

## Reproductive traits of an estuarine crab, *Neohelice* (= *Chasmagnathus*) *granulata* (Brachyura: Grapsoidea: Varunidae), in two contrasting habitats

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**SUMMARY:** Reproductive traits of an estuarine crab, *Neohelice* (= *Chasmagnathus*) *granulata* (Dana 1851), were compared between individuals living in contrasting habitats (mudflat and saltmarsh) of the same population in the brackish coastal lagoon of Mar Chiquita, Argentina. In both habitats, most measures of egg biomass decreased during embryogenesis, including total dry weight (DW) and organic matter (measured as ash-free dry weight, AFDW) per egg, the contents of ash, carbon, hydrogen and nitrogen (per egg and as percentage values of DW), the energy content (estimated from C; both per egg and per mg DW), and the C/N and C/H mass ratios. Egg size, wet weight (WW), and water content (in  $\mu\text{g}$  and % of WW), by contrast, increased significantly during the time of embryonic development. These parameters reached significantly higher final (near-to-hatching) values in mudflats than in saltmarshes (egg volume 0.0249 vs 0.0233 mm<sup>3</sup>; WW 36.5 vs 28.8  $\mu\text{g}$ ; water content 30.7 vs 23.2  $\mu\text{g}$  per egg or 84.2 vs 80.2% of WW, respectively). Fecundity and reproductive effort did not differ significantly between habitats. Habitat-specific differences in the water content and size of crab eggs are discussed in relation to small-scale local variation in environmental conditions.

**Keywords:** *Neohelice*, *Chasmagnathus*, estuary, fecundity, reproductive effort, egg volume, elemental composition (CHN).

**RESUMEN:** RASGOS REPRODUCTIVOS DE UN CANGREJO ESTUARINO *NEOHELICE* (= *CHASMAGNATHUS*) *GRANULATA* (BRACHYURA: GRAPSOIDEA: VARUNIDAE) EN DOS HÁBITATS CONTRASTANTES. – Los rasgos reproductivos del cangrejo estuarino, *Neohelice* (= *Chasmagnathus*) *granulata*, fueron comparados entre individuos que viven en hábitats contrastantes (planicie de marea y marisma) en una misma población de la laguna costera salobre de Mar Chiquita, Argentina. En ambos hábitats, la mayoría de las mediciones de biomasa de los huevos disminuyeron durante la embriogénesis, incluyendo el peso seco total (DW) y la materia orgánica (medida como peso seco libre de ceniza, AFDW) por huevo, el contenido de ceniza, carbón, hidrógeno y nitrógeno (por huevo y como valores porcentuales de DW), el contenido de energía (estimado a partir del C; por huevo y por mg DW), y la proporción de C/N y C/H. El tamaño del huevo, peso húmedo (WW), y contenido de agua (en % de WW), por el contrario, aumentaron significativamente durante el tiempo de desarrollo embrionario. Estos parámetros alcanzaron valores finales (cerca de la eclosión) significativamente más altos en la planicie de marea que en la marisma (volumen de los huevos 0.0249 vs 0.0233 mm<sup>3</sup>; WW 36.5 vs 28.8  $\mu\text{g}$ ; contenido de agua 30.7 vs 23.2  $\mu\text{g}$  por huevo y 84.2 vs 80.2% WW; respectivamente). La fecundidad y el esfuerzo reproductivo no difieren significativamente entre ambos hábitats. Las diferencias vinculadas al hábitat en el contenido de agua y tamaño de los huevos del cangrejo se discuten en relación a las variaciones a pequeña escala de las condiciones ambientales.

**Palabras clave:** *Neohelice*, *Chasmagnathus*, estuario, fecundidad, esfuerzo reproductivo, volumen del huevo, composición elemental (CHN).

## INTRODUCTION

The life cycles of most marine, coastal and estuarine species of decapod crustaceans comprise three principal phases prior to sexual maturity: embryonic, larval and juvenile development. Larvae of species living in estuaries can be retained in the estuary or exported to the sea, recruiting later as late larval or early juvenile stages (collectively referred to as “postlarvae”; see Sandifer, 1975). Embryos, in turn, normally experience the parental environmental conditions, unless the ovigerous females carry out catadromous spawning migrations towards the sea. Such reproductive behaviour occurs, for instance, in the blue crab, *Callinectes sapidus* (Tankersley *et al.*, 1998), but not in the species of crab which is the subject of the present study (see Spivak *et al.*, 1994, 1996). The nutritional state of females (Sastry, 1983; Harrison, 1990; Kennish, 1996; Palacios *et al.*, 1998, 1999; Wehrtmann and Kattner, 1998), temperature (Wear, 1974; Hartnoll and Paul, 1982; O’Leary Amstler and George, 1984; Péqueux, 1995; Wehrtmann and López, 2003; Sibert *et al.*, 2004) and salinity (Jones and Simons, 1982; Bas and Spivak, 2000; Giménez and Anger, 2001, 2003) may affect oogenesis and embryogenesis, and in consequence, influence initial larval biomass and the subsequent survival of early larvae in the field (Gomez Diaz, 1987; Laughlin and French, 1989; Giménez, 2006). In temporally and spatially variable environments such as estuaries, small-scale heterogeneity in those factors may therefore contribute to intraspecific variability in egg characteristics, causing intra- and inter-population variation in embryonic quality. There are many studies related to differences in size and biochemical composition of crustacean eggs comparing geographically separated populations (Jones and Simons, 1983; Wehrtmann and Kattner, 1998; Lardies and Castilla, 2001; Lardies and Wehrtmann, 2001; Bas *et al.*, 2007, Delgado and Defeo, 2008), whereas comparative studies between contrasting habitats of the same population are less commonly found in the scientific literature. This aspect of intraspecific variability is addressed in the present study.

