



Is there an indication of the origin of nutrient supply in different morphological structures of macrofauna at two different Brazilian southeastern sandy beaches? Comparison by C and N stable isotopes

Tito C. M. Almeida¹ · Pedro F. P. Rocha¹ · Ilana R. Zalmon² · Marcelo G. Almeida² · Carlos E. Rezende² · Claudemir M. Radetski¹

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Abstract

The goals of this study were to analyze if there is a difference in the stable isotopic ratio ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of macrobenthic species sampled at two sandy beaches (one close to a river mouth and the other far from any freshwater input) and to identify differences in the stable isotopic ratio ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in different body parts of three representative species of two Brazilian sandy beach macrofaunas: the polychaete *Hemipodia californiensis*, the mollusk bivalve *Donax hanleyanus*, and the crustacean decapod *Emerita brasiliensis*. No significant differences were detected in the $\delta^{13}\text{C}$ stable isotopic ratio between the two sites analyzed; however, in the $\delta^{15}\text{N}$ stable isotopic ratio, a significant difference was observed. Regarding the intraspecific response of stable isotopic ratio, *D. hanleyanus* showed a significant difference in carbon among different body part structures, while a trend for significance was observed for nitrogen isotopes. The differences were significant for both isotopes in *E. brasiliensis*, and no differences were observed among the body part structures in *H. californiensis*. There were significant differences in *E. brasiliensis* carapaces with regard to the $\delta^{15}\text{N}$ stable isotopic ratio between the muscle and the whole body. Although the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopic ratio differs significantly in the digestive tract, muscles, and whole body of *D. hanleyanus*, such differences were not enough to determine changes in their trophic levels and food sources. Similar stable isotopic ratios were observed in the whole body, proboscis, and teeth of *H. californiensis*, highlighting this species as the top predator. In conclusion, stable isotopic analysis of benthic trophic structure can be employed as a tool in coastal management plans or environmental impact studies.

Keywords Stable isotopes $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ · Macrofauna morphological structure · Sandy beach · Beach ecology · Trophic network

Introduction

The composition and structure of coastal ecosystems have been changing in the last few decades due to the increase of

anthropogenic activities, global climate change, and settlement of invasive species (UNEP 2002; Liao et al. 2015). The presence of the macrobenthic community in coastal ecosystems is recognized by the fundamental role of environmental processes, such as sediment remobilization and biomass availability to higher trophic levels. However, macrobenthic ecology can be modified by multiple sources of environmental change, including the origins of nutrient supplies. Thus, understanding the trophic structure of sandy beaches is an important issue for management actions in these areas (Corbisier et al. 2014; Linden 2017; McLachlan and Brown 2006; Thrush et al. 2006).

Stable isotope analyses are a good laboratory tool in physiological and trophic ecology studies (Olsen et al. 2013), and the common isotopes are usually carbon (^{13}C) and nitrogen (^{15}N) (Manetta and Benedito-Cecilio 2003; Michener and Lajtha 2008; Bongiorno et al. 2016). Both isotopes undergo

Responsible editor: Philippe Garrigues

✉ Tito C. M. Almeida
tito@univali.br

✉ Claudemir M. Radetski
radetski@univali.br

¹ Laboratório de Ecologia de Comunidades, Universidade do Vale do Itajaí (UNIVALI), Rua Uruguai 458, Itajaí, SC 88302-202, Brazil

² Centro de Biociências e Biotecnologia, Laboratório de Ciências Ambientais, Universidade Estadual do Norte Fluminense, Av. Alberto Lamego 2000, Campos dos Goytacazes, Rio de Janeiro 28013-602, Brazil

a consistent trophic enrichment pattern, autonomous of the organism position in the food web (Post 2002), and also present an increasing trend of about 3.4‰ for each trophic level in nitrogen, besides carbon enrichment at a smaller intensity of about 1.0‰. The isotope rate of C is used to trace the origin of primary sources of C in food webs, while the isotope ratio of N is used to determine the trophic level in organisms (Fry et al. 1999). These properties allow using these measurements to compare the chemical composition of the same species from different locations and to analyze the influence of environmental variables on the organism's diet within the food web.

Furthermore, the stable isotopic ratio in individuals is a good alternative when a limited quantity of biomass samples is available for analysis. When organisms reach a sufficient size for separating body parts, this can be used for stable isotopic analyses, as in muscle and mantle for mollusks (Antonio et al. 2010), abdominal muscle segments in decapod crustaceans (Bergamino et al. 2011), and even bivalve hemolymphs (Gustafson et al. 2007). When the biomass in a single organism is not sufficient for separating the body parts for isotopic analyses, it is possible to use the whole body, or in case of very small organisms, pooling animals from the same species (Bergamino et al. 2011).

