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**ECOLOGIA E INTERAÇÕES TRÓFICAS DE CETÁCEOS NO  
ATLÂNTICO SUL OCIDENTAL**

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## RESUMO

As águas costeiras e oceânicas do sudeste-sul brasileiro sustentam uma grande diversidade de espécies de cetáceos, e o principal objetivo do presente estudo é entender a ecologia e interações tróficas dessas espécies. Para este fim, foi analisada a composição isotópica do carbono ( $\delta^{13}\text{C}$ ) e nitrogênio ( $\delta^{15}\text{N}$ ) em uma espécie de cetáceo costeira (toninha, *Pontoboria blainvillei*) e suas presas, e em amostras de pele de dez espécies de cetáceos oceânicos e dos organismos que compõem os diferentes compartimentos da cadeia alimentar (material orgânico particulado – MOP, zooplâncton, peixes e lulas). As amostras de toninhas foram obtidas de animais acidentalmente capturados em redes de pesca na costa sul do Brasil entre 1994-2010. No primeiro capítulo avaliamos as diferenças ontogenéticas e sexuais em seus hábitos alimentares usando os valores de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  nos dentes de toninhas e aplicando modelos de mistura isotópica para estimar a contribuição proporcional de suas principais presas à sua dieta. Não foram observadas diferenças nos hábitos alimentares entre machos e fêmeas, nem entre adultos de diferentes idades. Além disso, houve diferença na importância das principais presas estimadas pelos modelos de mistura e estudos anteriores com base no conteúdo estomacal. As amostras de cetáceos e presas oceânicas, bem como a dos organismos que compõem a base das teias tróficas (MOP e zooplâncton) foram obtidas durante dez cruzeiros de pesquisa (2012-2015) realizados nos meses de primavera e outono. Caracterizar os padrões espaço-temporais em  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  na base das cadeias alimentares é essencial em estudos ecológicos para a avaliação dos hábitos alimentares e padrões de migração de animais marinhos com base nesses traçadores. Assim, no segundo capítulo desta tese foram construídos mapas isotópicos (*isoscapes*) descrevendo os padrões latitudinal (norte-sul), longitudinal (plataforma continental externa PCE - região oceânica) e sazonal (primavera-outono) nos valores de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  do zooplâncton. No terceiro capítulo, foram avaliados a ecologia trófica e as interações entre as espécies de cetáceos que habitam essas águas oceânicas por meio dos valores isotópicos. No capítulo 4 foram analisados os dados de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  em todos os organismos, desde os produtores e consumidores primários até os predadores de topo, em uma abordagem ecossistêmica. Além disso, a posição trófica das diferentes espécies de cetáceos oceânicos foi estimada através da análise dos valores de  $\delta^{15}\text{N}$  em seus aminoácidos. Os padrões de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  na base das teias tróficas pelágicas (zooplâncton) observados ao longo da área refletem o contraste entre as diferentes massas de água que influenciam sazonalmente as regiões sudeste-sul. Os valores de  $\delta^{13}\text{C}$  aumentam e os de  $\delta^{15}\text{N}$  diminuem entre a PCE e a região oceânica.  $\delta^{13}\text{C}$  diminui em direção a região sul na área oceânica enquanto os valores de

