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Geographic variation in the nutritional ecology of nominally herbivorous fishes in the southwestern Atlantic Ocean

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ABSTRACT: Spatial and temporal niche partitioning are the basis for the coexistence of species. Determining niche partitioning among species over broad geographical scales requires multiple approaches, as local environmental features may vary spatially. We analysed the trophic ecology of 3 nominally herbivorous fishes — the doctorfish *Acanthurus chirurgus*, the grey parrotfish *Sparisoma axillare*, and the brassy chub *Kyphosus vaigiensis* — to identify the drivers of variation in resource use at different locations along the Brazilian coast: Natal, Abrolhos Archipelago, and Arraial do Cabo. We used 2 complementary approaches — dietary (at 2 scales of magnification) and stable isotope analyses (SIA) — to investigate variation in the nutritional ecology of the study species. We observed interspecific dietary variation at each location. Doctorfish showed the highest dietary variability, with significant contributions from diatoms and cyanobacteria; grey parrotfish mainly ingested detritus, cyanobacteria, and green filamentous algae, while brassy chub mainly ingested brown algae but with some supplementation from red and green macroalgae. Interspecific isotopic niche overlap varied among locations following variation in the isotopic niche width of species. Trophic positions (TP) varied unexpectedly, with grey parrotfish displaying a relatively higher TP in one location and the lowest in the others. The use of complementary approaches, providing short- and comparatively long-term information (diet and SIA, respectively), and a detailed description of dietary items are essential to understand niche and resource partitioning when studying the nutritional ecology of nominally herbivorous fishes.

KEY WORDS: Geographic comparison · Herbivory · Diet · Stable isotopes · Herbivorous fishes · Nutritional ecology

1. INTRODUCTION

Dietary analyses provide a framework for the construction of the food webs that define ecosystems and their dynamics (Kartzinel et al. 2015). In the case of terrestrial herbivores, the combination of (1) dietary studies, (2) analysis of food processing and digestion mechanisms, (3) the distribution of diets and digestive mechanisms amongst taxa, and (4) their evolutionary history has provided a comprehensive picture of the ecology and evolution of this group. Although the term 'herbivore' typically indicates

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*Corresponding author: gabrielccf@gmail.com species that feed on plant material (Horn 1989), the complexity of the trophodynamics of piscine herbivores with the targeting, ingestion, and assimilation of a plethora of food items (Choat et al. 2002, 2004, Clements et al. 2009) led to an expansion of such classification to recognize them as 'nominal herbivores' (Choat et al. 2002). The diversity of food ingested by nominally herbivorous fishes ranges from nutrient-rich microscopic and filamentous taxa, including cyanobacteria, diatoms, dinoflagellates, and filamentous rhodophytes with a pre-Cambrian origin, to large structurally complex macroscopic algae, primarily fucoid and laminarian taxa, with a much more recent pattern of diversification (Bringloe et al. 2020, Choi et al. 2024). The autotrophs consumed by most herbivorous fishes are typically dominated by structurally simple taxa that are relatively rich in protein and lipids with low proportions of structural polysaccharides (Clements & Choat 2018). With such a wide range of food items, the dietary analysis of nominally herbivorous fishes can pose a significant challenge, especially when the identity of food items becomes visually unrecognizable following the initial stages of food processing (e.g. grinding in a pharyngeal mill or gizzard) and digestion. Some algae are quickly digested and/or fragmented due to their fragile physical structure, which differs from more robust material such as invertebrate shells or bones commonly found in the gut contents of omnivores and carnivores.

The doctorfish *Acanthurus chirurgus* (Bloch, 1787), the grey parrotfish *Sparisoma axillare* (Steindachner, 1878), and the brassy chub *Kyphosus vaigiensis* (Quoy & Gaimard, 1825) are representatives of the 3 major groups of herbivorous reef fishes in the southwestern Atlantic: acanthurids, scarine labrids (parrotfishes), and kyphosids. We investigated dietary and isotopic niche partitioning in these 3 taxa with disparate phylogenetic affinities and food-processing modes at all sites along the Brazilian coast. Doctorfish ingest a variety of algal taxa, including different Rhodophyta (Cardozo-Ferreira et al. 2023), but also a considerable amount of detritus within the epilithic turf assemblages on which they feed, and grind ingested food in a muscular gastric stomach (Mendes et al. 2018). The diet of grey parrotfish is dominated by detritus and calcareous red algae, often epiphytized by micro-photoautotrophs (Nicholson & Clements 2023b), along with filamentous algae and microalgae such as dinoflagellates, diatoms, and cyanobacteria (Cardozo-Ferreira et al. 2023). Many parrotfishes are known microphages that lack a stomach and grind their food in a pharyngeal mill before it reaches the foregut (Clements et al. 2017). Brassy chub are strict algivores that rely on symbiotic hindgut microbiota to digest and assimilate the refractory algal carbohydrates in dietary brown algae (Clements & Choat 1995, Mendes et al. 2018, Pisaniello et al. 2022, Cardozo-Ferreira et al. 2023). These 3 species are broadly distributed along the Brazilian coast (Ferreira et al. 2004) and, therefore, are exposed to a wide range of environmental and biological conditions that may impact their nutritional ecology (Johnson et al. 2020, Bellwood & Tebbett 2024). This variation spans from plant chemical defences (Goecker et al. 2005) to benthic composition (Cvitanovic & Hoey 2010, Francini-Filho et al. 2010), which is highly variable across the Brazilian coast (Aued et al. 2018). Although previous studies have assessed the local differences in diet among doctorfish and grey parrotfish, focusing on taxonomic and macronutrient composition of the diet (Mendes et al. 2018), and explored seasonal variation in diet and nutrient assimilations at a local scale (Cardozo-Ferreira et al. 2023), no study has yet examined their nutritional ecology across multiple locations in the light of spatial resource variation in the southwestern Atlantic.

As gut content analysis provides a temporal snapshot of diet, we employed estimates of both dietary and isotopic niches to obtain a more extended temporal framework of feeding targets. Isotope signatures in fish muscles can reflect feeding habits over a scale of 2 to 3 mo due to the long turnover rates of fish muscle (Fry 2002), although in some species turnover can be as long as ~5 to 6 mo (Madigan et al. 2012). Isotopic niche space (mostly using stable isotopes of carbon and nitrogen; δ^{13}C and δ^{15}N) has been used to describe and explore niche relationships within communities in different environments (Andrades et al. 2021), as well as to infer nutrient assimilation (Eurich et al. 2019, Cardozo-Ferreira et al. 2023). The concomitant use of these techniques allows the gathering of longer-term information on what these species ingest and assimilate from ingesta, providing a more complete picture of their resource use.

