UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO
CENTRO DE CIÊNCIAS HUMANAS E NATURAIS
PROGRAMA DE PÓS GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

Ecologia nutricional de peixes nominalmente herbívoros no Atlântico Sudoeste

Gabriel Costa Cardozo Ferreira

Vitória - ES
2019

UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO
CENTRO DE CIÊNCIAS HUMANAS E NATURAIS
PROGRAMA DE PÓS GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

Ecologia nutricional de peixes nominalmente herbívoros no Atlântico Sudoeste

Gabriel Costa Cardozo Ferreira

Orientador: Jean-Christophe Joyeux

Tese submetida ao Programa de Pós-graduação em Ciências Biológicas (Biologia Animal) da Universidade Federal do Espírito Santo como requisito parcial para obtenção do grau de Doutor em Ciências Biológicas (área Biologia Animal)

Vitória - ES
2019

Ficha catalográfica disponibilizada pelo Sistema Integrado de
Bibliotecas - SIBI/UFES e elaborada pelo autor

Ferreira, Gabriel Costa Cardozo, 1985-
F383e Ecologia nutricional de peixes nominalmente herbívoros no Atlântico Sudoeste / Gabriel Costa Cardozo Ferreira. - 2019.
74 f. : il.

Orientador: Jean-Christophe Joyeux.
Tese (Doutorado em Biologia Animal) - Universidade Federal do Espírito Santo, Centro de Ciências Humanas e Naturais.

1. Ecologia marinha. 2. Ecologia dos recifes de coral. 3. Peixes marinhos. 4. Carbono - Isótopos. I. Joyeux, Jean Christophe. II. Universidade Federal do Espírito Santo. Centro de Ciências Humanas e Naturais. III. Título.

CDU: 57

Gabriel Costa Cardozo Ferreira

"Ecologia nutricional de peixes nominalmente herbívoros no Atlântico Sudoeste"

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas do Centro de Ciências Humanas e Naturais, da Universidade Federal do Espírito Santo, como requisito parcial para obtenção do Grau de Doutor em Biologia Animal.

Aprovada em 26 de abril de 2019.

Comissão Examinadora:

Prof. Dr. Jean-Christophe Joyeux (UFES)
Orientador e Presidente da Comissão

Prof. Dr. Angelo Fraga Bernardino (UFES)
Examinador interno

RlAndrades
Prof. Dr. Ryan Carlos Andrades (PPGOAM/UFES)
Examinador externo

Prof. Dr. Jean-Christophe Joyeux (UFES) por
Prof. Dr. Ronaldo Bastos Francini - Filho (UFPB)
Examinador externo

Prof. Dr. Vinícius José Giglio (UNIFESP)
Examinador externo

AGRADECIMENTOS

Agradeço aos meus pais, Celino e Nete, e a minha irmã, Mariana, por me darem todo o apoio para ir em busca dos meus objetivos, por compreenderem a minha ausência em momentos importantes das nossas vidas, e por sempre me receberem com todo o amor do mundo e se despedirem com aperto no peito nas idas e vindas da vida.

A Larissa, minha linda, amiga e “Sra. minha esposa” (e editora nas horas vagas) por ser sempre minha companheira, estando todo o tempo ao meu lado, mesmo que distante. Pelas horas discutindo ciência levados por um bom vinho, cachaça, ou simplesmente por querer fazer ciência ao meu lado e por se empolgar muito com isso. E pelos seus olhos e sorrisos brilhantes todos os dias ao meu lado.

Ao belo francês Dr. Jean-Christophe Joyeux, meu orientador e grande incentivador na busca por conhecimento. Obrigado pelas conversas e discussões entre um café e uma caneca suja, por me transmitir um pouco do seu conhecimento e por aguentar minha insistência pelo ar-condicionado congelante nos últimos anos.

A todos os integrantes do Ictiolab pelas engrandecedoras conversas e gargalhadas nem sempre aliadas à ciência. Caio, Guabiroba, Maik e Larissa pelas incansáveis expedições ao Alaska (leia-se Arraial do Cabo). Ao Gaspa (Papai fofinho) e Raphael Macieira e outros que fizeram parte da infindável busca pelas pirajicas (entre outras amostras) em Guarapari.

Ao Dr. Carlos E. Ferreira (Cadu) por me confiar o desenvolvimento deste projeto e pela ‘sede do saber’. Aos amigos César, Thiago, Moysés, Matheus, Vinicius e toda a equipe do Laboratório de Ecologia e Conservação Recifal (LECAR) da Universidade Federal Fluminense pelo apoio logístico nas coletas em Arraial do Cabo.

Aos professores Dr. Kendall Clements, da *University of Auckland*, e Dr. John Howard Choat da *James Cook University (JCU)*, pelos ensinamentos transmitidos ao longo desta parceria. Em especial ao Howard por me receber em seu laboratório no *Marine and Aquaculture Research Facilities Unit* e no *Science Place* na JCU durante meu período de doutorado-sanduíche na Austrália.

Aos ‘Aussies’ Renato Morais, Alexandre Siqueira, Gus Crosbie, Hanaka Mera, Julia Mayr e outros tantos que fizeram da estadia em Townsville um ambiente de amizade e aprendizado.

À FAPES pela bolsa de doutorado concedida. Ao CNPq pela bolsa de doutorado-sanduíche e pelo financiamento de Professor Visitante Especial (PVE; Processo 401908/2013-5).

"What we observe is not nature in itself,

but nature exposed to our method of questioning."

Werner Heisenberg (1958)

SUMÁRIO

LISTA DE TABELAS

Capítulo 2 – Whole year nourished: seasonal overlap in diet and nutrient assimilation of nominally herbivorous fishes

Table 1: PERMANOVA statistics for dietary analysis comparisons on macro- (below diagonal) and micro-analysis (above diagonal) scales. For each cell, values represent Pseudo-*t* statistics (above) and *p*-value (below) for pair-wise comparisons. Uppercase acronyms represent each season: SM – Summer, AU – Autumn, WT – Winter, and SP – Spring. Bold values denote statistically significant comparisons ($\alpha < 0.05$). "–" not tested (see methods).

Table 2: PERMANOVA statistics for seasonal comparisons for concentrations of stable isotopes of carbon ($\delta^{13}C$ – above diagonal) and nitrogen ($\delta^{15}N$ – below diagonal) for the three studied fish species. For each cell, values represent Pseudo-*t* statistics (above) and *p*-value (below) for pair-wise comparisons. Uppercase acronyms represent each season: SM – Summer, AU – Autumn, WT – Winter, and SP – Spring. Bold values denote significantly different comparisons ($\alpha < 0.05$).

Table 3: PERMANOVA statistics for seasonal comparisons for concentrations of stable isotopes of carbon ($\delta^{13}C$ – above diagonal) and nitrogen ($\delta^{15}N$ – below diagonal) for algae groups: brown algae, red algae and turf. For each cell, values represent Pseudo-*t* statistics (above) and *p*-value (below) for pair-wise comparisons. Uppercase acronyms represent each season: SM – Summer, AU – Autumn, WT – Winter, and SP – Spring. Bold values denote significantly different comparisons ($\alpha < 0.05$). "–" not tested (see methods).

Table S1: Overlapping Schoener index (*Sh*i) calculated for dietary macro- (below diagonal) and micro-analysis (above diagonal) for the three herbivorous species. Uppercase acronyms represent each season: SM – Summer, AU – Autumn, WT – Winter, and SP – Spring. Bold denote higher *Sh*i overlap for seasonal comparisons on both analyses.

Capítulo 3 – Large-grain perspective may hinder the assignment of herbivorous fish functional roles

Table 4: Levels of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), C:N ratio, trophic position (TP), and number of samples (n) for each species in each sampled site. Superscript lowercase letters on $\delta^{13}C$ and $\delta^{15}N$ indicate similarities among species within each site.

Table S2: Collected number of specimens for each species, with ranges of length (mean total length, minimum - maximum) and weight (mean, minimum - maximum).

Table S3: Dietary items found in gut content of the three species of nominally herbivorous fishes: *Acanthurus chirurgus* (n = 51), *Sparisoma axillare* (n = 28) and *Kyphosus vaigiensis* (n = 27). Brown, green and red refer to algae classes Ochrophyta, Chlorophyta and Rhodophyta, respectively.

Table S4: Diet contribution (% diet) of dietary items found in gut contents of the three study nominally herbivorous fishes in the sampled four sites. Acronyms: *Aca chi – Acanthurus chirurgus*, *Spa axi – Sparisoma axillare* and *Kyp vai – Kyphosus vaigiensis*.

LISTA DE FIGURAS

Capítulo 2 - Whole year nourished: seasonal overlap in diet and nutrient assimilation of nominally herbivorous fishes

Figure 1: Principal Component Analysis (PCA) with comparative seasonal dietary macro-analysis data for the three species of nominally herbivorous fishes: *Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis*.

Figure 2: Principal Component Analysis (PCA) with comparative seasonal dietary micro-analysis data for *Acanthurus chirurgus* and *Sparisoma axillare*.

Figure 3: Stable isotope bi-plots (sample data in $\delta^{13}C$ and $\delta^{15}N$ bivariate space) illustrating the nutrient assimilation and isotopic niche through corrected standard ellipse area (SEAc, 95% C.I.; solid lines) and convex-hull area around extreme sample values (dashed lines) for A) *Acanthurus chirurgus*, B) *Sparisoma axillare* and C) *Kyphosus vaigiensis*, in each sampled season.

Figure 4: Seasonal small-sample size corrected Standard Ellipse Area (SEAc) for the three study species: A) *Acanthurus chirurgus*, B) *Sparisoma axillare* and C) *Kyphosus vaigiensis*. Shaded boxes represent the credible intervals of 50%, 75% and 95%, from dark to light grey. Black dots represent the sample mode and red ‘x’ is the mean value for each population.

Figure 5: $\delta^{13}C$ and $\delta^{15}N$ bivariate spaces with corrected standard ellipses area (SEAc – solid lines; C.I. 95%) and the convex-hull area around extreme sample values (dashed lines) illustrating the isotopic composition of primary producers sampled in each season.

Figure 6: Correlation between seasonal diet overlap and the seasonal isotopic niche overlap. Diet overlap is measured through Schoener’ index and isotopic niche overlap through the overlap area for the corrected Bayesian Standard Ellipse Area (SEAc). Colours indicate species and each point is a between-season comparison (e.g., Summer vs Winter) for each one of the overlap indexes in a bivariate space.

Figure S1: Dietary analysis comparison of *Acanthurus chirurgus* among the four seasons: Summer, Autumn, Winter and Spring – colours correspond to each season. Results from macro- (upper), and micro-analysis (lower). Lower case letters indicate: diet similarities in between-season comparisons for each item – similar letters in different seasons indicate non-significant comparisons (PERMANOVA: $\alpha > 0.05$), and mean (± C.I.) diet composition values (%) for each item, being a > b > c.

Figure S2: Dietary analysis comparison of *Sparisoma axillare* among the four seasons: Summer, Autumn, Winter and Spring – colours correspond to each season. Results from macro- (upper), and micro-analysis (lower). Lower case letters indicate: diet similarities in between-season comparisons for each item – similar letters in different seasons indicate non-significant comparisons (PERMANOVA: $\alpha > 0.05$), and mean (± C.I.) diet composition values (%) for each item, being a > b > c.

Figure S3: Dietary analysis comparison of *Kyphosus vaigiensis* among the four seasons: Summer, Autumn, Winter and Spring – colours correspond to each season. Lower case letters indicate: diet similarities in between-season comparisons for each item – similar letters in different seasons indicate non-significant comparisons (PERMANOVA: $\alpha > 0.05$), and mean (± C.I.) diet composition values (%) for each item, being a > b > c.

Figure S4: Minimum seasonal sea surface temperature (SST °C) recorded during the sampling period in Arraial do Cabo. Similar colours denote the same season in different years. Black middle dot and error bars represent mean ± standard deviation, respectively. Grey dots are minimum daily temperatures recorded. All seasons have three months pooled (see methods), except for Autumn 2015 (two months; April and May) and Winter 2017 (one month; June).

Figure S5: Yearlong stable isotope bi-plots (sample data in $\delta^{13}C$ and $\delta^{15}N$ bivariate space) illustrating the nutrient assimilation and isotopic niche through corrected standard ellipse area (SEAc, 95% C.I.; solid lines) and convex-hull area around extreme sample values (dashed lines) for the three study species: *Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis*, in each sampled season.

Capítulo 3 – Large-grain perspective may hinder the assignment of herbivorous fish functional roles

Figure 1: Sampled sites (A), diet composition for macro- (B) and micro-analyse (C), and corrected Standard Ellipses Area (SEAc ‰²) for small-sample size (D) based on stable isotopic composition. Acronyms between (A) and (B) indicate study species: *Acanthurus chirurgus* (*Aca chi*), *Sparisoma axillare* (*Spa axi*), and *Kyphosus vaigiensis* (*Kyp vai*). Lowercase letters at the right sides of (B) and (C) indicate diet dissimilarities among species within each site. Black contour in (B) refers to algal items. In (D), black dots represent the sample mode, red 'x' is the true mean value for each population and shaded boxes represent the credible intervals of 50 %, 75 % and 95 %, from dark to light grey.

Figure 2: Stable isotope bi-plots ($\delta^{13}C$ and $\delta^{15}N$ bivariate space – X and left Y axes, respectively) illustrating the isotopic niche of each nominally herbivorous fish species, and their trophic positions (TP; right Y axe) in each site. Ellipses correspond to small-sample corrected standard ellipse area (SEAc, solid lines) and convex-hull area around extreme sample values (dashed lines). Red circles for (A) *Acanthurus chirurgus*, (B) brown crosses for *Sparisoma axillare* and (C) green triangles for *Kyphosus vaigiensis*.

Figure 3: Stable isotope bi-plots (sample data in $\delta^{13}C$ and $\delta^{15}N$ bivariate space) illustrating the isotopic composition of algal sources in each sampled site with corrected standard ellipse area (SEAc, solid lines) and the convex-hull area around extreme sample values (dashed lines).

Figure S1: Dietary analysis comparison among species (left to right: *Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis* in Natal – colours correspond to each species. Results from macro- (A), and micro-analysis (B). Lower case letters indicate: diet similarities in between-species comparisons for each item (PERMANOVA: $\alpha > 0.05$), being a > b > c. Black dots are mean (±C.I.) diet composition (%; Tab. S3) for each item.

Figure S2: Dietary analysis comparison among species (left to right: *Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis* in the Abrolhos Archipelago – colours correspond to each species. Results from macro- (A), and micro-analyse (B). Lower case letters indicate: diet similarities in between-species comparisons for each item (PERMANOVA: $\alpha > 0.05$), being a > b > c. Black dots are mean (±C.I.) diet composition (%; Tab. S3) for each item.

Figure S3: Dietary analysis comparison among species (left to right: *Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis* in Guarapari – colours correspond to each species. Results from macro- (A), and micro-analyse (B). Lower case letters indicate: diet similarities in between-species comparisons for each item (PERMANOVA: $\alpha > 0.05$), being a > b > c. Black dots are mean (±C.I.) diet composition (%; Tab. S3) for each item.

Figure S4: Dietary analysis comparison among species (left to right: *Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis* in Arraial do Cabo – colours correspond to each species. Results from macro- (A), and micro-analyse (B). Lower case letters indicate: diet similarities in between-species comparisons for each item (PERMANOVA: $\alpha > 0.05$), being a > b > c. Black dots are mean (±C.I.) diet composition (%; Tab. S3) for each item.

Figure S1: Standard ellipse for each nominally herbivorous species independent of site. Ellipses correspond to small-sample corrected standard ellipse area (SEAc, 95% C.I.; solid lines) and convex-hull area around extreme sample values (dashed lines).

Figure S2: Standard ellipse for four sites without differentiating among the nominally herbivorous species. Ellipses correspond to small-sample corrected standard ellipse area (SEAc, 95% C.I.; solid lines) and convex-hull area around extreme sample values (dashed lines).

RESUMO

A ecologia trófica de peixes herbívoros é assunto de constante debate. Discussões permeiam entre como as espécies capturam seu alimento até quais são os seus verdadeiros alvos no substrato recifal e como isto implica em seus papeis funcionais no ambiente. Diferentes aspectos bióticos e abióticos podem influenciar na ecologia trófica deste grupo. Eventos sazonais como, por exemplo, a ressurgência, podem enriquecer o ecossistema com a entrada de água fria e rica em nutrientes vinda de regiões mais profundas. Da mesma forma, diferentes locais podem apresentar características particulares, como a composição bentônica, que têm efeito direto na ingestão e assimilação de nutrientes pelos peixes herbívoros recifais. Esta tese foi desenvolvida em quatro ambientes recifais ao longo da costa brasileira, sendo: Natal (Rio Grande do Norte), Arquipélago dos Abrolhos (Bahia), Guarapari (Espírito Santo) e Arraial do Cabo (Rio de Janeiro). Neste último local, os estudos foram conduzidos em uma escala sazonal, mas também latitudinal, quando o mesmo foi comparado com os outros locais citados acima. Verificou-se que os principais itens na dieta de cada espécie tendem a permanecer semelhantes em ambas as abordagens: sazonal e latitudinal. No entanto, os peixes nominalmente herbívoros apresentaram diferenças na composição da sua dieta e na diversidade de itens ingeridos em ambas as escalas. Similarmente, a assimilação de nutrientes e as relações tróficas entre as espécies também variaram na comparação latitudinal entre os locais. Este trabalho indica que os peixes nominalmente herbívoros possuem especificidades quanto à ecologia nutricional, e que as variações ambientais ou características dos habitats devem ser consideradas para evitar generalizações na ecologia de peixes tão importantes, diversificados e amplamente distribuídos. Finalmente, este estudo expande a compreensão de como os peixes herbívoros dividem os recursos disponíveis e reforça que a função de cada espécie no ecossistema não deve ser subestimada agrupando-as como unidades únicas sem análise específica para cada local /espécie.

Palavras-chave: ecologia nutricional, sazonalidade, herbivoria, peixes herbívoros, isótopos estáveis, análise de dieta.

ABSTRACT

The trophic ecology of herbivorous fishes is a constantly debated subject. Discussions permeate between how species capture their food till which are their real targets on the reef substrate and how it does imply on their functional roles on the environment. Different biotic and abiotic aspects may influence in this group trophic ecology. Seasonal events such as upwelling may, for example, enrich the whole system as a consequence of the input of cold and nutrient-rich deep waters. Similarly, different sites may present particular characteristics, as benthic composition, directly affecting ingestion and nutrient assimilation by herbivorous reef fishes. This thesis was conducted in four reef environments along the Brazilian coast: Natal (state of Rio Grande do Norte), Abrolhos Archipelago (state of Bahia), Guarapari (state of Espírito Santo) and Arraial do Cabo (state of Rio de Janeiro). In the latter, studies were conducted on a seasonal scale but also in a latitudinal approach when it was compared to the other three sites aforementioned. Was verified that the main items in each species diet trend to remain similar in both approaches: seasonal and latitudinal. However, the nominally herbivorous fishes have presented differences in diet composition and diversity of ingested items in both scales. Likewise, nutrient assimilation and trophic relationships among species also varied in the latitudinal comparison among sites. This work indicates that nominally herbivorous fishes have specificities regarding their nutritional ecology and that environmental variations or habitats characteristics must be considered to avoid generalizations on the ecology of such important, diverse, and widely-distributed fishes. Finally, this study expands the comprehension on how herbivorous reef fishes partition the available resources and reinforces that each species function in the ecosystem should not be underestimated by grouping them as single unities without site/species-specific analysis.

Keywords: nutritional ecology, seasonality, herbivory, herbivorous fishes, stable isotopes, dietary analysis.

CAPÍTULO 1

Introdução geral

Estudos acerca da herbivoria, seu impacto e relevância ecológica são necessários para a compreensão da dinâmica de ecossistemas terrestres e aquáticos (Meekan & Choat, 1997; McCook et al., 2001). A herbivoria marinha, assim como no ambiente terrestre, possui a capacidade de moldar as comunidades de plantas, influenciando, por exemplo, na segregação de habitat e o equilíbrio competitivo entre as diferentes espécies (Fine et al., 2004; Marquis, 2004). No ambiente marinho, a herbivoria é desempenhada por uma variedade de organismos, como peixes, ouriços-do-mar e tartarugas-marinhas (Choat & Clements, 1998; Marquis, 2004; Cordeiro et al., 2014; Santos et al., 2015).

Entretanto, cada organismo exerce sua própria função na dinâmica do ambiente e na efetividade da herbivoria, sendo os peixes os maiores representantes no que tange à biomassa relativa em ecossistemas marinhos tropicais (Floeter et al., 2005). Peixes herbívoros não apenas podem afetar a comunidade bentônica, como podem ser afetados por ela e por características abióticas do ambiente como a temperatura da água (Ferrari et al., 2012). Diferenças morfológicas e fisiológicas entre os peixes herbívoros refletem em sua preferência alimentar, mobilidade, impacto sobre a comunidade bentônica e consequente papel funcional (Choat et al., 2004; Clements et al., 2017). As diferentes preferências alimentares identificadas neste grupo levaram espécies como *Acanthurus chirurgus* (Bloch, 1787), *Sparisoma axillare* (Steindachner, 1878) e *Kyphosus vaigiensis* (Quoy & Gaimard, 1825) a serem reconhecidos como "nominalmente herbívoros", consumindo uma variada gama de itens, desde macroalgas a *turf*, detritos, esponjas, diatomáceas e até microalgas (Wilson & Bellwood, 1997; Wilson, 2000; Choat et al., 2002). Por vezes, mesmo espécies onívoras são consideradas nominalmente herbívoras devido à grande representatividade das algas em sua dieta (Mendes et al., 2018).

De acordo com seu papel funcional no ambiente, inferido a partir de dieta e observações diretas, peixes nominalmente herbívoros podem ser classificados como: raspadores (*scrapers*), escavadores (*excavators*), detritívoros (*grazers*) e pastadores (*browsers*) (Green & Bellwood, 2009). *Scrapers* e *excavators* possuem papeis funcionais semelhantes, mas diferem quanto à profundidade das mordidas e, consequentemente, à quantidade de substrato removido (Bellwood & Choat, 1990; Streelman et al., 2002). Uma mordida mais profunda permite explorar novos recursos, podendo representar especializações e papeis funcionais diferentes. Peixes-papagaio (família Labridae: tribo Scarini), por exemplo, são típicos *scrapers* e *excavators*, e investem no substrato não apenas em busca de algas epilíticas, mas também à intencional ingestão de microalgas (cianobactérias), diatomáceas e esponjas incrustantes (endolíticas) do substrato coralíneo (Clements et al., 2017). Já *grazers* e *browsers* diferem-se quanto à proximidade ao substrato quando investem no alimento. O primeiro chega a arranhar o substrato, enquanto o segundo não se aproxima dele, mas ambos limitam o crescimento e estabelecimento de macroalgas sem deixar cicatrizes no substrato onde mordem (Green & Bellwood, 2009).

De modo geral, os peixes herbívoros estão susceptíveis à disponibilidade de itens alimentares, os quais podem variar em escalas espaciais (Longo et al., 2015; Aued et al., 2018) e temporais (Ferreira et al., 1998; Ferrari et al., 2012), afetando o hábito alimentar destas espécies (Bennett & Bellwood, 2011). Assim, os nutrientes assimilados podem refletir os recursos alimentares ingeridos por cada indivíduo e indicar a origem da fonte alimentar utilizada. Portanto, a assimilação de nutrientes por peixes herbívoros pode ajudar na compreensão sobre as variações dos recursos alimentares disponíveis e, consequentemente, acerca das relações tróficas existentes entre as espécies em diferentes locais, ou em um mesmo local ao longo do tempo (McCutchan & Lewis, 2001; Hadwen et al., 2010). Por exemplo, espécies com maior ingestão de matéria vegetal tendem a apresentar maiores níveis de carboidratos (carbono) do que aquelas com maior ingestão de matéria animal, que apresentam níveis mais elevados de aminoácidos proteicos totais (nitrogênio) (Crossman et al., 2005), os quais podem indicar a posição trófica de uma espécie em relação às outras em uma cadeia trófica.

Estudos sobre a ecologia nutricional têm se beneficiado com o uso de técnicas cada vez mais robustas e abrangentes (Clements et al., 2017; Mendes et al., 2018). Entre estas, destaca-se o uso dos isótopos estáveis, principalmente de carbono e nitrogênio no que tange a estudos nutricionais e de cadeias tróficas (Jackson et al., 2011; Abrantes et al., 2014). A técnica de isótopos estáveis carece de uma fidelidade taxonômica devido às possíveis sobreposições na assinatura isotópica dos organismos (i.e., produtores e consumidores) de uma cadeia trófica (Lebreton et al., 2012). Em busca de uma maior confiabilidade, este método pode ser aliado a outros como, por exemplo, a análise de conteúdo estomacal. A dieta fornece uma visão imediata do hábito alimentar, enquanto os níveis de isótopos estáveis permitem verificar os nutrientes assimilados a partir dos recursos consumidos (Fry, 2006). Assim, a combinação de dois ou mais métodos permite melhor compreender a dinâmica trófica das espécies de determinado ambiente.

