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Trophic analysis of female mangrove crabs at two sites from Southeastern Brazil (Rio de Janeiro)

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Abstract

The mangrove crab *Ucides cordatus* (Linnaeus, 1763) is a burrowing crab with an important role in mangrove nutrient cycling. The species holds major socioeconomic importance, generating yield for traditional and low-income populations. Despite its ecological and economic importance, there are few experiments in Brazil applying stable isotope tools to trophic crab classification, and even fewer considering females in the reproductive period. Females have different energy demands than males; studies examining C and N can reveal details regarding the differences. Hence, the present study is the first analysis of the $\delta^{13}C$, $\delta^{15}N$, and the C/N ratio in ovigerous females of two populations from Southeastern Brazil (Caceribu River - Guanabara Bay and Gargaú mangrove - Paraiba do Sul River secondary estuary). The Caceribu mangrove is larger and is located in one of the most impacted bays in the world. In the Paraiba do Sul River region, the mangrove swamp is about ten times smaller, has a substantially lower population in the surrounding area, and is more influenced by agricultural activities. The $\delta^{13}C$ analysis confirmed mangrove leaves as their main food source. The significant $\delta^{13}C$ variation between the Guanabara and Paraiba do Sul estuary populations can be related to differences in food availability and nutritional value. However, the $\delta^{15}N$ values can be related to the ingestion of other food items during the breeding season as well as the influence of anthropogenic mangrove degradation. At both study sites, the δ15N values were higher than those observed in other mangrove crab populations and other herbivorous species. Significant ($p < 0.05$) isotopic differences were found among populations, suggesting variations in biogeochemical cycles that may be related to different environmental conditions between the sites.

Descriptors: Estuary, $\delta^{13}C$, $\delta^{15}N$, Feeding habit; *Ucides cordatus*.

INTRODUCTION

Ucides cordatus (Linnaeus, 1763) (Crustacea: Decapoda: Ocypodidae) is an endemic crab from mangrove areas, known as the ‘mangrove crab’ or ‘uçá crab’. Species occurence extends from Florida in Southeastern United States to Southern Brazil (OBIS, 2020); the species is commonly used for human consumption. On the Brazilian coast, *U. cordatus* fishing is of considerable socioeconomic importance, generating yield for traditional and low-income populations. In some regions, crab collecting is the main economic activity for most women in the riparian area (Magalhães et al., 2007).

The mangrove crab digs burrows in the intertidal zone and feeds mainly on fallen mangrove leaves (Nordhaus et al., 2009). According to Nordhaus

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and Wolff (2007), stomach contents also include roots, sediments, bark, and animal remains. The species presents a long and slow life cycle (about 17 years), low mobility in adulthood, and is indicated as an important agent of bioturbation (Pinheiro et al., 2005; Nordhaus et al., 2006). Reproduction occurs in the summer, and the females produce up to 220,800 eggs (Pinheiro et al., 2003). This crab is considered a keystone species in the mangrove, with an important role in nutrient cycling, and is indicated as a biomonitor of environmental pollution (Schories et al., 2003; Nordhaus et al., 2007; Almeida et al., 2016). Bioturbation by crabs affects the nutrients and physicochemical parameters of mangrove soil. Otero et al. (2020) showed lower nitrous oxide (N_2O) emission in soil naturally bioturbated by crabs compared to crab-exclusion areas.

Despite the mangrove crab's ecological and economic importance, few studies on stable isotopes have been conducted with this species, and data on the females are scarce. Authors such as Guerao et al. (2016) reported differences in isotopic composition between males and females of some crab species. Other authors did not find fractionation differences between sexes (Bodin et al., 2007), or between ovigerous and non-ovigerous females (Herbon and Nordhaus 2013). In the mangrove environment, isotopic analyses have been employed in food web studies, as well as in studies tracing the impact and species affected by anthropogenic pollution (Costa et al., 2018; Fragoso et al., 2018; Souza et al., 2018). The ^{13}C and ^{15}N isotopes have been used to identify carbon sources and trophic levels, respectively (Fry, 2006). Besides, stable isotopes such as ^{13}C and ^{15}N can be coupled to the C/N ratio to improve understanding of biogeochemical processes in an environment (Shilla and Routh, 2017). During crab life-history stages, the $\delta^{13}C$, $\delta^{15}N$ signature can change (Dittel et al., 2006). Knowing the variation of the isotopic signature throughout the entire life cycle is an important tool to assist in the management and conservation of species. Thus, this study investigated, for the first time, the $\delta^{13}C$, $\delta^{15}N$, and C/N ratio signals in *U. cordatus* ovigerous females.

