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Vol. 722: 125–143, 2023
https://doi.org/10.3354/meps14442

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published November 9

# Seasonal variation in diet and isotopic niche of nominally herbivorous fishes in subtropical rocky reefs

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ABSTRACT: Herbivorous fishes can be sensitive to environmental fluctuations, which influence both availability of food resources and metabolic rate, and thus nutritional requirements. Impacts on herbivore nutritional ecology may result from seasonal variations in temperature and meteoceanographic shifts such as wind-induced upwelling events. We evaluated the effects of seasonal changes on the nutritional ecology of 3 nominally herbivorous fishes (*Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis*) in a subtropical rocky reef on the southeastern Brazilian coast using a combination of gut content analysis (at 2 scales of magnification) and stable isotope analysis. Sampling of *in situ* water temperature covered both patterns of seasonal variation in sea surface temperature, and seasonal occurrence of upwelling. Local upwelling occurred throughout the year but less frequently in winter. Diet and isotopic niche displayed little seasonal variation. Species-specific patterns of seasonal variation indicated distinct responses to environmental fluctuations. Temperature alone cannot explain the locality-specific variation in the nutritional ecology of herbivorous reef fish, and contrary to predictions that digestion in herbivorous fishes is impaired by cooler temperatures, no significant shifts in species' nutritional ecology were detected.

KEY WORDS: Herbivory · Nutritional ecology · Dietary analysis · Seasonality · Stable isotopes · Trophic ecology

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## 1. INTRODUCTION

Seasonal changes in community structure and habitat use are prevalent in marginal reef systems experiencing significant environmental seasonality (Ateweberhan et al. 2006, Afeworki et al. 2013). Subtropical rocky reefs are subject to considerable seasonal environmental variation, as compared to more stable tropical reefs (Ferreira et al. 1998, Cordeiro et al. 2020), and upwelling events are especially important as they can exacerbate seasonal variation. Cold and nutrient-enriched waters from upwelling influence tropical and subtropical reef environments throughout the globe by substantially affecting water visibility, temperature and primary productivity (Perry & Larcombe 2003). Upwelling can influence herbivory in several ways, especially affecting the availability and nutritional

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quality of primary production, but also decreasing fish feeding rates due to low water temperature (Ferreira et al. 1998, Zemke-White & Clements 2004, Mendes et al. 2009). Herbivorous fishes can compensate for temporal changes in food sources (primary producers) by targeting different algal species or other benthic sources to satisfy nutritional requirements (Horn et al. 1986, Clements & Choat 1993, Zemke-White & Clements 1999).

Different techniques have been employed to track variation in ecosystem resources and its consequences for nutritional ecology. While dietary analysis provides a detailed description of ingested food items (Mendes et al. 2018, Pimentel et al. 2018), results generally reflect scales of hours or days. Problems with identifying the ingested food items in the guts of some herbivorous fishes, especially in parrotfishes, can also limit resolution of the diet (see Cocheret de la Morinière et al. 2003, Dromard et al. 2015, Clements et al. 2016). The complementary use of different approaches such as stable isotope analysis provides a broader perspective on diet and thus enables a more informed view of species' nutritional ecology and trophodynamic roles (Andrades et al. 2019a, Johnson et al. 2020, Grainger et al. 2023). Herbivorous fishes are conspicuous inhabitants of tropical and subtropical reef systems in terms of biomass (Choat & Clements 1998, Poore et al. 2012, Cordeiro et al. 2016), and have been the focus of intensive studies regarding their effects on benthic communities, especially their role as algal consumers (Bellwood et al. 2004, Tebbett et al. 2023). However, these fishes ingest a variety of items, from macroalgae and detritus (Choat et al. 2002) to epilithic and endolithic microscopic photoautotrophs (Clements et al. 2016, Clements & Choat 2017, Nicholson & Clements 2020). Low sea temperature was suggested as a factor limiting the capacity of herbivorous fishes to digest seaweed, i.e. the temperature constraint hypothesis (TCH) (Gaines & Lubchenco 1982, Floeter et al. 2005, Behrens & Lafferty 2007, 2012, Bennett & Bellwood 2011). The TCH predicts that cold water constrains the efficiency of digestion of macroscopic algae by herbivorous fishes, inducing a diet shift towards more protein-rich food sources (e.g. animal matter) so that species can meet nutritional demands (Floeter et al. 2005, Behrens & Lafferty 2007, 2012). However, sampling over latitudinal gradients has revealed little relationship between low temperature and digestive constraint in temperate herbivorous fishes (Trip et al. 2014, 2016, Johnson et al. 2020).

In Brazil, subtropical rocky reefs occur along ca. 2000 km of the south and southeastern coasts (Ferreira et al. 2004). The southeastern Brazilian coast is marked by significant seasonal variations in temperature, including upwelling events that are usually more frequent and stronger during spring and summer. Previous studies on these reefs indicated that temperature alone does not explain the seasonal variation observed in feeding rates of nominally herbivorous fishes (Ferreira et al. 1998), suggesting the need to investigate food sources and nutrient assimilation in more detail. Given that herbivores are important components of the reef fish fauna in the region, we examined seasonal variation in the nutritional ecology of 3 species with different diets and food-processing modes on a subtropical rocky reef subject to seasonal upwelling. We sought to understand how seasonal variation in sea surface temperature and associated upwelling events affected the nutritional ecology of these species. We used diet analysis at 2 magnifications and stable isotope analysis (SIA) to assess fish responses to seasonal variation. Specifically, we asked (1) do diet and stable isotope signatures of the 3 species vary among species and seasons, and (2) how do upwelling events influence variation in trophic niche?

## 2. MATERIALS AND METHODS

### 2.1. Study area

Fish and algae collections were carried out for 2 yr (April 2015 to June 2017), with sampling being done seasonally every 3 mo. Sampling was conducted at Arraial do Cabo, southeastern coast of Brazil (22° 58′ S, 42° 00′ W), in the state of Rio de Janeiro. The region is a multiple-use marine protected area and represents a hotspot for biodiversity, harbouring both tropical and subtropical biota (Ferreira et al. 2001, Cordeiro et al. 2016). Seasonal upwelling events associated with prevailing NE and N winds occur mainly from spring to autumn. Local rocky reefs are predominantly covered by a rich epilithic algal community and zoanthids, with a veneer of sponges, massive corals, fire corals and other sessile invertebrates (Cordeiro et al. 2014, Rogers et al. 2014). Several macroalgae, such as *Sargassum*, a common genus in the diet of some herbivorous fishes (Clements & Choat 1997, Mendes et al. 2018), are known for their seasonality in Arraial do Cabo (Yoneshigue-Valetin & Valentin 1992, Guimaraens et al. 2008).

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### 2.2. Water temperature

Temperature, as a proxy for upwelling occurrence, was monitored *in situ* during the sampling period using a HOBO TidbiT v2 temperature logger deployed (~6 m depth) at 2 sites (Anequim Bay: 22° 58′ 49.6″ S, 41° 59′ 01.4″ W, and Eastern Tip: 22° 58′ 42.8″ S, 41° 58′ 47.4″ W) within the sampling area. Temperature was registered hourly, and the data were pooled to obtain an average for each season: autumn (March to May), winter (June to August), spring (September to November) and summer (December to February). The occurrence of upwelling was inferred from recorded temperatures <18°C throughout the sampling period (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m722p125_supp.pdf. We used daily minimum temperature instead of mean because it is a better indicator of upwelling events (Valentin 2001).

### 2.3. Specimen selection and sampling

We selected 3 species of nominally herbivorous reef fishes: doctorfish *Acanthurus chirurgus* (Bloch, 1787) (Acanthuridae), endemic grey parrotfish *Sparisoma axillare* (Steindachner, 1878) (Labridae) and brassy chub *Kyphosus vaigiensis* (Quoy & Gaimard, 1825) (Kyphosidae). These are abundant and widely distributed species along tropical and subtropical Brazilian reefs (Ferreira et al. 2004) and display distinct diets and food-processing modes (Ferreira & Gonçalves 2006, Mendes et al. 2018). Specimens were collected by spear between 2 and 8 m depth and immediately preserved on ice. We minimised ontogenetic and size-related variation in diet and SIA by collecting only adult individuals (Table 1). In the laboratory, stomach contents (for dietary analysis) and dorsal muscle tissue (for SIA) were removed from all specimens. As parrotfishes lack a gastric stomach (Clements & Bellwood 1988), dietary content was removed from the proximal portion of the intestine in *S. axillare*. Stomach contents were preserved in 10% formalin, and muscle tissues were frozen, freeze-dried and ground to powder. Algae samples were collected throughout the seasons, concomitant with fish sampling, to represent the most abundant food items: brown algae (Phaeophyceae) *Dictyota* spp. and *Sargassum* spp.; red algae (Rhodophyta) *Gelidium pusillum* (Stackhouse) Le Jolis, 1863 and *Plocamium brasiliense* (Greville) M. Howe & W.R. Taylor, 1931; and turfs. Turfs in this system consist of epilithic filamentous algae and microalgae such as dinoflagellates, diatoms and cyanobacteria, a complex and heterogeneous matrix also rich in detrital components (Ferreira et al. 1998, Connell et al. 2014, Mendes et al. 2018). Turfs were scraped from the substratum using a spatula and stored in Falcon tubes for transport to the laboratory. To avoid confounding isotopic signatures, samples were washed to remove excess sediment, particulate matter and associated invertebrates and retain only the filamentous and calcareous