Esta tese apresenta duas abordagens acerca da ecologia trófica funcional dos peixes nominalmente herbívoros. O primeiro capítulo, intitulado "*Whole year nourished: seasonal overlap in diet and nutrient assimilation of nominally herbivorous fishes*" visou analisar, em um gradiente sazonal, as variações na ecologia nutricional de três espécies de peixes nominalmente herbívoros (*Acanthurus chirurgus* – Acanthuridae; *Kyphosus vaigiensis* – Kyphosidae; e *Sparisoma axillare* – Labridae, tribo Scarini). Neste capítulo foi investigada a existência de variações sazonais na dieta e assimilação de nutrientes destas espécies em um costão rochoso subtropical na costa brasileira – Arraial do Cabo (RJ). Esta região é afetada por eventos de ressurgência que ocorrem com maior intensidade nos meses de primavera e verão, afetando todo o ecossistema marinho local (Valentin, 2001). Ao longo de dois anos, amostras de produtores primários e consumidores (algas e peixes) foram coletadas buscando compreender como as variações ambientais em uma escala sazonal podem influenciar na ecologia trófica destas três espécies.

O segundo capítulo, intitulado "*Large-grain perspective may hinder the assignment of herbivorous fish functional roles*" investigou a existência de variações interespecíficas na dieta e assimilação de nutrientes das mesmas três espécies de peixes nominalmente herbívoros em uma larga escala espacial. As espécies estudadas são abundantes ao longo da costa brasileira e foram coletadas em quatro pontos: Natal – RN (5°47' S; 35°12' O), Arquipélago dos Abrolhos – BA (17°20' S; 39°30' O), Guarapari – ES (20°40' S; 40°23' O) e Arraial do Cabo – RJ (22°58' S; 42°00' O). Estes locais se diferem, principalmente, quanto a características bentônicas do substrato marinho e quanto à temperatura superficial da água, a qual diminui em direção às altas latitudes. Desta forma, considerando os diferentes habitats explorados por estas espécies, objetivou-se testar se existe uma variação nas relações tróficas entre elas e se o papel funcional dos peixes nominalmente herbívoros pode variar entre os distintos locais.

Referências

Abrantes, K. G., Barnett, A., & Bouillon, S. (2014). Stable isotope-based community metrics as a tool to identify patterns in food web structure in east African estuaries. *Functional Ecology*, *28*(1), 270–282. https://doi.org/10.1111/1365-2435.12155

Aued, A. W., Smith, F., Quimbayo, J. P., Cândido, D. V., Longo, G. O., Ferreira, C. E. L., … Segal, B. (2018). Large-scale patterns of benthic marine communities in the Brazilian Province. *PLOS ONE*, *13*(6), e0198452. https://doi.org/10.1371/journal.pone.0198452

Bellwood, D. R., & Choat, J. H. (1990). A functional analysis of grazing in parrotfishes (family Scaridae): the ecological implications. *Environmental Biology of Fishes*, *28*, 189–214.

Bennett, S., & Bellwood, D. R. (2011). Latitudinal variation in macroalgal consumption by fishes on the Great Barrier Reef. *Marine Ecology Progress Series*, *426*, 241–252. https://doi.org/10.3354/meps09016

Choat, J. H., & Clements, K. D. (1998). Vertebrate herbivores in marine and terrestrial environments: A nutritional ecology perspective. *Annual Review of Ecology and Systematics*, *29*, 375–403. https://doi.org/10.1146/annurev.ecolsys.29.1.375

Choat, J. H., Clements, K. D., & Robbins, W. D. (2002). The trophic status of herbivorous fishes on coral reefs I: Dietary analyses. *Marine Biology*, *140*(3), 613–623. https://doi.org/10.1007/s00227-001-0715-3

Choat, J. H., Robbins, W., & Clements, K. D. (2004). The trophic status of herbivorous fishes on coral reefs. II. Food processing modes and trophodynamics. *Marine Biology*, *145*(3), 445–454. https://doi.org/10.1007/s00227-004-1341-7

Clements, K. D., German, D. P., Piché, J., Tribollet, A., & Choat, J. H. (2017). Integrating ecological roles and trophic diversification on coral reefs: multiple lines of evidence identify parrotfishes as microphages. *Biological Journal of the Linnean Society*, *120*, 729–751. https://doi.org/10.1111/bij.12914

Cordeiro, C. A. M. M., Harborne, A. R., & Ferreira, C. E. L. (2014). Patterns of distribution and composition of sea urchin assemblages on Brazilian subtropical rocky reefs. *Marine Biology*, *161*(10), 2221–2232. https://doi.org/10.1007/s00227-014-2500-0

Crossman, D. J., Choat, J. H., & Clements, K. D. (2005). Nutritional ecology of nominally herbivorous fishes on coral reefs. *Marine Ecology Progress Series*, *296*, 129–142. https://doi.org/10.3354/meps296129

Ferrari, R., Gonzalez-Rivero, M., Ortiz, J. C., & Mumby, P. J. (2012). Interaction of herbivory and seasonality on the dynamics of Caribbean macroalgae. *Coral Reefs*, *31*(3), 683–692. https://doi.org/10.1007/s00338-012-0889-9

Ferreira, C. E. L., Peret, A. C., & Coutinho, R. (1998). Seasonal grazing rates and food processing by tropical herbivorous fishes. *Journal of Fish Biology*, *53*, 222–235. https://doi.org/10.1111/j.1095-8649.1998.tb01029.x

Fine, P. V. A., Mesones, I., & Coley, P. D. (2004). Herbivores promote habitat specialization by trees in Amazonian forests. *Science*, *305*, 663–665. https://doi.org/10.1126/science.1098982

Floeter, S. R., Behrens, M. D., Ferreira, C. E. L., Paddack, M. J., & Horn, M. H. (2005). Geographical gradients of marine herbivorous fishes: Patterns and processes. *Marine Biology*, *147*(6), 1435–1447. https://doi.org/10.1007/s00227-005-0027-0

Fry, B. (2006). *Stable Isotope Ecology*. New York: Springer.

Green, A. L., & Bellwood, D. R. (2009). *Monitoring functional groups of herbivorous reef fishes as indicators of coral reef resilience - A practical guide for coral reef managers in the Asia Pacific Region.* (D. R. Green, Alison L. and Bellwood, Ed.), *IUCN working group on Climate Change and Coral Reefs*. Gland, Switzerland: IUCN working group on Climate Change and Coral Reefs. Retrieved from http://cmsdata.iucn.org/downloads/resilience_herbivorous_monitoring.pdf

Hadwen, W. L., Spears, M., & Kennard, M. J. (2010). Temporal variability of benthic algal δ^{13}C signatures influences assessments of carbon flows in stream food webs. *Hydrobiologia*, *651*, 239–251. https://doi.org/10.1007/s10750-010-0303-y

Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, *80*, 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

Lebreton, B., Richard, P., Galois, R., Radenac, G., Brahmia, A., Colli, G., … Blanchard, G. F. (2012). Food sources used by sediment meiofauna in an intertidal *Zostera noltii* seagrass bed: a seasonal stable isotope study. *Marine Biology*, *159*(7), 1537–1550. https://doi.org/10.1007/s00227-012-1940-7

Longo, G. O., Morais, R. A., Martins, C. D. L., Mendes, T. C., Aued, A. W., Cândido, D. V., … Floeter, S. R. (2015). Between-habitat variation of benthic cover, reef fish assemblage and feeding pressure on the benthos at the only atoll in South Atlantic: Rocas Atoll, NE Brazil. *PLOS ONE*, *10*(6), e0127176. https://doi.org/10.1371/journal.pone.0127176

Marquis, R. J. (2004). Herbivores rule. *Science*, *305*, 619–621.

McCook, L. J., Jompa, J., & Diaz-Pulido, G. (2001). Competition between corals and algae on coral reefs: A review of evidence and mechanisms. *Coral Reefs*, *19*(4), 400–417. https://doi.org/10.1007/s003380000129

McCutchan, J. H., & Lewis, W. M. (2001). Seasonal variation in stable isotope ratios of stream algae. *Verhandlungen Des Internationalen Verein Limnologie*, *27*, 3304–3307.

Meekan, M. G., & Choat, J. H. (1997). Latitudinal variation in abundance of herbivorous fishes: a comparison of temperate and tropical reefs. *Marine Biology*, *128*(3), 373–383. https://doi.org/10.1007/s002270050103

Mendes, T. C., Ferreira, C. E. L., & Clements, K. D. (2018). Discordance between diet analysis and dietary macronutrient content in four nominally herbivorous fishes from the Southwestern Atlantic. *Marine Biology*, *165*(11), 1–12. https://doi.org/10.1007/s00227-018-3438-4

Santos, R. G., Martins, A. S., Batista, M. B., & Horta, P. A. (2015). Regional and local factors determining green turtle Chelonia mydas foraging relationships with the environment. *Marine Ecology Progress Series*, *529*(1981), 265–277. https://doi.org/10.3354/meps11276

Streelman, J. T., Alfaro, M. E., Westneat, M. W., Bellwood, D. R., & Karl, S. A. (2002). Evolutionary history of the parrotfishes: biogeography, ecomorphology, and comparative diversity. *Evolution*, *56*(5), 961–971. https://doi.org/10.1111/j.0014-3820.2002.tb01408.x

Valentin, J. L. (2001). The Cabo Frio upwelling system, Brazil. In U. Seeliger & B. Kjerfve (Eds.), *Coastal Marine Ecosystems of Latin America* (Vol. 144, pp. 97–105). Springer-Verlag. https://doi.org/10.1007/978-3-662-04482-7_8

Wilson, S. K. (2000). Trophic status and feeding selectivity of blennies (Blenniidae: Salariini). *Marine Biology*, *136*(3), 431–437. https://doi.org/10.1007/s002270050702

Wilson, S. K., & Bellwood, D. R. (1997). Cryptic dietary components of territorial damselfishes (Pomacentridae, Labroidei). *Marine Ecology Progress Series*, *153*(1–3), 299–310. https://doi.org/10.3354/meps153299

CAPÍTULO 2

WHOLE YEAR NOURISHED: SEASONAL OVERLAP IN DIET AND NUTRIENT ASSIMILATION OF NOMINALLY HERBIVOROUS FISHES

Whole year nourished: seasonal overlap in diet and nutrient assimilation of nominally herbivorous fishes

Cardozo-Ferreira G.C., Clements, K.D., Choat, J.H., Macieira, R.M., Mendes, T.C., Rezende, C.E., Ferreira, C.E.L. and Joyeux, J.-C.

Formatted for: Marine Ecology Progress Series

Abstract

Seasonal shifts on environmental features and the occurrence of oceanographic processes (e.g., upwelling) may affect the ecosystem energy flux and its inhabitant fauna. On this context, herbivorous fishes are subjected to fluctuations on water temperature and light exposition which could, for example, increase primary productivity in the whole system. We evaluated in a seasonal perspective the nutritional ecology of three nominally herbivorous fishes in an upwelling-affected rock reef. We used gut content and stable isotope analyses to access the seasonal variation on herbivorous fishes' nutritional ecology, and sea surface temperature (SST) as a proxy to measure the strength of the known upwelling events through seasons. Diet for each species, as well as stable isotope levels, presented differences across the seasons. However, a diet shift does not seem to be correlated with variations on stable isotope levels. The overlap on diet between seasons was not concomitant with the overlap observed on nutrient assimilation. Moreover, each species displayed different variations in their nutritional ecology. Despite seasonal changes, each species relies its diet on similar items along the year and their isotope levels displayed small seasonal variation. Diet shifts are likely to be a strategy to maintain the nourishment all year round. By doing so through selectively feeding on sources with different nutritional value, and thus constantly maximizing its fitness, herbivorous fishes display crucial functional roles for maintenance of community structure and ecosystem energy flux. Studying the nutritional ecology of herbivorous reef fishes may reveal new insights on the feeding ecology and evolutionary consequences on nutrient assimilation in a changing marine environment.

Keywords: dietary analysis; herbivory; nutritional ecology; seasonality; stable isotopes; trophic ecology.

Introduction

Marine seasonal fluctuations are discreet closer to the tropics, where solar irradiance and water temperature are less variable along the year. Primary productivity increases with insolation, and the stability on tropics may lead to a year-round enrichment (Beaver & Crisman 1991, Leberfinger et al. 2011). Yet, reef dynamics is subjected to particular effects as oceanographic and climatic processes such as upwelling, altering productivity and water temperature, and consequently influencing animal's consumption rates (Guimaraens & Coutinho 1996, Anthony et al. 2004, Borer et al. 2013).

Seasonal coastal upwelling events induce physiological changes on benthic algae due to increasing nutrients input on reef systems (Guimaraens & Coutinho 1996, Diaz-Pulido & Garzón-Ferreira 2002). Thus, fluctuation in nutrient level could be observed as these compounds vary both on biota and environment at temporal scales (McCutchan & Lewis 2001, Vander Zanden & Rasmussen 2001, Lefèvre & Bellwood 2011). As a result, this could affect the resource availability (Johnson et al. 2017) and, consequently, the nutritional ecology of nominally herbivorous reef fishes. These species are known to consume a variety of items including detrital aggregates, turf, macroscopic and endo/epilithic algae (Wilson & Bellwood 1997, Choat et al. 2002, Crossman et al. 2005, Clements et al. 2017). For example, fluctuations on algae abundance or biomass and its macronutrient compositions would affect herbivores once they feed mainly on algae (Lebreton et al. 2012, Johnson et al. 2017). Prior studies have showed that seasonal changes in plant size and condition can drive oscillations on herbivory rates mainly between summer and winter months (Lefèvre & Bellwood 2011). Likewise, if some dietary plasticity is allowed (e.g. omnivorous species), seasonal variation may occur by regulating between algal and animal-derived material throughout the year aiming to reach the nutritional demands (Raubenheimer et al. 2005). Moreover, evaluation of the seasonal effects on fish populations and habitat use revealed that differences are likely to be prevalent on reef systems experiencing well-marked seasonality (Afeworki et al. 2013).

On investigating such changes, different techniques have been employed to track the ecosystem nutritional enrichment and consequences on the nutritional ecology of herbivorous fishes. Whilst dietary analysis may provide a detailed description of ingested items (Pimentel et al. 2018, Mendes et al. 2018), the outcome is a short-term information and the sometimes-harsh identification of semi-digested items may conduct to misleading conclusions on species feeding habits (Cocheret de la Morinière et al. 2003, Dromard et al. 2015). On the other hand, stable isotope analysis (SIA) has been considered a powerful tool with increasing use at measuring nutrient compounds and investigating the trophodynamics over food chain webs. Trophic studies use mainly stable isotopes of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$, respectively) to describe food web relationships. Higher trophic groups tend to present higher levels of $\delta^{15}N$, being this isotope intimately related to trophic position (Post 2002). The available nutrients in the environment are absorbed by algae with a subsequent increase in the signal of $\delta^{15}N$ along the food webs (Fry 2006).

Meanwhile, $\delta^{13}C$ helps to distinguish between sources origin (e.g., freshwater, estuarine or marine), different groups of primary producers and to identify seasonal patterns of variation (Cabana & Rasmussen 1996, Abrantes et al. 2014).

SIA is useful in understanding long-term species trophic ecology, representing species' nutrient assimilation over the scale of few months, but still lacks a fine-scale taxonomic identification in the case of two different food sources present similar isotopic signatures (Johnson et al. 2002). Once any technique is subjected to flaws, the joint and concomitant use of different approaches to assess nutritional information has become a common strategy on trophodynamic studies in ecosystems (Fry 2006). The use of dietary analysis coupled with SIA allows, respectively, the identification of recent ingested items and the estimative of energy sources assimilated over a recent past, thus revealing information on species feeding habits and inferring species ecosystemic function (Dromard et al. 2015, Mendes et al. 2018).

We aimed to investigate seasonal variations in the diet and nutrient assimilation (through SIA) of three species of nominally herbivorous fishes an upwelling-affected rocky reef. In accordance, we have asked: 1) Do nominally herbivorous fishes exhibit variation on diet and nutrient assimilation among the seasons? 2) If so, are the seasonal variations concordant between both analytical approaches (i.e., diet and stable isotope analysis)? We hypothesised that each species of herbivorous fish will exhibit different diet and nutrient assimilation throughout the seasons, being the upwelling seasons (spring/summer) the most nutrient-enriched for all species and that nutrient assimilation will vary according to the food items ingested seasonally by each one.

Material and methods

Study area

Arraial do Cabo (22º 58' S; 42º 00' W), located on the southern coast of Brazil, state of Rio de Janeiro, represents an important area for ecological and biogeographical studies with marine communities due to upwelling events that give this place tropical and subtropical affinities (Mendes et al. 2009). Seasonally, upwelling events affect directly the southern region of Arraial do Cabo, while the local topography at the north prevents these effects to reach the sheltered places with great intensity (Valentin 2001). Consequently, it is possible to observe variations on water temperature and in the input of nutrients that enrich of the local food web (Guimaraens & Coutinho 1996, Ferreira et al. 2001, Valentin 2001). All samples were collected in upwelling-sheltered sites of Arraial do Cabo, characterised by blooms of red and green filamentous algae in summer, being *Jania* sp., *Amphiroa* sp., *Gelidium pusillum* and *Gelidiella* sp. the most abundant taxa (Ferreira, Gonçalves, et al. 1998).

Specimen selection and sampling

We selected three species of nominally herbivorous reef fishes: *Acanthurus chirurgus* (Bloch 1787) (Acanthuridae), *Sparisoma axillare* (Steindachner 1878) (Labridae: Scarinae), and *Kyphosus vaigiensis*

(Quoy & Gaimard 1825) (Kyphosidae). They represent reef fish families of significative impact on the benthic substrata at the Brazilian reefs (Ferreira et al. 2004, Cordeiro et al. 2016, Longo et al. 2018) and have distinct diets and food processing modes (Choat et al. 2002, 2004, Ferreira et al. 2004, Ferreira & Gonçalves 2006, Cordeiro et al. 2016). Specimens were collected (spearfished), pithed (when necessary), and immediately preserved in ice. In the laboratory, stomach contents (for dietary analysis) and fishes' dorsal muscle tissue (for stable isotopic analysis – SIA) were removed. As scarids lack a stomach, its content was removed from the gut's proximal portion (Clements & Bellwood 1988).

Material for dietary analysis was preserved in formalin 10% and muscle tissues were freeze-dried and ground to powder for posterior SIA. We collected only larger individuals to avoid ontogenetic and size-biased results on diet and stable isotope analysis. Algae samples were also collected along the seasons, concomitant with the fish sampling, aiming to represent the most abundant food items, i.e. turf, brown (Phaeophyceae) and red (Rhodophyta) algae. Collected algae were put on ice, frozen and freeze-dried and ground to powder for SIA. The collections lasted for two years (April/2015 to June/2017). During this period, four seasons were sampled and the data from the same season (in each of these years) were pooled to assure for interannual fluctuations. Notwithstanding, prior analysis on both diet and nutrient assimilation identified differences between the years for the same season (not for all species or comparisons), but evaluating the small-scale over time changes and interannual variations was beyond the scope of this work.

Characterizing diets

Grazing acanthurids usually ingest particulate material and scarids grind their food with pharyngeal mills, making the identification of ingested food a challenging task. Thus, gut contents were analysed in two steps for better clarification. First, using a stereomicroscope (step 1 – hereafter 'macro-analysis' –, 4x10 magnification), each gut content was spread in a Petri' dish marked with 50 fixed points for the three species. Secondly, aiming to identify the ingested micro-items, we used an optical microscope (step 2 – hereafter 'micro-analysis', 40x10 maximum magnification) in a slide marked with 30 fixed points for *S. axillare* and *A. chirurgus*, once these are known for their high amount of detritus intake (Choat et al. 2002, Ferreira & Gonçalves 2006, Mendes et al. 2018). In the micro-analysis, the material used was the same previously analysed on macro-analysis but after being filtered in a 60 µm mesh. For both approaches, dietary items were identified to the lowest possible taxonomic level. Identified algae were classified *a posteriori* into functional groups adapted from Steneck & Dethier (1994).

Sea surface temperature (SST)

To investigate the possible occurrence of the seasonal upwelling events in Arraial do Cabo (Valentin 2001) and the subsequent environmental changes, we gathered sea surface temperatures (SST) from a deployed sensor the near surface (~5 m deep) recording SST hourly during the approximate period of two years, encompassing the sampling period. SST data was pooled to obtain the average on each season:

Autumn (AU; March to May), Winter (WT; June to August), Spring (SP; September to November) and Summer (SM; December to February).

Statistical analysis

Dietary analysis

The dietary items recorded in each marked point had its percentage contribution to the whole diet calculated. These values were thus submitted to a permutational multivariate analysis of variance (PERMANOVA) using the PERMANOVA+ add-on for Primer (Anderson et al 2008) to access the dietary difference across the seasons for each species. PERMANOVA design was set as resemblance matrix with Euclidean distance, Type III sum of squares, of residuals under a reduced model and 9999 permutations. The results from macro and micro-analysis were compared across the year using a design with the factors 'Season', fixed with 4 levels (i.e. Summer, Autumn, Winter and Spring) nested within the fixed factor 'Species' (i.e. *A. chirurgus*, *S. axillare* and *K. vaigiensis*), and a posterior pair-wise tests among these were performed. PERMANOVA was also performed to verify the difference for each item that composes the fish diets among the seasons. A Principal Component Analysis (PCA) was performed to visualise the similarity/overlap among seasonal diet for the three species.

Nutrient assimilation: stable isotope analysis (SIA)

A Thermo Quest-Finnigan Delta Plus isotope ratio mass spectrometer (Finnigan-MAT) interfaced to an Elemental Analyser (Carlo Erba) was used in the ground-to-powder fish' muscle tissue and algae samples to measure the stable isotopes. Stable isotope ratios are expressed in delta notation (δ), defined as parts per thousand (‰) differences from a standard material following the formula: $\delta X‰ = [(R_{sample}/R_{standard}) - 1] \times 10^3$, where δ = the measure of heavy to light isotope in the sample, X = ^{13}C or ^{15}N and R = the corresponding ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$ – $\delta^{13}C$ and $\delta^{15}N$, respectively). International Standard references are Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric N^2 for nitrogen. Avoiding disruptive values on fish muscles induced by lipid content, $\delta^{13}C$ results were mathematically corrected when considered lipid-rich tissues (i.e. C:N > 3.5), following Post *et al.* (2007).

The isotopic composition of each species of fish and algae was evaluated using Stable Isotope Bayesian Ellipses in R (SIBER – Jackson *et al.* 2011) package for the R environment (R Core Team 2017). Bayesian ellipses (95% credibility interval) were calculated to better understand the isotopic composition of species over the seasonal gradient. Aiming to evaluate the isotopic variation of fishes and algae between the seasons, PERMANOVA was performed with isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$ concentrations, separately). The PERMANOVA design was the same as the dietary analysis followed by a pair-wise test when results returned significative ($\alpha < 0.05$). SST averages were tested between seasons also with PERMANOVA.

The following analysis were conducted in R (R Core Team 2017). PCA was performed using the function 'prcomp()' (Package 'stats' version 3.6.0, R Core Team 2017). To investigate the relationship between diet and nutrient assimilation, we first calculated the niche overlap among seasons for both macro- and micro-analysis through the Schoener' Index (Wallace 1981) using the 'spaa' package (Zhang 2016). For *A. chirurgus* and *S. axillare*, diet overlap was calculated pooling together the results for both diet resolution analysis. In addition, we used the SIBER package to calculate the isotopic niche overlap (Bayesian ellipses overlap area) between the seasons. Finally, we performed linear regressions for each species between both overlap results (diet and isotopic niches). It aimed to investigate whether diet and isotopic niche overlaps occur at the same time due to the influence of diet on assimilated nutrients.

Results

Dietary analysis

Differences were observed among seasons (macro-analysis: Pseudo-$F = 4.246$, $p = 0.0001$; micro-analysis: Pseudo-$F = 4.871$, $p = 0.0001$) but not for all between-season comparison (Table 1). It is probably a reflex of the high overlap observed among the seasons (Table S1). For *A. chirurgus*, all between-season comparisons for macro-analysis were different, and for the micro-analysis pairwise, only the comparison between Summer (SM) *vs.* Autumn (AU) had no differences. Macro-analysis diet of *S. axillare* was different between AU and Spring (SP), while the micro-analysis was different when compared SP against the other seasons, and Winter (WT) *vs.* SM. For *K. vaigiensis*, the diet was different between WT and the other seasons (Table 1).

Table 1: PERMANOVA statistics for dietary analysis comparisons on macro- (below diagonal) and micro-analysis (above diagonal) scales. For each cell, values represent Pseudo-*t* statistics (above) and *p*-value (below) for pair-wise comparisons. Uppercase acronyms represent each season: SM – Summer, AU – Autumn, WT – Winter, and SP – Spring. Bold values denote statistically significant comparisons ($\alpha < 0.05$). "–" not tested (see methods).

