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Relationships among salinity, egg size, embryonic development, and larval biomass in the estuarine crab *Chasmagnathus granulata* Dana, 1851

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Abstract

We studied interrelationships between initial egg size and biomass, duration of embryogenesis at different salinities, and initial larval biomass in an estuarine crab, *Chasmagnathus granulata*. Ovigerous females were maintained at three different salinities (15‰, 20‰ and 32‰); initial egg size (mean diameter), biomass (dry weight, carbon and nitrogen) as well as changes in egg size, embryonic development duration, and initial larval biomass were measured.

Initial egg size varied significantly among broods from different females maintained under identical environmental conditions. Eggs from females maintained at 15‰ had on average higher biomass and larger diameter. We hypothesise that this is a plastic response to salinity, which may have an adaptive value, i.e. it may increase the survivorship during postembryonic development. The degree of change in egg diameter during the embryonic development depended on salinity: eggs in a late developmental stage were at 15‰ significantly larger and had smaller increment than those incubated at higher salinities. Development duration was longer at 15‰, but this was significant only for the intermediate embryonic stages. Initial larval biomass depended on initial egg size and on biomass loss during embryogenesis. Larvae with high initial biomass originated either from those eggs that had, already from egg laying, a high initial biomass (reflecting individual variability under identical conditions), or from those developing at a high salinity (32‰), where embryonic biomass losses were generally minimum. Our results show that both individual variability in the provisioning of eggs with yolk and the salinity prevailing during the

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embryonic development are important factors causing variability in the initial larval biomass of *C. granulata*, and thus, in early larval survival and growth.

Keywords: *Chasmagnathus granulata*; Embryonic development; Larval biomass

1. Introduction

In most marine invertebrates, the newly laid eggs contain all the energy and reserves for embryonic development (Holland, 1978; Jaeckle, 1995). In species with complex life cycles, larval survival and growth may depend on the energy reserves that remain after hatching (Paschke, 1998; George, 1999). These, in turn, depend on the initial egg reserves and their utilization during embryogenesis. The embryonic development occurs in a variety of modes, e.g. free developing, encapsulated, incubated (reviewed in Sastry, 1983; Levin and Bridges, 1995), and under a particular combination of environmental factors that may affect the embryonic energy budget and hence, larval reserves. In higher decapod crustaceans, the embryos develop in broods that are carried by the females, and therefore, they experience the parental environmental conditions. Female nutritional and reproductive condition (Harrison, 1990; Palacios et al., 1998, 1999), temperature (McLaren et al., 1969; Wear, 1974; Steele and Steele, 1975; Paschke, 1998), and salinity (Crisp and Costlow, 1963; Bas and Spivak, 2000) may affect oogenesis, embryogenesis and larval quality. In heterogeneous environments (e.g. estuaries), these factors may contribute to natural variability in egg and larval characteristics.

In estuarine crabs, salinity may be one of the key factors affecting embryogenesis, energetics, and larval quality. Even if females actively select optimal microhabitats for embryonic development, embryos would experience the natural temporal variability and spatial heterogeneity in salinity that characterise estuarine environments (Kinne, 1964). Furthermore, since it is unlikely that adults migrate from one estuary to another, broods of the same species produced in different estuaries may develop under different environmental conditions. Thus, intra- and interpopulational differences in embryonic and larval quality may arise because of local heterogeneity in the environmental conditions of estuaries inhabited by a species. Interpopulational differences in egg energy content have been found in crustaceans and other invertebrates (Mashiko, 1983; George, 1990; Jaeckle, 1995; Odinetz-Collart and Rabelo, 1996; Wehrtmann and Kattner, 1998). Therefore, in estuarine crabs, the salinity experienced by the embryos and initial egg biomass may affect not only the survival and energy partitioning during embryogenesis, but also the initial larval quality and early larval survival.

Chasmagnathus granulata Dana, 1851, is a grapsid crab occurring in the estuaries of the southwestern Atlantic coast of Brazil, Uruguay and Argentina (Boschi, 1964). Embryonic development occurs in habitats where salinity may show wide fluctuations (Spivak et al., 1994). Effects of salinity on some aspects of the embryonic development of this species have been reported recently by Bas and Spivak (2000). Normal embryonic development occurs at salinities between 12‰ and 40‰; outside these

extremes, the development is arrested. Resistance against salinity stress appears to be linked with the formation of external membranes. Maximum embryonic development time to intermediate stages occurs at $< 24‰$ and $> 32‰$; duration of embryonic development (i.e. from egg laying to hatching) is independent of salinity.

Although egg volume and salinity are inversely related (Bas and Spivak, 2000), there is no information on variability in egg biomass, and how environmental factors and initial egg biomass affect the initial biomass of the larvae. We thus studied relationships among variability in egg size and biomass, salinity, and initial larval biomass.