As a model species, we studied the Varunid crab *Neohelice granulata* (Dana, 1851) (= *Chasmagnathus granulatus*; for recent taxonomic revision, see Sakai *et al.*, 2006). It is one of the most abundant semiterrestrial macro-invertebrates occurring in salt marshes of the southwestern Atlantic, where it excavates semi-permanent burrows in various types

of intertidal habitats, including bare mudflats and vegetated areas covered by cordgrass, *Spartina densiflora* (Anger *et al.*, 2008, and references therein). Size, shape and dynamics of their burrows, physical and chemical water characteristics, and periodicities of floods and feeding activity vary among habitats (e.g. Iribarne *et al.*, 1997, Botto and Iribarne, 1999, Bortolus and Iribarne, 1999). Ovigerous females carrying embryos in various developmental stages were simultaneously found in neighbouring but differential habitats, spending most of the time inside their burrows or nearby (Méndez Casariego *et al.*, 2006).

The reproductive biology of *N. granulata* has mostly been studied in Mar Chiquita, a temperate brackish coastal lagoon in Argentina (Anger *et al.*, 1994; Spivak *et al.*, 1996; Luppi *et al.*, 1997; Ituarte *et al.*, 2004; Silva *et al.*, 2007), including recent comparisons with a fully marine population (Bas *et al.*, 2007; Ituarte *et al.*, 2006). The main goals of the present study were (i) to quantify at Mar Chiquita developmental changes in biomass and elemental composition of eggs, comparing females from intertidal mudflats with those living nearby in relatively dry saltmarshes in the upper intertidal zone; and (ii) to compare fecundity, reproductive effort, and egg size between two contrasting types of habitats of the same population. Testing the significance of small-scale local heterogeneity in environmental conditions, the present study may enhance the understanding of the reproductive biology of this ecologically highly important estuarine crab.

## MATERIALS AND METHODS

**Study area**

This study was conducted in January-February 2006 and January 2007 at the coastal lagoon of Mar Chiquita, Argentina (37°32' to 37°45'S, 57°19' to 57°26'W). It has an area of 46 km<sup>2</sup>, its longest axis running parallel to the coastline. Water temperature and salinity show great seasonal, daily and local variations (Anger *et al.*, 1994; Reta *et al.*, 2001). Freshwater enters through creeks and rainfalls, while seawater enters from the adjacent ocean with tidal waves or due to easterly winds (Oliver *et al.*, 1972). The lower intertidal areas of Mar Chiquita lagoon are characterised by wet mudflats, where the sediment is constituted by silt and clay, showing

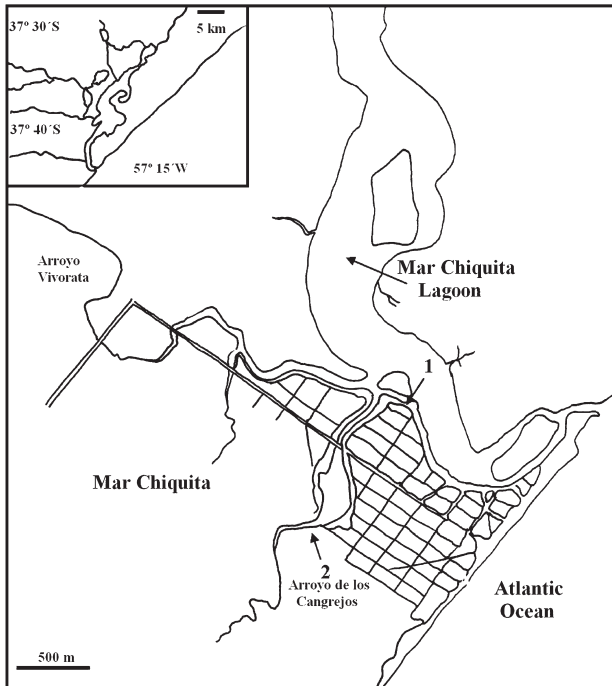


FIG. 1. – *Neohelice granulata*. Study area with sampling sites: (1) mudflats; (2) salt marsh. Inset: geographical position of Mar Chiquita lagoon.

high penetrability; the upper intertidal areas, by contrast, are characterised by relatively dry saltmarshes, which are covered by cordgrass (*Spartina densiflora*) and show lower sediment penetrability (Spivak *et al.*, 1994). The shape of crab burrows differed between areas, being large, straight and nearly vertical in saltmarshes, but oblique and short, with a funnel-shaped entrance and much larger diameter in mudflats. Saltmarshes are flooded only 1-2 times per week, while the mud flats are inundated by semi-diurnal tides 1-2 times per day (Iribarne *et al.*, 1997). Female *Neohelice granulata* were collected from two sampling sites: (1) a tidal mudflat, and (2) a saltmarsh at a distance of about 1300 m from the former location (Fig. 1).

#### Field measurement of physico-chemical features of water from burrows

From February 23 to 25, 2007, we measured temperature, dissolved oxygen and pH of water from burrows in mudflats and saltmarshes (5 per site) during each ebb and flood tide (four times per day) using a portable multi-parameter Hach sensION 156 (Hach Company, Loveland, USA). Salinity was measured in small water samples taken from crab burrows using a hand salinity refractometer (Atago Company,

Ltd., Tokyo, Japan). No data were obtained for the nocturnal ebb tide on February 24, 2007, due to lack of water inside the burrows.

#### Material processing

Ovigerous females (25 to 31 mm carapace width) were captured by hand during the period from January 15 to February 18, 2006, transported to the laboratory, and placed for 24 h into plastic aquaria filled with filtered and diluted seawater (23 psu).

