

**JÚLIO GUAZZELLI GONZALEZ**

**ECOLOGIA TRÓFICA DE DUAS ESPÉCIES DE CAMURIM (*Centropomus undecimalis* (BLOCH, 1792) E *Centropomus parallelus* (POEY, 1860)) EM QUATRO ESTUÁRIOS TROPICAIS, NORDESTE DO BRASIL.**

**RECIFE  
2017**



**UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO**  
**PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO**  
**PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA**

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Dissertação apresentada ao Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura da Universidade Federal Rural de Pernambuco como exigência para obtenção do título de Mestre.

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**Recife,**  
**Fevereiro/2017**

Dados Internacionais de Catalogação na Publicação (CIP)  
Sistema Integrado de Bibliotecas da UFRPE  
Biblioteca Central, Recife-PE, Brasil

G643e    Gonzalez, Júlio Guazzelli  
          Ecologia trófica de duas espécies de camurim (*Centropomus undecimalis* (Bloch, 1792) e *Centropomus parallelus* (Poey, 1860) em quatro estuários tropicais, nordeste do Brasil / Júlio Guazzelli Gonzalez. – 2017.  
          72 f. : il.  
  
          Orientador: Thierry Frédou.  
          Coorientador: Frédéric Ménard  
          Dissertação (Mestrado) – Universidade Federal Rural de Pernambuco, Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura, Recife, BR-PE, 2017.  
          Inclui referências e apêndice(s).  
  
          1. Estuário 2. Isótopos estáveis 3. Partição de recursos  
          4. Peixes 5. Recursos basais I. Frédou, Thierry, orient. II. Ménard, Frédéric, coorient. III. Título

CDD 639

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Dissertação julgada adequada para obtenção do título de mestre em Recursos Pesqueiros e Aquicultura. Defendida e aprovada em 22/02/2017 pela seguinte Banca Examinadora.

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## **Dedicatória**

Dedico este trabalho a meus pais, José e Mara, pelo apoio e incentivo, e aos meus avós, Alberto e Maria, cujo interesse pela pesca e ensino me colocaram neste caminho.

## **Agradecimentos**

Gostaria de agradecer primeiramente aos meus pais por me auxiliarem e incentivarem a minha escolha, mesmo sabendo que eu não estaria por perto em momentos importantes de suas vidas. Gostaria de agradecer também, à Thierry Frédou e Flávia Lucena Frédou, pelo apoio e confiança para a execução da pesquisa mesmo em tão curto tempo; e a todos os integrantes do Laboratório de Impactos Antrópicos na Biodiversidade Marinha e Estuarina (BIOIMPACT), pelo auxílio durante a execução do presente trabalho; em especial, à chefe Valdimere Ferreira, Andrea Pontes Viana e Alex Souza Lira, pelas discussões e interesse em aprimorar a área de ecologia estuarina. Agradeço a Gary Renato Vargas e Túlio Timoteo, pelo apoio, auxílio nas coletas e pontos cruciais nas horas de descontração. Por fim, agradeço à CAPES, pelo apoio financeiro viabilizado, bolsa de nível mestrado, para a execução do projeto e possibilitar minha estadia em Recife - PE.

## Resumo

A análise de isótopos estáveis de carbono ( $\delta^{13}\text{C}$ ) e nitrogênio ( $\delta^{15}\text{N}$ ) foi utilizada para acessar a ecologia trófica e o uso de recursos de dois peixes estuarinos economicamente importantes, o camurim açu (*Centropomus undecimalis*) e camurim branco (*Centropomus parallelus*), em quatro estuários tropicais, nordeste do Brasil. Fatores como a área de estudo e estação do ano foram analisados para verificar o possível efeito sobre estes processos. Os resultados de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  demonstram que os camurins exibem um dinâmico processo de partição de recursos entre os estuários estudados, com maior intensidade em estuários menores. De modo geral, os peixes foram classificados entre a segunda e terceira posição trófica e dependem de uma mistura de fontes disponíveis que varia de acordo com estuário. A sazonalidade também demonstrou ser um importante fator para a ecologia trófica das espécies, com maiores sobreposições no nicho isotópico e um uso mais amplo dos recursos disponíveis. Nossas descobertas sugerem que as duas espécies simpátricas de camurim compartilham, pelo menos em partes, os mesmos recursos. Entretanto, é improvável que isto represente um fator limitante para o estoque populacional, uma vez que os camurins apresentaram um comportamento oportunista entre os estuários e estações do ano. Ademais, nossos resultados chamam atenção para a importância da sazonalidade sobre a ecologia trófica dos camurins, proporcionando recursos similares e mais abundantes durante a estação chuvosa. Este estudo traz informações novas e intuitivas sobre a influência da morfologia do estuário sobre a interação de dois predadores com alto valor comercial.

**Palavras-chave:** Isótopos estáveis, estuário, partição de recursos, peixes, recursos basais.

**Abstract**

Stable isotopes analysis of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) was used to access the trophic ecology and resource use of two economically important estuarine fishes, the common (*Centropomus undecimalis*) and fat snook (*Centropomus parallelus*) at four tropical estuaries, northeast Brazil. Factors like studied location and season were analysed to verify potential influences on these process. Results of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  shows that snooks exhibits a dynamic resource use across studied locations, with stronger resource partitioning at smaller systems. Overall, fishes were classified between the second and third trophic position and rely on a mixture of available sources which varies according to estuary. Seasonality also showed to be an important factor for species trophic ecology, with greater overlaps in the isotopic niche and boarder use of resources available. Our findings suggests that the two sympatric snooks share, at least in part, the same resources. However, it is unlikely that this represents a limiting factor for the stock, since snooks present an opportunistic behaviour among estuaries and seasons. Further, our results highlights the importance of seasonality to snook's trophic ecology by providing similar and more abundant resources at the rainy season. This work provide new and insightful information about the influence of estuary's typology on the interaction of two high-value predators.

**Key words:** Basal resources, estuary, fishes, resource partitioning, stable isotopes.



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## 1. Introdução

Os estuários estão entre os ambientes mais produtivos e ameaçados ao redor do globo (KENNISH, 2002; MITRA e ZAMAN., 2016). Conhecidos como importantes ecossistemas de transição entre o meio terrestre e marinho, esses ambientes fornecem serviços ecossistêmicos essenciais para a vida humana e marinha (BARBIER et al., 2011). A alta taxa de sedimentação e produtividade primária encontrada nestes locais proporcionam condições favoráveis para diversas espécies de peixes residentes ou migratórias (BECK et al., 2001; SHEAVES et al., 2014), fornecendo alimento e proteção nos diferentes estágios de vida (BLABER e BLABER, 1980; VENDEL et al., 2003). Deste modo, compreender a dinâmica de recursos entre as espécies de peixes existentes nos estuários, assim como estes processos diferem entre sistemas distintos, é essencial para prevenir eventuais impactos que podem afetar a comunidade de peixes e pescarias locais (CROOK et al., 2014). Especialmente devido aos recorrentes impactos antrópicos (VALIELA et al., 2001; POLIDORO et al., 2010) e a recente preocupação com os efeitos das mudanças climáticas nestes ambientes (PRANDLE e LANE, 2015; ROSS et al., 2015).

Na última década, a área de pesquisa de peixes estuarinos obteve grandes avanços (BLABER, 2013), porém ainda carecem de informações acerca do funcionamento de habitats na América do Sul (BLABER e BARLETTA, 2016) e aspectos importantes de recursos marinhos com alto valor comercial, tais como os peixes Centropomidae. Os Centropomidae, mais especificamente os robalos (*Centropomus* spp.), são predadores com alto valor econômico para a pesca recreativa e comercial em estuários tropicais e subtropicais ao longo do continente americano (ORRELL, 2002; MULLER et al., 2015). Dentre as seis espécies simpátricas de robalo encontradas no oeste do Oceano Atlântico, o robalo flecha (*Centropomus undecimalis* (Bloch, 1792)) e o robalo peva (*Centropomus parallelus* (Poey, 1986)) podem ser distinguidos das outras quatro espécies por apresentarem maiores tamanhos, abundância e ocorrência em áreas estuarinas (FIGUEIREDO e MENEZES, 1980; RIVAS, 1986).

Os robalos são peixes estuarino-dependentes que apresentam um complexo uso de habitat ao longo de seu ciclo de vida, abrangendo um mosaico de ambientes estuarinos e costeiros (BARBOUR e ADAMS, 2012; BARBOUR et al., 2014; DANTAS e BARLETTA, 2016; DAROS et al., 2016). Por exemplo, indivíduos juvenis normalmente habitam as áreas próximas ao manguezal, movendo-se para ambientes mais profundos do

estuário a medida que atingem maiores tamanhos (STEVENS et al., 2007; BLEWETT et al., 2009). Além da similaridade no uso de habitat entre estas espécies de robalo, principalmente nos estágios juvenis, ambas se alimentam principalmente de pequenos peixes e crustáceos (ALIAUME et al., 2005; BLEWETT et al., 2006; CONTENTE et al., 2009; DUTKA-GIANELLI, 2014; LIRA, 2015), o que pode acarretar em processos interespecíficos importantes para a homeostase das populações (*e.g.* partição de recursos) (ALIAUME et al., 2005; DUTKA-GIANELLI, 2014). Apesar das informações existentes sobre alimentação (BLEWETT et al., 2006; CONTENTE et al., 2009; LIRA, 2015), reprodução (PETERS et al., 1998; TAYLOR et al., 2000) e uso de habitat (STEVENS et al., 2007; BARBOUR et al., 2014; DAROS et al., 2016), poucos são os trabalhos que visam investigar a interação entre duas espécies congêneres de robalo (*e.g.* ALIAUME et al. (2005); RABELO et al. (2009); LIRA (2015)) e necessitam de maior atenção.

O uso de recursos por um organismo normalmente é acessado através de métodos convencionais, como a análise de conteúdo estomacal (HYSLOP, 1980), embora, o recente desenvolvimento de técnicas de marcadores naturais, como a análise de isótopos estáveis, e estudos sobre o ciclo dos elementos no ambiente possibilitaram ter uma visão destes processos através de um ângulo diferente (LAYMAN, 2007; NEWSOME et al., 2007). A análise de isótopos estáveis, normalmente de carbono ( $\delta^{13}\text{C}$ ) e nitrogênio ( $\delta^{15}\text{N}$ ), proporciona uma visão integrada dos alimentos assimilados por um organismo (DENIRO e EPSTEIN, 1978; HERZKA, 2005), que pode variar de semanas a meses (HERZKA, 2005; ELSDON et al., 2010), em contraste ao recorrente *snapshot* fornecido pela análise de conteúdo estomacal (PINNEGAR e POLUNIN, 2000). Este método parte do pressuposto que (i) os valores isotópicos de um organismo refletem, de maneira previsível, os valores isotópicos de sua fonte (DENIRO e EPSTEIN, 1978); e que (ii) os elementos apresentam um comportamento cumulativo ao longo da cadeia trófica (FRY, 2006). Por exemplo, o nitrogênio ( $\delta^{15}\text{N}$ ) é comumente utilizado para acessar a posição trófica de um organismo, uma vez que possui um alto incremento por nível trófico (2,5 a 3,4‰ (VANDER-ZANDEN e RASMUSSEN, 2001; POST, 2002; VANDERKLIFT e PONSARD, 2003)). Em contrapartida, o carbono ( $\delta^{13}\text{C}$ ) tem maior eficiência em determinar a origem da matéria orgânica, de modo que produtores primários com pequenas diferenças no processo de fotossíntese (*e.g.* plantas C3 e C4) apresentam valores de ( $\delta^{13}\text{C}$ ) distintos (SMITH e EPSTEIN, 1971; FRY, 2006).

Deste modo, a análise de isótopos estáveis tem possibilitado identificar as fontes de matéria orgânica que suportam a cadeia trófica (CONNOLLY et al., 2005; GIARRIZZO

et al., 2011; CARREÓN-PALAU et al., 2013; CLAUDINO et al., 2015), expressar diferenças no uso de habitat por peixes (ADAMS et al., 2009a; SELLESLAGH et al., 2015), elucidar processos inter e intraespecíficos (POST et al., 2010; VAN DER LINGEN e MILLER, 2014; LE LOC'H et al., 2015) e evidenciar mudanças no ambiente que afetam a cadeia trófica (FRY, 1999; HOEINGHAUS et al., 2011; WANG et al., 2011).

### 1.1. Objetivos

O objetivo geral do presente trabalho foi acessar a ecologia trófica e o uso de habitat do robalo flecha (*Centropomus undecimalis*) e robalo peva (*Centropomus parallelus*), em quatro estuários tropicais do estado de Pernambuco – Brasil.

Como objetivos específicos, pretendeu-se (i.) acessar a partição de recursos entre as duas espécies, (ii.) determinar as principais fontes de matéria orgânica e (iii.) avaliar o efeito da sazonalidade e tipo de estuário sobre o uso de recursos das espécies.

### 1.2. Hipótese

O trabalho traz as hipóteses de que (i.) as espécies *Centropomus undecimalis* e *Centropomus parallelus* fazem uso de recursos similares e que (ii.) esta interação é influenciada pela sazonalidade e pela morfologia do estuário.

## **2. Sources of organic matter supporting two sympatric estuarine fishes at four tropical estuaries, northeast Brazil.**

### **Abstract**

Stable isotope analysis of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) was used to access the trophic position and determine the main basal sources supporting two economically important estuarine fishes, the common (*Centropomus undecimalis*) and fat snook (*Centropomus parallelus*) at four tropical estuaries, northeast Brazil. Factors like season and location were studied to verify potential influence on the base of the food chain of these species. For means of comparison, trophic positions were also estimated through stomach content analysis. Overall, snooks were classified between second and third trophic position, with both stable isotopes and stomach content estimates exhibiting similar results. Mixing models results showed a diverse use of available basal resources that varied according to estuary and season. Our results suggests that species exhibit closely related food chains, in which *C. parallelus* rely on broader range of sources than *C. undecimalis*. Furthermore, more similar and equal contributions from sources were observed during rainy season, and highlights the ecological importance of freshwater input on increasing the number of sources available during rainy season.

