Effects of osmotic stress on crustacean larval growth and protein and lipid levels are related to life-histories: The genus *Armases* as a model

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Abstract

In the larval stages of three euryhaline species of the genus *Armases*, we tested if changes in biomass (dry mass, W; protein; lipid) under hyposmotic stress were related to their salinity tolerance, capabilities of osmoregulation, and migration patterns. As model species, we compared *Armases miersii*, which breeds in supratidal rock pools, the riverine crab *Armases roberti* (showing a larval export strategy), and *Armases ricordi*, whose larvae probably develop in coastal marine waters. At each stage, larvae were exposed to different salinities (selected according to previous information on larval survival; range: 5‰–32‰ for *A. miersii*, 10‰–32‰ for *A. roberti*, and 15‰–32‰ for *A. ricordi*). Biomass was measured in early postmoult and intermoult. The larvae of the strongly osmoregulating species *A. miersii*, which develop in habitats with highly variable salinity conditions, showed the smallest variations in biomass. The effect on *A. roberti* varied during its ontogeny: the Zoea I and the Megalopa, which carry out downstream and upstream migrations, respectively, showed lower biomass variations than the intermediate zoeal instars, which develop in coastal waters. The larvae of *A. ricordi* showed generally the highest variations in biomass, reflecting poor adaptation to salinity variations. In addition, a common pattern was found for these estuarine species: the maximum of biomass shifted during ontogeny from 32‰ to 25‰, reflecting changes of the iso-osmotic point. The ontogeny of osmoregulation reflected ontogenetic migration patterns, which allow for avoiding detrimental effects of salinity variations.

1. Introduction

Variations in salinity affect the volume of water and the concentrations of soluble compounds in aquatic organisms, and in consequence, alter the performance of their internal systems. The maintenance of a stable cellular composition is achieved through ion-regulation strategies (for review see Péqueux, 1995). In coastal and estuarine waters, changes in salinity play therefore a central role for the survival and growth of planktonic larvae and other invertebrates, as well as fish (Kinne, 1971). In particular, biomass and chemical composition are affected by reduced salinities (Anger, 2001, 2003; Anger et al., 2000; Torres et al., 2002).

Fully marine decapod crustaceans (e.g. *Cancer pagurus*, *Hyas araneus*) are generally osmoconformers, which are restricted to habitats with little variability in salinity (Péqueux, 1995). Many other crustaceans, however, can live in environments with variable salinity conditions. The benthic juvenile and adult stages of estuarine, semiterrestrial and terrestrial decapods, for instance, have evolved numerous physiological and behavioural adaptations to their physically variable environments (Greenaway, 1999). Nevertheless, their planktonic larvae are in most cases osmoconformers and remain therefore vulnerable to osmotic stress. Most of those species evolved larval "export strategies" (Strathmann, 1982), i.e. their physiologically sensitive larvae are transported to waters with more stable conditions (e.g. *Armases roberti*: Diesel and Schuh, 1998; Torres et al., 2006; *Chasmagnathus granulata*: Anger et al.,

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1994; Charmantier et al., 2002; *Eriocheir sinensis*: Herborg et al., 2003; Cieluch et al., 2007). A lesser number of species develops through euryhaline, osmoregulating larval instars, which can successfully cope with strong salinity variations (e.g. *Armases miersii*, Charmantier et al., 1998). In these cases of advanced adaptation to non-marine conditions, the larval stages can be retained within the parental habitats ("retention strategy"; Strathmann, 1982).

Torres et al. (2002) found that larval biomass (dry mass, protein, lipid content) of weakly osmoregulating Zoea-I larvae was less affected by low salinity than the biomass of stenohaline osmoconforming zoeae. These results suggested that effects of low salinity on larval biomass (a) might vary with life-history strategies and ontogenetic patterns of osmoregulation, and (b) should be less detrimental in species with strongly osmoregulating larvae. In the present study, we tested these predictions. We expected that a reduced salinity should produce weaker effects on larval biomass in species,

which live in highly variable habitats and show a retention strategy, as compared to those exporting their larvae to coastal marine waters. In addition, we tested if larval instars that are involved, in their natural environment, in ontogenetic migrations between rivers and coastal waters are less susceptible to salinity reductions than the intermediate instars of the same species, which normally develop in coastal waters with higher and more stable salinities.

To test these hypotheses, we compared three species of *Armases*, which live in different environments and show different reproductive strategies: (1) The larval development of a strong osmoregulator, *A. miersii*, occurs in isolated supratidal rock pools, where a wide range of salinity variation from freshwater to concentrated seawater occurs (Anger, 1995; Schuh and Diesel, 1995). (2) The larvae of *Armases ricordi* are directly released into coastal marine waters (Diesel and Schuh, 1998). (3) *A. roberti* shows a larval export strategy consisting of a rapid passive transport of the Zoea I from the riverine parental habitat
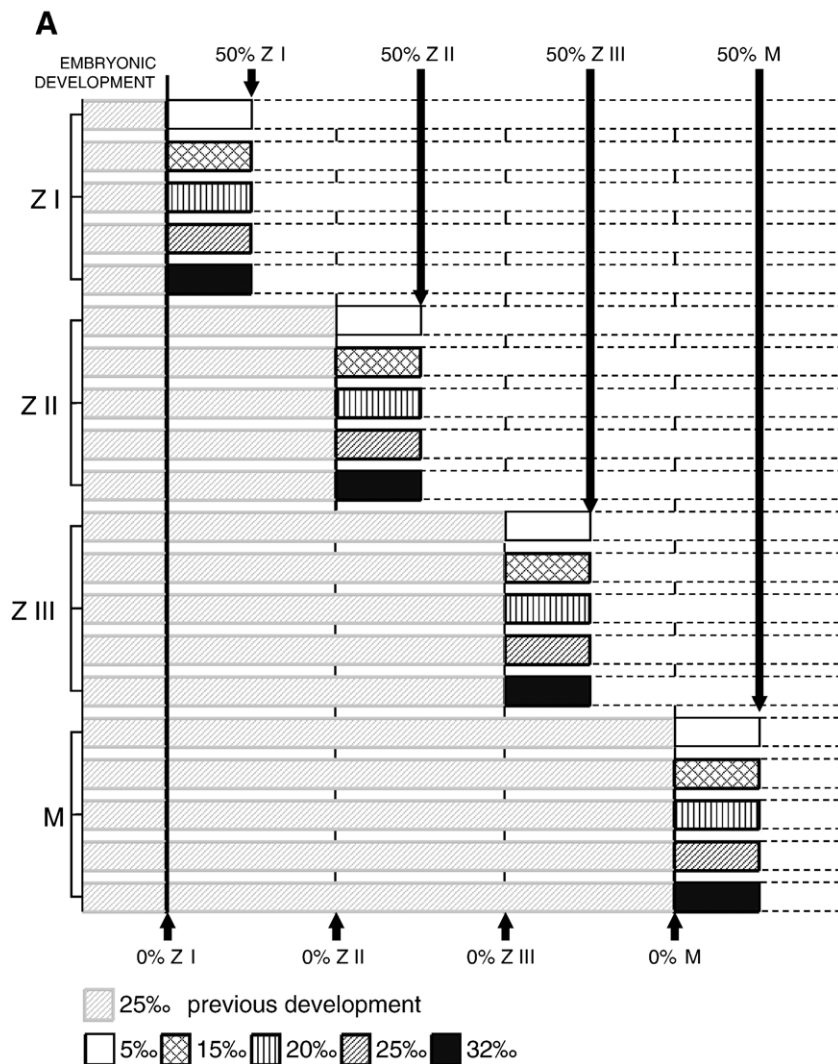

