Article

Is Latitudinal Variation Determinant in the Biology of *Neohelice granulata*? Reproduction and Relative Growth of a Coastal Wetland Key Specie at the Northern Limit of its Distribution

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Abstract: In order to test the hypothesis that local environmental features are more deterministic than latitudinal variation in crabs' life history, some aspects of the biology of the burrowing crab *Neohelice granulata* were studied in the northern limit of this species distribution (Caceribu River, Guapimirim, RJ, Brazil). The sex ratio was female-biased over the year. Males were significantly larger (t-test = 3.53, $p<0.05$) than females (CW mean = 29.14 mm and 27.32 mm, respectively). Females were predominant in the intermediary-size classes. The reproductive period took place from February to November, with a peak in May. Juveniles were found all over the year. The size at maturity was estimated at 19.0 mm for females and 23.9 mm for males. Average fecundity was 20,419 eggs (±12,627.12) and showed a decrease from the first to the last stage of embryonic development. Most of the results from this study do not corroborate the predictions of the latitudinal gradient in crabs' life history. This strengthens the hypothesis that the biology of brachyurans is mainly driven by environmental factors than latitudinal variation.

Keywords: relative growth; reproduction; fecundity; Decapoda

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1. Introduction

Mangrove ecosystems have characteristics that make them functionally and structurally unique. These areas are responsible for long-term geomorphological processes and continuous interactions with adjacent ecosystems (Alongi, 2002). The dynamic of these ecosystems is affected by abiotic and biotic factors, such as tidal flow, salinity and nutrient cycling (Lugo and Snedaker, 1974). Nutrient cycling is intrinsically related to the benthic fauna (e.g. mollusks and crabs), especially the herbivorous and detritivores species (Kristensen and Alongi, 2006). When it comes to the crabs, the burrowing habitat is essential for the sediment topography and biogeochemistry by modifying particle size distribution, drainage, redox conditions and organic matter as well as nutrient availability (Botto and Iribarne, 2000).

Neohelice granulata (Dana, 1851), previously known as *Chasmagnathus granulatus* (Sakai et al., 2006), is a semiterrestrial species, occurring in salt marshes and mangroves between Patagonia and Rio de Janeiro, southeast Brazil (Melo, 1996). It is considered a key species in the mangrove ecosystem due to its influence on substrate quality (Botto and Iribarne, 2000). During the low tide periods, their opened burrows remain full of water and act as passive traps of sediment and detritus that increase substrate penetrability and stability (Botto et al., 2006; Mendez-Casariego et al., 2011). Regarding their high abundance and ecological role in the mangrove forests, *N. granulata* has been recognized as a model for biochemical, physiological, and ecological research (Spivak, 2010).

Despite *N. granulata* being a dominant species at higher latitudes, it was included in the checklist of threatened fauna in Rio Grande do Sul State (South Brazil) (Marques et al., 2002). Regarding this, the study of N. Granulate biology can be useful for establishing management plans to preserve their natural populations (Hutchinson, 1981). With this knowledge, it is possible to infer the natality and mortality rates and, consequently, the ecological stability of the populations (Kennelly and Watkins, 1994). The biology of *N. granulata* is known in the Southeast (Greagati and Negreiros-Fransozo, 2007, 2009) and South Brazil (Ruffino et al., 1994; Barutot et al., 2009) and in Argentina (Ituarte et al., 2004; Bas et al., 2005; César et al., 2005; Barcelos et al., 2007). According to Barutot et al. (2009), as *N. granulata* shows a wide geographic range, is important to compare the biology from distinct populations in order to better understand the life history of this species.

In general, crabs' life histories can show a great intraspecific variation. For example, aspects such as growth, life expectancy, size of maturity and fecundity are closely related to a latitudinal gradient, since temperature variation affects animal metabolic rates (Hines, 1989). However, population differences are also observed between geographically close populations as a response to habitat conditions and ecological interactions (Vogt, 2011).

This study is detailed the biology of *N. granulata*, located at the northern limit of their distribution. Considering the geographical range of this species distribution, data from the present study are compared with the literature in order to discuss the effect of the latitudinal gradient on their life history. Finally, the present study aims to contribute with useful information regarding the future management concerning *N. granulata* conservation.

2. Material and Methods

Study area

The environmental protection area of Guapimirim (APA de Guapimirim) is located in Guanabara Bay, in the metropolitan area of Rio de Janeiro, Southeast Brazil. Almost 50% of its area (61.8 km^2) corresponds to the last continuous strip of mangroves in Guanabara Bay, in different conservation and regeneration status (Pires, 1986). Due to its localization, this mangrove area is especially vulnerable to anthropic action, particularly to urban expansion, deforestation, and domestic and industrial pollution. The existence of an environmental protection area ensures the maintenance of natural conditions, enabling the mangrove recuperation and allowing the permanence and survival of the local population that lives off its natural resources (IBAMA, 2001).

