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Salinity tolerance of the larvae and first juveniles of a semiterrestrial grapsid crab, *Armases miersii* (Rathbun)

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Abstract

The tropical crab *Armases miersii* breeds in supratidal rock pools, where great salinity variations occur. In laboratory experiments, all larval stages (zoea I–III, megalopa) and the first juvenile were studied as to their tolerance of acute salinity changes (5–55‰, intervals of 10‰) after preceding adaptation to ambient sea water (32–33‰). Our observations suggest that some osmoregulatory capability exists from hatching. Survival was frequently higher and development faster at 15–25‰ than in sea water (35‰), possibly indicating a phylogenetic adaptation of *A. miersii* to brackish water. Extremely low and high salinities (5, 45–55‰) caused prolonged development durations and increased mortality rates. Tolerance of very low salinity, but not that of hypersaline conditions tended to increase during development. This suggests that late stages attain an increasing capability for hyper-osmoregulation in dilute media, but probably remain poor regulators in concentrated media. The megalopa and crab I were, in some experiments, less affected by chronic than by acute exposure to unfavourable salinities, suggesting that non-genetic resistance adaptation (acclimatization) had taken place during earlier development. During continual exposure to extreme salinities, however, effects of severe osmotic stress outweighed those of acclimatization. The range which allowed for successful development through metamorphosis widened with consecutively later change of salinity: 15–35‰ (exposure from hatching), 15–45‰ (from zoea II), 5–45‰ (from zoea III), 5–55‰ (from megalopa or crab I). It is concluded that tolerance of variable, predominantly low salinities in larval and early juvenile *A. miersii* has evolved as an adaptation to breeding in a physically unstable environment, where rapidly decreasing or continually low salinities are more likely to occur than hypersaline conditions. The degree of salinity tolerance in its early life-cycle stages shows that this species has reached, within the Grapsidae, a phylogenetically intermediate stage of adaptation to conditions in freshwater and terrestrial environments.

Keywords: Crab larvae; Salinity tolerance; Acclimatization; Semiterrestrial; Tropical brachyura

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1. Introduction

The semiterrestrial tropical crab *Armases miersii* (formerly *Sesarma miersii*; for revision of generic taxonomy see Abele, 1992) has only recently been discovered to breed in supratidal coastal rock pools in Jamaica, where the larvae develop under extremely variable physical and nutritional conditions (Anger and Schultze, 1995; Anger, 1995b; Schuh and Diesel, 1995a). In spring, rainfall causes frequent sudden drops in salinity in rock pools where brackish conditions normally prevail (Anger, 1995b). Increased evaporation rates during dry periods, mainly in summer, sometimes lead to hypersaline conditions (Schuh and Diesel, 1995a).

In preliminary laboratory experiments, successful development from hatching to metamorphosis occurred at salinities from 5 to 60‰ (Schuh and Diesel, 1995b). When larvae were exposed later to different conditions, having developed to the megalopa stage in sea water, metamorphosis was occasionally observed even in freshwater (K. Schultze, M. Schuh and K. Anger, unpubl. results). This shows that salinity tolerance may change in subsequent ontogenetic stages, possibly following a genetic programme that is responsible for the development of gills or other osmoregulatory structures in later stages (Charmantier et al., 1988; Charmantier and Charmantier-Daures, 1991; Hong, 1988; Furigo, 1993; Bouaricha et al., 1994).

In the present study, laboratory experiments were conducted with *A. miersii*: (1) To ascertain possible ontogenetic changes in the tolerance of acute salinity changes in subsequent developmental stages. (2) To compare the salinity tolerance of late stages after chronic (earlier) exposure with those given acute (later) exposure. The comparison should allow the effects of non-genetic resistance adaptation (acclimatization), or those of prolonged osmotic stress, to be recognised. Acclimatization during the earlier stages should enhance the tolerance of the later stages (Kinne, 1964, 1971; Rosenberg and Costlow, 1979), whereas cumulative stress should cause the reverse effect. (3) All larvae were obtained from eggs that had been incubated under the same constant salinity conditions, in which the parent generation had been reared and maintained (natural sea water, 32–33‰). Hence, any shift in the salinity optimum of early stages should indicate a phylogenetic adaptation of this species to non-marine (brackish or hypersaline) conditions. Eventually, the overall pattern and degree of salinity tolerance in the early stages should help to clarify the possible phylogenetic directionality in salinity tolerances within the clade.

2. Materials and methods

2.1. Collection and handling of larvae

In April 1993, ovigerous *A. miersii* females were collected at night from supratidal rock pools near the Discovery Bay Marine Laboratory, Jamaica, and maintained in aquaria with sea water at 32–34‰ salinity and 25°C under a 12:12 h light:dark regime. Larvae were mass-reared at the same conditions in ca. 400 ml glass bowls, with *Artemia* sp. (San Francisco Bay Brand™) and sea water changed daily. Juvenile crabs from three

different hatches were transported to the Marine Biological Station Helgoland and reared to adulthood in 5-l aquaria at 24°C, 32–33‰ and a 12:12 h light cycle. The juvenile instars I to V were fed exclusively 2-day-old *Artemia* nauplii. Later stages were fed frozen isopods (*Idotea* sp.). Second-generation larvae have been frequently obtained under these conditions since December 1994. All later rearing experiments were carried out under the same temperature and light conditions as in juvenile rearing, in 400-ml glass bowls, with freshly hatched *Artemia* nauplii given as food, and a daily change of sea water.

2.2. Experiments

In five different series of experiments, all four larval stages and the first juvenile crab of *A. miersii* were each exposed to six different test salinities (5, 15, 25, 35, 45 and 55‰), after hatching or developing in local sea water (32–33‰). Transference of larvae or crabs to 25 and 45‰ was direct, whereas that to higher or lower salinities allowed for a stepwise adaptation (3 h per interval). Foskett (1977) and Charmantier and Charmantier-Daures (1991) have shown that an adaptation time of 1–2 h is sufficient for decapod larvae to reach a constant haemolymph concentration. Each experiment in a given stage was started with 25 individuals and was terminated when all surviving individuals had reached the crab II instar. Thus, this study comprised five experimental series beginning from five different stages, with six treatments each, and 25 individuals per treatment.

Salinities < 32‰ were obtained by dilution of filtered (1 μm) sea water with desalinated water, and higher concentrations by freezing sea water at -18°C. Salinities were checked with a temperature-compensated electric probe (WTW, Germany) or a hand refractometer (Krüss Salinometer S-28), to the nearest 0.1 or 1‰, respectively. During daily water change and feeding, larvae and juveniles were checked for moults or mortality. Freshly moulted individuals were transferred to new marked bowls, so that each rearing vessel contained exclusively larvae or juveniles with an identical moulting history in all preceding stages. Due to unsynchronized moulting, some late stage individuals were reared individually in 25-ml vials (Anger, 1995a).