Fig. 1. Experimental design to study effects of salinity on dry mass and biochemical composition during larval development of A: *Armases miersii*, B: *A. ricordi*, C: *A. roberti*. Postmoult and intermoult samples (see arrows) were taken immediately after hatching or moulting (0%) and at 50% of the moult cycle, respectively.
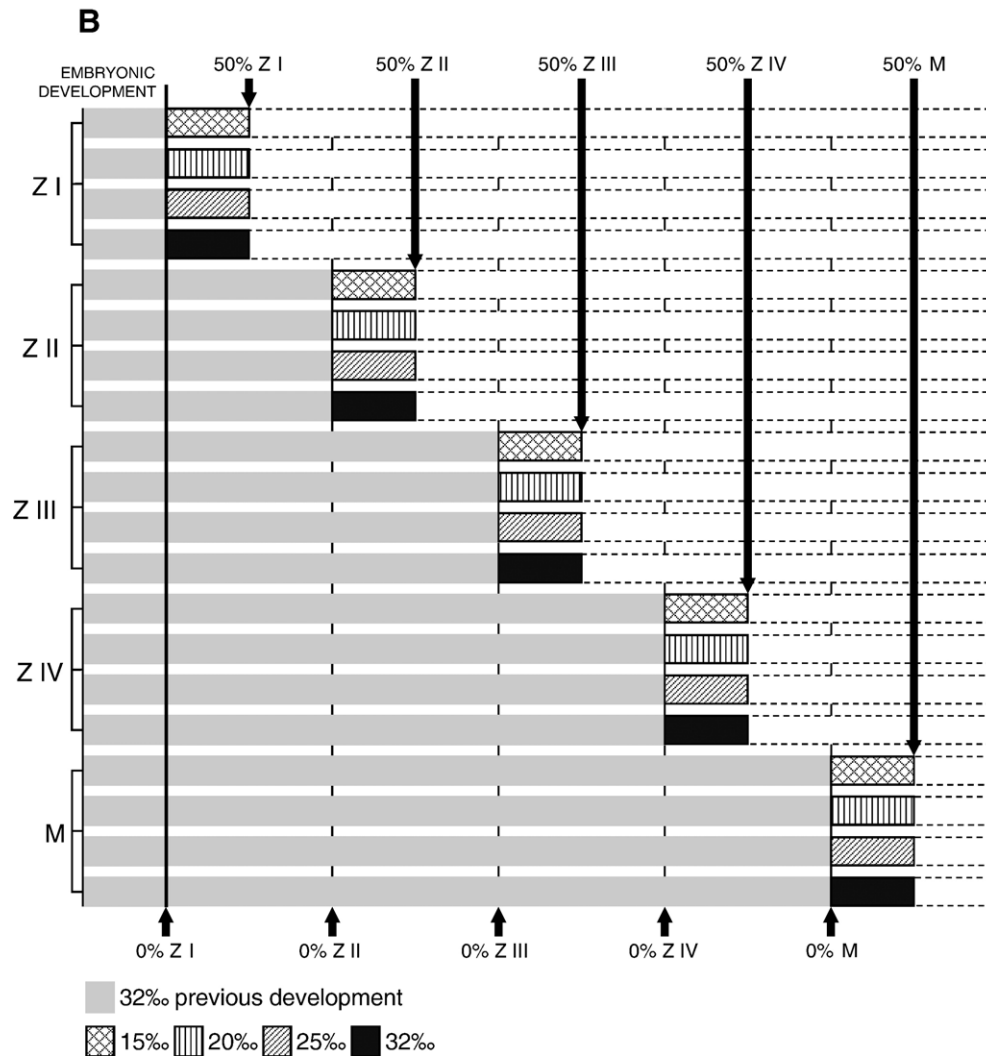
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to coastal waters and a later re-immigration of the Megalopa to freshwater habitats (Diesel and Schuh, 1998; Torres et al., 2006; Anger et al., 2006).

2. Materials and methods

2.1. Model species

2.1.1. A. miersii (Rathbun)

The distribution range of this semiterrestrial crab includes the Caribbean islands, Florida and the Bahamas (Abele, 1992). On the northern shore of Jamaica, the juvenile and adult crabs live hidden in crevices between mangrove roots or on subtidal rocks as well as in coastal limestone caves with brackish or fresh water, breeding in coastal mangrove swamps and in land-locked supratidal rock pools (Anger, 1995; Schuh and Diesel, 1995). Its larval development consists of three zoeal stages and a Megalopa (Cuesta et al., 1999). The physico-chemical conditions in the breeding pools are highly variable, particularly regarding salinity. Larval development in such

harsh environment is possible, because all larval stages are able to osmoregulate (Charmantier et al., 1998). The zoeae hyper-osmoregulate at low salinities and osmoconform at salinities higher than 25‰. The Megalopa and the subsequent juvenile stages reveal a weakly developed but gradually increasing capability of hyper-hypo-osmoregulation, i.e. hyper-regulation at low salinities and a slight hypo-regulation at salinities higher than 25‰. The adults show a strong capacity to hyper-hypo-osmoregulate in conditions ranging from freshwater to concentrated media (Charmantier et al., 1998; Schubart and Diesel, 1998).

2.1.2. A. ricordi (H Milne Edwards)

This highly terrestrial species ranges between Bermuda, Surinam and the Gulf of Mexico (Abele, 1992). It occupies typical dry coastal mangrove habitats as well as rocks and debris in the supralittoral fringe up to ca. 200 meters inland (Hartnoll, 1965; Chace and Hobbs, 1969; Abele, 1992). The ovigerous females migrate to the sea to release the larvae into coastal marine waters (Diesel and Schuh, 1998). All larval
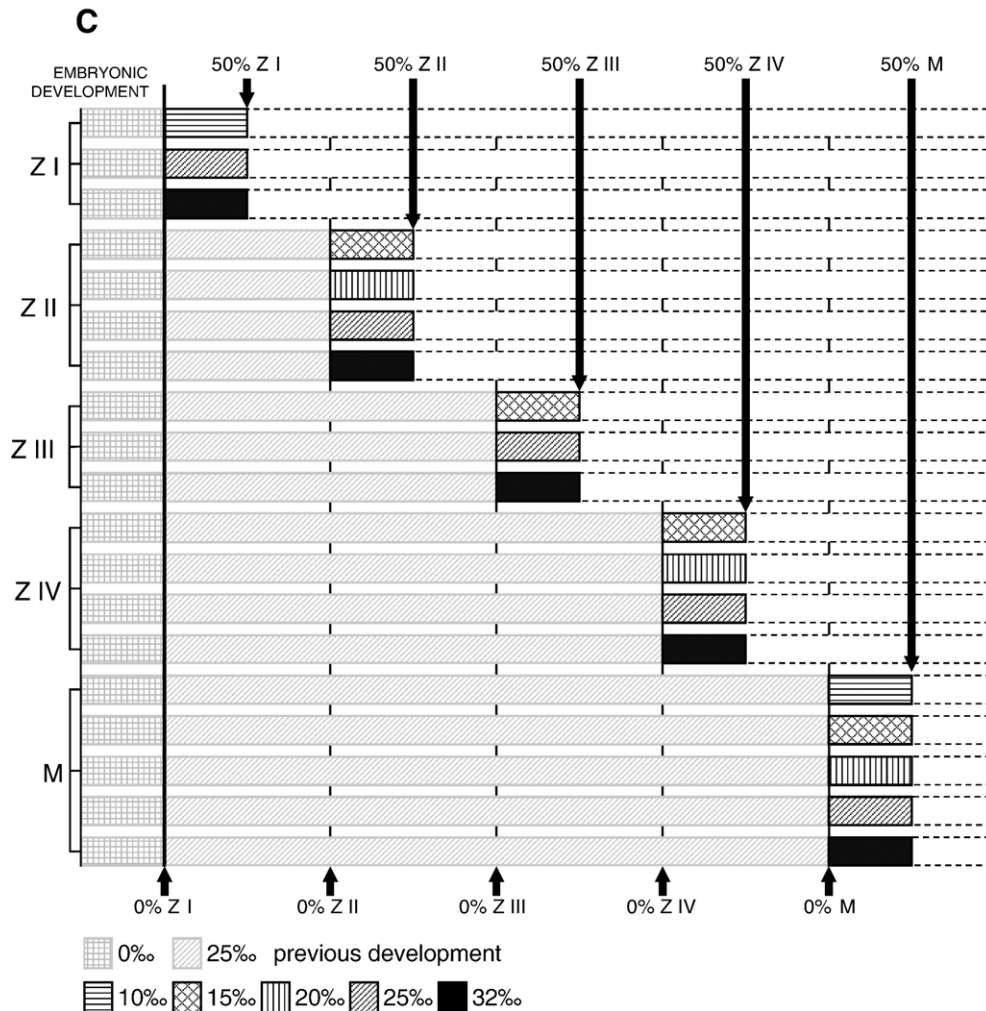
Fig. 1 (*continued*).

stages (four zoeae and a Megalopa; Guerao et al., in press) develop in the coastal planktonic environment (Díaz and Ewald, 1968; Alvarez and Ewald, 1990). They show a relatively narrow range of salinity tolerance, with high survival between 15‰ and 45‰ (G Torres, preliminary experiments). The four zoeal stages can slightly hyper-osmoregulate between 15‰ and 32‰ and osmoconform at higher salinities (Charmantier et al., unpublished data). The Megalopa, surviving in a wider salinity range (5‰–45‰), shows a fairly strong hyper-osmoregulatory capacity between 5‰ and 25‰ and an incipient hypo-regulation at higher salt concentrations. The adult crabs are strong hyper–hypo-osmoregulators (Schubart and Diesel, 1998).