METHODS

Study Sites

This study was conducted in two mangroves in Southeastern Brazil with markedly different environmental conditions, including floristic composition and level of anthropogenic impact. The study sites were Caceribu River mangrove (22°43'32.55"S; 43°00'48.03"W), in the Guapi-Mirim Environmental Protection Area - Guanabara Bay (GB), and the Gargaú mangrove (at 21°36'18.78"S; 41°03'12.65"W), Paraiba do Sul River secondary estuary (PSR), both in Rio de Janeiro state, Southeastern Brazil (Fig. 1).

Guanabara Bay is a highly eutrophic estuary that receives a large discharge of industrial and domestic sewage (Costa et al., 2018; Cotovicz et al., 2019). This bay has presented water and sediment contamination by toxic metals, hydrocarbons, nutrients, and pathogenic organisms, as well as changes in pelagic and benthic communities, since the middle of the last century (Soares-Gomes et al., 2016). Moreover, Guanabara Bay is surrounded by the metropolitan region of Rio de Janeiro, with about 11 million people and a large industrial center. The Guapi-Mirim Environmental Protection Area and the Guanabara Ecological Station are located in the eastern part of the bay, as where the Guapi-Macacu, Guaraí, Guaraí-Mirim, Guaxindiba/Alcântara, and Caceribu rivers flow. In these protected areas, the mangroves cover about 80 km^2. Densely populated cities with poor effluent treatment are located upstream of these rivers. At the site where the crabs were collected, there is a notable tidal influence, and saline intrusion can be significant during spring tides. Wasserman et al. (2006) showed higher hydrocarbon contamination in the sediments from the Guapi-Mirim EPA, with homogeneous Total Carbon (TOC) distribution in sediments in all areas (mean 22.2±7.3%, n=23). The sediments from this location are composed of fine grained mud (Wasserman et al., 2006). During our study, we observed salinity levels of 12.0 to 22.0 in surface waters of the Caceribu River and secondary channels.

The other sampling site, the Paraiba do Sul estuary, is classified as moderately mesotrophic to eutrophic (Borges, 2014). The upper portion of the Paraiba do Sul River watershed is highly urbanized and industrialized, and home to millions of people. But in the lower basin sector, where we conducted our study, extensive farming prevails, especially sugar cane and pineapple production. Compared to Guanabara Bay, this site is less urbanized (about 530,000 residents in the surroundings), and the mangrove is smaller (about 8.0 km^2) (Molisani

