Great unexpected differences between two populations of the intertidal crab *Neohelice granulata* inhabiting close but contrasting habitats (Crustacea: Decapoda: Brachyura)

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ABSTRACT. Two populations of the Southwestern Atlantic burrowing crab, *Neohelice granulata* (Dana, 1851), are separated by only 190 km. They inhabit bays that drain into a Patagonian gulf in the southernmost limit of the geographical distribution of the species: San Antonio Bay (SAO) and San José Gulf (RSJ). The population from SAO has been extensively studied, whereas that of RSJ has not. The main goal of this study is to compare the ecological and habitat characteristics of the two populations, based on samples collected on successive days during the middle of the reproductive season of the crab. The substrate of SAO is composed of a high proportion of gravel and scarce organic matter, whereas the substrate of RSJ presents twice as much silt and clay, and greater organic matter content. Crabs of both sexes were larger at RSJ. Fecundity and reproductive output were greater at RSJ. The size of females at maturity was smaller and their range was shorter at RSJ. Burrows were longer and wider at RSJ. Our results indicate that there are strong differences between the two populations, which might be correlated with local environmental differences, mainly substrate granulometry and food availability.

KEY WORDS. Argentine, estuaries, crab, fecundity, feeding, growth, maturity, sediment.

In crabs, size and morphology, physiology, life history and reproductive biology of larvae and adults are often affected by latitudinal gradients of temperature (e.g., Barria et al. 2014, Lagos et al. 2014, Kelley et al. 2015, and cites thereof). However, variability among populations of a crab species cannot always be explained by such gradients. In such cases, other environmental conditions that vary among habitats (salinity, photoperiod, and food availability) have to be considered (e.g., Ituarte et al. 2006, Bas et al. 2007, Silva et al. 2009). Herein we document, for the first time, strong differences in size, reproductive biology and burrow structure between two populations of the burrowing estuarine crab *Neohelice granulata* (Dana, 1851). These populations live in close but contrasting habitats in the southern limit of the geographic distribution of the species.

*Neohelice granulata* (formerly known as *Chasmagnathus granulatus*) is a key species in South American salt marshes, mudflats, and estuaries, which range from northern Patagonia, in Argentina, to the state of Rio de Janeiro, in Brazil (Fig. 1). The species is also an important model for ecology, physiology, neurophysiology and behavior studies (Spivak 2010). The Southwestern Atlantic burrowing crab has a complex life cycle with benthic adult and juvenile stages, and a planktonic phase (four or five zoeae and one megalopa stage). Zoeae from estuarine populations are exported to the sea, or to environments with moderately reduced (polyhaline) salinities, remaining close to the parental population and near the coastline (Anger et al. 1994, Bas et al. 2009); megalopae, in turn, re-immigrate to brackish and even limnetic habitats (Luppi et al. 2002, Anger et al. 2008). Juveniles and adults feed on saltmarsh plants and sediments, and have a disjunct geographical distribution, always associated with the halophytic grass *Spartina* spp. They inhabit areas with highly variable tidal range, salinity, temperature, sediment and biotic factors. As a consequence, they face many challenges, for instance: from a few cm up to 9 m semidiurnal tide amplitudes, which also vary in predictability; from near 0 up to 60 water salinities; from soft to hard substrata (muddy and cobble beaches, respectively), from high to low quality food and, also, to different predation pressures. In addition, these crabs live in mudflats and salt marshes placed at different intertidal levels, where adults of both sexes and juveniles are intermixed, with various emersion-submersion patterns, burrow size, shape and dynamics, physical and chemical characteristics of water inside them, feeding habits and activity (Luppi et al. 2013, Bas et al. 2014 and cites thereof). High levels of genetic structure were detected among populations of *N. granulata*.
inhabiting the complete geographic range of the species, indicating that connectivity between adjacent sites may be limited to scales of hundreds of kilometers. This, however, conflicts with morphometric analysis, which did not show a clear geographical pattern of morphological differentiation (although there were some differences among sites) (Iturarte et al. 2012). Life history traits, activity and feeding differed among several well-studied Argentinean habitats included in the genetic analysis (Iturarte et al. 2006, Bas et al. 2005, 2007, 2008, 2014, Silva et al. 2009, Luppi et al. 2013).

