differences between treatments ($P < 0.05$).

concentration caused consistently the highest mortality (statistically significant from the zoea II through the crab I stage; Fig. 1a), corresponding with significantly enhanced duration of development (Fig. 1b). Also 20‰ tended to cause a delay in development as compared with higher salinities, although this effect was weak and statistically significant only in the zoea II and, exclusively between 20 and 25‰, in the megalopa.

3.2. Osmoregulation

The two zoeal stages of *Sesarma curacaoense* did not survive a 24-h exposure to 5‰, but both already showed a fairly strong capability of hyper-osmoregulation in moderately dilute media (10–17‰; Fig. 2a). Their OC in these conditions was 140–172 mosm/kg (Fig. 2b). At higher salinities, the zoeae were hyper-osmoconformers, with OC values ranging from 13 to 60 mosm/kg.

In contrast to the zoeal stages, the megalopa also survived the exposure to 5‰, and it showed a steep increase in its capability of hyper-osmoregulation in dilute media (5–17‰; Fig. 2a). The OC reached in these treatments 183–265 mosm/kg (Fig. 2b). As a remarkable new trait, the megalopa also showed the capability of hypo-osmoregulation in concentrated media (Fig. 2a and b). Although this function was still weakly developed (OC = −27 mosm/kg at both 32 and 44‰), the difference between internal and external salt concentration was statistically significant ($P < 0.05$). Hence, the megalopa already showed in principle the adult pattern of hyper–hyporegulation.

In the juvenile and adult crabs, the capabilities of both hyper- and hypo-osmoregulation increased gradually, but no qualitative changes in the osmoregulatory pattern occurred (Fig. 2a and b). The adults survived also an experimental exposure to extremely low salinity (1‰), where they reached an average OC of 568 mosm/kg. Under hypersaline conditions (44‰), they were able to maintain their haemolymph concentration 238 mosm/kg below that of the external medium.

4. Discussion

As in previous experimental studies, which had been conducted with larvae obtained from field-caught ovigerous females on Jamaica (Schuh and Diesel, 1995), the larvae of our laboratory-cultivated *Sesarma curacaoense* were able to develop successfully through metamorphosis in a wide range of salinities. Compared with the previous observations, however, the larvae showed in the present study a less euryhaline response. At 15‰, they had poor survival and delayed development, while Schuh and Diesel (1995) reported for this condition a low mortality and occasionally some survival even at 10‰. Although these results do not differ in principle, the lower overall level of euryhalinity and the apparent optimum observed now at 25‰ may indicate an effect of differential embryonic acclimatization (or non-genetic resistance adaptation; Kinne, 1964). This phenomenon occurs in several aquatic invertebrates (Kinne, 1971), including the euryhaline crab species *Rhithropanopeus harrisii*, where temperature and salinity conditions prevailing during the period of egg development exerted a significant influence on later larval tolerance of these factors (Rosenberg and Costlow, 1979). This effect may explain the stronger tolerance of brackish water in *S. curacaoense* larvae obtained from field-caught ovigerous females, which were exposed to the full range of natural salinity fluctuations occurring in shallow mangrove puddles, due to heavy tropical rainfall and strong evaporation. The material (larvae and juveniles) used in the present study, in contrast, was cultivated at constant, supposedly optimal conditions of 25‰.

