

Research Article

Relative abundance and population biology of the non-indigenous crab *Charybdis hellerii* (Crustacea: Brachyura: Portunidae) in a southwestern Atlantic estuary-bay complex

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Received: 2 September 2011 / Accepted: 19 March 2012 / Published online: 26 March 2012

Handling editor: Mark Hanson, Fisheries and Oceans Canada, Canada

Abstract

Invasive species are potential threats to biodiversity, especially if they become established and outnumber native species. In this study, a population of the non-indigenous crab *Charybdis hellerii* was analyzed in an estuary-bay complex on the southeastern Brazilian coast, with respect to its abundance relative to sympatric native brachyuran species, as well as the size structure, sexual maturity, sex ratio, frequency of mutilation, reproductive period, and development of the reproductive system. Crabs were sampled monthly both in the intertidal zone of rocky shores and on sublittoral soft-bottom. Nine species were recorded on the rocky shores, where *C. hellerii* was the second most abundant species; only three individuals of *C. hellerii* were collected in the sublittoral samples. This population of *C. hellerii* showed a unimodal size structure composed mainly of mature individuals; males were larger than females, and the sex ratio was skewed toward males (3.1:1). About 46.9% of the individuals (75 of 160 crabs) had mutilated or regenerating appendages, more frequent in males (56.8%) than in females (28.2%), which may reflect both inter- and intraspecific agonistic interactions. A continuous reproductive pattern is suggested for this population, although ovigerous females occurred unevenly during the year, with 58.82% of them being collected in winter. There was evidence of multiple spawning, since the ovigerous females with an initial egg mass showed mature ovaries as well as seminal receptacles filled with sperm. *C. hellerii* is well established in the estuary-bay complex, but is concentrated in intertidal and shallow subtidal rocky shores, where it may compete with and replace other species such as the portunid *Cronius ruber*. This study also highlights the importance of systematic monitoring studies to evaluate the effects of the introduction of non-indigenous species on ecologically similar natives.

Key words: Brachyura, Portunidae, invader crab, estuary

Introduction

Many species around the world have successfully colonized new habitats, and this is an ongoing process. The rate of introductions of non-indigenous species in Brazil has increased in the last 20 years (Ferreira et al. 2009). Independent of the introduction pathway, bioinvasions threaten biodiversity (Morán and Atencio 2006). These exotic species may have negative impacts on benthic communities, especially indigenous species (Ferreira et al. 2009), leading to loss of biodiversity. Human activities may mediate the global dispersal of species (Barros et al. 2009),

as the high-intensity human activities in coastal waters, such as shipping (Ruiz et al. 1997), organisms transplanted for aquaculture purposes (Kerckhof et al. 2007), construction of inter-oceanic canals (Galil 2000), release of species associated with the pet industry or management practices (Ruiz et al. 1997), movement of fouling communities and transport of ballast water (Campos and Türkay 1989; Lemaitre 1995; Torchin et al. 2001; Ferreira et al. 2009), or marine litter (Derraik 2002). Human-induced global climate change may also be a factor in enlarging the geographical range of species (Kerckhof et al. 2007).

Non-indigenous species compete for resources (Rosson et al. 2006), spread diseases (Lowe et al. 2000), function as vectors of parasites (Bernier et al. 2009), and change the structure and function of species assemblages (Ruiz et al. 1997), and cause serious economic impacts (Hoagland and Jin 2006). Despite the importance of this phenomenon, quantitative evaluations of the ecological effects of non-natives are scarce, and most studies on invasive species merely report new records and range extensions.

In Brazilian marine ecosystems, 58 exotic species have now been recorded (Lopes et al. 2009). The portunid crab *Charybdis hellerii* (A. Milne-Edwards, 1867) is considered an invasive and established species (Lopes et al. 2009). *Charybdis hellerii* is native to the Indo-Pacific (Wee and Ng 1995; Lemaitre 1995; Mantelatto and Dias 1999; Dineen et al. 2001). This species has been recorded worldwide, including the Mediterranean Sea (Galil 2000) and Atlantic waters of the United States, Cuba, Colombia, Venezuela, French Guyana, and Brazil (Lemaitre 1995; Gómez and Martínez-Iglesias 1990; Campos and Türkay 1989; Hernández and Bolaños 1995; Mantelatto and Dias 1999; Tavares and Amouroux 2003). The presumed pathway of introduction is larval transport by ballast water (Mantelatto and Dias 1999; Dineen et al. 2001; Morán and Atencio 2006).