**Keywords:** Basal resources, mixing models, trophic position, snooks, stable isotope analysis.

### **Introduction**

Snooks are found on tropical and subtropical estuarine systems in America (ORRELL, 2002) and are highly valuable for commercial and sport fishing (RIVAS, 1986; ORRELL, 2002; MULLER et al., 2015). These species are estuarine-dependent and euryhaline exhibiting a complex habitat use along its life, moving between a mosaic of estuarine and nearshore marine habitats (BLEWETT et al., 2009; BARBOUR and ADAMS, 2012; BARBOUR et al., 2014; DAROS et al., 2016). Juvenile snooks usually inhabit mangrove creeks and estuarine ponds, moving to deeper and brackish waters as it reaches larger sizes (150 to 300 mm) (STEVENS et al., 2007; BLEWETT et al., 2009; BRAME et al., 2014). In Brazil, the common (*Centropomus undecimalis*) and fat snook (*Centropomus parallelus*) are the most recurrent species found in estuarine systems (FIGUEIREDO and MENEZES, 1980), and can be distinguished from other four sympatric species by exhibiting greater distribution and abundance, larger sizes and



higher economic value (FIGUEIREDO and MENEZES, 1980; RIVAS, 1986; ORRELL, 2002). Further, since snooks are reported as important apex predators that feed on a wide range of crustaceans and small fishes (ROSADO-SOLÓRZANO and GUZMÁN DEL PRÓO, 1998; VASCONCELOS FILHO et al., 2003; BLEWETT et al., 2006; CONTENTE et al., 2009), they may represent a relevant case to evaluate the consumption of different sources of organic matter through the food web. Nevertheless, although snooks do not present a proper fishery, they are under a constant threat due aquatic pollution, habitat loss, and degradation of nurseries areas (*e.g.* mangrove deforestation) (MUSICK et al., 2000).

Estuarine environments are among the most productive and threatened marine environments across the globe (KENNISH, 2002; MITRA and ZAMAN, 2016). Among the common impacts caused by anthropic pressure on estuaries, land use and urban expansion can be highlighted due to their great potential of altering habitat and watershed dynamics (SKLAR and BROWDER, 1998). Such impacts may alter the freshwater inflow and import of terrestrial nutrients that affect the food web from the basal levels up to high trophic consumers (SKLAR and BROWDER, 1998; ADAMS et al., 2009; ABRANTES et al., 2013). Therefore, determining the basal resources supporting species at landscape scale is essential to achieve better coastal management strategies, in a way that it will help to understand eventual impacts that might lead to top-down effects on food web structure and particularly important species (*e.g.* Abrantes et al. (2013)).

Developments on methods to trace the organic matter flux along the food web, such as the stable isotope analysis, has been successful in monitoring and evidencing anthropic impacts on aquatic environments. Stable isotope analysis, commonly for carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ), differs from other common methods, like stomach content analysis, by providing a reliable way to go further than the direct link of prey-consumer without an extensive sample effort. For example,  $\delta^{15}\text{N}$  have a considerable enrichment between trophic levels and provides a suitable estimate for the trophic position of an organism (POST, 2002; HUSSEY et al., 2014). In contrast, On the other hand,  $\delta^{13}\text{C}$  is better to outline the origin of organic matter in an ecosystem (FRY, 2006), since  $\delta^{13}\text{C}$  values have great variability between primary producers (*e.g.* C3 and C4 plants) (DEGENS et al., 1968; SMITH and EPSTEIN, 1971; POST, 2002). Hence, stable isotope analysis was able to elucidate the trophic structure of food webs and identify important basal resources for estuarine species.

The aim of the present work was to access the trophic position and determine the main organic sources that supports the populations of two congeneric snooks, the common (*Centropomus undecimalis*) and fat snook (*C. parallelus*), at four tropical estuaries, northeast Brazil. Stable isotope analysis of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) were used to investigate the hypotheses that basal resources assimilated by snook species differ between estuaries and seasons.

## Material and Methods

### Study area

The study area encompasses four estuarine systems on the coast of Pernambuco state, northeastern Brazil, two of them located in the north region and two in the south (Figure 1). Situated on the north coast, the estuaries of (1) Catuama (Figure 1a) and the (2) Santa Cruz channel (Figure 1b) are part of the largest estuarine complex of Pernambuco, the Itamaracá's estuary (SILVA et al., 2011). This system is characterised by the presence of a 22 km long U-shape channel, which connects to the sea by two entrances (MEDEIROS and KJERFVE, 1993; MONTES et al., 1998), each one associated to the estuaries of Catuama (north entrance) and Santa Cruz (south entrance) (MEDEIROS and KJERFVE, 1993).

The southern estuaries, namely (3) Suape and (4) Sirinhaém, are smaller systems and present greater morphological similarity among them than the ones in the north. Suape is formed by two main rivers that flow into a large brackish lagoon (CPRH, 2001), characterizing this system as an oligohaline-euhaline environment subject to great influences from seasonality (JÚNIOR and MONTES, 2014) (Figure 1c). In addition, this site differs from the other estuaries by exhibiting the highest levels of habitat alteration due to the development of an industrial harbour on its marine portion (MUNIZ et al., 2005). The estuary of Sirinhaém is the smallest and southern environment among the areas addressed. This system presents low depth and a small connection to the ocean, forming a few lagoons and flooded plains (CPRH, 2001) (Figure 1d).

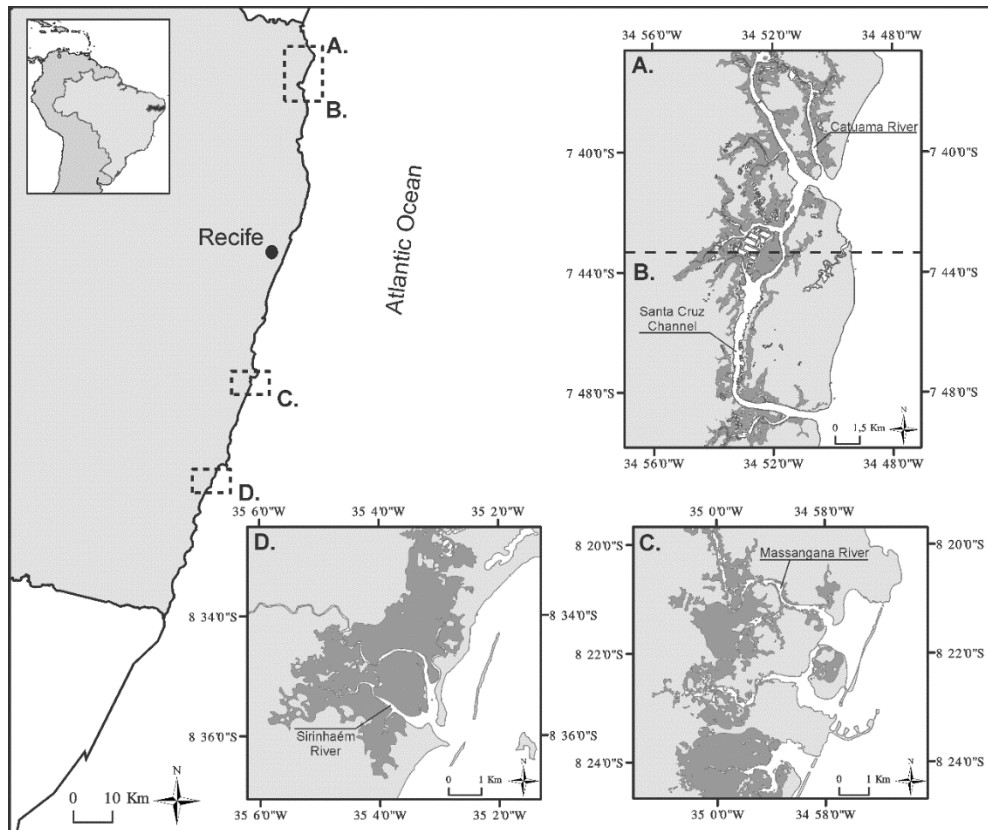


Figure 1: Estuaries of Catuama (a.), Santa Cruz (b.), Suape (c.) and Sirinhaém (d.) located at Pernambuco state, northeastern Brazil. The darker grey areas in each site represent the mangrove vegetated area.

#### Sampling procedures

Field campaigns were taken on each estuary from January to April and August to September of 2015, representing the local dry and rainy seasons respectively (MEDEIROS and KJERFVE, 1993), in which fishes and potential basal resources were collected. Snooks were collected using several fishing gears, like block nets placed near mangrove fringes (350 x 2.9 m, mesh 70 mm) and beach seine trawls (20 x 1.9 m, mesh 20 mm), that varied according to each site.

As basal resources, five representative sources of organic matter in estuaries were collected in each survey, and included a few primary producers, like the mangrove trees (leaves from *Rhizophora mangle*), macroalgae (*Sargassum* sp. and *Ulva* sp.) and benthonic microalgae, as well as the suspended particulate organic matter (POM) and the particulate organic matter in the sediment (SOM). Samples of mangrove leaves and macroalgae were collected by hand, whereas benthic microalgae was sampled from the sediment surface at low tide and extracted in the laboratory by a method modified from Riera and Richard (1996). Briefly, the samples were placed in clean vessels with 2 cm

deep, covered with a nylon screen (63 µm mesh) and added a thin layer of combusted sand (~2 mm) to avoid eventual contamination from the sample's sediment. Samples stayed under natural light until microalgae migrates to the surface of the nylon screen (usually 4 to 6 hours). Finally, the dense brown layer from the screen was removed and filtered in pre-combusted fibreglass filters (0.75 µm). SOM was collected from the first 2 mm layer of sediment and POM, which comprises a diverse number of organic sources present in the water column (*e. g.* phytoplankton), was obtained by filtering the water from the environment in fiberglass filters (0.75 µm). All fishes were identified using specific bibliography (FIGUEIREDO and MENEZES, 1980), stored in ice, measured to its standard length (SL) and then processed for isotopic analysis.

#### *Stable isotope analysis*

Up to five snook specimens from each season and environment were selected for isotope analysis. Fishes had their white muscle extracted before further procedures, whereas for other compartments (primary producers and organic matter sources) the whole sample was used in the analysis. Due to the high level of inorganic carbonates, SOM and POM were submitted to a decarbonation process through acidification (RYBA and BURGESS, 2002). Samples of SOM and POM were taken in duplicate, one non-acidified for  $\delta^{15}\text{N}$  and the another for  $\delta^{13}\text{C}$ , since this process might affect original  $\delta^{15}\text{N}$  values (PINNEGAR and POLUNIN, 1999).

After cleaning with distilled water to remove exogenous materials (*e.g.* remaining scales or sediment particles), samples were set to dry in an oven at 60°C for 48 hours, and grounded into a fine powder with a mortar and pestle. Each sample weighted between 0.35 and 0.45 mg and was analysed for carbon and nitrogen isotope ratios through a mass spectrometer (Thermo Delta V+) coupled to an element analyser (Thermo Flash 2000, interface Thermo ConFio IV) in the Laboratory of Marine Environment Science, Institute of Research for Development (LEMAR –IRD), France.

Results of stable isotope analysis for carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) are derived from the relation of the isotopic value from the sample and a known standard:  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3$ ; in which  $R$  corresponds to the ratio between  $^{13}\text{C}:^{12}\text{C}$  or  $^{15}\text{N}:^{14}\text{N}$ . Standards used for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were the PeeDee Belemnite (PDB) limestone and atmospheric nitrogen, respectively. The analytical precision of the analysis was monitored through a known standard (Thermo – Acétanilide) every six samples, and resulted in a standard error of  $\pm 0.11\text{‰}$  and  $\pm 0.07\text{‰}$  for carbon and nitrogen, respectively.

### Data analysis

Results from isotope analysis were first investigated through biplot diagrams ( $\delta^{13}\text{C}:\delta^{15}\text{N}$  scatterplot) to identify significant sources for snooks and verify potential overlap in isotope ratios of basal resources (FRY, 2006). Differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from snooks and basal resources between studied locations and seasons were accessed through an analysis of variance (ANOVA), once the assumptions of normality and homoscedasticity were met; otherwise, a Kruskal-Wallis test was applied.

The trophic position of snooks across estuaries and seasons was accessed through the formulae proposed by Post (2002):

$$\text{TP}_{\text{SIA}} = [(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}})/\text{TDF}] + \text{TP}_{\text{baseline}}$$

in which,  $\delta^{15}\text{N}_{\text{consumer}}$  and  $\delta^{15}\text{N}_{\text{baseline}}$  are the  $\delta^{15}\text{N}$  values of the target consumer and the baseline respectively; TDF is the trophic discrimination factor and was considered to be 2.54 ‰ (VANDERKLIFT and PONSARD, 2003); and  $\text{TP}_{\text{baseline}}$  is the trophic position of the baseline. Previous estimate of  $\text{TP}_{\text{SIA}}$  using a long living filter feeder as baseline (*Anomalocardia brasiliensis*), selected according to Post (2002) suggestion, revealed extremely low  $\text{TP}_{\text{SIA}}$  and was assumed to be non-representative primary consumer of snooks food chain. Therefore, we used as baseline the mean  $\delta^{15}\text{N}$  of all available basal resources on each site ( $\text{TP}_{\text{baseline}} = 1$ ).

For means of comparison, trophic positions were also estimated through stomach content analysis ( $\text{TP}_{\text{SCA}}$ ) of a dataset derived from Lira (2015), whom studied the feeding habits of snooks at Santa Cruz and Sirinhaém estuaries.  $\text{TP}_{\text{SCA}}$  was based on dietary items of individuals between 150 to 250 mm (SL) through the formulae:

$$\text{TP}_{\text{SCA}} = \sum (W_i T_i) + 1$$

in which,  $W_i$  and  $T_i$  is the relative weight and the trophic position of the  $i$ th prey item respectively (adapted from Winemiller (1990). Trophic positions of prey items were obtained from major functional groups (e.g. primary producers = 1; omnivorous = 2.5) based on dietary analysis (Table S1). Items with low accuracy in taxonomic identification (e.g. decapod crustaceans) were considered as omnivorous due the diverse feeding habits within each group (FAUCHALD and JUMARS, 1979; BRUSCA and BRUSCA, 2007). Fish prey items were classified according to its functional guild according to Elliott et al. (2007).