The following arbitrary developmental stages were chosen for the analyses:

Stage I: eggs recently produced, uniform yolk, no eye pigments visible, 100 to 90% of volume occupied by yolk.

Stage II: eyes appear as reddish lines, chromatophores visible, 80 to 40% of volume occupied by yolk.

Stage III: eyes entirely formed, less than 30% of volume occupied by yolk or yolk depleted.

In a previous study (Bas and Spivak, 2000), the embryonic development of *N. granulata* was subdivided into 9 stages based on yolk consumption and the appearance of embryonic structures (e.g. eyes, chromatophores) or activities (heartbeat, body movement). In the simplified system used in the present investigation, stage I corresponds to Bas and Spivak's stages 1-3 combined, stage II to stages 4-6, and our stage III to stages 7-9, respectively.

Egg volume ( $V$ ) was calculated from microscopic size measurements made in 15 eggs per brood, which had been separated from 60 females ( $n = 10$  per each embryonic stage and per habitat), assuming an ellipsoidal form ( $V = d^2 \cdot D \cdot \pi / 6$ ; where  $d$  is the minor, and  $D$  the major, diameter).

The number of eggs per female (fecundity, used here exclusively in its meaning of "realised fecundity"; see Steachey and Somers, 1995) was estimated for 30 females ( $n = 5$  per each embryonic stage and habitat). Samples of 300 freshly eggs were removed from each female, counted under a dissecting microscope, rinsed with distilled water, transferred to pre-weighed tin cartridges, oven-dried for 24 h at 80°C, and eventually weighed to the nearest 0.01 mg. Average values of dry weight per egg calculated from these 5 samples and total dry weight of the complete egg mass were used to estimate the total number of eggs per female.

In 120 females ( $n = 20$  per embryonic stage and habitat), the complete egg mass was separated and briefly rinsed in distilled water. The interstitial water

was subsequently removed with filter paper, before the egg mass was weighed to the nearest 0.01 mg (wet weight, WW). Females and egg masses were then separately oven-dried for 48 h at 80°C and dry weight (DW) was determined after cooling in a desiccator; they were then burned for 5 h at 500°C, and reweighed to obtain the ash weight (AW). Amounts of organic matter were estimated as ash-free dry weight (AFDW = DW – AW). The reproductive effort (RE) was estimated as DW ratio (egg mass DW / female DW without eggs), expressed as a percentage.

The elemental composition of eggs (contents of carbon, hydrogen, nitrogen; collectively referred to as CHN) was measured in 20 females with embryos in stages I and III only ( $n = 5$  per each embryonic stage and habitat), with five replicate samples (40 eggs each) taken per brood. Hence, our materials for determinations of DW and CHN comprised in total 100 analyses (2 stages X 2 habitats X 5 females X 5 replicates) of 4000 eggs. The samples were briefly rinsed in distilled water, blotted on filter paper, transferred to pre-weighed tin cartridges, stored frozen at –20°C, vacuum-dried for 28 h in a Labcono (Labcono Corporation, Kansas City, USA) Freeze Dry System, weighed on a Mettler UMT2 microbalance (precision 0.1 µg), and analysed in a Carlo Erba Elemental Analyser (EA 1108).

### Data analysis

Differences in salinity, temperature, dissolved oxygen and pH of water from crab burrows and size of ovigerous females were compared between habitats with Student's *t*-test, or with a Mann-Whitney *U* test when the data were not normally distributed after arc-sin transformations.

Differences in egg V, WW, DW, AW and contents of water and organic matter were compared with two-way ANOVA, after checking for normality and homocedasticity, with habitat (mudflats vs salt marshes) and embryonic stage (I, II, III) as factors. Differences between treatments (after ANOVA) were tested a posteriori with a Student-Newman-Keuls (SNK) test. Differences in WW, DW, AW, water content, organic matter, or RE observed between different females or habitats were tested with Student's *t*-test, or with a Mann-Whitney *U* test when data were not normally distributed after arc-sin transformation.

Differences in CHN (in µg/individual or % of DW), energy content (estimated from C content; Sa-

lonen *et al.*, 1976; in Joules per individual or per mg DW), and the C/N and C/H ratios of eggs were compared with two-way ANOVA, with habitat (mudflats vs saltmarshes) and embryonic stage (I vs III) as factors. Differences were subsequently tested with a Student-Newman-Keuls (SNK) test. Percentage values (e.g. CHN in % of DW) and ratios (e.g. C/N) were arc-sin transformed prior to statistical analysis. Standard analyses were based on Zar (1996) and Underwood (1997).

### RESULTS

Significant habitat-specific differences (mudflats vs saltmarshes) were observed in salinity, temperature, and pH of water collected from burrows of *N. granulata*, while dissolved oxygen concentrations did not differ between habitats (Fig. 2). The strongest differences were observed in average salinity, being in most cases significantly higher in burrows dug in a salt marsh than in those in a mudflat (30.8-35.8 vs 13.4-36.6 psu; Fig. 2A). Water temperature, by contrast, was mostly higher in mudflats (17.6-26.3 vs 19.1-22.3°C; Fig. 2B). As a general pattern, water temperature and salinity were more stable in burrows dug in saltmarshes than in mudflats. The pH was on average higher in mudflat burrows, although the range (5.61-8.29 vs 5.43-7.82 pH; Fig. 2C) was similar in the two habitat types. In most water samples taken from crab burrows, higher dissolved oxygen concentrations were measured in mudflats than in saltmarshes (0.48-2.1 vs 0.17-2.1 mg/l; Fig. 2D), but these differences were not statistically significant due to high variability.

