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Vertical distribution by demographic groups of ghost crab *Ocypode quadrata* (Crustacea: Brachyura)

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Abstract: The ghost crab *Ocypode quadrata* plays an important role in energy transfer between trophic levels, and has been widely used in evaluations of impacted environments. In order to provide data on the biology of this potential bioindicator species, the population structure and vertical distribution of individuals were studied on two beaches in southeastern Brazil. Each beach was divided into quadrants of 1000 m^2 with boundaries of upper, middle and lower levels in relation to the waterline. Collected monthly by active searching through one year, the specimens of *O. quadrata* were sexed, measured for carapace width, and returned to the beach. Of the total of 1904 specimens collected, the largest proportion (46.2%) were males, followed by 31.4% juveniles. The vertical distribution of the ghost crabs differed among age groups: males mostly occupied the middle and upper levels; adult females, ovigerous or not, were more abundant in the lower level; and juveniles were evenly distributed in all levels, with a slight tendency toward the middle. The sex ratio favored males in a few months of the year and in the larger size classes. The abundance of *O. quadrata* is limited by low temperatures, and its spatial and temporal distribution is controlled by food availability and ease of reproduction. Knowledge of the biology of these crabs is essential in order to use them as a bioindicator species; the vertical distribution patterns may reflect changes in the beach hydrodynamics or other environmental factors.

Key words: intertidal zone; *Ocypode quadrata*; Ocypodidae; population pattern; vertical migration

Introduction

The physical environment of sandy beaches is influenced by a number of factors including winds, currents, waves and tides, which interact and result in constant changes in the beach hydrodynamics and depositional patterns (Brown & McLachlan 1990). Crabs of the genus *Ocypode* (Weber, 1795), known as ghost crabs, are commonly found on sandy beaches of tropical and subtropical regions. This genus is represented in the western Atlantic by *Ocypode quadrata* (F., 1787), which occurs from Rhode Island, USA to Rio Grande do Sul, Brazil (Melo 1996). This species can be quite sensitive to climate change, such as variations in the intensity of winds, temperature and wave heights (Wolcott 1978; Alberto & Fontoura 1999).

Ecologically, the scavenger (see Wolcott 1978) *O. quadrata* plays an important role in energy transfer between different trophic levels of coastal ecosystems, acting as a predator of invertebrates on sandy beaches and as prey for a large number of consumers, and also consuming organic debris (Philips 1940). Similarly to all crabs of the genus, *O. quadrata* digs burrows regardless of its size and gender (De 2005). In many studies, the number of burrows is used to estimate population size, a simple, rapid and low-impact method (Warren 1990). Factors involving their rapid behavioral responses and the ease and low impact of studying ghost crabs have made these organisms popular subjects for studies that use them as indicators of human disturbance on beaches worldwide (Steiner & Leatherman 1981; Barros 2001), and have increased interest in their population characteristics.

According to Van Dam & Van Dam (2008), invertebrates are effective ecological indicators of human stressors in a wide range of environments, and usually respond to spatial disturbances on larger scales than vertebrates do (Carignan & Villard 2002). Their distributions and population sizes are often closely correlated with environmental conditions that are potentially subject to anthropogenic change (Schoener 1986). Several investigators have demonstrated the usefulness of crabs of the genus *Ocypode* as biological indicators of environmental impacts, and their feasibility for short-term

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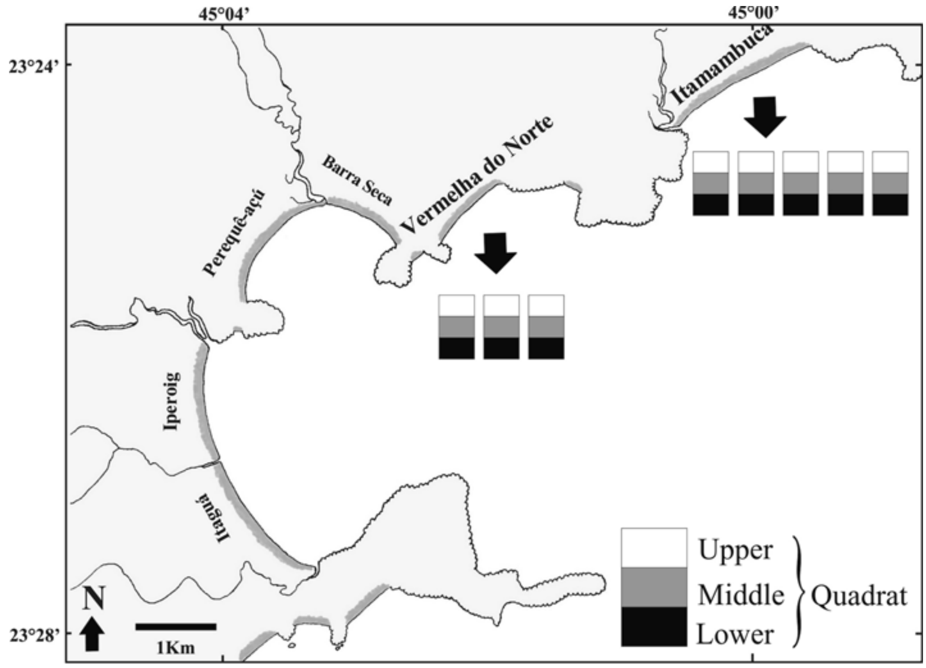

Fig. 1. Map of the beaches studied and scheme of the experimental design.

monitoring studies (Steiner & Leatherman 1981; Barros 2001; Blankensteyn 2006; Neves & Bemvenuti 2006; Lucrezi et al. 2009a, b). Studies by Wolcott & Wolcott (1984), Turra et al. (2005), Blankensteyn (2006) and Schlacher et al. (2011) showed that populations of *O. quadrata* have suffered a series of disturbances from the occupation of land near beaches, as well as continuous trampling by vacationers or vehicle traffic.