Despite the importance and long-range spread of *C. hellerii*, there are only a few studies on its biology and ecology, even in native populations. The natural history and larval stages were described in the western Atlantic Ocean (Florida, U.S.A.) (Dineen et al. 2001). In the southwest Atlantic, there are only the studies of Mantelatto and Dias (1999) and Mantelatto and Garcia (2001), which focused on morphological sexual maturity, population size classes, and sex ratio, and recorded established populations. According to Dineen et al. (2001) and Tavares and Amouroux (2003), this species possesses numerous life-history traits that are adaptive for invasion of new geographical regions, such as: (1) relatively long larval life span that facilitates dispersal; (2) rapid growth and maturation, contributing to a short renewal time; (3) ability to store sperm and produce multiple broods; (4) generalized carnivorous diet; and (5) ability to use a variety of habitats. Despite these studies, there is no information confirming that this species may be established in estuarine areas or in its preferred habitat. Indeed, no studies have compared the relative abundance of *C. hellerii*

with other sympatric (native) species, to provide data that may help in the understanding of the ecological and economic effects of this introduced crab. Therefore, the present study evaluated the abundance of *C. hellerii* relative to sympatric native brachyuran crabs, and also described its population biology in an estuary-bay complex.

Material and methods

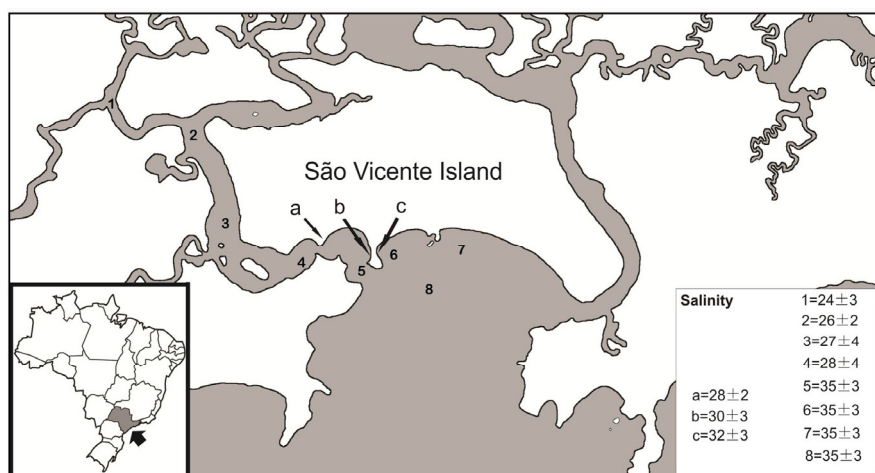
Field samples and laboratory measurements

The animals were collected monthly in two different habitats. On the rocky shore, three subareas were selected in intertidal and adjacent subtidal zones (interface). In subtidal soft-bottom habitats, 8 areas with different salinities were sampled along the estuary-bay complex of São Vicente, state of São Paulo, Brazil (Figure 1) from March 2007 through February 2008. This region is ecologically heterogeneous, including rocky shores, sandy beaches, mangroves, and subtidal areas with muddy sediments, sustaining a highly diverse marine community with many economically important species including portunid crabs (Severino-Rodrigues et al. 2001).

In the intertidal zone, the crabs were collected during low-tide periods from three locations: Biquinha (a), Ilha Porchat (b) and Itararé (c), from June 2009 through May 2010 (Figure 1). Two people collected the crabs by hand during two-hour periods, checking under rocks, in tide pools, and just below the water line. This time period was established to standardize the capture effort throughout the study. Together with the intertidal samples, crabs were also trapped in the interface between the rocky shore and soft bottom (about 5 meters off the rocky shore in the subtidal areas), using 5 ring nets with a 40 cm diameter, baited with fish. The traps were checked every ten minutes for two hours. All animals were collected and transported alive to the laboratory.