Average DW, WW, water content (in g/individual), ash content (both in g/individual and % WW), and RE values were similar in female crabs collected from the two habitats. By contrast, the content of organic matter (in g/individual, % WW, and % DW) was significantly higher in females from the saltmarsh (Table 1). The size of ovigerous females was similar in the two habitats ( $p = 0.6$ ).

While eggs in an early developmental stage did not differ between females from the two habitat types, embryos close to hatching were significantly larger and heavier in mudflats than in salt marshes (0.0249 vs 0.0233 mm<sup>3</sup>; 36.5 vs 28.8 µg WW; Table 2). DW, however, did not significantly vary among developmental stages and habitats (Tables 2, 4), which indicates that differences in egg volume and

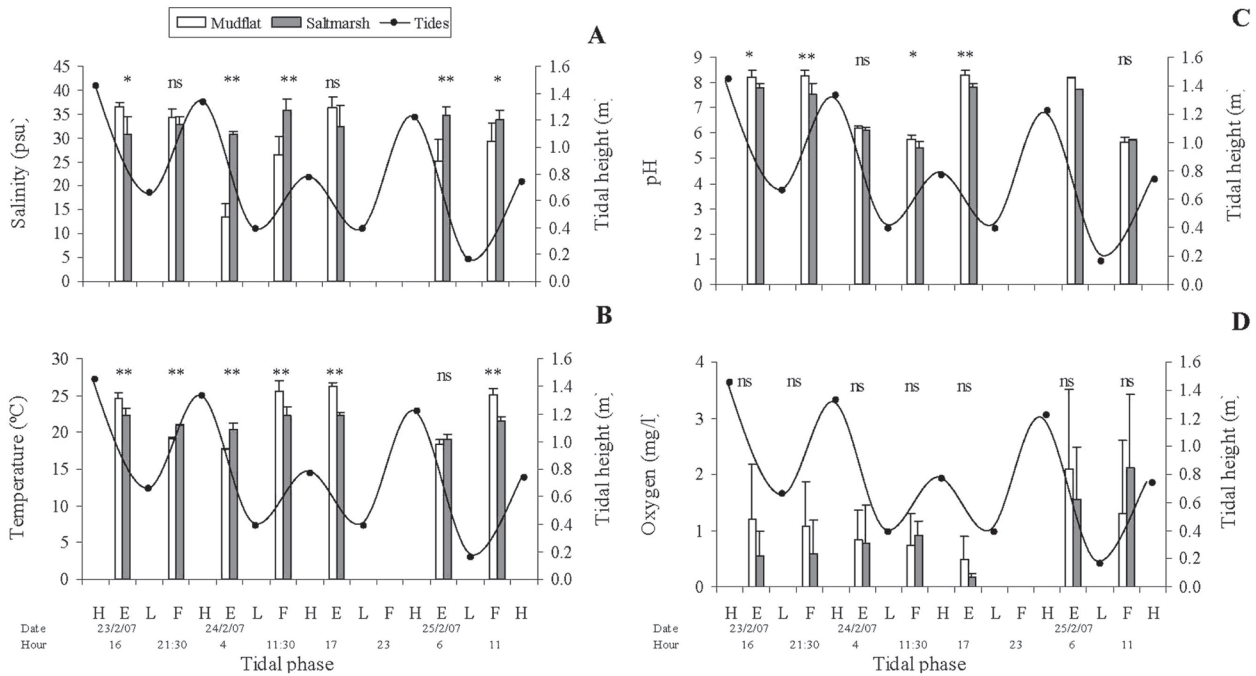


FIG. 2. – *Neohelice granulata*. (A) Temperature (°C), (B) salinity (psu), (C) pH and (D) dissolved oxygen (mg/l) of water sampled from burrows in a mudflat and a salt marsh; flood (F) vs ebb (E) tide; February 23, 4:00 PM to February 25, 2007, 11:00 AM; high (H) vs low (L) tide. Statistical test for differences between habitats (Student’s *t*-test):  $P < 0.001$  (\*\*),  $P < 0.05$  (\*), not significant (ns).

TABLE 1. – *Neohelice granulata*. Average biomass and reproductive effort (RE) of females from mudflats and salt marshes (mean  $\pm$  SD). Differences (D) between habitats tested with Student’s *t*-test:  $P < 0.001$  (\*\*\*),  $P < 0.05$  (\*\*), not significant (ns).

	Mudflats n = 60	Saltmarshes n = 60	D
Dry weight (g)	3.5 $\pm$ 0.7	3.6 $\pm$ 0.7	ns T=3459.000
Wet weight (g)	10.4 $\pm$ 2.1	10.3 $\pm$ 2.1	ns T=3664.000
Water content (g)	6.8 $\pm$ 1.5	6.7 $\pm$ 1.4	ns T=3778.000
Organic matter (g)	1.61 $\pm$ 0.3	1.75 $\pm$ 0.4	** t=-2.16
Organic matter (% DW)	45.7 $\pm$ 3.7	48.1 $\pm$ 3.7	*** t=-3.52
Organic matter (% WW)	15.7 $\pm$ 1.9	17.05 $\pm$ 1.8	*** t=-3.87
Ash content (g)	1.92 $\pm$ 0.4	1.90 $\pm$ 0.42	ns T=3718.500
Ash content (% WW)	18.6 $\pm$ 0.8	18.4 $\pm$ 1.2	ns T=3862.000
RE (%)	7.95 $\pm$ 1.84	8.47 $\pm$ 1.71	ns t=-1.59

WW were caused by differential water content. The egg volume always increased during embryogenesis, but the increase was significantly higher in mudflats than in salt marshes (90 vs 63%; Tables 3, 4). Concomitantly with changes in egg volume, the embryonic water content (both per egg and as a percentage of WW) increased steadily during incubation, reaching significantly higher final (near-to-hatching) values in mudflats than in saltmarshes (30.7 vs 23.2  $\mu$ g 84.2 vs 80.2%; Table 2 and 5). The proportion of organic matter within total egg dry mass (AFDW in % of DW) increased slightly during development in both habitats (significant only at the beginning of

TABLE 2. – *Neohelice granulata*. Number, volume, and biomass of eggs in three stages of embryonic development, comparing mudflats and saltmarshes (mean  $\pm$  SD). The symbol  $\neq$  indicates significant differences between successive stages of the same habitat, while the numbers in bold indicate significant differences between habitats for the same stage.