The contribution of each basal resource for snooks was accessed through bayesian mixing models with the *siar* package (“Stable Isotope Analysis in R” (PARNELL and

JACKSON, 2015)) in R 3.3.1 statistical software (R CORE TEAM, 2015). These models use Bayesian statistics to estimate the relative contribution of a source to the isotopic mixture of a target consumer (PARNELL et al., 2010). Input data included the isotope ratios of snooks and sources, and a trophic discriminant factor (TDF) derived from literature. Two different TDFs were used based on TP estimations since mixing models are sensitive to TDF. A TDF of  $0.47 \pm 1.23\text{‰}$  and  $2.54 \pm 0.11\text{‰}$ , for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively, was applied for fish with  $\text{TP}_{\text{SIA}}$  between 2 and 3 (VANDER-ZANDEN and RASMUSSEN, 2001; VANDERKLIFT and PONSARD, 2003). Snooks with  $\text{TP}_{\text{SIA}}$  higher than 3, and thus comprehending two trophic links (*i.e.* primary producer-primary consumer and primary consumer-snooks), required higher TDFs of  $0.94 \pm 1.73\text{‰}$  and  $5.08 \pm 0.15\text{‰}$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. Moreover, elements concentration (%C and %N) measured from each basal resource were used in the model to increase its accuracy (PHILLIPS et al., 2014). Previous scrutinizing of the isotope values obtained revealed a high overlap between SOM and POM, as well as SOM and benthic microalgae. SOM includes a broad range of organic sources like POM and pools of benthic microalgae, therefore, SOM was excluded from the model to avoid bias (PHILLIPS et al., 2014). Further, samples of *Ulva* sp. and *Sargassum* sp. also showed similar ratios and were grouped as one single source (macroalgae) (PHILLIPS et al., 2014).

## Results

A total of 170 samples were collected for stable isotope analysis, split into 66 snooks (*C. undecimalis* = 37; *C. parallelus* = 29; Table 1) and 134 basal resources (POM = 22; SOM = 24; *R. mangle* = 22; *Ulva* sp. = 6; *Sargassum* sp. = 12; Benthic microalgae = 18; Table 2). Fishes measured from 152 to 245 mm SL, except for Santa Cruz at the rainy season, where all *C. undecimalis* sampled sized over 300 mm SL. Since snooks show a strong relationship between  $\delta^{15}\text{N}$  and specimen's size (unpublish. data), further comparisons were not reliable, and rainy season Santa Cruz samples were excluded from the analysis. Moreover, a few basal resources could not be collected in every estuary and seasons due to some survey impairments, making it impossible to access their contributions in respective sites.

Table 1: Carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope ratios (mean  $\pm$  S.D.) and standard length (SL; mean  $\pm$  S.E.) of snook's species during the dry and rainy seasons at four tropical estuaries, northeastern Brazil.

| Species                        | Season |                  |                           |                           |       |                  |                           |                           |
|--------------------------------|--------|------------------|---------------------------|---------------------------|-------|------------------|---------------------------|---------------------------|
|                                | Dry    |                  |                           |                           | Rainy |                  |                           |                           |
|                                | N      | SL (mm)          | $\delta^{13}\text{C}$ (‰) | $\delta^{15}\text{N}$ (‰) | n     | SL (mm)          | $\delta^{13}\text{C}$ (‰) | $\delta^{15}\text{N}$ (‰) |
| Catuama (CAT)                  |        |                  |                           |                           |       |                  |                           |                           |
| <i>Centropomus undecimalis</i> | 5      | 21.18 $\pm$ 1.46 | -17.57 $\pm$ 2.5          | 10.27 $\pm$ 1.07          | 5     | 20.94 $\pm$ 0.85 | -20.34 $\pm$ 0.61         | 9.94 $\pm$ 0.6            |
| <i>Centropomus parallelus</i>  | 1      | 17.8             | -19.79                    | 8.97                      | 4     | 16.07 $\pm$ 0.53 | -19.82 $\pm$ 2.39         | 10.41 $\pm$ 0.99          |
| Santa Cruz (STC)               |        |                  |                           |                           |       |                  |                           |                           |
| <i>Centropomus undecimalis</i> | 5      | 21.04 $\pm$ 0.24 | -19.51 $\pm$ 1.95         | 10.4 $\pm$ 0.85           | 5     | 33.04 $\pm$ 1.22 | -18.81 $\pm$ 2.81         | 11.59 $\pm$ 1.13          |
| <i>Centropomus parallelus</i>  | 2      | 22.50 $\pm$ 2.0  | -21.98 $\pm$ 0.52         | 12.0 $\pm$ 1.02           | 5     | 17.82 $\pm$ 0.62 | -18.56 $\pm$ 0.94         | 11.55 $\pm$ 1.08          |
| SuaPe (SUA)                    |        |                  |                           |                           |       |                  |                           |                           |
| <i>Centropomus undecimalis</i> | 5      | 22.98 $\pm$ 0.67 | -20.43 $\pm$ 0.74         | 11.65 $\pm$ 0.28          | 2     | 20.75 $\pm$ 2.25 | -19.63 $\pm$ 0.25         | 11.45 $\pm$ 0.39          |
| <i>Centropomus parallelus</i>  | 5      | 19.98 $\pm$ 0.98 | -20.79 $\pm$ 2.39         | 10.19 $\pm$ 1.11          | 2     | 16.2 $\pm$ 0.5   | -18.53 $\pm$ 0.27         | 9.72 $\pm$ 0.2            |
| Sirinhaém (SIR)                |        |                  |                           |                           |       |                  |                           |                           |
| <i>Centropomus undecimalis</i> | 5      | 19.76 $\pm$ 0.4  | -21.72 $\pm$ 1.33         | 10.21 $\pm$ 0.2           | 5     | 18.7 $\pm$ 0.58  | -21.75 $\pm$ 0.73         | 9.59 $\pm$ 0.49           |
| <i>Centropomus parallelus</i>  | 5      | 20.34 $\pm$ 0.62 | -19.27 $\pm$ 2.01         | 11.47 $\pm$ 0.52          | 5     | 20.04 $\pm$ 0.54 | -21.80 $\pm$ 1.13         | 10.26 $\pm$ 0.19          |

n, number of samples. SL, standard length of specimens.

Snooks presented a wide range of  $\delta^{13}\text{C}$  (-24.84 to -15.28 ‰) and  $\delta^{15}\text{N}$  (8.47 to 13.14 ‰) values between studied locations, and could be distinguished from basal resources due to the high ratios of  $\delta^{15}\text{N}$ , with approximately 4.5 to 6.7 ‰ higher than the average  $\delta^{15}\text{N}$  from sources (Table 1, Figure 2). Significant differences were found in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from snooks among studied locations (ANOVA:  $\delta^{13}\text{C}$ ,  $F = 3.652$ ,  $p$ -value = 0.017;  $\delta^{15}\text{N}$ ,  $F = 3.277$ ,  $p$ -value = 0.027) rather than between seasons (ANOVA:  $\delta^{13}\text{C}$ ,  $F = 0.524$ ,  $p$ -value = 0.472;  $\delta^{15}\text{N}$ ,  $F = 0.805$ ,  $p$ -value = 0.373).

Average  $\delta^{13}\text{C}$  ratios from basal resources were similar between studied locations (Kruskal-Wallis:  $\chi^2 = 4.556$ ,  $p$ -value = 0.207), with mangrove trees and algae (macroalgae and benthic microalgae) responsible for depleted and enriched  $\delta^{13}\text{C}$  values, respectively (Table 2, Figure 2). Total variance in  $\delta^{13}\text{C}$  ratios from basal resources was greater at Catuama (-28.29 to -4.54 ‰), mainly due to the notably low values of benthic microalgae found during the rainy season, followed by Santa Cruz (-28.76 to -17.58 ‰), Sirinhaém (-29.24 to -15.76 ‰) and SuaPe (-27.90 to -14.50 ‰) (Figure 2). Although basal sources did not present significant differences in  $\delta^{13}\text{C}$  between seasons (Kruskal-Wallis:  $\chi^2 = 0.996$ ,  $p$ -value = 0.318), a general increase in  $\delta^{13}\text{C}$  variation was observed from the dry (-28.76 to -17.22 ‰) to the rainy season (-29.42 to -4.54 ‰). Likewise, a similar yet less intense seasonal increase on  $\delta^{15}\text{N}$  variability was found in Catuama and Sirinhaém (Figure 2). No significant differences were found in  $\delta^{15}\text{N}$  values from basal resources

between studied locations (ANOVA:  $F = 0.791$ ,  $p$ -value = 0.501) and seasons (ANOVA:  $F = 0.033$ ,  $p$ -value = 0.856).

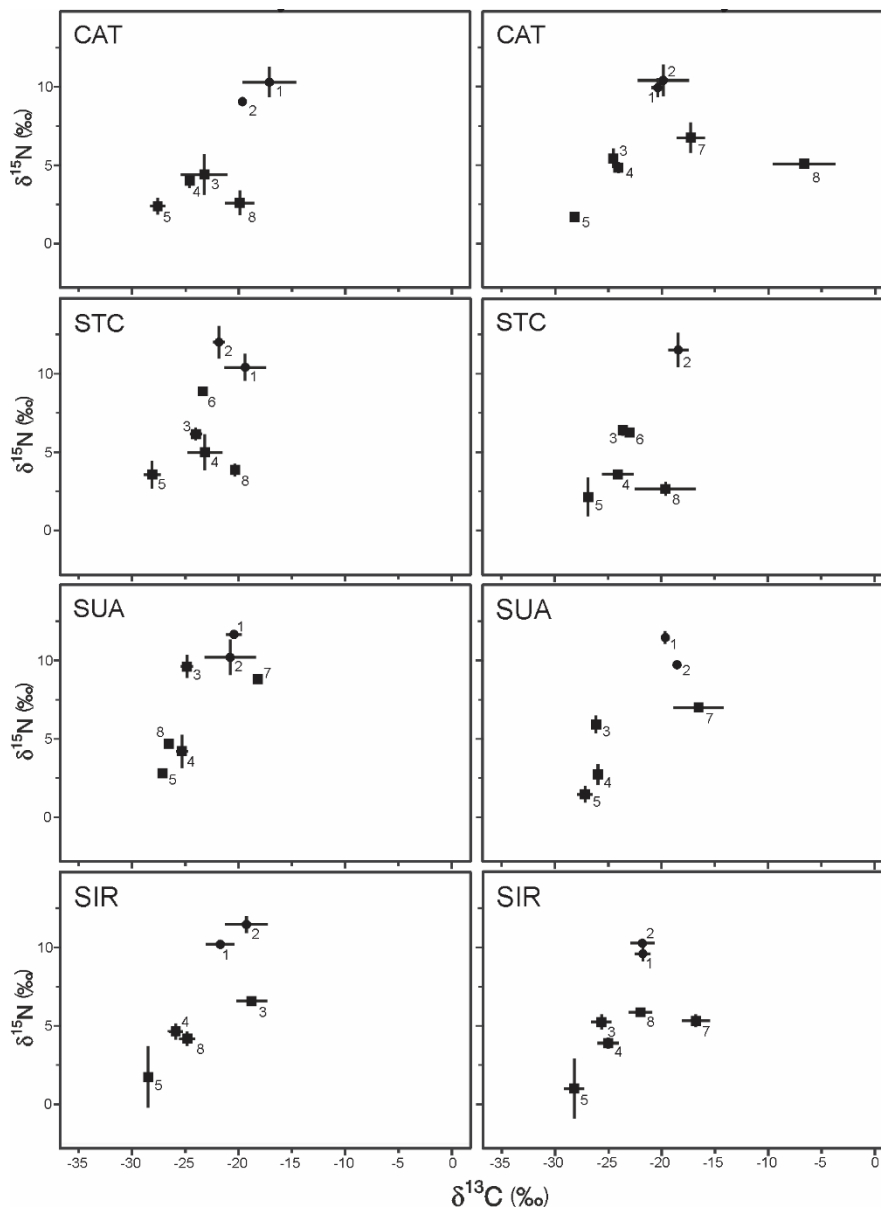


Figure 2: Distribution of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope ratios (mean  $\pm$  S.D.) from snooks (circles) and basal resources (squares) in four tropical estuaries, northeastern Brazil, at dry (left) and rainy seasons (right). Estuaries: CAT, Catuama; STC, Santa Cruz; SUA, Suape; SIR, Sirinhaém. Species: 1. *Centropomus undecimalis*; 2. *Centropomus parallelus*; 3. POM; 4. SOM; 5. *Rhizophora mangle*; 6. *Ulva* sp.; 7. *Sargassum* sp.; 8. Benthic microalgae.