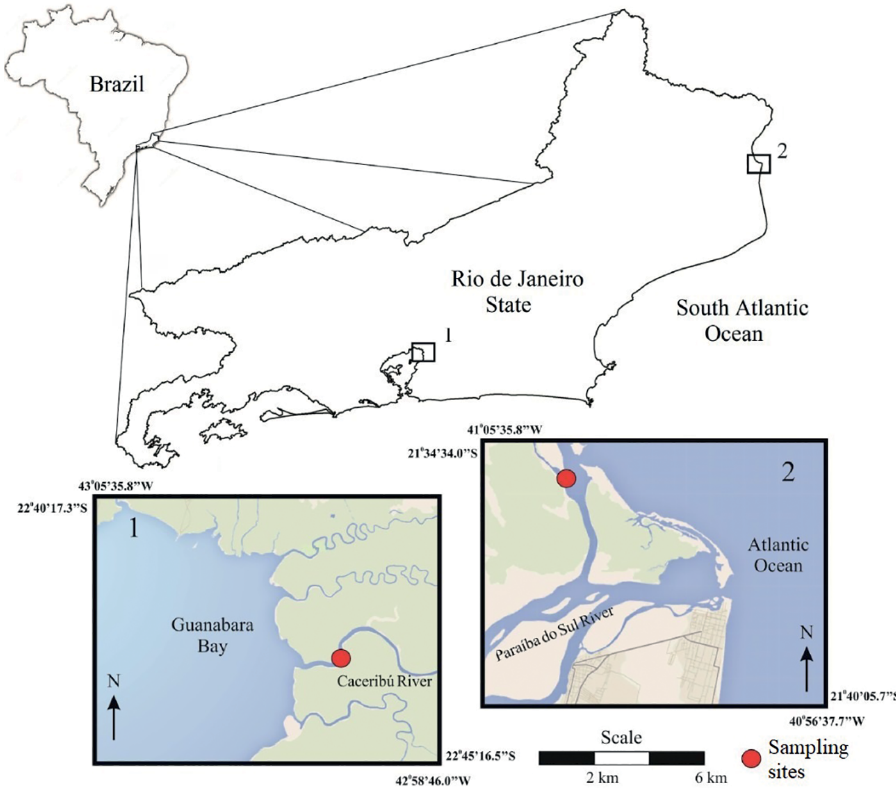

Figure 1. Sampling sites of the ovigerous females of U. cordatus crab: 1. Caceribu River mangrove - Guanabara Bay (GB) and 2. Gargaú mangrove - secondary estuary of Paraiba do Sul River (PSR). Modified from Almeida et al. (2016).

et al., 1999). As an effect of the many dams built along the river, water volume decreased in the last few decades and sediment exportation was reduced. Most of the year, this estuary is homogeneous, with a predominance of freshwater (Molisani et al., 1999). During our sampling, salinity in PSR and the mangrove channels varied between 0 and 8.0 in surface waters. In Gargaú mangrove, the sediments were predominantly composed of silt and clay (>60%). The organic carbon (range 2.7 to 32.7 %) and nitrogen (range 0.14 to 1.22%) were heterogeneously distributed (Fragoso et al., 2018).

In the Caceribu River mangrove (GB), the sampling area presented a predominance of *Rhizophora mangle L*. In the Gargaú mangrove (PSR), crabs were collected in a site with predominance of *Avicennia germinans L*.

Crab sampling and analyses

Crabs were obtained with the same field efforts of Almeida et al. (2016), a study about trace elements contamination that only sampled female mangrove crabs. Our study used 10 ovigerous females, 5 from each study site, with carapace widths between 6.0 and 7.0 cm, and collected under license 27753-3 (MMA/ICMBio). In each study area, the females were collected in the same place, by traps in an approximate area of 2,500 m^2 (50 x 50m). Immediately after the collection, animals were washed to remove mud and individually frozen at -18° C until analyses were carried out. Carapace width was measured with a caliper (±1.0 mm).

Crabs were defrosted and part of the chelipeds muscles was lyophilized for posterior assessments of total organic carbon, total nitrogen, and stable isotopes ($\delta^{13}C$, $\delta^{15}N$). The measurements were performed in an ANCA-GSL elemental analyzer, connected to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK), at the University of California, Davis, in the Stable Isotope Facility.

To assess differences in $\delta^{13}C$, $\delta^{15}N$ and C/N ratio signals between the two sampling sites, first we performed assumption checks on the homogeneity of variances (Levene's test) and normality (Shapiro-Wilk test). When data did not show homogeneity and normality, non-parametric tests were performed (Mann-Whitney U test). When the opposite occurred, Student's t-tests were applied. In all cases, results were considered significant at the 0.05 level.

To determine the food resource, the isotopic signature in crab tissue was compared to previous studies of isotopic signatures in sediments, mangrove leaves, and particulate organic matter (Fragoso et al., 2018; Monteiro et al., 2012; Carreira et al., 2002; Kalas et al., 2009).

RESULTS

The total organic C was slightly higher in Gargaú mangrove (PSR), but the C/N ratio was similar at both site (Tab. 1). At both study sites, $\delta^{13}C$ indicates herbivory (Fig. 2). However, the values showed significant variation among populations ($p < 0.05$). $\delta^{15}N$ values from both sampling areas were similar, indicating a similar trophic position (Fig. 2).

DISCUSSION

The range of $\delta^{13}C$ found in the present work (-25.6 to -24.4 ‰) was similar to observations from other Brazilian populations of *U. cordatus* and to the *Episesarma versicolor* (Tweedie, 1940) Malayan crab, which eats predominantly *Rhizophora* sp. leaves (Kristensen et al., 2010). Although $\delta^{13}C$ levels indicate herbivory in our two study areas, we detected a significant variation among $\delta^{13}C$

Table 1. Mean, standard deviation, range of isotopic values, C and N contents in the muscles of *U. cordatus* females from the Caceribu mangrove (Guanabara Bay), from the Gargaú mangrove (Paraiba do Sul River), and other Brazilian mangroves. Where Gender: F - female, nd - not determined.