The criteria of salinity tolerance used in the present paper are mortality rate and duration of development in individual postembryonic stages. Since this experimental design required a large amount of material (in particular a growth study that was run in parallel), the number of larvae produced by a single female did not suffice for carrying out the entire study with larvae from the same hatch. Thus, a different hatch was used in each series of experiments, in which effects of different salinities were tested in one developmental stage.

2.3. Statistical methods

Mortality data are given in percent of the number of individuals that survived to a given stage (instantaneous mortality rate), or in percent of the initial number of individuals in a given experimental treatment (cumulative mortality; $n = 25$). Average duration of development in a given stage (or in a longer period, for instance in total larval development from hatching to metamorphosis) is given as arithmetic mean $\pm 95\%$

confidence interval, for a given number, n, of survivors to the next stage. Statistical analysis of mortality and development data followed standard methods described by Sokal and Rohlf (1981); Sachs (1984). Since goodness-of-fit G-tests showed that the data deviated sometimes significantly from a normal distribution, non-parametric tests were consistently used for statistical analysis.

In contingency tables (mortality data), analysis of multiple data sets as well as pairwise comparisons were carried out with Pearson's log-likelihood test, using a Chi-square statistic to discern significant differences. In data sets of development duration, Kruskal-Wallis rank tests (H tests) were used for multiple comparisons of mean values (non-parametric ANOVA). The Tukey-Kramer HSD (Honestly Significant Difference) coefficient indicated significantly differing mean values within groups of means; pairwise comparisons were then carried out employing Wilcoxon-Mann-Whitney U-tests. Three levels of statistical significance are distinguished in this paper, with probabilities of error (P) for rejecting the null hypothesis of $P < 0.05$, $P < 0.01$, or $P < 0.001$.

3. Results

3.1. Experiment 1

In the first series of experiments (Expt. 1), the larvae were transferred, within a few hours after hatching, from natural sea water (32–33‰) to different test salinities (5–55‰). Lowest mortality (4%, i.e. 1 individual each) was recorded in sea water (35‰) and moderately reduced salinities (15–25‰), whereas significantly increased mortality (56%) occurred at 5‰ (Fig. 1). The zoea II survived well at all salinities (Fig. 1). In contrast, 80% of the zoea III died at 5‰, and all died at 55‰. In the intermediate range (15–45‰), mortality increased significantly with increasing salinity ($P < 0.001$ in multiple comparison). However, pairwise differences between nearest neighbouring conditions, e.g. 25 vs. 35‰, were not significant.

In the megalopa, the lowest mortality was again observed at 15–25‰, while significantly higher rates occurred at 5 and 35‰ (Fig. 1). The megalopa, like the zoea III, showed complete mortality in the highest salinity treatment remaining in the experimental series (here: 45‰). The last two larval stages showed significantly lower mortality in slightly diluted (15–25‰) compared with full-strength sea water (35‰), in spite of preceding adaptation to 32–33‰ during embryonic development. In the first crab stage, only a single individual died in Expt. 1.

Cumulative mortality from hatching to metamorphosis was at a minimum (24–28%) in the salinity range from 15–25‰, significantly elevated (68%) at 35‰, and almost complete in extreme conditions (5, 45 and 55‰). This pattern resulted from high mortality in the zoea I at 5‰ and in late stages (zoea III, megalopa) at 45–55‰. Intermediate salinities allowed for high survival in all developmental stages.

Development time showed, in general, the same tendencies as mortality, with delays at extremely low and high salinities (Fig. 2). However, the delay in the zoea I was significantly longer at 55‰ than at 5‰; an inverse pattern was observed in mortality

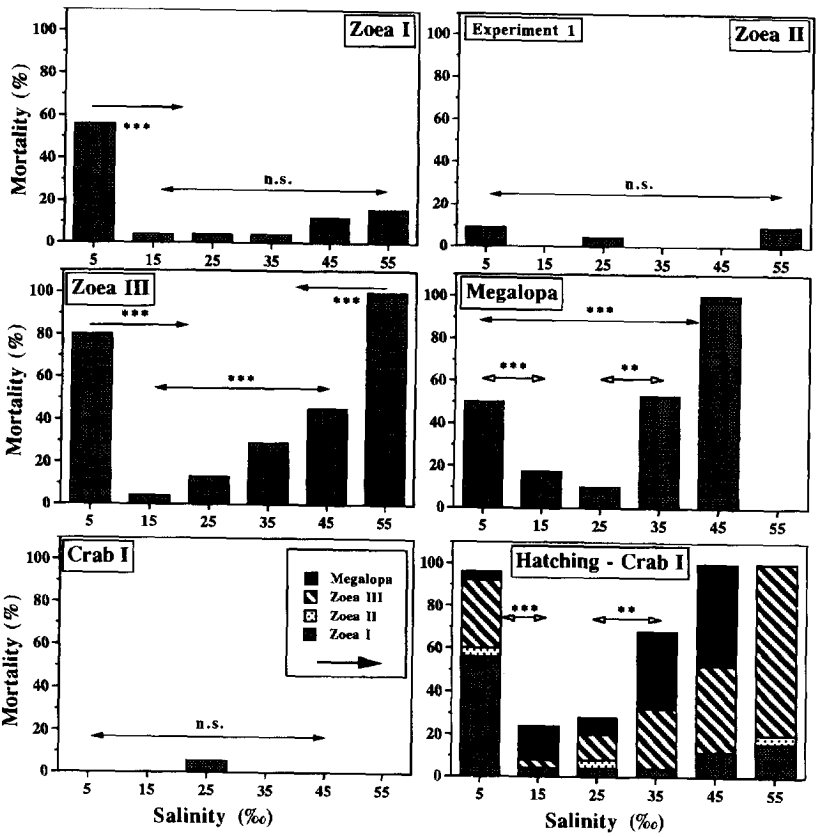

Fig. 1. Experiment 1: Development of larval and early juvenile *Armases miersii* at different salinities (‰), exposure from hatching. Mortality (%; initial $n = 25$ individuals per treatment). Statistically significant differences in pairwise or multiple comparisons (indicated by open or filled arrowheads, respectively) marked with asterisks: *, **, *** (= $P < 0.05$, < 0.01, < 0.001); NS = not significant, $P > 0.05$.

rates of the same stage (Fig. 1). In the zoea II, significantly prolonged development duration was found exclusively at 45 and 55‰, but not at 5‰ (Fig. 2). Since only a few individuals reached the megalopa stage at 5 and 55‰, most differences in development time of the zoea III were not statistically significant. In the megalopa, development was shortest at 15‰, but differences in the range 5–25‰ were not significant. It is remarkable that the moult cycle was significantly longer in sea water (35‰) than at lower salinities. Similar patterns were found in the duration of total larval development and in that of the first juvenile instar (Fig. 2).

