

## HABITAT RELATED VARIATION IN REPRODUCTIVE TRAITS AMONG INTERTIDAL CRABS FROM THE SOUTHWESTERN ATLANTIC

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### ABSTRACT

The reproductive traits of three coastal grapsoid crabs from the southwestern Atlantic, *Neohelice granulata* (Dana, 1851), *Cyrtograpsus angulatus* Dana, 1851 and *C. altimanus* Rathbun, 1914, were compared under the hypothesis that more energy is diverted to maintenance (and less to reproduction) in the upper intertidal and/or in estuaries than in the lower marine intertidal or subtidal, in order to cope with the harsh, and frequently variable, environmental conditions of semiterrestrial and brackish water habitats. Each species occupies a different habitat along intertidal, as well as estuarine gradients: *N. granulata* lives in the upper and middle intertidal of salt marshes and mud flats, especially in estuarine waters; *C. angulatus* lives in the low intertidal and subtidal of estuarine and marine habitats, and *C. altimanus* is predominantly an intertidal and subtidal marine crab. Results suggested that energy demands imposed by the harsh environmental conditions of the estuarine and semiterrestrial habitats resulted in less energy available for reproduction in *N. granulata*, evidenced by a shorter breeding season, a reduced reproductive output per clutch and an increased clutch interval. The opposite occurred with *C. altimanus*: less energy was necessary to cope with the stable marine water, allowing the presence of a long breeding season, a higher reproductive output per clutch and a very short clutch interval. Differences observed between marine and estuarine populations of *C. angulatus* are comparable with differences between *C. altimanus*, and *N. granulata*.

**KEY WORDS:** crabs, estuaries, intertidal, life history, reproduction

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### INTRODUCTION

The theory of life history evolution states that natural selection adjusts the partitioning of the energy budgets of organisms among reproduction, growth, and maintenance (Hirshfield and Tinkle, 1975). Consequently, one might expect that if more energy is used for maintenance, less is available for growth or reproduction. Marine coasts are especially suitable to test predictions based on this theory, since intertidal and/or estuarine areas have gradients for several environmental factors such as temperature, salinity, food supply, and air exposure. These gradients are mainly related to tidal levels in seashores and with distance to the sea in estuaries. Consequently, the costs of maintenance should vary along these gradients. Animals that live in the upper intertidal may experience a more prolonged daily air exposure and should divert more resources to regulate their osmotic, ionic, and thermal balances than those inhabiting the low intertidal or the subtidal (McDonald et al., 2006). On the other hand, the increase in respiration observed at low salinity in estuarine crabs may indicate greater costs due to osmoregulation, which are necessary for these animals to cope with intense, often unpredictable, salinity changes (Guerin and Stickle, 1997). In terms of energetic trade-offs, it is expected that less energy will be available for reproduction if more resources are allocated to maintenance.

Furthermore, there are additional costs of maintenance associated with reproduction, e.g., the metabolic cost of egg-bearing by ovigerous females (Taylor and Leelapiyanar, 2001).

Among intertidal animals, true crabs (Brachyura) have been excellent models to compare reproductive traits between contrasting habitats, e.g., tidal levels, estuarine and marine waters, and also along latitudinal and estuarine gradients. The comparisons have been carried on among populations of a single species, among species of a single genus, and among species of unrelated genera or families (Simons and Jones, 1981; Fukui and Wada, 1986; Kyomo, 1986, 2000; Seiple and Salmon, 1987; Fukui, 1988; Omori et al., 1997; Flores and Paula, 2002; Ituarte et al., 2006; Bas et al., 2007). The inter-specific comparisons may be useful to understand processes that involved habitat changes on an evolutionary timescale for a particular lineage, e.g., the conquest of freshwater and land by marine crabs. The grapsoid crabs have been especially successful invading freshwater and terrestrial environments (Anger, 1995), and the best example was provided by a “natural experiment”: the evolution to freshwater and terrestrial life in the Sesamididae endemic of Jamaica (Diesel et al., 2000, and references therein). Another grapsoid lineage that includes species from marine and freshwater habitats, the Varunidae (Yeo, 2007), has been less

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studied. On the other hand, the intra-specific comparisons allow for the examination of phenotypic plasticity found in many crab species living in habitat interfaces as the intertidal or estuaries (Weaver and Salmon, 2002; Brian et al., 2006).

The southwestern Atlantic coasts are inhabited by three intertidal grapsoid crabs, *Cyrtograpsus altimanus* Rathbun, 1914, *C. angulatus* Dana, 1851, and *Neohelice granulata* (Dana, 1851), with partially overlapped geographical and spatial distributions (Boschi, 1964). The three species are closely related phylogenetically and belong to the family Varunidae (Schubart et al., 2000; see Ng et al., 2008 for a systematic review of brachyuran crabs). Each species occupies a different habitat: *C. altimanus* is predominantly an intertidal and subtidal marine crab, *C. angulatus* occurs in the low intertidal and subtidal of estuarine and marine habitats, and *N. granulata* lives in the upper and middle intertidal of salt marshes and mud flats, especially in estuarine waters (Spivak, 1997). In fact, a single population of the last species may inhabit two contrasting types of habitats (frequently flooded mudflats in the middle intertidal and relatively dry saltmarshes in the upper intertidal); also, several differences in reproductive traits have been detected between them (Silva et al., 2009).