2. Methods

2.1. Handling of crabs and experimental setup

Juvenile *C. granulata* were collected in Mar Chiquita lagoon, Argentina (37°33′S, 57°20′W), and transported to the Helgoland Marine Station, Germany. They were reared to adulthood with constant temperature (21°C), salinity (32‰) and ad libitum food (shrimps or isopods).

Three groups of ovigerous females were maintained from egg laying until hatching of zoea 1 at salinities of 15‰, 20‰ and 32‰, respectively. Experiments were always run with embryos in vivo, i.e. the brood was not removed from the female. In preliminary experiments, we started always with females that laid eggs at 32‰. The females were checked daily, and on the day of egg laying, they were transferred to aquaria with the experimental salinities. In these experiments, embryonic development at 15‰ was not synchronised, i.e. within one brood, some embryos developed, while others arrested their development and died later, and most females dropped their eggs after a few days. Hence, we tested if eggs laid at 15‰ could survive at this salinity throughout embryonic development. We randomly took juvenile and adult crabs from the aquaria and maintained them at 15‰ until they laid eggs. As a result of this treatment, a higher percentage of embryos survived at 15‰ to the zoea 1. Therefore, our final experimental design consisted of three groups of broods. One was laid and developed thereafter at 15‰; the other two groups were laid at 32‰ and exposed during embryonic development to either 20‰ or 32‰ (Fig. 1).

Immediately after eggs were laid, 20 eggs per brood were removed to measure their size and biomass. Egg size was recorded as the mean egg diameter from two measurements made with a stereo microscope Olympus SZH ($4\times$) equipped with a micrometric eyepiece. Biomass was measured in five replicate samples of 40 eggs each per brood as dry weight (DW), carbon (C) and nitrogen content (N). Samples were rinsed for a few seconds in distilled water, dried on filter paper, transferred to tin cartridges, dried for 48 h in a vacuum drier (Finn-Aqua Lyovac GT2E), weighed on a microbalance (Mettler UMT2, precision: 0.1 μg), and analysed in a Carlo Erba Elemental Analyser (EA 1108). During the course of embryonic development, five eggs per brood were removed every second day for staging and measurements of egg size. Eggs were staged following Bas