In a previous study, we examined seasonal changes in the nutritional ecology of the same 3 species in one of the locations analysed here: the upwelling-affected reefs of Arraial do Cabo (Cardozo-Ferreira et al. 2023). The species maintained their dietary niches throughout the year, with little interspecific overlap. The seasonal stability observed in our previous work enabled us to conclude that the partitioning observed here was not seasonally variable despite the differing sampling times among locations. The present study builds on this previous work, expanding our sampling along the Brazilian coast to encompass both tropical and subtropical environments. We investigated (1) interspecific trophic variation across locations, hypothesizing that species would maintain similar relative trophic positions and niche partitioning (dietary and isotopic) among them, and (2) the relationship between diet and stable isotope signatures in muscle tissue.

2. MATERIALS AND METHODS

2.1. Study areas

Sampling took place at 3 locations on the Brazilian coast along ca. 2000 km: Natal (5° 47′ S, 35° 11′ W), Abrolhos Archipelago (17° 20′ S, 39°30′ W; hereafter Abrolhos) and Arraial do Cabo (22° 58′ S, 42° 00′ W; hereafter Arraial) during the austral summers of 2016 and 2017 (Fig. 1). Sampling lasted less than a week at each location to minimize temporal variation, although previous work indicated little seasonal variation in either diet or stable isotope signatures in the sampled fish species (Cardozo-Ferreira et al. 2023). During the sampling years, temperature variation showed distinct variation profiles at each location: Natal 27.7°C (range 26.0–29.2°C); Abrolhos 26.1°C (range 23.4–28.4°C); and Arraial 22.5°C (range 19.3–25.1°C). The tropical systems (Natal and Abrolhos) are warm and prone to little temperature variation, while the subtropical system (Arraial) is constantly affected by upwelling and has higher temperature variation, commonly dropping below 18°C. Sea surface temperature (SST) was obtained through monthly averages from January 2016 to December 2017, with a 0.1 degree resolution (~11 km; NASA Earth Observations), collected by the Aqua satellite (Moderate Resolution Imaging Spectroradiometer; https://neo.gsfc.nasa.gov/view.php?datasetId=MYD28M).

At Natal, the northernmost location, the benthic cover is mostly composed of macroalgae and turf, with some sites having high coverage of suspension/filter feeders (i.e. sponges). Abrolhos has the highest coral species diversity in the South Atlantic Ocean, with comparatively high coral cover interspersed with algal turfs (Francini-Filho et al. 2013, Aued et al. 2018, Longo et al. 2019). In contrast, the southernmost location, Arraial, is a subtropical granitic-based rocky reef where the benthic cover is mainly composed of turf

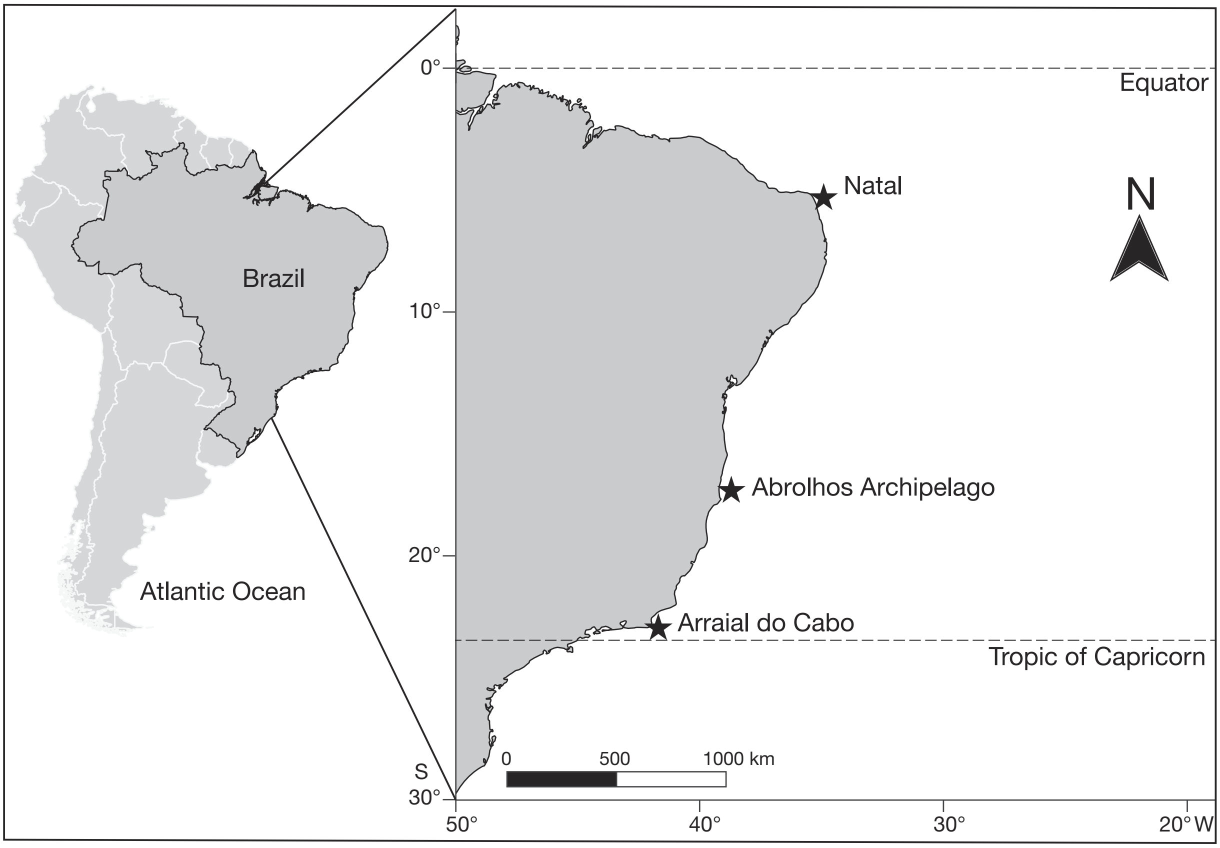

Fig. 1. Sampling locations (★) along the Brazilian coast: Natal, Abrolhos Archipelago, and Arraial do Cabo

algae, zoanthids, and crustose coralline algae (Aued et al. 2018). It is recurrently subjected to upwelling events. Sampling at each of the 3 locations was conducted at multiple reef sites, and in Natal at 4 reefs varying in distance from the shore. Although riverine input can influence isotopic baselines in coastal sites (Sammarco et al. 1999, Lapointe et al. 2005), we did not detect such variation in Natal (for further details, see Text S1 and Table S1 in the Supplement at www.int-res.com/articles/suppl/meps15012_supp.pdf).