3.2. Experiment 2

When larvae were reared in sea water to the zoea II and only then exposed to different salinities, mortality in the zoea II increased significantly at 55‰, but not in other conditions (Fig. 3). The zoea III showed, as in Expt. 1, enhanced mortality at both 5 and

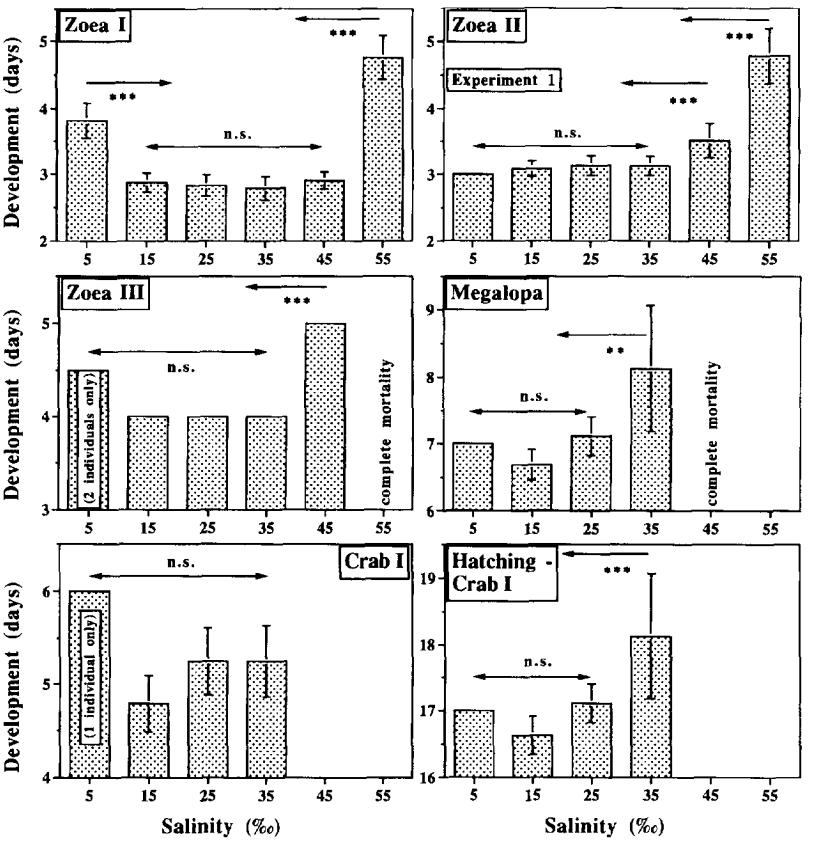

Fig. 2. Experiment 1: Development of larval and early juvenile *Armases miersii* at different salinities (‰), exposure from hatching. Duration of development (days; initial $n = 25$ individuals per treatment). Statistically significant differences in pairwise or multiple comparisons (indicated by open or filled arrowheads, respectively) marked with asterisks: *, **, *** (= $P < 0.05$, < 0.01, < 0.001); NS = not significant, $P > 0.05$.

55‰, reaching 100% in the latter treatment. The few larvae which survived at 5‰, died in the megalopa stage. In other treatments, the megalopa showed little mortality, without significant differences among salinities $\geq$ 15‰. As in Expt. 1, the first juvenile showed almost no mortality.

Patterns in cumulative mortality from the zoea II through the megalopa stage also appear to indicate that salinities of 15–25‰ are more suitable for *A. miersii* larvae than those of 35–45‰. Unlike Expt. 1, however, these differences were not statistically significant. The salinity range that allowed for survival through metamorphosis to the first juvenile appeared to have widened: it comprised practically only 15–35‰ in Expt. 1 (neglecting a single survivor at 5‰), but 15–45‰ in Expt. 2.

As in Expt. 1, the stage that was first exposed to acute salinity changes (here the zoea II) showed significantly delayed development at extreme salinities (5, 45–55‰; Fig. 4). A similar pattern was found also in the zoea III stage, which lasted significantly shorter

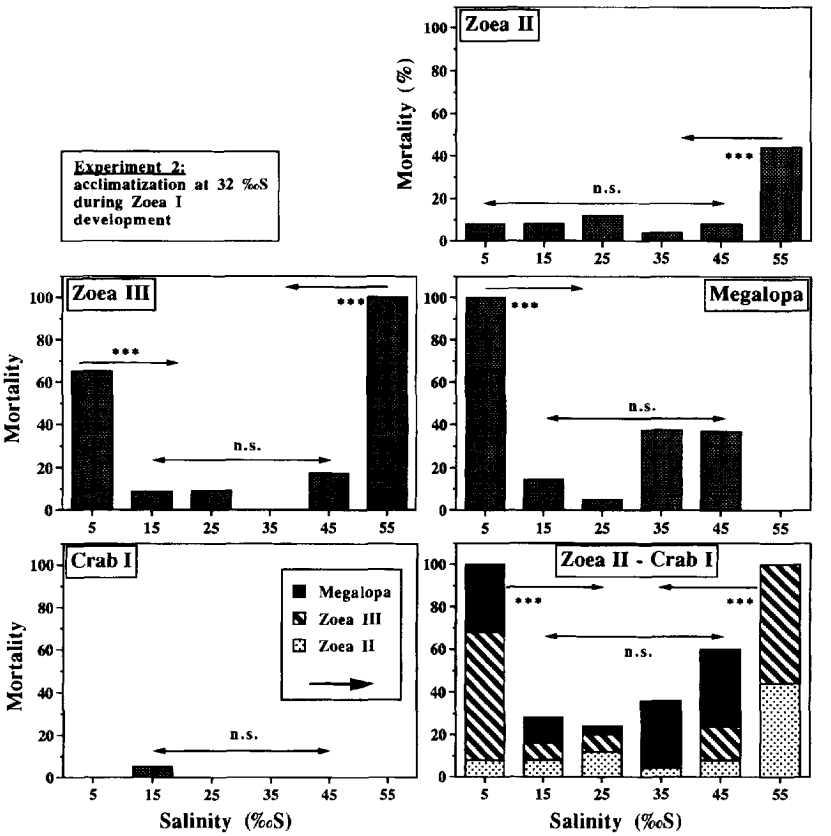

Fig. 3. Experiment 2: Development of larval and early juvenile *Armases miersii* at different salinities (‰), exposure from the beginning of the zoea II stage. Mortality (%; initial $n = 25$ individuals per treatment). Statistically significant differences in pairwise or multiple comparisons (indicated by open or filled arrowheads, respectively) marked with asterisks: *, **, *** ($= P < 0.05$, < 0.01, < 0.001); NS = not significant, $P > 0.05$.

at 25‰ than at any other salinity (Fig. 4). Larvae which survived through metamorphosis showed no differences in megalopa duration at salinities 15–35‰, but a highly significant delay at 45‰. In the crab I, again, the shortest development time was at 25‰, while slight but statistically significant delays were detected at 45 and 15‰. Patterns in total duration of larval development reflected those in the zoeal stages, which had a significantly shorter development at 15–25‰ than at higher salinities (Fig. 4; as in Expt. 1, cf. Fig. 2).