Table 2: Carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope ratios (mean  $\pm$  S.D.) of estuarine basal resources during the dry and rainy seasons at four tropical estuaries, northeastern Brazil.

| Estuary          | Species                  | Season |                           |                           |       |                           |                           |
|------------------|--------------------------|--------|---------------------------|---------------------------|-------|---------------------------|---------------------------|
|                  |                          | Dry    |                           |                           | Rainy |                           |                           |
|                  |                          | n      | $\delta^{13}\text{C}$ (‰) | $\delta^{15}\text{N}$ (‰) | n     | $\delta^{13}\text{C}$ (‰) | $\delta^{15}\text{N}$ (‰) |
| Catuama (CAT)    | <b>Mangrove trees</b>    |        |                           |                           |       |                           |                           |
|                  | <i>Rhizophora mangle</i> | 3      | -27.58 $\pm$ 0.67         | 2.39 $\pm$ 0.52           | 1     | -27.89                    | 1.7                       |
|                  | <b>Algae</b>             |        |                           |                           |       |                           |                           |
|                  | Benthonic microalgae     | 3      | -19.89 $\pm$ 1.36         | 2.6 $\pm$ 0.77            | 2     | -6.6 $\pm$ 2.91           | 5.08 $\pm$ 0.12           |
|                  | <i>Sargassum</i> sp.     | -      | -                         | -                         | 3     | -17.23 $\pm$ 1.32         | 6.75 $\pm$ 0.95           |
|                  | <b>Organic matter</b>    |        |                           |                           |       |                           |                           |
|                  | POM                      | 3      | -23.2 $\pm$ 2.18          | 4.41 $\pm$ 1.29           | 3     | -24.49 $\pm$ 0.13         | 5.43 $\pm$ 0.62           |
|                  | SOM                      | 3      | -24.45 $\pm$ 0.59         | 3.92 $\pm$ 0.07           | 3     | -23.85 $\pm$ 0.4          | 5.11 $\pm$ 0.06           |
| Santa Cruz (STC) | <b>Mangrove trees</b>    |        |                           |                           |       |                           |                           |
|                  | <i>Rhizophora mangle</i> | 3      | -28.1 $\pm$ 0.77          | 3.58 $\pm$ 0.88           | 3     | -26.87 $\pm$ 0.17         | 2.19 $\pm$ 1.22           |
|                  | <b>Algae</b>             |        |                           |                           |       |                           |                           |
|                  | Benthonic microalgae     | 3      | -20.33 $\pm$ 0.22         | 3.88 $\pm$ 0.4            | 2     | -19.6 $\pm$ 2.85          | 2.7 $\pm$ 0.44            |
|                  | <i>Ulva</i> sp           | 3      | -23.36 $\pm$ 0.42         | 8.88 $\pm$ 0.23           | 3     | -22.98 $\pm$ 0.38         | 6.3 $\pm$ 0.19            |
|                  | <b>Organic matter</b>    |        |                           |                           |       |                           |                           |
|                  | POM                      | 2      | -24.01 $\pm$ 0.56         | 6.17 $\pm$ 0.4            | 3     | -23.63 $\pm$ 0.26         | 6.42 $\pm$ 0.32           |
|                  | SOM                      | 3      | -22.5 $\pm$ 0.67          | 4.32 $\pm$ 0.67           | 3     | -23.34 $\pm$ 1.95         | 3.53 $\pm$ 0.06           |
| Suape (SUA)      | <b>Mangrove trees</b>    |        |                           |                           |       |                           |                           |
|                  | <i>Rhizophora mangle</i> | 3      | -27.12 $\pm$ 0.45         | 2.8 $\pm$ 0.22            | 3     | -27.16 $\pm$ 0.71         | 1.48 $\pm$ 0.53           |
|                  | <b>Algae</b>             |        |                           |                           |       |                           |                           |
|                  | Benthonic microalgae     | 1      | -26.48                    | 4.66                      | -     | -                         | -                         |
|                  | <i>Sargassum</i> sp.     | 3      | -18.21 $\pm$ 0.32         | 8.8 $\pm$ 0.9             | 3     | -16.51 $\pm$ 2.33         | 6.99 $\pm$ 0.21           |
|                  | <b>Organic matter</b>    |        |                           |                           |       |                           |                           |
|                  | POM                      | 2      | -24.84 $\pm$ 0.57         | 9.61 $\pm$ 0.71           | 3     | -26.11 $\pm$ 0.2          | 5.92 $\pm$ 0.56           |
|                  | SOM                      | 3      | -24.92 $\pm$ 0.3          | 3.47 $\pm$ 0.11           | 3     | -25.66 $\pm$ 0.22         | 3.15 $\pm$ 0.29           |
| Sirinhaém (SIR)  | <b>Mangrove trees</b>    |        |                           |                           |       |                           |                           |
|                  | <i>Rhizophora mangle</i> | 3      | -28.48 $\pm$ 0.04         | 1.74 $\pm$ 1.95           | 3     | -28.2 $\pm$ 0.91          | 1.08 $\pm$ 1.89           |
|                  | <b>Algae</b>             |        |                           |                           |       |                           |                           |
|                  | Benthonic microalgae     | 4      | -24.82 $\pm$ 0.73         | 4.19 $\pm$ 0.46           | 3     | -21.99 $\pm$ 1.09         | 5.95 $\pm$ 0.19           |
|                  | <i>Sargassum</i> sp.     | -      | -                         | -                         | 3     | -16.78 $\pm$ 1.31         | 5.4 $\pm$ 0.4             |
|                  | <b>Organic matter</b>    |        |                           |                           |       |                           |                           |
|                  | POM                      | 3      | -18.77 $\pm$ 1.45         | 6.58 $\pm$ 0.21           | 3     | -25.64 $\pm$ 0.93         | 5.33 $\pm$ 0.46           |
|                  | SOM                      | 3      | -25.59 $\pm$ 0.01         | 4.44 $\pm$ 0.69           | 3     | -24.16 $\pm$ 0.18         | 4.23 $\pm$ 0.15           |

n, number of samples.

Overall, snook species were classified between the second and third trophic position ( $\text{TP}_{\text{SIA}}$ ) (Table 3). Unexpected low  $\text{TP}_{\text{SIA}}$  ( $< 3.0$ ) were observed for *C. undecimalis* in Suape during dry season and for *C. parallelus* in Catuama during rainy season. Smooth differences were found between species within sites, in which *C. parallelus* presented higher  $\text{TP}_{\text{SIA}}$  at Santa Cruz and Sirinhaém, whereas *C. undecimalis* showed higher values in Suape. Seasonal effects on  $\text{TP}_{\text{SIA}}$  were weak yet present in most estuaries and no distinct pattern was found among sites. Differences in  $\text{TP}_{\text{SIA}}$  between seasons was greater at Catuama, in which *C. parallelus* showed a decrease ( $\sim 1.0$ ) from dry to rainy season.

TP<sub>SCA</sub> estimates exhibited similar values for snooks at Santa Cruz and Sirinhaem, characterising snooks as secondary consumers (Table 3).

Table 3: Trophic position estimates with stable isotopes (TP<sub>SIA</sub>) and stomach content analysis (TP<sub>SCA</sub>) of snook species during dry and rainy seasons at four tropical estuaries, northeastern Brazil.

| Species                        | Season |                   |       |                   |    |                   |
|--------------------------------|--------|-------------------|-------|-------------------|----|-------------------|
|                                | Dry    |                   | Rainy |                   | n  | TP <sub>SCA</sub> |
|                                | n      | TP <sub>SIA</sub> | n     | TP <sub>SIA</sub> |    |                   |
| Catuama (CAT)                  |        |                   |       |                   |    |                   |
| <i>Centropomus undecimalis</i> | 5      | 3.73              | 5     | 2.82              |    |                   |
| <i>Centropomus parallelus</i>  | 1      | 3.22              | 4     | 3.0               |    |                   |
| Santa Cruz (STC)               |        |                   |       |                   |    |                   |
| <i>Centropomus undecimalis</i> | 5      | 3.0               | -     | -                 | 8  | 3.45              |
| <i>Centropomus parallelus</i>  | 2      | 3.63              | 5     | 3.84              | 10 | 3.47              |
| Suape (SUA)                    |        |                   |       |                   |    |                   |
| <i>Centropomus undecimalis</i> | 5      | 3.32              | 2     | 3.78              |    |                   |
| <i>Centropomus parallelus</i>  | 5      | 2.74              | 2     | 3.1               |    |                   |
| Sirinhaém (SIR)                |        |                   |       |                   |    |                   |
| <i>Centropomus undecimalis</i> | 5      | 3.35              | 5     | 3.04              | 45 | 3.55              |
| <i>Centropomus parallelus</i>  | 5      | 3.85              | 5     | 3.31              | 30 | 3.54              |

n, number of samples.

Mixing models results revealed a complex resource use of fishes across estuaries and seasons (Figure 3). During dry season, higher contributions of benthic microalgae and POM were found at Catuama and Santa Cruz, whereas fishes had greater assimilation of nutrients from POM at Sirinhaém and a mixture of all sources in Suape (Figure 3). Sources contributions for snooks were of equal importance during rainy season and, although mixing model results presented great variability, seasonal differences within each estuary can be highlighted by higher assimilation of macroalgae in Catuama, Santa Cruz and Sirinhaém, and mangrove trees in Suape and Sirinhaém during rainy season. Overall, differences in resource use between species were more evident during dry season, in which *C. undecimalis* exhibited higher contributions of a few sources, like benthonic microalgae and POM, whereas *C. parallelus* had a more homogenous assimilation from distinct sources at Catuama and Santa Cruz (*e.g.* mangrove trees) and POM in Sirinhaém (95% Credible Interval, 0.9 to 1.0). Conversely, in Suape species exhibited an inverse behaviour, with *C. parallelus* showing higher contributions of macroalgae and POM and *C. undecimalis* a mixture of available sources.

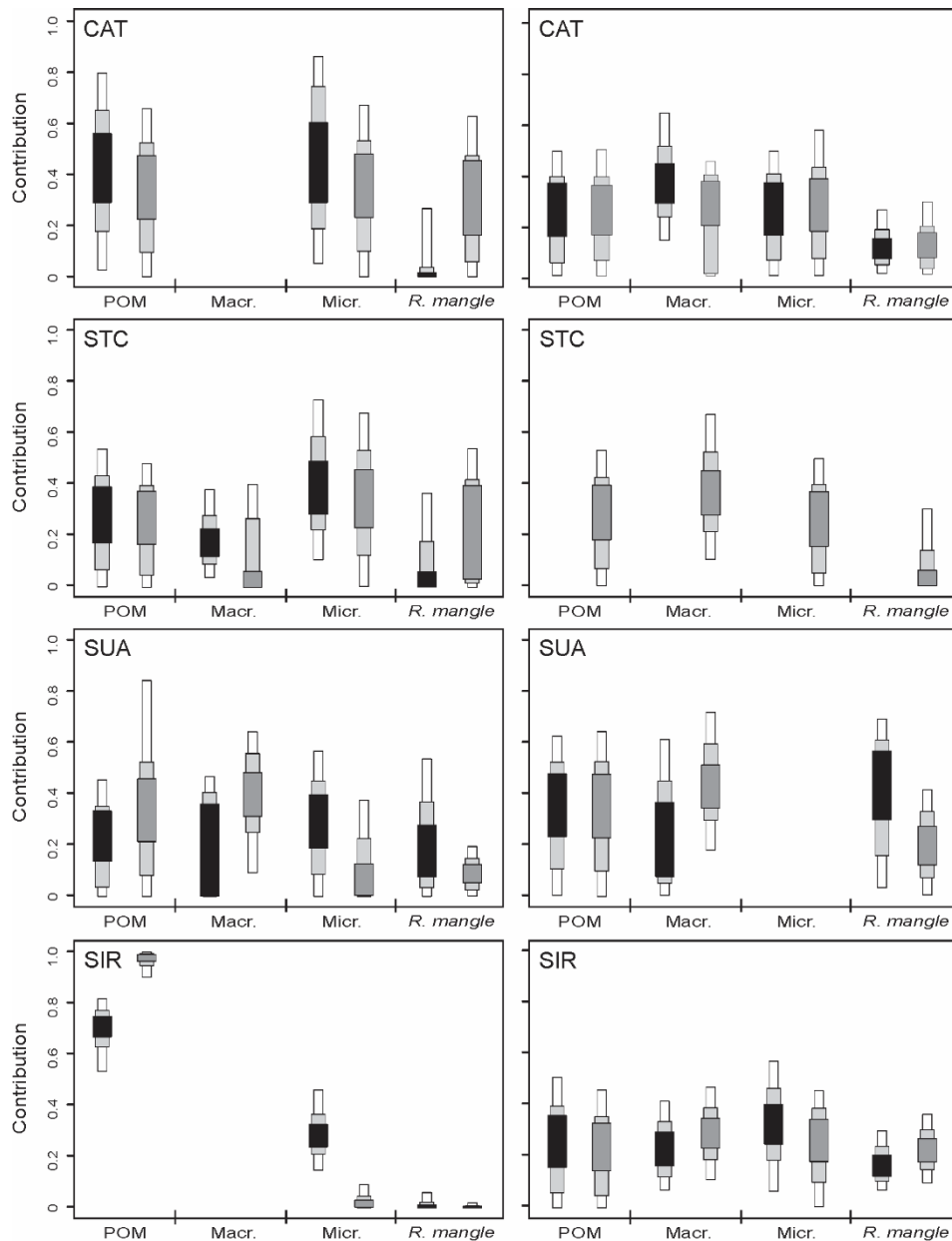


Figure 3: Estimated relative contribution of estuarine basal resources for *Centropomus undecimalis* (black boxes) and *Centropomus parallelus* (grey boxes) on four tropical estuaries, northeast Brazil, at the dry (left) and rainy seasons (right). Credibility intervals of 95, 75 and 50% are denoted by white, light grey and black/grey boxes, respectively. Estuaries: CAT, Catuama; STC, Santa Cruz; SUA, Suape; SIR, Sirinhaém. Sources: POM, POM; Macr., macroalgae; Micr., benthic microalgae; *R.mangle*, *Rhizophora mangle* mangrove trees.

## Discussion

Snooks are referred as recurrent predators from inshore areas that occupy the higher levels on food webs (FIGUEIREDO and MENEZES, 1980; ROSADO-SOLÓRZANO

and GUZMÁN DEL PRÓO, 1998; ORRELL, 2002; VASCONCELOS FILHO et al., 2003). Besides their importance as potential key species in estuarine systems (LIRA, 2017), snooks are well-prized fish for sport fishing and artisanal fisheries (ORRELL, 2002; MULLER et al., 2015), ensuring important ecological and socio-economic services at a local scale. To further extent the knowledge on habitat use and provide information of how hydrogeomorphological features of estuaries eventually influence the trophic ecology of snook species, stable isotopes of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) were used access the trophic position and identify main basal resources supporting two congeneric snooks in four tropical estuaries, northeast Brazil.

