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ARTICLE

Natural diet of *Ocypode quadrata* (Fabricius, 1787) (Crustacea, Decapoda, Brachyura) from the Northern Coast of São Paulo, Brazil

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Abstract. Decapod crustaceans have a wide variety of feeding habits, alternating among herbivory, predation, saprophagy, and filtration. The occupation of various trophic positions in the food web is a key feature in the evolution of the group. Thus, we analyzed the natural diet of the crab *Ocypode quadrata* (Fabricius, 1787), commonly known as the ghost crab and typically found on sandy beaches. The crabs were collected in the region of Ubatuba, between July 2016 and May 2017. The individuals were dissected in the laboratory, and each stomach was weighed and visually assessed in relation to the degree of repletion. After identification and classification, the items found were grouped for the analysis. In analyzing the stomach contents of *Ocypode quadrata,* 12 items were found: sand, Insecta, Hymenoptera, Hemiptera, Coleoptera, Crustacea, and other unidentified Arthropoda. We also found poriferans, Actnopterygii bones, plant pieces, non-organic material (plastic), and other non-identified materials. In the statistical analyzes, we observed seasonal differences in the composition of the diet, mostly related to the frequency of items consumed between dry and rainy seasons; the rainy season provided a greater diversity of items. Due to the great trophic spectrum of this species and tendency to feed on a wide range of items, *O. quadrata* is considered a generalist species, adapting according to the availability of prey in the wild.

Key-Words. Ghost crab; Natural diet; Ocypodidae; Seasonality; Trophic ecology.

INTRODUCTION

Decapod crustaceans are the characteristic fauna of coastal ecosystems, accelerating decomposition of organic matter and soil bioturbation, serving as food for various invertebrates and vertebrates (*e.g.,* fish and birds), and being exploited economically (Cowen, 1986; Schaeffer-Novelli, 1995; Hemmi, 2004). On sandy beaches, it is common to find resident decapod fauna that is highly sensitive to adverse conditions prevalent in the intertidal range (Veloso *et al.,* 1997). In this environment, *Ocypode quadrata* (Fabricius, 1787), popularly known as the ghost crab, is abundant with a wide geographical distribution, and it is easily found along the Brazilian coast, inhabiting regions of the supralittoral to the sand dune areas.

Ocypode quadrata is an ecologically important consumer of organic material (Phillips, 1940; Fales, 1976; Wolcott, 1978) and bioturbator (Branco *et al.,* 2010). Its ecological importance and high sensitivity to changes caused by anthropogenic processes make this species crucial to understand the processes affecting sand beaches. Populations of *O. quadrata* are very sensitive to temperature variations, intensity and direction of wind, and wave heights, as these directly affect their activities (Sawaya, 1939).

The diet plays an essential role in metabolic efficiency and homeostasis (Carew *et al.,* 1964). However, diets can change in response to environmental changes, such as seasonal variations (Rosalino *et al.,* 2005), species invasions (Griffen *et al.,* 2008), and climate changes (Kitaysky *et al.,* 2006). The feeding activity of Brachyura can be divided into processes, such as: food capture through chelipeds, grinding by mouth parts, the passage of the food from the mouth to the stomach, maceration of food by the ossicles of the stomach, and finally digestion. Understanding the feeding habits of crabs is fundamental because the availability and use of food play important roles in their distribution patterns, migration and ecdysis (Mclaughlin & Hebard, 1961).

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Understanding the diet of *Ocypode quadrata* is essential to comprehending the population dynamics in this environment. Analysis of the stomach contents, makes it possible to identify any variation in diet composition between the dry and rainy seasons, clarifying the role of this species in the trophic structure of the ecosystem. Thus, we analyzed the diet of *Ocypode quadrata,* identifying and classifying the food items according to the most inclusive taxonomic level possible.

MATERIAL AND METHODS

The Ubatuba region, in the northern coast of São Paulo, is characterized by a great diversity of beaches, ranging from rocky shores strongly influenced by waves to flat sandy bays and calm waters (Gondolo *et al.,* 2011). The Praia Vermelha do Norte (23°24'S, 45°01'W) is characterized by intensely pulsing waves, a vertical profile with slopes of 10 to 16° and sandy substrate composed mainly by medium (0.25-0.5 mm) to gross grain size (0.5-1.0 mm) (Nakagaki & Pinheiro, 1999). Four field trips were conducted at night during low tide – two in the dry and two in the rainy season – the first being in July 2016, the second in October 2016, the third in January 2017, and the fourth in May 2017.

