

Maíra Pombo

The Atlantic Ghost Crab *Ocypode quadrata* (Decapoda: Ocypodidae) as bioindicator of sandy beaches: assessment of the influence of environmental, behavioral and population factors

Thesis submitted to the Oceanographic Institute of the University of São Paulo in partial fulfillment of the requirements for the degree of Doctor of Science, program of Oceanography, Biological Oceanography area.

Advisor: Prof. Dr. Alexander Turra

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Abstract

The populations of the ghost-crab *Ocypode quadrata* have been evaluated as bioindicators at sandy beaches, but for this purpose some shortcomings need to be addressed. This study assessed several features of the species biology, from daily activity cycles to effectively its role as bioindicator. Between that, the occupation of burrows was evaluated at several morphodynamically distinct beaches and it differed temporally, among the areas and within beach strata, indicating that assessing these populations through the indirect method require more caution. The period of the year after major cold fronts showed the most similar values between individuals and burrows, followed by the greater entrance of young recorded. No trend according to beach type was identified for mean size, dissipative beaches tended to have higher densities, while body growth performance index was higher at reflective beaches, gathering therefore both similarities and differences concerning the trends known for other crustaceans from these environments. The use of the species as bioindicator was effective and gathered distinct areas, through the use of a new method. Increasing levels of erosion were associated to increasing mean densities and mean sizes, followed by a sharp decrease of both, especially mean size. Connectivity breakage, e.g., armoring and vegetation removal, proved to be a threat to the populations structure, worsened when associated to erosion process, to an extent that compromise the populations viability.

Keywords: marine ecology, population structure, spatial variability, sampling methods, climate change, erosion, connectivity breakage, environmental monitoring

Resumo

As populações do caranguejo *Ocypode quadrata*, conhecido no Brasil como guaruçá ou maria-farinha, têm sido exploradas como bioindicadores da qualidade de praias arenosas, mas para tal algumas lacunas precisam ser preenchidas. Este trabalho levantou diversas características da espécie no litoral sudeste brasileiro, desde ciclos diários de atividade até sua aplicação como bioindicador. Nesse entremeio, as taxas de ocupação das tocas foram avaliadas em diversas praias morfodinamicamente distintas, sendo que houve variação temporal, entre praias e mesmo entre estratos de uma mesma praia, indicando que avaliações dessas populações através do método indireto requerem maior cautela. O período pós frentes frias teve o número de tocas e de animais mais semelhante, seguido da maior entrada de recrutas. O tamanho médio dos indivíduos não apresentou tendências de acordo com o tipo praial, a densidade de indivíduos foi maior nas praias dissipativas e a performance de crescimento estimada foi maior em praias reflexivas, agregando características tanto similares quanto opostas aos outros crustáceos desses ambientes. O uso da espécie como bioindicador foi válido e incorporou áreas distintas, através do emprego de um novo método. Níveis crescentes de erosão foram associados a um inicial aumento de densidade e tamanho médios, seguido de um decréscimo severo de ambos, especialmente de tamanho. A quebra de conectividade, e.g. construções e remoção da vegetação, foi também responsável pela alteração da estrutura das populações, sendo a condição agravada em casos de associação com erosão, a ponto de comprometer a viabilidade das populações.

Palavras-chave: ecologia marinha, estrutura populacional, variação espacial, métodos amostrais, mudanças climáticas, erosão, quebra de conectividade, monitoramento ambiental

Introduction

In the face of the worrying scale of the global changing we presence nowadays, social and management paradigms have often been debated, reviewed and restructured in the last decades (COLBY, 1991; TURRA et al., 2013; WYNNE, 1992). Coastal areas are transitional environments that offer a huge range of ecosystem services, thus historically housing large human settlements responsible for alike large environmental impact, such as chaotic land occupation, generation and disposal of large volumes of waste, rising harbor activities, and many others (ASMUS; KITZMANN; LAYDNER, 2004; JABLONSKI; FILET, 2008). Out of the great diversity of environmental habitats, sandy beaches stand out on one hand for its fragility, and on the other for its remarkable tourist appeal. Their fragility is easily noticed when undergoing erosion processes, which have increased due to coastal armoring and climate change (DEFEO et al., 2009; SOUZA, 2009). Undoubtedly, one of the few options, if not the only, to minimize in the medium- to long term the pressures these environments are submitted to, is to improve and develop management strategies, what requires the integration of economic, government and conservation sectors, in addition to a constant knowledge development (BROWN; MCLACHLAN, 2002; TURRA et al., 2013)

In such a context, the demand for effective bioindicators have risen. Over the past few decades, researches on this matter reaches such a sophistication, that has allowed the implementation of the use of some bioindicators to normative levels, helping to build effective environmental policies (HEINK; KOWARIK, 2010; MARKERT; BREURE; ZECHMEISTER, 2003). Bioindicator is a comprehensive term, it may refer to an ecological component or to an ecological measures (HEINK; KOWARIK, 2010). Often the term biomonitor is used to name the ecological components that allow to quantify the quality of an environmental aspect (MARKERT; BREURE; ZECHMEISTER, 2003). Here, the word bioindicator is used to characterize a species that is often referred to as sandy beaches bioindicator, a developing issue, which potential is assessed and discussed.

To sandy beaches, identifying proper bioindicators is particularly challenging. These environments lack higher plants, widely used as indicators

in many ecosystems. Moreover, studies about its benthic macrofauna, mainly ecology, were neglected for a long while (FRÄNZLE, 2006; MCLACHLAN; BROWN, 2010). Few decades after sandy beaches ecology has deepened, especially at the intertidal region, many benthic organisms are target as potential indicators of environmental quality, and many are already fairly recognized (CAETANO; ABSALÃO, 2002; DEFEO; LERCARI, 2004; VELOSO et al., 2010).

From the three basic strata resulted from the transitional aspect of these environments, namely sub-, mid- and supralittoral, all closely associated, the supralittoral is historically the most neglected one concerning ecology, partly due to the low richness and abundance of organisms. This trend has been changing, and the need to understand its dynamic and responses to impacts starts to stand out. A recognized group that fulfill many requirements for bioindication in sandy beaches is comprised by the representatives of the subfamily Ocypodinae, the so called ghost-crabs. These are the most conspicuous invertebrates of these environments, the most readily observed and identified, either the animal or the burrow it digs in the sand. These burrows may greatly facilitate population assessments, making censuses easier, less costly, and non-destructive. Furthermore, they are important top-down control factors of sandy beaches, acting both as scavenger and predator (ROBERTSON; PFEIFFER, 1982; WOLCOTT, 1978). The fact that there is a single Ocypodinae species across the whole west Atlantic, making their identification even easier, and preventing the overlap of species in population studies assessed from burrows. All these have been leading to the investigation of the answers of the group to environmental changes. Once these answers would be recognized mainly through alteration in the population structure, the species meets an indication category whose stress response time take from month to years. This raises the difficulty to identify the stressor but also raises the ecological relevance of the response (FRÄNZLE, 2006). In the context of beaches quality, this bioindication category is a quite convenient one, since the main environment stressors found within are chronic (additive and long-lasting):

over-exploitation of resources, e.g. tourism and land occupation, erosion, climate change.

In order that the answers to these stressors may be recorded and understood, it is important to gather knowledge about the species biology and ecology. Nevertheless, before the 1950s, the studies were primarily behavioral and quantitative; later physiological and developmental studies stand (DIAZ; COSTLOW, 1972; MILNE; MILNE, 1946; SANTOS; MOREIRA, 1999; SCHÖNE; SCHÖNE, 1961; WEINSTEIN; FULL; AHN, 1994). In Brazil, for example, after the behavioral studies of Sawaya (1939), studies about *Ocypode quadrata* were taken back on 1999, focusing on population structure and distribution, by Alberto and Fountoura (1999). In summary, until the 20th century, the knowledge about *O. quadrata* ecology was limited. From then on, many advances concerning population aspects of the species, and its environmental role, were achieved. Some patterns are fairly recognized, such as its occurrence from oceanic to estuarine beaches, exposed or sheltered; the occupation, by individuals, from the upper intertidal, across the whole supralittoral, reaching dunes and vegetation (ALBERTO; FONTOURA, 1999; BRANCO et al., 2010; ROSA; BORZONE, 2008; TURRA; GONÇALVES; DENADAI, 2005). A size gradient is well recognized, with smaller individuals closer to the waterline and increasing sizes towards the vegetation (ALBERTO; FONTOURA, 1999; TURRA; GONÇALVES; DENADAI, 2005). The species exhibits reproductive peaks during warmer periods, but has been identified throughout the year, with varying intensity, at Southeastern Brazil (HALEY, 1972; NEGREIROS-FRANSOZO; FRANSOZO; BERTINI, 2002).

Most of the recent ecological studies of *O. quadrata* aimed to identify effects of anthropogenic impacts on the populations (BARROS, 2001; BLANKENSTEYN, 2006; FISHER; TEVESZ, 1979; STEINER; LEATHERMAN, 1981). Indeed, the group as a whole has showed some positive answers on this regard. For instance, the better assessed human impact on ghost crab populations so far is the off-road vehicles (ORV) traffic on beaches, what reduces population abundance, mean size, and burrows structure. Burrows

depth and control the transit time are effective factors to minimize this damage (HOBBS; LANDRY; PERRY, 2008; LUCREZI; SCHLACHER, 2010; SCHLACHER; THOMPSON; PRICE, 2007; WOLCOTT; WOLCOTT, 1984). Also, reduction of these populations have already been shown to occur due to beach nourishment and bulldozing (PETERSON; HICKERSON; JOHNSON, 2000). Regarding the most frequent human actions to sandy beaches, specially at the present study area, Southeastern Brazil, intense trampling and armoring were also identified as factors negatively affecting *Ocypode* populations. For tourism alone, it hasn't been recognized a clear effect (LUCREZI; SCHLACHER; WALKER, 2009; SCHLACHER; DE JAGER; NIELSEN, 2011; STEINER; LEATHERMAN, 1981; TURRA; GONÇALVES; DENADAI, 2005; WOLCOTT; WOLCOTT, 1984).

Comparing the *Ocypode* populations of distinct beaches, though, is still a major constraint to ecological and impact studies. There is no agreement about the influence of natural factors on population parameters and thus, how these vary spatially. This is largely because virtually all ecological studies are based on burrow counting and measures, thus aggregating a noise that is not of the population itself, but may be of differences in the burrows occupation rate over areas. Such difference may due a higher or lower ability to dig in given the sediment features, e.g. coarseness and compacting. These features may also act on the duration of the burrows, among many other possible factors. There are studies on this regard, i.e., accounting for geological and physical factors in attempt to exclude them as sources of population variation (HOBBS; LANDRY; PERRY, 2008; NORIEGA; SCHLACHER; SMEUNINX, 2012; QUIJÓN; JARAMILLO; CONTRERAS, 2001). However, there is a long way to go to a proper understanding of this features, concerning proper study areas, temporal variations, assessed abiotic factors, and investigation of the occupation rate.

Aiming to contribute to this matter, this study addressed several biological aspects of *O. quadrata*, mainly ecological aspects, covering behavioral and methodological matters whenever necessary to lead to the finest results possible. The initial proposal was to understand spatial sources of

variation in burrows occupation rate and in the population itself. Having these information, it would be possible to broaden the use of the species to assess the magnitude of environmental changes on sandy beaches, because it would enable comparison among geographically distinct areas through the use of the indirect method (burrows assessment). To this end, it was considered important to understand the daily cycle of activity of the species, since the knowledge about its high and low peaks may guide studies to which finding the individuals inside or out their burrow is of prime importance. Another relevant matters were to evaluate the effectiveness of capture methods and the choice of proper study areas. Choosing adequate study areas is essential to isolate the influence of natural from anthropogenic factors, allowing further assessments of impacts itself. Furthemost, taking into account temporal sources of variation of population parameters is also of prime relevance, since reproductive, recruitment and mortality events would periodically reshape the populations structure. This chain of considerations defined the structure of this manuscript.

The chapters distribution reflects this study building process. The first chapter, "Tidal and daily cycles trigger the activity of the ghost crab *Ocypode quadrata*", discourses precisely about the investigation of high and low daily peaks of activity of the crab, and potential natural elements that guide it. Although there is a general agreement on the greatest activity occurs at nighttime, the main study that conducted to it was not quantitative (MILNE; MILNE, 1946); a more recent study on this regard has not identified relevant differences between day- and nighttime (VALERO-PACHECO et al., 2007). The existence of this certain contradiction, plus the fact that there were no such studies developed in the study area, led to this accomplishment. The choice of a preserved area with restricted access, coupled to image recording rather than direct observation, assured that human influence on the animal patterns of activity was mostly excluded.

The following two chapters also comprise the choice of pristine areas so to exclude anthropogenic effects, but here with the aim to explore the influence of the environmental characteristics on population features, including the ratio

between individuals and burrows. First, the best method to identify the presence or not of a crab within a burrow was investigated, and surrounding features that could facilitate such identification in future studies were recorded. This is, thus, the subject of the second chapter, "Issues to be considered in counting burrows as a measure of Atlantic ghost crab populations, an important bioindicator of sandy beaches". The third chapter, "Natural drivers of spatio-temporal distribution patterns of the ghost crab *Ocypode quadrata*: differences between burrow and Individuals", follows the aim of understanding differences in the burrows occupation rate over areas, but includes the temporal factor, in order to meet the influence of not only space, but also of time, on patterns of distribution and burrows occupation rate of the Atlantic ghost crab. Beaches previously classified regarding morphodynamics were chosen, and for density and size of individuals and burrows.

Attempting to better understand the life cycles of these individuals the fourth chapter was developed, "Variation in the life-history traits of the ghost crab *Ocypode quadrata* in morphodynamically distinct sandy beaches". The body growth parameters had not yet been assessed for the species, and knowledge about population dynamics is quite relevant to guide conservation actions, as it offers information about recruitment period, growth rate, and longevity, among others.

Finally, it was assessed how this ghost crab populations respond to erosion and connectivity breakage. Since individuals use the whole beach upper areas, the lack of connection between the waterline and the vegetation was considered an important factor for evaluation, as well as the loss of space, in face of the rising intensity of erosion processes, due to anthropogenic and climate change. Given the dramatic influence that these elements may have on these populations dynamics, both were combined (erosion level and the presence of connectivity breakage) for the selection of beaches among which the structure of populations was compared. The last chapter, "Effects of habitat connectivity and beach erosion on population structure of the Atlantic ghost crab *Ocypode quadrata*: implications for beach conservation", therefore, aims to

effectively evaluate the potential of the species as bioindicator. A new and simple method was proposed, that eliminates sources of error due to the use of the indirect method. From this method, it was still possible to gather distinct areas to assess how the spatial differences may affect the identification of population responses. The impacts of concern here, were levels of coastal erosion and the presence of coastal armoring (representing connectivity breakage), essential aspects to be investigated in front of the current scenario of increasing coastal occupation and climate change.

A section of final conclusions closes the document, connecting all previous chapters, and making a critical evaluation of the knowledge advances they have provided.

Chapter 1

Tidal and daily cycles trigger the activity of the ghost crab *Ocypode quadrata*

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Abstract

Several studies using ghost crabs as an indicator of impact on sandy beaches have been conducted lately. They are mainly developed through burrow assessments, but recent studies have demonstrated that the ratio between the number of burrows and crabs might not be consistent over different beaches. The activity period of crabs may influence these estimates since the chance to record them inside the burrow increases when they are inactive. However, in literature, the few studies on daily activity of *Ocypode* impede generalization. This study thoroughly assessed the activity intensity of *O. quadrata* over six repeated 24-hour cycles, using filming devices on a beach with restricted access, thus excluding the influence of the observer as well as other possible human influences. The amount of individuals and time spent outside the burrows were recorded for every ten minutes filming, and these data were confronted with temperature and tide height. Higher activity peaks concerning number of individuals and time outside the burrow were recorded in the mornings. Fewer individuals were recorded at night although they tended to remain stationary for longer periods by the burrow opening. Activity variations were more related to tidal range than temperature, with higher activity associated to low tides in the morning. Our results showed that the activity pattern of this species is influenced by several factors and, therefore, does not support the nocturnal habit described in literature.

Keywords: activity periods, filming, behavior, number, time, tide, temperature

Introduction

Sandy beaches are among the most threatened coastal habitats due to erosion, pollution, and tourism (SCHLACHER et al., 2007). There are several strategies available to manage and monitor their quality, but most of them have been performed using physical or microbiological features, mainly those associated with human use, such as bathing conditions (GRAY, 2010; CETESB, 1991; 1992). The increasing concern with broader aspects of environmental quality has demanded more systematic uses of ecological indicators (DAUVIN, 2007; ZHOU et al., 2008). The representatives of the genus *Ocypode*, commonly known as ghost crabs, and which are found on sandy beaches of the tropics and subtropics around the globe (MILNE; MILNE, 1946), are among the most promising bioindicators for sandy beaches. They are the most conspicuous invertebrates on sandy beaches, inhabiting from the upper intertidal zone to dunes and vegetated areas (FISHER; TEVESZ, 1979).

The most frequently studied species of the genus is *Ocypode quadrata*, probably because of its wide distribution in the Western Atlantic coast, where it is also the only species of *Ocypode*. By the mid-1950s, studies on the species were basically descriptive and most of them used behavioral approaches (COWLES 1908; SAWAYA 1939; MILNE; MILNE, 1946). By the end of the century more quantitative studies on the population biology started to be developed (ALBERTO; FONTOURA, 1999; BRANCO et al., 2010; TURRA; GONÇALVES; DENADAI, 2005) and since then, many studies have been considering the use of population features to assess anthropogenic impacts (BARROS, 2001; MAGALHÃES et al., 2009; NEVES; BEMVENUTI, 2006; STEINER; LEATHERMAN, 1981).

It is interesting that, although this species is attractive to study, some important information concerning different aspects of the species biology is still scarce, such as the influence of abiotic factors on population biology and the relationship between number of burrows and individuals, among others (POMBO; TURRA, 2013; SILVA; CALADO, 2013). This is probably a consequence of the difficulty to directly study the animals in their natural

environment, as opposed to the ease of working with the burrows they build. However, recent studies have demonstrated that the ratio between the number of burrows and crabs might not be consistent over different beaches (POMBO; TURRA, 2013; SILVA; CALADO, 2013). Thus, the activity period of crabs may influence estimates depending on the methodology used, i.e., whether assessments encompass capturing individuals that are outside their burrows, for example. Still, there is no consensus in literature on the activity cycle of ghost crabs. This topic was first addressed in studies developed in the Northern hemisphere (MILNE; MILNE, 1946), which did mention that ghost crabs were mostly nocturnal but lacked systematic assessment. Later, a circadian rhythm investigation under laboratorial conditions supported that they were mostly active at night (PALMER, 1971). This information has been used since then to justify nocturnal assessments of *Ocypode* and to discuss many field results (WEINSTEIN, 1995; WOLCOTT, 1978). Nevertheless, the only systematic study performed in the tropics did not identify differences between day and night, stating only that ghost crabs were less active at higher temperatures (VALERO-PACHECO et al., 2007). Differences between the above mentioned reports could be due to environmental differences, since the latter was developed in the tropics, but it is important to note that it used systematic field assessments.

The present study addressed the activity of the Atlantic ghost crab *O. quadrata* regarding the influence of time (daily hours), temperature, and tide. The presence of a circatidal rhythm for *Ocypode* has never been assessed, although it is widely recognized for other crabs (PALMER, 1971; REID; NAYLOR, 1989). Furthermore, anthropogenic influences were excluded as much as possible, once field assessments were fully performed with the help of filming devices on a beach with restricted access.

Material and Methods

Study area

The present study was carried out on Guaraúzinho Beach, a preserved, dissipative, moderately exposed subtropical sandy beach in southeastern

Brazil. This beach is located in an environmental protected area, and thus comprises preserved dunes and vegetation, as well as the adjacent promontory. The beach is about 900-m long; the intertidal zone is about 100-m wide, and the supralitoral zone at the filming area was approximately 20-m wide (Figure 1.1).

The entire beach has restricted access (Juréia-Itatins Ecological Station), limited to station staff and authorized people who go there for teaching or scientific purposes. Along with the total lack of artificial lighting, the above mentioned features ensured strictly natural conditions for ghost crabs, which are abundant in the area.

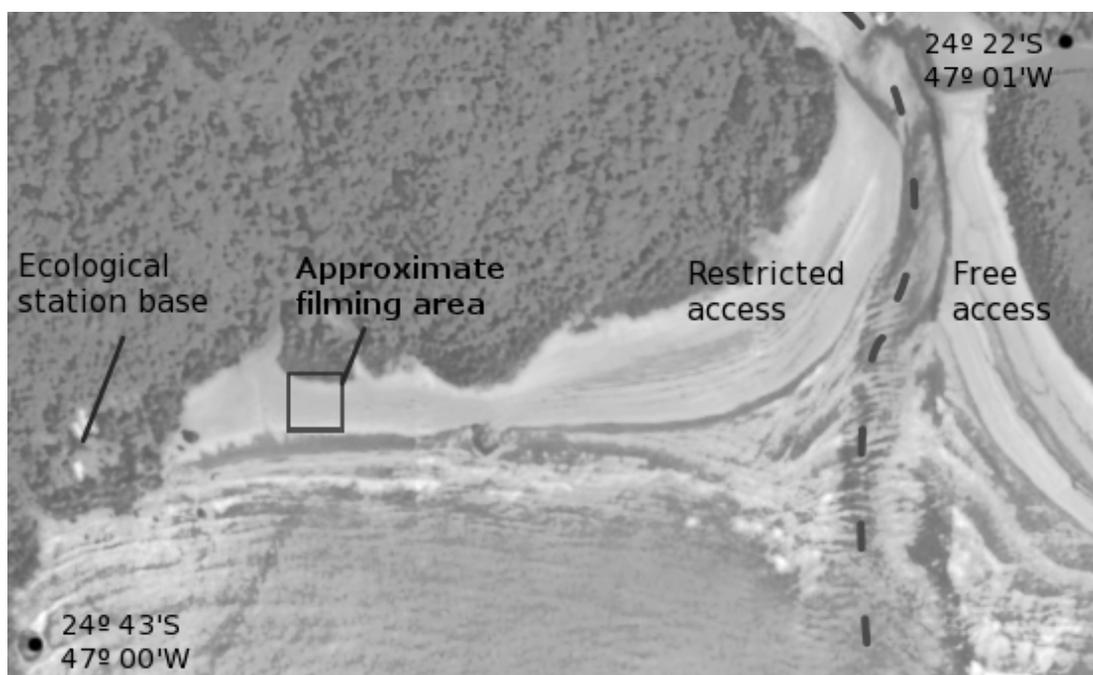


Figura 1.1. Study area located at Guaraú beach, a dissipative and moderately exposed subtropical sandy beach at southeastern Brazil. The beach is located inside the Ecological Station Juréia-Itatins, comprising preserved dunes and vegetation. The beach is about 900 m long, the intertidal zone is about 100 m wide and the supralitoral zone is about 20 m wide. Dashed line over the Guaraú river indicates the division between free and restricted access areas. Map data modified from: Google, CNES/Astrium, Cnes/Spot Image, DigitalGlobe, TerraMetrics.

Methodological Procedures

Filming device

In order to develop this study, we set a device with basic safety equipment. An infrared sensitive video camera was placed on a tripod and

connected to a DVR (digital video recorder) with an 80GB hard disk. The DVR was protected from the rain by a plastic box with strategic perforations. This set was connected to a 12V/150Ah battery; this and all other connections were enveloped by tapes and plastic to prevent damage by heavy rains. Since this device was not manufactured to be used without a source of electricity, the energy plug had to be replaced by a 12V adapter. A mini-playscreen was used during each installation to ensure a precise fit of the recording area.

Filming and analyses

Filming was performed in six different, randomized days, comprising different tides (moon phases), from October 1st to October 21th, 2012. The coverage of the entire moon cycle allowed for the observation of distinct tidal cycles, and therefore, for possible inferences on the presence and significance of the circatidal rhythm of these individuals, corresponding to a rhythmic behavior associated to a tidal cycle, which has not been assessed, yet.

We chose a small area of about 10 m² in the supralitoral and adjacent to the vegetation to place the camera throughout the studied period. Within this area, the position of the camera could differ between sampling days, with the purpose of focusing on the highest possible number of burrows within the predetermined sampling area. The camera was positioned so that it would cover the area ranging from the vegetation to the intertidal area (Figure 1.2).

All videos were fully examined. Every ten minutes, we recorded (i) the maximum number of individuals observed simultaneously (variable name: number), and (ii) the time of permanency of at least one individual in the observed area (variable name: time).

The amount of individuals was not directly comparable between days, because the position of the camera each day was not strictly the same, and also due to eventual behavioral differences between days. Therefore, these values were converted to relative values within each day. The mean number of individuals within one hour (calculated based on six ten-minute values) was divided by the sum of the means within a day. Therefore, for each day, the lowest and the highest amount of individuals were represented by values from 0

to 1, respectively. The variable time was also expressed as the ratio of an hour, i.e., the sum of time (in minutes) spent within each ten-minute assessment contained in an hour and divided by 60, corresponding to a value from 0 to 1, where 1 corresponds to one full hour.

Temperature and tidal data were gathered from public databases. Temperature data were specifically taken from *SINDA* (Portuguese acronym for Integrated System for Environmental Data); information was available for every three hours during the studied period, obtained from a 2m high weather station, located in a neighboring coastal city, about 32km from the studied area (ID:30889). Tidal data were taken from the *BNDO* (Portuguese acronym for National Bank of Oceanography Data) for the nearest station available, which was Santos Harbour, about 85km from the studied area. To obtain hourly data for both environmental factors, linear interpolations were performed.

Graphs were provided gathering information on the relative number and observation time of individuals, within each of the 24 daily hours, along with the corresponding environmental data (temperature and tide). Because there is a correlation between daily hours and temperature and also, to some extent, between daily hours and tide height (considering that closer days have similar peak hours), we performed a more descriptive analysis instead of applying predictive models.

Results

Six 24-hour cycles, named “days”, were filmed and quantified. Only two of these cycles were shorter, day 1 (with 19 hours filmed) and day 6 (with 22 hours filmed). The days in question and their respective lunar phases, described as moon percentage of illumination, were all in October 2012: Day 1, 1st to 2nd (96%), Day 2, 3rd to 4th (85%), Day 3, 10th to 11th (22%), Day 4, 15th to 16th (0%), Day 5, 18th to 19th (20%), and Day 6, 20th to 21st (41%).

The higher the number of individuals observed within an hour, the higher was the tendency towards spending a higher amount of time observing individuals within that hour. However, this was not simply a direct relationship,

since many of the higher values of time spent observing animals actually resulted from recording a single individual.

Activity concerning the variable time showed more than one mode over each cycle in most of the cases. On the other hand, activity peaks concerning the amount of individuals (variable number), were mostly unimodal and occurred mainly during daytime; most of these peaks were recorded in the morning, from about 7AM to 10 AM; except for day 2, when the peak occurred from 11 AM onwards, and day 5, when very few individuals were recorded and no clear peak was identified.

Temperature ranged from 14 to 26°C over the sampling period. Figure 1.2 shows there isn't a clear, common, activity pattern related to temperature over the filmed cycles. On days 1, 4, and 6, the activity peaks were related to milder temperatures, decreasing during warmer periods. These three days also showed wider temperature ranges (Figure 1.2). On days 2 and 5 this trend was not clear, although temperature variations on these days were among the lowest ones observed (temperature ranges 4.5°C and 3°C, respectively). On day 3 the temperature variation range was also very slight (3°C), and this weather condition was associated to the fact that most of the day was rainy.