3.3. Experiment 3

As in the two previous experimental series, the zoea III suffered maximum mortality at 5 and 55‰, whereas mortality in the megalopa did not vary significantly among

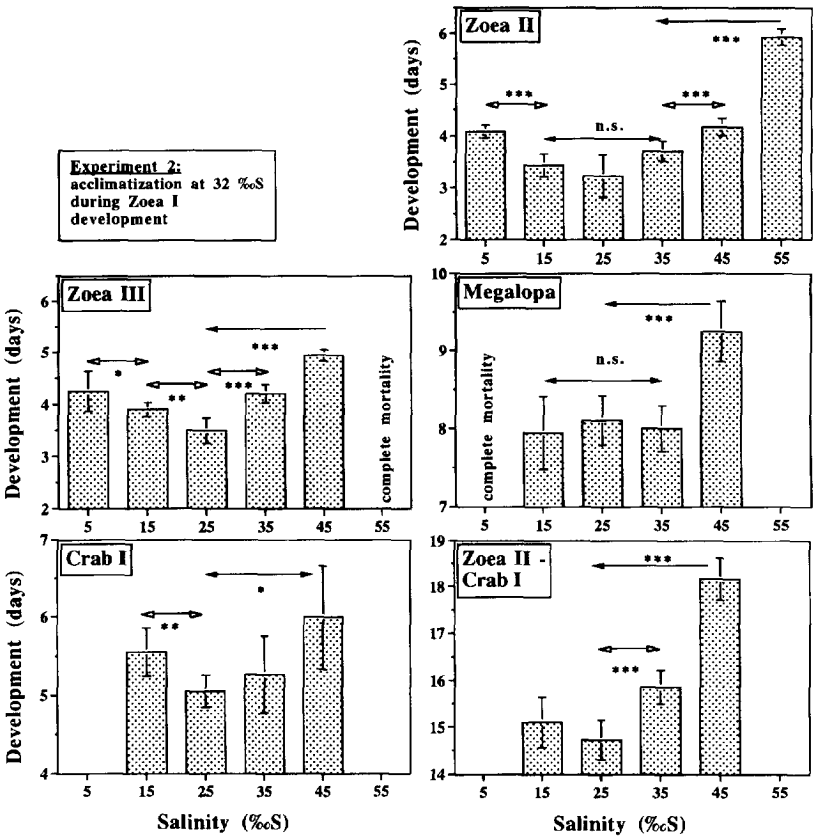

Fig. 4. Experiment 2: Development of larval and early juvenile *Armases miersii* at different salinities (‰), exposure from the beginning of the zoea II stage. Duration of development (days; initial $n = 25$ individuals per treatment). Statistically significant differences in pairwise or multiple comparisons (indicated by open or filled arrowheads, respectively) marked with asterisks: *, **, *** ($= P < 0.05$, < 0.01, < 0.001); NS = not significant, $P > 0.05$.

treatments (Fig. 5). Again, the first crab stage survived well, regardless of salinity. Cumulative larval mortality (zoea III and megalopa combined) was generally without significant differences at 15–45‰. Metamorphosis was possible in the salinity range 5–45‰, a wider range than in Expts. 1 and 2 (15–35 and 15–45‰, respectively).

The time of development in the zoea III stage was shortest at 15–35‰, and significant delays occurred at 5 and 45‰ (Fig. 6), but no larva survived to the megalopa at 55‰. In the megalopa, a significant delay was observed only at 45‰ (group-wise statistical comparison with other salinity conditions, or pairwise 45 vs. 25‰). The duration of the crab I stage did not show significant differences between treatments. Cumulative larval development time from the beginning of the zoea III to metamorphosis was shortest at 25‰, and this was significantly different from 35 and 5‰, but not from 15‰. Again, the longest delay was observed at the highest remaining salinity (45‰).

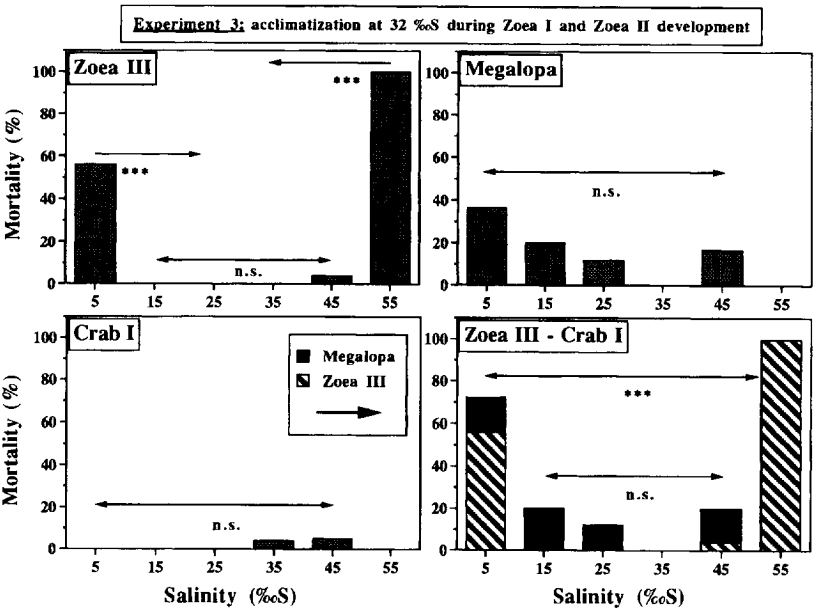

Fig. 5. Experiment 3: Development of larval and early juvenile *Armases miersii* at different salinities (‰), exposure from the beginning of the zoea III stage. Mortality (%; initial $n = 25$ individuals per treatment). Statistically significant differences in pairwise or multiple comparisons (indicated by open or filled arrowheads, respectively) marked with asterisks: *, **, *** ($= P < 0.05$, < 0.01, < 0.001); NS = not significant, $P > 0.05$.

3.4. Experiment 4

When only the megalopa was exposed to salinity changes, its mortality was significantly enhanced only at 55‰, and metamorphosis was possible in the full experimental range (5–55‰; Fig. 7). This is a further indication that the effects of osmotic stress decreased as the beginning of the exposure became consecutively later. In the first crab stage, again no significant influence of salinity on survival could be detected; maximum mortality (15%) was observed at 55‰.