The APA de Guapimirim hydrographic bay is the second major draining area in Guanabara Bay, corresponding to 20.7% of this hydrographic zone. It is about 60 km long and flows into APA de Guapimirim, on the East side of Guanabara Bay (Negreiros et al., 2002). The samples were taken in the fringe and neighborhoods of the Caceribu River, one of the most important rivers in APA de Guapimirim.

Fieldwork

Samples were taken monthly, between January and December (2010), in the intertidal zone of the Caceribu River´s mangrove (Fig 1). One researcher manually collected crabs on the surface and in the interior of the substratum, during 25 minutes in the low tide (capture per unit of effort – CPUE). The specimens were placed in plastic bags and kept in a cooler for transportation.

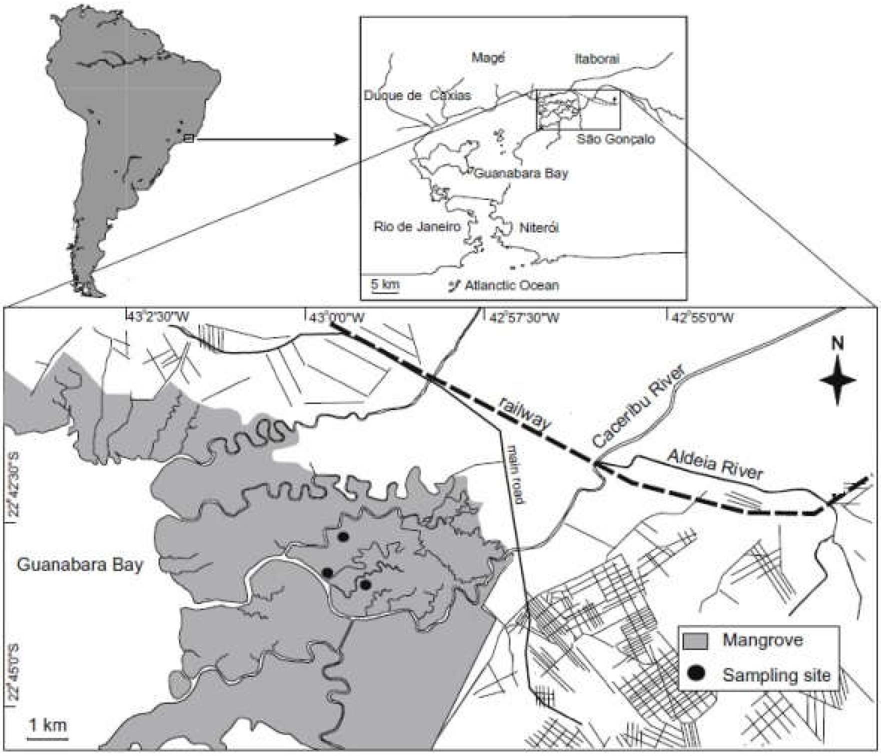

Figure 1. Sample site: Caceribu River, Guanabara Bay, Rio de Janeiro State, Brazil. Modified from Costa et al. (2014).

Laboratory procedures

In the laboratory, crabs were sexed and the following biometric measures were taken using a vernier caliper (0.01 mm): carapace width (CW), right and left cheliped propodus length (RQL, LQL), abdomen width (AW) for females, and gonopod length (GL) for males (Fig. 2). Ovigerous females had their individual fecundity estimated through the volumetric method (Díaz et al., 1983). These females had their egg mass (with the pleopods) removed and immersed for a few seconds in a sodium hypochlorite (70%) solution for egg dissociation and then quickly washed in tap water. After that, the eggs were taken into a becker, supplementing the volume to 40 mL. After homogenization, five sub-samples of 1 mL each were taken (with reposition) and the eggs were counted using a stereoscopic microscope, a mechanical counter with four digits, and a petri dish with a grid. The average fecundity of the five sub-samples was performed to estimate individual fecundity. The embryonic stage of the eggs in each ovigerous female was determined according to the following features: Stage I – freshly extruded egg mass sponge, a large quantity of yolk filing all egg volume; Stage II – a large quantity of yolk, marginal areas of the eggs without yolk fill; Stage III – compound eyes of the larvae are differentiated, the embryo occupies 1/3 of the volume of the egg and the segmentation is not visible; Stage IV – the larvae is totally formed, the segmentation is well visible.