Knowledge of the biology of bioindicator species may aid in recognizing an impacted environment, providing data that can be used to assess the environmental balance and the spatial scope and severity of human impacts, and also to evaluate the effectiveness of management interventions (Niemi & McDonald 2004). The southeastern coast of Brazil is widely studied because it is a zone of hydrological and wildlife transition, with a combination of biological characteristics and fauna of tropical, subtropical and subantarctic origins (Sumida & Pires-Vanin 1997). The present study investigated the sex ratio, population structure and ecological distribution of demographic groups of *O. quadrata* on two beaches in southeastern Brazil. The results obtained here can be used for future comparisons aimed at assessing possible environmental impacts in the region.

Material and methods

Characterization of the study area

The study was conducted in Ubatuba Bay, northern coast of São Paulo, on Itamambuca Beach (23°23′48″ S and 44°00′36″ W), which faces the open sea, and the sheltered Vermelha do Norte Beach (23°24′48″ S and 44°02′18″ W). Itamambuca (ITA) is one of the most pristine beaches of Ubatuba; is famous for its excellent waves for surfing, and also provides a remarkable opportunity for ecotourism through the locally well-preserved Atlantic Forest (Mata Atlântica) vegetation. It has "restinga" vegetation (coastal dune forest) along its entire length, and to the south, a very productive estuary. Vermelha do Norte (VER) is a sloping beach popular for surfing. It is located at the edge of a highway, opposite a stand of "caxetal" vegetation (dominated by the caxeta tree *Tabebuia cassinoides*) that, unlike the estuary, is influenced only by the nearby river. No restinga vegetation is present along this beach.

The beaches were divided into quadrants of 1000 m^2, with an interval of 250 m between each quadrant, in order to avoid data skewing. Itamambuca (ITA), which is 2000 m long, was sectioned into five quadrants of 10 × 100 m, parallel to the shoreline. Vermelha do Norte (VER), 1300 m long, was divided into three quadrants with the same dimensions and spacing. These quadrants were used as replicates within each beach. The quadrants were further divided into three levels (upper, middle and lower); the upper level extended to 3.3 m above the highest boundary of the strandline (or high-water mark), and the other two levels, each 3.3 m wide, were located below the strandline (Fig. 1). Tidal fluctuations were always taken into consideration.

Collection of Ocypode quadrata

The samples were obtained in a systematic way, from March 1999 through February 2000, during the night, and always on a spring tide. Specimens of *O. quadrata* were collected through active capture for 20 minutes on each level of each quadrant, with an effort by four people carrying two flashlights and dip nets. Burrows were not excavated, since these crabs are active on the beach at night (Wolcott 1978). The specimens were placed in buckets marked with the levels where they were collected. At the end of each quadrant collection, the specimens were sexed and measured for carapace width (CW), and then returned to the beach. Individuals without apparent sexual dimorphism were taken to the laboratory, where they were sexed according to the shape of the pleopods, and were later returned to the collection points. Young and adult individuals were distinguished based on a study by Negreiros-Fransozo et al. (2002), which estimated carapace widths at which 50% of the subjects were adult (CW50), of 20 mm for males and 23 mm for females.

Environmental factors
Environmental measurements were taken at the beginning and at the end of each level of each quadrant sampling; i.e., two measurements were taken during each evaluation of 20 minutes. The relative humidity (RH) (measured in the crab burrows) and the air temperature, on each beach level, were measured with an aspiration psychrometer (accuracy ± 3–5% RH); the water temperature with an analog thermometer, with a precision of 0.1 °C; and salinity with a refractometer (level of the beach were obtained with two corers, approximately 15 cm long with an opening of 5 cm in diameter. Thus, monthly, each environmental variable was assessed twice in each quadrant and at each level of the beach, totaling at the end of the study 216 samples of each variable for VER and 360 samples of each variable for ITA.

The samples of sediment were placed in plastic bags with identification tags and refrigerated for later analysis. In the laboratory, the sediment was placed on trays and oven-dried for 24 h at 70 °C. When completely dry, two 50 g aliquots per level were passed through a series of sieves with decreasing mesh sizes, with stirring for 5 min to separate the grains. For particle size analysis, the Wentworth (1922) scale was used to determine the pattern of sediment texture from the weights of particles retained in each sieve. Two additional 10 g aliquots per quadrant level were placed in porcelain crucibles and incinerated in a muffle furnace at 500 °C for 2 h. The samples were re-weighed, and the percentage of organic matter was obtained from the difference of the ash-free dry weight (Mantelatto & Fransozo 1999).

Statistical analysis
All analyses were performed using the software R (R Development Core Team 2009). Initially data were tested for univariate normality through the Shapiro-Wilk test (Shapiro & Wilk 1965), and multivariate normality through the skewness and kurtosis test (Mardia 1970, 1980) (with modifications proposed by Doornik & Hansen 1994; omnibus test). To test the homogeneity of variances, the Brown-Forsythe test (Brown & Forsythe 1974) was performed. The Brown-Forsythe test was chosen because of the unequal n of groups compared. For multivariate data, Box's M test was performed to evaluate the equivalence (multivariate homogeneity) among data covariance matrices, with Monte Carlo permutations to estimate the significance (McCune and Grace 2002).