Development duration in the megalopa was shortest at 35‰, showing a gradual increase towards brackish water conditions (significant both in a group-wise comparison, 5–35‰, or pairwise, 35 vs. 5‰). The longest delays, however, occurred at high salinities (Fig. 7). The crab I showed no significant response in the range 5–45‰, but a conspicuous delay at 55‰.

3.5. Experiment 5

In this series of experiments, complete larval development took place in sea water (32–33‰), and only the first juvenile was exposed to other salinities. Average survival was lower than in all other experiments, but without significant differences in the range

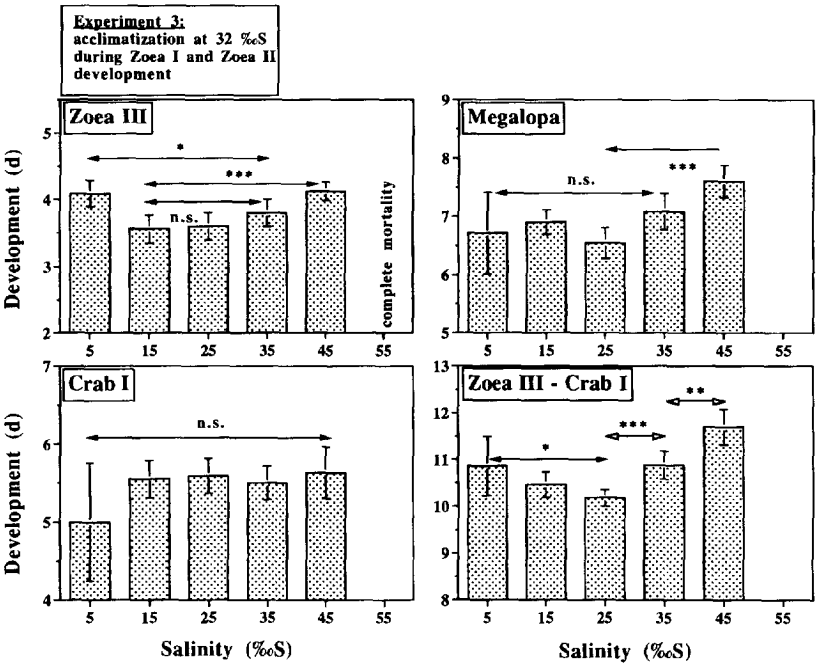

Fig. 6. Experiment 3: Development of larval and early juvenile *Armases miersii* at different salinities (‰), exposure from the beginning of the zoea III stage. Duration of development (days; initial $n = 25$ individuals per treatment). Statistically significant differences in pairwise or multiple comparisons (indicated by open or filled arrowheads, respectively) marked with asterisks: *, **, *** (= $P < 0.05$, < 0.01, < 0.001); NS = not significant, $P > 0.05$.

5–45‰. Only 55‰ caused a significantly increased mortality (84%; Fig. 8). The duration of the crab I stage was significantly shorter at 15–25‰ than at all other salinities and, again, longest delays occurred in hypersaline conditions (45–55‰; Fig. 8).

4. Discussion and conclusions

All larval stages and the first juvenile of *A. miersii* show an unusually euryhaline response (cf. early life-cycle stages of those decapod crustaceans for which data are available; see review by Charmantier et al., 1988). Since salinity tolerance is in general associated with osmoregulation (e.g. Charmantier et al., 1988; Bouaricha et al., 1991; Charmantier and Charmantier-Daures, 1991), a limited capability for regulation from the first larval stage must be expected in *A. miersii*, as in some other decapod species that are able to develop at low salinities (*Rhithropanopeus harrisii*: Kalber and Costlow, 1966; *Sesarma reticulatum*: Foskett, 1977; *Uca subcylindrica*: Rabalais and Cameron, 1985; *Macrobrachium amazonicum*: Zanders and Rodríguez, 1992). As gills are absent in early larval stages (Hong, 1988; Furigo, 1993; Bouaricha et al., 1994), other, less

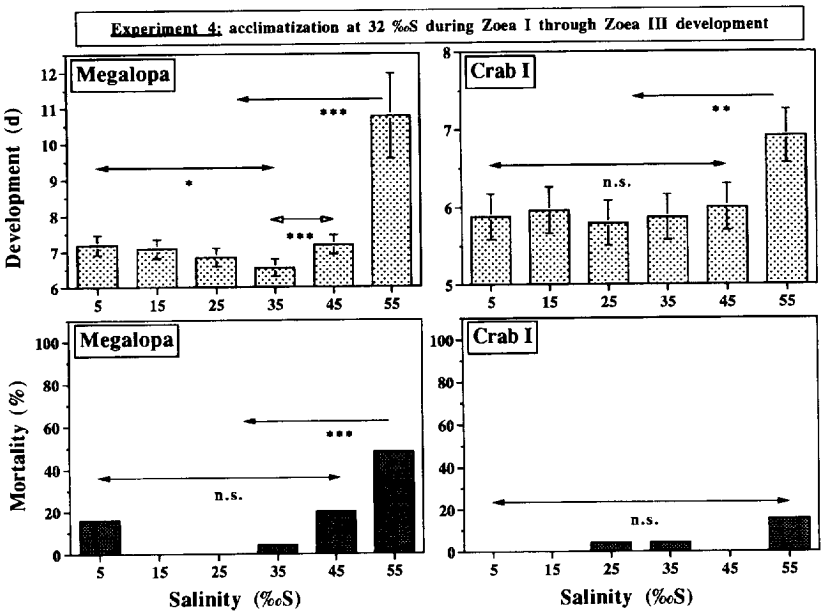

Fig. 7. Experiment 4: Development of larval and early juvenile *Armases miersii* at different salinities (‰), exposure from the beginning of the megalopa stage. Lower graphs: mortality (%); upper graphs: duration of development (days) (initial $n = 25$ individuals per treatment). Statistically significant differences in pairwise or multiple comparisons (indicated by open or filled arrowheads, respectively) marked with asterisks: *, **, *** ($= P < 0.05$, < 0.01, < 0.001); NS = not significant, $P > 0.05$.

efficient, osmoregulatory structures (probably located in the branchiostegite tissues: Bouaricha et al., 1991, 1994; Furigo, 1993) as well as a partially-functional neuroendocrine control system must be present (review: Kamemoto, 1991).

When acute effects of salinity change are compared in subsequent postembryonic stages, ontogenetic changes in short-term salinity tolerance are discernible. Fig. 9 shows percentage differences in relation to figures observed in sea water (35‰, reference salinity) in mortality or development duration found at different test salinities. This summary comparison indicates that the most stenohaline responses occurred in the zoea I and III stages, while the zoea II, the megalopa and the crab I were, in general, the most euryhaline stages.