Specimens of *Ocypode quadrata* were manually captured through line transects along the entire beach. The specimens were euthanized in ice baths and subsequently preserved in 70% ethanol. In the laboratory, the morphometric data were recorded (carapace maximum width: CW, and carapace length: CL, measured from the anterior region of the front to the posterior margin of the carapace) with a digital caliper (0.01 mm). Each animal was weighed and sexed. For dissection, a U-shaped cut on the gastric region of the carapace was made. The digestive organs, gonads and muscle were removed, and then the esophagi were cut to allow the removal of the stomachs.

After dissected, the stomachs were wet weighed in a precision balance 0.01g and classified into four categories according to the stomach repletion index – SRI; *i.e.,* the amount of food present in the stomach: full (< 100% and > 70%), almost full (< 70% and > 30%), almost empty (< 30% and > 1%) and empty (< 1%) (Kapusta & Bemvenutti, 1998). This index indicates the weight of the stomach contents and is represented by the ratio of stomach to the total weight of the specimen (Zavala-Camim, 1996), obtained by the following formula:

$$IR = PC/PT*100,$$

where PC is the total weight of stomach and PT is total weight of the specimen.

After categorization, the stomachs were preserved in 70% alcohol. To analysis of the stomach contents, we made incisions in the stomachs and the contents were removed, deposited in aqueous solution in Petri dishes, and observed in the stereo microscope Motic K Series to identify the lowest taxonomic level of food items found.

After screening each sample, for the analysis of food items, the following statistical tests were used:

- Frequency of occurrence (Williams, 1981) according to the equation:

$$FO = (bi/n).100,$$

where bi is number of stomachs containing the item and n is total stomachs analyzed.

- Percentage of occurrence (Ackerman, 1984) according to the equation:

$$PO = (Ni/\Sigma Ni).100,$$

where Ni represents the occurrence number of each item, and ΣNi represents a summation of occurrence of all items.

- Test T (parametric and non-parametric) were used to calculate seasonal differences in food (total number of items) and SRI (total number of stomachs). The hypotheses tested on the diet composition between seasons were: Ha = there is a significant difference in the diet composition between rainy and dry seasons; H0 = There is no difference in diet composition between rainy and dry seasons.The hypotheses tested for the SRI were: Ha = there is a significant difference in the SRI between the dry and rainy seasons; H0 = no difference in the SRI between the dry and rainy seasons.

- The Bray-Curtis coefficient was used to analyze the similarity of diet composition between dry and rainy season.

- Species accumulation curve was obtained through the Bootstrap wealth estimator based on species incidence.

- Niche amplitude was calculated using the Levins index (Krebs, 1989) equation:

$$Ba = [(\Sigma p_{ij}^2)^1-1]/(n-1),$$

where Ba is the standardized niche amplitude, p*ij* is the proportion of the volume of food item *j* in the total diet of species *i,* and n is the total number of food items consumed.

RESULTS

From July 2016 to May 2017, a total of 189 *Ocypode quadrata* stomachs were analyzed, 114 specimens collected in the dry season and 75 in the rainy season. According to the index of repletion, 17.9% were empty, 41.2% almost empty, 28.1% almost full and 12.6% full for the whole period. From these numbers, we evaluated the index of repletion through seasons (*i.e.,* rainy and dry) and compared the values (Fig. 1). Although we observed small differences in the SRI between seasons, these differences are not significant (df = 3, t = 3.182, $p > 0.05$).

From the analysis of the stomach contents, it was possible to identify 12 items composing the diet of *O. quadrata,* classified as non-food items (sand and plastic pieces) and food items (animal and plant material). The food items were identified at various taxonomic levels according to the degree of maceration of the items: Insecta, Hymenoptera, Hemiptera, Coleoptera, Crustacea, and other unidentified Arthropoda, poriferans, Actnopterygii bones, plant pieces, non-organic material (plastic), and other non-identified materials (NIM).