	Stage I n = 20	Mudflats Stage II n = 20	Stage III n = 20	Stage I n = 20	Saltmarshes Stage II n = 20	Stage III n = 20
Egg number	47151 $\pm$ 12060 =	49707 $\pm$ 12048 =	44684 $\pm$ 13297	53977 $\pm$ 12919 =	49006 $\pm$ 13274 =	48116 $\pm$ 12393
Egg volume (mm <sup>3</sup> )	0.0132 $\pm$ 0.001 $\neq$	0.0183 $\pm$ 0.002 $\neq$	<b>0.0249 <math>\pm</math> 0.002</b>	0.0143 $\pm$ 0.0009 $\neq$	0.0188 $\pm$ 0.0021 $\neq$	<b>0.0233 <math>\pm</math> 0.002</b>
Dry weight ( $\mu$ g)	6.26 $\pm$ 0.5 =	5.72 $\pm$ 0.24 =	5.75 $\pm$ 0.26	6.35 $\pm$ 1.6 =	6.34 $\pm$ 0.36 =	5.7 $\pm$ 0.2
Wet weight ( $\mu$ g)	19.5 $\pm$ 2.2 $\neq$	26.5 $\pm$ 4.5 $\neq$	<b>36.5 <math>\pm</math> 2.1</b>	18.1 $\pm$ 3.4 $\neq$	26.5 $\pm$ 2.6 =	<b>28.8 <math>\pm</math> 3.3</b>
Water content ( $\mu$ g)	<b>13.3 <math>\pm</math> 2.2 <math>\neq</math></b>	20.8 $\pm$ 4.5 $\neq$	<b>30.7 <math>\pm</math> 2.1</b>	<b>11.7 <math>\pm</math> 3.3 <math>\neq</math></b>	20.1 $\pm$ 2.6 =	<b>23.2 <math>\pm</math> 3.3</b>
Water content (%)	<b>67.7 <math>\pm</math> 3.6 <math>\neq</math></b>	<b>77.8 <math>\pm</math> 3.8 <math>\neq</math></b>	<b>84.2 <math>\pm</math> 0.9</b>	<b>63.9 <math>\pm</math> 4.7 <math>\neq</math></b>	<b>75.8 <math>\pm</math> 2.6 <math>\neq</math></b>	<b>80.2 <math>\pm</math> 1.9</b>
Organic matter ( $\mu$ g)	5.41 $\pm$ 0.2 $\neq$	<b>5.03 <math>\pm</math> 0.47 =</b>	5.1 $\pm$ 0.07	5.4 $\pm$ 0.2 $\neq$	<b>5.6 <math>\pm</math> 0.15 <math>\neq</math></b>	5.1 $\pm$ 0.1
Organic matter (% DW)	86.47 $\pm$ 2.5 $\neq$	87.46 $\pm$ 7.57 =	88.21 $\pm$ 1.41	84.9 $\pm$ 2.99 $\neq$	89.11 $\pm$ 2.46 =	89.65 $\pm$ 0.65
Ash content ( $\mu$ g)	0.85 $\pm$ 0.2 $\neq$	0.7 $\pm$ 0.5 =	0.7 $\pm$ 0.07	0.9 $\pm$ 0.2 $\neq$	0.7 $\pm$ 1.2 =	0.6 $\pm$ 0.04
Ash content (% DW)	13.52 $\pm$ 2.5 $\neq$	12.53 $\pm$ 7.56 =	11.78 $\pm$ 1.14	15.09 $\pm$ 2.99 $\neq$	10.89 $\pm$ 2.46 =	10.34 $\pm$ 0.65

TABLE 3. – *Neohelice granulata*. Percentage egg loss and increase in egg volume during embryonic development, comparing mudflats and saltmarshes (mean  $\pm$  SD). The symbol  $\neq$  indicates significant differences between successive values of the same habitat, while the numbers in bold indicate significant differences between habitats for the same value.

	Stage I-II n = 20	Stage II-III n = 20	Stage I-III n = 20
Mudflat	40.25 $\pm$ 0.22 =	36.6 $\pm$ 0.13 $\neq$	<b>89.6 <math>\pm</math> 0.20</b>
Saltmarsh	31.4 $\pm$ 0.17 =	24.9 $\pm$ 0.13 $\neq$	<b>62.9 <math>\pm</math> 0.16</b>

embryogenesis, comparing stages I – II). When absolute amounts of AFDW/egg were compared between habitats, embryos in stage II showed significantly higher values in salt marshes, while no significant differences were found in the initial and late stages (I, III). The ash content (both in g/individual and in

% of DW) was in all embryonic stages similar between mudflats and saltmarshes, tending to decrease during embryonic development (significant between Stages I-II; Tables 2, 5).

The number of eggs carried per female decreased slightly during embryonic development but the differences observed between embryonic stages and habitat were not statistically significant (Tables 3, 4).

The contents of CHN (in  $\mu\text{g}/\text{ind}$  and % of DW), energy contents (in  $\text{J}/\text{ind}$  and  $\text{J}/\text{mg}$  DW, estimated from C), as well as C/N and C/H mass ratios differed significantly between embryos in stages I and III, being consistently higher at the beginning of embryonic development, except for N (in % of DW), which was higher during late embryogenesis. These patterns did not significantly differ between saltmarshes and mudflats (Figs. 3, 4; Tables 6, 7).

