UNIVERSIDADE FEDERAL FLUMINENSE
INSTITUTO DE GEOCIÊNCIAS
DEPARTAMENTO DE GEOLOGIA E GEOFÍSICA/LAGEMAR
PROGRAMA DE PÓS-GRADUAÇÃO EM DINÂMICA
DOS OCEANOS E DA TERRA

PEDRO VIANNA GATTS

Ecologia trófica do nécton demersal da plataforma da Bacia de Campos, Sudeste do Brasil

Niterói, RJ

2019

ECOLOGIA TRÓFICA DO NÉCTON DEMERSAL DA PLATAFORMA DA BACIA DE CAMPOS, SUDESTE DO BRASIL

PEDRO VIANNA GATTS

Tese apresentada ao Programa de Pós-Graduação em Dinâmica dos Oceanos e da Terra da Universidade Federal Fluminense, como requisito parcial da obtenção do Título de Doutor. Área de Concentração: Ecologia Marinha

Orientador: Prof. Dr. Paulo Alberto Silva da Costa

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Aprovada em 31 de outubro de 2019.

BANCA EXAMINADORA

Prof. Dr. Paulo Alberto Silva da Costa – Orientador
Universidade Federal Fluminense – UFF

Prof. Dr. Carlos Eduardo Rezende – Co-orientador
Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF

Prof[a]. Dr[a]. Alejandra Filippo Gonzalez Neves dos Santos
Universidade Federal Fluminense – UFF

Prof[a]. Dr[a]. Ana Paula de Castro Rodrigues
Universidade Federal do Rio de Janeiro – UFRJ

Dr. Bruno Pereira Masi
Universidade Federal do Rio de Janeiro – UFRJ

Ficha catalográfica automática - SDC/BIG
Gerada com informações fornecidas pelo autor

G262e   Gatts, Pedro Vianna
Ecologia trófica do nécton demersal da plataforma da Bacia de Campos, Sudeste do Brasil / Pedro Vianna Gatts ; Paulo Alberto Silva da Costa, orientador ; Carlos Eduardo de Rezende, coorientador. Niterói, 2019.
186 f. : il.

Tese (doutorado)-Universidade Federal Fluminense, Niterói, 2019.

DOI: http://dx.doi.org/10.22409/PPGDOT .2019.d.13249682730

1. Megafauna demersal. 2. Plataforma continental. 3. Isótopos estáveis. 4. Mercúrio total. 5. Produção intelectual. I. Costa, Paulo Alberto Silva da, orientador. II. Rezende, Carlos Eduardo de, coorientador. III. Universidade Federal Fluminense. Instituto de Geociências. IV. Título.

CDD -

Bibliotecário responsável: Debora do Nascimento - CRB7/6368

Aos meus pais, Carlos e Renata

A minha avó, Higia

A minha madrasta, Denise

As minhas irmãs, Fernanda e Juliana

AGRADECIMENTOS

Ao meu orientador Prof. Paulo Alberto Silva da Costa pela orientação, confiança, parceria, incentivos e paciência no transcorrer desses anos.

Ao meu orientador Dr. Carlos Eduardo de Rezende pela orientação, confiança, parceria, oportunidades, incentivos e ensinamentos dentro e fora da vida acadêmica ao longo dos 6 anos de convivência.

Ao Dr. Marcelo Gomes de Almeida por estar sempre disposto a ajudar nas etapas laboratoriais, nas discussões importantes e pelos seus ensinamentos.

Ao amigo e Dr. Marcos Franco pela parceria desde o início ao final do projeto, com discussões importantes para minha formação acadêmica e pessoal.

A prof[a]. Alejandra Filippo Gonzalez Neves dos Santos por me acompanhar, avaliar e contribuir significativamente no meu processo de doutorado desde a banca de seleção do Programa de Pós-Graduação em Dinâmica dos Oceanos e da Terra à banca de avaliação da tese.

A CAPES pelo auxílio da bolsa de doutorado. A FAPERJ e CNPq pela viabilização do desenvolvimento do projeto.

Ao Programa de Pós-Graduação em Dinâmica dos Oceanos e da Terra. Ao Laboratório de Ciências Ambientais (LCA) da UENF pela realização de todos os procedimentos laboratoriais.

Aos amigos Braulio Cherene, Diogo Quitete, Thiago Rangel, Andressa Batista, Diego Lacerda, Tassiana Gonçalves, Beatriz Araujo, Cynara Fragoso, Luciana Vidal e Leandro Drummond que fizeram e/ou ainda fazem parte do Grupo de Biogeoquímica de Ecossistemas Aquáticos (GBEA) por toda a ajuda em coletas, análises laboratoriais, discussões acadêmicas e pelo convívio.

A Anna Hautequestt, Annaliza Meneguelli, Bruno Ramos, Danielle Awabdi, Igor Broggio, Inácio Pestana, Karoline Ferreira, Keltony Aquino, Luisa Viana e Lucas Azevedo, amigos da Nata do LCA que deram leveza e tranqüilidade nos momentos mais difíceis nesta jornada.

A minha família que sempre me deu força nos momentos difíceis. São pessoas que me incentivaram a continuar no caminho certo.

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Lista de Siglas

^{13}C – Isótopo de carbono

$\delta^{13}C$ – Assinatura isotópica de carbono

$\Delta^{13}C$ – Carbon discriminate factor

^{15}N – Isótopo de nitrogênio

$\delta^{15}N$ – Assinatura isotópica de nitrogênio

$\Delta^{15}N$ – Nitrogen discriminant factor

ACAS – Águas Centrais do Atlântico Sul

AJE – American Journal Experts

Al – *Aspistor luniscutis*

ANOVA – Analysis of variance

ATCY – *Atlantoraja cyclophora*

ATPL – *Atlantoraja platana*

Av. kg h^{-1} – Average kilograms per hour

Bb – *Bagre bagre*

BC – Bacia de Campos

Bent – Bentophagus

BN – Bentophagus-nektophagus

C – Carbono

°C – Celsius degrees

CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

CB – Campos Basin

CCA – Canonical Correspondence Analysis

CD – Centroid distance

Chl*a* – Chlorophyll a

CI – Confidence interval

CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico

C/N – Ratio of carbon and nitrogen

(C:N)*a* – Atomic ratio of carbon and nitrogen

cm – Centimeter

CorgSOM – Organic carbon of sediment organic matter

CorgSPM – Organic carbon of suspended particle matter

CPUE – Catch per unit effort

CR – Carbon range

CS – Coarse sand

Csp – *Callinectes* sp.

d – Diameter

DAVO – *Dactylopterus volitans*

DD – Data deficient

Dis – PSR Discharge

DO – Dissolved oxigen

DOC – Dissolved organic carbon

DORM – Dogfish reference material

Ds – *Doryteuthis sanpaulensis*

DUAU – *Dules auriga*

dw – Dry weight

FAPERJ – Fundação de Amparo à Pesquisa do Rio de Janeiro

FO – Frequence of occurence

g – Gram

Gg – *Genidens genidens*

Hg – Mercúrio

Hg^0 – Elemental mercury

Hg^{+2} – Ion mercury

HgT – Mercúrio total

IUCN – International Union for Conservation of Nature

kg – Kilogram

kg g^{-1} – Kilogram per gram

kg h^{-1} – Kilogram per hour

km – Kilometers

km^2 – Square kilometers

$km^2\ d^{-1}$ – Square kilometers per day

LC – Least Concern

Log_{10} – Logarithm base 10

logN – Logarithmic number of individuals

logWt – Logarithmic total weight

Lt – Total length

M – Molar

MeHg – Methylmercury

mg – Milligram

mg L^{-1} – Milligram per liter

Mw – Megawatt

mS cm^{-1} – Millisimens per centimeter

MS – Medium sand

m^3 s^{-1} – Cubic meters per second

n – Number of individuals

N – Nitrogênio

NE – Not Evaluated

Nekt – Nektophagus

ng – nanogram

ng g^{-1} – nanogram per gram

ngHg g^{-1} – nanogram of Hg per gram

N h^{-1} – Number of individuals per hour

NND – Nearest neighbour distance

NR – Nitrogen range

ns – Non-significant

NT – Near threatened

NTU – Nephelometric Turbidity Unit

max – Maximum

mg – Milligram

min – Minimum

Ml – Mantle length

mm – Millimeter

µmol L^{-1} – Micromol per liter

µm – Micrometer

OAS – Organic Analytical Standard

OM – Organic matter

PEBR – *Percophis brasiliensis*

Pp – *Porichthys porosissimus*

PDB – Pee Dee Belemnite

Phyto – Phytoplankton

POM – Particulate organic matter

PRNU – *Prionotus nudigula*

PSR – Paraíba do Sul River

RPS – Rio Paraíba do Sul

S – South

SACA – *Saurida caribbaea*

SACW – South Atlantic Center Water

Sc – Sciaenidae

SCA – Stomach contents analysis

SD – Standard deviation

SDNND – Standard deviation of nearest neighbor distance

SEA – Standard ellipse area

SEAc – Standard ellipse area corrected for small sample sizes

SIAR – Stable Isotope Analysis in R)

SIBER – Stable Isotope Bayesian Ellipses in R

SIMPER – Similarity of percent

SOM – Sediment organic matter

T – Temperature

TA – Total isotopic niche area

TEF – Trophic enrichment factor

TEF^{13}C – Carbon isotope trophic enrichment factor

TEF^{15}N – Nitrogen isotope trophic enrichment factor

Temp – Temperature

THg – Total mercury

THg_{STD} – Total mercury standardized by length

TN – Total nitrogen

Turb – Turbidity

UENF – Universidade Estadual do Norte Fluminense Darcy Ribeiro

VU – Vulnerable

W – West

WHO – World Health Organization

Wt – Total wieght

ww – Wet weight

Xk – *Xiphopenaeus kroyeri*

ZABR – *Zapteryx brevirostris*

Zoo – Zooplankton

Resumo Geral

O objetivo do presente estudo foi investigar as relações entre os organismos de teias tróficas demersais da plataforma continental da Bacia de Campos (BC). Este estudo é pioneiro em utilizar as ferramentas de isótopos estáveis de C e N e a concentração de HgT para compreender as relações tróficas entre organismos de 10 a 60 m de profundidade do norte da BC, assim como a importância do Rio Paraíba do Sul e origem da matéria orgânica (MO) que sustenta as teias alimentares investigadas. Os bagres marinhos de águas costeiras (10 m de profundidade) particionam os recursos alimentares abundantes na região. A espécie *Genidens genidens* apresentou a maior amplitude de nicho isotópico, portanto foi o bagre marinho mais oportunista. Enquanto a bioacumulação de Hg foi observada para *Bagre bagre* e *G. genidens*, a biomagnificação de Hg ocorreu ao longo da teia alimentar. A assembleia demersal da plataforma continental da BC (40 a 60 m de profundidade) é composta por espécies residentes. Sua sazonalidade está relacionada a padrões de migração e de desova das espécies. A guilda trófica dos bentófagos foi dominante entre as guildas por se beneficiar da diponibilidade de presas na região, a macrofauna bêntica. A MO aderida ao sedimento, composta pela mistura de fontes de origem marinha e terrestre, sustenta as teias alimentares da plataforma continental da BC (40 a 60 m de profundidade). As guildas tróficas dos bentófagos e betófagos-nectófagos oscilaram sazonalmente de áreas bênticas e costeiras para pelágicas e de mar aberto. As concentrações de HgT estão dentro do permitido pela legislação para consumo humano. A bioacumulação do Hg foi observada para as três guildas e a biomagnificação do metal ressaltou a importância da MO como base da teia alimentar.

Palavras-chave: Megafauna demersal, plataforma continental, estrutura de assembléias, isótopos estáveis, nicho isotópico, mercúrio.

Abstract Geral

The objective of the present study was to investigate the relationships among demersal food web organisms of Campos Basin (CB) continental shelf. This study is pioneer in using C and N stable isotopes and total mercury (THg) concentration tools to understand the trophic relationships among organisms from 10 to 60 m deep of the north of CB, as well the importance of Paraíba do Sul river and the organic matter (OM) origin that sustains the investigated food webs. Marine catfishes if coastal waters (10 m deep) partitionate the most abundant feeding resources in the region. The species *Genidens genidens* presented the widest isotopic niche breath, thus it was the most opportunistic marine catfish. While bioaccumulation of Hg was observed for *Bagre bagre* and *G. Genidens*, biomagnification of Hg occurred through the food web. The demersal assemblage of CB continental shelf (40 to 60 m deep) is composed by resident species. Their seasonality is related to species migration and spawning patterns. Bentophagus trophic guild was dominant among guilds due to benefits the availability of prey in the region, the benthic macrofauna. The OM adhered to the sediment, composed by a mixture of marine and terrestrial sources, sustains the food webs of CB continental shelf (40 to 60 m deep). Bentophagus and bentophagus-nektophagus trophic guilds oscillated seasonally, from benthic and inshore to pelagic and offshore areas. The THg concentrations are under the regulatory limit for human consumption. Bioaccumulation of Hg was observed in the three guilds and the biomagnification of the metal highlighted the importance of OM as the base of the food web.

Palavras-chave: Demersal megafaunal, continental shelf, assemblage structure, stable isotopes, isotopic niche, mercury.

Introdução Geral

Organismos demersais são estudados no mundo inteiro em relação a padrões espaciais (Peristeraki et al., 2017), variações interanuais incluindo efeitos de mudanças climáticas (Dulvy et al., 2008; Queirós et al., 2018), impacto pesqueiro (Labropoulou e Papaconstantinou, 2004), bem como as dinâmicas alimentares (Willis et al., 2017) e sazonais de comunidades (Martins e Haimovici, 2016).

A fauna demersal das regiões Sul e Sudeste do Brasil é a mais conhecida, devido à elevada produtividade marinha que leva ao desembarque pesqueiro de peixes e invertebrados demersais e pelágicos, muitos deles comercialmente importantes, (Haimovici, 1998; Haimovici, 2006), assim como pela maior concentração de universidades e centros de pesquisa nas duas regiões. Enquanto em águas costeiras rasas (10 m de profundidade) as famílias Sciaenidae e Ariidae compõem os principais organismos demersais, as assembleias de peixes e cefalópodes são as mais representativas ao longo do gradiente batimétrico, ocorrendo durante o ano inteiro (Fagundes-Netto, 1991; Gatts et al., 2014; Costa et al., 2015)

Na Bacia de Campos (BC), região Sudeste do Brasil, o conhecimento acerca da fauna demersal na plataforma continental e de águas costeiras é extenso (Fagundes-Netto e Gaelzer, 1991; Costa et al., 2015; Haimovici et al., 2017; Mincarone et al., 2017). Esta região é caracterizada por eventos de ressurgência bem como pelo aporte de águas continentais, importantes fontes de matéria orgânica para o sistema, que por sua vez sustentam uma enorme diversidade de organismos marinhos (Corbisier et al., 2014; Carreira et al., 2015; Cordeiro et al., 2018). Na região Norte da BC (22° S, 40° W), enquanto a ressurgência transporta águas oceânicas frias e ricas em nutrientes provenientes das Águas Centrais do Atlântico Sul (ACAS) sazonalmente para águas costeiras regidas pela Corrente do Brasil, o estuário do Rio Paraíba do Sul (RPS) exporta águas continentais quentes e ricas em nutrientes originadas e regidas pelo regime de chuvas ao longo da sua bacia de drenagem (Almeida et al., 2007; Palóczy et al., 2014), integrando, portanto, cadeias alimentares costeiras e marinhas (Zalmon et al., 2015; Almeida et al., 2019).

Além do fornecimento de nutrientes de origem continental, os estuários também são fontes relevantes de elementos potencialmente tóxicos de águas costeiras e da plataforma continental (Souza et al., 2010). No Norte da BC o mercúrio (Hg) é amplamente reportado como um dos principais metais pesados, que além de ser exportado pelo RPS para o ambiente marinho, atingindo o talude (Araujo et al., 2017), também é assimilado pelos organismos a partir da ingestão de presas, desde produtores primários a predadores de topo de cadeia (Lacerda et al., 1993; Di Beneditto et al., 2012; Kehrig et al., 2013).

Neste sentido, uma vez que isótopos estáveis de carbono ($\delta^{13}C$) são utilizados na determinação de fontesde energia (De Niro & Epstein, 1978), e isótopos estáveis de nitrogênio ($\delta^{15}N$) e a determinação das concentrações de mercúrio total (HgT) são definidores de posições tróficas (Post, 2002; Fry & Chumchal, 2012), estas ferramentas vem sendo utilizadas em estudos na região costeira do norte da BC. São aplicadas para a compreensão da origem da matéria orgânica e de produtores primários que sustentam cadeias alimentares (Di Beneditto et al., 2013), da dinâmica trófica entre espécies costeiras (Di Beneditto et al., 2011, 2016) e das relações entre tamanho corporal, posição trófica e processos de magnificação do Hg ao longo das cadeias alimentares (Di Benedito et al., 2012; Kehrig et al., 2013).

Entretanto, o conhecimento adquirido com a aplicação de traçadores isotópicos de $\delta^{13}C$ e $\delta^{15}N$ e das concentrações de HgT em organismos é limitado a profundidade de 30m. Logo, com a perspectiva de ampliar a aplicação destas ferramentas em organismos marinhos demersais de 10 a 60 m de profundidade, a hipótese desta tese é que as análises de isótopos estáveis de carbono ($\delta^{13}C$) e nitrogênio ($\delta^{15}N$) e a determinação do HgT sejam indicadores da estrutura trófica da assembléia demersal da BC.

A tese está dividida em quatro capítulos em formato de artigos científicos:

Capítulo 1: Trohic ecology of marine catfishes in Southeastern Brazil.

O capítulo 1 avaliou o nicho isotópico dos três principais bagres marinhos ao longo da costa norte do Rio de Janeiro. Nossos dados contemplaram os três bagres marinhos, assim como, suas presas no ecossistema estuarino costeiro, levando a respostas de questões relevantes sobre disponibilidade de recursos, o uso de habitat e a competição por recursos em relação a isótopos estáveis de carbono e nitrogênio, além de processos de bioacumulação e biomagnificação de mercúrio total. Este capítulo foi aceito para publicação no periódico Journal of the Marine Biological Association of the United Kingdom.

Gatts PV, Franco MAL, Almeida MG, Zalmon IR, Di Beneditto APM, Costa PAS, Rezende CE. Trophic Ecology of marine catfishes in Southeastern Brazil. Manuscript ID: JMBA-07-19-OA-0160.R1.

Capítulo 2: Demersal assemblage structure and dynamics off southwestern Atlantic continental shelf, north of Campos Basin, Brazil.

No capítulo 2 identificamos a estrutura e dinâmica sazonal da assembleia demersal da plataforma continental da Bacia de Campos na costa Norte do Rio de Janeiro. As espécies que ocorreram em maiores abundância e freqüência foram classificadas em guildas tróficas. Análises multivariadas permitiram a compreensão da relação entre a assembléia demersal com os principais fatores abióticos que influenciam nos padrões sazonais dos organismos. Este capítulo será submetido para o periódico Estuarine, Coastal and Shelf Science.

Capítulo 3: Isotopic niche breadth of coastal fish and cephalopods off Campos Basin, Southeastern Brazil.

No capítulo 3 os isótopos estáveis de carbono e nitrogênio das três principais guildas tróficas da assembléia demersal foram explorados para compreender suas relações tróficas na plataforma continental. Inferências acerca da origem da matéria orgânica que sustenta a cadeia trófica demersal, bem como a amplitude e sobreposição dos nichos isotópicos das guildas foram interpretadas de acordo com a os padrões de sazonalidade da região. Este capítulo será submetido ao periódico Science of the Total Enviroment.

Capítulo 4: Mercury and nitrogen stable isotope in fish and cephalopods from the southwestern Atlantic.

No capítulo 4 analisamos as relações entre as concentrações de mercúrio total e isótopos estáveis de nitrogênio entre as três principais guildas tróficas da assembléia demersal da plataforma continental. Aspectos relacionados aos processos de bioacumulação em cada guilda e de biomagnificação na cadeia trófica demersal, bem como a limites estabelecidos para o consumo humano de espécies comerciais foram abordados. Este capítulo será submetido ao periódico Environmental Science and Pollution Research.

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Capítulo 1 – aceito para publicação no Journal of the Marine Biological Association of the United Kingdom, JMBA-07-19-OA-0160.R1.

The trophic ecology of marine catfishes in Southeastern Brazil.

Pedro V. Gatts[1*], Marcos A.L. Franco[2], Marcelo G. Almeida[2], Ilana R. Zalmon[2], Ana Paula M. Di Beneditto[2], Paulo A. S. Costa[3], Carlos E. Rezende[2].

[1]Instituto de Geociências, Universidade Federal Fluminense (UFF), Niterói, RJ, Brazil; [2]Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes, RJ, Brazil; [3]Departamento de Ecologia e Recursos Marinhos, Instituto de Biociências, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Rio de Janeiro, RJ, Brazil.

Abstract

The stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) and total mercury concentrations (THg) of the three marine catfish species *Aspistor luniscutis*, *Bagre bagre* and *Genidens genidens* were evaluated to understand their trophic relationship in northern Rio de Janeiro state, Southeastern Brazil. The $\delta^{13}C$ was similar among the three marine catfishes, whereas $\delta^{15}N$ was similar in *A. luniscutis* and *B. bagre* and lower in *G. genidens*. THg was higher in *G. genidens* and lower in *B. bagre*. The greater assimilation of Sciaenidae fishes and squids by *A. luniscutis* and *B. bagre* resulted in smaller isotopic niche areas and trophic diversity but higher isotopic niche overlap, trophic redundancy and evenness. For *G. genidens,* the similar assimilation of all prey items resulted in the broadest isotopic niche among the marine catfishes. The higher mercury content in *G. Genidens* is consistent with an increased important contribution of the preys with higher Hg burden. The bioaccumulation process was indicated by significant correlations of $\delta^{15}N$ and THg with total length and total mass. Additionally,

a significant correlation between THg and $\delta^{15}N$ reflected the biomagnification process through the food web.

Key words:
Southwestern Atlantic; Stable isotopes; Isotopic niche; Mercury; Bioaccumulation; Biomagnification.

INTRODUCTION

Food webs are models of trophic interactions among species, usually simplified into networks of species and energy links between them (Thompson *et al*., 2012). The trophic dynamic theory supports the understanding of food web structure and species interactions that ultimately shape modern marine ecosystem ecology, conservation and management (Treblico *et al*., 2013). Therefore, the analysis of diet to identify the role of each predator species on prey resource sharing relationships within the fish community is of great value (Svanbäck *et al.*, 2015).

The coexistence of ecologically similar fishes in high abundance in an ecosystem is possible because of the development of strategies that allow not only spatial and/or temporal separation of the species (Azevedo *et al.*, 1999) but also ecological variations in the use of available niches and/or partitioning of the available resources (Herder & Freyhof, 2006; Sandlund *et al*., 2010). On the other hand, the functional or morphological homology of sympatric species can potentially increase competition (Wootton, 1990). If the food resources are limited, this competition can result in exclusion of the less adapted species. Nevertheless, according to the principles of competitive exclusion, interspecific competition also favours trophic niche diversification or resource partitioning across species (Schluter, 1996; Svanbäck & Bolnick, 2008).

The isotopic niche concept proposed by Newsome *et al*. (2007), a refinement of Hutchinson's idea (Hutchinson, 1957, 1978), suggests the use of isotopic tools to assess the ecological characteristics of organisms that ecologists aim to investigate, such as coexistence and resource sharing. Stable isotopic signatures of carbon (C) and nitrogen (N) have been widely used in this sense (Jackson *et al*., 2011; Yasue *et al*., 2014; Gallagher *et al*., 2017; Jensen *et al*., 2017; Rader *et al*., 2017), as the values measured in the consumer's tissues are closely related to the values from its diet. Stable nitrogen isotope ratios in consumers are typically enriched in the heavier isotope (^{15}N) by 2 to 4 ‰ per trophic level (Minagawa & Wada, 1984; Peterson & Fry, 1987), making $\delta^{15}N$ values useful for the definition of trophic positions of consumers (Post 2002). In contrast, the fractionation of carbon isotopes (^{13}C) is lower (0 to 1‰) and is typically used to define energy sources (De Niro & Epstein, 1978).

The determination of total mercury (THg) in organisms is a complementary tool for isotopic analysis that is widely used in trophic ecology investigations (Eagles-Smith *et al*., 2008; Fry & Chumchal, 2012; Pouilly *et al*., 2013). This tool has been used in ecological studies as a good indicator of the trophic level of organisms (Fry & Chumchal, 2012), but it can also be used to identify spatiotemporal patterns of fish bioaccumulation (Buckman *et al*., 2017; Liu *et al*., 2017) and biomagnification processes (Cresson *et al.*, 2015; Chouvelon *et al*., 2018). Tropical estuaries are known as source of anthropogenic Hg for fish feeding in coastal areas (Le Croizier *et al*., 2019). In estuarine and coastal environments, it is estimated that 90% of the river-derived Hg is buried in sediments on ocean margins (Chester, 1990), highlighting the contribution of estuaries to the transport of Hg to coastal marine waters and, consequently, to the associated food webs.

In Brazil, the Ariidae family is represented by demersal marine catfishes usually found in coastal and estuarine ecosystems (Marceniuk & Menezes, 2007; Schmidt *et al*., 2008; Denadai *et al*., 2012; Pereyra *et al*., 2016). Along the coast of São Paulo and Rio de Janeiro, their spatial distribution in estuarine systems is governed by salinity (Denadai *et al*., 2012; Bizerril 1999). They usually have wide trophic niches that, in general, overlap with each other's (Denadai *et al*., 2012; Bruton 1996) as many other sympatric fish species (Svanbäck *et al*., 2015; Cachera *et al*., 2017) and have been classified as generalist opportunistic omnivorous predators, with crustaceans, fishes, molluscs, annelids, detritus and algae commonly observed as the main groups of food items (Chaves & Vendel, 1996; Denadai *et al*., 2012; Pinheiro-Sousa *et al*., 2015; Tavares & Di Beneditto, 2017; Di Beneditto *et al*., 2018; Di Beneditto & Tavares, 2019).

The amount of information generated by stomach assessments by scientists all over Brazil report the high importance of fishes and invertebrates (mainly crabs) as prey items of ariids (Chaves & Vendel, 1996; Denadai *et al*., 2012; Tavares & Di Beneditto, 2017). To date, the trophic ecology of marine catfishes has been intensively studied over the last two decades (Chaves & Vendel, 1996; Bizerril 1999; Denadai *et al*., 2012; Pinheiro-Sousa *et al*., 2015; Tavares & Di Beneditto, 2017; Di Beneditto *et al*., 2018, Di Beneditto & Tavares, 2019). However, few studies off the Brazilian coast have focused on isotopic tracers (Giarrizzo *et al*., 2011; Claudino *et al*., 2015; Pereyra *et al*., 2016; Di Beneditto *et al*., 2018), and no data are available regarding stable isotopes coupled with Hg analyses in marine catfishes.

In this sense, the present study used isotopic tracers of carbon (^{13}C) and nitrogen (^{15}N) coupled with mercury (Hg) to understand the trophic relationships among three marine catfishes *Aspistor luniscutis* (Valenciennes, 1840), *Bagre bagre* (Linnaeus,

1766) and *Genidens genidens* (Cuvier, 1829) in the Southeastern Brazil, leading to an improvement in the knowledge of their trophic dynamics in Brazilian shallow waters. Thus, the questions to be answered are as follows: 1) Do the isotopic niches of these catfish species overlap? 2) Are there any spatial and/or temporal patterns in habitat usage among these sympatric marine catfishes? and 3) Do these catfish species compete for the same resource items?

MATERIAL AND METHODS

Study site and sampling

Samples were collected off Manguinhos Beach (21° 29'S, 41° 01'W), District of São Francisco do Itabapoana, at approximately 10 m depth (Figure 1). This area is under the influence of the Itabapoana (north) and Paraíba do Sul (south) rivers. The shelf is naturally depleted of rock substratum or other hard substrates, and it is covered by extensive sandy beaches with variable amounts of mud and calcareous nodules, such as rhodolites (Zalmon *et al*., 2002).

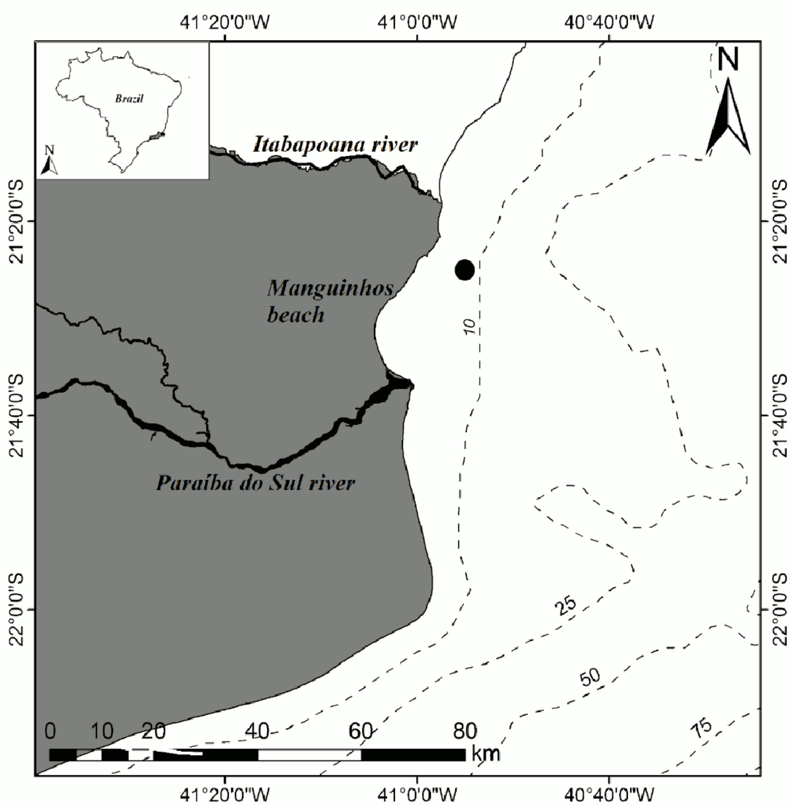

Fig.1. Study area and sampling location (●) off Manguinhos Beach, northern Rio de Janeiro state, Southeastern Brazil.