Habitat differences among the three species, between marine and estuarine *C. angulatus*, and between *N. granulata* inhabiting mud flats and salt marshes, made this group of crabs an interesting model to study both the life history evolution of the species within a lineage of varunids, and the intraspecific variability between contrasting environments. The aim of this study was to compare the reproductive traits of *N. granulata*, *C. angulatus*, and *C. altimanus* under the hypothesis that more energy is diverted to maintenance (and less to reproduction) in order to cope with the harsh, and frequently variable, environmental conditions of semiterrestrial and brackish water habitats. The specific predictions are that the breeding season would be shorter, the reproductive output per clutch would be lower and the clutch interval would be longer in the upper intertidal than in the low intertidal or the subtidal, and/or in estuaries than in marine waters. These differences would not only occur among the three species but also intraspecifically, between habitats of *N. granulata* and *C. angulatus*.

## MATERIAL AND METHODS

### The Species

*Neohelice granulata* (Dana, 1851) (= *Chasmagnathus granulatus*, see Sakai et al., 2006) inhabits estuaries and salt marshes of the temperate southwestern Atlantic, from Río de Janeiro, Brazil, to Golfo Nuevo, Argentina (Spivak, 2010). Its maximum carapace width (MCW) is 40 mm (Spivak et al., 1994). This semiterrestrial crab digs burrows, sometimes deep and ramified, in two contrasting microhabitats: tidal flats and salt marshes (Boschi, 1964; Spivak et al., 1994). This is the most studied crab species in the region (Spivak, 2010) and many reproductive traits were described in several sites through its geographical range (Ruffino et al., 1994; Spivak et al., 1996; Stella et al., 1996; Luppi et al., 1997; López Greco and Rodríguez, 1998, 1999; Ituarte et al., 2004, 2006; Bas et al., 2007; Barutot et al., 2008; Gregati and Negreiros Fransozo, 2009). Burrow size and shape,

the frequency and duration of floods, physical and chemical characteristics of the water covering burrows during floods, as well as crab feeding and activity, varies between microhabitats (Silva et al., 2007, 2009 and references thereof).

*Cyrtograpsus angulatus* lives both in the Pacific (Peru and Chile) and the Atlantic (from Río de Janeiro, Brazil, to Santa Cruz, Argentina) coasts of South America (Spivak, 1997). This relatively large (MCW = 60 mm; Spivak et al., 1994) epibenthic species inhabits rocky, sandy or muddy substrata in marine and estuarine waters. Several reproductive traits were described in different sites along the Atlantic coasts (Cracco and Fontoura, 1996; Spivak et al., 1996; Luppi et al., 1997; Silva et al., 2003; López Greco and Rodríguez, 2004).

*Cyrtograpsus altimanus* has a more restricted geographical distribution in the temperate southwestern Atlantic, from Río Grande do Sul, Brazil, to Puerto Madryn, Argentina (Spivak, 1997; Lezcano, 2008). This smaller species (MCW = 20 mm; Lezcano, 2008) mainly inhabits marine coasts (occasionally the mouth of estuaries), occupying rocky intertidal and subtidal areas rich in refuges. This is the least studied species regarding reproductive traits (Gavio, 2003; López Greco and Rodríguez, 2004).

### Collection Sites

Crabs were collected in Mar Chiquita lagoon (37°45'S, 57°19'W) and in Mar del Plata Harbor (38°03'S, 57°31'W). Mar Chiquita lagoon is connected with the open sea through a narrow channel about 5 km long (Fig. 1). The freshwater input comes from small rivers and artificial channels. Semidiurnal tides (0.3-1 m amplitude) influence water salinity in an extremely variable way depending on the wind direction and intensity, tidal phase, and freshwater runoff (Reta, 2001). For example, in a December afternoon, salinity decreased from 35 to 6 in 6 h near the mouth of the lagoon (Spivak et al., 1994). The subtidal and low intertidal are bare mud flats; the relatively dry upper intertidal is a salt marsh dominated by *Spartina densiflora*; saltmarshes are flooded only 1-2 times per week, while the intertidal mud flats are inundated 1-2 times per day (Silva et al., 2009). Samples were obtained in three habitats: salt marshes, mud flats and subtidal grounds. The other sampling location was Mar del Plata Harbor (Fig. 1), an oceanic harbor limited by two artificial breakwaters including a commercial sector (the main activity is deep-sea and coastal fishing), an Argentine Navy base, and facilities for nautical sports. The tidal regime is semidiurnal (0.5-1.6 m amplitude) and salinity ranges from 33.5 to 34 (Martos et al., 2004). Samples were obtained in an intertidal rocky area.

### Sampling Procedures

Thirty morphologically mature females (characterized by a wide pleon that reaches the coxae of pereopods) were caught monthly by hand in the following sites and periods: *N. granulata*, Mar Chiquita salt marshes and mud flats, from September 2004 to April 2006; *C. angulatus*, Mar Chiquita subtidal, from July 2002 to May 2005, and Mar del Plata Harbor, from May 2003 to May 2005; and *C. altimanus*, Mar del Plata Harbor, from May 2003 to June 2005. All samples were taken during diurnal low tides. *N. granulata* were obtained from their burrows, a procedure that was suc-