In this study, trophic position estimates with stable isotopes and stomach content analysis revealed that snooks occupy between the second and third trophic position in estuarine systems addressed. These results corroborate with previous diet (GARCIA and GIARRIZZO, 2014), stable isotopes (GIARRIZZO et al., 2011; WINEMILLER et al., 2011) and ecosystem modelling (ROSADO-SOLÓRZANO and GUZMÁN DEL PRÓO, 1998) studies concerning *Centropomus* species, which reported snooks with trophic positions ranging from 2.5 to 4.5 within estuarine areas. Moreover, unexpected low values in  $\text{TP}_{\text{SIA}}$  (2.7 to 2.8) observed at Catuama and Suape may reflect occasional ingestion and assimilation of vegetal detritus by snooks. Alternatively, low  $\text{TP}_{\text{SIA}}$  also could be associated to the usage of basal sources as baseline and eventual contrasting  $\delta^{15}\text{N}$  between pelagic and benthonic sources (*e.g.* enriched  $\delta^{15}\text{N}$  values of POM at Suape during dry season). Besides the disadvantages on selecting primary producers for  $\text{TP}_{\text{SIA}}$  estimation (POST, 2002), in our case, this approach yield better results than previous estimate with a primary consumer as baseline (not shown) and similar to those obtained with stomach content analysis.

Snooks exhibited a diverse use of available basal resources that varied according to estuary and season. Overall, differences between species were more evident during dry season, in which *C. parallelus* tend to have a more homogeneous contribution from distinct sources than *C. undecimalis*. These results corroborates with the statements of *C. parallelus* being a more generalist predator than *C. undecimalis* (BLEWETT et al., 2006; LIRA, 2015). Further, the recurrent contribution of benthic microalgae for *C. undecimalis* found in all estuaries emphasises the relationship between this species and the benthic and near mangrove fringed habitats (PETERS et al., 1998; STEVENS et al., 2007). These finding are in accordance with those reported by Brame (2012), which reports that young *C. undecimalis* rely mainly on POM and microalgae on estuarine creeks in Florida.

Although the long standing paradigm assumes mangroves as important sources of carbon for estuarine fishes (ODUM and HEALD, 1972), recent studies have shown that in fact it provides little or even no contribution to organic matter assimilated by consumers (BOUILLON et al., 2008; NAGELKERKEN et al., 2008), and its contribution seems to be entangled with geomorphological and environmental features of each system (PINEDA, 2003). For instance, Sirinhaém has the higher ratio between cover of vegetated mangrove and estuary's area among studied locations, and even so, the contribution of mangrove litter to consumers was notably low during dry season. It is clear that the presence of such vegetation improves the habitat complexity increasing species richness (NAGELKERKEN and FAUNCE, 2008; NAGELKERKEN et al., 2008, 2010). However, in our case, it does not appear to have a direct link between mangrove biomass and assimilation of this source by snooks. At Curaçá bay, northern Brazil, Giarrizzo et al. (2011) observed that mangrove contributes to local consumer directly or indirectly via POM, which was derived from the resemblance of depleted  $\delta^{13}\text{C}$  values from POM and vascular plants. The studied area in question comprehends a mangrove creek that presents no freshwater input than direct rainfall (GIARRIZZO et al., 2011). Similarly, Bouillon et al. (2004) observed that the contribution of mangrove-derived organic matter to epifaunal communities was higher in estuaries with less material exchange with adjacent waters. Therefore, the contribution of mangrove trees to consumers could be associated with the time that leaves spend in the system before incorporating organic matter pool sources (e.g. POM and SOM). This would explain why Suape, a coastal lagoon estuary with restricted connection to the sea, exhibited similar values between organic sources and *R. mangle* and higher contributions of mangrove among studied locations.

Estuarine consumers usually rely on multiple basal sources that are locally abundant (CLAUDINO et al., 2015). In addition, the relative contribution of autotrophic sources are often linked to morphological features and hydrologic dynamics of each estuary, such as the estuarine area, water turbidity and freshwater inflow (HOEINGHAUS et al., 2011; ABRANTES et al., 2013). As expected, stable isotope results and mixing model's contribution of four representative basal resources varied between studied locations. Higher assimilation of benthonic microalgae was observed in Santa Cruz, in accordance with the detritivore based food web found at this site and the predominant occurrence of species associated to muddy substrates (e.g. Gobiidae fishes) (SILVA-JÚNIOR et al., 2016). Likewise, Catuama exhibited a similar yet less intense pattern, once this estuary is inserted in the same complex as Santa Cruz (i.e. Itamaracá's estuary) but differs in

freshwater discharge and depth (MEDEIROS and KJERFVE, 1993, 2005). On the other hand, snooks at Sirinhaém, the smallest and shallowest estuary, appears to rely mainly on POM, which is probably due to the low depths and restricted connection to the sea that reduces water turbidity and enable a higher productivity of pelagic primary producers (phytoplankton) (ABRANTES et al., 2013). Suape was the only system where snooks exhibited a mixture of distinct basal resources. The low water exchange with marine realm at this site may enhance the productivity and assimilation of bottom-fixed primary producers, like macroalgae and mangrove trees.

Seasonality shows to be an important factor influencing available basal sources for snook species. A more similar and homogeneous contribution of basal resources was found at the rainy season, suggesting that these species utilize similar and more diverse food chains. The freshwater input at rainy season might carry terrestrial nutrients that increase the productivity and the mixture of autotrophic sources available in the environment (ABRANTES et al., 2013, 2014). Such pattern can be easily observed through the increase of mangrove assimilation at this period, probably due to the transport of mangrove litter to the aquatic environment. Alternatively, the higher contribution of mangrove trees could also be mistaken by the presence of terrestrial C3 producer's inputs at the rainy season, which show similar  $\delta^{13}\text{C}$ -depleted values (SMITH and EPSTEIN, 1971; BOUILLON et al., 2008). Although the actual factor responsible for a broader use of basal resources remains unclear, it highlights the importance of freshwater flow patterns for snooks at studied locations. This finding is particularly interesting for snook species, once adult fish exhibit a seasonal migration toward riverine habitats in rainy season (BLEWETT et al., 2009; TROTTER et al., 2012), which might increase competition for resources and mortality of young juveniles (BARBOUR and ADAMS, 2012).

Although stable isotope mixing models are useful tools to investigate trophic relationships on food webs, its limitations fall into a vast number of requirements, such as robust sampling designs and distinct isotopic compositions among sources, and may produce doubtful results if these assumptions are not met (PHILLIPS et al., 2014). In this study, the absence of sources at some sites and the similarity in isotopic ratios between sources might have biased model's results and weakened comparisons between sites. Yet, our results were able to highlight at some point important resources at the base of food webs and increase previous knowledge of two economically important species. These findings are particularly important for tropical estuaries at South America, where the

habitat use of high value marine resources and functionality of estuarine systems are poorly understood (BLABER and BARLETTA, 2016). Moreover, this study exemplifies the difficulties in working with complex habitats that usually present a diverse range of basal sources and biological process such as the estuaries. In summary, our findings revealed that juvenile snooks are between the second and third trophic position and rely on a mixture of available sources, which will vary according to hydromorphological features of each estuary. Furthermore, our results have also shown the importance of seasonality in providing a wider range of basal resources for snooks, and rises the concern on current anthropic impacts that might affect freshwater flow at such places. Future laboratory experiments to obtain an accurate trophic discriminant factor for snooks that will enhance mixing models accuracy, as well as further studies about the contribution of marine basal sources at northeast estuaries in Brazil are needed to improve our understanding of tropical estuarine food webs.

### Acknowledgements

This study was funded in part by the INCT (National Institute of Science and Technology, CNPq n° 610013/2011-4) in Tropical Marine Environments – INCT-AmbTropic, the CNPq (National Council for Scientific and Technological Development, CNPq n° 479845/2013-1 and n° 407125/2013-2), and by CAPES (Coordination for the Improvement of Higher Education Personnel) for providing the scholarship to the first author during the MSc graduation. We thanks to the members of BIOIMPACT Laboratory for their efforts on field campaigns and sampling procedures.

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### **3. Resource partitioning of two congeneric snooks revealed by stable isotopes analysis.**

#### **Abstract**

Resource partitioning of two economically important fishes, the common (*Centropomus undecimalis*) and the fat snook (*Centropomus parallelus*), at four tropical estuaries, northeastern Brazil were analysed through stable isotope analysis of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ). Potential influences of location and seasonality on the interspecific process between species were studied. Isotope ratios of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  indicate that snooks inhabit similar environments but feed at different trophic levels in most estuaries. Further, a recurrent, yet low, overlap was observed. Higher overlaps and wider isotopic niches were found within larger estuaries, whereas smaller systems with restricted connections to the sea presented low or even no overlap. Seasonality also played an important role in the partitioning process, with higher overlap during the rainy season. Our findings suggest that snook species share in some degree the same resources. However, it is unlikely this represents a limiting factor for the stocks once they express a clear opportunistic feeding behaviour. This study provides new and insightful information about the resource partitioning of two high-value predators and the effect of estuary's typology on this process.

**Keywords:** Carbon, estuary, fish, isotopic niche, nitrogen.

#### **Introduction**

The niche concept is one of the central baselines for ecological studies. Briefly, it consists on the space occupied by species in an  $n$ -dimensional plane and predicts that two species cannot coexist by exhibiting the same niche (*e.g.* resource partitioning process)(HUTCHINSON, 1957; ODUM, 1976). Understanding the different resource uses by species in a community structure, along with the respective factors that drive them, is essential to underline important ecological and evolutionary processes (BEARHOP et al., 2004), especially when dealing with important and threatened marine resources, such as the fish from the Centropomidae family (MUSICK et al., 2000; MULLER et al., 2015).

Snooks (*Centropomus* spp.) are predators with a great economic importance for recreational and commercial fisheries in tropical and subtropical estuaries along the American continent (ORRELL, 2002; MULLER et al., 2015). Among the six sympatric species occurring in the western Atlantic Ocean, the common (*Centropomus undecimalis*

(Bloch 1792)) and the fat snook (*Centropomus parallelus* (Poey 1986)) can be distinguished from other species by reaching greater economic value, larger sizes and having broader and more abundant distribution along estuarine areas (FIGUEIREDO and MENEZES, 1980; RIVAS, 1986).

The snooks are protandric, euryhaline and estuarine-dependent species that exhibit a complex habitat shift over their life cycle, encompassing a mosaic estuarine and nearshore habitats (BARBOUR and ADAMS, 2012; BARBOUR et al., 2014; DANTAS and BARLETTA, 2016; DAROS et al., 2016). Juvenile snooks usually inhabit mangrove creeks and move to deeper brackish waters as they reach larger sizes (150 to 300 mm standard length) (STEVENS et al., 2007; BLEWETT et al., 2009), and integrate in the adults population (*i.e.*  $\geq 330$  mm for *C. undecimalis* (PETERS et al., 1998) and  $\geq 280$  mm for *C. parallelus* (RODRIGUES et al., 2006)). These two snooks species feed mainly on crustaceans and small fish (BLEWETT et al., 2006; CONTENTE et al., 2009; DUTKA-GIANELLI, 2014; LIRA, 2015), although fish has been reported as the dominant item for *C. undecimalis* while crustaceans prevail in *C. parallelus* diet (ALIAUME et al., 2005; LIRA, 2015). The similarities in habitat use, occurrence and feeding habits, especially during juvenile stages, may be a limiting factor for each snook population, leading to an overlap on their trophic niche and resource partitioning processes (ALIAUME et al., 2005; DUTKA-GIANELLI, 2014). There are valuable information on aspects of biology (PETERS et al., 1998; TAYLOR et al., 2000), feeding ecology (BLEWETT et al., 2006; CONTENTE et al., 2009; LIRA, 2015) and habitat use (STEVENS et al., 2007; BARBOUR et al., 2014; DAROS et al., 2016), but very few studies attempt to investigate the interaction between snook species (*e.g.* Aliaume et al. (2005); Rabelo et al. (2009); Lira (2015)).

The trophic niche of a species is one of the easier quantifiable dimensions among the many facets regarding the niche concept. It is usually accessed using stomach content analysis coupled with other descriptive indexes like diversity and evenness (BEARHOP et al., 2004), however, recent developments on methods to trace the organic matter flux through the food web, such as the stable isotope analysis (SIA), have provide insightful information on the species trophic ecology (NEWSOME et al., 2007). The stable isotope analysis, commonly for carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ), takes advantage by providing a time integrated information of food assimilated by an organism (DENIRO and EPSTEIN, 1978; HERZKA, 2005), which may vary from weeks to months (HERZKA, 2005; ELSDON et al., 2010), instead of the common *snapshot* of a dietary analysis

(PINNEGAR and POLUNIN, 2000). The  $\delta^{15}\text{N}$  exhibits a considerable enrichment per trophic transfers allowing to estimate the trophic position of an organism (POST, 2002; HUSSEY et al., 2014). On the other hand,  $\delta^{13}\text{C}$  is better to outline the origin of organic matter (FRY, 2006), once it presents low enrichment across the food web and great variability between primary producers (*e.g.* C3 and C4 plants) (DEGENS et al., 1968; SMITH and EPSTEIN, 1971; POST, 2002).

Our goals in the present study is to assess the resource partitioning of the common (*C. undecimalis*) and fat snook (*C. parallelus*) in four tropical estuaries in the northeast of Brazil. Stable isotope analysis was used to verify the hypothesis that (i.) snook species share the same resources and (ii.) such process is affected by seasonality and estuary's structure.

