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Cumulative effects of low salinity on larval growth and biochemical composition in an estuarine crab, *Neohelice granulata*

Gabriela Torres[1,2,3,*], Luis Giménez[3], Klaus Anger[1]

[1]Biologische Anstalt Helgoland, Foundation Alfred Wegener Institute for Polar and Marine Research, 27498 Helgoland, Germany
[2]AG Zoosystematik & Morphologie, Carl von Ossietzky Universität Oldenburg, 26129 Oldenburg, Germany
[3]School of Ocean Sciences, Bangor University, Menai Bridge, Anglesey LL59 5AB, UK

ABSTRACT: Throughout the larval development of a highly euryhaline crab, *Neohelice* (= *Chasmagnathus*) *granulata*, we studied effects of reduced salinities (15 and 25‰ vs. seawater control at 32‰) on larval dry mass (*W*) and biochemical composition (total lipid and protein). In the early zoeal stages (I, II), reduced salinity caused delayed moulting, while *W* and biochemical composition at ecdysis remained unaffected. This suggests that the capability of the Zoea I for hyper-osmoregulation (demonstrated in a previous study) mitigated potential effects of hypo-osmotic stress on biomass accumulation and biochemical composition, but did not prevent a developmental delay. After continued rearing at low salinities, later larval stages showed increasingly negative effects on growth. The Zoea IV, for instance, revealed at 15‰ significantly reduced *W*, lipid and protein contents compared to higher salinities, probably as a consequence of weak osmoregulatory capabilities in the zoeal stages II and III. Likewise, average daily rates of biomass accumulation (taking into account salinity-induced variations in development duration) were lower at reduced salinities. These negative effects on larval growth persisted throughout the Megalopa, in spite of a strong capability in this stage to hyper-osmoregulate. This indicates that the mitigating force of osmoregulation in the final larval stage was not strong enough to offset cumulative effects of a continuous exposure to osmotic stress lasting from hatching throughout the more stenohaline zoeal stages. Osmoregulation in *N. granulata* has implications for population dynamics in the field, where salinity conditions prevailing during the development in the plankton may influence larval condition and survival at metamorphosis, eventually affecting recruitment.

KEY WORDS: *Neohelice granulata* · *Chasmagnathus granulata* · Biochemical composition · Cumulative osmotic stress · Growth · Lipid · Protein

INTRODUCTION

Benthic decapod crustaceans such as crabs and lobsters develop through complex life cycles comprising a series of planktonic larval instars. This life-history pattern involves dramatic ontogenetic changes in morphology and physiology, which are also influenced by variations in environmental conditions. In the case of coastal and estuarine crustaceans, reduced salinities affect larval metabolism, growth and survival (Anger 2001, 2003). The strength of such effects depends on physiological adaptations, in particular on species-specific capabilities of osmoregulation. In fully marine species, whose larvae are stenohaline and osmoconforming, even moderately reduced salinities affect the rate of biomass accumulation, while larvae of euryhaline, osmoregulating species reveal significantly weaker responses to salinity variation (Torres et al. 2002, 2007b). Besides interspecific differences, some species show ontogenetic changes in their osmoregulatory ca-

*Email: g.torres@bangor.ac.uk

pacity and salinity tolerance (Charmantier 1998, Charmantier et al. 2002, Torres et al. 2007b). We may therefore expect that stenohaline, osmoconforming larval stages of such species should exhibit a stronger response to salinity variation than other, more euryhaline and osmoregulating stages of the same species.

Larval development and growth are not only affected by present salinity conditions, but also by previous experience (Giménez & Torres 2002, Giménez & Anger 2003, Giménez et al. 2004). Such effects of previous history should depend on ontogenetic changes in salinity tolerance and osmoregulatory capacity. For instance, a physiologically strong larval stage may have poor growth, reflecting cumulative effects of continued osmotic stress during previous (physiologically weaker) developmental stages. On the other hand, a weak (i.e. osmoconforming) larval stage may benefit from osmoregulatory capabilities in previous stages, so that it may appear little affected by osmotic stress.

In order to test this hypothesis, we studied sublethal effects of continued osmotic stress on larval biomass in a species with both osmoconforming and osmoregulating larval instars, namely the South American salt-marsh crab *Neohelice* (= *Chasmagnathus*) *granulata* (Dana, 1851; for recent taxonomic revision, see Sakai et al. 2006). In nature, the larvae of this species hatch in estuarine waters, from where they are rapidly transported towards osmotically more stable coastal marine environments (Anger et al. 1994). While the Zoea I stage reveals a moderate capability of hyper-osmoregulation at reduced salinities, the following zoeal stages (II to IV) lose this function and become stenohaline osmoconformers; a conspicuous capability of hyper-osmoregulation reappears in the Megalopa stage, increasing throughout subsequent juvenile development (Charmantier et al. 2002). Hence, the first and the last larval stage of this species are physiologically prepared to hatch and to settle, respectively, in oligohaline and occasionally limnic habitats where the benthic juveniles and adults live (Luppi et al. 2002).

Under continued conditions of reduced salinity, we may therefore expect that: (1) due to the hyper-osmoregulatory ability of the Zoea I stage, cumulative stress effects should still be weak or absent in Zoea II, although this stage is a stenohaline osmoconformer; (2) continued osmotic stress should increasingly affect the osmoconforming Zoea III and IV; (3) this condition of continuously reduced salinity should eventually also affect the Megalopa, although this stage is capable of osmoregulation.

MATERIALS AND METHODS

Handling of ovigerous females and obtaining of larvae. Larvae of *Neohelice granulata* were obtained from ovigerous females maintained since 1990 at the Helgoland Marine Biological Station (BAH, Helgoland, Germany) under controlled conditions of temperature (21°C), salinity (32‰), photoperiod (12:12 h dark:light) and food (ad libitum feeding with isopods *Idotea* sp.). This laboratory population was established with animals captured in the Mar Chiquita lagoon, Argentina (1990), and later complemented with specimens from Mar Chiquita (1997) and from the Solís Chico River, Uruguay (2006). When females laid eggs, they were isolated in individual aquaria and kept under otherwise identical conditions.