The marine catfishes *Aspistor luniscutis*, *Bagre bagre* and *Genidens genidens* and their potential prey groups were collected every three months between April 2010 and January 2011. The captured ariid specimens were classified as adults from 180 mm total length for both sexes of *A. luniscutis* (Froese & Pauly, 2019), from 159 and 212 mm total length for females and males, respectively, of *B. bagre* (Véras & Almeida, 2016), and from 55 and 85 mm total length for females and males, respectively, of *G. genidens* (Mazzoni *et al*., 2000).

Fishes were sampled at 13 randomized sampling points with bottom gillnets (n = 78), measuring 25 m in length and 3 m in height and mesh sizes of 20 mm (n = 18), 30 mm (n = 42) and 40 mm (n = 18), measured between adjacent nodes, submerged for 24 h.

Potential food resources identified based on previous stomach content studies of ariids (Chaves & Vendel, 1996; Denadai *et al*., 2012; Tavares & Di Beneditto, 2017; Di Beneditto & Tavares, 2019) off the Southeastern Brazilian coast were also sampled in their natural habitat in the same sampling area. Corers (15 cm diameter, 20 cm height) were used to collect sediment and invertebrates. Phytoplankton and zooplankton were captured by surface trawl nets (30 cm mouth, 1.10 m length and of 20 and 70 µm mesh sizes, respectively) to sample local pelagic trophic end members.

All megafauna (fishes and invertebrates) collected were identified to the lowest taxonomic level, counted, measured (total length) and weighed (total body mass). Dorsal muscle tissues of fish, mantles of cephalopods and pieces of soft tissues (avoiding gastric tracts) of crustaceans as well as total organic matter in the sediment (SOM) and phytoplankton and zooplankton were dried and homogenized for stable isotope and THg analysis. All laboratory analyses were performed at the Laboratório de Ciências Ambientais from Universidade Estadual do Norte Fluminense Darcy Ribeiro.

Stable isotope analysis – $\delta^{13}C$ and $\delta^{15}N$

All samples were stored in clean transparent plastic bags in iceboxes and then transported to the laboratory where they were kept frozen (-18ºC) in dry sterile vials prior to analysis. Freeze-dried samples were ground with a mortar and pestle to a homogeneous fine powder. Approximately 0.5 to 1 mg of animal and phytoplankton tissues and 10 mg of sediment were used in the analysis.

For the elemental composition of carbon and nitrogen as well for $\delta^{13}C$ and $\delta^{15}N$ of sediment, approximately 10 mg was weighed in silver capsules, followed by acidification through the addition of HCl (2 M) to remove inorganic carbon (Kennedy *et al*., 2005; Brodie *et al*., 2011).

The elemental and isotopic composition of all samples were determined using a Flash 2000 Elemental Analyzer with a CONFLO IV interface coupled to a Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific, Germany) in Laboratório de Ciências Ambientais. Samples were analysed using analytical blanks and urea analytical standards (IVA Analysentechnik-330802174; CH4N2O Mw = 60, C = 20%, N = 46%) using certified isotopic compositions ($\delta^{13}C$ = -39.89‰ and $\delta^{15}N$ = -0.73‰). For biota samples, analytical control was performed for every 10 samples using certified isotopic standards (Elemental Microanalysis Protein Standard OAS: 46.5 ± 0.78% for C; 13.32 ± 0.40% for N; -26.98 ± 0.13‰ for $\delta^{13}C$; +5.94 ± 0.08‰ for $\delta^{15}N$). The accuracy of the sediment analysis verified using the Elemental Microanalysis Low Organic Soil Standard (1.52 ± 0.02% for C; 0.13 ± 0.02% for N; -27.46 ± 0.11‰ for $\delta^{13}C$; +6.70 ± 0.15‰ for $\delta^{15}N$).

Carbon and nitrogen contents were expressed as percent elements (%) and the detection limits were 0.05% and 0.02%, respectively. Carbon and nitrogen isotope ratios were expressed in δ notation as ‰ relative to Pee Dee Belemnite (PDB) and atmospheric nitrogen, respectively, and were calculated using the following equation:

$$\delta = (Rsample/Rstandard) \times 10^3$$

where $\delta = \delta^{13}C$ or $\delta^{15}N$ and R = $\delta^{13}C:\delta^{12}C$ or $\delta^{15}N:\delta^{14}N$. Analytical reproducibility was based on triplicates for every 10 samples: ± 0.3‰ for $\delta^{15}N$ and ± 0.2‰ for $\delta^{13}C$. There was no prior lipid extraction from the fish muscle samples, but the C/N ratios were lower than 3.5, indicating a low lipid level that did not compromise the carbon isotope results and their interpretation (Post *et al*., 2007).

Total mercury analysis – THg

Dry muscle samples were digested according to Bastos *et al*. (1998). Briefly, the digestion of tissue samples was performed in a digestion block (60 °C, 4 h) with a mixture of 3 ml HNO3:H2SO4 (1:1) and 1 ml of concentrated H2O2 and allowed to stand overnight. The addition of 5 ml of 5% KnMnO4 and subsequent 30 min of heating were followed by titration with hydroxylamine hydrochloride (HONH3Cl + NaCl 12%). THg was determined with a QuickTrace M-7500 CETAC (CV-AAS). The detection limit was 0.25 ng g^{-1}. The analytical control of the method was determined and monitored using replicate analysis, blank solutions, and certified reference material from the National Research Council-Canada (DORM-2 dogfish *Squalus acanthias* muscle sample). The analytical coefficient of variation between replicates was less than 10%, and the recovery of THg was in agreement with certified values (higher than 90%). Our results are expressed in mg kg^{-1} dry weight (dw).

Data treatment and analysis

The differences between species regarding total length and $\delta^{15}N$ were assessed via one-way parametric ANOVA followed by Tukey's HSD *post hoc* test. For total mass, $\delta^{13}C$, C/N and THg, normality (Shapiro-Wilk test) and homoscedasticity (Levene test) were not achieved, and therefore non-parametric analysis of variance (Kruskal-Wallis) was applied followed by Dunn's *post hoc* test. Linear regressions were performed to check for possible relationships between variables (total length and $\delta^{15}N$, total mass and $\delta^{15}N$, total length and $\log_{10}$THg and total mass and $\log_{10}$THg). Correlation between $\delta^{15}N$ and $\log_{10}$THg was also used with the organisms that compose the food web. Results were considered significant at $P < 0.05$.

Isotopic niche breadth was quantified for each marine catfish using the standard ellipse area corrected for small sample sizes (SEAc), calculated with the Stable Isotope Bayesian Ellipses tool in R (SIBER) (Jackson *et al*., 2011). To evaluate the relative contribution of the different prey, *Xiphopenaeus kroyeri* (Heller, 1862), *Callinectes* sp., *Doryteuthis sanpaulensis* (Brakoniecki, 1984), *Porichthys porosissimus* (Cuvier, 1829) and Sciaenidae, that represent a pool of species composed by *Isopisthus parvipinnis* (Cuvier, 1830), *Paralonchurus brasiliensis* (Steindachner, 1875) and *Stellifer rastrifer* (Jordan, 1889), were used in the Bayesian stable isotope mixing model in the software package SIAR (Stable Isotope Analysis in R) (Parnell & Jackson, 2013), which allows the inclusion of isotopic signatures and fractionation together with the uncertainty of these values within the model. To reduce the uncertainty in the interpretation of the mixing model, the potential prey were reduced/grouped to a total of five sources, similarly to the previous study of Di Beneditto *et al.* (2018).

The trophic enrichment factors (TEFs) are key parameters in isotopic mixing models, representing the isotopic differences between consumers' tissues and their prey items after they reached equilibrium (Parnell *et al*., 2010). In the absence of species-specific TEF values from controlled diet experiments, these values can be obtained in meta-analyses for phylogenetically related species, considering the same tissue (Newsome *et al*., 2007). In this sense, we calculated $TEF^{15}N$ and $TEF^{13}C$ based on equations from a meta-analysis of isotopic studies that considered muscle of fish species (Caut *et al*. 2009) ($\Delta^{15}N = -0.281\ \delta^{15}N + 5.879$ and $\Delta^{13}C = -0.248\ \delta^{13}C - 3.4770$). The calculated values for our data were +1.8 ± 0.2‰ for $TEF^{15}N$ and +0.5 ± 0.2‰ for $TEF^{13}C$, similar to those of marine catfishes of the same region (Di Beneditto *et al*., 2018).

The isotopic niche metrics that relate the characteristics of the isotopic space filled by each marine catfish species in this study were calculated and consisted of the following: NR (maximum $\delta^{15}N$ range – larger range suggests more trophic levels and greater degree of trophic diversity); CR (maximum $\delta^{13}C$ range – increased range suggests higher diversity of basal sources); TA (total area of isotopic niche – representing the total amount of niche space occupied); SEAc (corrected standard ellipses area for small sample size – isotopic niche width); CD (mean Euclidean distance from the centroid – higher distances suggests high average degree of trophic diversity within the food web); NND (mean Euclidean distance to the nearest neighbor – similar trophic ecology will exhibit small NND); and SDNND (standard deviation of NND – low values suggests more even distribution of trophic niche) (Layman *et al*., 2007).

One-way ANOVAs were also used to evaluate differences and interactions between species considering CD and MNND, because they involve comparisons of means. The statistic SDNND, being a standard deviation, was compared between groups by an F-ratio test. The *P* values were interpreted as strengths of evidence towards null hypotheses rather than on the dichotomic scale of significance testing (Hurlbert & Lombardi, 2009).

All statistics and models were fitted using R version 3.5.2 (2018 – 12 – 20, "Eggshell Igloo") for Linux in the RStudio software (Version 1.1.436) (R Core Team, 2018).

RESULTS

The potential basal sources and prey groups are presented in Table 1. The lowest values of $\delta^{13}C$ and $\delta^{15}N$ were observed for particulate organic matter in sediment (SOM) and phytoplankton, respectively, while the highest values were found for Sciaenidae and *Doryteuthis sanpaulensis*, respectively. THg concentrations were lowest for phytoplankton and highest for *Callinectes* sp.

Table 1. Potential basal sources and prey group size range (mm), mean ± standard deviation (SD) of $\delta^{13}C$ (‰), $\delta^{15}N$ (‰) and THg (ng g^{-1} dw) sampled in northern Rio de Janeiro state, Southeastern Brazil.

Basal sources/Prey group	Size range*	$\delta^{13}C$	$\delta^{15}N$	THg
SOM	-	-20.67 ± 0.93	9.03 ± 2.66	-
Phytoplankton**	-	-19.02	7.50	0.02
Zooplankton**	-	-17.68 ± 0.74	8.44 ± 0.12	0.04
X. kroyeri	30.00 – 70.00	-17.19 ± 0.40	11.96 ± 0.21	3.06 ± 1.37
Callinectes sp.	88.00 – 162.00	-16.32 ± 1.44	11.91 ± 0.39	1087.20 ± 290.00
D. sanpaulensis	-	-17.33 ± 0.31	13.25 ± 0.55	2.48 ± 1.17
*P. porosissimus****	180.00	-18.00 ± 0.80	10.60 ± 0.50	630.00
Sciaenidae	125.00 – 175.00	-15.77 ± 0.86	13.20 ± 0.32	171.60 ± 64.65

*For fish species, the size range is standard length; for *X. kroyeri*, the size range is total length; for *Callinectes* sp., the size range is carapace width.
**Composite sample.
***One single specimen.

Overall, 65 muscle samples were analysed for $\delta^{13}C$, $\delta^{15}N$ and THg representing the three ariid species in the present study. For *Aspistor luniscutis*, *Bagre bagre* and *Genidens genidens* similar $\delta^{13}C$ values (Kruskal-Wallis, $X^2 = 4.545$, $P > 0.05$) and C/N ratios (Kruskal-Wallis, $X^2 = 4.029$, $P > 0.05$) were observed (Table 2). The species *A. luniscutis* is the smallest (total length – ANOVA, $F = 26.962$, $P < 0.001$) and lightest (mass – Kruskal-Wallis, $X^2 = 17.610$, $P < 0.001$) catfish species. Nitrogen isotope signatures ($\delta^{15}N$) were similar for *A. luniscutis* and *B. bagre*, while a lower value ($\delta^{15}N$ – ANOVA, $F = 18.089$, $P < 0.001$) was found for *G. genidens*. Total mercury (THg) was significantly different (Kruskal-Wallis, X^2 = 21.892, $P < 0.001$) for all three species, with the highest concentrations in *G. genidens* and lowest concentrations in *B. bagre*.

Table 2. Number of individuals (n), mean ± standard deviation (SD) of total length (mm), total mass (kg), $\delta^{13}C$ (‰), $\delta^{15}N$ (‰), C/N (%) and THg (ng g^{-1} dw) of the marine catfishes *Aspistor luniscutis*, *Bagre bagre* and *Genidens genidens* in northern Rio de Janeiro state, Southeastern Brazil. Letters indicate significant differences ($P < 0.05$).

	Aspistor luniscutis	***Bagre bagre***	***Genidens genidens***
n	25	21	19
Total length	269.85 ± 48.70^a	354.74 ± 49.63^b	353.35 ± 26.65^b
Total length (min – max)	200 – 343	282 – 424	302 – 393
Total Mass	0.229 ± 0.138^a	0.313.83 ± 0.148^b	0.454 ± 0.147^c
Total Mass (min – max)	0.070 – 0.550	0.127 – 0.550	0.132 – 0.700
$\delta^{13}C$	-15.89 ± 0.71^a	-16.16 ± 0.40^a	-15.91 ± 0.45^a
$\delta^{15}N$	14.62 ± 0.28^a	14.62 ± 0.46^a	13.84 ± 0.71^b
C/N	3.18 ± 0.06^a	3.20 ± 0.04^a	3.17 ± 0.06^a
THg	524.58 ± 191.58^a	310.00 ± 175.50^b	693.33 ± 269.07^c

The $\delta^{13}C$ and $\delta^{15}N$ values of the invertebrates *Doryteuthis sanpaulensis*, *Callinectes* sp., *Xiphopenaeus kroyeri* and of the bony fishes *Porichthys porosissimus* and Sciaenidae, all prey items for marine catfishes, ranged from – 18.00 ± 0.80‰ for *P. porosissimus* to – 15.77 ± 1.44‰ for Sciaenidae and 10.60 ± 0.60‰ for *P. porosissimus* to 13.25 ± 0.55‰ for *D. sanpaulensis*, respectively. The $\delta^{13}C$ of organic matter (OM) basal sources (SOM and phytoplankton) and zooplankton ranged from – 20.67 ± 0.93‰ for SOM to – 17.68 ± 0.75‰ for zooplankton while $\delta^{15}N$ values ranged from 7.05 ‰ for phytoplankton to 9.03 ± 2.66‰ for SOM (Figure 2).

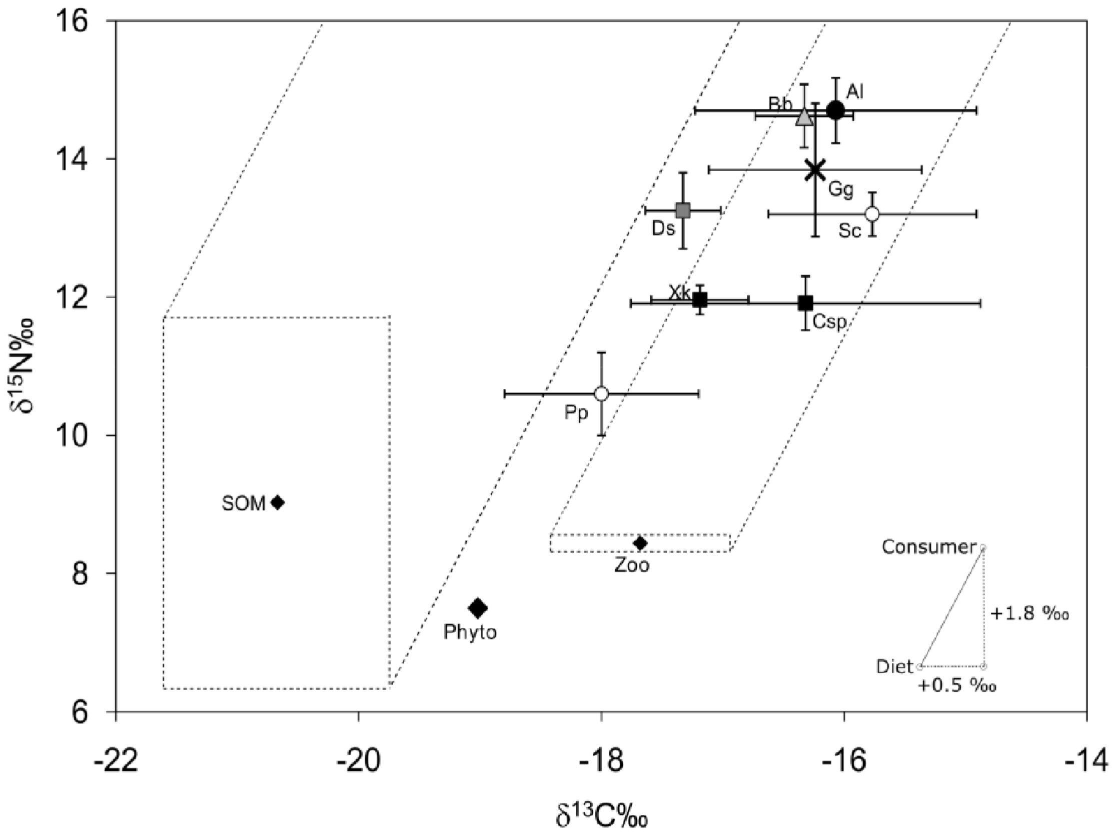

Fig.2. Relationship between $\delta^{13}C$ and $\delta^{15}N$ considering *Aspistor luniscutis* (Al), *Bagre bagre* (Bb), *Genidens genidens* (Gg), *Doryteuthis sanpaulensis* (Ds), *Xiphopenaeus kroyeri* (Xk), *Callinectes* sp. (Csp), Sciaenidae (Sc), *Porichthys porosissimus* (Pp), zooplankton (Zoo), phytoplankton (phyto) and organic matter in sediment (SOM) in northern Rio de Janeiro state, Southeastern Brazil. The range of isotopic ratios measured for the potential SOM and phytoplankton (through zooplankton) sources is represented by small hatched squares. Hatched zones represent the zone influenced by the OM sources.

The $\delta^{15}N$ was significantly correlated with total length and total mass only for *B. bagre* (Figure 3A–B). THg also showed correlations, but with total length, for both *B. bagre* and *G. genidens*, and with total mass, for *B. bagre* only (Figure 3C–D). No correlations between variables were observed for *A. luniscutis*.

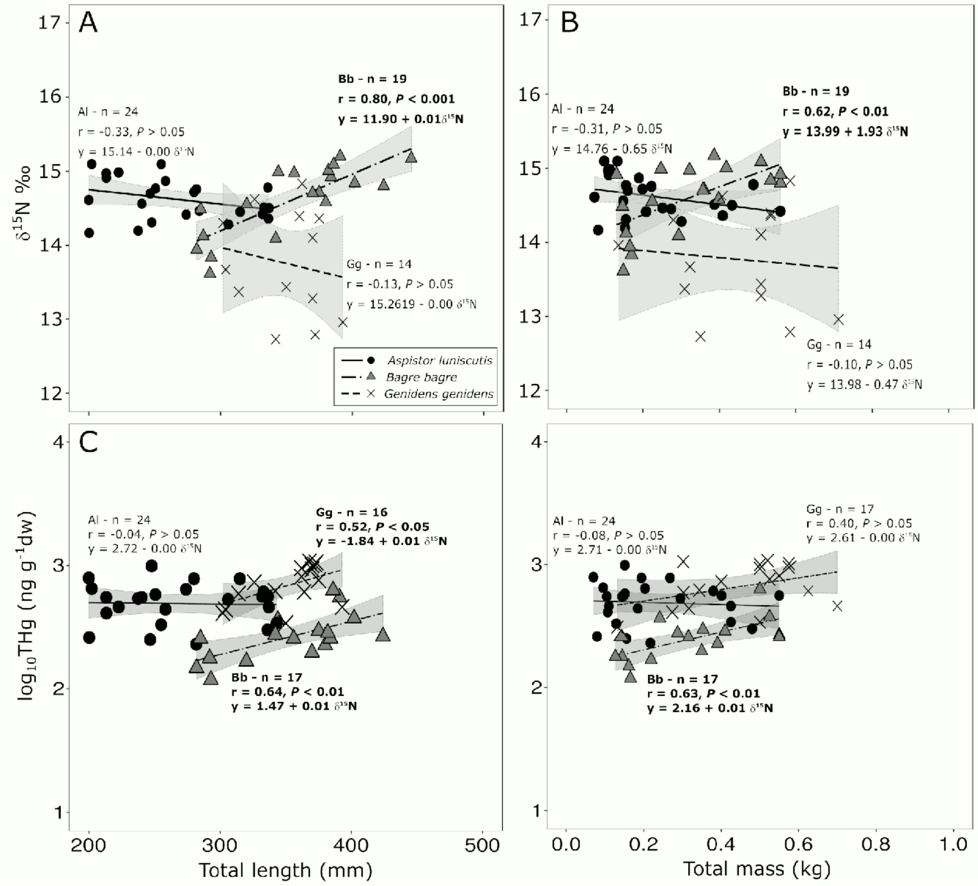

Fig 3. Relationships between: (A) $\delta^{15}N$ (‰) and total length (mm), (B) $\delta^{15}N$ (‰) and total mass (kg), (C) log-transformed total mercury (THg) and total length (mm) and (D) log-transformed total mercury (THg) and total mass (kg) of the three marine catfishes *Aspistor luniscutis* (Al), *Bagre bagre* (Bb) and *Genidens genidens* (Gg) in northern Rio de Janeiro state, Southeastern Brazil. The results from the linear models (lines + 95% confidence interval grey polygons) are plotted on observed log-transformed data. The r and *P*-values of the Pearson correlation as well as the equations of the lines (derived from linear model output) are indicated. Bold values indicate significant (P < 0.05) relationships.

The relationship between $\delta^{15}N$ and THg was highly significant. The slope was 0.34, indicating biomagnification of Hg over the increase in $\delta^{15}N$ in this marine food web (Figure 4).

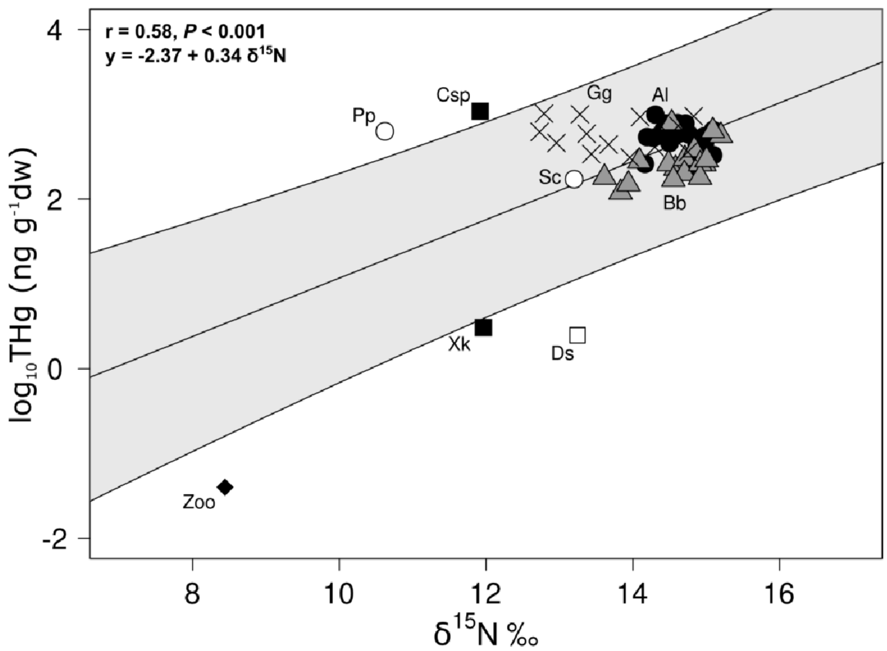

Fig. 4. Relationships between $\delta^{15}N$ (‰) and log-transformed THg considering *Aspistor luniscutis* (Al), *Bagre bagre* (Bb), *Genidens genidens* (Gg), *Doryteuthis sanpaulensis* (Ds), *Xiphopenaeus kroyeri* (Xk), *Callinectes* sp. (Csp), Sciaenidae (Sc), *Porichthys porosissimus* (Pp) and zooplankton (Zoo) in northern Rio de Janeiro state, Southeastern Brazil. The results from the linear model (lines + 95% confidence interval) are plotted on observed log-transformed data. The r and *P*-values of the Pearson correlation as well as the equation of the line (derived from linear model output) are indicated. Bold values indicate significant ($P < 0.05$) relationships.

The isotopic niche area represented by SEAc revealed a broader trophic niche for *G. genidens* (1.88‰²) than for *A. luniscutis* (0.55‰²) and *B. bagre* (0.47‰²) (Figure 5). The greatest isotopic niche overlap was observed for the ellipses of *B. bagre* on *A. luniscutis*, followed by *A. luniscutis* overlapping the ellipse of *B. bagre*, covering an area of 0.29‰^{2}. The lowest SIBER ellipse overlaps were observed for *G. genidens* on *A. luniscutis* and *B. bagre*, with overlapping areas of 0.21‰^{2} and 0.15‰^{2}, respectively (Figure 5, Table 3).

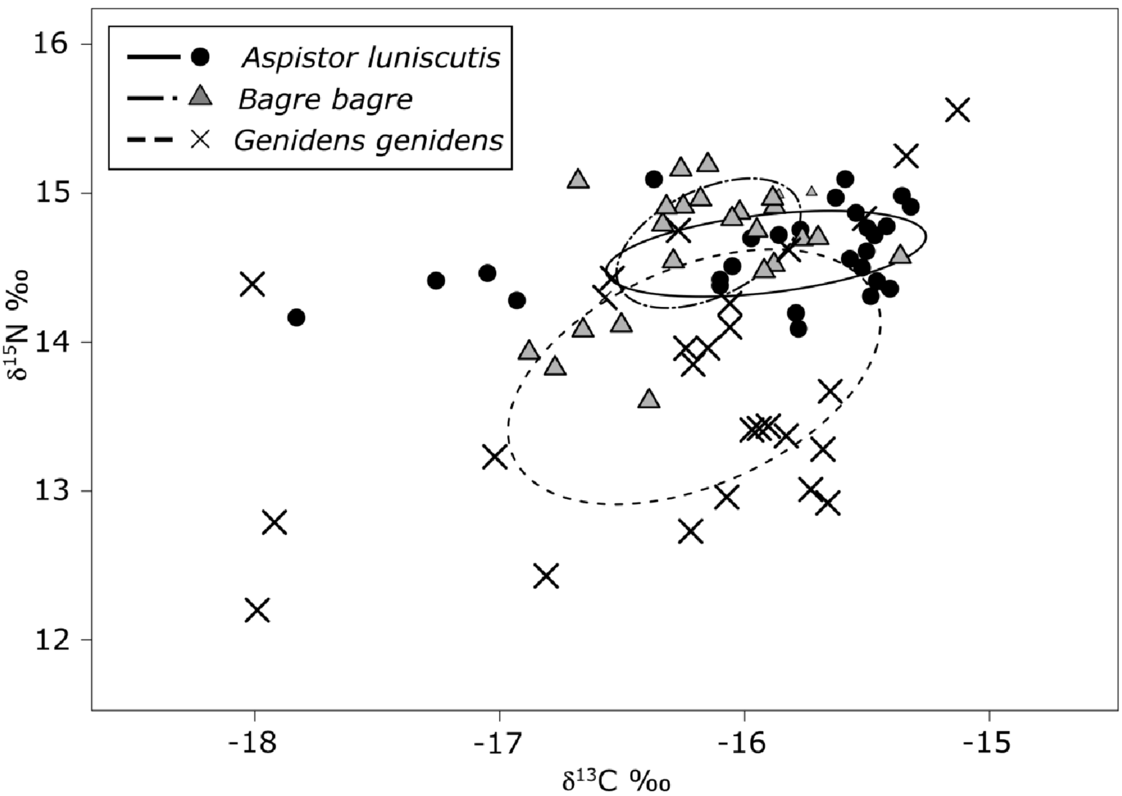

Fig. 5. The standard ellipse areas corrected for small sample size (SEAc) for the three marine catfishes *Aspistor luniscutis*, *Bagre bagre* and *Genidens genidens* in northern Rio de Janeiro state, Southeastern Brazil.