On the subtidal soft bottom substrate, samples were taken from March 2007 through February 2008, using a shrimp trawler equipped with an otter trawl 4.0 m wide, 2.0 m high, and 9.5 m long, with a 15-mm mesh body and 10-mm mesh cod liner. The tows lasted 20 minutes (speed varied from 1.85 to 2.7 km/h) and were made along eight transects parallel to the shore, four in the estuary and four in the bay (Figure 1). After

Figure 1. Estuary-bay complex of São Vicente, state of São Paulo, Brazil, showing sampling locations on subtidal soft bottom substrates, in the estuary (transects 1, 2, 3 and 4), and in the bay (transects 5, 6, 7 and 8); and on three rocky shores in intertidal and adjacent shallow subtidal areas with the corresponding salinity (a - Biquinha; b - Ilha Porchat and c - Itararé), located between transects 4 and 6.



the crabs were collected, three samples of bottom water were collected with a Nansen bottle for salinity measurements (Figure 1).

The native species were identified according to Melo (1996) and *C. hellerii* according to Lemaitre (1995), and all animals were counted. The carapace width (CW, without spines) of *C. hellerii* individuals was measured by digital calipers (0.01 mm). The sex was determined by inspection of abdominal morphology, and the crabs were classed as males, non-ovigerous or ovigerous females. The presence of mutilated or regenerating appendages was also recorded.

Reproductive system development and histology

Non-ovigerous and ovigerous females of *C. hellerii* were anesthetized by thermal shock and then dissected. Ovarian development was macroscopically classified as rudimentary (RUD), developing (DE), intermediate (INT), and mature (MAT) (Costa and Negreiros-Fransozo 1998; Zara et al. 2011). The egg masses were also classified according to the stage of embryonic development, characterized by the amount of yolk present in the egg, in three stages: initial (large amount of yolk, orange color), intermediate (less yolk, brownish), and final (less yolk, dark brown) adapted from Costa and Negreiros-Fransozo (1998).

The seminal receptacle from ovigerous females was dissected for histological examination, in order to evaluate the occurrence of multiple spawning. These organs were fixed in 4% paraformaldehyde in 0.2 M phosphate

buffer (pH 7.4) for 24 h, and washed twice for 1 h with the same buffer. The material was dehydrated in an ascending ethanol series (70-95%) for 20 min and embedded in glycol methacrylate resin (Leica® historesin kit) for 72 h at 4°C. After polymerization, the blocks were sectioned in a Leica RM2245 microtome. The 5-7 μ m sections were mounted on slides and stained with hematoxylin and eosin (HE) (Junqueira and Junqueira 1983, modified), avoiding the use of xylene and ethanol.

The male reproductive system of crabs from all size classes was dissected, and the posterior vas deferens (PVD) was examined for the presence of spermatophores. The PVD was squashed on slides, and inspected under a light microscope for the presence or absence of spermatophores.

Data analysis

As *C. hellerii* occurred mostly on the rocky shore and only occasionally on the subtidal soft-bottom (see below), the statistical analyses of population characteristics and comparisons of species abundance were conducted using only the former data. Size-class intervals of 5 mm CW were determined according to the equation of Sturges (1926). The Komolgorov-Smirnov (KS) test was used to verify the normality of the size-frequency distribution of *C. hellerii* for each population category (sex), and their sizes were compared by the Kruskal-Wallis test. Departures from the expected sex-ratio of 1:1 (M:F) were detected using a Chi-square test.

Table 1. Number of individuals per species sampled in three rocky shore areas (Biquinha, Ilha Porchat and Itararé) of the estuary-bay complex of São Vicente, state of São Paulo, Brazil. Data for *Charybdis hellerii* in bold.

Species	Biquinha	Ilha Porchat	Itararé	Total
<i>Menippe nodifrons</i> (Stimpson, 1859)	97	43	52	192
<i>Charybdis hellerii</i> (A. Milne-Edwards, 1867)	30	42	88	160
<i>Pachygrapsus transversus</i> (Gibbes, 1850)	10	10	24	44
<i>Callinectes danae</i> (Smith, 1869)	3	24	5	32
<i>Eriphia gonagra</i> (Fabricius, 1781)	1	0	21	22
<i>Callinectes exasperatus</i> (Gerstaecker, 1856)	2	1	7	10
<i>Arenaeus cribrarius</i> (Lamarck, 1818)	0	0	2	2
<i>Callinectes sapidus</i> (Rathbun, 1896)	1	0	0	1
<i>Cronius ruber</i> (Lamarck, 1818)	0	0	1	1
<i>Panopeus austrobesus</i> (Williams, 1983)	0	0	1	1

Table 2. Number of individuals per species sampled on each transect (T) on subtidal soft bottom habitats of the estuary-bay complex of São Vicente, state of São Paulo, Brazil. Data for *Charybdis hellerii* in bold.