Laboratory experiments. Freshly hatched larvae were assigned to 3 groups reared at 15, 25 and 32‰ (Fig. 1), 18°C and a photoperiod of 12:12 h, with freshly hatched *Artemia* sp. nauplii provided ad libitum as food. Salinities of 15 and 25‰ were obtained by mixing filtered seawater (Orion, pore size = 1 µm), with appropriate amounts of deionized tap water. These treatments represent conditions of severe, moderate and no hypo-osmotic stress (control condition), respectively. Water and food were changed daily. Cultures were controlled daily for moulted or dead larvae.

A total of 70 Zoea I were group-reared in 5 replicate 400 ml bowls without aeration, until 50% of the moulting cycle (intermoult) was reached. For the production of subsequent stages, larvae were mass-reared with slight aeration in 5 l bottles (approximate density: 200 ind. l^{-1}). Early postmoult larvae were separated from the cultures and reared in 400 ml bowls until intermoult, under identical conditions as the Zoea I. Thus, each set of experiments was performed with individuals having the same moulting history (Table 1). The stocking density decreased in successive larval instars (70, 50, 40, 30 and 20 $bowl^{-1}$, respectively).

Samples of larvae were taken for biochemical analyses at early postmoult (<24 h after hatching of Zoea I or

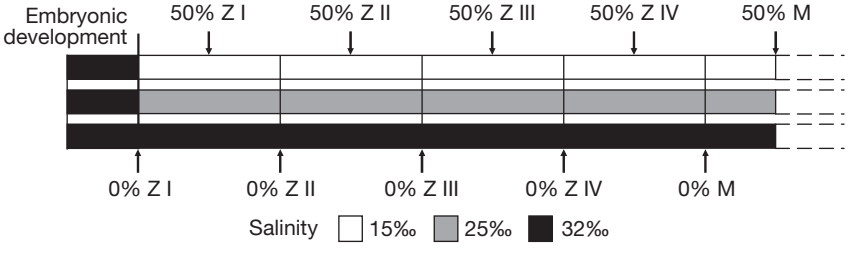

Fig. 1. Experimental design to study cumulative effects of salinity on dry mass and biochemical composition during larval development in *Neohelice granulata*. Postmoult and intermoult samples (arrows) were taken shortly after moulting (0%) and at 50% of the moult cycle, respectively

Table 1. *Neohelice granulata*. Cumulative duration (d) of larval development from hatching to early postmoult (PM) and intermoult (IM) of successive stages (different females were used for experiments with different stages)

Salinity	Moult stage	Zoea II	Zoea III	Zoea IV	Megalopa
15	PM	8	12	19	27
	IM	11	15	23	31
25	PM	6	10	17	23
	IM	9	13	21	27
32	PM	6	10	17	23
	IM	9	13	21	27

moulting to later stages, respectively) and again at intermoult (after ca. 50% of each moulting cycle).

Biochemical analyses. Samples for biochemical analyses were gently rinsed in distilled water for 10 s and blotted on filter paper. Then they were transferred to an Eppendorf vial and frozen at –80°C. These 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, placed in ice, and each homogenate was divided in 2 aliquots to perform lipid and protein determinations.

The lipid content of the homogenate was determined after the sulphophosphovanillin method following Zöllner & Kirsch (1962), modified for microplates (Torres et al. 2007b). The protein content of the homogenate was determined using a modified method after Lowry et al. (1951; kit: BioRad D_C Protein Assay), adapted for microplates (Torres et al. 2007a).

Data analyses. We expected 2 types of effects of salinity: (1) a stage-dependent change, i.e. the effect on biomass observed at a particular developmental stage (e.g. Zoea II, intermoult) and (2) an effect on the rate of biomass accumulation per unit of time, due to combined effects on the time of development and biomass reached. The stage-dependent change was evaluated using larval dry mass (W) and biochemical composition (protein, lipid) per individual as response variable. The effect of salinity on biomass accumulation rates was evaluated using the biomass per individual reached at a given developmental stage, divided by the cumulative number of days required from hatching to reach the time of sampling (see Table 1).

Statistical analyses were performed following Zar (1996). All datasets were analysed separately with ANOVA for each larval instar, because each instar originated from a different female. We were not able to conduct all experiments with larvae from 1 hatch, as a large number of larvae was required for the biochemical methods. Zoea I data were analysed with a 1-way ANOVA, with salinity (15, 25 and 32‰) as factor; planned comparisons were made in order to test for significant growth from hatching to intermoult. Data from Zoea II to Megalopa were analysed with a 2-way ANOVA, with salinity and moult-stage (postmoult and intermoult) as factors. The number of replicates was 4 or 5 for each stage–salinity combination.

Comparisons between different factors, after finding significant differences by ANOVA, were performed with the Student-Newman-Keuls test (SNK). The critical level α to reject the null hypothesis was 0.05. Before performing ANOVAs, normality and variance homogeneity (normal plots and Cochran's C-test, respectively) were checked. In case of failing to meet the assumptions (i.e. variance homogeneity), data were logarithmically transformed. On a few occasions, variances were not homogeneous, even after data transformation. In these cases, differences were significant even after we reduced the critical level below the level of significance of variance heterogeneity.

RESULTS

In all larval instars, there was always a significant increase in W, lipid and protein content per individual between early postmoult and intermoult (Table 2). Rates of biomass accumulation from postmoult to intermoult increased significantly in most successive instars (Table 2).