Sediment characteristics, crab density and size, reproductive traits and burrow dimensions were studied from samples collected in the middle of the reproductive season of N. granulata (22 and 23 January 2013, respectively) in Riacho San José (42°25.5'S, 64°36'W) and San Antonio Oeste (40°46'S, 64°50'W). The timing was selected in order to avoid the effect of variations in reproductive parameters during the entire season, which had been previously observed in the population from San Antonio Oeste (Bas et al. 2007). Both sites are located in two bays that drain into the much larger San Matías Gulf in northern Patagonia, San José Gulf and San Antonio Bay respectively, and are separated by ca. 190 km (Fig. 1). The pattern of water circulation at the San Matías Gulf (Rivas & Beier 1990, Amoroso et al. 2011, Crespi-Abril et al. 2014) suggests that the populations of N. granulata in both sites are likely connected by larval transport. San Antonio Oeste (SAO) is a large intertidal area (143 km²) placed in the western bank of San Antonio Bay and is characterized by extensive sand-cobble intertidal flats; Riacho San José (RSJ) is a smaller intertidal area (2 km²) located in the southwestern extreme of San José Gulf; this is the southernmost population of this species registered so far. The life history, reproductive cycles and behavior of N. granulata in SAO are well known (Iturarte et al. 2006, Bas et al. 2005, 2007, 2008, 2014, Sal Moyaño et al. 2012, Luppi et al. 2013) but the population biology of this crab in RSJ has not been previously studied. These two sites have similar climatic and hydrographic characteristics: tidal amplitude and predictability, mean water temperature from November to April, a period that includes the whole reproductive season (Fig. 1, redrawn from Rivas 1990), scarce rains, absence of freshwater input and, consequently, high and constant salinity (Rivas, 1990, Rivas & Beier 1990). However, it differs in sediment granulometries (Bas et al. 2005), vegetation composition, and distribution (Isacchi et al. 2006).

Crabs from SAO and RSJ are genetically alike, although they differ genetically from their conspecifics at all other sites studied within the wide geographic distribution of N. granulata (Iturarte et al. 2012). Despite their genetic similarity, crabs from the two populations have marked morphometric differences (Iturarte et al. 2012) and according to preliminary observations, there are dramatic differences in their maximal size.

The organic matter content (ignition loss after five hours at 500°C; three replicate measurements) of the sediment surface (5 mm thickness) was measured. To determine the granulometric composition, samples from the 20 cm upper layer of soil were taken, dried at 60°C to weight constancy, sieved, sorted into three major fractions (gravel: >2.0 mm; sand: from 0.062 to 2 mm; and silt combined with clay: <0.062 mm), following the terms of the Wentworth scale (Wentworth, 1922), and weighted.

Crab samples consisted of all N. granulata individuals present at the surface and in burrows in 15-20 areas delimited by square metal frames (0.25 m²), randomly placed along transects parallel to the shore, in the salt marsh and in the mudflat, at each site (total: 75 areas). Crabs on the surface or from burrows were collected following Luppi et al. (2013). All crabs were measured to the nearest 0.1 mm with a Vernier caliper (carapace width,
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Crab density was higher in saltmarsh than in mudflat in both sites and higher in mudflat in SAO than in RSJ; it did not differ between salt marshes in spite of the higher proportion of gravel found in SAO, which may cause difficulties for digging, and the lower content of organic matter (SAO saltmarsh: 21.85 ± 5.31 individuals/0.25 m$^2$; RSJ saltmarsh: 29.50 ± 11.93 individuals/0.25 m$^2$; SAO mudflat: 8.93 ± 2.87 individuals/0.25 m$^2$ and RSJ mudflat: 4.36 ± 2.36 individuals/0.25 m$^2$; Two way ANOVA: interaction $F_{1,14} = 23.8$, $p < 0.001$). Differences between mudflat densities could be explained by a recruitment event inferred for SAO (see below). Interestingly, the maximum mean densities ever recorded for *N. granulata* correspond to both salt marshes: 120 crabs/m$^2$ (RSJ; this study) and 136 crabs/m$^2$ (SAO; Bas et al. 2005), confirming the observed trend of increased values towards the southern limit of its geographical range (Bas et al. 2005).