The relationships between the abundance of crabs in the different demographic groups and environmental factors were evaluated by a Canonical Correspondence Analysis (CCA) with environmental vectors fitted through the functions "cca" and "envfit" of the "vegan" package (Oksanem 2001). To test the hypothesis of differences in abundance among demographic groups, levels of vertical distribution, beaches, and the interaction of these three factors, a multifactorial variance analysis (multiway ANOVA) was performed.

A heat map was generated from the abundances of demographic groups according to the vertical levels and the beaches, using the function "heatmap.2" of the package "gplots" (Gregory et al. 2010). The heat map is generated by combining original abundance data in two paired dendrograms, the first grouping them by sampling site, and the second grouping the same data by demographic groups. The result is a density gradient to which a color key is associated and generates an easily interpretable visual quantitative and qualitative result (Eisen et al. 1998). In this case, a visual association between groups and areas was generated.

Dendrograms of the heat map were generated by the binding function of the Ward method and by the Bray-Curtis dissimilarity distance. This binding function and dissimilarity index were chosen by determining the Agglomeration index (AC) and the Cophenetic Correlation Coefficient (CPCC) through the functions "agnes" of the "cluster" and "mass" packages, respectively (see additional details in Lessig 1972; Maechler et al. 2005).

After the determination of the dissimilarity distance and binding method, groupings were made using the "pvclust" function of "pvclust" package (Suzuki & Shimodaira 2006), which generates probability values using the bootstrap resampling technique (see additional details in Suzuki & Shimodaira 2006; Shimodaira 2008).

For size data, a bifactorial variance analysis was performed with data transformed in ranks, due to their non-parametric character. The factors were Beaches vs. Demographic groups, and in a second approach, Levels vs. Demographic groups. It was not possible to perform multiway ANOVA with transformed data because there were no records of this approach for the respective sampling design. Simultaneously, as suggested by Zar (2010), a bifactorial ANOVA was performed with the same, non-transformed data, taking quadrats of each beach as a repeat analysis of the levels (3 quadrats in VER and 5 quadrats in ITA). According to Quade (1979), when ANOVA results with original data and rank-transformed data are similar, an ANOVA with ranked data can be used for non-parametric data as a solution for the absence of robust bifactorial tests. Due to the large number of treatments in each factor, the bifactorial ANOVA post-hoc results were not included in the final work. Rather, a Kolmogorov-Smirnov test (KS) was performed to compare the medians of each demographic group between the beaches. The KS test is used when medians of two groups are to be compared, considering skewness and kurtosis aspects of data distribution in each group or the treatment level. The significance level adopted for all analyses was 5% (Zar 2010).

To demonstrate the population structure during the year, the absolute values of spatial and temporal abundance were transformed into catch per unit effort (CPUE) in 10 m^2, since the beaches had different dimensions and therefore different total sampling efforts. We used the Chi-square test (χ^2) of goodness of fit to test whether the observed sex ratio conformed to the theoretical ratio of 1:1, and if the sex ratio differed through space and time and among size classes.

Results

Distribution, abundance and relationships to environmental variables
A total of 1904 crabs were collected, 1294 of them on ITA beach and 610 on VER beach. ITA beach had a relatively high percentage of medium sand (49.5%), followed by fine sand (34.5%) and grit (14.3%) and a higher organic-matter content (0.52 ± 0.07%) than VER. VER had a higher percentage of medium sand (54.1%), followed by grit (40.6%) and fine sand (5.1%), with a lower mean organic-matter content than ITA (0.24 ± 0.17%); on this beach, the percentage of organic matter peaked in March (0.5%). The remaining fractions refer to biodetritus: 1.7% at ITA and 0.2% at VER. The relative humidity in the burrows

(RH) and water salinity (SAL) were similar on both beaches; i.e., in ITA RH = 92.68 ± 4.32% and SAL = 34.05 ± 1.76 and VER with RH = 91.70 ± 3.98% and SAL = 31.04 ± 2.72.

The canonical correlation analysis related the abundance of demographic groups only to relative humidity ($R^2 = 0.9065$, $P < 0.05$; Fig. 2); the relationships to organic matter, air temperature and salinity were not significant ($P > 0.05$). The triplot inspection indicated that adult females (AF) and ovigerous females (OF) showed a closer relationship to relative humidity than did the other demographic groups. Relative humidity appeared to form an environmental gradient with three groupings, the first related to the mean and upper ranges of VER (first quadrant), followed by the grouping of the mean and upper ranges of ITA (fourth quadrant), and finally, the grouping of the lower levels of both beaches (second quadrant). The same grouping pattern appeared in the heat map (Fig. 3). These results indicate that there was an association of Demographic groups/Beach vs. Beach Levels, marked by a gradient of relative humidity.