	Acanthurus chirurgus				*Sparisoma axillare*				*Kyphosus vaigiensis*			
	Micro-analysis				Micro-analysis				Micro-analysis			
	SM	AU	WT	SP	SM	AU	WT	SP	SM	AU	WT	SP
SM		1.267	2.019	2.477		1.459	2.187	1.896		–	–	–
		0.183	**0.023**	**0.003**		0.105	**0.008**	**0.030**				
AU	2.757		2.417	2.040	1.398		0.951	3.766	0.696		–	–
	0.002		**0.003**	**0.026**	0.139		0.422	**0.001**	0.716			
WT	2.865	2.859		4.194	1.580	1.703		3.851	2.714	2.108		–
	0.001	**0.001**		**0.001**	0.084	0.066		**0.001**	**0.001**	**0.001**		
SP	2.014	3.485	1.657		1.006	2.294	0.629		1.407	1.261	1.575	
	0.012	**0.001**	**0.044**		0.329	**0.019**	0.706		0.117	0.181	**0.039**	
	Macro-analysis				Macro-analysis				Macro-analysis			

Diet of each species was characterised by one or two main items (Figure 1), being *A. chirurgus*' diet dominated by red algae and invertebrates, *S. axillare* by detritus and *K. vaigiensis* by brown leathery (*Sargassum* spp.) and foliose (mainly *Dictyota* spp.) algae. Moreover, the micro-analysis (Figure 2) pointed to a higher contribution of diatoms in the diet of *A. chirurgus* whereas sediment, sponge spicules (Porifera) and green filamentous algae were the main items for *S. axillare*'. The each-item seasonal comparison (Figs. S1, S2 and S3, respectively) revealed that for macro- and micro-analysis, about one-third of the identified items was different among seasons for *A. chirurgus* and *S. axillare*, but five out of nine items presented a seasonal variation on *K. vaigiensis* diet.

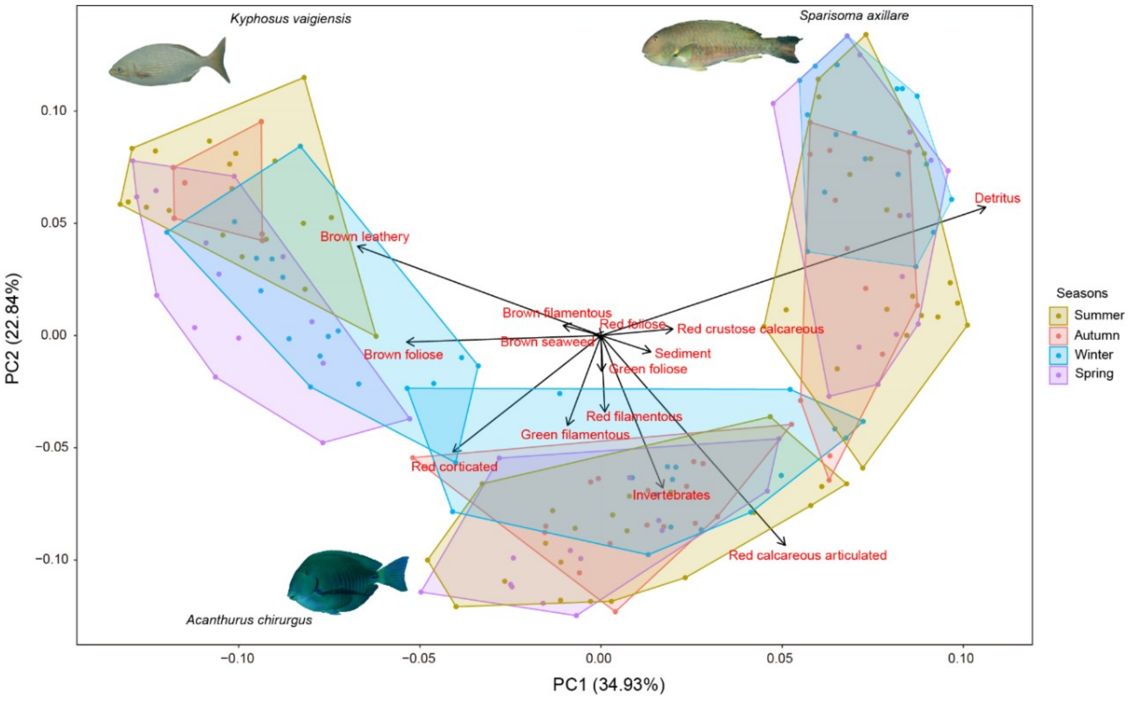

Figure 1: Principal Component Analysis (PCA) with comparative seasonal dietary macro-analysis data for the three species of nominally herbivorous fishes: *Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis*.

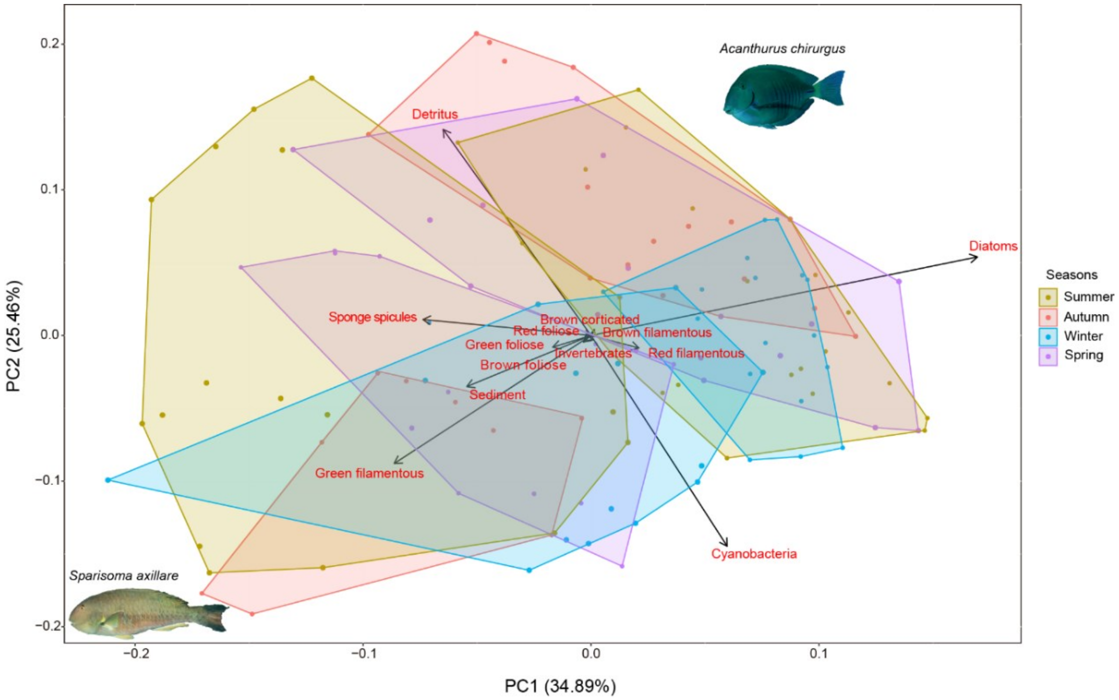

Figure 2: Principal Component Analysis (PCA) with comparative seasonal dietary micro-analysis data for *Acanthurus chirurgus* and *Sparisoma axillare*.

Consumers stable isotope signatures

Stable isotope concentrations among fishes were different (δ^{13}C: Pseudo-F = 2.042, p = 0.041; δ^{15}N: Pseudo-F = 3.767, p = 0.001), but the pair-wise comparisons revealed that it happened only in a few between-season comparisons (Table 2). For *A. chirurgus*, nutrient assimilation (i.e. isotopic signatures) was different between SM and SP for δ^{13}C and between AU and SP for δ^{15}N (Table 2; Figure 3). No seasonal difference was found on *S. axillare* isotopic signatures of δ^{13}C, but δ^{15}N levels were different between SM and AU. For *K. vaigiensis,* δ^{13}C signature was different between AU and SM, whilst for δ^{15}N signature in AU was different from the other seasons (Table 2; Figure 3). The Standard Ellipse Area corrected for small sample sizes (SEAc ‰^{2}) revealed that isotopic niche breadth varied among the study species and seasons. *Acanthurus chirurgus* and *S. axillare* displayed broader isotopic niches in SP (SEAc: 1.8 ‰^{2} and 0.6 ‰^{2}, respectively), while *K. vaigiensis* did it in WT (SEAc: 5.2 ‰^{2}). The season where isotopic niche was more restricted also differed, being in WT for *A. chirurgus* (SEAc: 0.4 ‰^{2}), in SM for *S. axillare* (SEAc: 0.3 ‰^{2}) and in the AU for *K. vaigiensis* (SEAc: 1.7 ‰^{2}) (Figure 4).

Table 2: PERMANOVA statistics for seasonal comparisons for concentrations of stable isotopes of carbon ($\delta^{13}C$ – above diagonal) and nitrogen ($\delta^{15}N$ – below diagonal) for the three studied fish species. For each cell, values represent Pseudo-*t* statistics (above) and *p*-value (below) for pair-wise comparisons. Uppercase acronyms represent each season: SM – Summer, AU – Autumn, WT – Winter, and SP – Spring. Bold values denote significantly different comparisons ($\alpha < 0.05$).

	Acanthurus chirurgus				*Sparisoma axillare*				*Kyphosus vaigiensis*			
	$\delta^{13}C$				$\delta^{13}C$				$\delta^{13}C$			
	SM	AU	WT	SP	SM	AU	WT	SP	SM	AU	WT	SP
SM		0.422	1.312	2.732		0.147	0.123	1.163		2.609	0.316	0.228
		0.686	0.191	**0.009**		0.886	0.905	0.257		**0.016**	0.755	0.822
AU	1.365		0.600	1.615	2.281		0.044	0.857	2.775		1.984	1.901
	0.186		0.549	0.118	**0.029**		0.965	0.403	**0.010**		0.058	0.067
WT	0.416	0.922		1.322	0.316	1.726		1.205	0.611	3.189		0.442
	0.681	0.367		0.192	0.757	0.092		0.229	0.560	**0.006**		0.655
SP	1.708	2.272	1.961		1.777	0.284	1.658		0.608	3.510	0.021	
	0.095	**0.032**	0.056		0.079	0.776	0.115		0.555	**0.002**	0.984	
	$\delta^{15}N$				$\delta^{15}N$				$\delta^{15}N$			

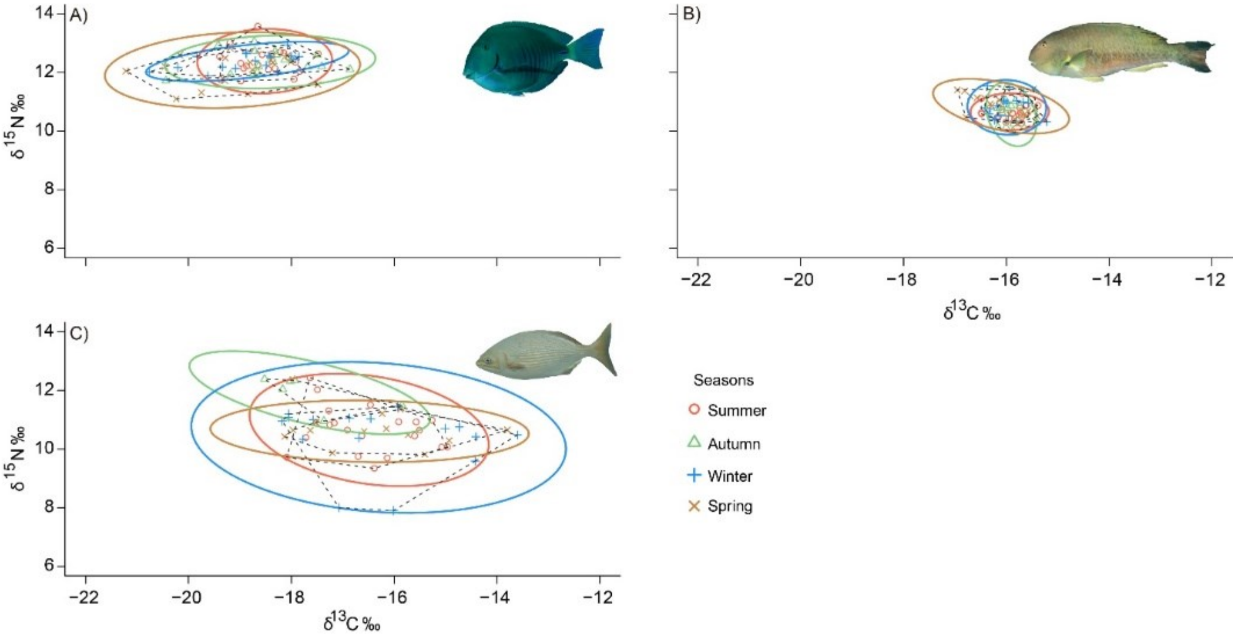

Figure 3: Stable isotope bi-plots (sample data in $\delta^{13}C$ and $\delta^{15}N$ bivariate space) illustrating the nutrient assimilation and isotopic niche through corrected standard ellipse area (SEAc, 95% C.I.; solid lines) and convex-hull area around extreme sample values (dashed lines) for A) *Acanthurus chirurgus*, B) *Sparisoma axillare* and C) *Kyphosus vaigiensis*, in each sampled season.

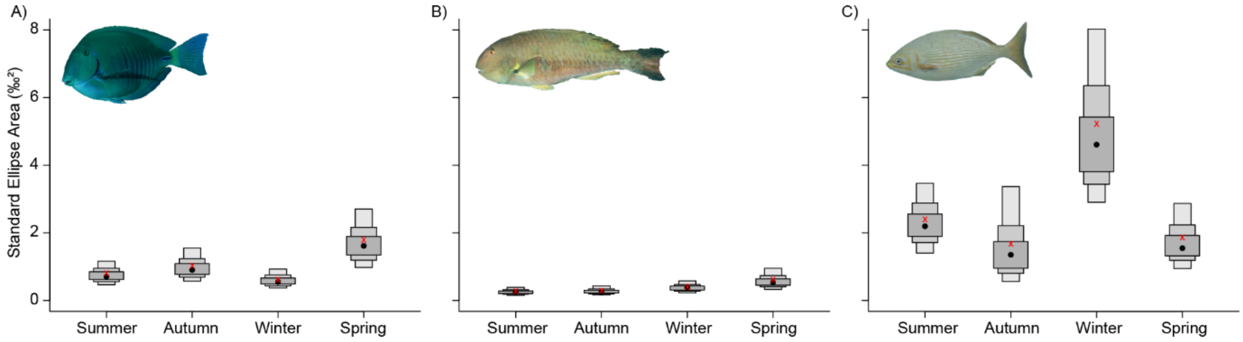

Figure 4: Seasonal small-sample size corrected Standard Ellipse Area (SEAc) for the three study species: A) *Acanthurus chirurgus*, B) *Sparisoma axillare* and C) *Kyphosus vaigiensis*. Shaded boxes represent the credible intervals of 50%, 75% and 95%, from dark to light grey. Black dots represent the sample mode and red 'x' is the mean value for each population.

Primary producer's stable isotope signatures

Seasonal differences within algae groups were identified ($\delta^{13}C$: Pseudo-$F = 7.478$, $p = 0.001$; $\delta^{15}N$: Pseudo-$F = 3.702$, $p = 0.002$) but not among all seasons or for both stable isotopes (Table 3). Regarding $\delta^{13}C$ levels, 'brown algae' were significantly enriched in SM than AU and SP (Figure 5; Table S3), and the $\delta^{15}N$ levels had no difference between any of the seasonal comparisons. 'Red algae' $\delta^{13}C$ composition was more depleted in SP than in AU and WT, while SM was $\delta^{15}N$-enriched than WT. Finally, 'turf algae' was more $\delta^{13}C$-depleted in SM than in SP, whilst the $\delta^{15}N$ composition was enriched on WT than SP and SM, and slightly higher in AU than in SP (Figure 5; Table S3).

Table 3: PERMANOVA statistics for seasonal comparisons for concentrations of stable isotopes of carbon ($\delta^{13}C$ – above diagonal) and nitrogen ($\delta^{15}N$ – below diagonal) for algae groups: brown algae, red algae and turf. For each cell, values represent Pseudo-*t* statistics (above) and *p*-value (below) for pair-wise comparisons. Uppercase acronyms represent each season: SM – Summer, AU – Autumn, WT – Winter, and SP – Spring. Bold values denote significantly different comparisons ($\alpha < 0.05$). "–" not tested (see methods).

	Brown algae				Red algae				Turf			
	$\delta^{13}C$				$\delta^{13}C$				$\delta^{13}C$			
	SM	AU	WT	SP	SM	AU	WT	SP	SM	AU	WT	SP
SM		4.151	-	6.100		3.505	1.504	2.459		1.486	1.388	2.046
		0.001		**0.001**		0.101	0.294	0.060		0.150	0.194	**0.049**
AU	1.007		-	1.944	1.233		0.270	11.59	1.051		0.514	1.368
	0.342			0.067	0.296		0.801	**0.037**	0.314		0.610	0.225
WT	-	-		-	0.855	1.873		3.480	2.367	2.019		1.732
					0.401	0.192		**0.013**	**0.037**	0.069		0.111
SP	1.008	0.486	-		5.408	1.647	7.012		0.450	2.096	3.386	
	0.331	0.640			**0.012**	0.244	**0.012**		0.634	**0.041**	**0.004**	
	$\delta^{15}N$				$\delta^{15}N$				$\delta^{15}N$			

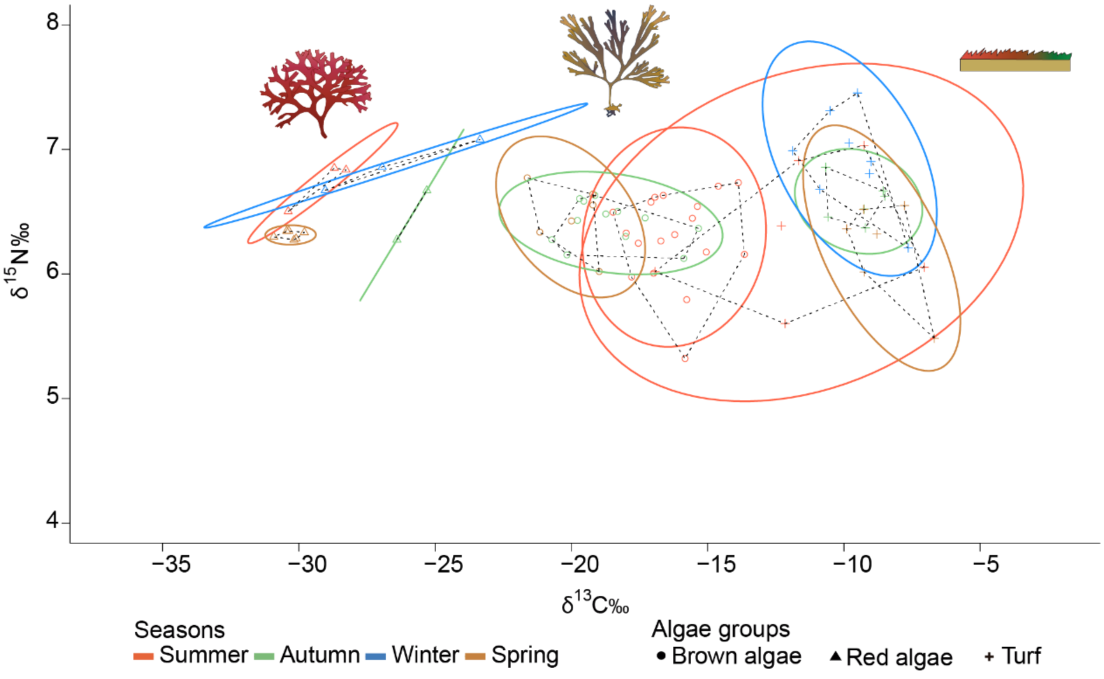

Figure 5: $\delta^{13}C$ and $\delta^{15}N$ bivariate spaces with corrected standard ellipses area (SEAc – solid lines; C.I. 95%) and the convex-hull area around extreme sample values (dashed lines) illustrating the isotopic composition of primary producers sampled in each season.

Seasonal niche overlapping

Dietary analyses presented seasonal overlap higher than 60% (Shoener' index > 0.600) in all between-seasons comparison for *A. chirurgus* and *S. axillare* (Table S1). However, only one of these comparison for each one of the species reached such high overlap on stable isotope ellipses (Table S2). Diet macro- and micro-analysis showed to have a consistent overlap, with the higher values happening between the same seasons for these two species. Overlap was lower for *K. vaigieinsis* on both analysis (diet and assimilated nutrients; Table S1 and S2). Nutrient assimilation' overlap was different among seasons in each species, although higher values occurred involving the SM in all three species (Table S2). Similarly, the comparisons in which the overlap reached the lower values within each species was observed in one specific season, the SP (Table S2).

An overlapping discordance between both metrics was also verified (linear regressions, $p > 0.05$; Figure 6), indicating that seasonal overlap on diet and nutrient assimilation were not concomitant.

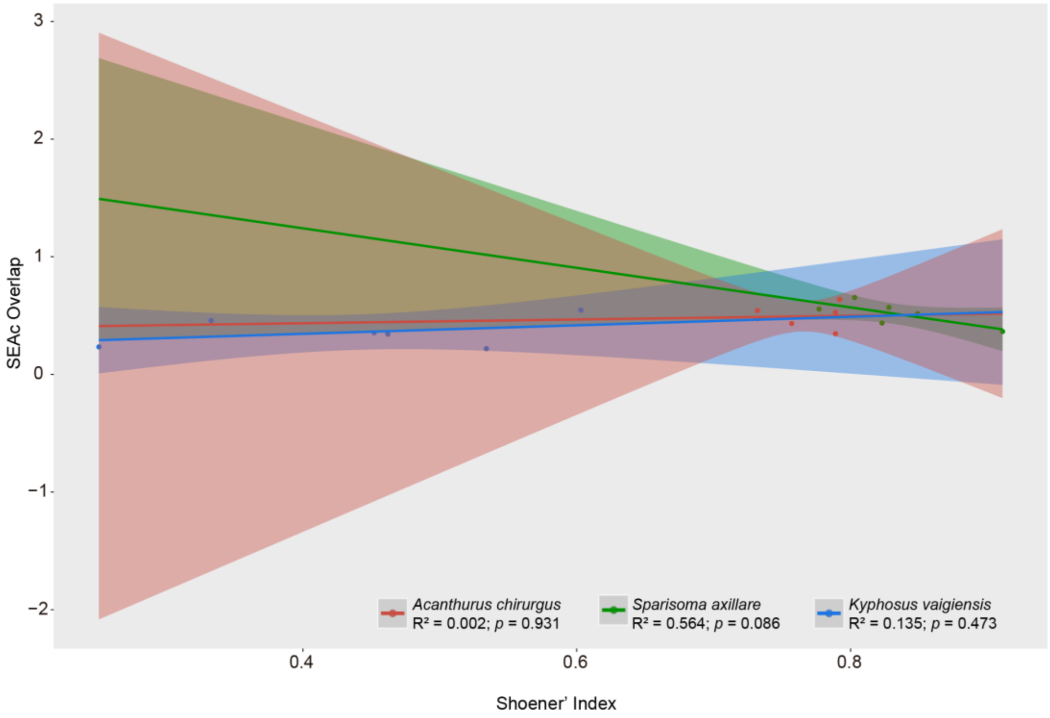

Figure 6: Correlation between seasonal diet overlap and the seasonal isotopic niche overlap. Diet overlap is measured through Schoener' index and isotopic niche overlap through the overlap area for the corrected Bayesian Standard Ellipse Area (SEAc). Colours indicate species and each point is a between-season comparison (e.g., Summer *vs* Winter) for each one of the overlap indexes in a bivariate space.

Sea surface temperature (SST)

SST showed differences throughout the year, with AU presenting higher average temperature when compared to other seasons (*p* = 0.016). The difference from the coldest season (WT) to the warmest (AU) was of 1.2 °C (SM = 22.1 °C; AU = 22.6 °C; WT = 21.4 °C; SP = 21.6 °C). SM presented the higher SST variation (11.5 ºC), whilst the smallest was observed in WT (8.2 °C), indicating that overall temperature is more stable in WT. Moreover, the peaks of low temperatures used as a proxy for identifying upwelling events occurrence revealed some similarity between the seasons (Figure S4). SM, AU and SP were the seasons with the lower temperatures, about ~15.5 ºC, while the minimum temperature observed in WT was 17.8 ºC. Small variation was also observed on higher averages: 25 °C in SP, 26 ºC in AU and WT, and 27 °C in SM.

Discussion

This work investigated the seasonal shifts on diet and nutrient assimilation of three nominally herbivorous fishes in an upwelling-affected rocky reef. *Acanthurus chirurgus* has fed mainly on red algae, *S. axillare* displayed high intake of detritus, while *K. vaigiensis* has relied its diet mainly on brown algae. All these items remained important during all seasons for each species. The nutrient assimilation showed particular differences between-seasons for each species but with high seasonal overlap. However, the observed overlap in each of the analytical approaches did not match in which between-season it occurred. Diet varied more frequently than nutrient assimilation, indicating that dietary shifts may be intentionally performed by the fishes to their nutritional requirements and stay the whole year nourished.

Acanthurus chirurgus displayed the most diversified diet (i.e., the higher number of items) within study species. Such diet plasticity may allow the exploitation of different food sources to cope with the known seasonal fluctuation in macroalgae availability (Yoneshigue-Valetin & Valentin 1992). Thus, the high variability of items and its proportions may have induced the dietary differences found across most seasons. Seasonal differences in nutrient assimilation were expected to present a similar pattern of variation than those observed on diet, once nutrients come from fishes food sources (Fry 2006). Nevertheless, *A. chirurgus* seems to be able to adapt its diet over macroalgal seasonal oscillation, reaching a better balance of absorbed nutrients from ingested food to keep a stable and optimal nutrition.