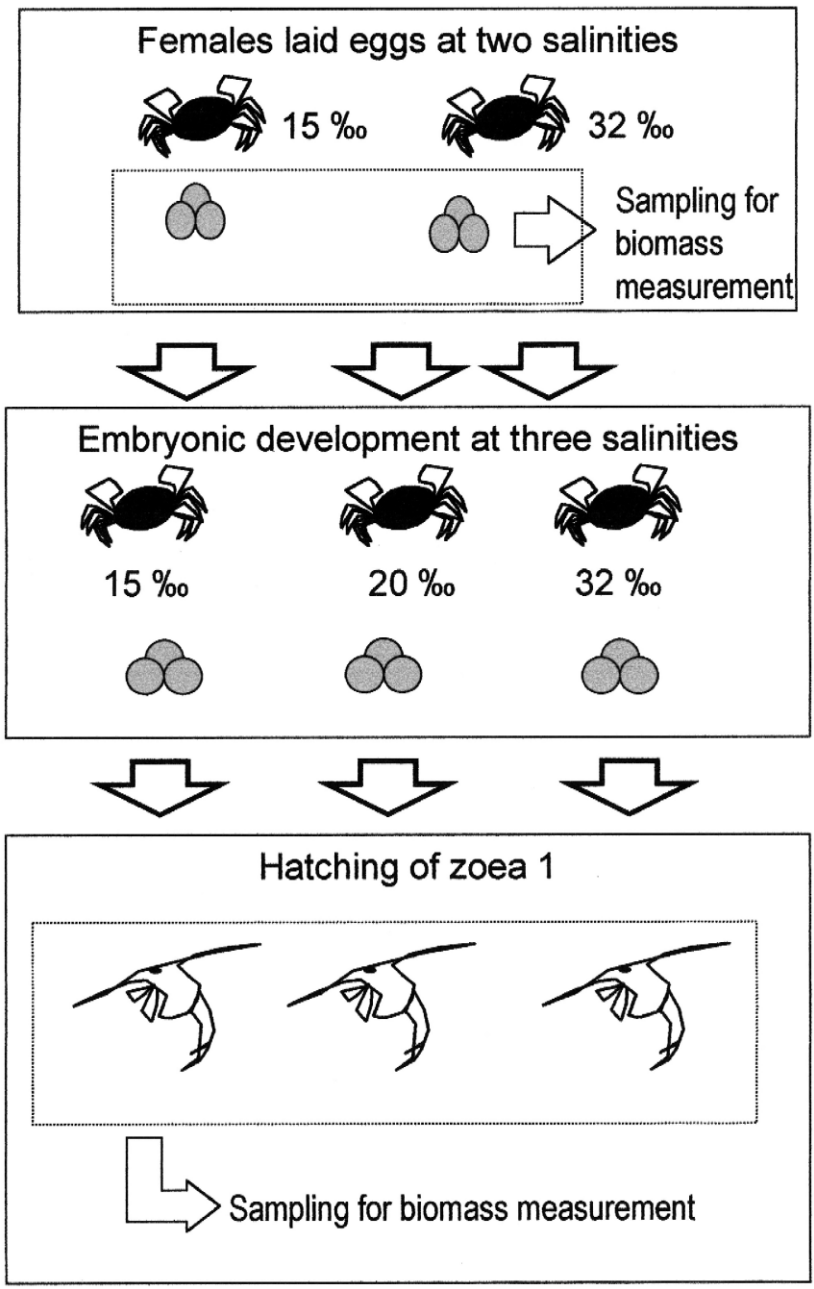

Fig. 1. *C. granulata*. Experimental design to study the effect of prehatching salinity on embryonic development and initial biomass of zoea 1.

Table 1
C. granulata. Stages of embryonic development
Summarised after Bas and Spivak (2000). The stages considered in this study are highlighted.

Stage	Description
1	100% of volume occupied by yolk, only one cell
2	Two white folds appeared at the end
3	Folds join and form a white bend
4	Appendage buds are recognisable
5	Eyes appear as reddish lines
6	Eyes begin to be rounded, 40% occupied by yolk
7	30% occupied by yolk
8	Eyes entirely formed, less than 20% occupied by yolk
9	Yolk largely depleted, embryo ready to hatch

and Spivak (2000), with nine developmental stages (Table 1), and development time to selected stages was measured (Table 1).

2.2. Data analysis

Standard statistical analyses were done following Day and Quinn (1989), Zar (1996) and Underwood (1997). Normality was checked with normal plots and variance heterogeneity with the Cochran test. Differences among treatments after a significant ANOVA were tested with Student–Newman–Keuls (SNK) test. We first evaluated if salinity at egg laying affected initial egg size and biomass. A nested ANOVA was used with two factors: (1) salinity at egg laying with two levels (15‰ and 32‰), and (2) brood, nested in salinity, with 11 broods. The number of replicates per brood was 20 for egg size and 5 for egg biomass. Changes in C in relation to N were evaluated with ANCOVA (N as a covariate) on the mean values of C and N (Packard and Boardmann, 1998). Possible effects of female size on egg size and biomass were investigated with polynomial and linear regressions.

We then evaluated the effect of the salinity during the embryonic development (thereafter called prehatching salinity) on egg size increment, maximum and final egg size, and development duration. For each brood, a linear model, with size depending on time, was adjusted by linear regression, and the slope of the regression line was used as an estimate of size increment per brood. Effects of prehatching salinity on egg size increment and on maximum and final egg size were evaluated with a one-way ANOVA (three levels: 15‰, 20‰ and 32‰; 11 replicates per level). The effect of prehatching salinity on embryonic development time was evaluated with a one-way ANOVA in selected developmental stages (Table 1). Relationships between development time and initial egg biomass were investigated with parametric correlation and linear regression.

Effects of prehatching salinity and the initial egg biomass on mean initial larval biomass were evaluated by repeated-measures ANOVA. The factors were prehatching salinity (15‰, 20‰ and 32‰) and life phase as a repeated-measures factor (egg and