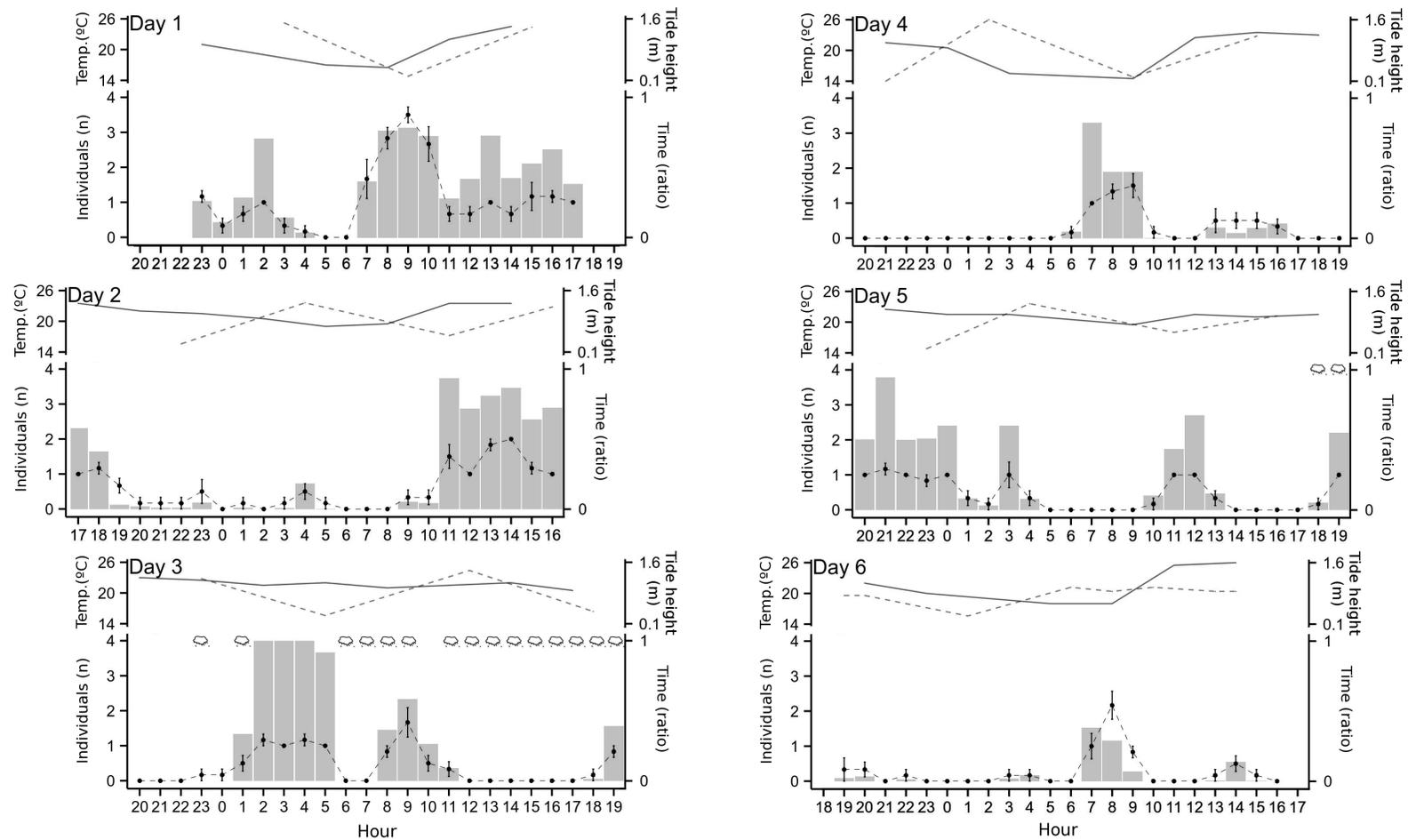


Figure 1.2. Twenty-four hours assessments of intensity of activity of *Ocypode quadrata*, developed in Guarauzinho beach, Southeastern Brazil. Videos were produced and quantified every 10 minutes. The proportion of time with at least one crab recorded out of the burrow, within each one-hour filming, is represented by gray bars (right axis); points and bars represent mean and standard error of the hourly number of individuals. Number values were relativized within each day: the mean number of individuals within one hour (calculated based on ten-minutes intervals) was divided by the sum of the means within a day. Above each daily cycle, the graphs represent environmental conditions of temperature (left axis; solid line) and tide height (right axis; dashed line). Icons represent rainy periods within the one-hour observation period.

It is interesting, however, that many activity peaks, whether based on time or number, tended to be related to low tides (in Figure 1.2, days 1 to 5). The only exception was day 6, which had a neap tide regime, and therefore, a lower tidal range. The opposite was also observed: long periods with low or no activity were mostly related to high tide peaks, mainly after the high tide peak (see Figure 1.2, from day 2 to 5). Again, these associations were clearer on days with wider tidal ranges.

The number of active individuals clearly decreased as tides increased (Figure 1.3A). One outlier was identified, and this was associated to a neap tide regime. There was also a decreasing tendency with increase in temperature, but slighter than it was with tide (Figure 1.3B).

The values of the variable time were scattered all over the graph region, considering tide or temperature (Figure 1.3 C and D), although a shift towards higher temperature values was observed. This lack of clear patterns related to the amount of time spent on observation of activity, mostly represented by a very low number of individuals, suggests that environmental variables were not a determining factor for situations with point activities.

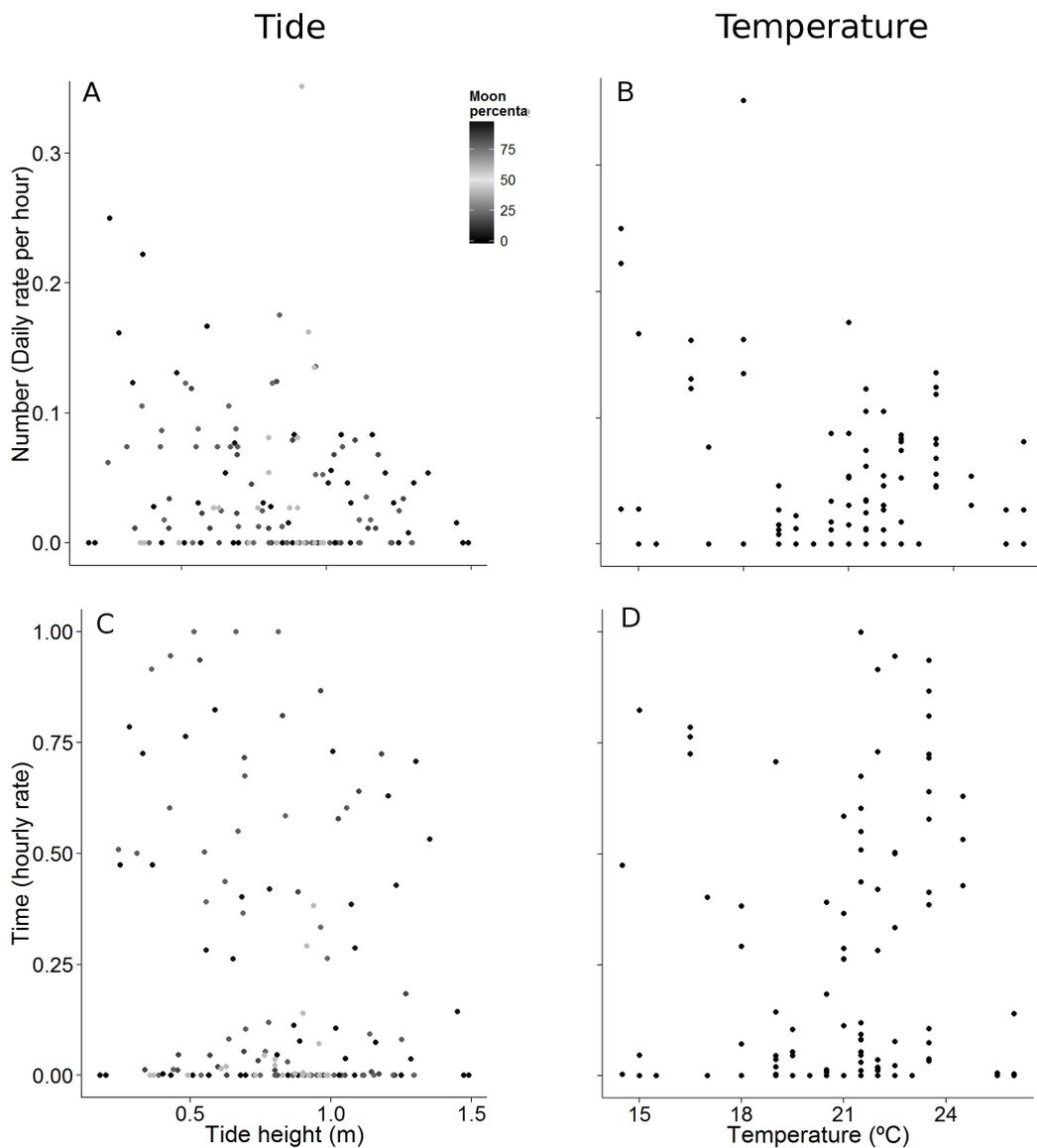


Figure 1.3. Scatterplots of relative values of number of individuals (A, B) and of time observing individuals (C, D) of *Ocypode quadrata*, according to tide height (m) and temperature (°C) respectively. Data was collected at Guaraú Beach, Southeastern Brazil, through October 2012.

Discussion

Behavioral aspects of species of the genus *Ocypode*, such as investigations about daily activity patterns, were among the first topics addressed on this species around the globe. For *O. ceratophthalmus*, studies showed predominant nocturnal habits and, for *O. kuhlii*, no differences between day and night periods were demonstrated (JONES, 1972). For *O. quadrata*, major studies have concluded so far that the species is either predominantly

nocturnal or that there is no great difference (MILNE; MILNE, 1946; PALMER, 1971; VALERO-PACHECO et al., 2007). The present study is the first developed in the field without the influence of an observer on the natural activities of crabs. Moreover, our methodology allowed for quantification through a continuous observation of the cycles assessed.

Peaks of time spent outside the burrow were mostly represented by single individuals, while peaks of higher number of individuals were grouped in narrower time ranges. This result suggests that during the time they are outside their burrows, a small period of time is spent covering longer distances and interacting with other individuals, the latter being represented by moments with higher number of individuals. The narrower time range with higher amount of crabs was represented basically by morning periods. Therefore, although most of the literature available reported more activity of individuals at night, this cannot be generalized for *O. quadrata*. The number of active crabs during some nocturnal periods was lower compared to daytime, and not all nights had active individuals, while all days did.

It is very likely that regional differences in these patterns may occur. For example, Valero-Pacheco et al. (2007) showed more distinctly the absence of individuals during warmer periods than the present study. However, temperature was considerably milder in our subtropical study area than the former, equatorial study area. Nevertheless, concerning the supposedly nocturnal habits of ghost crabs, other questions may be raised. First, we must highlight that most previous studies were influenced by the observers, which might seriously compromise the observation. Crabs are very sensitive to human presence, and this may considerably change the behavior of individuals, which would remain in an alert attitude at the presence of observers. Milne (1946), who reported more activity of *O. quadrata* individuals during the night, mentioned interestingly enough that they are not afraid at night. It is possible that they behave differently in relation to the observers during day and during the night, and they may also stand out at night, thus being more easily observed. Palmer (1971) recognized the negative influence of the observer's

proximity, and studied *O. quadrata* circadian rhythms in the laboratory, also identifying more activity during periods without light. However, important factors, such as temperature and tide, were not tested in the laboratory-controlled conditions. Although tide is an extremely important factor for coastal organisms, there are no studies on the circatidal rhythm of *O. quadrata* or any other species of the genus. For some intertidal crabs the association of circadian and circatidal rhythms are well described (PALMER, 1971; REID; NAYLOR, 1989) but it has not been assessed for species of *Ocypode* so far. We could assume that the influence of tide is less overt for these backshore species, but in the present study, the influence of tide on the intensity of activities of *O. quadrata* was even more noticeable than the influence of temperature. An important adaptive feature of this crab consists in building mostly “Y”-shaped burrows, of which one branch is closed and acts as an anti-collapsing structure (DE, 2005), working precisely as a refuge to protect them from raising water. Therefore, tide is expected to influence other behavioral elements of *O. quadrata*. Because they do not predominantly occupy intertidal areas, the decreasing tide might be perceived from the bottom of the burrows, which would become less humid due to the lowering of ground water.

Lower tides may be associated to more extensive foraging areas. Furthermore, lowering water is also associated to renewed resources left behind during high tides. On the other hand, increasing tide would reduce foraging areas, leading to higher competition and more occurrences of agonistic events.

Ocypode quadrata is the top resident predator of sandy beach environments (WOLCOTT, 1978). However, this doesn't mean they don't have predators. The most frequently mentioned predators of *Ocypode* are birds, which are most active during sunrise and sunset (DAAN; ASCHOFF, 1975), and opossums were observed in the videos circling around the burrows during the night in two of the six recorded days. Higher daily activity periods of potential predators (birds, mammals, and reptiles) may influence the activity period of ghost crabs. This would correspond to early mornings and evenings, mainly

concerning birds, and nights concerning most mammals and reptiles. But, Valero-Pacheco et al (2007) found, through direct observation, activity peaks of *O. quadrata* precisely during sunrise and sunset. The activity peaks during daylight recorded here suggest that, in addition to tide, individuals prefer intermediate temperatures; because daily hours are directly associated to temperature, there would be a lower number of active individuals during the night (lowest temperatures) and at midday (highest temperatures). Temperature cycles vary regionally and seasonally, which may lead to different behavior patterns in the individuals observed here. Biological cycles may also influence behavior patterns, such as reproductive peaks, and therefore, it is important that they are assessed in broader scales of space and time to consolidate the issue.

This study suggests that the activity rhythm of *O. quadrata* is not likely random but is influenced by several factors apart from light and temperature. Possibly rain and potential predator activity, among other factors, also play their role controlling the activity of individuals, and should be assessed. In this study, low tides during daylight and preferably intermediate temperatures (around 19 to 24°) proved to be the conditions with higher activity from the crabs, while high tides, especially during spring tides, and extreme temperature conditions were the situations when most crabs remained almost entirely burrowed and inactive.

Chapter 2

Issues to be considered in counting burrows as a measure of Atlantic ghost crab populations, an important bioindicator of sandy beaches

Issues to be considered in counting burrows as a measure of Atlantic ghost crab populations, an important bioindicator of sandy beaches

Abstract

The use of indirect estimates of ghost-crab populations to assess beach disturbance has several advantages, including non-destructiveness, ease and low cost, although this strategy may add some degree of noise to estimates of population parameters. Resolution of these shortcomings may allow wider use of these populations as an indicator of differences in quality among beaches. This study analyzed to what extent the number of crab burrows may diverge from the number of animals, considering beach morphology, burrow depth and signs of occupation as contributing factors or indicators of a higher or lower occupation rate. We estimated the occupation rate of crabs in burrows on nine low-use beaches, which were previously categorized as dissipative, intermediate or reflexive. Three random 2-m-wide transects were laid perpendicular to the shoreline, where burrows were counted and excavated to search for crabs. The depth and signs of recent activity around the burrows were also recorded. The occupation rate differed on the different beaches, but morphodynamics was not identified as a grouping factor. A considerable number of burrows that lacked signs of recent activity proved to be occupied, and the proportions of these burrows also differed among beaches. Virtually all burrows less than 10 cm deep were unoccupied; the occupation rate tended to increase gradually to a burrow depth of 20-35 cm. Other methods (water, smoke, and traps) were applied to measure the effectiveness of excavating as a method for burrow counts. Traps and excavation proved to be the best methods. These observations illustrate the possible degree of unreliability of comparisons of beaches based on indirect measures. Combining burrow depth assessment with surrounding signs of occupation proved to be a useful tool to minimize biases.

Keywords: burrow occupation rate, ghost crab, environmental factors, population parameters, beach type, indirect method.

Introduction

The attractiveness of sandy-beach environments and their particular geological traits make beaches highly susceptible to a wide range of local- to global-scale stressors, from tourism and vegetation removal to erosion and rising sea levels. Together with the limited number of marine reserves, the manifold stress factors make sandy beaches among the most threatened ecosystems in the world (BROWN; MCLACHLAN, 2002; DEFEO et al., 2009). According to Brown and McLachlan (2002), improvements in the understanding and management of sandy-shore ecosystems may considerably minimize the effects of increasing human pressures. Defeo et al. (2009) highlighted the prime importance of long-term monitoring to identify and quantify global-scale impacts on sandy beaches.

Assessing key groups of bioindicator organisms is an active area of sandy-beach studies. Some groups of supralittoral macrofauna, such as Talitridae, Cirolanidae and Ocypodidae (DEFEO; MCLACHLAN, 2011) are particularly sensitive to impacts from tourism activities (trampling, beach cleaning), vehicle traffic, and shoreline armoring, erosion and nourishment (FANINI; CANTARINO; SCAPINI, 2005; FANINI et al., 2007; LUCREZI; SCHLACHER; ROBINSON, 2010; NORIEGA; SCHLACHER; SMEUNINX, 2012; SCHLACHER; DE JAGER; NIELSEN, 2011; SCHLACHER; THOMPSON; PRICE, 2007; VELOSO; NEVES; DE ALMEIDA CAPPER, 2011; VELOSO et al., 2010). Recent efforts of Research Networks, e.g. ReBentos (acronym for, in Portuguese, *Monitoring Network for Coastal Benthic Habitats*), also highlight the importance of improving and organizing knowledge of key organisms to aid in emerging management requirements (TURRA et al., 2013).

The use of Atlantic ghost-crab populations as an indicator of beach quality and a tool for monitoring these environments has been widely proposed (BARROS, 2001; BLANKENSTEYN, 2006; LUCREZI; SCHLACHER; WALKER, 2009; MAGALHÃES et al., 2009; NEVES; BEMVENUTI, 2006; STEINER; LEATHERMAN, 1981). Ghost crabs are the most conspicuous invertebrates of sandy beaches in the tropics and subtropics, and an important top-down control

factor (WOLCOTT, 1978). They inhabit sandy beaches from the upper intertidal zone to dunes and vegetated areas, where they build burrows and maintain territories around them (FISHER; TEVESZ, 1979; MILNE; MILNE, 1946). The vast majority of burrows are Y-shaped, composed of a main branch and an alternative, closed one (rarely more), which may also open to the surface (DE, 2005). Reports on the main daily period of activity of these crabs state either that they are mostly active at night, or that no difference was detected over a 24-h cycle (MILNE; MILNE, 1946; VALERO-PACHECO et al., 2007).

The crabs' excellent camouflage, quickness and sensitivity to observers have necessitated wide use of indirect methods, which basically use the number and size of active burrows as the parameters for population assessments (ALBERTO; FONTOURA, 1999; BARROS, 2001; FISHER; TEVESZ, 1979; VALERO-PACHECO et al., 2007; WOLCOTT, 1978). An active burrow is identified by the observation of typical traces left by the crabs, which include recently excavated sand and tracks surrounding the burrow, and also the definition of the perimeter of the burrow opening (FISHER; TEVESZ, 1979; WOLCOTT; WOLCOTT, 1984). The ease, non-destructiveness, and low cost of this censusing approach make these crabs even more attractive to study.

The Atlantic ghost crab *Ocypode quadrata* is the only member of the genus along the entire western Atlantic coast (MILNE; MILNE, 1946), so that interspecific competition, spatial overlapping and the difficulty of distinguishing between burrows of different species cannot bias population studies. Altogether, these crustaceans offer many advantages for estimating disturbances, and the great majority of recent research on *Ocypode* has focused on its use as a valuable impact-assessment tool.

However, certain shortcomings may impede comparisons among beaches or over time (TURRA; GONÇALVES; DENADAI, 2005). Although these are territorial animals, there is no definite information about the number of burrows that a single crab might actively maintain, for example, to increase the safety of its foraging area. Burrow counts might overestimate crab populations, and may also be subject to different ranges of errors in different locations.

There is evidence that population density varies according to beach type, but differently from other, smaller macrofaunal species, populations of *O. quadrata* do not change much in abundance according to the grain size of the sediment (DEFEO; MCLACHLAN, 2011). However, considering that grain size and soil compaction might significantly affect the effort needed to dig a burrow, and how long the burrow would last until it collapsed, beach morphodynamics could, in part, affect the proportion of burrows that are occupied. However, since this evidence is based essentially on burrow counts, it could partially reflect differential occupation rates resulting from natural environmental factors.

Not only external environmental factors, but some internal characteristics are interesting to consider along with the occupancy or not of a burrow. The signs of occupation surrounding burrows, i.e., fresh tracks, excavated sand and/or preserved perimeter definition, which are traditionally used to decide whether to count a burrow (FISHER; TEVESZ, 1979; WOLCOTT; WOLCOTT, 1984), probably last for different periods of time in different beaches, being affected by grain size and slope. Sediment compactness, for example, may affect the number and depth of burrows.

Here, we attempted to assess to what extent the number of burrows might diverge from the number of animals (i) over different beaches, with different morphodynamics; and (ii) how some internal factors may indicate a higher or lower occupation rate, specifically (ii.1) signs of recent activity surrounding the burrow and (ii.2) burrow depth. Further, we (iii) attempted to measure the effectiveness of the excavation method used to estimate the occupation rate, by quantifying and comparing its results with several related methods. This study resulted in some simple but important observations on basic features that may be useful for further indirect studies of ghost-crab populations.

Methodology

Nine low-use beaches were selected on the coast of São Paulo State, southeastern Brazil (Figure 2.1). By studying preserved, low-use beaches, we

attempted to focus on natural environmental factors rather than human impacts. The beaches (from 24°31'S, 47°10'W to 23°22'S, 44°W50') were previously categorized as dissipative (Costa, Fazenda and Una), intermediate (Félix, Justa and Brava da Fortaleza) or reflexive (Figueira, Prumirim and Puruba). Fieldwork was conducted during daylight hours in late November and early December 2011, when the weather is typically warm with scattered rain showers. A homogeneous area 200 m long was selected on each beach, where three 2-m-wide transects were randomly laid out perpendicular to the shoreline, and every 2 m (in 4-m² quadrats), burrows were counted, measured (depth, using a semiflexible steel cable), and excavated to search for the occupying crab. All burrows on a transect were counted, from the low intertidal zone to the vegetated area, until it was certain that no further burrows remained. All measurement activities and collections complied with the license from the appropriate federal environmental agency (*Ministério do Meio Ambiente (MMA) – Instituto Chico Mendes de Conservação da Biodiversidade (ICMbio)* No. 31629-1; acronyms for, in English: Ministry of the Environment – Chico Mendes Biodiversity Conservation Institute).

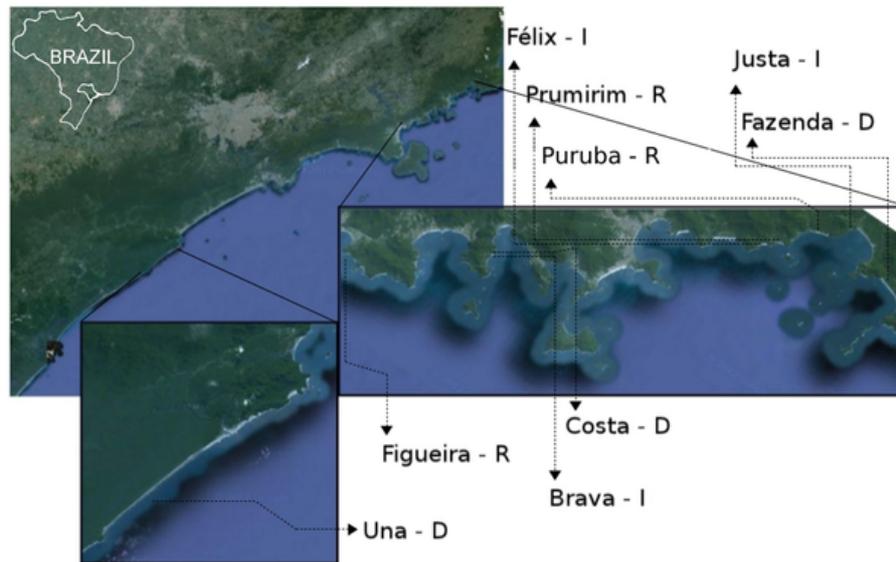


Figure 2.1. Study area. Satellite image of southeastern Brazil and detail of the study area. The nine beaches are between 24°31'S, 47°10'W and 23°22'S, 44°W50'. Each is named and followed by a letter indicating its morphodynamics: D – dissipative, I – intermediate and R – reflexive. Map data: Google, Cnes/Spot Image, DigitalGlobe, Landsat, TerraMetrics.

Since the study concerned a methodological approach, all burrows were assessed, including covered or semi-covered burrows. The signs of occupation around the burrow were recorded for Fazenda, Una, Brava, Justa and Figueira beaches, and later classified into four categories (Figure 2.2): none – no sign of occupation; subtle – barely recognizable tracks and/or excavated sand; only noting the definition of the internal perimeter of the burrow; moderate – obvious presence of sand, or tracks, and a well-defined internal perimeter; strong – presence of more than one clear sign and/or a very prominent sign.

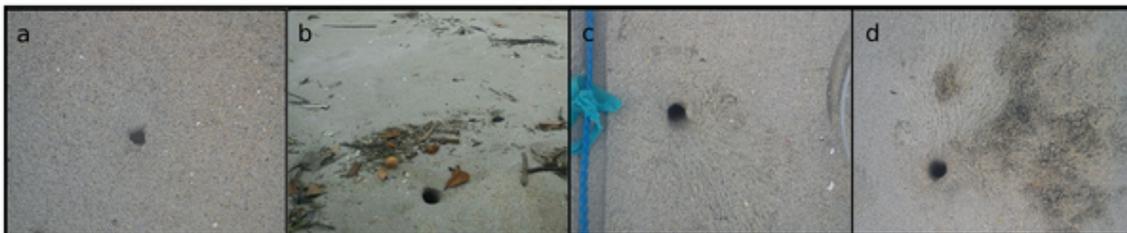


Figure 2.2. Categories of signs of occupation. Examples of each of the four categories of intensity of signs of occupation of *Ocypode quadrata*: a- none (no sign of occupation); b- subtle (barely recognizable tracks and/or excavated sand; only noting the definition of the internal perimeter); c- moderate (obvious presence of sand, or tracks, and internal perimeter definition); d- strong (presence of more than one clear sign and/or a very prominent one).

Considering that the upper portion of a burrow is funnel-shaped, due to the unconsolidated sediment, which changes from beach to beach, we used “internal perimeter” for the perimeter of the lower circumference of the funnel, since from that point on the shape of the burrow best matches a perimeter modelled by the crab.

All burrows were excavated to the bottom whenever possible. The steel cable was helpful to locate the bottom of the burrow; probing around while excavating helped to detect an occasional continuation or side branch. Differences in soil compaction made it easier or harder to be sure that the crab was not there, but usually, it was easy to establish that the burrow was in fact empty, in the case of larger, shallower burrows. This led us to consider, in most cases where the crab was not found, two situations: (i) the burrow was empty in all cases – *minimum rate*; and (ii) the burrow was occupied in all cases – *maximum rate*. The difference between these values (minimum and maximum rate) represents an “uncertainty rate”.

For the purpose of evaluating the excavation method as a measure of occupation rate, three other methods, described below, were applied along with excavation, following the same transect pattern (three replicates of 2-m-wide transects comprised of 4-m² quadrats). Samples were taken during three consecutive days, each comprising 4 transects, one of each treatment, in August 2012. The method giving the highest mean values of occupation and the lowest standard deviation was considered the most appropriate. The chosen area is a dissipative beach with restricted access (Arpoador Beach), within a Conservation Unit (EEJI - acronym for, in English: Juréia-Itatins Ecological Station), where tourist activity would not be a possible source of bias. These methods were:

- 1 – Flooding the burrow (water): a 5-L container was used to flood all identified burrows. Sign of occupation: crab left the burrow.

- 2 – Smudging (smoke): fumigator. Sign of occupation: crab left the burrow.

3 – Setting traps (trapping): a piece of cotton lint was used to close each burrow opening, which was also surrounded (by burying a thick cylindrical cardboard panel, around 60 cm in diameter). Traps were left in place for 24 h. Sign of occupation: the lint was disturbed.

4 – Excavating (excavate): The main duct was traced with the aid of a flexible steel cable. Moving the cable around while excavating enabled us to locate any alternative openings around the main duct. Sign of occupation: finding the crab.

All these methods are inexpensive and easily replicated, although trapping will increase in efficiency if a longer response time is allowed than for the other methods, and if the traps cannot be dismantled by visitors. Values were compared using one-way ANOVA, after assessing for its premises, and a Tukey post-hoc test.

Three other methods were applied, under different, more exploratory, conditions, attempting to credibly establish the crabs' presence or absence: the use of a bronchoscope, stethoscope, and ground-penetrating radar (GPR), with simpler experimental designs, to assess their feasibility and value. The bronchoscope and stethoscope were used in randomly chosen burrows and in burrows where a crab was known to be present. The GPR consisted of an armored antenna with 200 MHz frequency range and high resolution, and reached depths up to 6 m. Two sampling procedures were used: moving in straight lines across-shore, every 5 m in a 50-m beach section; and focusing on burrows where crabs were observed.

Results

The mean minimum occupation rate ranged from 20 to 60% for the study areas, and did not reach 40% in four areas (Fazenda, Una, Justa, Figueira). The mean maximum occupation rate showed a larger range over areas, from 20 to 90%, exceeding 80% in only two areas (Costa and Brava beaches). No pattern distinguishing rates by morphodynamics could be determined (Figure 2.3), but the degree of uncertainty was somewhat greater

for beaches with more dissipative profiles, except for Fazenda Beach, where the low abundance of individuals allowed exceptionally high accuracy.

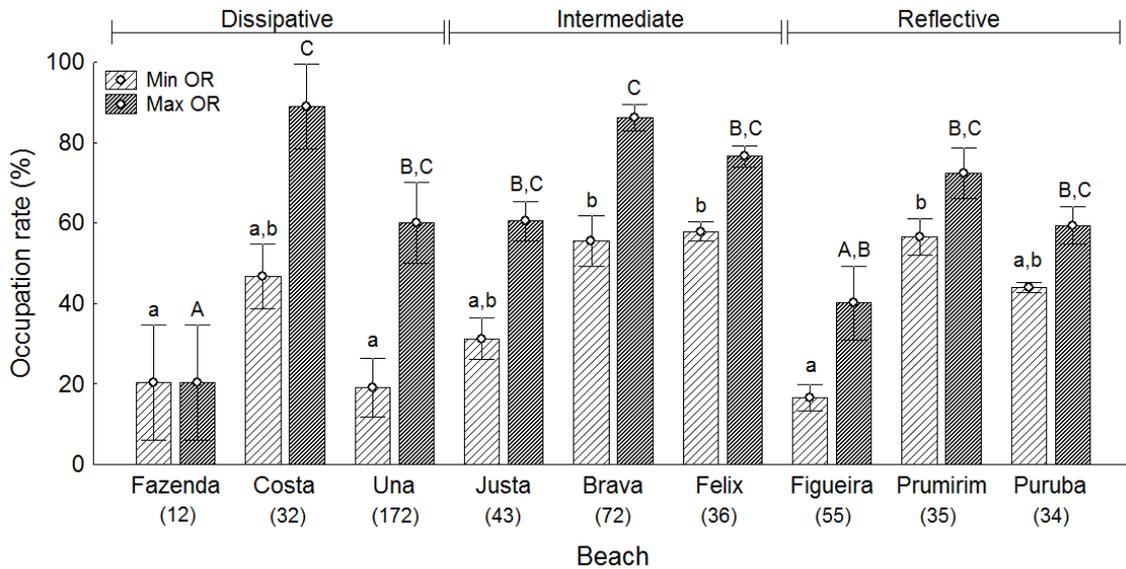


Figure 2.3. Minimum and maximum occupation rate by beach. Mean and standard error of occupation rate of *Ocyropsis quadrata* burrows (minimum and maximum rates), by beach. The total number of burrows assessed and the number of uncertainty cases, on each beach, are shown below the x axis.