2.1.3. A. roberti (H Milne Edwards)

This little known tropical species is found in the West Indies, but not on the American mainland (Abele, 1992). The adult crabs live on the banks of rivers and streams from the mouth to ca. 10 km upstream (Chace and Hobbs, 1969; Abele, 1992; Anger et al., 2006). According to Diesel and Schuh (1998), the first zoea is released directly into freshwater near the semiterrestrial habitats of the adult population. Hence, the freshly hatched larvae are carried downstream by the river currents, so that they soon reach estuarine or coastal waters. The exported larvae develop in osmotically more stable waters through four zoeal stages to the megalopa (Guerao et al., in press). The latter is presumed to migrate back into estuaries and, probably, further upstream into riverine habitats (Torres et al., 2006; Anger et al., 2006). In agreement with this tentative migration pattern, the Zoea I shows a wide range of salinity tolerance (0–50‰; Diesel and Schuh, 1998), hyper-osmoregulating between 5‰ and 32‰ and osmoconforming at

Table 1
Time of exposure to experimental salinities (*ca.* 50% of each moulting cycle)

Stage	*Armases miersii*	*Armases ricordi*	*Armases roberti*
Zoea I	24 hs	2 days	2 days
Zoea II	2 days	2 days	36 h
Zoea III	2 days	2 days	2 days
Zoea IV	–	36 h	3 days
Megalopa	2 days	5 days	4 days

Table 2
Armases miersii. Two-way ANOVA to evaluate the effect of salinity and larval instar on dry mass, protein and lipid content (log-transformed data)

Factor	Dry mass			Protein			Lipids		
	df	MS	F	df	MS	F	df	MS	F
Instar (I)	3	821	3747***	3	1394	7470***	3	380	355***
Salinity (S)	4	1.6	7.0***	4	1.1	5.9***	4	0.9	0.8[ns]
I x S	12	1.8	8.0***	12	0.4	1.9***	12	1.2	1.1[ns]
Error	85	0.2		81	0.2		85	1.1	

Symbols: *df*: degrees of freedom, MS: means squares. Mean squares are (1×10^{-2}). Significant levels are indicated as follows: ns: non-significant; *: <0.05, **:<0.01, ***:<0.001.

higher salinities (Charmantier et al., unpublished data). The following larval stages have a narrower range of salinity tolerance (15–45‰; Diesel and Schuh, 1998), coinciding with a lower hyper-osmoregulatory capacity. The tolerance of salinity variation increases again after metamorphosis to the Megalopa, which is able to strongly hyper-osmoregulate between 0‰ and 25‰ and slightly hypo-osmoregulate at higher salinities. The iso-osmotic point shifts during the course of larval development from 32‰ to 25‰ (Charmantier et al., unpublished data). As in the previous species of *Armases*, the adult crabs are strong hyper-hypo-osmoregulators (Schubart and Diesel, 1998).

2.2. Handling of ovigerous females and larval rearing

Larvae of *A. miersii*, *A. roberti*, and *A. ricordi* were obtained from ovigerous females, which had been collected near the Discovery Bay Marine Laboratory (DBML), Jamaica. After transport to the Helgoland Marine Biological Station (BAH, Helgoland, Germany), the crabs were maintained in an aerated circulating water system, under controlled conditions of temperature (24 °C), salinity (25‰ for *A. miersii*; 32‰ for *A. ricordi*; 0.2‰ for *A. roberti*), a light/dark cycle of 12L:12D, and feeding *ad libitum* with frozen isopods, *Idotea* sp. (for further details of habitat characteristics, collection, and long-
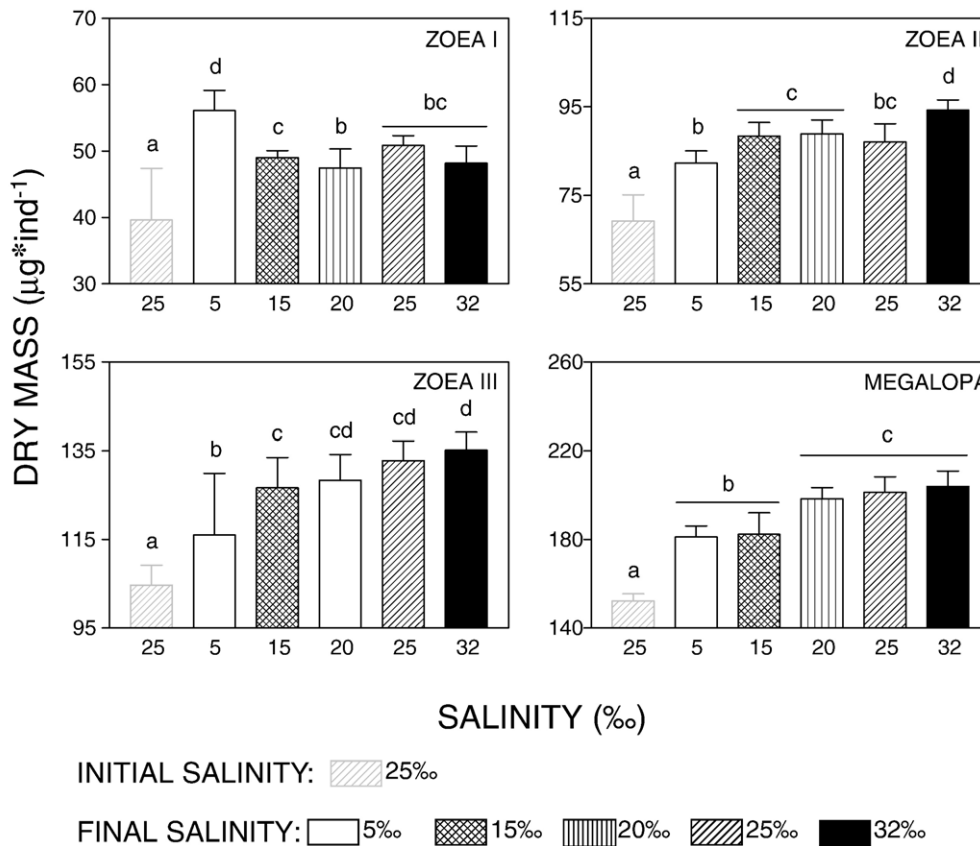

Fig. 2. *Armases miersii*. Changes in dry mass (expressed as $\mu g * ind^{-1}$) after exposure to 5‰, 15‰, 20‰, 25‰ and 32‰. Column drawn in grey: biomass at hatching or early postmoult; columns drawn in black: biomass at intermoult. Different letters show significant differences (after SNK tests) between salinities at hatching and intermoult.

Fig. 3. *Armases miersii*. Changes in protein content (expressed as $\mu g * ind^{-1}$) after exposure to 5‰, 15‰, 20‰, 25‰ and 32‰. Column drawn in grey: biomass at hatching or early postmoult; columns drawn in black: biomass at intermoult. Different letters show significant differences (after SNK tests) between salinities at hatching and intermoult.
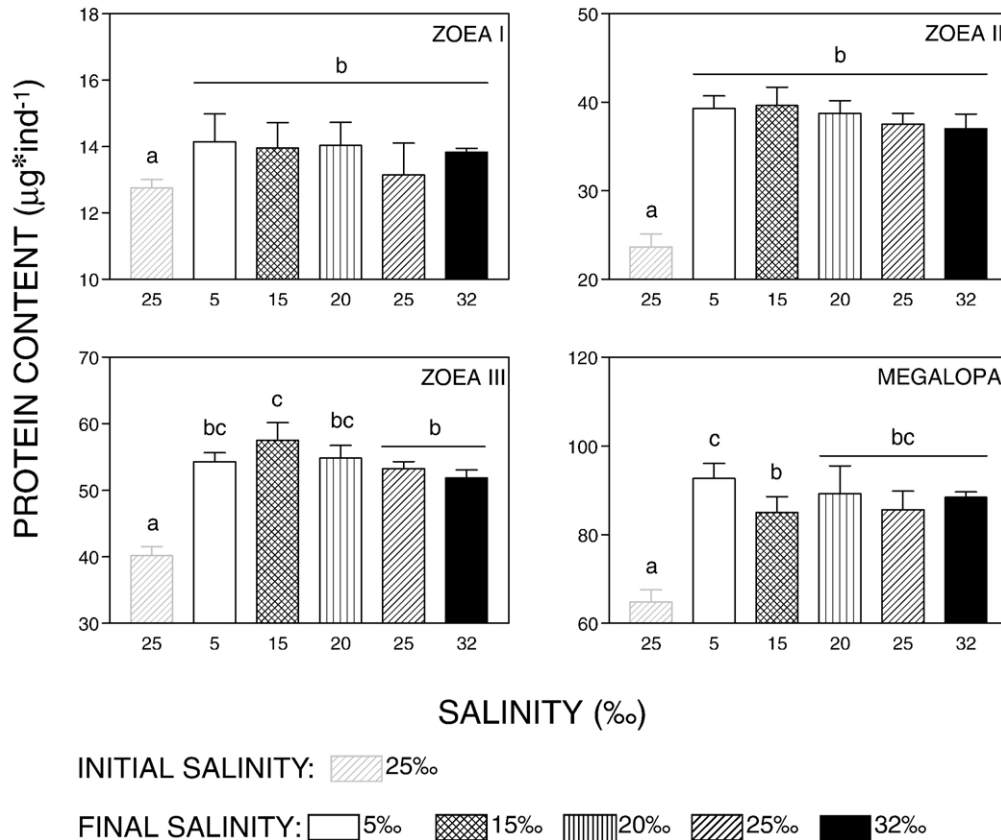
term cultivation, see Anger et al., 2000; Torres et al., 2006). Ovigerous females were isolated in individual aquaria and kept under otherwise identical conditions until larvae hatched.