Minimum tolerance in the first and the last zoeal stage has also been observed in larvae of other crab species (Rosenberg and Costlow, 1979; Anger, 1991). While little tolerance of abrupt salinity changes may not be surprising in freshly hatched zoea I larvae, the particular sensitivity of the last zoeal stage seems more difficult to explain. Imminent metamorphosis to the megalopa may be a possible explanation, as it requires great morphological and behavioural changes, probably accompanied by increased metabolic needs at the end of its moulting cycle. In a stage of development, in which the formation of an osmoregulatory system is not yet complete, this might increase the vulnerability against osmotic stress. In the zoea II, lack of dramatic morphogenetic and

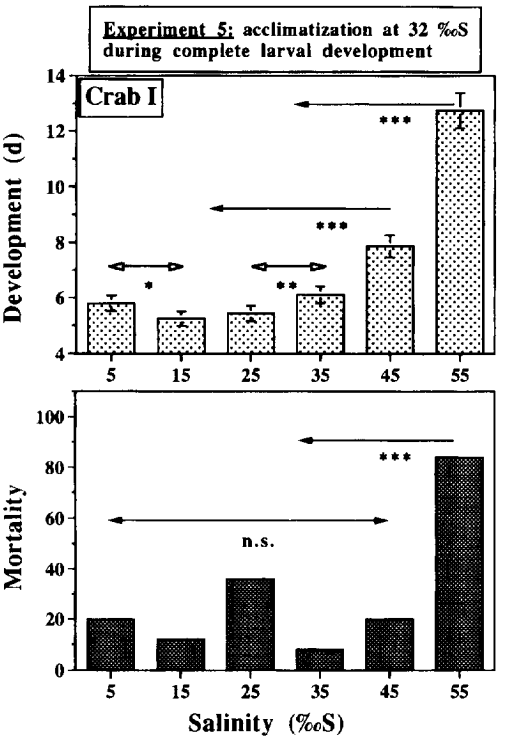

Fig. 8. Experiment 5: Development of early juvenile *Armases miersii* at different salinities (‰), exposure from metamorphosis. Lower graph: mortality (%); upper graph: duration of development (days) (initial $n = 25$ individuals per treatment). Statistically significant differences in pairwise or multiple comparisons (indicated by open or filled arrowheads, respectively) marked with asterisks: *, **, *** (= $P < 0.05$, < 0.01, < 0.001); NS = not significant, $P > 0.05$.

metabolic changes, and a gradually increasing capability of regulation, may be a plausible explanation for particularly high salinity tolerance.

The megalopa and crab I which, in contrast to the zoeal stages, have a thick cuticle and functional gills (Hong, 1988; Furigo, 1993; Bouaricha et al., 1994), are physically much better protected against osmotic changes, and they should possess a greater physiological capability of osmoregulation. This is also suggested by the results of Expts. 4 and 5, in which a very euryhaline response was observed in these late stages. However, successive developmental stages showed in particular an increasing tolerance of low salinities, while that of hypersaline conditions remained weak. In the first juvenile stage, for instance, direct exposure to 45 or 55‰ caused a significant delay in development, and mortality increased significantly at 55‰ (Figs. 8 and 9), but no such effects occurred at other salinities. This pattern suggests a substantially increasing capability of osmoregulation in dilute media, but not in high salt concentrations. As in other larval decapods (review: Charmantier et al., 1988), the larvae of *Armases miersii* are probably hyper-osmoconformers, at least at reduced salinities, i.e. they should be

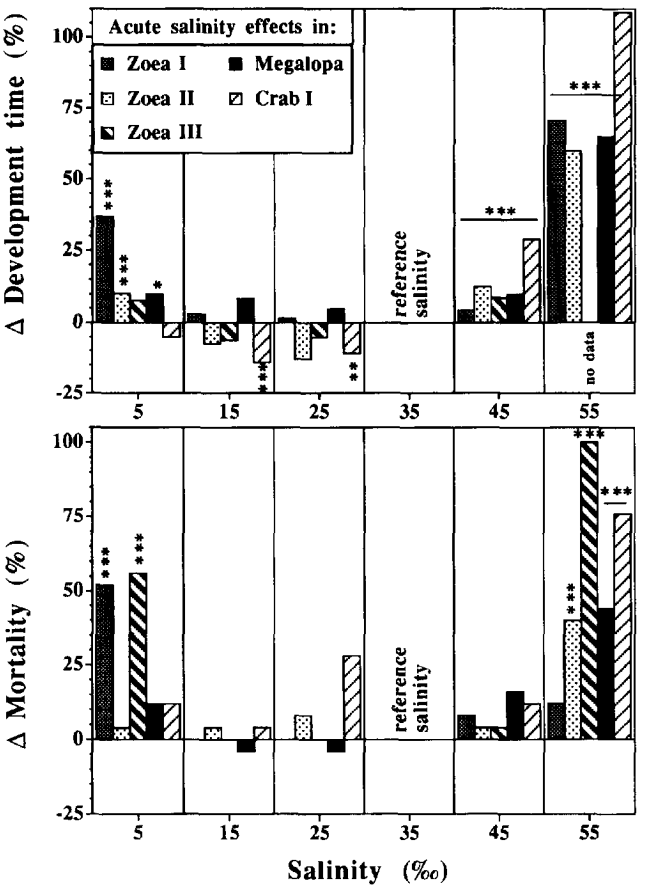

Fig. 9. Differences (Δ) in mortality (lower graph) and duration of development (upper graph) of larval and early juvenile *Armases miersii* after acute exposure to salinity changes. Δ given in % of values observed in a seawater control (35‰ = reference salinity). Statistically significant differences in pairwise or multiple comparisons marked with asterisks: *, **, *** (= $P < 0.05$, < 0.01, < 0.001); NS = not significant, $P > 0.05$.

capable of a partial upward-regulation of haemolymph concentration. The same response, hyper-regulation, might be found also in hypersaline conditions, possibly as a mechanism that maintains the turgor in swimming appendages (Foskett, 1977; Charmantier et al., 1988). Late, predominantly benthic stages (megalopa, crab I), in contrast, may already exhibit the adult-type of response (hyper-hypo-osmoregulation; Charmantier et al., 1988; Charmantier and Charmantier-Daures, 1991), explaining their greater tolerance of osmotic stress.

On average, hypersaline conditions had, in all developmental stages of *A. miersii*, much stronger detrimental effects than very low salinity, in spite of a greater osmotic change in the latter case. Significantly enhanced mortality at 5‰ was found only in the first and the last zoeal stage, while 55‰, and frequently also 45‰, caused severe effects in all stages, except for the zoea I (Fig. 9, lower graph). This response pattern may be

explained as an adaptation to the salinity conditions that are likely to occur in the natural environment, where evaporation in rock pools is always a slow, gradual process, which leads to high salinities only after prolonged dry periods (Schuh and Diesel, 1995a) and thus, gives enough time for gradual acclimatization during larval development. Heavy rainfall, on the other hand, can cause sudden drops in salinity, which may be followed by extended periods of oligohaline conditions (Anger, 1995b; Schuh and Diesel, 1995a). Hence, in supratidal rock pools, the larvae of *A. miersii* have much more time to adapt to high than to low salinities. After gradual acclimatization, their actual tolerance of hypersaline conditions might thus be considerably higher in natural rock pools than in our laboratory experiments, where only a few hours were allotted for stepwise acclimatization. Thus, *A. miersii* is well-adapted in its ontogeny to the conditions in its particular breeding habitat, where tolerance of low salinities is far more important for successful development through metamorphosis than tolerance of rapidly increasing or continually hypersaline conditions.