The heat map did not show visually significant differences in the distribution of demographic groups of *O. quadrata* by beach, in agreement with the results of the mixed multifactorial ANOVA (Table 1). How-

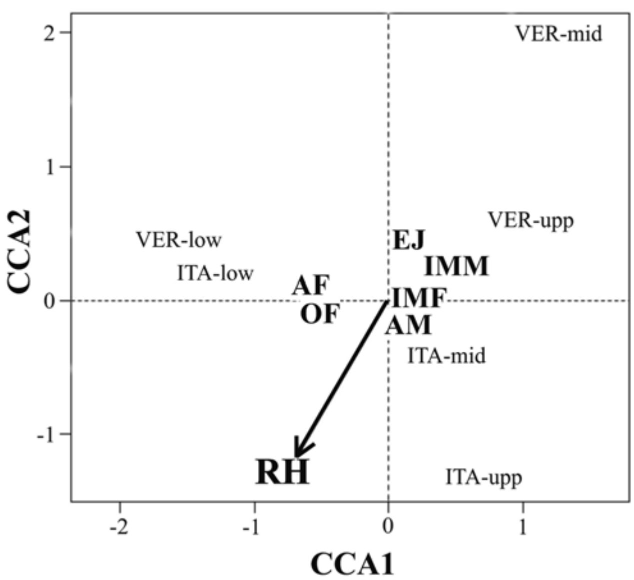

Fig. 2. Canonical Correlation Analysis of the abundance of different demographic groups of *Ocypode quadrata* from Itamambuca (ITA) and Vermelha do Norte beaches (VER), northern coast of São Paulo state, Brazil, at three levels of vertical distribution (low – Lower; mid – middle; upp – Upper). AM – Adult males; AF – Adult females; IMM – Immature males; IMF – Immature females; EJ – Immature juveniles, sex undetermined; OF – Ovigerous females; RH – humidity.

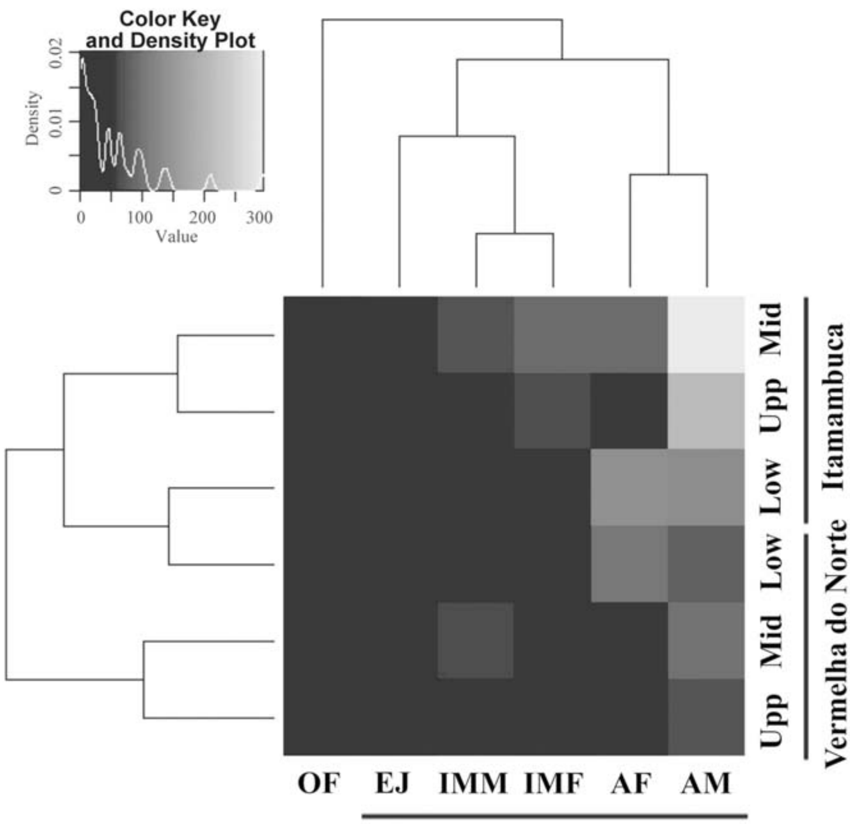

Fig. 3. Heat map of abundances of different demographic groups of *Ocypode quadrata* on beaches (Itamambuca and Vermelha do Norte) and levels of vertical distribution (low – Lower; mid – Middle; upp – Upper), using Bray-Curtis distance as distance measurement and Ward as binding method. AM – Adult males; AF – Adult females; IMM – Immature males; IMF – Immature females; EJ – Immature juveniles, non-sexed; OF – Ovigerous females.

Table 1. Multifactorial ANOVA of the abundance of demographic groups (DG) of *Ocypode quadrata* from Itamambuca and Vermelha do Norte beaches and vertical distribution levels (Lower, Middle and Upper).

	SS	df	MS	*F*	*P*
Intercept	31220.16	1	31220.16	196.2183	0.00
Beach	288.91	1	288.91	1.8158	0.18
Level	983.53	2	491.76	3.0907	0.05
DG	28021.11	5	9340.37	58.7041	0.00
Beach*Level	204.65	2	102.33	0.6431	0.52
Beach*Maturity	382.86	5	127.62	0.8021	0.49
Level* DG	3832.71	8	638.78	4.0148	0.00
Beach*Level* DG	336.08	8	56.01	0.3520	0.90
Error	11455.87	72	159.11		

Explanations: SS – Sum of squares, df – degrees of freedom, MS – mean square, *F* – F Statistics ANOVA, *P* – *P* value.

Table 2. Abundance of demographic groups (DG) of *Ocypode quadrata* from Itamambuca and Vermelha do Norte beaches and vertical distribution levels.

Maturity	Demographic group	Itamambuca			Vermelha do Norte		
		Lower	Middle	Upper	Lower	Middle	Upper
Adults	AF	141	91	44	102	25	14
	OF	5	3	1	1	0	1
	AM	133	295	210	77	98	66
Juveniles	IMF	45	91	62	19	47	22
	IMM	14	66	46	12	61	29
	EJ	16	27	4	7	22	7

Explanations: AF – adult females; OF – ovigerous females; AM – adult males; IMF – immature females; IMM – immature males; EJ – early juveniles.

ever, a slight difference was perceptible in the density of demographic groups by beach levels, with adult males (AM) present in higher densities in ITA, while immature males (IMM) were present in higher densities in VER (Table 2). There were marked density differences among the vertical levels, independent of the beach, in agreement with the visual difference shown by the correspondence analysis (Fig. 4) and with the statistical difference detected by ANOVA (Table 1).