Zoea I

W and protein content per individual measured at intermoult were not affected by salinity. However, larvae exposed to 15‰ showed a significantly lower lipid content than those at 25 and 32‰ (Table 2, Fig. 2B).

Zoea II

In Zoea II, W per individual was, both at postmoult and at intermoult, significantly lower at 15 than at 32‰, while intermediate values were measured at 25‰ (Table 2, Fig. 3A). The effect of salinity on dry mass increased from postmoult to intermoult. No significant effects were detected on the lipid content (Fig. 3B). The protein content was consistently higher at 15‰ than at the other test salinities (Fig. 3C). Although this pattern appeared to be clearer at intermoult than at early postmoult, the interaction term Salinity × Moult stage (Table 2) was not significant.

Biomass accumulation rates were significantly affected by salinity, with lower levels in W and lipid

Table 2. 2-way ANOVA for dry mass, lipid and protein contents per individual *Neohelice granulata* (log-transformed data) and accumulation rate in relation to salinity and moulting stage (postmoult, intermoult) in successive larval instars. MS: mean squares; significant effects are in **bold**

	Dry mass			Lipid			Protein		
	MS	*F*	p	MS	*F*	p	MS	*F*	p
Content per individual									
Zoea I									
Salinity (S)	0.012	3.65	0.058	0.053	15.13	**<0.0006**	0.012	2.4	0.13
Error	0.003			0.003			0.005		
Zoea II									
Salinity (S)	0.010	5.9	**0.0087**	0.019	2.60	0.096	0.009	8.3	**<0.002**
Moult-stage (M)	0.122	68.7	**<0.0001**	0.044	5.84	**0.024**	0.115	101.3	**<0.0001**
S × M	0.005	2.7	0.085	0.002	0.34	0.717	0.002	2.0	0.16
Error	0.002			0.007			0.001		
Zoea III									
Salinity (S)	0.0027	2.6	0.10	0.016	14.8	**<0.0001**	0.0134	27.1	**<0.0001**
Moult-stage (M)	0.1165	112.8	**0.0001**	0.216	202.9	**<0.0001**	0.1791	363.3	**<0.0001**
S × M	0.0005	0.5	0.59	0.002	2.2	0.13	0.0174	35.2	**<0.0001**
Error	0.0010			0.001			0.0005		
Zoea IV									
Salinity (S)	0.0479	129	**<0.0001**	0.032	31.8	**<0.0001**	0.0124	9.0	**<0.002**
Moult-stage (M)	0.2011	541	**<0.0001**	0.095	94.6	**<0.0001**	0.4758	345.9	**<0.0001**
S × M	0.0001	<0.1	0.83	0.004	4.0	**0.032**	0.0237	17.2	**<0.0001**
Error	0.0004			0.001			0.0014		
Megalopa									
Salinity (S)	749.7493	977445	**<0.0001**	0.0209	15.4	**<0.0001**	0.0274	17.7	**<0.0001**
Moult-stage (M)	0.0258	33	**<0.0001**	1.3208	969.4	**<0.0001**	0.1288	83.2	**<0.0001**
S × M	0.2220	289	**<0.0001**	0.0613	45.0	**<0.0001**	0.0076	4.9	**<0.02**
Error	0.0056			0.0014			0.0015		
Accumulation rate									
Zoea II									
Salinity (S)	2.43	42.48	**<0.0001**	0.0082	11.87	**<0.0003**	0.054	12.62	**0.0002**
Moult-stage (M)	0.26	4.61	**0.042**	0.0058	8.42	**0.008**	0.044	10.42	**<0.004**
S × M	0.19	3.37	0.052	0.0012	1.68	0.21	0.029	6.61	**<0.005**
Error	0.06			0.0007			0.004		
Zoea III									
Salinity (S)	2.09	30.24	**<0.0001**	0.016	56.30	**<0.0001**	0.37	86.16	**<0.0001**
Moult-stage (M)	0.15	2.30	0.14	0.008	29.50	**<0.0001**	0.17	40.40	**<0.0001**
S × M	0.03	0.39	0.68	0.0006	2.16	0.14	0.09	23.37	**<0.0001**
Error	0.07			0.0003			0.004		
Zoea IV									
Salinity (S)	9.91	217.97	**<0.0001**	0.048	45.25	**<0.0001**	0.58	25.23	**<0.0001**
Moult-stage (M)	5.55	122.03	**<0.0001**	0.003	3.04	0.09	3.52	152.25	**<0.0001**
S × M	0.04	0.96	0.391	0.003	3.29	0.06	0.41	17.88	**<0.0001**
Error	0.04			0.001			0.02		
Megalopa									
Salinity (S)	17.90	64.47	**<0.0001**	0.069	35.40	**<0.0001**	1.14	17.31	**<0.0001**
Moult-stage (M)	26.79	96.49	**<0.0001**	0.712	365.22	**<0.0001**	1.36	20.68	**<0.0002**
S × M	2.19	7.91	**0.0025**	0.066	33.86	**<0.0001**	0.22	3.31	0.054
Error	0.28			0.002			0.06		

growth at the lowest salinity; this effect was observed both at postmoult and intermoult (Fig. 3D,E, Table 2). For the protein content, this pattern was found only at postmoult (Table 2, Fig. 3F).

Zoea III

No effects of salinity were found in *W* (Table 2, Fig. 4A). For the lipid fraction, the growth-reducing effect of low salinity (15‰) was similar in early postmoult and intermoult, with lower values (lipid content ind^{-1}) in larvae exposed to 15 and 25‰ as compared to those kept at 32‰. The effect of salinity on the protein content depended on the moult stage, with no significant effects observed at early postmoult, but reduced protein levels at intermoult in larvae exposed to 15‰ (Fig. 4C).