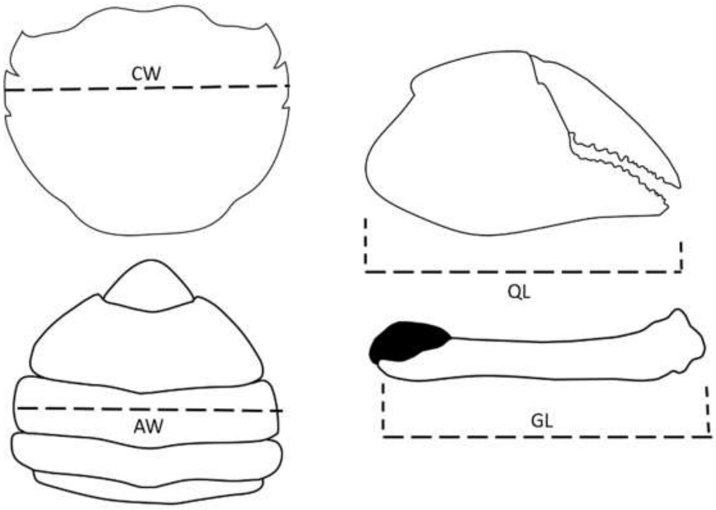

Figure 2. *Neohelice granulata*. Biometrics measures. CW: carapace width; QL: right and left cheliped propodus length; AW: abdomen width for females and GL: gonopod length for males.

Data analysis

Individuals were distributed in twenty-eight CW classes of 1 mm amplitude each, ranging from 11 to 39 mm. The sex ratio significance was tested monthly using the Chi-Square test ($\alpha = 0.05$) as a function of size (CW). The size at maturity was estimated through the discontinuity (breakpoint - BP) of the relationships CWxAW for females and CWxGL for males using the software REGRANS (Pezzuto, 1993). These biometrics measures (AW and GL) were chosen because they represent sexual characters that present allometric growth when crabs attain maturity (Hartnoll, 1974). The linear regression [egg number (EN) = a + b (CW)] and Pearson´s correlation index (r) were employed to verify the correlation between fecundity and CW. Analyses of relative growth were performed to describe the growth ratio of the reference measure (CW) and the dependent variables (AW, RQL, LQL, and GL) through the logarithmic form ($\log y = \log a + b \log CW$) of the power function $y = ab^{CW}$ (Hartnoll, 1978). The Student´s T-test ($\alpha = 0.05$) was used to test the significant deviation of the allometric constant (b) from 1, determining the isometric or allometric condition between CW and the dependent variables.

3. Results

Population Structure

Sampling added up to 551 crabs, 164 males, and 387 females (169 ovigerous) during a year of fieldwork. The overall sex ratio (M: F) was female-biased in all months ($p < 0.05$). The sex ratio as a function of size (CW) was female-biased ($p < 0.05$) in the intermediary size classes (Fig.3). The CW of males ranged from 11.11 to 38.03 mm (mean ± SD = 29.14 ± 5.66), with higher frequency in the 30-31 and 32-33 mm CW classes. In females, CW ranged from 12.21 to 33.87 mm (27.32 ± 4.03), with higher frequency in the 29-30 mm CW class. CW values for males were significantly higher than the females' results.

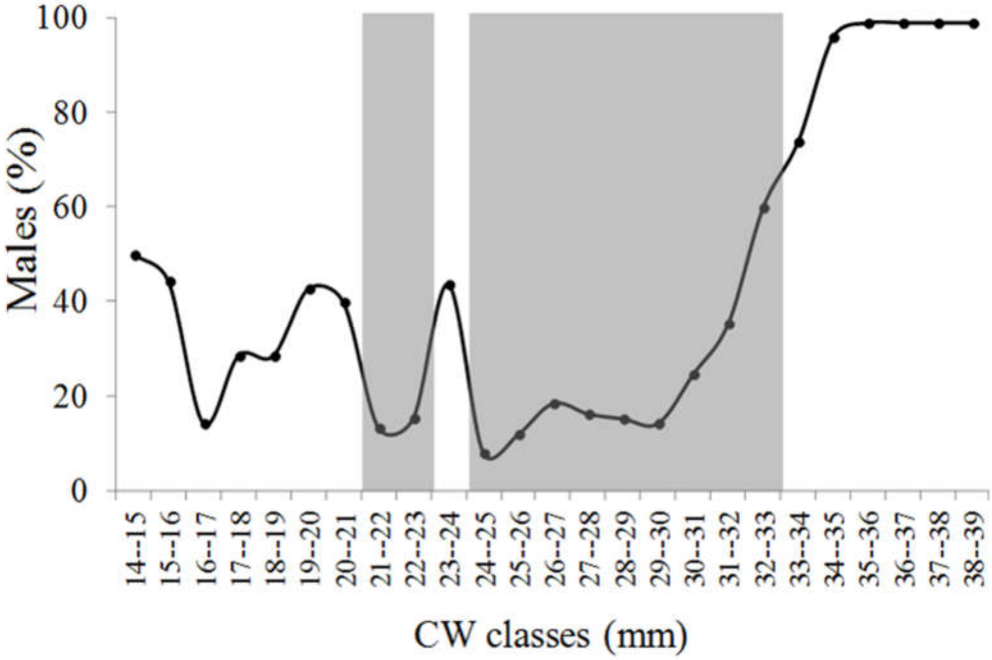

Figure 3. *Neohelice granulata*. Relative frequency of males by size classes. The intervals marked in grey mean significant deviations from the sex ratio 1:1, towards to females ($p < 0.05$).