Attempts for solving discrepancies in stable isotopic ratio using different sampling preparations are always in progress. For example, for minimizing chances in changing the stable isotopic ratio from the food in the digestive tract, some authors place organisms in individual aquariums with filtered marine water for cleaning them, and in this way, the contents do not affect the stable isotopic ratio (Olsen et al. 2013). There are other important aspects of sampling preparation, such as acidification, distilled water use, and lipid and fat removal. For example, Mateo et al. (2008) compared results between the whole body of marine invertebrates to those using soft tissues, showing that the stable isotopic ratio of ^{13}C and ^{15}N changed depending on the method used.

Thus, stable isotopic ratio measurements can be used to compare, for example, differences between food preferences in species living in different places and to analyze the influence of environmental variables on the organism's diet within the food web. The beach locations analyzed in the present study, i.e., close to the organically polluted Paraíba do Sul River's mouth and to the upwelling zone of the South Atlantic Central Water (SACW) intrusion at Cabo Frio, could be considered as the most important environmental variables in determining the food sources, since the water physicochemical characteristics of these two locations are quite different, as previously reported (Corbisier et al. 2014; Carreira et al. 2015; Cordeiro et al. 2018).

There were two main objectives in this study: (i) to determine differences in the stable isotopic ratio ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in macrobenthic species sampled at two sandy beaches (one

close to a river mouth and another far from any freshwater input) and (ii) to identify differences in the stable isotopic ratio ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in different body parts of three representative species of sandy beach macrofauna: the polychaete *Hemipodia californiensis*, the mollusk bivalve *Donax hanleyanus*, and the crustacean decapod *Emerita brasiliensis*.

Materials and methods

Sampling and preparation for analyses

The *H. californiensis*, *D. hanleyanus*, and *E. brasiliensis* species were collected from two sandy beaches in the state of Rio de Janeiro: Praia do Farol in São Thomé (PF), located at Paraíba do Sul River's mouth, in the municipality of São João da Barra (21° 37' S and 41° 00' W), and Praia Grande (PG) in the municipality of Arraial do Cabo (22° 58' S and 42° 01' W). PF is a reflective beach with a high nutrient contribution from the continent provided by the Paraíba do Sul River. This local seasonality includes rainy and dry periods, and according to Krüger et al. (2006), the difference in the nutrient supply changes slightly between both seasons, and 94% to 95% of the dissolved inorganic nitrogen (DIN) in the river is related to NO_3^- , with concentrations of this compound ranging from 7.41 to 32.8 μM .

As PG is a dissipative beach, there is no continental influence, and it is affected by the upwelling at Cabo Frio; the main intrusion is from the SACW. According to Corbisier et al. (2014), the variations in the DIN values range from < 0.05 to 3.20 μM NH_4^+ , from < 0.05 to 1.11 μM NO_2^- , and from < 0.05 to 11.93 μM NO_3^- . The organisms were sampled using a 20-cm-diameter PVC cylinder, buried at a 20 cm depth in the sediment. Two sampling campaigns were carried out on each beach in December 2016 (favorable to upwelling), and organisms from the same species were collected at different beach locations to achieve spatial representativeness from each beach. Thus, for comparison of stable isotopic ratio ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in macrobenthic species sampled at two sandy beaches, 50 *D. hanleyanus* and 18 *H. californiensis* organisms were sampled at different locations and freeze-dried for the stable isotopic ratio determination in muscles; 14 *D. hanleyanus*, 25 *E. brasiliensis*, and 12 *H. californiensis* individuals were sampled to identify differences in the stable isotopic ratio ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in different body parts. It should be mentioned that not all individuals were used in the analyses. Each one of the following body parts was thawed, weighed, and dissected: in *Hemipodia*, the proboscis teeth (4 per animal), proboscis (without contents), whole body with full digestive tract; in *Donax*, the adductor muscle, all viscera including gills, and whole body; and in *Emerita*, the carapace, muscle bundles below the carapace, and whole body. After the body parts were separated, the samples were immediately

washed with distilled and deionized water, and the wet weight was determined after acidic decarbonation. After the frozen-lyophilization process of the body parts, the dry weight was also determined. The mean elemental composition of the C:N ratio of the samples used in this study was ≤ 3.5 . In that context, the lipid extraction was not considered as a restriction on body part $\delta^{13}\text{C}$ value interpretation (Kiljunen et al. 2006; Post et al. 2007).