All larval stages were mass-reared at constant salinities (25‰ for *A. miersii* and *A. roberti*, 32‰ for *A. ricordi*) and otherwise identical conditions as the adults (see above). They were fed *ad libitum* with freshly hatched *Artemia* sp nauplii. Newly hatched larvae were used for experiments (for details see Fig. 1) or mass-reared to subsequent stages in 400-mL bowls without aeration. Larval density was initially 50 per bowl (Zoea I), thereafter decreased according to increasing size in the following zoeal stages (30, 20, 10, respectively). Filtered (pore size 1 μm) seawater (32‰) was mixed with appropriate amounts of desalinated freshwater to obtain the experimental salinities (5, 10, 15, 20 and 25‰). In daily intervals, water was changed, dead larvae were removed, new food was added, and cultures were controlled for ecdyses. Freshly moulted larvae were transferred to new bowls to ensure that all larvae used in experiments had the same moulting history and the same age within each moulting cycle.

2.3. Experimental design

Each set of experiments comprised treatments with a series of salinities (ranging from 5‰ to 32‰). Larval exposure lasted from early postmoult (<12 h after hatching or moulting) to the intermoult stage (ca. 50% of the moulting cycle, see Table 1; Fig. 1). These treatments represent conditions of optimal salinity, severe, and moderate osmotic stress, respectively.

In each treatment, five replicate groups of larvae were reared in 400-mL bowls. Samples of larvae were taken at hatching (Zoea I) or at early postmoult (later larval stages), and again at the end of each experiment. In all samples, larval dry mass (W), and contents of total lipid and protein were measured (see below).

2.4. Biochemical analyses

Samples for biochemical analyses were gently rinsed in distilled water for 10 s and blotted on filter paper. Subsequently, they were transferred to 1.5 mL microcentrifuge vials and frozen at −80 °C. The samples were left in a vacuum drier (Finn-Aqua Lyovac GT2E) for 48 h and their dry mass was determined in a Sartorius MC1 RC 210 S balance (precision: 0,01 mg, capacity 210 g). Afterwards, they were homogenized by sonication (Branson, Sonifier, Cell Disruptor B 15) with 5 strokes of 5 s, on ice and each homogenate was divided in two aliquots to perform lipid and protein content determinations.
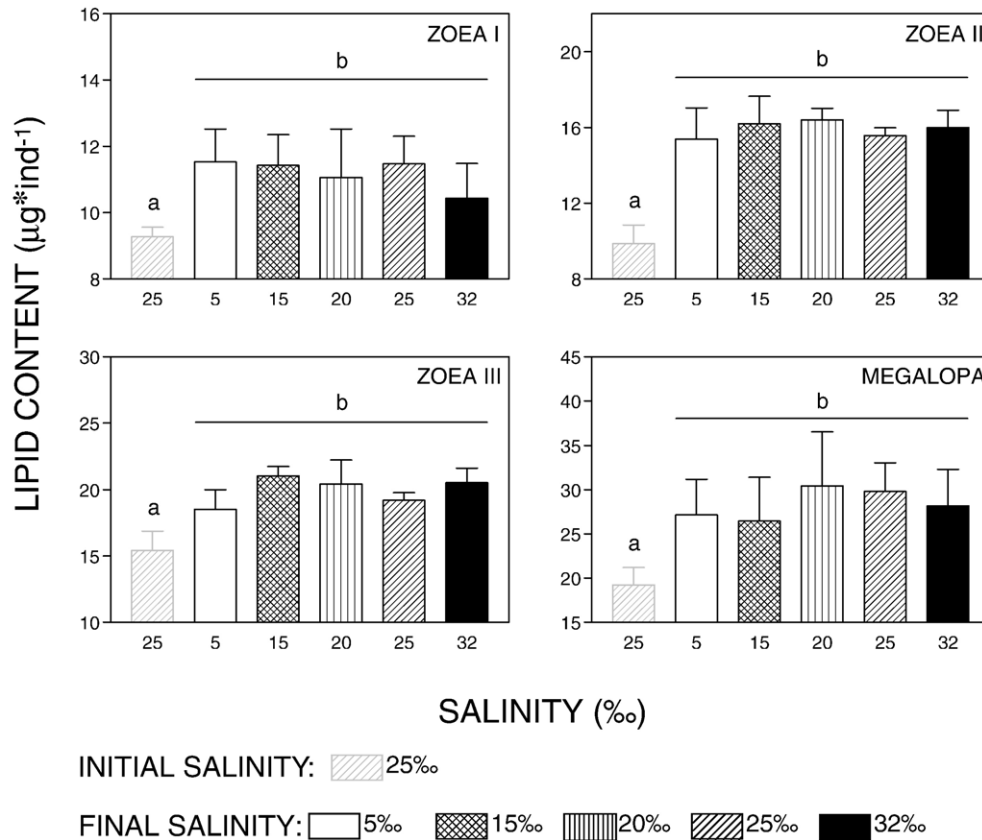
Fig. 4. *Armases miersii*. Changes in lipid content (expressed as μg*ind^{-1}) after exposure to 5‰, 15‰, 20‰, 25‰ and 32‰. Column drawn in grey: biomass at hatching or early postmoult; columns drawn in black: biomass at intermoult. Different letters show significant differences (after SNK tests) between salinities at hatching and intermoult.

2.4.1. Protein determination

The total protein content of the homogenate was determined using a modified method (Torres et al., 2007) after Lowry et al. (1951)(BioRad D$_C$ Protein Assay).

2.4.2. Lipid determinations

The total lipid content of the homogenate was determined after the sulphophosphovanillin method following Zöllner and Kirsch (1962), modified for microplates. 40 μL of homogenate were mixed with 300 μL of ice-cold CHCl$_3$/CH$_3$OH (2:1). After 15 min incubation at room temperature, the samples were centrifuged at 10.000 g for 20 min at 4 °C.

180 μL of the lower phase were transferred to new tubes. These were left open to dry in a Thermomixer Eppendorf for 90 min at 56 °C with shaking at 700 rpm. The dried pellet was dissolved in 200 μL of H$_2$SO$_4$ conc. and incubated for 10 min at 95 °C with shaking at 1400 rpm (in a Thermomixer Eppendorf, with the tubes closed). After cooling for 20 min at room temperature, 8 replicates of 20 μL from each sample were distributed in two 96-well microplates. In the first plate (Blank: Plate 0) 300 μL of H$_3$PO$_4$ conc. were added; in the second (Plate 1), 300 μL of vanillin solution (8 mM H$_3$PO$_4$ conc.). The microplates were incubated for colour development, 40 min at room temperature and subsequently

Table 3

Armases ricordi. Two-way ANOVA to evaluate the effect of salinity and larval instar on dry mass, protein and lipid content (log-transformed data)

Factor	Dry mass			Protein			Lipids		
	df	MS	F	df	MS	F	df	MS	F
Instar (I)	4	1344	7171***	4	2025	4101***	4	1368	3242***
Salinity (S)	3	24.7	131***	2	4.1	8.3***	3	15	37***
I×S	12	0.6	3.2***	12	1.3	2.7 **	12	1.8	4.0**
Error	77	0.2		80	0.5		77	0.4	

Symbols: *df*: degrees of freedom, MS: means squares. Mean squares are (1×10^{-2}). Significant levels are indicated as follows: ns: not significant; *: <0.05, **:<0.01, ***:<0.001.
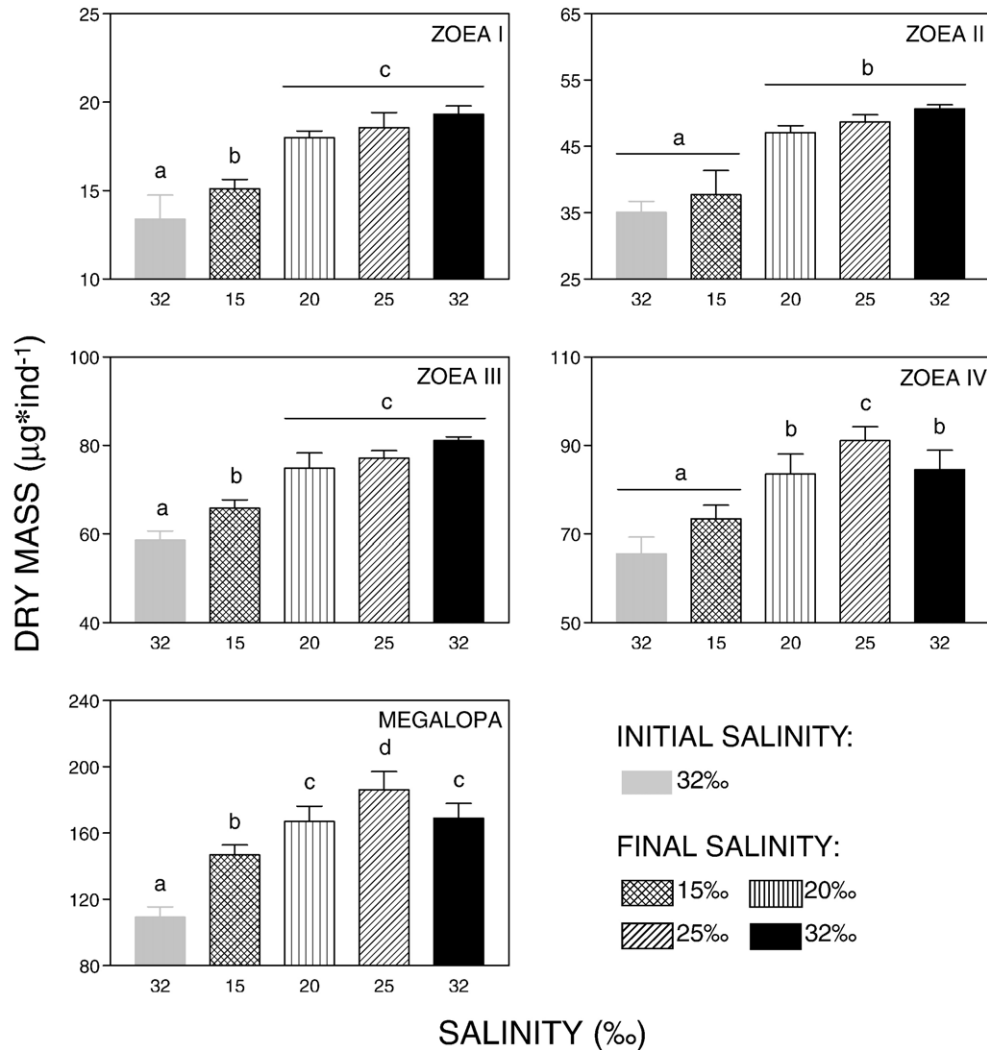
Fig. 5. *Armases ricordi*. Changes in dry mass (expressed as $\mu g * ind^{-1}$) after exposure to 15‰, 20‰, 25‰ and 32‰. Column drawn in grey: biomass at hatching or early postmoult; columns drawn in black: biomass at intermoult. Different letters show significant differences (after SNK tests) between salinities at hatching and intermoult.