Table 3. Overlapping SEAc (%) between the three marine catfishes *Aspistor luniscutis*, *Bagre bagre* and *Genidens genidens* in northern Rio de Janeiro state, Southeastern Brazil.

	A. luniscutis	***B. bagre***	***G. genidens***
A. luniscutis	-	63.0	10.9
B. bagre	53.3	-	7.7
G. genidens	37.2	31.3	-

Table 4 presents the quantitative metrics to estimate the isotopic niche breadth for the three marine catfishes. The highest values of food web length (NR), variability of food resources (CR), total occupied niche area (TA) and small sample size corrected standard ellipse area (SEAc) were observed for *G. genidens*. Additionally, trophic diversity within the demersal food web (CD - ANOVA, F = 7.515, P = 0.001), trophic redundancy (MNND - ANOVA, $F = 6.842$, $P = 0.001$) and evenness (SDNND - F-ratio test, $P < 0.001$) were higher for *G. genidens* than for *A. luniscutis* and *B. bagre*. Similar to *G. genidens*, the species *A. luniscutis* also presented elevated variability of food resources compared to *B. bagre*.

Table 4. Isotopic niche metrics of the marine catfishes *Aspistor luniscutis*, *Bagre bagre* and *Genidens genidens* in northern Rio de Janeiro state, Southeastern Brazil. Letters indicate significant differences ($P < 0.05$).

	NR	CR	TA	SEAc	CD	MNND	SDNND
A. luniscutis	1.01	2.51	1.65	0.55	0.57^{a}	0.14^{a}	0.14^{a}
B. bagre	1.59	1.52	1.48	0.47	0.49^{a}	0.13^{a}	0.11^{a}
G. genidens	3.36	2.88	6.21	1.88	0.94^{b}	0.32^{b}	0.30^{b}

The SIAR analysis revealed similar relative contributions of all prey items in *A. luniscutis* and *B. bagre*, highlighting higher assimilations of Sciaenidae fishes and *D. sanpaulensis* (73 and 70%, respectively) compared to *G. genidens*, which presented no preference for any prey (Figure 6).

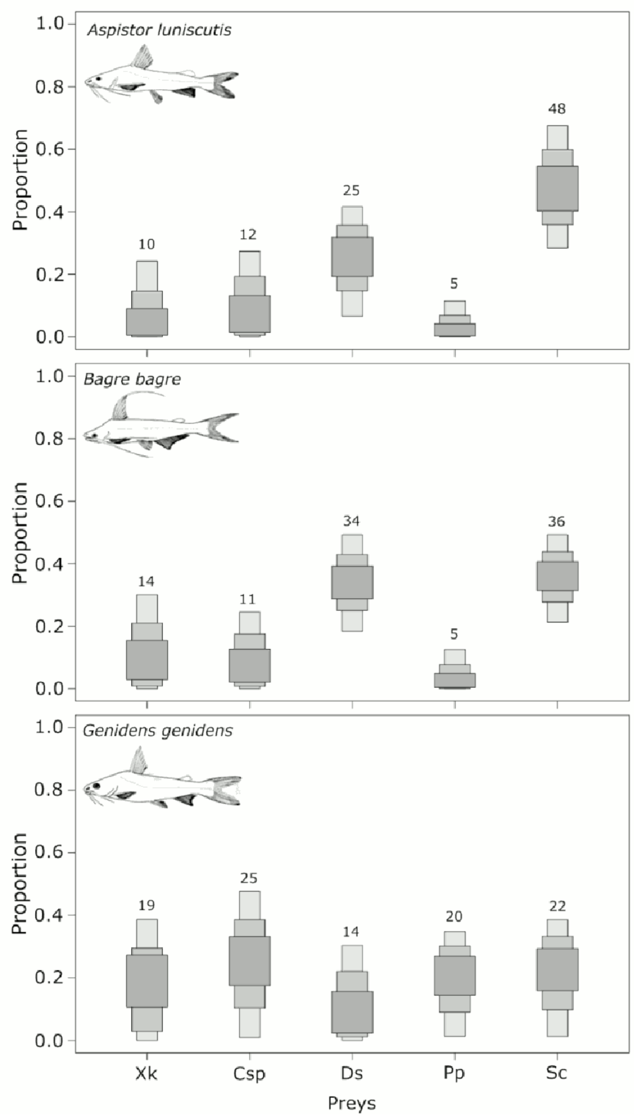

Fig. 6. Results of SIAR (Stable Isotope Analysis in R) showing 95% (dark grey), 75% (intermediate grey) and 25% (light grey) credibility intervals of prey items contributions to the diet of the catfishes *Aspistor luniscutis*, *Bagre bagre* and *Genidens genidens* diets. Xk = *Xiphopenaeus kroyeri*; Csp = *Callinectes* sp.; Ds = *Doryteuthis sanpaulensis*; Pp = *Porichthys porosissimus*; and Sc = Sciaenidae). Numbers above credibility intervals are the percentage of contribution of each prey to the respective predator.

DISCUSSION

This study revealed the extent of sharing and segregating food resources of the three sympatric marine catfish species *Aspistor luniscutis*, *Bagre bagre* and *Genidens genidens* based on comparisons of food assimilation using SIAR, SIBER, isotopic niche breadth metrics and THg concentrations. Diet overlaps observed between the three marine catfish species were explained by proportions in the assimilation of Sciaenidae fishes (*Isopisthus parvipinnis*, *Paralonchurus brasiliensis* and *Stellifer rastrifer*), *Doryteuthis sanpaulensis*, *Xiphopenaeus kroyeri* and *Callinectes* sp.

The present study corroborated using a stable isotope approach to classify marine catfish trophic guilds as omnivorous, as previously determined by stomach contents and stable isotope studies (Chaves & Vendel, 1996; Denadai *et al*., 2012; Tavares & Di Beneditto, 2017; Di Beneditto *et al*., 2018; Di Beneditto & Tavares, 2019). In Southeastern Brazil, Denadai *et al*. (2012) analysed stomach contents and intestine remains in juveniles, sub adults and adults, concluding that marine catfishes have wide trophic plasticity during their lifetimes. Juveniles feed mainly on soft and small food items, such as algae and molluscs; adults forage on harder and larger items, such as crustaceans, molluscs and fishes; and sub-adults feed on both prey types.

The SIBER analysis revealed overlaps between the isotopic niches of *A. luniscutis*, *B. bagre* and *G. genidens*. The greater overlaps (> 53%) between the isotopic niches of *A. luniscutis* and *B. bagre* indicate that these two species rely on similar prey proportions of assimilation, specifically on Sciaenidae and *D. sanpaulensis*, as observed by SIAR analysis. The species *A. luniscutis* and *B. bagre* presented lower trophic diversity within the demersal food web (CD) and higher trophic redundancy (MNND) and evenness (SDNND) when compared to *G. genidens*, reinforcing its more restricted assimilation of prey resources. Based on isotopic niche metrics, *G. genidens* showed the broadest niche breadth among the catfishes, as evidenced by the highest isotopic niche metrics observed (NR, CR, TA, SEAc, CD, MNND, SDNND). Similar to what other authors found through stomach content analysis (Denadai *et al*., 2012), the isotopic data suggest that diet of *G. genidens* is the most diversified, based on the higher proportion of assimilation of *Porichthys porosissimus* and *Callinectes* sp. in the PSR estuary compared to *A. luniscutis* and B. *bagre*.

The higher trophic plasticity of *G. genidens* and the similar isotopic niches of *A. luniscutis* and *B. bagre* may be a result of three situations, individually or combined: 1. the high abundance of shrimps, crabs and neritic squids in the study area; 2. different spatial habitat usage by catfishes in the estuarine system; and 3. foraging of other prey items to avoid competition.

Situation 1 can be sustained, as lolignid squids (*Doryteuthis plei* and *D. sanpaulensis*) have a strong association with the sea bottom and are considered abundant in the inner shelf from 10 to 50 m (Robin *et al*., 2014; Costa *et al*., 2015). A short distance south (100 km), in the Cabo Frio upwelling region, Soares *et al*. (2014) found similar $\delta^{15}N$ values (higher than for other invertebrates of the present study) of loliginid species (*D. plei* – 12.5‰ and *D. sanpaulensis* – 11.0‰) compared to ours results (13.3‰). Shrimps (*Xiphopenaeus kroyeri*) and crabs (*Callinectes* sp.) are considered to be as abundant as squids on the northern coast off Rio de Janeiro (Di Beneditto *et al*., 2010; Santos & Menegon, 2010; Fernandes *et al*., 2014). Thus, the local abundance of prey is high to consumers. Nevertheless, the mean $\delta^{15}N$ values of decapods (*Callinectes* sp. and *X. kroyeri*) and *D. sanpaulensis* are higher than that of *P. porosissimus* (Table 2), reflecting the nitrogen isotope values of the consumers. In this sense, it is noteworthy that the $\delta^{15}N$ values of the catfishes represented differences in their prey isotopic values rather than in their trophic levels.

Denadai *et al*. (2012) observed a slight overlap between *A*. *luniscutis* and *G*. *genidens* because of crustacean fragments and fish scales in both diets and associated the small overlap (37.6%) with differences in spatial usage of the estuary. However, situation 2 may not be plausible. Pereyra *et al*. (2016) found that *G. genidens* adults consumed freshwater-derived carbon sources, and Bizerril (1999) also observed spatial differences governed by salinity in the habitat usage of marine catfishes, allowing their coexistence. The stable isotopes of the present study revealed no variation in habitat usage. The $\delta^{13}C$ results were similar among the three ariids, suggesting not only the absence of distinctions in basal OM assimilated sources but also similarity in habitat usage (Bouillon *et al*., 2011). Nevertheless, our $\delta^{13}C$ values are in accordance with the previous study of Di Beneditto *et al.* (2018), reinforcing that marine catfishes are characteristically widespread in the coastal waters influenced by the Paraíba do Sul river (PSR).

Although the focus of the present study was on trophic ecology rather than habitat usage variations, the THg concentrations observed in the present study coupled with previous knowledge of ariids suggesting different spatial usage in estuaries (Azevedo *et al.*, 1999; Bizerril, 1999; Schmidt *et al.*, 2008; Denadai *et al.*, 2012), can lead to misunderstandings about their spatial dynamics in the estuarine habitat. Regarding THg, a decrease in its concentration in fishes along the river-ocean gradient is expected (Liu *et al.*, 2017). As adult catfishes usually live in adjacent coastal waters influenced by estuaries (Mishima & Tanji, 1983; Denadai *et al*., 2012) and only seek riverine waters during the spawning period (Schmidt *et al.*, 2008), the differences in THg concentrations between marine catfishes observed in the present study seem to be related to biological factors, not to spatial segregation.

Previous studies developed along this area support the Hg assimilation related to predator-prey interactions (Carvalho *et al*., 2008; Kehrig *et al*., 2009; Di Beneditto *et al*., 2012), suggesting that the past practices of gold-mining and the use of mercurial fungicides on sugarcane plantations in the PSR drainage basin are the main input sources of Hg in this fluvial ecosystem, which is largely exported to the adjacent marine areas, coupled with Hg atmospheric deposition (Lacerda *et al*., 1993; Lacerda, 1996; Araujo *et al.*, 2017; Azevedo *et al.*, 2018). Reinfelder *et al*. (1998) indicated that the assimilation of dissolved Hg in the water column is an important route for bioaccumulation by aquatic organisms with small body sizes and greater surface areas. The input of Hg in food webs through phytoplankton and the successive increase in metal concentrations to the next trophic levels (Pickhardt *et al*., 2002) were already observed in coastal areas influenced by PSR in other fish species (Di Beneditto *et al.*, 2012; Kehrig *et al*., 2013).

The reasons for the elevated mean THg values observed in larger ariid individuals seem to be three-fold: first, it is related to the bioaccumulation of THg during the catfish lifespan, evidenced by the significant correlations ($P < 0.05$) between THg and total length and mass. The accumulation might occur in ariids in the adjacent marine waters of the PSR estuary in accordance with the findings of other authors that suggest that larger and/or older fish have higher THg concentrations due to longer exposure and the difficulty of eliminating mercury, as THg has a very slow excretion rate due to the metal's high affinity for thiol groups that constitute the protein fraction of the muscles (Nakao *et al.*, 2007). Second, the elevated assimilation of species closely related to the bottom sediment, that provides higher Hg bioavailability than water column (Muto *et al*., 2014; Le Croizier *et al*., 2019), by *G. genidens* (*Callinectes* sp. - highest THg in prey and *P. porosissimus* - highest THg in prey fishes) and *A. luniscutis* (Sciaenidae fish) resulted in the higher THg than in *B. bagre*. Finally, the seasonality of the PSR discharge (input of Hg to the ocean) may also be an influencing factor. As observed by Rocha *et al*. (2015), at times of higher PSR discharge, *G. genidens* and *A. luniscutis* were more abundant, thus contributing to higher mean THg values for both species compared to *B. bagre*. At times of lower PSR discharge, *B. bagre* is more abundant; thus as suggested by authors worldwide and for PSR estuary, during low river discharge the main source of Hg is the direct deposition of the contaminant from the atmosphere to surface waters, resulting in higher concentrations of THg in fish due to the rapid methylation and absorption by biota, becoming more bioavailable (Harris *et al*., 2007; Mason *et al*., 2012; Araujo *et al*., 2017; Azevedo *et al*., 2018).

Biomagnification was also indicated by the significant correlations of THg and $\delta^{15}N$. Since diet is the main source of THg assimilation in fishes (Hall *et al*., 1997), it might be occurring in this food web, as already observed for other fish species influenced by the PSR plume in the region (Bisi *et al*., 2012; Di Beneditto *et al*., 2012; Kehrig *et al*., 2013) and worldwide (Al-Reasi *et al*., 2007; Liu *et al*., 2017; Chouvelon *et al*., 2018). In the present study, the slope of the linear regression between THg and $\delta^{15}N$ (0.34), used as measurement of biomagnification across food webs (Kidd *et al*., 1995), was higher than those observed by Al-Reasi *et al*. (2007) in Gulf of Oman (0.07), Muto *et al*., (2014) in the Santos continental shelf (0.13), and Di Beneditto *et al*. (2012) (0.25) and Kehrig *et al*. (2013) (0.21) in the same region as our study. Although biomagnification studies in tropical marine ecosystems are limited, this range (0.07 to 0.34), already observed by Lavoie *et al.* (2013) in tropical marine food webs, reflects the different composition and vertical position in the water column (higher Hg bioavailability in the bottom sediment for benthic/demersal organisms) of the tropical food webs and/or differences in the growth rate of organisms (Muto *et al*., 2014; Le

Croizier *et al*., 2019). It is already known that the biomagnification of Hg can vary greatly between marine ecosystems (Cossa *et al*., 2012), or even between environments of the same ecosystem (Cresson *et al*., 2015).

Competitive exclusion (situation 3) seems not to regulate the preference of Sciaenidae fishes and *D. sanpaulensis* by *A. luniscutis* and *B. bagre*. As mentioned above, Rocha *et al*. (2015) observed *A. luniscutis* and *G. genidens* in the region mainly during the rainy season (higher discharge of PSR - October to April), while *B. bagre* was observed during the dry season (lower discharge of PSR - May to September). In this sense, the elevated level of partitioning of resources between *A. luniscutis* and *B. bagre*, reflected by high isotopic niche overlaps, seems to exclude none of the species. Thus, based on the high abundance of prey coupled with the different seasonal abundances in the region of the catfishes, it seems they are not competing for the same resources, at the same time, in the same place, leading us to conclude that there is low evidence of interspecific resource competition.

In conclusion, we found that *G. genidens* has a broader trophic niche breadth, is considered generalist and is the most opportunistic marine catfish, due to assimilation of organisms in similar proportions and with lower $\delta^{15}N$. The prey preference for Sciaenidae fishes and *D. sanpaulensis* was observed for *A. luniscutis* and *B. bagre*, resulting in the highest isotopic niche overlap and $\delta^{15}N$ signatures, answering the first question of the present study. The coexistence of these three sympatric marine catfishes is probably regulated by different temporal patterns in the coastal area influenced by the PSR plume (question 2) and the high abundance of prey items, leading to resource partitioning with no evidence of competition among the species (question 3). The THg highlighted the different proportions of assimilation of prey as well as the seasonality in the habitat usage by the species. Bioaccumulation of Hg was observed for *B. bagre* and

G. genidens and Hg biomagnification also occurred in their food web. The understanding of the trophic dynamics of sympatric fishes enhances the knowledge of ecological and environmental forces driving their coexistence. More studies incorporating multiple tools are important for the advancement of knowledge of the trophic dynamics, life cycles, habitat usage and environmental role of marine catfishes in the continent-ocean interface.

ACKNOWLEDGEMENTS

We are grateful to DSc. Bruno P. Masi and MSc. Thiago P. Rangel for diving and geoprocessing, respectively. The authors thank the American Journal Experts (AJE) for the English revision of the manuscript. Finally, we would like to express our thanks to Dr. Vlad Laptikhovsky and the anonymous reviewers for their comments and suggestions on the document.

FINANCIAL SUPPORT

This research was supported by Fundação de Amparo à Pesquisa do Rio de Janeiro – FAPERJ (Rezende CE, grant number E-26/110.437/2010) and by Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (INCT Material Transfer at the Continent-Ocean Interface, Rezende CER, grant number 573.601/08-9; Zalmon IR, grant number 470997/2010-9). Likewise, this research study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001.

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Capítulo 2 – a ser submetido para Estuarine, Coastal and Shelf Science

Demersal assemblage structure and dynamics off the southwestern Atlantic continental shelf, north of Campos Basin, Brazil.

Gatts, P.V.[a] *, Franco, M.A.L.[b], Rezende, C.E.[b], Costa, P.A.S.[c].

[a] Instituto de Geociências, Universidade Federal Fluminense, Programa de Pós Graduação de Dinâmica dos Oceanos e da Terra, Niterói RJ CEP 24210-346,Brazil. pedrogatts90@gmail.com.
[b] Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes RJ CEP 28013-602,Brazil.malfranco@yahoo.com.br. crezende@uenf.br.
[c] Departamento de Ecologia e Recursos Marinhos, Instituto de Biociências, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro RJ CEP 22290-240, Brazil. paulocosta.unirio@gmail.com.

ABSTRACT

The demersal nekton assemblage in the northern continental shelf of Campos Basin was accessed with focus on its seasonality in the dry and rainy seasons. Overall, 63 species belonging to four main taxa were identified: teleosts were represented by 54 species, crustaceans by four species, elasmobranchs by three species and cephalopods by two species. Teleosts, elasmobranchs and cephalopods represented 94% of the 63 taxa. Several species (43) were recorded in only one season, and the species accumulation curves reached asymptotic values for biomass, with higher number of species in the rainy season. The 10 most important species contributed 82% to the global similarity between seasons and were classified in three trophic guilds: bentophagus, bentophagus-nektophagus and nektophagus. Bentophagus species were the dominant trophic guild, regardless seasonality. The relationships between the demersal assemblages with (C:N)*a* and DOC were most important in the dry season, whereas with temperature and PSR discharge in the rainy season. This study provides information about seasonal

environmental influences on the dominant demersal nekton assemblage species related to terrigenous and upwelling nutrient supply into the shelf shallow tropical waters.

Keywords: megafauna; seasonality; nekton; organic nutrients.

INTRODUCTION

The marine demersal nekton represents a broad group of organisms that lives on or close to the bottom and usually swims near the ocean floor (Gordon, 2009; Pearcy and Brodeur, 2009). It is one of the main fishing targets in the world since the beginning of 1900s due to bottom trawling activity (Watson and Tidd, 2018). In Brazil, the Southeastern region is responsible for 15.8% of the total production of marine fisheries (553,670 tons), with Rio de Janeiro state representing in 2011 the 3rd position (78,933 tons) in fish production of the country (ICMBIO, 2011). From 2012 to 2015 the mean landings of marine fishes of Rio de Janeiro state coast depleted from 90,000 tons to 60,684 tons, respectively, with demersal fishes accounting for a total of 1,838 tons landed in 2015 (FIPERJ, 2015).

Several aspects of demersal assemblages are studied all over the world, such as spatial patterns (Peristeraki et al., 2017), interannual variations including climate change effects (Dulvy et al., 2008; Queirós, 2018), fishing impacts (Labropoulou and Papaconstantinou, 2004), as well as trophic dynamics (Schaal et al., 2016) and seasonality (Martins and Haimovici, 2017). The knowledge of the processes that drive the distribution and dynamics of biological assemblages is essential to evaluate its structure and, consequently, in predict scenarios related to global change and anthropogenic influence (Guisan and Rahbek, 2011; Coll et al., 2012). Variations in richness and diversity coupled with species attributes, such as trophic guilds approach (*i.e.* groups of organisms with similar feeding preferences and food webs; de Sylva, 1975), generates information of the organization and dynamics of demersal assemblages within and between habitats and ecosystems, as well how organisms interact with the environment (Godoy et al., 2002; Elliot et al., 2007; Bosman et al., 2011).

In Campos Basin (CB), southeast Brazil, the knowledge about the demersal nekton off the continental shelf is historically documented in correlation to depth gradients (Costa et al., 2015) and seasonal dynamics (Rossi-Wongtshowski and Paes, 1993; Haimovici et al., 1994; Pinheiro et al., 2009; Martins and Haimovici, 2017). Since 1990s Sciaenid species are reported as predominant in the shallow coastal waters (0 to 30 m) (Fagundes-Netto and Gaelzer, 1991; Di Beneditto et al., 1998; Pinheiro et al., 2011). Along the bathymetry gradient a community of fishes and cephalopods are reported as representative and occurring all over the year (Fagundes-Netto and Gaelzer, 1991; Costa et al., 2015).

In the northernmost area of CB shelf, the intrusion of the cold and nutrient-rich South Atlantic Center Water (SACW) (Calado et al., 2010; Palóczy et al., 2014) is the major enrichment factor for primary producers and, consequently, for demersal nekton assemblages (Carbonel and Valentin, 1999; Quintana et al. 2010). Secondly, the effect of continental river discharge resultant from the estuary of Paraíba do Sul River (PSR) (Souza et al., 2010; Rudorff et al., 2011) off the continental shelf, also affects the hydrochemistry of the environment. In this sense, the combination among several environmental parameters (depth, temperature, transparency, salinity, nutrients, grain size) along with biological interactions (competition, predation) of organisms usually determines assemblage spatial and seasonal distributions (Muto et al., 2000; Azevedo et al., 2007; Costa et al., 2015).

An increasing number of studies provided information of the structure and dynamics of nekton assemblages in CB associated with environmental parameters, such as depth, temperature, salinity, dissolved oxygen and sediment grain size (Fagundes-Netto and Gaelzer, 1991; Costa et al., 2007; Costa et al., 2015; Haimovici et al., 2017; Martins et al., 2017; Mincarone et al., 2017; Rotundo et al., 2019). However, researches investigating the relationships of the demersal nekton with a broader set of environmental parameters are still scarce.

The present study aims to determine the structure of the demersal nekton assemblages off the northernmost CB continental shelf (≤50 m depth). Additionally we relate a broader set of environmental parameters with trophic behaviour of the species. This was the first study to describe the relationship of demersal nekton assemblages with continental PSR and marine upwelling nutrient supplies in the region.

The following three hypothesis were tested: 1- even after 32 km from the river the PSR influences much beyond the plume boundary and achieve a much greater area in marine bottom waters reflecting on higher conductivity, temperature, dissolved organic carbon, total nitrogen and chlorophyll in the rainy season; 2- the terrigenous nutrient inputs from PSR coupled with the intrusion of nutrient-rich upwelling waters of SACW increase the variability of nutrients in coastal marine waters leading to an increase in richness and diversity of demersal nekton in the rainy season; and 3- due to the riverine input in coastal marine waters the demersal nekton assemblage composition shifts between dry and rainy seasons.

MATERIAL AND METHODS

Study site

This study was performed on the continental shelf of Campos Basin, in the southwest Brazilian coast. The region is characterized by two seasons (Ovalle et al., 2013), considering the rainfall in the drainage basin of the Paraíba do Sul River (PSR). Almeida et al. (2007) linked rainfall seasons to the discharge rates of the river and classified as: rainy season (October to April), with higher rainfall and discharge; and dry season (May to September), with lower rainfall and discharge. The PSR discharge is measured twice a month all over the year since 1995 by the Laboratório de Ciências Ambientais from Universidade Estadual do Norte Fluminense Darcy Ribeiro. In Figure 1, PSR discharges from each sampling month September (2015), July (2016) and April (2017) as well from 1995 to 2017 is represented.

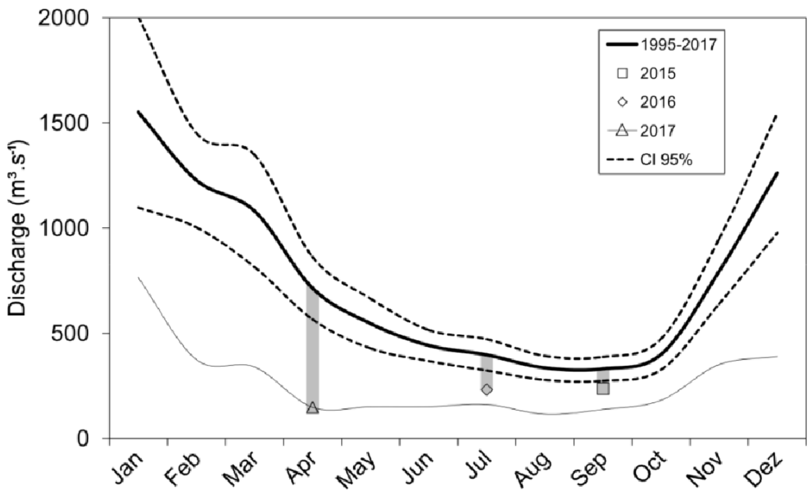

Figure 1. Discharge of PSR during the time series of 1995 - 2017 and the sampling periods on 2015, 2016 and 2017. Highlighted in grey are the months when sampling was performed. CI: confidence interval. Source: Laboratório de Ciências Ambientais – Universidade Estadual do Norte Fluminense Darcy Ribeiro.

The PSR drains an area of 57,300 km² in the states of São Paulo, Minas Gerais and Rio de Janeiro and is located between the latitudes of 20°26' and 23°38'S and the longitudes of 41°00' and 46°30'W. The total length of the river channel is approximately 1,150 km (Ovalle et al., 2013). The Paraíba do Sul estuary is located at the coastal plain formed by the PSR delta in the North of Rio de Janeiro state, near São João da Barra, and is the major river in the hydrographic basin of this region.

Souza et al. (2010) observed the mixture of the continental PSR with coastal marine waters and showed that the water mixture ratio in the dry season ranges from 23 to 38 $km^2\ d^{-1}$, whereas in the rainy season, it ranges from 65 to 68 $km^2\ d^{-1}$. Still, seasonality of environmental parameters is usually observed off the coastal waters influenced by PSR. While temperature, salinity and organic nitrogen are higher in the rainy season, dissolved organic carbon (DOC) and (C:N)*a* are lower (Zalmon et al., 2013, 2015; Suzuki et al., 2015).

The PSR drains one of the most populated region of Brazil – the southeast (ANA, 2018). Studies evaluating the PSR influence off the northern coast of Rio de Janeiro point that the plume can reach 32 km in the ocean from the river mouth (Souza et al., 2010). However, its real influence must be greater than in surface water, reaching more extensive areas and depths for other parameters, such as phytoplankton (Rudorff et al., 2011) and nutrients (Carter, 1990), and consequently the organisms that depends on these sources.

Along the continental margin in the southeast Brazil (20°S to 28°S), there is also a constant coastal current-eddy-upwelling systems that develops in the vicinities of Cabo de São Tomé (22°S) and Cabo Frio (23°S) (Calado et al., 2010), that is intensified during summer, when the dominating regime of northeastern wind induce the subsurface shoreward intrusion of oceanic South Atlantic Central Waters (SACW). This process results on the enhancement of productivity in both pelagic and benthic systems and the availability of resources for demersal fish and shellfish stocks (Borzone et al., 1999; Sumida et al., 2005; Rossi-Wongtschowski et al., 2006).