TABLE 4. – *Neohelice granulata*. Two-way ANOVA evaluating differences in egg number, egg volume, percentage increments in egg volume, dry weight (DW), wet weight (WW) comparing different stages of embryonic development (I, II, III) and habitats (mudflats vs saltmarshes); dff: degrees of freedom of factors, MSf: mean squares of factors, dfe: degrees of freedom of errors, MSe: mean squares of errors, F: MSf / MSe, *p*: probability.

Variable	Factor	dff	MSf	dfe	MSe	F	<i>p</i>
Egg number	Habitat	1	304479172.6	114	160692347.81	1.895	= 0.171
	Development stage	2	183525771.7	114	160692347.81	1.142	= 0.323
	Habitat x Development stage	2	142086604.6	114	160692347.81	0.884	= 0.416
Volume	Habitat	1	0.000000004	54	0.00000284	0.0017	= 0.97
	Development stage	2	0.0005	54	0.00000284	187.3	< 0.001
	Habitat x Development stage	2	0.00001	54	0.00000284	3.52	= 0.037
Volume Increase (%)	Habitat	1	0.0652	54	0.00789	8.26	= 0.006
	Development stage	2	0.233	54	0.00789	29.56	< 0.001
	Habitat x Development stage	2	0.0025	54	0.00789	0.317	= 0.73
DW	Habitat	1	2.816E-013	22	4.589E-013	0.61	= 0.44
	Development stage	2	7.911E-013	22	4.589E-013	1.72	= 0.2
	Habitat x Development stage	2	3.248E-013	22	4.589E-013	0.71	= 0.5
WW	Habitat	1	0.000000003	114	9.776E-012	28.9	< 0.001
	Development stage	2	0.000000002	114	9.776E-012	198.4	< 0.001
	Habitat x Development stage	2	9.711E-012	114	9.776E-012	16.8	< 0.001

TABLE 5. – *Neohelice granulata*. Two-way ANOVA evaluating differences in embryonic contents of water, organic matter, ash (expressed in  $\mu\text{g}$  and % of DW), comparing different stages of development (I, II, III) and habitats (mudflats vs saltmarshes). Symbols as in Table 4.

Variable	Factor	dff	MSf	dfe	MSe	F	<i>p</i>
Water content ( $\mu\text{g}$ )	Habitat	1	0.000004	114	1.2E07	28.54	< 0.001
	Development stage	2	0.00003	114	1.2E07	223.92	< 0.001
	Habitat x Development stage	2	0.000001	114	1.2E07	10.05	< 0.001
Water content (%)	Habitat	1	0.0435	114	0.00132	32.9	< 0.001
	Development stage	2	0.369	114	0.00132	278.98	< 0.001
	Habitat x Development stage	2	0.0019	114	0.00132	1.5	= 0.23
Organic matter ( $\mu\text{g}$ )	Habitat	1	0.00000006	114	2.4E-09	24.87	< 0.001
	Development stage	2	0.00000005	114	2.4E-09	22.21	< 0.001
	Habitat x Development stage	2	0.00000007	114	2.4E-09	28.25	< 0.001
Organic matter (%)	Habitat	1	0.0009	114	0.00267	0.357	= 0.55
	Development stage	2	0.0279	114	0.00267	10.428	< 0.001
	Habitat x Development stage	2	0.0058	114	0.00267	2.197	= 0.116
Ash content ( $\mu\text{g}$ )	Habitat	1	0.000000002	114	1.3E-08	0.0179	= 0.894
	Development stage	2	0.00000003	114	1.3E-08	19.62	< 0.001
	Habitat x Development stage	2	0.00000003	114	1.3E-08	2.56	= 0.082
Ash content (%)	Habitat	1	0.0009	114	0.00267	0.357	= 0.552
	Development stage	2	0.0279	114	0.00267	10.42	< 0.001
	Habitat x Development stage	2	0.00587	114	0.00267	2.19	= 0.116

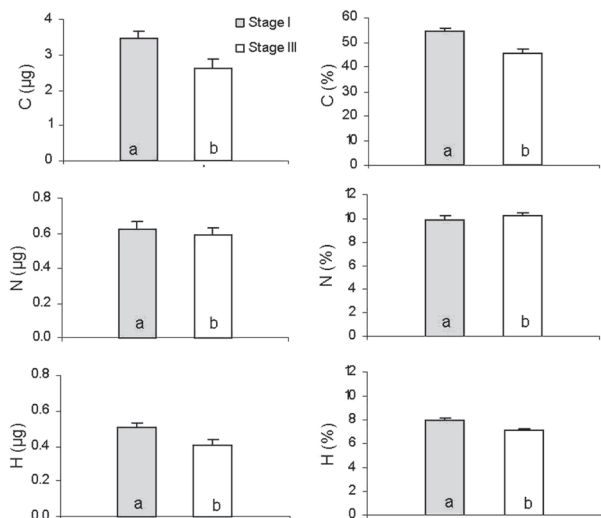


FIG. 3. – *Neohelice granulata*. Embryonic contents of carbon (C), nitrogen (N), hydrogen (H) (in µg/individual, % of DW; mean ± SD); beginning vs end of embryonic development (stages I vs III); data from mudflats and salt marshes pooled; different letters indicate significant differences (see Tables 6 and 7 for ANOVA).