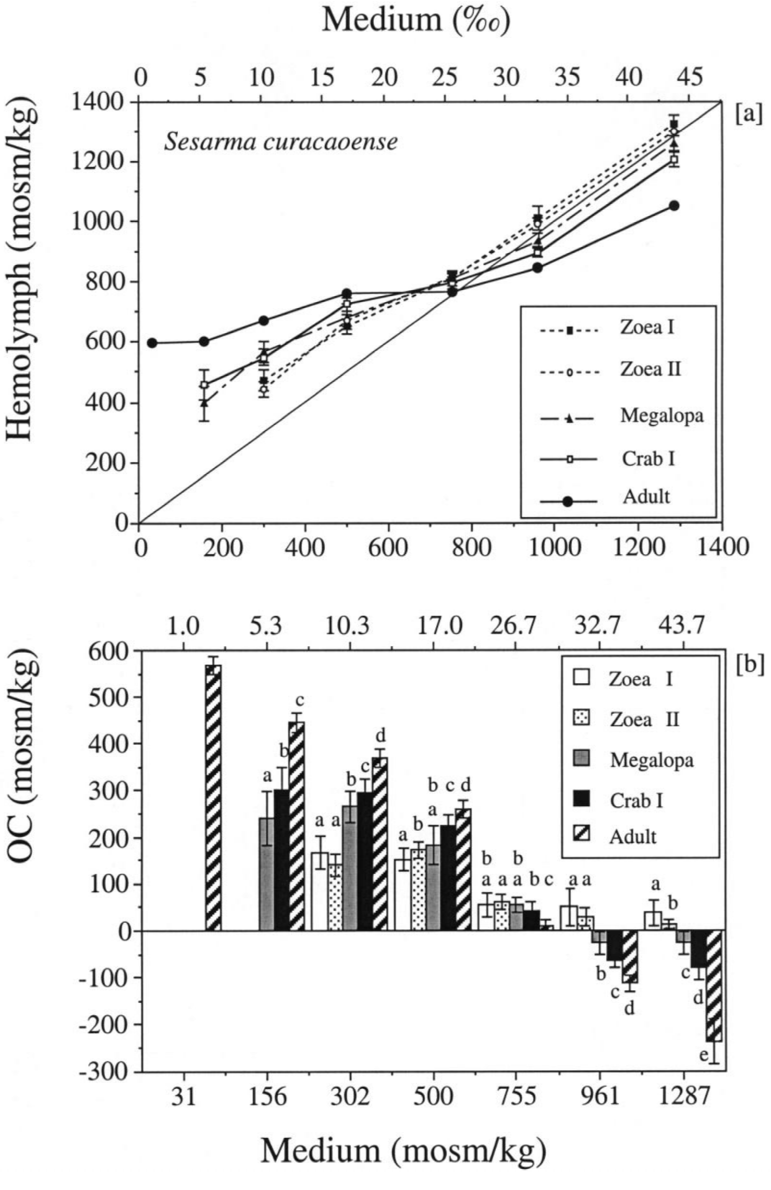

Fig. 2. Hemolymph osmolality (a) and osmoregulatory capacity, OC (b) in different life history stages (larvae, first juveniles, adults) of *Sesarma curacaoense* exposed to various external salinities (media); straight line in (a): isosmotic line (internal = external osmolality); different letters above error bars indicate statistically significant differences between stages ($P < 0.05$).

As should be expected from observations in many other aquatic crustaceans (for review see Charmantier, 1998), the euryhalinity of the larval stages of *S. curacaoense* corresponds with an early appearance of osmoregulatory functions. Already at hatching from the eggs, the zoeae are fairly strong hyper-osmoregulators in moderately dilute media (10–17‰). Although their OC was not quite as high as in another extremely euryhaline grapsid crab species from Jamaica, *Armases miersii*, the difference in osmotic pressure between their hemolymph and the surrounding medium was higher than in most other brachyuran crab larvae studied so far (Charmantier et al., 1998). As a consequence, the zoeae are able to survive and develop under conditions of variable or low salinities.

More remarkable than the strong larval capacity for hyper-osmoregulation in brackish water, *S. curacaoense* shows also an unusually early appearance of the capability of hypo-osmoregulation in concentrated media ($\geq$ 32‰). In most euryhaline species, the pattern of hyper-hyporegulation is normally found only in juveniles or adults, while in *S. curacaoense* it becomes functional in the megalopa stage (Fig. 2). The same type of ontogenetic change was observed also in an ocypodid crab, *Uca subcylindrica* (Rabalais and Cameron, 1985), and in *A. miersii* (Charmantier et al., 1998). All these species have in common that they breed in land-locked, non-marine environments (temporary rainfall puddles and supratidal rock pools, respectively), where highly variable and widely unpredictable physical conditions select for an early expression of physiological mechanisms that allow for an enhanced salinity tolerance.

As in *U. subcylindrica* and *A. miersii*, the capability of hyper-osmoregulation appears in *S. curacaoense* earlier and increases more rapidly than the function of hypo-osmoregulation. At least in *A. miersii*, this is consistent with changes in the salinity tolerance of successive larval stages (Anger, 1996). This maybe widespread ontogenetic pattern should be explained by differential selection pressures for an evolutionary acquisition of a fast-increasing ability to hyper-regulate and a slow-growing ability to hyporegulate: while heavy rainfall can reduce the salinity in a small land-locked water body within a few minutes or hours, the process of evaporation due to solar radiation and wind is much slower (taking days or weeks). This allows for gradual acclimation or, probably in most cases, an escape from increasingly hypersaline conditions through a fast and abbreviated zoeal development. Since the megalopa stage requires a much longer time to develop through metamorphosis to the first juvenile crab, exposure to hyper-osmotic stress is more likely to occur during this stage rather than during the brief zoeal stages. Hence, we may hypothesize that the function of hypo-osmoregulation occurs only when it is most necessary, i.e. in the megalopa and in later life-history stages.

If we accept that *S. curacaoense* is the closest relative and hence, a suitable model of adaptations that should have occurred also in the ancestor of the endemic Jamaican land and freshwater crabs, then we may postulate that its larvae already showed the following traits (Anger, 1995a): (1) an abbreviated type of development, (2) a high level of nutritional independence and (3) euryhalinity. Our present results suggest that the latter feature was based upon an early ontogenetic appearance of osmoregulatory capabilities; this should include, already from hatching, the occurrence of specialised ion-transporting epidermal structures and enzymes, namely Na^+–K^+-ATPase (Charmantier, 1998). We suggest that these traits represent important prerequisites for the evolutionary transition from the marine intertidal through transitional habitats with a highly variable salinity regime (e.g. estuarine mangrove swamps), and eventually, into limnic and terrestrial habitats.

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