From the 274 burrows assessed for the intensity of signs of recent activity (excavated sand, fresh tracks, internal perimeter definition) 86 were proven to be occupied, of which 20 completely lacked any sign of activity. For the reflective beach, all occupied burrows showed at least subtle signs, although none showed strong signs (Figure 2.4). However, combining all burrows without obvious signs of activity (no signs or subtle signs) revealed a tendency toward increasing occupation of these apparently inactive burrows, from dissipative to reflective beaches. Considering that subtle signs may be easily overlooked, intentionally or not, we observed that, for dissipative beaches, over 8.82% of burrows that lacked any signs of activity were in fact occupied, and this proportion reached 50% when burrows without obvious signs of activity were included. On intermediate beaches, this value increased from 36.17% to 59.57%, and on reflective beaches from 0% to 80%. On the reflective beach, few crabs were captured, but the data provided good evidence that a

small proportion of the occupied burrows would show evident signs of activity in this beach.

Many burrows reached considerable depths, some over 1 m. The occupation rates increased gradually with burrow depth until 20 (minimum OR) or 40 cm (maximum OR) (Figure 2.5), but virtually all burrows less than 10 cm deep were empty. With increasing depth the maximum rate tended toward 100%, and the minimum rate tended to decrease slightly.

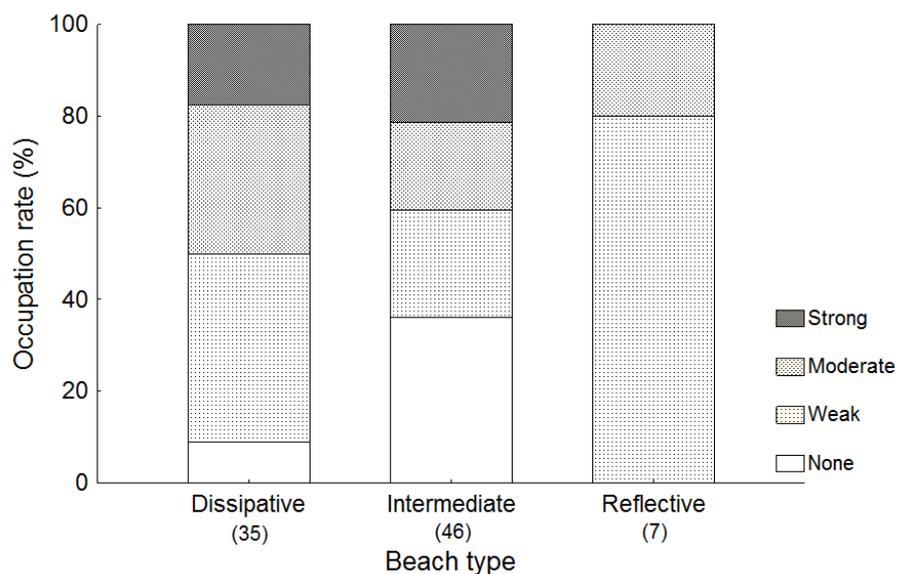


Figure 2.4. Relative intensity of signs around occupied burrows by beach type. Intensity of signs of *Ocypode quadrata* activity around occupied burrows. None – no sign of occupation; Subtle – only the internal perimeter definition and/or any other, barely recognizable sign could be noted; Moderate – Visible presence of sand and/or tracks; Strong – one of the above signs very obvious and/or presence of more than one visible sign. Covered or semi-covered burrows were also assessed. The number of animals caught on each type of beach is shown in parentheses below the x axis.

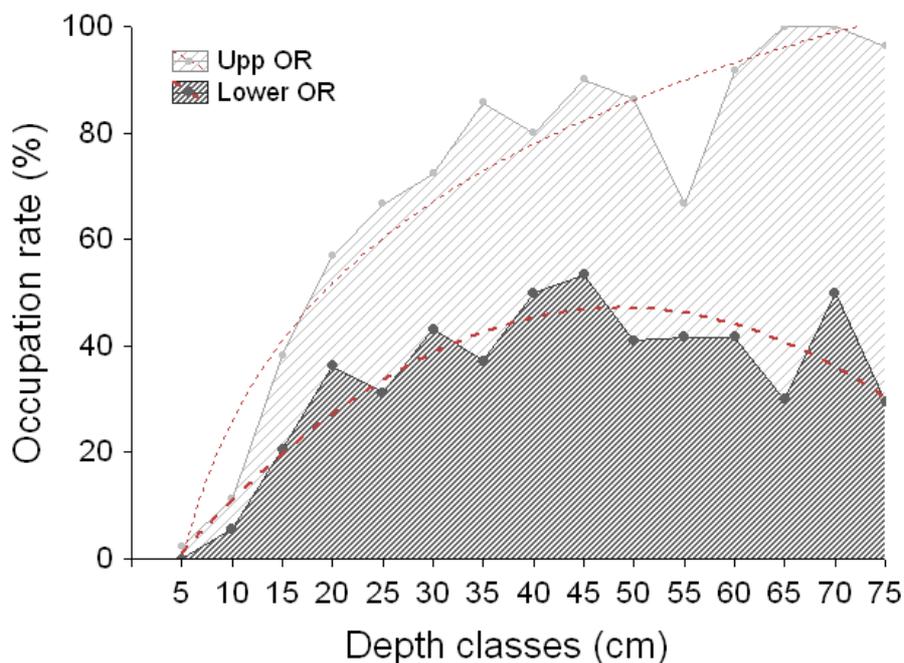


Figure 2.5. Minimum and maximum occupation rate according to depth. Occupation rates (minimum, dark gray; maximum, light gray) of burrows of *Ocyropsis quadrata*, according to their depth ($n=491$); dashed red lines represent the curve trend of each rate with increasing depth.

A total of 169 burrows were assessed to compare methods for estimating the occupation rate. The homogeneity of variances ($C = 0.78$, $d.f.=3$, $p=0.15$) allowed the use of a one-way ANOVA, which indicated a significant difference among the methods ($F=8.17$, $d.f.=3$, $p<0.01$). A Tukey post-hoc test discriminated three similarity groups (Figure 2.6), of which flooding showed the worst result. The difference between smudging and trapping was marginal, and traps were significantly similar only to excavating (Table 2.1). Surprisingly, excavating showed the highest mean values and the lowest standard error. The bronchoscope, stethoscope and GPR did not give satisfactory results. The bronchoscope was used without the monitor, and therefore the light was not adequate and the image too small. Because the bronchoscope was about 60 cm long and some burrows exceeded 1 m, it was still necessary to excavate to some extent. The presence of branching tunnels may also impede the use of a bronchoscope, although this method may merit further attention. The stethoscope probably was not sensitive enough, and the ambient noise of water and wind are likely to interfere with the use of this tool regardless of its

sensitivity. The GPR distinguished the air column in the burrows from the sand, rather than from the crabs inside. The use of different frequencies might improve the resolution of the objects, but would probably not change the actual results.

Table 2.1. Tukey post-hoc test between methods. Approximate probabilities for Tukey post-hoc test to compare the occupation rate of *Ocypode quadrata* burrows by means of different treatments: water, smoke, trapping and excavation. Samples were taken at Arpoador Beach in August 2012.

Probabilities for Tukey post-hoc test			
Treatment	Smoke	Trap	Excavate
Water	0.9997	0.04815	0.0216
Smoke	-	0.0544	0.0243
Trap	-	-	0.9356

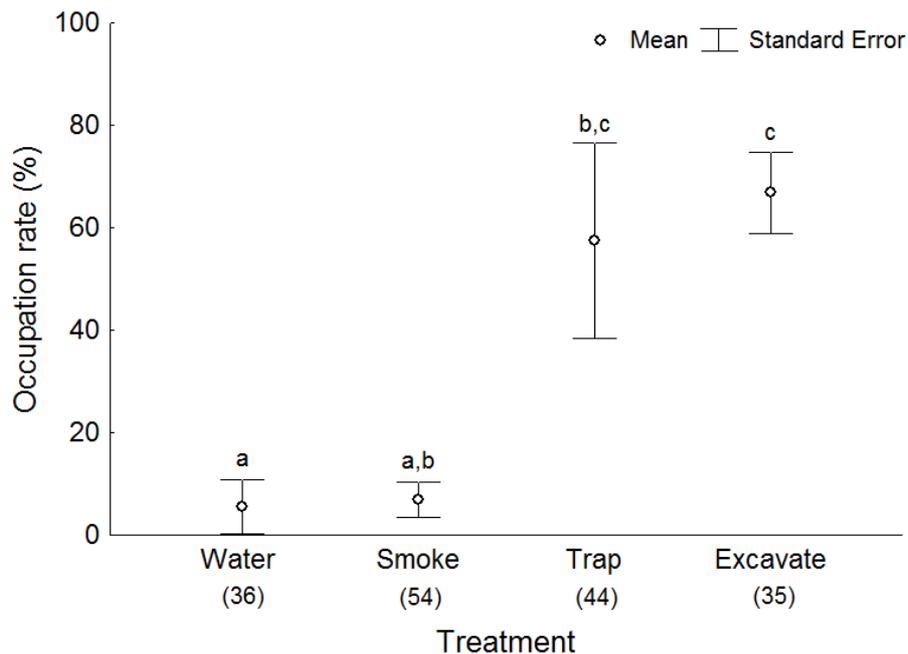


Figure 2.6. Occupation detected by means of different treatments. Mean and standard error of occupation rate of *Ocypode quadrata* burrows, determined by means of water, smoke, trapping and excavation methods, at Arpoador Beach in August 2012. Different letters denote significant differences discriminated by Tukey test. The number of burrows assessed for each treatment is shown in parentheses below the x axis.

Discussion

Estimating the rates of occupied burrows may change the perspective on some parameters of *O. quadrata* populations over different beach types. The relationship between grain size and organism abundance and richness is well known; the supralittoral crustacean community is usually more abundant with increasing grain size. However, this pattern is not evident for *O. quadrata* (DEFEO; MCLACHLAN, 2011). Since ghost-crab population data are most often estimated by burrow counts (ALBERTO; FONTOURA, 1999; BARROS, 2001; FISHER; TEVESZ, 1979; VALERO-PACHECO et al., 2007; WOLCOTT; WOLCOTT, 1984), comparing abundance estimates among different beaches also involves the assumption that the relationship between burrows and crab density is similar among beaches.

According to the results obtained here, the occupation rate is indeed likely to differ among areas. Therefore, although differences in population density over different beaches may exist, they are likely to differ to some extent from those predicted through burrow counts. Better understanding of these differences can improve the usefulness of *O. quadrata* as a bioindicator or monitoring tool. Undoubtedly, the method of rate detection needs refinement, given the uncertainty of the excavation method, but no other methods, despite the additional investment in time (e.g., traps) or technology (e.g., GPR), were as effective as excavating to measure the occupation rate.

It seems reasonable that occupation rates may differ because of natural characteristics of the environment. On some beaches, or even in different zones of the same beach, burrows must collapse more rapidly than in others, especially when we consider that *O. quadrata* individuals exploit the entire supralittoral zone as well as dunes and vegetated coastal flats (BRANCO et al., 2010; FISHER; TEVESZ, 1979; MILNE; MILNE, 1946; TURRA; GONÇALVES; DENADAI, 2005). Previous observations showed that an abandoned burrow can persist for over a week where the sediment is wetter or more compact, and especially if sheltered by vegetation (unpublished data). If this is so, the indirect method of assessing population density and age structure would be likely to

produce higher estimates for some strata of the population than for others, since these crabs show size stratification across the beach (ALBERTO; FONTOURA, 1999; TURRA; GONÇALVES; DENADAI, 2005). Therefore, abandoned burrows of animals of a length class that occupies drier regions would be prone to disappear faster than burrows constructed in wetter regions, and especially faster than those in vegetated areas, where the effects of wind and water are considerably reduced.

Combining these considerations, it seems reasonable that the minimum rate predicted by the present results is more reliable than the maximum rate. By this criterion, we have a scenario where at least 20% (this value is the same for the maximum OR), and probably not more than 60%, of the burrows of *O. quadrata* would be occupied.

An interesting, cautionary result emerged from the analysis of the intensity of signs of recent activity around burrows, since, in general, nearly a quarter of burrows lacking any sign were in fact occupied. Considering also subtle signs, i.e., those that are easily overlooked, this proportion tended to increase toward reflective environments, increasing from dissipative to intermediate beaches. On the reflective Figueira Beach, obvious signs of occupation were rare. This may reflect the ease with which the crabs leave tracks on finer-grained and more-compacted sediments. Also, it may be one more piece of evidence that burrows tend to close more easily on beaches with coarser sediment: if the signs disappear faster, the burrows should also close faster. If so, then dissipative beaches would tend to show lower rates of occupation of burrows of *O. quadrata* than reflective beaches, which partially agrees with the present results for minimum occupation rates.

The measurement of burrow depth showed a markedly different pattern for the maximum and minimum estimates of occupation rate. The differences between the curve trends may result from the increasing difficulty of reaching the crab; alternatively, the occupation rate of these deeper burrows may indeed be lower, since deeper burrows would last longer before collapsing. Furthermore, deeper burrows tend to be located farther from the waterline,

where the sediment is less compact. Nevertheless, this measurement proved to be a helpful and easy tool to minimize biases arising from taking decisions of whether or not to consider a burrow based on signs of recent activity. Depth measurement helped, for example, in a location with high activity of crabs and a large number of burrows, to distinguish which burrows were started but abandoned from those that the crabs continued to dig (Figure 2.7). Because virtually all burrows less than 10 cm deep were unoccupied, despite the intensity of signs around them, these burrows should not be counted in population assessments. Similarly, given the previous results, when no sign of activity is observed but the burrow depth is considerable, there is a greater chance that the burrow is occupied and therefore should be included in estimates.

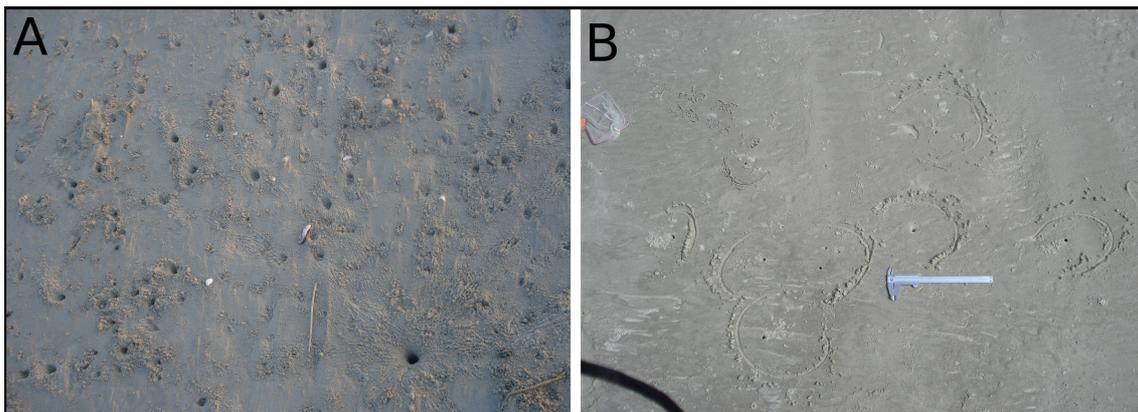


Figure 2.7. Examples of misleading situations. Examples of sets of burrows of *Ocypode quadrata* at Una Beach, southeastern Brazil. All these burrows showed signs of recent occupation, but only one was more than 10 cm deep and was the only occupied burrow (arrows). This may be more (a) or less (b) obvious from a superficial assessment.

Standardizing protocols that allow the use of ghost crabs as bioindicators is a challenge for researchers and managers of coastal environments. Although the results presented here illustrate the difficulties and noise in estimates and comparisons of crab density among beaches, stemming from differences in the occupation density, monitoring of ghost crabs through indirect methods may still be a powerful tool to evaluate temporal variations in crab densities in a given area, considering that the physical attributes

(sediment, slope) of an area will not vary much over time. The results also show that noise can be reduced using simple biological features, such as depth, which should be associated with the traditionally used "signs of recent activity" in assessments of the Atlantic ghost crab.

Chapter 3

Natural drivers of spatio-temporal distribution patterns of the ghost crab *Ocypode quadrata*: differences between burrow and individuals

Natural drivers of spatio-temporal distribution patterns of the ghost crab *Ocypode quadrata*: differences between burrows and individuals

Abstract

A large amount of knowledge on sandy beach ecology has been developed over the past few decades, and this is of key importance to understand the threats this fragile ecosystem has been facing. Ghost crabs are important inhabitants of sandy beaches and have a high bioindicator potential, which depends on a broad understanding of their spatial distribution patterns. Since population parameters such as density and individual size may be influenced by beach morphodynamics and season, variation in distribution patterns should not directly indicate the presence of a given environmental impact. Even so, the general tendency of these ecological population parameters according to beach type is not quite understood, yet. This study aimed to understand the influence of beach features and periods of the year on vertical distribution, abundance, size, and sex ratio of the ghost crab *Ocypode quadrata*. We assessed the effect of burrow and individual counts on the above mentioned relationships since population assessments through indirect measurements are widespread in literature. Nine pristine beaches comprising different degrees of wave exposure and morphodynamics were sampled seasonally. Across-beach distribution varied over beaches with different morphodynamic states and width, showing periodical sexual segregation and emphasizing the importance of vegetated areas as part of the area inhabited by *Ocypode quadrata*. General sex-ratio was mostly deviated towards males, but there were persistent seasonal differences between areas, with females predominating in the winter, followed by autumn. Mean abundance and burrow size were recurrently higher than those of individuals. Beaches and seasonality influenced burrow occupation rate, which means that indirect methods underestimated the number of individuals differently over areas and seasons. Higher burrow occupation rates were observed in autumn, associated to cold front entries, which means it would be more suitable to extrapolate data from

indirect measures to the effective population in this period. Higher proportions of empty burrows were surveyed for larger crab sizes; hence, this population stratum is more prone to overestimations. Both mean burrow densities and mean individual densities became higher towards dissipative beaches, a trend in the opposite direction to the expected for other supralitoral crustaceans. Mean size showed no trend according to beach type, although it differed over time and space. Equal-sized burrows housed larger individuals in dissipative beaches than in reflective beaches, meaning that different relationships should be used to estimate size of individuals from burrows on beaches with different grain sizes.

Keywords: environmental factors, morphodynamics, beach exposure, abundance, size, spatio-temporal, across-beach

Introduction

Social and environmental benefits of sandy beaches have been recognized for some time now, but until the mid-20th century, these ecosystems were considered marine deserts. Sandy beach ecology only emerged as a particular field of coastal science in the early 1980's (MCLACHLAN; BROWN, 2010). Since then, substantial knowledge has been built on the issue, as is the case of the habitat harshness hypothesis, which has been recurrently supported over the globe (DEFEO; MARTÍNEZ, 2003; MCLACHLAN et al., 1993). According to this hypothesis, intertidal biota is favored by gentler slopes and finer grains due to water retention and greater amounts of organic matter; consequently, higher species richness, diversity, and biomass are observed towards dissipative conditions (CELENTANO; DEFEO, 2006; DEFEO; DELGADO, 2007).

Backshore individuals tend towards the opposite, since steeper slopes and less compact sediment would offer shelter for organisms, mainly burrowing crustaceans that are not under direct contact with wave action (DEFEO; GÓMEZ, 2005; DEFEO; MARTÍNEZ, 2003). However, knowledge on backshore biology is far behind intertidal ecology, partially due to its scarcer richness. Thus, knowledge on supralitoral organisms requires further development to achieve the level of understanding available for intertidal species. This also applies to the ecology of the most conspicuous organism on sandy beach backshores around the globe, the ghost crabs - common name for individuals of the genus *Ocypode*. Similar to what happened with sandy beach ecology, most of the information on *Ocypode* was behavioral and mainly qualitative until the mid-20th century (Sawaya 1939; Milne and Milne 1946). Physiological, developmental, and energetic studies were fairly developed during the late XXth (DIAZ; COSTLOW, 1972; SANTOS; MOREIRA, 1999; SCHÖNE; SCHÖNE, 1961; WEINSTEIN; FULL; AHN, 1994; WOLCOTT, 1978).

Ecological research on *Ocypode* gained more space from the late 80's on, with many studies developed on its population structure for very distinct environments around the globe, such as low-energy, high-energy, and estuarine

beaches, both dissipative and reflective (ALBERTO; FONTOURA, 1999; BRANCO et al., 2010; QUIJÓN; JARAMILLO; CONTRERAS, 2001; ROSA; BORZONE, 2008; STRACHAN et al., 1999; TURRA; GONÇALVES; DENADAI, 2005). General spatial patterns, however, are not as well defined for ghost crabs as for other sandy beach crustaceans. For *O. gaudichaudii*, Quijón et al. (2001) did not find differences in density to be directly related to Dean's parameter, and Lucrezi (2015) found higher densities in more dissipative beaches, considering burrows of three different species combined. For *O. quadrata*, Turra et al. (2005) observed lower densities associated to very fine and poor- to moderately-sorted grains in low-energy beaches. The relationship between basic environmental parameters, e.g., increase in number with increased grain size, noticeable for small-sized beach crustaceans, has not been recognized for *Ocypode* species so far (CELENTANO; DEFEO, 2006; DEFEO; MARTÍNEZ, 2003; DEFEO; MCLACHLAN, 2011). Therefore, we still lack understanding on how natural factors drive the abundance and structure of different populations of ghost crabs, as well as burrow occupation rate. In addition, local impacts may influence these relationships, preventing more accurate population studies as well as their wider use with indicative purposes.

Many studies on the effects of anthropogenic activities on ghost crab distribution have been carried out recently and the negative effect of vehicles, trampling, urbanization, and nourishment on these populations have been described (BLANKENSTEYN, 2006; HOBBS; LANDRY; PERRY, 2008; LUCREZI; SCHLACHER; WALKER, 2009; MAGALHÃES et al., 2009; NEVES; BEMVENUTI, 2006; NORIEGA; SCHLACHER; SMEUNINX, 2012; PETERSON; HICKERSON; JOHNSON, 2000; SCHLACHER; THOMPSON; PRICE, 2007; VALERO-PACHECO et al., 2007). In fact, recognition and proper use of information provided by bioindicators is of great value in a context of increasing human pressure, as is the case of sandy beaches. On the other hand, understanding the effects of environmental variables, i.e. beach type, as well as other factors that influence beach characteristics, crab population density, and individual size, such as seasonality, is essential to use ghost crabs

as indicators of sandy beach quality.

In addition, the great majority of studies on ghost crabs have compared populations from different periods or areas using indirect measures, i.e. burrow counts. The use of indirect measures is actually one of the greatest advantages in using these species for bioindication purposes, since assessing burrows is easier, faster, and less destructive than directly assessing individuals. Nevertheless, the relationship between abundance and burrow size and size of animals, i.e., how direct and indirect measures are related through different beach types and seasons, is not quite understood (POMBO; TURRA, 2013; SILVA; CALADO, 2013).

Therefore, this study aims to shed some light on the investigation of the population biology of ghost crabs, assessing spatio-temporal distribution patterns of *O. quadrata* in pristine habitats (minimizing the influence of human activity), to single out the influence of beach features and period of the year on vertical distribution, abundance, size, and sex ratio of the ghost crab *Ocypode quadrata*.

This study covered spatial and temporal changes using a detailed, descriptive analysis of across-beach distribution patterns to model the influence of abiotic factors on biological factors. More specifically, we assessed:

- I. the across-shore distribution patterns of ghost crab burrows and individuals, regarding abundance and sex ratio.
- II. general spatio-temporal patterns of (i) abundance of ghost crab burrows and individuals, (ii) size of burrows and individuals, and (iii) sex ratio;
- III. influence of environmental factors (namely, season, beach slope, grain size and sorting, level of exposure, and crab range of occurrence) on (i) the abundance of burrows and individuals and (ii) on the relationship between size of individuals and burrows.

Methodology

Study areas

Nine pristine, low-use beaches were chosen so that anthropic influence was minimized as much as possible. These areas were distributed along São Paulo coast, in southeastern Brazil (S24°30'60", W47°10'40" and S23°21'15", W44°55'60"), comprising approximately 350 km of coastline with a great variety of sandy beach types. Beaches were also selected so as to ensure a wide gradient of abiotic factors concerning morphodynamics and degree of exposure to waves. To support these choices, we referred to a previous geological study of the local beaches for morphodynamic characterization (ROCHA, 2008), while visual assessments in the field and satellite images were used for a preliminary assessment of beach exposure. For organization purposes, the study areas are further arranged by morphodynamic states (dissipative, intermediate, and reflexive) and, within each group, by degree of exposure (low, intermediate, high), as follows: Costa, Arpoador and Una; Justa, Brava and Felix; Figueira, Puruba, and Prumirim.

Sampling procedures and environmental characterization

All sampling procedures complied with the license from the appropriate federal environmental agency (*Ministério do Meio Ambiente (MMA) – Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio)* No. 31629-1; acronyms for: Ministry of the Environment – Chico Mendes Biodiversity Conservation Institute).

From February 2012 to December 2012 beaches were assessed seasonally (in the central month of each season to avoid the influence of adjacent seasons). For each beach a homogeneous section of about 200m was selected so that eventual influences of rivers, promontories or any other possible causes of noise or disruption were avoided. In each section of each month, three 2-meter-wide transects were laid perpendicular to the water line, and assessed from the water line landwards, until the last observed burrow, including those inside the vegetation of the coastal dunes. Transects were divided into 2m sequential quadrats, i.e., 4m² each, so that their number varied according to beach length.

In each quadrat, burrow diameters were measured. The diameter considered was that of the bottom of the tunnel shaped portion of burrows (upper portion), presumably closely related to animal size; thus, bias due to sediment deformation would be reduced. Burrows were then excavated to search for the crab, with the help of a steel cable to locate and guide us to the bottom of the burrow. Probing around while excavating helped us detect occasional continuations or side branches of the galleries. Captured crabs were also counted and measured.

The sum of quadrats from the first to the last observed burrow, in each transect, multiplied by 2 (adapting the width of the last quadrat, which could be less than 2m) was considered, in meters, as the range of occurrence of individuals per transect. The slope was recorded in each quadrat of the range of occurrence. Therefore, the aim was not to characterize the beach slope itself, but the profile of the area where the animals live. The slope was expressed as the traditional “rise over run”, i.e., in one transect, it was the tangent of the angle opposed to the leg corresponding to the sum of the rise registered in each quadrat.

Sediment was collected in each transect, in the backshore, intertidal area and sub-terrestrial fringe. The mean grain size and its standard deviation (sorting coefficient), in phi units, were obtained following the screening procedure as described in Day (1965).

Wave exposure levels have been defined for the sampled beaches based on the dominant wave conditions for each month of 2012. In order to obtain the local wave conditions that reach each of the sampling sites, a numerical model has been applied in order to propagate the waves onshore according to the nearshore morphology. The applied numerical model is the Delft3D (open source version from Deltares) WAVE module. The Delft3D WAVE module simulates the propagation and transformation of wind-generated waves as they move over varying bottom morphology (HOLTHUIJSEN et al., 1993). It simulates effects such as wave refraction, diffraction, shoaling and set-up in coastal areas. The model used in this application covers the whole São Paulo

state continental shelf, including adjacent areas, keeping the areas of interest well inside the model domain. Grid resolution varies in the domain, with increased resolution onshore. Offshore boundary conditions representing the dominant wave climate (significant wave height and correspondent wave period) for each month has been defined from the information extracted from the global wave generation model WaveWatch III (NCEP/NOAA – TOLMAN et al., 2002), at a point close to the shelf break. After running the model for each period, wave data has been extracted in front of the sampled beaches (at depths of about 3 – 4 m) providing the local wave conditions for the definition of exposure levels during sampling.

A common question in abundance assessments of *O. quadrata* lies on whether tide height according to sampling time may influence the results. The field work of the present study was very time consuming, usually lasting all day; hence, it did not allow any restriction to a specific tide height. Therefore, its influence was assessed by correlating the ranges of occurrence of *O. quadrata* to the tide height of each transect (sampling time was regularly recorded) per beach. The Pearson's correlation tests were not significant for any beaches (Costa $r=-0.27$, $p=0.43$; Arpoador $r=-0.29$, $p=0.37$; Una $r=-0.22$, $p=0.49$; Justa $r=0.23$, $p=0.47$; Brava $r=0.27$, $p=0.46$; Felix $r=-0.16$, $p=0.62$; Figueira $r=0.47$, $p=0.12$; Puruba $r=0.11$, $p=0.73$; Prumirim $r=-0.57$, $p=0.05$). On Prumirim, a marginally significant result was identified, but even taking this into account, the correlation coefficient was very low. Therefore, the influence of tide may be disregarded in the validation of further results.