Breeding season

Ovigerous females were observed between February and November, with the highest relative frequency in May. Sampling added up to 169 ovigerous females but, unfortunately, the ones collected in February and March could not be analyzed due to poor conservation of the specimens. In this category, CW ranged from 19.01 to 33.87 mm (mean ± SD = 28.97 ± 2.5), with higher frequency observed in the 29-30 CW class. The juvenile individuals were observed all over the year but with a higher frequency between October and December (Tab. 1). Juveniles were found in all months (in the exception of May) with the higher frequency between October and December, probably due to the high reproductive activity observed in the previous months.

Table 1. *Neohelice granulata*. Relative frequency of ovigerous females and juveniles, mean temperature, and total precipitation over the sampling period (2010). Data on temperature and precipitation were provided by INMET (Instituto Nacional de Metereologia do Brasil).

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Ago	Sep	Oct	Nov	Dec
Total Precipitation (mm^3)	287	87	325	280	45	42	79	20	36	118	67	291
Mean Temperature (^{o}C)	30	30	27	26	24	21	23	22	23	24	25	27
Ovigerous Females (%)	0	2.3	12.5	18.7	85	73	34	51	54	24	18	0
Juveniles (%)	4.7	2.3	6.25	4.16	0	2.04	2.12	7.3	6.8	30.9	15	25

Size of sexual maturity

The breakpoint was estimated at CW = 19.0 mm for females [(SSR): F (3.03) = 67.3] and in CW = 23.9 mm in males [(SSR): F (3.01) = 44.5], here we considered it as an indication of size at maturity.

Relative Growth

The relative growth analyses showed that the relationships between CWxAW and CWxGL changed from the juvenile to the adult phase. For females, the relationship CWxAW showed positive allometry in juveniles and negative allometry in adult specimens. For males, the relationship CWxGL showed positive allometry in juveniles and isometry in adults. The remaining relationships did not change from the juvenile to the adult phase (Tab. 2).

Table 2. *Neohelice granulata*. Relative growth of carapace width (CW) and the dependent variables (CW: carapace width; AW: abdomen width; RCL: right cheliped length; LCL: left cheliped length; GL: gonopod length; n: sample size; r: correlation coefficient; R^2: determination coefficient; a: intercept axis; b: allometric constant; I.C.: confidence interval; t: Student's T-test result; AL: allometry; *: significant difference ($p < 0.05$); +: positive allometry; -: negative allometry; and 0: isometry).

		Regression	n	r	R^2	a	b (C.I. 95%)	t(α=0.05)	AL
Females	Juvenile	CW/AW	26	0.903	0.811	-0.781	1.426 (1.19 - 1.65)	3.80*	+
	Adult	CW/AW	530	0.336	0.11	0.554	0.889 (0.86 - 0.91)	-9.50*	-
Males	Juvenile	CW/GL	26	0.956	0.914	-0.715	1.868 (1.73 - 2.00)	14.46*	+
		CW/RQL	26	0.963	0.927	-0.307	1.11 (1.10 - 1.31)	12.06*	+
		CW/LQL	26	0.974	0.865	-0.238	1.21 (1.17 - 1.32)	11.3	+
	Adult	CW/GL	396	0.916	0.839	-0.473	0.876 (0.82 - 0.94)	1.96	0
		CW/RQL	132	0.969	0.939	-0.39	1.09 (0.95 - 1.21)	-1.83*	+
		CW/LQL	138	0.875	0.833	-0.362	1.12 (1.01 - 1.26)	-1.64*	+

Fecundity

Individual fecundity ranged from 336 to 82592 eggs/brood (20419.36 ± 12627.12). Table 3 shows the fecundity found in each stage of egg development, which had a decrease from stage I to IV. Females with eggs in the last stage of development were found throughout the breeding season, except in September (Fig. 4). The linear regression between CW and fecundity was significant (egg number = 37.07CW - 586.0; $r = 0.5$; $p < 0.01$).

Table 3. *Neohelice granulata*. Fecundity parameters in each stage of egg development. I-IV: stages of egg development. n: number of sampled females. SD: standard deviation.