2.2. Specimen collection

We sampled 170 individuals distributed among species and locations (Table 1). Fishes were collected from 10:00 to 17:00 h to encompass the peak of feeding activity of herbivorous fishes and ensure that gut content was present for dietary analysis. Although some herbivorous species exhibit peak grazing activity around noon (Ferreira et al. 1998), kyphosids are crepuscular feeders (Choat & Clements 1998). Individuals were collected up to 20 m deep with spearguns (under collection permits SISBIO 48094 and 48112) and immediately put on ice to slow digestive processes and preserve the integrity of the ingested material for dietary analysis. At each location, we aimed at minimizing variation in body size to avoid ontogenetic bias in both diet and stable isotope analysis. Therefore, we collected larger individuals to keep body size consistent. Individuals were measured to the nearest millimetre and weighed in grams (Table 1). Specimens collected and analysed from Arraial are part of the samples used in our recently published work on the seasonal variation in the nutritional ecology of nominally herbivorous fishes (Cardozo-Ferreira et al. 2023).

2.3. Characterizing diets

After collection, sampled individuals were removed from ice, and their gut contents were extracted following a ventral incision (from anus to operculum), carefully exposing the intestinal tract to avoid damage. For both doctorfish and brassy chub, cuts were made from the lower oesophageal sphincter to the pylorus. As parrotfishes lack a gastric stomach (Choat et al. 2002), content was removed from the foregut in grey parrotfish. Gut contents were removed manually from the gut, then fixed in 10% formalin for dietary analysis. We flushed the stomach walls so that all the content was removed. Gut content was analysed at 2 magnifications (see Choat & Clements 1992). For the macro-analysis, the content was spread in a Petri dish marked with 50 equidistant (10 mm) points and identified using a stereomicroscope Leica MZ7.5 with a 7.5:1 zoom ration (4× maximum magnification). For the analysis of the microscopic items — hereafter 'micro-analysis' — the macro-analysed material was filtered through a 60 µm mesh, spread over a slide marked with 30 equidistant (5 mm) points and identified under an optical binocular Marte microscope MIC-100 1600× (40× maximum magnification). For both steps, the dietary item above each marked point was identified to the lowest possible taxonomic level and the percentage contribution of each item was cal-

Table 1. Number of collected individuals (N), total length (mm) and weight (g), $\delta^{13}C$ and $\delta^{15}N$ signatures, and trophic position (TP) for each species and location. Superscript letters on $\delta^{13}C$ and $\delta^{15}N$ denote homogenous groups for comparisons among species within each location as detected by pair-wise tests (Kruskal-Wallis, $p < 0.05$; matching letters indicate statistical similarity among species; a > b)

Location and species	N	Total length (mean ± SD; range)	Total weight (mean ± SD; range)	$\delta^{13}C$ (mean ± SE)	$\delta^{15}N$ (mean ± SE)	TP
Natal						
Acanthurus chirurgus	23	211 ± 20 (169–239)	226 ± 56 (125–325)	-17.1 ± 0.1^{a}	$7.9 \pm 0.1^{a,b}$	2.77
Sparisoma axillare	18	281 ± 65 (195–447)	420 ± 232 (152–950)	-16.9 ± 0.2^{a}	8.9 ± 0.4^{a}	3.05
Kyphosus vaigiensis	13	409 ± 59 (342–538)	1498 ± 874 (685–3365)	-16.0 ± 0.2^{a}	7.4 ± 0.3^{b}	2.61
Abrolhos Archipelago						
Acanthurus chirurgus	22	241 ± 21 (183–284)	328 ± 99 (140–595)	-13.6 ± 0.4^{b}	6.8 ± 0.1^{a}	2.23
Sparisoma axillare	21	268 ± 29 (225–327)	369 ± 108(220–580)	-13.7 ± 0.2^{b}	5.4 ± 0.1^{b}	1.90
Kyphosus vaigiensis	23	351 ± 20 (300–390)	888 ± 141 (560–1160)	-11.9 ± 0.2^{a}	5.7 ± 0.1^{b}	1.96
Arraial do Cabo						
Acanthurus chirurgus	20	307 ± 25 (261–341)	752 ± 142 (424–945)	-18.7 ± 0.1^{b}	12.3 ± 0.1^{a}	3.21
Sparisoma axillare	21	319 ± 58 (237–430)	638 ± 333 (258–1355)	-16.0 ± 0.1^{a}	10.7 ± 0.1^{b}	2.62
Kyphosus vaigiensis	9	312 ± 36 (252–359)	597 ± 207 (312–902)	-16.8 ± 0.3^{a}	10.7 ± 0.4^{b}	2.64

culated — separately for macro- and micro-analysis — based on the total number of points. The percentage contribution of each item was calculated as the proportion of points at which it occurred relative to the total number of points analysed in each sample, multiplied by 100. Only doctorfish and grey parrotfish required analysis at a microscopic scale due to the presence of detritus and other elements that could not be identified at lower magnification. Only 2 individuals, both grey parrotfish, had empty guts. Algal items were assigned into functional groups (see Cardozo-Ferreira et al. 2023).

2.4. Stable isotope analysis

A portion of dorsal fish muscle was removed from each specimen to determine the isotopic composition of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$). Primary producers (i.e. species of red, green, and brown algae and turf) were collected at each location as potential food sources. Both algae and muscle tissue samples were frozen, freeze-dried, and ground to powder for stable isotope analysis (SIA). The stable isotope ratio was determined using a Delta V isotope ratio mass spectrometer interfaced with a Conflo IV and linked to a Flash 2000 Elemental Analyzer (Thermo Scientific). International standard references Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen were used in the analyses. The analytical precisions were ±0.1‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{15}N$ (triplicate samples of every fifth sample). The accuracy for elemental and isotopic compositions was determined by certified standard (Protein OAS/114859, Elemental Microanalysis). Mean recovery values were always ≥95% of the certified value. Stable isotope ratios were expressed in δ notation as parts per thousand (‰) deviations from the international calibration standards. Fish $\delta^{13}C$ values were mathematically corrected when considered lipid-rich (i.e. C:N > 3.5) (see Post et al. 2007). Calcareous material was not acidified (1) to avoid possible disruption in $\delta^{15}N$ values (acidification can affect the estimation of trophic position), and (2) because post-treatment changes in $\delta^{13}C$ values tend to be minor (Ng et al. 2007, Pires-Teixeira et al. 2021) and therefore unlikely to interfere in our inter- and intra-specific comparisons (Cardozo-Ferreira et al. 2023).