Table 1. Number (N) of sampled individuals; mean (range) of total length (TL) and total weight (TW); mean ± SE of stable isotope signatures of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$); and the calculated range of variation for both $\delta^{13}C$ (CR) and $\delta^{15}N$ (NR) for each species in each season

| Family<br>Species | Season | N | TL (mm) | TW (g) | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) | CR | NR |
|---|---|---|---|---|---|---|---|---|
| Acanthuridae | | | | | | | | |
| *Acanthurus chirurgus* | Summer | 22 | 309 (262–369) | 762 (490–1226) | −18.5 ± 0.5 | 12.4 ± 0.4 | 1.91 | 1.23 |
| | Autumn | 17 | 302 (279–320) | 685 (570–805) | −18.6 ± 0.7 | 12.5 ± 0.4 | 3.58 | 1.33 |
| | Winter | 17 | 307 (270–360) | 686 (440–1031) | −18.7 ± 0.8 | 12.3 ± 0.3 | 2.92 | 1.70 |
| | Spring | 14 | 279 (235–328) | 476 (240–720) | −19.1 ± 1.0 | 12.1 ± 0.5 | 3.74 | 1.74 |
| Labridae | | | | | | | | |
| *Sparisoma axillare* | Summer | 20 | 344 (237–500) | 772 (258–1610) | −15.9 ± 0.3 | 10.7 ± 0.3 | 1.10 | 1.01 |
| | Autumn | 13 | 340 (277–422) | 832 (385–1479) | −16.0 ± 0.2 | 10.9 ± 0.3 | 0.49 | 0.94 |
| | Winter | 19 | 333 (267–450) | 619 (267–1280) | −15.9 ± 0.3 | 10.6 ± 0.4 | 1.21 | 1.60 |
| | Spring | 13 | 356 (260–475) | 686 (115–1985) | −16.1 ± 0.5 | 10.9 ± 0.4 | 1.55 | 1.74 |
| Kyphosidae | | | | | | | | |
| *Kyphosus vaigiensis* | Summer | 20 | 307 (241–359) | 510 (260–902) | −16.5 ± 0.9 | 10.7 ± 0.8 | 3.13 | 3.09 |
| | Autumn | 6 | 350 (261–389) | 984 (404–1317) | −17.6 ± 0.9 | 11.9 ± 0.5 | 2.67 | 1.43 |
| | Winter | 17 | 274 (190–338) | 400 (120–841) | −16.3 ± 1.4 | 10.4 ± 1.0 | 4.58 | 3.55 |
| | Spring | 16 | 275 (177–394) | 392 (50–986) | −16.5 ± 1.2 | 10.6 ± 0.4 | 4.33 | 1.53 |

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algae. Turf algae mostly comprised the red calcareous *Amphiroa* spp. and *Jania* spp. These were often associated with filamentous epiphytes (*Herposiphonia* spp., *Polysiphonia* spp., *Bryopsis* spp. and *Cladophora* spp.) and cyanobacteria, which were retained for analysis due to their potential importance for the nutrition of the study species (Clements & Choat 2017, Nicholson & Clements 2022, 2023).

For each season, data were pooled across years, thereby excluding interannual variation and avoiding single-season bias in describing and characterizing species' nutritional ecology. Evaluating interannual variation was beyond the scope of this work.

### 2.4. Characterizing diets

Grazing acanthurids usually ingest small-sized particulate material which is triturated in a gizzard-like stomach, and parrotfishes grind ingested food using a pharyngeal mill. This makes the identification of items challenging (Clements et al. 2016). To minimise this problem, contents were analysed in 2 steps (adapted from Choat & Clements 1992). The first step (hereafter 'macro-analysis') used a stereomicroscope at 4× magnification for the 3 species. The gut content was spread in a Petri dish marked with 50 equidistant (10 mm) fixed points. The second step (hereafter 'micro-analysis') aimed to identify items ingested by *A. chirurgus* and *S. axillare* at 40× maximum magnification under an optical microscope. Micro-analysis was unnecessary for *K. vaigiensis*, as all dietary items could be resolved with the macro-analysis. The content used in the macro-analysis was filtered through a 60 µm mesh, and what passed through the mesh was analysed using a microscope slide marked with 30 equidistant (5 mm) fixed points. For both approaches, items at each marked point were identified to the lowest possible taxonomic level and used to calculate percentage contribution to the whole diet. Identified algae were classified *a posteriori* into functional groups adapted from Steneck & Dethier (1994). Sponge spicules comprised ~41% of 'sediment' in the macro-analysis and ~95% in the micro-analysis. No sponge tissue was observed in either the macro- or micro-analysis (sediment was not detected in *K. vaigiensis*). Spicules were included in the sediment category as they are mostly composed of silicate, have no nutritional value and were likely ingested as sediment while targeting epilithic material.

### 2.5. Stable isotope analysis

Stable isotope ratios were determined in dry samples (~0.4 mg), using a Delta V Advantage isotope ratio mass spectrometer interfaced with a Conflo IV and linked to a Flash 2000 Elemental Analyzer (Thermo Scientific). Pee Dee Belemnite carbonate and atmospheric nitrogen were used as standard values for the carbon and nitrogen analyses, respectively. The analytical precisions were ±0.1‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{15}N$ (triplicate samples of every fifth sample). Accuracy was assessed through the analysis of Elemental Microanalysis Protein Standard OAS/isotope certified material 114859. 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. To avoid biased values from fish muscle caused by high lipid content, $\delta^{13}C$ results were corrected following Post et al. (2007) when considering lipid-rich tissues (i.e. C:N > 3.5). Calcareous material was not acidified to avoid possible disruption in $\delta^{15}N$ signatures, which would affect trophic position estimations, and because post-treatment changes in the $\delta^{13}C$ signatures tend to be too discrete (Ng et al. 2007, Pires-Teixeira et al. 2021) to interfere in our inter- and intraspecific comparisons.

### 2.6. Data analysis

#### 2.6.1. Water temperature

Daily minimum temperature data were compared among seasons (summer, autumn, winter and spring) using a Kruskal-Wallis test followed by Dunn's multiple comparison test.

#### 2.6.2. Dietary analysis

Percentage contribution of each item was submitted to permutational multivariate analysis of variance (PERMANOVA) to determine seasonal dietary differences within and between species. PERMANOVA design was set as a resemblance matrix with Euclidean distance, Type III sum of squares, with residuals under a reduced model and 9999 permutations. Results from macro- and micro-analyses were compared among seasons using a design with 2 factors: 'Season,' fixed with 4 levels (i.e. summer, autumn, winter, spring), and 'Species,' fixed with 3 levels (i.e. *A. chirurgus*, *S. axillare*, *K. vaigiensis*). Posterior

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pairwise tests among levels of each factor were performed. PERMANOVA was also used to conduct a seasonal comparison for each item comprising the diet of each species. This analysis was performed separately for each species.

Principal component analysis (PCA) was performed on dietary data using percentage composition of each item separately for macro- and micro-analyses to visualize and understand seasonal and interspecific dietary variation. In addition, we calculated the Shoener index (Wallace 1981) to verify possible dietary overlap between each pair of species within seasons and between each pair of seasons for each species.

#### 2.6.3. Isotopic niche analysis

The isotopic signature of each fish species and algal group was evaluated using Stable Isotope Bayesian Ellipses in R (SIBER). Bayesian ellipses (95% credibility interval) were calculated to describe isotopic variation among seasons. We also used SIBER to calculate the overall range (variation) in $\delta^{13}C$ and $\delta^{15}N$ signatures (CR and NR, respectively) (Layman et al. 2007). Isotopic composition ($\delta^{13}C$ and $\delta^{15}N$ concentrations, separately) was tested among seasons with PERMANOVA using the same design used for dietary analysis.

PERMANOVAs were performed in Primer 6 with PERMANOVA+ add-on (Anderson et al. 2008). All other analyses were conducted in the R environment (R Core Team 2020) using the packages 'stats' (R Core Team 2020) and 'FSA' (Ogle et al. 2019) for seawater temperature tests; 'SIBER' (Jackson et al. 2011) for SIA; 'stats', 'ggplot2' (Wickham 2016) and 'ggfortify' (Tang et al. 2016) for PCA; and 'spaa' (Zhang 2016) for the Schoener overlap index.

## 3. RESULTS

### 3.1. Water temperature

Temperatures below 18°C are commonly used as a proxy for identifying upwelling occurrence (Valentin 2001) and were recorded in all seasons (Fig. S1). The lowest minimum temperatures occurred from spring (15.50°C) to summer (15.50°C) as expected, but also in autumn (15.51°C), with temperatures <18°C being less frequent during winter (2 occurrences: 17.82°C and 17.96°C). The average minimum temperature was similar across the seasons (summer = 20.84°C; autumn = 21.58°C; winter = 21.11°C; spring = 20.58°C). The minimum temperatures registered varied significantly among seasons (Kruskal-Wallis, p = 0.01).

### 3.2. Seasonal variation in diet

We collected and analysed the gut contents of 194 individuals (Table 1). Diets of all 3 species differed from each other (PERMANOVA; macro-analysis: pseudo-$F$ = 105.58, p = 0.0001; micro-analysis: pseudo-$F$ = 66.35, p = 0.001) and seasonally (PERMANOVA, macro-analysis: pseudo-$F$ = 15.65, p = 0.0001; micro-analysis: pseudo-$F$ = 255.26, p = 0.002; Tables S1 & S2), but with substantial overlap (see Figs. 1 & 2). Detritus was a common component in the diets of *Acanthurus chirurgus* and *Sparisoma axillare*, although it should be noted that its composition may differ between species. Grinding by the pharyngeal jaws of parrotfishes reduces ingested food, whereas *A. chirurgus* relies on a gizzard-like muscular stomach to grind food. Consequently, the term 'detritus' used in our analysis refers to an amorphous organic material that could have been ingested or produced by mechanical trituration.

PCA for macro-analysis of *A. chirurgus* showed that PC1 (which explained 47.92% of variation) was positively associated with red calcareous algae, particularly in autumn, while on PC2, invertebrates and red filamentous algae were opposed to red corticated algae (Fig. 1A). Mostly, diet varied seasonally except when comparing summer vs. autumn (PERMANOVA: pseudo-$t$ = 1.721, p = 0.0512; Table S3). For *S. axillare*, PC1 (81.4% of variation) showed a trend from detritus to red calcareous algae. PC2 was negatively associated with red filamentous, red corticated and red crustose calcareous algae (Fig. 1B). For *Kyphosus vaigiensis*, PC1 (44.65% of variation) was negatively associated with brown leathery algae (i.e. *Sargassum* spp.), while PC2 contrasted brown foliose algae (i.e. *Dictyota* spp.) to red corticated algae (Fig. 1C). In both *S. axillare* and *K. vaigiensis*, winter diet clearly differed from that in all other seasons (Fig. 1; Table S3).