The embryos, and in most experiments the early larval stages of *A. miersii* also, were acclimatized to undiluted sea water (32–33‰), before they were exposed to other salinities. Hence, highest survival and fastest development should be expected, in general, at 35‰ (Kinne, 1964, 1971; Rosenberg and Costlow, 1979). However, the zoea II and III as well as the crab I stage developed, after acute exposure, faster at 15–25‰ than at 35‰ (statistically significant only in juvenile development; Fig. 9). In experiments with chronic exposure, the zoea III and the megalopa showed an accelerated development or a reduced mortality at 15–25‰ compared with sea water (Figs. 10 and 11). Also cumulative larval mortality in Expts. 1 and 2 was lower at 15–25 than that at 35‰ (Figs. 1 and 3). These patterns may be explained by the existence of phylogenetic constraints to acclimatization, in *A. miersii* there may be a species-specific preference for moderately reduced salinities. This would facilitate an acclimatization to brackish water and outweigh effects of preceding embryonic acclimatization at full-strength sea water.

Besides ontogenetic changes in salinity tolerance and a possible phylogenetic adaptation to brackish water, cumulative effects of continual stress and signs of a gradual acclimatization (non-genetic resistance adaptation) could also be detected. In order to recognize the two latter, inversely directed effects, the response patterns in late stages must be analysed, after successively longer periods of chronic exposure to different salinites. Figs. 10 and 11 Fig. 12 summarize percent differences in mortality rate or development duration, respectively, found in late stages (zoea III–crab I) after differential periods of chronic exposure to different test salinities, all in relation to sea water (35‰; reference salinity).

The salinity range, which allowed successful development to the first juvenile stage, comprised practically only 15–35‰ in Expt. 1 (disregarding a single survivor at 5‰; Fig. 1). This range increased, when exposure began later in development: 15–45‰ (from zoea II; Fig. 3), 5–45‰ (from zoea III; Fig. 5), 5–55‰ (from megalopa or crab I; Figs. 7 and 8). Since the tolerance of acute salinity changes tended to increase in successive developmental stages, the decreasing tolerance of chronic exposure indicates a predominant influence of continued osmotic stress, outweighing both the ontogenetic increase in osmoregulatory capabilities, and possible acclimatization. Developmental

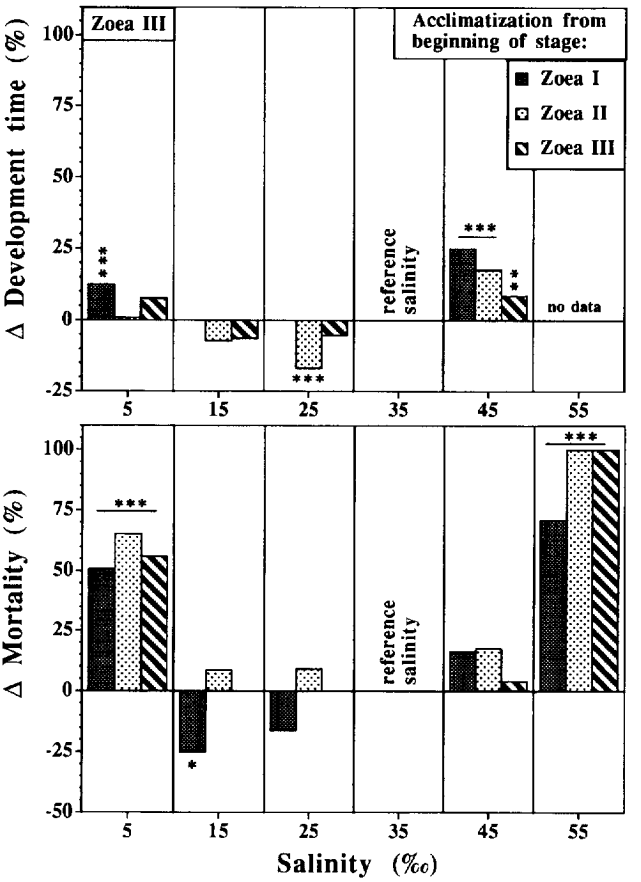

Fig. 10. Differences (Δ) in mortality (lower graph) and duration of development (upper graph) in the zoea III stage of *Armases miersii* after differential periods of chronic exposure to different salinities (from the beginning of the zoea I, II, or III stage, respectively). Δ given in % of values observed in a seawater control (35‰ = reference salinity). Statistically significant differences in pairwise or multiple comparisons marked with asterisks: *, **, *** (= $P < 0.05$, < 0.01, < 0.001); NS = not significant, $P > 0.05$.

delay and high mortality were observed also in the zoea III exposed to 5 or 45–55‰, with a decreasing tendency in an order of successively later onset of exposure (Fig. 10; cf. zoea III in Figs. 1,3,5). This trend shows again that, at extreme salinities, cumulative effects of continued osmotic stress may outweigh the influence of acclimatization during earlier stages. Similar patterns have been observed also in other larval decapods (Anger, 1985, Anger, 1991; Anger et al., 1990; Charmantier and Charmantier-Daures, 1991).

Effects of acclimatization (Kinne, 1964, 1971) can be identified, when responses in single salinity conditions and developmental stages are analysed separately. After a relatively short period of chronic exposure (from hatching), mortality rates in the zoea II stage did not vary significantly among different treatments (Fig. 1), but an acute exposure to 55‰ in this stage caused a significantly increased mortality (Fig. 3). In