The correspondence analysis showed a significant association of the abundance of the demographic groups of *O. quadrata* with the beaches, on all three vertical levels of distribution ($\chi^2 = 297.48$, $P = 0.0004998$, n permutations = 9999; Fig. 4). The multifactorial variance analysis showed a significant difference in the mean abundances of the demographic groups by vertical level (Beach level vs Demographic groups, $F = 4.014$, df = 8, $P < 0.05$). There was no significant difference regarding the "Beach" factor (Table 1).

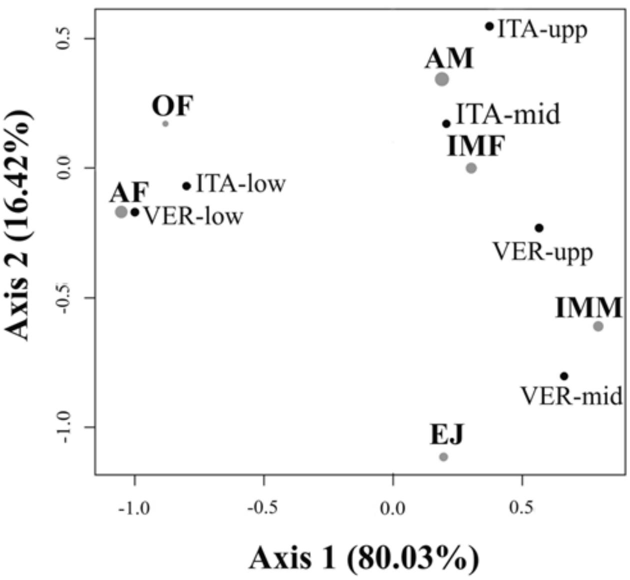

Fig. 4. Correspondence Analysis of abundances of different demographic groups of *Ocypode quadrata* from Itamambuca (ITA) and Vermelha do Norte beaches (VER), northern coast of São Paulo state, Brazil, at three levels of vertical distribution (low – Lower; mid – middle; upp – Upper). AM – Adult males; AF – Adult females; IMM – Immature males; IMF – Immature females; EJ – Immature juveniles, non-sexed; OF – Ovigerous females.

Population structure and sex ratio

The crabs were separated in 12 size classes with an interval of 3 mm. Individuals without sexual dimorphism, called early juveniles, measured from 6.1 to 10 mm CW. The smallest collected ovigerous female measured 23 mm CW, and the largest 32.6 mm CW. The largest young crab reached 22.9 mm CW. Because of the non-parametric nature of the data and the absence of non-parametric tests for multifactorial design, the analyses were performed in bifactorial models separately, with rank-transformed data. The bifactorial variance analysis showed a difference in carapace width among the demographic groups in relation to the beaches, and separately, in relation to the levels of vertical distribution (Table 3). There was a significant difference in the me-

Table 3. Bifactorial ANOVA with demographic groups (DG) data transformed in ranks of carapace width of *Ocypode quadrata* between (A) beaches (Itamambuca and Vermelha do Norte), and separately among (B) levels of vertical distribution (Lower, Middle and Upper).

	SS	df	MS	*F*	*P*
A – Beach vs. Demographic group					
Intercept	119058241.76	1.00	119058241.76	1130.37	0.00
Beach	37914.85	1.00	37914.85	0.36	0.55
DG	358262742.24	5.00	71652548.45	680.29	0.00
Beach*DG	3025016.20	5.00	605003.24	5.74	0.00
Error	199277935.58	1892.00	105326.60		
B – Level vs. Demographic Group					
Intercept	149769913.04	1.00	149769913.04	1436.14	0.00
Level	282996.85	2.00	141498.42	1.36	0.26*
DG	294415315.35	5.00	58883063.07	564.63	0.00
Level*DG	2369181.53	10.00	236918.15	2.27	0.01
Error	196684383.14	1886.00	104286.52		

Explanations: SS – Sum of squares, df – degrees of freedom, MS – mean square, *F* – F Statistics ANOVA, *P* – *P* value.

Table 4. Descriptive statistics of median values of carapace width for each demographic group of *Ocypode quadrata* present on the two beaches.

	Itamambuca					Vermelha do Norte					
	N	Median	SE	Skewness	Kurtosis	N	Median	SE	Skewness	Kurtosis	KS
AF	276	27.00	0.18	0.74	0.22	141	28.40	0.35	0.76	–0.04	0
AM	638	26.50	0.17	0.53	–0.37	241	27.60	0.36	0.48	–1.02	0
EJ	47	8.30	0.14	–0.23	–0.29	36	8.80	0.13	–0.82	–0.50	0.1
IMF	198	16.00	0.31	–0.06	–1.33	88	12.65	0.43	0.87	–0.39	0
IMM	126	14.35	0.29	0.03	–1.27	102	12.45	0.33	0.53	–1.04	0.1

Explanations: AF – adult females; AM – adult males; EJ – early juveniles; IMF – immature females; IMM – immature males. N – number of individuals; SE – Standard error; KS – Kolmogorov-Smirnov test of median differences for two samples.

dian size of crabs between beaches and among vertical levels, and at VER the highest median values were found for adults (AF, AM) (Tables 3, 4). At ITA the highest median values of carapace width were observed for the immature stages (IMF, IMM) (Table 4).

