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ARTICLE

# Stable Isotope Analysis of Oysters as a Tool for Environmental Monitoring in a Marine Extractive Reserve

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## ABSTRACT

The stable isotopes $\delta^{13}C$ and $\delta^{15}N$ are widely recognized and utilized as biomarkers for analysing trophic links, paleoenvironmental reconstruction, biogeography and nutrient sources. However, it is essential to further develop their applications, as their use in marine environmental monitoring is not as prominent. $^{13}C$ and $^{15}N$ have distinct signatures in organic compounds, which can be utilized to identify potential carbon and nitrogen sources. Marine bivalves are often employed in environmental studies as efficient bioindicators because sessile filter feeders tend to bioaccumulate pollutants. The present study analysed $\delta^{13}C$ and $\delta^{15}N$ in seston and oysters inhabiting two areas with different environmental conditions in a marine extractive reserve. The isotopic values were compared for two trophic levels and three oyster tissues, resulting in a broad view of local dynamics. Seston samples from Forno Beach (FB) exhibited depleted $\delta^{13}C$ values, possibly reflecting a terrigenous carbon contribution in this area. Considering oyster tissues, $\delta^{13}C$ and $\delta^{15}N$ values in the hepatopancreas were similar to seston, possibly due to oysters' role as filter feeders, supporting the use of digestive tissues for assessing short-term changes of environmental conditions. Moreover, isotope values for oyster gills and muscles suggest long-term homogeneous conditions for Anjos Beach (AB) and FB, with a predominance of marine carbon and nitrogen sources. Our results underline the relevance of analysing bivalve tissues separately because they display different turnover rates and depict variable time frames of environmental conditions. This article provides valuable information on the variables that must be considered when applying stable isotope analysis in coastal environmental monitoring, highlights knowledge gaps and recommends best practices for future work in this area.

## 1 | Introduction

The uncontrolled population growth along the coast represents a constant challenge to environmental management (Zhai et al. 2020; Pena et al. 2020). Marine pollution monitoring usually relies on indicator organisms, which experience physiological, biochemical or molecular changes in response to seawater contamination (Ferreira, Coutinho, and de Oliveira 2023).

Bivalve molluscs are often selected as bioindicators because they are sessile filter feeders that absorb and accumulate seawater pollutants (Fiori et al. 2018; Phan et al. 2019). These motivated the continuous search for pollution biochemical markers in bivalve tissues (Sardi et al. 2017; López-Landavery et al. 2019; Araújo et al. 2021).

Recently, several studies have applied stable isotopes $^{13}C$ and $^{15}N$ as pollution biomarkers due to their particular signature in a variety of organic compounds, which can be used to identify the type and potential feeding sources in marine organisms (Wang et al. 2020; Vezzone et al. 2021; Felizardo et al. 2021; Srinivas, Sukumaran, and Babu 2022). Besides, the $\delta^{13}C$ isotopic signatures in pollutants are expected to be transferred from producers to higher trophic levels (Zanden and Rasmussen 2001; Post 2002). Graham et al. (2010) employed the $\delta^{13}C$ signature as a marker of oil pollution and observed that the lighter $\delta^{13}C$ of petrogenic sources led to the depletion of values in planktonic communities. Regarding the $^{15}N$ signature, several studies reported that a high input of urban effluents in coastal environments resulted in the change of $\delta^{15}N$ values in suspended particulate organic material and marine organisms (Gaston and Suthers 2004; Rožič et al. 2015; Ke et al. 2020; González-De Zayas et al. 2020). One challenge in interpreting the results of these studies is the variety of sources contributing to these effluents, which can result in either an increase or a decrease in isotopic signatures (Betti et al. 2011; Rožič et al. 2015; Ke et al. 2020).