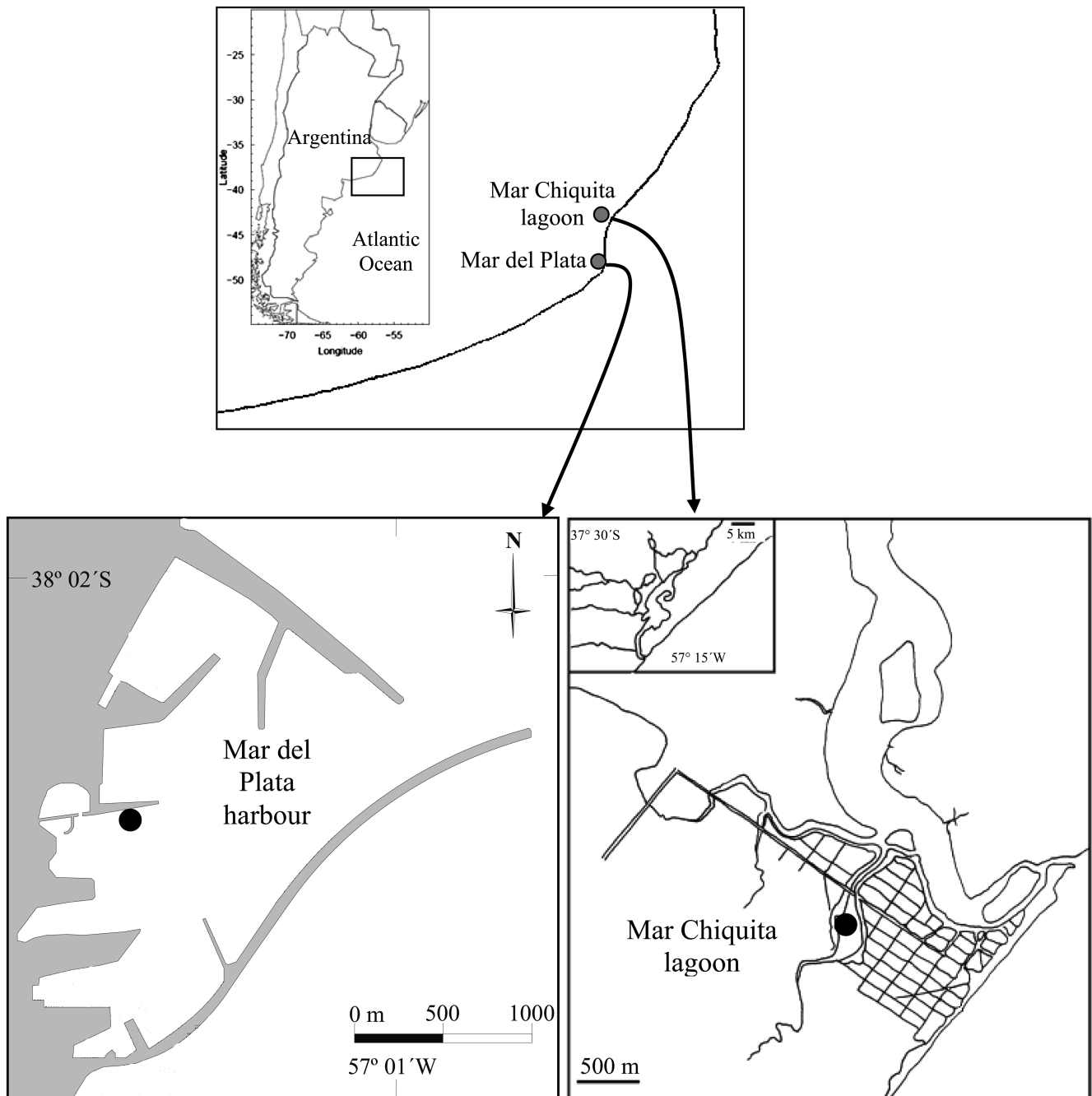


Fig. 1. Study area.

cessfully used in previous studies (Ituarte et al., 2004, 2006). Mar Chiquita *C. angulatus* were collected while moving in the shallow subtidal (>50 cm depth) and both *Cyrtograpsus* from Mar del Plata Harbor were found under boulders. Data of air and water temperature for Mar del Plata coasts were obtained from the Servicio Meteorológico Nacional and from the Servicio de Hidrografía Naval (Argentina) respectively.

#### Laboratory Procedures

Each female was transported to the laboratory, frozen to death at  $-10^{\circ}\text{C}$ , measured (carapace width) with a Vernier

caliper (precision 0.1 mm) and dissected. Few embryos were extracted from the ovigerous females in order to establish the degree of embryonic development on the basis of the following arbitrary scale:

E1 or initial embryos, recently laid, with uniform vitellum that occupied from 100 to 80% of egg volume and without ocular pigment.

E2 or intermediate embryos with 80 to 30% of egg volume occupied by vitellum and with eye primordia visible as a red line.

E3 or late embryos with less than 30% of egg volume occupied by vitellum and eyes well formed.

The ovaries of all females were dissected, examined and classified in one of the following 5 stages:

O1: filiform and translucent; pale grey.

O2: both ovarian filaments tubular and narrow; orange, red or brown.

O3: both ovarian filaments tubular, wider but without differences between the anterior and the posterior part; sometimes granular; brown to violet.

O4: the anterior part expanded, not covering neither the cardiac stomach nor the hepatopancreas; the posterior part with both filaments already visible; always granular orange to dark violet.

O5: the entire organ expanded, covering the cardiac stomach and the hepatopancreas; always granular; burgundy to dark violet.

The percentage of females with ovaries in each stage was calculated.