Species	T - 1	T - 2	T - 3	T - 4	T - 5	T - 6	T - 7	T - 8	Total
<i>Callinectes danae</i>	113	345	289	120	374	199	486	347	2273
<i>Callinectes ornatus</i>	37	1	50	9	67	82	182	190	618
<i>Arenaeus cribrarius</i>	0	0	0	0	49	53	131	213	446
<i>Callinectes sapidus</i>	5	4	12	9	6	4	8	2	50
<i>Callinectes bocourti</i>	2	3	3	18	1	1	0	0	28
<i>Panopeus austrobesus</i>	5	1	0	0	0	0	0	0	6
<i>Charybdis hellerii</i>	0	1	1	0	0	1	0	0	3
<i>Hepatus pudibundus</i> (Herbst, 1758)	0	0	0	0	0	1	1	0	2
<i>Callinectes exasperatus</i>	0	0	1	0	0	0	0	0	1
<i>Persephona punctata</i> (Linnaeus, 1758)	0	0	0	0	0	0	0	1	1

The abundances of crab species were compared using the monthly data for the study period as temporal replicates, using an analysis of variance (ANOVA), followed by the Tukey's range test for post-hoc comparisons (Sokal and Rohlf 1995). Only species with more than 10 individuals in the study period were included. A 5% significance level was adopted for all statistical analyses (Sokal and Rohlf 1995) with the statistical package Bio Estat 5.0 (Ayres et al. 2007). The individual samples from the three rocky-shore areas were pooled.

Results

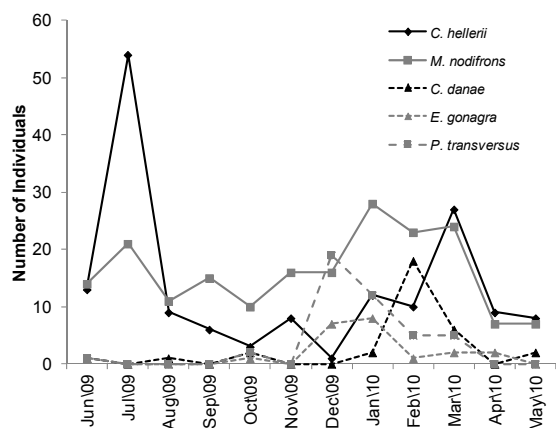
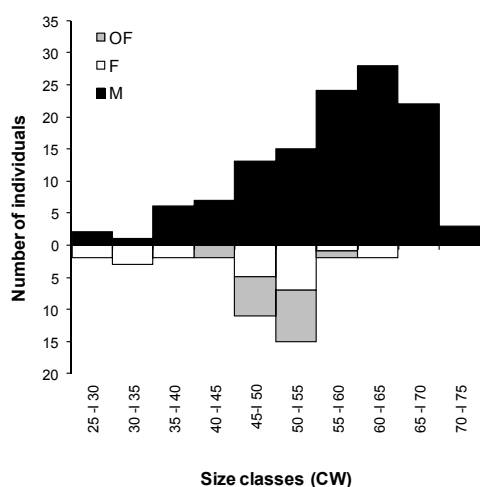
A total of 3,887 crabs were collected in the estuary-bay complex during the two sampling periods: 465 from rocky shores and 2422 from subtidal soft-bottom habitats. On the rocky shores, 10 brachyuran species were collected, including the invasive species *C. hellerii*, which comprised 34.41% of the total number of individuals. The average abundance differed

significantly among species (ANOVA, $F = 8.22$; $df = 4,55$; $P < 0.0001$) and the abundance of *C. hellerii* was significantly greater (Tukey's test, $P < 0.05$) than that of all species except *Menippe nodifrons* (Stimpson, 1859) (Tukey's test, $P > 0.05$) (Table 1, Figure 2). The relative abundance of species varied over time, with *C. hellerii* being more abundant than *M. nodifrons* only in July 2009 (Figure 2). *C. hellerii* showed two peaks during the year (July 2009 and March 2010), interspersed with periods of very low abundances. The distribution of the crab species also varied among rocky-shore areas. The relative abundance of *C. hellerii* increased from Biquinha (low salinity) to Itararé (high salinity) (Table 1).