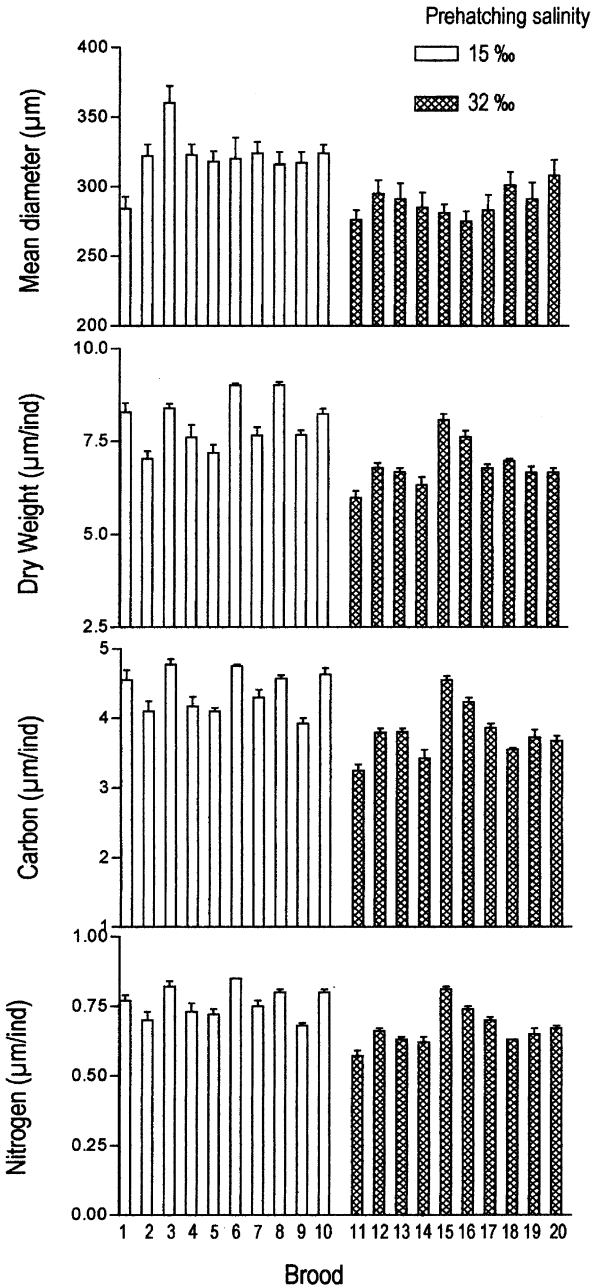

Fig. 2. *C. granulata*. Initial egg size (mean diameter), dry weight, carbon and nitrogen content for eggs laid at different salinities. Error bars: standard deviation.

larva). Changes in C in relation to N were investigated with linear regressions, test of homogeneity of slopes and ANCOVA (N as a covariate).

3. Results

3.1. Initial egg size and biomass

Mean egg size ranged between 270 and 370 μm (Fig. 2). There was a significant variability of egg diameter among broods laid at the same salinity ($F_{18,\ 380} = 48.27$; $p < 10^{-4}$), and a significant effect of salinity at egg laying ($F_{1,\ 18} = 23.32$; $p < 10^{-3}$). Eggs laid at 15‰ were larger than those laid at 32‰. There was a significant nonlinear correlation between egg diameter and female size, but only for eggs laid at 32‰, with a maximum for intermediate female size ($y = 138.90 + 13.98\,x - 0.32\,x^2$; $R^2 = 0.25$; $p < 0.01$).

Initial biomass ranged between 5.70 and 9.10 μg DW, 3.20 and 4.80 μg C, and 0.55 and 0.85 μg N (Fig. 2). As in egg diameter, there was a significant variability among broods laid at the same salinity, and a significant effect of salinity at egg laying. The highest biomass values were found in eggs laid at 15‰ (Table 2). The C/N ratio varied among broods but not salinities. The relationship between female size and initial egg biomass was significant only for DW when eggs were laid at 15‰ ($y = -17.74 + 2.25\,x - 0.048\,x^2$; $R^2 = 0.22$; $p < 0.05$), with a maximum for intermediate female size. There was a significant correlation between initial egg biomass and egg size (Table 3); ANCOVA showed also significant variability in egg size independently of egg biomass (Table 3).

3.2. Egg size increment and development duration

During embryonic development, eggs increased in size and reached their maximum a few days before hatching. Linear regressions to estimate the rate of increase were always

Table 2
C. granulata. One-way ANOVA to evaluate differences in initial egg biomass among broods and between salinities at egg laying

Variable (μg/ind) (covariate)	Factor	dff	MSf	dfe	MSe	*F*	*p*
DW	Brood	18	2.09	80	0.03	74.43	$< 10^{-4}$
	‰	1	33.12	18	2.09	15.86	$< 10^{-3}$
C	Brood	18	0.59	80	7×10^{-3}	81.80	$< 10^{-4}$
	‰	1	9.12	18	0.59	15.48	$< 10^{-3}$
N	Brood	18	0.02	80	3×10^{-4}	69.62	$< 10^{-4}$
	‰	1	0.23	18	0.02	11.90	< 0.01
C (N)	Brood	18	0.04	79	7×10^{-4}	53.81	$< 10^{-4}$
	‰	1	0.04	18	0.04	1.12	0.30

DW: dry weight; C: carbon; N: nitrogen; ‰: salinity at egg laying; dff, MSf, dfe, MSe: degrees of freedom and mean squares of factors and errors, respectively.

Table 3
C. granulata. Linear regression, correlation, probability of homogeneity of slopes (P) and ANCOVA between initial size and biomass of eggs laid at different salinities

	Intercept	Slope	R^2	p
DW (μg / ind)				
Total	180.90	15.50	0.628	$< 10^{-6}$
15‰	231.67	9.90	0.441	< 0.01
32‰	214.14	10.25	0.327	< 0.01
p				0.94
ANCOVA				$< 10^{-3}$
C (μg / ind)				
Total	175.84	29.48	0.605	$< 10^{-6}$
15‰	214.01	22.23	0.401	< 0.01
32‰	215.57	18.18	0.339	< 0.01
p				0.22
ANCOVA				$< 10^{-3}$
N (μg / ind)				
Total	183.86	155.91	0.480	$< 10^{-6}$
15‰	210.32	132.62	0.496	< 0.01
32‰	229.85	81.24	0.208	< 0.05
p				0.39
ANCOVA				$< 10^{-4}$

significant ($R^2 > 0.80$; $n > 12$; $p < 0.05$). Significantly lower rates ($F_{1,\ 56} = 27.40$; $p < 10^{-5}$) occurred in eggs that were laid and developed at 15‰ as compared to those laid at 32‰ and developing at 20‰, or 32‰ (Table 4). A similar result was found for

Table 4
C. granulata. Mean rate of increment in egg size (μm/day), maximum size reached by eggs (μm), and size at hatching (μm) at different salinities
The number of broods were as follows: 15‰ = 19, 20‰ = 15, 32‰ = 26. Different letters: significant differences ($p < 0.05$) after Student–Newman–Keuls (SNK) test.