Significant effects of salinity were found in the accumulation rates of *W*, lipid and protein at both early postmoult and intermoult, with lower levels in larvae maintained at 15‰ (Table 2, Fig. 4D to F). In addition,

larvae exposed to 25‰ also showed a significantly lower lipid accumulation rate than those at 32‰. The effect on the rate of accumulation of proteins was stronger at intermoult than at early postmoult.

Zoea IV

Continuous exposure to reduced salinities affected *W* as well as the lipid and protein contents, depending also on moult stage (Fig. 5A to C, Table 2). At early postmoult, *W* and the lipid content were lower at 15‰ compared to the other test salinities. By contrast, the postmoult protein content was significantly higher at 15 and 25‰ compared to 32‰. At intermoult, larvae exposed to 15‰ showed significantly lower *W*, lipid and protein contents compared to higher salinities (Fig. 5A to C). At 25‰, the larvae showed a significantly lower intermoult lipid content than those at 32‰.

Zoea IV showed reduced daily accumulation rates of *W* and lipids at 15‰, both at postmoult and intermoult (Fig. 5D to F, Table 2). The accumulation rate of proteins in larvae exposed to 15‰ was reduced only at intermoult. At early postmoult, accumulation rates differed only slightly among salinity treatments, with a maximum at 25‰.

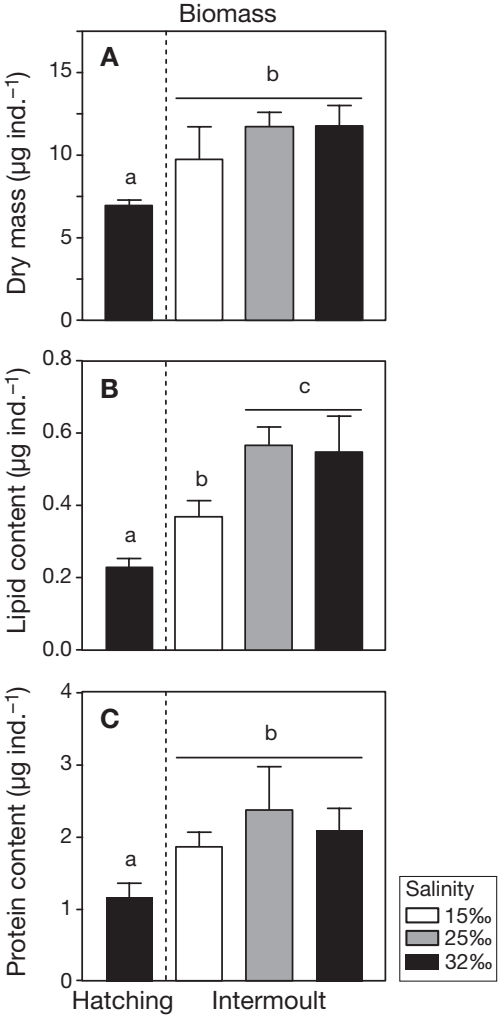

Fig. 2. *Neohelice granulata* Zoea I. Changes in biomass after exposure to 15, 25 and 32‰. Different lower case letters show significant differences between salinities (Student-Newman-Keuls test)

Megalopa

Effects of salinity on *W*, lipid and protein contents depended on the moulting stage (Fig. 6A to C, Table 2). Continuous exposure to 15‰ caused a decreased *W*, both at early postmoult and at intermoult, compared to larvae maintained at 32‰. *W* at postmoult was also lower at 25‰ than at 32‰. At postmoult, the lipid and protein contents were slightly lower at 32‰ than at the other test salinities. At intermoult, 15‰ caused very low lipid content, but it did not significantly affect the protein level.

The effects of salinity on daily accumulation rates of *W* and lipid content also depended on moult stage (Table 2, Fig. 6D to F). For *W*, the growth rates were lower at 15‰ than at any other salinity, both at postmoult and intermoult. Accumulation rates of lipids were, at early postmoult, slightly affected by salinity, with a maximum at 25‰. At intermoult, they were significantly reduced only at 15‰. The protein accumulation rate was significantly higher at 25‰ than at the other salinities, regardless of the stage in the moulting cycle.

Lipid–protein relationships

Overall patterns in the lipid and protein data are shown in Fig. 7 as a lipid–protein plot. Cumulative effects of low salinities on the lipid and protein contents were conspicuous at intermoult in Zoea IV and Megalopa stages (Fig. 7A). Within a developmental phase or stage (circled groups of values), shifts to the left or upward indicate low lipid:protein ratios (L:P), while plot symbols in the right or lower part of a cluster indicate high L:P values. The L:P ratios at intermoult of the Megalopa were lower at 15‰ (0.12) compared to those at higher salinities (0.18 to 0.21). The same pattern was found in Zoea IV at postmoult (15‰: 0.21; 32‰: 0.41). However, the same stage showed at intermoult a higher L:P at 15‰ (0.18) than at 25 and 32‰ (0.10 to 0.11).

Effects of salinity were also visible at intermoult in Zoea II and III (Fig. 7B). In Zoea II, there was a consis-