2.5. Statistical analysis

For dietary comparisons among species, percent contribution of each item was tested through permutational multivariate analyses of variance (PERMANOVA) with 2 fixed factors (Species, with 3 levels: *Acanthurus chirurgus*, *Sparisoma axillare*, and *Kyphosus vaigiensis*; and Locations, with 3 levels: Natal, Abrolhos, and Arraial), built on a Euclidean distance-based matrix, Type III sum of squares, and 9999 permutations of residuals under a reduced model to calculate the significance of pseudo-*F* statistics. PERMANOVAs were also used to compare each ingested item among locations. For each comparison, the results of both macro- and microscopic analyses were tested separately: macro-analysis among doctorfish, grey parrotfish, and brassy chub, and micro-analysis between doctorfish and grey parrotfish. Similarity percentage (SIMPER) (Clarke & Warwick 1994) analysis was also conducted to understand which dietary items led to similarity or dissimilarity among species.

Isotopic and elemental composition data (i.e. levels of $\delta^{13}C$, $\delta^{15}N$, and C:N ratio) were compared among species using the Kruskal-Wallis rank test, followed by Dunn's multiple comparisons test. Isotopic composition was evaluated using a multivariate Bayesian ellipse-based model, which measures the isotopic niche through the standard ellipse area corrected for small samples (SEAc; 95% CI) and calculates the overlap between each pair of ellipses (see Jackson et al. 2011). Baseline enrichment is expected to influence the isotopic values of consumers (Solomon et al. 2008, Lorrain et al. 2015). For an accurate and reliable estimation of the trophic position (TP) of consumers, we computed the site-specific trophic discriminant factors (TDF) for $\delta^{15}N$ and $\delta^{13}C$ using consumer isotope signatures for each location separately, applying the following formulas (Caut et al. 2009):

$$\Delta^{13}C = -0.248 \times \delta^{13}C - 3.477 \tag{1}$$

$$\Delta^{15}N = -0.281 \times \delta^{15}N - 5.879 \tag{2}$$

TDFs were calculated for each individual and the mean values by location were used for TP calculation. We calculated the TP of each species within each location using the 'one-baseline model', which performs a link between $\delta^{15}N$ enrichment per trophic level and the trophic position of the baselines (i.e. algal species in the present study) (see Quezada-Romegialli et al. 2018). To ensure that our data were unbiased due to a possible contamination by inorganic C, we performed a linear regression with $\delta^{13}C$ and C:N.

We used the software Primer 6+ (see Anderson et al. 2008) for PERMANOVA and SIMPER, the R environment (R Core Team 2024) and the R packages 'SIBER' (Jackson et al. 2011) for the estimation of isotopic niches, 'tRophicPosition' (Quezada-Romegialli et al. 2018) for TP estimation, 'FSA' (Ogle et al. 2019) for multiple comparison tests, and 'ggplot2' (Wickham 2016) and 'circlize' (Gu et al. 2014) for plotting.

3. RESULTS

3.1. Dietary analysis

Diet composition differed among locations for each species (PERMANOVA; pseudo-$F_{2,4}$ = 24.53, p = 0.001) for most comparisons in both the macro- and micro-analyses (Fig. 2; Table S2), the exceptions being the macro-analysis of grey parrotfish (PERMANOVA; pseudo-t = 0.820, p = 0.494) and the micro-analysis of doctorfish (PERMANOVA; pseudo-t = 1.510, p = 0.093) between Abrolhos and Natal (Fig. 2; Table S2). The Species × Location interaction term was also significant for both macro- (PERMANOVA; pseudo-$F_{2,4}$ = 16.35, p = 0.001) and micro-analysis (PERMANOVA; pseudo-$F_{2,2}$ = 7.244, p = 0.001), indicating dietary differences among species between locations and among locations for each species (Table S2). SIMPER indicated an average dietary similarity of 59.6% among locations in the diet of brassy chub, dominated by brown algae at all 3 locations (Fig. 2; Figs. S1–S3), with brown foliose algae being responsible for the most variation (91.6%). Food items and their proportion in the diet of each species are given in Tables S3 & S4. For doctorfish, SIMPER showed the lowest similarity (greatest variation) among locations (37.5%) of the 3 species, with percentage contribution being higher for red calcareous articulated algae (24.6%) than invertebrates (14%), red crustose calcareous (11.3%), and detritus (10.6%). In doctorfish, sediment was lower in Natal than in Abrolhos and Arraial but did not differ between the 2 latter locations. Diatoms were the most important item in the micro-analysis in Natal, and the second most important item (after sediment) at both other locations (Fig. 2; Table S4). Sediment increased in proportion with latitude in grey parrotfish, but detritus exhibited the greatest variation (84.4%), and its contribution and that of cyanobacteria decreased from Natal to Arraial (Fig. 2). SIMPER analysis revealed an overall similarity of 65.1% among locations for grey parrotfish.

Diet composition differed significantly among species within locations (PERMANOVA; pseudo-$F_{2,4}$ = 135.0, p = 0.001). Overall, a diet dominated by macroscopic brown algae (but shifting from foliose to leathery algae at different locations) was recorded for brassy chub, confirming a similar dietary pattern for this species across its circumglobal distribution (Clements & Choat 1997, Knudsen et al. 2019, Cardozo-Ferreira et al. 2023). The diet of grey parrotfish was dominated by micro-photoautotrophs and detrital material, corroborating a microphagous diet (Clements et al. 2017), but with the main items varying in contribution at different locations. Doctorfish ingested the greatest proportion of smaller rhodophytes among the 3 species, although the microscopic analysis indicated that their diet was also abundant in cyanobacteria and diatoms. SIMPER analysis yielded a dissimilarity of 91.4% between doctorfish and brassy chub, with brown foliose algae, more abundant in the stomachs of brassy chub, contributing to most of this dissimilarity (37.9%). A dissimilarity of 75.4% was identified between doctorfish and grey parrotfish, led mostly by the higher abundance of detritus in the gut contents of grey parrotfish, which contributed 39.0% of the overall dissimilarity. The higher proportion of brown foliose algae in brassy chub and of detritus in grey parrotfish led to a dissimilarity of 97.1% between these species, with brown foliose algae and detritus contributing, respectively, to 36.9 and 33.8% of their dissimilarity. Sponge spicules contributed the most to the diet of both species (44.9% for doctorfish and 46.6% for grey parrotfish). However, the second-most contributing dietary item was diatoms for doctorfish (38.5%) and green filamentous algae for grey parrotfish (26.9%). Between these species, the dissimilarity was 54.5%, driven mostly by the higher abundance of diatoms in doctorfish, which contributed 25.1% of the variation between these species.

Sponge spicules found in the gut contents of doctorfish and grey parrotfish were included within the category 'sediment' as these silica spicules have no nutritional value. Spicules composed between 95 and 100% of the sediment category (Table S4). Both doctorfish and grey parrotfish contained a higher proportion of animal matter in their diet at Arraial: doctorfish (Natal: 5.0%; Abrolhos: 4.9%; Arraial: 28.6%) and grey parrotfish (Natal: 1.0%; Abrolhos: 0.6%; Arraial: 7.1%). However, no decrease in algal ingestion was observed from northernmost—warmer to southernmost—colder locations. Algae ingestion in grey parrotfish increased southwards (Natal: 14.1%; Abrolhos: 15.7%; Arraial: 29.6%), while it did not follow a linear pattern in the doctorfish (Natal: 65.5%; Abrolhos: 80.7%; Arraial: 61.8%). For brassy chub, the proportion of algae barely changed among locations (Natal: 99.4%; Abrolhos: 100%; Arraial: 98.2%).