Crabs were smaller in SAO than in RSJ; the maximum CW of males registered during this study was 32.8 mm in SAO and 38.3 mm in RSJ, whereas the maximum CW of females was 29 mm in SAO and 35.5 mm in RSJ. Consequently, the maximum size of SAO crabs was 85.6% (males) and 81.7% (females) of RSJ values, although a female measuring 39 mm CW was caught in a preliminary survey in the latter (Luppi not published data). In fact, RSJ crabs are the largest ever recorded for *N. granulata*, even taking into account the wide knowledge about the life history of MC and SAO populations.

Size frequency distributions of sexually differentiated crabs illustrated the marked size differences between SAO and RSJ: an additional modal class (5th) was detected in RSJ mudflat males and the 4th modal class of females, corresponded to relatively larger crabs (Figs. 2-5, Table 1). The proportion of adult males and females larger than 30 mm CW was ca. 30% in RSJ, but only 8% of males (and no females) in SAO. In addition, the two smaller modal classes (1st and 2nd) of both males and females were not found in the mudflat of RSJ. The proportion of undifferentiated crabs was higher in SAO (n = 81, 28.4%) than in RSJ (n = 14, 4.2%) and may represent a recent event of recruitment only in the former site.

The size range of females at morphological maturity differed between sites, being larger in SAO (14.2-24.9 mm CW) than in RSJ (14.5-18.9 mm CW), and points of morphometric maturity were 20 ± 0.12 mm CW and 17 ± 0.91 mm CW respectively. Consequently, the relative size of females at maturity, estimated according to Hibose et al. (2012) also differed: 0.61 and 0.45, in **Table 1.** Modal components of size frequency distributions of males and females from mudflats and salt marshes of San Antonio Oeste (SAO) and Riacho San José (RSJ) estimated fitting observed data to the expected values of a mixture of normal distributions by the least squares method (see Material and Methods).

<table>
<thead>
<tr>
<th>Site</th>
<th>Habitat</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
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<td>4</td>
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<tr>
<td>SAO</td>
<td>Saltmarsh</td>
<td>8.6 ± 1.4</td>
<td>13.68 ± 1.95</td>
<td>23.31 ± 2.4</td>
<td>29.86 ± 2.4</td>
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<tr>
<td></td>
<td>Mudflat</td>
<td>8.56 ± 1.2</td>
<td>13.88 ± 1.9</td>
<td>23.42 ± 1.9</td>
<td>29.38 ± 1.9</td>
</tr>
<tr>
<td>RSJ</td>
<td>Saltmarsh</td>
<td>8.8 ± 1.4</td>
<td>12.76 ± 2.1</td>
<td>19.71 ± 2.3</td>
<td>29.90 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>Mudflat</td>
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<td>21.78 ± 1.97</td>
<td>30.94 ± 2.5</td>
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</table>
SAO and RSJ, respectively. In addition, the size of the smallest ovigerous mature female was similar in both populations (15.9 mm in SAO and 15 in RSJ) but the interval of maturity size was larger in SAO, reinforcing the idea of a maturity delay in at least a portion of the females in this site. Intraspecific variation in the relative size at sexual maturity was considered a consequence of the great plasticity of this crab’s life history under different environmental conditions (Hirose et al. 2012 and references hereof). The size at sexual maturity of several crabs varies with latitude but “small-scale factors such as food availability, population density or subtle changes in substrate, rather than latitudinal factors, are important in regulating size at maturity” in other species (Hines 1989). The observed differences in female size at maturity could be related with food supply: according to Wenner et al. (1974), individuals should grow more quickly and reach maturity earlier in a food-rich habitat (e.g. RSJ) than in a food-limited habitat (e.g. SAO). This hypothesis, however, needs to be tested experimentally.