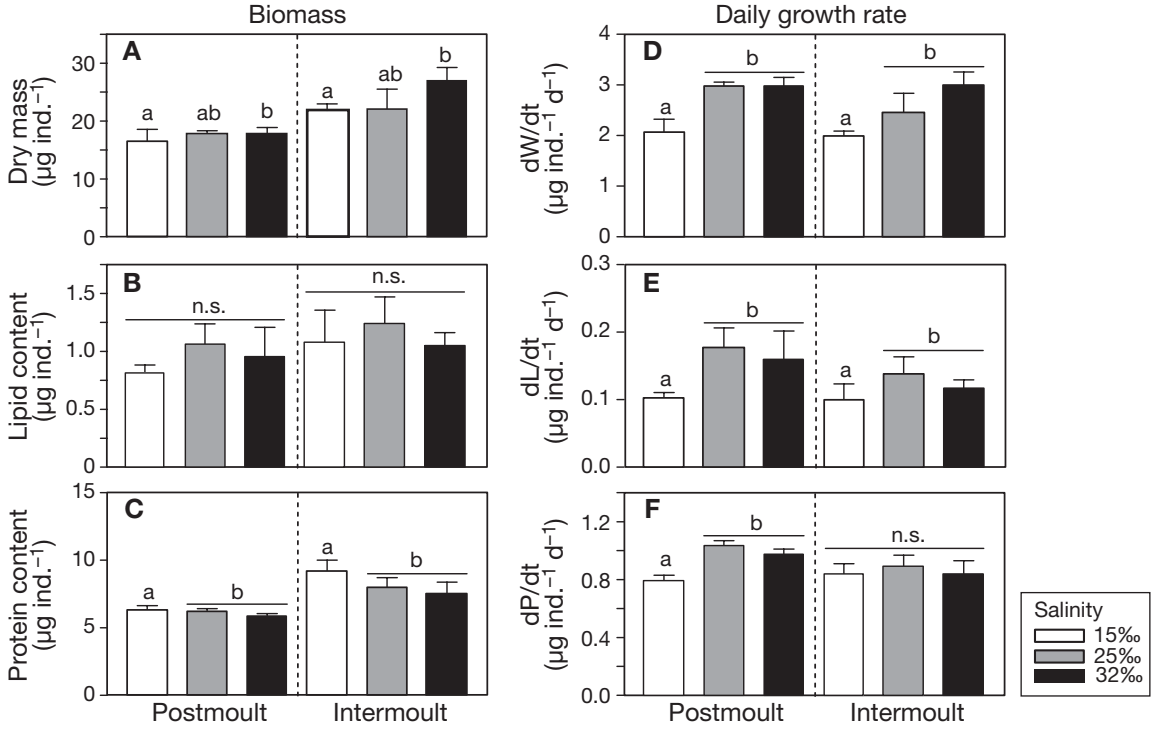

Fig. 3. *Neohelice granulata* Zoea II. (A to C) Changes in biomass after continuous exposure to 15, 25 and 32‰. (D to F) Changes in accumulation rates. Different lower case letters show significant differences between salinities (SNK test), separately for postmoult and intermoult

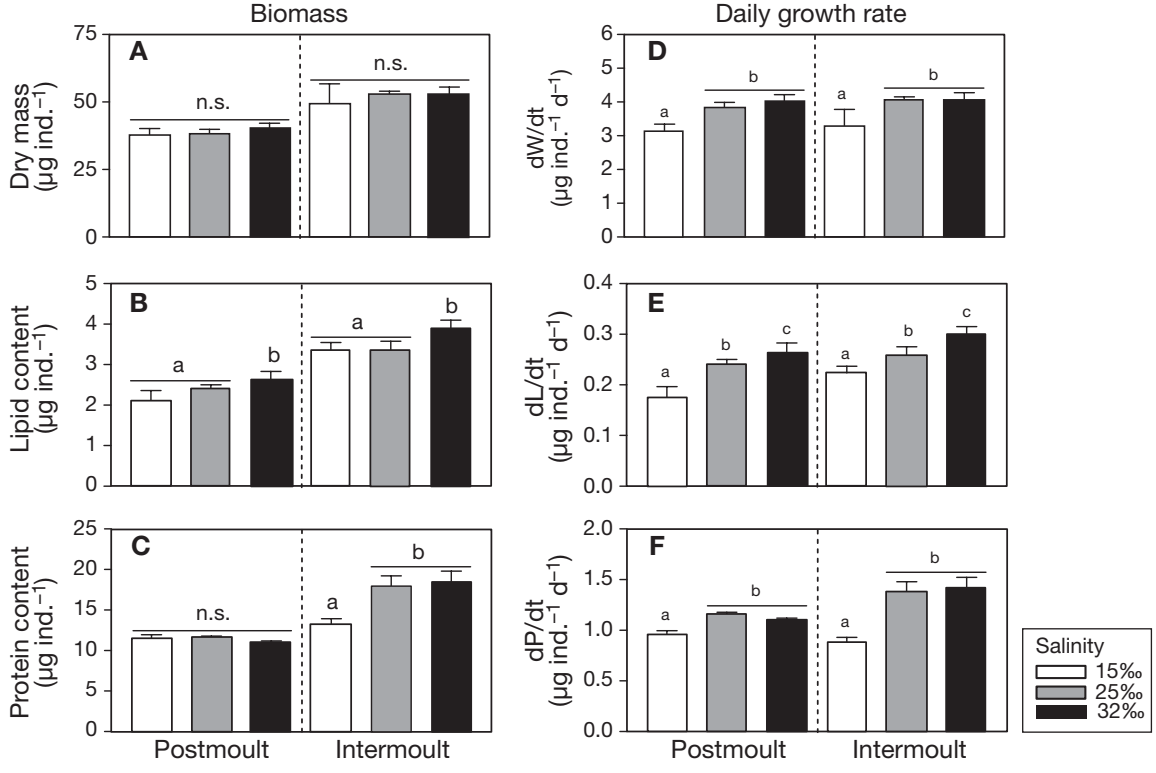

Fig. 4. *Neohelice granulata* Zoea III. (A to C) Changes in biomass after continuous exposure to 15, 25 and 32‰. (D to F) Changes in accumulation rates. Symbols as in Fig. 3