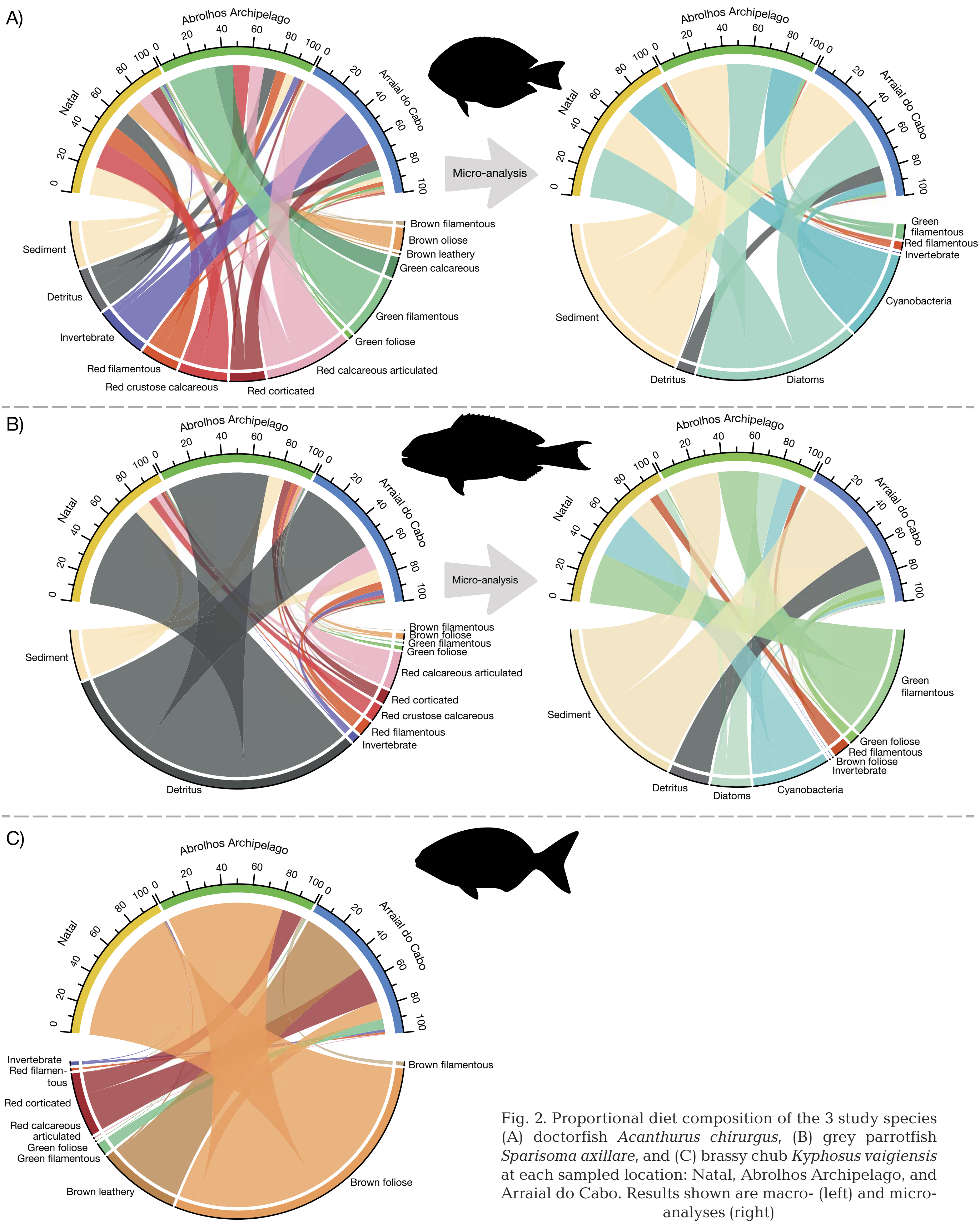

Fig. 2. Proportional diet composition of the 3 study species (A) doctorfish *Acanthurus chirurgus*, (B) grey parrotfish *Sparisoma axillare*, and (C) brassy chub *Kyphosus vaigiensis* at each sampled location: Natal, Abrolhos Archipelago, and Arraial do Cabo. Results shown are macro- (left) and micro-analyses (right)

3.2. Isotopic variation

TDFs differed among locations (Natal: $\delta^{15}N = 3.6 \pm 0.4$ and $\delta^{13}C = 0.7 \pm 0.4$; Abrolhos: $\delta^{15}N = 4.2 \pm 0.2$ and $\delta^{13}C = -0.2 \pm 0.4$; Arraial: $\delta^{15}N = 2.7 \pm 0.3$ and $\delta^{13}C = 0.8 \pm 0.3$). The variation in isotopic niche width resulted in a variable isotopic niche overlap among species at each location. Conversely, relative TP showed an unexpected inversion, being higher for grey parrotfish than doctorfish in the northernmost location, while doctorfish displayed higher TP in the other locations. Isotopic niches were most restricted at Arraial for doctorfish and grey parrotfish, and at Abrolhos for brassy chub (Fig. 3). Each species showed the widest isotopic niche in distinct locations: doctorfish in Abrolhos, grey parrotfish in Natal, and brassy chub in Arraial (Table S5). The lowest TP of all 3 fish species was observed in Abrolhos. The highest TP for doctorfish occurred in Arraial and for grey parrotfish in Natal; the brassy chub TP was similar in Arraial and Natal (Table 1). Relative trophic positions differed within each location (Fig. 4). The C:N ratio was lower for grey parrotfish than for doctorfish in 2 out of 3 locations: Natal and Arraial (Table S5). Overlap of the isotopic niche was observed among species within each location (Fig. 4; Table S6). Overlap is given as a proportion of the non-overlapping area between the corresponding 95% prediction intervals of 2 ellipses (Jackson et al. 2011). At all 3 locations, the lowest overlap occurred between doctorfish and grey parrotfish (being zero at Arraial), while the highest overlap occurred between brassy chub and grey parrotfish at Natal and Abrolhos, and between doctorfish and brassy chub at Arraial (Fig. 4; Table S6).