Moreover, stable isotope analyses are broadly used to infer potential sources of dissolved organic matter and particulate organic matter (DOM/POM) in coastal environments. Indeed, multiple origins were associated with organic matter in coastal waters, including local primary production and microbial decomposition of algal biomass; terrestrial sources by decomposition of soil and plant matter; and anthropogenic sources, such as industrial and domestic sewage (Lee, Kim, and Kim 2020). Another important factor to consider is that the isotopic composition in a consumer's tissue reflects their current diet and consumption in the preceding weeks and months, allowing for medium-term evaluations (Buchheister and Latour 2010). $\delta^{13}C$ and $\delta^{15}N$ fractioning also provide valuable information for the study of ecological niches and animal interactions in wild communities. In that sense, Shipley and Matich (2020) reviewed the important factors that interfere with data interpretation, such as physiological conditions, sampling and storage procedures and statistical analysis. In this context, the physiology of bioindicators can complicate the interpretation of the obtained data, as it may vary according to environmental characteristics and, in turn, influence them (Bearham et al. 2023).

The understanding of the dynamics of stable isotopes in marine organisms is expanding. A series of studies utilized these isotopes intensively for paleoenvironmental reconstruction (Milano, Schöne, and Gutiérrez-Zugasti 2020; Das et al. 2021; Schöne and Huang 2021; Peharda et al. 2022; Wichern et al. 2023), food web analysis (Zhao, Yang, and Shan 2022; Amiraux et al. 2023; Whippo et al. 2024), investigation of the diets of various species (Schoo et al. 2018; Srinivas, Sukumaran, and Babu 2022; Jiao et al. 2024) and marine biogeography (Andrades et al. 2019; Raoult et al. 2020; Ainis et al. 2021; Tatsch et al. 2024). However, studies directly employing this tool for marine environmental monitoring are less frequent (Kanduč et al. 2018; González-De Zayas et al. 2020; Liénart et al. 2022). The present study applies $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis of seston and bivalve tissues with varying turnover rates (muscle, gill and hepatopancreas) to assess the environmental conditions of the coastal region in Arraial do Cabo, a tourist destination situated within a marine extractive reserve (RESEXmar-AC).

## 2 | Methodology

### 2.1 | Study Area

Samples were collected from two sites with varying anthropogenic interference in Arraial do Cabo, a marine extractive reserve characterized by multiple uses of the sea, including boat traffic, diving, swimming and artisanal fishery (de Melo et al. 2009; Sarmento et al. 2020; ICMBIO 2020). Site 1 is located at the Anjos Beach (AB) pier (Figure 1), the primary nautical support point for Arraial do Cabo, housing the Fisher's Marina, the floating pier of AB and a commercial harbour (ICMBIO 2020). The frequent transit and docking of hundreds of boats could be a source of chronic release of oil at this location (Warnken and Byrnes 2004; Lin, Lin, and Jong 2007). This area also experiences occasional sewage discharge events triggered by heavy rainfall (ICMBIO 2020). Site 2 is located on the rocky shore of Forno Beach (FB), which is subject to a low degree of anthropogenic disturbance, restricted to the presence of tourists and a small bivalve mollusc farm (Galvao et al. 2012; ICMBIO 2020). This site is situated within Forno Inlet (Figure 1), with oceanographic characteristics similar to Site 1. Despite the proximity of the selected sample sites, which could promote occasional water mixing, the most frequent circulation is characterized by strong currents from the northeast border with the trend of water from inside the enclosed areas (Forno and Anjos bays) to flow outwards (Batista et al. 2017). During our sampling, we observed higher values at the AB site for nitrate (AB = 0.44 μM; FB = 0.09 μM), nitrite (AB = 0.21 μM; FB = 0.09 μM) and phosphate (AB = 0.29 μM; FB = 0.10 μM). Furthermore, Coelho-Souza et al. (2013) demonstrated that heterotrophic bacterial production is significantly higher at AB compared with FB and attributed this difference to anthropogenic pressures at AB, particularly due to harbour activities and sporadic sewage discharges.