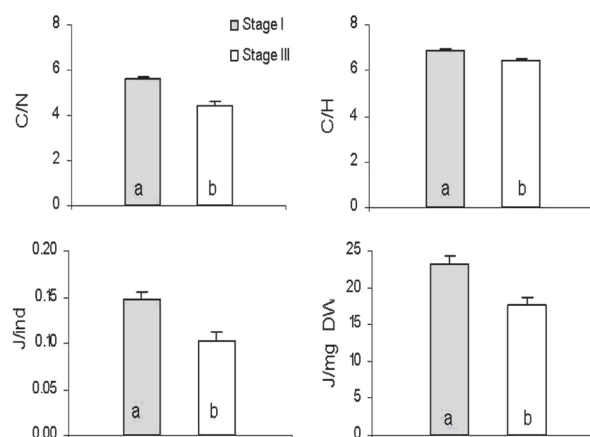


FIG. 4. – *Neohelice granulata*. Individual, weight-specific energy contents (J/ind, J/mg DW), C/N and C/H ratio of eggs (mean ± SD); beginning vs end of embryonic development (I vs III); data from mudflats and salt marshes pooled; different letters indicate significant differences (see Tables 6 and 7 for ANOVA).

TABLE 6. – *Neohelice granulata*. Two-way ANOVA evaluating differences in embryonic carbon (C), nitrogen (N), hydrogen (H), energy content, C/N and C/H ratios, comparing different developmental stages (I, III) and habitats (mudflats vs saltmarshes). Symbols as in Table 4.

Variable	Factor	dff	MSf	dfe	MSe	F	p
C content (µg/ind)	Habitat	1	0.039	16	0.0463	0.86	= 0.367
	Development stage	1	3.668	16	0.0463	79.21	< 0.001
	Habitat x Development stage	1	0.177	16	0.0463	3.83	= 0.068
N content (µg/ind)	Habitat	1	0.00006	16	0.00151	0.046	= 0.833
	Development stage	1	0.0073	16	0.00151	4.89	= 0.042
	Habitat x Development stage	1	0.0094	16	0.00151	6.24	= 0.024
H content (µg/ind)	Habitat	1	0.0009	16	0.000851	1.11	= 0.30
	Development stage	1	0.049	16	0.000851	57.8	< 0.001
	Habitat x Development stage	1	0.0047	16	0.000851	5.59	= 0.031
Energy content (Joule/ind)	Habitat	1	0.00006	16	0.0000941	0.707	= 0.41
	Development stage	1	0.0106	16	0.0000941	112.9	< 0.001
	Habitat x Development stage	1	0.00016	16	0.0000941	1.72	= 0.208
C/N	Habitat	1	0.029	16	0.0236	1.25	= 0.28
	Development stage	1	6.12	16	0.0236	259.21	< 0.001
	Habitat x Development stage	1	0.0015	16	0.0236	0.63	= 0.43
C/H	Habitat	1	0.0014	16	0.00717	0.20	= 0.65
	Development stage	1	0.98	16	0.00717	136.9	< 0.001
	Habitat x Development stage	1	0.008	16	0.00717	1.14	= 0.30

TABLE 7. – *Neohelice granulata*. Two-way ANOVA evaluating differences in the proportions of embryonic contents of carbon (C%), nitrogen (N%), hydrogen (H%), DW-specific energy (J/mg DW), comparing different developmental stages (I, III) and habitats (mudflats vs saltmarshes). Symbols as in Table 4.

Variable	Factor	dff	MSf	dfe	MSe	F	p
C (%)	Habitat	1	0.00000	16	0.000189	0.0068	= 0.93
	Development stage	1	0.04	16	0.000189	215.96	< 0.001
	Habitat x Development stage	1	0.0013	16	0.000189	7.21	= 0.016
N (%)	Habitat	1	0.00002	16	0.0000217	1.34	= 0.26
	Development stage	1	0.0002	16	0.0000217	11.07	= 0.004
	Habitat x Development stage	1	0.00002	16	0.0000217	2.59	= 0.12
H (%)	Habitat	1	0.00000	16	0.0000122	0.101	= 0.75
	Development stage	1	0.0013	16	0.0000122	108.5	< 0.001
	Habitat x Development stage	1	0.00001	16	0.0000122	6.061	= 0.026
Energy (Joule/mg DW)	Habitat	1	0.028	16	0.653	0.043	= 0.83
	Development stage	1	155.3	16	0.653	237.7	< 0.001
	Habitat x Development stage	1	4.98	16	0.653	7.62	= 0.014

## DISCUSSION

Our study provides the first comparison of volume, biomass and elemental composition (CHN) of eggs produced by crabs in different habitats with contrasting environmental conditions. Egg WW and volume increased in *Neohelice granulata* during embryonic development, presumably due to an uptake of water through the egg membrane. This process is more pronounced near the end of the incubation period (see Anger *et al.*, 2002, and references therein). Different percentage increases in egg volume (comparing stages I - III) observed in different habitats (90% in mudflats vs 63% in salt-marshes) may thus be a consequence of differential water uptake (48% vs 15% increase in the water content per egg; Table 2).

Temperature and salinity may affect egg production, embryogenesis and larval quality in crustaceans (McLaren *et al.*, 1969; Steele and Steele, 1975; Giménez and Anger, 2001). These factors are highly variable in Mar Chiquita (Spivak *et al.*, 1994), but the degree of variation differed between habitats, being more stable in salt marshes than in mudflats (Fig. 2). The higher average water content of crab eggs produced in mudflats, especially near the end of embryonic development, could mitigate effects of environmental fluctuations (Pandian, 1970a). The larger egg size in mudflats may be related to the lower average salinity of this habitat. It has been demonstrated that *N. granulata* produces larger eggs at lower salinity, due to an increase in water uptake (Giménez and Anger, 2001). However, the differences in egg volume observed between habitats during the present study persisted after 24 h of acclimatisation to 23 psu. In summary, the possible adaptive significance of differential egg size in different habitats remains to be further studied both in the field and in physiological laboratory experiments.