Laboratory measurements

Elemental (C and N) and isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) compositions were determined by weighing approximate 0.5-mg samples in tin capsules and analyzed by a continuous-flow isotope-ratio mass spectrometer (Delta V Advantage; Thermo Scientific, Germany) coupled with an elemental analyzer (Flash 2000) (Fry 2006). The results are expressed in the conventional delta (δ) notation related to Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric N_2 for $\delta^{15}\text{N}$, according to Eq. (1), where R_{sample} and R_{standard} are the corresponding ratios of rare to common isotopes ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) in the sample and international standards, respectively (Peterson and Fry 1987). Samples were run using blank cups and known analytical isotope standards of urea of IVA Analysentechnik 330802174 ($\text{CH}_4\text{N}_2\text{O}$, Mw = 60, C = 20%, N = 46%) with the certified isotopic composition ($\delta^{13}\text{C} = -39.79\text{‰}$ and $\delta^{15}\text{N} = -0.73\text{‰}$). Data quality control was checked by performing a reference standard run (Elemental Microanalysis Protein Standard OAS of certified isotopic composition: $\delta^{13}\text{C} = -26.98\text{‰}$ and $\delta^{15}\text{N} = 5.94\text{‰}$) after every ten samples. Reproducibility was based on triplicate analysis of samples as $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

$$\delta_{(\text{sample})} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \quad (1)$$

Statistics

Two sites were compared by bi-factorial ANOVA considering beaches (PG and PF) and species (*H. californiensis* and *D. hanleyanus*) as orthogonal and fixed factors, using the stable

isotopic ratio determined in the muscle of both species (Underwood 1997). One-way ANOVA was applied to compare the body structures on each species, considering different body structures as factors (Underwood 1997). Normality and homogeneity variances were verified by the Kolmogorov-Smirnov and Bartlett’s tests, respectively, and when these requirements were not met, transformations were applied to $\log_{10}(X + 1)$.

Results and discussion

The ecosystem data are analyzed by applying temporal and spatial scales and play a critical role in shaping our understanding of their structure and function (Levin 1992; Estes et al. 2018). A comparison between two specific places was intended in this study, and temporal replication of data was contemplated at different times when the organisms were sampled. The objective emphasized in this study was to verify if different sites or body part structures presented differences in the stable isotopic ratio of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Spatial comparison between Praia Grande and Praia do Farol beaches

The reflective beach of Praia do Farol in São Thomé is characterized by a high nutrient contribution from the continent, in the mouth of Paraíba do Sul River, while Praia Grande is a dissipative beach, without any continental influence, but it is affected by the upwelling at Cabo Frio (intrusion of the SACW). There were differences in the stable isotopic ratio ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of macrobenthic species sampled at these two sandy beaches. Table 1 shows the mean stable isotopic ratio on the muscle of organisms sampled at PG and PF, while Table 2 shows the ANOVA results from these data.

No significant differences were detected for the $\delta^{13}\text{C}$ stable isotopic ratio between the two sites; however, a significant difference was observed for the $\delta^{15}\text{N}$ stable isotopic ratio (Table 2). In fact, the two sampled species at the PF beach showed lower $\delta^{15}\text{N}$ stable isotopic ratio than the sampled organisms at the PG beach. While the $\delta^{13}\text{C}$ stable isotopic

Table 1 Mean isotopic ratio of the organisms of Praia Grande (PG) and Praia do Farol (PF) and their respective standard errors (SEs) and lower limit (LL) and upper limit (UL) of the confidence interval

Local	Species	$\delta^{13}\text{C}$ (‰)				$\delta^{15}\text{N}$ (‰)				N
		Mean	SE	95% UL	95% LL	Mean	SE	95% UL	95% LL	
PF	<i>Donax hanleyanus</i>	-16.8	0.1	-16.9	-16.7	7.8	0.1	7.6	7.9	36
	<i>Hemipodia californiensis</i>	-16.8	0.2	-17.3	-16.2	13.8	0.3	13.0	14.5	8
PG	<i>Donax hanleyanus</i>	-16.7	0.1	-17.0	-16.5	9.0	0.2	8.7	9.3	42
	<i>Hemipodia californiensis</i>	-16.9	0.3	-17.6	-16.1	14.5	0.3	13.8	15.1	8