### 2.2 | Sample Collection and Preparation

Nitrite, nitrate and phosphorus concentrations were measured in the seawater using a multi-parameter sonde (U-50 Series; Horiba®). Seawater was collected in 4 L triplicate samples at each site to analyse stable isotopes in the seston. Samples were pre-filtered using a 200 μm mesh to exclude large organisms and limit the size of the seston to be analysed and then vacuum filtered using 0.7 μm GF/F filters (Schoo et al. 2018). The filters were previously dried and decontaminated in a muffle furnace (8 h at 450°C) and individually weighed on a precision scale (Mettler Toledo; 1 μg resolution). After filtration, the filters were dried at 60°C until they reached a constant weight and then stored for isotopic analysis. Six individuals from the Ostreidae

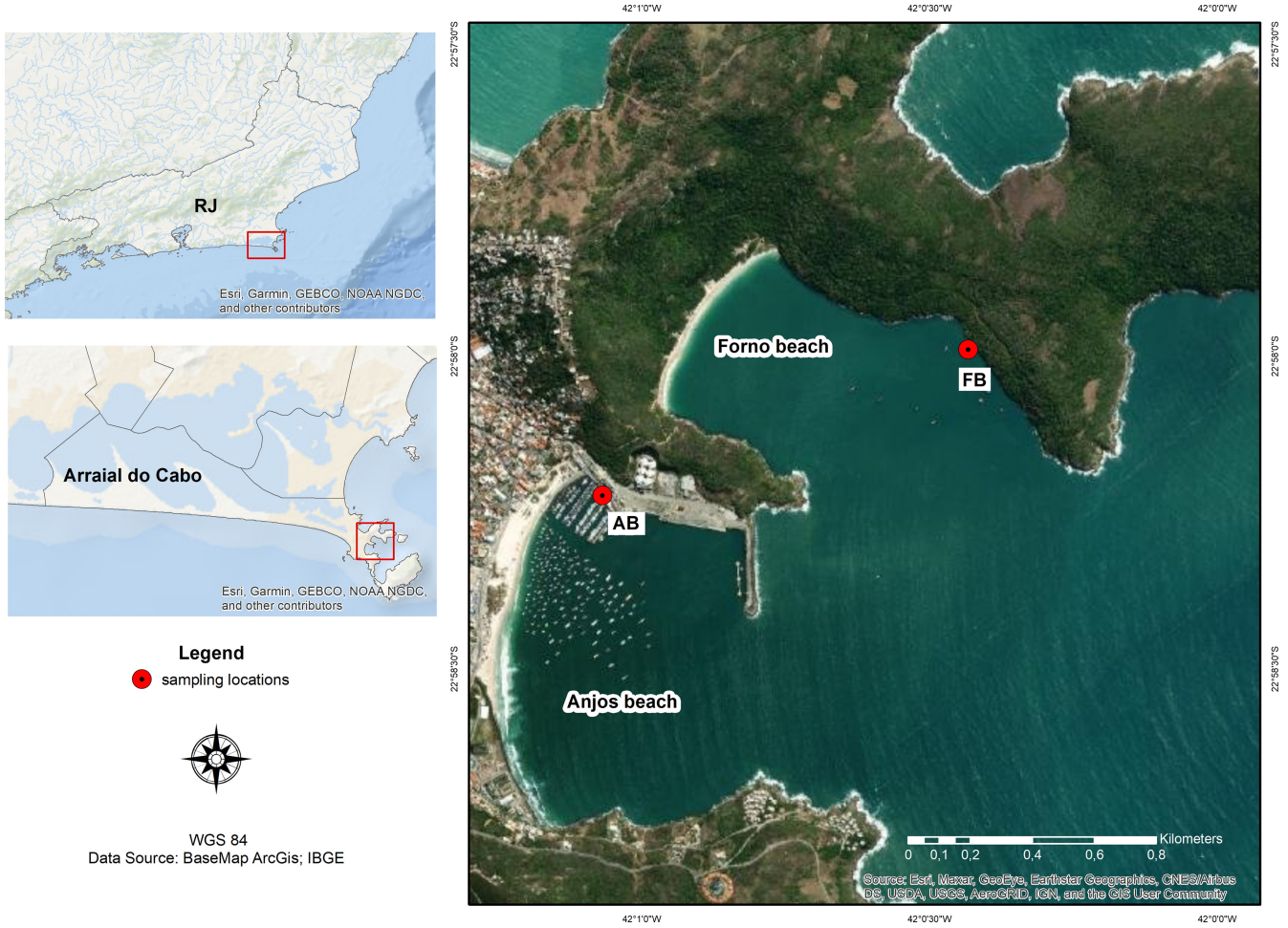


**FIGURE 1** | Location of sampling sites at Arraial do Cabo, RJ, Brazil (AB = Anjos Beach; FB = Forno Beach).

family were collected at each sampling point and identified as *Saccostrea cucullata* and *Crassostrea brasiliana*. The specimens were transported in a refrigerated container to the laboratory and then frozen for later dissection. Each specimen's gills, hepatopancreas and muscle tissues were separated, weighed, placed in 2 mL Eppendorf tubes and individually frozen at −20°C. The tissues were then freeze-dried and macerated in an agate mortar and pestle. All samples were collected during the low spring tide in December 2021.