## Material and Methods

### *Study area and sampling procedures*

Located in the west coast of South Atlantic Ocean, the study area includes four estuarine systems along the coast of Pernambuco state, northeastern Brazil (Figure 1). The largest estuarine complex in Pernambuco is Itamaracá which exhibits a U-shape channel that connects to the sea through two entrances (SILVA et al., 2011). Due to its size and morphological differences (MEDEIROS and KJERFVE, 1993), Itamaracá was subdivided in two smaller systems, (1) the estuary of Catuama (Figure 1a), and (2) the Santa Cruz Channel (Figure 1b), which are associated to the north and south entrances respectively. Overall the Itamaracá system presents a mean depth of 5 m in the inner part of the channel and reaches up to 20 m deep near the mouth (MEDEIROS and KJERFVE, 1993; MONTES et al., 1998).

The estuaries of (3) Suape and (4) Sirinhaém, are distinct in size and shape when compared to the ones in the north coast. Suape, located 40 km from the state capital Recife, is formed by two main rivers that flow into a large brackish lagoon (CPRH, 2001) (Figure 1c). With an average depth of 3 m and a restrict connection to the ocean (BORGES, 2011), this estuary can be distinguished from the others because there is an industrial harbour on its marine portion. Sirinhaém located in the very south part of Pernambuco is the smallest among the estuaries addressed. This system presents low depth, which varies from 1 to 5.2 m (mean of 2.6 m), and a small connection to the ocean, forming few lagoons and flooded plains (CPRH, 2001; SILVA, 2009) (Figure 1d).



Sampling surveys on each estuary were taken between January to April and August to September of 2015, representing local dry and rainy seasons respectively (MEDEIROS and KJERFVE, 1993). Block nets placed near the mangrove creeks (350 x 2.9 m, mesh 70 mm) and beach seine trawls (20 x 1.9 m, mesh 20 mm) were used to collect *C. undecimalis* and *C. parallelus* snooks. All specimens were identified using specific bibliography (FIGUEIREDO and MENEZES, 1980), stored in ice, measured and then processed for isotopic analysis.

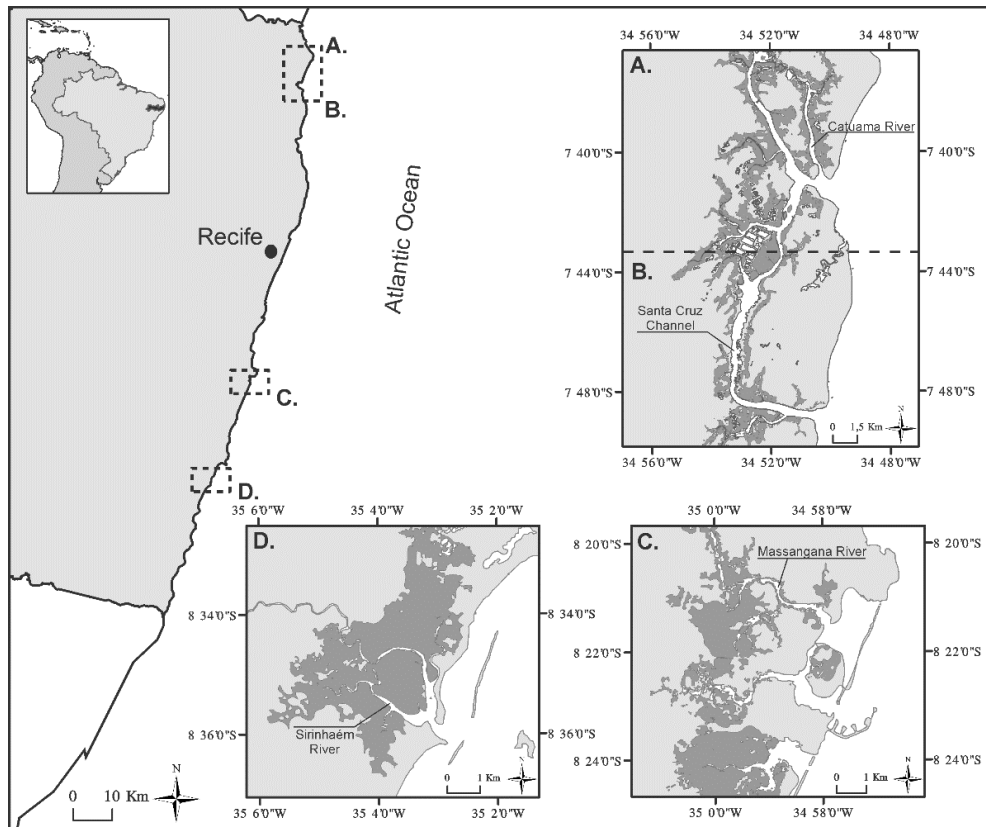


Figure 1: Estuarine systems of Catuama (a.), Santa Cruz (b.), Suape (c.) and Sirinhaém (d.) located in Pernambuco state, northeastern Brazil. Mangrove areas are presented as the darker area in each estuary.

#### *Stable isotope analysis*

A minimum of three specimens of each species from each season and environment were sampled for isotope analysis, in which, the muscle of individuals was sampled, cleaned with distilled water to remove further exogenous materials (*e. g.* remaining scales or bones), and set to dry in an oven at 60°C for 48 hours. After dried, the samples were grounded into a fine powder with a mortar and pestle. Each sample weighted between 0.35 and 0.45 mg and were analysed for carbon and nitrogen isotope ratios through a mass

spectrometer (Thermo Delta V+) coupled to an element analyser (Thermo Flash 2000, interface Thermo ConFio IV) in the Laboratory of Marine Environment Science, Institute of Research for Development (LEMAR –IRD), France.

Results for carbon and nitrogen are reported as  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively, and are derived from the relation of the isotopic value from the sample and a known standard:  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3$ ; in which  $R$  corresponds to the ratio between  $^{13}\text{C}$ : $^{12}\text{C}$  or  $^{15}\text{N}$ : $^{14}\text{N}$ . Standards used for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were the PeeDee Belemnite (PDB) limestone and atmospheric nitrogen, respectively. The analytical precision of the analysis, monitored through a known standard (Thermo – Acétanilide) every six samples, had a standard error of  $\pm 0.11\text{‰}$  and  $\pm 0.07\text{‰}$  for carbon and nitrogen respectively.

#### Data analysis

Due to a great variability on the specimens' length, preliminary analysis of the relationships between the estimations of isotope ratios and fish size (SL) were assessed using linear regressions to verify potential effects of size on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . Further, two generalized linear models (GLM) were performed, one for  $\delta^{15}\text{N}$  and other for  $\delta^{13}\text{C}$ . The first one looked at the relationship between fish size and  $\delta^{15}\text{N}$  values for the two species across seasons and environments. The response variable was  $\delta^{15}\text{N}$ , while the explanatory variables were specimen's size (SL), estuary, season, and specie. Size was set as continuous, while the remaining variables were classified as categorical ones: specie (two levels – *C. undecimalis*/ *C. parallelus*), season (two levels – dry/ rainy) and estuary (four levels - Catuama/ Santa Cruz/ Suape/ Sirinhaém). Main effects as well as first order interactions were considered. The best model was selected based on the Akaike's criterion (AIC). This criterion is useful to identify the model with best trade-off between bias and variance (BURNHAM and ANDERSON, 2002). Statistical inference (test F) was conducted to assess if the factors considered are important to explain the variability of  $\delta^{15}\text{N}$ , based on deviance table analyses.

A second model was built to evaluate differences of  $\delta^{13}\text{C}$  between species, estuaries and seasons. The selection of the model was also based on AIC, and similarly, inference was based on deviance table. Overall, the procedures were the same concerning the models for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ .

We were particularly interested in differences concerning estuaries, seasons and species but not fish size. Hence, the selected models were used to estimate expectations of  $\delta^{15}\text{N}$  (or  $\delta^{13}\text{C}$  if necessary) for the two species in the different estuaries and months but

for a fixed fish size of 200 mm standard length (SL), which is the approximate mean and mode of specimens analysed. The variability in  $\delta^{15}\text{N}$  among individuals was accounted by adding the model's residuals to the predicted  $\delta^{15}\text{N}$  calculated by fixing fish size as 200 mm. For example, if a residual of 0.25 was calculated for a 150 mm (SL) fish of one species in a given estuary and month, and the prediction for the same species, estuary and month but for an overall fixed fish size of 200 mm was 10‰, then we have 10.25‰ (10‰+0.25‰). This approach allowed us to exclude the size effect retaining the inherent variability of each specimen.

Resource partitioning processes were evaluated by looking at the overlap of the isotopic niche. The isotopic niche of both snooks species in each estuary and season was computed using the standard ellipses area method with  $\delta^{13}\text{C}$  and estimated  $\delta^{15}\text{N}$  values. The standard ellipses area (SEA) represents the mean core of the isotopic niche and encompasses approximately 40% of the available data (JACKSON et al., 2011). This method has showed to be less sensitive to sample size than other conventional methods (*e.g.* convex hull area) (JACKSON et al., 2011; SYVÄRANTA et al., 2013). In order to deal with biases that can occur in comparisons between groups with unequal sample size (*i.e.* underestimation of population's SEA for small samples), Jackson et al. (2011) proposed a corrected standard ellipse area (SEA<sub>C</sub>), which maintains the same geometrical aspects but with a slight increase in the ellipses area for small sample sizes. In addition, for comparison purpose, the isotopic space was standardized following Cucherousset and Villéger (2015).

All statistical procedures were made upon R 3.3.1 statistical software (R CORE TEAM, 2015), with the packages *siar* ("Stable Isotope Analysis in R" (PARNELL and JACKSON, 2015)) and *SIBER* ("Stable Isotope Bayesian Ellipses in R" (JACKSON and PARNELL, 2016)) for the estimation of isotopic niche areas and overlaps.

## Results

A total of 138 individuals were collected for stable isotope analysis, split in 62 *C. undecimalis* and 76 *C. parallelus*. Fishes ranged from 57 to 392 mm (SL) with a predominance of specimens in size classes 150 to 250 mm. Stable isotope ratios showed a broad range of  $\delta^{13}\text{C}$  (-25.38 to -14.22 ‰) and  $\delta^{15}\text{N}$  values (6.93 to 13.63 ‰) across sites and seasons, with *C. parallelus* exhibiting a slightly higher  $\delta^{15}\text{N}$  in most of the cases (Table 1).

Table 1: Mean values of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope ratios ( $\pm$ S.D.), predicted  $\delta^{15}\text{N}$  ( $\pm$  S.D.) and standard length (SL;  $\pm$  S.E.) of snook species and the baseline during dry and rainy seasons at four tropical estuaries, northeastern Brazil.

| Estuary          | Species               | Season |                  |                           |                           |                                 |       |                  |                           |                           |                                 |
|------------------|-----------------------|--------|------------------|---------------------------|---------------------------|---------------------------------|-------|------------------|---------------------------|---------------------------|---------------------------------|
|                  |                       | Dry    |                  |                           |                           |                                 | Rainy |                  |                           |                           |                                 |
|                  |                       | n      | SL (mm)          | $\delta^{13}\text{C}$ (‰) | $\delta^{15}\text{N}$ (‰) | Pred. $\delta^{15}\text{N}$ (‰) | n     | SL (mm)          | $\delta^{13}\text{C}$ (‰) | $\delta^{15}\text{N}$ (‰) | Pred. $\delta^{15}\text{N}$ (‰) |
| Catuama (CAT)    | <i>C. undecimalis</i> | 6      | 20.28 $\pm$ 1.49 | -17.1 $\pm$ 2.51          | 10.30 $\pm$ 0.96          | 10.18 $\pm$ 0.79                | 9     | 19.92 $\pm$ 0.93 | -20.09 $\pm$ 2.30         | 10.07 $\pm$ 0.51          | 10.13 $\pm$ 0.49                |
|                  | <i>C. parallelus</i>  | 8      | 9.15 $\pm$ 1.26  | -20.06 $\pm$ 0.92         | 9.55 $\pm$ 0.56           | 10.24 $\pm$ 0.66                | 8     | 14.09 $\pm$ 1.06 | -19.79 $\pm$ 1.61         | 9.91 $\pm$ 0.92           | 10.18 $\pm$ 0.87                |
| Santa Cruz (STC) | <i>C. undecimalis</i> | 10     | 22.71 $\pm$ 0.67 | -19.03 $\pm$ 2.0          | 10.89 $\pm$ 0.95          | 10.59 $\pm$ 0.92                | 8     | 31.04 $\pm$ 3.62 | -18.95 $\pm$ 3.32         | 10.94 $\pm$ 1.88          | 9.79 $\pm$ 1.28                 |
|                  | <i>C. parallelus</i>  | 5      | 23.92 $\pm$ 1.17 | -20.83 $\pm$ 3.97         | 12.23 $\pm$ 1.25          | 12.03 $\pm$ 1.14                | 8     | 16.54 $\pm$ 0.83 | -19.39 $\pm$ 1.73         | 11.05 $\pm$ 1.12          | 11.24 $\pm$ 1.09                |
| Suape (SUA)      | <i>C. undecimalis</i> | 7      | 25.03 $\pm$ 1.48 | -20.2 $\pm$ 0.72          | 11.86 $\pm$ 0.43          | 11.41 $\pm$ 0.13                | 3     | 16.97 $\pm$ 4.0  | -19.28 $\pm$ 0.62         | 10.88 $\pm$ 1.02          | 11.0 $\pm$ 0.3                  |
|                  | <i>C. parallelus</i>  | 14     | 17.59 $\pm$ 1.65 | -20.06 $\pm$ 1.54         | 10.27 $\pm$ 0.79          | 10.37 $\pm$ 0.73                | 11    | 13.27 $\pm$ 0.7  | -19.88 $\pm$ 1.72         | 9.52 $\pm$ 0.38           | 9.96 $\pm$ 0.37                 |
| Sirinhaém (SIR)  | <i>C. undecimalis</i> | 9      | 20.81 $\pm$ 0.55 | -21.58 $\pm$ 1.16         | 10.36 $\pm$ 0.23          | 10.39 $\pm$ 0.17                | 12    | 21.25 $\pm$ 1.26 | -22.37 $\pm$ 0.9          | 10.17 $\pm$ 0.35          | 9.55 $\pm$ 0.51                 |
|                  | <i>C. parallelus</i>  | 10     | 19.97 $\pm$ 1.56 | -20.25 $\pm$ 2.31         | 11.14 $\pm$ 0.61          | 11.03 $\pm$ 0.58                | 10    | 19.69 $\pm$ 2.17 | -22.09 $\pm$ 0.88         | 9.62 $\pm$ 0.52           | 10.18 $\pm$ 0.27                |

Despite the determination coefficients were not high the correlation between size of fish and  $\delta^{15}\text{N}$  values were significant for both species (linear regression: *C. undecimalis*  $r^2 = 0.37$ ,  $F = 36.74$ ,  $p < 0.001$  *C. parallelus*  $r^2 = 0.28$ ,  $F = 29.29$ ,  $p < 0.001$ ) (Figure 2). Conversely, the correlation between  $\delta^{13}\text{C}$  and fish size were not significant (linear regression: *C. undecimalis*  $r^2 < 0.01$ ,  $F = 0.03$ ,  $p = 0.85$ ; *C. parallelus*  $r^2 = 0.04$ ,  $F = 3.72$ ,  $p = 0.05$ ) (Figure 2).