The first axis of the micro-analysis PCA (which explained 58.25% of variation) for *A. chirurgus* showed a trend from diatoms to detritus and sediment. PC2 contrasted cyanobacteria and detritus from sediment and mostly characterized the diet in winter (Fig. 2A). Seasonal differences were only found between consecutive seasons, but not between spring and summer (PERMANOVA: pseudo-$t$ = 1.338, p = 0.152; Table S3). For *S. axillare,* PC1 (51.91% of variation) trended from sediment to cyanobacteria and diatoms, especially in winter. PC2 was associated with detritus. Together

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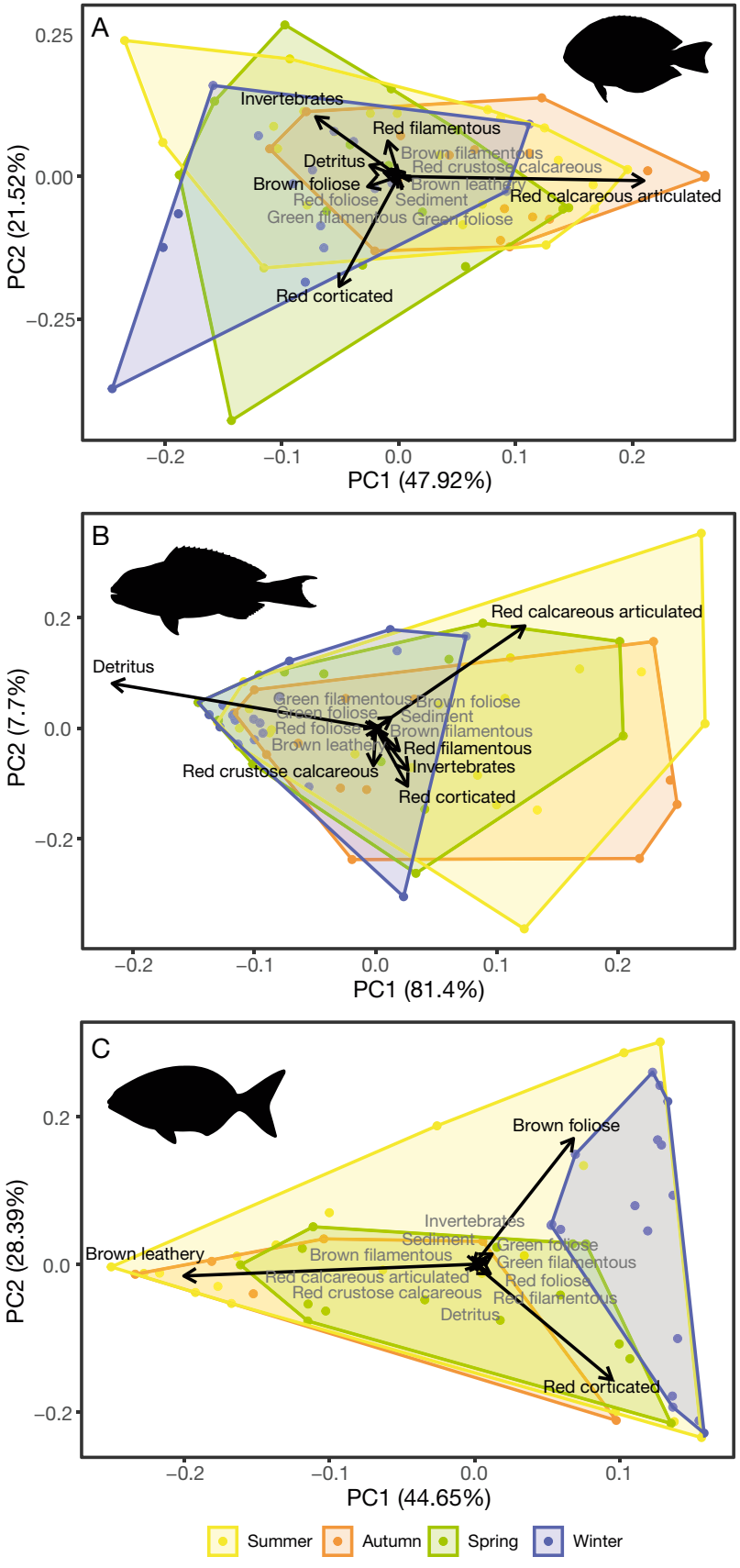


Fig. 1. Seasonal comparison of diet macro-analysis data for (A) *Acanthurus chirurgus*, (B) *Sparisoma axillare* and (C) *Kyphosus vaigiensis*. Each dot represents an individual in each season (principal component analysis, PCA). Loading labels in grey depict items with lower influence (eigen-values <0.100) in both PC1 and PC2

with sediment, these 2 items were responsible for the distinctiveness of summer diet (Fig. 2B). Significant differences in the diet micro-analysis of *S. axillare* were found between winter and the other seasons, similarly to the macro-analysis (PERMANOVA: winter vs. summer – pseudo-$t$ = 3.207, p = 0.0001; winter vs. autumn – pseudo-$t$ = 2.704, p = 0.0009; winter vs. spring – pseudo-$t$ = 2.350, p = 0.004; Fig. 2; Table S3).

In all seasons, the main macro-items in *A. chirurgus* diet were red corticated and red calcareous articulated algae (Fig. 3A; Fig. S2). While these same algae represented at least 10% of the diet of *S. axillare* year-round (Fig. S3), the main item consumed by this parrotfish was detritus (Fig. 3B). Micro-material in *A. chirurgus* mainly comprised diatoms, while sediments dominated in *S. axillare*. *K. vaigiensis* mainly ingested brown algae (Fig. 3C) throughout the year. However, during winter, *Sargassum* spp. was absent and replaced by brown foliose *Dictyota* spp. and red corticated algae (Fig. S4). Also during winter, 3 out of the 17 individuals of *K. vaigiensis* sampled in that season ingested a disproportionally high (>80%) amount of Copepoda.

Dietary overlap was identified among seasons and species (Table S4). In *A. chirurgus* and *S. axillare*, all seasonal comparisons had Schoener values above 0.71, ranging from 0.72 to 0.79 in the former and 0.77 to 0.91 in the latter species. Overlap was more variable in *K. vaigiensis* (Schoener = 0.31–0.84). For all 3 species, lowest seasonal overlaps were between winter and any other seasons (except between winter and spring in *A. chirurgus*; Table S4). Within seasons, overlap was consistently low (≤0.081) between *S. axillare* and *K. vaigiensis* and ranged from 0.39 to 0.43 between *A. chi-*

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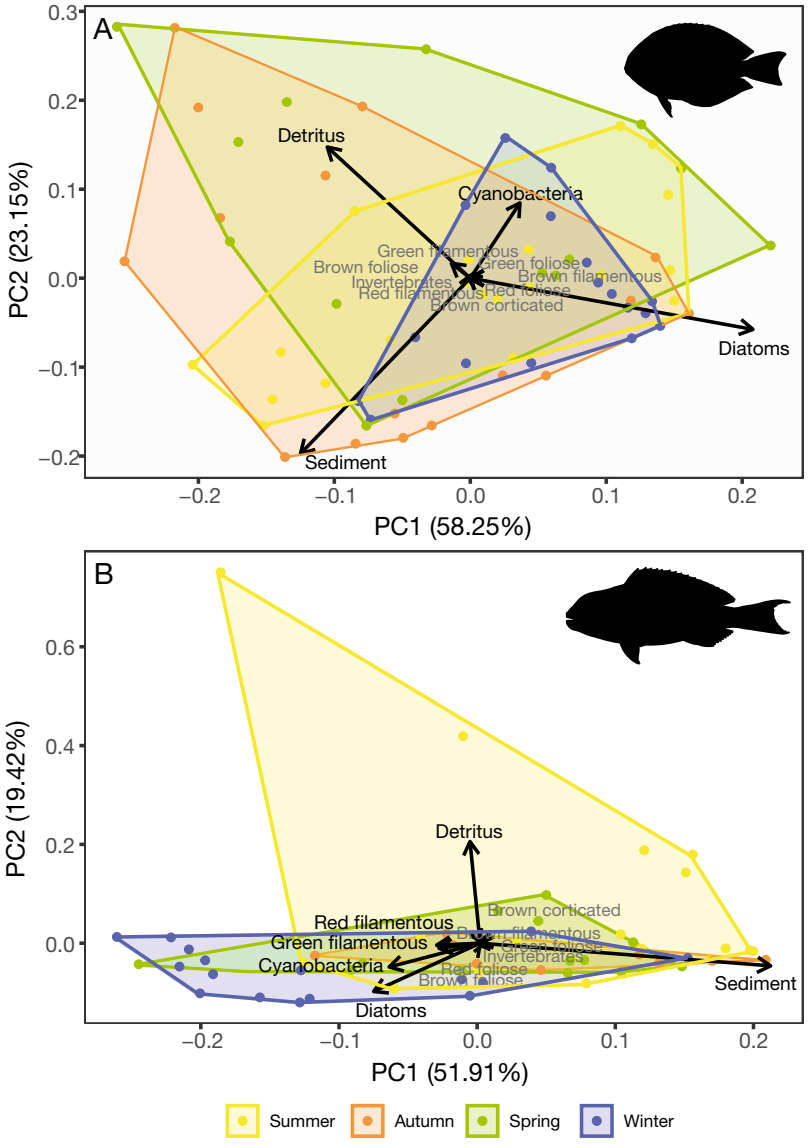


Fig. 2. Seasonal comparison of the diet micro-analysis data for (A) *Acanthurus chirurgus* and (B) *Sparisoma axillare*. Each dot represents an individual in each season (principal component analysis, PCA). Loadings labels in grey depict items with lower influence (eigenvalues < 0.100) in both PC1 and PC2

*rurgus* and *S. axillare*. Dietary overlap between *A. chirurgus* and. *K. vaigiensis* varied seasonally from 0.15–0.16 in summer and autumn to 0.31–0.32 in winter and spring (Fig. S4).

### 3.3. Seasonal variation in isotopic niche

Stable isotope signatures of the 3 species showed only small variation among seasons (Table 1; Table S5). In *A. chirurgus*, $\delta^{15}N$ in spring differed from winter, and $\delta^{13}C$ differed between summer and autumn. No seasonal differences were found in *S. axillare* in either $\delta^{15}N$ or $\delta^{13}C$. In *K. vaigiensis*, $\delta^{15}N$ signature was higher (11.9‰) and differed (PERMANOVA, $p < 0.05$) between autumn and other seasons (PERMANOVA: autumn vs. summer – pseudo-$t = 3.656$, $p = 0.002$; autumn vs. winter – pseudo-$t = 3.339$, $p = 0.003$; autumn vs. spring – pseudo-$t = 5.648$, $p = 0.0003$; range $\delta^{15}N$: 10.4 – 10.7‰; Table 1; Table S5). The isotopic standard ellipse area corrected for small sample size (SEAc ‰$^2$; niche breadth) varied among seasons, but with high overlap (Fig. 4). Isotopic niche in *Acanthurus chirurgus* and *S. axillare* was broader in spring and restricted in summer (Fig. 4A,B), while in *K. vaigiensis*, the isotopic niche was broader in winter and more restricted in autumn (Fig. 4C). $\delta^{15}N$ range (NR) and $\delta^{13}C$ range (CR) varied seasonally in each species (Table 1). NR in *A. chirurgus* increased from summer to spring, while CR oscillated throughout the year, being higher in spring and lower in summer. CR and NR were lower in *S. axillare*, being lower in autumn and higher in spring, while NR was also lower in autumn but higher in winter. CR and NR in *K. vaigiensis* were higher in winter and lower in autumn.