## 2.3 | Stable Isotope Analysis

The quantification of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), total carbon (TC) and total nitrogen (TN) was performed on aliquots of dry filters (12 mg) and of each freeze-dried oyster tissue (0.7 mg), weighed using tin capsules and a precision scale. A blank analysis accounted for the percentage of inorganic carbon and nitrogen in the filters. TC, TN, $\delta^{13}C$ and $\delta^{15}N$ levels were quantified using an elemental analyser coupled to an isotope ratio mass spectrometer (EA Flash 2000 coupled to an IRMS Delta Advantage; Thermo Electron Corp., Bremen, Germany), as described by Vezzone et al. (2021). To calculate the analysis error, reference materials were used, along with empty tin capsules: B2155 PROTEIN ($\delta^{13}C = -26.98 \pm 0.13$, $\delta^{15}N = 5.94 \pm 0.08$), USGS65 GLYCINE ($\delta^{13}C = -20.29 \pm 0.04$, $\delta^{15}N = -20.68 \pm 0.06$) and IVA33802174 UREA ($\delta^{13}C = -41.3 \pm 0.04$, $\delta^{15}N = -0.32 \pm 0.02$). The measured analytical errors were ±0.4‰ for $\delta^{13}C$, ±0.4‰ for $\delta^{15}N$, ±0.5% for TC and ±0.6% for TN.

## 2.4 | Statistical Analysis

The data were determined to be normally distributed through Kolmogorov–Smirnov and Shapiro–Wilk tests and compared through analysis of variance (ANOVA), followed by Tukey multiple comparison tests (Zar 1996). Significant differences were identified by a coefficient $p \leq 0.05$. The results were represented in boxplot graphics depicting the mean ± standard deviation. All statistical analyses were performed with the software Excel and Statistica 7.0.

## 3 | Results

Stable isotope analyses in seston samples revealed a significant depletion of $\delta^{13}C$ at FB compared with the AB pier (Figure 2a, $p = 0.048$). $\delta^{13}C$ in oyster tissues showed the same trend observed for seston (Figure 2b, $p > 0.05$). When comparing oyster tissues, the hepatopancreas exhibits $\delta^{13}C$ values significantly lower than gills and muscle and closer to the values observed for seston (Figure 2b). The values related to the discrepancies observed for

$\delta^{13}C$ between the tissue types in AB and FB were quite similar (Table S1). The difference between muscle and hepatopancreas was 2.88‰ for AB and 2.56‰ for FB; the difference between muscle and gill was 0.98‰ for AB and 0.79‰ for FB; and the difference between gill and hepatopancreas was 1.9‰ for AB and 1.71‰ for FB.

The $\delta^{15}N$ mean values were similar between AB and FB, considering both seston and oyster samples (Figure 3). When comparing different oyster tissues, the hepatopancreas had the lowest $\delta^{15}N$ values (Figure 3b), closely resembling those observed in the seston, whereas the values for muscles and gills exceed the seston by more than 2‰.

The mean TC percentages were significantly higher for seston in AB than in FB (Figure 4a, $p = 0.025$). Oyster tissues did not exhibit significant variations in TC values between sampling sites (Figure 4b).

A significant difference was also observed between sampling sites for seston TN levels, with higher values in AB than in FB (Figure 5a, $p = 0.047$). TN levels for oyster tissues were similar between sampling sites and among tissue types (Figure 5b).