On the other hand, *S. axillare* presented a less varied diet, showing high detritus intake in all seasons. Parrotfishes harbour a denticulated pharyngeal mill that finely grounds the ingested food before reaching the foregut (Liem & Sanderson 1986, Clements & Bellwood 1988). It can be perceived by the amount small fragments of calcarean articulated and crustose red algae found in its gut contents. Such algae groups are important components of the turf and usually harbour different filamentous algae as epiphytes. Overall, turf is an heterogenous resource highly complex and may harbour a diversity of components that contribute to the nutrition of grazing fishes like, for example, microalgae (diatoms, dinoflagellate and cyanobacteria) and detritus (Choat 1991, Crossman et al. 2001). Parrotfishes feed less frequently than acanthurids (Ferreira, Peret, et al. 1998, Francini-Filho et al. 2010), and lower bite rates are linked with the intake of more energetic (i.e., protein-rich) food resources (Bowen et al. 1995). It would indicate that *S. axillare* targets are indeed nitrogen-richer, such as microscopic autotrophs, selectively targeted by parrotfishes thanks to their morpho- and physiological adaptations (Clements et al. 2017, Clements & Choat 2018). In addition, the most restrict isotopic niche observed for *S. axillare* all year long suggests that parrotfishes are a very selective group and can develop a unique function on reef systems.

Kyphosus vaigiensis, known for its preference for brown algae (Clements & Choat 1997) shifted its diet mainly between two brown algae categories through the seasons. The negligible presence of *Sargassum* on winter's diet is probably due to its seasonality at the sampling region induced by its association with lower water temperature from the upwelling increasing effect (Yoneshigue-Valetin & Valentin 1992, Ferreira et al. 2001). Thus, the variable availability of *Sargassum* reflected the differences found in diet between winter and the other seasons. Previous works have identified seasonal dietary shifts on the brown algae feeder *Odax pullus* at New Zealand's temperate rocky reefs with a high seasonal intake of reproductive structures of fucoid algae (Clements & Choat 1993, Johnson et al. 2017). On that, it is possible that *K. vaigiensis* has fed on *Sargassum* reproductive structures, known for its high nutritional value due to the elevated levels of polysaccharides and protein (Kaur & Vijayaraghavan 1992), during summer, leading to its δ^{15}N-enrichment in autumn, only clearly observed in *K. vaigiensis*.

The dietary analysis represents roughly a photograph of a species feeding behaviour. It can vary within hours, days or more typically among seasons (Keast & Welsh 1968, Ferreira, Peret, et al. 1998, Johnson et al. 2017) led by temporal variations in their habitats, algae abundance and biomass, plus fish grazing rates (Ferreira, Peret, et al. 1998, Diaz-Pulido & Garzón-Ferreira 2002, Ateweberhan et al. 2006). Upwelling events are one of the forces capable of altering individuals diet by modifications on the ecosystem dynamics (Diaz-Pulido & Garzón-Ferreira 2002, Bode et al. 2003). At the subtropical rocky reefs of Arraial do Cabo, although upwelling events being particularly reported to be more frequent and intense in Summer/Spring months due to more intense and constant northeast and east winds (Valentin 2001), these triggers also occur around all the year as we saw here. Thus, despite periodic N pulses/upwelling events are occurring frequently in the region, such enrichment was not particularly observed on nutrient composition of the primary producers. The noticed stability on fish's nutrient assimilation is hence a consequence of repeated upwelling events that kept primary producers' composition similar over the seasons. Thereby, fishes seemed to keep their nutrition steadfast across the seasons instead of showing a better or worse nutritional status in a specific time of the year.

A discordance between dietary analysis and macronutrient composition (C% and N%) on gut contents was recently reported for nominally herbivorous fishes in the same region (Mendes et al. 2018). The authors have noticed that although presenting similar diets with high detritus intake, *A. chirurgus* and *S. axillare* gut contents exhibit highly different nutritional dietary profiles, being the latter richer in carbon and nitrogen. The authors argue that the heterogeneity of the ingested detritus by both species could be the cause of such discordance, but we have found different results for *A. chirurgus* diet. However, as pointed by Mendes et al. (2018), the different nutritional profiles of each species indicate that although they explore similar reef habitats (Bonaldo et al. 2006, Francini-Filho et al. 2010), there is a clear ecological role separation (e.g. feeding behaviour). Indeed, our data on diet (Figs. 1 and 2) and SIA (Figure S5) supports the niche partitioning between both species all year long.

The poorly-variable levels of nutrients of the study fish species over the seasons may have other two possible explanations. First, the seasonal dietary overlap may indicate some consistent food selectivity where changes would happen with one aim, supply the species' nutritional requirements (Johnson et al. 2017, Clements & Choat 2018) according to the seasonal availability. A second explanation would be the presence all year round of upwelling enriching events. Albeit the isotopic variations may be driven by different causes (Marshall et al. 2007), it would explain the absence of the expected alterations on $\delta^{13}C$ and $\delta^{15}N$ observed on primary producers (Cifuentes et al. 1988, McCutchan & Lewis 2001, Koch 2007).

We provide here a seasonal perspective and a comprehensive study on the nutritional ecology of nominally herbivorous fishes. With a two-resolution dietary analysis and nutrient assimilation accessed through SIA, our results revealed that each species tend to keep diet and nutrient assimilation similar across a seasonal gradient, indicating that they can selectively feed to reach its nutritional requirements. Optimal diet models assume that fitness is maximized when the energy gain of some nutrients, or the food value, is maximized (Pyke 1984). Thus, the amount of food types and the consequent amount of energy obtained by these fishes shows that they decide whether or not to eat a particular food item without mistaking them. Marine herbivorous fishes have key functional roles in reef ecosystems community structure and carbon flux (Clements et al. 2009). Still, little attention has been given to how much energy gained with the fish nutritional processes are going through higher trophic levels. The study of nutritional ecology in these animals has the potential to open a new light beyond the feeding ecology, integrating the ecological and evolutionary consequences of an adaptation on nutrient assimilation in a constantly disturbed marine environment.

References

Abrantes KG, Barnett A, Bouillon S (2014) Stable isotope-based community metrics as a tool to identify patterns in food web structure in east African estuaries. Funct Ecol 28:270–282

Afeworki Y, Videler JJ, Bruggemann JH (2013) Seasonally changing habitat use patterns among roving herbivorous fishes in the southern Red Sea: The role of temperature and algal community structure. Coral Reefs 32:475–485

Anthony KRN, Ridd P V., Orpin AR, Larcombe P, Lough J (2004) Temporal variation of light availability in coastal benthic habitats: Effects of clouds, turbidity, and tides. Limnol Oceanogr 49:2201–2211

Ateweberhan M, Bruggemann JH, Breeman AM (2006) Effects of extreme seasonality on community structure and functional group dynamics of coral reef algae in the southern Red Sea (Eritrea). Coral Reefs 25:391–406

Beaver JR, Crisman TL (1991) Temporal variability in algal biomass and primary productivity in Florida lakes relative to latitudinal gradients, organic color and trophic state. Hydrobiologia 224:89–97

Bode A, Carrera P, Lens S (2003) The pelagic foodweb in the upwelling ecosystem of Galicia (NW Spain) during spring: Natural abundance of stable carbon and nitrogen isotopes. ICES J Mar Sci 60:11–22

Bonaldo RM, Krajewski JP, Sazima C, Sazima I (2006) Foraging activity and resource use by three parrotfish species at Fernando de Noronha Archipelago, tropical West Atlantic. Mar Biol 149:423–433

Borer ET, Bracken MES, Seabloom EW, Smith JE, Cebrian J, Cleland EE, Elser JJ, Fagan WF, Gruner DS, Harpole WS, Hillebrand H, Kerkhoff AJ, Ngai JT (2013) Global biogeography of autotroph chemistry: is insolation a driving force? Oikos 122:1121–1130

Bowen SH, Lutz E V., Ahlgren MO (1995) Dietary protein and energy as determinants of food quality: trophic strategies compared. Ecology 76:899–907

Cabana G, Rasmussen JB (1996) Comparison of aquatic food chains using nitrogen isotopes. Proc Natl Acad Sci 93:10844–10847

Choat JH (1991) The biology of herbivorous fishes on coral reefs. In: Sale PE (ed) The Ecology of Fishes on Coral Reefs. Elsevier Inc., p 120–155

Choat JH, Clements KD, Robbins WD (2002) The trophic status of herbivorous fishes on coral reefs I: Dietary analyses. Mar Biol 140:613–623

Choat JH, Robbins W, Clements KD (2004) The trophic status of herbivorous fishes on coral reefs. II. Food processing modes and trophodynamics. Mar Biol 145:445–454

Cifuentes LA, Sharp JH, Fogel ML (1988) Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. Limnol Oceanogr 33:1102–1115

Clements KD, Bellwood DR (1988) A comparison of the feeding mechanisms of two herbivorous labroid fishes, the temperate *Odax pullus*, and the tropical *Scarus rubroviolaceus*. Aust J Mar Freshw Res 39:87–107

Clements KD, Choat JH (1993) Influence of season, ontogeny and tide on the diet of the temperate marine herbivorous fish *Odax pullus* (Odacidae). Mar Biol 117:213–220

Clements KD, Choat JH (1997) Comparison of herbivory in the closely-related marine fish genera *Girella* and *Kyphosus*. Mar Biol 127:579–586

Clements KD, Choat JH (2018) Nutritional ecology of parrotfishes. In: Hoey AS, Bonaldo RM (eds) Biology of Parrotfishes. CRC Press - Taylor & Francis Group, New York, p 420

Clements KD, German DP, Piché J, Tribollet A, Choat JH (2017) Integrating ecological roles and trophic diversification on coral reefs: multiple lines of evidence identify parrotfishes as microphages. Biol J Linn Soc 120:729–751

Clements KD, Raubenheimer D, Choat JH (2009) Nutritional ecology of marine herbivorous fishes: Ten years on. Funct Ecol 23:79–92

Cocheret de la Morinière E, Pollux BJA, Nagelkerken I, Hemminga MA, Huiskes AHL, Velde G van der (2003) Ontogenetic dietary changes of coral reef fishes in the mangrove-seagrass-reef continuum: Stable isotopes and gut-content analysis. Mar Ecol Prog Ser 246:279–289

Cordeiro CAMM, Mendes TC, Harborne AR, Ferreira CEL (2016) Spatial distribution of nominally herbivorous fishes across environmental gradients on Brazilian rocky reefs. J Fish Biol 89:939–958

Crossman DJ, Choat JH, Clements KD (2005) Nutritional ecology of nominally herbivorous fishes on coral reefs. Mar Ecol Prog Ser 296:129–142

Crossman DJ, Choat JH, Clements KD, Hardy T, McConochie J (2001) Detritus as food for grazing fishes on coral reefs. Limnol Oceanogr 46:1596–1605

Diaz-Pulido G, Garzón-Ferreira J (2002) Seasonality in algal assemblages on upwelling-influenced coral reefs in the Colombian Caribbean. Bot Mar 45:284–292

Dromard CR, Bouchon-Navaro Y, Harmelin-Vivien M, Bouchon C (2015) Diversity of trophic niches among herbivorous fishes on a Caribbean reef (Guadeloupe, Lesser Antilles), evidenced by stable isotope and gut content analyses. J Sea Res 95:124–131

Ferreira CEL, Floeter SR, Gasparini JL, Ferreira BP, Joyeux J-C (2004) Trophic structure patterns of Brazilian reef fishes: a latitudinal comparison. J Biogeogr 31:1093–1106

Ferreira CEL, Gonçalves JEA (2006) Community structure and diet of roving herbivorous reef fishes in the Abrolhos Archipelago, south-western Atlantic. J Fish Biol 69:1533–1551

Ferreira CEL, Gonçalves JEA, Coutinho R (2001) Community structure of fishes and habitat complexity on a tropical rocky shore. Environ Biol Fishes 61:353–369

Ferreira CEL, Gonçalves JEA, Coutinho R, Peret AC (1998) Herbivory by the Dusky Damselfish *Stegastes fuscus* (Cuvier, 1830) in a tropical rocky shore: effects on the benthic community. J Exp Mar Bio Ecol 229:241–264

Ferreira CEL, Peret AC, Coutinho R (1998) Seasonal grazing rates and food processing by tropical herbivorous fishes. J Fish Biol 53:222–235

Francini-Filho RB, Ferreira CM, Coni EOC, Moura RL, Kaufman L (2010) Foraging activity of roving herbivorous reef fish (Acanthuridae and Scaridae) in eastern Brazil: influence of resource availability and interference competition. J Mar Biol Assoc United Kingdom 90:481

Fry B (2006) Stable Isotope Ecology. Springer, New York

Guimaraens MA, Coutinho R (1996) Spatial and temporal variation of benthic marine algae at the Cabo Frio upwelling region, Rio de Janeiro, Brazil. Aquat Bot 52:283–299

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. J Anim Ecol 80:595–602

Johnson JS, Clements KD, Raubenheimer D (2017) The nutritional basis of seasonal selective feeding by a marine herbivorous fish. Mar Biol 164:201

Johnson BM, Martinez PJ, Stockwell JD (2002) Tracking trophic interactions in coldwater reservoirs using naturally occurring stable isotopes. Trans Am Fish Soc 131:1–13

Kaur I, Vijayaraghavan MR (1992) Oogonial development, maturation and release in *Sargassum vulgare* C. Agardh and *S. johnstonii* Setchell & Gardner. Aquat Bot 42:173–185

Keast A, Welsh L (1968) Daily deeding periodicities, food uptake rates, and dietary changes with hour of day in some lake fishes. J Fish Res Board Canada 25:1133–1144

Koch PL (2007) Isotopic study of the biology of modern and fossil vertebrates. In: Michener R, Lajtha K (eds) Stable isotopes in ecology and environmental science, 2nd edn. Blackwell Publishing, p 99–154

Leberfinger K, Bohman I, Herrmann J (2011) The importance of terrestrial resource subsidies for shredders in open-canopy streams revealed by stable isotope analysis. Freshw Biol 56:470–480

Lebreton B, Richard P, Galois R, Radenac G, Brahmia A, Colli G, Grouazel M, André C, Guillou G, Blanchard GF (2012) Food sources used by sediment meiofauna in an intertidal *Zostera noltii* seagrass bed: a seasonal stable isotope study. Mar Biol 159:1537–1550

Lefèvre CD, Bellwood D (2011) Temporal variation in coral reef ecosystem processes: herbivory of macroalgae by fishes. Mar Ecol Prog Ser 422:239–251

Liem KF, Sanderson SL (1986) The pharyngeal jaw apparatus of labrid fishes: A functional morphological perspective. J Morphol 187:143–158

Longo GO, Hay ME, Ferreira CEL, Floeter SR (2018) Trophic interactions across 61 degrees of latitude in the Western Atlantic. Glob Ecol Biogeogr:1–11

Marshall JD, Brooks JR, Lajtha K (2007) Sources of variation in the stable isotopic composition of plants. In: Michener R, Lajtha K (eds) Stable Isotopes in Ecology and Environmental Science, 2nd edn. Blackwell Publishing, p 22–60

McCutchan JH, Lewis WM (2001) Seasonal variation in stable isotope ratios of stream algae. Verhandlungen des Int Verein Limnol 27:3304–3307

Mendes TC, Ferreira CEL, Clements KD (2018) Discordance between diet analysis and dietary macronutrient content in four nominally herbivorous fishes from the Southwestern Atlantic. Mar Biol 165:1–12

Mendes TC, Villaça RC, Ferreira CEL (2009) Diet and trophic plasticity of an herbivorous blenny *Scartella cristata* of subtropical rocky shores. J Fish Biol 75:1816–1830

Pimentel CR, Soares LSH, Macieira RM, Joyeux J-C (2018) Trophic relationships in tidepool fish assemblages of the tropical Southwestern Atlantic. Mar Ecol:e12496

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG (2007) Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–189

Pyke GH (1984) Optimal foraging theory: a critical review. Annu Rev Ecol Syst 15:523–575

R Core Team (2017) R: A language and environment for statistical computing. R Found Stat Comput

Raubenheimer D, Zemke-White L, Phillips RJ, Clements KD (2005) Algal macronutrients and food selection by the omnivorous marine fish *Girella tricuspidata*. Ecology 86:2601–2610

Steneck RS, Dethier MN (1994) A functional group approach to the structure of algal-dominated communities. Oikos 69:476–498

Valentin JL (2001) The Cabo Frio upwelling system, Brazil. In: Seeliger U, Kjerfve B (eds) Coastal Marine Ecosystems of Latin America. Springer-Verlag, p 97–105

Wallace RK (1981) An assessment of diet-overlap indexes. Trans Am Fish Soc 110:72–76

Wilson SK, Bellwood DR (1997) Cryptic dietary components of territorial damselfishes (Pomacentridae, Labroidei). Mar Ecol Prog Ser 153:299–310

Yoneshigue-Valetin Y, Valentin JL (1992) Macroalgae of the Cabo Frio upwelling region, Brazil: Ordination of communities. In: Ulrich Seeliger (ed) Coastal plant communities of Latin America. Academic Press, Inc., New York, p 31–50

Zanden MJ Vander, Rasmussen JB (2001) Variation in δ^{15}N and δ^{13}C trophic fractionation: Implications for aquatic food web studies. Limnol Oceanogr 46:2061–2066

Zhang J (2016) spaa: SPecies Association Analysis. R package version 0.2.2. https://cran.r-project.org/package=spaa

Supplementary material

Table S1: Overlapping Schoener index (*Sh*i) calculated for dietary macro- (below diagonal) and micro-analysis (above diagonal) for the three herbivorous species. Uppercase acronyms represent each season: SM – Summer, AU – Autumn, WT – Winter, and SP – Spring. Bold denote higher *Sh*i overlap for seasonal comparisons on both analyses.

	Acanthurus chirurgus				*Sparisoma axillare*				*Kyphosus vaigiensis*			
	Micro-analysis				Micro-analysis				Micro-analysis			
	SM	AU	WT	SP	SM	AU	WT	SP	SM	AU	WT	SP
SM		0.818	**0.924**	0.838		0.865	0.689	0.839		-	-	-
AU	0.808		0.755	0.870	0.863		0.709	**0.886**	0.462		-	-
WT	**0.818**	0.745		0.816	0.780	0.790		0.785	0.333	0.251		-
SP	0.734	0.676	0.672		0.842	**0.869**	0.840		**0.603**	0.534	0.452	
	Macro-analysis				Macro-analysis				Macro-analysis			

Table S2: Values of Bayesian Standard Ellipse Area (SEAc) overlapping calculated between the seasons for the three herbivorous species. Uppercase acronyms represent each season: SM – Summer, AU – Autumn, WT – Winter, and SP – Spring. Bold denote the higher overlap value for seasonal comparisons in each species.

	Acanthurus chirurgus				*Sparisoma axillare*				*Kyphosus vaigiensis*			
	SM	AU	WT	SP	SM	AU	WT	SP	SM	AU	WT	SP
SM												
AU	**0.638**				0.516				0.343			
WT	0.474	0.543			**0.654**	0.556			0.458	0.234		
SP	0.433	0.527	0.346		0.437	0.365	0.570		**0.547**	0.219	0.355	

Table S3: Mean and standard error (mean ± S.E.) for the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) for each fish species and algae group in each season. "–" inserted where samples were not collected.

	Summer	Autumn	Winter	Spring
$\delta^{13}C$				
Acanthurus chirurgus	-18.5 ± 0.5	-18.6 ± 0.7	-18.7 ± 0.8	-19.1 ± 1.0
Sparisoma axillare	-15.9 ± 0.3	-16.0 ± 0.2	-15.9 ± 0.3	-16.1 ± 0.5
Kyphosus vaigiensis	-16.5 ± 0.9	-17.6 ± 0.9	-16.3 ± 1.4	-16.5 ± 1.2
$\delta^{15}N$				
Acanthurus chirurgus	12.4 ± 0.4	12.5 ± 0.4	12.3 ± 0.3	12.1 ± 0.5
Sparisoma axillare	10.7 ± 0.3	10.9 ± 0.3	10.6 ± 0.4	10.9 ± 0.4
Kyphosus vaigiensis	10.7 ± 0.8	11.9 ± 0.5	10.4 ± 1.0	10.6 ± 0.4
$\delta^{13}C$				
Brown algae	-16.2 ± 1.38	-18.6 ± 1.68	-	-20.0 ± 1.12
Red algae	-29.1 ± 1.12	-25.9 ± 0.78	-26.4 ± 2.87	-30.3 ± 0.38
Turf	-10.7 ± 2.45	-9.5 ± 0.96	-9.8 ± 1.31	-8.6 ± 1.18
$\delta^{15}N$				
Brown algae	6.7 ± 0.20	6.2 ± 0.17	-	6.5 ± 0.26
Red algae	6.4 ± 0.17	6.5 ± 0.28	6.9 ± 0.20	6.3 ± 0.03
Turf	6.5 ± 0.26	6.3 ± 0.03	6.9 ± 0.38	6.2 ± 0.40

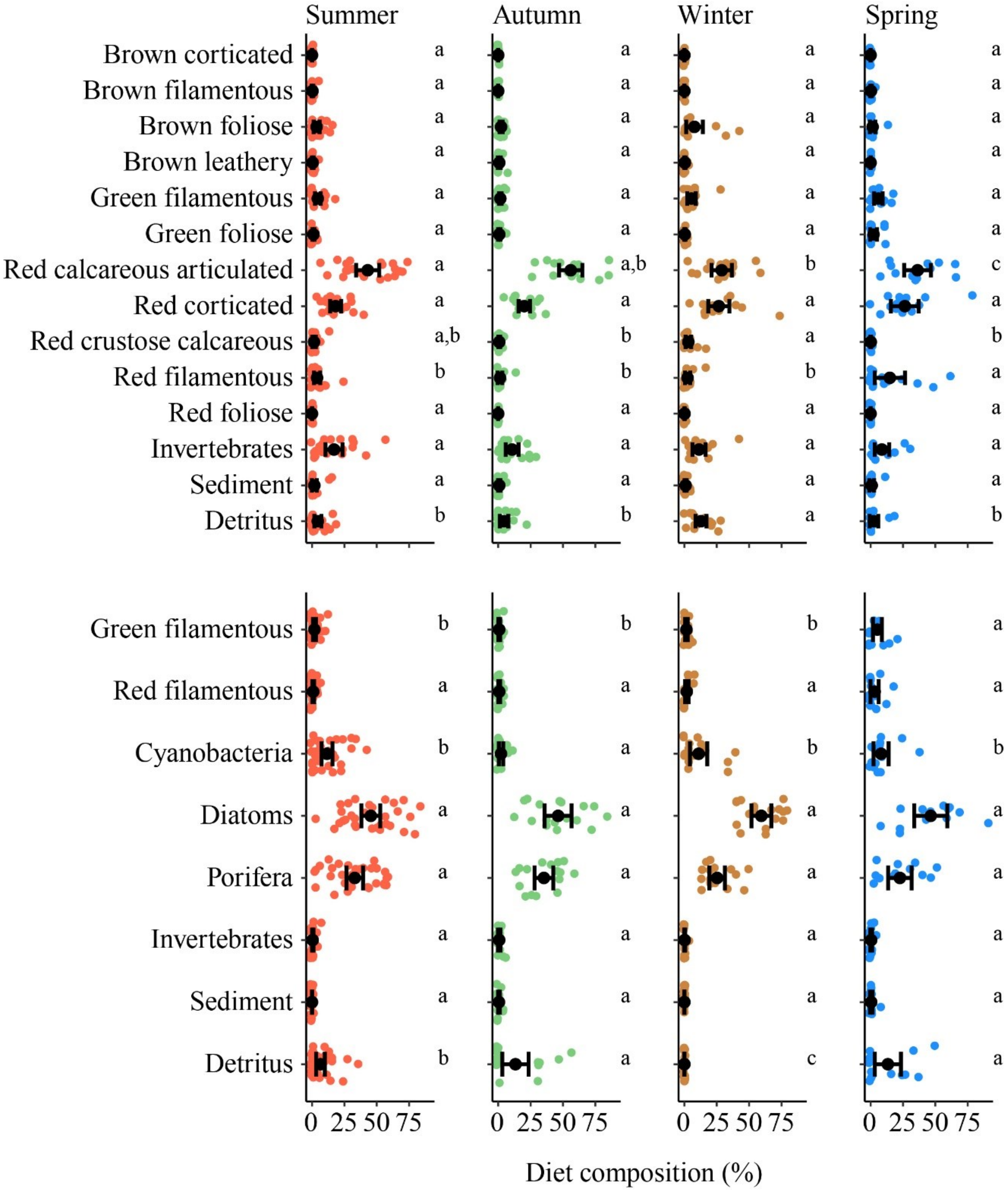

Figure S1: Dietary analysis comparison of *Acanthurus chirurgus* among the four seasons: Summer, Autumn, Winter and Spring – colours correspond to each season. Results from macro- (upper), and micro-analysis (lower). Lower case letters indicate: diet similarities in between-season comparisons for each item – similar letters in different seasons indicate non-significant comparisons (PERMANOVA: $\alpha > 0.05$), and mean (± C.I.) diet composition values (%) for each item, being a > b > c.