	Mean	S.D.	SNK
Increment			
15‰	2.42	0.55	A
20‰	3.01	0.48	B
32‰	3.35	0.43	B
Maximum			
15‰	390	17.66	A
20‰	375	16.43	B
32‰	377	15.62	B
Hatching			
15‰	386	16.54	A
20‰	369	12.00	B
32‰	371	13.22	B

Table 5
C. granulata. One-way ANOVAs to evaluate the effect of prehatching salinity on development time to selected stages of the embryonic development

Stage	dff	MSf	dfe	MSe	*F*	*p*
2	2	11.22	55	2.94	3.82	< 0.05
5	2	21.36	55	3.769	5.67	< 0.01
8	2	41.55	55	5.92	7.02	< 0.01
9	2	15.85	55	13.09	1.21	0.30

Symbols as in Table 2. See Table 1 for description of stages of development.

maximum egg size (ANOVA: $F_{1, 56} = 8.37$; $p < 0.01$) and size at hatching (ANOVA: $F_{1, 56} = 13.73$; $p < 10^{-3}$); eggs developing at 15‰ were larger than those incubated at other salinity conditions (Table 4).

Embryos maintained at high prehatching salinities (20‰ and 32‰) developed faster than those at 15‰, but significant differences were only found in embryonic stages 2–8 (Table 5; Fig. 3). Development duration was not correlated with initial egg biomass ($p > 0.05$).

3.3. Initial larval biomass: dependence on initial egg biomass and prehatching salinity

The initial larval biomass was significantly correlated with the initial egg biomass (Fig. 4; Table 6). Differences in larval biomass among prehatching salinities were

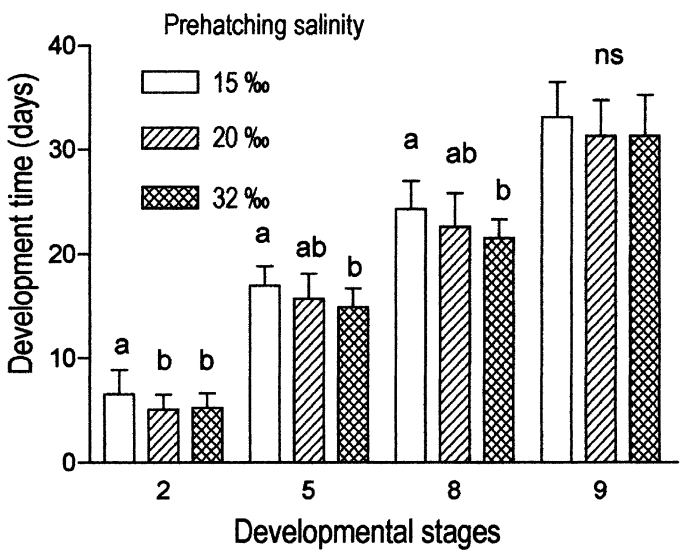

Fig. 3. *C. granulata*. Development time to selected stages, for embryos developing at different prehatching salinities. Error bars: standard deviation. Different letters: significant differences ($p < 0.05$) between prehatching salinities; ns: no significant differences.