Data analysis

To assess the abundance of burrows and individuals, the behavior of two variables was primarily investigated: the raw number of individuals per transect, namely individuals per strip transect (IST; DEFEO; RUEDA, 2002), and density (ind.m⁻²). To calculate density, the largest range of occurrence from all transects and seasons within a beach was used as the pattern area to calculate the density of all other transects. Both variables behave very similarly

through exploratory analysis, but IST showed more noticeable correlations with the range of occurrence, as expected (Pearson's correlation tests of range of occurrence with: $IST_{burrows} r= 0.53, p< 0.001$; $IST_{individuals} r= 0.51, p< 0.001$; $density_{burrows} r= -0.29, p= 0.002$; $density_{individuals} r= -0.22, p= 0.021$). Therefore, the relativized measure of abundance, density, was used in further analyses. This approach may be understood as a modification of individuals per transect unit (IST), intended to avoid result masking due to differences in width between beaches, although without compromising comparisons over time within each study area, i.e. avoiding over- or underestimations due to temporal variation of distribution ranges.

Regarding size, the individual measures that showed better correlation with burrow diameter were width (Pearson's $r= 0.71, p< 0.01, n= 531$) and length ($r= 0.70, p< 0.01, n= 531$). Height did not have a strong correlation coefficient ($r= 0.09, p= 0.03, n= 531$). Because width is the most frequently studied measure in literature, it was chosen for further analyses.

Across-beach distribution

The across-beach analysis comprised mainly visual comparisons; the limited amount of individuals at some points did not allow for the development of quantitative analyses. The terrestrial fringe position, i.e., the vegetation line, was defined as 0 for all transects to standardize sampling positions within a beach. The abundance values of the three replicate transects were individually represented in weighted dot graphs, for each beach and season. Dots were placed for each relative quadrat, thus representing the across-distribution of abundance of burrows (blank dots) and individuals (filled portion). Sex ratio was calculated by quadrat, summing up all transects, and added to the plot.

General spatio-temporal distribution patterns

General aspects of distribution through areas and seasons were assessed, as well as how these aspects differ between burrows and individuals. Three-way ANOVAs were performed separately for density and size,

considering the following factors: beaches, season, and “subject” (a factor name for measures of burrows or individuals).

Both ANOVAs were followed by Tukey post-hoc tests and the results were plotted inside mean and standard deviation graphs. Seasonal sex ratios of each beach and season were provided, as well.

Influence of abiotic variables

Regressions were performed to assess the relation between the sampled abiotic factors and (i) ghost crab burrow density (ind/m²), (ii) ghost crab individual density (ind/m²), and (iii) estimation of individual size based on burrow size. For each transect the assessed abiotic variables comprised: slope (average slope of quadrats), mean grain size (for each of the three beach levels), standard deviation of mean grain size (for each of the three beach levels), and range of occurrence. The exposure level could only be calculated as a general value for each beach seasonally, and these values were repeated for the replicates of a beach/season (transects). Concerning categorical variables, the first sampling season, summer, was used as baseline. In summary, for each beach 12 values were plotted (4 seasons, three replicates), totaling 108 values for each variable. For size analysis, the *burrow diameter* was the factor used to model the size of individuals. Initially, additive linear regressions were performed including all factors. The factors considered significant were then assessed for interaction, and model selection was basically performed thorough stepwise regression. If differences in the Akaike information criteria (AIC) values were very small, hypothesis testing was used to decide which model would be kept. Finally, residuals were checked for model validation.

Results

Environmental factors characterization

Distribution range

The study areas showed an across-beach range of occurrence of *O. quadrata* from 10 to 60 m. The narrowest ranges were observed on Costa and

Justa beaches throughout the year, while the widest ones were observed on Una and Brava beaches (Figure 3.1). Range variability within each season and beach was generally low, but noticeable differences were recorded over seasons. For most areas the largest ranges of occurrence were observed during the summer and the shortest ones occurred during autumn.

Slope

The mean slope of the areas inhabited by *Ocypode* did not always correspond to the whole beach slope, previously characterized by Rocha (2008). Costa beach, previously classified as dissipative, showed some of the steepest slopes throughout the areas inhabited by individuals. Arpodor, Una (previously classified as dissipative), and Brava (previously classified as intermediate) showed gentler slopes, followed by Felix beach (intermediate). Along with Costa beach, Justa (intermediate), Figueira, Puruba, and Prumirim showed the steepest slopes among the areas inhabited by *Ocypode*.

Mean grain size and standard deviation

Mean grain size matched the previous characterization of beach morphodynamics, i.e., decreasing values of phi from dissipative to reflective beaches. Costa beach, probably because it is a tide-dominated dissipative beach, was an exception, showing the greatest seasonal variation in mean grain size, as well as its standard deviations. With a few exceptions, the mean values of phi did not have considerable standard deviations within and among seasons; dissipative beaches tended to show higher deviations during the summer.

Exposure

The mean values of wave energy generally confirmed the visual choice of areas according to their exposure degree. This was mostly evident on dissipative beaches (Figure 3.1, exposure increasing from Costa to Una), followed by reflective beaches (exposure increasing from Figueira to Prumirim), and it was less evident on intermediate beaches. As opposed to the distribution range pattern, wave energy was less intense during the summer and more intense during autumn for all study areas.

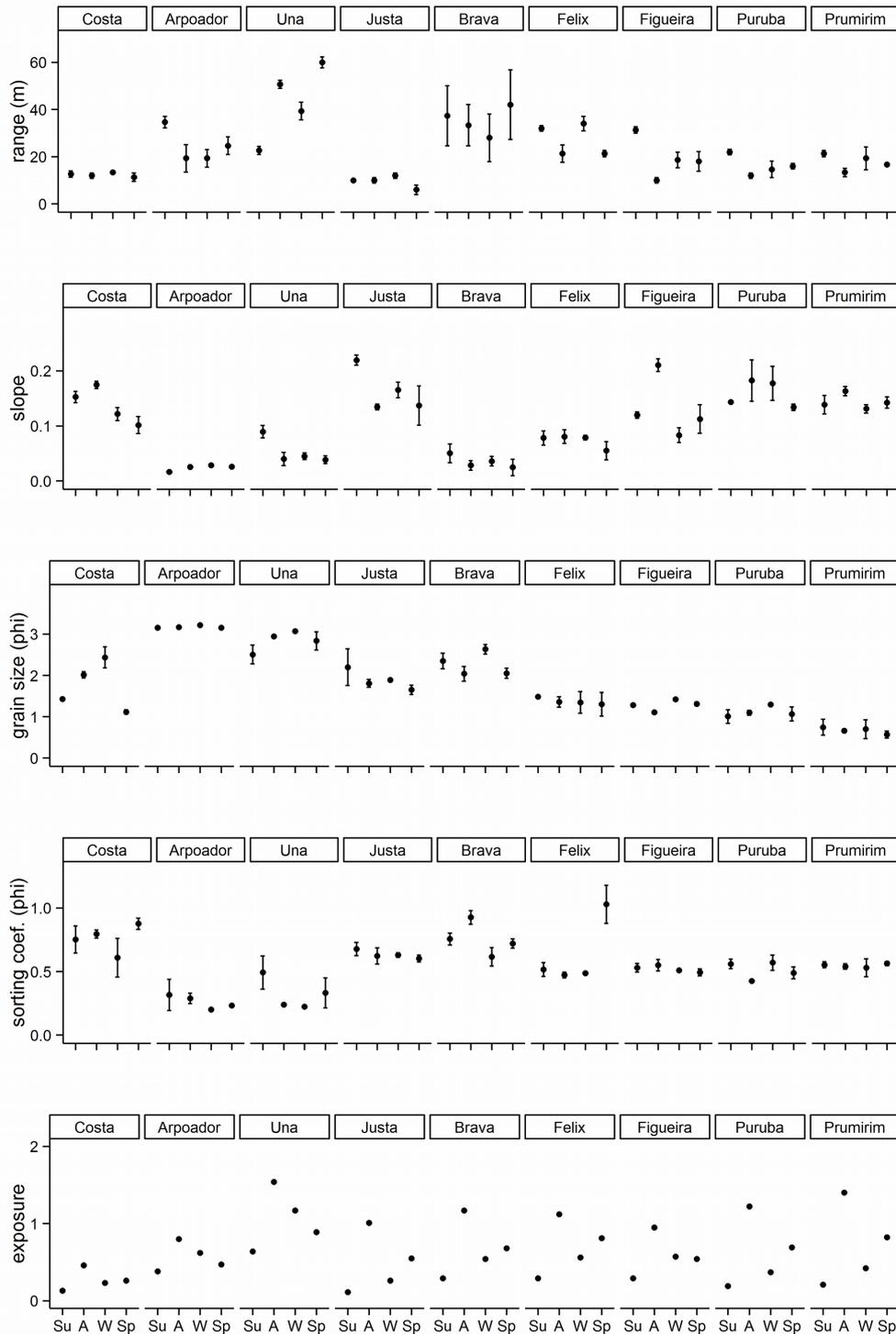


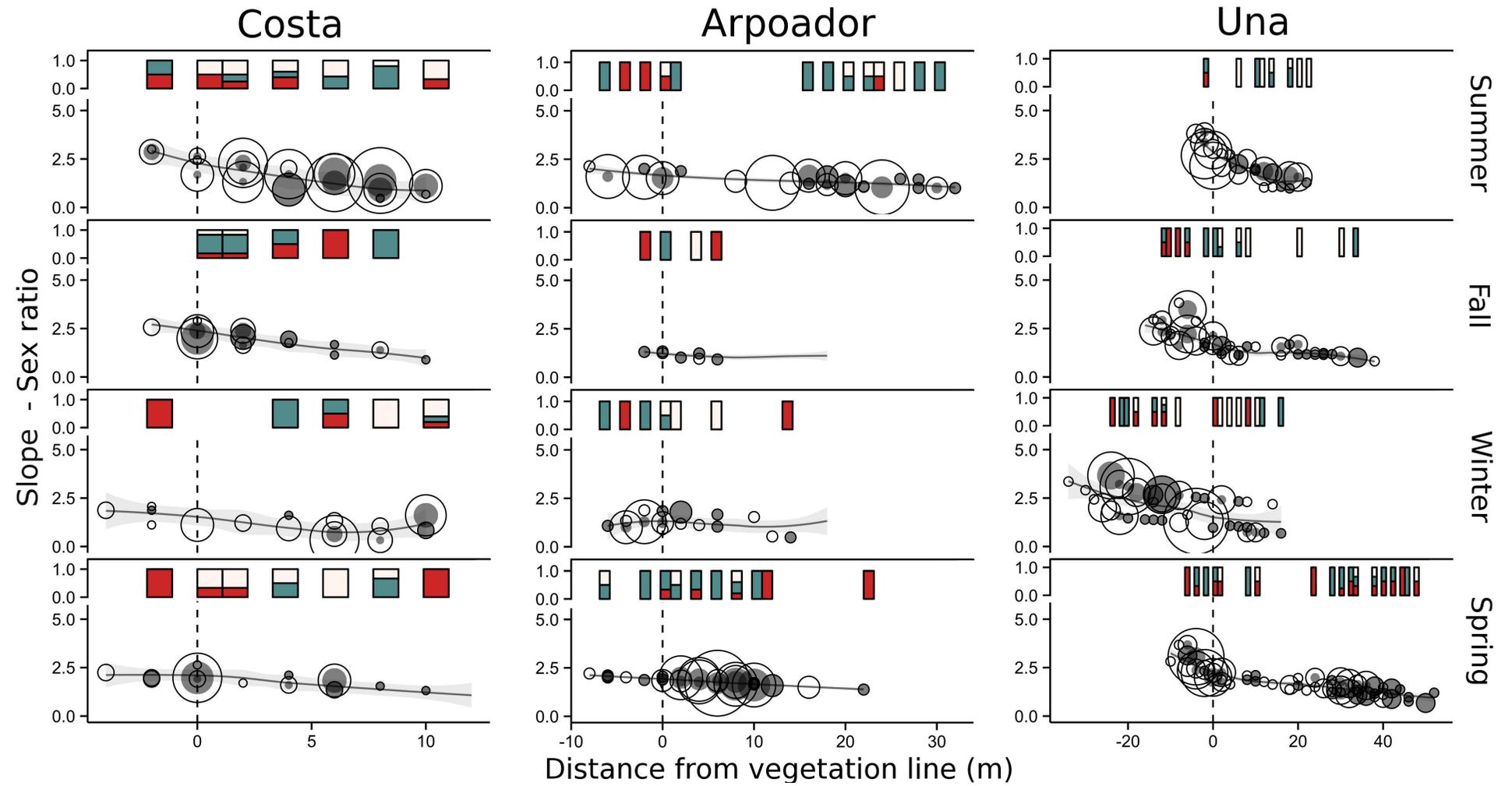
Figure 3.1. Beaches seasonal mean and standard error of: range of occurrence of *Ocyode quadrate* (m) slope, grain diameter (phi), grain diameter standard deviation (phi). Data derived from three transects sampled seasonally from February to November 2012. Exposition degree is based in monthly main waves' height; only one value was provided per season within each beach.

Across-beach distribution

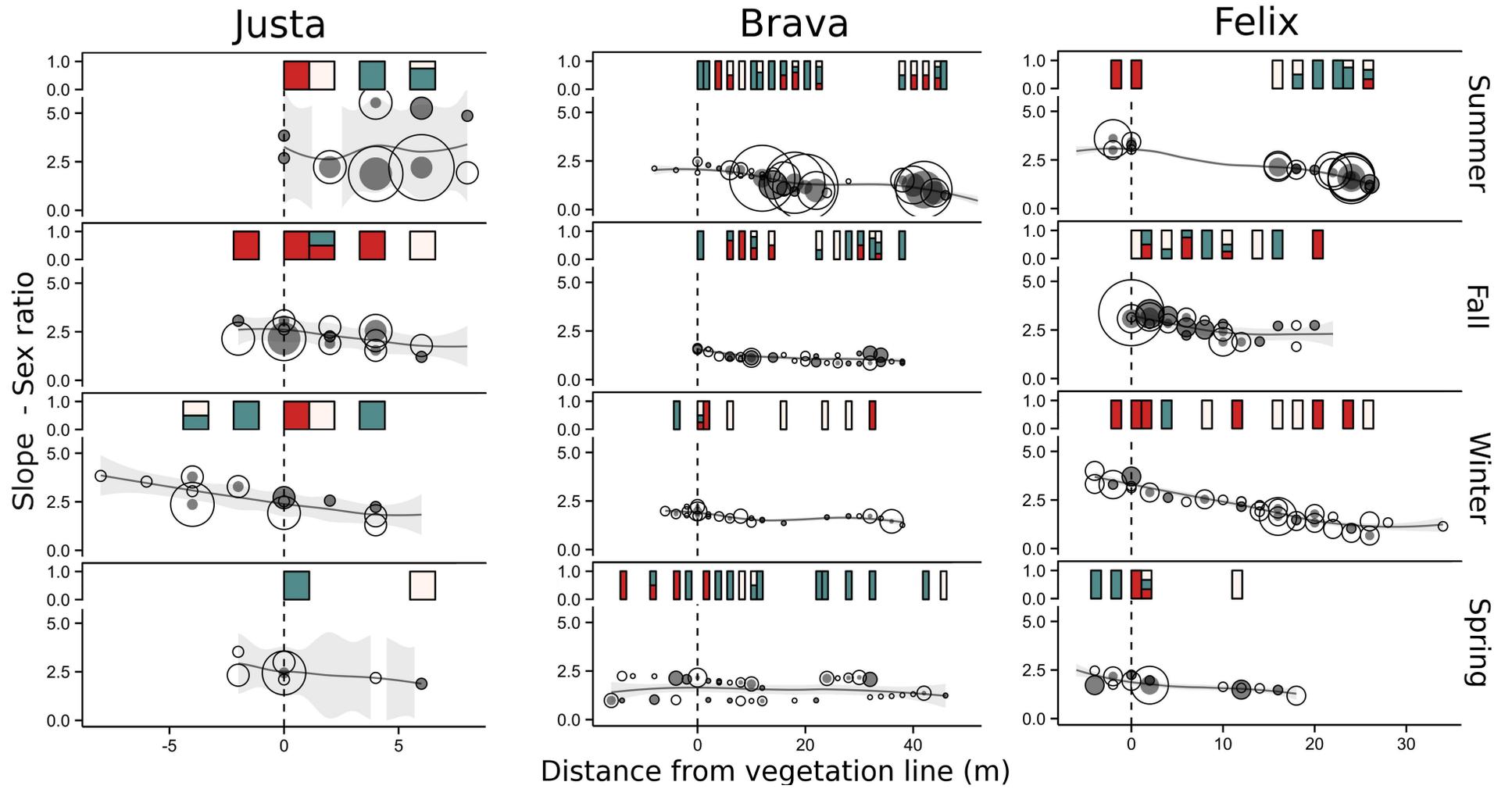
Clear across-shore distribution patterns were identified throughout time, while over areas, patterns depended on beach features, specially length and slope (Figure 3.2). During the warmest periods (summer and spring) the range of occurrence tended to be larger (Figure 3.1), although with less occupied mid-areas, and this pattern increased as ranges and slopes increased (see Arpoador, Una, Brava, Felix, Figueira, Puruba). In addition, this pattern was more evident for individuals rather than for burrows. The majority of burrows where no crabs were found are farther from the water, which implies there are usual higher occupation rates closer to the waterline. The vegetation line and beyond was an important dwelling area throughout the study period. In autumn and/or winter, the distribution moved backwards in many study areas (see Figure 3.2: Arpoador, Una, Justa, Felix, Puruba).

Regarding sex ratios, the across-beach distribution during the summer was clearly skewed, with a noticeable concentration of males closer to the waterline and of females closer to or inside the vegetated area. This pattern was observed in 7 of the 9 studied beaches. For the other 2 areas (Costa and Figueira) this pattern was not as clear. During the coldest seasons there was no clear sexual distribution pattern over the study areas and individuals tended to concentrate closer to the vegetation. This retraction in the distribution range was especially clear for reflective beaches during autumn. In the winter there was no clear pattern of across-shore sexual segregation, but this occurred when the higher proportion of females was closer to the waterline in 3 of the studied areas, two of which had only females identified. In the spring the pattern reversed once again, tending to be more similar to the somewhat less marked pattern observed during the summer.

A - Dissipative beaches



B - Intermediate beaches



C - Reflective beaches

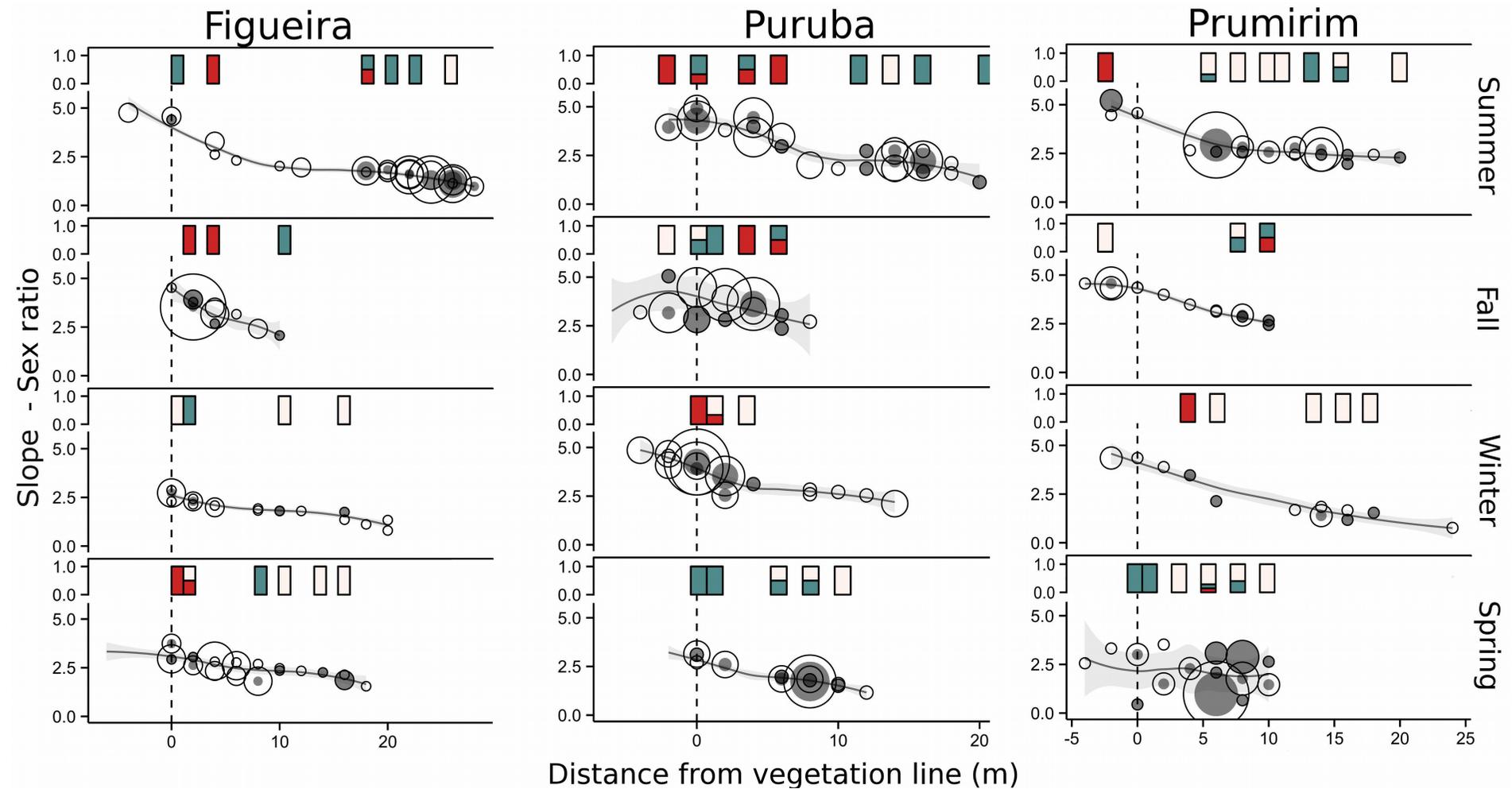


Figure 3.2. Across-beach seasonal distribution of *Ocyropsis quadrata* sampled from February to November, 2012, in 9 different beaches in Southeastern Brazil, previously classified as (A) dissipative, (B) intermediate, and (C) reflective. The gray line and the smooth area represent slope mean and standard deviation of the slope, in meters (Y-axis), for every two meters of beach width (X-axis), where 0 (dashed vertical line) represents the vegetation line, with increasing values towards water line. White dots are weighted according to the number of burrows in each transect and quadrat, the gray centered portion is weighted according to the number of animals captured in these burrows. Upper bars represent the sexual proportion recorded (blue=males; red= females), regarding quadrats, for the set of transects ($n=3$).

General patterns of spatio-temporal distribution

Number of individuals and burrows

According to mean spatio-temporal distribution of burrows and individuals, we noticed that temporal fluctuations in number of individuals were considerably lower than that of burrows. Standard deviations also tended to be higher for burrows (Figure 3.3).

Density three-way ANOVA accused interactions between all terms but no 3-way interaction. Single terms were all significant. There were differences between areas ($F= 26.21$; d.f.=8,207; $p<0.01$), concentrating more dissipative beaches in higher mean groups and reflective beaches in lower mean groups, except for Arpodor (a dissipative beach with low mean values). Regarding seasons, summer had significant higher density values than all others, which did not differ between each other ($F= 30.08$; d.f.=3,212; $p<0.01$), and regarding subject the overall burrow density was significantly higher than density of individuals ($F= 170.05$; d.f.=1,214; $p<0.01$).

However, there were significant interactions between these terms. The term beach and season ($F= 4.46$; d.f.=35,180; $p<0.01$) showed that regardless of the subject assessed (burrows or individuals), some areas and certain seasons had higher values than others, concentrating dissipative beaches, mainly during warmer seasons, at the higher mean groups. But, most cases did not differ from each other (33 out of the 36 combination were also part of the lowest group, *d*). The interaction in density between season and subject ($F= 5.64$; d.f.=3,212; $p<0.01$) showed higher values of burrows in the summer (tukey group *a*) than burrows in the winter, spring and autumn (*b*, *b*, and *bc* groups, respectively). The density of individuals composed the lowest four groups which did not differ from each other (summer – *bcd*, spring and autumn – *cd* and winter – *d*). The interaction term beach and subject ($F= 3.25$; d.f.=17,198; $p<0.01$) concentrated burrows on more dissipative beaches in groups with maximum values and, on the other hand, individuals on reflective beaches were concentrated in groups with minimum values; a high overlap of

groups was observed for intermediate values (Figure 3.3). To sum up, the biases in estimates of density of individuals from burrows were different between seasons. These temporal biases were constant over areas, although there were significant differences in bias magnitude between different beaches regardless of the season.

Size

The 3-way ANOVA for mean size indicated significant differences according to each factor alone and to one interaction, between beach and season. More specifically, mean burrow size was significantly larger than size of individuals in general ($F= 4.90$; $d.f.=1,214$; $p<0.01$) and, despite the subject, size values varied over beaches and seasons ($F= 2.25$; $d.f.=35,180$; $p<0.01$), showing that there are differences in mean size over areas, and size values also depend on recruitment and temporal mortality patterns. Despite the difference between areas, there was no increasing tendency in mean size towards a beach morphodynamic type. For instance, the highest mean values of the interaction were observed to Arpoador (dissipative), Justa (intermediate) and Prumirim (reflective), all in the autumn, as well as for Una (dissipative) during the spring and Puruba (reflective) during the summer and also autumn. The lowest mean values of the interaction were recorded for Prumirim during winter and spring; other mean values did not significantly differ from any other.

Temporal size patterns followed similar trends over most studied beaches, although significant differences were not precisely the same for all areas (Figure 3.4). An increase in size was generally observed from summer to autumn, decreasing again in the winter and remaining the same or increasing in spring. This pattern was generally more evident for individual size than for burrow size. Thus, mean sizes tended to reach higher values in autumn, mainly regarding individual width.

Regarding size class frequencies, summer and autumn showed size frequency distribution tending to be more unimodal over areas than the other seasons, with a larger size mode in autumn. Histograms also show that winter

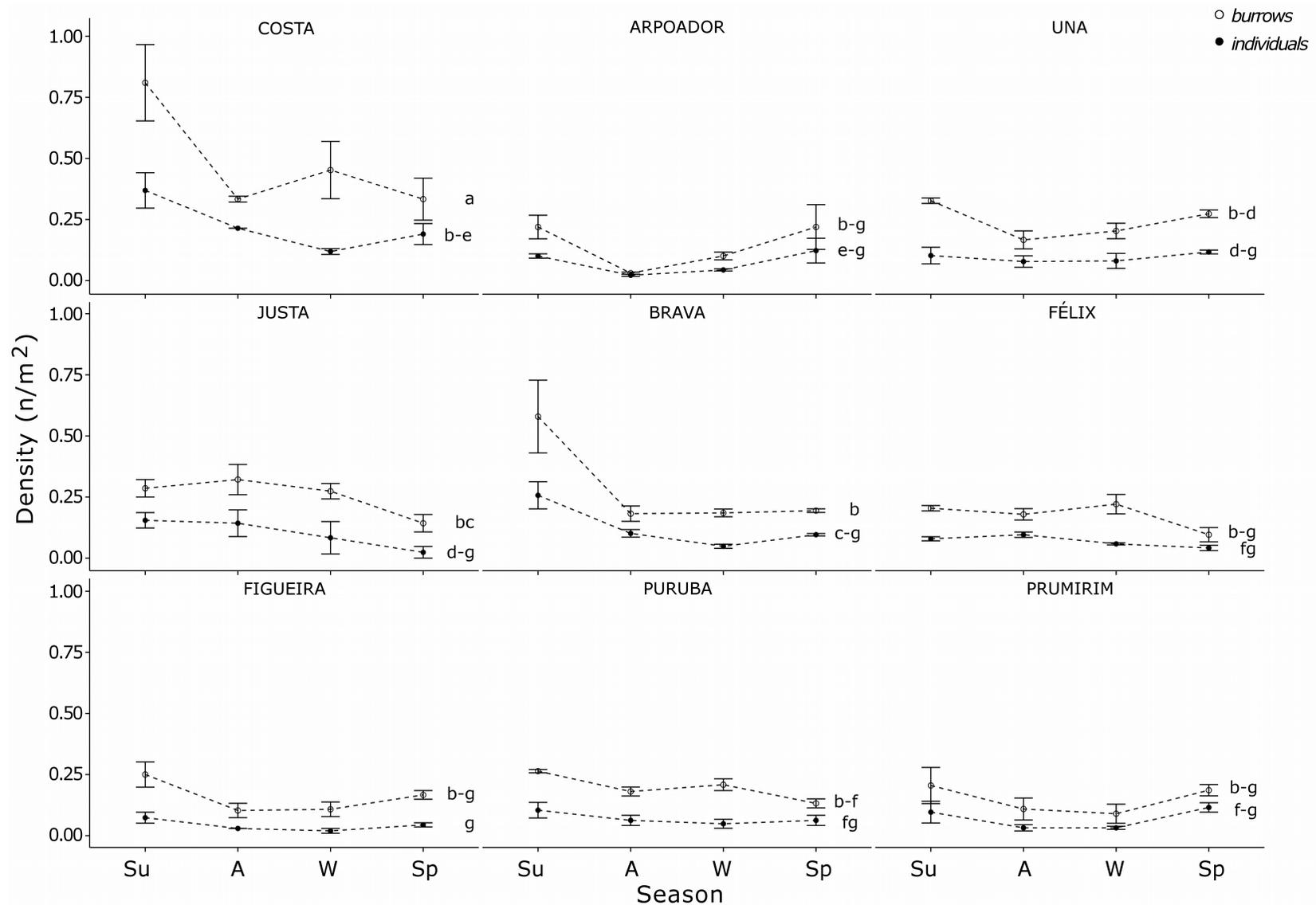


Figure 3.3. Seasonal mean and standard deviation of density of *Ocypode quadrata*: burrows (white symbols) and captured individuals (black symbols) in each study area. Sampling was carried out from February to November, 2012. A factorial 3-way ANOVA was performed and all two-way interactions were significant; lower case letters following each graph denote results of the Tukey test for the interaction between beach and subject (burrow, above, or individuals, below).