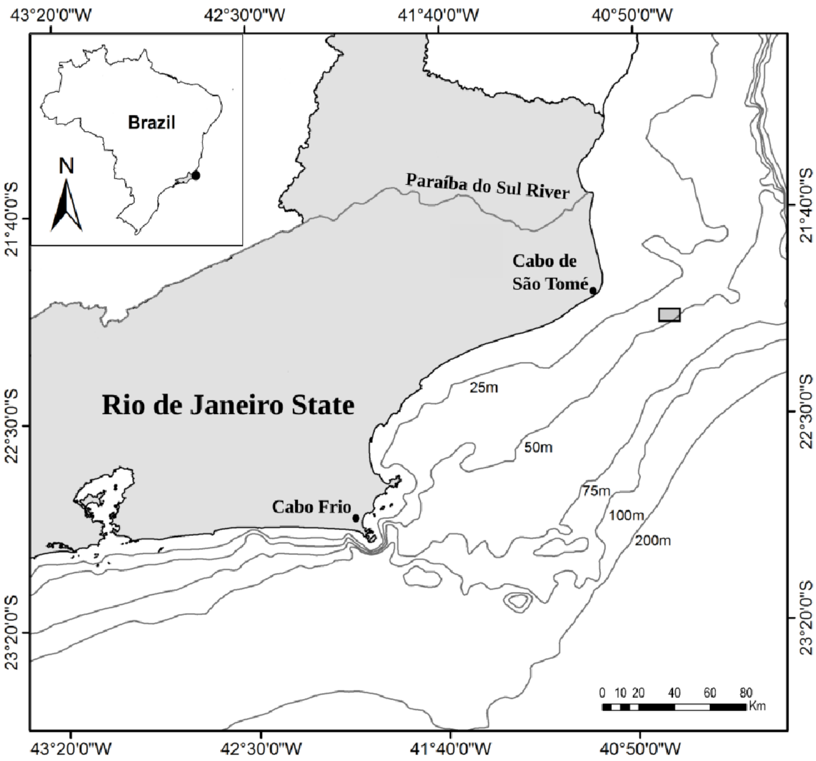

Figure 2. Study site at the continental shelf in southwestern Atlantic on Campos Basin, Brazil. The grey rectangle represents the sampling area.

Sampling

The epibenthic megafauna, including fish, cephalopods and decapod crustaceans, was sampled with bottom trawls performed over sandy and muddy bottoms off the northernmost Campos Basin (Figure 2). The trawl was a 12 m ground rope, 1.2 m height and 15 mm stretch mesh in the cod-end, towed by a single warp cable aboard the fishing boat *Karen I* (12 m, 156 HP), specifically rented for the samplings.

Three bottom trawls were performed at the same study site on the continental shelf, during September (2015), July (2016) and April (2017), representing the dry season and the end of the rainy season respectively. A total of nine trawls were performed between 43 and 56 m, with a mean speed of 3.0 knots, 20 minutes each, corresponding to a triplicate on each sampling period.

All specimens (fishes, cephalopods and decapods crustaceans) were identified using regional specific literature (Figueiredo, 1977; Figueiredo & Menezes, 1978; 1980; FAO, 2002a,b). The specimens were maintained in low temperatures on ice, and transported to the laboratory and kept frozen up to biometry and weighting to the nearest mm and 0.1kg for total length (Lt) and total weight (Wt), respectively.

Previously to the bottom trawls, depth was recorded and sediment and environmental parameters were collected. Sediment was collected with a steel corer of 20 cm of diameter and 40 cm of height on each bottom trawl for grain size analysis. A sub-aliquot of each sediment sample (< 2.0 mm) was used for grain size analysis. The determination of the grain size was measured through a particle analyzer by laser diffraction (*Shimadzu* – SALD-3101) in several fractions of the Wentworth scale (Suguio, 1973). Organic carbon of sediment organic matter (C_{org}SOM) was estimated by Total Organic Carbon analyzer (TOC-V CPH).

Samples from the bottom water were collected with a *Van Dorn* bottle in order to record pH, temperature (T), turbidity (Turb), dissolved oxygen (DO), conductivity, dissolved organic carbon (DOC), total nitrogen (TN), atomic ratios of carbon and nitrogen ratio (abbreviated as (C:N)*a*), organic carbon of suspended particle matter (C_{org}SPM) and chlorophyll *a* (Chl *a*). On the field, pH was measured with a peagameter YSI 60 – 10FT (0.001), the turbidity with a turbidimeter LaMotte 2020WE (0.01), the temperature and dissolved oxygen with an oxymeter YSI 55 – 12FT (0.01) and conductivity with a conductivimeter YSI EcoSense EC300A (0.01). Bottom water samples were maintained in low temperatures and transported to the laboratory to measure DOC, TN, (C:N)*a*, C_{org}SPM and Chl *a* in triplicates for each sampling period and later analyzed by a Total Organic Carbon analyzer (TOC-V CPH), a coupled measurer of total nitrogen unity (TMN – 1) and a fluorimeter (Turner Designs TD-700), respectively.

Data of the average monthly discharge of PSR in the region were obtained from the Laboratório de Ciências Ambientais, Universidade Estadual do Norte Fluminense Darcy Ribeiro. All the environmental parameters were correlated to the sampling periods. All water and sediment samples were kept on ice until the transport to the laboratory. Water samples were kept refrigerated and the sediment samples frozen until its respective processing.

Data analysis

The percentage proportions of each fraction of the sediment were determined and classified according to the diameter grain size fractions as follows: silt+clay ($d < 0.06$ mm), fine sand ($0.06 < d < 0.2$ mm), medium sand ($0.2 < d < 0.6$ mm) and coarse sand ($0.6 < d < 2$ mm). Normality and homoscedasticity of grain size fractions were tested by Shapiro-Wilk and Kolmogorov-Smirnoff tests, respectively, at a level of significance of 5 %. One-Way ANOVA was applied followed by Tukey HSD test.

For all other environmental parameters (depth, temperature, conductivity, discharge, turbidity, pH, DO, DOC, TN, (C:N)*a*, C_{org}SOM, C_{org}SPM and Chl *a*) mean and 95% confidence intervals were calculated for dry and rainy seasons. The t-student test was estimated and seasonal differences were observed when *p* level was lower than 5%.

The total number of individuals (N), total weight (Wt), frequency of occurrence (FO) and total length (Lt) were recorded for all nekton species. Biomass was converted to weight per hour (kg h^{-1}). The relative weight of the species was elected to describe the assemblage's attributes, since biomass represents better the functional aspects of communities and, thus, adequate for the trophic guild approach. The species that accumulated more than 80% of total biomass and occurred in more than 20% of the trawls were used to describe the structure of the demersal assemblage and classified into the following trophic guilds, as determined by Martins et al. (2017): bentophagus (benthic invertebrates representing > 80% of the diet); bentophagus-nektophagus (benthic invertebrates representing > 50% and fish and cephalopods representing > 20%); and nektophagus (fish and cephalopods representing > 80% of the diet).

Species rank curve were used to compare evenness of log transformed numbers and weight related to species richness. Rarefaction curves were estimated to assess the expected number of species as a function of the accumulated assemblage biomass. Each point of the curve is a result of a mean based on sample randomization, and confidence intervals of 95% were attributed for each curve (Gotelli and Colwell, 2010).

Hierarchical cluster analysis, based on Bray-Curtis similarity (Clarke & Warwick, 2001) of the weight catch per unit effort (CPUE in kg h^{-1}) of the most frequent and abundant species was used to evaluate the temporal distribution of demersal nekton assemblage. Species were selected for analysis with basis on its biomass (accumulated 80%) and occurrence (at least 20%).

Analysis of similarities (ANOSIM) was used to determine significant differences between the groups (Clarke, 1993), obtaining a global R value and considering groups as highly separated if $R > 0.75$, overlapped if $R > 0.5$ and poorly separated if $R < 0.25$ (Clarke et al. 2014). Identification of species responsible for dissimilarity between seasons was estimated with similarity percentage analysis (SIMPER).

The relationships between nekton assemblage structure with the environmental variables that varied significantly between dry and rainy seasons in the prior t-student test (temperature, PSR discharge, DOC, (C:N)*a*, C_{org}SOM, coarse and medium sand) were elucidated using canonical correspondence analysis (CCA) (ter Braak, 1986) based on the CPUE (kg h^{-1}) of the most frequent (> 30%) and abundant species (> 80%). A Monte-Carlo randomization test (999 permitations) (McCune and Grace, 2002) was used to assess the probability of the observed pattern being due to chance. Besides cluster analysis, estimated with Primer 6.0 statistical software (Clarke and Warwick, 2001), all statistics were estimated with R 3.5.0 "*Joy in playing*" for Windows (R Core Team 2018) using vegan package (Oksanen et al., 2019).

RESULTS

Environmental variables

Details of the environmental parameters collected at the two sampling seasons are listed in Table 1. Temperature, DOC, (C:N)*a* and C_{org}SOM presented significant variations between dry and rainy seasons. While temperature was significantly higher in the rainy season, DOC, (C:N)*a* and C_{org}SOM were higher in the dry season. For depth, conductivity, turbidity, pH, DO, TN, C_{org}SPM and Chl*a* there was no significant differences. The sediment was heterogeneous between the seasons. While it was mainly composed by medium sand in the dry season (ANOVA, $F = 63.9$, $P < 0.05$), the coarse sand grain size predominated in the rainy season (ANOVA, $F = 24.5$, $P < 0.05$). In these sense, neglecting the first hypothesis.

Table 1. Mean ± 95% confidence interval and *P* level values of the environmental parameters measured in the dry and rainy seasons in the continental shelf of Campos Basin, southwestern Atlantic. Bold values mean significant seasonal differences ($P < 0.05$).

	Dry (Sep/15 and July/16)	Rainy (April/17)	*P* level
Depth (m)	49.83 ± 6.51	49.67 ± 5.58	0.972
Temperature (°C)	22.08 ± 0.48	25.83 ± 0.13	**0.003**
Conductivity (mS cm^{-1})	53.57 ± 0.24	53.40 ± 0.71	0.544
PSR discharge (m^3 s^{-1})*	200.17 ± 41.87	244.00 ± 108.63	0.522
Turbidity (NTU)	0.86 ± 0.04	1.70 ± 0.90	0.208
pH	7.67 ± 0.47	8.09 ± 0.16	0.221
DO (mg L^{-1})	7.74 ± 0.22	7.84 ± 0.42	0.723
DOC (mg L^{-1})	2.00 ± 0.09	1.50 ± 0.11	**0.002**
TN (mg L^{-1})	0.16 ± 0.02	0.20 ± 0.05	0.199
(C:N)*a*	13.58 ± 0.23	7.53 ± 1.43	**0.012**
Chl*a* (µg L^{-1})	1.15 ± 0.89	1.36 ± 0.58	0.730
Coarse Sand (%)	26.27 ± 13.95	54.90 ± 16.80	**0.052**
Medium Sand (%)	59.77 ± 11.18	36.23 ± 14.99	**0.058**
Fine Sand (%)	8.44 ± 4.61	2.85 ± 1.76	0.068
Silt-Clay (%)	5.53 ± 4.55	5.44 ± 4.62	0.975
C_{org}SOM	0.33 ± 0.03	0.24 ± 0.02	**0.007**
C_{org}SPM	5.97 ± 1.70	5.46 ± 1.81	0.992

DO: dissolved oxigen; DOC: dissolved organic carbon; TN: total nitrogen; (C:N)*a*: atomic ration of carbon and nitrogen; Chl*a*: chlorophyll *a*; C_{org}SOM: organic carbon of sediment organic matter; C_{org}SPM: organic carbon of suspended particle matter.* Source: Laboratório de Ciências Ambientais of Universidade Estadual do Norte Fluminense Darcy Ribeiro.

Assemblage composition and structure

The total catch in numbers was 2,239, consisting mainly of bony fishes (1,920) followed by cephalopods (141), elasmobranchs (126) and benthic crustaceans (52) (Table 2). The total weight of fish and cephalopods and benthic crustaceans were 137.7 and 0.7 kg, respectively. Teleosts (Actinopterygii) were represented by 54 species. The other groups included a smaller number of species: elasmobranchs (3), crustaceans (4) and cephalopods (2). Most taxa (43) were recorded exclusively on a single season. The other 23 taxa (34.85%) were observed in both dry and rainy seasons.

The six teleost species (*D. volitans*, *P. brasiliensis*, *Lophius gastrophysis*, *Prionotus nudigula*, *S. caribbaea* and *D. auriga*), three elasmobranchs (*Z. brevirostris*, *Atlantoraja cyclophora* and *A. platana*) and one octopuses species (*Octopus vulgaris*) comprised 90.38 % and 79.01% of biomass in dry and rainy seasons, respectively. The biomass of nekton varied between 76.17 kg h^{-1} (dry season) and 62.28 kg h^{-1} (rainy season) accounting for a total effort of 138.44 kg h^{-1}. As these species also occurred in at least 30% of the catches were used to describe the assemblages (Table 2).

In Table 2 the Red List score of threatened species of IUCN is also presented. A total of six species (*Z. brevirostris*, *A. cyclophora*, *A. platana*, *Hippocampus erectus*, *Ballistes capriscus* and *Hyporthodus niveatus*) considered as vulnerable, representing a total of 136 individuals (6.50%) and 57.80 kg (44.30%) of the sampled demersal nekton (Table 2). Only one species (*H. reidi*) is considered near threatened, while the other 59 taxa are classified not threatened to extinction.

Table 2. Fish species, conservation status in the IUCN Red List (IUCN 2019), frequency of occurrence (%FO), weight per hour (Wt h^{-1}) and number of individuals per hour (N h^{-1}) of the demersal nekton from the continental shelf of Campos Basin, southwestern Atlantic, during dry and rainy seasons. VU: Vulnerable; NT: Near threatened; LC: Least Concern; DD: Data Deficient; NE: Not Evaluated; Lt = total length).

Order	**Family**	**Species**	**IUCN**	**Lt range (mm)**	**%FO**		**Wt h^{-1}**		**N h^{-1}**	
					Dry	Rainy	Dry	Rainy	Dry	Rainy
FISHES										
Anguiliformes	Muraenidae	*Gymnothorax ocellatus* (Agassiz, 1831)	LC	139 – 500	33.3	66.7	0.36	0.75	2	4
Aulopiformes	Synodontidae	*Saurida caribbaea* (Breder, 1927)	LC	41 – 209	66.7	100.0	0.01	3.73	2	560
		Synodus myops (Forster, 1801)	LC	123 – 222	16.7	66.7	0.07	0.11	1	7
		Synodus foetens (Linnaeus, 1766)	LC	356 – 372	16.7	-	0.48	-	1	-
		Synodus sp.	LC	184 – 220	16.7	-	0.08	-	2	-
Batrachoidiformes	Batrachoididae	*Porichthys porosissimus* (Cuvier, 1829)	NE	151 – 230	-	33.3	-	0.19	-	2
Clupeiformes	Clupeidae	*Sardinella brasiliensis* (Steindachner,	DD	153	-	33.3	-	0.04	-	1
	Pristigasteridae	*Odontognathus mucronatus* (Lacepède,	LC	55 – 115	-	100.0	-	0.10	-	34
Lophiiformes	Antennariidae	*Antennarius striatus* (Shaw, 1794)	LC	102 – 124	33.3	-	0.08	-	1	-
	Lophidae	*Lophius gastrophysus* (Miranda Ribeiro,	LC	395 – 576	33.3	-	4.88	-	3	-
	Ogcocephalidae	*Ogcocephalus vespertilio* (Linnaeus,	NE	36 – 113	33.3	-	0.02	-	2	-
Perciformes	Carangidae	*Caranx latus* (Agassiz, 1831)	LC	54 – 80	-	100.0	-	0.11	-	47
		Caranx ruber (Bloch, 1793)	LC	57 – 81	-	66.7	-	0.12	-	41
		Caranx sp. (Cuvier, 1833)	LC	114	-	66.7	-	0.01	-	1
		Decapterus sp.	LC	176	16.7	-	0.02	-	1	-
		Oligoplites saliens (Bloch, 1793)	LC	273	-	33.3	-	0.19	-	1
	Gerreidae	*Eucinostomus gula* (Quoy & Gaimard,	LC	125 – 188	-	33.3	-	0.41	-	8
	Haemulidae	*Orthopristis ruber* (Cuvier, 1830)	LC	98 – 316	-	33.3	-	2.16	-	11
	Mullidae	*Upeneus parvus* (Poey, 1852)	LC	173	16.7	-	0.03	-	1	-
	Percophidae	*Percophis brasiliensis* (Quoy &	NE	152 – 548	66.7	100.0	6.57	0.90	29	11
	Pinguipidae	*Pinguipes brasilianus* (Cuvier, 1829)	NE	197 – 235	33.3	-	0.29	-	2	-
	Priacanthidae	*Cookeolus japonicus* (Cuvier, 1829)	LC	231	16.7	-	0.08	-	1	-
		Priacanthus arenatus (Cuvier, 1829)	LC	41 – 115	33.3	100.0	0.09	0.29	4	74
	Sciaenidae	*Ctenosciaena gracilicirrhus* (Metzelaar,	LC	117 – 202	-	66.7	-	0.33	-	4
		Cynoscion sp.	LC	288 – 332	-	66.7	-	1.05	-	2
		Cynoscion striatus (Cuvier, 1829)	NE	472	-	33.3	-	1.20	-	1
		Micropogonias furnieri (Desmarest,	LC	300	-	33.3	-	0.45	-	1
		Umbrina canosai (Berg, 1895)	NE	302 – 310	-	33.3	-	0.70	-	2
	Serranidae	*Diplectrum* sp.	LC	68 – 98	-	66.7	-	0.04	-	7
		Diplectrum formosum (Linnaeus, 1766)	LC	48 – 83	16.7	66.7	0.06	0.01	1	2
		Diplectrum radiale (Quoy & Gaimard,	LC	89 – 160	16.7	33.3	0.03	0.03	1	3

		Dules auriga (Cuvier, 1829)	NE	26 – 140	100.0	100.0	0.89	1.84	41	117
		Hyporthodus niveatus (Valenciennes,	VU	130	16.7	-	0.01	-	1	-
Pleuronectiformes	Bothidae	*Bothus ocellatus* (Agassiz, 1831)	LC	55 – 93	16.7	66.7	<	0.05	1	10
		Bothus robinsi (Topp & Hoff, 1972)	LC	53 – 86	-	100.0	-	0.03	-	6
	Cynoglossidae	*Symphurus jenynsi* (Evermann &	NE	161 – 240	-	66.7	-	0.53	-	14
		Symphurus kyaropterygium (Menezes &	NE	141	16.7	-	0.01	-	1	-
		Symphurus trewavasae (Chabanaud,	NE	172	16.7	-	0.02	-	1	-
	Paralichthydae	*Citharichthys macrops* (Dresel, 1885)	LC	156	-	33.3	-	0.04	-	1
		Etropus longimanus (Norman, 1933)	NE	52 – 115	66.7	100.0	0.15	0.12	23	26
		Paralichthys isosceles (Jordan, 1891)	NE	225	-	33.3	-	0.09	-	1
		Paralichthys patagonicus (Jordan, 1889)	NE	110 – 379	50.0	33.3	0.19	1.20	3	2
		Syacium papillosum (Linnaeus, 1758)	LC	254	16.7	-	0.08	-	1	-
		Verecundum rasile (Jordan, 1891)	NE	196 – 245	-	66.7	-	0.46	-	6
		Xystreurys rasile (Jordan, 1891)	NE	241	-	33.3	-	0.12	-	1
Rajiformes	Arhynchobatidae	*Atlantoraja cyclophora* (Regan, 1903)	VU	113 – 570	50.0	100.0	2.55	7.92	4	12
		Atlantoraja platana (Günther, 1880)	VU	215 – 550	50.0	100.0	1.36	4.18	3	10
	Rhinobatidae	*Zapteryx brevirostris* (Müller & Henle,	VU	161 – 550	83.3	100.0	8.53	21.07	30	32
Scorpaeniformes	Dactylopteridae	*Dactylopterus volitans* (Linnaeus, 1758)	LC	66 – 339	100.0	100.0	7.05	1.06	302	26
	Triglidae	*Prionotus nudigula* (Ginsburg, 1950)	NE	44 – 633	83.3	100.0	0.11	3.61	6	6
		Prionotus punctatus (Bloch, 1793)	LC	122	-	33.3	-	0.02	-	1
Syngnathiformes	Fistularidae	*Fistularia tabacaria* (Linnaeus, 1758)	LC	385 –	33.3	66.7	0.06	0.77	1	4
	Sygnathidae	*Hippocampus erectus* (Perry, 1810)	VU	121 – 123	16.7	33.3	0.01	0.01	1	1
		Hippocampus reidi (Ginsburg, 1933)	NT	86 – 122	16.7	66.7	0.01	0.01	2	3
Tetradontiformes	Balistidae	*Balistes capriscus* (Gmelin, 1789)	VU	137 – 160	16.7	-	0.27	-	3	-
	Diodontidae	*Chilomycterus spinosus* (Linneaus,	LC	71 – 239	66.7	-	0.69	-	2	-
	Tetraodontidae	*Lagocephalus laevigatus* (Linnaeus,	LC	352	-	33.3	-	0.60	-	1
INVERTEBRATES										
Bivalvia (Class)			-	22*	16.7	-	< 0.1	-	1	-
Cidaroida	Cidaridae	*Cidaridae* sp. (Gray, 1825)	-	46 – 55*	16.7	-	0.8	-	2	-
Decapoda	Paguridae	*Paguridae* sp. (Latrielle, 1802)	-	11 – 39*	33.3	-	0.04	-	4	-
	Portunidae	*Achelous spinicarpus* (Stimpson, 1871)	NE	11 – 46*	16.7	33.3	0.01	0.13	1	23
	Scyllaridae	*Scyllaridae* sp. (Latrielle, 1825)	LC/DD	43 – 60*	33.3	33.3	0.03	0.08	1	3
Gastropoda (Class)			-	41 – 66*	-	66.7	-	< 0.1	-	3
Myopsida	Lolignidae	*Doryteuthis pleii* (Blainville, 1823)	NE	14 –	83.3	100.0	0.21	0.52	38	40
Octopoda	Octopodidae	*Octopus vulgaris* (Cuvier, 1797)	LC	56 –	66.7	100.0	2.03	4.88	7	13
Ostreida	Ostreidae	*Ostreidae* sp. (Rafinesque, 1815)	LC/DD	54 – 69*	16.7	-	0.02	-	3	-
Total							**76.17**	**62.28**	**1053**	**1186**
Total (Dry + Rainy)								**138.44**		**2239**

*carapace length, **mantle length

Catch in numbers and weight were more even in the rainy season. There was dominance in numbers of one species in both seasons, with *D. volitans* (n = 604, 57%) as dominant in the dry season and *S. caribbaea (*n = 560, 47%) in the rainy season (Table 2; Figure 3A). Four species (*Z. brevirostris*, *D. volitans*, *P. brasiliensis* and *L. gastrophysus*) represented most of the catch in the dry season while only two ray species (*Z. brevirostris* and *A. cyclophora*) dominated in the rainy season in terms of weight (Figure 3B).

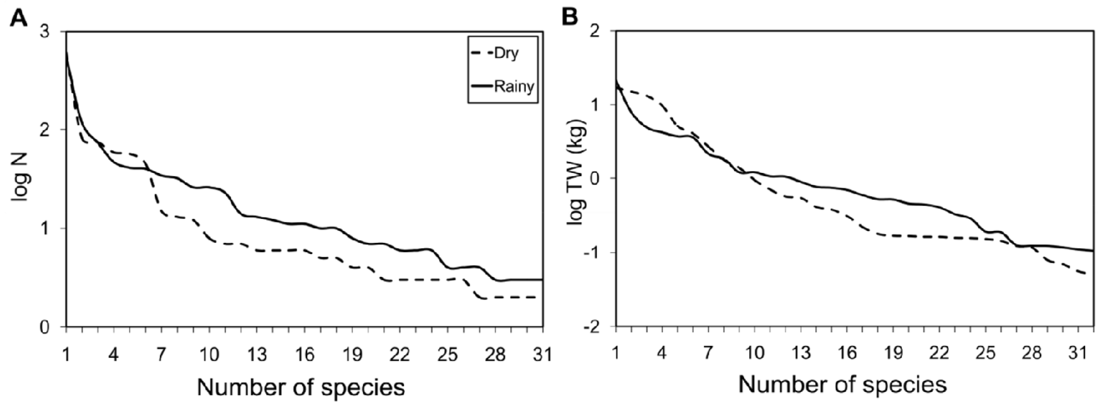

Figure 3. Total number of individuals (A) and total weight (B) in log scale related to species rank of demersal nekton from the continental shelf of Campos Basin, southwestern Atlantic, during dry and rainy seasons.

Assemblage attributes

The rarefaction curves based on numbers and weight revealed higher richness in the rainy season, confirming the second hypothesis. The high rarity and high nekton richness related to numbers was observed in species rarefaction curves that did not appear to approach their asymptotes (Figure 4A). In terms of biomass, both dry and rainy seasons curves achieved their respective asymptotes (Figure 4B). Thus, biomass was considered as the most representative parameter of the assemblage and consequently used in further analysis.

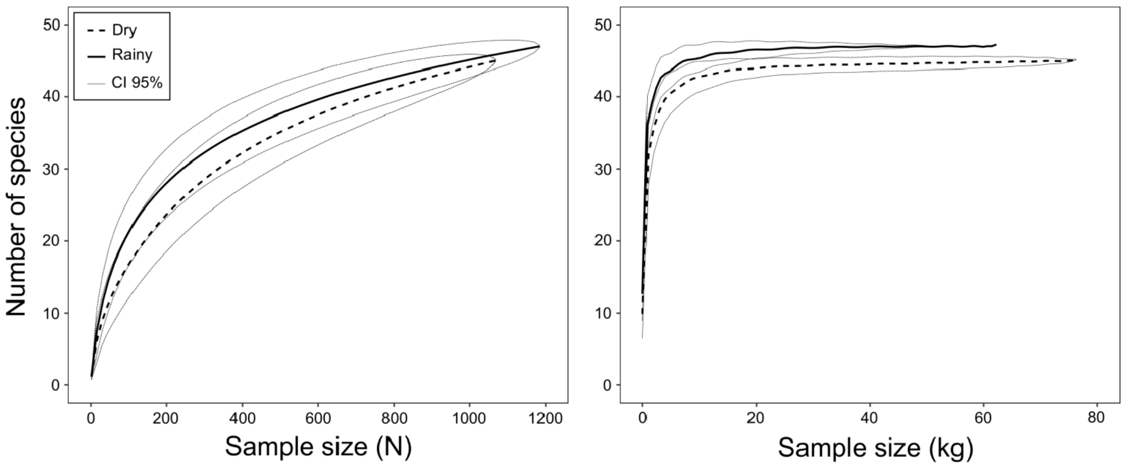

Figure 4. Species accumulation curve related to the total weight of dry and rainy seasons of the demersal nekton from the continental shelf of Campos Basin, southwestern Atlantic, during dry and rainy seasons. CI: confidence interval.

Nine of the 10 most frequent and abundant species occurred in both dry and rainy seasons revealing no shift in assemblage composition, thus rejecting the third hypothesis. Five species are bentophagus (*Z. brevirostris*, *D. volitans*, *A. platana*, *P. nudigula* and *D. auriga*), three are nektophagus (*P. brasiliensis*, *L. gastrophysus* and *S. caribbaea*) and two are bentophagus-nektophagus (*A. cyclophora* and *O. vulgaris*) (Table 3). Bentophagus species were more abundant in both seasons, followed by nektophagus and bentophagus-nektophagus in the dry and rainy seasons, respectively.

Within the most frequent and abundant species, six are of commercial importance. The four species *Z. brevirostris* and *A. platana* (bentophagus) and *O. vulgaris* and *A. cyclophora* (bentophagus-nektophagus) were related to the rainy season, while *L. gastrophysus* and *P. brasiliensis* (nektophagus) were related to the dry season (Table 3).

Table 3. Ranking of the dominant species according to the relative biomass (kg h^{-1}) ± 95% confidence interval and *P* level from the continental shelf of Campos Basin, southwestern Atlantic, on dry and rainy seasons. Species code and trophic guilds are also presented. Bold values mean significant seasonal differences ($P < 0.05$).

				CPUE (kg h^{-1})		
	Species	**Code**	**Guild**	**Dry**	**Rainy**	***P* level**
1	*Zapteryx brevirostris*[a]	ZABR	Bent	8.53 ± 8.73	21.07 ± 22.13	0.262
2	*Dactylopterus volitans*	DAVO	Bent	7.50 ± 3.48	1.06 ± 1.81	**0.015**
3	*Percophis brasiliensis*[a]	PEBR	Nekt	6.57 ± 7.07	0.90 ± 0.22	**0.057**
4	*Atlantoraja cyclophora*[a]	ATCY	BN	2.55 ± 4.44	7.92 ± 4.87	0.612
5	*Lophius gastrophysus*[a]	LOGA	Nekt	4.88± 6.45	-	-
6	*Octopus vulgaris*[a]	OCVU	BN	2.03 ± 3.09	4.88 ± 4.22	0.542
7	*Atlantoraja platana*[a]	ATPL	Bent	1.36 ± 1.48	4.18 ± 1.02	0.119
8	*Prionotus nudigula*	PRNU	Bent	0.11 ± 0.06	3.61 ± 7.05	0.548
9	*Saurida caribbaea*	SACA	Nekt	0.01 ± 0.00	3.73 ± 2.75	**0.036**
10	*Dules auriga*	DUAU	Bent	0.89 ± 0.75	1.84 ± 1.54	0.356

[a]Species of commercial value (Garcez et al., 2007, FIPERJ 2015).

Species assemblages

The cluster and ANOSIM (global R = 0.77; $P = 0.01$) analysis identified two seasonal groups based on the main important species of fish and cephalopods in terms of weight per hour (Figures 5). The dry season group was mainly composed by *D. volitans*, *P. brasiliensis* and *L. gastrophysus*, whereas *A. platana, O. vulgaris, A. cyclophora, S. caribbaea* and *P. nudigula* contributed the most to rainy season group (Figure 5; Table 4). *Dules auriga* and *Z. brevirostris* contributed in lower proportions (< 50% similarity) to the rainy season and, thus, were not seasonally grouped.