measured (Thermoelectron Multiskan® Spectrum spectral-photometer, wavelength: 530 nm). The final values were obtained as the difference between Plate 1 and Plate 0. The calibration curve was obtained by dilutions of a standard solution of cholesterol.

2.5. Statistical analyses

Statistical analyses were performed following Zar (1996). Data sets from *A. miersii* and *A. ricordi* were analysed by two-way ANOVA with developmental stage and salinity as fixed factors. Data from *A. roberti* were analysed for each stage separately using one-way ANOVA with salinity as factor, because different test salinities were used in different larval instars. For all stages, planned comparisons were made in order to test for significant growth from hatching or early postmoult to intermoult. The number of replicates was 4–5 for each species and stage-salinity combination.

When significant differences were detected in the ANOVAs, comparisons between different factors were performed with the Student–Newman–Keuls test (SNK). The critical level (α) to reject the null hypothesis was fixed at 0.05. Before performing ANOVAs, normality (normal plots) and variance homogeneity (Cochran test) were checked. When raw data failed to meet the assumptions logarithmically transformed data showed normal errors and variance homogeneity.

3. Results

3.1. A. miersii

During the period from postmoult to intermoult, significant increments in larval dry mass (i.e. W growth) were found in all larval stages and at all salinities. However, salinity affected the rate of developmental increase in W, and these salinity effects varied significantly among instars (Table 2). In the Zoea I, the

intermoult level of W was significantly higher at 5‰ than at 15‰–32‰ (Fig. 2). The following larval stages, by contrast, grew at 5‰ less than in treatments with higher salinities, while maximum W growth was achieved at 32‰.

The levels of both the protein and the lipid fraction increased during development from postmoult to intermoult in all larval stages and in all salinity treatments (Figs. 3, 4). The protein contents measured at intermoult were in the first and second zoeal instar not significantly affected by salinity. The Zoea III, by contrast, showed maximum protein growth at 15‰, i.e. significantly lower increments in treatments with either higher or lower salinities. In the Megalopa, maximal protein growth was observed at 5‰ (Fig. 3).

The lipid content reached at intermoult was generally not affected by salinity (Table 2, Fig. 4).

3.2. A. ricordi

Effects of salinity on intermoult levels of W, protein and lipid varied among larval instars (Table 3). In all stages,

intermoult W was lowest at the lowest test salinity (15‰; Fig. 5). W growth in the early stages (Zoea I -III) did not vary in the range 20–32‰, but later instars showed a maximum at 25‰ (Fig. 5). The Zoea II and IV did not show significant developmental increments in W at 15‰, while significant W growth occurred in all treatments with higher salinities (Fig. 5).

The increments in protein occurring during development from postmoult to intermoult were in all stages and treatments significant (Fig. 6). However, the protein level at the intermoult stage showed in the Zoea I and Zoea II a significantly increasing trend with increasing salinity, while the Zoea III showed a peak at 20‰, and no salinity effect was found in the Zoea IV (Fig. 6). In contrast to the zoeal stages, the megalopae showed a higher protein gain in the 15, 20, and 25‰ treatments compared to the control condition (32‰).

In the lipid fraction, the rate of growth (increment from postmoult to intermoult) increased significantly in treatments with increasing salinity, except for the Megalopa stage (Fig. 7). In the range 20–32‰, significant salinity effects were found
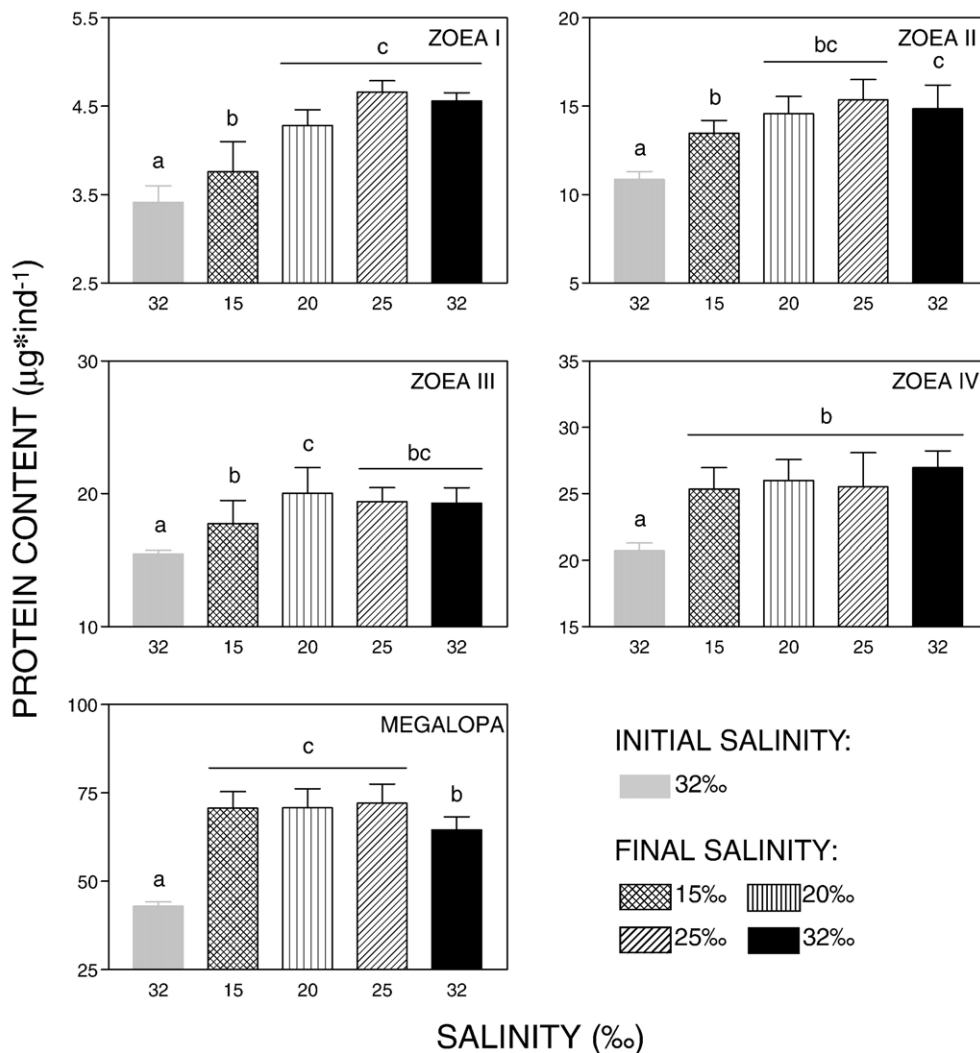

Fig. 6. *Armases ricordi*. Changes in protein content (expressed as $\mu g * ind^{-1}$) after exposure to 15‰, 20‰, 25‰ and 32‰. Column drawn in grey: biomass at hatching or early postmoult; columns drawn in black: biomass at intermoult. Different letters show significant differences (after SNK tests) between salinities at hatching and intermoult.