In general, when compared with previous studies (Tables S2 and S3), the isotopic signatures obtained for seston and oysters were similar to the values found in coastal regions (Figure 6). Moreover, seston samples from FB showed variable $\delta^{15}N$ levels, compatible with coastal and estuary signatures (Figure 6a). In contrast, $\delta^{13}C$ values for oyster muscle tissues are out of the range observed for inner estuary samples and similar to the observed for coastal and outer estuary samples (Figure 6b).

## 4 | Discussion

$\delta^{13}C$ and TC values for seston suggest different environmental conditions between the AB and FB stations during collection (Roth et al. 2016; Srinivas, Sukumaran, and Babu 2022). However, the enrichment of $\delta^{13}C$ at station AB is contrary to the trend of $\delta^{13}C$ depletion observed in areas with a significant input of urban effluents (Rogers 2003; Gaston and Suthers 2004) or subjected to oil spill incidents (Graham et al. 2010). Indeed, local dynamics may favour the influence of enriched carbon sources of marine origin at AB, supplanting the effects of occasional oil or sewage pollution (Bardhan et al. 2015; Kopprio et al. 2018).

Moreover, the depletion of $\delta^{13}C$ at FB could be attributed to greater terrigenous carbon input because this station is located next to rocky shores in a cove surrounded by terrestrial vegetation. A similar trend in $^{13}C$ signature was observed by Bearham et al. (2023) and Bardhan et al. (2015), which demonstrated that depleted $\delta^{13}C$ values in POM are characteristics of environments where terrestrial carbon sources predominate. In contrast, the

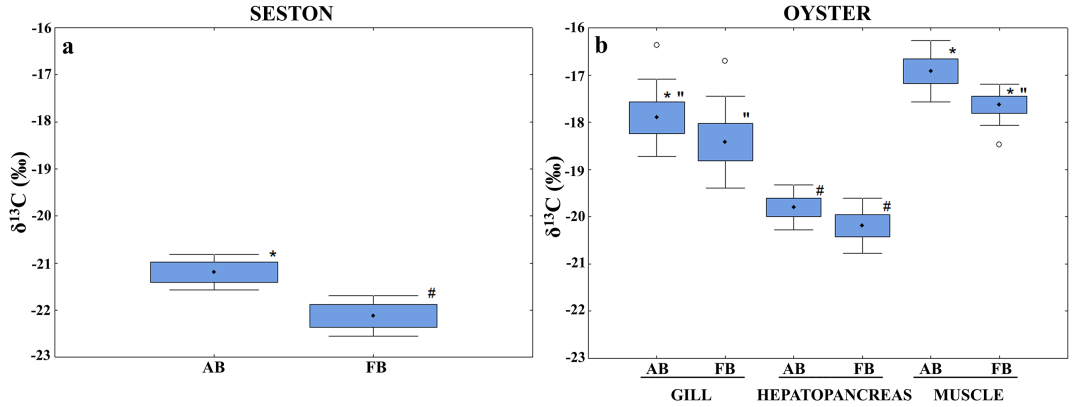


**FIGURE 2** | $\delta^{13}C$ levels in (a) seston ($n = 3$) and (b) oyster tissues ($n = 6$) at sampling sites in Arraial do Cabo. Mean ± standard deviation; boxes indicate standard errors. AB = Anjos Beach pier; FB = Forno Beach. Equal symbols (*, ", #) indicate no significant differences.