Ten brachyuran species were also recorded from soft bottoms, but only three individuals of *C. hellerii* were sampled, two in the estuary (mean salinity 26 ± 2) and one in the bay (Table 2), indicating that *C. hellerii* uses this microhabitat less frequently. Portunid crabs of the genera *Callinectes* and *Arenaeus* were more abundant, and *Callinectes danae* (Smith, 1869)

Table 3. Numbers and size (CW, with range mean, and standard deviation) of *Charybdis hellerii* in the estuary-bay complex of São Vicente, state of São Paulo, Brazil. N, number of individuals; Min, minimum; Max, maximum; x, mean; SD, standard deviation.

Sex	N	Min	Max	x±SD
Males	121	27.2	72.7	56.4±9.7
Nonovigerous females	22	28.5	63.1	45.7±10.7
Ovigerous females	17	40.4	59.5	49.4±4.7

**Figure 2.** Number of individuals of different crab species sampled on the rocky shores from June 2009 through May 2010 in the estuary-bay complex of São Vicente, state of São Paulo, Brazil. Only the more abundant species were included (see Table 1).**Figure 3.** Size-frequency distribution (CW, carapace width in millimeters) of males (M, n=121), ovigerous females (OF, n=17), and non-ovigerous females (F, n=20) of *Charybdis hellerii* collected in the estuary-bay complex of São Vicente, state of São Paulo, Brazil from June 2009 through May 2010.

was the most numerous, comprising 93% (2273) of the total number of crabs caught.

Of the 160 specimens of *C. hellerii* collected from the rocky shores, 121 were males, 22 nonovigerous females, and 17 ovigerous females. Males were significantly larger than all females ($Z=4.0653$; $P<0.05$) and ovigerous females ($Z=3.0962$; $P<0.05$); non-ovigerous and ovigerous females did not differ in size ($Z=0.4344$; $P>0.05$) (Table 3), although the latter tended toward larger sizes. The overall sex ratio of the population was skewed toward males (3.1:1; M:F), significantly different from the expected 1:1 proportion ($\chi^2=42.025$; $P=0.0001$).

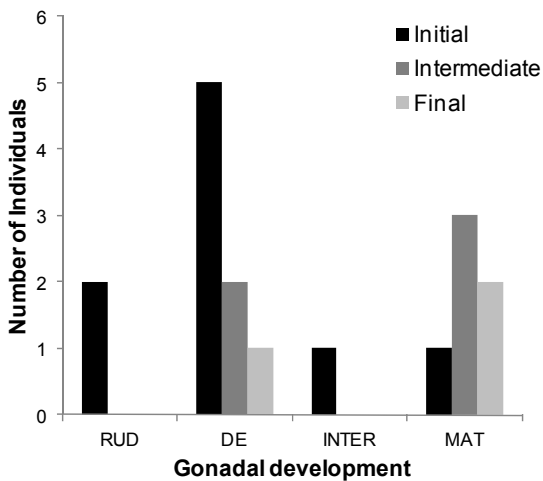
The total count of mutilated or regenerating individuals was 75 of 160 crabs. Most of the males had some injured or regenerating parts (52.9%), whereas females showed fewer injuries than males (28.2%).

The overall size-frequency distribution of the population showed a normal distribution ($KS(MD)=0.1009$; $N=160$; $P>0.05$), as did non-ovigerous females ($KS(MD)=0.2553$; $N=22$; $P>0.05$) and ovigerous females ($KS(MD)=0.1398$; $N=17$ $P>0.05$). However, the male size structure was not normally distributed ($KS(MD)=0.139$; $N=121$ $P<0.05$) (Figure 3). The majority of males were large, whereas non-ovigerous and ovigerous females were less numerous and smaller-sized. Males larger than 30 mm produced sperm and their abdomen was not attached to the carapace, indicating that even the small-sized males are capable of fertilizing females.

Ovigerous females were collected almost year-round, although their abundance varied widely; 58.8% were collected in the winter months. Despite the absence of ovigerous females in October and December 2009 and April 2010, and the small total number of females (37 individuals) and ovigerous females (17 of 37) in the study area, the sparse data suggested a heterogeneously continuous reproductive pattern. Macroscopic analysis of the gonads of these ovigerous females demonstrated

Table 4. Size (mean carapace width, in millimeters) of *Charybdis hellerii* recorded around the world.

