A food source analysis for the swimming crab *Callinectes ornatus* (Portunidae) in Ubatuba Bay (Brazil), using carbon isotopes.

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Abstract

We describe the isotopic carbon composition of *Callinectes ornatus* Ordway, 1863 tissues and its principal prey items collected in Ubatuba Bay, Brazil. The preys were mollusk shells, echinoderms, crustaceans, squid, fish, algae and polychaetes. Hepatopancreas and ovaries presented lower values of $\delta^{13}C$ than other tissues and the food items $\delta^{13}C$ values variated from -0.84 to -17.94‰. This result differs from that of other crabs obtained in different areas, probably due to $\delta^{13}C$ values variations according the environment caracteristics. This crab is an opportunistic omnivorous with a considerably diversified diet performing an important function in the trophic chain in the Ubatuba region.

Key words: *Callinectes ornatus,* carbon, crustacean, trophic chain, Brazil, subtropical area

Introduction

The diets of the Portunidae have been especially well studied (see Mantelatto and Christofoletti, 2001 in review) because of their ecological importance in marine and freshwater habitats. In the Ubatuba region – an important area for general crustacean investigations because it represents a transition zone - *Callinectes ornatus* Ordway, 1863 has received special attention in recent years (see Mantelatto and Christofoletti 2001 for references) because it is the most abundant species in the area, representing about 60% of the total brachyuran community on non-consolidated substrates in shallow waters (Fransozo *et al.* 1992, Mantelatto and Fransozo, 2000). The success of this species in the Ubatuba region depends, in large part on the dietary habits of the crabs during the juvenile and reproductive stages. Although it is assumed that brachyuran crabs play an important role in the feeding dynamics of the mega benthos of the Ubatuba region because of their abundance Pires 1992; (Mantelatto and Petracco 1997), the data available on the natural diet of crabs are still insufficient to evaluate the trophic organization in this benthic system (Mantelatto and Christofoletti, 2001).

Ecological studies have utilized stable isotope carbon ($\delta^{13}C$) to focus on the sources and the dynamics of food chains. The $\delta^{13}C$ values of marine and freshwater animals are derived from the carbon isotope composition of their food sources (Martinelli *et al.*, 1988; DeNiro and Epstein, 1978; Fry and Sherr, 1984; Peterson and Fry, 1987; Michener and Schell, 1994). Besides, the carbon isotope technique is a quick and precise method, and the conservative transference has proved useful in a variety of trophic chain studies concerning differences in $\delta^{13}C$ from food sources (DeNiro and Epstein, 1978).

Some studies involving isotope analysis have been conducted on brachyuran, principally portunids crabs (Lacerda *et al.,* 1991, Raz-Guzman and de la Lanza, 1993; Raz-Guzman *et al.,* 1993; Raz-Guzman and Sanchez, 1996; France, 1998; Fantle *et al.*, 1999; Dittel *et al.,* 2000). However, considering the number of brachyuran species detected along the Brazilian coast, there is a severe lack of information.

The objective of the present study was to determine the carbon isotope composition of *C. ornatus* tissues and the principal prey available in its habitat area (Ubatuba Bay). This was done directly by analysis of animals and prey using $\delta^{13}C$ values.

Material and Methods

Study area and sampling - Ubatuba Bay (23° 26'S and 45° 02'W), is located along the northern coast of São Paulo State, Brazil. The mainly algae of Ubatuba region are composed by *Sargassum cymosum* Agarth, *Soliera tenera* Whynne and Taylor, *Galaxaura oblongata* Ellis and Loander, and *Callithamnium felipponei* Howe, among others (Fransozo and Mantelatto, 1998). This region has a high amount of phytoplankton that is the primary producers of the region. The total area of the bay is 8km^2, and the width is 4.5km at the entrance and decreases landward. This Bay can be divided into an inner and outer section (Costa *et al.*, 2000). The inner section is affected by direct fresh water drainage from four small rivers with subtropical forest cover and consequently receives a continuous input of domestic sewage and a considerable deposition of organic matter. The outer section is exposed to oceanic influence. The environmental factors of the Bay, obtained at bottom area, were studied by Mantelatto and Fransozo (1999) who observed a mean salinity of 33.2 ± 0.35‰ during the study period after present work. There was no significant difference among the sites sampled, but variations in mean salinity were recorded over the months. The annual mean temperature was 23.8 ± 0.62°C, and there was no significant difference in water temperature among the sites sampled. The mean values of organic matter content of the sediment (11.3 ± 5.71%) did not differ significantly throughout the study period, but differed between the sites sampled.

Specimens were collected from different subareas in the Bay, chosen according to trawler viability and to the principal sites of occurrence of *C. ornatus* within the bay (Mantelatto, 2000). Crabs and prey were collected during daylight with double-rigged trawl nets (3.5 m wide at the mouth, 10 mm mesh size at the cod end) from June to September 1999.