The number of eggs carried by a female was linearly related with CW in both sites (SAO: \( y = 2510x - 42478, R^2 = 0.37; \) RSJ: \( y = 2590x - 34309, R^2 = 0.86; \)) the slope of the respective equations did not differ but the y-intercept was higher in RSJ (ANCOVA, slope: \( F_{1,64} = 0.022, p = 0.88; \) y-intercept: \( F = 36.86, p < 0.0001, \)) indicating that females in this site had from 25 to 55% more eggs than in SAO, according to size. The dry weight of a recently laid egg did not differ between SAO (DW = 10.50 ± 2.08 µg) and RSJ (DW = 9.50 ± 1.01 µg; t-test, \( t = 1.411, p = 0.169 \)) However, the reproductive output was higher in RSJ (12.15% ± 1.54) than in SAO (8.74% ± 2.33; \( t = 5.668, p < 0.001 \)) and it was not related with female size. A pattern of latitudinal decrease in fecundity and increase in egg mass had been found in several crab species and related to temperature gradients (e.g., Lardies & Castilla 2001). Previously, it was found that fecundity in Neohelice granulata was lower, and egg dry mass higher, in SAO than in Mar Chiquita (MC), regardless of season. The reproductive output was sometimes similar, and sometimes higher in one of them, since it varied within the reproductive season and between successive years; however, on average it never exceed 10 (Bas et al. 2007). Since temperature did not differ between the sites during the reproductive season (Fig. 1, Rivas 1990, Rivas & Beier 1990), a latitudinal temperature gradient should be discarded as the main explanation for the differences in the reproductive investment of Neohelice granulata, since the fecundity and RO > 12 found in RSJ, the southernmost population, the
highest ever reported for this species, is more similar to the MC population values than to the closer SAO population. The effect of other environmental factors, including salinity, quality or quantity of benthic food sources and productivity, had been suggested as possible causes of the differences between MC and SAO (Bas et al. 2007). The effect of salinity cannot explain the observed differences between SAO and RSJ, since both sites have a high and constant salinity, and the notorious variation in the quality of food available for females seems to be a more plausible interpretation: it has been shown that the main food source for females is the organic matter of sediment (Bas et al. 2014). If so, and since RSJ and MC had similar food quality, an interesting question remains open: why are fecundity and reproductive output higher in the former?

The length of burrows did not differ between the sexes in RSJ, but male's burrows were wider than females' burrows (length, males: 235.8 ± 62.6 mm; females: 198.3 ± 58.45 mm; t-test, t = 1.07, p = 0.3; width, males: 30.17 ± 16.07 mm; females: 12.17 ± 7.14 mm; t-test, t = 2.49, p = 0.03). Burrows were longer and wider in RSJ than those recorded from SAO by Sal Moyano et al. (2012; length, males: 76.4 ± 14.07 mm; females: 66.6 ± 13.97 mm; width, males: 15.76 ± 9.16 mm; females: 5.52 ± 6.16). The fine sediments found at MC allow for the building of deep burrows with copulatory chambers constructed along their length. The coarse sediments of SAO, by contrast, prevent burrows from being so deep and their widened entries may serve as open copulatory chambers (Sal Moyano et al. 2012). As documented in MC, burrows of RSJ are deep and complex, probably due to sediment characteristics. Burrow structure was associated with different mating strategies used by males in MC and SAO (Sal Moyano et al. 2012). RSJ should be considered in future studies that aim to test the generality of the observed relationships between burrows and behavior.

Summarizing, RSJ had environmental similarities with SAO (climate, tidal amplitude and predictability, salinity) and with MC (sediment texture, high organic matter content). Maximum size, reproductive output, size at maturity and burrow size and shape, recorded in the middle of the reproductive season of N. granulata were more similar between RSJ and MC than between RSJ and the nearer SAO population. Since SAO and RSJ crabs did not differ genetically (Iruarte et al. 2012), likely as a result of larval connectivity, phenotypic plasticity seems to be a plausible working hypothesis for searching for an explanation for the differences between the populations described here. This hypothesis merits a thoroughly experimental test comparing the performance of individuals of MC, SAO and RSJ reared with different diets and at different salinities.

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