### 3.4. Stable isotope signatures of primary producers

Differences in isotopic signatures of primary producers were more evident for carbon ($\delta^{13}C$) than for nitrogen ($\delta^{15}N$; Table 2, Fig. 5). Red algae (*Gelidium pusilum* and *Plocamium brasiliense*) were $^{13}C$-depleted, turf was $^{13}C$-enriched, and brown algae (*Sargassum* spp. and *Dictyota* spp.) were intermediate (Fig. 5). While isotopic composition of species (including 'turf') differed from each other (Table S6), no overall effect of season on composition was detected. Differences in $\delta^{13}C$ and/or $\delta^{15}N$ were restricted to spring vs. autumn *G. pusillum* (PERMANOVA: pseudo-$t = 11.26$, $p = 0.035$) and to summer vs. spring and vs. autumn *Dictyota* spp. (PERMANOVA: pseudo-$t = 5.676$, $p = 0.001$, and pseudo-$t = 3.343$, $p = 0.003$, respectively) (Table S7). No seasonal variation was detected within *P. brasiliense*, *Sargassum* spp. and turf.

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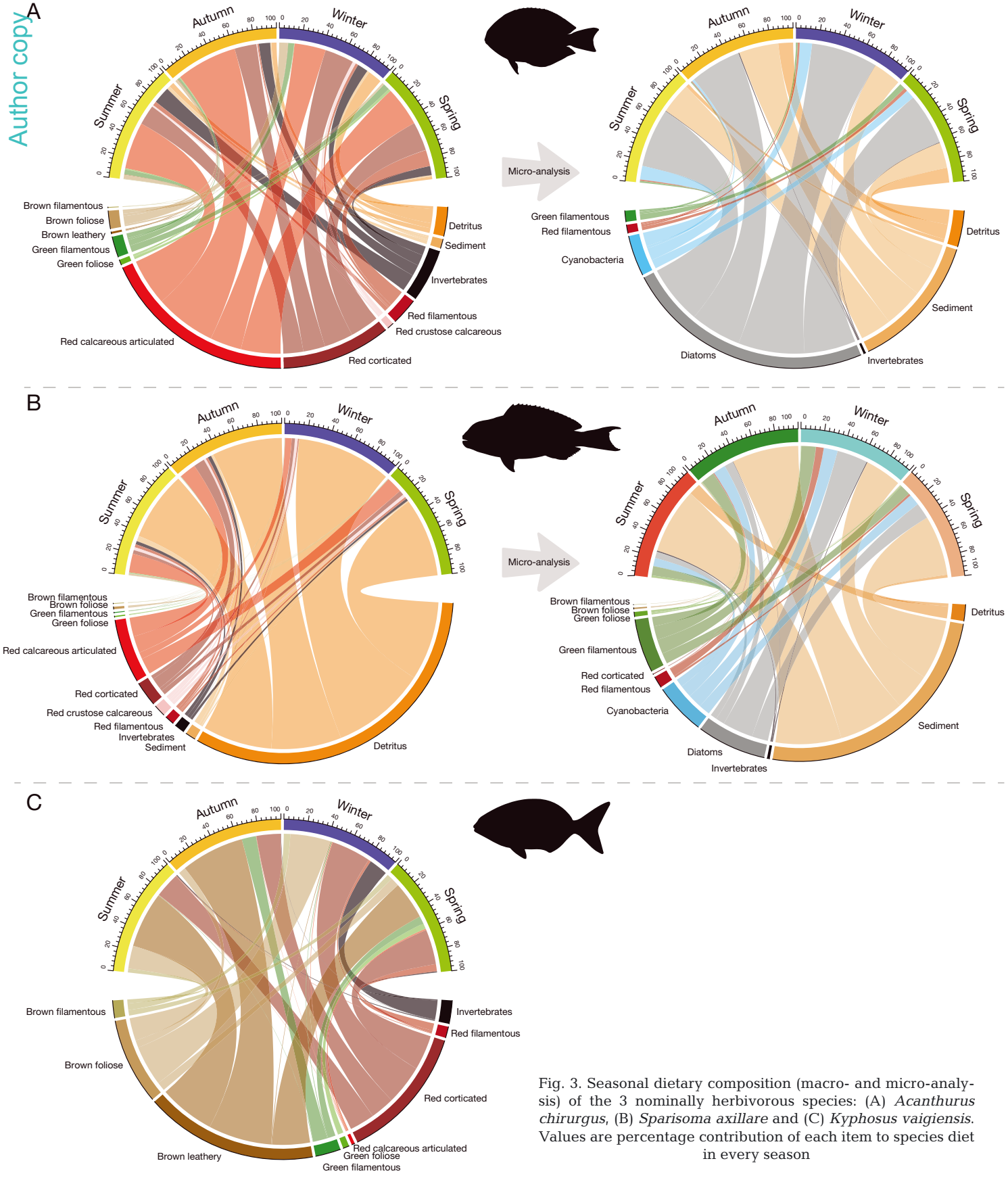


Fig. 3. Seasonal dietary composition (macro- and micro-analysis) of the 3 nominally herbivorous species: (A) *Acanthurus chirurgus*, (B) *Sparisoma axillare* and (C) *Kyphosus vaigiensis*. Values are percentage contribution of each item to species diet in every season

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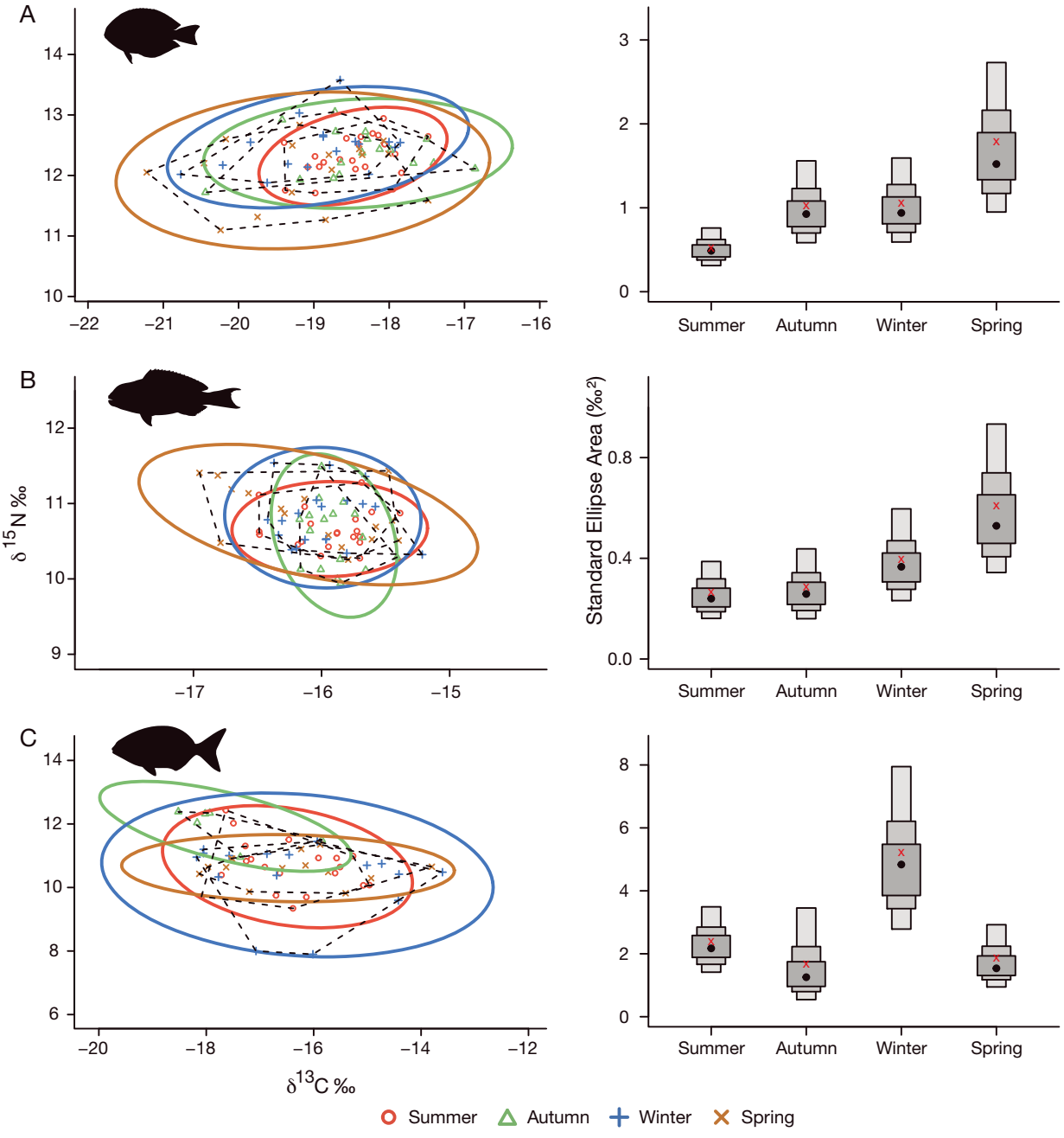


Fig. 4. Seasonal comparisons of stable isotope bivariate space (left column; $\delta^{13}C$ and $\delta^{15}N$) and seasonal standard ellipse area (right column) among the 3 study species: (A) *Acanthurus chirurgus*, (B) *Sparisoma axillare* and (C) *Kyphosus vaigiensis*. Ellipses in the left column are the 95% credible interval, and each symbol represents an individual. Dashed lines are total hull area for each season for the species. Shaded boxes in the right column represent credible intervals at 50, 75 and 95%, from dark to light grey, respectively. Black dots represent the sample mode, and red 'x' shows the mean value for each season. Note that numerical axes are plotted on distinct scales