Table 2 Result of the ANOVA for the comparisons between the beaches (local) through the isotopic ratio of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in *D. hanleyanus* and *H. californiensis*

Factors	Isotope			
	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
	F_{calc}	p	F_{calc}	p
Local	0.001	0.976	16.91	< 0.001**
Species	0.091	0.764	520.74	< 0.0001***
Local \times species	0.402	0.528	3.39	0.0689

The calculated F values and p values with asterisks denote statistical significance for the tested factors

ratio was similar in both species and sites, the $\delta^{15}\text{N}$ stable isotopic ratio varied between the sites and species. The SE values were lower when the N value was higher regarding the measurement data quality.

The *D. hanleyanus* and *H. californiensis* species are not clearly subject to the influence of marine organic matter origin in their $\delta^{13}\text{C}$ stable isotopic ratio (Table 1) when comparing the stable isotopic ratio in the organisms at PG and PF. *D. hanleyanus* is a filtering bivalve, incorporating unicellular organisms with or without photosynthetic capacity, placing it at the base of the trophic web (Post 2002; Gustafson et al. 2007). Since there is no significant difference related to the $\delta^{13}\text{C}$ isotopic signal among the *D. hanleyanus* individuals sampled at both beaches, we can infer that organisms do not have a differentiated influence on the organic matter source, namely the upwelling of the SACW at the PG beach or the supply of the river organic matter from the PF beach. However, a variation of $\delta^{13}\text{C}$ stable isotopic ratio was reported from the same sources of organic matter (i.e., zooplankton, suspended organic matter, and phytoplankton). They are the most potential source of the $\delta^{13}\text{C}$ stable isotopic ratio for the macrobenthic organisms (Corbisier et al. 2006; Petracco 2008; Franco 2013; Di Benedetto et al. 2013; Corbisier et al. 2014). Thus, according to our results, it is still important to determine if the influence of the different phenomena occurring in these two places is equivalent as a source of organic matter, at least for the $\delta^{13}\text{C}$ stable isotopic ratio. Higher values were found in the PG-sampled organisms related to the $\delta^{15}\text{N}$ stable isotopic ratio. That may be an indication of the greater influence of the SACW upwelling on the constitution of nitrogenous organic matter. Seasonal upwelling leads to strong and variable shifts, and they were reported in production sources cascading up food webs in the region. According to France (1994), the $\delta^{15}\text{N}$ stable isotopic ratio of invertebrates reflects that both trophic-dietary and habitat source fractionation and the relative position of statistical mode are different for marine and freshwater organisms. Thus, if we consider the results from

Table 1, organisms from the PG beach clearly showed marine food source influence, while organisms from the PF beach showed continental food source influence. Stable isotope analyses offer a reliable approach for defining these changes; however, in relation to generated data, small temporal variability sampling is likely to provide different information than longer ones, and thus, conclusions obtained from specific studies should be put into perspective (Corbisier et al. 2014).

Comparisons of stable isotopic ratio among species morphological structures

Three species were chosen due to the sampling facility, for easy separation of the body part structures, and mainly because they represent organisms feeding on sediment (e.g., *Hemipodia*) and in the water column (e.g., *Donax* and *Emerita*). Thus, these organisms play distinct ecological functions at different levels of the trophic web in sandy beaches. *Hemipodia* is a top predator (carnivorous) (Pinotti et al. 2014), and *Donax* and *Emerita* are both consumers (McLachlan and Brown 2006; Bergamino et al. 2011; Pinotti et al. 2014).

D. hanleyanus showed a significant difference for $\delta^{13}\text{C}$ in the body part structures, but only a tendency to be significant for the $\delta^{15}\text{N}$ stable isotopic ratio. In *E. brasiliensis*, the differences were significant for both isotopes, and in *H. californiensis*, no differences were observed among the body part structures (Tables 3 and 4).

For *D. hanleyanus*, $\delta^{13}\text{C}$ mean values ranged from -16.7‰ in the muscle to -17.9‰ in the whole animal, while $\delta^{15}\text{N}$ ranged from 8.4‰ in the viscera to 9.2‰ in the muscle. For *E. brasiliensis*, the carapace displayed a notable reduction for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, ranging from -15.7 to -18.9‰ for $\delta^{13}\text{C}$ and from 3.2 to 8.1‰ for $\delta^{15}\text{N}$, respectively. In the case of *H. californiensis*, which presented no differences among its body part structures, the values for $\delta^{13}\text{C}$ ranged from -16.4‰ to -17.2‰ and those for $\delta^{15}\text{N}$ from 13.7 to 14.5‰ (Table 3).