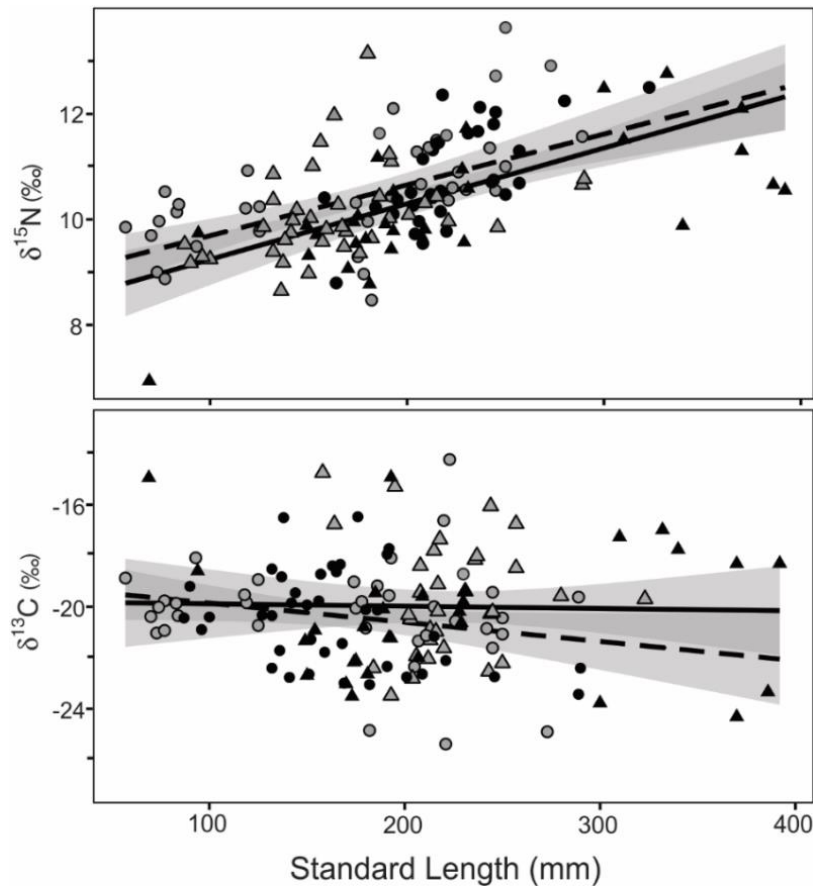


Figure 2: Linear regressions between estimations of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) isotope ratios and size of *Centropomus undecimalis* (triangles) and *Centropomus parallelus* (circles) from four tropical estuaries, northeastern Brazil, during dry (grey points) and rainy (black points) seasons. Linear regressions for *C. undecimalis* and *C. parallelus* are present as the solid and dashed lines on each graph respectively, with the shaded area representing the standard error of each regression.

The generalized linear model selected using AIC criterion explained about 60% of  $\delta^{15}\text{N}$  variation and included the factors species, estuary, and season, the covariate size, and the interactions species x estuary, species x size and estuary x season (Table 2). Deviance decrease significantly as estuary, season, size and interaction between

explanatory variables were included in the model (Table 2). Corrected values of  $\delta^{15}\text{N}$  for a 200 mm of standard length fish were higher in Suape, for *C. undecimalis*, and in Santa Cruz for *C. parallelus* (Table 1). Further,  $\delta^{15}\text{N}$  values were slightly higher during dry season ( $< 1\text{‰}$ ). Species within each estuary showed distinct  $\delta^{15}\text{N}$  levels, with differences higher than  $1\text{‰}$  in most of the cases. Catuama was the only area in which both snooks exhibited similar  $\delta^{15}\text{N}$  values (Figure 3).

Variability of  $\delta^{13}\text{C}$  were high, but there was not a distinct pattern between species and seasons (Table 3). However, significant differences of  $\delta^{13}\text{C}$  were found across studied locations (Table 3), with more depleted mean  $\delta^{13}\text{C}$  at Sirinhaém. Besides the interaction of season and estuary was not statistically significant (Table 3), average  $\delta^{13}\text{C}$  values were higher during dry than rainy season for Sirinhaém (Figure 3).

Table 2: Deviance analysis table of variables selected to the final model for  $\delta^{15}\text{N}$  prediction of two sympatric estuarine fishes (*Centropomus undecimalis* and *Centropomus parallelus*), northeastern Brazil.

| Variable             | d.f. | Deviance | <i>F</i> | <i>p</i> -value |
|----------------------|------|----------|----------|-----------------|
| Specie               | 1    | 1.24     | 2.50     | 0.115           |
| Estuary              | 3    | 24.04    | 16.09    | <0.001          |
| Season               | 1    | 10.04    | 20.16    | <0.001          |
| Fish's size          | 1    | 32.20    | 64.65    | <0.001          |
| Specie x Estuary     | 3    | 19.45    | 13.01    | <0.001          |
| Specie x Fish's size | 1    | 1.17     | 2.36     | 0.126           |
| Estuary x Season     | 3    | 3.27     | 2.18     | 0.092           |

d.f., degrees of freedom.

Table 3: Deviance analysis table of factors affecting  $\delta^{13}\text{C}$  of two sympatric estuarine fishes (*Centropomus undecimalis* and *Centropomus parallelus*), northeastern Brazil.

| Variable         | d.f. | Deviance | <i>F</i> | <i>p</i> -value |
|------------------|------|----------|----------|-----------------|
| Specie           | 1    | 4.86     | 1.31     | 0.254           |
| Estuary          | 3    | 119.50   | 10.73    | <0.001          |
| Season           | 1    | 8.62     | 2.32     | 0.129           |
| Specie x Estuary | 3    | 13.97    | 1.25     | 0.292           |
| Specie x Season  | 1    | 1.37     | 0.37     | 0.543           |
| Estuary x Season | 3    | 27.81    | 2.49     | 0.062           |

d.f., degrees of freedom.

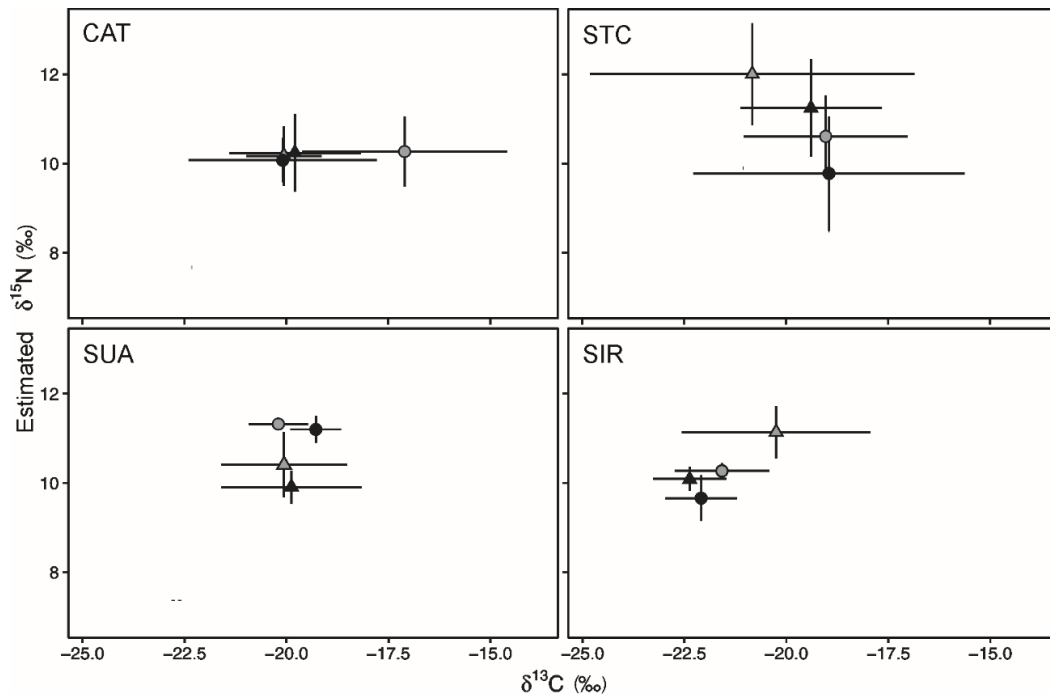


Figure 3: Distribution of carbon ( $\delta^{13}\text{C}$ ) and estimated nitrogen ( $\delta^{15}\text{N}$ ) isotope ratios (mean  $\pm$  S.D.) from *Centropomus undecimalis* (circles) and *Centropomus parallelus* (triangles) at four tropical estuaries, northeastern Brazil, during dry (grey points) and rainy (black points) seasons. Estuaries: CAT, Catuama; STC, Santa Cruz; SUA, Suape; SIR, Sirinhaém.

The isotopic niche of snook species varied greatly across areas and seasons (Table 4). The estimated standard ellipse area ( $\text{SEA}_C$ ) of snooks communities were larger in the northern areas (Santa Cruz and Catuama) than southern estuaries (Sirinhaém and Suape) (Figure 4). Northern estuaries also exhibited higher overlaps than the other systems (Table 4).  $\text{SEA}_C$  overlaps between species were found in every estuaries but Suape. No overlap was observed for Sirinhaém at the dry season. Overlaps were higher during the rainy season (Table 4, Figure 4). The  $\text{SEA}_C$  of *C. undecimalis* in Suape during the rainy season was not computed due to its low sample size ( $n = 3$ ).

Table 4: Standard ellipse area ( $SEA_C$ ) and overlap on the isotopic niche of two sympatric estuarine fishes (*Centropomus undecimalis* and *Centropomus parallelus*), during dry and rainy seasons at four tropical estuaries, northeastern Brazil.

| Estuary          | Specie                | Season  |             |           |         |             |           |         |             |           |
|------------------|-----------------------|---------|-------------|-----------|---------|-------------|-----------|---------|-------------|-----------|
|                  |                       | Total   |             |           | Dry     |             |           | Rainy   |             |           |
|                  |                       | $SEA_C$ | Overlap (%) | Total (%) | $SEA_C$ | Overlap (%) | Total (%) | $SEA_C$ | Overlap (%) | Total (%) |
| Catuama (CAT)    | <i>C. undecimalis</i> | 9.41    | 44.89       | 39.27     | 13.0    | 7.41        | 6.0       | 6.83    | 69.85       | 45.0      |
|                  | <i>C. parallelus</i>  | 5.57    | 75.81       |           | 3.97    | 24.29       |           | 8.54    | 55.9        |           |
| Santa Cruz (STC) | <i>C. undecimalis</i> | 17.37   | 33.31       | 19.82     | 10.09   | 39.61       | 12.54     | 23.96   | 18.8        | 14.22     |
|                  | <i>C. parallelus</i>  | 17.61   | 32.86       |           | 25.79   | 15.5        |           | 12.23   | 36.86       |           |
| Suape (SUA)      | <i>C. undecimalis</i> | 0.86    | 0.0         | 0.0       | 0.59    | 0.0         | 0.0       | -       | -           | -         |
|                  | <i>C. parallelus</i>  | 5.54    | 0.0         |           | 5.41    | 0.0         |           | 3.96    | -           |           |
| Sirinhaém (SIR)  | <i>C. undecimalis</i> | 2.54    | 32.24       | 13.08     | 1.17    | 0.0         | 0.0       | 2.06    | 9.57        | 6.37      |
|                  | <i>C. parallelus</i>  | 4.55    | 18.05       |           | 5.18    | 0.0         |           | 1.23    | 16.03       |           |

$SEA_C$ , standard ellipse area ( $\% \times 10^3$ ). Overlap, proportion of overlapped  $SEA_C$  by a given specie (%). Total, percentage of intersection area from the sum of species  $SEA_C$  (%).