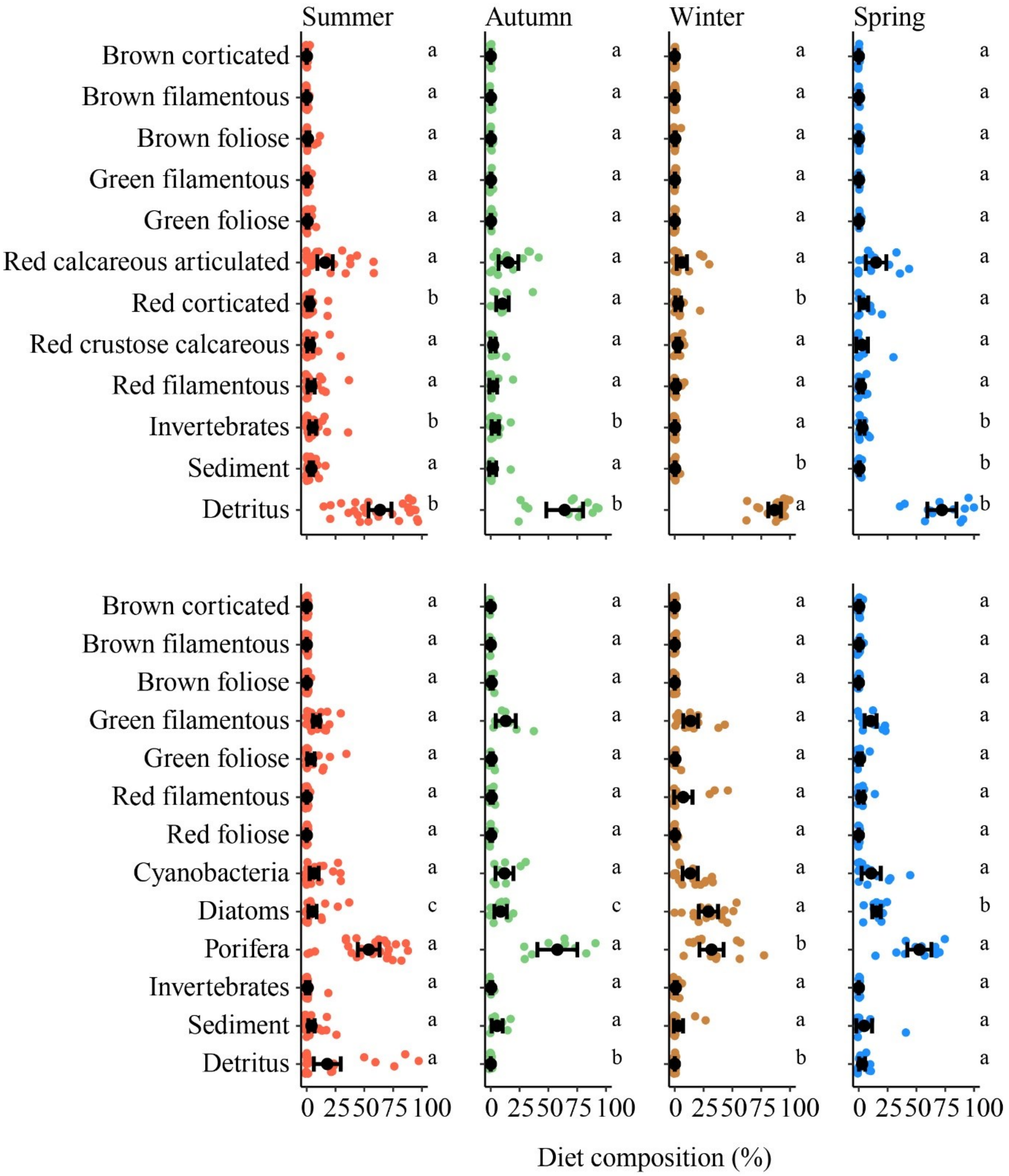

Figure S2: Dietary analysis comparison of *Sparisoma axillare* among the four seasons: Summer, Autumn, Winter and Spring – colours correspond to each season. Results from macro- (upper), and micro-analysis (lower). Lower case letters indicate: diet similarities in between-season comparisons for each item – similar letters in different seasons indicate non-significant comparisons (PERMANOVA: $\alpha > 0.05$), and mean (± C.I.) diet composition values (%) for each item, being a > b > c.

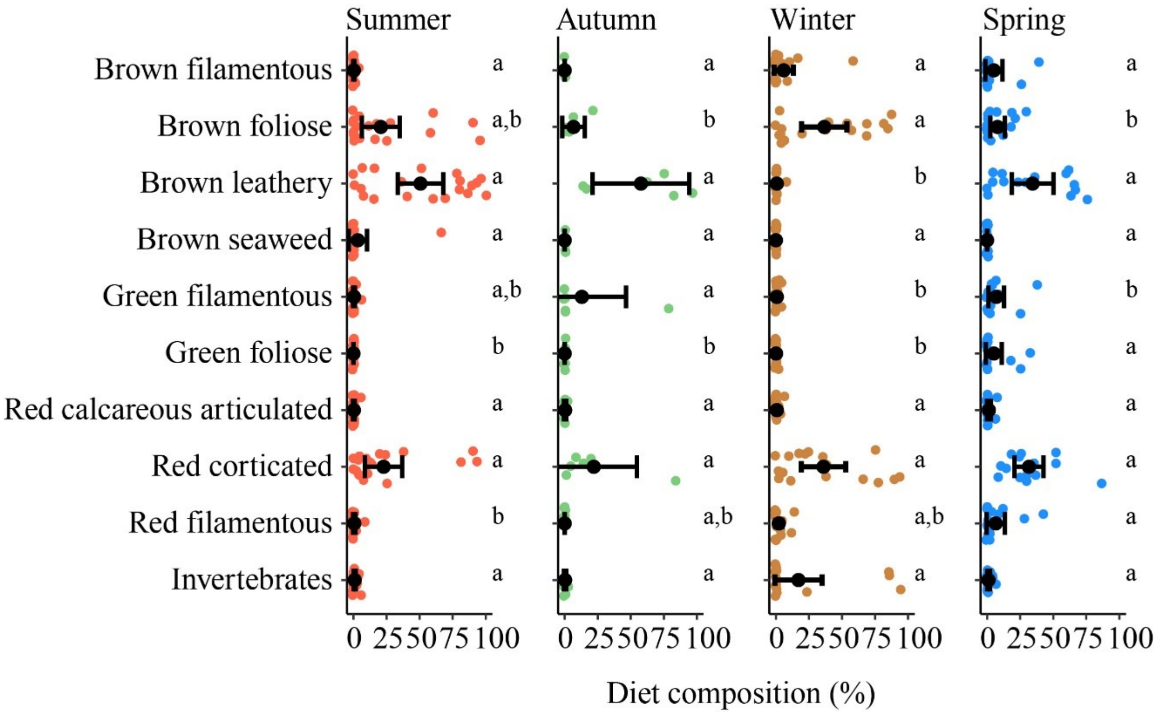

Figure S3: Dietary analysis comparison of *Kyphosus vaigiensis* among the four seasons: Summer, Autumn, Winter and Spring – colours correspond to each season. Lower case letters indicate: diet similarities in between-season comparisons for each item – similar letters in different seasons indicate non-significant comparisons (PERMANOVA: $\alpha > 0.05$), and mean (± C.I.) diet composition values (%) for each item, being a > b > c.

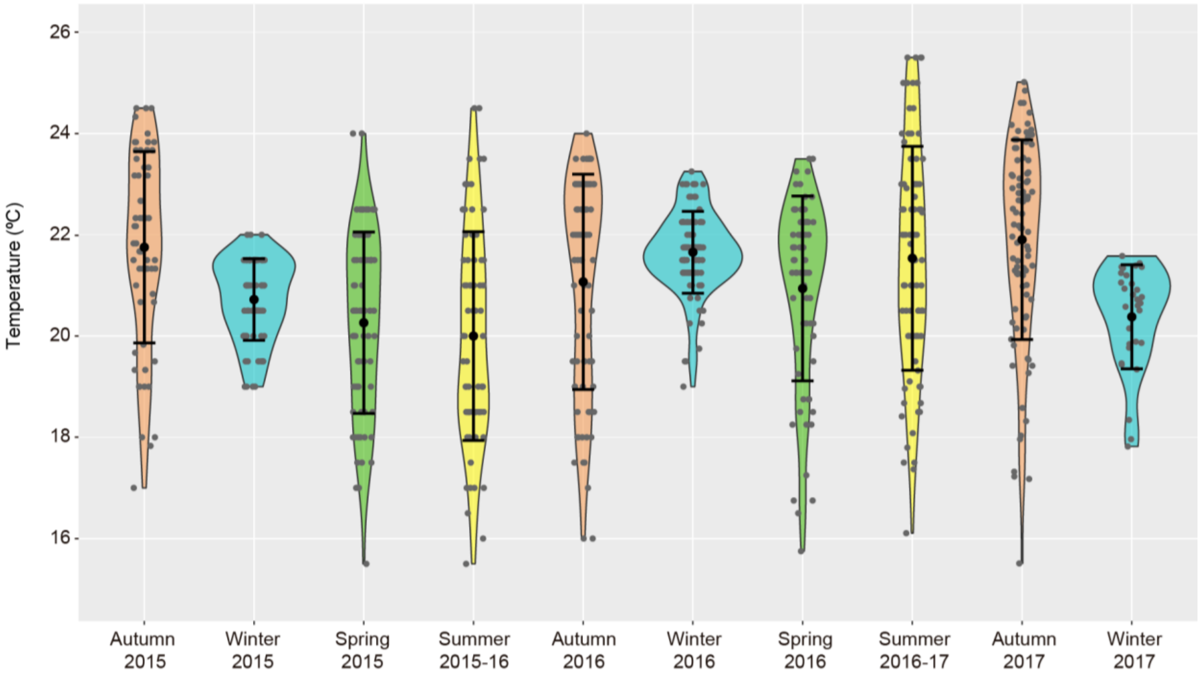

Figure S4: Minimum seasonal sea surface temperature (SST °C) recorded during the sampling period in Arraial do Cabo. Similar colours denote the same season in different years. Black middle dot and error bars represent mean ± standard deviation, respectively. Grey dots are minimum daily temperatures recorded. All seasons have three months pooled (see methods), except for Autumn 2015 (two months; April and May) and Winter 2017 (one month; June).

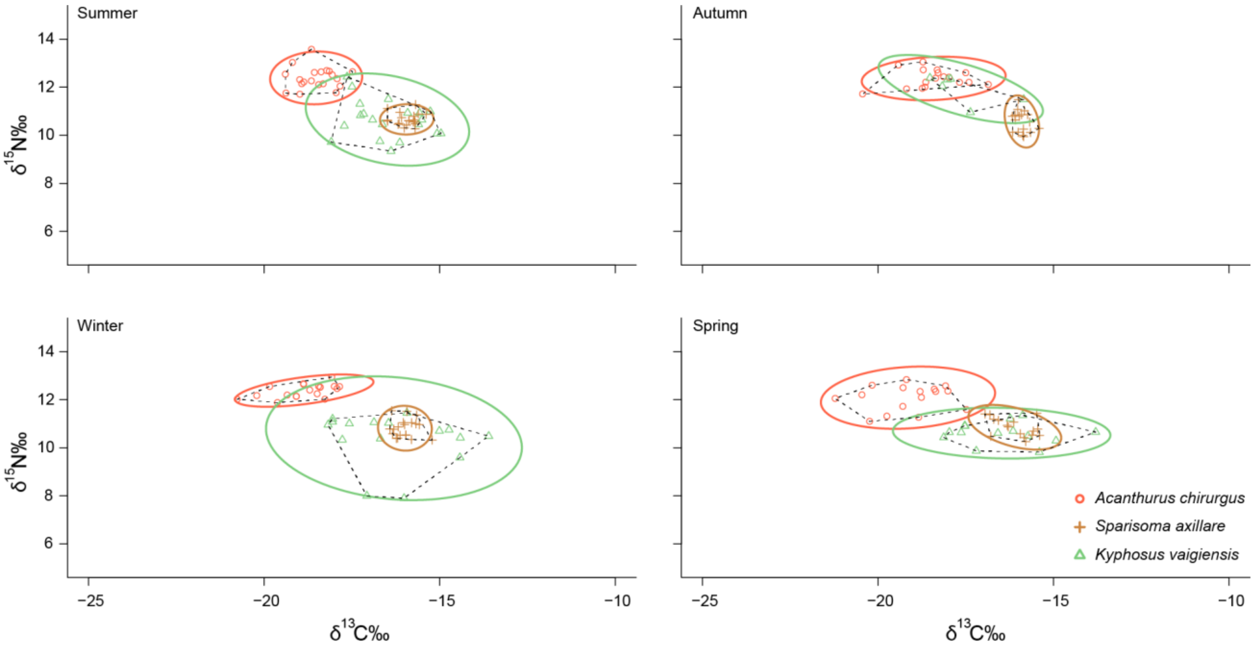

Figure S5: Yearlong stable isotope bi-plots (sample data in δ^{13}C and δ^{15}N bivariate space) illustrating the nutrient assimilation and isotopic niche through corrected standard ellipse area (SEAc, 95% C.I.; solid lines) and convex-hull area around extreme sample values (dashed lines) for the three study species: *Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis*, in each sampled season.

CAPÍTULO 3

LARGE-GRAIN PERSPECTIVE MAY HINDER THE ASSIGNMENT OF HERBIVOROUS FISH FUNCTIONAL ROLES

Large-grain perspective may hinder the assignment of herbivorous fish functional roles

Cardozo-Ferreira G.C., Clements, K.D., Choat, J.H., Macieira, R.M., Mendes, T.C., Rezende, C.E.,
Joyeux, J.-C. and Ferreira, C.E.L.

Formatted for Journal of Animal Ecology

Abstract

1. The diet plasticity of nominally herbivorous fishes allows them to explore different feeding sources. Distributed along a continuum between yield and rate maximisers, the nutritional ecology of these species may be subjected to environmental oscillations such as on food availability or water temperature.

2. We analysed here the nutritional ecology of three nominally herbivorous fishes using complementary approaches: i) a two-resolution dietary analysis, and ii) stable isotope analysis (SIA) within a latitudinal scale of 17º and a four-site temperature gradient between 22.9ºC and 27.8ºC.

3. Variation in diet among species revealed a partitioning of available resources. Moreover, each species kept their main food items among sites. Quantity of algal intake was stable across sampled sites in *Acanthurus chirurgus* and *Kyphosus vaigiensis* and increased toward lower latitudes in *Sparisoma axillare*, with calcareous articulated red algae, brown foliose algae and detritus the most frequent items consumed by each species, respectively. Shifts in trophic position among species occurred across the sites.

4. Ecologists commonly seek for patterns to explain the functioning of communities. However, patterns may underestimate important details on species ecology. Particular feeding preferences and nutrient assimilation observed in nominally herbivorous fishes indicate that each species displays a different functional role in the reef system and also that such role can vary among locations. Looking over ecosystem trophodynamics on a large scale may lead to a loss of important details that would clarify the subtle role(s) played by each species. Instead, a small-grain view should be used in future studies and by decision-makers to improve efforts toward the conservation of widely distributed herbivorous species.

Key-words: dietary analysis, feeding ecology, herbivory, nominally herbivorous fishes, nutritional ecology, stable isotopes.

Introduction

Herbivorous fishes on reef systems exhibit diverse diets and can be broadly classified along a continuum between yield and rate maximisers (Clements, German, Piché, Tribollet, & Choat, 2017; Crossman, Choat, & Clements, 2005). The former depend on endosymbionts to break down refractory macroalgal carbohydrates while the latter target cyanobacteria and protein-rich eukaryotic algae, and digest soluble components without hindgut fermentation (Clements et al., 2017; Crossman et al., 2005; Johnson, Clements, & Raubenheimer, 2017). On this continuum, species have been classified as 'nominally herbivorous' due to their varied diet, including turfing and macroscopic algae but also detrital conglomerates or even macrozooplankton (Choat, Clements, & Robbins, 2002; Crossman et al., 2005; Wilson & Bellwood, 1997). All this has profound implications on how each species react to environmental variations and how they use and partition the available resources (Clements et al., 2017).

However, despite this range of food items, some studies broadly classify species into functional groups as exerting the same functions and pressure over the benthic community (Ferreira, Floeter, Gasparini, Ferreira, & Joyeux, 2004; Longo, Hay, Ferreira, & Floeter, 2018). On the other hand, some authors indicate that the particular role of these species is not only shaped by where individuals forage or what they are ingesting, but also what they are really targeting and have been assimilating (Clements & Choat, 2018; Clements et al., 2017; Johnson et al., 2017; Mendes, Ferreira, & Clements, 2018). Species with algae-richer diet usually present higher macronutrient levels of assimilated carbohydrate while those feeding on detrital aggregates and animal matter assimilate more protein total amino acids (Crossman et al., 2005).

Such macronutrients can be measured through stoichiometry, as the levels of the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), respectively, that accumulate in consumers tissues. Therefore, food web relationships has been described using mainly $\delta^{13}C$ and $\delta^{15}N$ to track food sources origin, differing between groups of primary producers, helping to elucidate the environment trophodynamics (Abrantes, Barnett, & Bouillon, 2014; Jackson, Inger, Parnell, & Bearhop, 2011; Parnell, Inger, Bearhop, & Jackson, 2010). Nevertheless, comprehensive studies that use complementary approaches are required on investigating how ecosystem trophodynamics are influenced by pre- and post-ingestive processes of herbivorous reef fishes (Choat, Robbins, & Clements, 2004; Clements et al., 2017; Clements, Raubenheimer, & Choat, 2009; Mendes et al., 2018).

Importantly, environmental characteristics such as water temperature and sunlight levels, which change rapidly along temporal and spatial gradients (Gaines & Lubchenco, 1982), can influence food resource availability (e.g., macroalgae), affecting herbivory rates, algae consumption (Bennett & Bellwood, 2011) and thus nutrient assimilation. Opposite to that, previous studies predict that temperature may not restrict growth rate, body size and reproductive schedule of herbivorous fishes over gradients of latitude and temperature (Trip, Clements, Raubenheimer, & Choat, 2014; Trip, Clements, Raubenheimer, & Howard Choat, 2016). Hence, there is no consensus regarding the extension in which environmental and site-specific characteristics could trigger diet changes and so affect the herbivorous fishes' nourishment.

We examined the nutritional ecology of three nominally herbivorous fishes using macroscopic and microscopic dietary analysis, and stable isotopes to describe their interspecific relationship within a latitudinal range of 17º and a water temperature between 22.9ºC and 27.8ºC along the Brazilian coast. Having distinct feeding behaviour and living in habitats with particular environmental characteristics (including water temperature and benthic composition), we have asked: 1) Do herbivorous species explore different resources and thus present different nutrient levels in different sites? and 2) Are the interspecific trophic relationships maintained in each site? We hypothesized that each nominally herbivorous fish has dietary preferences and thus a resource partitioning occur among them. Additionally, we expected that levels of assimilated stable isotopes and so nutrition vary among species but the trophic position would be maintained in the different sites.

Material and methods

Study areas

The tropical and subtropical environments on the Brazilian coast comprise a variety of reef types. The tropical north-eastern coast includes mainly coral and algal reefs, while the south-eastern and southern coasts are subtropical rocky shores, some seasonally affected by upwelling that enriches all trophic levels through high nutrient input from deeper colder waters (Leão & Dominguez, 2000; Leão, Kikuchi, & Testa, 2003; Pinheiro et al., 2018; Valentin, 2001). Four sites were sampled along 17º of latitude: (i) Natal (NT), state of Rio Grande do Norte (5º 47' S; 35º11' W), (ii) Abrolhos Archipelago (AB), state of Bahia (17°20' S; 39°30' W), Guarapari (GR), state of Espírito Santo (20°40' S; 40°23' W), and Arraial do Cabo (AC), state of Rio de Janeiro (22°58' S; 42°00' W). The former two represent tropical locations (NT and AB) while the latter two (GR and AC) represent subtropical environments (Fig. 1A).

Sampling in all sites was conducted during summer in 2016 and 2017. The sites differ in key environmental characteristics including substratum composition (Aued et al., 2018), mean sea surface temperature (SST) and fish composition (Ferreira & Gonçalves, 2006; Kikuchi, Leão, Sampaio, & Telles, 2003; Moura & Francini-Filho, 2005; Simon, Joyeux, & Pinheiro, 2013). SST data consist in monthly averages over the last ten years (from January 2009 to December 2018) and were provided by Nasa Earth Observations (NEO) from the Aqua MODIS database (retrieved from https://neo.sci.gsfc.nasa.gov/view.php?datasetId=MYD28M). The tropical sites consist of biogenic reefs harbouring a great diversity of benthic organisms and fish species (Bruce et al., 2012; Ferreira & Gonçalves, 2006; Moura & Francini-Filho, 2005), and the coastline is characterized by a transition from siliciclasticdominated sediments, shifting to carbonates from the middle to outer shelf (Leão & Dominguez, 2000). The subtropical sites involve granitic-base rocky reef with bryozoan and some crustose coralline algae (CCA) but dominated by algal turfs (Aued et al., 2018; Cordeiro, Harborne, & Ferreira, 2014; Simon, Pinheiro, & Joyeux, 2011). Although AB and AC are only separated by three degrees of latitude, they present distinct and unique environments. The region of GR has often been considered as a transitional zone between tropical and subtropical (Floeter et al., 2001; Joyeux, Floeter, Ferreira, & Gasparini, 2001; Pinheiro et al., 2018) as it does not harbour the coral reef environments present in AB or the colder upwelling-waters characteristics of AC (Valentin, 2001).

Specimen collection

Three species of nominal herbivorous fish were selected as a target for this study: *Acanthurus chirurgus* (Bloch 1787) (Acanthuridae), *Sparisoma axillare* (Steindachner 1878) (Labridae), and *Kyphosus vaigiensis* (Quoy & Gaimard 1825) (Kyphosidae). These species represent important families of reef fishes that exhibit a variety of diets and food processing modes (Choat, Clements, & Robbins, 2002; Choat et al., 2004; Cordeiro, Mendes, Harborne, & Ferreira, 2016; Ferreira, Floeter, Gasparini, Ferreira, & Joeux, 2004; Ferreira & Gonçalves, 2006) and inhabit shallow biogenic and rocky reefs all along the Brazilian coast (Ferreira et al., 2004; Floeter, Behrens, Ferreira, Paddack, & Horn, 2005). *Acanthurus chirurgus* and *S. axillare* are grazing herbivores that target mainly rhodophytes and detrital aggregates, although the composition of the dominant detrital component appears to differ between the two species (Mendes et al., 2018). Both species display mechanisms for mechanical disruption of ingesta: *A. chirurgus* with a gizzard-like stomach and *S. axillare* with a pharyngeal mill (Horn, 1989). *Kyphosus vaigiensis* is an algivorous species that feeds mainly on brown macroalgae and harbours a symbiotic hindgut microbiota that converts refractory algal carbohydrates into short-chain fatty acids (Clements & Choat, 1995, 1997).

Specimens were collected in each study site through spearfishing, pithed, and immediately put on ice to stop digestive processes. In the laboratory, stomach contents (for dietary analysis) and fishes dorsal muscle tissue (for stable isotopic analysis – SIA) were removed. As *S. axillare* lacks a stomach, content was removed from the anterior intestine (Choat et al., 2002). The collection of *K. vaigiensis* was not possible in GR. We targeted only large individuals to avoid ontogenetic and/or size bias (Tab. S1). Along with fish sampling, the algae species most abundant were collected (except in GR) as potential food resources for study fishes. Algae species were assigned to functional groups (adapted from Steneck & Dethier, 1994) and represent brown, red and green algae (Ochrophyta, Rhodophyta and Chlorophyta, respectively). In addition, we collected turf – an epilithic multi-species algal community that is prevalent along the Brazilian coast (Aued et al., 2018), once it is reported to be target of *A. chirurgus* and *S. axillare* (Ferreira & Gonçalves, 2006; Mendes et al., 2018). After collection, algae samples were frozen, freeze-dried and ground to powder for SIA.

Characterizing diets

Formalin-preserved contents were analysed in two steps to resolve diet at a fine scale. In the first step (hereafter 'macro-analysis'), content was spread in a Petri dish marked with 50 fixed points (following Mendes, Ferreira, & Clements, 2018) and identified using a stereomicroscope at 40X magnification. The second step (hereafter 'micro-analysis') was used to identify microscopic items ingested by *S. axillare* and *A. chirurgus*. To do so, the macro-analysed content was filtered through a 60 μm mesh, spread over a slide marked with 30 fixed points and identified under an optical microscope (magnification 400X). For both steps, dietary items above each of the marked points were identified to the lowest possible taxonomic level. Percentage contribution of each dietary item to the whole diet was calculated separately for macro- and micro-analysis.

Stable isotope analysis (SIA)

A Thermo Quest-Finnigan Delta Plus isotope ratio mass spectrometer (Finnigan-MAT) interfaced to an Elemental Analyzer (Carlo Erba) was used to determine the levels of stable isotopes of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$, respectively). Stable isotope ratios are expressed in delta notation (δ), defined as parts per thousand (‰) differences from a standard material following the formula: $\delta X‰ = [(R_{sample}/R_{standard}) - 1] \times 10^3$, where δ = the measure of heavy to light isotope in the sample, X = ^{13}C or ^{15}N and R = the corresponding ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$ – $\delta^{13}C$ and $\delta^{15}N$, respectively). International Standard references are Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric N^2 for nitrogen. Following Post *et al.* (2007), to avoid bias potentially caused by lipid content in fish muscles, individual $\delta^{13}C$ values were mathematically corrected when, after SIA analysis, considered lipid-rich (i.e. C:N > 3.5),.