Baselines (primary producers) were 2 to 6 times richer in ^{15}N in Arraial than in Natal or Abrolhos (Fig. S4; Table S7) due to local upwelling enrichment in the former. There was some variation among algae species in $\delta^{15}N$ in Abrolhos and Natal, but not in Arraial (Table S7). Within locations, algae species differed mostly in $\delta^{13}C$ values (Fig. S4, Table S7). No decrease in $\delta^{13}C$ was observed at Arraial as an influence of upwelling ^{13}C-depleted deep water (Kroon & Ganssen 1989). Linear regressions tested the bias of samples regarding inorganic carbon enrichment and showed a weak negative but significant relationship (adjusted $R^2 = 0.097$, F-statistic $= 19.18$, $p < 0.01$).

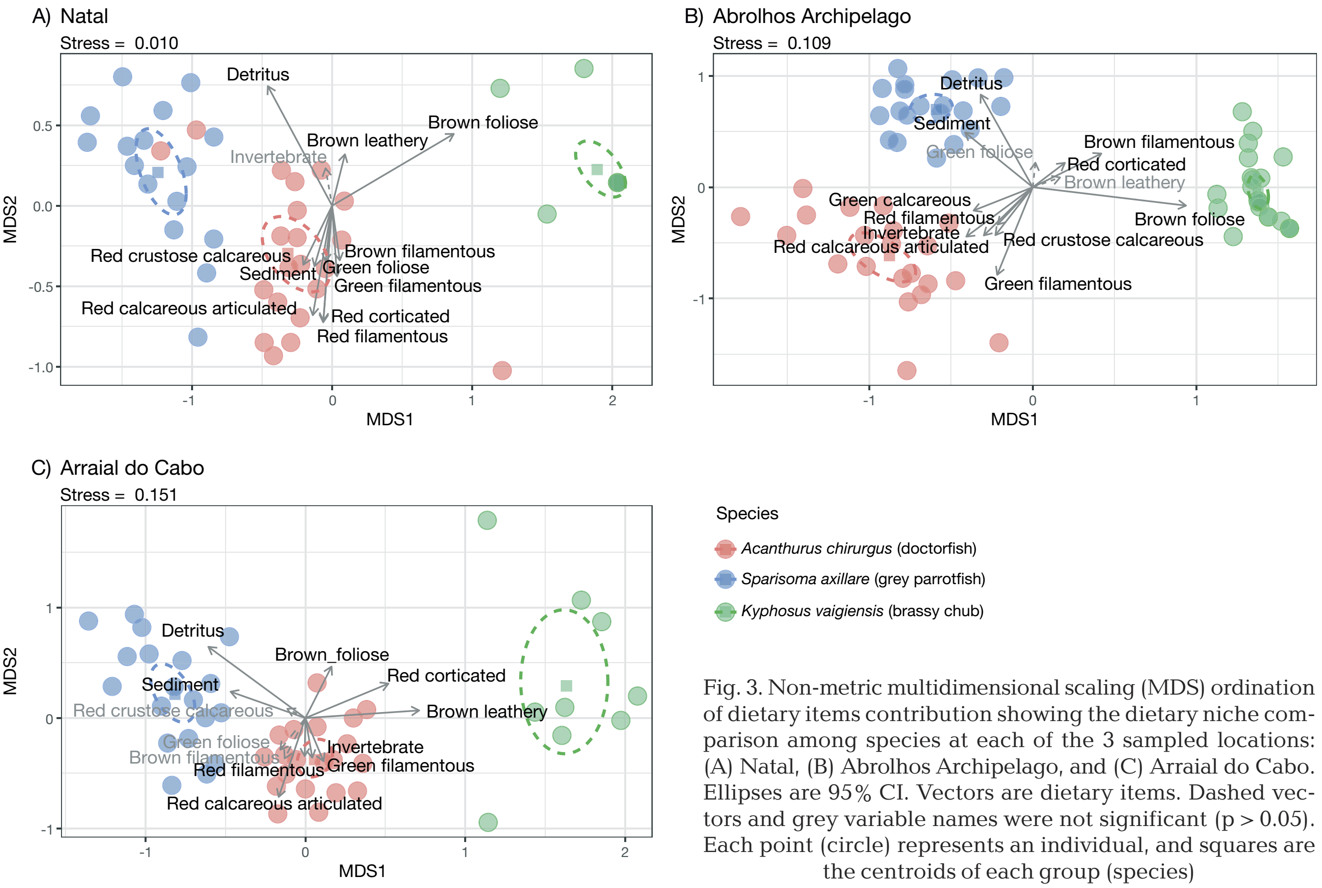

Fig. 3. Non-metric multidimensional scaling (MDS) ordination of dietary items contribution showing the dietary niche comparison among species at each of the 3 sampled locations: (A) Natal, (B) Abrolhos Archipelago, and (C) Arraial do Cabo. Ellipses are 95% CI. Vectors are dietary items. Dashed vectors and grey variable names were not significant ($p > 0.05$). Each point (circle) represents an individual, and squares are the centroids of each group (species)