Ovaries and hepatopancreas of females were carefully dissected, dried 48 h at 80°C, and weighed individually to 0.0001 g; the exoskeleton and remaining organs were also dried 48 h at 80°C and weighed individually to 0.01 g. Eggs of ovigerous females were previously removed. The gonadosomatic and hepatosomatic index (GSI and HSI) were calculated as the ratio between the dry weight of the organ and the dry weight of the whole crab, including the organ, and expressed as a percentage. The reproductive output was calculated as the ratio between the dry weight of the egg mass and the dry weight of the mother, without eggs, and expressed as a percentage (Hines, 1982).

#### Data Processing and Statistical Methods

The breeding season and the non-breeding season were defined on the basis of the presence or absence of ovigerous females, respectively. The differences in the monthly proportions of ovigerous females between consecutive breeding seasons were tested by the Z test (Zar, 2009). The significance of differences in reproductive output was tested by one-factor ANOVA. When a significant effect was detected, the Tukey test for multiple comparisons was applied (Zar, 2009). The frequency of females with ovaries in each level of development was compared by a log-lineal analysis (Norman and Streiner, 1996; Zar, 2009), with species and developmental stage of embryos mass as factors. The significance of differences in GSI and HSI among ovigerous females carrying eggs in the 3 embryonic stages and the non ovigerous females of both breeding and non breeding season were tested by one-factor ANOVA for each species and habitat. When a significant effect was detected, the Holm-Sidak multiple comparison was applied (Sokal and Rohlf, 2001). In some cases, normality or homoscedasticity assumptions were not met despite arcsin or logarithmic transformations; the analysis of variance, however, is robust to heterogeneity of variances and non-normality, particularly with large samples in each treatment (Underwood, 1997).

#### RESULTS

Reproduction is seasonal in the three species studied, with ovigerous females present mainly in spring and summer;

however, strong differences were observed among years. The annual breeding cycle was related to the environmental temperature (Fig. 2). Water temperature was higher than air temperature from February to July (from midsummer to midwinter), at least in Mar del Plata, but both values were similar during most of the breeding season (August to January; Fig. 2). The breeding season started earlier, and was longer, in *Cyrtograpsus* spp. (9-11 months) than in *N. granulata* (7 months). In Mar del Plata Harbor, the percentage of ovigerous females of *C. altimanus* and *C. angulatus* rapidly increased in July, when water and air temperature were minimal (air: 7.9°C; water: 9.3°C), reaching the maximum value one month later. Ovigerous *C. angulatus* from Mar Chiquita were present even earlier, in May, they reached higher percentages (ca. 50%) in August and a maximum value in October. Ovigerous *N. granulata* appeared in September, when temperature began to rise (both habitats; air: 10.2°C; water: 10.9°C) and reached a maximum value in October. Ovigerous females of all species and habitats disappeared at the same time in April, after a strong temperature reduction (air: from 18.5 to 14.9°C; water: from 20 to 16.7°C; Fig. 2).

The reproductive output differed among species and habitats (Fig. 3;  $F = 78.36$ ,  $p < 0.001$ ). The highest value was observed in *C. altimanus* (10.7) followed by *C. angulatus* from Mar del Plata Harbor (8.14). The lowest value was observed in *C. angulatus* from Mar Chiquita (5.36); the values of *N. granulata* were slightly greater, with no differences between the mudflat and salt marsh crabs.

Overlapping between ovarian and embryonic development differed between species and habitat (Fig. 4, ovarian development  $\lambda^2 = 835.8$ ,  $p < 0.001$ , interaction ovarian development vs. species  $\lambda^2 = 231.9$ ,  $p < 0.001$ , interaction ovarian development vs. embryonic development  $\lambda^2 = 619$ ,  $p < 0.001$ ). The proportion of ovigerous females with late embryos (E3) with ripe ovaries (O5) was higher in *C. altimanus*, lower in *C. angulatus* from Mar del Plata, and null in *C. angulatus* from Mar Chiquita and in *N. granulata* (both habitats). However, the last 3 cases strongly differed: almost half of the females of *C. angulatus* with late embryos had stage O4 ovaries whereas ovigerous *N. granulata* never surpassed ovarian stage O3. On the other hand, Mar Chiquita non ovigerous females frequently reached stage O4 in all species and sites (and occasionally stage O5), both in the breeding and non breeding seasons (Fig. 4).

The gonadosomatic index varied among non-ovigerous and ovigerous females with embryos in different stages in all species and habitats ( $p < 0.001$ ). It increased during all the embryonic development in *C. altimanus* and from E1 to E2 in *C. angulatus*, but remained low in *N. granulata* (Fig. 5). In the latter species and in *C. angulatus* from Mar Chiquita, the GSI of non ovigerous females was higher in the non breeding season than in the breeding season; in *N. granulata* it was also higher in non ovigerous than in ovigerous females. Instead, in *C. altimanus* and *C. angulatus* from Mar del Plata, the GSI of non ovigerous females was higher in the breeding season and always lower than in ovigerous females with advanced embryos.