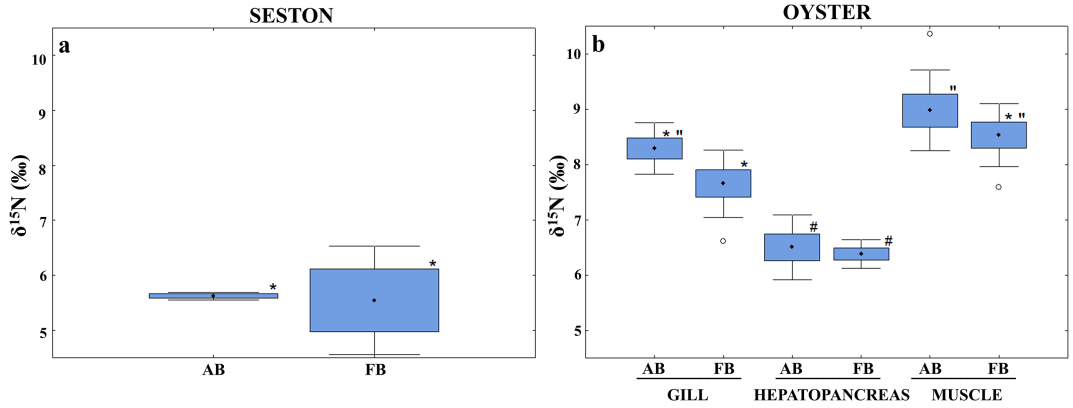


**FIGURE 3** | $\delta^{15}N$ levels in (a) seston ($n = 3$) and (b) oyster tissues ($n = 6$) at sampling sites in Arraial do Cabo. Mean ± standard deviation; boxes indicate standard errors. AB = Anjos Beach pier; FB = Forno Beach. Different symbols (*, ", #) indicate statistically significant differences.

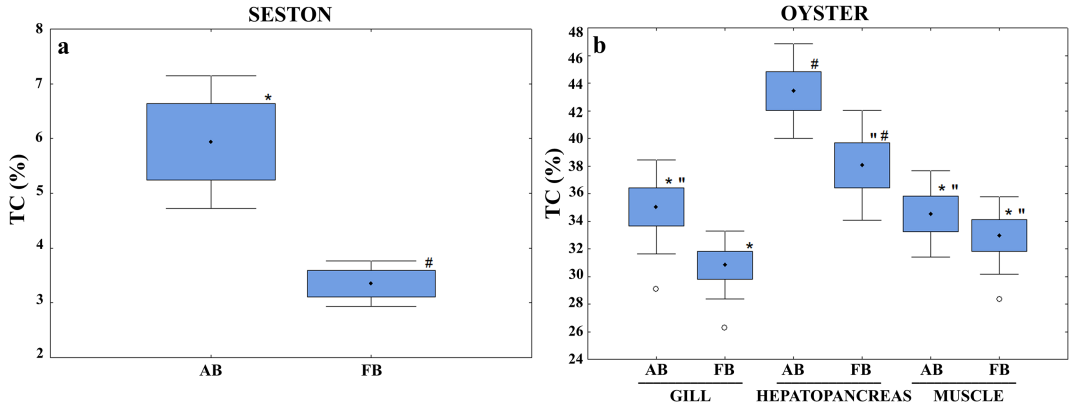


**FIGURE 4** | Total carbon percentage (TC%) in (a) seston ($n = 3$) and (b) oyster tissues ($n = 6$) at sampling sites in Arraial do Cabo. Mean ± standard deviation; boxes indicate standard errors. AB = Anjos Beach pier; FB = Forno Beach. Different symbols (*, ", #) indicate statistically significant differences.

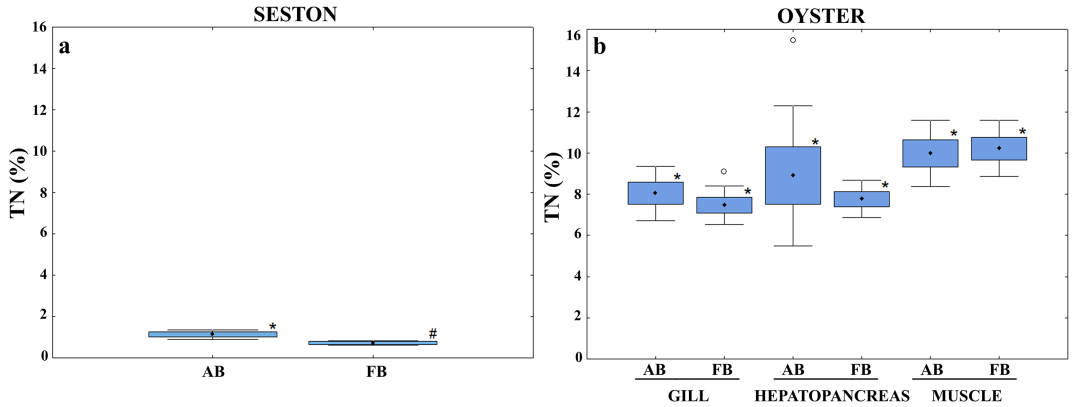


**FIGURE 5** | Total nitrogen percentage (TN%) in (a) seston ($n = 3$) and (b) oyster tissues ($n = 6$) at sampling sites in Arraial do Cabo. Mean ± standard deviation; boxes indicate standard errors. AB = Anjos Beach pier; FB = Forno Beach. Different symbols (*, ", #) indicate statistically significant differences.