Fig. 7. *Armases ricordi*. Changes in lipid content (expressed as μg * ind^{-1}) after exposure to 15‰, 20‰, 25‰ and 32‰. Column drawn in grey: biomass at hatching or early postmoult; columns drawn in black: biomass at intermoult. Different letters show significant differences (after SNK tests) between salinities at hatching and intermoult.
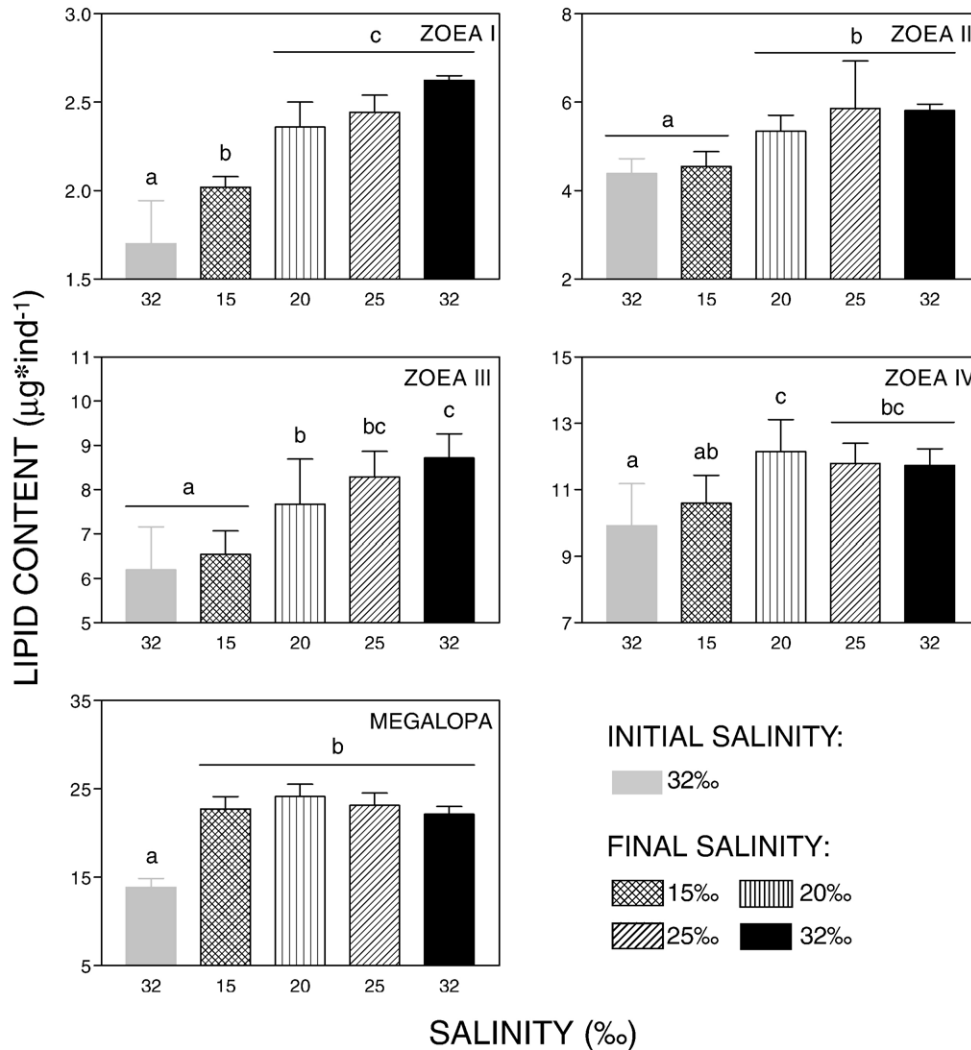
only in the Zoea III, where the lipid content at intermoult was higher at 32‰ than at 20‰. No lipid growth was found in the zoeal stages II, III and IV exposed to 15‰; at higher salinities, lipid growth was always significant.

3.3. A. roberti

In this species, all larval stages revealed a significant W growth from postmoult to intermoult (Fig. 8). Salinity affected the rate of growth in larval W only in the early instars (Zoea I–III, Table 4). W measured at intermoult was lower at 10–15‰ than at higher salinities. In the zoeal stages I and II it was maximum at 25–32‰, in the Zoea III at 25‰ (Fig. 8). Intermoult W in the Zoea IV and Megalopa tended to be higher in treatments with 10‰–25‰ compared to higher salinities, but the differences were statistically not significant.

Protein growth was significant in all stages and at all salinities. The effect of salinity was statistically significant only in the Zoea IV and Megalopa (Table 4). In the Zoea IV, the

lowest developmental protein increment was found at 15‰, while the Megalopa showed a minimum at 10‰ and maximal increments in the range 15‰–25‰ (Fig. 9).

In the Zoea I stage, only the control condition (32‰) allowed for a significant lipid growth during development from postmoult to intermoult. All other larval instars showed at all salinities significant increments in their lipid contents. Lipid growth was in the Zoea I, III and Megalopa affected by salinity (statistically not significant in the Zoea II; Table 4). While the zoeal stages I and III showed at intermoult maximal lipid contents at a condition of 32‰, the Megalopa reached maximum lipid growth at 25‰ (Fig. 10).

3.4. Interspecific comparisons

While detailed statistical analyses show significant salinity effects on growth rates and intermoult levels of total W, protein and lipid contents in individual species and larval stages (see sections above), an index of relative (biomass-specific) changes
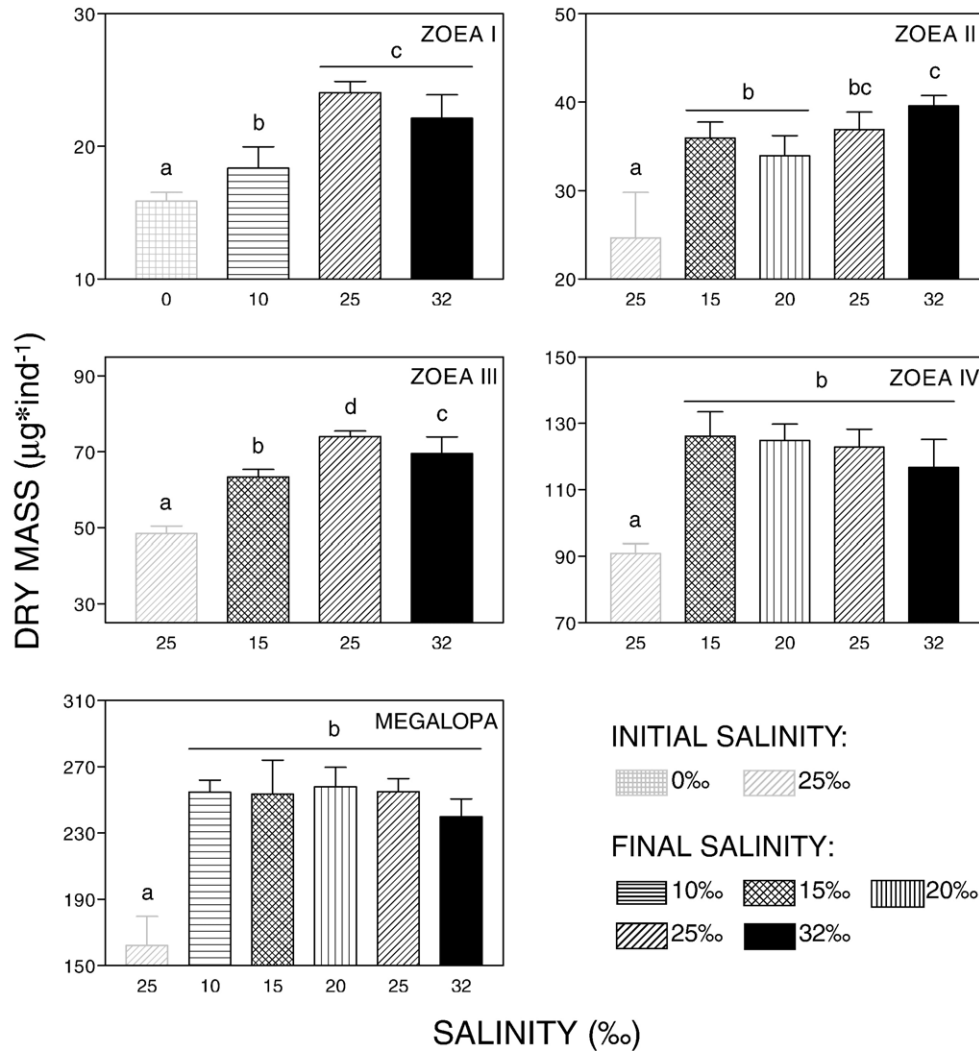
Fig. 8. *Armases roberti*. Changes in dry mass (expressed as µg*ind^{-1}) after exposure to 10–32‰. Column drawn in grey: biomass at hatching or early postmoult; columns drawn in black: biomass at intermoult. Different letters show significant differences (after SNK tests) between salinities at hatching and intermoult.

in developmental growth rates (B'_{H-L}) removes the confounding effects of interspecfic differences in stage-specific biomass, allowing for a comparative quantification and visualization of salinity effects:

$$B'_{H-L} = (B_H - B_L) \cdot 100 / (B_{25} \cdot (S_H - S_L))$$

where B_H is individual biomass (W, protein or lipid content per larva) measured at the end of the experiment (at intermoult) at the higher salinity (S_H) in an interval of salinities ($S_H - S_L$), and B_L is the biomass at the lower salinity (S_L). The dimension is a percentage of variation in biomass (%B per ‰ salinity change) in relation to the biomass at the reference condition, 25‰ (B_{25}).