In all species and habitats ( $p < 0.001$ ), except in *C. altimanus*, the hepatosomatic index varied among non-

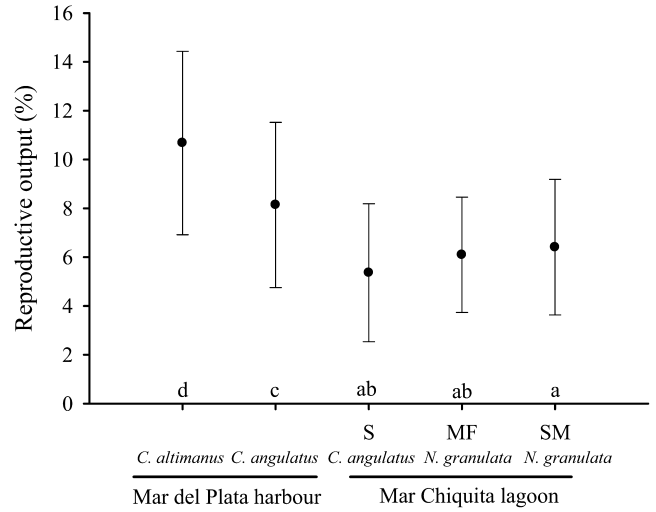
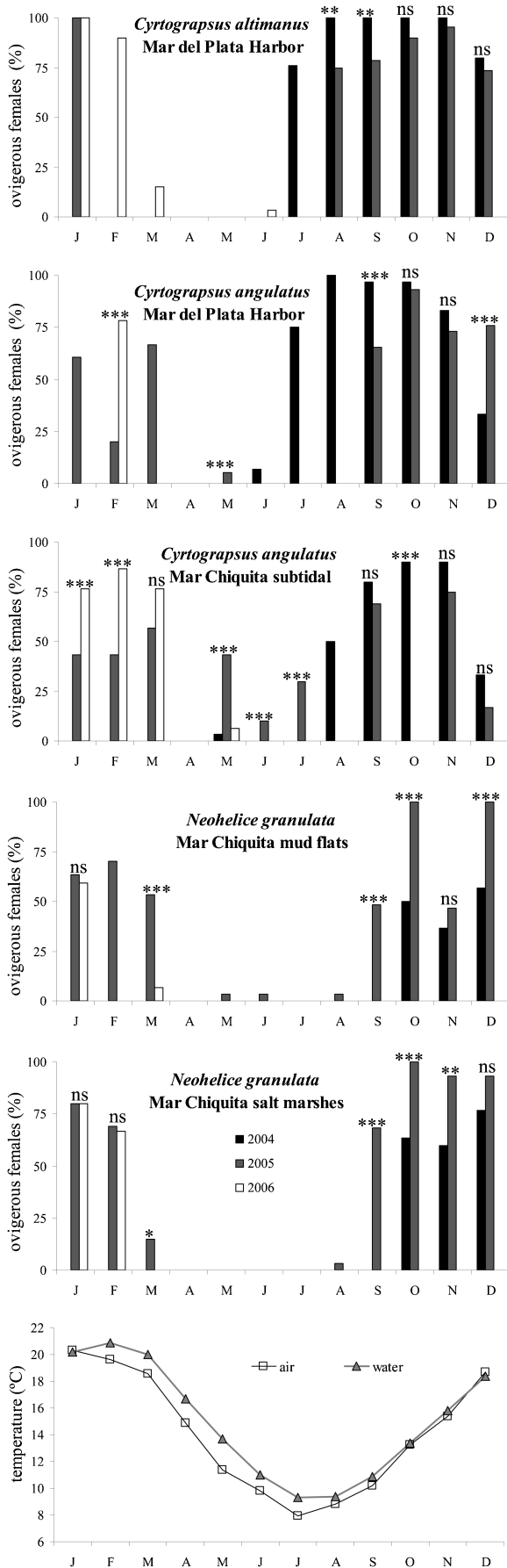


Fig. 3. Reproductive output (mean  $\pm$  standard deviation) of *Cyrtograpsus altimanus* and *C. angulatus* from Mar del Plata Harbor, *C. angulatus* from Mar Chiquita Lagoon subtidal grounds (S) and *Neohelice granulata* from Mar Chiquita Lagoon salt marshes (SM) and mud flats (MF), calculated as the ratio between dry weight of egg mass and dry weight of mother, without eggs and expressed as a percentage. Different letters (a-d) indicate significant differences according to the Tukey test for multiple comparisons after one-factor ANOVA.

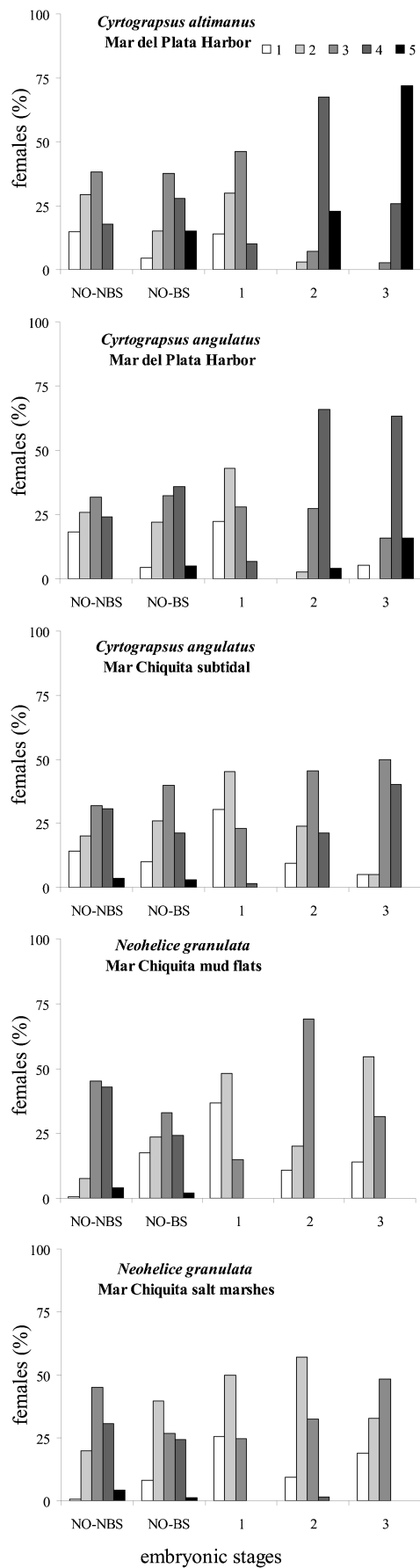
ovigerous and ovigerous females with embryos in different stages. In *C. angulatus* from Mar del Plata, the HSI varied without a clear pattern, but in Mar Chiquita crabs (*C. angulatus* and *N. granulata*) it was higher in non ovigerous females, especially in the non-breeding season (Fig. 5).