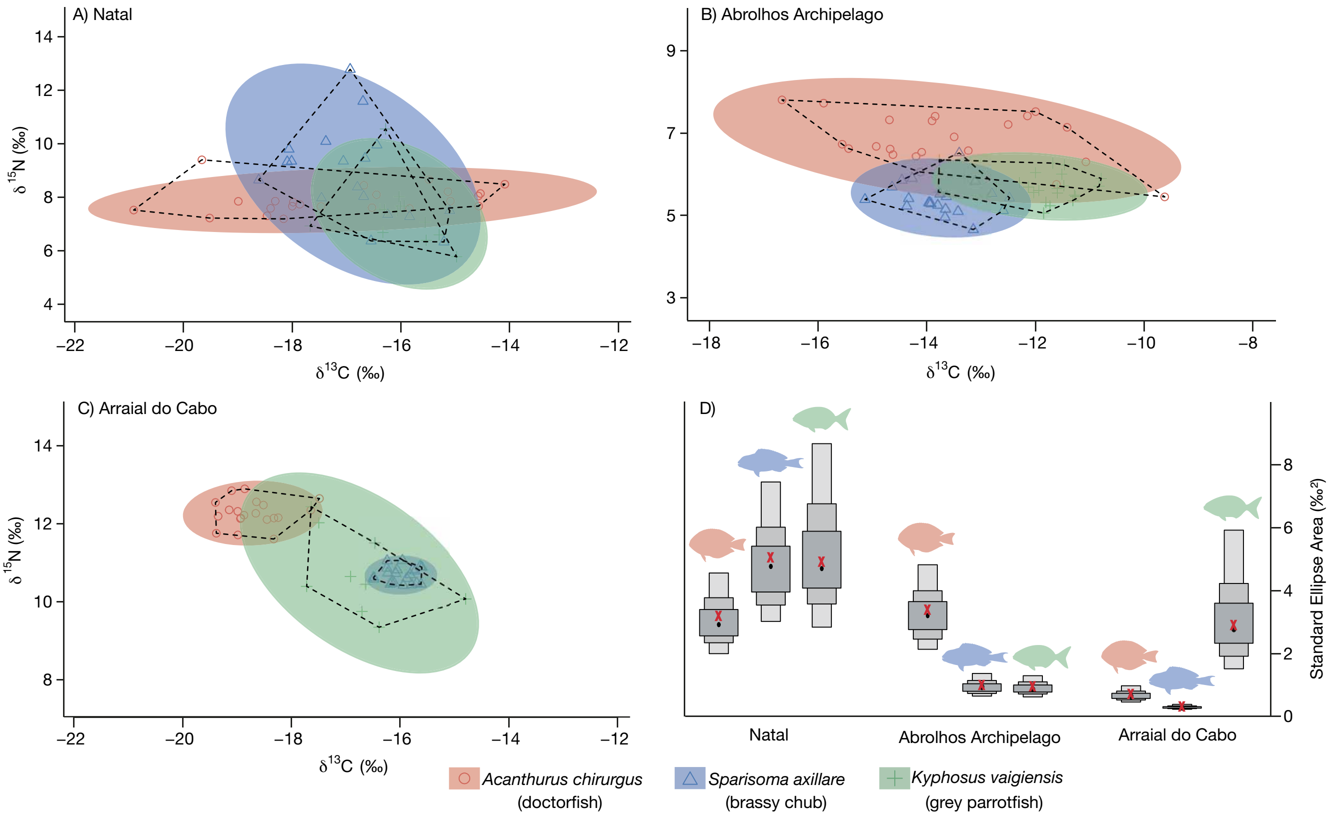

Fig. 4. Isotopic niche for the 3 species at each location: (A) Natal, (B) Abrolhos Archipelago, and (C) Arraial do Cabo. Ellipses (SEAc, ‰^{2}; 95% CI) and convex hulls (dashed lines) show the niche area. (D) SEAc values with sample mode (black dot), true mean (red x), and credible intervals of 50, 75, and 95% as shaded boxes from dark to light grey. Note that the axes have different scales for better visualization

4. DISCUSSION

We analysed the nutritional ecology of 3 nominally herbivorous reef fishes using diet and SIA at 3 locations in the southwestern Atlantic. Our hypothesis that species would maintain similar niche differentiation (dietary and isotopic) among these locations was supported, although the relative TPs showed some unexpected shifts at one location. The diets of the 3 species were consistent, with the main items remaining the same in each location. Enrichment in muscle $\delta^{15}N$ levels was not connected to higher ingestion of more proteinaceous food. For example, the higher TP and $\delta^{15}N$ values of grey parrotfish in Natal were not associated with greater ingestion of animal matter, as doctorfish ingested a greater proportion of animal material. Unsurprisingly, all samples (fish and primary producers) showed higher values of $\delta^{15}N$ in Arraial, as a consequence of the frequent upwelling events at that location (Cardozo-Ferreira et al. 2023) that enrich the whole reef community. Therefore, our results indicate that local characteristics influence the nutritional ecology of nominally herbivorous fishes and should be considered in study designs for species-specific habitat use and diet.

Some herbivorous fishes graze over turf assemblages, leading some previous studies to group species into target-based functional groups (Ferreira et al. 2004, Floeter et al. 2004). Some turf-associated elements (i.e. detritus, crypto- and meiofauna, and microalgae such as dinoflagellates, diatoms, and cyanobacteria) are generally more protein-rich than filamentous algae and can constitute a nutritious source for grazing fishes (Crossman et al. 2001, Wilson et al. 2003, Clements & Choat 2018). Both grey parrotfish and doctorfish are commonly pooled when trophic classifications are based on observational data, as they feed over similar substrata at a macroscopic scale (Francini-Filho et al. 2010). Although they show similar macro-scale feeding substrata and foraging time, and are both highly mobile (Ferreira et al. 2004, Francini-Filho et al. 2010), doctorfish and grey parrotfish differ greatly both in anatomy (Clements et al. 2017, Cardozo-Ferreira et al. 2018, 2021) and gut microbiomes (Thompson et al. 2024), indicating that they may target different food sources. We observed greater variation in the diet of doctorfish, which ingested a significantly greater proportion of diatoms than grey parrotfish. The latter ingested more nitrogen-enriched items, particularly detritus and associated cyanobacteria (Mendes et al. 2018, Cardozo-Ferreira et al. 2023), a pattern that was consistent across the 3 sampled sites. Our diet and SIA results over a wider geographic scale corroborate the niche differentiation observed between these species at Arraial alone (Mendes et al. 2018, Cardozo-Ferreira et al. 2023). Clearly, appearing to graze over the same substrata cannot be used to infer diet in such grazing herbivorous fishes. Rather, multiple, more fine-grained analyses are required to describe food targets and niche partitioning (Clements et al. 2017, Nicholson & Clements 2023a).

Sponge spicules (but no sponge tissue) were found in considerable amounts within the gut contents of the study fish species. Sponges can be an important food source for reef fishes (de Goeij et al. 2013, McMurray et al. 2018) and their ingestion has been recorded for parrotfishes in Brazil (Pereira et al. 2016), the Great Barrier Reef (Nicholson & Clements 2023b, 2024), and the Caribbean, including the sister species of the grey parrotfish, the redfin parrotfish *Sparisoma rubripinne* (Valenciennes, 1840) (Burkepile et al. 2019, Wulff 2021). However, parrotfishes might be targeting sponge-associated micro-photoautotrophic organisms (e.g. cyanobacteria and dinoflagellates) rather than sponge tissue itself (Clements et al. 2017, Nicholson & Clements 2023b, 2024). It is unlikely that sponge tissues were digested before the gut content analysis, since our methods aimed to collect specimens at their peak feeding time and to preserve the gut content to reduce digestion after collection (see Section 2.2). Sponges make dissolved organic carbon available to higher trophic levels in the form of expelled filter cells as detritus (de Goeij et al. 2013), which are consumed by fishes. Some sponges can harbour symbiotic nitrogen-fixing photoautotrophic cyanobacteria (Wilkinson & Fay 1979, Hudspith et al. 2022, Nicholson & Clements 2024), which corroborates the microphage hypothesis for parrotfishes (Clements et al. 2017, Clements & Choat 2018). Therefore, the most probable causes of the occurrence of sponge spicules in the sampled stomachs are both the targeting of sponge-associated cyanobacteria and the incidental ingestion of spicules present in the sediment while feeding.