The analysis of the skewness and kurtosis values of the frequency distribution of each demographic group between the beaches revealed that for adults of both sexes, the kurtosis values were more platykurtic for distributions on VER, indicating fewer adult individuals in intermediate size classes (Table 4). A positive asymmetric and mesokurtic direction was observed for juveniles from ITA compared with VER. The Kolmogorov-Smirnov test indicated a significant difference only between the frequency distribution of adults (AF, AM) and immature females (IMF) (Table 4). In this analysis we used only five demographic groups, since the low abundance of ovigerous females at VER (2 crabs) made it impossible to estimate skewness and kurtosis.

Immature and adult females and males were captured in all the collection months (Fig. 5), but were less abundant during the cooler season (Fig. 6). Ovigerous females and immature individuals were found mainly during the summer. They showed a restricted distribution in March and April 1999 on both beaches, and in May 1999 and February 2000 on VER and ITA, respectively (Fig. 5). The size-frequency distribution revealed a clear input of recruits (early juveniles) in March and April 1999 on both beaches (Fig. 6). After the recruitment period the population declined in the following months, returning to the previous levels of abundance in January and February 2000 (Fig. 6).

Throughout the sampling period on both beaches, the sex ratio of *O. quadrata* favored males (χ^2, $P < 0.05$) in some months, and in others followed the Mendelian proportion of 1:1 (Fig. 7A). Testing the sex ratio among size classes revealed that males predominated over females beginning at 20 mm carapace width. Among beach levels, females were more abundant (χ^2, $P < 0.05$) on the lower level, with a ratio of 1:1.3 females on ITA and 1:1.4 females on VER (Fig. 7B); while on the middle and upper levels, males predominated.

Discussion

ITA showed higher organic-matter contents than VER, probably due to the smaller proportion of coarse sand in the sediment, and to the presence of an estuary that receives large amounts of nutrients and organic matter from the local rivers. However, since VER is located inside Ubatuba Bay, it receives organic input from rivers, which increases the availability of food. The texture of the substrate is highly important for the distribution and composition of benthic communities of coastal environments (Buchanan 1963), and the organisms are closely associated with the granulometric composition

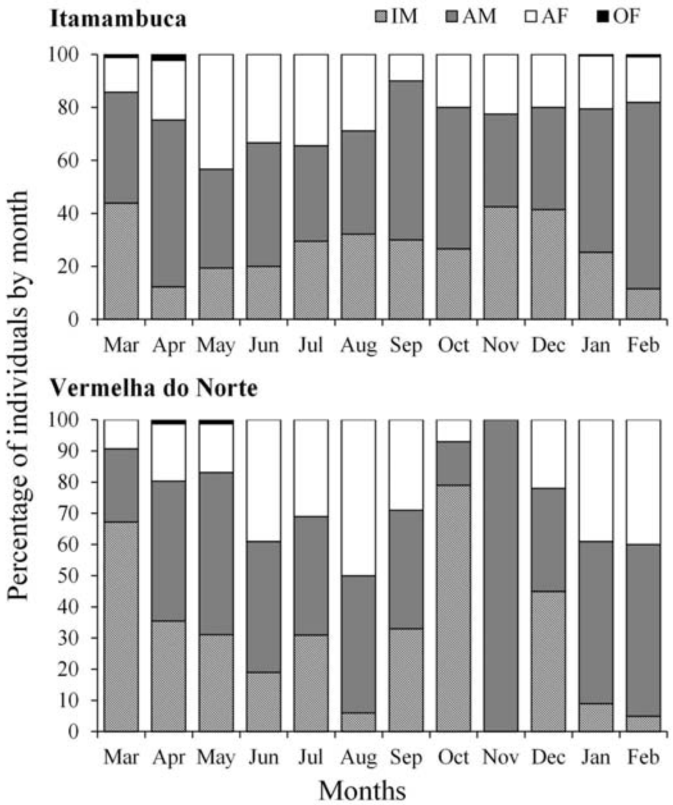

Fig. 5. Percentage of adult females (AF), ovigerous females (OF), adult males (AM) and immature males (IM) of *Ocypode quadrata* on Itamambuca and Vermelha do Norte beaches.