DISCUSSION

Evidence presented here suggested the existence of habitat related variation in reproductive traits among the three species of intertidal crabs that live in marine and/or estuarine shores of the southwestern Atlantic. These traits were: length of the breeding season, reproductive output per clutch, clutch interval associated with differences in the degree of overlapping between ovarian and embryonic development, and mass relationships between ovary and hepatopancreas. The observed variation agreed with the hypothesis that if more energy is used for maintenance, as supposedly occurs in estuarine and more terrestrial habitats, less energy is available for reproduction. Reproductive traits of *N. granulata* did not differ between mud flats and salt marshes, as was expected on the basis of previous studies (Silva et al., 2009); consequently, the results from both habitats were considered together in this section.

As it occurs with most decapods of temperate littoral areas, the four species had a well-defined breeding season that coincided with the higher temperatures, and a winter period with less or none ovigerous females. The reproduction of tropical and sub-tropical marine species, instead, is continu-

Fig. 2. Breeding periods of *Cyrtograpsus altimanus* and *C. angulatus* from Mar del Plata Harbor, *C. angulatus* from Mar Chiquita Lagoon subtidal grounds and *Neohelice granulata* from Mar Chiquita Lagoon salt marshes and mud flats, and annual variation of sea and air temperature in Mar del Plata. Asterisks indicate significant differences in monthly proportions of ovigerous females between consecutive breeding seasons after a Z test.



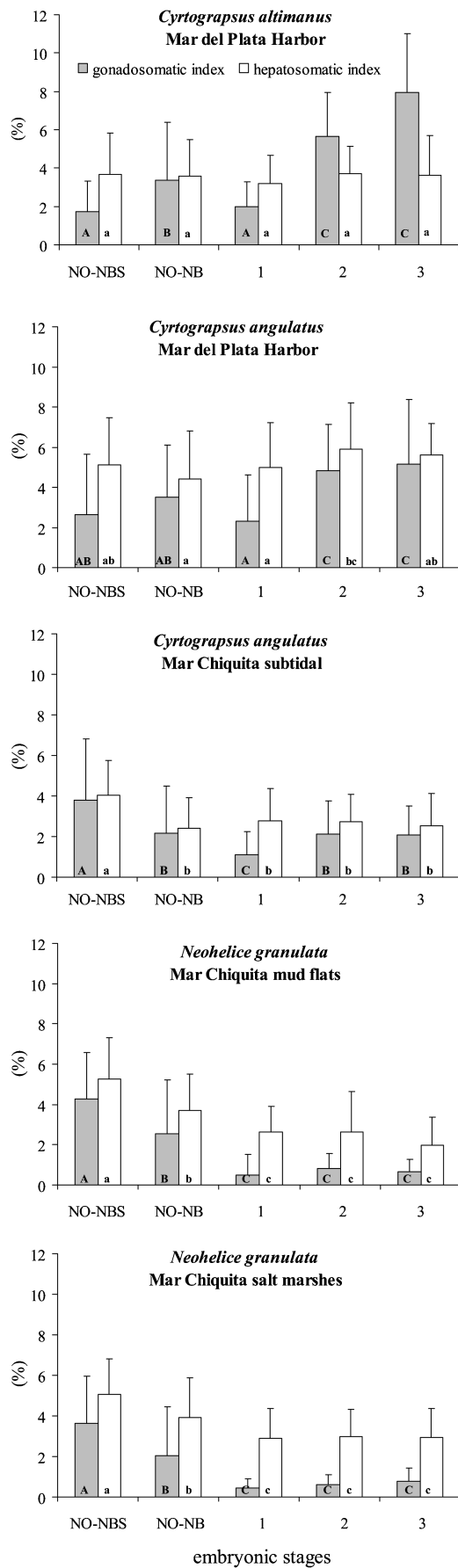
ous throughout the year (Bauer, 1989) and, in fact, ovigerous females of *C. angulatus* and *N. granulata* had been observed all year round in populations of these species that inhabit lower latitudes (*C. angulatus*: Tramandai estuary, 29°59'S, Cracco and Fontoura, 1996; *N. granulata*: Jabaquara Beach, 23°13'S, Gregati and Negreiros Fransozo, 2009). On the other hand, the breeding season of a southern population of *N. granulata* (San Antonio Bay, 40°46'S) was shorter than in Mar Chiquita (from October to February) indicating a latitudinal trend for this species (Ituarte et al., 2006).

Interspecific differences in length of the breeding period were observed. The winter resting period was longer in the more terrestrial species *N. granulata*, as was expected from the available literature: the negative correlation between length of the breeding season and height in the shore was described by Sastry (1983), and later confirmed in different species (Emmerson, 1994). This trend may be explained by a complex interaction of endogenous and exogenous factors (Sastry, 1983) and the temperature differences between air and water observed in winter (Fig. 2) may have a direct and indirect influence, through the feeding activity.