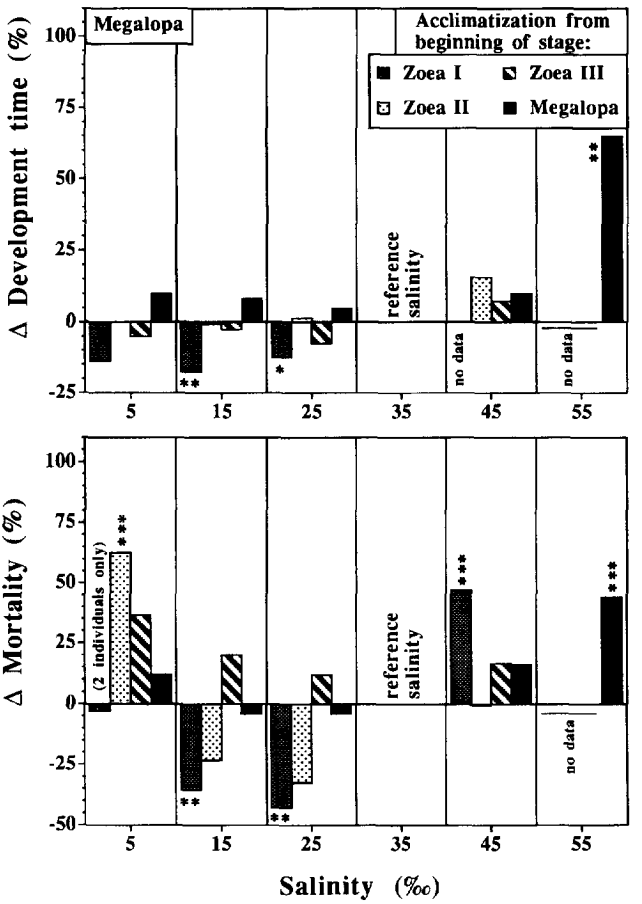

Fig. 11. Differences (Δ) in mortality (lower graph) and duration of development (upper graph) in the megalopa stage of *Armases miersii* after differential periods of chronic exposure to different salinities (from the beginning of the zoea I, II, III, or megalopa stage, respectively). Δ given in % of values observed in a seawater control (35‰ = reference salinity). Statistically significant differences in pairwise or multiple comparisons marked with asterisks: *, **, *** (= $P < 0.05$, < 0.01, < 0.001); NS = not significant, $P > 0.05$.

development duration of the zoea II, significant effects of acute exposure were recognized at 5, 45 and 55‰ (Fig. 4), but these delays were weaker after preceding development at these salinities (Expt. 1; Fig. 2). These response patterns in the zoea II indicate that an acclimatization had taken place in zoea I larvae surviving at extreme salinities. Another example of results that suggests a non-genetic resistance adaptation during larval development may be seen in the first juvenile stage: while acute exposure to 45–55‰ caused significant negative effects on its survival and development, there were signs of a successful acclimatization in individuals that had developed earlier at these conditions (Figs. 7 and 8,12).

In supratidal rock pools, where *A. miersii* breeds throughout spring and summer,

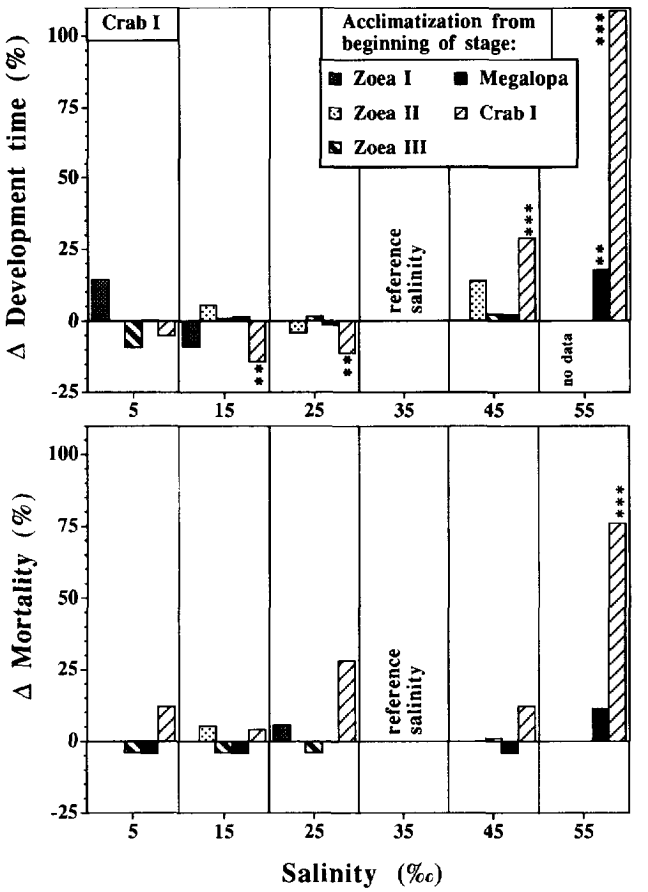

Fig. 12. Differences (Δ) in mortality (lower graph) and duration of development (upper graph) in the crab I stage of *Armases miersii* after differential periods of chronic exposure to different salinities (from the beginning of the zoea I, II, III, megalopa, or crab I stage, respectively). Δ given in % of values observed in a seawater control (35‰ = reference salinity). Statistically significant differences in pairwise or multiple comparisons marked with asterisks: *, **, *** (= $P < 0.05$, < 0.01, < 0.001); NS = not significant, $P > 0.05$.

extreme temporal and local variation in physical conditions should select for an early appearance of osmoregulatory capabilities. Moreover, this species shows ontogenetic adaptations to unpredictable nutritional conditions, namely facultative lecithotrophy (Anger, 1995a; Anger and Schultze, 1995; Schuh and Diesel, 1995b). Experimental evidence of food-limitation in the breeding habitat, at least in spring, was recently presented by Anger (1995b), who showed in situ that artificial food enrichment enhanced larval growth when compared with untreated controls. In contrast, Schuh and Diesel (1995a) found potential food organisms in rock pools during summer and concluded from this observation that food should, in general, not be a limiting factor for *A. miersii* larvae. However, the amounts of potential food were highly variable and quite unpredictable, and in some rock pools, food was practically absent. Thus, the data

presented by Schuh and Diesel (1995a) confirm rather than contradict food-limitation in supratidal rock pools.

Ontogenetic adaptations to unpredictable physical and nutritional conditions were found also in another grapsid crab from Jamaica, *Sesarma curacaoense*, which lives in coastal mangrove swamps (Anger and Schultze, 1995; Anger, 1995c,d; Schuh and Diesel, 1995c). This species is considered the closest relative of an ancestor, from which, in a process of adaptive radiation, inhabitants of rivers, streams, mountain forests, subterranean caves, terrestrial snail shells, or bromeliads have evolved (Hartnoll, 1964; Abele and Means, 1972; Diesel and Horst, 1995). All these limnic and terrestrial species of Sesarminae species show, as far as is known, ontogenetic adaptations to their specific breeding habitats (Hartnoll, 1964; Anger and Schuh, 1992; Diesel and Schuh, 1993; Diesel and Horst, 1995). Since some but not all adaptations to development under non-marine conditions can be found in species that live in mostly brackish transitional habitats (mangroves, rock pools, coastal caves), the semiterrestrial crabs *Armases miersii* and *Sesarma curacaoense* should be suitable model species for the reconstruction of the course of life-cycle evolution in crabs that have successfully adapted to freshwater and land.

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