Biomass-specific effects of salinity on the intermoult levels of larval biomass are shown in Fig. 11, comparing in each stage and species two salinity ranges, 15–25‰ and 25–32‰ (B'_{25-15} and B'_{32-25}). Positive B' values indicate a higher

biomass increment at the higher as compared to the lower salinity (B_H vs. B_L), while negative values indicate that the biomass increment (from postmoult to intermoult) was lower at the higher salinity.

A. miersii showed generally very small values of B'_{25-15} and B'_{32-25} (−1.3 to +1.1% per salinity unit), indicating only a weak response of larval biomass to variations in salinity (Fig. 11). For W, a developmental change from negative to positive B'_{32-25} values indicated an ontogenetic shift in the optimal salinity, where increments in biomass were maximal (25‰ in earlier larval stages, 32‰ in later stages).

A. ricordi showed a stronger susceptibility to salinity variations than the other two species. The B'_{25-15} values were >2% per salinity unit, except for protein growth in the Zoea IV and both protein and lipid growth in the Megalopa (Fig. 11). Thus, salinity-induced changes in the protein and lipid contents at intermoult tended, in this species, to decrease with development. The B'_{32-25} values for protein and lipid growth

Table 4
Armases roberti. One-way ANOVAs per stage to evaluate the effect of salinity and larval instar on dry mass, protein and lipid content (all data log-transformed except Lipids in Megalopa)

Factor	Dry mass			Protein			Lipids		
	df	MS	F	df	MS	F	df	MS	F
Zoea I									
Salinity	2	9.72	19.8***	2	0.29	0.66^{ns}	2	10.3	5.85*
Error	12	0.49		12	0.45		12	1.8	
Zoea II									
Salinity	3	2.06	7.6**	3	0.04	0.13^{ns}	3	1.4	0.36^{ns}
Error	15	0.27		15	0.32		15	3.9	
Zoea III									
Salinity	2	3.05	16.4***	2	0.50	1.04^{ns}	2	4.4	6.1*
Error	12	0.19		12	0.48		11	0.7	
Zoea IV									
Salinity	3	0.62	2.0^{ns}	3	2.29	9.80**	3	3.4	1.11^{ns}
Error	16	0.31		16	0.23		15	3.1	
Megalopa									
Salinity	4	0.40	1.7^{ns}	4	1.20	4.60**	4	102.2	3.70*
Error	20	0.24		20	0.26		19	27.6	

Symbols: df: degrees of freedom, MS: means squares. Mean squares are (1×10^{-2}). Significance levels are indicated as follows: ns: not significant, *: <0.05, **:<0.01, ***:<0.001.

indicated an ontogenetic shift in the salinity allowing for maximum growth, from 32‰ in the early stages (Zoea I–IV) to 25‰ in later stages (Megalopa).

The larvae of *A. roberti* showed generally little sensitivity of growth to salinity variations, albeit a stronger response than in *A. miersii* (Fig. 11). The strongest effect was found in the lipid content, where the B'_{25-15} values of the Zoea IV and the Megalopa as well as the B'_{32-25} values of the Zoea I and Zoea III were ca. 2% per salinity unit. As in *A. ricordi*, maximum W, protein and lipid growth shifted from 32‰ (Zoea I–III) to 25‰ (Zoea IV–Megalopa), indicated by changes in the sign of B'_{32-25}.

4. Discussion

Our experiments have shown that, within the genus *Armases*, effects of salinity on larval biomass vary among species and ontogenetic stages. These differences appear to be related to differential osmoregulatory capacities and ecological characteristics in each species and larval stage.

The larval development of *A. miersii* occurs in isolated supratidal rock pools, which are characterized by strong variations in salinity due to evaporation and rainfalls (Anger, 1995; Schuh and Diesel, 1995). The larvae of this species hatch with strong hyper-osmoregulatory capacities within the range 5-25‰ (Charmantier et al., 1998). This capability is most probably based on an increase in the level of mRNA encoding for the $Na^+–K^+$-ATPase, one of the key enzymes in osmoregulatory mechanisms (Torres et al., in prep.). These physiological traits can be considered as adaptive, because they enhance the larval tolerance of naturally occurring salinity variations, reducing osmotic stress and physiological damage. This should explain why *A. miersii* showed, in the present study, the smallest salinity-induced changes in biomass growth,

especially in the fractions of protein and lipid. The increase in total W growth at higher salinities may reflect an uptake of inorganic substances (Anger, 2003). No effects were detected in the intermoult lipid content, and protein growth showed in the Zoea III a peak at 15‰ rather than in full-strength seawater. These observations indicate that reduced salinities did not negatively affect, or sometimes even favoured larval growth in this species.

Larval *A. ricordi* and *A. roberti* exposed to salinity variations showed compared to *A. miersii*, wider variations in biomass. The larvae of *A. ricordi*, which develop in coastal marine waters (Díaz and Ewald, 1968; Alvarez and Ewald, 1990, Diesel and Schuh, 1998), experience in nature normally lesser variations in salinity than *A. miersii*. This corresponds with a weaker osmoregulatory capacity (Charmantier et al., unpublished data) and greater salinity-induced changes in biomass; especially in the Zoea I (present study). In the natural environment, a direct release of the larvae into coastal waters prevents osmotic stress and reduced growth. In later larval instars, the condition allowing for maximum biomass growth shifted from 32‰ to 25‰. This ontogenetic change in the salinity optimum may be related to the peculiar osmoregulatory patterns in the Megalopa, which reveals a strong hyper-osmoregulatory capacity, accompanied by a shift of the iso-osmotic point from 32‰ to 25‰ (Charmantier et al., unpublished data).

The physical conditions experienced by the larval instars of *A. roberti* vary greatly during ontogeny in the natural environment. The Zoea I faces freshwater after hatching in riverine habitats, while the following zoeal stages develop in estuarine or coastal marine waters, and finally, the Megalopa encounters during its upstream re-immigration brackish or freshwater conditions (Torres et al., 2006; Anger et al., 2006). These changes in the surrounding media experienced by
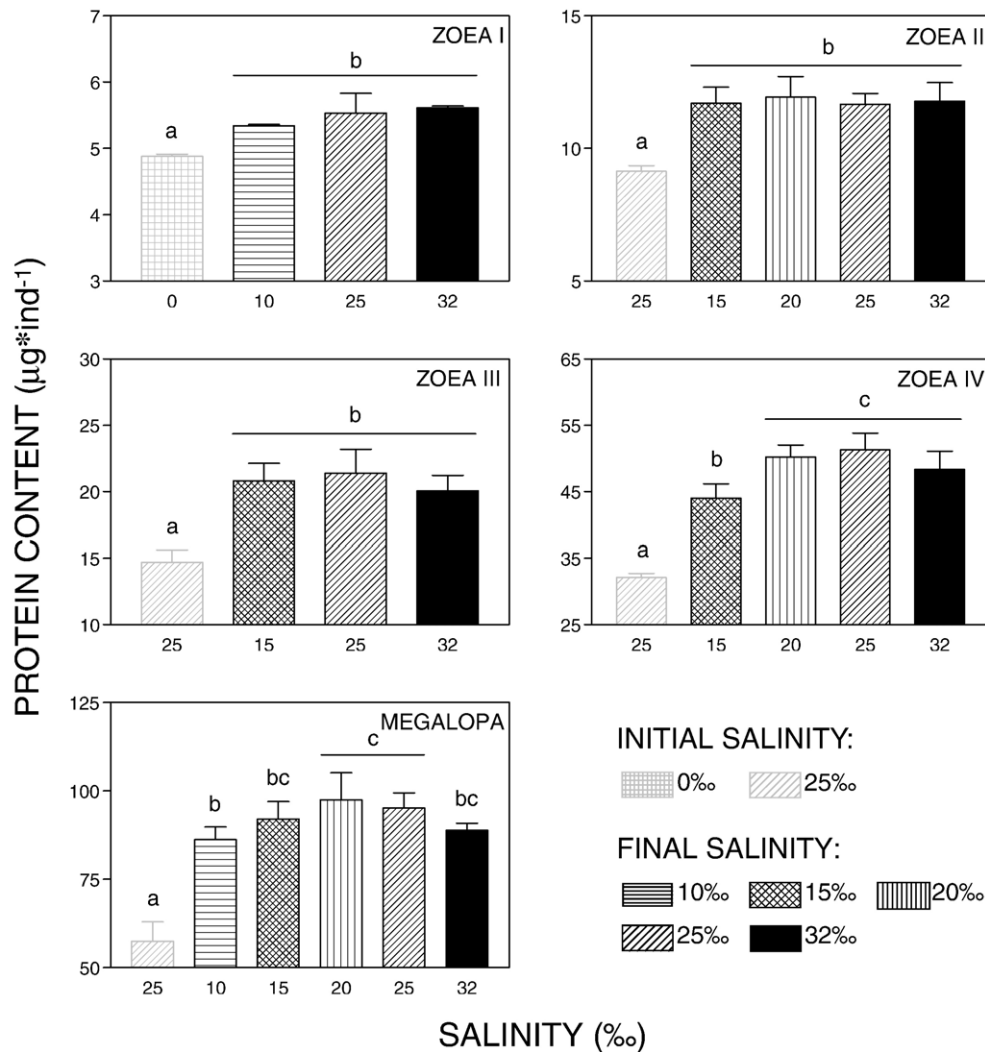
Fig. 9. *Armases roberti*. Changes in protein content (expressed as µg*ind^{-1}) after exposure to 10–32‰. Column drawn in grey: biomass at hatching or early postmoult; columns drawn in black: biomass at intermoult. Different letters show significant differences (after SNK tests) between salinities at hatching and intermoult.

successive larval stages in the field correspond with changes in the ontogenetic pattern of osmoregulation under constant laboratory conditions (Charmantier et al., unpublished data). In agreement with these patterns, the variations of biomass in the Zoea I exposed to salinities between 10‰ and 32‰ were low, while the following zoeal stages showed a stronger response within a narrower salinity range (15‰–32‰).