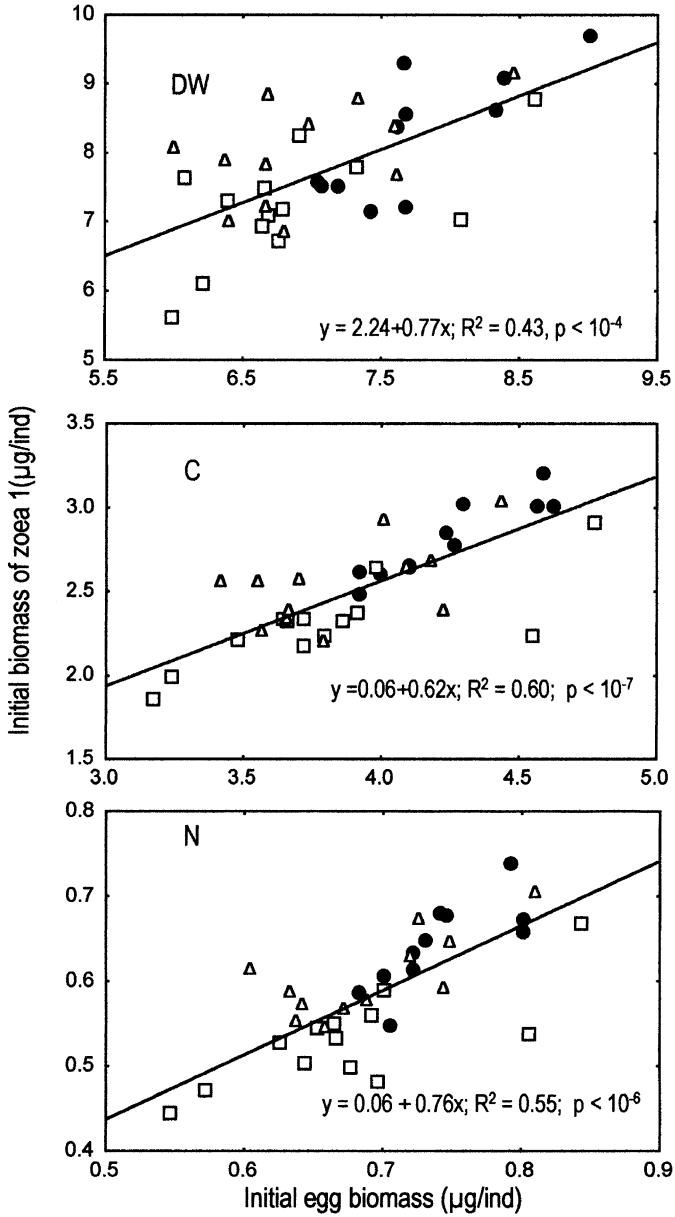

Fig. 4. *C. granulata*. Relationships between initial biomass, as dry weight (DW), carbon (C) and nitrogen (N), of eggs and zoea 1 from different prehatching salinities (15‰: ●, 20‰: □ and 32‰: △).

independent of initial egg biomass (ANCOVA, Table 6). Freshly hatched larvae had significantly higher DW and lower values of C and N (Fig. 5, Table 6) and C/N than freshly laid eggs (Table 7). The slope of the regression C on N was significantly higher

Table 6
C. granulata. Linear regression between biomass of eggs and larvae from different prehatching salinities, homogeneity of slopes and ANCOVA with egg biomass as covariate

	Intercept	Slope	R^2	p
DW (μg / ind)				
15‰	−0.52	1.13	0.78	< 0.01
20‰	2.61	0.67	0.32	< 0.05
32‰	4.12	0.56	0.19	0.09
p				0.40
ANCOVA				< 0.05
C (μg / ind)				
15‰	−0.62	0.81	0.84	$< 10^{-4}$
20‰	0.57	0.45	0.57	< 0.01
32‰	0.79	0.45	0.27	< 0.05
p				0.31
ANCOVA				$< 10^{-4}$
N (μg / ind)				
15‰	−0.08	0.97	0.53	< 0.01
20‰	0.16	0.55	0.56	< 0.01
32‰	0.19	0.60	0.49	< 0.01
p				0.40
ANCOVA				$< 10^{-5}$

Symbols as in Table 3.

for eggs than for zoea 1 (eggs: $C = -0.07 + 5.75N$, $R^2 = 0.94$, $p < 10^{-6}$; zoea 1: $C = 0.02 + 4.26N$, $R^2 = 0.86$, $p < 10^{-6}$; homogeneity of slopes: $p < 10^{-4}$). This indicates that large larvae (in terms of biomass) tended to have lower C/N (i.e. proportionally more N than C content) than large eggs.

Developmental changes in DW, C and N of the eggs depended on the prehatching salinity (see significant interactions in Table 8). DW increased significantly at 32‰; at lower salinities, the DW increments were very small and not significant (Table 7). The highest C and N decrements were detected at 20‰, followed by those observed at 15‰ and 32‰ (Table 7). At 20‰, the C loss was 40% and the N loss was 21%; at 15‰ and 32‰, the C loss was ca. 34% and the N loss was 13% (15‰) and 12% (32‰). The C

Table 7
C. granulata. C/N ratio of eggs and larvae, and changes in biomass (negative values are biomass losses) from egg laying to hatching, at different salinities

	C/N_{egg}	C/N_{larvae}	DW (μg/ind)	C (μg/ind)	N (μg/ind)
15‰	5.72	4.37	0.50	−1.43	−0.10
20‰	5.64	4.33	0.37	−1.50	−0.14
32‰	5.59	4.22	1.03	−1.32	−0.08