There is plenty of information on the reproductive output per clutch of crabs that allows some generalizations: brood size is constrained to about 11% of female body weight by the space available within the cephalothorax, although there are some exceptions, as in pinnotherids (Hines, 1982, 1992; reviewed by Hartnoll, 2006). Values calculated here ranged from 5.5 to 10.6 depending on species and habitat. In all cases, these values were lower than previously reported data both in estuarine (including Mar Chiquita) and marine habitats (Stella et al., 1996; Luppi et al., 1997; López Greco and Rodríguez, 2004; Silva et al., 2009). However, it should be considered that the reproductive output of *N. granulata* varies markedly between the beginning and the end of the breeding season, between consecutive years and between populations (Bas et al., 2007) and the data presented here included the complete season.

As it occurred with the length of the breeding season, evident differences between habitats were observed in reproductive output (representing clutch size relative to female size). Lower values observed in the estuarine habitat of Mar Chiquita (Fig. 3) could be explained by the needs of more energy to cope with low and variable salinities and temperatures (*C. angulatus* and *N. granulata*) and to aerial life (*N. granulata*). On the other hand, higher values observed in the marine habitat could be the result of more energy available for growth and/or reproduction. In Mar del Plata, the difference in reproductive output between both *Cyrtograpsus* (Fig. 3) could also be explained in terms of energy budget: *C. altimanus*, the smaller species, would invest more in reproduction and less in growth than *C. angulatus*. In fact, although the relationship between body size and crab instar was similar between juveniles of both species in a labora-

Fig. 4. Proportion of female crabs of *Cyrtograpsus altimanus* and *C. angulatus* from Mar del Plata Harbor, *C. angulatus* from Mar Chiquita Lagoon subtidal grounds and *Neohelice granulata* from Mar Chiquita Lagoon salt marshes and mud flats, in different ovarian stages (1-5, see text). Ovigerous females with embryos in different stage of development (1-3, see text) and non-ovigerous females collected during both reproductive (NO-BS) and non-reproductive (NO-NBS) seasons, were separately considered.



tory study, females of *C. altimanus* reached the maturity size (~6 mm) at instar 6, and females of *C. angulatus* at a larger size (~20 mm) and after instar 11 (Spivak 1999). The competition for resources between reproduction and molt was demonstrated in many decapod crustaceans (Nelson, 1991; Raviv et al., 2008).

A female crab can produce several broods during a breeding season, sometimes after a single mating (Morgan et al., 1983). The number of broods produced during the season is another reproductive trait that shows high interspecific variability in crabs (Hines, 1982). An indirect evidence of the ability of species to produce several broods per year is the so-called "clutch interval". The simultaneous development of ovary and embryos allows a reduction of this interval to a minimum and the production of a new clutch soon after the previous one has hatched. A shorter clutch interval may be directly related to a higher number of broods per season.

Clutch intervals varied according to habitat: they were minimum in the more marine species, *C. altimanus*, and maximum in the more terrestrial and estuarine one, *N. granulata*. In the former, ovary morphology (Fig. 4) and an increase in the gonadosomatic index (Fig. 5) showed that these gonads had completed their development when embryos were ready to hatch; in the latter, ovaries reached only stage O3 and the gonadosomatic index did not increase during embryonic development. On the contrary, the non ovigerous females had more developed ovaries and higher gonadosomatic index in *N. granulata* than in *C. altimanus* (Figs. 4 and 5). The clutch interval was shorter in the marine population of *C. angulatus* than in the estuarine one. In the sea, most ovigerous females with late embryos had stage O4 ovaries and some also reached stage O5; in the estuary, a similar proportion of these females had ovaries in stages O3 or O4 and none reached stage O5 (Fig. 4); the gonadosomatic index increased during embryonic development in the former habitat but not in the latter, indicating a simultaneous development of ovaries and embryos and the possibility of a shorter clutch interval (Fig. 5).

Trade-offs between brood size and brood interval had been reported for various animals (Forsman, 2001; Wada et al., 2008). Henmi (2003) demonstrated the existence of such trade-off among fiddler crabs of three subgenera of *Uca* that differ in habitat and feeding behavior during brooding. Specifically, ovigerous females that feed actively during incubation had shorter brood intervals (Henmi, 2003). Results of the present study showed that *C. altimanus*, the species with the lowest fecundity (López Greco and Rodríguez, 2004), had the shorter brood interval, and also had the highest reproductive output; the other two species, that carried similar number of eggs (Luppi et al., 1997), differed in the brood interval. A decrease of feeding activity was ob-

Fig. 5. Gonadosomatic index (GSI) and hepatosomatic index (HSI) of female crabs of *Cyrtograpsus altimanus*, *C. angulatus* from Mar del Plata Harbor, *C. angulatus* from Mar Chiquita Lagoon subtidal grounds and *Neohelice granulata* from Mar Chiquita Lagoon salt marshes and mud flats. Ovigerous females with embryos in different stage of development (1-3, see text) and non-ovigerous females collected during both reproductive (NO-NBS) and non reproductive (NO-NB) seasons, were separately considered. Different letters indicate differences in GSI (A-C) and in HIS (a-c) among females after one-factor ANOVA and Holm-Sidak multiple comparisons applied to each species and habitat.

served in the field in ovigerous females of *N. granulata*, the species with the longest brood interval, (Claudia Bas, pers. comm.); unfortunately no information is available on the feeding behavior of females in *Cyrtograpsus* spp. In future studies, the system *C. altimanus* – *C. angulatus* – *N. granulata* will be helpful to enhance knowledge on the relationship between feeding during incubation and brood.