According to the frequency of occurrence, the most frequent item in the samples was sand at 75.4%. Arthropoda represented 51.6%, Insecta at 35.4%, followed by the other items at 30.6%, 20.9%, and 14.4% respectively (Table 1). There was a significant difference among food items consumed between the seasons (df = 22 t = 3.112, p = 0.00506). In the rainy season the frequency of occurrence of the items composing the species diet is distinctly higher (Fig. 2). This may be associated with the greater availability of these items in the area during this period of the year.

A comparison of the composition of seasonal diets performed through the Bray-Curtis similarity analysis showed that both samples from dry periods were similar

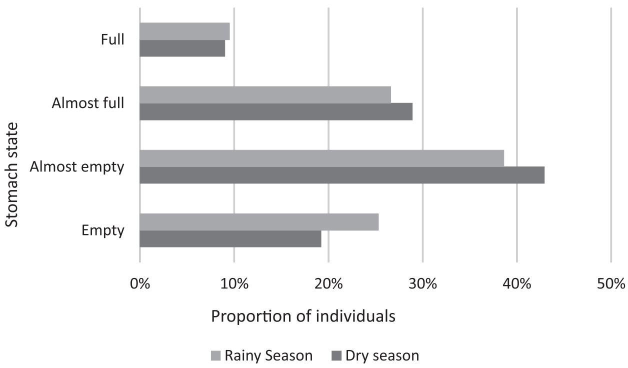

Figure 1. Percentage of individuals classified into categories according to the stomach repletion index.

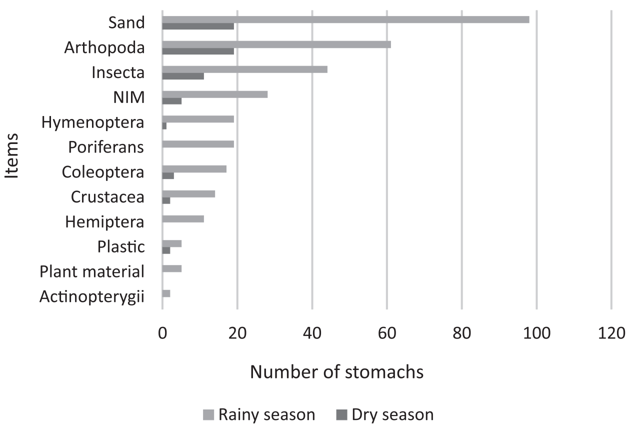

Figure 2. Consumption of items according to the number of stomachs in different seasons.

Table 1. Food items found in the stomach content of *Ocypode quadrata* (Fabricius, 1787). Number of stomachs (N) in which the food items were found, frequency of occurrence (FO) and percentage of occurrence (PO).

Food Items	FO	PO
Sand	75.4%	30.6%
Arthropoda	51.6%	20.9%
Insecta	35.4%	14.4%
NIM	21.2%	8.6%
Coleoptera	12.9%	5.2%
Poriferans	12.2%	4.9%
Hymenoptera	12.2%	4.9%
Crustacea	10.3%	4.1%
Hemiptera	7.1%	2.8%
Plastic	4.5%	1.8%
Plant material	3.2%	1.3%
Actnopterygii	1.2%	0.5%
Total		**100%**

(± 65% similarity), as were the samples from rainy season (± 60% similarity). However, the similarity between dry and rainy seasons was lower (approximately 45%), highlighting the differences in the diet between seasons. Via the similarity matrix, also performed in this analysis, the distance (or dissimilarity) was calculated that at approximately 54%, that is, the distance between the dry and rainy seasons was greater than the similarity between them (Fig. 3).

The food item accumulation curve (collector curve) shows us the level of success in the sample effort and the probability of finding new items in the diet. We found 12 items in the diet of *O. quadrata* in the four sampling periods. According to the Bootstrap non-parametric estimator, which clearly demonstrates a great tendency to stabilize the curve, a sufficient sample size was used (Fig. 4).

The Levins index for *O. quadrata* was 1.00966. The niche amplitude is expressed in a scale from 0 to 1, and an index value close or equal to 1 indicates a greater niche amplitude (generalists), while values close to 0 indicate that few types of prey are consumed at high frequency (specialists). This value demonstrates that this species is a generalist with a food habit consisting of a variety of items according to availability.