Site (number of samples)	Environmental conditions	Gender	Mean ± SD (min-max)					Ref.
			$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C (%)	N (%)	C/N ratio (molar)	
Caceribu mangrove (n= 5) SW Brazil	Highly impacted: domestic and industrial sewage	F	24.6±0.14 (-24.4 to -24.8)	8.3±0.17 (8.2 to 8.7)	38.5± 2.31 (34.7 to 40.8)	11.4±0.67 (10.2 to 12.2)	3.94±0.09 (3.81 to 4.06)	Present study
Gargaú mangrove (n=5) SW Brazil	Moderately impacted: farming and domestic sewage	F	25.5±0.06 (-25.5 to -25.6)	8.7±0.59 (7.8 to 9.6)	41.1±1.52 (38.2 to 42.6)	12.2±0.45 (11.5 to 12.9)	3.91±0.05 (3.85 to 3.97)	Present study
Mãe Bá Lagoon (n=1) SW Brazil	Lowly impacted, sewage from iron ore mining and pelletizing plant	nd	-27.7	6.0	-	-	-	Pereira et al. 2010
Jaguaribe estuary, Ceará (n=39) NE Brazil	Highly impacted, shrimp farms and agricultural sewage	nd	-25.8±0.5 (-25.0 to -27.3)	7.1±0.7 (5.4 to 8.6)	-	-	-	Pereira et al. 2019
Pacoti mangrove, Ceará (n=39) NE Brazil	Pristine área	nd	-24.5±0.6 (-23.5 to -25.5)	3.0±1.5 (0.5 to 6.0)	-	-	-	Pereira et al. 2019
Curuçá mangrove, Pará (n=2) N Brazil	Pristine área	nd	-24.8±1.0	5.0±1.0	-	-	-	Giarrizzo et al. 2011

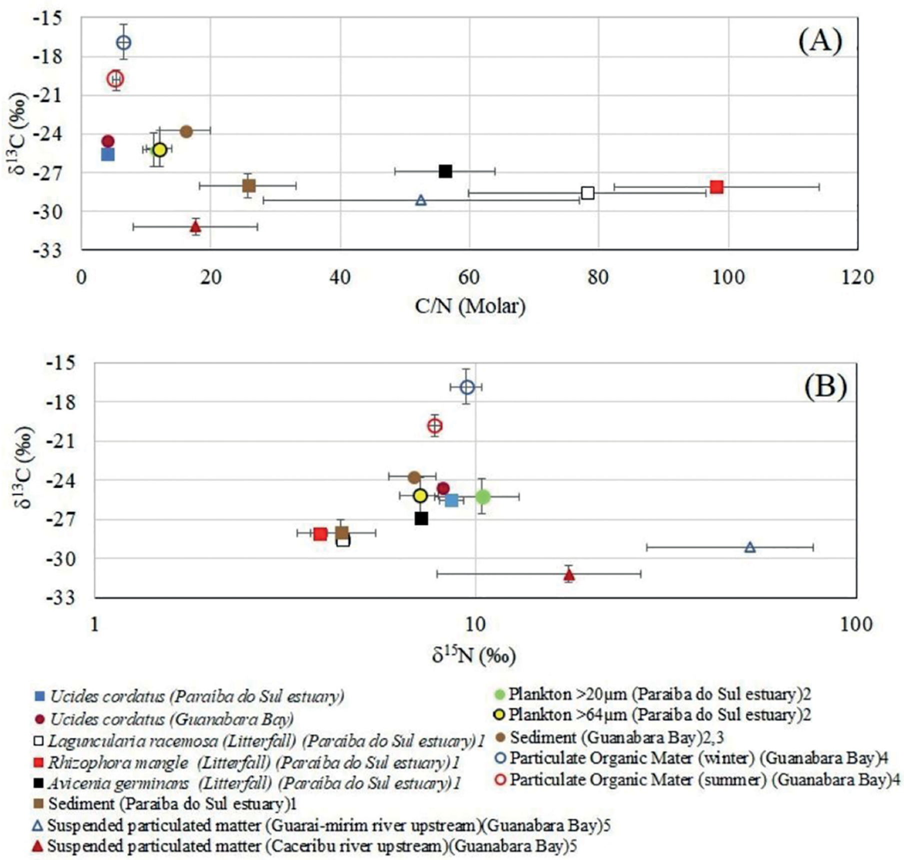

Figure 2. Distribution of $\delta^{13}C$ and C/N ratio (A), and of $\delta\ ^{13}C$ and $\delta\ ^{15}N$ (B) in Guanabara Bay (GB) and Paraiba do Sul River (PSR), Southeastern Brazil. Sources: 1. Fragoso et al. (2018); 2. Monteiro et al. (2012); 3. Carreira et al. (2002); 4. Kalas et al. (2009).