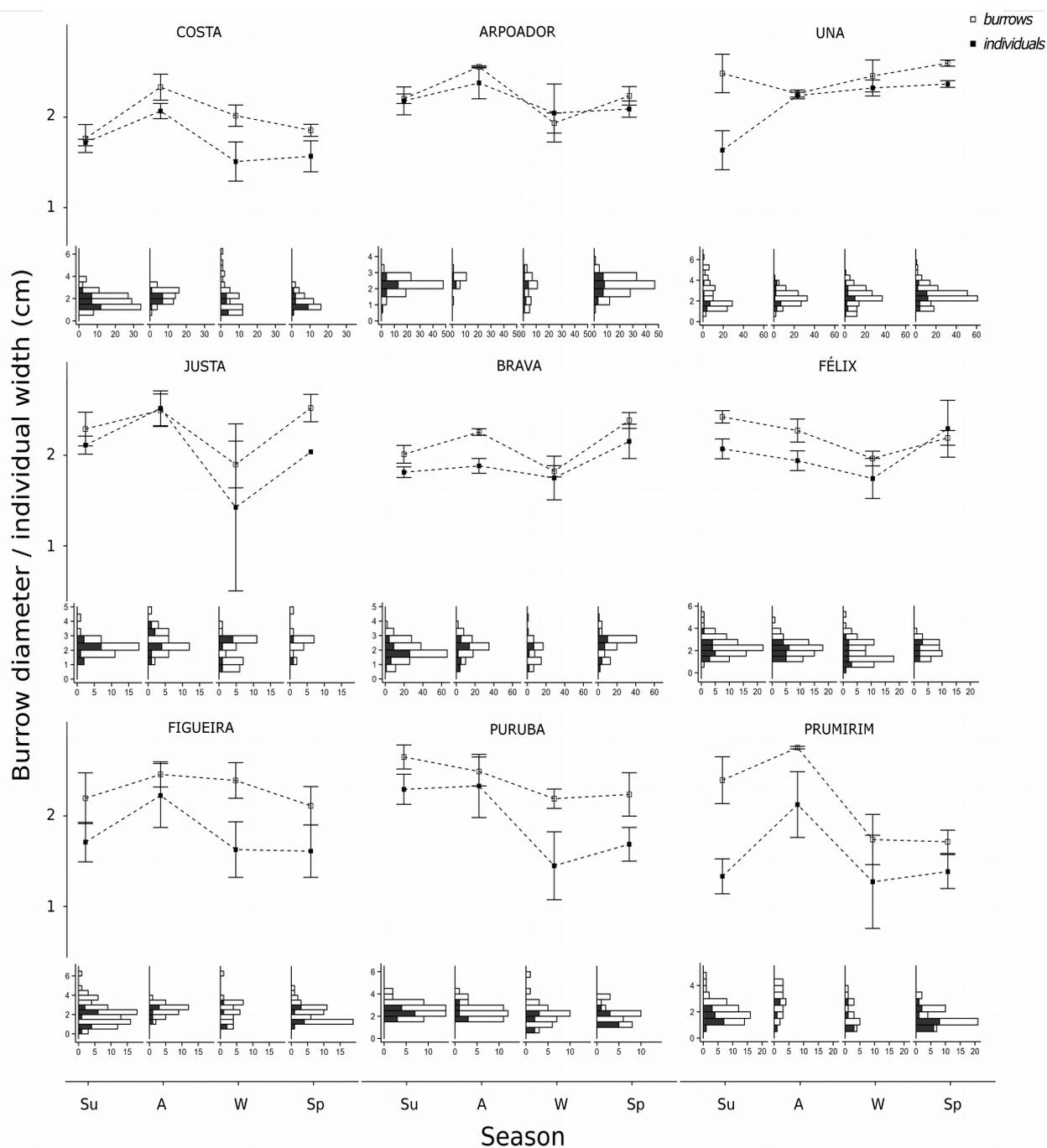


Figure 3.4. Seasonal mean and standard deviation of width (cm) of *Ocypode quadrata*: burrows (white symbols) and captured individuals (black symbols) in each study area. Below are the corresponding histograms (white bars=number of burrows; black bars=number of individuals); scales of histograms are different between beaches to highlight proportions. Samples were performed from February to November 2012.

had the greatest entrance of (captured) smaller individuals for most of the studied areas. Periods when crabs were larger showed the highest rate of empty burrows.

The analysis of mean size, in summary, showed that mean burrow size was generally larger than that of individuals. According to spatio-temporal patterns, these differences reflected mostly events of population dynamics, which were observed similarly both through burrows and individuals.

Sex-ratio

In general, the overall ratio males:females was higher than 1.0, except for Felix and Figueira beaches (Table 3.1). However, in the winter, the number of captured males was lower than females in most of the areas. Autumn and spring had, respectively, 3 and 2 study areas with smaller amounts of captured males than females, while all areas in the summer had more captured males than females.

Table 3.1. Ratio between the number of males and the number of females of *Ocypode quadrata*, captured and identified for each season and study area, from February to November 2012. Values lower than 1.0 are highlighted.

	Summer	Autumn	Winter	Spring	General
Costa	1.67	2.5	1	0.75	1.53
Arpoador	3.25	0.5	1.5	2.5	2.29
Una	6.00	2	1	1.07	1.37
Justa	8.00	0.14	1.5	1/0	1.30
Brava	1.77	1.62	0.67	2.5	1.77
Felix	1.40	1	0.14	2.00	0.90
Figueira	3.00	0.2	1/0	0.33	0.90
Puruba	2.25	1.50	0/4	5/0	1.70
Prumirim	2	2	0/1	6.00	2.40

Influence of environmental factors

In the preliminary assessments of the variables involved, before the regression analysis, no clear case of non-linearity was evident. Despite the collinearity between mean grain size and slope, both variables were maintained;

firstly, because the general model adjustment value was not compromised by multicollinearity and, secondly, because since the relationship between these variables usually follows a known regular pattern, it does not affect the model extrapolation (GUJARATI; PORTER, 2003).

Regarding burrow density, all seasons had significantly lower values than the summer; grain size (in phi units) showed a positive relation with number of burrows, i.e., the thinner the grain the higher the burrow densities. On the other hand, slope did not show the same relation. The sorting coefficient also had a significant and positive influence on burrow density (Figure 3.5A), i.e., the greater the variability in sand grains the higher the density. For the number of individuals the results were very similar, except for winter, which was the only season that had a significantly lower number of captured individuals than summer, reinforcing that seasonal fluctuations were sharper for burrows than for individuals. Grain size and sorting coefficient behaved more similarly to number of individuals than to number of burrows, i.e., both had a positive relation with abundance (Figure 3.5B).

For burrow diameter analyses the significant variables, both with positive relations, were size of individuals (width, cm) and grain diameter (Figure 3.6). Since grain size is expressed in phi units, it corresponds to an increase in burrow size compared to size of individuals as grain coarseness increases. The interaction between both variables was not significant ($F= 1.10$, d.f.= 1, $p=0.29$). A final model describing the size of the individual, considering only significant factors, was significant and resulted in the following formula: *Individual width* = $0.24 + 0.71 \cdot \textit{burrow diameter} + 0.09 \cdot \textit{grain diameter}$ ($F= 363.4$, d.f.= 2, 509, $p < 0.01$, $r^2 = 0.59$ $r^2_{\text{adjusted}} = 0.59$).

Discussion

Ghost crabs are sandy beach organisms whose clear spatial distribution patterns ecological studies have failed to observe, as opposed to other species

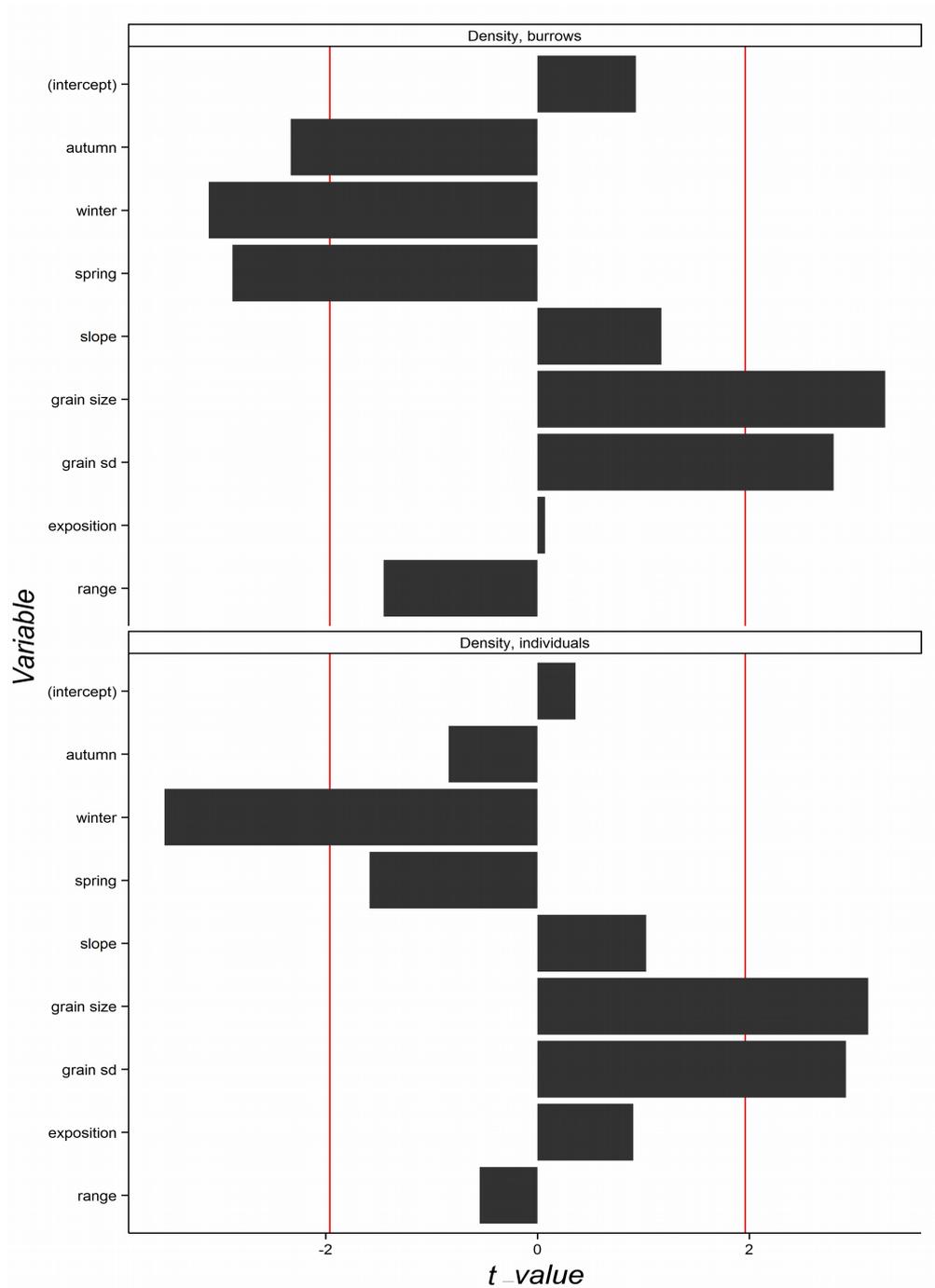


Figure 3.5. Values of the *Student t* test for each factor of the multiple linear regression compared to the baseline season, summer. Analyses were performed for burrow density and for number of individuals of *Ocypode quadrata* separately. Data was collected seasonally on 9 different beaches (covering a variety of morphodynamic classifications and energy levels). Red vertical lines represent the critical *t* value (± 1.96).

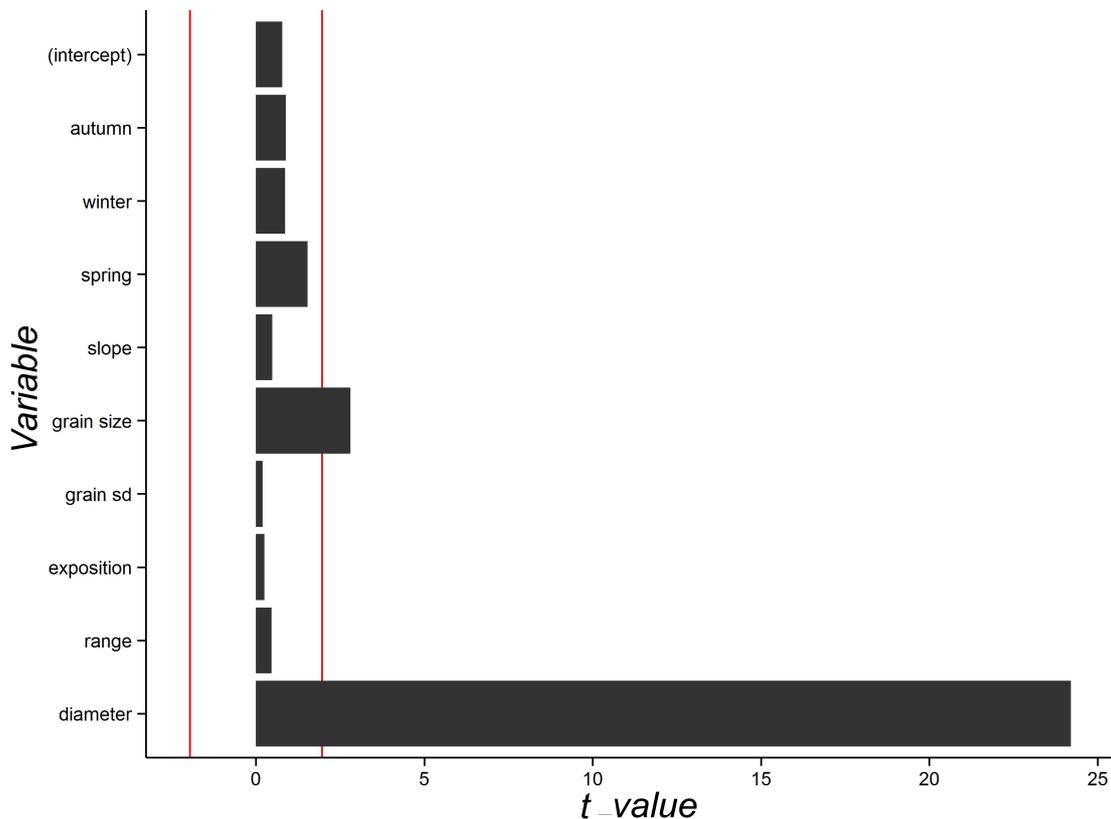


Figure 3.6. Values of the *Student t* test for each factor of the multiple linear regression compared to the baseline season, summer, performed for the width of *Ocypode quadrata* individuals. Data was collected seasonally on 9 different beaches (covering a variety of morphodynamic classifications and energy levels). Red vertical represent lines represent the critical *t* value (± 1.96).

(DEFEO; MCLACHLAN, 2011). Based on available studies, possible explanations involve anthropogenic influence on the environment, non-controlled source of variations or, mostly, the use of indirect measures without understanding how they relate to real population measures (BRANCO et al., 2010; HOBBS; LANDRY; PERRY, 2008; LUCREZI, 2015; QUIJÓN; JARAMILLO; CONTRERAS, 2001; ROSA; BORZONE, 2008; TURRA; GONÇALVES; DENADAI, 2005). In fact, our study showed that the latter is undoubtedly a major factor. Furthermore, all population parameters assessed (across-beach distribution, density, size, and sex ratio) showed either similarities or differences related to beach characteristics and study periods.

Across-beach distribution showed some similar temporal patterns regardless of beach type. In autumn, individuals tended to contract their

distribution and move backwards. Thus, smaller individuals, to whom mobility to higher areas is more costly, would be more jeopardized during these periods. Indeed, it was a period with high mortality of individuals (mainly younger individuals, according to size results) and also of a natural reset of burrows, mainly of those in the upper beach level. Other studies have argued that individuals migrate further into foredunes for protection and it is essential to include these areas in population analyses (ALBERTO; FONTOURA, 1999; BARROS, 2001).

In the winter, once again the number of burrows is recurrently higher than during autumn, while the number of individuals did not increase in the same proportion. This corroborates to some extent the previously stated idea that during lower abundances individuals may cover larger areas individually maintaining and using additional burrows. During warmer periods, the across-beach distribution of individuals was wider, with central gaps of occurrence, increasing towards wider and/or more reflective beaches. This partially agrees with the findings for *O. gaudichaudii* (QUIJÓN; JARAMILLO; CONTRERAS, 2001), a different species in a different area, whose indirect relationship between abundance and the slope of the dry zone was also found. However, unlike the present study, they found a direct relationship between dry zone length and abundance. Although burrows of *O. quadrata* may be over one meter deep (POMBO; TURRA, 2013), and so are likely to protect animals from desiccation, they probably prefer less arid areas, where shadow (upwards) and water (downwards) may also favor the abundance of food and humidity. Maybe even more important is the energy they would have to spend in maintaining their burrows in these wetter, windy areas. Burrow openings tend to vanish much faster in central areas than in areas with more compacted sediment, i.e., closer to water, and areas inside the vegetation, which are more hardly reached by the water.

Across-beach distribution patterns over time probably played a role on the great differences recorded for the ranges of occurrence over seasons. Range tends to be wider in periods of higher abundance and during

reproductive period peaks, which occurred in the warmer seasons (NEGREIROS-FRANSOZO; FRANSOZO; BERTINI, 2002) and also when males tended to be closer to the waterline. However, these differences in range did not have a significant influence on density values, for which a fixed range by area was used. Therefore, this approach is likely to eliminate this influence factor, enhancing the feasibility of comparisons between areas without compromising differences over time.

There was also a differential across-beach distribution of sexes in some periods, which behaved similarly across areas. During warmer periods males tended to be closer to the waterline, probably due to courtship display. Our results contradict the findings of Corrêa et al. (2014), who found males concentrated closer to the vegetation line than females, but the amount of females found by the authors was very low, and another important factor is that their methodology was very different from the present study, with active capturing of surfacing crabs.

Sex-ratio also changed over the seasons. Higher relative numbers of captured females coincided with periods of higher recruitment of young individuals, mostly winter, followed by autumn. Branco et al. (2010) also observed differences in the sex ratio of *O. quadrata* throughout a year. A possible explanation is that females have higher rates of mortality during warmer periods, which are also important reproductive periods (HALEY, 1972; NEGREIROS-FRANSOZO; FRANSOZO; BERTINI, 2002). And, if so, hatching and settlement rates would not be higher for males (although they might be for females), but sexual rates of mortality would be higher for females due to their exposure while incubating (jeopardized mobility) and spawning, when they have to get to the water and are exposed to a greater range of predators.

Autumn, and the associated cold fronts, defined some of the sharpest patterns observed in across-beach distributions, but also in general spatio-temporal aspects. The high wave energy observed across areas during autumn was very likely the main cause for higher occupation rates (number of burrows much closer to number of captured individuals), and it is very likely that this

period is when indirect measures better represent the effective population. Also during autumn, higher mean values of size were observed (together with higher occupation rates), followed by a great decrease in the winter, which, according to the histograms, is mainly due to the entrance of young individuals. Considering that this ghost crab species was reported to reproduce continuously (HALEY, 1972; NEGREIROS-FRANSOZO; FRANSOZO; BERTINI, 2002), the absence of younger individuals during some periods may be due to the fact that individuals that do not settle minimally before the main cold front periods are very unlikely to survive. Developmental timing in *O. quadrata* is poorly known (DIAZ; COSTLOW, 1972; HALEY, 1972), and it is important to thoroughly assess this issue to better understand how the reproduction peaks and recruitment are linked.

Histograms also indicate that larger individuals are more prone to be overestimated, since the highest proportion of empty burrows was among the largest individuals, which tend to inhabit upper beach levels (ALBERTO; FONTOURA, 1999; TURRA; GONÇALVES; DENADAI, 2005). Consequently, the current across-beach distribution results also showed lower occupation rates in the upper levels than closer to the waterline. More protected burrows probably are rarely reached by the water and last longer, because they are closer to or inside the vegetation, i.e., more protected from collapsing due to wind and substrate hardness. Although post-cold front periods had a lower number of small-sized animals, for the purposes of impact assessments this may be an advantage, since it would reveal the presence of individuals that were able to survive harsh events.

The presence of ghost crabs on a beach at a random moment does not necessarily mean that this is a viable population: it may also reflect a genetic “sink”, not “source” (DIAS, 1996). Hobbs et al. (2008) also registered high mortality rates during high-energy oceanographic events, stating that these fall events “*essentially remove all of the ghost crabs*”. The present study corroborates that autumn was the highest mortality period for the tropics.

Statistical tests reflected and summarized these patterns. The

relationship between burrow density and density of individuals or, in other words, the occupation rate, depended on the beach and on the time of the year. Because there was no interaction between the three variables (subject:beach:season), there was a spatial difference in occupation rate identified regardless of the time of the year, but there were also seasonal differences regardless of the study area. Regarding size, mean burrow size was generally larger than size of individuals, as expected, and spatio-temporal interaction indicated that there were differences in mean size over areas, but they were not constant over time.

Regarding the other environmental features, it is worth noting that Costa beach was previously characterized as dissipative and showed more consistent density values of *O. quadrata* than other dissipative beaches despite having the steepest slope. The other steeper beaches, which have a general reflective pattern, showed much lower density values, mainly of burrows. Grain size, on the other hand, corresponded better to general characterization, and that is probably why grain showed a significant influence on the abundance of *O. quadrata* and slope did not. Although slope and grain size are correlated variables, the slope measured here was restricted to the individual range of occurrence. This means that general beach profile was more likely to influence animal density than the slope of the dwelling area specifically. That is somewhat different from the findings for *O. gaudichaudii* (QUIJÓN; JARAMILLO; CONTRERAS, 2001), on the Pacific coast of South America, where they found no significant relationship between morphodynamics (using Dean's parameter) and burrow density, although there was a relationship to other factors that are linked to beach morphodynamics, such as retention and dry zone slopes and drift line heights. It is possible that some divergences among studies may be due to different density calculations or due to some levels of anthropogenic influence on study areas, an aspect controlled here by working with pristine beaches. Interestingly enough, the standard deviation in grain size also influenced both the abundance of burrows and animals. Substrate composition is very likely to influence the maintenance of burrows, but if grain size and

deviation influence the number of individuals they might also be parameters for settlement. This pattern opposes the abundance of other semi-terrestrial crustaceans, which tend to increase in abundance and fitness towards more reflective beaches (DEFEO; MCLACHLAN, 2011). Finally, the relationship between burrow size and size of individuals, which had already been estimated previously for the species (ALBERTO; FONTOURA, 1999; BRANCO et al., 2010; TURRA; GONÇALVES; DENADAI, 2005), was reinforced here considering different environmental characteristics. The result showed that it is important to consider beach granulometry to convert burrow size into size of individuals, especially if comparisons among areas are being considered.

In synthesis, the across-beach distribution showed temporal patterns that indicate that individuals used backshore areas for protection during harsh event prone periods, e.g. cold fronts, emphasizing the importance of vegetated areas as part of the habitat of *O. quadrata*. Mean abundance and burrow size were recurrently higher than those of individuals. Beaches and seasonality influenced burrow occupation rate independently, which means that indirect methods underestimated the number of individuals differently over areas and seasons. The highest burrow occupation rates and largest burrow sizes were observed in autumn, associated to cold front entries. Therefore, it would be advisable to extrapolate data from indirect measures in this period. Differences in density over areas, of both burrows and individuals, were related to beach grain features (diameter and sorting coefficient), tending to increase towards dissipative beaches, an opposite trend to the predicted for other supralitoral crustaceans. For mean size, no trend towards a beach type was identified. Higher proportions of empty burrows were surveyed for larger crab sizes, suggesting that larger population strata are more likely overestimated through indirect assessment methods. The size of individuals was better predicted by burrow size and mean grain size, whereas equal-sized burrows housed larger individuals on dissipative beaches than on reflective beaches, meaning that different relationships to estimate individual size from burrows should be used in beaches with distinct granulometry.

Chapter 4

Variation in the life-history traits of the ghost crab *Ocypode quadrata* in morphodynamically distinct sandy beaches

Variation in the life-history traits of the ghost crab *Ocypode quadrata* from morphodynamically distinct sandy beaches

Abstract

The patterns of distribution of sandy beach macrofauna have been widely investigated across the globe, showing that dissipative beaches tend to concentrate higher values of richness and biomass at the intertidal zone than reflective beaches. Dissipative beaches intertidal populations tend also to show better fitness and higher size of its species, while for supralittoral crustaceans this trend has been shown to behave oppositely. For ghost-crabs, an often conspicuous group within these environments, it has been seldom investigated the way population features respond to different beach morphologies. Here, variation in crab size and growth was evaluated using indirect measures from three morphodynamically distinct and low-impact beaches over a one year period: Costa (dissipative beach), Felix (intermediate) and Puruba (reflective), all in Southeastern Brazil. At each site, five 2m wide transects were randomly selected each month and laid perpendicularly to the water line. The burrows were assessed along their entire area of occurrence. The fit of the von Bertalanffy body growth model was done and compared among sites. A growth performance index was used to compare the growth performance among sites. For all three beaches a main mode due the entrance of juveniles (mm) was identified in August and September, reaching summer about the same size of the main mode (mm) recorded during the previous summer. Specifically, the growth parameters estimated were: Costa $L_{\infty} = 46.36\text{mm}$, $K = 0.90\text{year}^{-1}$ and $t_0 = -0.19$; Félix $L_{\infty} = 57.95\text{mm}$, $K = 0.87\text{year}^{-1}$ and $t_0 = -0.12$; Puruba $L_{\infty} = 53.01\text{mm}$, $K = 1.11\text{year}^{-1}$ and $t_0 = -0.07$. The body growth adjustments were similar between Puruba and Felix, both being distinct from Costa. Growth performance indexes ranged from 3.29 (Costa) to 3.49 (Puruba), while to Félix beach it was 3.46, indicating homogeneity of this parameter among distinct beaches, but mainly between the intermediate and reflective ones.

Key-words: sandy beach, growth, performance, longevity, spatial variability

Introduction

Beach ecology is in increasing development during the last decades, and some theories concerning the influence of environmental factors on community and population dynamics are recurrently supported across the globe (MCLACHLAN et al., 1993). Intertidal species richness, diversity and biomass have been shown to increase under more dissipative conditions, corresponding to a paradigm of sandy beach ecology known as the habitat harshness hypothesis (DEFEO; MARTÍNEZ, 2003). The steeper the slope, the coarser the grains, and therefore the smaller the soil water content (SHORT, 1996); these basic features, particularly for exposed sandy beaches allows greater accumulation of organic matter, favouring higher abundance and individuals size in dissipative sandy beaches (CELENTANO; DEFEO, 2006; DEFEO; DELGADO, 2007). On the other hand, for crustaceans species, especially for supralittoral species, an opposite trend has been demonstrated (DEFEO; GÓMEZ, 2005; DEFEO; MARTÍNEZ, 2003). For these species, steeper slopes, narrower swash zones and even the higher soil permeability are considered more favourable conditions.

Investigations of ghost crab populations, involving members of the genus *Ocypode*, which occupy the upper intertidal and backshore zones of sandy beaches, have important peculiarities when compared to other macrofauna species. Taking representative, random samples of the population directly can be very difficult since they construct burrows that may reach depths of over a meter. Capturing them outside of their burrows cannot be considered a precise random sample of the population, and thus the most commonly used approach by which to extract them from their burrows has been digging (POMBO; TURRA, 2013). Hence, most studies rely on burrow features, which are assumed to correspond in size and number to population features (ALBERTO; FONTOURA, 1999; BARROS, 2001; FISHER; TEVESZ, 1979; VALERO-PACHECO et al., 2007). The relationship between the size of the burrows and their occupant crabs have been supported, but abundance relationships are not similarly well defined, and comparisons of abundance of

burrows over distinct areas might be particularly tricky (ALBERTO; FONTOURA, 1999; POMBO; TURRA, 2013; TURRA; GONÇALVES; DENADAI, 2005). Thus, *Ocypode* assessments using burrows make field studies more feasible, but with possible constraints that must be taken into account.