The isotopic composition of each species of fish and algae was evaluated using Stable Isotope Bayesian Ellipses in R (SIBER), a multivariate ellipse-based model (Jackson *et al.* 2011). Isotopic niche was measured through the standard ellipse area corrected (SEAc; 95% C.I.), a measurement employed to avoid bias due to small sample sizes (Jackson et al., 2011). The Bayesian trophic position (TP) of each species in each site in relation to community baselines (algal resources) was obtained using the tRophicPosition package (Quezada-Romegialli et al., 2018). TP is calculated for the consumer at the population level using consumers and baseline $\delta^{15}N$ values. We used the 'one-baseline model' which performs a link between ^{15}N enrichment per trophic level and the trophic position of the baseline (i.e. algae in the present study). Linear regressions were calculated to evaluate isotopic variation among sampled sites. All analyses were conducted in the R environment (R Core Team, 2017).

Statistical analysis

Dietary and isotopic composition data (i.e. levels of $\delta^{13}C$, $\delta^{15}N$ and C:N ratio) were submitted to a permutational multivariate analysis of variance (PERMANOVA) using the PERMANOVA+ add-on for

Primer (Anderson, Gorley, & Clarke, 2008). PERMANOVAs were performed using Euclidean distance, Type III sums of squares, and 9,999 permutations of residuals under a reduced model to calculate the significance of Pseudo-F statistics. For each site, diet and isotopic composition of fishes and algae ($\delta^{13}C$ and $\delta^{15}N$ separately) were compared among species using a design with 'Species' as a fixed factor with three levels (i.e. *A. chirurgus*, *S. axillare* and *K. vaigiensis*), nested in the fixed factor 'Site' with four levels (i.e. NT, AB, GR and AC). Posterior pair-wise tests were performed among levels to verify differences among species.

Results

Sea surface temperature (SST)

We used monthly average SST retrieved from the Aqua MODIS database to calculate mean SST for each site. Mean SST increased from the southernmost to the northernmost site, being 22.9 ± 1.6 ºC (mean ± S.D.) for Arraial do Cabo (AC), 23.5 ± 1.1 ºC for Guarapari (GR), 26.2 ± 1.0 ºC for Abrolhos Archipelago (AB) and 27.8 ± 1.0 ºC for Natal (NT). SST was different between all the sites (Pseudo-F = 418.16, p = 0.0001).

Dietary analysis

Diet composition was different among species in all sites (Fig. 1B-C) for both macro- (Pseudo-F = 61.10, p = 0.0001) and micro-analyses (Pseudo-F = 13.91, p = 0.0001).

Acanthurus chirurgus showed the highest diversity of dietary items in all sites (Tab. S2). When analysed in a macro-scale, its diet relied mainly on calcareous red algae (crustose and articulated) and invertebrates. As also revealed by the macro-analysis, *S. axillare* had a diet dominated by 'detritus'. *Kyphosus vaigiensis* ate predominantly brown algae, mostly *Sargassum*, *Dictyota* and *Dictyopteris*. In addition, the micro-analysis performed in *A. chirurgus* and *S. axillare* revealed that the former ingests more frequently sponge spicules, diatoms and cyanobacteria, and the latter targets mainly green filamentous algae and cyanobacteria in inverse proportions. Overall, species kept similar targets across the sampled sites but varying the proportional amount of each item (Figs. S1-S4).

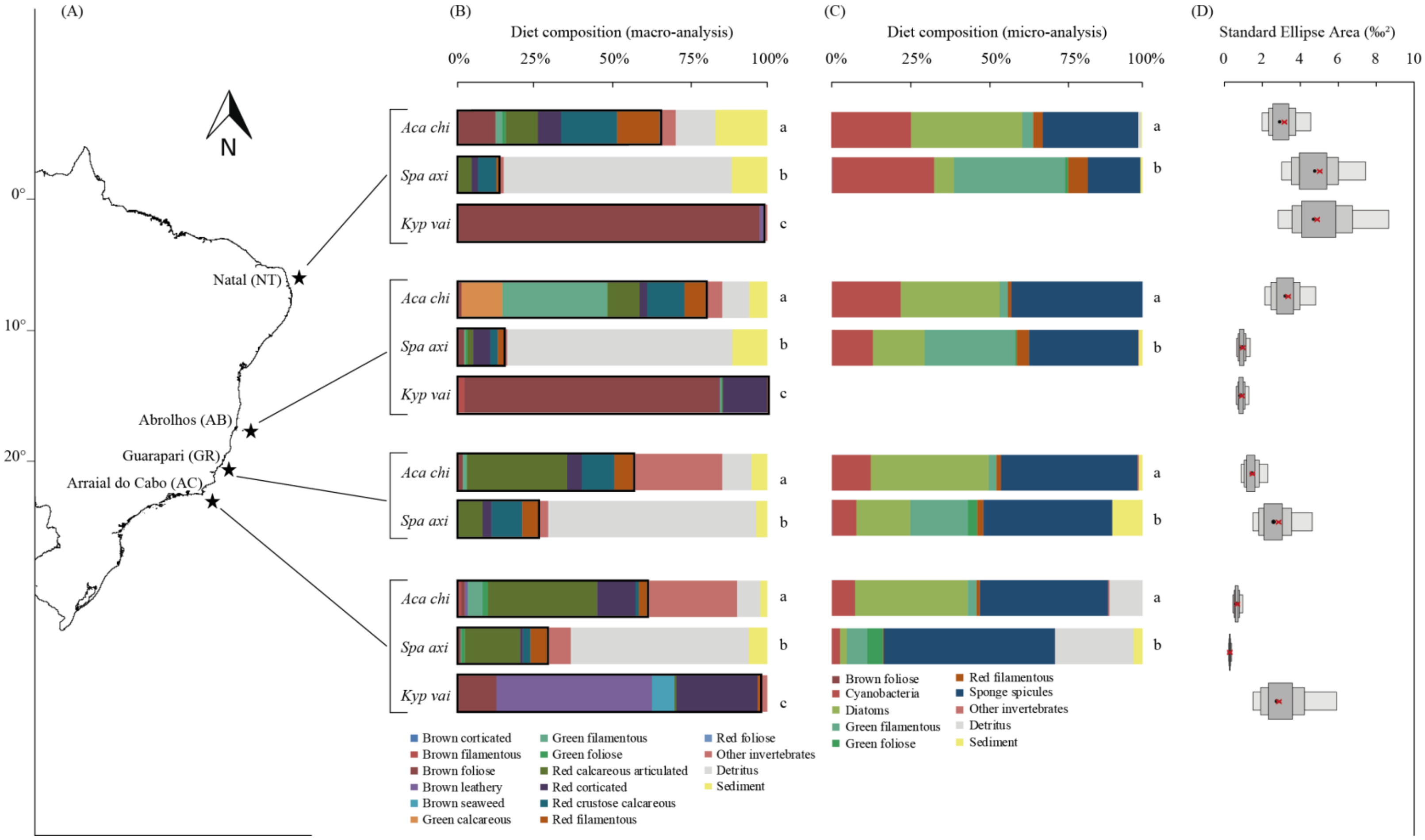

Figure 1: Sampled sites (A), diet composition for macro- (B) and micro-analyses (C; except for *K. vaigiensis*, see methods), and corrected Standard Ellipses Area (SEAc ‰²) for small-sample size (D) based on stable isotopic composition. Acronyms between (A) and (B) indicate study species: *Acanthurus chirurgus* (*Aca chi*), *Sparisoma axillare* (*Spa axi*), and *Kyphosus vaigiensis* (*Kyp vai*). Lowercase letters at the right sides of (B) and of (C) indicate homogenous groups of species within each site detected by PERMANOVA pair-wise tests (different letters indicate species belong to different groups). Black contour in (B) refers to algal items. In (D), black dots represent the sample mode, red 'x' is the true mean value for each population and shaded boxes represent the credible intervals of 50 %, 75 % and 95 %, from dark to light grey.

Isotopic variation

Different scenarios were observed in each site and for each species regarding levels of $\delta^{13}C$ and $\delta^{15}N$. Levels of $\delta^{13}C$ were only different between *S. axillare* and *K. vaigiensis* in NT (Pseudo-*t* = 3.818; *p* = 0.0001), being the latter's slightly $\delta^{13}C$-depleted (Tab. 1; Fig. 2). Still in NT, *Sparisoma axillare* showed the highest $\delta^{15}N$-enrichment, significantly different from those observed in *A. chirurgus* (Pseudo-*t* = 6.610; *p* = 0.01) and *K. vaigiensis* (Pseudo-*t* = 2.740; *p* = 0.01) (Tab. 1; Fig. 2). This lead *S. axillare* to occupy the higher trophic position (TP) among the species. Also, *S. axillare* presented the wider isotopic niche (SEAc, ‰²), followed by *K. vaigiensis* and *A. chirurgus* (Fig. 2).

In AB, levels of $\delta^{13}C$ in *S. axillare* differed from those in *A. chirurgus* (Pseudo-*t* = 5.076; *p* = 0.001) and *K. vaigiensis* (Pseudo-*t* = 5.219; *p* = 0.001); no difference was detected between the last two. The three species differed in $\delta^{15}N$ levels, being *A. chirurgus* the most enriched, with higher TP and wider isotopic niche (Tab. 1; Fig. 2).

In GR, only *A. chirurgus* and *S. axillare* were sampled, and differed in levels of $\delta^{13}C$ (Pseudo-*t* = 20.84; *p* = 0.001) and $\delta^{15}N$ (Pseudo-*t* = 2.080; *p* = 0.049). TP was not calculated in GR due the lack of baseline (i.e., algae) sampling. However, the lower $\delta^{13}C$ values and higher $\delta^{15}N$ (Tab. 1; Fig. 2) indicates that *A. chirurgus* may present higher TP than *S. axillare*.

Finally, in AC the three species differed in levels of $\delta^{13}C$. *Acanthurus chirurgus* presented higher levels of $\delta^{15}N$ than *S. axillare* (Pseudo-*t* = 18.18; *p* = 0.001) and *K. vaigiensis* (Pseudo-*t* = 6.018; *p* = 0.001). *Acanthurus chirurgus* presented higher TP. *Sparisoma axillare* presented the most restricted isotopic niche.

The isotopic niche of the three species presented some degree of overlap (Fig. 2). However, the clear dietary separation leads to conclude that explored algal sources may have similar isotopic signatures. For example, red and brown algae in NT have similar $\delta^{13}C$ signatures but brown algae are $\delta^{15}N$-depleted (Tab. 2). Accordingly, brown-algae-feeder *K. vaigiensis* signature exhibits lower levels of $\delta^{15}N$ when compared to *A. chirurgus* and *S. axillare*, evidencing the occupation of a different dietary niche.

Table 1: Levels of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), C:N ratio, trophic position (TP), and number of samples (n) for each species in each sampled site. Superscript lowercase letters on $\delta^{13}C$ and $\delta^{15}N$ indicate similarities among species within each site.

Site *Species*	**n**	**$\delta^{13}C$ (mean ± S.E.)**	**$\delta^{15}N$ (mean ± S.E.)**	**C:N (mean ± S.E.)**	**SEAc (‰²)**	**TP**
Natal						
Acanthurus chirurgus	23	$-17.12 \pm 0.41^{a,b}$	7.89 ± 0.79^{b}	3.36 ± 0.03^{a}	3.07	3.24
Sparisoma axillare	18	-16.88 ± 0.23^{a}	8.86 ± 3.55^{a}	3.21 ± 0.01^{b}	4.92	3.51
Kyphosus vaigiensis	13	-16.83 ± 0.28^{b}	8.09 ± 4.11^{b}	3.88 ± 0.11^{c}	4.81	3.37
Abrolhos Archipelago						
Acanthurus chirurgus	22	-13.64 ± 0.38^{a}	6.82 ± 1.01^{a}	3.29 ± 0.02^{a}	3.27	2.79
Sparisoma axillare	21	-13.74 ± 0.15^{b}	5.42 ± 1.01^{b}	3.29 ± 0.01^{a}	0.86	2.40
Kyphosus vaigiensis	23	-11.92 ± 0.17^{a}	5.71 ± 0.81^{c}	3.24 ± 0.01^{b}	0.85	2.48
Guarapari						
Acanthurus chirurgus	16	-19.19 ± 0.25^{a}	10.5 ± 1.32^{a}	3.58 ± 0.09^{a}	1.39	-
Sparisoma axillare	12	-18.68 ± 0.34^{b}	9.89 ± 1.45^{b}	3.37 ± 0.06^{a}	2.75	-
Arraial do Cabo						
Acanthurus chirurgus	20	-18.78 ± 0.12^{a}	12.5 ± 0.97^{a}	3.44 ± 0.03^{a}	0.59	3.77
Sparisoma axillare	21	-15.99 ± 0.06^{b}	10.7 ± 0.04^{b}	3.33 ± 0.01^{b}	0.18	3.28
Kyphosus vaigiensis	9	-16.81 ± 0.28^{c}	10.3 ± 3.35^{b}	3.41 ± 0.04^{a}	2.80	3.13

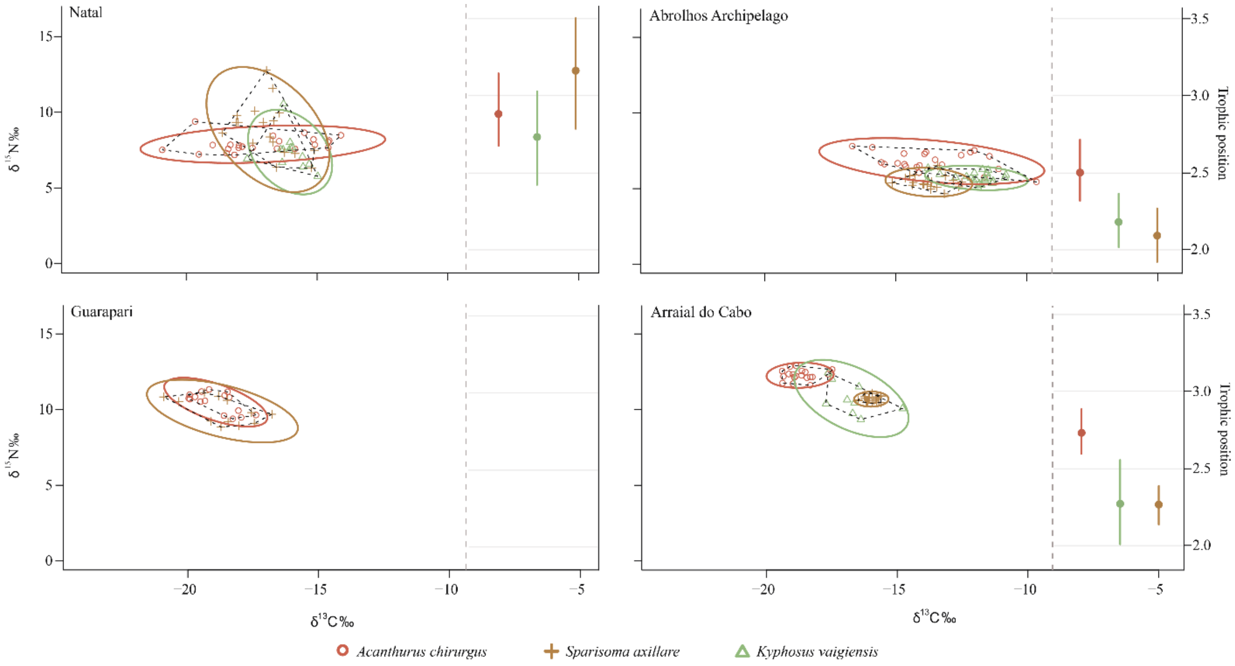

Figure 2: Stable isotope bi-plots ($\delta^{13}C$ and $\delta^{15}N$ bivariate space – X and left Y axes, respectively) illustrating the isotopic niche of each nominally herbivorous fish species in each site. Ellipses correspond to small-sample corrected standard ellipse area (SEAc – 95% C.I.; solid lines) and convex-hull of total niche area around extreme sample values (dashed lines). Red circles for (A) *Acanthurus chirurgus*, (B) brown crosses for *Sparisoma axillare* and (C) green triangles for *Kyphosus vaigiensis*. When estimated (see methods), trophic positions (TP) are shown on the right Y axes as mean (± 95% C.I.)

Algae mostly differed within sites regarding $\delta^{13}C$ (lowercase letters Tab. 2). However, some differences were also observed on levels of $\delta^{15}N$ (Tab. 2; Fig.3). In NT, the red foliose algae *Ochtodes secundiramea* was the most $\delta^{15}N$-enriched algae species and *Dictyopteris jamaicensis* the most depleted. In AB, the most $\delta^{15}N$-enriched algae was brown foliose algae *Dictyopteris delicatula* while the most depleted was the turf algae. AC was the only site where algae sources did not differ in $\delta^{15}N$ levels, but all sampled species were $\delta^{15}N$-richer than those from other sites. Brown foliose algae presented the same levels of $\delta^{13}C$, as well as the red calcareous articulated *Amphiroa beauvoisii* and the turf algae (Fig. 3).

Table 2: Isotopic composition ($\delta^{13}C$; mean ± S.E. and $\delta^{15}N$; mean ± S.E.), functional group and number of samples (n) for algae species and turf sampled in each site. *adapted from Steneck & Dethier (1994). Superscript lowercase letters on $\delta^{13}C$ and $\delta^{15}N$ denote homogenous groups within each site detected by PERMANOVA pair-wise tests (different letters indicate species belong to different groups).

	Functional group*	**n**	**$\delta^{13}C$ (mean ± S.E.)**	**$\delta^{15}N$ (mean ± S.E.)**
Natal				
Dictyopteris jamaicensis	Brown foliose	6	-19.35 ± 0.26 ^{a}	0.63 ± 0.14 ^{a}
Dictyopteris jolyana	Brown foliose	6	-17.41 ± 0.25 ^{b}	0.84 ± 0.15 ^{b}
Dictyota mertensii	Brown foliose	6	-19.63 ± 0.14 ^{a,c}	1.11 ± 0.19 ^{a}
Ochtodes secundiramea	Red foliose	5	-19.79 ± 0.33 ^{a,c}	3.75 ± 0.17 ^{c}
Abrolhos Archipelago				
Dictyopteris delicatula	Brown foliose	6	-14.8 ± 0.48 ^{b}	4.04 ± 0.06 ^{a}
Dictyota sp.	Brown foliose	4	-12.9 ± 0.26 ^{a}	1.32 ± 0.45 ^{b}
Sargassum sp.	Brown leathery	6	-20.23 ± 0.62 ^{c}	1.93 ± 0.25 ^{b,d,f}
Spatoglossum sp.	Brown foliose	6	-17.08 ± 0.37 ^{d}	1.02 ± 0.27 ^{b,e,f,g}
Halimeda tuna	Green calcareous	4	-10.66 ± 0.93 ^{a}	1.83 ± 0.56 ^{b,d,e}
Tricleocarpa cylindrica	Red calcareous	6	-9.38 ± 0.32 ^{a}	0.85 ± 0.14 ^{b,e,g}
Turf	Turf	4	-5.76 ± 0.43 ^{e}	0.13 ± 0.03 ^{c}
Arraial do Cabo				
Dictyota sp.	Brown foliose	6	-17.19 ± 0.4 ^{b}	6.25 ± 0.12 ^{a}
Dictyota mertensii	Brown foliose	6	-16.31 ± 0.38 ^{b}	6.14 ± 0.17 ^{a}
Sargassum sp.	Brown leathery	6	-15.17 ± 0.5 ^{b}	6.49 ± 0.09 ^{a}
Amphiroa beauvoisii	Red calcareous	6	-9.3 ± 0.11 ^{a}	6.45 ± 0.14 ^{a}
Plocamium brasiliense	Red foliose	3	-29.12 ± 0.53 ^{c}	6.73 ± 0.09 ^{a}
Turf	Turf	6	-11.56 ± 1.24 ^{a}	6.33 ± 0.21 ^{a}

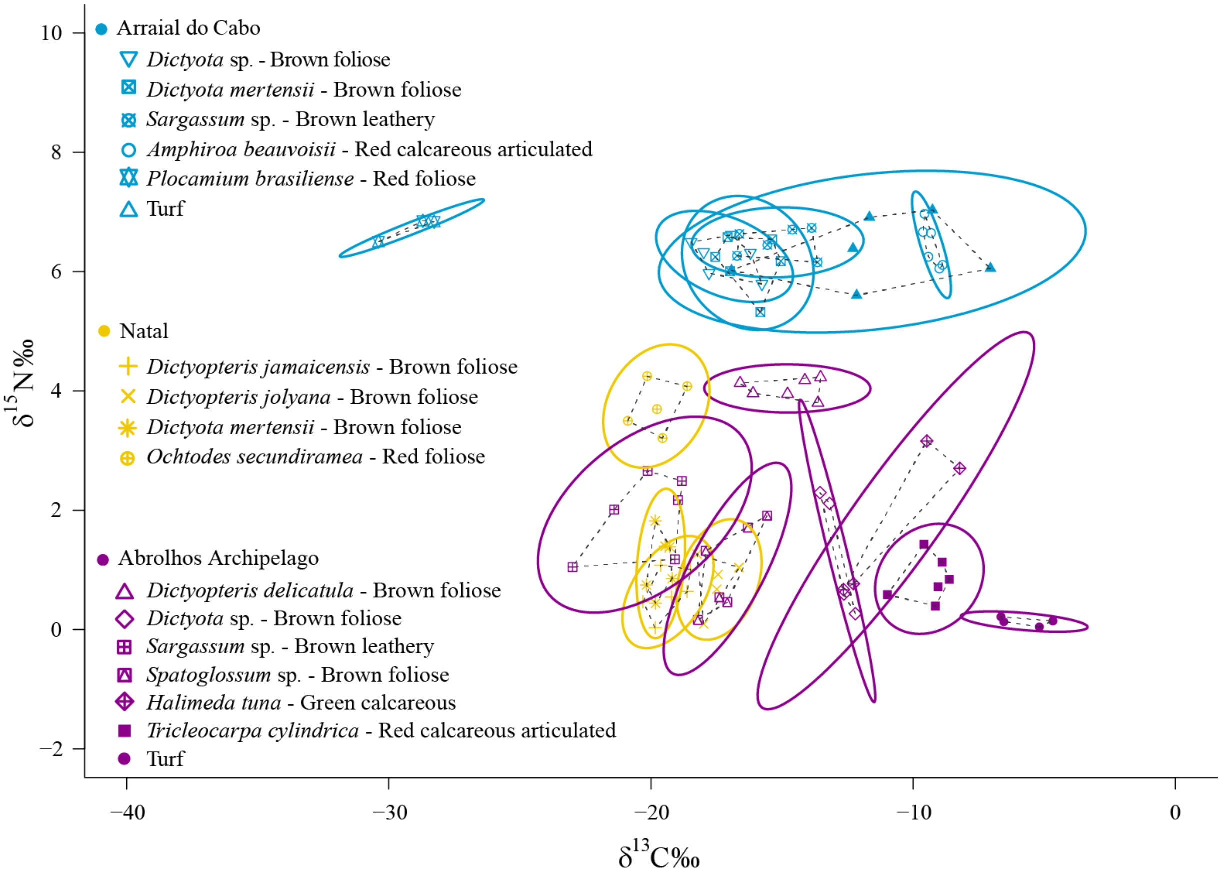

Figure 2: Stable isotope bi-plots (δ^{13}C and δ^{15}N bivariate space) illustrating the isotopic composition of algal source in each site with corrected standard ellipse area (SEAc – 95% C.I.; solid lines) and the convex-hull of total niche area around extreme sample values (dashed lines).

Discussion

The assignment of herbivorous fishes into similar functional groups might be useful to infer ecosystem function but it could also underestimate the particular role of each species. Nominally herbivorous fishes in our study exhibited dietary differences, maintaining specific feeding preference and revealing a clear resource partitioning in each site. Alongside, their levels of δ^{13}C and δ^{15}N also varied relatively to each other, with *S. axillare* the most δ^{15}N-enriched in Natal and *A. chirurgus* at all other sites. The latter was the more δ^{13}C-depleted species at all sites but AB, where *S. axillare* presented the lowest values. Moreover, the nominally herbivorous fishes have shown a tendency to trade-off nutritional requirements and food resource availability rather than have an immutable ecological role over distinct sites. Our work suggests that large-grain view of the nutritional ecology of herbivorous fishes may underestimate their functional role in the reef systems.

Do herbivorous species explore different resources and thus present different nutrient levels at different sites?

Contrary to the other two species, *A. chirurgus* does not show strong specificity for a particular type of food item and lives upon the most variable diet. This feeding diversity demonstrate the species' large capacity for diversifying its diet according to the situation (e.g., variation in algae availability). Such dietary plasticity could lead to an overlap with the two other species, especially *S. axillare* once both feed over the same substrata (Francini-Filho, Ferreira, Coni, Moura, & Kaufman, 2010). However, *S. axillare* and *K. vaigiensis* display a strong preference for specific items (detritus and brown algae, respectively) that are ingested in lesser amounts by *A. chirurgus* at all sampled sites (Figs. 1 and S1-S4).