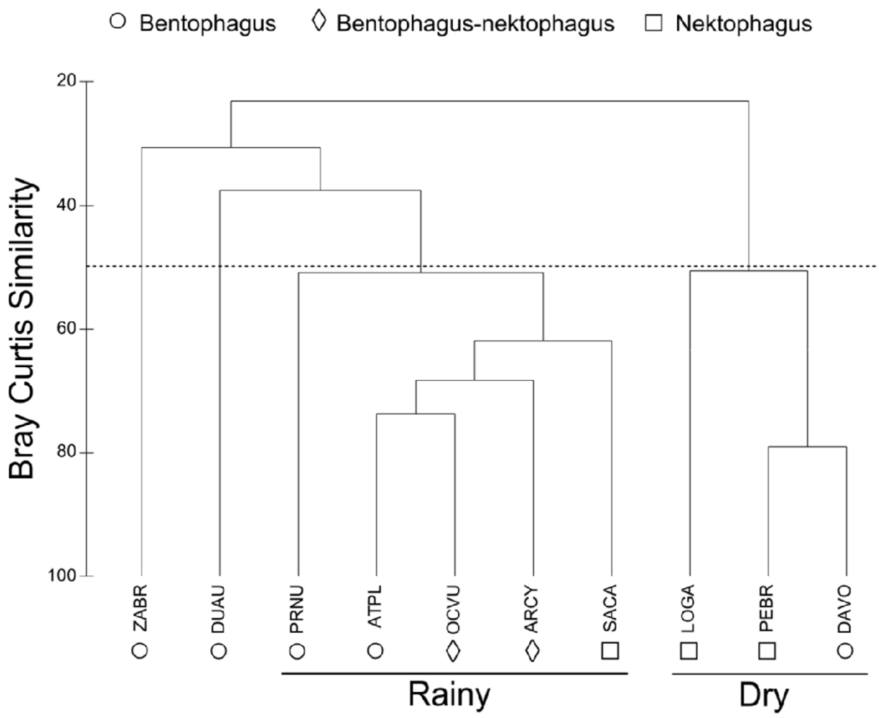

Figure 5. Dendrogram constructed using the Bray-Curtis similarity of the most important species in terms of total catch (kg h^{-1}) used to define nekton assemblages at Campos Basin, south-western Atlantic. Dotted line represents 50% level of similarity. Codes for species according to Table 3.

Table 4. One-way similarity of percent (SIMPER) analysis in terms of total weight per hour (kg h^{-1}) of the species contributions that summed cumulatively to > 80% of dissimilarity. Av. kg h^{-1} Dry: average total weight per hour during dry season; Av. kg h^{-1} Rainy: average total weight per hour during rainy season; Diss ± SD average dissimilarity ± standard deviation; Codes for species according to Table 3.

	Av. kg h^{-1}				
Species	**Dry**	**Rainy**	**Diss ± SD**	**Contribution %**	**Cumulative %**
DAVO	2.13	0.54	7.46 ± 2.07	16.62	16.62
SACA	0.01	1.46	6.45 ± 5.30	14.38	31.00
ATCY	0.88	2.10	6.25 ± 1.66	13.93	44.93
PEBR	1.93	0.64	6.13 ± 2.16	13.65	58.58
ZABR	2.00	2.85	4.76 ± 1.35	10.61	69.18
OCVU	1.09	1.49	4.14 ± 4.17	9.24	78.42
ATPL	0.76	1.63	4.10 ± 1.77	9.12	87.54
PRNU	0.10	0.83	3.31 ± 0.77	7.37	94.91
DUAU	0.62	0.97	2.28 ± 1.10	5.09	100.00
LOGA	1.39	0.00	-	-	-

The environmental-species relationship related to dry and rainy seasons was summarized by CCA analysis. Axis I significantly explained 42.81% (eigenvalue = 0.45; Monte-Carlo, F = 6.76, P = 0.03) whereas Axis II explained 24.46%, but with no significance (eigenvalue = 0.25; Monte-Carlo, F = 3.92, P = 0.17), the total variation (total inertia = 1.04). Monte-Carlo test was significant (F = 2.15, P = 0.03), with 999 permutations (Figure 6). According to parsimonious forward selection by CCA, C_{org}SOM contributed most to species distribution (P < 0.01), followed by temperature (P < 0.01), DOC (P = 0.01) and (C:N)*a* (P = 0.01), respectively. Axis 1 was positively correlated with C_{org}SOM, DOC and (C:N)*a* as well as inversely correlated with temperature.

The CCA biplot showed a seasonal shift in species composition, with *A. platana, S. caribbaea*, *P. nudigula*, *A. cyclophora*, *Z. brevirostris* and *O. vulgaris* associated to higher temperatures, characteristic of the rainy season, whereas *L. gastrophysus*, *P. brasiliensis* and *D. volitans* were associated to C_{org}SOM, DOC and CN in the dry season (Figure 6).

The bentophagus and nektophagus trophic guilds were correlated to both seasons. Most of the bentophagus species were correlated with the high temperature, characteristic of rainy season, except for *D. volitans*, the unique correlated to higher DOC in the dry season. Most of nektophagus species were correlated to high C_{org}SOM, DOC and (C:N)*a* observed in the dry season. Only *S. caribbaea* was correlated to rainy season. The bentophagus-nektophagus guild was correlated to high temperatures (Figure 5 and 6).

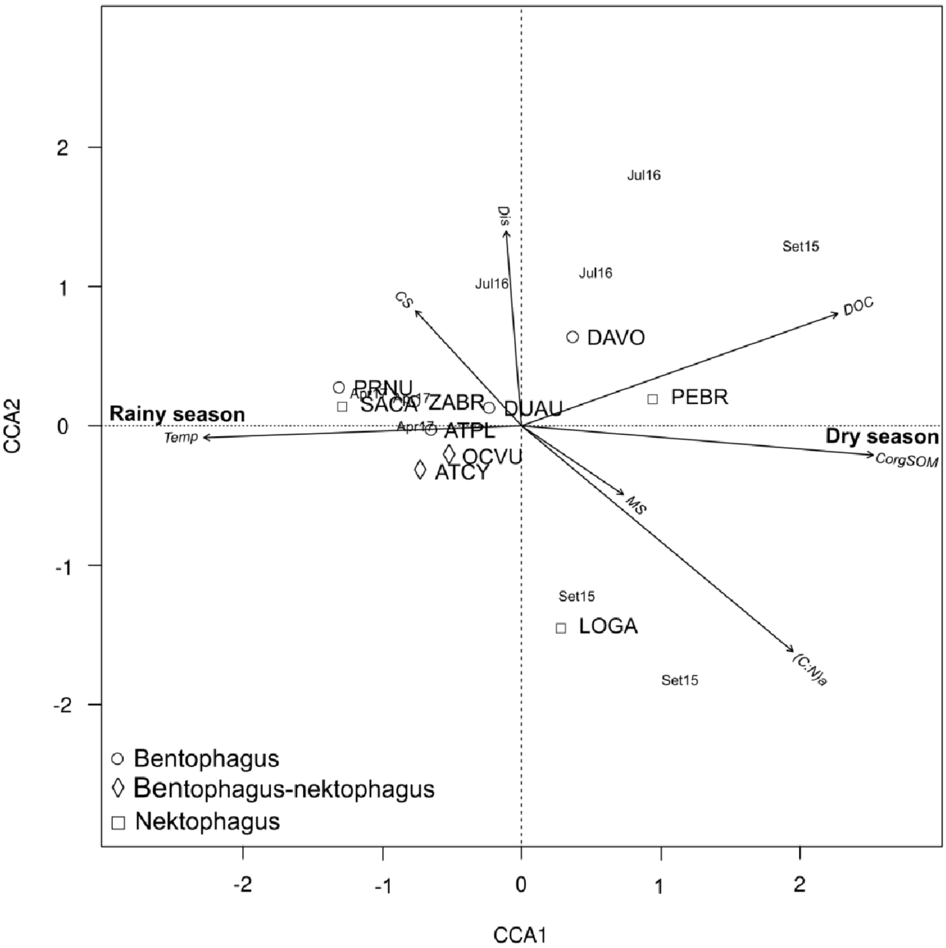

Figure 6. Canonical Correspondence Analysis (CCA) of the most important species in terms of total weight per hour (kg h^{-1}) of the demersal nekton at Campos Basin, south-western Atlantic. (C:N)*a* – atomic ratio of carbon and nitrogen; Dis – PSR discharge; DOC – dissolved organic carbon; Temp - temperature; CS – coarse sand; MS – medium sand; C_{org}SOM – organic carbon of sediment organic matter; Codes for species according to Table 3.

DISCUSSION

This study examined the nekton composition and richness in a coastal area in the continental shelf of Campos Basin (CB), and provided evidence of bentophagus species dominance regardless seasonality. The assemblages were mostly similar to those studied elsewhere in the same basin by Fagundes-Netto and Gaelzer (1991), Pinheiro et al. (2011) and Costa et al. (2015), reinforcing that the demersal nekton assemblage is basically composed by the same species all year-round (*i.e.* shelf resident species) and refuting our first hypothesis. While *Z. brevirostris* and *D. auriga* where similarly abundant in the dry and rainy seasons, differential seasonality in abundances occurred for some species, such as *Dactylopterus volitans*, *Percophis brasiliensis* and *Lophius gastrophysus*, which were associated with the dry season, and *A.* cyclophora, *A. platana*, S. *caribbaea*, *P. nudigula* and *O. vulgaris* which were associated with the rainy season.

The seasonal relationship between nekton assemblage and predominant environmental parameters in northernmost CB shelf was observed by clustering analysis (UPGMA) and ANOSIM multivariate techniques. The predominant species in terms of total weight per hour trawled (kg h^{-1}) in the dry (8.53 kg h^{-1}) and rainy (21.07 kg h^{-1}) seasons was *Z. brevirostris*, that exploits areas with high abundance of prey items, such as crustaceans and polychaetes (Marion et al., 2011). *Dules auriga* that feeds mainly on crustaceans (Cussac and Molero, 1987; Soares et al., 1993) occurred in lower abundance (0.89 ± 0.75 and 1.84 ± 1.54 kg h^{-1} in dry and rainy seasons, respectively) but was also less influenced by seasonality. Crustaceans and polychaetes are abundant macrofaunal species in the same region (Zalmon et al., 2013; 2015) occurring all over the year, thus are constantly available in both seasons, explaining the low evidence of seasonality of *Z. brevirostris* and *D. auriga*.

The elevated demersal nekton species was related to higher temperature in the rainy season, which is widely reported in Brazilian and worldwide coastal marine waters as a driver of entire group of species distribution (e.g. assemblages, communities, associations) (Kordas et al., 2011; Martins & Haimovici, 2016). This species pattern might also be explained by hydrodynamics and sediment type coupled with upwelling and biological factors, such as interspecific feeding, migration and reproduction behaviors. It is known that the sediment grain size largely delineates the favorable living niches of demersal organisms, either directly (providing shelter) or indirectly (providing food) (Damalas et al., 2010). The coarse sand observed in the rainy season indicates a lower hydrodynamic environment in the bottom of CB continental shelf (Zalmon et al., 2013), thus due to the higher environmental stability (lower current influence compared to dry season) and high abundance of sand associated prey species (polychaetes, crustaceans, mollusks, echinoderms, nemerteans and sipunculans), an elevated number of demersal nekton species was observed in the rainy season compared to dry season in the present study. Additionally, the short time upwelling events, that are intensified during summer (rainy season), may also be favorable for higher nutrients in the waters, attracting prey fishes and invertebrates and, consequently the demersal predators (Quintana et al., 2010; Palóczy et al., 2014).

Regarding migration and reproduction behaviors, *D. volitans*, *P. brasiliensis* and *L. gastrophysus* that occurred predominantly in the dry season usually reproduces between spring and summer. While *D. volitans* and *P. brasiliensis* migrate to shallower waters for breeding and juveniles use these waters as nursery areas for development before recruitment, *L. gastrophysus* migrate to deeper waters of the slope for breeding whereas juveniles develop in continental shelf before recruitment in slope waters (Machado et al., 2002; Militelli and Macchi, 2001; Laurenson et al., 2001; Valentim et al., 2007). For nekton species associated with the rainy season, *A. cyclophora*, *A. platana* and *O. vulgaris* reproduces all year-round across the continental shelf with absence of migration patterns. *Prionotus nudigula* breeding occurs from spring to early fall, also with no evidence of migration, while adult females of *S. caribbaea* usually occurs in deeper waters (100 – 900 m depth) than that of the present study, indicating that juveniles occupies the shelf shallower waters as nursery and development areas (Teixeira and Haimovici, 1989; Cergole et al., 2005; Bernardes et al., 2007; Oddone et al., 2008a,b).

The bentophagus species (*Z. brevirostris*, *D. volitans*, *A. platana*, *P. nudigula* and *D. auriga*) dominated the demersal nekton assemblage, regardless seasonality. On the northernmost continental shelf of Campos Basin polychaetes and crustaceans are the dominant taxa of benthic invertebrates on the sandy soft bottom, followed by mollusks, echinoderms, nemerteans and sipunculans (Zalmon et al., 2013; 2015), justifying the bentophagus demersal nekton dominance found in the present study in the dry and rainy seasons. Still, according to Martins et al. (2017) the high complexity of habitats in the shelf is characteristic of the CB region and can attract a higher availability (number of individuals and richness) of prey items and, thus, justify the bentophagus species dominance.

The seasonality observed for bentophagus-nektophagus (*A. cyclophora* and *O. vulgaris*) and nektophagus (*P. brasiliensis*, *L. gastrophysus* and *S. caribbaea*) species resulted in similar importance (17.37 and 16.09 kg h^{-1} of the demersal assemblage, respectively) of both trophic guilds in the region. The graduate reduction of organic matter and detritus with the increase in depth, leads to an increment in nekton prey items from upper slope to deeper areas, reflecting in the predominance of nektophagus organisms in deep waters (Martins et al., 2017). Still, the authors argue that nektophagus species characteristic from upper slope area are dependent of lower water temperatures than that in shelf waters.

Overall, CCA indicated a clear seasonal pattern for environmental variables, mainly for temperature, C_{org}SOM, DOC and (C:N)*a* discriminating between the sampling seasons, but not as hypothesized. Previous studies in the northern sector of the CB continental shelf found terrigenous allochthonous (through stable isotope of ^{13}C) OM inputs in the sediment of shallow waters (25 and 150 m depth) from PSR, regardless seasonality, that significantly influences the structural pattern and composition of the benthic megafauna (Zalmon et al., 2013; 2015; Cordeiro et al., 2018). Additionally, based on lipid biomarkers, Cordeiro et al. (2018) observed that marine autochthonous OM are also important in the northernmost CB coastal waters, resulted from mesoscale events of water circulation, reflected by upwelling events at low intensities.

Suzuki et al. (2015) observed that DOC represent more than 90% of the carbon pool and, similarly to the present study, with higher DOC in the biolytic layer (bottom) higher in the dry season (winter - 79 µmol L^{-1}) compared to the rainy season (summer - 35 µmol L^{-1}). The authors highlighted DOC as an important link between autotrophic and heterotrophic metabolisms in biogenic (superficial) and biolytic (bottom) layers, respectively, since the marine trophic chains obtains most of the required N nutrients from the dissolved fraction of organic material through bacteria assimilation (Thingstad and Rassoulzadegan, 1995; Karl, 2014). This process reflects on plankton production in scale of days to weeks and consequently, on upper trophic levels of the chain (Karl, 2014).

In this sense, the significant higher C_{org}SOM, DOC and (C:N)*a* concentrations in the dry season coupled with the association of (C:N)*a* with the nektophagus species *L. gastrophysus*, raise the possible SACW intrusions resulting in increased concentrations of nutrients a few days before the field trips, even with no evidence of upwelling characteristic temperature (> 20°C; Table 1). This process, that occurs all year-round (with higher intensity during summer) on a reduced time scale (days) and rapidly increase the nutrient concentration in the photic zone promoting primary production that is consumed quickly by herbivore organisms (Carbonel and Valentin, 1999), markedly alter all environmental and ecological parameters (Mahiques et al., 2005). Thus, as phytoplankton DOC excretion can reach 30% of the total assimilated in oligotrophic waters (Vieira and Teixeira, 1981), it is likely to explain the DOC accumulation (higher values) on dry season. Still, Suzuki et al. (2015) attributed the organic (C:N)*a* difference between the dry (winter – 20) and rainy (summer – 10) seasons to upwelling events in the region. Thus, as in the present study (C:N)*a* presented similar proportion (approximately 14 and 8 on dry and rainy seasons, respectively), *i.e.* organic (C:N)*a* almost doubled from rainy to dry season, the results reinforce the supposed upwelling influence in the demersal nekton of the region.

In conclusion, the present study allowed the observation of shelf resident demersal nekton with seasonal variations related to migration and spawning behaviors of the species between dry and rainy seasons. Despite the fifteen environmental parameters, only temperature as well as C_{org}SOM, DOC and (C:N)*a* were significantly associated with demersal nekton species abundances. The coupling effects of terrestrial and upwelling inputs of nutrients in the continental shelf of Campos Basin was observed, however not ruling the assemblage dynamics. It was possible to observe influence higher than 32 km from the mouth of the river, as proposed by Rudorff et al. 2011, but not as hypothesized. Apparently, bentophagus species benefits the availability of the most abundant benthic macrofaunal prey items in the region (polychaetes, crustaceans, mollusks, echinoderms, nemerteans and sipunculans).

The understanding of the assemblage dynamics of demersal fishes enhances the knowledge of ecological and environmental forces driving their coexistence. More studies are important for the advancement of knowledge of the trophic dynamics, life cycles, habitat usage and environmental role of demersal nekton in the continental shelf habitats.

ACKNOWLEDGEMENTS

We are greatful to MSc. Thiago P. Rangel for geoprocessing.

FINANCIAL SUPPORT

This research was supported by Fundação de Amparo à Pesquisa do Rio de Janeiro – FAPERJ (Rezende CE, Grant number E-26/110.437/2010) and by Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPQ (INCT Material Transfer at the Continent-Ocean Interface, Rezende CE, grant number 573.601/08-9). Likewise, this research study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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Capítulo 3 – a ser submetido para Science of the Total Environment

Isotopic niche breadth of coastal fish and cephalopods off Campos Basin, Southeastern Brazil

Gatts, P.V.[a] *, Franco, M.A.L.[b], Almeida, M. G.[b], Rezende, C.E.[b], Costa, P.A.S.[a,c].

[a] Instituto de Geociências, Universidade Federal Fluminense, Programa de Pós Graduação de Dinâmica dos Oceanos e da Terra, Niterói RJ CEP 24210-346, Brazil. pedrogatts90@gmail.com.
[b] Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes RJ CEP 28013-602, Brazil. malfranco@yahoo.com.br, crezende@uenf.br.
[c] Departamento de Ecologia e Recursos Marinhos, Instituto de Biociências, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro RJ CEP 22290-240, Brazil. paulocosta.unirio@gmail.com.

ABSTRACT

The stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) three demersal nekton guilds (bentophagus, bentophagus-nektophagus and nektophagus) were evaluated to understand their trophic relationships in the continental shelf of Campos Basin, north of Rio de Janeiro State, Southeastern Brazil. The organic matter (OM) pool that fuels the demersal food web is composed by a mixture of autochthonous (marine) and allochthonous (terrigenous) sources, highlighting the Paraíba do Sul River (PSR) influence in the shallow coastal waters, regardless seasonality. Highest $\delta^{15}N$ values were observed for nektophagus guild (*Doryteuthis plei*, *Lophius gastrophysus*, *Percophis brasiliensis* and *Saurida caribbaea*) and reflected in the most elevated vertical position in the demersal food web in the dry (P < 0.001) and rainy (P < 0.001) seasons. Bentophagus guild (*Atlantoraja platana*, *Dactylopterus volitans*, *Dules auriga*, *Etropus longimanus*, *Prionotus nudigula* and *Zapteryx brevirostris*) presented the widest isotopic niche breadth (highest total niche area and trophic diversity) due to the broad groups of assimilated invertebrates. The assimilation of similar groups of

crustaceans by bentophagus and bentophagus-nektophagus (*A. cyclophora*, *Octopus vulgaris* and *Priacanthus arenatus*) guilds reflected in isotopic overlaps (< 40%) among them. Seasonal trends from benthic and inshore to pelagic and offshore associated food web were observed for bentophagus and bentophagus-nektophagus guilds.
Keywords: demersal nekton, stable isotopes, niche breadth, trophic guilds.

INTRODUCTION

Food web structure and functioning have been subjects of increasing interest in science during the last decades to understand and predict the response of ecosystems to environmental change (Dunne and Williams, 2009; Woodward et al., 2010). The understanding of how trophic interactions affect ecosystem functioning is the main questions to be answered by food web researches (Ings et al., 2009). Therefore, studies on the trophic functioning are essential to predict the response of ecosystems to the effect of environmental variability on biodiversity (Keyl and Wolff, 2008).

The food web approach in order to compare different habitats promotes the identification of fundamental relationships of the structure and the dynamic of assemblages (Bascompte et al., 2003; Guimarães et al., 2007). The understanding of trophic guilds, *i.e.* groups of organisms that explore the same classes of feeding resources, are usually organized spatially and/or temporally in response to environmental, physiological and biological drivers. Therefore, improving the knowledge of assemblage functioning and the degree of connection and independence among species groups allows the comparison between trophic webs in different localities, even with distinct species composition (Luczovich et al., 2002; Kondoh et al., 2010).

Researchers have been using stable isotope approach to understand food web trophic organization, allowing comparisons among ecosystems (Grangeré et al., 2012; Kopp et al., 2013). Carbon (C) and nitrogen (N) are the most commonly employed in trophic ecological studies (Layman et al., 2012). The incorporation of stable isotopic analysis has been widely applied to the determination of energy sources ($\delta^{13}C$) and vertical position ($\delta^{15}N$) of the organisms in their food webs (Vander Zanden et al., 1999; Boekclen et al., 2011; Fry and Davis, 2015; Krumsick et al., 2019). Stable isotope proportions of nitrogen in consumers are usually enriched in the heavier isotope (^{15}N) in 3‰ by trophic level, leading to useful the interpretation of $\delta^{15}N$ for consumer trophic position in the food web. The fractionation of carbon isotopes (^{13}C) is lower (1‰) and usually used to define sources of organic matter (OM) (Post, 2002; Layman et al., 2015).

The coastal zone off the northernmost Campos Basin shelf is largely influenced by marine autochthonous and terrestrial allochthonous organic matter (OM) inputs. The seasonal intrusion of the cold and nutrient-rich South Atlantic Center Water (SACW) (Calado et al., 2010; Palóczy et al., 2014) acts as an enrichment factor for primary producers and, consequently, for demersal nekton assemblages (Carbonel and Valentin, 1999; Quintana et al. 2010). Costa et al. (2015) suggested that SACW significantly affects the composition and distribution of megafauna assemblages in Campos Basin shelf and slope. Martins et al. (2017) used the same data to establish the trophic guilds based on stomach contents analysis (SCA).

The Paraíba do Sul River (PSR) is the major terrigenous OM supply in the region, where the estuarine system acts as a transition zone from fresh to shallow marine coastal waters, presenting a seasonal discharge pattern linked to rainfall, with dry (May to September) and a rainy (October to April) seasons (Almeida et al., 2007), as well interannual discharges of suspended particle matter (0.8 to 2.0×106 t/year) and dissolved organic carbon (0.3×106 t/year) (Carvalho et al., 2002; Figueiredo et al. 2011; Ovalle et al. 2013). Carreira et al. (2015) and Cordeiro et al. (2018) found carbon isotopic signals of terrigenous origin up to 150 m depth, indicating the PSR influence in the OM buried in bottom sediments. Additionally, studies off the continental shelf shallow waters reported the river plume can achieve 32 km from the coast and is highly correlated to phytoplankton distribution (Souza et al., 2010; Rudorff et al., 2011), indicating OM delivered by PSR discharge as trophic resources for aquatic organisms (Bittar and Di Beneditto, 2009; Zalmon et al., 2015), thus being a favorable region to investigate the OM influence in the trophic ecology of demersal guilds.

To date, the amount of information generated by stable isotope approach related to trophic studies in Brazil has been increasingly conducted (Bisi et al., 2012; Muto et al., 2014), rising new insights to trophic guild researches based on SCA (Marques et al., 2018; Garcia et al., 2018). Martins et al. (2017) identified 6 trophic guilds over the Campos Basin continental shelf and slope, between 13 and 2000 m (bentophagus, bentophagus-nektophagus, generalists, nektophagus, nektophagus-bentophagus and zooplanktophagus-bentophagus), but information about the trophic ecology and origin of the OM that fuels this demersal food web is scarce.

In this sense, the present study used isotopic tracers of carbon (^{13}C) and nitrogen (^{15}N) to understand the trophic relationships among the bentophagus, bentophagus-nektophagus and nektophagus guilds of demersal nekton in the Campos Basin shelf, leading to an improvement in the knowledge of their trophic dynamics in Brazilian shallow waters. Thus, the following hypothesis were tested: 1) due to the differences in dietary sources that support each trophic guild of the fishes and cephalopods, the isotopic niche breadths vary among themselves leading to low niche overlaps; 2) if the influence of Paraíba do Sul River (PSR) on phytoplankton distribution can reach more than 32 km, then the $\delta^{13}C$ of an entire trophic chain dependent on the phytoplankton will reflect such terrigenous OM input; and 3) Due to the seasonality of the terrigenous OM supply, there will be alternation in the basal resources of the trophic web and, consequently, the $\delta^{13}C$ values will reflect the differences in guild isotopic niche breadth between dry and rainy seasons.

MATERIAL AND METHODS

Study site

This study was developed over the continental shelf of Campos Basin, in the southwest Brazilian coast. It is located in the north of Rio de Janeiro State and is adjacent to the mouth of the Paraíba do Sul River (Figure 1).

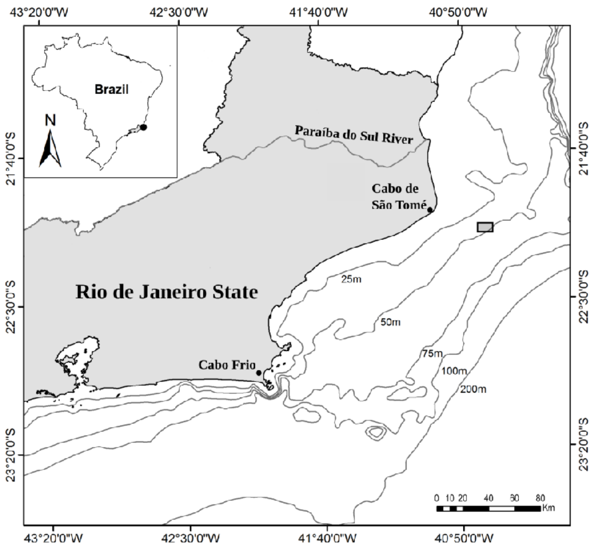

Figure 1. Study site at the continental shelf in southwestern Atlantic on Campos Basin, Brazil. The grey rectangle represents the sampling area.

The region is characterized by two seasons (Ovalle et al., 2013), considering the rainfall in the drainage basin of the Paraíba do Sul River (PSR). Almeida et al. (2007) linked rainfall seasons to the discharge rates of the river and classified as: rainy season

(October to April), with higher rainfall and discharge; and dry season (May to September), with lower rainfall and discharge. The PSR discharge is measured twice a month all over the year since 1995 by the Laboratório de Ciências Ambientais from Universidade Estadual do Norte Fluminense Darcy Ribeiro. In Figure 2, PSR discharges from each sampling month September (2015), July (2016) and April (2017) as well from 1995 to 2017 is represented. PSR discharges were similar between sampling seasons, with lower mean values (234.3 and 148.0 m^3 s^{-1} in dry and rainy seasons, respectively) compared to the historical pattern of the river (410.3 and 1004.5 m^3 s^{-1} in dry and rainy seasons, respectively), especially in the rainy season. The PSR drains one of the most populated region of Brazil – the southeast (ANA, 2018). Studies evaluating the influence of PSR off the northern coast of Rio de Janeiro point that the PSR plume can reach 32 km in the ocean from the river mouth (Souza et al., 2010). However, its real influence must be greater than in surface water, reaching more extensive areas and depths for other parameters, such as phytoplankton (Rudorff et al., 2011) and nutrients (Carter, 1990), and consequently the organisms that depends on these sources.