It is well documented that these populations are distributed over the entire upper extension of both oceanic and estuarine sandy beaches, from the upper intertidal zone to dunes and vegetation, across which a gradient of size of the individuals is observed, with increasing sizes towards upper beach levels (ALBERTO; FONTOURA, 1999; BRANCO et al., 2010; ROSA; BORZONE, 2008; TURRA; GONÇALVES; DENADAI, 2005). Reproductive activity has been shown to exhibit peaks, during warmer periods, but recruitment was observed throughout the year in Southeastern Brazil (HALEY, 1972; NEGREIROS-FRANSOZO; FRANSOZO; BERTINI, 2002). Still, there are no reports on body growth parameters for this species, important descriptors in population ecology, specifically for those of management concern (SPARRE; VENEMA, 1998). Modal progression analysis is a powerful tool for this purpose once it assumes the development of a cohort, and has already been applied to other crustacean groups (DEFEO; MARTÍNEZ, 2003; GOMEZ; DEFEO, 1999; TURRA; LEITE, 2000).

Therefore, we aimed to contribute to the knowledge on *O. quadrata* population descriptors by assessing individual growth parameters through the von Bertalanffy model in three distinct, low-use beaches in an effort to exclude the influence of anthropogenic impacts. The study was based on the identification of modal progressions; information based on the abundance of crabs was disregarded because the rate of occupation of burrows is likely to differ over areas and over beach strata (POMBO;TURRA, *in prep.*). Environmental perturbations may alter population structure and even certain life-history parameters (BENTON; GRANT, 1999). For ghost crabs, beach type, as well as human disturbance may change the occupation rate of the burrows constructed by individuals. Therefore it was considered of primary importance to exclude these factors in order to understand how population parameters behave

naturally among beaches with distinct morphodynamics. In fact, the existence of a trend on population features according to beach dynamics is not supported for ghost crabs, as it is for many other sandy beach species (DEFEO; MCLACHLAN, 2011). Whether differences in the fitness of individuals are apparent for morphodynamically distinct areas is an important issue, given that these species have been proposed as an indicator of rapid assessment of environmental quality (BARROS, 2001; BLANKENSTEYN, 2006; LUCREZI; SCHLACHER; WALKER, 2009). For this reason, the beaches chosen for the present study were close spatially but different morphodynamically, in an attempt to add information on the influence of some physical factors to the ecological parameters of this potential indicator species.

Methodology

Study area and sampling

Three beaches were selected on the northern coast of São Paulo State, southeastern Brazil, in an attempt to represent the three basic types of morphodynamics: dissipative (Costa beach), intermediate (Felix beach) and reflexive (Puruba beach), based on the previous characterizations of Rocha (2008). These beaches were selected to avoid additional sources of variation, such as beach use.

From December 2011 to November 2012, each of these areas was sampled monthly, during daylight hours, to count and measure *O. quadrata* burrows. For this purpose, a homogeneous area 200 m long was defined at each beach and, during every sampling procedure, five 2-m-wide transects were randomly laid out perpendicular to the shoreline.

In every 2 m of the transect (in 4 m² quadrats), burrows were assessed from the low intertidal zone to the vegetated area, until the uppermost burrow was recorded. For a general environmental characterization of each area, seasonal samples of the sediment were taken for granulometric assessments. These samples were taken from the intertidal zone in three out the five transects.

Following the methodology proposed by Pombo and Turra (2013), all burrows deeper than 10cm were considered (identified using a steel semi-flexible cable), regardless of direct signs of occupation. Closely separated burrows were checked for connectivity to ensure that these were not concurrent openings to a single burrow and, if so, the shorter was discarded. Size measurements considered the smaller diameter of the bottom of the funnel-shaped portion of the burrow openings.

All measurement activities and collections complied with the permit from the appropriate federal environmental agency (*Ministério do Meio Ambiente (MMA) – Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio)* No. 31629-1; acronyms for, in English: Ministry of the Environment – Chico Mendes Biodiversity Conservation Institute).

Data analysis

A general assessment of spatio-temporal size distribution was performed. The mean general size (independent from transect) was compared among areas and months by means of a factorial ANOVA, followed by the Tukey post-hoc test, once the required assumptions were properly met.

Transect data were gathered for each month and beach and then apportioned in 5 mm size classes. For each of these sets of samples, frequencies distributions were decomposed, with the aim of NORMSEP package, by identifying the main modes. Mean values, standard deviations and number of individuals were computed, not allowing superposition indexes under 2.00, over curves within a month.

A settlement age for lowest size class was estimated based on Diaz and Costlow (1972) and Haley (1972) and used to build the age-size key. The values from this key were then applied to the von Bertalanffy body growth model, to estimate the asymptotic length (L_{∞}), growth constant (K) and age of length 0 (t_0). For each study area the convergence of the parameters to the von Bertalanffy body growth model was performed using the R software, essentially the *nls* function of the stats packages, used to determine weighted least

squares estimates of the parameters of non-linear models (R CORE TEAM, 2014).

An analysis of the residual sum of squares (ARSS) was performed to assess if the three curves were coincident. In cases where this hypothesis was rejected, likelihood ratio pair wise tests were performed to assess what details differed (CHEN; JACKSON; HARVEY, 1992; KIMURA, 1980), using the *vblrt* function available at the *fishmethods* package from R (NELSON, 2014). Growth performances over areas were compared based on the values of L_{∞} and K adjusted (phi-prime performance index), calculated as $\log K + 2\log L_{\infty}$; because K and L_{∞} tend to be inversely proportional. This index compound both parameters to indicate how close the growth fitness is over populations (MUNRO; PAULY, 1983).

Results

At Costa beach, from 904 identified, measured burrows, 172 (19.02%) were excluded because of insufficient depth or double openings. For Félix beach, from 836 burrows, 151 were excluded (18.06%); for Puruba beach, from 606 burrows 130 were not used (21.45%). In total, 1983 valid burrows were measured throughout the study period. The beach granulometric analyses were in agreement with previous characterisations, in which Costa is considered a dissipative beach (1.75 ± 0.57 phi), Félix an intermediate (1.37 ± 0.31 phi) and Puruba a reflective beach (1.11 ± 0.21 phi).

General patterns of size distribution were similar among beaches referring to be unimodal, with mode values around 20 mm and slightly skewed to larger sizes (Figure 4.1). It is possible, however, to observe a slight deviation from the main mode towards a reflective trend, a pattern emphasized by the ANOVA of size among beaches ($F=9.35$, d.f.=2, $p<0.01$). Specifically, the mean value of burrow diameter observed for Costa beach ($21.01\text{mm} \pm 7.75$ SD) was significantly lower than both other beaches, Félix ($22.40\text{mm} \pm 8.02$ SD) and Puruba ($22.84\text{mm} \pm 7.78$ SD), which did not differ significantly between each other. There was, however, a significant interaction between the factors beach

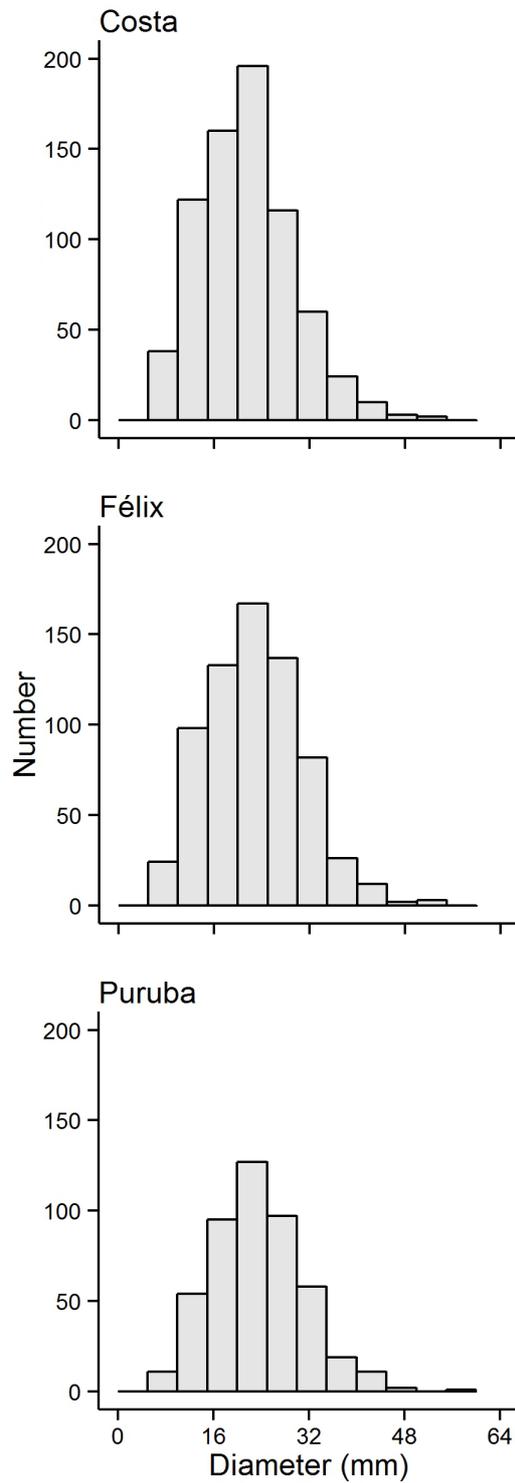


Figure 4.1. Diameter (mm) classes distribution for *Ocypode quadrata* burrows, sampled in three beaches with different morphodynamics (Costa-dissipative, Félix-intermediate and Puruba-reflexive) from December/2011 to November/2012.

and month ($F=3.82$, $d.f.=22$, $p<0.01$), with means varying from 17.42 to 26.5 mm. The lowest means were observed in February, June and November for Costa beach and September for both Félix and Puruba, while the highest mean values comprised February for both Félix and Puruba and April only for Puruba. The results of the Tukey test for temporal differences were provided for each beach separately, to facilitate the identification of common patterns over areas (Figure 4.2). With the aim of the histograms it is identifiable that the months with the records of the smallest size classes, which were common among beaches, were August and September (Figure 4.3). For Puruba, this condition lasted until October.

Values from age-length key obtained from modal progression analysis converged to the von Bertalanffy body growth model, computing output values with biological meaning for the three parameters (L_{∞} , K , t_0). For the dissipative beach Costa, the estimated value of L_{∞} was the lowest out of the three areas, along with an intermediate value of K (46.36 mm and 0.90 yr^{-1} respectively; Table 4.1). Félix, the intermediate beach, had the largest L_{∞} and the lowest K (57.95 mm and 0.87 yr^{-1}). Finally, Puruba, the reflective beach, showed an intermediate L_{∞} and the highest value of K (53.01 mm and 1.11 yr^{-1}). The parameters estimated, for each beach, are summarized in table 4.1 and the respective growth curves were included in the monthly histograms (Figure 4.3) for the main modal progressions identified. Residuals were tested for normal

Table 4.1. Values of the parameters from von Bertalanffy individual growth model adjusted from *Ocypode quadrata* burrows sampled from December 2011 through November 2012, in three different beaches in Southeastern Brazil. * L_{∞} =asymptotic length; K = growth parameter; t_0 =theoretical point in time when the individual has zero length; Φ = growth performance index (phi-prime). Each parameter is followed by respective estimative, for each studied area, and its standard error when available (SE). r^2 values are presented for each fitted model.

	Costa			Félix			Puruba		
	Estimate	SE	r^2	Estimate	SE	r^2	Estimate	SE	r^2
L_{∞} (mm)	46.36	16.12	0.99	57.95	69.76	0.96	53.01	26.90	0.98
K (yr^{-1})	0.90	0.54		0.87	1.78		1.11	0.98	
t_0 (year)	-0.19	0.08		-0.12	0.26		-0.07	0.9	
Φ	3.29	-	-	3.46	-	-	3.49	-	-

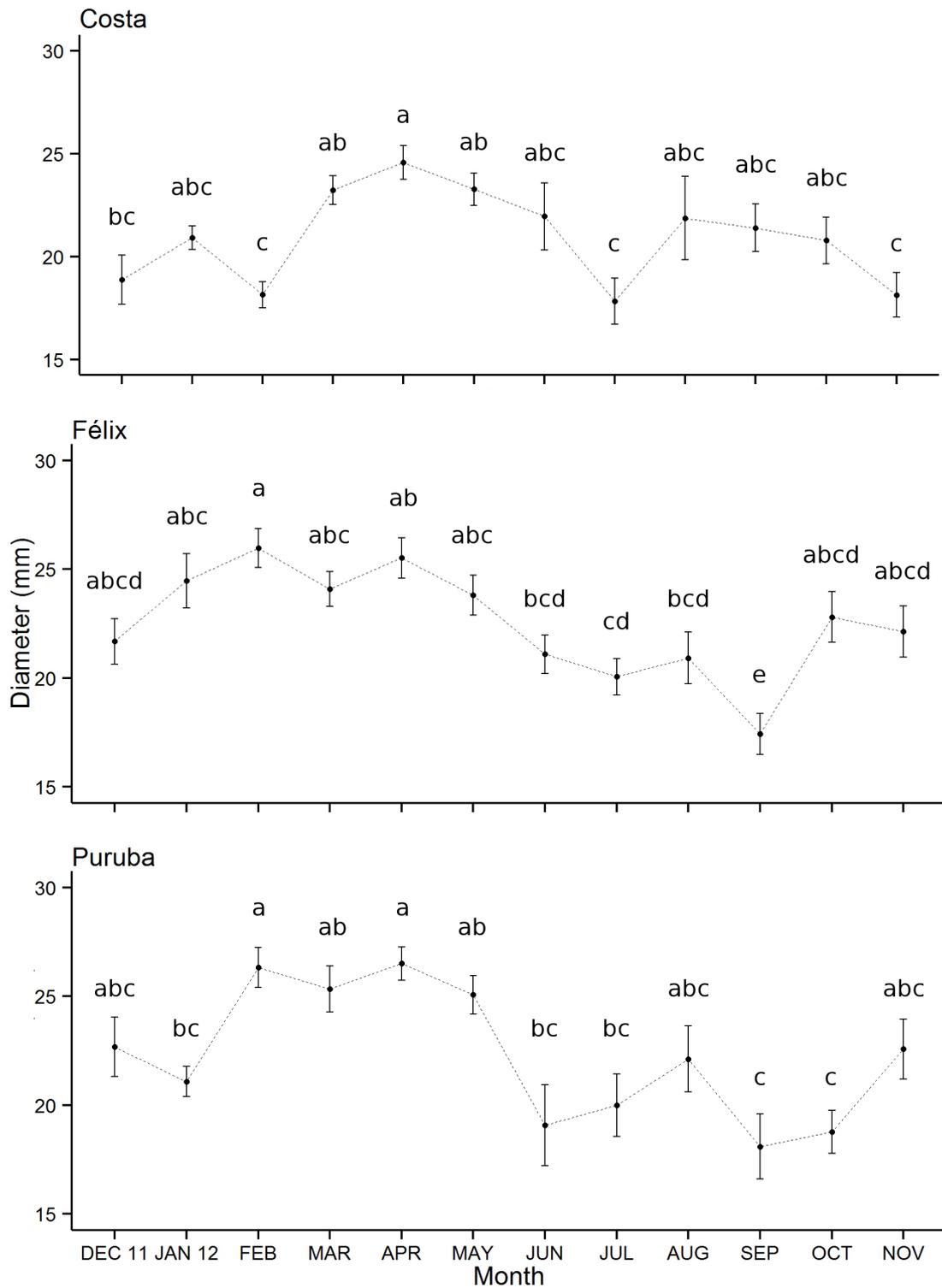


Figure 4.2. Temporal variation of the diameter (mm; mean \pm standard error, $n=5$, $\alpha=0.05$) for *Ocypode quadrata* burrows, sampled in three beaches with different morphodynamics (Costa-dissipative, Félix-intermediate and Puruba-reflexive) from December/2011 to November/2012. Different letters denote significant differences among months by beach, discriminated by Tukey test.

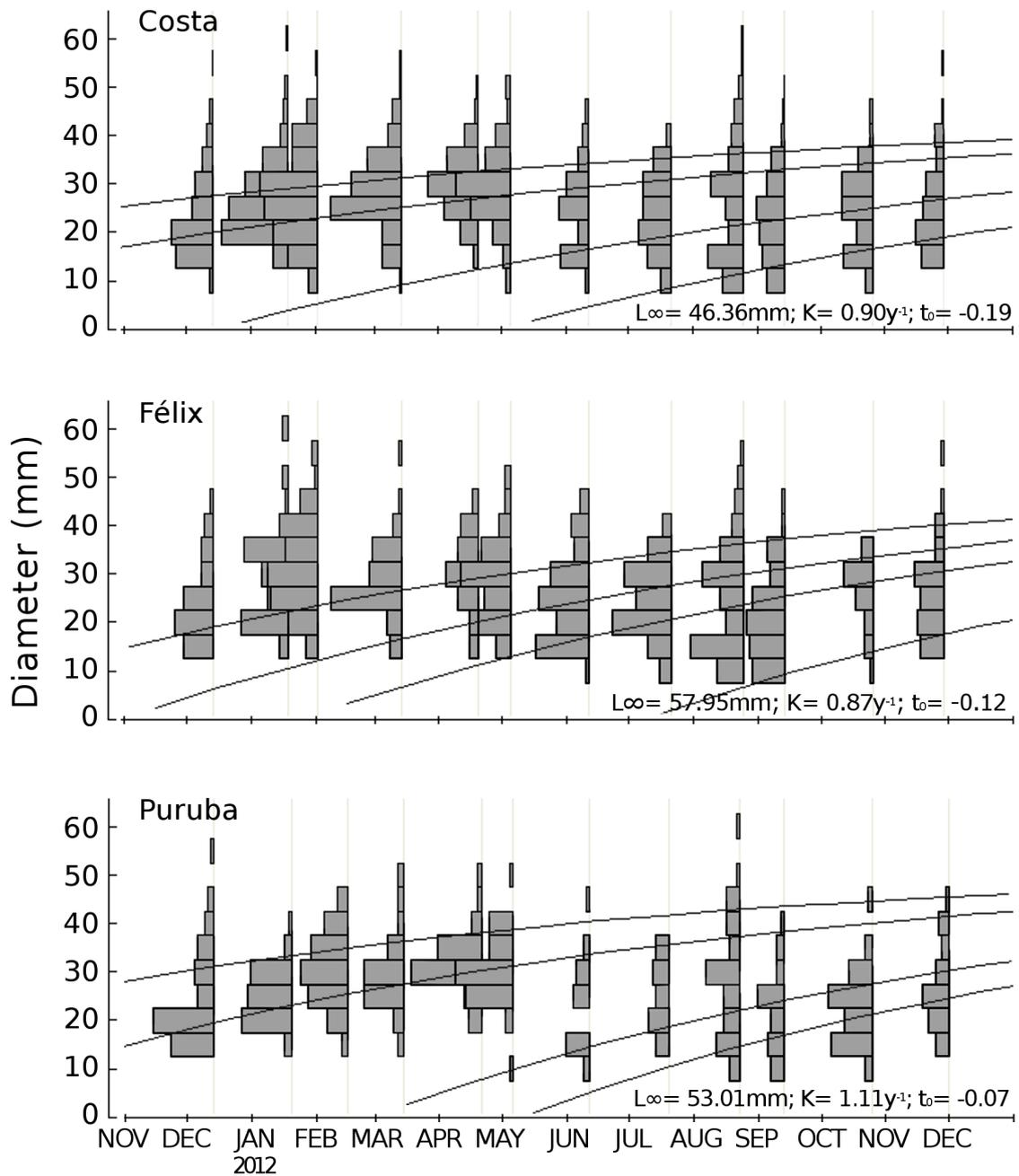


Figure 4.3. Monthly histograms with curves representing the estimated parameters of the von Bertalanffy growth model, within the main identified modal progressions, for *Ocypode quadrata* burrows, sampled in three beaches with different morphodynamics (Costa-dissipative, Félix-intermediate and Puruba-reflexive) from December/2011 to November/2012.

distribution and the results supported the analysis (Costa: $W=0.843$, $p=0.034$; Félix: $W=0.868$, $p=0.177$; Puruba: $W=0.956$, $p=0.792$).

The ARSS test results point to significant differences among von Bertalanffy growth curves ($F=13.02$; d.f.=6,15; $p<0.01$), and therefore a common adjustment gathering the three beaches data was not considered suitable. According to the likelihood ratio tests, Costa beach curve was the only one that differed from each of the other beaches, considering overall parameters (Table 4.2). The phi-prime performance index calculated was lower for Costa beach (3.29), and very similar between Félix and Puruba beach (3.46 and 3.49 respectively).

Table 4.2. Matrix of results of the likelihood ratio tests comparing estimates of the von Bertalanffy body growth parameters of *Ocypode quadrata* between beaches (Costa, Félix and Puruba). Columns show parameters confronted and lines the tests results.

Beaches		Costa				Félix			
		L_{∞}	K	t_0	<i>all</i>	L_{∞}	K	t_0	<i>all</i>
Félix	<i>chi</i> ²	0.08	0.00	0.15	19.00	-			
	<i>d.f.</i>	1	1	1	3				
	<i>p</i>	0.77	1.00	0.70	<0.01				
Puruba	<i>chi</i> ²	0.08	0.08	1.72	34.38	0.01	0.03	0.06	2.18
	<i>d.f.</i>	1	1	1	3	1	1	1	3
	<i>p</i>	0.77	0.77	0.19	<0.01	0.92	0.86	0.80	0.54

Discussion

The patterns of size distribution of *O. quadrata* had both noticeable similarities and differences over the study areas. A positive skew in the general size structure (histograms) reflected the presence of over-sized burrows at the three beaches. Because they correspond to individuals larger than those described in previous literature using direct measures (ALBERTO; FONTOURA, 1999; HALEY, 1969; NEGREIROS-FRANSOZO; FRANSOZO; BERTINI, 2002), the size of these burrows may have been distorted over time, after abandoned. Examining the monthly histograms, it is interesting to note that all areas had

higher numbers of these larger burrows, simultaneously, during August 2012. This coincides with the period shortly after the main cold fronts in the region, and the previous months tended to have lower amounts of these burrows, mainly at Costa at Puruba beaches. During periods of intense cold fronts the individuals retreat to back dunes and vegetation, followed by an increase in the frequency of abandoned burrows, which remain there after a while (POMBO; TURRA, *in prep.*). All this corroborates the hypothesis that the largest burrows are abandoned and remain a certain period of time before collapsing completely, justifying the acceptance of L_{∞} values under the largest sampled burrows.

This common feature in August was reflected in mean size values in relation to the adjacent months, for all areas. According to both mean values and histograms it is possible to observe that from June to September the greatest amount of entrance of young individuals was recorded for all areas. This fact indicates that the major entrance of individuals occurred soon after the main cold front period. Also, according to the growth parameters parametrized, represented in the histograms growth curves, it is noticed that this main entrance would correspond in December 2012 to a size very similar than that recorded as the main mode in December 2011. Further, a noticeable modal progression could be identified from the December 2011 main mode, which could be followed until April/May. Therefore, although the species has been shown to reproduce during most of the year (HALEY, 1972; NEGREIROS-FRANSOZO; FRANSOZO; BERTINI, 2002), our data support the idea that there is a single main cohort within a year, derived from the entrance of recruits after cold fronts. The main peak in reproductive activity has been recorded during summer for the area (corresponding to December-February), but also many ovigerous females were found through Spring and Fall, not only during Winter (NEGREIROS-FRANSOZO; FRANSOZO; BERTINI, 2002). Haley (1972) estimated 45 days from ovulation to hatching, and Diaz and Costlow (1972) recorded, under experimental conditions at 25°C, that minimally 34 days were required for the development of individuals to megalopa stage, summing

estimated 79 days until the megalopa stage. Experiments with a similar species, *Cyclograpsus cinereus*, demonstrate that development is slower at lower temperatures, while the survival rate is considerably increased (COSTLOW; FAGETTI, 1967). The information for the time expended as megalopa is not available because, according to the previous authors, the survival of individuals at this stage under artificial conditions is extremely low. Nevertheless, the experiments of Costlow and Fagetti (1967) corroborate the view that during winter the survival rates of the larvae would be higher, as well as the estimated period (79 days) of larval development would be actually longer. In this way, the greater entrance in August-September would better correspond to the corresponding reproductive period in autumn. Over 6 months of larval development, from fecundity to settlement, would be needed to correspond to the peak of reproductive period in summer, what seems overestimated. Indeed, the aforementioned study identified one of two pulses of ovigerous females from February to May. It is also important to take into account that, even if in higher amounts, the young settling right after, or during, intense cold front periods would probably have few chances to survive.

The predominance of low size modes was important to define the body-growth parameter estimation. Frequent records of higher size classes, obtained from indirect measures, should be considered with caution with respect to this species. Firstly, because the number of larger burrows, in relation to the real population, tend to be overestimated. Secondly, because size deviations for the burrows in relation to the individuals are disproportionally higher for larger burrows (POMBO; TURRA, *in prep.*). Therefore, following the shorter size classes cohorts, whose burrows are reseted more frequently, may be actually the best approach to estimate population parameters from indirect measures.

For the species as a whole, the present results suggest that the growth constant was around 1 year^{-1} for *O. quadrata* populations in the region, with growth performance indexes being around 3.30-3.50. However, the study areas were classified as morphodynamically distinct (ROCHA, 2008) and such differences have been shown to affect population abundance and the mean size

of individuals (DEFEO; DELGADO, 2007; DEFEO; GOMEZ; LERCARI, 2001; MCLACHLAN et al., 1993). For supralittoral species, this effect has been shown to be positive towards more reflective beaches (DEFEO; GÓMEZ, 2005; DEFEO; MARTÍNEZ, 2003). For *Ocypode* this relationship is not yet well established (DEFEO; MCLACHLAN, 2011), but from the present results, with respect to growth parameters and potential maximum sizes, individuals from reflective beaches seems to have advantages. The differences estimated, namely lower values for both growth parameter and asymptotic length for Costa beach, were not due to a lack of larger size classes in any of the areas. Rather, the modal progressions identified did not reach higher classes, particularly for Costa beach, to which the lowest asymptotic length was estimated. Therefore, if this population does not reach larger sizes like in the other areas, but has similar growth performances, there may be differential mortality among beaches, with lower longevity for this population. If so, it would be important to understand the reason. There is also the possibility that the asymptotic length for the other areas is overestimated. It has been observed that the size of burrows are larger with respect to individuals in coarser grained areas (POMBO; TURRA, *in prep.*), but because this was an additive model, with no interactions, this would not alter the slope of the curve through modes. In this way, further efforts to understand such population dynamics are needed, given the importance of this crustacean group for beach management and conservation across the globe. This study provides the first overview of the species population dynamics of ghost crabs for sub-tropical coastlines, and suggests that the development of individuals may mirror that seen for other supralittoral crustaceans and in this way provide further evidence for the habitat safety hypothesis (DEFEO; GÓMEZ, 2005; DEFEO; MARTÍNEZ, 2003).

Chapter 5

Effects of habitat connectivity and beach erosion on population structure of the Atlantic ghost crab *Ocypode quadrata*: implications for beach conservation

Effects of habitat connectivity and beach erosion on population structure of the Atlantic ghost crab *Ocypode quadrata*: implications for beach conservation

Abstract

The individuals of the genus *Ocypode* have a great bioindicator potential, with plenty to explore in order to expand its use for conservation purposes. Since their habitat is one of the most threatened by the rising sea level, one of the most prominent effects of the climate change, which along with coastal urbanization, is among the main reasons of the intensification of erosion processes of that habitat. Thus, it is important to understand how these populations behave in relation to space loss and connectivity breakage, i.e., interruption of the connection between backshore and the coastal dune/vegetation. This study evaluated the effect of space loss and connectivity breakage on abundance and size structure of populations of *O. quadrata* in, 24 beaches with different levels of erosion, with or without coastline armoring and encompassing a wide granulometric range. To minimize eventual differences of the burrow occupation rate over the study areas, a new methodological approach was proposed and used. It consisted in inducing the resetting of all burrows from predetermined transects and take measurements about 12 to 24 hours after that. This and traditional sampling generated significant differences in both abundance and size estimates. From this new methodological approach, models were constructed attempting to estimate density and mean size of *O. quadrata* populations, according to morphodynamically distinct beaches to different levels of connectivity breakage and erosion (from 1, low, to 3, high). Models significantly predicted density and mean size of these populations, but it is important that these two variables are used together to predict the real impacts individuals are submitted to. For dissipative beaches, intermediate levels of erosion were associated to increase of mean values of density and size of crabs, attributed to loss of space and a consequent higher competition, which is likely to favor larger individuals. High levels of erosion reduced the

values of density close to those of ideal conditions, but mean size showed a dramatic decrease. If natural backshore interruption was present, the density patterns were kept similar through erosion levels, and mean size decreased severely in relation to beaches without connectivity breakage and natural vegetation present. Ghost crabs population from beaches of coarser sand grains have shown to be more resilient than those from dissipative beaches, the responses in these areas tended to be later in relation to progressive levels of impact, when compared to more dissipative areas. Notwithstanding, connectivity breakage affected size structure in all study areas and, especially in areas subjected to high erosion, this effect might cause a functionally extinct population. Due to the climate change, these areas are subject to increasing erosion process. Thus, keeping the connectivity between water, dunes and vegetation should be considered essential to maintain viable populations of ghost crabs.

Key-words: burrows reset, density, size, morphodynamic, erosion, natural vegetation

Introduction

The use of biological or ecological indicators has been intensively developed during the last decades, and researches reached a quantitative sophistication that allowed the implementation of these descriptive approaches to normative levels, i.e., effective environmental policies (HEINK; KOWARIK, 2010). For sandy beaches this approach hasn't been so successful although much needed, given the increasing pressures these environments have been submitted to. Partially, it stems from the long time sandy beach macrofauna (mainly its integrated ecology) has been neglected, but the amount of studies of the last decades has largely compensated it (MCLACHLAN; BROWN, 2010). Another considerable reason is that these environments lack higher plants, which are the most intensively used in biological quantification of environmental stress in terrestrial habitats (FRÄNZLE, 2006).