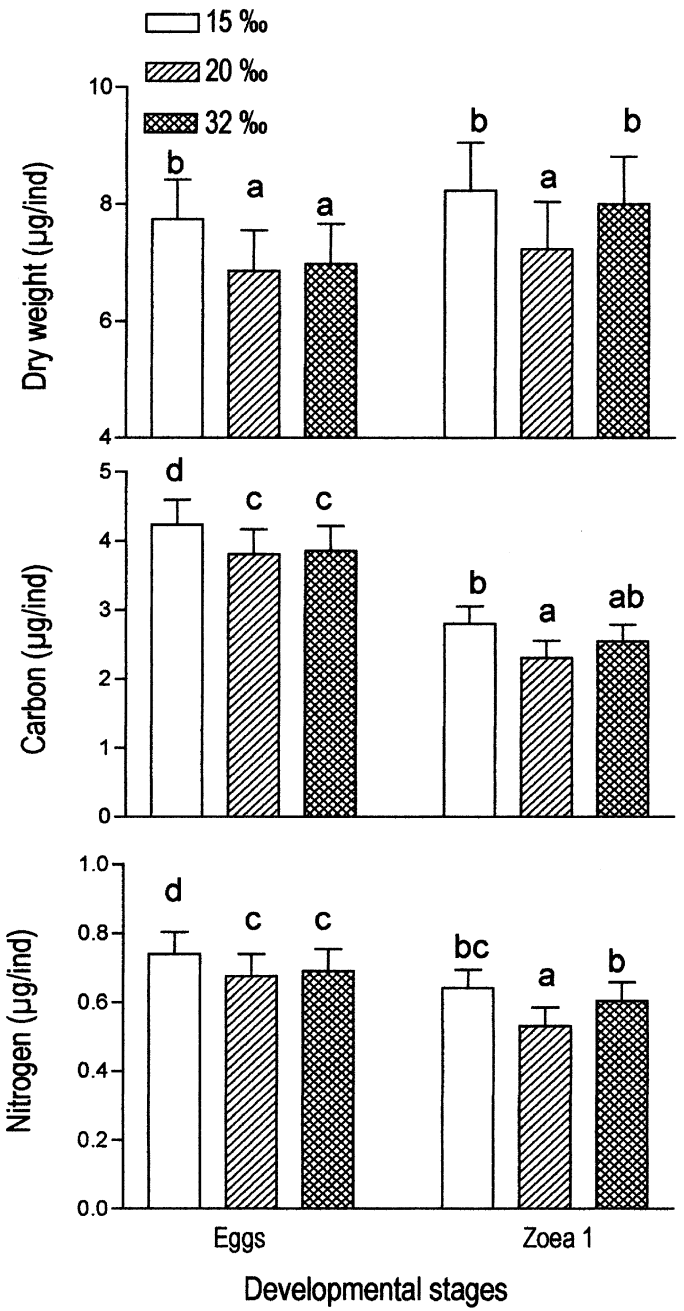

Fig. 5. *C. granulata*. Changes in biomass, as dry weight, carbon and nitrogen, freshly laid eggs and hatched zoea 1 from different prehatching salinities. Error bars: standard deviation. Different letters: significant differences ($p < 0.05$) between prehatching salinities.

Table 8
C. granulata. Repeated-measures ANOVA to evaluate changes in biomass between egg and zoea 1 (Phase) and the effect of prehatching salinity (*E*‰)

Variable (μg/ind)	Factor	dff	MSf	dfe	MSe	*F*	*p*
DW	*E*‰	2	5.32	33	0.93	5.70	< 0.01
	Phase	1	7.19	33	0.22	32.66	$< 10^{-5}$
	E‰ × Phase	2	0.75	33	0.22	3.40	< 0.05
C	*E*‰	2	1.33	33	0.16	8.30	< 0.01
	Phase	1	36.04	33	0.03	1218	$< 10^{-6}$
	E‰ × Phase	2	0.06	33	0.03	1.91	0.16
N	*E*‰	2	0.04	33	0.006	7.64	< 0.01
	Phase	1	0.21	33	0.0009	227.51	$< 10^{-6}$
	E‰ × Phase	2	0.005	33	0.0009	6.27	0.01

Symbols as in Table 2.

and N contents of the zoea 1 hatching from a prehatching salinity of 15‰ were similar to those from 32‰, but higher than in those from 20‰ (Fig. 5).

4. Discussion

4.1. Egg size, biomass and embryonic development time

We found significant variability in egg size and biomass among broods that were laid under identical conditions of salinity (15‰ or 32‰), temperature (21°C) and food. Under constant environmental conditions, the variability in egg size and biomass has been attributed to variation in female size or age (Qian and Chia, 1992; Stella et al., 1996; Ito, 1997) and genetic factors (Eyster, 1979; Glazier, 1992; Mashiko, 1992). For *C. granulata*, there was a weak correlation between egg size or biomass and female body size, which only explained a minor part of the observed variability. Therefore, we cannot entirely attribute variability in egg size or biomass to variability in female size. Other factors, as female age or genetic factors may be responsible for most of the observed variability. Whatever the underlying causes, our results show a significant intraspecific variability in egg size and egg biomass of *C. granulata* maintained under identical conditions.