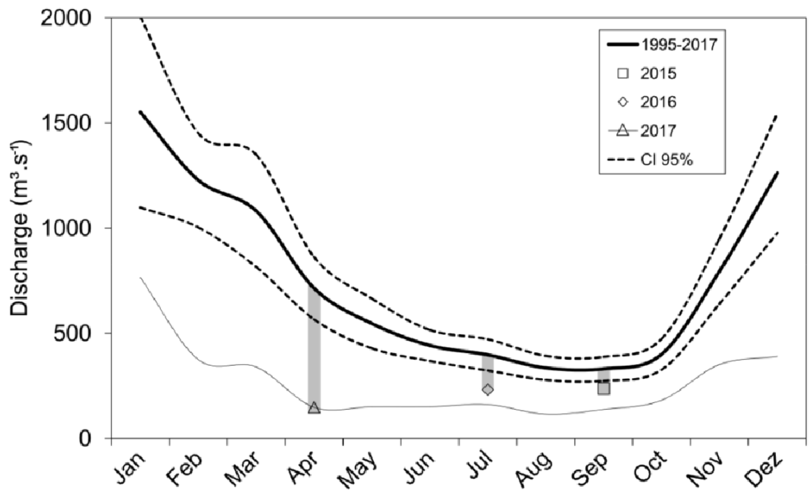

Figure 2. Discharge of PSR during the time series of 1995 - 2017 and the sampling periods on 2015, 2016 and 2017. Highlighted in grey are the months when sampling was performed. CI: confidence interval. Source: Laboratório de Ciências Ambientais – Universidade Estadual do Norte Fluminense.

Along the continental margin in the southeast Brazil (20°S to 28°S), there is also a constant coastal current-eddy-upwelling systems that develops in the vicinities of Cabo de São Tomé (22°S) and Cabo Frio (23°S) (Calado et al., 2010), that is intensified during summer, when the dominating regime of northeastern wind induce the subsurface shoreward intrusion of oceanic South Atlantic Central Waters (SACW). This process results on the enhancement of productivity in both pelagic and benthic systems and the availability of resources for demersal fish and shellfish stocks (Borzone et al., 1999; Sumida et al., 2005; Rossi-Wongtschowski et al., 2006).

Sampling

Fishes and cephalopods were sampled with bottom trawls performed over sandy and muddy bottoms off the northernmost Campos Basin (Figure 1). The trawl was a 12 m ground rope, 1.2 m height and 15 mm stretch mesh in the cod-end, towed by a single warp cable aboard the fishing boat *Karen I* (12 m, 156 HP), specifically rented for the samplings.

Three bottom trawls surveys were performed at the same study site during September (2015), July (2016) and April (2017), representing the dry season and the end of the rainy season, respectively. A total of nine trawls were performed between 43 and 56 m, with a mean speed of 3.0 knots, 20 minutes each, corresponding to a total sampling effort of 180 minutes (3 hours) and a triplicate on each sampling period.

All specimens (fishes and cephalopods) were identified using regional specific literature (Figueiredo, 1977; Figueiredo & Menezes, 1978; 1980; FAO, 2002a,b). During the samplings, the specimens were maintained fresh on ice, transported to the laboratory and kept frozen. Fishes and cephalopods were measured in total length (Lt, mm) and mantle length (Ml, mm) respectively, and had their total weight (Wt) recorded.

A fragment of the dorsal muscle and mantle of each specimen was collected and lyophilized (L10, Liotop) for isotopic analysis.

Previously to the bottom trawls, depth was recorded and water and sediment samples were collected to determine the organic matter that fuels the demersal food web. The water samples were kept refrigerated and the sediment samples frozen until its respective processing in the laboratory. Samples from the bottom water were collected with a *Van Dorn* bottle in order to record particle organic matter (POM). Two liters of the marine bottom water was collected in polyethylene flasks in triplicates at each bottom trawl site. POM was collected by saturation of 0.7-μm of porosity, ϕ 47 mm GF/F filters and lyophilized (L10, Liotop).

A box-corer was used to collect undisturbed 0-2 cm layers of the sediment on each bottom trawl. A sub-aliquot of each sediment sample was used to record total organic matter in the sediment (SOM) in triplicates. Samples were sieved and the passing fraction (< 63 μm) was lyophilized.

Dorsal muscle tissues of fish and mantles of cephalopods as well as POM filters and sediment were dried and homogenized for stable isotope analysis. All laboratory analyses were performed at the Laboratório de Ciências Ambientais from Universidade Estadual do Norte Fluminense Darcy Ribeiro.

Stable isotope analysis – $\delta^{13}C$ and $\delta^{15}N$

All samples were stored in clean transparent plastic bags in iceboxes and then transported to the laboratory where they were kept frozen (-18°C) in dry sterile vials prior to analysis. Lyophilized samples (except POM filters) were ground with a mortar and pestle to a homogeneous fine powder. Approximately 0.5 to 1.0 mg animal muscle tissue and 10 mg sediment were used in the analysis.

POM was collected with pre-combusted 25 mm GF/F filters, and fumed in a desiccator with HCl (32%, analytical grade) for 48 hours to carbonates removing after drying. Filters were placed in tin disks and remained in the desiccator until stable isotopes analysis (Cloern et al. 2002). For elemental composition of carbon and nitrogen as well as $\delta^{13}C$ and $\delta^{15}N$ of sediment, approximately 10 mg was weighed in silver capsules, respectively, followed by acidification through the addition of HCl (2M) to remove inorganic carbon (Kennedy et al. 2005; Brodie et al. 2011).

The elemental and isotopic composition of all samples were determined by using an Elemental Analyzer (Flash 2000) with interface CONFLO IV coupled to an isotope ratio mass spectrometer Delta V Advantage (Thermo Scientific, Germany) in Laboratório de Ciências Ambientais. Samples were analysed using analytical blanks and urea analytical standards (IVA Analysentechnik-330802174; CH4N2O Mw=60, C= 20%, N= 46%), using certified isotopic compositions ($\delta^{13}C$= -39.89‰ and $\delta^{15}N$ = -0.73‰). For biota samples, analytical control was done for every 10 samples using certified isotopic standard (Elemental Microanalysis Protein Standard OAS: 46.5 ± 0.78% for C; 13.32 ± 0.40% for N; -26.98 ± 0.13‰ for $\delta^{13}C$; + 5.94 ± 0.08‰ for $\delta^{15}N$). Accuracy for POM filters and sediment was verified using Elemental Microanalysis Low Organic Soil Standard (1.52 ± 0.02% for C; 0.13 ± 0.02% for N; -27.46 ± 0.11‰ for $\delta^{13}C$; +6.70 ± 0.15‰ for $\delta^{15}N$).

Carbon and nitrogen contents were expressed as percent element (%) and the detection limits were 0.05% and 0.02%, respectively. Carbon and nitrogen isotope ratios were expressed in δ notation as ‰ relative to Pee Dee Belemnite (PDB) and atmospheric nitrogen, respectively, and were calculated using the following equation:

$$\delta = (Rsample/Rstantard) \text{ X } 10^3,$$

where $\delta = \delta^{13}C$ and $\delta^{15}N$ and $R = \delta^{13}C:\delta^{12}C$ or $\delta^{15}N:\delta^{14}N$. Analytical reproducibility was based on triplicates for every 10 samples: ± 0.3‰ for $\delta^{15}N$ and ± 0.2‰ for $\delta^{13}C$. There was no prior lipids extraction from samples, but C/N ratios were lower than 4.0, indicating low lipid level that does not compromise the carbon isotope results and its interpretation (Hoffman et al., 2015).

Data treatment and analysis

Species that accumulated more than 80% of total biomass and occurred in more than 20% of the trawls were used to describe the structure of the demersal assemblage and were classified into trophic guilds adapted from Martins et al. (2017): bentophagus (benthic invertebrates representing > 80% of the diet); bentophagus-nektophagus (benthic invertebrates representing > 50% and fish and squids representing > 20% of the diet); and nektophagus (fish and cephalopods representing > 80% of the diet).

Normality and homoscedasticity of data was tested using Shapiro-Wilk and Levene tests, respectively. The differences in $\delta^{13}C$, $\delta^{15}N$ and C:N ratio were analyzed using ANOVA (type III), applied for unbalanced data (Shaw and Mitchell-Olds, 1993), followed by the Tukey *post hoc* test. When normality and homoscedasticity were not achieved non-parametric analysis of variance (Kruskal-Wallis) was applied followed by Dunn's *post hoc* test. The Pearson correlation was used to correlate $\delta^{13}C$ and $\delta^{15}N$ with Lt and logWt. Results were considered significant at $P < 0.05$. F-ratio test was applied to test the significance of regressions (Best and Roberts, 1975).

Isotopic niche breadth was quantified for each trophic guild of demersal nekton using standard ellipse area (SEA), calculated with the Stable Isotope Bayesian Ellipses tool in R (SIBER) (Jackson et al., 2011). For sample size lower than 30, standard ellipse area corrected for small sample sizes (SEAc) was estimated. The isotopic niche metrics that relate the characteristics of the isotopic space filled by each trophic guild in this study were calculated and consisted of the following: NR (maximum $\delta^{15}N$ range – larger range suggests more trophic levels and greater degree of trophic diversity); CR (maximum $\delta^{13}C$ range – increased range suggests higher diversity of basal sources); TA (total area of isotopic niche – representing the total amount of niche space occupied); SEAc (corrected standard ellipses area for small sample size – isotopic niche width); CD (mean Euclidean distance from the centroid – higher distances suggests high average degree of trophic diversity within the food web); NND (mean Euclidean distance to the nearest neighbour – similar trophic ecology will exhibit small NND); and SDNND (standard deviation of NND – low values suggests more even distribution of trophic niche) (Layman *et al*., 2007).

One-way ANOVAs were also used to evaluate differences and interactions between species considering CD and MNND, because they involve comparisons of means. The statistic SDNND, being a standard deviation, was compared between groups by an F-ratio test. The *P* values were interpreted as strengths of evidence towards null hypotheses rather than on the dichotomic scale of significance testing (Hurlbert & Lombardi, 2009). All statistics and models were fitted using R version 3.5.2 (2018 – 12 – 20, "Eggshell Igloo") for Linux in the RStudio software (Version 1.1.436) (R Core Team, 2018).

RESULTS

A total of 337 specimens, distributed in fishes (314) and cephalopods (17) were analyzed (Table 1). Teleosts (Actinopterygii) were represented by eight species, whereas elasmobranchs and cephalopods included smaller number of species (three and two, respectively). Bentophagus was composed by four teleost species (*Dactylopterus* *volitans*, *Dules auriga*, *Etropus longimanus* and *Prionotus nudigula*) and two elasmobranch species (*Atlantoraja platana* and *Zapteryx brevirostris*) comprised 179 and 37 specimens in dry and rainy seasons, respectively. Bentophagus-nektophagus was composed by one teleost (*Prionotus nudigula*), one elasmobranch (*A. cyclophora*) and one cephalopod (*Octopus vulgaris*) species, comprising 26 and 19 specimens in dry and rainy seasons, respectively. Three teleost species (*Lophius gastrophysus*, *Percophis brasiliensis* and *Saurida caribbaea*) and one cephalopod (*Doryteuthis plei*) composed nektophagus guild and comprised 52 and 18 specimens in dry and rainy seasons, respectively.

Interguild significant differences of $\delta^{13}C$, $\delta^{15}N$ and C:N ratio were observed within seasons (Table 1). In dry season, while C:N was similar among guilds (ANOVA, $F = 0.42$, $P > 0.05$), bentophagus-nektophagus and nektophagus presented highest $\delta^{13}C$ values (ANOVA, $F = 24.72$; $P < 0.001$) whereas nektophagus highest $\delta^{15}N$ (ANOVA, $F = 75.32$, $P < 0.001$). In rainy season, bentophagus and nektophagus presented the highest $\delta^{13}C$ (ANOVA, $F = 7.40$, $P = 0.001$), whereas nektophagus the highest $\delta^{15}N$ (ANOVA, $F = 56.42$, $P < 0.001$) and C:N (ANOVA, $F = 6.75$, $P < 0.01$).

Intraguild significant differences of $\delta^{13}C$, $\delta^{15}N$ and C:N ratio were also observed between seasons (Table 1). For bentophagus the highest $\delta^{13}C$ values (Kruskal-Wallis, $X^2 = 23.15$, $P < 0.001$) were observed in the rainy season whereas $\delta^{15}N$ (Kruskal-Wallis, $X^2 = 2.73$, $P > 0.05$) and C:N ratio (ANOVA, $F = 2.22$, $P > 0.05$) were similar. For bentophagus-nektophagus while the highest $\delta^{13}C$ values (Kruskall-Wallis, $X^2 = 4.52$, $P < 0.05$) and $\delta^{15}N$ (Kruskall-Wallis, $X^2 = 4.67$, $P < 0.05$) were observed in dry season, C:N ratio was similar (Kruskall-Wallis, $X^2 = 1.19$, $P > 0.05$). For nektophagus the highest $\delta^{13}C$ (Kruskall-Wallis, $X^2 = 9.81$, $P < 0.01$) and C:N ratio (Kruskall-Wallis,

$X^2 = 29.89$, $P < 0.001$) were in dry and rainy seasons, respectively, whereas $\delta^{15}N$ was similar (ANOVA, $F = 1.88$, $P > 0.05$).

Table 1. Trophic guilds, number of individuals (n), total length (Lt) and total weight (Wt) ranges and the mean ± 95 % confidence interval of $\delta^{13}C$ (‰), $\delta^{15}N$ (‰) and C:N ratios sampled in the dry and rainy seasons in the northern Rio de Janeiro state, Southeastern Brazil. Upper case and lower case letters indicate significant differences between trophic guilds in the same season and of trophic guilds between seasons, respectively ($P < 0.05$).

Guilds/Species	Season	*n*	Lt	Wt	$\delta^{13}C$	$\delta^{15}N$	C:N
Bentophagus	Dry	179	26 – 480	0.003 – 0.800	-18.3 ± 0.1^{Aa}	11.3 ± 0.1^{Aa}	3.6 ± 0.0^{Aa}
	Rainy	37	62 - 522	0.001 – 1.100	-17.7 ± 0.2^{Ab}	11.1 ± 0.2^{Aa}	3.6 ± 0.1^{Aa}
Atlantoraja platana	Dry	2	215 – 410	0.067 – 0.400	-17.4 ± 0.3	11.5 ± 0.0	3.3 ± 0.3
	Rainy	6	304 – 485	0.123 – 0.750	-18.0 ± 0.2	10.8 ± 0.4	3.3 ± 0.2
Dactylopterus volitans	Dry	74	66 – 339	0.013 – 0.443	-18.8 ± 0.2	11.1 ± 0.2	3.6 ± 0.0
	Rainy	4	139 – 330	0.023 – 0.450	-18.9 ± 0.9	11.6 ± 0.8	4.0 ± 0.0
Dules auriga	Dry	67	26 – 131	0.003 – 0.038	-18.1 ± 0.1	11.5 ± 0.1	3.5 ± 0.0
	Rainy	7	73 – 139	0.007 – 0.042	-17.9 ± 0.3	11.4 ± 0.4	3.9 ± 0.1
Etropus longimanus	Dry	15	72 – 111	0.003 – 0.011	-18.4 ± 0.2	11.3 ± 0.4	3.5 ± 0.1
	Rainy	6	62 – 115	0.001 – 0.013	-17.8 ± 0.3	10.7 ± 0.3	3.9 ± 0.1
Prionotus nudigula	Dry	8	44 – 130	0.013 – 0.023	-18.3 ± 0.3	11.8 ± 0.5	3.6 ± 0.1
	Rainy	-	-	-	-	-	-
Zapteryx brevirostris	Dry	13	228 – 480	0.067 – 0.800	-17.5 ± 0.1	11.2 ± 0.2	3.4 ± 0.1
	Rainy	14	305 – 522	0.350 – 1.100	-17.1 ± 0.3	11.1 ± 0.3	3.4 ± 0.1
Bentophagus-nektophagus	Dry	26	56 – 570	0.007 – 1.500	-17.9 ± 0.1^{Ba}	11.0 ± 0.2^{Aa}	3.5 ± 0.1^{Aa}
	Rainy	19	45 - 550	0.002 – 1.400	-18.3 ± 0.3^{Bb}	10.6 ± 0.3^{Bb}	3.6 ± 0.1^{Aa}
Atlantoraja cyclophora	Dry	9	287 – 570	0.092 – 1.500	-17.7 ± 0.1	11.0 ± 0.1	3.2 ± 0.1
	Rainy	9	273 – 550	0.119 – 1.400	-17.9 ± 0.5	10.9 ± 0.3	3.4 ± 0.1
Octopus vulgaris	Dry	10	56 – 151*	0.034 – 0.920	-17.9 ± 0.1	10.7 ± 0.2	3.6 ± 0.1
	Rainy	-	-	-	-	-	-
Priacanthus arenatus	Dry	7	75 – 108	0.007 – 0.020	-18.1 ± 0.1	11.5 ± 0.7	3.8 ± 0.1
	Rainy	10	45 – 110	0.002 – 0.021	-18.7 ± 0.2	10.2 ± 0.4	3.8 ± 0.1
Nektophagus	Dry	52	74 – 548	0.009 – 2.450	-17.7 ± 0.1^{Ba}	12.6 ± 0.2^{Ba}	3.6 ± 0.0^{Aa}
	Rainy	18	75 – 360	0.002 – 0.193	-17.9 ± 0.2^{ABb}	12.4 ± 0.3^{Ca}	3.9 ± 0.1^{Bb}

Doryteuthis plei	Dry	7	74 – 109*	0.009 – 0.026	-18.8 ± 0.2	11.2 ± 0.3	3.7 ± 0.1
	Rainy	-	-	-	-	-	-
Lophius gastrophysus	Dry	4	395 – 475	1.056 – 2.450	-17.5 ± 0.2	13.1 ± 0.4	3.5 ± 0.0
	Rainy	-	-	-	-	-	-
Percophis brasiliensis	Dry	41	225 – 548	0.044 – 0.662	-17.5 ± 0.1	12.8 ± 0.1	3.5 ± 0.0
	Rainy	8	199 – 360	0.024 – 0.193	-17.8 ± 0.2	12.3 ± 0.3	3.9 ± 0.1
Saurida caribbaea	Dry	-	-	-	-	-	-
	Rainy	10	75 – 130	0.002 – 0.015	-18.1 ± 0.2	12.3 ± 0.6	3.9 ± 0.1
Total Dry		257					
Total Rainy		74					
Total		331					

***Mantle length**

The mean ± 95% confidence interval $\delta^{13}C$ and $\delta^{15}N$ values of organic matter in sediment (SOM), particle organic matter (POM), phytoplankton and zooplankton ranged from -25.8 ± 0.7‰ for POM to -17.7 ± 1.4‰ for zooplankton and from 2.4 ± 0.6‰ for SOM to 8.4 ± 0.2‰ for phytoplankton, respectively, in the dry season. A similar pattern was observed in the rainy season, when $\delta^{13}C$ and $\delta^{15}N$ ranged from -24.6 ± 1.0‰ for POM to -17.7 ± 1.4‰ for phytoplankton and from 2.3 ± 0.1‰ for SOM to 8.4 ± 0.2‰ for phytoplankton, respectively. Macroinvertebrates $\delta^{13}C$ ranged from – 19.82 ± 0.1‰ for ostreidae to 17.8 ± 0.4‰ for paguridae whereas $\delta^{15}N$ ranged from 4.8 ± 0.1‰ for ostreidae to 11.3 ± 0.0‰ for paguridae in the dry and rainy seasons (Figure 3).

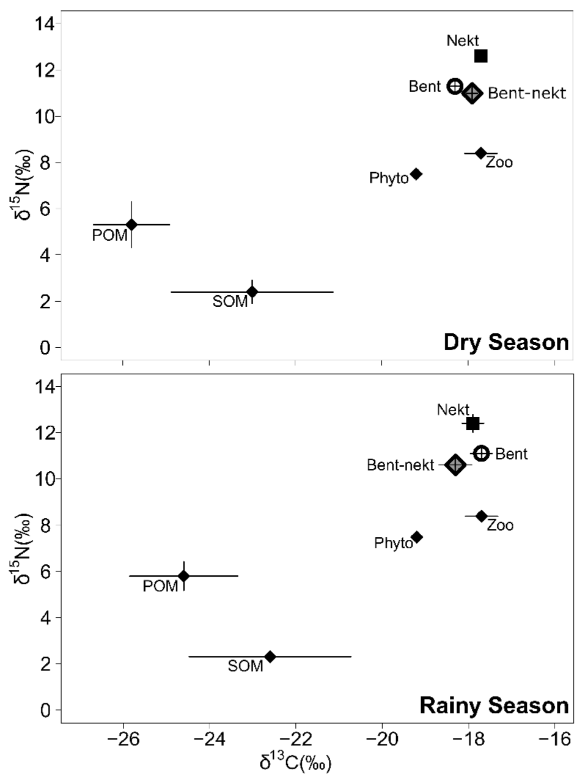

Figure 3. Relationship between mean ± 95% confidence interval of $\delta^{13}C$ and $\delta^{15}N$ in the dry and rainy seasons considering bentophagus, bentophagus-nektophagus (Bent-Nekt), nektophagus trophic guilds of demersal nekton, zooplankton (Zoo)*, phytoplankton (Phyto)*, suspended organic matter (POM) and organic matter in sediment (SOM) in the northern Rio de Janeiro State, Southeastern Brazil. *Gatts et al., (*in prep*).

The $\delta^{13}C$ was significantly correlated (slope = 0.23, adjusted R^2 = 0.39, $F_{1,24}$ = 16.95, P < 0.001) with log Wt only for bentophagus-nektophagus in the dry season whereas was significantly correlated with Lt and log Wt for bentophagus (slope = 0.02, adjusted R^2 = 0.24, $F_{1,33}$ = 11.86, P < 0.01 and slope = 0.17, adjusted R^2 = 0.19, $F_{1,33}$ = 9.11, P < 0.01, respectively), bentophagus-nektophagus (slope = 0.01, adjusted R^2 = 0.41, $F_{1,8}$ = 7.33, P < 0.05 and slope = 0.19, adjusted R^2 = 0.42, $F_{1,18}$ = 14.84, P = 0.001, respectively) and nektophagus (slope = 0.02, adjusted R^2 = 0.49, $F_{1,17}$ = 18.27, P < 0.01 and slope = 0.15, adjusted R^2 = 0.44, $F_{1,17}$ = 15.20, P = 0.001, respectively) in the rainy season (Figures 4A, B, E and F). $\delta^{15}N$ also showed significant correlations with Lt and log Wt for bentophagus-nektophagus (slope = 0.01, adjusted R^2 = 0.37, $F_{1,7}$ = 6.55, P = 0.05 and slope = 0.09, adjusted R^2 = 0.32, $F_{1,14}$ = 8.14, P = 0.01, respectively) only in the rainy season (Figures 4D and H). Additionally, $\delta^{15}N$ was correlated (slope = 0.01, adjusted R^2 = 0.48, $F_{1,43}$ = 41.61, P < 0.001) with Lt for nektophagus only in the dry season and with log Wt in dry (slope = 0.35, adjusted R^2 = 0.57, $F_{1,40}$ = 55.54, P < 0.001) and rainy (slope = 0.18, adjusted R^2 = 0.16, $F_{1,16}$ = 4.13, P = 0.06) seasons (Figures 4C, G and H).

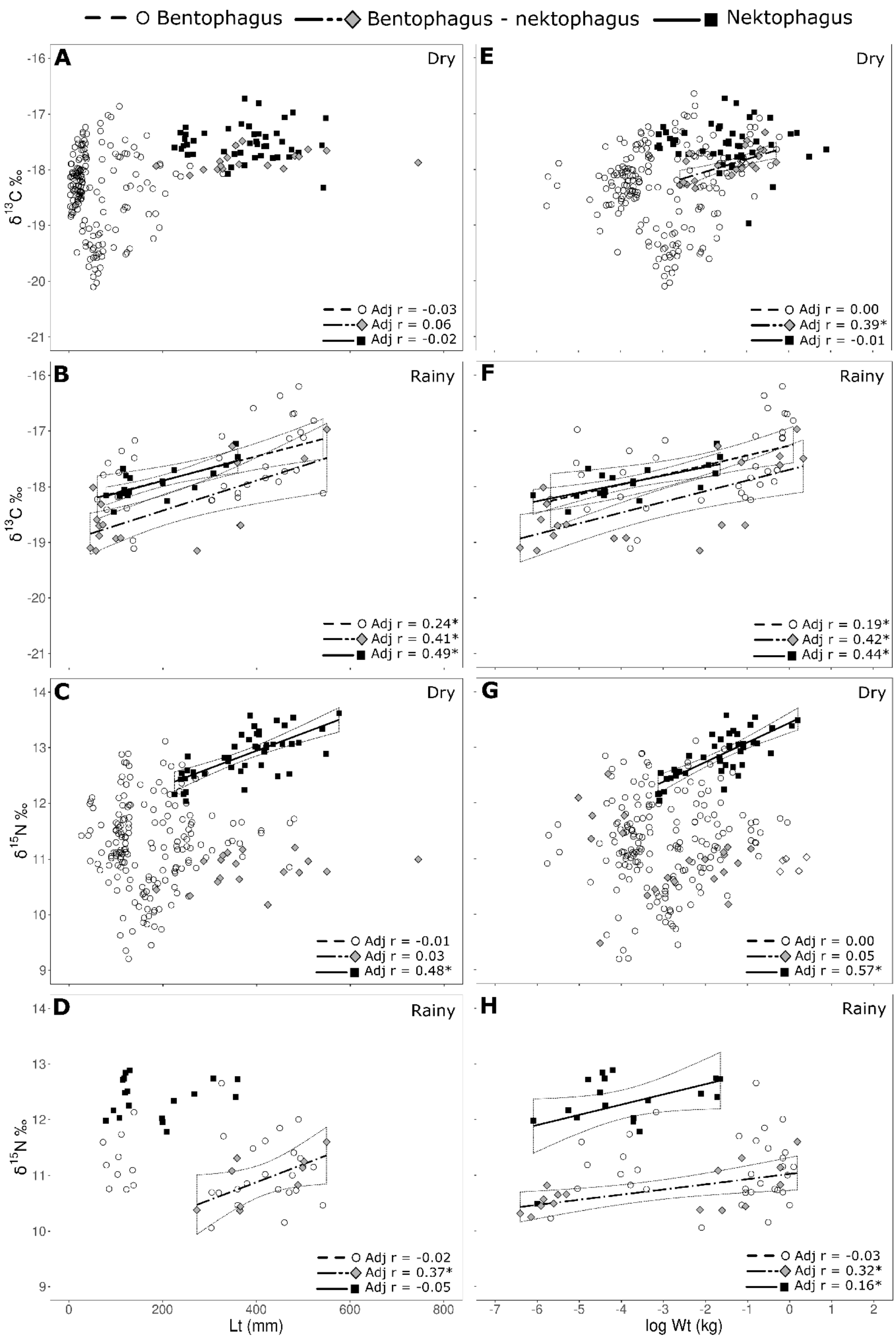

Figure 4. Relationships between: (A and B) $\delta^{13}C$ and Lt (mm), (C and D) $\delta^{15}N$ and Lt (mm), (E and F) $\delta^{13}C$ and Wt (kg), (G and H) $\delta^{15}N$ and Wt (kg) in the dry and rainy seasons, respectively, of the three trophic guilds bentophagus, bentophagus-nektophagus and nektophagus of demersal nekton in the northern Rio de Janeiro State, Southeastern Brazil. The results of linear models (lines + 95% confidence interval), equations and r values are only reported to statistically significant Pearson correlations. * indicates significant correlations (*P* < 0.05).

The isotopic niche areas represented by SEA revealed a broader trophic niche for bentophagus in both dry (1.22‰^{2}) and rainy (1.05‰^{2}) seasons (Figure 5). Bentophagus-nektophagus presented the smaller trophic niche in the dry season (0.51‰^{2}) whereas nektophagus was the smaller trophic niche in the rainy season (0.30‰^{2}). The greatest isotopic niche overlap was observed for the ellipse of bentophagus on bentophagus-nektophagus in the dry and rainy seasons, covering areas of 0.17 and 0.40‰^{2}, respectively. The lowest SIBER ellipse overlaps were observed for nektophagus on bentophagus in the dry season, covering an area of 0.03‰^{2}, whereas no overlap of nektophagus on bentophagus and bentophagus-nektophagus was observed in the rainy season (Table 2).

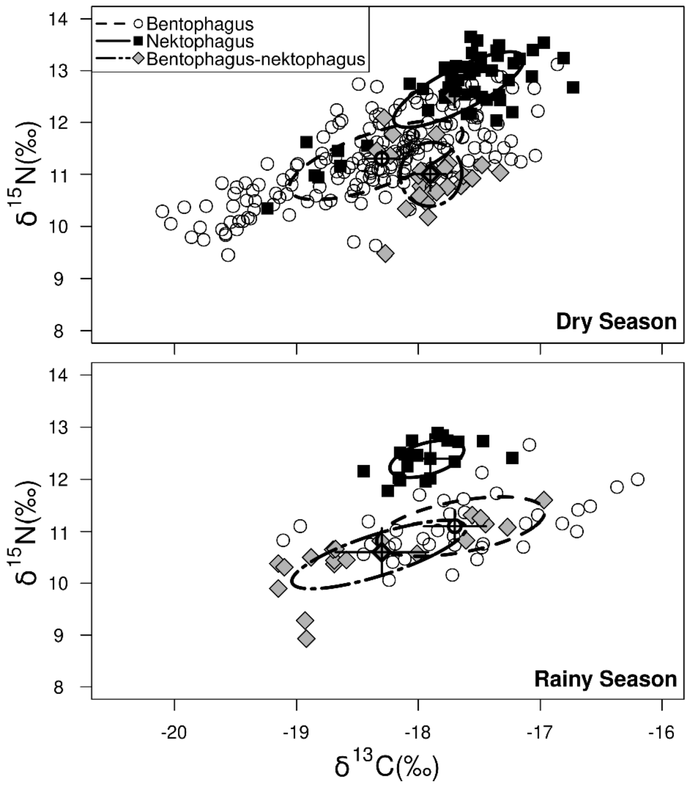

Figure 5. The standard ellipses area (SEA) in the dry and rainy seasons for the three trophic guilds bentophagus, bentophagus-nektophagus and nektophagus of demersal nekton in the northern Rio de Janeiro State, Southeastern Brazil. Bold symbols represent mean ± 95% confidence interval.