There are several aspects indicate that ghost crabs population parameters (genus *Ocyopode*) can be used as indicators for assessment and monitoring purposes. This is the most conspicuous animal from the macrofauna of sandy beaches around the globe. Of the whole community, they are the easiest to identify visually, whether the animal itself or the burrows they construct as shelter. Further, the possibility of using the burrows for the assessment of ghost-crab populations reduces considerably the expenses and efforts required, allowing wider sampling areas and replicates. Moreover, the animals are spared from being collected and fixed for identification and measurement purposes, inevitable for most other macrofauna populations (MCLACHLAN; BROWN, 2010).

The use of the structure (burrow) instead of the individuals, which is called indirect assessment, has many advantages, but must be used with caution. As many authors have discussed, natural and man-induced features of sandy beaches may influence the relationship between the number of burrows and animals in different ways (BARROS, 2001; TURRA; GONÇALVES; DENADAI, 2005). Presence of fresh tracks surrounding the burrows or good correlations found for other Ocypodidae have been used as arguments to

justify the method feasibility (BARROS, 2001; LUCREZI; SCHLACHER; WALKER, 2009; SCHLACHER; THOMPSON; PRICE, 2007; WOLCOTT, 1978), but a few studies have dealt with the relationship of burrows and individuals, and some of these have signalized towards differences inter areas and within beach strata (POMBO; TURRA, 2013; SILVA; CALADO, 2013; POMBO; TURRA, *in prep.*). But the technique merit has been long overcoming potentially negative points, and considering its results with caution have increased considerably our knowledge about the genus (ALBERTO; FONTOURA, 1999; HOBBS; LANDRY; PERRY, 2008; QUIJÓN; JARAMILLO; CONTRERAS, 2001; ROSA; BORZONE, 2008).

Another aspect that highlights the species potential to conservation purposes, is that the group has a unique ecological niche in their environment, due to a particularly great body size and feeding behavior, both scavenger and predatory (ROBERTSON; PFEIFFER, 1982; WOLCOTT, 1978). Although birds may predate them, as well as lizards and other occasional visitors of sandy beaches, they represent the typical top-down controllers of these environments, also notably capable of diminishing the amount of organic matter discharged in these areas (BRANCO et al., 2010). The whole West Atlantic coast has also the particularity of having a single species of ghost-crab, *Ocypode quadrata*. This avoids interspecific overlapping in ecological studies, and mainly classification problems when working with burrows (SCHLACHER; DE JAGER; NIELSEN, 2011), i.e., the indirect method of population assessment.

Therefore, these individuals fulfill many preconditions to be a candidate of a biological indicator. The environmental pressures that the group would suitable indicate (tourism, trampling, traffic, erosion) is still at issue. The impacts of off-road vehicles has been more largely studied (HOBBS; LANDRY; PERRY, 2008; LUCREZI; SCHLACHER, 2010; SCHLACHER; THOMPSON; PRICE, 2007; WOLCOTT; WOLCOTT, 1984). Trampling and urbanization have been shown to decrease abundance of ghost crabs burrows (LUCREZI; SCHLACHER; WALKER, 2009; SCHLACHER; DE JAGER; NIELSEN, 2011), and tourism has still ambiguous reflects on these populations (STEINER;

LEATHERMAN, 1981; TURRA; GONÇALVES; DENADAI, 2005). In lesser extent, nourishment and bulldozing have also been assessed concerning impacts to ghost crabs (PETERSON; HICKERSON; JOHNSON, 2000).

The parameters used to indicate environmental stress in the case of *O. quadrata* are derived from their population structure, with abundance being the mostly widely used sign of stress (BLANKENSTEYN, 2006; NEVES; BEMVENUTI, 2006; STEINER; LEATHERMAN, 1981). Responses on an ecological level may take months to years, what means it is likely to reveal more complex changes, of greater magnitude (FRÄNZLE, 2006). Thus, ghost crabs are convenient bioindicators for areas that are suffering of chronic stress, such as sandy beaches. Besides the intense exploitation of these areas for anthropogenic uses, given the variety of services it offers, sandy beaches habitats are among the most threatened by the rising sea level, one of the most prominent effects of the climate change, adding even greater importance to the knowledge of this bioindicator responses, which could guide environmental policy and decision making (JONES et al., 2007; TURRA et al., 2013).

This study had two general objectives. First, to propose a new approach to assess ghost crabs population features through indirect measures, aiming to reduce potential biases of this method in relation to the effective population measures. Second, from this proposal, to assess general aspects, namely abundance (density) and size, of populations associated to different levels of connectivity breakage and erosion. As connectivity breakage we mean increase in difficulty, or impossibility, to ghost crabs to move from beaches' down- to upwards, including dunes and vegetation, a frequent consequence of armoring. Therefore, for this purpose we enclosed both, erosion level and beach backwards alteration as factors. Connectivity breakage is an important impact for ghost crab species since they usually use the beach-dune environment (FISHER; TEVESZ, 1979; TURRA; GONÇALVES; DENADAI, 2005). Furthermore, to extend these assessments, contradistinguishing and broadening the understanding of effects under variable natural conditions, both objectives were carried out on beaches with different morphodynamics.

Methodology

Pilot study

The first objective of the study was to evaluate how population structure, assessed using a new indirect sampling method, differs from the traditional assessment of directly counting the burrows. The proposed method comprises the re-sampling a given area a period after carefully covering all burrows with sand. This approach aims to minimize possible biases in areas where resetting of empty burrows by natural processes (wind and tides) takes longer time, avoiding discrepancies between number of burrows and number of animals (POMBO; TURRA, 2013).

To establish the period taken for crabs to re-open the burrows, a pilot study was done at São Francisco Beach, in São Sebastião, Southeastern Brazil. This beach has short across-shore extension, has a backward boardwalk and is moderately used for tourism and fisheries. Therefore, the burrows of this area tend to be recurrently resetted, what is an ideal condition to identify the time it lasts for all the occupied burrows to re-open.

Three transects 10m long, covering the whole beach width (from water line to the break-wall), were randomly allocated at the beach. All burrows were measured, flagged and covered. Covering of burrows was superficial to avoid possible harm to animals, but enough to avoid natural collapse. Each burrow of each transect was accompanied after 3, 6, 12, 24 hours, awaiting the stabilization in the number of burrows re-opened. It was assumed that the stabilization time reasonably represents the period of time taken to all animals restructured the burrows in use. Because all burrows were flagged and accompanied, it was possible to assure that the approach did not damage the crabs or prevent them to reopen their burrows to the surface.

The stabilization time registered was from 12- to 24-hours, what means that by 12-hours after covering the burrows, all ghost crabs had reopened them and the amount remained the same for the next 12 hours, i.e., 24-hours after covering the burrows. Therefore, this interval of 12-24 h was used before the re-

sampling of all further areas.

Sampling and analyzes

Twenty-four beaches, along the Brazilian southeastern coast, were chosen based on the morphodynamic classification of Rocha (2005), the risk of erosion identified by Souza (2009), and visual assessments. These intended to cover areas of three distinct erosion levels: low, intermediate and high; each of these levels encompassed beaches both with and without coastal armoring, i.e., beaches with connectivity breakage between backshore and dunes/vegetation. In addition, each combination of the previous cases encompassed beaches tending to morphodynamic extremes, dissipative or reflective, with replicates whenever possible.

Estimates of crab population structure were based on the traditional and the new (reset) method using three transects 10m wide perpendicular to water line, covering the whole beach width, randomly allocated within a homogeneous beach sector of at least 100m. All burrows were counted, measured, using a caliper, and superficially covered with sand. Measures of burrows and slope were taken every two meters across the transect width, i.e., transects were divide into quadrats (10 x 2 m). Slopes measures were restricted to the area of occurrence of individuals, performed and calculated following the Emery method (EMERY, 1961), as the traditional rise over run, i.e., for a transect, the tangent of the angle opposed to the cathet correspondent to the sum of the registered rise at each quadrat. For each transect, intertidal sediment was collected to get the mean grain size (ϕ) for each beach. These variables were assessed to be used to a more precise beach characterization.

These beaches are all located in São Paulo state, southeastern Brazil, from coordinates S23° 48' 3.395" W46° 0' 52.715" and S23° 21' 44.795" W44° 50' 46.702", corresponding to about 130 km of coast line. Their locations, names and the respective coordinates are found in Table 5.1. To facilitate data display, beaches were numbered from 1 to 24, according to, sequentially: (i) no connectivity breakage between waterline and dunes/vegetation, (ii) level of

erosion and (iii) grain size (as an indicative of beach morphodynamics); this sequence was used throughout the text.

Assessments were all done during the winter, specifically throughout August 2012, to exclude temporal differences. Winter was chosen over warmer seasons because, during the latter, the crab abundance, or at least abundance of burrows, tends to be greater (ALBERTO; FONTOURA, 1999; NEGREIROS-FRANZOSO et al. 2002, POMBO; TURRA, *in prep.*). After high-mortality events, which are usually during winter cold-fronts, there are consequently lower abundances, so that less safe habitats may be better recognizable, because the lack of older, surviving individuals would be evidenced, thus revealing more threatened populations.

Regarding the methodological approach assessment, a student *t*-test for dependent samples were applied to compare differences between days (before and after *resetting* burrows), of both mean size and abundance. The measure used for size comparisons was burrow diameter (cm) and for abundance both individuals per strip transect (IST), that represents the raw amount of burrows sampled within each transect, and density (ind/m²).

Concerning the assessment of impacts, the results from the methodological approach were used to define if both or, which sampling day would be used as standard during the further analyses. The dependent variables (i.e. size and abundance), were treated similarly but separately. Here, though, the abundance measure used was density (occurrences per squared meter). First, to relativize the beach width, minimizing the influence of this natural factor as an influence in population feature (POMBO; TURRA, *in prep.*). Second because one of our objectives is to assess the influence of habitat loss on population features, to what density is very likely to respond. The number of individuals at a beach (IST) is influenced by the beach width, while a relativized measure, such as density, is more suitable to exclude this factor. Density was calculated as the number of burrows divided by the largest transect area at the respective beach. To calculate the transect area, the 10m width was multiplied by the range of occurrence of burrows (in meters, distance from the first to the

last observed burrows across the beach recorded in all samplings conducted). In other words, the largest range of occurrence within a beach was used in the calculation of the density of the three transects.

After general assessments, the variables density and size were analyzed separately in regression models with the environmental factors assessed. A linear model or the appropriate generalized model was used in each case. After the identification of the appropriate regression method, the selection of the most parsimonious model was performed testing the significance of the terms by hypothesis testing, in backward stepwise selection. For mean size analysis, the presence of the value 0 (zero) means no occurrence; these values are shown in the general assessments but were removed for models development.

Table 5.1. List of study areas and the respective coordinates. Twenty four beaches from Southeastern Brazil, encompassing distinct levels of impact caused by erosion and armoring. Beaches were numbered from 1 to 24, according to, sequentially: (i) no connectivity breakage between waterline and dunes/vegetation, (ii) level of erosion and (iii) grain size (as an indicative of beach morphodynamics). This sequence was used throughout the text to facilitate reading (see figure 5.3 for more details).

Number	Name	Coordinate
1	Prumirim	S23° 22' 47.982" W44° 57' 36.022"
2	Figueira	S23° 35' 10.475" W45° 16' 24.413"
3	Boraceia	S23° 45' 20.675" W45° 49' 38.978"
4	Capricornio	S23° 36' 41.015" W45° 21' 3.031"
5	Lagoa Azul	S23° 37' 14.578" W45° 21' 22.522"
6	Itaguá (vegetation)	S23° 26' 49.772" W45° 4' 1.063"
7	Fazenda	S23° 21' 44.795" W44° 50' 46.702"
8	Brava da Almada	S23° 21' 47.038" W44° 52' 46.477"
9	Puruba	S23° 21' 21.24" W44° 56' 20.512"
10	Itaguare (vegetation)	S23° 47' 27.388" W45° 59' 23.906"
11	Dura	S23° 29' 58.258" W45° 10' 30.943"
12	Sununga	S23° 30' 32.641" W45° 7' 58.094"
13	Martim de Sá	S23° 37' 41.736" W45° 22' 59.848"
14	Toninhas	S23° 29' 21.041" W45° 4' 28.549"
15	Riviera São Lourenço	S23° 48' 3.395" W46° 0' 52.715"
16	Vermelha do Centro	S23° 27' 49.691" W45° 2' 59.186"
17	Toque-Toque Grande	S23° 50' 2.44" W45° 30' 39.1"

18	Itagua (armoring)	S23° 26' 40.027" W45° 4' 5.113"
19	Massaguaçu	S23° 35' 16.228" W45° 19' 56.348"
20	Fortaleza	S23° 31' 39.047" W45° 10' 1.376"
21	Sapê	S23° 31' 47.01" W45° 13' 15.672"
22	Lázaro	S23° 30' 23.501" W45° 8' 3.656"
23	Itaguare (armoring)	S23° 47' 46.241" W45° 59' 37.982"
24	Barequecaba	S23° 49' 39.356" W45° 26' 7.552"

Results

Methodological assessment

Both the mean values of abundance and size of burrows differed between day 1 (before covering the burrows) and day 2 (resample day 1 areas 12 to 24 hours after covering its burrows). The mean number of burrows sampled at the first day was 19.46 (± 15.98), while at the second day it was 14.37 individuals (± 12.16), a significant lower mean (pairwised $t=2.46$, d.f.=71, $p=0.002$). The second day also showed a lower variance and a considerable decrease of maximum values (Figure 5.1A). During both days, though, the distributions were skewed to the right (Figure 5.1A).

The mean burrow size at the first day was 1.44cm (± 0.57), what was significantly greater than the second day, 1.32cm (± 0.57) (pairwised $t=2.46$, d.f.=71, $p=0.016$). Also, similarly to abundance values, the first day showed a higher variance, but the distribution was more skewed towards larger sizes only for the first sampling day. In figure 5.1B it is noticeable that size distribution was more normally distributed in the second day. The outliers in the figure (length=0) denote areas with no observation of burrows.

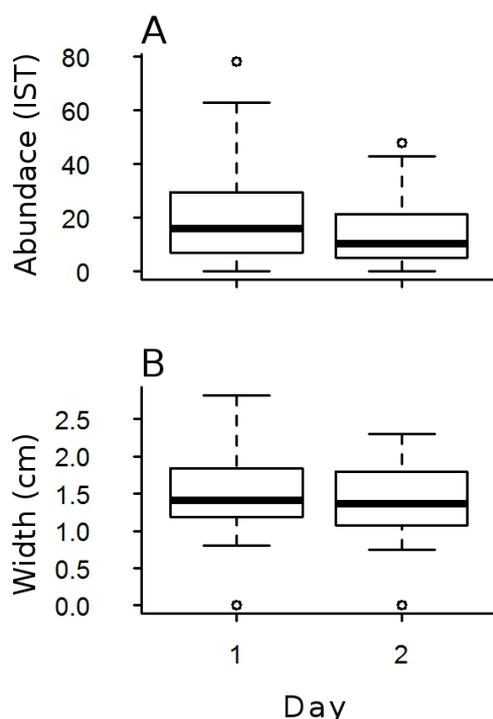


Figure 5.1. Median and quartiles of (A) individuals per strip transect (IST) and (B) width, in cm, of burrows of *Ocypode quadrata*. Measures were taken at 24 beaches at Southeastern Brazil, during August 2013, in two consecutive days. After traditional sampling (day 1) all recorded burrows were superficially covered, and the same areas were then resampled between 12 to 24 hours later (day 2).

The level of the assessed impacts influenced the differences between days (Figure 5.2). Conversely, the indirect method used influenced the estimate of the effects of connectivity breakage and erosion on the density and size structure of *Ocypode*. The difference in abundance (IST and density) between days was specially magnified in the absence of connectivity breakage, where vegetation is present to shelter individuals. Larger amounts of burrows in day 1, in relation to day 2, were observed where there was connectivity rather than where it was not (Figure 5.2, A and B). Concerning the size of burrows, this measure showed a lesser difference of patterns between days than abundance. Nevertheless, mean size values were lower for day 2 in almost all cases, and the difference between days was more clearly influenced by the degree of erosion in this case (Figure 5.2C).

The resetting methodological approach has shown to alter results

significantly compared to traditional sampling, and these differences did not vary constantly through beaches. Thus, further analysis only encompassed samples from day 2, after resetting *O. quadrata* burrows from predetermined transects. It assumes that results after resetting burrows better reflect the values correspondent to direct assessments, i.e. crab individuals directly.

Assessment of impacts encompassing areas comparison

To evaluate how erosion and armoring influence crab (burrow) population parameters on distinct beaches, some beach variables were preliminary assessed. Mean grain size of the sampling areas showed a broad range, from 0.46 to 3.48phi units, and in general low deviations, which correspond to the sorting coefficient (Figure 5.3A). The slope measures, which were focused to the area of occurrence of animals, covered a range from 3.00 to 42.10 and showed an opposite pattern in relation to grain size in phi units (Figure 5.3B). The range of occurrence of burrows per transect varied from less than 1 to 44m, with the majority of mean values per beach around 10 to 20m; some of these mean values showed noticeable deviations (Figure 5.3C).

To comply with further analysis, these environmental factors were assessed for collinearity. There was a significant and strong correlation between the mean grain diameter and the mean slope of the range of occurrence of *O. quadrata* (Pearson $r = -0.77$, d.f.=70, $p < 0.01$), and grain size was chosen over slope to characterize the beaches nature (morphodynamic). The range of occurrence had a significant correlation with the level of erosion (Pearson $r = -0.32$, d.f.=70, $p < 0.01$). As this last correlation was relatively low, because this measure was already included in the density calculation, it was also suppressed from further analysis.

A preliminary assessment of the density showed, as main identifiable features, that both largest and lowest values are related to higher levels of erosion (Figure 5.4). The largest values were associated to lower values of phi (coarser grain beaches), while the lowest ones were associated to higher values of phi (smaller grain beaches). For intermediate values of erosion (level

2), an ascending tendency towards finer grains was observed. Beaches with low levels of erosion showed low to intermediate density values.

To assess more thoroughly the relationship between burrow density and environmental conditions, a generalized least squares regression was applied, to allow the identified differences in variance per stratum of factors combination (erosion and armoring). The lowest variance was observed at beaches with the lowest levels of erosion and presence of natural vegetation (assumed to be the best condition), while the largest variance was observed at the highest level of erosion associated to natural vegetation removal (Table 5.2).

The final model, obtained through maximum likelihood backwards selection, encompassed the three explanatory variables (grain diameter, erosion level and connectivity breakage) and the interaction of grain size with both erosion level and absence of connectivity breakage. All intercepts and slopes were significantly different from the baseline conditions, i.e., erosion 1 or absence of armoring (Table 5.3). Figure 5.5 illustrates the results of the model effects.

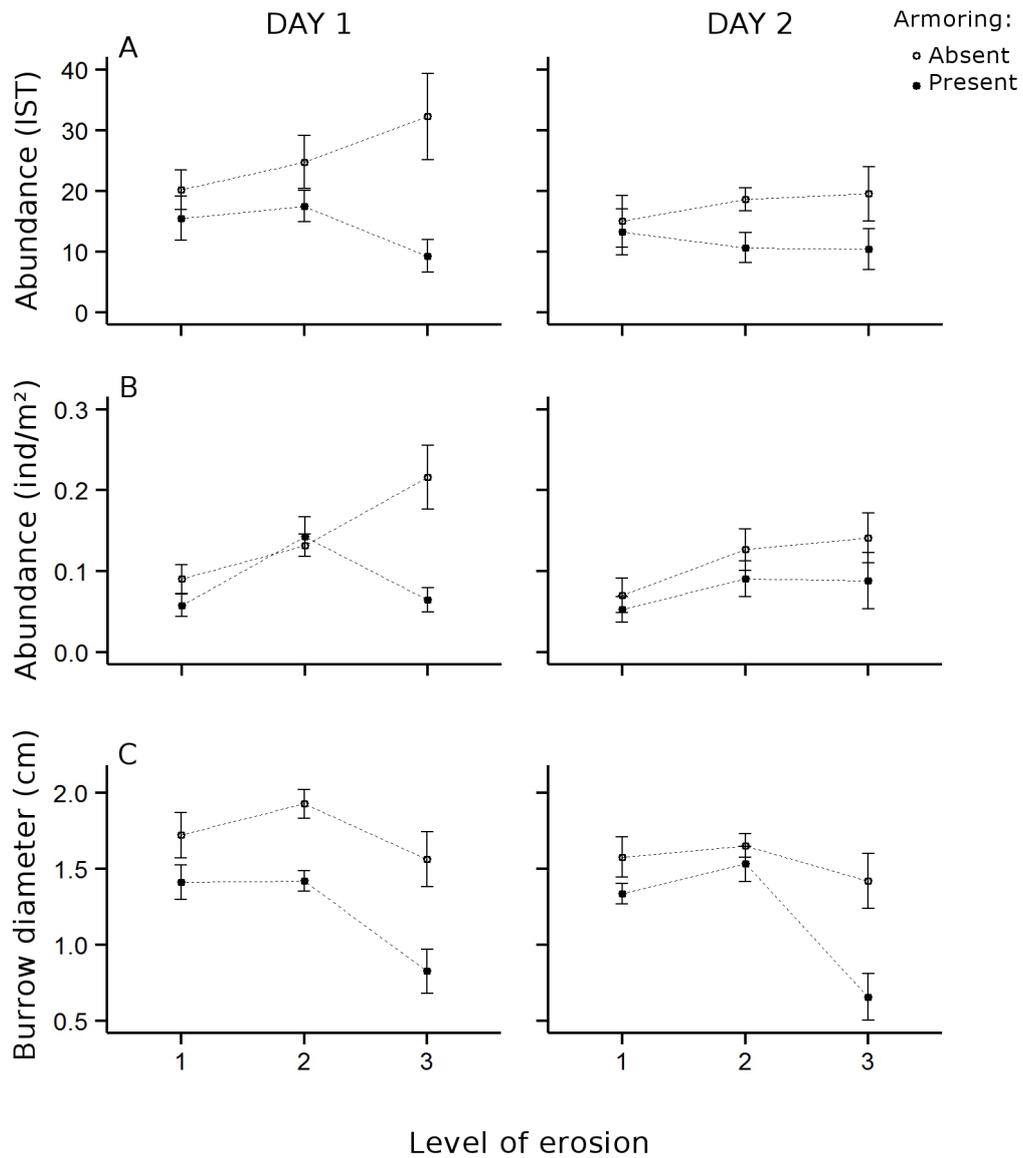


Figure 5.2. Mean and standard errors of abundance (A: individuals per transect; B: individuals per square meter) and size (C: cm) of burrows of *Ocypode quadrata* considering (i) the erosion level (1: low – 3: high) and (ii) the armoring (absent or present). Samples were taken in two consecutive days. Day 1: traditional sampling; Day 2: resampled areas from Day 1, between 12 to 24 hours after covering all recorded burrows. Data originates from 24 beaches at Southeastern Brazil, during August 2013.

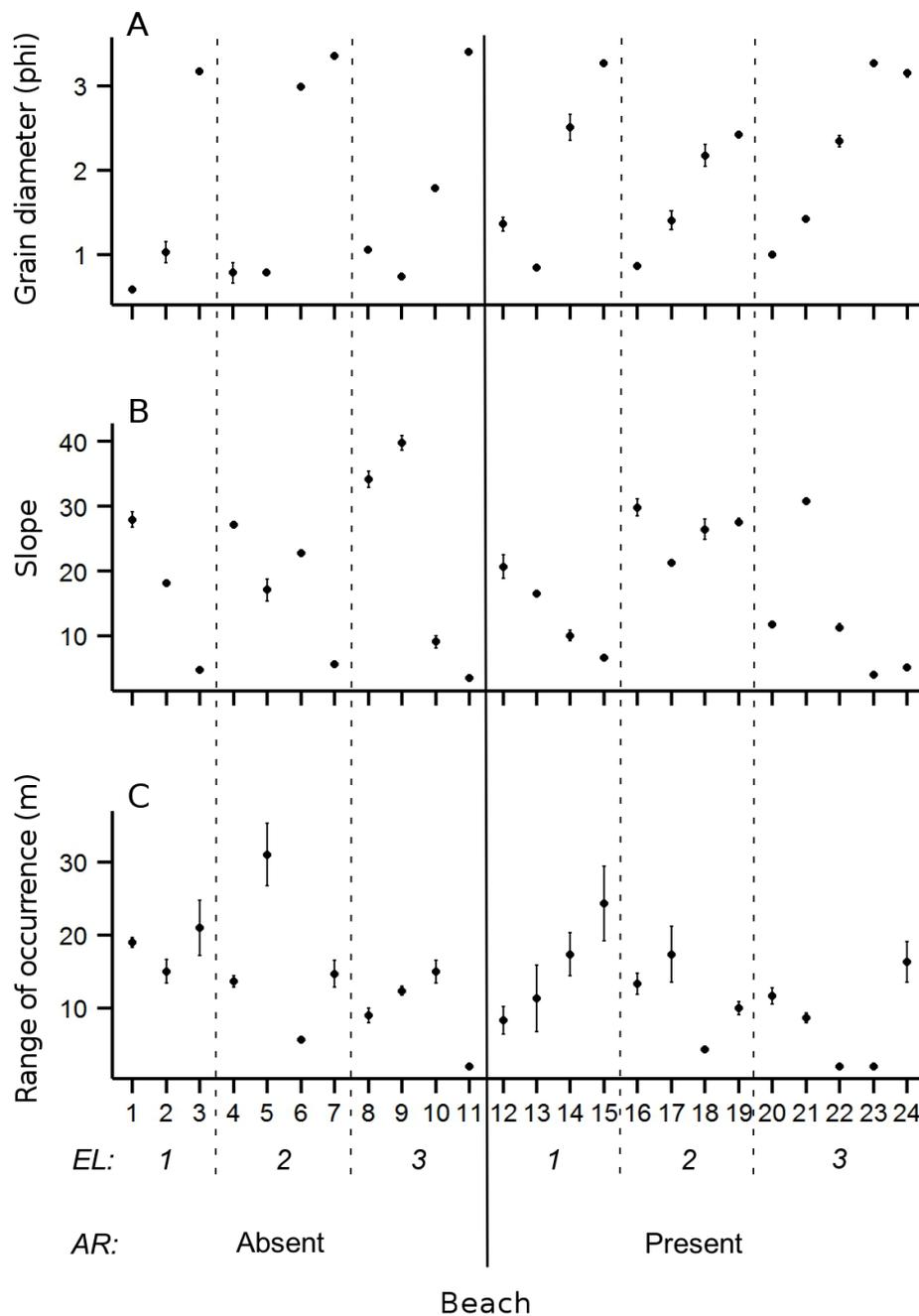


Figure 5.3. Study areas characterization (numbered from 1-24) regarding (A) grain diameter (phi units), (B) beach slope and (C) range of occurrence (m) of *Ocypode quadrata*. Areas were assessed for abundance and size of *O. quadrata* burrows through a wide granulometric range, comprising different levels of connectivity breakage, namely a combination of three levels of erosion and the absence or not of armoring. EL= erosion level (1: low – 3: high); AR= armoring (absent or present).

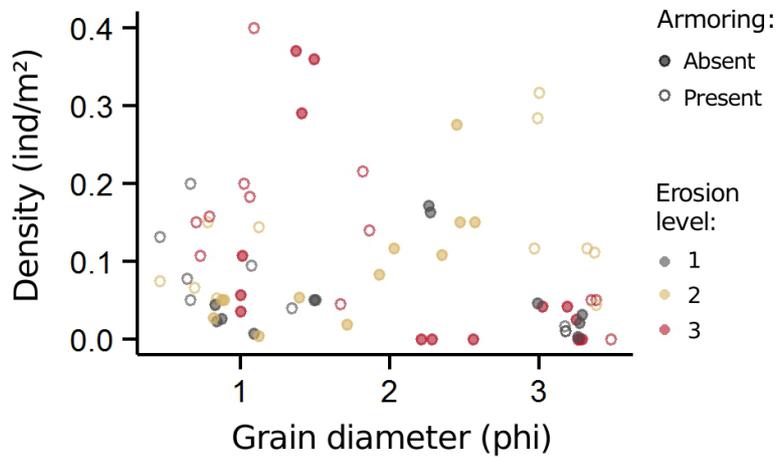


Figure 5.4. Density of individuals of *Ocypode quadrata* (ind/m²) through a granulometric gradient. Dots represent each of three transect measures, for each of 24 assessed beach, and are distinguished by the erosion level (coloured, 1: low – 3: high) and the absence or not of armoring (filled and blank, respectively).

Table 5.2. Variance structure of the terms of the generalized least squares model applied to the density of *Ocypode quadrata*, sampled at 24 beaches in Southeastern Brazil throughout August 2012, versus the explanatory variables: grain size (continuous), level of erosion (three levels: low, intermediate and high) and connectivity breakage (two levels: absent or present).