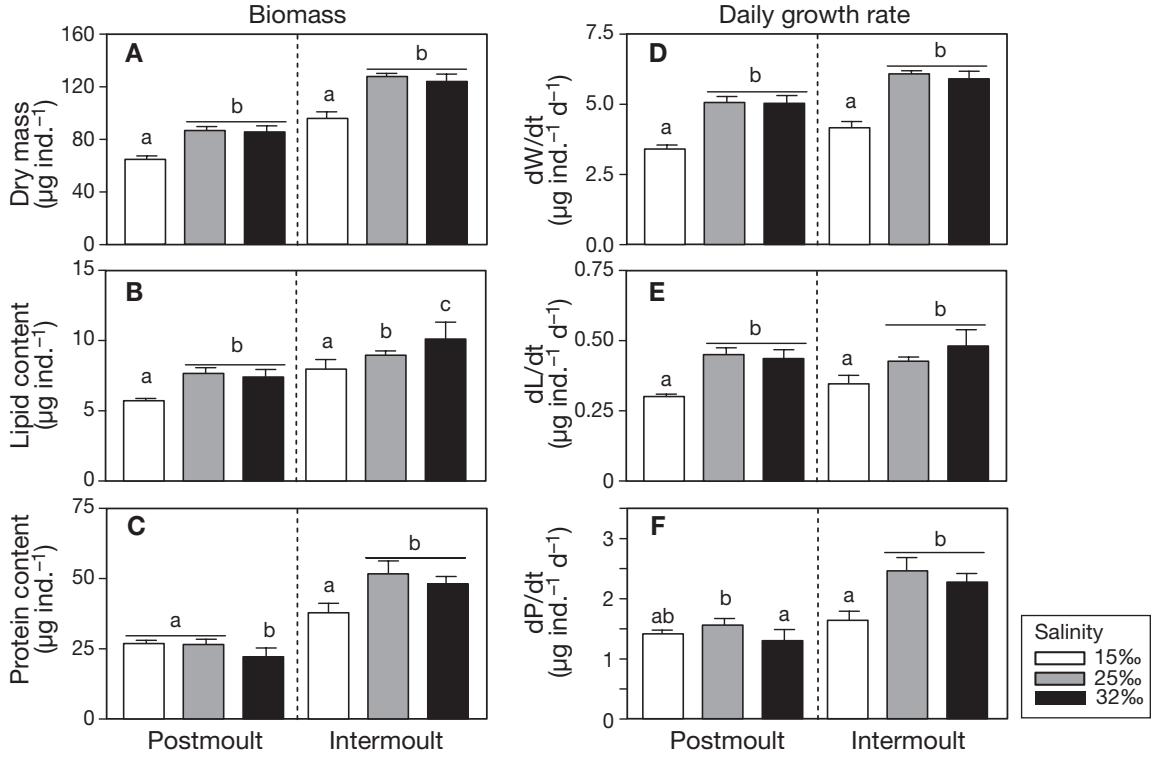

Fig. 5. *Neohelice granulata* Zoea IV. (A to C) Changes in biomass after continuous exposure to 15, 25 and 32‰. (D to F) Changes in accumulation rates. Symbols as in Fig. 3

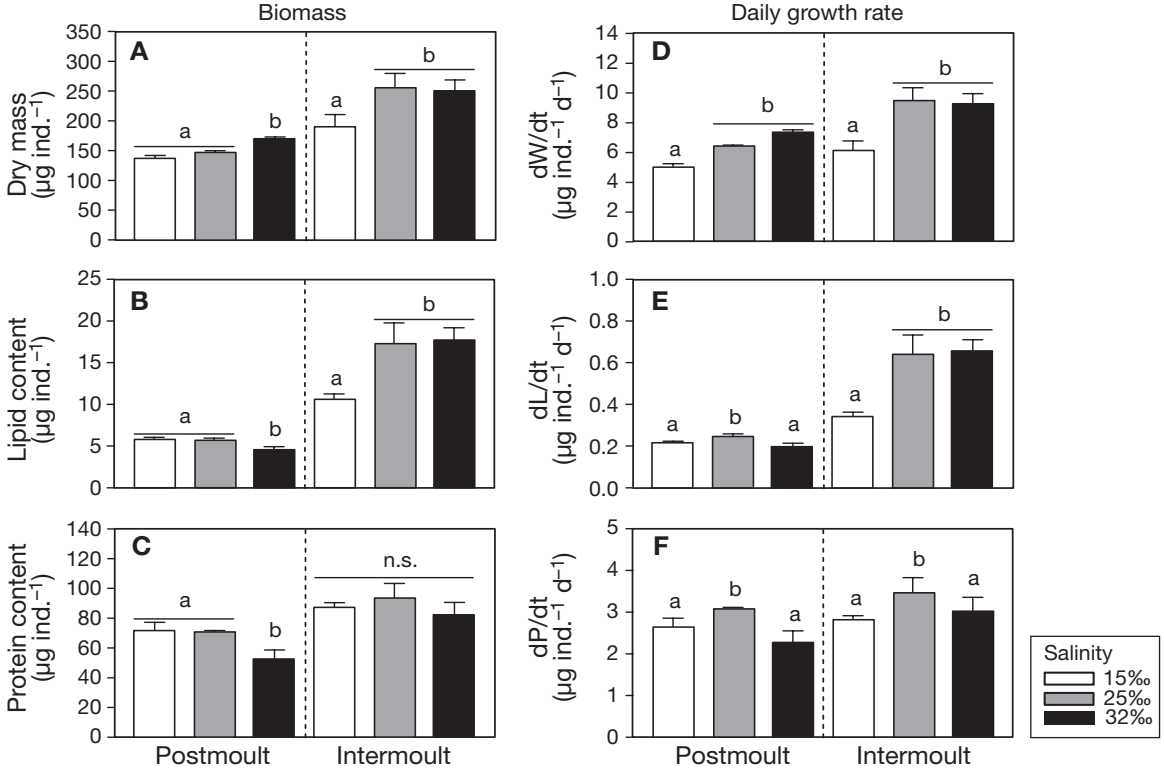

Fig. 6. *Neohelice granulata* Megalopa. (A to C) Changes in biomass after continuous exposure to 15, 25 and 32‰. (D to F) Changes in accumulation rates. Symbols as in Fig. 3