Table 2. Overlapping SEA (%) between the demersal nekton trophic guilds in the dry and rainy seasons in the northern Rio de Janeiro state, Southeastern Brazil.

	Bentophagus	**Bentophagus-nektophagus**	**Nektophagus**
Dry season			
Bentophagus	-	32.9	3.3
Bentophagus-nektophagus	13.6	-	0.0
Nektophagus	2.0	0.0	-
Rainy season			
Bentophagus	-	39.7	0.0
Bentophagus-nektophagus	36.3	-	0.0
Nektophagus	0.0	0.0	-

Table 3 presents the quantitative metrics to estimate the isotopic niche breadth for the three trophic guilds of demersal nekton. The highest values of trophic length (NR), variability of food resources (CR), total occupied niche area (TA) and standard ellipse areas (SEA) were observed for bentophagus regardless seasonality. The trophic diversity within the demersal food web (CD – ANOVA, F = 7.77, P < 0.001) was higher for bentophagus whereas trophic redundancy (MNND – ANOVA, F = 12.214, P < 0.001) and evenness (SDNND – F-ratio test, P < 0.01) were higher for bentophagus-nektophagus in the dry season. In the rainy season the highest CD (ANOVA, F = 7.01, P < 0.01) was observed for bentophagus and bentophagus-nektophagus and the highest SDNND (F-ratio test, P < 0.05) was observed only for bentophagus-nektophagus.

Seasonal significant differences in the isotopic niche metrics were also observed (Table 3). While CD was higher in the dry season for bentophagus (ANOVA, F = 5.64, P < 0.05), it was higher for bentophagus-nektophagus in the rainy season (ANOVA, F = 4.09, P < 0.05). The MNND (ANOVA, F = 58.84, P < 0.001) and SDNND (F-ratio test, P < 0.001) metrics varied only for bentophagus, with the highest values in the rainy season.

Table 3. Isotopic niche metrics of the demersal nekton trophic guilds in the dry and rainy seasons in the northern Rio de Janeiro state, Southeastern Brazil. Upper case and lower case letters indicate significant differences between trophic guilds in the same season and of trophic guilds between seasons, respectively ($P < 0.05$).

Trophic guild	NR	CR	TA	SEA	CD	MNND	SDNND
Dry season							
Bentophagus	3.67	3.24	6.84	1.22	0.89^{Aa}	0.10^{Aa}	0.07^{Aa}
Bentophagus-nektophagus	3.04	1.01	1.82	0.49*	0.51^{Ba}	0.19^{Ba}	0.18^{Ba}
Nektophagus	3.30	2.51	3.08	0.74	0.69^{Ba}	0.13^{Ca}	0.12^{Ca}
Rainy season							
Bentophagus	2.60	2.91	3.96	1.05	0.77^{Aa}	0.21^{Ab}	0.12^{Ab}
Bentophagus-nektophagus	2.67	2.18	1.93	0.99*	0.82^{Ab}	0.21^{Aa}	0.17^{Ba}
Nektophagus	1.11	1.22	0.70	0.28*	0.41^{Bb}	0.15^{Aa}	0.11^{ABa}

*Standard ellipse areas corrected for small sample size (SEAc).

DISCUSSION

This study evaluated the isotopic niche breadth of three demersal trophic guilds of the continental shelf of the Campos Basin in the northern Rio de Janeiro state for the first time, showing that SOM fuels the OM input into the food web and to what extent bentophagus, bentophagus-nektophagus and nektophagus guilds are probably sharing dietary resources, indicating resource competition.

In Santos continental shelf, Muto et al. (2014) observed sediment (-22.0 ± 1.27‰) and bottom POM (-20.9 ± 1.2 ‰) ^{13}C-enriched values than ours and attributed the OM source of the food web to phytoplankton pelagic primary production. In the present study, the nature of the bulk OM observed in sediment (median $\delta^{13}C$ = -22.5 and -22.4 ‰ in the dry and rainy seasons, respectively) seem to be related to autochthonous (marine) sources, regardless seasonality. However, the low $\delta^{13}C$ values observed in SOM (-27.1 to -19.8 ‰ and -25.5 to -19.3 ‰ in the dry and rainy seasons, respectively) are characteristic of estuarine areas (Bouillon et al., 2011) and indicates a mixture of autochthonous and allochthonous (terrigenous) OM inputs, as already reported for the coastal waters of Campos Basin (Carreira et al., 2015; Cordeiro et al., 2018). The authors observed similar $\delta^{13}C$ ranges (-26.8 to -21.6 ‰ and -26.1 to -22.9 ‰, respectively) and related the lower $\delta^{13}C$ values to PSR input of OM in the marine coastal sediment up to 150 m depth. They argue that both autochthonous and allochthonous sources fuel the OM pools in the sediment. While the PSR delivers reworked OM with preserved isotopic signal, the labile lipid biomarkers, also analyzed by the authors, reflected on marine OM origin. Additionally, the low $\delta^{13}C$ values of POM (-25.8 ± 0.7 and -24.6 ± 1.0 ‰ in the dry and rainy seasons, respectively) also indicate terrigenous influence in the OM pool off the coastal waters, reinforcing the PSR role in the demersal food web of continental shelf already reported for benthic invertebrates by Zalmon et al. (2013). In this sense, even with low PSR discharges in the dry and rainy seasons during the present study (Figure 2), the terrestrial influence in POM and SOM was observed, reflecting in a demersal food web fueled by a mixture of OM pool. Volumes of PSR discharges were exceptionally low during 2017 (samplings) and very similar in both dry (234.3 m^3 s^{-1}) and rainy seasons (148.0 m^3 s^{-1}). This may have reduced the contribution of PSR to food web.

Regarding the isotopic niche breadth, absence of overlaps and overlap' percentages below 40% were observed for all trophic guilds (Table 2). Nektophagus occupied the highest vertical positions in the demersal food web (as evidenced by higher average $\delta^{15}N$) in the dry and rainy seasons with lowest and absent overlaps with bentophagus and bentophagus-nektophagus, respectively. The highest $\delta^{15}N$ values concur with existing literature, which indicates that its diets rely mainly on fishes, other nektophagus (*Trichiurus lepturus*, *S. caribbaea*, *P. brasiliensis*) and bentophagus (*D.*

auriga, *E. longimanus*, *D. volitans*), with higher $\delta^{15}N$ than invertebrate prey consumed by bentophagus and bentophagus-nektophagus, thus, reflecting on the observed highest $\delta^{15}N$ values among the three trophic guilds (Valentim et al., 2008; Gasalla et al., 2010; Milessi and Mary, 2012; Sharp, 2017; Froese and Pauly, 2019). The higher $\delta^{15}N$ values for species that feed on fishes was already observed in tropical costal food webs by Bisi et al. (2012). The feeding behavior of *D. plei* throughout the water column might have influenced the variance in the isotopic niche metrics for nektophagus guild. The lolignid squid diet rely on fish prey with different habits, *i.e.* demersal (*Merluccius hubbsi*, *Trachurus lathami* and *Ctenosciaenna gracilicirrhus*), benthic (flatfish) and pelagic (*Anchoa* spp. and *Sardinella brasiliensis*), that directly affects the predator isotopic composition (Gasalla et al., 2010; Bouillon et al., 2011). In this sense, the higher NR, CR, TA, SEA and CD values in the dry season, compared to the rainy season, might be related the natural demerso-pelagic feeding habit of *D. plei*.

Bentophagus presented the broadest $\delta^{13}C$ space in dry and rainy seasons (highest CR values among guilds), suggesting a multiple isotopically distinct bottom-associated exploitation of resource pools (Table 3; Layman et al., 2007). The bentophagus species of the present study are reported by literature to feed on benthic invertebrates with different preferences and proportions. While *A. platana D. volitans*, *E. longimanus* and *P. nudigula* diets rely mainly on a single invertebrate group (crustaceans), *D. auriga* and *Z. brevirostris* exploits crustaceans and polychaetes (Kawakami and Amaral, 1983; Cussac and Molero, 1987; Schwingel and Assunção, 2009; Marion et al., 2011; São Clemente et al., 2014; Martins et al., 2017; Segadilha et al., 2017). The $\delta^{13}C$ range of the invertebrates (2.1 ‰) of the present study can explain, in part, the wide variation in source assimilation by bentophagus, since amphipods, isopods and polychaetes were not able to be sampled. Thus, as all invertebrate prey are common year-round off the northernmost Campos Basin shelf (Zalmon et al., 2013; 2015) and, consequently, highly available for consumers, the different types of invertebrates that compose bentophagus diets may explain its broadest $\delta^{13}C$ range, as well the high niche metrics values (NR, CR, TA, SEA and CD), among trophic guilds.

The overlaps among bentophagus-nektophagus and bentophagus guilds indicate a degree of resource partitioning off the shelf, as they might be assimilating different prey items of the same invertebrate groups with similar $\delta^{13}C$. The crustaceans (amphipods, decapods and isopods) reported for the three bentophagus-nektophagus species of the present study as the main prey items in stomach content studies might be a plausible explanation for the overlaps (Viana and Vianna, 2014; Idrissi et al., 2016; Cardozo et al., 2018). Crustaceans, along with polychaetes, constitute the most abundant macrofaunal invertebrate groups, been widely available for predators in the continental shelf sea floor (Lavrado et al., 2017). However, since *A. cyclophora* diet rely mainly on a single decapod species (*Achelous spinicarpus*), *O. vulgaris* on decapods and *P. arenatus* on amphipods, decapods, teleost fishes, isopods, cephalopods and polychaetes, the differences in prey consumption compared to bentophagus guild reflected on the distinctness of the isotopic niche breadth and metrics (NR, CR, TA, SEA and CD), regardless seasonality. Additionally, the lowest trophic niche redundancy (MNND) and evenness (SDNND) observed for bentophagus-nektophagus guild in the dry and rainy seasons might be related to *P. arenatus* diet that is reported to have the most diverse prey types among bentophagus-nektophagus and bentophagus guilds.

No intraspefic shifts in sources neither in trophic level (+1.0 and +3.4 ‰, respectively; Post et al., 2002) was observed for bentophagus, bentophagus-nektophagus and nektophagus species, due to low $\delta^{13}C$ and $\delta^{15}N$ ranges. The correlations of Lt and Wt with $\delta^{13}C$ and $\delta^{15}N$ indicated interspecific different dietary prey assimilation within trophic guilds (Figure 4). The wide body size variation observed between species within each trophic guild coupled with the continuous rise of $\delta^{13}C$ and $\delta^{15}N$ as body size increases indicated differences in gape sizes within each trophic guild are affecting the consumption and, consequently, the assimilation of prey, with larger nekton species consuming resources of greater average prey size enriched in ^{13}C and ^{15}N (Jennings et al., 2002; Segura et al., 2015).

Additionally, differences in $\delta^{13}C$ values seem to reflect seasonal differences in habitat use and trophic behavior by demersal fish and cephalopods related to intra and interspecific levels. The $\delta^{13}C$ values are usually applied to distinguish different dietary-based carbon sources, with benthic and inshore primary resources enriched in $\delta^{13}C$ compared to pelagic and offshore ones (Fry, 2008; Bouillon et al., 2011). In the Campos Basin, Martins et al. (2017) argued that the presence of benthic and pelagic crustaceans in the diet of benthophagus and bentophagus-nektophagus species off the continental shelf is related to a lower segregation of the benthic and pelagic compartments in the water column coupled with high habitat complexity that provides higher quantity and variety of prey when compared to outer shelf and slope areas. Thus, in the present study the benthophagus $\delta^{13}C$ values trended from benthic and inshore associated food chain in the dry season to a pelagic and offshore one in the rainy season, whereas the inverse pattern was observed for bentophagus-nektophagus guild, similarly to the observed $\delta^{13}C$ patterns in three Brazilian tropical coastal food webs (Bisi et al., 2012).

The present study was aimed to assess trophic relationships through $\delta^{13}C$ and $\delta^{15}N$ of the main demersal nekton trophic guilds off the northernmost Campos Basin continental shelf. Measured SOM values were similar between dry and rainy seasons, indicating that the demersal food web is fueled by a mixture of marine and terrestrial OM and highlighting the importance of PSR in the trophic relations off the adjacent coastal waters. Thus, our second hypothesis was partially accepted whereas the third one was refuted. Nektophagus was the most $\delta^{15}N$ enriched guild due to the assimilation of nekton prey occupying higher vertical positions in the food web. Bentophagus and bentophagus-nektophagus (<40 %) isotopic niche overlaps were related to a degree of similarity in invertebrate resources, rejecting the first tested hypothesis and rising evidence of resource competition. The high abundance of invertebrates all year-round due to habitat complexity and low segregation of benthic and pelagic compartments seem to have led to seasonal trends in benthic and inshore to pelagic and offshore behaviors for bentophagus and bentophagus-nektophagus guilds. Interspecific variations were detected and related to differences in body and gape sizes between species of the same guilds, reflecting in enriched $\delta^{13}C$ and $\delta^{15}N$ prey assimilation by larger specimens. The understanding of the trophic dynamics of demersal nekton enhances the knowledge of ecological and environmental forces driving their coexistence. More studies incorporating stable isotopes are important for the advancement of knowledge of the trophic dynamics, habitat usage and environmental role of demersal nekton off Campos Basin continental shelf.

ACKNOWLEDGEMENTS

We are greatful to MSc. Thiago P. Rangel for geoprocessing.

FINANCIAL SUPPORT

This research was supported by Fundação de Amparo à Pesquisa do Rio de Janeiro – FAPERJ (Rezende CE, Grant number E-26/110.437/2010) and by Conselho

Nacional de Desenvolvimento Científico e Tecnológico – CNPQ (INCT Material Transfer at the Continent-Ocean Interface, Rezende CE, grant number 573.601/08-9). Likewise, this research study was financedin part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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Capítulo 4 – a ser submetido para Environmental Science and Pollution Research

Mercury and nitrogen stable isotope in fish and cephalopods from the southwestern Atlantic

Authors names and affiliations:
Gatts, P.V.[a] *, Franco, M.A.L.[b], Almeida, M. G.[b], Rezende, C.E.[b], Costa, P.A.S.[c].

[a] Instituto de Geociências, Universidade Federal Fluminense, Programa de Pós Graduação de Dinâmica dos Oceanos e da Terra, Niterói RJ CEP 24210-346, Brazil. pedrogatts90@gmail.com.
[b] Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes RJ CEP 28013-602, Brazil. malfranco@yahoo.com.br, crezende@uenf.br.
[c] Departamento de Ecologia e Recursos Marinhos, Instituto de Biociências, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro RJ CEP 22290-240, Brazil. paulocosta.unirio@gmail.com.

ABSTRACT

The total mercury concentrations standardized by length (THg_{STD}) and stable isotope ratios of nitrogen ($\delta^{15}N$) of three demersal guilds (bentophagus, bentophagus-nektophagus and nektophagus) were evaluated to tests the hypothesis that predators with highest $\delta^{15}N$ of demersal nekton's food web over the continental shelf of Campos Basin, from 40 to 60 m depth, presents the highest THg_{STD} concentrations and are under the limits regulated for human consumption. The highest THg_{STD} values (310.8 ± 121.4 ng g^{-1} dw and 46.6 ± 18.2 ng g^{-1} ww) were observed within bentophagus guild, whereas the lowest (46.6 ± 6.6 ng g^{-1} dw and 7.0 ± 1.0 ng g^{-1} ww) found in bentophagus-nektophagus guild, representing no threat for human consumption. $\delta^{15}N$ highest values were observed in bentophagus and nektophagus and the lowest in bentophagus-nektophagus. Bioaccumulation process was indicated by significant correlations of $\delta^{15}N$ and THg_{STD} with total length (Lt) and total weight (Wt). The significant relationship between $\delta^{15}N$ and THg_{STD} reflected in biomagnifications process through the food web

only with benthic sources, indicating that organic matter in sediment is the main input of Hg in the demersal food web.

Keywords: bioaccumulation, biomagnification, trophic guilds, food web.

INTRODUCTION

Mercury (Hg) is a non-essential metal and also an element with high mobility and toxicity (Förstner e Wittmann, 1983; He e Wang, 2011). In aquatic ecosystems, the two main oxidized states of this element are the elemental (Hg^0) and the ion mercury (Hg^{+2}), most abundant form with high affinity with organic binders (Selin et al., 2009). Although the diversity of chemical forms that Hg can assume (Harris et al., 2003; George et al., 2008), methylmercury (MeHg) is the most toxic one due to its properties of elevated neurotoxicity, easy penetration in biological membranes, efficient bioaccumulation, high stability and long term retention in biological tissues (Cabañero et al., 2007; Cabañero et al., 2004; Chapman and Chan, 2000).

This metal is transported to aquatic ecosystems through natural and anthropogenic sources and can be accumulated over the lifetime of organisms (bioaccumulation) and trend to increase from lower to higher trophic levels (biomagnification) across food webs (Lawson e Mason, 1998). In fishes (bony and cartilaginous), as well in aquatic invertebrates, the MeHg (*i.e.* the Hg organic form) is absorbed and concentrated preferentially on muscle tissue (Francesconi & Lenanton, 1992; Wagemann et al., 1998; Baeyens et al., 2003). Consequently, the highest Hg concentrations are observed in high trophic level species, such as piscivores and carnivores (Buck et al., 2019).

As trophic level is closely correlated with nitrogen isotope ratios ($\delta^{15}N$), under the assumption that the heavier isotope (^{15}N) is 3 to 4‰ enriched by trophic level, this marker has been intensively used in food web studies (Post, 2002; Layman et al., 2015).

Researchers have been using $\delta^{15}N$ coupled with Hg concentrations to understand food web vertical organization and metal-related processes (McKinney et al., 2001; Chouvelon et al., 2018).

In Campos Basin (CB), Paraíba do Sul River (PSR) is reported as important to Hg supply in continental shelf coastal waters. It is the main input of terrigenous waters in the southeast Brazilian coast, exporting organomercurial fungicides used in sugarcane plantations and the mining activity (in the 1980s) due to anthropogenic activities throughout its drainage basin (Lacerda et al., 1993; Araujo et al., 2017). The river is the major terrigenous OM supply in the region, acting as a transition zone from fresh to shallow marine coastal waters, presenting a seasonal discharge pattern linked to rainfall, with dry (May to September) and rainy (October to April) seasons (Almeida et al., 2007), as well interannual discharges of suspended particle matter (0.8 to 2.0×106 t/year) (Carvalho et al., 2002; Figueiredo et al. 2011; Ovalle et al. 2013). Due to seasonality in river water discharge, riverine water take about 10 days to reach open ocean waters (salinity ~35) in the dry season, whereas 6 days in the rainy season (Souza et al., 2010). However, regardless seasonality, Hg concentrations in bottom sediments over the continental shelf rapidly decreases below 30 m depth, increasing its concentration in slope (Araujo et al., 2017).

Over the CB continental shelf and slope, Fagundes-Netto and Gaelzer (1991) and Costa et al. (2015) observed demersal teleosts and squids as the most abundant shelf groups. In 2017, Martins et al. established 6 trophic guilds (bentophagus, bentophagus-nektophagus, generalists, nektophagus, nektophagus-bentophagus and zooplanktophagus-bentophagus) between 13 to 2000 m depth based on stomach content analysis. In the CB shelf, previous researches had already reported Hg exported by the PSR riverine waters in marine coastal food webs, accumulating at basal sources (phytoplankton) and magnifying to top predators (fishes) (Kehrig et al., 2009; Di Beneditto et al., 2012; Kehrig et al., 2013). The authors observed elevated total mercury (THg) values (1,400 ngHg g^{-1}) for the top predator fish *Trichiurus lepturus* by associating the concentration of THg with nitrogen stable isotopes ($\delta^{15}N$) of its food web on the coast of Rio de Janeiro State. The elevated THg in predators, higher than the maximum permissible limit (1,000 ngHg g^{-1} for predators) for human consumption regulated by World Health Organization (WHO, 1976) and Brazilian legislation (ANVISA, 2013), rise concern regarding the consumption of commercial fisheries commonly represented by demersal marine species (Watson and Tidd, 2018).

In this sense, the present study tested two hypothesis: 1) as Hg concentrations decrease in bottom sediment at depths under 30 m regardless seasonality, negligible Hg variations are observed among trophic guilds between dry and rainy seasons; and 2) predators with highest $\delta^{15}N$ of demersal food web in the continental shelf of CB, from 40 to 60 m depth, presents the highest THg concentrations and are under the limits regulated for human consumption, following the Hg concentration reduction already observed for bottom sediment. Thus, the objective was to evaluate and compare the relationships of THg concentrations and $\delta^{15}N$ in different trophic guilds of demersal nekton from 40 to 60 m depth in the northern coast of Rio de Janeiro State.

MATERIAL AND METHODS

Study site

The Paraíba do Sul River (PSR) is located in the southeast Brazil, flowing across the Rio de Janeiro State, where finally drains off to the Atlantic Ocean (21° 36’ S, 41° 05’ W) (Rudorff et al., 2011) (Figure 1).

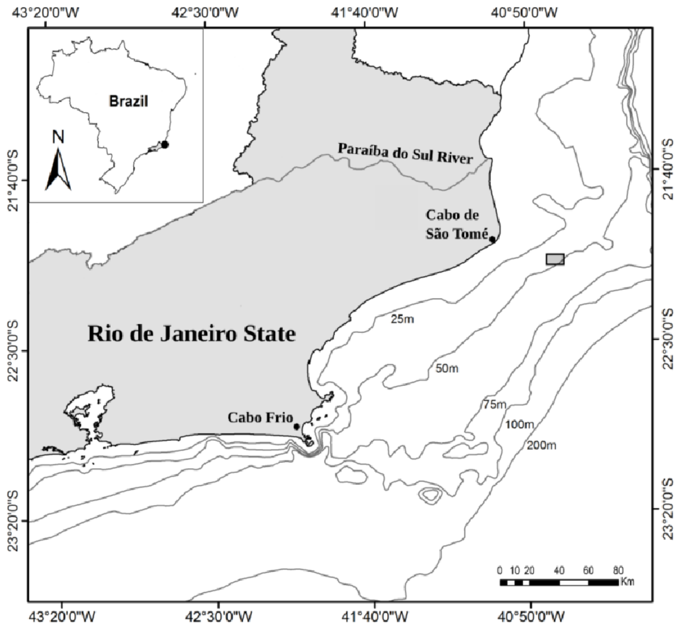

Figure 1. Study site at the continental shelf in southwestern Atlantic on Campos Basin, Brazil. The grey rectangle represents the sampling area.

The PSR drainage basin has an approximate area of 58,400 km² and an extension of 1,145 km (Almeida et al., 2007). The PSR discharge is measured twice a month all over the year since 1995 by the Laboratório de Ciências Ambientais from Universidade Estadual do Norte Fluminense Darcy Ribeiro. In Figure 2, PSR discharges from each sampling month September (2015), July (2016) and April (2017) as well from 1995 to 2017 is represented. The historical highest riverine discharge reached 1,004.5 m^3 s^{-1} in the summer (October to April) whereas the lowest discharge in the winter (May to September) reached 410.3 m^3 s^{-1}. In our samplings, PSR discharges were similar between sampling seasons, with lower mean values (234.3 and 148.0 m^3 s^{-1} in dry and rainy seasons, respectively) compared to the historical pattern of the river, especially in the rainy season. The PSR plume, that can reach 32 km from the coast, transport to the ocean nutrients, inorganic and organic compounds that play important roles in coastal ecosystems (Souza et al., 2010; Rudorff et al., 2011).

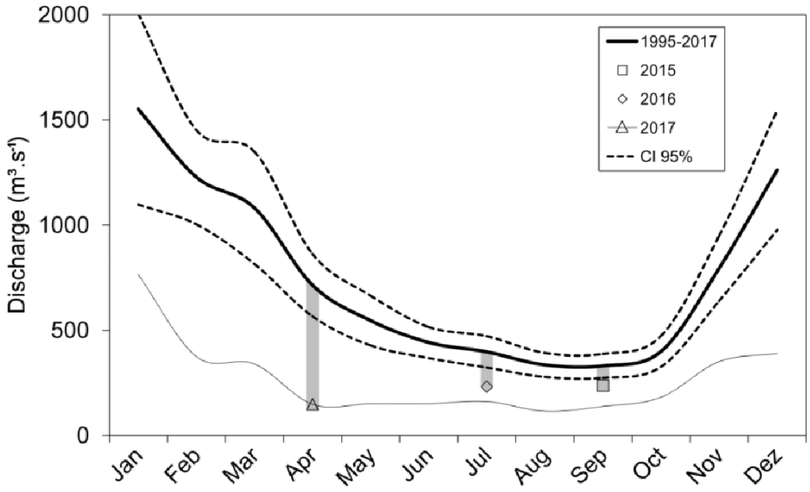

Figure 2. Discharge of PSR during the time series of 1995 - 2017 and the sampling periods on 2015, 2016 and 2017. Highlighted in grey are the months when sampling was performed. CI: confidence interval. Source: Laboratório de Ciências Ambientais – Universidade Estadual do Norte Fluminense Darcy Ribeiro.

Along PSR many anthropic activities have already been and some are still practiced (Lacerda et al., 1993; Rudorff et al., 2011; Ovalle et al., 2013). The main sources of Hg are related to the use of organomercurial fungicides in sugar cane plantations, gold extraction and industrial productions of steel, chemicals, food and paper.

Sampling

Fishes and cephalopods off the northernmost continental shelf of Campos Basin (22°S, 40°W) were sampled with bottom trawls performed over sandy and muddy bottoms between 43 and 53 m. The trawl was a 12 m ground rope, 1.2 m height and 15 mm stretch mesh in the cod-end, towed by a single warp cable aboard the fishing boat Karen I (12 m, 156 HP), and specifically rented for the samplings.

Three bottom trawls were performed at the same study site on the continental shelf, during September (2015), July (2016) and April (2017), representing the dry season and the end of the rainy season, respectively. A total of nine trawls were performed, with a mean speed of 3.0 knots, 20 minutes each, corresponding to a triplicate on each sampling period.

All specimens (fishes and cephalopods) were identified using specific literature (Figueiredo, 1977; Figueiredo & Menezes, 1978; 1980; Carpenter, 2002a,b). During the samplings, the specimens were maintained fresh on ice, transported to the laboratory and kept frozen. Fishes and cephalopods were measured in total length (Lt, mm) and mantle length (Ml, mm) respectively, and had their total weight (Wt) recorded. Epibenthic macroinvertebrates, were also collected by bottom trawls, identified using specific literature (Carpenter, 2002a) and included in further analyses to compose the demersal food web.

Previously to the bottom trawls, depth was recorded and sediment collected to determine the organic matter that fuels the demersal food web. A box-corer was used to collect undisturbed 0-2 cm layers of the sediment on each bottom trawl. A sub-aliquot of each sediment sample was used to record total sedimentary organic matter (SOM) in triplicates. Samples were sieved and the passing fraction (< 63 µm) was lyophilized.

A fragment of the dorsal muscle of fish, mantle of cephalopods and pieces of soft tissues (avoiding gastric tracts) of macroinvertebrates as well as sediment were collected, lyophilized (L10, Liotop) and homogenized for mercury and stable isotope analysis at the Laboratório de Ciências Ambientais from Universidade Estadual do Norte Fluminense Darcy Ribeiro.

Total mercury analysis – THg

Dry muscle samples were digested according to Bastos *et al*. (1998). Briefly, the digestion of tissue samples was performed in a digestion block (60 °C, 4 h) with a mixture of 3 ml HNO_3:H_2SO_4 (1:1) and 1 ml of concentrated H_2O_2 and allowed to stand overnight. The addition of 5 ml of 5% KnMnO4 and subsequent 30 min of heating were followed by titration with hydroxylamine hydrochloride (HONH3Cl + NaCl 12%). THg was determined with a QuickTrace M-7500 CETAC (CV-AAS). The detection limit was 0.25 ng g^{-1}. The analytical control of the method was determined and monitored using replicate analysis, blank solutions, and certified reference material from the National Research Council-Canada (DORM-2 dogfish *Squalus acanthias* muscle sample). The analytical coefficient of variation between replicates was less than 10%, and the recovery of THg was in agreement with certified values (higher than 90%).Values of commercial species were compared with the Brazilian legislation and WHO values for mercury intake by humans (WHO, 1976; ANVISA, 2013). The dry weight basis concentration of Hg was converted to wet weight considering 75% of water lost during freeze-dry (Di Beneditto et al., 2013). THg results were expressed in ng g^{-1} in dry weight (dw) and wet weight (ww).

Stable isotope analysis – δ^{13}C and δ^{15}N

All samples were stored in clean transparent plastic bags in iceboxes and then transported to the laboratory where they were kept frozen (-18°C) in dry sterile vials prior to analysis. Lyophilized samples were ground with a mortar and pestle to a homogeneous fine powder. Approximately 0.5 to 1.0 mg of animal muscle tissue was used in the analysis.