The dietary shift from foliose (*Dictyota* spp.) to leathery (*Sargassum* spp.) brown algae in the algivore brassy chub across our sampling sites follows a change in relative abundance along the Brazilian coast, with *Dictyota* spp. being more abundant in the northern tropical locations and *Sargassum* spp. dominating more southern subtropical reefs (Aued et al. 2018). An increase in *Sargassum* spp. abundance could be linked with colder nutrient-rich water environments such as upwelling zones (Magaña-Gallegos et al. 2023). We did not sample food availability at each location and consequently cannot draw conclusions on feeding preference. Chubs are functional algivores targeting red and brown algae, despite the structural and storage carbohydrates in the latter that are indigestible for most fish species. Digestion of brown algae involves endosymbiotic bacteria in the hindgut that ferment mannitol and complex carbohydrates into short-chain fatty acids that are metabolically useful to the host (Clements & Choat 1995, 1997, Choat & Clements 1998, Mountfort et al. 2002, Pisaniello et al. 2022, Stevenson et al. 2022).

The consistent ingestion of algae by brassy chub across the studied locations was expected given the known dietary targets of chubs and their specialized diet and food-processing mode (Clements & Choat 1995, 1997, Choat et al. 2002, 2004, Mendes et al. 2018, Cardozo-Ferreira et al. 2023). Such specialization led to a low dietary overlap between this and the other species. The observed dietary variation supports the capacity of this species to maintain the ability to extract energy and nutrients from a range of macroalgae in the face of strong environmental variation (e.g. between tropical and subtropical reefs influenced by cold upwelling waters).

Isotopic values of consumers generally reflect diet and local environmental biochemical profiles (Fry 2006, Andrades et al. 2019, Cardozo-Ferreira et al. 2023). Sharing the same resources could result in overlapping isotopic niches, indicating competition among species. However, isotopic niche overlap in our study was greater than that indicated by the dietary analysis. Isotopic overlap can also be caused by different food sources sharing similar isotopic values, leading to discordance between dietary and isotopic profiles (Mendes et al. 2018) and an overestimation of isotopic niche overlap.

The unexpected wider isotopic niche of grey parrotfish in Natal could be due to the greater ingestion of cyanobacteria, an item known for its highly variable isotopic values, mostly in $\delta^{13}C$ (Vuorio et al. 2006). Moreover, disparate isotopic values or nitrogen content between the most dominant items in the micro-analysis (green filamentous algae and cyanobacteria) (Mendes et al. 2018) could play a role in widening the isotopic niche of grey parrotfish. Higher $\delta^{13}C$ values could be associated with the selection of organic particles and the consumption of ^{13}C-enriched benthic primary producers such as microphytobenthos (i.e. photoautotrophic diatoms, cyanobacteria, dinoflagellates, and green algae) (Underwood 2001, Bouillon et al. 2002). However, factors other than feeding on ^{13}C-rich benthic resources can also explain enriched carbon ratios in consumers, as detrital particles in planktonic food webs affected by upwelling may be enriched in heavy carbon isotopes after the depletion of light isotopes due to microbial reworking (Bode et al. 2020), affecting the whole community.

The 3 study fish species and the benthic sources in Arraial presented a 2-fold δ^{15}N-enrichment compared to Abrolhos and a 2‰ δ^{15}N increase in comparison to Natal. This was expected as a trend for both consumers and primary producers at locations affected by upwelling (Docmac et al. 2017, Radice et al. 2019). Although recent work has not identified seasonal variation in δ^{13}C and δ^{15}N in Arraial during the upwelling season compared to the non-upwelling season (Cardozo-Ferreira et al. 2023), shallow systems can carry δ^{15}N values consistent with a deep-water nitrate source carried up via upwelling (Radice et al. 2019). This δ^{15}N-enrichment affects lower trophic levels such as coral hosts, symbionts, and particulate organic matter (Radice et al. 2019), likely enriching the whole ecosystem when compared to non-upwelling locations. As the isotopic signal of a consumer reflects diet, an increase in δ^{15}N would also be expected if species targeted more δ^{15}N-enriched sources. In this context, the authors of previous studies (Gaines & Lubchenco 1982, Floeter et al. 2005, Behrens & Lafferty 2012, Longo et al. 2019, Hardison & Eliason 2024) have argued that nominally herbivorous fishes experience a constraint on the digestion of algae at high latitudes (Temperature Constraint Hypothesis, TCH), and compensate by increased consumption of animal matter. However, the dietary variation at the Arraial location reflects the influence of upwelling, not an increase in animal matter as would be expected under the TCH.

Widely distributed species display site-specific variation in diet and isotope signatures (Andrades et al. 2019, Johnson et al. 2020), making it difficult to develop general classifications of their diets. Differences between surgeonfishes and parrotfishes in the sources of dietary protein and detrital inputs have been noted previously (Clements et al. 2017, Clements & Choat 2018, Mendes et al. 2018). The enriched δ^{15}N observed in surgeonfish muscle compared to that of parrotfishes could be the result of either greater ingestion of invertebrates by the former (Carassou et al. 2013, Dromard et al. 2015, Zhu et al. 2019) or the assimilation of diazotrophic cyanobacteria by the latter. Detritus can be highly variable on different spatial scales as its origin can vary, for example, from algal detritus to fish faeces (Wilson et al. 2003). When the benthic composition varies among locations, so can the nutritional composition of detritus. The role and composition of detritus in the nutritional ecology of nominally herbivorous fishes is a critical issue that is yet to be resolved (Crossman et al. 2001, Wilson et al. 2003).

We emphasize that more detailed description and analytical approaches investigating the nutritional targets of nominally herbivorous fishes are required to understand their niche partitioning. Taxonomic resolution of the components within the food resources utilized by herbivorous fishes is essential to understand dietary targets and specialization, such as the selective feeding of parrotfishes on microbial elements (Clements et al. 2017, Nicholson & Clements 2020, 2023b). Furthermore, habitat features and resource availability at different spatial scales can yield a more complete understanding of food consumption and nutritional ecology of species. Although sampling at geographical scales can provide important general patterns, local environmental factors can be a better predictor of the ecology of reef fishes (Bellwood & Tebbett 2024). Future studies would benefit from a thorough nutritional characterization of detrital aggregates in turf assemblages, but also an investigation of the taxonomic composition of these resources along large spatial gradients, including tropical, subtropical, and temperate environments.

Our study highlights consistency in the trophodynamics of the study species at different tropical and subtropical reefs along the Brazilian coast. By integrating temporal analyses of 2 components (diet at 2 scales of magnification and SIA), we were able to elaborate trophic resource use among these species. Besides the dietary stability observed for each species at different locations, isotopic values and TPs varied with location. However, nitrogen enrichment was not observed to be linked to an increase in ingestion of animal matter. Refining the understanding of trophic interactions among coexisting species, by analysing complementary feeding and nutritional approaches, could improve knowledge on species ecology and, therefore, guide priority in management policies.

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