The food items with more than a 20% rate of occurrence in the natural diet of *C. ornatus* reported for this bay (Mantelatto and Christofoletti, 2001), *i.e.* crustaceans, mollusks, echinoderms, fishes, polychaetes and algae, were separated for individual isotope analysis. Digested material and sediments were not included in the analysis. Immediately after capture, all crabs and prey were placed on ice. About 2 h after collection, all individuals were stored at -15°C. In the laboratory, all analyses were carried out at room temperature after thawing the material.

Isotope analysis – Five tissues (muscle, hepatopancreas, testis, ovary and ocular peduncle) of 20 males and 20 females of *C. ornatus* of adult specimens (carapace width > 45 mm) of different sizes (intermolt stage and non ovigerous conditions) were analyzed separately as a function of the differences in isotopic composition. The same tissues from different individuals were pooled for analysis to avoid individual differences (DeNiro and Epstein, 1978). All prey and tissues were dried at 60°C for 72 h and macerated before the isotope analysis. Parts of the prey (*i.e*, muscle, legs, arms), not including stomach parts, were utilized in this analysis and no acidification was performed for calcium carbonate before analysis.

The isotope analyses were performed following the method of Victória *et al.* (1992) and Forsberg *et al.* (1993), with replicates and 0.3‰ of maximal analytic variation. The carbon isotope composition of the samples was determined by combustion in an Elementary Analysis equipment Carlo Erba (CHN-1110) attached to a Mass Spectrometer Finnigan Delta Plus. The pattern was based on calcareous rock (PDB) from the PeeDee formation (USA), and the results are reported as $\delta^{13}C$ values (‰). Details on sample treatment and reagents used here have been described by Martinelli *et al.* (1988).

Results and Discussion

The food items presented a higher variation in the $\delta^{13}C$ values (-0.84 to -17.94‰) than the tissues of *C. ornatus* (-13.75 to –18.62‰). The values of $\delta^{13}C$ samples are listed in Table I.

The isotope values of prey items were similar to those obtained in other investigations with a $\delta^{13}C$ variability between -6.4 and -29.4‰, and peaks from -13 to -25‰ (Lacerda *et al.,* 1986, Lacerda *et al.* 1991; Raz-Guzman and de la Lanza, 1991; Raz-Guzman *et al.* 1992; Ráz-Guzmán and de la Lanza, 1993; Raz-Guzman *et al.,* 1993; France, 1998; Fantle *et al.,* 1999), and corroborated the higher trophic level of *C. ornatus* in the communities of Ubatuba Bay.

Table I: Isotopic composition ($\delta^{13}C$ - ‰) of food items and tissues of *C. ornatus* from Ubatuba Bay.

Group	Material analyzed	$\delta^{13}C$
Crab tissues	Muscle	-14.48
	Ocular peduncle	-13.75
	Hepatopancreas	-18.62
	Testis	-15.21
	Ovary	-17.27
Food items	Mollusks - Bivalves (*Perna perna*) and gastropod shells (*Stramonita haemastoma*)	-0.84
	Mollusks - Squid (*Loligo brasiliensis*) (whole body)	-17.94
	Crustaceans - Shrimp (*Xyphopenaeus kroyeri*) (whole body)	-16.87
	Echinoderms - Sea star (*Luidia senegalensis*) (whole body)	-11.19
	Fishes - (*Paralonchurus brasiliensis*) (whole body)	-17.38
	Algae - (*Sargassum cymosum*)	-17.78
	Polychaetes - (*Phragmatopoma lapidosa*)	-16.48

None of the cited references presented the isotopic characterization of shells. There is a report for marine mussels by Fantle *et al.* (1999), with $\delta^{13}C$ values between -14.6 and -18.5‰, and by Newell *et al.* (1995) on muscle of gastropods and bivalves with values between -26.01 and -17.78‰. These values correspond to protein from viscera, and the calcium carbonate of the shells probably has values of $\delta^{13}C$ close to 0‰.

The general $\delta^{13}C$ value for *C. ornatus* tissues was different from that detected in other crabs (Table II). This variation resulted from the different food chains of the studied areas. The lowest and highest $\delta^{13}C$ values were registered for crabs from mangrove and marine areas, respectively. The vegetation influence can be considerate. The vegetation and related organic matter from mangrove areas presents C3 photosynthetic cycle (Calvin cycle for soil plants). In fresh water conditions, *i.e.* low salinity, the photosynthetic substrate (HCO_3) exhibited $d^{13}C$ from -5 to -10‰. Consequently, the lowest $\delta^{13}C$ values registered for crabs from mangrove. In another way, marine vegetation presents, in all almost cases, C4 photosynthetic cycle (Hatch-Slack). During high salinity conditions, the photosynthetic substrate (HCO_3) exhibited $\delta^{13}C$ close to 0‰. Consequently, the highest $d^{13}C$ values registered for crabs from marine areas.