While resource partitioning among species has a clear diet-basis, micro-analysis revealed a similar composition for *S. axillare* and *A. chirurgus* diets mainly differing in item proportions. Sponge spicules (including endolithic ones) and cyanobacteria were constantly present in both species gut content. Cryptic and encrusting sponges make dissolved organic matter (DOM) available to higher trophic levels by expelling filter cells as detritus, that is consumed by reef fauna, a process known as the 'sponge loop' (de Goeij et al., 2013). Whether *S. axillare* is targeting sponges or ingesting them while targeting highly nutritious sources require further investigations, although cyanobacteria may comprise large proportions of sponge's cellular volume (Erwin & Thacker, 2008). Even so, scarid species harbour a series of adaptations that allows the exploitation of microscopic autotrophs, either embedded in the detritus or selected in the epilithic and endolithic matrix (Clements & Choat, 2018; Clements et al., 2017). The importance of diatoms for *A. chirurgus*, that represent more than 30% of its diet (micro-analysis; Fig. S3) in all locations, is also notable. It reinforces that both species are likely to represent distinct functional groups (Mendes et al., 2018), especially through a micro-scale view.

Kyphosus vaigiensis differs from *A. chirurgus* and *S. axillare* in morphology and physiology and exhibits a preference for non-palatable brown algae such as *Dictyota* spp., that are known for their high lipid contents (Mcdermid, Stuercke, & Balazs, 2007). Kyphosids are able to break down these algae molecules and maximize nutrient absorption thanks to endosymbionts bacteria and a fermentative chamber in the foregut (Choat et al., 2002; Clements & Choat, 1995, 1997; Mountfort, Campbell, & Clements, 2002). In contrast, parrotfishes' digestive system is refractory to the brown algae sought after by *K. vaigiensis*, and so no absorption results from an occasional ingestion by *S. axillare* while targeting epiphytes (Clements et al., 2017). Additionally, the three species presented a consistent pattern of algae ingestion across the different locations. *Kyphosus vaigiensis* have ingested higher amounts of total algae material in each site, followed by *A. chirurgus* and *S. axillare*. These results do not support previous studies that, based on the temperature constraint hypothesis – TCH (Gaines & Lubchenco, 1982), have suggested that herbivorous fishes would reduce algal intake as moving to higher latitudes (Behrens & Lafferty, 2012; Floeter et al., 2005; Longo et al., 2018).

By exploring different food sources and presenting different isotopic signatures, each species should be individually analyzed, in a closest as possible scale, prior to assigning them into functional groups or to stating wide generalizations around their ecology. It would avoid overestimate functional redundancy within species, once even a single species may display distinct roles in the ecosystem (Cardozo-Ferreira, Macieira, Francini-Filho, & Joyeux, 2018).

Are the interspecific trophic relationships maintained at different sites?

Previous studies run at local scales indicated that acanthurids typically present higher $\delta^{15}N$ levels than parrotfishes (Carassou, Kulbicki, Nicola, & Polunin, 2008; Dromard, Bouchon-Navaro, Harmelin-Vivien, & Bouchon, 2015). However, widely distributed species may present food habits and nutrient assimilation varying according to each-site specific characteristics. For example, the herbivore dusky damselfish *Stegastes fuscus,* known for consuming the total algal production of their territories (Ferreira, Gonçalves, Coutinho, & Peret, 1998), may act as omnivores while in intertidal environments (Pimentel, Soares, Macieira, & Joyeux, 2018). High $\delta^{15}N$ levels, low C:N ratio and high trophic levels are commonly observed in species feeding over more nutrient-rich food sources. In an herbivorous fish perspective, this would mean a shift toward more nutritious sources, e.g. from algae to detritus and microscopic autotrophs (Crossman et al., 2005; Wilson, Bellwood, Choat, & Furnas, 2003).

No pattern was observed in the size of the isotopic niche of species in each site (see SEAc, Fig. 1D). Niche broadening can be a response to the low availability of palatable algae (Thacker, Nagle, & Paul, 1997) due to the exploitation of different food sources (Jackson et al., 2011). Unpalatable brown macroalgae are more abundant in NT than in the other sites (pers. obs.; Aued et al., 2018). The broader isotopic niche observed for *S. axillare* in NT and more restrict in AC is likely to be due to the reported heterogeneity of the detritus (Crossman, Choat, Clements, Hardy, & McConochie, 2001; Moore et al., 2004), the dominant food category in *S. axillare* all sites and with higher contribution as moving north. Therefore, we may infer that the ingestion of different detritus concomitant with the balanced ingestion of cyanobacteria and green filamentous algae has influenced the *S. axillare* isotopic niche breadth. Yet, the real composition of detritus and turf, where detritivores acanthurid and parrotfish feed in the Atlantic Ocean, require further investigation.

The grain size issue

Herbivorous fishes have long been recognized for their role on algae removal and subsequent coral reef resilience (Hughes, Bellwood, Folke, McCook, & Pandolfi, 2007; Mumby, 2006). But if we have a closer look, are they only removing algal matter?

Indeed, to achieve optimal nourishment some nominally herbivorous fishes may select difficult-to-detect nutrient-rich food (Clements et al., 2017; Mendes et al., 2018). Had we compared the fish species independently of site (Fig. S5), or the sites through the functional guild bin (Fig. S6), we would in both cases have inferred that species display similar roles and develop the same ecosystemic function, in complete opposition to the exquisite details provided by the fine-grained study (e.g., Fig. 2). The core point is: can we ingenuously infer about herbivorous fish ecological role solely based on a large-scale view?

Our work demonstrate that the intraspecific feeding ecology of nominally herbivorous fishes is more complex than previously thought and reinforces that species function in the ecosystem should not be underestimated or misinterpreted by grouping species in single units or bins without prior site/species specific analysis. Finally, our study expands the comprehension on how nominally herbivorous reef fishes partition the available resources at a nutritional small-grain scale. Furthermore, we suggest that site- and species-specific analysis must be conducted when providing information on species ecology in order to improve decision-making attitudes and conservation programs of wide-distributed herbivorous species.

References

Abrantes, K. G., Barnett, A., & Bouillon, S. (2014). Stable isotope-based community metrics as a tool to identify patterns in food web structure in east African estuaries. *Functional Ecology*, *28*(1), 270–282. doi:10.1111/1365-2435.12155

Anderson, M. J., Gorley, R. N., & Clarke, K. R. (2008). PERMANOVA + for PRIMER: Guide to software and statistical methods. Plymouth, UK: PRIMER-E.

Aued, A. W., Smith, F., Quimbayo, J. P., Cândido, D. V., Longo, G. O., Ferreira, C. E. L., … Segal, B. (2018). Large-scale patterns of benthic marine communities in the Brazilian Province. *PLOS ONE*, *13*(6), e0198452. doi:10.1371/journal.pone.0198452

Behrens, M. D., & Lafferty, K. D. (2012). Geographic variation in the diet of opaleye (*Girella nigricans*) with respect to temperature and habitat. *PLoS ONE*, *7*(9), e45901. doi:10.1371/journal.pone.0045901

Bennett, S., & Bellwood, D. R. (2011). Latitudinal variation in macroalgal consumption by fishes on the Great Barrier Reef. *Marine Ecology Progress Series*, *426*, 241–252. doi:10.3354/meps09016

Bruce, T., Meirelles, P. M., Garcia, G., Paranhos, R., Rezende, C. E., Moura, R. L., … Thompson, F. L. (2012). Abrolhos bank reef health evaluated by means of water quality, microbial diversity, benthic cover, and fish biomass data. *PloS One*, *7*(6), e36687. doi:10.1371/journal.pone.0036687

Carassou, L., Kulbicki, M., Nicola, T. J. R., & Polunin, N. V. C. (2008). Assessment of fish trophic status and relationships by stable isotope data in the coral reef lagoon of New Caledonia, southwest Pacific. *Aquatic Living Resources*, *21*(1), 1–12. doi:10.1051/alr:2008017

Cardozo-Ferreira, G. C., Macieira, R., Francini-Filho, R. B., & Joyeux, J.-C. (2018). Inferring labrid functional roles through morphological and ecological traits. *Marine Ecology Progress Series*, *588*, 135–145. doi:10.3354/meps12422

Choat, J. H., Clements, K. D., & Robbins, W. D. (2002). The trophic status of herbivorous fishes on coral reefs I: Dietary analyses. *Marine Biology*, *140*(3), 613–623. doi:10.1007/s00227-001-0715-3

Choat, J. H., Robbins, W., & Clements, K. D. (2004). The trophic status of herbivorous fishes on coral reefs. II. Food processing modes and trophodynamics. *Marine Biology*, *145*(3), 445–454. doi:10.1007/s00227-004-1341-7

Clements, K. D., & Choat, J. H. (1995). Fermentation in tropical marine herbivorous fishes. *Physiological Zoology*, *68*(3), 355–378. doi:10.2307/30163774

Clements, K. D., & Choat, J. H. (1997). Comparison of herbivory in the closely-related marine fish genera *Girella* and *Kyphosus*. *Marine Biology*, *127*(4), 579–586. doi:10.1007/s002270050048

Clements, K. D., & Choat, J. H. (2018). Nutritional ecology of parrotfishes. In A. S. Hoey & R. M. Bonaldo (Eds.), *Biology of Parrotfishes* (p. 420). New York: CRC Press - Taylor & Francis Group.

Clements, K. D., German, D. P., Piché, J., Tribollet, A., & Choat, J. H. (2017). Integrating ecological roles and trophic diversification on coral reefs: multiple lines of evidence identify parrotfishes as microphages. *Biological Journal of the Linnean Society*, *120*, 729–751. doi:10.1111/bij.12914

Clements, K. D., Raubenheimer, D., & Choat, J. H. (2009). Nutritional ecology of marine herbivorous fishes: Ten years on. *Functional Ecology*, *23*(1), 79–92. doi:10.1111/j.1365-2435.2008.01524.x

Cordeiro, C. A. M. M., Harborne, A. R., & Ferreira, C. E. L. (2014). Patterns of distribution and composition of sea urchin assemblages on Brazilian subtropical rocky reefs. *Marine Biology*, *161*(10), 2221–2232. doi:10.1007/s00227-014-2500-0

Cordeiro, C. A. M. M., Mendes, T. C., Harborne, A. R., & Ferreira, C. E. L. (2016). Spatial distribution of nominally herbivorous fishes across environmental gradients on Brazilian rocky reefs. *Journal of Fish Biology*, *89*(1), 939–958. doi:10.1111/jfb.12849

Crossman, D. J., Choat, J. H., & Clements, K. D. (2005). Nutritional ecology of nominally herbivorous fishes on coral reefs. *Marine Ecology Progress Series*, *296*, 129–142. doi:10.3354/meps296129

Crossman, D. J., Choat, J. H., Clements, K. D., Hardy, T., & McConochie, J. (2001). Detritus as food for grazing fishes on coral reefs. *Limnology and Oceanography*, *46*(7), 1596–1605. doi:10.4319/lo.2001.46.7.1596

de Goeij, J. M., van Oevelen, D., Vermeij, M. J. A., Osinga, R., Middelburg, J. J., de Goeij, A. F. P. M., & Admiraal, W. (2013). Surviving in a Marine Desert: The Sponge Loop Retains Resources Within Coral Reefs. *Science*, *342*(6154), 108–110. doi:10.1126/science.1241981

Dromard, C. R., Bouchon-Navaro, Y., Harmelin-Vivien, M., & Bouchon, C. (2015). Diversity of trophic niches among herbivorous fishes on a Caribbean reef (Guadeloupe, Lesser Antilles), evidenced by stable isotope and gut content analyses. *Journal of Sea Research*, *95*, 124–131. doi:10.1016/j.seares.2014.07.014

Erwin, P. M., & Thacker, R. W. (2008). Phototrophic nutrition and symbiont diversity of two Caribbean sponge-cyanobacteria symbioses. *Marine Ecology Progress Series*, *362*, 139–147. doi:10.3354/meps07464

Ferreira, C. E. L., Floeter, S. R., Gasparini, J. L., Ferreira, B. P., & Joyeux, J.-C. (2004). Trophic structure patterns of Brazilian reef fishes: a latitudinal comparison. *Journal of Biogeography*, *31*(7), 1093–1106. doi:10.1111/j.1365-2699.2004.01044.x

Ferreira, C. E. L., & Gonçalves, J. E. A. (2006). Community structure and diet of roving herbivorous reef fishes in the Abrolhos Archipelago, south-western Atlantic. *Journal of Fish Biology*, *69*(5), 1533–1551. doi:10.1111/j.1095-8649.2006.01220.x

Ferreira, C. E. L., Gonçalves, J. E. A., Coutinho, R., & Peret, A. C. (1998). Herbivory by the Dusky Damselfish *Stegastes fuscus* (Cuvier, 1830) in a tropical rocky shore: effects on the benthic community. *Journal of Experimental Marine Biology and Ecology*, *229*(2), 241–264. doi:10.1016/S0022-0981(98)00056-2

Floeter, S. R., Behrens, M. D., Ferreira, C. E. L., Paddack, M. J., & Horn, M. H. (2005). Geographical gradients of marine herbivorous fishes: Patterns and processes. *Marine Biology*, *147*(6), 1435–1447. doi:10.1007/s00227-005-0027-0

Floeter, S. R., Guimarães, R. Z. P., Rocha, L. A., Ferreira, C. E. L., Rangel, C. A., & Gasparini, J. L. (2001). Geographic variation in reef-fish assemblages along the Brazilian coast. *Global Ecology and Biogeography*, *10*, 423–431. doi:10.1046/j.1466-822X.2001.00245.x

Francini-Filho, R. B., Ferreira, C. M., Coni, E. O. C., Moura, R. L., & Kaufman, L. (2010). Foraging activity of roving herbivorous reef fish (Acanthuridae and Scaridae) in eastern Brazil: influence of resource availability and interference competition. *Journal of the Marine Biological Association of the United Kingdom*, *90*(03), 481. doi:10.1017/S0025315409991147

Gaines, S. D., & Lubchenco, J. (1982). A unified approach to marine plant-herbivore interactions. II. Biogeography. *Annual Review of Ecology and Systematics*, *13*(1), 111–138. doi:10.1146/annurev.es.13.110182.000551

Horn, M. H. (1989). Biology of marine herbivorous fishes. *Oceanography and Marine Biology*, *27*, 167–272.

Hughes, T. P., Bellwood, D. R., Folke, C. S., McCook, L. J., & Pandolfi, J. M. (2007). No-take areas, herbivory and coral reef resilience. *Trends in Ecology & Evolution*, *22*(1), 1–3. doi:10.1016/j.tree.2006.10.009

Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, *80*, 595–602. doi:10.1111/j.1365-2656.2011.01806.x

Johnson, J. S., Clements, K. D., & Raubenheimer, D. (2017). The nutritional basis of seasonal selective feeding by a marine herbivorous fish. *Marine Biology*, *164*(10), 201. doi:10.1007/s00227-017-3223-9

Joyeux, J.-C., Floeter, S. R., Ferreira, C. E. L., & Gasparini, J. L. (2001). Biogeography of tropical reef fishes: the South Atlantic puzzle. *Journal of Biogeography*, *28*, 831–841. doi:10.1046/j.1365-2699.2001.00602.x

Kikuchi, R. K. P., Leão, Z. M. A. N., Sampaio, C. L. S., & Telles, M. D. (2003). Rapid assessment of the Abrolhos reefs, eastern Brazil (Part 2: Fish Communities). *Atoll Research Bulletin*, *496*, 188–203. doi:10.5479/si.00775630.496-10.188

Leão, Z. M. A. N., & Dominguez, J. M. L. (2000). Tropical coast of Brazil. *Marine Pollution Bulletin*, *41*(1–6), 112–122. doi:10.1016/S0025-326X(00)00105-3

Leão, Z. M. A. N., Kikuchi, R. K. P., & Testa, V. (2003). Corals and coral reefs of Brazil. In J. Cortés (Ed.), *Latin American Coral Reefs* (pp. 9–52). Elsevier. doi:10.1016/B978-044451388-5/50003-5

Longo, G. O., Hay, M. E., Ferreira, C. E. L., & Floeter, S. R. (2018). Trophic interactions across 61 degrees of latitude in the Western Atlantic. *Global Ecology and Biogeography*, (March), 1–11. doi:10.1111/geb.12806

Mcdermid, K. J., Stuercke, B., & Balazs, G. (2007). Nutritional composition of marine plants in the diet of the green sea turtle (*Chelonia mydas*) in the Hawaiian Islands. *Bulletin of Marine Science*, *81*(1), 55–71.

Mendes, T. C., Ferreira, C. E. L., & Clements, K. D. (2018). Discordance between diet analysis and dietary macronutrient content in four nominally herbivorous fishes from the Southwestern Atlantic. *Marine Biology*, *165*(11), 1–12. doi:10.1007/s00227-018-3438-4

Moore, J. C., Berlow, E. L., Coleman, D. C., De Suiter, P. C., Dong, Q., Hastings, A., … Wall, D. H. (2004). Detritus, trophic dynamics and biodiversity. *Ecology Letters*, *7*(7), 584–600. doi:10.1111/j.1461-0248.2004.00606.x

Mountfort, D. O., Campbell, J., & Clements, K. D. (2002). Hindgut fermentation in three species of marine herbivorous fish. *Applied and Environmental Microbiology*, *68*(3), 1374–1380. doi:10.1128/AEM.68.3.1374-1380.2002

Moura, R. L., & Francini-Filho, R. B. (2005). Reef and shore fishes of the Abrolhos Region, Brazil. In F. G. Dutra, G. R. Allen, T. Werner, & S. A. McKenna (Eds.), *Rapid Marine Biodiversity Assessment of the Abrolhos Bank, Bahia, Brazil.* (pp. 40–55). Washington, DC: RAP Bulletin of Biological Assessment, Conservation International.

Mumby, P. J. (2006). The impact of exploiting grazers (Scaridae) on the dynamics of Caribbean coral reefs. *Ecological Applications*, *16*(2), 747–769. doi:https://doi.org/10.1890/1051-0761(2006)016[0747:TIOEGS]2.0.CO;2

Parnell, A. C., Inger, R., Bearhop, S., & Jackson, A. L. (2010). Source partitioning using stable isotopes: Coping with too much variation. *PLoS ONE*, *5*(3), 1–5. doi:10.1371/journal.pone.0009672

Pimentel, C. R., Soares, L. S. H., Macieira, R. M., & Joyeux, J.-C. (2018). Trophic relationships in tidepool fish assemblages of the tropical Southwestern Atlantic. *Marine Ecology*, (March), e12496. doi:10.1111/maec.12496

Pinheiro, H. T., Rocha, L. A., Macieira, R. M., Carvalho-Filho, A., Anderson, A. B., Bender, M. G., … Floeter, S. R. (2018). South-western Atlantic reef fishes: Zoogeographical patterns and ecological drivers reveal a secondary biodiversity centre in the Atlantic Ocean. *Diversity and Distributions*, 1–20. doi:10.1111/ddi.12729

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., & Montaña, C. G. (2007). Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, *152*, 179–189. doi:10.1007/s00442-006-0630-x

Quezada-Romegialli, C., Jackson, A. L., Hayden, B., Kahilainen, K. K., Lopes, C., & Harrod, C. (2018). tRophicPosition, an R package for the Bayesian estimation of trophic position from consumer stable isotope ratios. *Methods in Ecology and Evolution*, *9*, 1592–1599. doi:10.1111/2041-210X.13009

R Core Team. (2017). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*. Viena, Austria. Retrieved from https://www.r-project.org/

Simon, T., Joyeux, J.-C., & Pinheiro, H. T. (2013). Fish assemblages on shipwrecks and natural rocky reefs strongly differ in trophic structure. *Marine Environmental Research*, *90*, 55–65. doi:10.1016/j.marenvres.2013.05.012

Simon, T., Pinheiro, H. T., & Joyeux, J.-C. (2011). Target fishes on artificial reefs: evidences of impacts over nearby natural environments. *Science of the Total Environment*, *409*(21), 4579–84. doi:10.1016/j.scitotenv.2011.07.057

Steneck, R. S., & Dethier, M. N. (1994). A functional group approach to the structure of algal-dominated communities. *Oikos*, *69*(3), 476–498. doi:10.2307/3545860

Thacker, R., Nagle, D., & Paul, V. (1997). Effects of repeated exposures to marine cyanobacterial secondary metabolites on feeding by juvenile rabbitfish and parrotfish. *Marine Ecology Progress Series*, *147*, 21–29. doi:10.3354/meps147021

Trip, E. D. L., Clements, K. D., Raubenheimer, D., & Choat, J. H. (2014). Temperature-related variation in growth rate, size, maturation and life span in a marine herbivorous fish over a latitudinal gradient. *Journal of Animal Ecology*, *83*(4), 866–875. doi:10.1111/1365-2656.12183

Trip, E. D. L., Clements, K. D., Raubenheimer, D., & Howard Choat, J. (2016). Temperate marine herbivorous fishes will likely do worse, not better, as waters warm up. *Marine Biology*, *163*(2), 24. doi:10.1007/s00227-015-2790-x

Valentin, J. L. (2001). The Cabo Frio upwelling system, Brazil. In U. Seeliger & B. Kjerfve (Eds.), *Coastal Marine Ecosystems of Latin America* (Vol. 144, pp. 97–105). Springer-Verlag. doi:10.1007/978-3-662-04482-7_8

Wilson, S. K., & Bellwood, D. R. (1997). Cryptic dietary components of territorial damselfishes (Pomacentridae, Labroidei). *Marine Ecology Progress Series*, *153*(1–3), 299–310. doi:10.3354/meps153299

Wilson, S. K., Bellwood, D. R., Choat, J. H., & Furnas, M. J. (2003). Detritus in the epilithic algal matrix and its use by coral reef fishes. *Oceanography and Marine Biology: An Annual Review*, *41*, 279–309.

Supplementary material

Table S1: Collected number of specimens for each species, with ranges of length (mean total length, minimum - maximum) and weight (mean, minimum - maximum).

Family **Species**	**Site**	**N**	**Total length (mm)** **Average (min – max)**	**Weight (g)** **Average (min – max)**
Acanthuridae				
Acanthurus chirurgus	Natal	23	211 (169 - 239)	226 (125 - 325)
	Abrolhos Archipelago	22	241 (183 - 284)	328 (140 - 595)
	Guarapari	16	244 (207 - 292)	374 (207 - 690)
	Arraial do Cabo	20	307 (261 - 341)	752 (424 - 945)
Labridae				
Sparisoma axillare	Natal	18	281 (195 - 447)	420 (152 - 950)
	Abrolhos Archipelago	21	268 (225 - 327)	369 (220 - 580)
	Guarapari	12	245 (178 - 305)	286 (101 - 455)
	Arraial do Cabo	21	319 (237 - 430)	638 (258 - 1355)
Kyphosidae				
Kyphosus vaigiensis	Natal	13	409 (342 - 538)	1498 (685 - 3365)
	Abrolhos Archipelago	23	351 (300 - 390)	888 (560 - 1160)
	Arraial do Cabo	9	312 (252 - 359)	597 (312 - 902)

Table S2: Dietary items found in gut content of the three species of nominally herbivorous fishes: *Acanthurus chirurgus* (n = 51), *Sparisoma axillare* (n = 28) and *Kyphosus vaigiensis* (n = 27). Brown, green and red refer to algae classes Ochrophyta, Chlorophyta and Rhodophyta, respectively.

Dietary items	**Species**		
	Acanthurus chirurgus	***Kyphosus vaigiensis***	***Sparisoma axillare***
Brown corticated			
Colpomenia sp.	X	–	–
Undetermined	X	–	–
Brown filamentous			
Ectocarpales	X	–	–
Hincksia sp.	–	X	–
Undetermined	X	–	X
Brown foliose			
Dictyopteris sp.	X	X	–
Dictyopteris deliculata	–	X	–
Dictyopteris jamaicensis	–	X	–

Dietary items	**Species**		
	Acanthurus chirurgus	***Kyphosus vaigiensis***	***Sparisoma axillare***
Dictyopteris justii	−	X	−
Dictyopteris lucida	−	X	−
Dictyota sp.	X	X	X
Dictyota menstrualis	−	X	−
Dictyota mertensii	−	X	−
Brown leathery			
Padina sp.	−	X	−
Sargassum sp.	X	X	−
Stypopodium zonale	−	X	−
Brown seaweed			
Fucoid	−	X	−
Green calcareous			
Halimeda tuna	X	−	−
Green filamentous			
Bryopsis sp.	X	−	X
Chaetomorpha	X	−	−
Cladophora sp.	X	X	−
Rhizoclodium sp.	X	−	
Undetermined	X	−	X
Green foliose			
Enteromorpha flexuosa	X	X	−
Enteromorpha sp.	X	X	X
Undetermined	X	−	X
Red calcareous articulated			
Amphiroa beauvoisii	X	X	X
Corallina sp.	X	−	X
Jania capillacea	X	−	X
Undetermined	−	−	X
Red crustose calcareous			
Calcareous detritus	X	−	X
Red corticated			
Gelidiella acerosa	X	X	X
Gelidiella sp.	X	−	X
Gelidiopsis sp.	X	−	−
Gelidium pusillum	X	X	X

Dietary items	**Species**		
	Acanthurus chirurgus	***Kyphosus vaigiensis***	***Sparisoma axillare***
Hypnea spinella	X	X	X
Plocamium brasiliense	X	−	−
Polysiphonia sp.	−	−	X
Undetermined	X	X	X
Red filamentous			
Acrothamnion sp.	X	−	−
Ceramium diaphanum	X	−	X
Herposiphonia sp.	X	X	X
Heterosiphonia sp.	X	−	−
Hypnea spinella	−	−	X
Ophidocladus simpliciusculus	X	−	−
Polysiphonia sp.	X	−	X
Stylonema sp.	X	−	−
Undetermined	−	−	X
Red foliose			
Undetermined	X	−	−
Invertebrates			
Ascidiacea	X	−	−
Didemnum sp.	X	−	−
Bryozoa	X	−	X
Echinodermata	X	−	X
Foraminifera	X	−	X
Hydrozoa	X	X	−
Nematoda	−	X	X
Polychaeta	X	X	−
Sponge spicules	X	−	X
Arthropoda	X	−	−
Crustacea	X	−	−
Amphipoda	X	X	−
Brachyura	X	−	−
Cirripedia	X	−	−
Mollusca	X	−	X
Bivalvia	X	−	−
Piece of shell	X	X	X

Table S3: Diet contribution (% diet) of dietary items found in gut contents of the three study nominally herbivorous fishes in the sampled four sites. Acronyms: *Aca chi – Acanthurus chirurgus*, *Spa axi – Sparisoma axillare* and *Kyp vai – Kyphosus vaigiensis*.