### 3.5. Interspecific variation

Diet differed among species in all seasons in both macro- (PERMANOVA: pseudo-$F$ = 105.58, p = 0.0001) and micro-analyses (PERMANOVA: pseudo-$F$ = 66.35, p = 0.0001) (Tables S1 & S8) except for overlap between *A. chirurgus* and *K. vaigiensis* in winter. PCA variation clearly separated species based on diet (Fig. 6). The first principal component (PC1) explained most of the variation (summer: 39.97%;

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Table 2. Mean ± SE of the stable isotope ratios (‰) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) for sampled algae in each season. '–': species not encountered

| | Summer | | Autumn | | Winter | | Spring | |
|---|---|---|---|---|---|---|---|---|
| | $\delta^{13}C$ | $\delta^{15}N$ | $\delta^{13}C$ | $\delta^{15}N$ | $\delta^{13}C$ | $\delta^{15}N$ | $\delta^{13}C$ | $\delta^{15}N$ |
| **Foliose brown algae** | | | | | | | | |
| *Dictyota* spp. | −16.8 ± 1.1 | 6.2 ± 0.4 | −18.7 ± 1.5 | 6.5 ± 0.1 | – | – | −20.0 ± 1.1 | 6.5 ± 0.3 |
| **Leathery brown algae** | | | | | | | | |
| *Sargassum* spp. | −15.2 ± 1.3 | 6.5 ± 0.2 | −18.0 ± 3.0 | 6.1 ± 0.1 | – | – | – | – |
| **Corticated red algae** | | | | | | | | |
| *Plocamium brasiliense* | −29.1 ± 1.1 | 6.7 ± 0.2 | – | – | −26.4 ± 2.9 | 6.9 ± 0.2 | – | – |
| *Gelidium pusillum* | – | – | −25.9 ± 0.8 | 6.5 ± 0.3 | – | – | −30.3 ± 0.4 | 6.3 ± 0.1 |
| **Turf** | −11.6 ± 3.3 | 6.3 ± 0.6 | −9.5 ± 1.0 | 6.6 ± 0.2 | −9.5 ± 1.0 | 6.9 ± 0.4 | −8.6 ± 1.2 | 6.2 ± 0.4 |

autumn: 49.66%; winter: 55.84%; and spring: 49.5%), and involved a trend from detritus in *S. axillare* (positively influencing variation in summer and autumn, but negatively in winter and spring) to brown algae (either leathery or foliose, the latter mostly in winter) in *K. vaigiensis*, or red algae (calcareous articulated or corticated) in *A. chirurgus* (Fig. 6). Information provided by the second axis (PC2, summer: 27.87%; autumn: 31.33%; winter: 17.05%; and spring: 22.41%) was essentially restricted to further differentiating *A. chirurgus* from the other species based on the trend of articulated red calcareous algae (positively associated in summer, autumn and spring) or red corticated algae (winter).

The PCA on dietary micro-analysis revealed clear dietary partitioning with little overlap between *A. chirurgus* and *S. axillare* within seasons (Fig. 7). PC1 explained most variation (summer: 69.46%; autumn: 59.93%; winter: 51.42%; spring: 62.78%) with a trend of diatoms characterizing *A. chirurgus*, while sediment (plus green filamentous algae in winter) characterized *S. axillare*. PC2 in summer (17.48% of variation) was mostly positively influenced by detritus, and negatively associated with it in autumn and

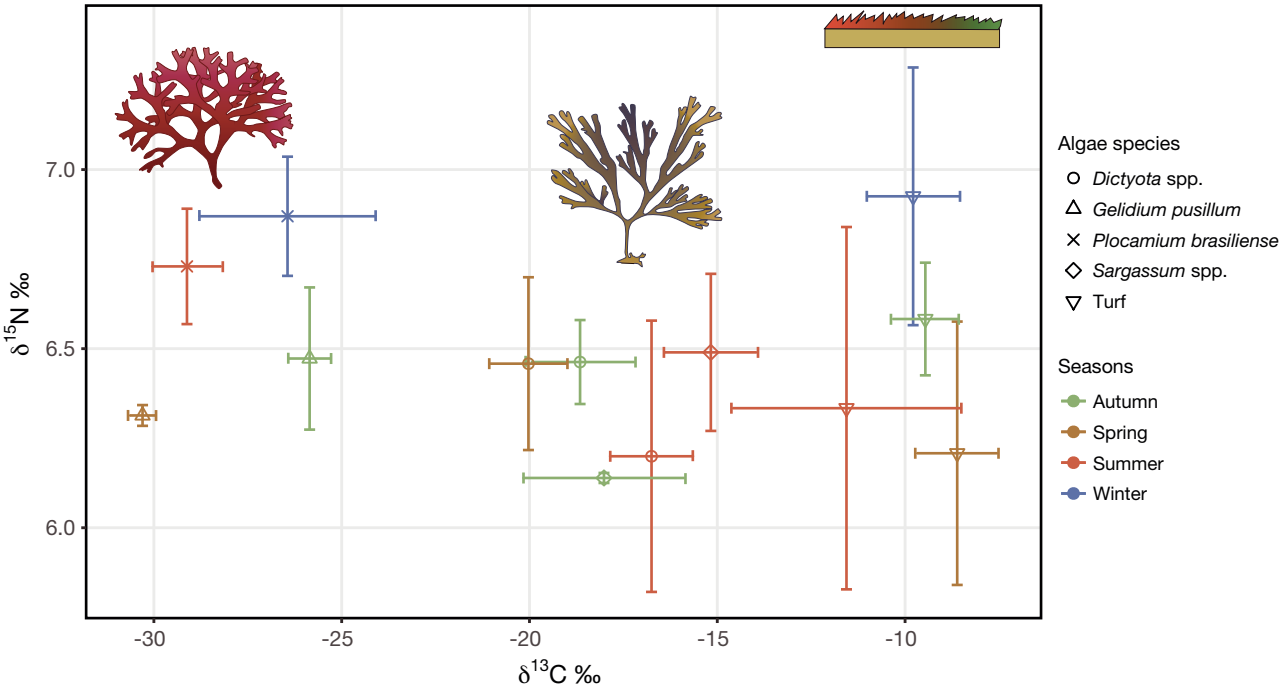


Fig. 5. $\delta^{13}C$ and $\delta^{15}N$ bivariate space with mean (symbols) and standard deviation (error bars) illustrating the isotopic composition of primary producers sampled in each season: corticated red algae (*Gelidium pusillum* and *Plocamium brasiliense*), foliose brown algae (*Dictyota* spp.), leathery brown algae (*Sargassum* spp.) and turf

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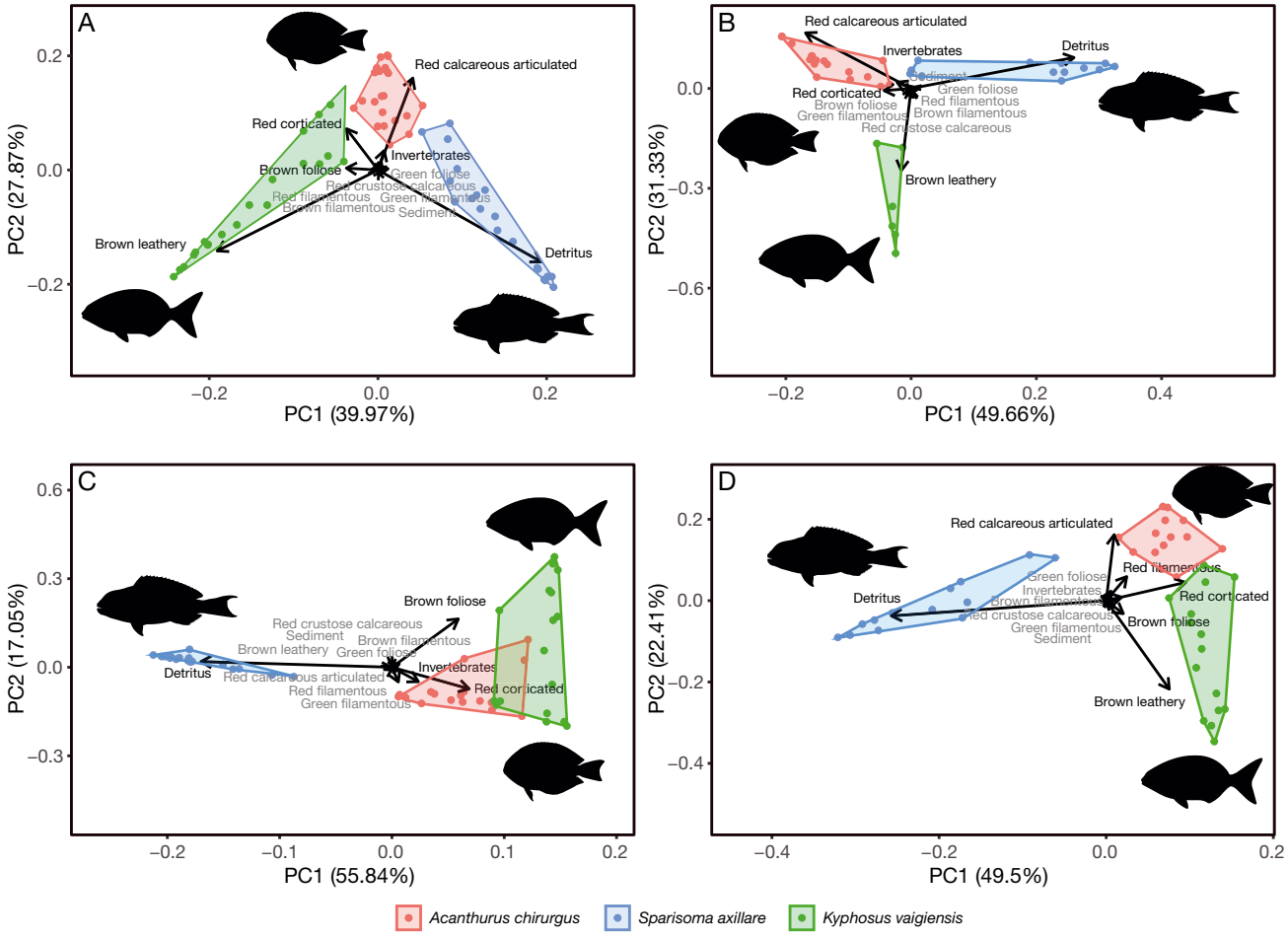


Fig. 6. Comparison of dietary macro-analysis among studied species *Acanthurus chirurgus*, *Sparisoma axillare*, and *Kyphosus vaigiensis* within each season: (A) summer, (B) autumn, (C) winter and (D) spring. Each dot represents an individual (principal component analysis, PCA). Loadings labels in grey depict items with lower influence (eigenvalues < 0.100) on both PC1 and PC2 in differentiating species regarding their diet

spring (26.93 and 19.98% of variation, respectively) (Fig. 7). In winter, PC2 (29.73% of variation) was positively associated with sediment, while negatively associated with cyanobacteria and red filamentous algae.