According to France (1998), the high values for marine species may result from the diminishing out-welling of respiratory CO_2 that is produced by the heterotrophic activity associated with decomposing mangrove detritus. Despite this information, a comparative analysis with different species is difficult because of the lack of information about the type of tissues analyzed in the cited studies. Also, the assimilation of $\delta^{13}C$ can be different in each tissue (DeNiro and Epstein, 1978 and 1981), as confirmed in the present study (Table II).

The enrichment of $\delta^{13}C$ values in *C. ornatus* may be a result of the natural characteristics of Ubatuba Bay. This area presents high water dynamics, with the influence of three important currents (Sumida and Pires-Vanin, 1997) causing a shallow water area. Besides, differences between areas should also be considered. Raz-Guzman and Sanchez (1996) found strong variability in carbon isotopes between sites from the same area that was attributed to the physical complexity of habitats, such as organic matter and primary producer composition and salinity. These factors were not analyzed in the present study, but organic matter has been reported to be frequently present at high frequency in the natural diet of *C. ornatus* (Mantelatto and Christofoletti, 2001), and the isotopic composition of this organic matter could be responsible for these differences.

The higher $\delta^{13}C$ values (-8.1 to -8.65‰) found in *C. sapidus* from Términos Lagoon (México) by Raz-Guzman and de la Lanza (1993) were attributed to the differences on diet of the components throughout the food chain. Raz-Guzman *et al.* (1993) reported a reduction in $\delta^{13}C$ values in *C. sapidus* as a function of the increase in animal size, as well the salinity, $\delta^{13}C$ of HCO_3, $\delta^{13}C$ of prey, and vegetation origin. Another hypothesis concerns the difference in the ingestion of prey with calcium carbonate, resulting in increased $\delta^{13}C$ values. This condition was observed in the present study, and was confirmed by the natural diet of *C. ornatus* determined by Mantelatto and Christofoletti (2001) studied in this bay. Also is important to note that different species of crabs may simply have different isotopic signature regardless on environmental differences.

Table II: Isotopic composition ($\delta^{13}C$ - ‰) of brachyuran species (* the mean values were calculated from data of different subareas studied by the authors cited).

Species	$\delta^{13}C$ *	Local	Authors
Aratus pisonii (H. Milne Edwards, 1837)	-21,5	Sepetiba Bay (Brazil)	Lacerda *et al.* (1991)
Uca vocator	-21,0	Joyuda Lagoon (Porto Rico)	France (1998)
C. sapidus	-8,7	Términos Lagoon (Mexico)	Raz-Guzman and de la Lanza (1993)
C. sapidus (juveniles)	-8,1		
Callinectes similis Williams, 1896	-18,4	Alvarado Lagoon (Mexico)	Raz-Guzman *et al.* (1993)
C. sapidus	-22,3		
Callinectes rathbunae Contreas	-24,1		
C. sapidus (megalopa)	-19.3	Delaware Estuary (USA)	Fantle *et al.* (1999)
C. sapidus (crab I)	-17.1		
C. sapidus (juveniles)	-15.9		
C. ornatus	-13.75 to -18.62	Ubatuba Bay (Brazil)	present study

Callinectes ornatus is an opportunistic omnivorous swimming crab, with a considerably diversified diet. It tends to prey on animals, especially crustaceans and mollusks. This habit was confirmed by the stable isotope analysis, which showed a considerable fidelity between the isotopic composition of crabs and that of their food sources. Our results offer evidence that Ubatuba Bay provides a source of food that supports early growth, development and reproduction for this species, as proposed by Mantelatto (2000). Despite no published data on source of food in this bay (*i.e.*, primary source, macro and micro invertebrates and plankton diversity), personal observation on local fauna and flora was made during the last ten years in function of our long-term effort to study crustacean decapod ecology. In this way, carbon isotopes represent an important tool for diet analysis. Of particular interest are predatory species that may provide top-down effects on the community structure of the Ubatuba region. We recommend that information be provided on different types of tissues analyzed in future studies in order to facilitate comparative analysis.

Acknowledgements

RAC received a Scientific Initiation Grant from FAPESP (Grant # 98/05470-3). FLM is grateful to FAPESP (Grant # 95/02833-0) for financial support during the sampling work and to CNPq (Grant # 300279/95-7) for a research scholarship. Special thanks are due to Daniela Mariano Silva for help with isotope analysis. We are grateful to Andrea Raz-Guzman (UNAM – Mexico) and anonimous referee for useful suggestions and corrections on an early version of the manuscript. All experiments conducted in this study comply with current applicable state and federal laws.

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Received: 31th Oct 2001
Accepted: 15th Jan 2002