Country	State, locality	Male	Female	Reference
Brazil	Rio de Janeiro, Rio de Janeiro	75.0	62.0	Tavares and Mendonça (1996)
Brazil	São Paulo, Ubatuba	64.9	57.2	Mantelatto and Dias (1999)
Brazil	São Paulo, São Vicente	72.7	63.1	Present study
Brazil	Santa Catarina, Florianópolis	72.7	-	Mantelatto and Dias (1999)
Venezuela	Falcón, Paraguana	83.2	65.6	Morán and Atencio (2006)
Colombia	Caribbean coast	75.0	55.6	Campos and Tukey (1989)
USA	Florida, Fort Pierce	79.0	77	Dineen (2001)
Turkey	Iskenderum Bay	78.0	62.1	Ozcan et al. (2010)
India	West coast	63.7	51.0	Kathirvel and Gopalakrishnan (1974), Ozcan et al. (2010)

**Figure 4.** Macroscopic ovarian development for the ovigerous females (RUD = rudimentary, DE = developing, INT = intermediate, MAT = mature) separated according to the embryo (egg mass) development, categorized into initial, intermediate and final stages.

that ovarian development is continuous and independent of the egg development stage (Figure 4). About 35% of the ovigerous females displayed mature or ripe ovaries, including females with an initial egg mass and all ovigerous females had sperm stored in the seminal receptacle (Figure 5).

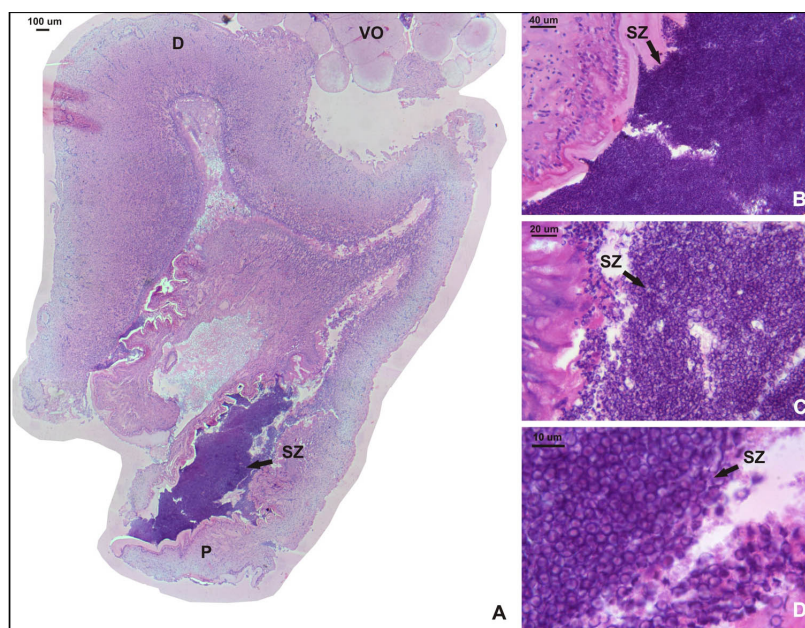
Discussion

Charybdis hellerii is reported to prefer soft-bottom habitats, but also occurs in mangroves and on coral reefs and other hard bottoms, from

the intertidal zone to 51 m depth (Stephenson et al. 1957; Galil 1992; Lemaitre 1995; D'Udekem d'Acoz 1999; Dineen et al. 2001). In North Atlantic waters (Florida, U.S.A.), *C. hellerii* has been found associated with hard or artificially constructed substrates near inlets and lagoons of the Indian River in Florida (Lemaitre 1995; Dineen et al. 2001). In the Caribbean Sea off Venezuela, it was recorded as an established and breeding population, from both soft and hard bottom habitats (Morán and Atencio 2006). Mantelatto and Dias (1999) recorded the species from soft-bottoms in some areas and on rocky shores in another (southern) area along the Brazilian coast. In South Atlantic waters, only one study discusses biological aspects of *C. hellerii*, using samples from soft bottoms and rocky shores (Mantelatto and Garcia 2001), similar to those investigated in the present study. However, none of these studies has focused on the highly dynamic estuarine systems where this species may also be established, nor has any compared these two habitats in the same region.