$\delta^{13}C$ values in oyster tissues were similar between sampling sites, potentially reflecting long-term environmental conditions. The $\delta^{13}C$ values for oysters are directly linked to their dietary intake and indicate feeding patterns over weeks or months (Zanden and Rasmussen 2001; Post 2002). These results suggest the contribution of terrigenous carbon sources at FB during sampling and the trend to long-term homogenization of carbon sources between FB and AB.

The nitrogen isotopic ratio observed in both seston and oyster tissues was similar across the sampling stations, indicating similar nitrogen sources, at least on a scale of days to months preceding the collections, despite the occasional discharge of sewage at AB.

Studies using $\delta^{15}N$ values to assess the effects of anthropogenic effluents in coastal areas show contrasting results, mainly due to the variable composition of these effluents. Ke et al. (2020), for example, attributed a depletion of recorded nitrogen isotopic signatures (below 2‰) to the impact of urban sewage, while Rožič et al. (2015) observed the opposite result (enriched $\delta^{15}N$ signatures above 5‰). These contrasting results reinforce the need to standardize methods in studies that apply $\delta^{15}N$ analysis for environmental monitoring. Moreover, the higher standard deviation observed for seston $\delta^{15}N$ values in FB suggests a greater variability of nitrogen sources in this location when compared with AB. Indeed, $\delta^{15}N$ values for seston at FB are compatible with marine and terrestrial sources (Table S2).

The $\delta^{15}N$ values for oyster muscles and gills are more than 2‰ higher than those of seston, reflecting the higher trophic position of oysters in the food chain in relation to seston (Zanden and Rasmussen 2001; Post 2002; Layman et al. 2012). Similarly, previous studies demonstrated that consumers have isotopic signatures higher than those of their diets, with an average value of about 3.0 ± 1.0‰ per trophic level, considering only the feeding factor (Post 2002; McCutchan et al. 2003). Recent studies emphasize that each environment must be carefully evaluated because trophic structures are unique and intrinsically linked to environmental conditions (Shipley and Matich 2020; Kjeldgaard, Hewlett, and Eubanks 2021).

The comparison between oyster tissues showed that the values of $\delta^{13}C$ and $\delta^{15}N$ were lower in the hepatopancreas than in the other tissues and closer to those recorded for seston. This difference in isotopic signatures for digestive tissues is attributed in part to their higher recycling rate, which reflects feeding behaviour (Raikow and Hamilton 2001; Deudero et al. 2009;