Female crabs obtain materials and energy for ovarian development directly from ingested food or indirectly through reserves, mainly accumulated in the hepatopancreas (Nelson, 1991). The second strategy should be detected when an increase in the hepatosomatic index precedes an increase in the gonadosomatic index or when there is a negative correlation between HSI and GHI (Kyomo, 1988; Omori et al., 1997; Spivak et al., 2010). These trends were not observed in the studied species. Values of the HSI neither varied between non-ovigerous and ovigerous females of *C. altimanus* nor during the ovarian development that took place simultaneously with the embryonic development, when the GSI increased continuously. On the other hand, HSI values were higher in non-ovigerous females than in ovigerous females of *N. granulata*, especially during the non breeding season; in these non ovigerous females, characterized by a high GSI (Fig. 5), ovarian development could be completed. The low values of HSI in ovigerous females of *N. granulata* could be explained by a decrease of their feeding activity; this decrease was probably related to an increase in the time passed in the more stable and less stressful environmental conditions inside burrows which are better suited for embryonic development (Silva et al., 2009). Again, the pattern of HSI variations observed in *C. angulatus* depended on the habitat, being more similar to *C. altimanus* in marine populations, and to *N. granulata*, in estuarine populations. The hypotheses of a continuous feeding in ovigerous females of the marine species, as opposed to the apparent reduced activity of the estuarine one, should be addressed by a sampling of stomach content of the former.

Summarizing, observations reported in this paper suggest that the metabolic demands imposed by the harsh environmental conditions of the estuarine and semiterrestrial habitats, e.g., osmoregulation and aerial exposure, result in less energy available for reproduction in *N. granulata* evidenced by a shorter breeding season, a reduced reproductive output per clutch and an increased clutch interval. The opposite happened with *C. altimanus*: less energy is needed to cope with the osmotic and thermal stability allowing for the presence of a long breeding season, a higher reproductive output per clutch and a very short clutch interval. Differences observed between marine and estuarine populations of *C. angulatus* are consistent with differences between *C. altimanus*, and *N. granulata*.

There are several intra and interspecific comparisons of crab reproductive traits between habitats, but the results are not consistent. For example, the length of the breeding season was longer in the marine than in the estuarine population of one species but did not differ among populations in another (Kyomo, 1986, 2000; Simons and Jones, 1981, respectively). However, interspecific surveys showed that estuarine species had longer breeding periods in the most stressful and fluctuating habitats (Fukui and Wada, 1986; Omori

et al., 1997), but the opposite was observed in this work. On the other hand, the reproductive output may be similar among species that live at different heights in the shore (Seiple, 1979; Fukui and Wada, 1986; Seiple and Salmon, 1987; Flores and Paula, 2002), even between both species of *Cyrtograpsus* in a southern marine habitat (López Greco and Rodríguez, 2004). Finally, the number of clutches per year was higher in the more terrestrial habitat of several species (Seiple, 1979; Fukui and Wada, 1986; Seiple and Salmon, 1987; Flores and Paula, 2002). Instead, results in this study suggested the opposite trend regarding the reproductive output and the annual number of clutches, the latter on the basis of the degree of overlapping in breeding and ovarian development.

When the length of the breeding season, the reproductive output (egg production per clutch) and the number of broods per season are considered together, it is possible to obtain an idea of the reproductive effort (annual egg production). Data collected in this study do not match with the findings of other authors regarding a direct relationship between fluctuating and unstable habitat and high reproductive effort (Fukui and Wada, 1986). On the contrary, they support the role of energetic constraints that may explain why a higher reproductive effort corresponded to the relatively more stable habitat.

The big problem that still remains unsolved is how the relatively minor annual egg production of *N. granulata* matches the presence of such dense populations of adult crabs observed in Mar Chiquita lagoon (Spivak et al., 1994), a species well adapted to estuarine conditions. The answer is likely related to juvenile mortality. Despite the different habitats of adults, zoea larvae that hatch from eggs of the three species develop in coastal waters (Anger et al., 1994; Bas et al., 2009). Although no information on zoeal mortality is available at present, no interspecific differences are expected for larvae of similar size and shape, living together in plankton patches. Instead, a differential mortality should be expected during the return of megalopae to the parental habitat near metamorphosis. Megalopae of *N. granulata* and *C. angulatus* should find the narrow entrance of Mar Chiquita lagoon, a rather difficult task, whereas those of the marine species or populations could settle in many habitats along seashores; however, this bottleneck would decrease recruitment in the estuarine habitat. Consequently, it is necessary to consider mortality after metamorphosis and recruitment: burrows of *N. granulata* and reefs of *Ficopomatus enigmaticus* facilitate settlement of both Mar Chiquita species and protect early juveniles from predators; late juveniles of *N. granulata* and *C. angulatus* dig their own burrows or migrate to the subtidal, respectively (Luppi et al., 2002; Méndez Casariego et al., 2007), but the second species should face a new bottleneck when leaving the recruitment habitat (Méndez Casariego et al., 2004). Finally, after a successful recruitment and a low juvenile mortality, *N. granulata* inhabits a typical estuarine environment with high production and low competition (Olivier et al., 1972a, b; De Marco et al., 2005) that would contribute to explain the existence of dense populations.



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