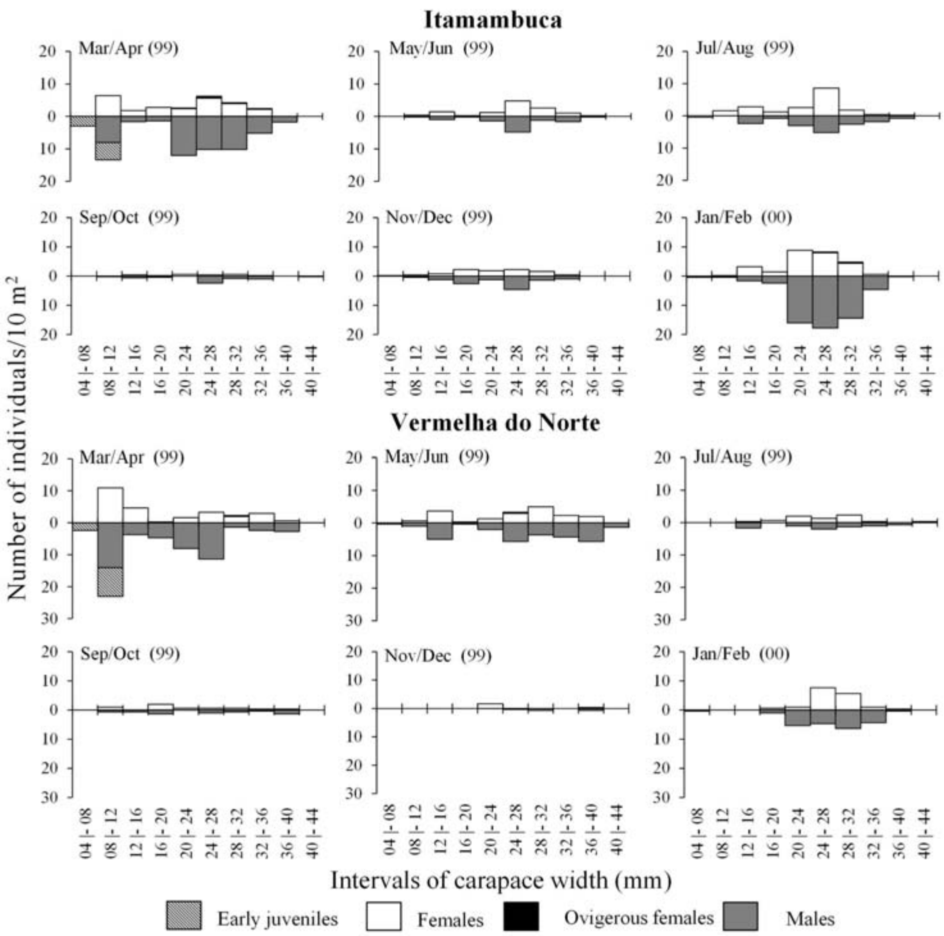

Fig. 6. Bimonthly size-frequency histograms of *Ocypode quadrata* on Itamambuca and Vermelha do Norte beaches.

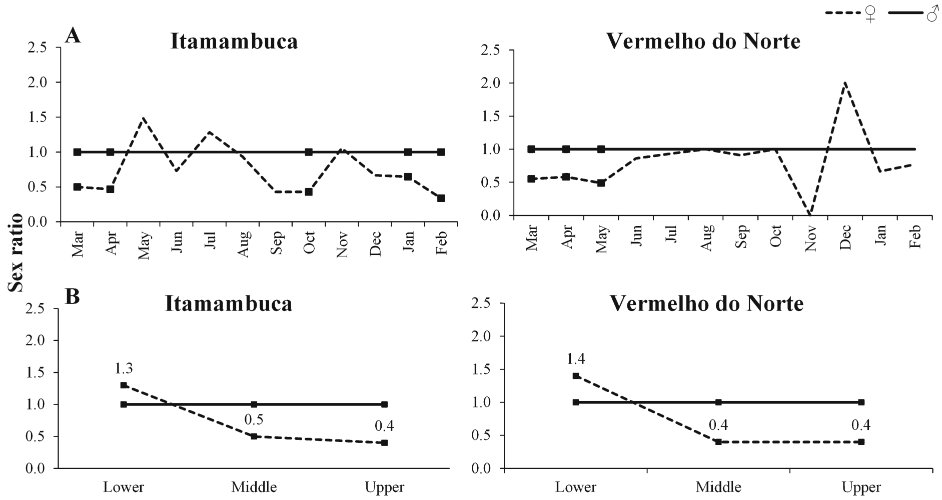

Fig. 7. Sex ratio by months (A) and beach levels (B), of *Ocypode quadrata* on Itamambuca and Vermelha do Norte beaches. Labels on lines indicate significant difference, $P < 0.05$.

of the sediments (Buchanan & Stoner 1988). Substrates constituted mainly of finer sand grains have a higher organic content than deposits with coarser grains, and this feature is a major determinant of the number of living organisms that can be supported (Moore 1958). According to Robertson & Pfeifer (1982), deposit-feeding is an important component in the foraging repertoire of *O. quadrata*, which can extract up to 70% of the available algae. These authors stated that the feeding rate of these crabs depends on the temperature, relative humidity and composition of the substrate. These factors seem to affect not only feeding rates, but also the distribution of demographic groups of this species.

Although we found no significant correlation between crab abundance and organic-matter content, Henmi (1989) observed for the ocypodid crab *Macrophthalmus japonicus* (De Haan, 1835) in Japan, that a larger number of crabs can be supported when more food is available. In addition to the sediment type and organic-matter content, which may influence the abundance, the temperature may also affect the population behavior, because the activity level of individuals is directly related to the temperature. Working with *O. quadrata* in South Africa, Haley (1972) reported that temperatures around 16 ℃ were sufficient to induce the crabs to remain dormant in their burrows. Atkinson & Taylor (1988) noted that temperatures in the burrows are considerably more stable than in the open air, and do not exceed the lethal limits for ocypodids.

Alberto & Fountoura (1999) reported that not only variations in temperature but also wind and intense trampling will induce the crabs to close the entrances to their burrows, making them impossible to locate. This behavior of closing burrows must be taken into account in evaluating the low number of ovigerous females collected in this study. Other investigators have not succeeded in capturing these females (Haley 1972; Alberto & Fontoura 1999; Negreiros-Fransozo et al. 2002; Graf et al. 2008), since they can remain in their burrows while they are incubating their eggs, as do other ocypodids (Christy 1982). This behavior may also be related to the maintenance of the humidity level inside the burrow. In this study, we observed a close relationship between relative humidity and female abundance, which possibly controls the females' preference for the lower zone of the beach. Both ovigerous and non-ovigerous adult females were more abundant on the lower beach, possibly because they are closer to the water, which in addition to be a site less vulnerable to predation when the spawning process begins, provides milder temperatures and protection from desiccation.