$\delta^{15}\text{N}$  são mais altos na região sul. Além disso, o valor de  $\delta^{13}\text{C}$  foi significativamente mais alto no outono e o do  $\delta^{15}\text{N}$  foi mais alto na primavera. Os valores isotópicos nas espécies de cetáceos seguiram os padrões da base das teias tróficas das regiões sudeste e sul. Os dados isotópicos permitiram a identificação de duas potenciais subpopulações de *Stenella frontalis*. Uma alta sobreposição de nicho isotópico entre *S. frontalis* da região sul com *Delphinus delphis* sugere que eles compartilham recursos similares, porém utilizando a segregação espaço-temporal como uma forma de diferenciação de nicho para minimizar competição. Os dados isotópicos apontaram alta similaridade entre *Tursiops truncatus*, *S. frontalis* e *Globicephala melas* no uso de recursos. *Steno bredanensis* apresentou os maiores valores de  $\delta^{15}\text{N}$  e  $\delta^{13}\text{C}$ , e os valores mais altos em  $\delta^{15}\text{N}$  observados nos aminoácidos do tipo *fonte* ajudaram a corroborar a sua ocorrência em ambientes neríticos. Em contraste, os baixos valores de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  de *S. longirostris* sugerem alimentação em águas oceânicas em níveis tróficos relativamente baixos. Além disso, seu nicho isotópico se sobrepôs apenas ao de *S. attenuata*. Os baixos valores de  $\delta^{15}\text{N}$  em *Orcinus orca* sugerem que o ecótipo amostrado neste estudo ocupa posição trófica relativamente baixa, que foi confirmado com a análise de  $\delta^{15}\text{N}$  em aminoácidos. Por fim, os valores de  $\delta^{15}\text{N}$  na pele e nos aminoácidos de *Physeter macrocephalus* sugerem que os indivíduos se alimentam de níveis tróficos relativamente altos e semelhantes entre si. Os dados apresentados aqui fornecem informações originais sobre os hábitos alimentares, as interações tróficas e o nicho ecológico dos cetáceos que habitam as águas costeiras e oceânicas do Atlântico Sul ocidental.

**Palavras-chave:** cetáceos, isótopos estáveis, aminoácidos, isoscapes, ecologia alimentar, interações tróficas, Oceano Atlântico Sul Ocidental

## ABSTRACT

The main aim of the present study was to investigate the trophic ecology of the cetacean species occurring in the Brazilian coastal and oceanic waters. To this end, we have analyzed the natural carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotopic composition in a coastal cetacean species (franciscanas, *Pontoboria blainvillei*) and their prey, and in ten oceanic cetacean species and the organisms comprising the different compartments of the food chain (particulate organic matter - POM, zooplankton, fish and squid). Franciscanas' samples were obtained from animals by-caught in fishing nets off the southern coast of Brazil between 1994-2010. In the first chapter we evaluated the ontogenetic and sexual differences in their feeding habits using the values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in franciscanas' teeth and applying isotopic mixing models to estimate the proportional contribution of their main prey to their diet. Data showed no differences in the feeding habits between males and females, nor between adults of different ages classes. In addition, there was a difference between the importance of the main prey estimated by the mixing models and other studies based on the analysis of stomach content. The samples from the oceanic cetacean species and prey, as well as from the organisms at the base of the food webs (POM and zooplankton) were obtained during ten research cruises (2012-2015) in autumn and spring months. Characterizing the spatiotemporal patterns in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  at the base of the food webs (baseline) is essential in ecological studies for the assessment of the feeding habits and migration patterns of marine predators. Therefore, in the second chapter of this thesis we have constructed isotopic maps (*isoscapes*) describing the latitudinal (north-to-south), longitudinal (outer continental shelf-to-offshore) and seasonal (spring-autumn) patterns in zooplankton  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . In the third chapter we discuss the trophic ecology and interactions among the cetacean species that inhabit these oceanic waters. In chapter four we analyzed the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in all organisms from primary producers and consumers to top predators in an ecosystem approach. Additionally, the trophic position of the cetacean species was estimated with the analysis of  $\delta^{15}\text{N}$  in individual amino acids. The patterns in baseline organic  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  observed along the area reflect the contrast between the different water masses that influence each of the two regions. We found an increase in  $\delta^{13}\text{C}$  and a decrease in  $\delta^{15}\text{N}$  from the shelf break towards the offshore waters, a latitudinal (north-south) decrease in  $\delta^{13}\text{C}$  along the offshore, and an increase in  $\delta^{15}\text{N}$  towards the south along both shelf break and offshore waters. Additionally,  $\delta^{13}\text{C}$  was significantly higher in autumn and  $\delta^{15}\text{N}$  was higher in spring. The isotopic values in cetacean species followed the baseline patterns of the southeast and southern regions. Additionally, our isotopic analysis allowed us to identify two