Eggs laid at 15‰ in general were larger in terms of size and biomass than those laid at 32‰. There are two possible explanations for this difference: (1) salinity was directly responsible; (2) there were changes in other environmental conditions associated with salinity. Although all females were kept at the same temperature, salinity, and feeding conditions, further factors related to the culture conditions must be considered as well. The crabs kept at 32‰ were maintained in larger aquaria with a flow-through system, while those at 15‰ were kept in a closed system with water change every 2 days, due to limited amounts of water with 15‰ and constant 21°C. However, salinity could be responsible for the patterns of egg size and biomass. Larger egg size at 15‰ independently of biomass (Table 2), must be a consequence of a greater difference between internal and external osmolality and, in consequence, in the water uptake at egg laying

(see Krogh, 1966; Lange and Mostad, 1967). Water uptake must have also been responsible for increments in egg size during embryonic development, and may play an important role in the hatching process (Pandian, 1967; Wear, 1974; Clarke et al., 1990; Saigusa, 1992; Petersen and Anger, 1997). Also in other decapod species, larger eggs were found in freshwater populations (Thorson, 1950; Mashiko, 1983; Odinetz-Collart and Rabelo, 1996). In our material of *C. granulata*, egg size, but also biomass appears to be a plastic character responding to variation in salinity. Increments in egg biomass at low salinity may allow the embryo to face subsequent salinity stress with higher energy reserves.

Low prehatching salinity increased the duration of embryonic development to stages 2, 5 and 8, but no significant difference was detected in other embryonic stages or in total embryonic development time to hatching. This is consistent with the report by Bas and Spivak (2000). Egg biomass has been invoked to explain embryonic development time for several other crustaceans (Wear, 1974; Steele and Steele, 1975; Paschke, 1998), but this was not the case in *C. granulata*. The timing of hatching may be influenced by a counteracting influence of low salinity on embryonic development rate and hatching. At low salinities, embryos must have developed more slowly, but later the enhanced water content of the eggs might have accelerated the hatching process. Alternatively, the timing of hatching may be set by other factors, as chemical signals between embryos and the female (see Saigusa, 1996). This is suggested also by the fact that the larvae hatch predominantly at night (personal observation).

4.2. Initial larval biomass: dependence on initial egg biomass and prehatching salinity

We found variability in the initial larval biomass among broods, and we evaluated the roles of initial egg biomass (at egg laying) and salinity. This comprised (a) correlations of all broods incubated at 15‰, 20‰ and 32‰ (pooled in a single data set) as well as separate correlations in individual treatments; (b) two-way ANOVA with salinity and life phase as factors. The effect of initial egg biomass on initial larval biomass was evidenced by a significant overall correlation: regardless of pooling data from different salinities, this correlation was significant. The effect of salinity on biomass loss during embryogenesis was indicated by the fact that C and N losses were higher during embryonic development at 15‰, and especially at 20‰, than at 32‰, leading to a disappearance of initial biomass differences by the time of hatching. The potential importance of variability in egg and larval biomass in the life history of *C. granulata* is associated with a significant correlation between initial larval biomass and later larval survival, as observed by Giménez (2000). This may be an important factor for larval survival in the field.

Losses of C and N occur due to yolk utilization (Holland, 1978), with C primarily correlated with lipid, and N with the protein content (Anger and Harms, 1990; Petersen and Anger, 1997). A proportionally greater loss of C thus suggests that *C. granulata* utilises more lipids than proteins during embryogenesis, as other crustaceans (see Holland, 1978; Petersen and Anger, 1997; Paschke, 1998). Higher C and N losses at low salinities should be a consequence of a lower efficiency of yolk utilization, probably due to physiological disturbance. Physiological disturbance must have been particularly high

for eggs developing at 20‰ as, in this case (unlike in that of eggs incubated at 15‰), there was no previous acclimation. Laughlin and French (1989) found that crab larvae from low prehatching salinities had a lower DW, and suggested compensatory modifications of the N metabolism, reducing the concentration of osmotically active organic molecules (Schoffeniels and Gilles, 1970). C and N losses in *C. granulata* could have occurred also due to embryonic energy utilization for osmoregulation. In this case, higher C losses may have occurred because of a higher respiration rate at low salinities, as proposed for osmoregulating shrimp larvae (Agard, 1999). However, this remains to be a speculation, as long as the osmoregulatory capacity of the crab embryos has not been shown experimentally. Since the zoea 1 of this species is a fairly strong hyper-osmoregulator (Charmantier et al., in preparation), it is possible that some regulatory functions appear already in late embryos.

Changes in DW includes changes in minerals that were not discussed above. Higher DW in larvae than in embryos must have been a consequence of an increase in minerals during late embryonic development and at the beginning of larval life (Clarke et al., 1990; Petersen and Anger, 1997). Lower DW increments at 15‰ and 20‰ may occur because of lower salt absorption as compared with development in seawater.

In summary, *C. granulata* shows intrapopulational variability in the biomass of eggs and freshly hatched larvae. Salinity at egg laying appeared to influence initial egg size and biomass (larger at low salinities). This suggests both a plastic response (higher yolk reserves) and passive physical changes (higher water uptake) at lower salinities. Total embryonic development time to hatching was not affected by salinity. Initial larval biomass depended on initial egg biomass and on the C and N losses occurring during embryonic development. Since other experiments showed that survival is positively correlated with initial larval biomass (Giménez, 2000), variability in initial egg biomass and effects of salinity may be important for the subsequent survival of early larvae in the field.

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