values and postulated some hypotheses. The first is linked to variations in the floristic composition of the sampling areas. In the PSR sampling location, we observed a predominance of *A. germinan*, while in the Caberibu mangrove (GB), *R. mangle* predominates. Due to the lack of data about the feeding habits of the mangrove crab in these areas, we did not categorically postulate the food preference.

The mangrove crab can feed on several types of mangroves, such as *R. mangle* and *A. germinans*, but also *Avicennia schaueriana* Stapf & Leechman ex Moldenke, *Laguncularia racemosa* (L.) C.F. Gaertn., and others, mainly due to its ability to digest tannins, as indicated by Nordhaus and Wolff (2007). These authors reported *R. mangle* as the preferred food in a population of crabs in northern Brazil. According to them, *U. cordatus* prefers *R. mangle* to *A. germinans*, even though the latter presents higher nitrogen content, a lower C/N ratio, and a lower concentration of tannins. The authors noted that the assimilation rates of *R. mangle* leaves were higher and that the composition of the crab feces indicated more difficulty in mastication and mechanical digestion of *A. germinans* leaves.

Regarding the disparities among populations, previous studies have pointed out differences in $\delta^{13}C$ between crabs from different mangroves (Giarrizzo et al., 2011). Christofoletti et al. (2013) stated that food selection among mangrove species can vary depending on environmental conditions and on the age of the crab. While studying the fiddler crab *Minuca pugnax* (Smith, 1870) (detritivore), Haines (1976) found linear correlations between vegetation type and $\delta^{13}C$ values. This species belongs to the same family as *U. cordatus* and lives in intertidal zones. The author observed variations in $\delta^{13}C$ among food sources, directly affecting the values observed in crabs. The publications of Doi et al. (2005) and Bodin et al. (2007) with coastal crabs also reported differences in $\delta^{13}C$ among individuals of the same species but from different habitats.

In mangrove trees, the production of phenolic compounds and tannins can be induced by pollution, with an increase of these compounds in metals and hydrocarbon contaminated environment (Jiang et al., 2017). The mangrove sediments of Guanabara Bay are more contaminated than Paraiba do Sul secondary estuary, and indicating they may have more phenolic compounds and tannins in the mangrove leaves. Lacerda et al. (1986) found a higher quantity of phenolic compound in *L. racemosa* from Catalão Island in Guanabara Bay in comparison to a lower polluted site. Furthermore, Numbere and Camilo (2017) reported a decrease in leaf decomposition in sediments contaminated by hydrocarbons compared to uncontaminated sites.

The lower $\delta^{13}C$ seen in Paraiba do Sul female crabs may also be explained by the more evident influence of freshwater in this region. Due to the decomposition of organic matter both from the drainage basin and originating in situ, freshwater environments are richer in dissolved carbon species (DOC). As a result, maximum fractionation occurs between the cells and the carbon source (18 to 19 ‰) (Degens et al., 1968). Beside this, the stomatal conductance of CO_2 is affected by environmental conditions, such as soil salinity and humidity, and air temperature. This conductance affects the concentration of CO_2 inside the leaf in C3 plants, including mangrove trees, and has an influence on the fractionation of stable isotopes (Whooler et al., 2003). Marques et al. (2017) demonstrated high exportation of dissolved organic carbon (at a range of 72 to 529 µmol L^{-1}) and dissolved black carbon (5 to 35 µmol L^{-1}) in the Paraiba do Sul River. In freshwater environments, both the particulate and dissolved organic matter reflect the soil and plants in the drainage basin. Lower $\delta^{13}C$ values were observed both in the present study (in PSR) and in Mãe Bá Lagoon, Southeast Brazil (Pereira et al., 2010), both environments with lower salinities. Table 1 shows a tendency for lower values of $\delta^{13}C$ in *U. cordatus* from areas with more significant freshwater influence (Jaguaribe estuary, Mãe Bá lagoon, and Paraiba do Sul estuary) when compared to more saline environments.