potential subpopulations of *Stenella frontalis*. A high core isotopic niche overlap between *S. frontalis* from the southern range of the study area and *Delphinus delphis* suggests that they share similar resources and rely on spatiotemporal segregation to achieve niche differentiation and thus minimize competition. Our isotopic data pointed to high similarity of *Tursiops truncatus*, *S. frontalis* and *Globicephala melas* in the use of resources. *Steno bredanensis* had the highest  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , and the high  $\delta^{15}\text{N}$  in source amino acids helped corroborate their occurrence in neritic environments. In contrast, *S. longirostris*' low  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  suggest feeding in oceanic waters at relatively lower trophic levels. Additionally, their isotopic niche overlapped only with that of *S. attenuata*. The low  $\delta^{15}\text{N}$  values in *Orcinus orca* suggest they occupy relatively lower trophic position. Lastly, bulk and amino acids  $\delta^{15}\text{N}$  in *Physeter macrocephalus* suggested that individuals occupy relatively high and similar trophic positions. The data presented here provide a first insight into the feeding habits, trophic interactions and ecological niche of cetaceans that inhabit the coastal and oceanic waters of the subtropical western South Atlantic.

**Key words:** cetaceans, stable isotopes, isoscapes, feeding habits, trophic interactions, Southwestern Atlantic Ocean

## INTRODUÇÃO

Os cetáceos são componentes-chave na estrutura e funcionamento dos ecossistemas em que habitam, sendo considerados importantes nos processos de controle “bottom-up” e “top-down” (Bowen 1997; Estes et al. 1998; Roman et al. 2014, 2016). Através dos seus materiais fecais e excrementos os cetáceos disponibilizam nitrogênio e outros nutrientes aos ambientes aquáticos intensificando, assim, a produção primária e a transferência de energia para os níveis tróficos mais altos (Nicol et al. 2010; Roman & McCarthy 2010; Lavery et al. 2014; Roman et al. 2016). Além disso, contribuem transferindo energia de águas superficiais para águas mais profundas através de movimentos verticais para alimentação ou quando suas carcaças afundam, servindo de alimento ou abrigo para diversos micro e macro organismos em grandes profundidades (Bennett et al. 1994; Jones et al. 1998). Seus efeitos no controle top-down foram evidentes quando a redução de espécies-chave gerou efeito cascata afetando a estrutura e dinâmica de ecossistemas (Estes et al. 1998; Springer et al. 2003). Além disso, os cetáceos influenciam a abundância de espécies simpátricas competidoras que exploram os mesmos recursos, refletido, por exemplo, no aumento da abundância de aves e pinípedes em águas Antárticas devido a menor pressão de cetáceos sobre o krill após a caça exploratória às baleias (Laws 1985; Ballance et al. 2006). Portanto, é evidente que para compreender o papel ecológico dos cetáceos é necessário avaliar as suas relações com seu ambiente, incluindo as principais

Uma grande diversidade de espécies de cetáceos habita as águas costeiras e oceânicas do sudeste-sul do Brasil (Moreno et al. 2005; Di Tullio et al. 2016). A co-existência dessas espécies requer comportamentos alimentares distintos que podem incluir o consumo especializado ou generalista, de presas solitárias ou que formam densas agregações, de diferentes composições específicas, níveis tróficos ou classes de tamanho. Além disso, espécies de presas demersais são frequentemente relatadas como itens importantes na dieta de espécies de cetáceos costeiras (e.g., Secchi et al. 2016; Tellechea et al. 2017), enquanto presas dos compartimentos epi- e meso-pelágicos compõem a dieta de espécies de ocorrência oceânica (e.g., Silva-Jr. et al. 2007; Wang et al. 2003; 2012). A maioria das informações disponíveis sobre os hábitos alimentares dos cetáceos provém de estudos com dados de conteúdo estomacal de animais encontrados encalhados ou capturados acidentalmente, ou através da observação de comportamentos alimentares de animais costeiros. A análise de conteúdo estomacal oferece informações valiosas sobre as espécies consumidas, porém pode superestimar a importância de presas mais resistentes à digestão, enquanto subestima a importância de presas com maior