Stage	n	Mean	Min	Max	SD
I	21	28,694.1	8,936	82,592	16,158.26
II	43	24,996.5	7,760	46,336	9,540.27
III	8	16,028.0	7,808	28,024	7,440.15
IV	38	11,666.9	336	42,944	7,967.34

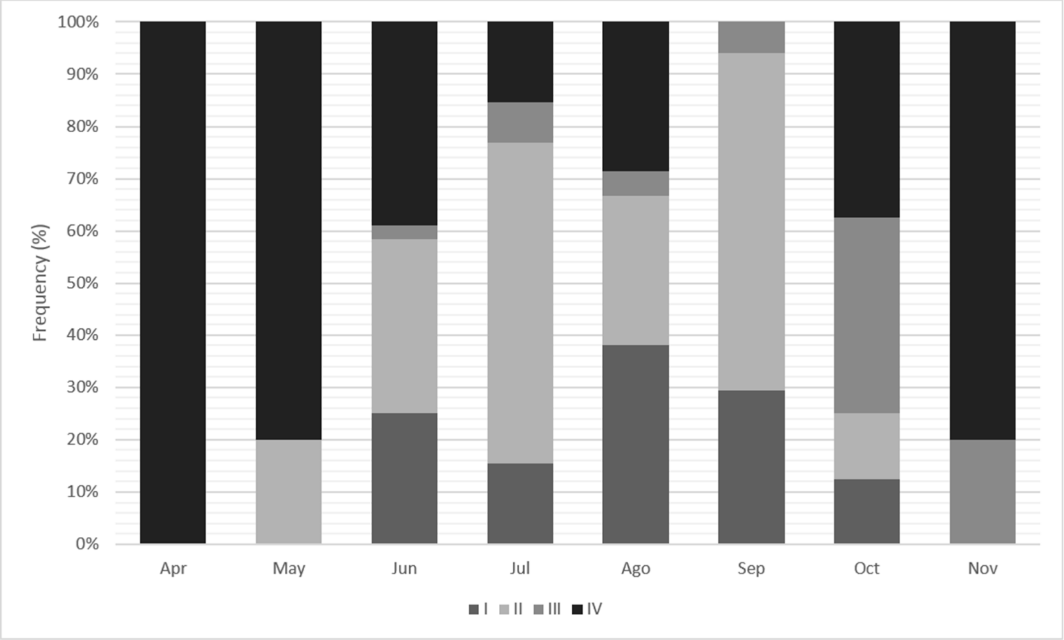

Figure 4. *Neohelice granulata*. Relative frequency of females with eggs in different stages of development (I-IV) during the breeding season.

4. Discussion

In this study, the sex ratio was female-biased, as already noticed by other populations from Argentina as Salado River (Botto and Irigoyen, 1979), Mar Chiquita Lagoon (Spivak et al., 1996) and Samborombón Bay (Cesar et al., 2007). In contrast, Bas et al. (2005) and Angeletti and Cervellini (2015) found a male-biased sex ratio throughout the year in Santo Antonio Bay and Bahia Blanca Estuary (South Argentina), respectively. According to Botto and Irigoyen (1979) and Spivak et al. (1996), *N. granulata* presents a variation in the spatial distribution related to sex and age. The sex ratio as a function of the size was female-biased in the intermediary size classes. The dominance of males in the largest size classes is a common pattern for *N. granulata* as other studies have demonstrated (Gregati and Negreiros-Fransozo, 2007; Angeletti and Cervellini, 2015). One reasonable explanation for this could be the difference in energy investment after reaching maturity as usually mature females apply more energy for reproduction, presenting a decrease in growth rates (Hartnoll, 1982).

Generally, brachyuran's populations from tropical areas have enlarged reproductive periods, mainly during the warmest months, while populations from higher latitudes show more restricted reproductive periods (Sastry, 1983; Adiyodi, 1988). The reproductive period of *N. granulata* follows this pattern of longer reproduction period in populations from subtropical regions, including the population here studied (Tab. 4). In this study, the breeding season of *N. granulata* was more intense during the dry season when a higher frequency of ovigerous females was observed. The precipitation seems to exert an influence on reproduction once breeding decreases in the months of the rainy season (September to March) (Tab. 1). However, further studies are necessary to confirm this statement. Long-term studies have observed that the reproductive period changes between two consecutive years, suggesting that the temperature is correlated with the start and duration of the breeding season (Ituarte et al., 2004; Ituarte et al., 2006). Bas et al. (2005) raised the possibility that the reproductive period should be related to the temperature range seen among seasons. The difference between the extreme temperatures found during winter and summer affects the reproductive process in populations from higher latitudes. However, in the present study, the population experienced a small amplitude of temperature variation (Tab. 1) which suggests that other environmental factors have considerable influence on the rhythm of reproduction, at least when *N. granulata* inhabit subtropical regions. Spivak et al. (2016) found similar results comparing two populations in Southern Argentina. They inhabit relatively close habitats (190 km), with no difference in temperature during the reproductive season, but other environmental conditions were contrasting and led to consistent differences in reproductive aspects.

Relative growth analyses here described were comparable to those presented by Gragati and Negreiros-Fransozo (2007) who studied another *N. granulata* subtropical population (Paraty, RJ, Brazil). The only exception was the relationship between CWxAW in adult females, which showed negative allometry in the present study while the previous authors found isometry. Therefore, in both studies, this species followed the predictions for Brachyura growth (Hartnoll, 1978).