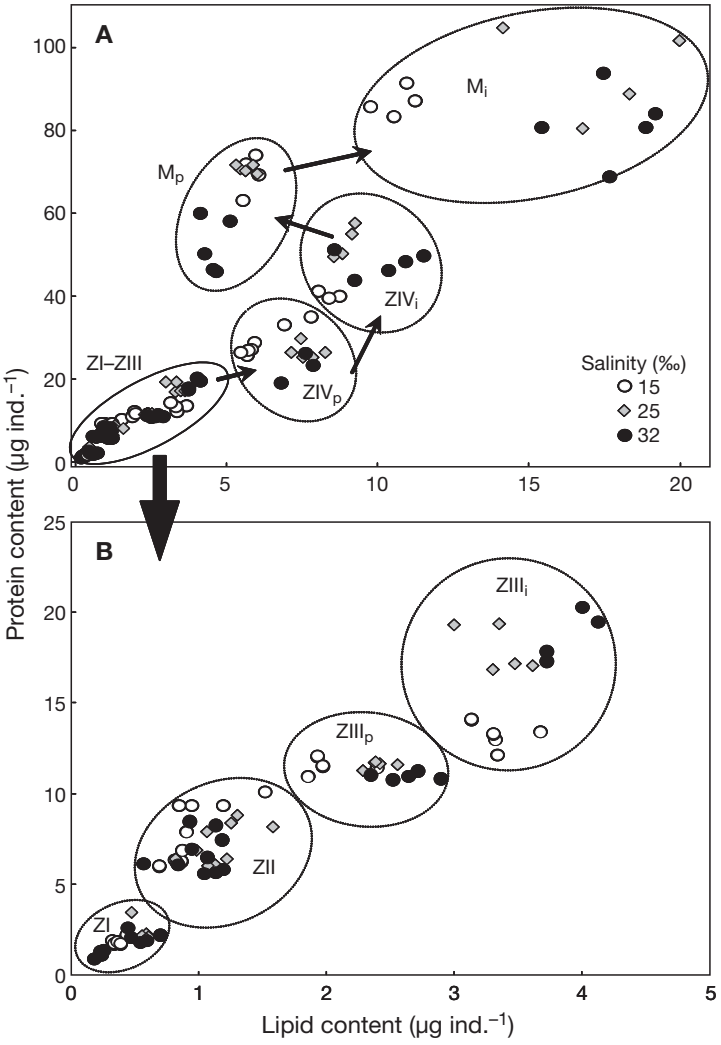

Fig. 7. *Neohelice granulata*. Plot of lipid versus protein content (µg ind.$^{-1}$), showing overall salinity effects throughout larval development (arrows show successive stages): (A) all data and (B) detail of Panel A, showing the early larval stages. ZI to ZIV: zoeal stages; M: Megalopa; subscripts p and i: postmoult and intermoult, respectively

tent reduction of the L:P ratio at 15‰ compared to that at the other salinities, both at early postmoult (0.13 vs. 0.16 to 0.17) and at intermoult (0.11 vs. 0.14 to 0.15). In Zoea III the L:P ratio showed no consistent response to salinity. In early postmoult, it was lower at 15‰ than at 32‰ (0.19 vs. 0.32); however, at intermoult, the low protein content at 15‰ (Fig. 7B) reversed the pattern in L:P ratios (0.25 vs. 0.18).

DISCUSSION

Our data show that salinity may affect biomass (measured as *W*, protein and lipid content per individual) and average daily growth rates. The latter reflect effects on both the biomass reached at a particular developmental stage and the duration of a developmental period. As in previous experiments (Giménez & Anger 2003), larvae kept at 15‰ revealed a delayed development and a reduced average daily accumulation of biomass. At this low salinity, the larvae also reached lower maximal *W* and lipid contents, compared to siblings reared at high salinity (32‰). The differences in maximum biomass were small in the early zoeal instars (6 to 7% in *W*, 10 to 20% in the lipid content), but they increased after continued exposure to 15‰ in Zoea IV and the Megalopa to 20–25% in *W* and to 20–40% in the lipid fraction. Reduced *W* and lipid contents at low salinities are consistent with previous investigations on early decapod larvae (Torres et al. 2002, 2007b). Lower *W* is caused by reduced accumulation of both organic and inorganic substances (Anger 2001). Within the organic larval biomass, osmotic stress affects, in particular, the accumulation of lipids.

Effects of reduced salinity (15‰ vs. 32‰) on larval biomass were small or absent in the early zoeal instars (I and II), but increased in later instars. Effects on the average daily growth rates, by contrast, became conspicuous already in early postmoult Zoea II larvae, with reduced accumulation of *W*, lipids and proteins at 15‰. In this larval stage, moulting was delayed by low salinity, while little change occurred in biochemical composition. We conclude that these generally weak effects on the biomass of Zoea II were due to the previously strong hyper-osmoregulating capability during the Zoea I stage (see Charmantier et al. 2002, Torres et al. 2002).

From the Zoea III stage, continued hypo-osmotic stress caused not only an increasing delay in the timing of successive moults, but also reduced maximal biomass and altered biochemical composition (Fig. 7). This increase in stress effects is consistent with poor osmoregulatory capabilities in Zoea II to IV (Charmantier et al. 2002) and with the observation that an exposure of osmoconforming instars to low salinity causes reduced larval biomass (Torres et al. 2002, 2007b).