The $\delta^{15}N$ values observed in the present study were above those observed in other *U. cordatus* populations from Brazil and in other herbivorous crabs, such as *N. versicolor* (6.7 to 7.6) (Kristensen et al., 2010). $\delta^{15}N$ values were closer to those observed in secondary consumer crabs, such as *Goniopsis cruentata* (Latreille, 1803) (9.2 ± 0.7), *Eurytium limosum* (Say, 1818) (10.0 ± 0.3), and in predators/ necrophages such as the swimming crab *Callinectes bocourti A.* Milne-Edwards, 1879 (9.3 ± 0.4) (Giarrizzo et al., 2011). A possible explanation for that difference is linked to the analysis of females in the reproductive season, which may present different assimilation rates when compared to males or non-reproductive females. As stated earlier, the metabolism in females is slightly different than in males, and these differences are probably accentuated in reproductive periods.

In Northeast Brazilian mangroves, Pereira et al. (2019) observed lower nitrogen assimilation in *U. cordatus* when compared to the crab *G. cruentata* (mean 11.75 ± 0.83%). These authors attributed the difference to the faster growth of *G. cruentata*, as opposed to the lower growth rates of *U. cordatus*. If we consider that females of *U. cordatus* need to assimilate more C and N during the reproductive season, this could explain our higher values of $\delta^{15}N$. Egg production explains this increase in demand, especially when in large quantities such as is observed in *U. cordatus*. As previously mentioned, Nordhaus and Wolff (2007) detected the ingestion of other types of food besides mangrove leaves. These authors pointed out that algae ingestion can supplement the diet. However, females may also increase their ingestion of other types of food, such as small invertebrates, including mollusks and other crustaceans. López and Conde (2013) observed, for instance, that the mangrove tree crab *Aratus pisonii* is mainly herbivorous - eating live leaves, but using alternative food resources when the quality of foliar resources decreases. These authors observed cannibalism through detection of conspecific eggs in adult stomachs, mainly in females from mangroves under conditions of hypersalinity and low leaf quality.

On the other hand, Herbon and Nordhaus (2013) found no difference between ovigerous and non-ovigerous females of the mangrove crabs *E. versicolor* and *Episesarma singaporense* (Tweedie, 1936). These authors proposed two possible explanations: 1 - Egg incubation time is shorter than the time it takes to renew muscle tissue and any possible differences in metabolism have not yet been reflected in the females' isotopic composition and 2 - Ovigerous crabs balance the discharge of light nitrogen (^{14}N) in their eggs by the selective capture of specific food items. The latter explanation fits with our hypothesis of changes in the feeding habits of *U. cordatus* females during the reproductive season.

Table 1 shows that $\delta^{15}N$ tends to have more positive values for *U. cordatus* in areas that suffered more anthropogenic influence (Jaguaribe estuary, Guanabara Bay, and the Paraiba do Sul estuary) when compared to more preserved environments. Almeida et al. (2016), in a study on trace-element contamination performed in the same sites as the present study, found higher contamination rates in ovigerous females from the Paraiba do Sul River. Their Cd, Cr, Mn, Ni, and Cu concentrations in the hepatopancreas were higher in comparison with the Guanabara Bay females. Other studies pointed out the influence of environmental contamination in isotopic composition; differences among populations, sexes, and life cycles can be employed to understand the foraging strategies associated with seasonal cycles and environmental changes, including degradation (Mackenzie et al., 2020). Pereira et al. (2019) studied the different food sources and partitioning in two crab species from Northern Brazil; in the areas where the litterfall presented the highest C/N ratios, they observed that *U. cordatus* and *G. cruentata* ingested larger proportions of soil and small invertebrates to meet their nutritional needs. This may be another possible explanation for the high $\delta^{15}N$ values we found, considering that the two areas in our study have suffered an anthropogenic impact.

The current results of the research in Guanabara Bay and Paraiba do Sul River can serve as a baseline for future studies, as degradation of the two sites is expected to continue to increase over time. We strongly recommend further analysis of stable isotopes in *U. cordatus*, with females and males, in the reproductive periods and between them as well as increasing the sample size and using multiple sampling sites within each mangrove.

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AUTHOR CONTRIBUTIONS

E.V.A.: Conceptualization, Data curation, Formal analysis, Writing - original draft;

V.T.K.: Conceptualization, Data curation, Methodology, Writing - review & editing;

E.V.S.F.: Resources, Methodology, Project administration, Supervision, Writing - review & editing.

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