For δ^{15}N of sediment, approximately 10 mg was weighed in silver capsules, respectively, followed by acidification through the addition of HCl (2M) to remove inorganic carbon (Kennedy et al. 2005; Brodie et al. 2011).

The elemental and isotopic composition of all samples were determined by using an Elemental Analyzer (Flash 2000) with interface CONFLO IV coupled to an isotope ratio mass spectrometer Delta V Advantage (Thermo Scientific, Germany) in Laboratório de Ciências Ambientais. Samples were analysed using analytical blanks and urea analytical standards (IVA Analysentechnik-330802174; CH4N2O Mw= 60, N= 46%), using certified isotopic composition ($\delta^{15}N$ = -0.73‰). Analytical control was done for every 10 samples using certified isotopic standard (Elemental Microanalysis Protein Standard OAS: 13.32 ± 0.40% for N; +5.94 ± 0.08‰ for $\delta^{15}N$).

Nitrogen isotope ratios were expressed in δ notation as ‰ relative to atmospheric nitrogen, respectively, and were calculated using the following equation:

$$\delta = (Rsample/Rstantard) \times 10^3,$$

where $\delta = \delta^{15}N$ and $R = \delta^{15}N:\delta^{14}N$. The analytical reproducibility was based on triplicates for every 10 samples were ± 0.3‰.

Data standardization

THg concentrations were standardized by fish/cephalopod length as proposed by Sccuder-Eikenberry et al. (2015) as variability in contaminant levels can be reduced by standardization based on fish length (Wiener et al., 2007). Hereafter, contaminant concentration standardized by fish and cephalopod lengths will be referred as THg_{STD}.

Data treatment and analysis

Species that accumulated more than 80% of Wt and occurred in more than 20% of the trawls were used to describe the fish and cephalopods and were classified into three trophic guilds adapted from Martins et al. (2017) in the Campos Basin continental shelf and slope: bentophagus (benthic invertebrates representing > 80% of the diet); bentophagus-nektophagus (benthic invertebrates representing > 50% and fish and cephalopods representing > 20%); and nektophagus (fish and cephalopods representing

> 80% of the diet). The THg_{STD} and $\delta^{15}N$ means and 95% confidence intervals as well as Lt and Wt ranges were estimated for the three demersal trophic guilds bentophagus, bentophagus-nektophagus and nektophagus in the dry and rainy seasons.

Normality and homoscedasticity of data was tested using Shapiro-Wilk and Levene tests, respectively. The differences in THg_{STD} and $\delta^{15}N$ were analyzed using ANOVA (type III), applied for unbalanced data (Shaw and Mitchell-Olds, 1993) and followed by the Tukey HSD *post hoc* test. When normality and homoscedasticity were not achieved non-parametric analysis of variance (Kruskal-Wallis) was applied followed by Dunn's *post hoc* test. The Pearson correlation was used to correlate log-transformed THg_{STD} with Lt, logWt and $\delta^{15}N$. Results were considered significant at $P < 0.05$. F-ratio test was applied to test the significance of regressions (Best and Roberts, 1975). THg and $\delta^{15}N$ means and 95% confidence intervals of the macroinvertebrates Cidaridae (n = 1, THg = 118.7 ng g^{-1},$\delta^{15}N$ = 9.2 ‰), Ostreidae (n = 4, THg = 169.3 ± 7.1 ng g^{-1} dw, $\delta^{15}N$ = 4.8 ± 0.3‰), Paguridae (n = 2, THg = 48.0 ± 5.1 ng g^{-1} dw, $\delta^{15}N$ = 11.3 ± 0.1‰), Scyllaridae (n = 2, THg = 84.4 ± 3.2 ng g^{-1} dw, $\delta^{15}N$ = 7.2 ± 0.2‰) and Squillidae (n = 2, THg = 245.3 ± 144.3 ng g^{-1} dw, $\delta^{15}N$ = 11.1 ± 0.4‰) were only included in food web correlations.

RESULTS

A total of 274 specimens (dry = 223; rainy = 51), distributed in fishes (n = 254) and cephalopods (n = 20) were analyzed. The demersal nekton assemblage was represented by bentophagus (dry = 156; rainy = 25), bentophagus-nektophagus (dry = 13; rainy = 8) and nektophagus (dry = 54; rainy = 18) trophic guilds (Table 1).

Interguild significant variations of THg_{STD} and $\delta^{15}N$ were observed in both dry and rainy seasons (Table 1). Bentophagus THg_{STD} concentrations were highest in the dry season (ANOVA, $F = 4.27$, $P < 0.05$) and in the rainy season (ANOVA, $F = 8.96$, $P < 0.001$). Bentophagus and nektophagus presented the highest $\delta^{15}N$ values in the dry season (Kruskal-Wallis, $X^2 = 25.48$, $P< 0.001$), whereas only nektophagus $\delta^{15}N$ was highest in the rainy season (Kruskal-Wallis, $X^2 = 25.11$, $P < 0.001$).

Intraguild significant differences of THg and $\delta^{15}N$ were observed between seasons (Table 1). For bentophagus the highest THg (ANOVA, $F = 48.48$, $P < 0.001$) was observed in the rainy season whereas $\delta^{15}N$ (ANOVA, $F = 68.10$, $P < 0.001$) was higher in the dry season. For bentophagus-nektophagus while THg (ANOVA, $F = 21.46$, $P < 0.001$) was highest in the dry season, $\delta^{15}N$ (Kruskal-Wallis, $X^2 = 0.76$, $P > 0.05$) was similar between seasons. For nektophagus THg (ANOVA, $F = 5.85$, $P < 0.05$) and $\delta^{15}N$ (Kruskal-Wallis, $X^2 = 13.26$, $P < 0.001$) were both highest in the dry season.

Table 1. Trophic guilds, sample size (n), mean ± 95% confidence interval of total length (Lt; mm), total weight (Wt; g), total mercury (THg; ng g^{-1}) in dry weight (dw) and wet weight (ww) and $\delta^{15}N$ (‰) of the three trophic guilds in the northern Rio de Janeiro State, Southeastern Brazil. Upper case and lower case letters indicate significant differences between trophic guilds in the same season and of trophic guilds between seasons, respectively ($P < 0.05$).

	Season	*n*	Lt(minimum – maximum)	Wt(minimum – maximum)	THg_{STD} dw	THg_{STD} ww	$\delta^{15}N$
Bentophagus	Dry	156	26 – 470	3.3 – 650.0	114.2 ± 11.1^{Aa}	17.1 ± 1.7	13.6 ± 0.2^{Aa}
	Rainy	25	111 – 542	12.1 – 1000	310.8 ± 121.4^{Ab}	46.6 ± 18.2	11.1 ± 0.3^{Ab}
*Atlantoraja platana***	Dry	2	215 – 470	67.3 – 650.0	300.5 ± 272.6	45.1 ± 40.9	16.3 ± 3.0
	Rainy	6	304 – 542	123.1 – 1000.0	53.0 ± 26.6	8.0 ± 4.0	10.8 ± 0.4
Dactylopterus volitans	Dry	75	66 – 290	17.4 – 258.5	82.7 ± 9.4	12.4 ± 1.4	13.5 ± 0.3
	Rainy	4	138 – 330	22.8 – 450.0	216.9 ± 206.2	32.5 ± 30.9	11.9 ± 0.8
Dules auriga	Dry	56	26 – 131	3.3 – 39.2	134.2 ± 12.0	20.1 ± 1.8	13.8 ± 0.3
	Rainy	–	–	–	–	–	–
Etropus longimanus	Dry	3	103 – 105	8.5 – 9.2	28.4 ± 7.5	4.3 ± 1.1	12.1 ± 0.3
	Rainy	1	111	12.1	32.0	4.8	10.4
Prionotus nudigula	Dry	7	44 – 113	12.5 – 18.6	114.2 ± 35.5	17.1 ± 5.3	14.5 ± 0.1
	Rainy	–	–	–	–	–	–
*Zapteryx brevirostris***	Dry	13	225 – 370	70.3 – 480.0	201.2 ± 27.4	30.2 ± 4.1	12.0 ± 1.0
	Rainy	14	305 – 500	350.0 – 1000.0	468.0 ± 166.1	70.2 ± 24.9	11.1 ± 0.3
Bentophagus-Nektophagus	Dry	13	56 – 510	1.9 – 920.0	112.1 ± 21.1^{ABa}	16.8 ± 3.2	11.0 ± 0.7^{Ba}
	Rainy	8	273 – 550	118.7 – 1200.0	46.6 ± 6.6^{Bb}	7.0 ± 1.0	10.9 ± 0.3^{Aa}
*Atlantoraja cyclophora***	Dry	2	287 – 510	1.9 – 850.0	65.2 ± 12.8	9.8 ± 1.9	12.9 ± 3.7
	Rainy	8	273 – 550	118.7 – 1200.0	46.6 ± 6.6	7.0 ± 1.0	10.9 ± 0.3
*Octopus vulgaris***	Dry	11	56 – 151*	34.0 – 920.0	120.7 ± 21.2	18.1 ± 3.2	10.6 ± 0.2
	Rainy	–	–	–	–	–	–
Nektophagus	Dry	54	74 – 548	8.6 – 2450	84.1 ± 14.4^{Ba}	12.6 ± 2.2	13.2 ± 0.4^{Aa}
	Rainy	18	108 – 360	6.3 – 192.8	52.7 ± 8.8^{Bb}	7.9 ± 1.5	12.3 ± 0.2^{Bb}
*Dorytheutis plei***	Dry	9	74 – 109*	8.6 – 25.7	55.4 ± 7.2	8.3 ± 1.1	10.9 ± 0.7

	Rainy	–	–	–	–	–	–
*Lophius gastrophysus***	Dry	2	459 – 475	1215.8 – 2450	243.7 ± 173.7	36.6 ± 26.1	13.7 ± 0.1
	Rainy	–	–	–	–	–	–
*Percophis brasiliensis***	Dry	43	225 – 548	44.0 – 643.2	82.7 ± 12.9	12.4 ± 1.9	13.7 ± 0.3
	Rainy	10	195 – 360	24.3 – 192.8	51.3 ± 10.3	7.7 ± 1.5	12.2 ± 0.4
Saurida caribbaea	Dry	–	–	–	–	–	–
	Rainy	8	108 – 130	6.3 – 14.9	54.4 ± 15.9	7.6 ± 2.4	12.6 ± 0.2
Total Dry		223					
Total Rainy		51					
Total		274					

*Mantle length; ** species of commercial value (Garcez et al., 2007; FIPERJ 2015).

The THg_{STD} was significantly correlated with Lt and logWt for bentophagus only in the rainy season, whereas with bentophagus-nektophagus and nektophagus only in the dry season. THg_{STD} also showed correlation in the rainy season with $\delta^{15}N$, but only for bentophagus-nektophagus guild (Table 2).

Table 2. Linear regressions (α = 0.05) between log-transformed THg_{STD} and Lt, log-transformed Wt and $\delta^{15}N$ in bentophagus, bentophagus-nektophagus and nektophagus demersal guilds in the dry and rainy seasons. ns: non-significant.

Guild	Season	Slope	Adjusted R^2	*df*	*F*	*P*-value
Bentophagus						
Lt *vs* $\log_{10}THg_{STD}$	Dry	0.0005	-0.0062	156	0.03	ns
	Rainy	0.0027	0.3028	23	11.43	**
logWt *vs* $\log_{10}THg_{STD}$	Dry	0.0001	-0.0041	154	0.37	ns
	Rainy	0.0012	0.5160	23	26.59	***
$\delta^{15}N$ *vs* $\log_{10}THg_{STD}$	Dry	-0.0046	-0.0058	155	0.10	ns
	Rainy	0.2761	0.0910	23	3.40	ns
Bentophagus-Nektophagus						
Lt *vs* $\log_{10}THg_{STD}$	Dry	-0.0008	0.3872	11	8.58	**
	Rainy	0.0001	-0.1068	6	0.33	ns
logWt *vs* $\log_{10}THg_{STD}$	Dry	-0.0003	0.6443	10	20.93	***
	Rainy	0.0001	0.0245	6	1.18	ns
$\delta^{15}N$ *vs* $\log_{10}THg_{STD}$	Dry	-0.0530	0.0988	11	2.32	ns
	Rainy	0.1214	0.4311	6	6.31	*
Nektophagus						
Lt *vs* $\log_{10}THg_{STD}$	Dry	0.0006	0.1113	53	7.76	**
	Rainy	0.0005	0.0291	17	1.54	ns
logWt *vs* $\log_{10}THg_{STD}$	Dry	0.0005	0.1870	54	13.65	***
	Rainy	0.0007	0.0259	17	1.48	ns
$\delta^{15}N$ *vs* $\log_{10}THg_{STD}$	Dry	0.0497	0.0745	52	5.27	*
	Rainy	0.0273	-0.0540	16	0.13	ns

*** *P*-value $\leq$ 0.001.
** *P*-value $\leq$ 0.01.
* *P*-value $\leq$ 0.05.

The positive relationships between THg_{STD} and $\delta^{15}N$ in the dry and rainy seasons were not significant ($P > 0.05$) for the food webs with the pelagic sources phytoplankton, zooplankton and ostreids (Figure 3A and B). However, when pelagic sources are removed and only benthic sources are included in the linear model significant ($P \leq 0.05$) relationships were observed, with the positive slopes of 0.12 and 0.13 and 64 and 43% of the THg_{STD} concentrations variations as function of $\delta^{15}N$ values in the dry and rainy seasons, respectively. This indicates Hg biomagnification over the increase in $\delta^{15}N$ in the demersal marine food web (Figure 3C and D).

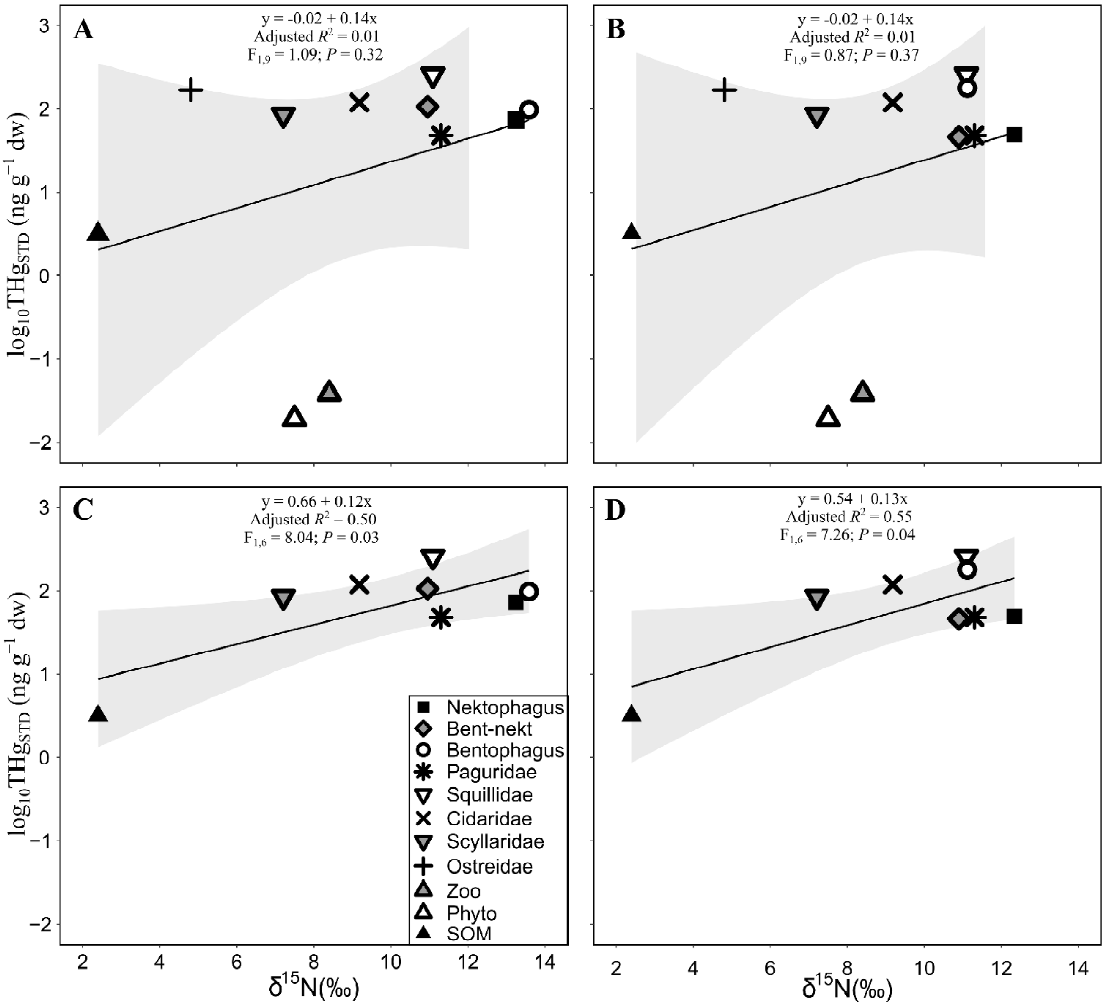

Figure 3. Relationships between: (A-B) $\delta^{15}N$ and log-transformed THg_{STD} of demersal food web with pelagic sources and (C-D) $\delta^{15}N$ and log-transformed THg_{STD} of demersal food web without pelagic sources in the dry and rainy seasons, respectively, of Campos Basin shelf, northern Rio de Janeiro. The results from the linear models (lines ± 95% confidence interval) are plotted on observed log-transformed data. The adjusted R^2, statistic F and P-values of the Pearson correlations as well as the equations of the lines (derived from linear models output) are indicated. Bent-nekt = bentophagus-nektophagus.

DISCUSSION

This study evaluated the THg_{STD} and $\delta^{15}N$ relationships between three demersal trophic guilds in the southwestern Atlantic (22°S, 40°W). For the first time, information on THg_{STD} concentrations and bioaccumulation in demersal fish and cephalopods, as well biomagnification across the food web was documented for 30 m deeper waters in the region.

Bentophagus presented higher THg_{STD} than the other guilds, which may be a consequence of its bottom associated prey assimilation (mainly crustaceans and polychaetes) (Martins et al., 2017). As food resource consumption is the most important route of Hg assimilation for organisms (Hall et al., 1997), and this demersal food web is fueled (at least in part) by the organic matter buried in the sediment (Gatts et al., *in prep.*), the higher Hg bioavailability compared to water column will influence metal concentration in potential bottom associated prey and, consequently, the demersal nekton predators (Muto et al., 2014; Le Croizier et al., 2019).

The bioaccumulation of THg_{STD} was observed in bentophagus only in the rainy season and in bentophagus-nektophagus and nektophagus only in the dry season seems to be three-fold: first, biological characteristics such as age, body size and the metal excretion physiology can affect Hg bioaccumulation in organisms (Kasper et al., 2007). Although age is the best biological parameter in Hg concentration predictions, length and weight usually are useful to understand accumulation and magnification processes related to Hg in fishes (Ikingura and Akagi, 2003). In this study, the wide body size variation observed in species that compose the trophic guilds affected the Hg concentrations, in accordance to reports from other authors (Kehrig et al., 2013; Chouvelon et al., 2018). The range of THg_{STD} within the trophic guilds was proportional to the Lt and Wt variability of demersal fish and cephalopods. In this sense, large bodied specimens presented higher THg_{STD} concentrations due to Hg accumulation over lifetime as MeHg, the organic form that likely binds with sulfhydryl groups of muscular proteins, and to the difficulty of eliminating mercury, as MeHg has a very slow excretion rate of the muscles (Nakao et al., 2007). Secondly, as above mentioned another plausible explanation might be related to assimilation of prey with high THg that are closely related to the bottom sediment or that occupies higher vertical positions ($\delta^{15}N$) in the food webs. The increased THg_{STD} with continuous rise in body size (Lt and logWt) in bentophagus (rainy season) and bentophagus-nektophagus and nektophagus (dry season) may be a consequence of differences in gape sizes, resulting in larger species with higher gapes assimilating THg enriched resources, such as squillids (245.3 ngHg g^{-1} dw) and cidarids (118.7 ngHg g^{-1} dw) crustaceans by bentophagus and *Trichiurus lepturus* (1,400 ngHg g^{-1}) by nektophagus (Segura et al., 2015; Buck et al., 2019). At last, the PSR influence in the Hg exportation to adjacent coastal waters has already been reported as the metal transference in predator-prey relations (Carvalho et al., 2008; Di Beneditto et al., 2012), thus also influencing the demersal Hg bioaccumulation by organisms. However, the volumes of PSR discharges were exceptionally low during 2017 (samplings) and very similar in both dry (234.3 m^3 s^{-1}) and rainy seasons (148.0 m^3 s^{-1}), leading to decreased dissolved Hg in the coastal waters, explain in part the bentophagus bioaccumulation in rainy season as well bentophagus-nektophagus and nektophagus bioaccumulation in dry season.

Biomagnifications of THg_{STD} were observed in the dry and rainy seasons only when benthic sources (SOM, benthic invertebrates) were included in linear regressions indicating its role as main feeding resources of the demersal nekton off the continental shelf in the northernmost Campos Basin. The relationship between THg_{STD} concentration and $\delta^{15}N$ values of the demersal food web was clear, *i.e.* organisms of higher trophic positions, estimated by $\delta^{15}N$, exhibited higher THg_{STD} concentrations than those of lower positions (Carvalho et al., 2008; Eagles-Smith et al., 2008). The slopes of the linear regressions between THg_{STD} and $\delta^{15}N$ (0.12 and 0.13 in dry and rainy seasons, respectively), used as measurement of biomagnifications across food webs (Kidd et al., 1995), was similar than those observed by Muto et al. (2014) in the Santos continental shelf (0.13), whereas lower than Di Beneditto et al. (2012) (0.25) and Kehrig et al. (2013) (0.21) in shallower waters of the same geographic region as our study. The decrease in THg concentrations buried in sediment from 25 to 50m depth (Araujo et al., 2017) seems to be plausible for explaining these differences. Finally, the absence of biomagnification when pelagic related sources (phytoplankton, zooplankton and ostreidaeans) included in the linear regressions may be related to PSR discharges during samplings. The atypical low PSR discharge (especially in the rainy season) seems to have resulted in low input of Hg in the continental shelf water column, leading to assimilation of dissolved Hg by phytoplankton lower than expected, and consequently in metal through the food web (Reinfelder et al., 1998; Pickhardt et al., 2002).

The THg_{STD} concentrations of the most ^{15}N-enriched guilds and species (including the commercial ones) are under the limit of 500 THg ng g^{-1} regulated for human consumption regardless seasonality (WHO, 1976; ANVISA, 2013). The decrease in the sediment THg concentrations from 13.3 to 3.3 ng g^{-1} (25 to 50 m depth, respectively) compared to fish and cephalopod species in shallower waters up to 30 m depth (Di Beneditto et al., 2012; Araujo et al., 2017) coupled with the decreased PSR Hg input in coastal waters might have reflected the general pattern of lower THg_{STD} in demersal nekton organisms of the present study.

In conclusion, Hg concentrations of all demersal species were under the regulatory limit representing no potential threat of Hg intoxication for humans. Mercury levels varied considerably among guilds, with the lowest THg_{STD} (46.6 ± 6.6 ng g^{-1} dw and 7.0 ± 1.0 ng g^{-1} ww) found in bentophagus-nektophagus and highest (310.8 ± 121.4 ng g^{-1} dw and 46.6 ± 18.2 ng g^{-1} ww) found in bentophagus. The initial hypothesis that no seasonal differences would be observed for Hg concentrations among guilds was rejected, as bentophagus presented higher THg_{STD} in the rainy season whereas bentophagus-nektophagus and nektophagus in dry season. However, organisms with highest $\delta^{15}N$ presented the highest THg_{STD} values, thus the second hypothesis was accepted. The observation of bioaccumulation processes for bentophagus and nektophagus seem to be related to body and gape sizes leading to Hg enriched prey assimilation. The assessment of THg_{STD} and $\delta^{15}N$ relationships indicated SOM as an organic matter important source for the demersal food web, reinforced by the biomagnifications through the food web only when benthic sources were present.

ACKNOWLEDGEMENTS

We are greatful to MSc. Thiago P. Rangel for geoprocessing.

FINANCIAL SUPPORT

This research was supported by Fundação de Amparo à Pesquisa do Rio de Janeiro – FAPERJ (Rezende CE, Grant number E-26/110.437/2010) and by Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPQ (INCT Material Transfer at the Continent-Ocean Interface, Rezende CE, grant number 573.601/08-9). Likewise, this research study was financedin part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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Conclusão Geral

- A espécie *Genidens genidens* apresentou a maior amplitude de nicho trófico, é considerado generalista e o mais oportunista bagre marinho, devido a assimilação de organismos em proporções similares e de baixo δ^{15}N.
- A preferência por peixes Sciaenidae e *Doryteuthis sanpaulensis* foi observada para *Aspistor luniscutis* e *Bagre bagre*, resultando nas maiores sobreposições de nicho isotópico e maiores valores de δ^{15}N.
- A coexistência destes três bagres marinhos simpátricos é provavelmente regulada por diferentes padrões temporais na área costeira influenciada pela pluma do Rio Paraíba do Sul e elevada abundância de presas, levando ao particionamento de recursos sem evidências de competição entre as espécies.
- O mercúrio total (HgT) ressaltou as diferentes proporções de assimilação de presas assim como a sazonalidade no uso do habitat pela espécies. A bioacumulação de Hg foi observada para *B. bagre* e *G. genidens* e a biomagnificação do Hg também ocorreu na teia alimentar.
- A assembléia demersal da plataforma continental de 40 a 60 m de profundidade da Bacia de Campos é representada por três guildas tróficas: bentófagos (*Atlantoraja platana*, *Dactylopterus volitans*, *Dules auriga*, *Prionotus nudigula* e *Zapterix brevirostris*), bentófagos-nectófagos (*A. cyclophora* e *Octopus vulgaris*) e nectófagos (*Lophius gastrophysus*, *Percophis brasiliensis* e *Saurida caribbaea*).
- As espécies bentófogas foram dominantes, independente da sazonalidade, devido à disponibilidade de presas que são as mais abundantes da macrofauna bêntica na plataforma continental.

- As principais espécies que compõem as três guildas tróficas são residentes da plataforma continental, apresentando padrões sazonais relacionados a comportamentos migratórios e de reprodução entre as estações de seca e de chuva.
- A abundânca das espécies da assembléia demersal foram associadas à temperatura, carbono orgânico do sedimento (C_{org}SOM), carbono orgânico dissolvido (COD) e razão atômica entre carbono e nitrogênio (C:N)*a*, sugerindo a entrada de nutrientes de origem terrestre e de ressurgência no sistema.
- O presente estudo é pioneiro em utilizar ferramentas de isótopos estáveis de carbono e nitrogênio para compreender as relações tróficas das principais guildas tróficas da plataforma continental da Bacia de Campos.
- A matéria orgânica (MO) do sedimento, composta pela mistura de MO de origem marinha e terrestre, sustenta toda a teia alimentar demersal da plataforma continental de 40 a 60 m de profundidade, destacando a importância do Rio Paraíba do Sul nas relações tróficas em águas costeiras.
- Os nectófagos foram a guilda mais enriquecida em $\delta^{15}N$ devido a assimilação de presas (espécies bentófogas e nectófogas) que ocupam posições verticais superiores na cadeia alimentar.
- As sobreposições dos nichos isotópicos aliadas aos hábitos alimentares dos bentófagos e bentófagos-nectófagos sugerem evidências de competição de recursos na plataforma continental.
- Os valores de $\delta^{13}C$ de bentófagos e bentófagos-nectófagos sugerem variações sazonais de hábitos alimentares de costeiros e bênticos para marinhos e pelágicos.

- As variações interespecíficas de cada guilda trófica foram relacionadas a diferenças entre tamanho corporal e de boca, refletindo na assimilação de presas enriquecidas em $\delta^{13}C$ e $\delta^{15}N$ por espécimes maiores.

- O presente estudo também foi pioneiro em abordar as relações entre a determinação de mercúrio total (HgT) com o $\delta^{15}N$ na em águas de 40 a 60 m de profundidade da plataforma continental da BC.

- As concentrações de Hg observadas foram abaixo do limite máximo para consumo humano determinado por agências regulatórias para todas as espécies demersais aqui investigadas, inclusive as de valor comercial, portanto não representando risco de intoxicação humana do metal.

- Os organismos com maiores valores de $\delta^{15}N$ apresentaram maiores valores de HgT, assim como, já reportado pela literatura para organismos costeiros em águas até 30 m de profundidade da mesma região geográfica.

- A bioacumulação do Hg foi observada nas teias alimentares demersais no período de seca e de chuva, que pode estar relacionada aos diferentes tamanhos corporais e de boca das espécies de cada guilda trófica, resultando na assimilação mais elevada do metal em organismos maiores que se alimentam de presas maiores e mais enriquecidas em Hg.

- As relações de HgT e $\delta^{15}N$ também indicaram a MO do sedimento como importante fonte base para as teias alimentares demersais. Sendo assim, corroborando as biomagnificações observadas apenas quando fontes bênticas foram incluídas nas regressões lineares, independente da sazonalidade.