The estimated size of sexual maturity was higher for males (23.9 mm) than for females (19.0 mm) as predicted by Shine (1988) for brachyurans. However, the size at the onset of maturity in the present study was higher than the values found by other authors in southern locations (Tab. 4), contradicting the pattern proposed by Sastry (1983). This author claims that populations inhabiting tropical habitats show smaller size at maturity than populations in higher latitudes but our data showed the opposite. Barutot et al. (2009) found similar incongruences in the size at the onset of maturity that does not support the latitudinal prediction when comparing their results (Patos Lagoon, Southeast Brazil) with populations found Northwards and Southwards. It seems that *N. granulata* does not follow the latitudinal prediction expected for brachyurans considering the size of sexual maturation. This may mean that, apart from temperature, other factors are determinants for the maturation size of this species.

Table 4. *Neohelice granulata*. Comparative table of the breeding season and size of maturity in different latitudes.

Author	Study area/Latitude	Breeding season	Size of maturity (CW)	
This study	Caceribu River, Guapimirim, RJ, Brazil (22°57'S / 43°19'W)	Feb - Nov	Male	23.9 mm
			Female	19 mm
Gregati & Negreiros-Fransozo (2007, 2009)	Jabaquara Bay, Paraty, RJ, Brazil (23°13'04''S / 44°42'47''W)	continuous	Male	19.7 mm
			Female	19.2 mm
Ruffino *et al*. (1994)	Patos Lagoon, RS, Brazil (32°S)	continuous	Male	-
			Female	16.5 mm
Barutot *et al*., (2009)	Prainha, Patos Lagoon, RS, Brazil (32°09'011''S 52°06'02''W)	continuous	Male	-
			Female	14.1 mm
López *et al*., (1997)	Faro San Antonio Beach, Samboronbon Bay, Argentina (36° 18'S 56° 48'W)	Oct - Feb	Male	16.5 mm
			Female	22.7 mm
Luppi *et al*. (2004)	Mar Chiquita Lagoon, Mar del Plata, Argentina (37°32'S / 57°19W)	-	Male	17.2 mm
			Female	18 mm
Ituarte *et al*. (2006)	Mar Chiquita Lagoon, Mar del Plata, Argentina (37°32'S / 57°19W)	Sep - Mar	-	

The average fecundity in this study seems related to the female's size as already reported by Luppi et al. (1997) and César et al. (2007). This may be due to the fact that the body cavity space restricts the yolk accumulation, being a limiting factor in the brood size (Hines, 1982). However, fecundity can vary largely between populations as a result of different life conditions (Ruffino et al., 1994; Silva et al., 2008; Barutot et al., 2009). According to Hines (1982), the reproductive effort exhibits significant intraspecific variation with respect to limited resources, molting stage, tidal height, and latitude (especially in large distribution species). Moreover, the study of reproductive traits provides the basis for theoretical considerations concerning life history and offers clues about the evolution of reproductive strategies. The decrease in the number of eggs along the embryonic development stages observed in this study may be a response to abiotic variations, pollution, egg predation, and infection by parasites or microbes (Balsundaran and Pandian, 1982; Ruffino et al., 1994; Luppi et al., 1997).

The population biology of *N. granulata* has been extensively studied, especially in the Southern portion of its distribution, namely in Southern Brazil and Argentina. Data from this study indicate that local environmental factors are the main drivers to determine features of the biology of *N. granulata*, especially reproduction (size at onset of the first maturity and reproductive period). Considering the relevant ecological role of *N. granulata* in bioturbation and the increasing degradation of their habitats along the Southern West Atlantic coast, further studies addressing the conservation status and potential use of this crab as an indicator of the environmental state of mangrove and salt marshes is welcome.