During the year, a distinct subset of food items was important for each species (Fig. 8), indicating strong diet partitioning: red calcareous articulated algae in *A. chirurgus*, detritus in *S. axillare* and brown and red corticated algae in *K. vaigiensis* (Fig. 8). Diatoms and sediment also characterized the diets of *A. chirurgus* and *S. axillare*, respectively.

The widest isotopic niche was observed in *K. vaigiensis*, and the narrowest in *S. axillare*, with a large overlap between them. This suggests that *S. axillare* had a diet based on less isotopically variable items than *K. vaigiensis* (Fig. 9) despite feeding at similar $\delta^{13}C$ and $\delta^{15}N$ isotopic levels. *A. chirurgus* displayed a $^{13}C$-depleted and $^{15}N$-enriched signature compared to *S. axillare* (Fig. 9) and, at least in autumn, to *K. vaigiensis*. This might be a consequence of the higher intake of corticated red algae by *K. vaigiensis* (Table 2, Fig. 5).

## 4. DISCUSSION

We used diet and stable isotope analyses to investigate seasonal shifts in the nutritional ecology of 3 nominally herbivorous fishes in a subtropical rocky reef affected by upwelling events. We found upwelling effects on water temperature in all seasons, but less frequently in winter. Locally, upwelling is typically stronger during spring and summer (Valentin 2001) and is an important driver of seasonal changes in our study area (Yoneshigue-Valetin &

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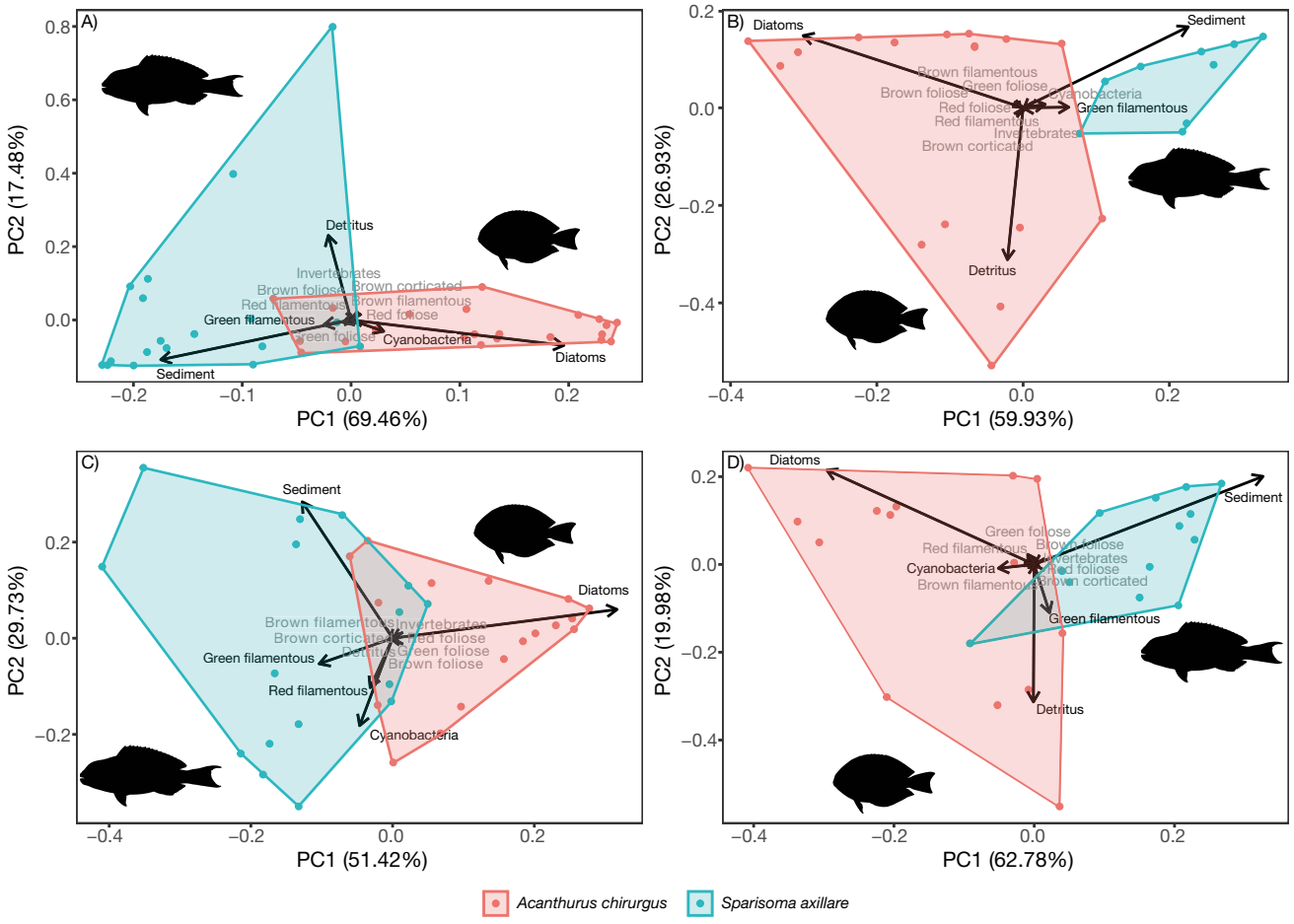


Fig. 7. Comparison of dietary micro-analysis among study species *Acanthurus chirurgus* and *Sparisoma axillare* within each season: (A) summer, (B) autumn, (C) winter and (D) spring. Dots represent the individual dietary composition in each season (principal component analysis, PCA). Grey loadings labels depict items with lower influence (eigenvalues < 0.100) on both PC1 and PC2 in differentiating species regarding their diet

Valentin 1992, Soares et al. 2014). Despite these subtropical reefs being constantly affected by temporal changes in water temperature influencing metabolic rates (Ferreira et al. 1998), a continuous nutrient input from upwelling events drove the low variation in the nutritional ecology of the nominally herbivorous fishes. Diet of all species varied across seasons, while stable isotope signatures mostly remained stable, suggesting that species switch among isotopically similar foods to meet their nutritional demands, as seen for other herbivorous fishes worldwide (Horn 1989, Choat & Clements 1993, Clements & Choat 1997, Moran & Clements 2002, Raubenheimer et al. 2005, Clements et al. 2009).

Seasonal variation in isotopic signatures was only observed in spring in *A. chirurgus* and in autumn in *K. vaigiensis*. Diet variation in *K. vaigiensis* was not reflected in isotopic variation, indicating a balance in the ingestion of isotopically different food sources to meet nutritional requirements or feeding on different food items with similar isotopic signatures. During upwelling events, shallow waters increase their nitrate concentrations, and therefore nitrogen fractionation, leading to changes in $\delta^{15}N$ of photoautotrophic organisms (e.g. algae) (Bradley et al. 2016). Isotope $\delta^{15}N$ values of primary producers in Arraial do Cabo are 50 to >100% higher than those in non-upwelling locations along the Brazilian coast (Andrades et al. 2019b, G. Cardozo-Ferreira et al. unpubl. data). However, the almost-constant occurrence of upwelling events homogenised the isotopic signatures of both primary producers and consumers. Potential effects of nutrient-rich cold waters on fish nutritional ecology include the nitrogen enrichment and/or availability of their food. Our results on the $\delta^{15}N$ signatures of food sources (ranging from 6.1 to 6.9‰) were similar to those previously found for macroalgae (6.18‰), suspended particulate matter (6.28–6.5‰)

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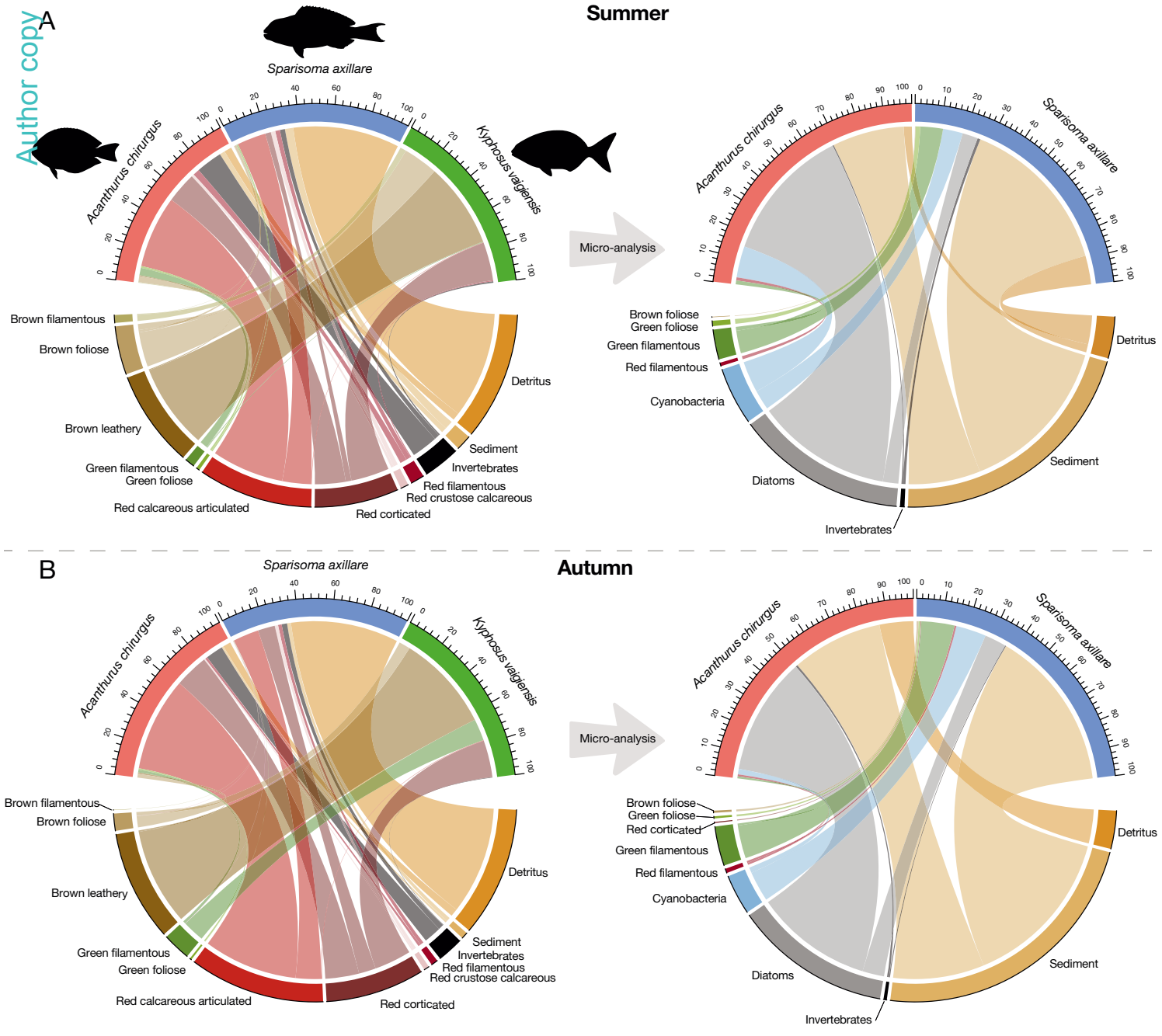