The quality and quantity of nutrients stored in egg yolk depend on maternal body reserves, capacity for biosynthesis and dietary intake during ovary maturation (Harrison, 1990). While ovigerous females of *N. granulata* spend most of the time inside or near their burrows, non-ovigerous females (where oocytes are developing) show active migrations within or between habitats (Méndez Casariego *et al.*, 2006; Luppi, own observations). The latter may thus obtain sufficient food from either *Spartina* or soft-bottom fauna, irrespective of the place where their burrows are located (Iribarne *et al.*, 1997). As a consequence,

their dietary intake is not restricted during oogenesis, so similar amounts of organic matter can be invested into newly laid eggs. During embryogenesis, the organic matter content (AFDW, in % of total DW) decreased in both habitats at similar rates, indicating similar energy demands for embryonic metabolism (Holland, 1978; Pandian, 1994).

The ash content represents inorganic salts that are absorbed with water taken up across the embryonic membrane (Amsler and George, 1984). Since a gradual increase in the water content during embryogenesis is common in decapods, a similar increase in inorganic constituents should be expected both per egg and as a percentage of DW. Such patterns have been reported for embryos of many species of decapods (Pandian, 1970a, b; Amsler and George, 1984; Clarke *et al.*, 1990; Valdés *et al.*, 1991). In some other species, however, relatively stable or even decreasing inorganic matter contents have been observed (Wehrtmann and Kattner, 1998; Lardies and Wehrtmann, 1996, 1997). In *N. granulata*, we found a slight decrease in the ash content during development (see Table 2). This suggests that the concomitant increase in the water content may be, at least partially, due to retention of metabolic water produced by the embryo rather than an uptake of external seawater. Alternatively, the ion permeability of the egg membrane may be low, while water may enter passively due to an osmotic process. Future studies of osmoregulation in the embryonic stages of *N. granulata* may clarify the mechanisms of changes in the contents of water and minerals during egg development.

The fecundity of *N. granulata* can vary greatly among populations, seasons, and years. While the values observed in this study coincided with those recently reported by Bas *et al.* (2007), they were higher than those found previously by Luppi *et al.* (1997) in Mar Chiquita. Also, the present values were higher than those found by Stella *et al.* (1996) in Samborombón Bay and by Bas *et al.* (2007) in San Antonio Bay. On the other hand, fecundity in Mar Chiquita did not significantly differ between closely neighbouring habitats.

Egg loss in decapods was associated with extreme temperatures, salinities and the effect of egg parasites, predators, and microbial infections (Bas and Spivak, 2003; Silva *et al.*, 2007 and references therein). Ovigerous females of *N. granulata* lost eggs, perhaps as a consequence of an incomplete closing of the pleon, so part of the egg mass is exposed to



environmental conditions (Luppi *et al.*, 1997). Since these conditions varied between habitats, we expected to see variations in the degree of egg loss. Moreover, a higher percentage of females with abnormal embryos had been found in mudflats than in saltmarshes and correlated with a higher epibiosis (bacteria, fungi, and peritrichid ciliates), as well as more detritus in the incubation chamber, especially in the periphery of egg masses (Silva *et al.*, 2007). However, no statistically significant differences in egg loss were detected during this study, suggesting that the protection provided by ovigerous females is similarly effective in these two different habitats.

Brood weight in marine brachyuran crabs is generally constrained to a maximum of approximately 10% of the body weight of the female (Hines 1991, 1992). In the present study, we found similar RE values in mudflats and salt marshes (7.9 and 8.5%, respectively). Stella *et al.* (1996) reported an RE of 7.1% for a population living in Samborombón Bay, while Luppi *et al.* (1997) found an RE of 9.0% in the Mar Chiquita lagoon. Bas *et al.* (2007) compared populations in San Antonio Bay and the Mar Chiquita lagoon and found average RE values of 8.4 and 8.8%, respectively. In summary, the RE appears to vary only very little within or among different populations of *N. granulata* from Argentina.

The chemical composition of the eggs of *N. granulata* changed during the final phase of embryonic development, with decreasing organic matter contents prior to hatching in both salt marshes and mudflats. These patterns reflect metabolic losses of yolk (Holland, 1978), which were more pronounced in the elemental fractions of C and H than in the N content of egg biomass. As a consequence, the percentage values of C and H, the C/N ratio, and the DW-specific energy content also decreased considerably. These patterns suggest that *N. granulata* utilises proportionately more lipids than proteins as a fuel for embryonic energy metabolism. The same pattern has also been observed in other studies on the same species (Giménez and Anger, 2001; Bas *et al.*, 2007), as well as in other crustaceans (Holland, 1978; Amsler and George, 1984; Petersen and Anger, 1997; Anger *et al.*, 2002; Anger and Moreira, 2004), although the the egg yolk (lipovitellin) is composed of approximately equal amounts of lipids and proteins (Walker *et al.*, 2006). Preferential lipid degradation, accompanied by preservation or accumulation of structural proteins, may thus be considered a general pattern in crustacean egg development

(e.g. Clarke *et al.*, 1990; Petersen and Anger, 1997; Heras *et al.*, 2000; Anger *et al.*, 2002).

Although developmental changes in egg characteristics vary among decapod taxa, ontogenetically increasing tendencies in egg volume and ash and water contents, and a decrease in organic fractions may be general patterns (cf. Anger *et al.*, 2002; Anger and Moreira, 2004; Bas *et al.*, 2007). In *N. granulata*, intrapopulation variability in the water content and size of eggs may be caused by small-scale variations in physical factors such as salinity, and possibly pH and temperature.

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