References

1. Adiyodi RG. 1988. Reproduction and development. *In:*Burggren, W. W., & McMahon, B. R. (Eds). Biology of the land crabs. Cambridge University Press.
2. Alongi DM. 2002. Present state and future of the world's mangrove forests. Environmental conservation, 29(03): 331-349.
3. Angeletti S, Cervellini, PM. 2015. Population structure of the burrowing crab *Neohelice granulata* (Brachyura, Varunidae) in a southwestern Atlantic salt marsh. Latin American Journal of Aquatic Research, 43 (3): 539-547.
4. Balsundaran C, Pandian TJ. 1982. Egg loss during incubation in *Macrobrachium nobilii* (Henderson & Mathias). Journal of Experimental Marine Biology and Ecology 59(2,3):289-299.
5. Barcelos DF, Castigliono DS, Barutot R, Santos S. 2007. Crescimento de *Chasmagnathus granulatus* (Crustacea, Decapoda, Varunidae) na Lagoa do Peixe, Rio Grande do Sul, Brasil. Iheringia, 97 (3): 263-267.
6. Barutot RA, D'Incao F, Fonseca DB. 2009. Reproductive biology of *Neohelice granulata* (Decapoda: Varunidae) in two salt marshes of the estuarine region of the Lagoa dos Patos Lagoon, southern Brazil. Journal of the Marine Biological Association of the United Kingdom, 89 (4): 769-774.
7. Bas C, Luppi T, Spivak E. 2005. Population structure of the South American Estuarine crab, *Chasmagnathus granulatus* (Brachyura: Varunidae) near the southern limit of its geographical distribution: comparison with northern populations. Hidrobiologia, 537: 217-228.
8. Botto F, Iribarne O. 2000. Contrasting effects of two burrowing crabs (*Chasmagnathus granulata* and *Uca uruguayensis*) on sediment composition and transport in estuarine environments. Estuarine, Coastal and Shelf Science, 51: 141–151.
9. Botto F, Iribarne O, Guitierrez J, Bava J, Gagliardini A. 2006. The ecological importance of passive deposition of meiofauna and organic matter into burrows of the SW Atlantic burrowing crab *Chasmagnathus granulatus*. Marine Ecology Progress Series, 312: 201-2010.
10. Botto JL, Irigoyen HR. 1979. Bioecologia de la comunidad del cangrejal. I. Contribucion al conocimiento biologico del cangrejo de estuario, *Chasmagnathus granulata* Dana (Crustacea, Decapoda, Grapsidae) en la desembocadura del rio Salado, Provincia de Buenos Aires. Seminario Latinoamericano de Ecologia Bentonica y Sedimentologia de la Plataforma Continental del Atlantico Sur. UNESCO, Montevideo: 161–169.
11. César II, Armendáriz LC, Becerra RV. 2007. Fecundity of *Uca uruguayensis* and *Chasmagnathus granulatus* (Decapoda: Brachyura) from the "Refugio de Vida Silvestre" Bahía Samborombón, Argentina. Brazilian Journal of Biology, 67 (4): 749-753.
12. Costa TMM, Pitombo FB, Soares-Gomes A. 2014. The population biology of the exploited crab *Ucides cordatus* (Linnaeus, 1763) in a southeastern Atlantic Coast mangrove area, Brazil. Invertebrate Reproduction & Development, 58(4), pp.259-268.
13. Conde JE, Bevilacqua M. 1983. A volumetric method for estimating fecundity in Decapoda. Mar. Ecol. Progr. Ser, 10, 206-210.
14. Gregati RA, Negreiros-Fransozo ML. 2007. Relative growth and morphological sexual maturity of *Chasmagnathus granulatus* (Crustacea, Varunidae) from a mangrove area in southeastern Brazilian coast. Iheringia, Sér. Zool., 97(3): 268-272
15. Gregati RA, Negreiros-Fransozo ML. 2009. Population biology of the burrowing crab *Neohelice granulata*, (Crustacea: Decapoda: Varunidae) from a tropical mangrove in Brazil. Zoologia, 26 (1):31-37
16. Hartnoll RG. 1974. Variation in growth patterns between some secondary sexual characters in crabs (Decapoda: Brachyura). Crustaceana, 27: 131-136.
17. Hartnoll RG. 1978. The determination of relative growth in Crustacea. Crustaceana, 34 (3):281-293.
18. Hartnoll RG. 1982. Growth, *p.III-196.1n:* L.G. ABELE (Ed.). The Biology of Crustacea. 2. Embryology, morphology and genetics. New York, Academic Press New York, 440p.
19. Hines AH. 1982. Allometric constraints and variables of reproductive effort in Brachyuran crabs. Marine Biology, 69:309-320
20. Hines AH. 1989. Geographic variation in size at maturity in brachyuran crabs. Bulletin of Marine Science, 45 (2): 356-368.