DISCUSSION

The Praia Vermelha do Norte presents a wide variety of food items available for *Ocypode quadrata,* especially insects from the sand dune vegetation and animal remains washed up on the sand during high tides. From the analysis of the stomach contents, it was possible to observe 12 food items as part of the *O. quadrata* diet. However, it was not possible to classify the items found to lower taxonomic levels (*i.e.,* genus or species level), as identifications was restricted to more inclusive taxonomic groups. That is due to the high degree of maceration of the food; the stomach of most Brachyura have a strong gastric mill (Branco & Verani, 1997).

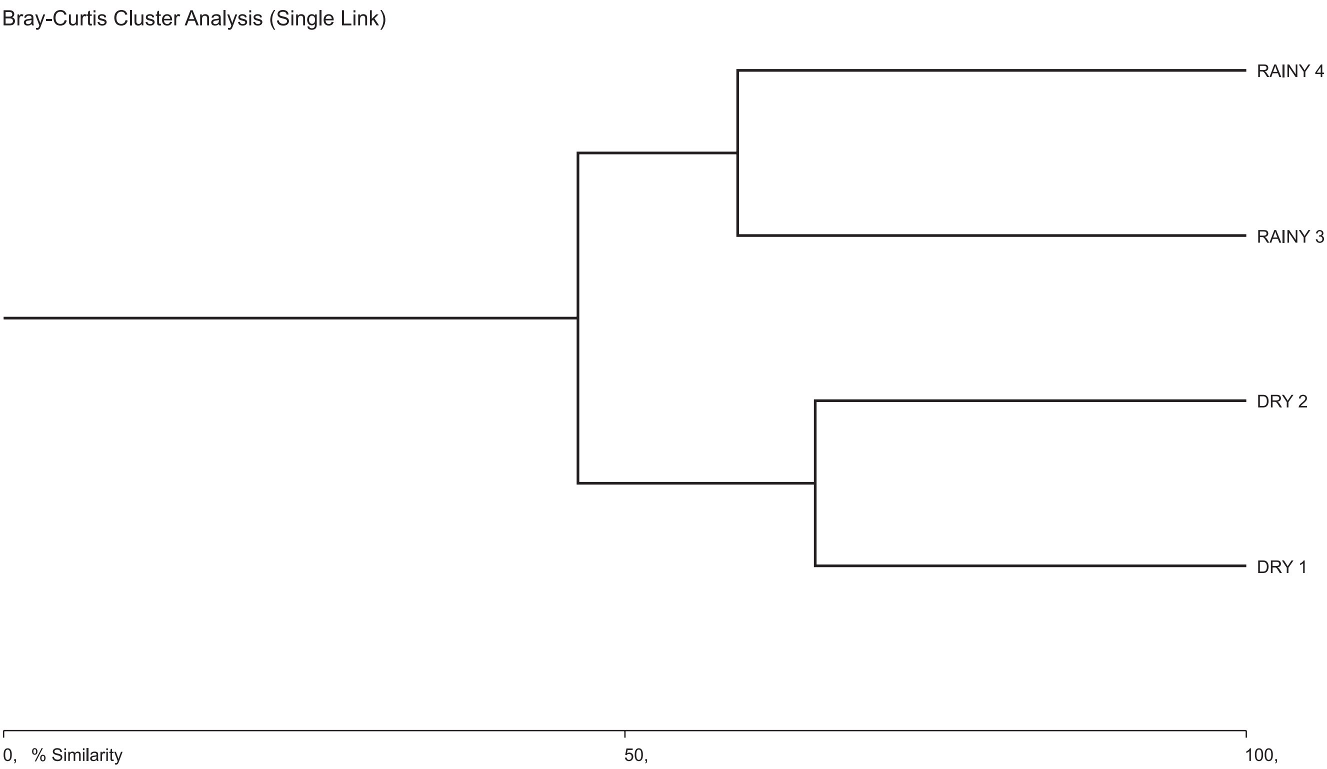

Figure 3. Dendogram of similarity of diet composition between the seasonal periods performed through the Bray-Curtis similarity analysis.