Fig. 8. Intraspecific comparison of dietary composition (macro- and micro-analysis) of the 3 herbivorous study species (*Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis*) in (A) summer, (B) autumn, (C) winter and (D) spring. Values are percentage contribution of each item to the diet of each species in every season. *Kyphosus* is not included in the micro-analysis

(*Fig. 8 continued on next page*)

and sediment organic matter (6.44–7.14‰) at the same location (Soares et al. 2014), highlighting the almost constant presence of upwelling-derived nutrients. Whether temperature variation was insufficient to affect herbivore diets and isotopic signatures or the yearlong stability of species' nutritional ecology is a product of adaptation to cope with lower water temperatures requires further work. It is important to acknowledge that temperature alone cannot explain the site-specific variation in the nutritional ecology of herbivorous reef fish. Although the present study focused on the impacts of temperature arising from seasonal patterns and episodic upwelling events, the potential but unknown influence of primary produc-

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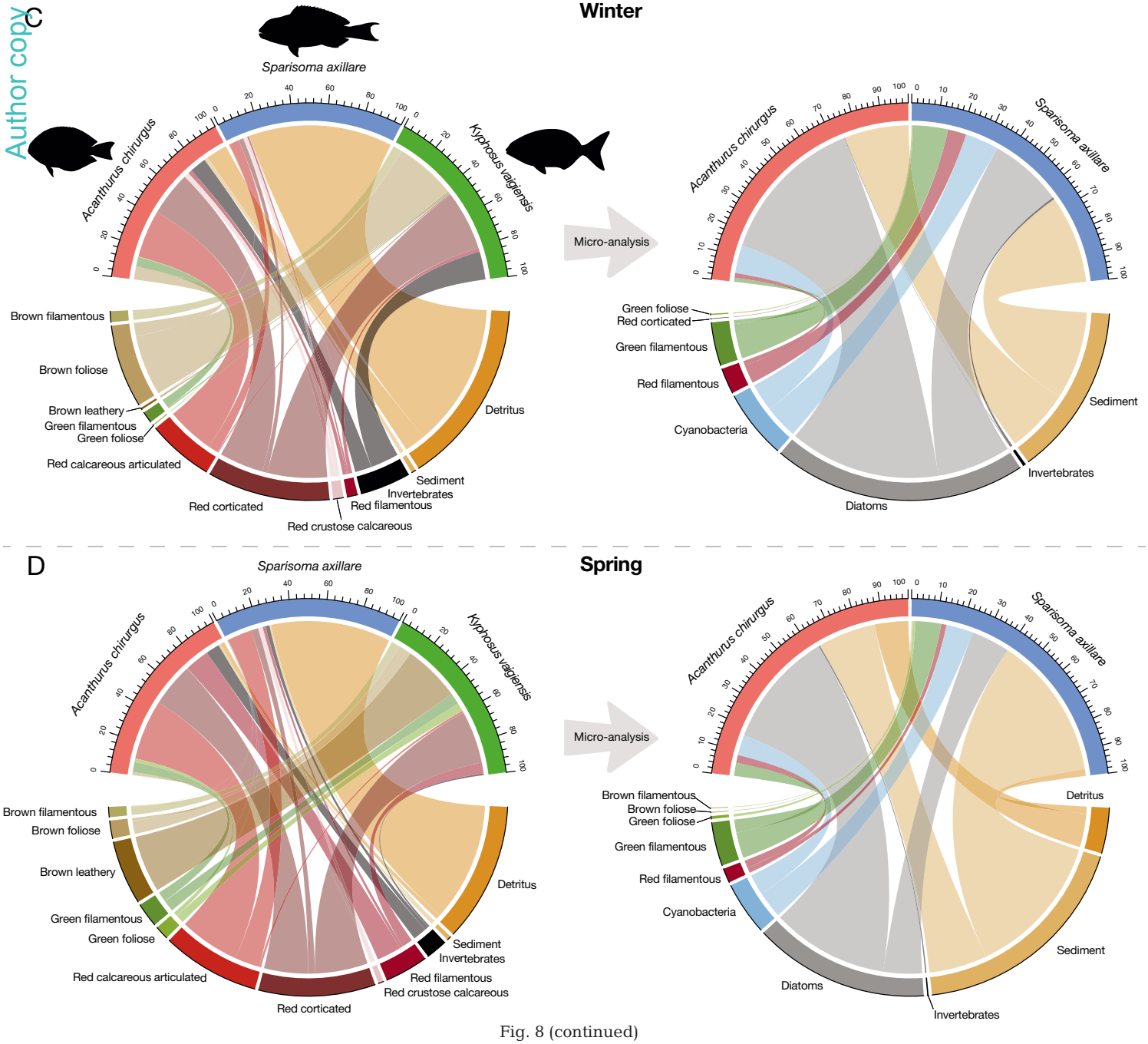


Fig. 8 (continued)

tivity variation is acknowledged (Huey & Kingsolver 2019, Vinton & Vasseur 2022).

*K. vaigiensis* mainly selected *Sargassum* spp., except during winter, when these annual algae are virtually absent (Yoneshigue-Valetin & Valentin 1992, Guimaraens et al. 2008, Cordeiro et al. 2020). In New Zealand, the algivorous *Odax pullus* (Labridae) shifts its diet seasonally, varying the ingested proportions of thallus and reproductive structures of brown algae in response to phenological shifts (Clements & Choat 1993, Johnson et al. 2017). However, such structures were not found in the gut contents of either *K. vaigiensis* in the present study, or *K. sydneyanus* in New Zealand (Moran & Clements 2002). During winter, *K. vaigiensis* consumed a wider variety of food items, replacing *Sargassum* spp. with *Dictyota* spp., and ingesting red corticated algae such as *Plocamium brasiliense*, *Gelidiella* spp. and *Gelidium pusillum*. A similar pattern of dietary supplementation with red algae during winter was also observed

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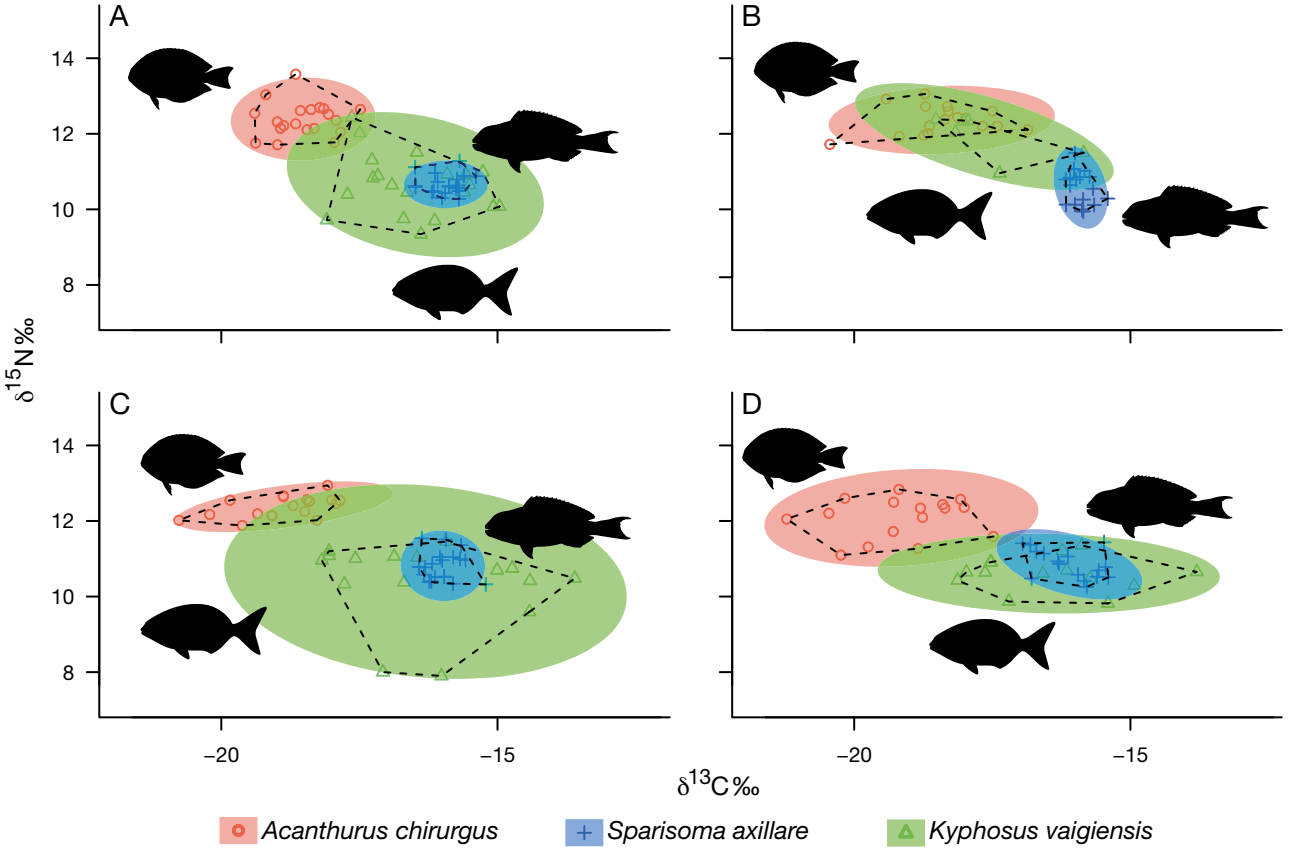