The euryhaline larval stages of the three species of *Armases* studied here showed smaller salinity-induced changes in biomass than the Zoea I of the stenohaline species *Cancer pagurus* and *Homarus gammarus* (Fig. 11; cf. Torres et al., 2002). Hence, a low sensitivity of growth to salinity variations appears to be related to euryhalinity. Unfavourably low salinity may cause a reduction of the average rate of feeding or of growth efficiency, presumably due to metabolic disadjustments induced by osmotic stress, and such effects should be stronger in osmoconformers or weak osmoregulators as compared to strong osmoregulators. For instance, significant reduction in the

metabolism of the Zoea I stage of *C. maenas* exposed to low salinities was observed to cause reduced net growth efficiency (Anger et al., 1998).

The juvenile stages of the portunid crabs *Callinectes sapidus*, *C. similis* and *Scylla serrata* tolerate a wide range of salinities and their growth is weakly affected by salinity variations in the range 10–40‰ (Guerin and Stickle, 1997; Ruscoe et al. 2004). In contrast, juveniles of another portunid, *Portunus pelagicus*, tolerate a narrower range of salinities, exhibiting also a reduced growth at low salinities (Romano and Zeng 2006). As in the present study, these interspecific differences in the responsiveness of survival and growth to changes in salinity correspond with differences in the species-specific osmoregulatory capabilities (Romano and Zeng 2006). This suggests that effects of salinity variations on juvenile growth are physiologically similar to effects observed in larval stages: strong osmoregulatory capabilities buffer detrimental effects of variable salinity. In summary, our results corroborate our previous prediction that
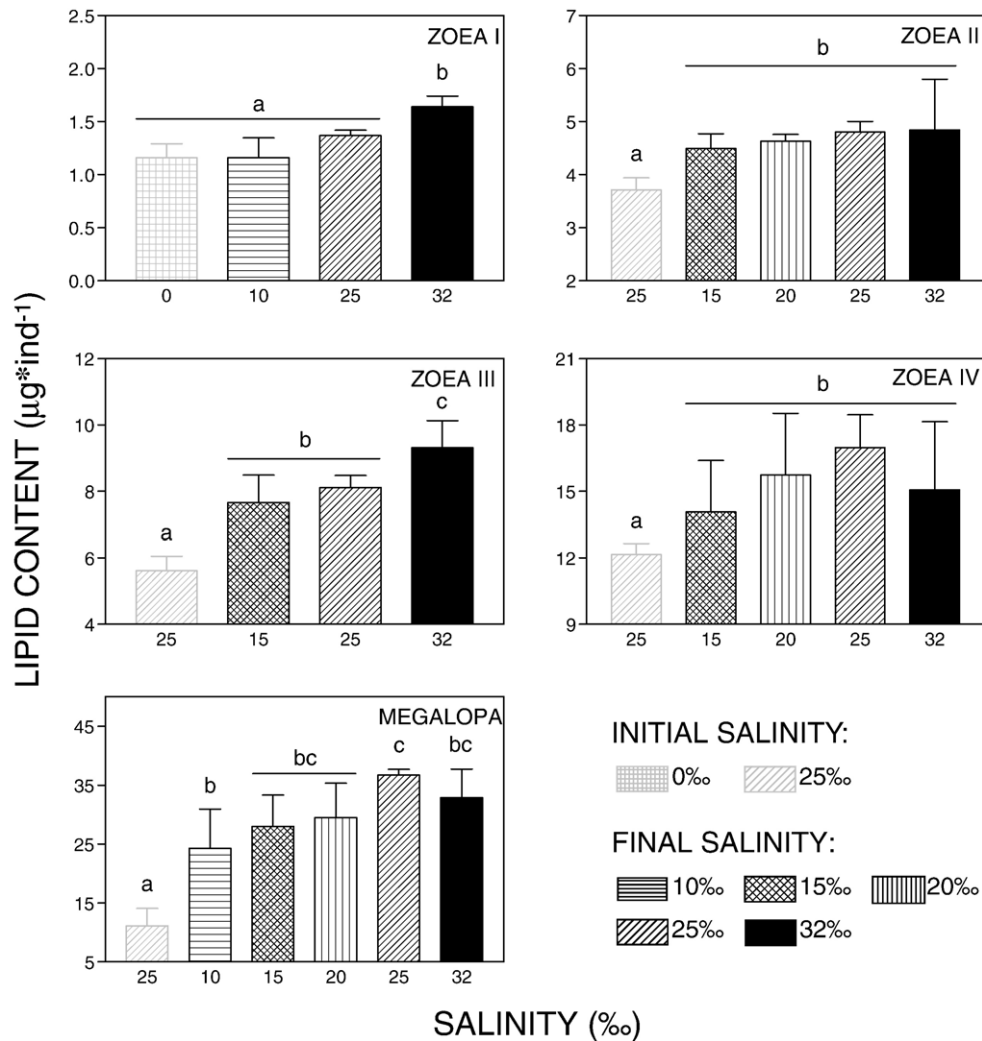
Fig. 10. *Armases roberti*. Changes in lipid content (expressed as $\mu g * ind^{-1}$) after exposure to 10–32‰. Column drawn in grey: biomass at hatching or early postmoult; columns drawn in black: biomass at intermoult. Different letters show significant differences (after SNK tests) between salinities at hatching and intermoult.

ontogenetic changes in the capability of osmoregulation are reflected not only in differential larval survival, but also in responses of growth to reduced salinities.

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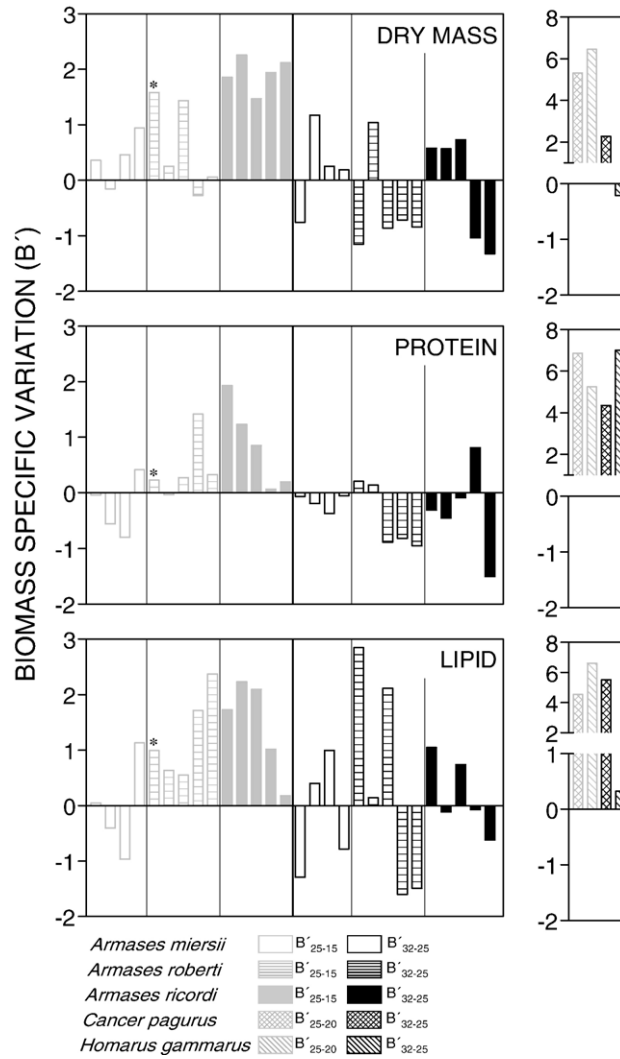
Fig. 11. Biomass-specific variation for each stage and species studied, exposed to different salinities. Variation at the interval 15‰–25‰ or 20‰–25‰: Columns drawn in grey; at the interval 25‰–32‰: columns drawn in black. *Armases miersii* (ZI–M): blank columns; *A. roberti* (ZI–M): horizontally striped columns; *A. ricordi* (ZI–M): full columns; *Cancer pagurus* (ZI): crossed columns; *Homarus gammarus* (ZI): diagonally striped columns. * For Zoea I of *A. roberti*, we calculated B'_{25-10} (i.e. using 10‰), since 15‰ was not measured. Data from *C. pagurus* and *H. gammarus*, after Torres et al. (2002).