Differences in the SRI between seasons were not significant, meaning that the amount of food in the stomachs was quite similar between seasons. However, comparing the number of items, the dry and rainy seasons were considerably different. The rainy season presented the highest number of items consumed. Among climatic factors, rainfall is one of the main factors affecting the distribution and abundance of insect populations (Wolda, 1988), which are the most important items in the diet of *O. quadrata*. In the dry season, which coincides with the winter and brings lower temperatures, we observed a lower consumption of items, which was expected due to the reduced mobility of these animals during this time of the year.

Although sand had a significant frequency of occurrence (75.4%) in the stomachs analyzed, its intake was probably accidental and occurred together with other food items, since *Ocypode* collects its food mostly from the substrate. In addition, many authors point out the presence of sand in Brachyura stomachs but do not classify them as a food component, only some authors such as Haefner (1990) considered sand as a source of carbonate for crabs of the genus *Callinectes*. Other authors also attributed this item as being of intentional intak e and source of minerals to be used in the formation of a new exoskeleton (Williams, 1981).

Arthropods are the main food source for *O. quadrata,* a finding supported by previous studies from other areas

Figure 4. Curve of accumulation of food items according to the Bootstrap wealth stimulator.

of Brazil (Branco *et al.,* 2010). Unidentified Arthropoda, and the more inclusive taxonomic groups (*i.e.,* Insecta, Coleoptera, Hymenoptera, Hemiptera and Crustacea), account for 52.3% in percentage of occurrence of the diet of *O. quadrata*. These items may be associated with a higher availability and/ or the ease of capture and processing of these preys by the ghost crabs (Hillesheim, 2005).

Several authors have reported the predominance of crustaceans in the Brachyura diet, and for some of them, Crustacea is the most important group in the crab diet (Wolcott, 1978; Paul, 1981; Williams, 1981). However, for *O. quadrata* from the Praia Vermelha do Norte, Crustacea occurred at a frequency only 10.3%. Although Crustacea's contribution to the diet was lower than other items, the presence of this item was expected due to the existence of cannibalism and interspecific predation among brachyurans, especially in juvenile, ecdystic or diseased individuals (Williams, 1981).

The composition of the *O. quadrata* diet in the Praia Vermelha do Norte corresponds in great majority to animal protein and only a small percentage to vegetable matter, a value similar to other studies (*e.g.,* Wolcott, 1978). The occurrence of plant material in several species of Brachyura stomachs is usually associated with accidental ingestion, since most of them are predatory animals. Generally, their main food component is other crustaceans (Wolcott, 1978; Branco *et al.,* 2010). We recorded a 3.2% frequency of plants, a percentage similar to other crab species, which may represent selective intake from the digestive tract of its prey or incidental intake (Mantelatto & Petracco, 1997).

Non-identified materials (NIM) account for a high frequency in the stomachs of *O. quadrata* (21.2%). However, NIM can be a plethora of items with a very high degree of maceration/digestion, which potentially includes vegetal and animal remains or even non-organic materials.

Actnopterygii exhibited lower frequency (1.2%). Dead fish are commonly found on the beaches of Ubatuba, a city with high fishery activity. Saprophagic activity is common in *O. quadrata,* the remains of dead fish supply the ghost crab a good source of protein (Hillesheim, 2005).

The presence of plastic, although lower (*i.e.,* around 4% of frequency of occurrence), can be a result of the constant human presence in the Praia Vermelha do Norte. Plastic waste that accumulates in the marine environment and beaches generally fragments into smaller pieces, which increases the potential for ingestion by marine and shore animals (Browne *et al.,* 2008). The buoyancy of these small pieces of plastic increases the likelihood of their mixing with food sources available in the environment. Plastic is found in the stomach contents of several groups of marine animals, causing several problems in the biota and affecting food (Savoca *et al.,* 2017; Chatterjee & Shivika, 2019). The accumulation of non-nutritive elements in the digestive tracts of these animals can lead to malnutrition and may decrease the crab's size. Plastic was found in the stomachs of seven individuals during the samples.

The diet of decapod crustaceans, such as *Ocypode quadrata,* may vary according to availability and food search processes. Seasonal dietary variations reflect how prey availability changes throughout the year; *O. quadrata* consumes more arthropods and insects during the rainy season, which coincides with the highest incidence of these species. Thus, this study made it possible to observe the plasticity in the diet of the species and its importance in the trophic ecology of its environment.

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