Young individuals were distributed equally among the levels, with a preference for the middle level. Williams (1984) stated that burrows of young crabs are located near the waterline. This seems reasonable, since immature individuals are less resistant to desiccation and are less efficient burrowers than adults. However, the beaches analyzed in this study are relatively narrow and the upper levels are relatively close to the water. Importantly, because of the crabs' detritivorous habit, the proximity to food must also be considered in analyzing their distribution. Despite the greater difficulty in burrowing, individuals on the middle and upper beach can obtain food more easily because they are closer to the restinga. Furthermore, these levels are preferred by bathers who spend all day on the beach, which increases the possibility of scavenging garbage. The competition for food in this zone may also be a factor that selects individuals by size. The middle and upper levels of the beach were mainly occupied by adult males, which occurred on all the levels but preferred to be higher on the beach. Here can be seen the importance of assessing the distribution of individuals not only by demographics but also by size (CW) compared to the levels of the beach. In other words, larger juveniles also advance to upper levels of the beach in search of more food. Likewise, smaller adults compete with the larger adults, which prevent the first occupying the upper level, keeping them in the middle level. The same pattern was found by Hill & Hunter (1973), Fisher &

Tevesz (1979) and Alberto & Fontoura (1999), who observed that the larger burrow openings, made by larger crabs, are more distant from the waterline, and the burrows with smaller openings, which belong to younger individuals, are nearer the water.

This vertical distribution of *O. quadrata* was also reported by Turra et al. (2005), suggesting that the strip of sand occupied varies according to the state of ontogenetic development of the crabs. In a study by Neves & Bemvenuti (2006) in Rio Grande do Sul, on beaches impacted by human activities, in addition to a reduction in density, there was a change in the vertical distribution of the crabs. These authors stated that on pristine beaches the population tends to be near the dunes (restinga); whereas on highly visited beaches, with bars and snack bars, the crabs congregate around these places (Neves & Bemvenuti 2006). Steiner & Leatherman (1981) suggested that ghost crabs become accustomed to increased levels of recreational use of a beach, and perform vertical migrations. However, even the positive effects of recreation such as food left by visitors can cause intense and continuous disturbance that may have negative impacts on the population of *O. quadrata* in the long term (Lucrezi et al. 2009b). According to Turra et al. (2005), it is believed that changes in beach morphodynamics alter the availability of food, influencing the size, distribution and abundance of the species.

Crabs in environments disturbed by intense tourist visitation or anthropic impacts also may alter their reproductive patterns. According to Fisher & Tevesz (1979), *O. quadrata* are less capable of reproducing in constantly trampled locations, because they feel threatened upon exiting the burrows to copulate. This species' reproductive period was studied by means of histological analysis of gonads by Graf et al. (2008), who found that the period of gonadal development is slow and discontinuous, with intense vitellogenesis from October onward and enlarged gonads by the end of November. This period coincides with the tourist season and consequently with the increase in the intensity of disturbances, trampling, and closed burrows, which prevents the couples from meeting. Haley (1972) observed cyclical copulatory activities with peaks in spring and summer. In the present study, precocious juveniles and ovigerous females were abundant during the summer months. The coincidence of this reproductive period with the peak tourist season may reduce the survival of the species, since, according to Lucrezi et al. (2009a), frequent disturbance and trampling on beaches can crush post-larval forms and limit the duration of foraging activity on the surface.

The temporal distribution was similar to other studies with *O. quadrata*, in indicating the greater sensitivity of these animals to a decrease in temperature. This phenomenon, previously described by other authors (Sawaya 1939; Milne & Milne 1946; Wolcott 1978; Alberto & Fontoura 1999; Negreiros-Fransozo et al. 2002; Turra et al. 2005; Blankensteyn 2006; Branco et al. 2010), suggests that the crabs hibernate in the colder months. During these months, there was a drastic reduction in young individuals, although some were still present, indicating continuous recruitment. The sex ratio also seems to be influenced by temporal variations. In this study, the sex ratio calculated monthly and by size classes differed from Mendelian proportions in some cases, with a larger number of males. These data differ only from the observations by Branco et al. (2010) at Praia Brava (Santa Catarina, Brazil), who caught females of *O. quadrata* in higher densities during several months of the year. Similar results were found in several other studies of ocypodid populations (Milne & Milne 1946; Strachan et al. 1999; Negreiros-Fransozo et al. 2002), which according to Milne & Milne (1946), are usually skewed toward males. However, according to Trott (1998), in these crabs mating may be polygamous, favoring females, because normally this group prefers the intertidal zone and males remain in the upper portion. Trott also stated that the sex ratio in the upper third, i.e., the area of the beach excavation, may reach 2 males for each female, while in the intertidal zone this ratio is skewed toward females in the same proportion.

The present study revealed that *O. quadrata* has a well-defined spatial and temporal distribution that is limited by temperature, food availability, and ease of reproduction. The presence of females at the lowest level of the beach is important for evaluating possible impacts, since the absence of females from the level closest to the water could indicate changes in hydrodynamic patterns or human impacts from neighboring areas. Ghost crabs were numerous on both beaches throughout the study period, allowing us to identify biological patterns and environmental factors that influence the ontogenetic development of the species, and providing information to identify affected regions, as well as for preparation of management plans.

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