Optimal model (d.f.=72): density ~ grain diameter + erosion level + armoring + grain diameter * erosion level + grain diameter * natural vegetation		
Residual standard error (d.f.=64): 0.11		
Term		Parameters estimates
Armoring	Erosion level	
Absent	Low	0.36
	Intermediate	0.70
	High	0.75
Present	Low	0.47
	Intermediate	0.41
	High	1.00

Table 5.3. Coefficients structure applied to the density of *Ocypode quadrata* burrows, sampled in 24 different beaches. The explicative variables are grain diameter (ϕ), level of erosion (1-3) and connectivity breakage. Model was determined by generalized least squares; variance structure as described in Table 5.2. Baseline parameters of the categorical, independent variables are level of erosion 1 and absence of connectivity breakage (armoring). Significant interactions were between (i) grain diameter : erosion level and (ii) grain diameter : armoring.

Effects	Intercept				Slope			
	Value	SE	t value	p value	Value	SE	t value	p value
Baseline	0.132	0.024	5.513	<0.001	-0.036	0.012	-3.071	0.003
Erosion level								
2	-0.084	0.038	-2.239	0.029	0.074	0.018	4.097	<0.001
3	0.148	0.048	3.092	0.003	-0.045	0.022	-2.072	0.042
Armoring								
Presence	-0.085	0.034	-2.497	0.015	0.038	0.016	2.360	0.021

Concerning the effects of erosion, the lowest level, 1, showed a slight tendency of increase in density towards coarser grains. To areas in advancing erosion process density increased towards finer sediment, with a steeper and positive slope. Both coefficients significantly differing from the baseline conditions means that density in extreme reflexive areas was lower (intercept), while for extreme dissipative beaches it was much higher, in relation to those of erosion level 1. In areas of advanced erosion process, the slope became again negative, but considerably steeper than in erosion level 1. This situation, with both coefficients being significantly different from the baseline conditions, means that extreme reflexive beaches showed a sharply increase of concentration of individuals, while extremely dissipative beaches sharply decreases towards initial condition. Because reflective beaches show further, within a progression of impact level, the response observed for dissipative beaches earlier, this indicates that populations from the former are likely to be more resilient. Concerning the armoring effects, its presence was associated with a sharp decrease in abundance in extreme reflexive beaches, increasing towards dissipative beaches, i.e. slope towards finer grains was gentler and reversed.

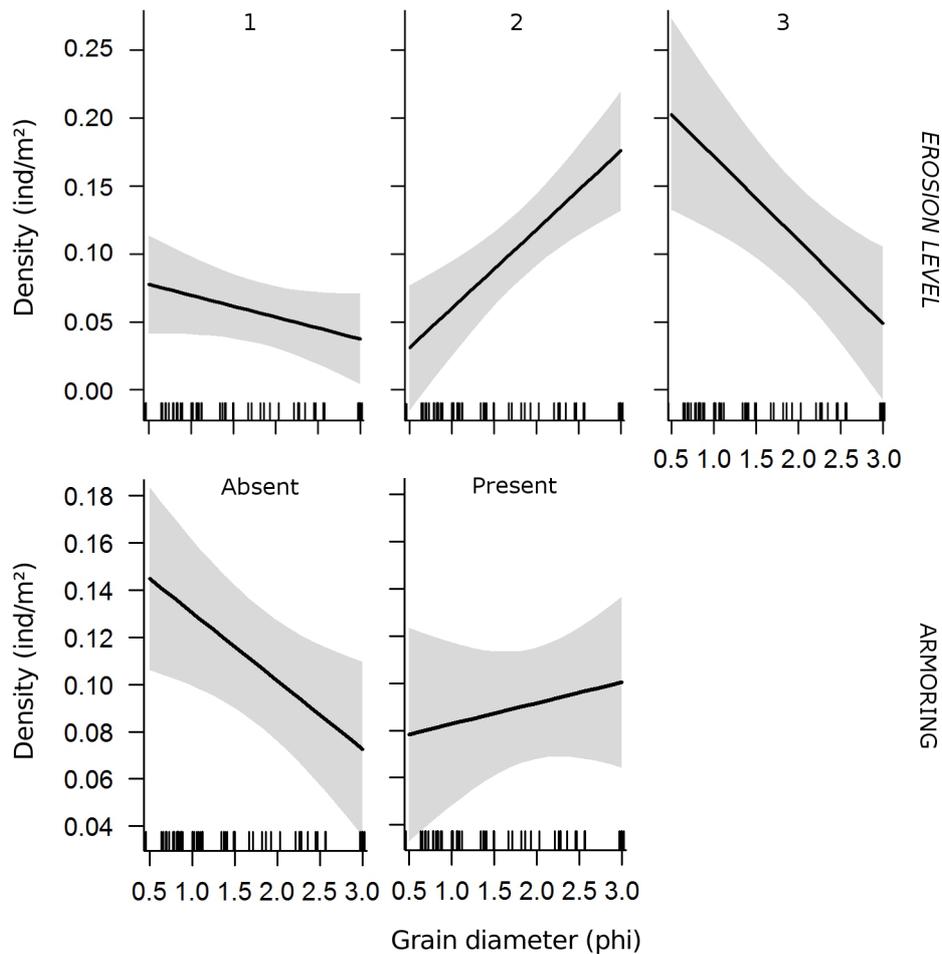


Figure 5.5. All effects of the model constructed to explain the density of *Ocypode quadrata* burrows considering (i) grain diameter (ϕ), (ii) the erosion level (1: low – 3: high) and (iii) the armoring (absent or present), through a generalized least squares regression. The model was statistically significant and encompassed the interaction of grain diameter with each of the two categorical variables. Effects are displayed along with 95% confidence interval (gray area) and number of observation (x-axis).

Regarding the burrow diameter measurements, a preliminary, general assessment, indicated a general decrease of size towards finer grains (Figure 5.6). The largest sizes observed were associated to the intermediate level of erosion, while the lowest values, including total absence of individuals (represented as 0.00cm in figure 5.6), were all associated to the highest level of erosion and dissipative beaches.

The residual distribution of the linear model applied to size measures showed regular variances and, although the distribution was slightly positive skewed, premises to perform an ordinary linear model were accomplished. The

global test was significant ($F=8.18$, $d.f.=11,53$; $p<0.01$) and the backwards selection indicated that the three way interaction was significant ($F=9.714$, $d.f.=2$; $p<0.01$). All effects results are shown in Table 5.4 and illustrated in Figure 5.7.

For the less impact condition, which is the lowest erosion level and absence of armoring, a decrease in size was observed towards finer grains, i.e., a negative slope (Figure 5.7). The progression of the erosion level did not result in significant differences of intercept, but it did for slope, wherein for erosion level 2 the slope became slightly positive and for level 3 it was significantly lower. That means that for extreme reflexive beaches no differences in mean size were identified, but there was sufficient evidence that towards dissipative conditions the mean size increase for intermediate erosion levels and decrease abruptly for elevated erosion levels.

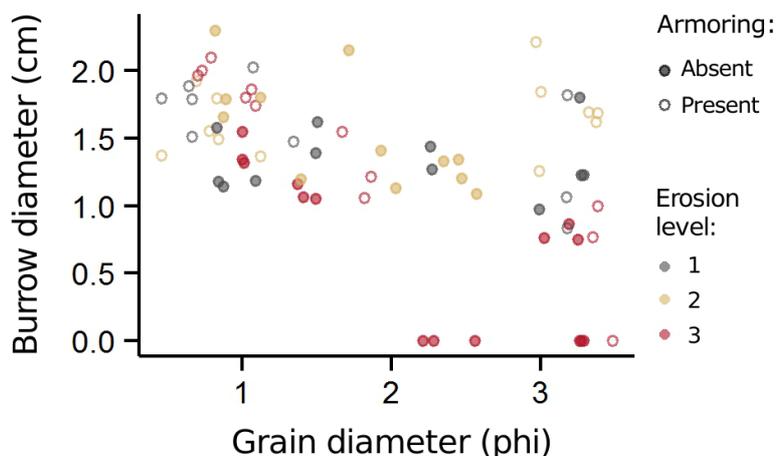


Figure 5.6. Mean diameter of burrows of *Ocypode quadrata* (cm) through a granulometric gradient. Dots represent each of three transect measures, for each of 24 assessed beach, and are distinguished by the erosion level (coloured, 1: low – 3: high) and the absence or not of armoring (filled and blank, respectively).

When subjected to the removal of the vegetation, beaches of the lowest erosion level showed a decrease in mean size (Figure 5.7). For beaches of erosion level 2 and presence of armoring, the intercept was significantly higher and the slope significantly lower, i.e., reflexive beaches had the mean size

significantly enlarged, and the decrease of size towards dissipative beach is significantly more abrupt. For the last, more severe case, high erosion and armoring, there was no significant difference detected from the baseline condition. However, it is important to reinforce here that zero values were removed from data to model construction, and these were all enclosed in this situation (erosion level 3, armoring). This fact not only reduces the number of observation, and therefore the power of the test for this situation, but also biases its results. Although including the zeros could soften this situation, it would be an incorrect approach, so the best way to deal with this condition is to consider the effects on size always together with the effects on abundance.

Table 5.4. Coefficients structure of mean size of *Ocypode quadrata* burrows, sampled in 24 different beaches. The explicative variables are grain diameter (ϕ), level of erosion (1-3) and presence of armoring. Model was determined by multiple linear regression. Baseline parameters of the categorical, independent variables are level of erosion 1 and presence of natural vegetation. There was significant interaction among the three parameters.

Effects combination		Intercept				Slope			
Erosion level	Armoring	Value	SE	t value	p value	Value	SE	t value	p value
1	No (baseline)	1.917	0.146	13.117	<0.01	-0.212	0.074	-2.854	<0.01
2	No	-0.361	0.203	-1.776	0.08	0.261	0.096	2.714	<0.01
3	No	0.332	0.211	1.570	0.122	-0.227	0.111	-2.039	0.046
1	Yes	-0.587	0.223	-2.631	0.011	0.216	0.106	2.028	0.047
2	Yes	1.281	0.337	3.803	<0.01	-0.682	0.167	-4.077	<0.01
3	Yes	-0.098	0.331	-0.298	0.767	-0.027	0.163	-0.167	0.868

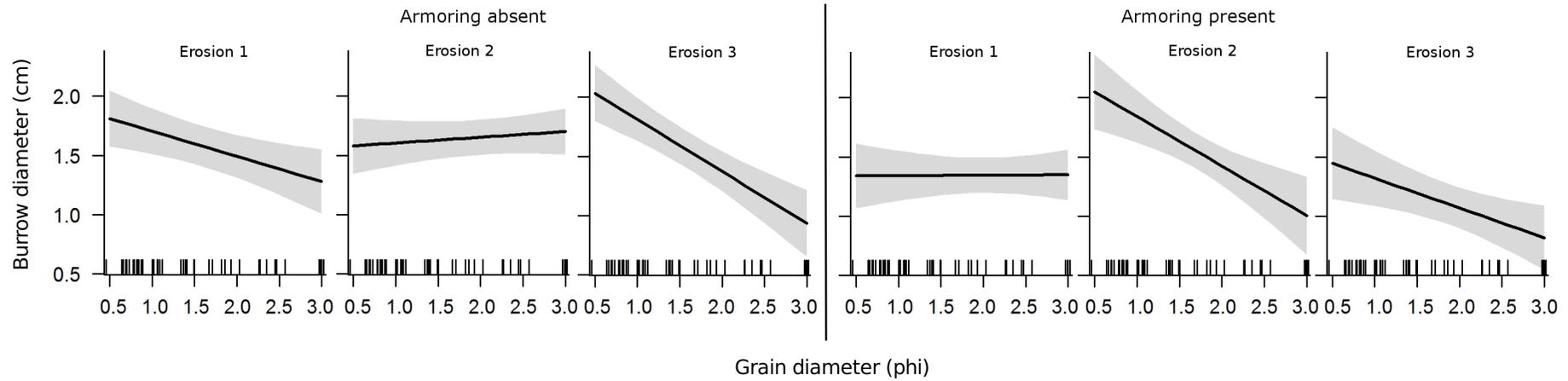


Figure 5.7. All effects of the model constructed to explain the mean size of *Ocypode quadrata* burrows considering (i) grain diameter (ϕ), (ii) the erosion level (1: low – 3: high) and (iii) armoring (absent or present), through a multiple linear regression. The model was statistically significant and encompassed the interaction of the three terms. Effects are displayed along with 95% confidence interval (gray area) and number of observation (x-axis).

Discussion

Methodological assessment

Most of what is known on ghost crabs population ecology was assessed from burrow abundance and size (ALBERTO; FONTOURA, 1999; BARROS, 2001; FISHER; TEVESZ, 1979; VALERO-PACHECO et al., 2007). Studies based on direct assessments usually encompass visualizing and following the individuals that are out of the burrows and, if possible, capture them, thus depending on what is more visible, or easily reachable / capturable (HALEY, 1969; NEGREIROS-FRANSOZO; FRANSOZO; BERTINI, 2002). Therefore, direct assessments are hardly random. This may not prevent some scientific objectives, but surely affect others. The best approaches to bring direct measures closer to randomness have only recently been assessed (POMBO; TURRA, 2013), but the expenses of these methods may difficult population ecology studies, a price that sandy beaches ecology cannot afford at a moment of increasing pressures on the habitat (SCHLACHER et al., 2007).

Thus, a good option is to work towards reducing potential differences between the number of burrows and of individuals, i.e., developing and applying more realistic methods. An usual method encompasses the assessment of only burrows surrounded by fresh tracks (WOLCOTT, 1978), but this has been shown to work poorly for some areas (POMBO; TURRA, 2013). The present results showed that pre-resetting sampling areas reduces significantly measures of abundance and mean size. Because the pilot study assured animals were not prejudiced, this difference between days is an excellent candidate to smooth (at least) biases from indirect measures.

Beyond the clear general difference between days, the influence of the beach condition on these differences impels some interesting interpretations. Because in the presence of connectivity (waterline to dunes/vegetation) day 1 had higher differences of abundance and size of burrows in relation to day 2, it is assumed that the vegetation present in these areas lacking armoring is very likely to retard the burrows collapsing. There is a segregation of size of these individuals across beach, where larger ones tend to be upwards (ALBERTO;

FONTOURA, 1999; TURRA; GONÇALVES; DENADAI, 2005). Consequently, the longer lasting of burrows in the vegetation would entail that these tend to be larger burrows. Indeed, that corroborates other findings, about across beach distribution of *O. quadrata*, that showed a lower occupation rates backwards on the beach (POMBO; TURRA, *in prep.*). Therefore, not only natural and artificial features could possibly influence occupation rates (BARROS, 2001; TURRA et al., 2005; POMBO; TURRA, *in prep.*), but also these would be biased through distinct population strata.

The long lasting of some burrows opposes to the comment at Hobbs et al. (2008), that abandoned burrows rapidly collapsed. The lasting of abandoned burrows is probably longer on more compact sediment zones than, for example, in dunes. Therefore, either the climatic regions of different studies (windy, wetter) or different beach zones (dunes, intertidal or vegetation), will influence the extent of the effect of a calculated resetting.

The simple approach of covering the burrow of a pre-determined area some hours before sampling was shown to be very effective at the present study. The significant decrease in abundance and size, and specially the differences between approaches regarding different level of impacts, indicate that important biases were eliminated from analysis. Ghost crab abandoned burrows are naturally covered, by wind and water rising, at a speed that depends on the characteristics of each beach and their location within the beach. With this method, despite the local conditions, excessive burrows, that did not represented the real population were eliminated, most of it larger burrows, probably more protected from the actions of wind and water. It is very important to smooth as most as possible differences that may lead to biased results and wrong conclusions, not only with respect to the level of impact, but also with respect to the nature of beaches. This approach is a prime candidate to refine assessments through indirect measures. Furthermore, because the rate of occupation of burrows differs over areas, comparison of ghost crabs population over distinct beaches, through indirect measures, are limited, and this limitation was considerably reduced by this methodology, of inducing

burrows reset.

Assessment of impacts encompassing areas comparison

The need for monitoring and assessment tools to evaluate the extent of anthropogenic influence on environmental quality has long been recognized and discussed, having as an important ally the use of bioindicators for conservation purposes, which have been widely improved in the last decades (FRÄNZLE, 2006; HEINK; KOWARIK, 2010). For sandy beaches, *Ocypode* has been a strong candidate, due to a number of benefits, namely conspicuity, possibility of assessment from indirect methods, and trophic importance, among others (MILNE; MILNE, 1946; ROBERTSON; PFEIFFER, 1982; WOLCOTT, 1978). Ghost crabs in general have been shown to respond effectively to some environmental pressures, while to other impacts, such as tourism, a standard response has not been yet identified.

From the first assessment of anthropogenic damages to ghost crab populations, the influence of off- road vehicles (ORV) has been the most widely investigated, from alteration on number and size to burrows structure. Burrows depth, time of the day, and beach strata were shown to be important factors of protection of ghost crabs from ORV, and this information aided the constructions of ORV corridors (HOBBS; LANDRY; PERRY, 2008; LUCREZI; SCHLACHER, 2010; SCHLACHER; THOMPSON; PRICE, 2007; WOLCOTT; WOLCOTT, 1984). Other kinds of trampling have also been assessed, but it is usually associated to other impacts as shoreline armoring and other consequences of tourism; tourism alone has shown imparity concerning reflects on ghost crab abundance (LUCREZI; SCHLACHER; WALKER, 2009; SCHLACHER; DE JAGER; NIELSEN, 2011; STEINER; LEATHERMAN, 1981; TURRA; GONÇALVES; DENADAI, 2005; WOLCOTT; WOLCOTT, 1984) Also bulldozing has been shown to decrease the abundance of burrows (PETERSON; HICKERSON; JOHNSON, 2000).

The importance of the species as bioindicator, and of the indirect measures to actual and further knowledge are clear, as well as the relevance of

burrows resetting to each of these approaches. Importantly, comparing beaches has been also a challenge, because burrowing and resetting dynamics are very likely to change according to natural features, such as climate and morphodynamic. Here, the effects of connectivity breakage were assessed after a complete, induced resetting, and a step was given towards the comparison of different beaches.

A lower variance of density was generally observed at beaches in better conditions (low erosion, vegetation present), suggesting that the clustering patterns tend to be more similar under natural conditions than under space loss conditions. Along with habitat loss, despite the increase in mean values or not, variances increased. The models encompassing the influence of connectivity breakage and grain size on density and mean size, were both significant, what means that erosion levels and armoring influence clustering and the structure of population of *O. quadrata*, throughout a large grain size range. Therefore, both abundance and size must be considered together in order to assess the extent of these impacts.

To illustrate the models predictions, consider a dissipative beach (3.20 phi units of grain diameter), in a baseline condition (low erosion and presence of natural vegetation) with density of *O. quadrata* 0.015 ind/m² and mean width 1.24 cm. In an advancing process of erosion (level 2), according to the previous models, these values would increase respectively to 0.17 ind/m² and 1.71 cm. A severe level of erosion (level 3) would turn out these values to 0.017 and 0.84. This example alone shows the importance of considering the population variables, density and size combined to assess the effect of this impact. Initially, within an increasing erosion process, a large increase in the concentration of burrows was predicted to occur, but also an increase in mean size. If greater concentration means greater competition for resources, then it is likely that the larger individuals would be favored, and/or the smaller more jeopardized, complying with the model prediction. In a further step, a severe erosion state would lead to a density not that different from the baseline condition, but the size of burrows was predicted to decrease severely with the reduction in grain

size, since individuals are not expected to reach larger sizes due to impossibility to occupy dune or vegetated habitats due to connectivity breakage.

Supposing that the beach from the previous example was still in a low erosion condition, but the natural vegetation had been removed, the values of density and size would be 0.050 ind/m² and 1.34 cm. Again, both variables increase. If now this beach presents an intermediate erosion state these values turn out to be 0.204 ind/m² and 0.91 cm. Observe that, compared to the situation without armoring and where vegetation was kept, values of density had a relatively small difference, but the mean size would be relatively much lower. The next step, the worst scenario, would lead to values of 0.053 ind/m² and 0.76 cm.

Consider another condition, of a beach with mean grain diameter of 0.80 in phi units, at baseline conditions, with mean density 0.102 ind/m² and mean diameter 1.75 cm. The second level of erosion would lead to 0.078 ind/m² and 1.59 cm respectively, while the third, most severe erosion level, these would be 0.214 ind/m² and 1.90 cm. It is noticeable that the reduced area is causing an elevated concentration of individuals that are likely to be the larger ones, living towards and inside the vegetation (POMBO; TURRA, *in prep.*). The armoring of this reflexive beach, would drastically change these values, either of density or of mean size. Even if not eroded, density values would drop to average 0.047 ind/m², and of size to 1.33 cm. The following step would decrease sharper the density, while increasing the mean size to the highest predict value (0.023 ind/m² and 1.91 cm). The last condition reflects a trend of elevating the density, of crabs of increasingly smaller sizes (0.159 ind/m² and 1.36 cm, respectively).

It all illustrates that increase in density indicates increasing severity of space availability. Even when density tends to recede to earlier (baseline) values within an increasing severity of space loss, there will be a concomitant decrease in mean size of individuals that compromises the success of the population. That because, the lower the mean size of individuals, the higher the evidence that the population does not have a satisfactory reproductive success.

Negreiros-Fransozo et al. (2002) estimated the onset size of females' sexual maturation as 23 mm of carapace width. That would require an age of about six months (POMBO; TURRA, *in prep.*), what is a considerable length of time for a sandy beach macro-invertebrate, especially for those submitted to loss of space in areas subjected to frequent harsh conditions, as are cold fronts. This alteration of age structure due to environmental alteration, identified here indicates a level of complexity for *O. quadrata* as bioindicator that is beyond changes in abundance (FRÄNZLE, 2006).

The armoring and absence of vegetation significantly worsens the situation for larger individuals, and this worsening increase with increasing levels of erosion. At first, competition would compromise survival of young, elevating means sizes. But, when submitted to space loss, the size of individuals was severely reduced, indicating that utilization of vegetated areas is essential to maintain the survival of reproductively active individuals (the larger ones). In fact, the initial results of the present study showed how unlikely it was, for beaches without connectivity, that individuals reach this maturing size before dying, due to periodical harsh events, such as cold fronts. Thus, these areas with armoring would be more prone to function only as a genetic "sink" for the species, thus representing functionally extinct populations (DIAS, 1996).

It is also interesting to note that beaches of different morphodynamic showed similar patterns throughout the processes, but responses were dislocated through the levels of impact to distinct beaches, regarding grain size. The populations from more reflexive beaches showed a trend towards greater resilience than the more dissipative ones. It is likely that higher backwards (steeper slopes) offer better shelters at places at risk, especially flooding. In this regard, the results match the hypothesis that for supralittoral crustaceans, environmental harshness behaves oppositely than do for most of the intertidal macrofauna (DEFEO; GÓMEZ, 2005; DEFEO; MARTÍNEZ, 2003).

Results showed that the continuity of beach backwards, is essential in a conservation panorama, considering that the species represent an important top-down control at sandy-beaches ecosystems. Erosion process often cannot

be avoided, but the presence of sandy beaches with natural vegetation, and the possibility for individuals to get to it, may shield populations even in some drastic erosion conditions. The models of this study were shown to significantly predict density and mean size of animals considering the grain size, level of erosion and presence of natural vegetation. This is an important advance towards the use of *O. quadrata* as a bioindicator, since, for the first time it was shown to be feasible to gather data from very distinct beaches together to predict the influence of connectivity breakage on its population measures.

These, demonstrate the possibility of using that specie as bioindicators in a much broader context and, bring new opportunities to use such information to support conservation strategies and set coastal occupation plans.

Closing Remarks

The central purpose of this study was, from the beginning, to advance as most as possible on methodological issues, in order to assess, and ideally to broaden, the potential of the group to bioindication. By the end, undeniably, important methodological issues were reviewed, and patterns were identified in the responses of distinct populations to common impacts. In addition, other relevant aspects about the biology of *O. quadrata* have been addressed.

Over the course of this research, many behavioral and evolutionary questions have raised, which could guide, or answer, certain elements of the ongoing study. One of the surprises was related to the period of activity of the individuals. Although it was expected the influence of the tide to some extent, it was not expected to observe the major peaks of activity during the daylight, even though the available literature lack strict correspondence, identifying from greater nocturnal activity (MILNE; MILNE, 1946) to equally distributed over a 24-hours cycle, except during higher local temperatures (VALERO-PACHECO et al., 2007). Here, the avoidance of higher temperature was also observed, but the peaks of activity occurred all during daylight, and mostly associated to low tides. It is important to highlight that nocturnal activity was recorded, but recurrently the presence of few individuals, minimally moving or interacting with others. It is also worth noting that this is the first time that this matter is assessed excluding the influence of observers in the field.

There wasn't, after all, the need to restrict the field activity to the lower activity times of the individuals. First, because during the samplings, when the team was around, the animals remained inside the burrows; second, field times were randomized between the areas, thus supporting that the individuals activity pattern was not a factor of influence on the results. Some of the consideration of chapter 2, concerning methodological issues and spatial variation, were decisive for the continuity of the study. Surveys were as thoroughly as possible, assessing every burrow. By the end, it was concluded that a relevant amount of occupied burrows would be disregarded due to their

weak surrounding signs of recent activity, as well as many burrows that have strong signs of occupation are actually empty, the later usually quite shallow. Moreover, it was observed that beach type had a influence regarding the intensity of the surrounding signs of activity of a burrows and its occupation. Thus, using signs of recent activity requires caution, and preferably associated to burrows depth assessment (here estimated as at least 10 cm) to assist the decision of whether or not to include a burrow to population studies.

Because excavating was the most efficient to determine the occupation rate, the following samplings were performed using this technique. The number of burrows in relation to individuals differed over time, but a similar temporal trend over areas was observed. Therefore, population quantification through the indirect method naturally overestimate the number of individuals, and this difference depends on time and beach type. But, even more important, is that this rate of occupation of burrows differs across a beach; burrows in the upper regions, that are usually larger, tend to show lower occupation rates, i.e., the indirect method is prone to overestimate the older strata of a population. The difference of sex ratios over time and across a beach, what probably related to reproductive factors, also drew attention. A plausible explanation for what was observed here, is that mortality rate are higher among females, specially during incubation and spawning periods. In addition, certain periods concentrated individuals of distinct sex at distinct beach regions. This reinforces the importance of using transects perpendicular to the waterline, and that dunes and vegetation must be included to population assessments.

Autumn was the season that, in general, showed the highest occupation rates, sometimes also winter. In these periods, closest values of burrows and individuals were recorded. Also, this period showed higher mean length values. These features were assigned to the concomitant occurrence of intense cold fronts, which are likely to lead to high mortality rates. Therefore, post cold front periods are good candidates for more accurate population assessments, using the indirect method, that aims to compare distinct areas or to assess environmental quality. This statement is based on chapter 3, which shows the

highest mean size during these periods, and also on chapter 5, which shows higher mean sizes in areas with preserved upper portions. Thus, preserved areas have greater potential to shelter *O. quadrata* individuals during periods of intense mortality, during which largest individuals would have greater chances to survive, probably because they can move more efficiently to higher areas, less affected by the cold fronts effects.

Size differences followed a similar pattern over areas, all well preserved. The higher abundance of recently settled individuals was recorded right after the period of cold fronts, in winter, suggesting that individuals settling right before it have low survival chances. These abundant, low size, modes were important to define the body-growth parameter estimation. These results indicate that the recruits arriving on winter, correspond to the predominant cohort of the next summer. Furthermore, the higher body growth constant on reflective beaches, sign towards more successful development history at these environments, even though abundance was higher at dissipative beaches. The former (body growth trend) is in accordance to the habitat safety hypothesis, according to which crustaceans of reflective beaches show adaptive advantages in relation to those of more dissipative areas, but the later (abundance trend) should behave oppositely according to the same paradigm. This ambiguity may be one of the reasons why the identification of patterns for this species has been historically so puzzling.

The influence of abiotic factors analysis showed a greater influence of granulometry than of any other factor, in the density of burrows and of individuals, and on the influence of size estimation of animals from burrows. This means that a simple sediment analysis can improve population studies based on burrows measure. Population surveys through burrows assessments are essential for *Ocypode* studies, in contrast to other macrofauna representatives of sandy beaches. Thus, this better understanding of the relationship between burrows and individuals is essential.

A key point of this study, undoubtedly, was the methodological proposal that largely eliminates sources of biases intra- and inter-areas: to induce a reset

of all burrows in predetermined transects and perform the assessments a while later (12-24 hours). This method allowed successfully to cross information from very distinct areas, in search of patterns of response to common impacts. In addition, this methodology showed that occupation rates differ according to the level of impact on an area. The presence of initial levels of erosion were associated to an increase in density as well as in mean size, probably due to the competition advantage for space that larger individuals would have. In the most severe cases of habitat loss, densities were reduced back to similar values than non eroding beaches, but this abundance decline was, in all cases, associated with the lowest mean size recorded. When prevented to reach the vegetation, a population subjected to erosion process totally lack larger individuals, and are likely to be genetically extinct populations. Even at non eroding beaches, connectivity breakage, between waterline and vegetation, caused dramatic changes in the population density. The trend of responses according to the assessed impacts were similar despite the beaches morphodynamic, but populations of reflective beaches were more resilient than those of dissipative beaches, showing a delayed response to the impacts as compared to correspondent dissipative beaches.

Lastly, after dealing with various aspects of the biology of *O. quadrata*, spatially and temporally, and considering the influence of natural factors only, the use of these populations structure to assess the level of impacts, related to habitat loss, has succeeded in finding a pattern of response, allowing even the comparison of distinct areas, a scenario that broadens the range of the use of the species as bioindicator.

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