In the Megalopa, reduced biomass values were found after continued exposure to 15‰, although this stage is a fairly strong osmoregulator (Charmantier et al. 2002). This indicates that its osmoregulatory capacity did not suffice to compensate previous cumulative effects of low salinity on larval growth and biochemical composition. This may also explain enhanced megalopal mortality after previous zoeal exposure to 15‰ (Giménez & Anger 2003). Moreover, continuous salinity stress may lead to prolonged developmental pathways including an additional zoeal stage (Giménez & Torres 2002), and reduced larval fitness may tran-

scend through metamorphosis into the benthic phase, leading to carry-over effects with reduced benthic juvenile survival and growth (Giménez et al. 2004, Giménez 2006). Together, such cumulative effects of continued osmotic stress during the period of larval development should act as a physiological barrier that impedes larval retention in upper estuaries or brackish coastal lagoons, where the adults commonly live under oligohaline conditions. This barrier should therefore select for larval export towards the sea (Anger et al. 1994, Giménez 2003). However, retention of the larvae in lower estuarine waters with moderately reduced salinities, for instance in the Bay of Sanborombón on the southern shore of the Rio de la Plata estuary (Argentina), is certainly possible, especially after gradual acclimation to slowly decreasing salinities (Anger et al. 2008). On the other hand, there are also coastal marine populations of *Neohelice granulata*, which are not normally exposed to reduced salinities (Bas et al. 2005, 2007). Future comparative investigations may show if genetically isolated metapopulations (see Giménez 2003) differ significantly in their larval salinity tolerance and ontogeny of osmoregulation, i.e. in their adaptability to estuarine conditions. Altogether, *N. granulata* is a suitable model for studies of ecophysiological limits and mechanisms associated with evolutionary transitions of marine invertebrates towards brackish and limnic environments.

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LITERATURE CITED

Anger K (2001) The biology of decapod crustacean larvae. Crustacean Issues, Vol 14. Balkema, Lisse

Anger K (2003) Salinity as a key parameter in the larval biology of decapod crustaceans. Invertebr Reprod Dev 43: 29–45

Anger K, Spivak E, Bas C, Ismael D, Luppi T (1994) Hatching rhythms and dispersion of decapod crustacean larvae in a brackish coastal lagoon in Argentina. Helgol Meersunters 48:445–466

Anger K, Spivak E, Luppi T, Bas C, Ismael D (2008) Larval salinity tolerance of the South American salt-marsh crab, *Neohelice* (*Chasmagnathus*) *granulata*: physiological constraints to estuarine retention, export and reimmigration. Helgol Mar Res (in press) doi:10.1007/s10152-007-0076-5

Bas C, Luppi T, Spivak E (2005) Population structure of the South American estuarine crab, *Chasmagnathus granulatus* (Brachyura: Varunidae) near the southern limit of its geographical distribution: comparison with northern populations. Hydrobiologia 537:217–228

Bas C, Spivak ED, Anger K (2007) Seasonal and interpopulational variability in fecundity, egg size, and elemental composition (CHN) of eggs and larvae of a grapsoid crab, *Chasmagnathus granulatus.* Helgol Mar Res 61:225–237

Charmantier G (1998) Ontogeny of osmoregulation in crustaceans: a review. Invertebr Reprod Dev 33:177–190

Charmantier G, Giménez L, Charmantier-Daures M, Anger K (2002) Ontogeny of osmoregulation, physiological plasticity, and larval export strategy in the grapsid crab *Chasmagnathus granulata* (Crustacea, Decapoda). Mar Ecol Prog Ser 229:185–194

Giménez L (2003) Potential effects of physiological plastic responses to salinity on population networks of the estuarine crab *Chasmagnathus granulata.* Helgol Mar Res 56: 265–273

Giménez L (2006) Phenotypic links in complex life cycles: conclusions from studies with decapod crustaceans. Integr Comp Biol 46:615–622

Giménez L, Anger K (2003) Larval performance in an estuarine crab, *Chasmagnathus granulata*, is a consequence of both larval and embryonic experience. Mar Ecol Prog Ser 249:251–264

Giménez L, Torres G (2002) Larval growth in the estuarine crab *Chasmagnathus granulata*: the importance of salinity experienced during embryonic development, and the initial larval biomass. Mar Biol 141:877–885

Giménez L, Anger K, Torres G (2004) Linking life history traits in successive phases of a complex life cycle: effects of larval biomass on early juvenile development in an estuarine crab, *Chasmagnathus granulata.* Oikos 104:570–580

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Luppi TA, Spivak ED, Anger K, Valero JL (2002) Patterns and processes of *Chasmagnathus granulata* and *Cyrtograpsus angulatus* (Brachyura: Grapsidae) recruitment in Mar Chiquita Coastal Lagoon, Argentina. Estuar Coast Shelf Sci 55:287–297

Sakai K, Türkay M, Yang SL (2006) Revision of the *Helice/Chasmagnathus* complex (Crustacea: Decapoda: Brachyura). Abh Senckenb Natforsch Ges 565:1–76

Torres G, Giménez L, Anger K (2002) Effects of reduced salinity on the biochemical composition (lipid, protein) of Zoea 1 decapod crustacean larvae. J Exp Mar Biol Ecol 277:43–60

Torres G, Charmantier-Daures M, Chifflet S, Anger K (2007a) Effects of long-term exposure to different salinities on the location and activity of Na^+-K^+-ATPase in the gills of juvenile mitten crab, *Eriocheir sinensis.* Comp Biochem Physiol A 147:460–465

Torres G, Anger K, Giménez L (2007b) Effects of osmotic stress on crustacean larval growth and protein and lipid levels are related to life-histories: the genus *Armases* as a model. Comp Biochem Physiol B 148:209–224

Zar J (1996) Biostatistical analysis. Prentice Hall, Upper Saddle River, NJ

Zöllner N, Kirsch K (1962) Über die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasmalipoiden) gemeinsamen Sulfophosphovanillin-Reaktion. Z Gesamte Exp Med 135:545–561

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