Fig. 9. Stable isotope bivariate space (for $\delta^{13}C$ and $\delta^{15}N$) illustrating the comparative isotopic niche through corrected standard ellipse area (ellipses; SEAc, 95% credible interval) and the convex-hull area around extreme sample values (dashed lines) for *Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis*, in each season: (A) summer, (B) autumn, (C) winter and (D) spring

for *K. bigibbus* in Japan (Yatsuya et al. 2015). This resulted in a wider isotopic niche in *K. vaigiensis* during winter and, consequently, in a higher overlap of isotopic niche with the other 2 fish species. The enriched $\delta^{15}N$ values during autumn might indicate some seasonal variation in diet of *K. vaigiensis*, as it ingested a higher proportion of green filamentous algae which usually display higher $\delta^{15}N$ signatures than other macroalgae (Abrantes et al. 2013, Pinheiro et al. 2017). As the isotopic signatures of our sampled algae species differed mainly in $\delta^{13}C$, we expected to see greater variation in species' $\delta^{13}C$ signatures while feeding on a greater variety of algal taxa. *K. vaigiensis*, for example, displayed a greater range of $\delta^{13}C$ variation (CR) in winter and spring (CR-summer = 3.1‰; CR-autumn = 2.7‰; CR-winter = 4.6‰; CR-spring = 4.3‰), when this species ingested similar amounts of red and brown algae. A significant ingestion of invertebrates in winter by *K. vaigiensis* resulted in a greater range of $\delta^{15}N$ variation (NR) in that season (NR-summer = 3.1‰; NR-autumn = 1.4‰; NR-winter = 3.6‰; NR-spring = 1.5‰), as its diet comprised a mixture of isotopically different sources. However, such higher ingestion occurred in 3 out of the 17 individuals sampled during winter, coupled with other algal taxa that were eaten by *Kyphosus* in winter because *Sargassum* is unavailable, reflected in the increased NR. The prediction of the TCH that lower temperatures would lead to increased feeding on nitrogen-rich animal sources (i.e. invertebrates) was not supported, as temperature during winter was higher than in the other seasons. Thus, the interaction between food availability and temperature better explains the variations in the species' nutritional ecology than solely temperature (Theus et al. 2022, Vinton & Vasseur 2022).

The diet of *Sparisoma axillare* was largely dominated by detritus and calcareous articulate algae, conspicuous components that, along with the associated epiphytic filamentous algae and microalgae such as dinoflagellates, diatoms and cyanobacteria, form the turf assemblage in the study region (Ferreira et al. 1998, Mendes et al. 2018). Filamentous turf forms a complex and spatially heterogeneous

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assemblage with detrital components that contribute to the nutrition of grazing fishes (Choat 1991, Crossman et al. 2001, Connell et al. 2014). Detritus and cyanobacteria were both important in the diet of *S. axillare*, as well as sediment (which is not a nutritional source, except when it contains epilithic or endolithic microorganisms). The observation of sponge spicules, but not tissues, in *S. axillare* gut contents suggests incidental ingestion while targeting epilithic photoautotrophic microorganisms (Clements et al. 2016). We noted a high abundance of tiny sponges within turf in our study area (pers. obs.). The importance of sponges as a source of nutrients for marine environments is well established (de Goeij et al. 2013, McMurray et al. 2018). Sponges may support or contain high densities of cyanobacteria and zooxanthellae (Patten et al. 2011, Easson & Thacker 2014), i.e. nutrient-rich items known to be a target for parrotfishes (Clements et al. 2016, Clements & Choat 2017, Nicholson & Clements 2020). While spongivory has previously been recorded for parrotfishes in Brazil (Pereira et al. 2016) and the Caribbean (Burkepile et al. 2019, Wulff 2021), the full extent of their nutritional potential is yet to be determined.

The red calcareous articulated algae in the diet of *A. chirurgus* decreased from summer to spring while the isotopic niche increased correspondingly. As calcareous articulated algae contain high amounts of inorganic carbon and as a result are considered nutritionally poorer than other red algae, nutrients obtained by fishes from these algae are possibly associated with epiphytic or endolithic filamentous algae and microscopic photoautotrophs commonly colonizing this living substrate (Nicholson & Clements 2020). Although we did not detect any seasonal variation in the diet of *A. chirurgus* in the proportion of filamentous epiphytes, seasonal variation in the composition of turfs in Arraial do Cabo remains to be tested and could reveal important details on its availability for herbivorous reef fishes. Detritus was frequently observed in the diet of *A. chirurgus* (Ferreira & Gonçalves 2006, Dromard et al. 2015, Mendes et al. 2018). However, this category substantially differs in composition between for *A. chirurgus* and *S. axillare*. Grinding by the pharyngeal jaws of parrotfishes reduces ingested food into a fine, whitish mass, that also includes the remains of crustose coralline algae ingested while targeting epilithic and endolithic photoautotrophic organisms (Clements et al. 2016, Nicholson & Clements 2020). The micro-analysis revealed that the detrital component of *S. axillare* intestinal content comprises sediment (and non-nutritious sponge spicules), cyanobacteria, diatoms, and some green and red filamentous algae. On the other hand, *A. chirurgus* relies on a gizzard-like muscular stomach to triturate food. The detritus observed in the stomach contents of *A. chirurgus* had a more dense and greyish aspect and comprised mainly diatoms. *S. axillare* and *A. chirurgus* are syntopic (Cordeiro et al. 2016) and feed within the same habitats (Francini-Filho et al. 2010) yet appear to target different food resources (Mendes et al. 2018). Mendes et al. (2018) reported detritus-rich diets in both *A. chirurgus* and *S. axillare* that nonetheless differed in C:N ratio. We found a higher proportion of detritus in *S. axillare* than in *A. chirurgus*, and the greater proportion of cyanobacteria we found in the gut contents of *S. axillare* supports the nitrogen-richer dietary profile of this species (Mendes et al. 2018).

Diet studies based on feeding behaviour tend to group herbivorous grazing species into broad functional groups, an approach leading to simplistic or superficial views of the trophic role played by these species in their environments. Describing dietary preference based solely on visual observations (i.e. using feeding behaviour as a proxy for trophic niche) fails to resolve on the taxonomic composition the items being ingested, digested and assimilated by grazing fishes, and can thus fail to detect trophic niche partitioning among coexisting species (Kent & Sherry 2020). Recent studies revealed a high level of niche partitioning even over fine spatial scales among Indo-Pacific parrotfishes (Nicholson & Clements 2020, 2021, 2023), highlighting that trophic diversification in parrotfish classification goes beyond the functional 'scraper' and 'excavator' categories. For example, *S. rubripinne* is reported to be a macroalgal browser based on its feeding behaviour in the Caribbean (e.g. McAfee & Morgan 1996, Adam et al. 2015, 2018, Duran et al. 2019), while its gut contents appear mainly composed of algae, 'unidentified material' and sediment, and it differs in isotopic signature from syntopic surgeonfishes (Dromard et al. 2015). Our macro- and micro-analysis of diet in *S. axillare*, the Brazilian sister-species of *S. rubripinne*, revealed considerable proportions of protein-rich foods other than macroalgae, especially cyanobacteria and other microscopic photoautotrophs (see also Table 3 in Mendes et al. 2018).

Resolving which food sources are ingested, digested and assimilated requires the use of complementary tools (Andrades et al. 2019a, Grainger et al. 2023). Combining our 2-level resolution approach to diet with SIA revealed a high degree of partitioning (on a multi-niche basis) between *A. chirurgus* and *S.*

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*axillare*, usually grouped into the same functional and trophic groups. However, additional methods, such as compound-specific stable isotope analysis (CSIA) and fatty acid analysis, could provide greater detail into the nutritional ecology of these nominally herbivorous fishes.

The TCH predicts that marine herbivorous fishes are largely absent from temperate and polar areas because they cannot meet their energetic demands in cold water due to a physiological constraint on algal digestion (Gaines & Lubchenco 1982, Floeter et al. 2005). Our evaluation of the nutritional ecology of 3 species differing in diet, food-processing modes and phylogenetic affinities found no support for constraints on digestion driven by low temperature. This adds the mounting evidence that temperature does not directly influence digestive processes in ectotherms (Trip et al. 2014, 2016, Johnson et al. 2020). Future studies should also focus on evaluating the nutritional ecology of nominally herbivorous fishes over larger temperature and/or latitudinal gradients, making use of complementary methods such as SIA, fatty acids and CSIA. This combination will certainly provide more refined details on dietary and isotopic niches, and a better understanding of how fishes cope with environmental fluctuations while meeting nutritional demands.

*Acknowledgements*. We are thankful to L. Benevides, R. Andrades, A. Bernardino, V. Giglio and R. Francini-Filho for their valuable comments on the manuscript, and to L. Benevides, T. Calazans, C. Pimentel, H. Guabiroba, M. da Hora, L. Eggertsen, R. Freitas, M. Pacheco, M. Barbosa, C. Cordeiro, M. Almeida, M. Franco, J. Gonçalves and J. Gasparini for their help with field and lab work. This project was funded by Brazilian National Council for Scientific and Technological Development (CNPQ 401908/2013-5) and Rio de Janeiro State Foundation for Support to Research (FAPERJ E-26/111.168/2014 and E-26/200.638/2016) grants to C.E.L.F., to T.C.M. (FAPERJ E-26/202.372/2021) and to G.C.C.F. (FAPERJ E-26/204.522/2021). G.C.C.F. also received financial support from Espírito Santo State Foundation for Support to Research and Innovation (FAPES 292/2015-70223165/15) and CNPQ (SWE 208399/2017-8). This research was conducted under SISBIO Collection of Species Permit (#48094-3) issued by ICMBio (RESEXMar – AC), Brazilian Environmental Agency.

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*Editorial responsibility: Konstantinos Stergiou, Thessaloniki, Greece*
*Reviewed by: 2 anonymous referees*

*Submitted: December 2, 2022*
*Accepted: September 21, 2023*
*Proofs received from author(s): November 5, 2023*