Sites	**Natal**			**Abrolhos Archipelago**			**Guarapari**		**Arraial do Cabo**		
Species	*Aca chi*	*Spa axi*	*Kyp vai*	*Aca chi*	*Spa axi*	*Kyp vai*	*Aca chi*	*Spa axi*	*Aca chi*	*Spa axi*	*Kyp vai*
Macro-analysis											
Brown corticated	-	-	-	-	-	-	0.1	-	0.1	-	-
Brown filamentous	0.7	-	-	0.2	0.1	2.5	-	-	1.4	0.2	-
Brown foliose	11.8	0.1	97.5	1.3	2.4	82.0	2.0	-	1.2	1.1	12.9
Brown leathery	-	-	1.5	-	-	0.3	-	-	0.9	-	50.0
Brown seaweed	-	-	-	-	-	-	-	-	-	-	7.3
Green calcareous	-	-	-	13.3	-	-	-	-	-	-	-
Green filamentous	2.4	-	-	33.6	0.4	0.4	1.1	-	4.9	0.4	-
Green foliose	1.0	0.5	-	0.2	0.7	0.3	0.2	-	1.6	1.1	-
Red calcareous articulated	10.4	4.3	-	10.3	1.7	0.1	32.3	8.5	35.1	17.8	0.7
Red corticated	7.4	2.0	0.3	2.5	5.6	14.3	4.5	2.8	12.4	0.8	26.0
Red crustose calcareous	18.0	5.9	-	12.1	2.4		10.5	9.8	1.1	2.4	-
Red filamentous	13.6	1.4	-	7.1	2.4	0.1	6.5	5.2	3.1	5.8	1.3
Red foliose	0.2	-	-	-	-	-	-	-	-	-	-
Invertebrate	5.0	1.0	0.6	4.9	0.6	-	28.2	3.2	28.6	7.1	1.8
Detritus	12.7	73.5	-	8.7	72.4	-	9.6	66.9	7.5	57.4	-
Sediment	16.8	11.4	-	5.8	11.3	-	5.0	3.5	2.1	6.0	-
Micro-analysis											
Brown foliose	-	0.2	-	-	-	-	-	0.3	-	0.2	-
Cyanobacteria	25.8	32.9	-	22.4	13.3	-	12.7	7.8	7.5	2.6	-
Diatoms	35.7	6.3	-	31.7	16.7	-	37.9	17.2	36.3	2.1	-
Green filamentous	3.7	35.8	-	2.7	29.2	-	2.5	18.6	2.8	6.7	-
Green foliose	-	0.8	-	-	0.3	-	-	3.1	-	5.1	-
Red filamentous	3.0	6.5	-	1.0	4.0	-	1.4	1.9	1.0	0.2	-
Sponge spicules	30.8	16.9	-	42.2	35.2	-	44.0	41.4	41.3	55.1	-
Invertebrate	-	-	-	-	0.2	-	0.5	-	0.5	0.0	-
Detritus	0.8	-	-	-	-	-	-	-	10.5	25.3	-
Sediment	0.2	0.6	-	-	1.2	-	1.1	9.7	-	2.8	-

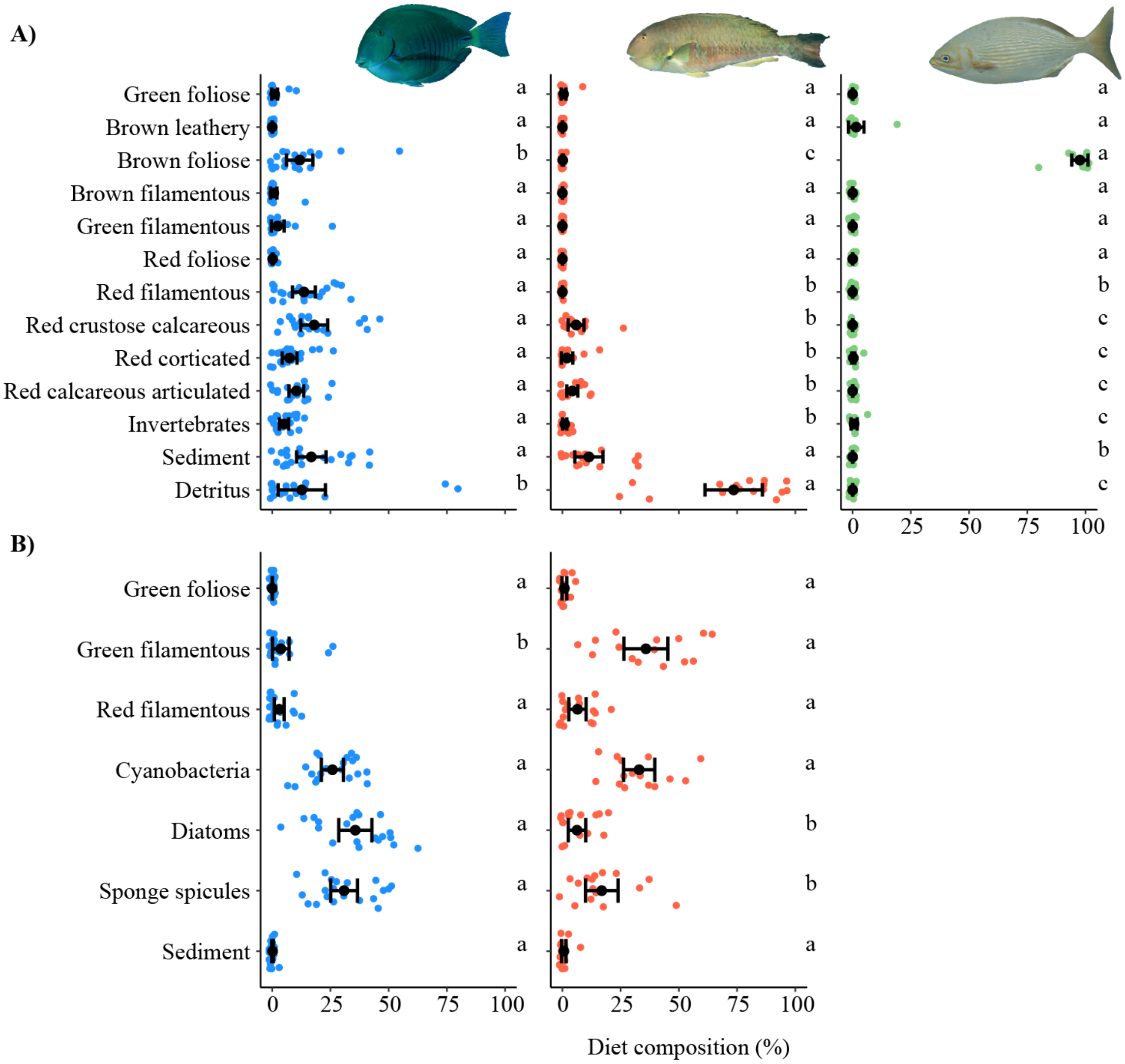

Figure S1: Dietary analysis comparison among species (left to right: *Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis* in Natal – colours correspond to each species. Results from macro- (A), and micro-analysis (B). Lower case letters indicate: diet similarities in between-species comparisons for each item (PERMANOVA: α > 0.05), being a > b > c. Black dots are mean (±C.I.) diet composition (%; Tab. S3) for each item.

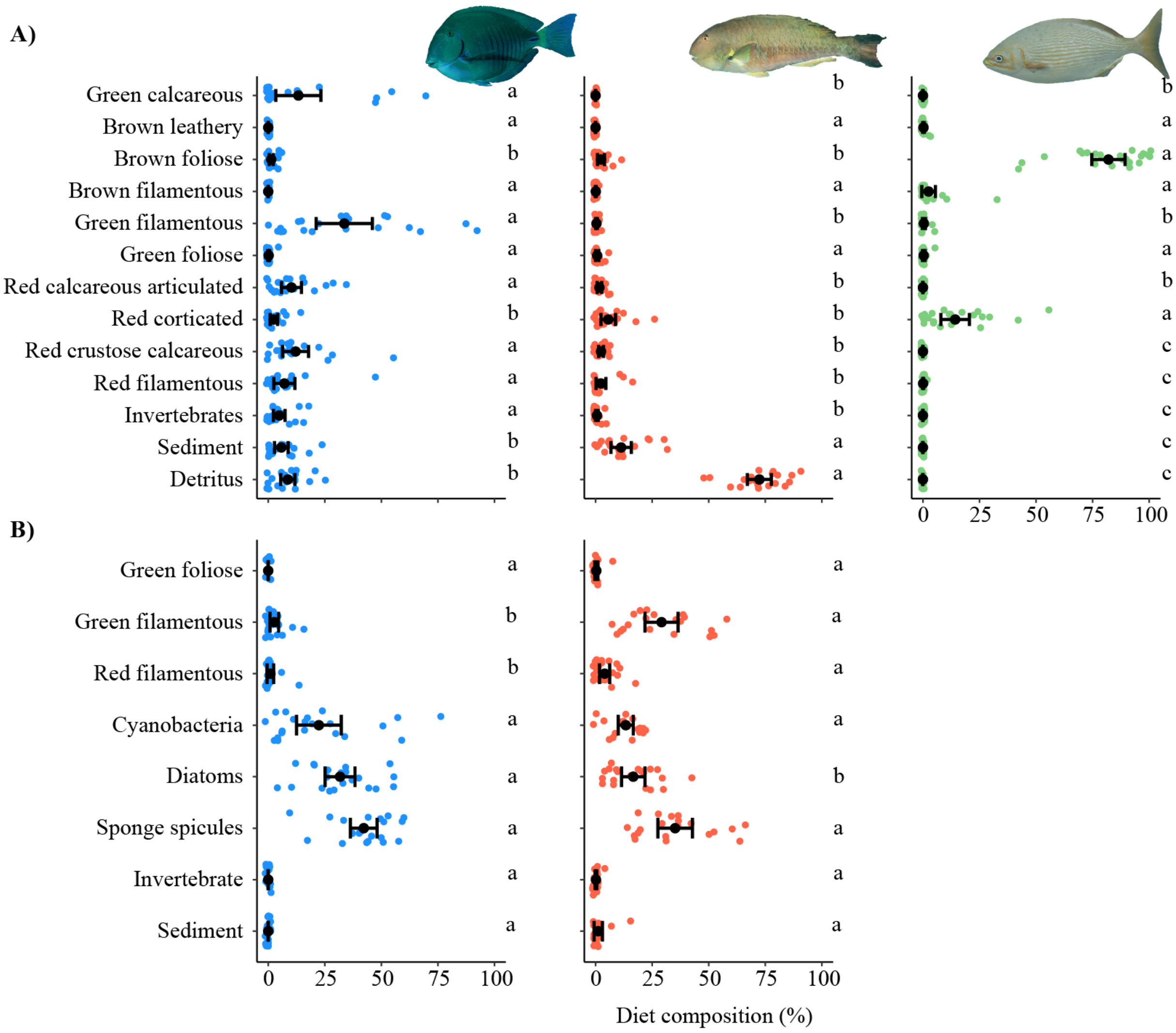

Figure S2: Dietary analysis comparison among species (left to right: *Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis* in the Abrolhos Archipelago – colours correspond to each species. Results from macro- (A), and micro-analyse (B). Lower case letters indicate: diet similarities in between-species comparisons for each item (PERMANOVA: α > 0.05), being a > b > c. Black dots are mean (±C.I.) diet composition (%; Tab. S3) for each item.

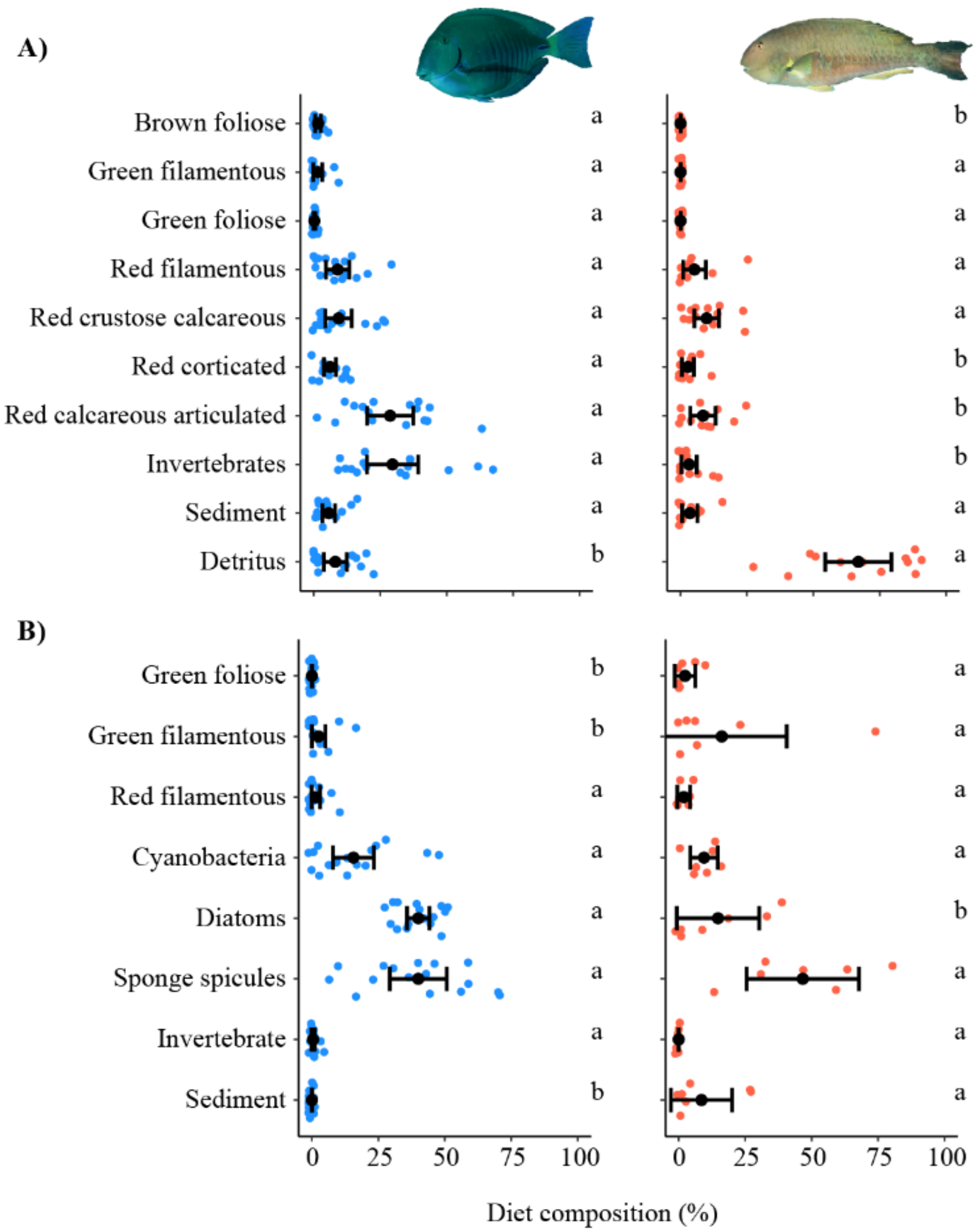

Figure S3: Dietary analysis comparison among species (left to right: *Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis* in Guarapari – colours correspond to each species. Results from macro- (A), and micro-analyse (B). Lower case letters indicate: diet similarities in between-species comparisons for each item (PERMANOVA: α > 0.05), being a > b > c. Black dots are mean (±C.I.) diet composition (%; Tab. S3) for each item.

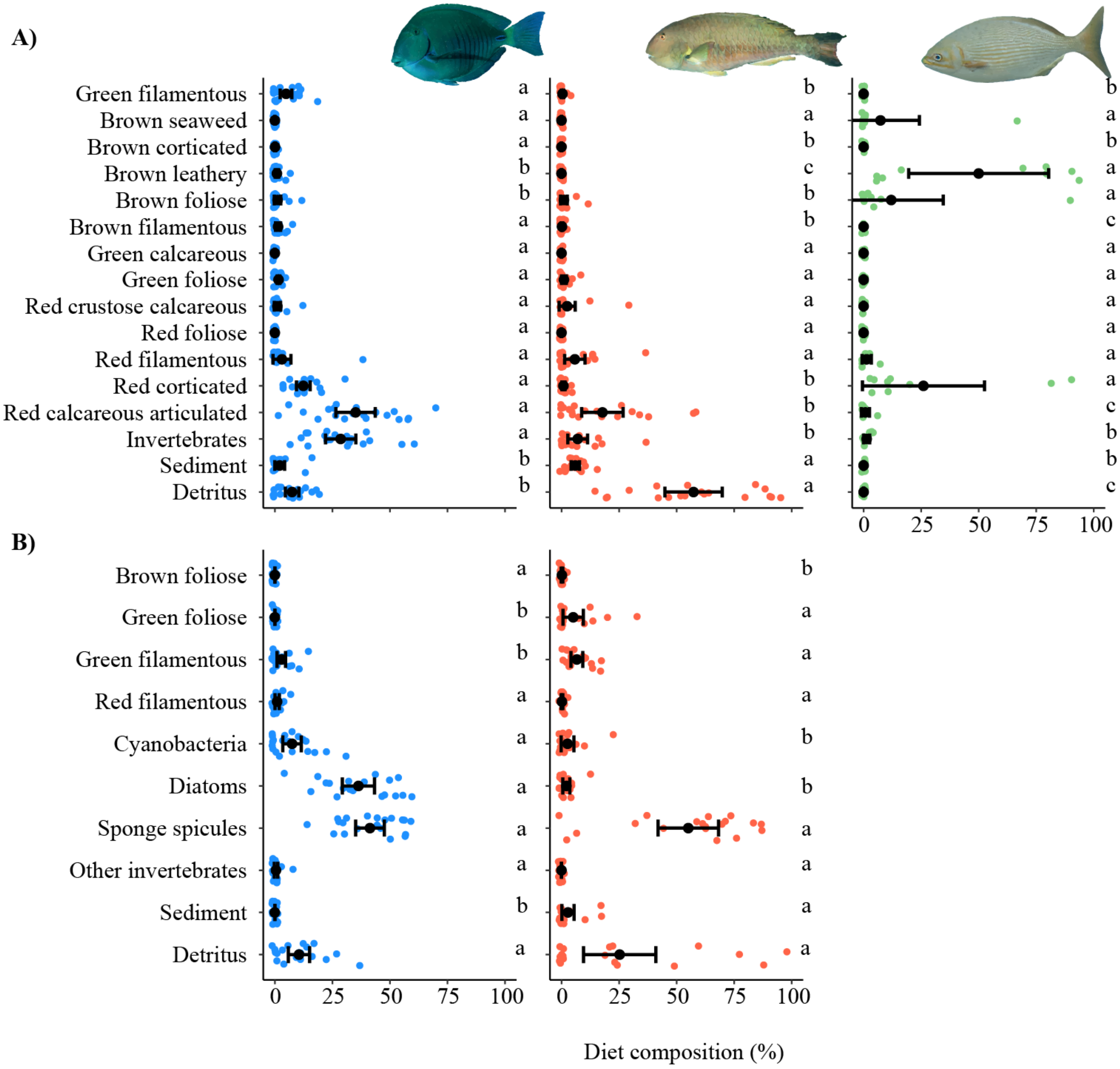

Figure S4: Dietary analysis comparison among species (left to right: *Acanthurus chirurgus*, *Sparisoma axillare* and *Kyphosus vaigiensis* in Arraial do Cabo – colours correspond to each species. Results from macro- (A), and micro-analyse (B). Lower case letters indicate: diet similarities in between-species comparisons for each item (PERMANOVA: α > 0.05), being a > b > c. Black dots are mean (±C.I.) diet composition (%; Tab. S3) for each item.

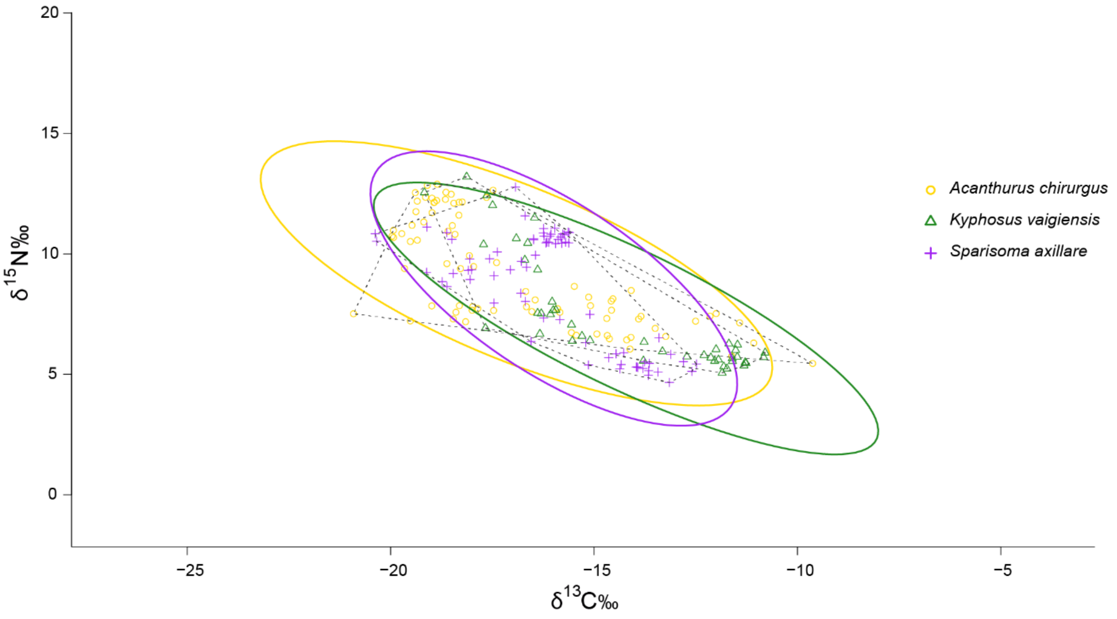

Figure S5: Standard ellipse for each nominally herbivorous species independent of site. Ellipses correspond to small-sample corrected standard ellipse area (SEAc, 95% C.I.; solid lines) and convex-hull area around extreme sample values (dashed lines).

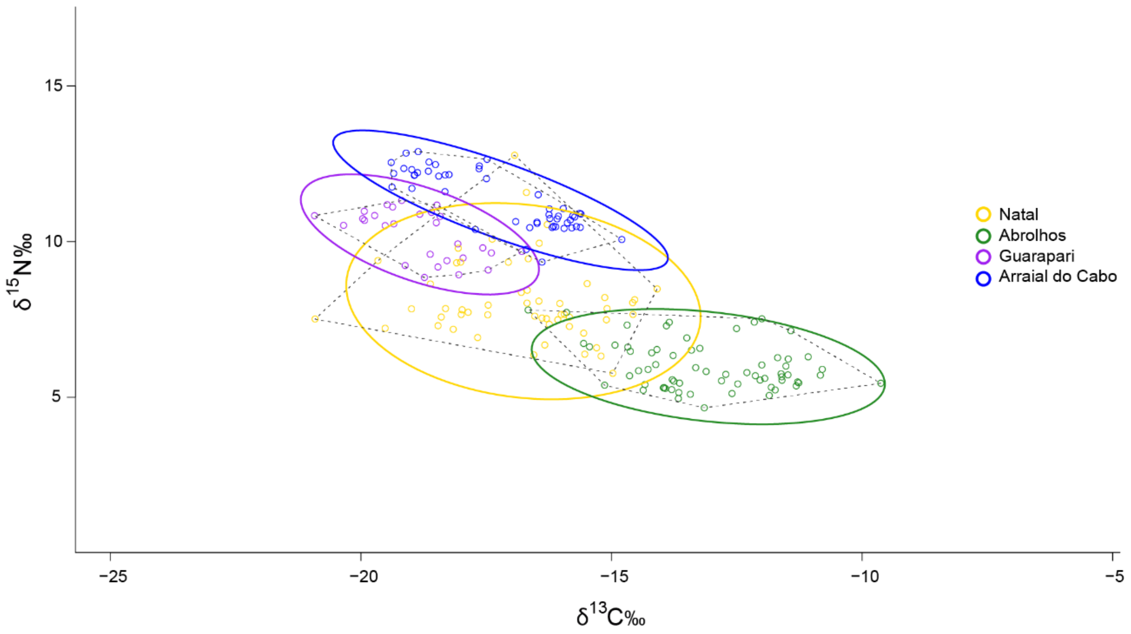

Figure S6: Standard ellipse for four sites without differentiating among the nominally herbivorous species. Ellipses correspond to small-sample corrected standard ellipse area (SEAc, 95% C.I.; solid lines) and convex-hull area around extreme sample values (dashed lines).