The $\delta^{15}\text{N}$ stable isotopic ratio of the bivalve *D. hanleyanus* was close to that of several other suspended bivalves. In *E. brasiliensis*, a suspensivore crustacean, the $\delta^{15}\text{N}$ stable isotopic ratio is similar to that in other crustaceans with the same feeding habit (Corbisier et al. 2006; Han et al. 2015; Franco 2013). Finally, the $\delta^{15}\text{N}$ isotopic signature of *H. californiensis* was similar to that of other predators, as polychaetes of the same class and other polychaetes, and even similar to carnivorous fishes, such as *Trichiurus lepturus* (Corbisier et al. 2006; Petracco 2008; Bergamino et al. 2011; Di Benedetto et al. 2013; Corbisier et al. 2014).

H. californiensis, a polychaete of the *Glyceridae* family, has developed jaws made up by four pointed teeth with the capacity to inject paralyzing poison produced by glands at the base of the teeth. Also, a long muscular proboscis, which can reach three fourths of the body, is everted for projecting the teeth and catching its prey. The internal digestive tract may be

Table 3 Mean values of the isotopic ratio of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and respective standard errors in the determinations of *Donax hanleyanus*, *Emerita brasiliensis*, and *Hemipodia californiensis*

	$\delta^{13}\text{C}$ (‰)	Standard error (‰)	$\delta^{15}\text{N}$ (‰)	Standard error (‰)
<i>Donax</i>				
Whole organism	-17.9	0.2	8.5	0.2
Viscera	-17.4	0.1	8.4	0.2
Muscle	-16.7	0.1	9.2	0.2
<i>Emerita</i>				
Whole organism	-18.9	0.6	8.1	0.3
Muscle	-16.5	0.1	7.7	0.1
Carapace	-15.7	0.3	3.2	0.3
<i>Hemipodia</i>				
Teeth	-16.5	0.2	13.7	0.2
Pharynx	-16.7	0.3	13.7	0.4
Soft parts	-16.7	0.1	14.5	0.1
Whole organism	-17.2	0.3	14.3	0.2

visible to the naked eye in the rest of the body because it is often filled (Fauchald and Jumars 1979). *H. californiensis* presented no significant difference for $\delta^{15}\text{N}$ signal, but the values were lower than the ones presented by Bergamino et al. (2011) for the same species. However, our results corroborate with those of Corbisier et al. (2014) for several benthic predators, placing the polychaete in the same trophic position as an octopus or a carnivorous fish.

D. hanleyanus is a filtering bivalve feeding by capturing suspended particulate matter in the water column. There is a system in its gills like eyelashes that select particles compatible with the opening of the mouth, while the larger particles are led to pseudofeces production. The data obtained for $\delta^{15}\text{N}$ indicate this species is a secondary consumer, and the

variations found for the structures did not indicate a trophic position variation based on the values found in the literature (Bergamino et al. 2011). The reduced values in the viscera, compared to those in the muscle, could be a result of the unassimilated material in the inhalant siphon.

E. brasiliensis presented a marked reduction for $\delta^{15}\text{N}$ signal in the carapace compared to the muscle, with almost a three times lower mean value. Also, Yokoyama et al. (2005) reported that the carapaces present much lower values of $\delta^{15}\text{N}$ than other parts of crustaceans. The lower values of $\delta^{15}\text{N}$ found in carapaces were similar to those found by Kogure (2004) in benthic diatoms, phytoplankton, and zoobenthos. The $\delta^{15}\text{N}$ stable isotopic ratio found in the muscle of this species is similar to those obtained by other authors. That is

Table 4 Results of the one-way analysis of variance (ANOVA) with the isotopic ratio of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ found in the body parts of *Donax hanleyanus*, *Emerita brasiliensis*, and *Hemipodia californiensis*

	SS	Degree of freedom	MS	F_{calc}	p	Significance level
<i>D. hanleyanus</i>						
$\delta^{13}\text{C}$	0.005	2	0.003	12.0	0.0002	***
Error	0.006	27	0.000			
$\delta^{15}\text{N}$	3.233	2	1.616	3.440	0.047	*
Error	12.686	27	0.470			
<i>E. brasiliensis</i>						
$\delta^{13}\text{C}$	62.051	2	31.025	6.699	0.005	***
Error	115.784	25	4.631			
$\delta^{15}\text{N}$	136.235	2	68.118	62.955	<0.00001	***
Error	27.050	25	1.082			
<i>H. californiensis</i>						
$\delta^{13}\text{C}$	0.0012	3	0.0004	1.9	0.1697	ns
Error	0.0044	20	0.0002			
$\delta^{15}\text{N}$	0.0025	3	0.0008	2.18	0.1221	ns
Error	0.0078	20	0.0004			