21. Hutchinson GE. 1981. Introduccíon a la ecologia de poblaciones. Barcelona Blume Editorial, 492p.
22. IBAMA 2001. Área de Proteção Ambiental de Guapi-Mirim - Plano de manejo.
23. Ituarte RB, Spivak ED, Luppi TA. 2004. Female reproductive cycle of the Southwestern Atlantic estuarine crab *Chasmagnathus granulatus* (Brachyura: Grapsoidea: Varunidae). Scientia Marina, 68 (1): 127-137.
24. Ituarte RB, Bas C, Luppi TA, Spivak ED. 2006. Interpopulational differences in the female reproductive cycle of the southwestern Atlantic estuarine crab *Chasmagnathus granulatus* Dana, 1851 (Brachyura: Grapsoidea: Varunidae). Scientia Marina, 70(4), 709-718.
25. Kennelly SJ, Watkins D. 1994. Fecundity and reproductive period, and their relationship to cacth rates of spanner crabs, *Ranina ranina* of the East coast of Australia. Journal of Crustacean Biology14 (1): 146-150
26. Kristensen E, Alongi DM. 2006. Control by fiddler crabs (*Uca vocans*) and plant roots (*Avicennia marina*) on carbon, iron and sulfur biogeochemistry in mangrove sediment. Limnol. Oceanogr. 51, 1557–1571.
27. López LS, Stella VS, Rodríguez EM. 1997. Size at onset of sexual maturity in *Chasmagnathus granulate* (Decapoda, Brachyura). Nauplius, 5 (2): 65-75
28. Lugo AE, Snedaker SC. 1974. The ecology of mangroves. Annual review of ecology and systematics, 39-64.
29. Luppi T, Bas C, Spivak E, Anger K. 1997. Fecundity of two grapsid crab species in the laguna mar Chiquita, Argentina. Arch. Fish. and Mar. Res., 45: 149-166.
30. Luppi TA, Spivak ED, Bas CC, Anger K. 2004. Molt and growth of an estuarine crab, *Chasmagnathus granulatus* (Brachyura: Varunidae), in Mar Chiquita coastal lagoon, Argentina. J.Appl.Ichthyol. 20: 333-344.
31. Marques ABM, Fontana CS, Vélez E, Bencke GA, Schneider M, Reis RE. 2002. Lista das espécies da fauna ameaçadas de extinção no Rio Grande do Sul. Decreto nº41.672 de 11 de junho de 2002.
32. Melo GAS. 1996. Manual de identificação dos Brachyura (caranguejos e siris) do litoral brasileiro. São Paulo, Plêiade. 604p.
33. Mendez-Casariego A, Luppi T, Iribarne O, Daleo P. 2011. Increase of organic matter transport between marshes and tidal flats by the burrowing crab *Neohelice (Chasmagnathus) granulata* Dana in SW Atlantic salt marshes. Journal of Experimental Marine Biology and Ecology, 401: 110-117.
34. Negreiros DHD, Araújo FP, Coreixas MA. 2002. Nossos rios. Niterói: Instituto Baía de Guanabara. 34 p.
35. Ng P, Guinot D, Davie P. 2008. *Systema Brachyururum*: Part I. An Annotated Checklist of Extant Brachyuran Crabs of the World. The Raffles Bulletin of Zoology, 17:1-286.
36. Pezzuto PR. 1993. Regrans: a “basic” program for an extensive analysis of relative growth. Atlântica, Rio Grande, 15: 93-105.
37. Pinheiro MAA, Santana W, Boos H, Matsunaga AMF, Lianos L. 2016. Avaliação do Caranguejo Neohelice granulata (Dana, 1851) (Decapoda: Varunidae). Cap. 34: p. 459-466. In: Pinheiro, M. & Boos, H. (Org.), Livro Vermelho dos Crustáceos do Brasil: Avaliação 2010-2014. Porto Alegre, RS, Sociedade Brasileira de Carcinologia - SBC, 466 p.
38. Pires IO. 1986. Mapeamento de manguezais do recôncavo da Baía de Guanabara, RJ, através de técnicas de sensoriamento remoto. Dissertação. Instituto de Pesquisas Espaciais, São José dos Campos, 86 p.
39. Ruffino ML, Telles MD, D'Incao F. 1994. Reproductive aspects of *Chasmagnathus granulata* Dana, 1851 (Decapoda, Grapsidae) in the Patos Lagoon Estuary - Brazil. Nauplius, 2: 43-52
40. Sakai K, Turkay M, Yang SL. 2006. Revision of the *Helice/Chasmagnathus* complex (Crustacea: Decapoda: Brachyura). AbhSenckenbergNaturforschGes, 565:1–76
41. Sastry AN. 1983. Ecological aspects of reproduction. *In*: Bliss, D.E. (Eds). The Biology of Crustacea (vol.8) Environmental adaptations, 179-270.
42. Shine R. 1988. The evolution of large body size in females: a critique on Darwin’s “fecundity advantage” model. The American Naturalist, 131 (1): 124-131.
43. Silva PV, Luppi TA, Spivak ED, Anger K. 2009. Reproductive traits of an estuarine crab, *Neohelice* (= *Chasmagnathus*) *granulata* (Brachyura: Grapsoidea: Varunidae), in two contrasting habitats. Scientia Marina, 73(1), 117-127.
44. Spivak ED. 2010. The crab *Neohelice* (= *Chasmagnathus*) *granulata*: an emergent animal model from emergent countries. Helgoland Marine Research, 64: 149-154.
45. Spivak ED, Bas CC, Luppi TA. 2016. Great unexpected differences between two populations of the intertidal carb *Neohelice granulata* inhabiting close but contrasting habitats (Crustacea: Decapoda: Brachyura). Zoologia, 33 (6): e20160020.
46. Spivak ED, Anger K, Bas CC, Luppi TA, Ismael D. 1996. Size structure, sex ratio, and breeding season in two intertidal grapsid crab species from Mar Chiquita Lagoon, Argentina. Neritica 10: 7–26.

47. Vogt G. 2011. Ageing and longevity in the Decapoda (Crustacea): A review. Zoologischer Anzeiger, 251 (1): 1-2
48. Wenner AM. 1972. Sex ratio as a function of size in marine Crustacea. American Naturalist, pp.321-350.