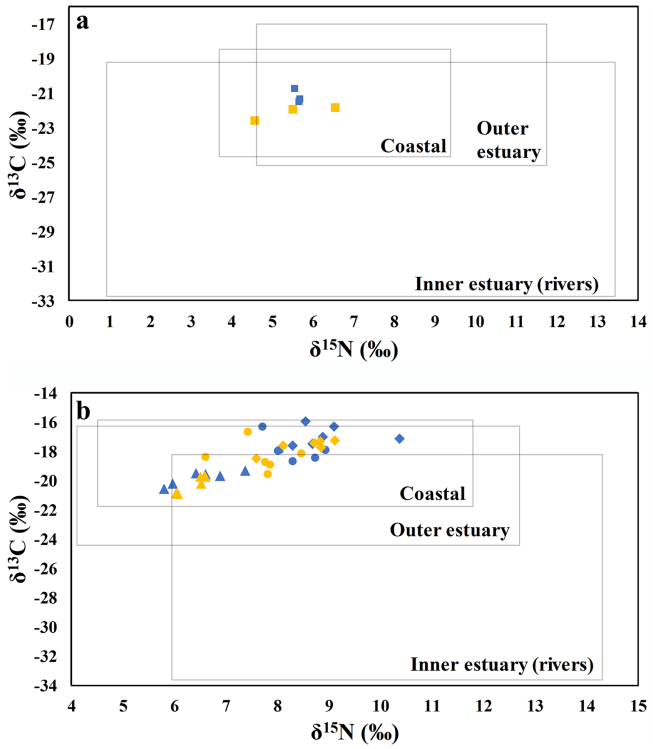


**FIGURE 6** | $\delta^{13}C$ and $\delta^{15}N$ in seston (a) and oyster tissues (b) were compared with those reported in previous studies for coastal areas, including external (deltas, lagoons and bays) and internal (rivers) estuarine areas. Symbols: ■ = seston; ▲ = hepatopancreas; ● = gills; ◆ = muscle. Blue = Anjos Beach pier; yellow = Forno Beach. Grey rectangles represent values obtained from previous studies (references at Tables S2 and S3).

Özdilek, Demir, and Gürkan 2019). On the other hand, gill and muscle tissues exhibit a more intricate isotopic fractionation, leading to higher values for the studied isotopes (Yokohama, Ishihi, and Yamamoto 2008; de Barros Ferraz et al. 2009). Similarly, previous isotopic studies have demonstrated the importance of using different bivalve tissues to enhance the robustness of the analyses and integrate multiple time scales, taking into account the metabolic properties of each tissue (Paulet et al. 2006; Malet et al. 2007; Deudero et al. 2009; Fertig et al. 2010; Emmery et al. 2017; Özdilek, Demir, and Gürkan 2019; Bearham et al. 2023).

Considering the variations of isotope fractioning among different ecological niches and consumer tissues, the decrease in $\delta^{13}C$ in the seston and hepatopancreas at FB reflects the short-term environmental conditions, which might indicate the influence of low $\delta^{13}C$ sources at the sampling moment, such as terrigenous material (Riera and Richard 1996; Bardhan et al. 2015; Bearham et al. 2023). In contrast, $\delta^{13}C$ values for oyster muscle tissue are higher than the hepatopancreas and out of the range observed for inner estuary samples (Table S3 and Figure 3), which could indicate the long-term predominance of marine sources. The difference in isotopic signature between fast and slow turnover tissues was considered by Bearham et al. (2023) as an important tool to evaluate environmental variability. In their analysis of the diets of oysters and mussels across a gradient of environmental conditions, the authors inferred that the discrepancy in $\delta^{13}C$ values between tissues with short and long turnover rates would indicate variations in carbon assimilation. They further argue that discrepancies in assimilation may complicate comparisons between locations if they are not consistent across all studied environments. In the present study, data analysed at both sampling points revealed minor differences between hepatopancreas tissues compared with muscles and gills, indicating that carbon assimilation by oysters did not present a problem in the analysis conducted.

Indeed, the interpretation of environmental isotopic data is complex and challenging because of the myriad of biotic and abiotic factors influencing isotopic turnover (Liu et al. 2018; Bauer et al. 2021; Yang et al. 2021). The isotopic signature in indicator organisms is influenced not only by the current environmental conditions but also by their diet and the recycling rate for carbon and nitrogen in the analysed tissues. The recycling speed is influenced by life stage, metabolism (Herzka and Holt 2000) and abiotic factors, such as temperature (Dattagupta et al. 2004). The present study enhances the current understanding of stable isotope fractionation and turnover in natural marine environments, as well as in bivalve tissues. Further research is necessary to evaluate the effects of physicochemical and biological factors on isotope signature and to expand the application of this tool for environmental monitoring.

## 5 | Conclusion

The $\delta^{13}C$ signature was successfully applied to differentiate two areas with particular environmental characteristics in the RESEXmar-AC and to identify a possible influence of terrigenous carbon sources at FB. The values obtained for $\delta^{13}C$ and $\delta^{15}N$ from seston and oyster hepatopancreas reflect this short-term difference. Still, the isotopic signature of oyster gills and muscles depicts a long-term scenario of homogeneous environmental conditions for AB and FB. The present results reinforce the importance of analysing bivalve tissues separately because the different turnover rates could lead to misinterpretation of isotopic values.

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### Ethics Statement

The authors have nothing to report.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available in a public data repository and can be accessed through DOI: https://doi.org/10.6084/m9.figshare.28279058.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.