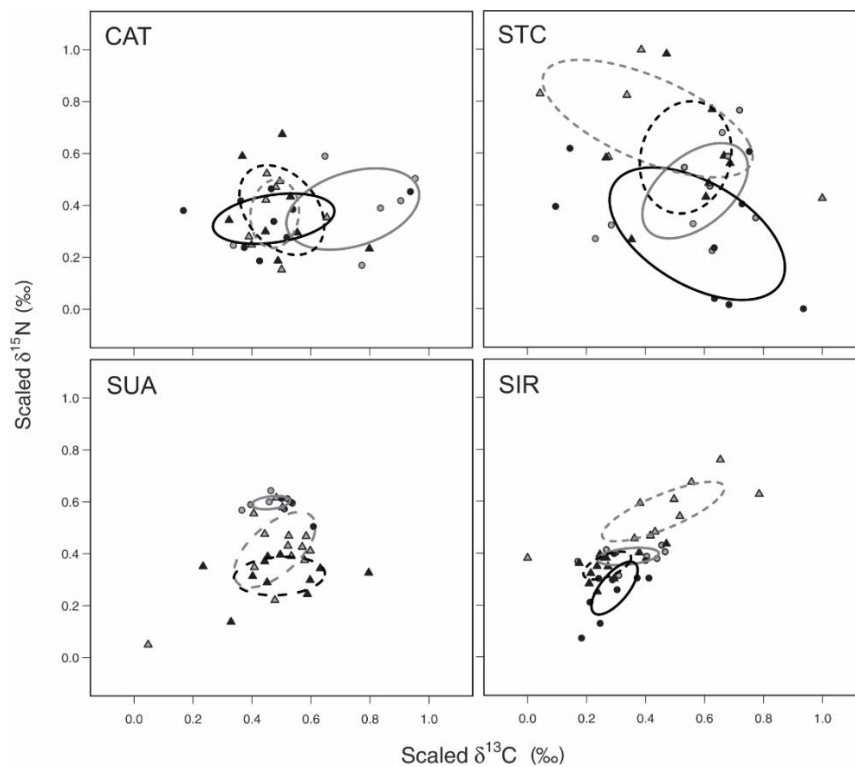


Figure 4: Scaled isotopic niche of *Centropomus undecimalis* (circles) and *Centropomus parallelus* (triangles) at four tropical estuaries, northeastern Brazil, during dry (grey points) and rainy (black points) seasons. Solid and dashed ellipses represent the corrected standard ellipses area ( $SEA_C$ ) of *C. undecimalis* and *C. parallelus* respectively. Scaled  $\delta^{15}N$  values are driven from the estimated  $\delta^{15}N$  at 200 mm of standard length. Estuaries: CAT, Catuama; STC, Santa Cruz; SUA, Suape; SIR, Sirinhaém.



## Discussion

In essence, the niche characteristics will respond to changes in intraspecific and interspecific competition, prey abundance (BEARHOP et al., 2004) as well as changes throughout the different life cycle stages (WARD-CAMPBELL et al., 2005; CONTENTE et al., 2009). In the present study, the significant relationship between fish size and  $\delta^{15}\text{N}$  indicates that both snooks, *C. undecimalis* and *C. parallelus*, feed on more nutritive preys with higher  $\delta^{15}\text{N}$  values as they reach larger sizes (BLEWETT et al., 2006; CONTENTE et al., 2009; ARAÚJO et al., 2011). Conversely, size and  $\delta^{13}\text{C}$  did not show any correlation which suggests that those two species occupies the estuaries long enough to assimilate local  $\delta^{13}\text{C}$  values. Larval migration usually occurs at 40 mm SL (BRAME et al., 2014) and the sampled individuals ranged from 57 to 392 mm SL.

Differences in isotope ratios are expected when comparing environments with distinct structures (e.g. Lugendo et al. (2007) and Hoeinghaus et al. (2011)). Indeed, both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  had significant differences between estuaries. Higher  $\delta^{15}\text{N}$  values were found at Santa Cruz, although it does not necessarily indicate higher trophic levels, but rather the presence of detritivorous species with low trophic position (e.g. Gobiidae fish) observed through stomach content analysis (LIRA, 2015). Alternatively, differences in  $\delta^{15}\text{N}$  on basal sources in the Santa Cruz Channel suggest a greater input of nitrogen in this system (MCCLELLAND and VALIELA, 1998), probably due to the recurrent discharge of domestic sewage from closer cities and the presence of aquaculture ponds nearby (MEDEIROS et al., 2001). Diet analysis of snook species from the Santa Cruz Channel and Sirinhaém revealed that snooks, especially *C. parallelus*, have different diet between estuaries. Higher proportions of decapod crustaceans were found in Sirinhaém, whereas fish and crustaceans prevailed in Santa Cruz (LIRA, 2015). Moreover, Sirinhaém, the smallest and shallower estuary out of the 4 sampled areas, presented a depleted mean  $\delta^{13}\text{C}$  and was the only area which appears to have seasonal influences on  $\delta^{13}\text{C}$ , illustrating the potential importance of freshwater runoff on providing depleted  $\delta^{13}\text{C}$  nutrients (terrestrial and C3 plants input) (SMITH and EPSTEIN, 1971; FRY, 2006).

*Centropomus* species presented a dynamic process of resource partitioning among studied locations. In general, the significant differences found in  $\delta^{15}\text{N}$ , rather than  $\delta^{13}\text{C}$ , between snooks within each estuary suggest that they inhabit similar environments but feed on different trophic levels. Higher overlaps and larger isotopic niches were observed at Catuama and, in a lesser extend Santa Cruz, which may be attributed to the estuary typology and food web structure. Stomach content analysis of *C. parallelus* and *C.*

*undecimalis* at Santa Cruz shows that snooks feed on similar and abundant preys and have wider niche breadth than Sirinhaém (LIRA, 2015), enhancing the overlap pattern observed for those species. The Itamaracá system (Santa Cruz Channel and Catuama) is the largest estuary in Pernambuco and exhibits a more abundant and closely related taxonomic fauna than Suape and Sirinhaém (MERIGOT et al., 2016) that results in a more similar diet. Conversely, a wider connection to the sea allows the entrance of allochthonous sources (e.g. marine nutrients and phytoplankton) and marine species that elevate the productivity and isotopic diversity of the environment (WALTERS et al., 2009; OLIVEIRA et al., 2014). Moreover, larger connections also facilitate the entrance of occasional large bodied predators (e.g. jacks and adult snooks (ADAMS and WOLFE, 2006; ADAMS et al., 2006)), which might increase predation risk and competition for resources between juvenile snooks. Kondoh (2003) reports that one of the essential features to the stability and resilience of high complexity food webs, such as the estuaries, is the capability of forager consumers to adapt to eventual fluctuations on community's abundance. Thereby, the variability observed in  $\delta^{15}\text{N}$ , as well as in  $\text{SEA}_C$ , from snooks among studied locations, especially for *C. undecimalis*, highlights its ecological importance as predator on estuarine ecosystems.

Seasonal variations on isotopic niche were weak yet significant. A greater  $\text{SEA}_C$  overlap between species was observed during rainy season. Abrantes et al. (2014) observed an increase in the abundance of different basal sources, like benthonic microalgae and epiphytes, after the wet season on tropical estuaries in Africa. Hence, the input of riverine nutrients on estuaries at the rainy season, as well as the greater availability of different basal sources, probably increase the production rate at lower trophic levels (NEUMANN-LEITÃO et al., 2001; GOMES et al., 2002; SILVA et al., 2003), providing enough resources for both species. In addition, migratory movements might also be an important factor for the seasonal trend observed. At summertime, adult snooks move to more saline and coastal habitats to spawn (PETERS et al., 1998; ADAMS et al., 2009b; DAROS et al., 2016), entering in riverine and estuarine areas during the cold months (BLEWETT et al., 2009; TROTTER et al., 2012). The presence of adult fishes collected in the estuary during the rainy season may increase the competition between juvenile snooks that are restricted to a smaller area in order to avoid the interaction with larger fishes (SCHOENER, 1982; ADAMS and WOLFE, 2006; BARBOUR et al., 2014).

Understanding the effect of estuary's typology on the interspecific process of estuarine fishes is essential to achieve better management strategies, especially on environments with recurrent anthropic pressures, such as urban expansion, which might leads to habitat degradation and collapse of ecologically important predators (LAYMAN et al., 2007b; ADAMS et al., 2009a). Stable isotopes metrics, like the  $\text{SEA}_C$ , were first described by Layman et al. (2007a) and have received much attention in recent years due to their capability of evidence disturbances in food web structure from a different point of view than classical methods (*e.g.* dietary analysis) (BEARHOP et al., 2004; NEWSOME et al., 2007; JACKSON et al., 2012; CUCHEROUSSET and VILLÉGER, 2015). On the other hand, besides the advances in modelling framework, these metrics are still sensitive to sample size and subjective without a prior knowledge on feeding habits (PARNELL et al., 2010; JACKSON et al., 2011). Despite the low sample size, the results indicated that there are resource partitioning by snooks and provide insightful information about the effect of estuary's morphology on this process. Juvenile snooks of the two species do share in some degree the same resources. Although it is unlikely that this competition represents a limiting factor for the stocks, since they show an opportunistic behaviour (ALIAUME et al., 2005) and represent some of the most abundant species in the estuaries addressed (MERIGOT et al., 2016). The resource partitioning process between the congener snook species seems to reflect the local features of estuary's typology and food web structure, with a strong resource partitioning on smaller estuaries. Further studies regarding the ontogenetic changes in habitat use of these species might fill the gaps found in this study and help to elucidate potential resource partitioning between species at different life stages.

### **Acknowledgements**

This study was funded in part by the INCT (National Institute of Science and Technology, CNPq n° 610013/2011-4) in Tropical Marine Environments – INCT-AmbTropic, the CNPq (National Council for Scientific and Technological Development, CNPq n° 479845/2013-1 and n° 407125/2013-2), and by CAPES (Coordination for the Improvement of Higher Education Personnel) for providing the scholarship to the first author during the MSc graduation. We thank to the members of BIOIMPACT Laboratory for their efforts on field campaigns and sampling procedures, and to A. Bertrand for personal advices during the preparation of the manuscript.

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#### 4. Considerações finais

Apesar das falhas encontradas neste trabalho, especialmente os problemas no deliniamento amostral, os resultados da análise de isótopos estáveis puderam elucidar a ecologia trófica e processos de partição de recursos entre as espécies de camurim, provendo informações importantes de como a morfologia dos estuários tende a afetar estes processos. De modo geral, nossos resultados sugerem que juvenis de robalo compartilham, pelo menos em algum nível, os mesmos recursos. Entretanto, esta interação se mostrou mais intensa em estuários com maior área estuarina e conexões mais largas com o meio marinho, de modo que estes fatores aparentam estar ligados a uma maior produtividade e diversidade nos valores isotópicos. Portanto, a partição de recursos entre juvenis de *C. undecimalis* e *C. parallelus* tendem a refletir características locais do tipo de estuário e estruturação da cadeia trófica. Estes resultados corroboram com a grande divergência na contribuição de fontes basais em cada local, e enfatizam o comportamento oportunista apresentado por estas espécies. Tais descobertas são de extremo valor para alcançar medidas de manejo eficientes, de modo a conseguir prever possíveis impactos no ambiente que podem levar a alterações na cadeia trófica (*e.g.* top-down) e colapso de espécies particularmente importantes.

Estudos futuros acerca do uso de habitat e mudanças ontogênicas para estas espécies, assim como trabalhos que visem compreender a relação entre a morfologia do ambiente e possíveis efeitos na biota local preencherão as lacunas encontradas aqui, fornecendo uma visão ecossistêmica mais eficaz para a gestão costeira atual.

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## ANEXO

Table S1: Trophic position (TP) estimate of prey items used to calculate the trophic position of *Centropomus undecimalis* and *Centropomus parallelus* from Santa Cruz and Sirinhaém estuaries, northeastern Brazil, and references used to evaluate respective functional feeding groups.

| Prey item category |                |                          | Functional feeding group  | TP  | Ref.                                                 |
|--------------------|----------------|--------------------------|---------------------------|-----|------------------------------------------------------|
| Polychaeta         | Polychaeta     |                          | Onivorous                 | 2.5 | (FAUCHALD and JUMARS, 1979)                          |
| Gastropoda         | Neritidae      | <i>Neritina virgínea</i> | Herbivorous/Detritivorous | 2   | (VILARDY and POLANÍA, 2002)                          |
| Hexapoda           | Insecta        |                          | Onivorous                 | 2.5 | (BRUSCA and BRUSCA, 2007)                            |
| Crustacea          | Alpheidae      | Alpheus sp.              |                           | 2   | (PALOMAR et al., 2004)                               |
|                    | Amphipoda      |                          | Onivorous                 | 2.5 | (BRUSCA and BRUSCA, 2007)                            |
|                    | Caridae        |                          | Onivorous                 | 2.5 | (BRUSCA and BRUSCA, 2007)                            |
|                    | Decapod        |                          | Onivorous                 | 2.5 | (BRUSCA and BRUSCA, 2007)                            |
|                    | Isopoda        |                          | Onivorous                 | 2.5 | (BRUSCA and BRUSCA, 2007)                            |
|                    | Ocypodidae     |                          | Onivorous                 | 2.5 | (BRUSCA and BRUSCA, 2007)                            |
|                    | Penaeidae      |                          | Onivorous                 | 2.5 | (BRUSCA and BRUSCA, 2007)                            |
|                    | Portunidae     | <i>Callinectes</i> sp.   | Carnivorous               | 3   | (BRANCO and VERANI, 1997; OLIVEIRA et al., 2006)     |
|                    | Sesarmidae     | <i>Aratus</i> sp.        | Herbivorous               | 2   | (BEEVER et al., 1979)                                |
|                    | Xanthidae      |                          | Onivorous                 | 2.5 | (BRUSCA and BRUSCA, 2007)                            |
| Teleostei          | Ariidae        |                          | Carnivorous               | 3   | (MENDOZA-CARRANZA, 2003; DENADAI et al., 2012)       |
|                    | Carangidae     | <i>Caranx</i> sp.        | Carnivorous               | 3   | (TIMÓTEO et al., 2015)                               |
|                    | Eleotridae     | <i>Eleotris pisonis</i>  | Carnivorous               | 3   | (TEIXEIRA, 1994)                                     |
|                    | Gerreidae      | <i>Eucinostomus</i> sp.  | Onivorous                 | 2.5 | (LEÃO, 2016)                                         |
|                    | Gobiidae       | <i>Ctenogobius</i> sp.   | Onivorous                 | 2.5 | (ZANLORENZI and CHAVES, 2011; CONTENTE et al., 2012) |
|                    | Mugilidae      | <i>Mugil</i> sp.         | Onivorous                 | 2.5 | (RUEDA, 2002)                                        |
|                    | Teleostei      | Unidf. Fish              | Onivorous                 | 2.5 |                                                      |
| Plant detritus     | Plant detritus |                          | -                         | 1   |                                                      |

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