In the present study, *C. hellerii* clearly predominated on the rocky shores and in the interface with the adjacent soft bottoms, rather than on the subtidal soft bottoms in the estuary or bay, where it occurred only occasionally. Thus, no direct negative impacts are expected on other soft-bottom portunids or other brachyuran species in these areas. Populations of commercially important Portunidae on soft-bottom habitats (*Callinectes* and *Arenaeus*) showed similar frequencies to those reported by Pita et al. (1985). Given these differences in

Figure 5. Seminal receptacle of ovigerous female of *Charybdis hellerii* with mature ovary. (A) General morphology of the seminal receptacle depicting the ventral ectodermal region (P) filled with sperm (SZ). The dorsal-mesoderm region (D) contains mature vitellogenic oocytes (VO). The sperm (SZ) is found in different regions of the seminal receptacle, such as ventrally (B), in the dorso-ventral transition area (C), and dorsally (D).



habitat use, the possibility of a negative impact on the *Callinectes* populations from competition with *C. hellerii* (Morán and Atencio 2006) should be evaluated with caution. Mantelatto and Garcia (2001) suggested that large portunids such as *Callinectes ornatus* (Ordway, 1863), *C. danae*, *Portunus spinimanus* (Latreille, 1819) and *Arenaeus cribrarius* (Lamarck, 1818) may limit the impact of *C. hellerii*, since they are numerically dominant and may be potential predators of this invasive species. The results presented here support this hypothesis, although the relationships between *C. hellerii* and soft-bottom portunid crabs were not tested experimentally; such relationships may explain the predominance of *C. hellerii* on the rocky shores. This differential use of the rocky shore by *C. hellerii* and its relatively higher abundance in relation to other crabs, such as *Cronius ruber* (Lamarck, 1818), deserve attention regarding the possible impact on the native fauna.

Nevertheless, the impacts of this invasive crab will depend on several factors, as mentioned above, and may change depending on microhabitat use, which may vary among localities. Therefore, *C. hellerii* may have different (or no) impacts in different areas or microhabitats, a possibility that should be investigated locally. One indication of a possible negative impact on the rocky-shore brachyuran assemblage is related to the very low frequency

of the portunid *C. ruber* in the study area. On the rocky shores of the São Vicente estuary-bay complex, only one individual of *C. ruber*, which is characteristic of this microhabitat, was collected during the study period. Recent studies using trawling, conducted on the northern and southern coasts of São Paulo have reported very few individuals of *C. ruber* (two and four, respectively) (Braga et al. 2005; Severino-Rodrigues et al. 2009). In contrast, in a study done in the mid-1980s, 16 individuals of *C. ruber* were collected by trawling in the São Vicente estuary-bay complex, a period before the introduction of *C. hellerii* into Brazilian waters (Pita et al. 1985). A checklist by Reigada et al. (1998) in the same area of this study listed individuals of *C. hellerii* and none of *C. ruber*. A further relevant observation is that during transport and laboratory manipulation, males of *C. hellerii* displayed aggressive behavior, especially against *C. ruber* (Fernando José Zara, personal observations). Taken together, these observations may suggest the decline of the sympatric *C. ruber* in Brazilian waters, as predicted by Mantelatto et al. (2009), although further comparative studies of different rocky-shore assemblages are required to confirm this tendency.

The impact of *C. hellerii* as a possible consequence of its aggressiveness is supported by Morán and Atencio (2006), who reported a

high percentage of males with mutilated appendages (47%), roughly comparable to the 57% recorded here. This apparent aggressive behavior could be related to interspecific competition, although predation and intraspecific competition may also cause such damage.

With respect to population parameters, the size range of this crab was similar to that described for other parts of Brazil, except for data from Ubatuba (Table 1), where individuals were smaller than those found here. The size of *C. hellerii* here is slightly smaller than in other countries except India (Table 4). Sexual dimorphism, with males larger than females, was also observed by Mantelatto and Garcia (2001) in another location. This common pattern in portunid crabs (Avila and Branco 1996; Pinheiro and Fransozo 1999; Batista-Metri et al. 2005; Fernandes et al. 2006) is associated with the puberty molt, when metabolic energy is directed to somatic growth in males, and to ovary development in females (Hartnoll 1985). The larger size of males facilitates manipulation of the female during copulation (Santos et al. 1995). Nevertheless, we detected sperm production in very small individuals, suggesting that males are fertile and have a free abdomen at a similar CW to the onset of morphological maturity measured for females by Mantelatto and Garcia (2001). Therefore, the female reproductive system should be investigated to check if sperm transfer with the typical portunid sperm plug (Hartnoll 1968, 1969) also occurs in this species.