SS sum of squares, MS average of the sum of squares, ns no significance

***Error probability of less than 0.001%

*Tendency to be significant (5% error)

Table 5 Summary of the isotopic results in the different parts of the organisms and in the different beaches

Species	$\delta^{13}\text{C}$ isotopic signal		$\delta^{15}\text{N}$ isotopic signal	
	Part of body comparison	Beach comparison	Part of body comparison	Beach comparison
<i>D. hanleyanus</i>	Differs	Does not differ	Differs	Differs
<i>E. brasiliensis</i>	Differs	–	Differs	–
<i>H. californiensis</i>	Does not differ	Does not differ	Does not differ	Tendency to be significant

in benthic crustaceans with the same feeding habits as *E. brasiliensis* (Corbisier et al. 2006; Han et al. 2015). There were differences found in the $\delta^{13}\text{C}$ isotopic signals in this species, especially when the whole organism was compared to specific body part structures that possibly occurred because the complete digestive tract was analyzed in the organism. Thus, the food that was not assimilated by the organism may affect the stable isotopic ratio, whereas in the muscle, the stable isotopic ratio reflects only what the organism has already assimilated. The difference may have occurred because both organic and inorganic carbons are present in their exoskeletons in carapaces, and this fact may reflect a decrease in the carbon isotope signal (Mateo et al. 2008) (Tables 3 and 4). The bivalve mollusk *D. hanleyanus* presented a similar situation to *E. brasiliensis*, in which the carbon isotopic signal was more enriched in the whole organism, probably because the digestive tract was filled with undigested matter. *H. californiensis* did not follow this pattern, suggesting that predators do not have this differentiation. Here, it should be noted that the stable isotopic ratio is species-specific and tissue-specific and that the accepted fractionation values may not be universally applicable (Yokoyama et al. 2005). Therefore, it is possible to identify contrasts in the stable isotopic ratio according to the body structure, as well as to the sampled location (Table 5).

Conclusions

Stable isotope analyses are a good tool in trophic ecology studies related to the chemical composition of the same species found in different locations, making it possible to analyze the influence of environmental variables on the organism's diet. Also, isotope ratio quantification can be a good alternative analysis when little organic matter is available for testing. No significant differences were detected for the $\delta^{13}\text{C}$ stable isotopic ratio between the two studied sites (i.e., PF and PG); however, for the $\delta^{15}\text{N}$ stable isotopic ratio, a significant difference was observed. Also, it was possible to identify differences in the stable isotopic ratio according to the body structure. Significant differences were detected in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signals for *D. hanleyanus* and *E. brasiliensis*. According to the $\delta^{13}\text{C}$ isotope analysis,

D. hanleyanus and *H. californiensis* displayed a great impact of marine organic matter in their feeding habits. More specifically, as there is no significant difference related to the $\delta^{13}\text{C}$ isotopic signal among individuals of *D. hanleyanus*, we could infer that two beaches do not have different contributions of organic matter, and in both, the diet organism contribution is predominantly from zooplankton. However, the values obtained for $\delta^{15}\text{N}$ stable isotopic ratio indicated that the variations found for the different structures did not show trophic position variations. *E. brasiliensis* presented a marked reduction in the $\delta^{15}\text{N}$ ratio in the carapace compared to muscle, probably due to the sample acidification. Its average value was almost three times lower. The difference found between $\delta^{13}\text{C}$ stable isotopic ratio in this species showed a significant difference comparing its body parts (complete digestive tract, muscle, and carapace), which may have occurred because of both organic carbon and the remaining inorganic carbon in the exoskeletons, reflecting the decrease in the $\delta^{13}\text{C}$ stable isotopic ratio. The results also indicated a similar stable isotopic ratio in the whole body (including digestive tract), proboscis, and teeth of *H. californiensis*, highlighting this species as a top predator. In conclusion, there is an indication of the nutrient supply origin in different morphological structures allowing to understand the trophodynamic of benthic communities, which can be employed as a tool in coastal management plans or environmental impact studies.

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