The main difference between the São Vicente and Ubatuba populations was the predominance of males (Mantelatto and Garcia 2001). A similar sex ratio in favor of males was found for *C. hellerii* in Venezuela (Morán and Atencio 2006). In the U.S.A., the sex ratio was nearly even, although slightly in favor of males (Dineen 2001). The skewed sex ratio may be a sampling artifact because females tend to be cryptic in habit and less active than males. As well as that, females may occur in different microhabitats than males. Small-sized and immature individuals, not recorded in the study areas, may also prefer different microhabitats.

Despite their low frequency of occurrence, ovigerous females were recorded year-round, indicating a continuous, but heterogeneous, reproductive pattern. According to Mantelatto and Garcia (2001), ovigerous females were found throughout the year but with higher frequencies in autumn, winter, and spring. In the present

study, the rather sparse data showed the highest incidence of ovigerous females during the winter months. Reproduction of portunid crabs of the genus *Callinectes* inhabiting soft bottoms in this estuary-bay complex often peaks in the summer (Sant'Anna et al. 2012). As suggested by Mantelatto and Garcia (2001), this difference in reproductive periods between *C. hellerii* and the native portunid species may minimize competition. Nevertheless, reproductive periods have only been studied for the commercially important Brazilian portunids of soft bottoms (Costa and Negreiros-Fransozo 1998; Pinheiro and Fransozo 2002; Mantelatto and Fransozo 1999). Also, the reproductive biology of *C. ruber* is still largely unknown. In the Pacific Ocean, the related species *Charybdis bimaculata* (Miers 1886) reproduces throughout the year, showing peaks in the warmer seasons (Doi et al. 2008), similar to other native portunid crabs in Brazil. The high reproductive activity in the winter may be an endogenous characteristic of *C. hellerii*, or perhaps excessively warm waters may not be appropriate for their reproduction. In southwest Atlantic coastal waters, temperatures nearest to their original environment occur only in the winter. Therefore, it is expected that this species will move to deeper waters in summer months or in low latitudes, a hypothesis that should be carefully evaluated.

Ovigerous females of *C. hellerii* with developed gonads showed sperm stored in the seminal receptacle. These features may suggest the occurrence of multiple spawning, because ovigerous females can produce a new clutch of eggs just after the incubation period, as in other portunid crabs (Costa and Negreiros-Fransozo, 1998). Dineen et al. (2001) suggested that this strategy increases larval production. These reproductive characteristics observed here, similar to observations by Dineen et al. (2001), may explain the success of this invasive species.

In conclusion, knowledge of the comparative abundance of the non-indigenous crab *C. hellerii* and how it interacts with native species is important in predicting its ecological impact. The population structure and successful reproduction of *C. hellerii* in the southwestern Atlantic waters showed that this crab is well established in different habitats along the Brazilian coast. On the Brazilian coast, there is no evidence of an effect of *C. hellerii* on economically important species (*Callinectes* and *Arenaeus*). On the other hand, our data suggest that the rocky-shore portunid *Cronius ruber* (and

Cronius tumidulus (Stimpson, 1871)) may be in decline, and this possible effect should be further investigated. Thus, integrative actions to combat invasive species, such as the Interagency Monitoring Action Plan (I-MAP) developed in the U.S.A., to control the quagga mussel *Dreissena bugensis* (Andrusov, 1897) (Turner et al. 2011), should be followed in other countries, including Brazil, to control and monitor environments and invasive species

Acknowledgements

F.J. Zara thanks FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, JP Proc. 2005/04707-5 and Biota Proc. 2010/50188-8), PROPe UNESP – Programa Primeiros Projetos, and CNPq (PQ Proc. 308215/2010-9) for financial support, and T.T. Watanabe thanks CNPq (Proc. 138732/2011-6) for a scholarship. Thanks are due to Dr. Alvaro L. D. Reigada and the undergraduate students of the Invertebrate Morphology Laboratory (IML – UNESP) for field assistance. Our gratitude to Gerson R.R. Pereira for providing some of his histological data for this study, Dr. Gustavo A. S. Melo for help in the identification of the species, and Dr. Janet W. Reid (JWR Associates) for editing the English text.

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