digestibilidade (Bowen & Iverson 2013). Além disso, oferece uma visão da última refeição do animal que pode não representar a dieta da população e de animais saudáveis quando o número amostral for muito baixo ou quando o período do estudo for curto (Bowen & Iverson 2013). Além disso, quando se trata de animais oceânicos devido à sua ocorrência a longas distâncias da costa, pouco se sabe sobre a ecologia trófica ou o nível de interações intra- e interespecíficas. Nos raros eventos em que espécimes de populações oceânicas encalham em regiões costeiras (Prado et al. 2016) permitindo a recuperação das carcaças para seu estudo, informações provenientes do conteúdo estomacal provavelmente não refletem a dieta de animais saudáveis, pois pode incluir presas costeiras consumidas antes da morte do animal que estava fora do seu ambiente natural. Portanto, caracterizar essas interações ecológicas é particularmente dificultado pelo acesso limitado a informações sobre essas populações, o que impede a avaliação de seus hábitos alimentares.

Neste contexto, os isótopos estáveis vêm sendo utilizados para inferir os hábitos alimentares de mamíferos marinhos (Newsome et al. 2010), pois as razões isotópicas de carbono ( $\delta^{13}\text{C}$ ) e nitrogênio ( $\delta^{15}\text{N}$ ) dos alimentos assimilados são refletidas nos tecidos dos seus consumidores (DeNiro & Epstein 1978; 1981; Tieszen et al. 1983). Há um efeito trófico nos valores isotópicos entre predador e suas presas, ou fator de discriminação trófica, que na pele de cetáceos foi estimado em  $\sim 1,0\text{‰}$  ( $\pm 0,37$ ) para  $\delta^{13}\text{C}$  e  $1,6\text{‰}$  ( $\pm 0,52$ ) para  $\delta^{15}\text{N}$  (Gimenez et al. 2016). Assim, os valores isotópicos dos indivíduos dentro de uma população representam suas áreas de alimentação e recursos utilizados ( $\delta^{13}\text{C}$ ), bem como suas posições tróficas relativas ( $\delta^{15}\text{N}$ ) (Bearhop et al. 2004; Layman et al. 2012). Além disso, isótopos estáveis podem ser usados para estimar a área do nicho isotópico de espécies ou populações (Jackson et al. 2011), que permite aproximações quantitativas de seu nicho ecológico (Bearhop et al. 2004; Layman et al. 2012). Aspectos importantes da estrutura trófica de populações ou comunidades podem ser avaliados através de métricas estimadas usando os valores de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$ . O nicho isotópico pode ser aplicado para fazer inferências sobre o nível de sobreposição (ou partição) espacial ou trófica entre diferentes espécies dentro de comunidades (Jackson et al. 2011). Além disso, as distribuições de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  dentro do espaço  $\delta$  permitem avaliar o nível de espaçamento das espécies, oferecendo informações sobre a posição relativa de indivíduos entre si dentro do espaço do nicho, e sendo usadas para inferir o nível da redundância trófica dentro dessas populações ou comunidades (Layman et al. 2007; Jackson et al. 2012).

Assim, esses traçadores químicos têm sido amplamente utilizados em estudos sobre as relações tróficas de predadores (Newsome et al. 2010; Seyboth et al. 2018), descrevendo o

fluxo de matéria e energia dentro de ecossistemas e teias alimentares marinhas (Botto et al. 2011), e avaliando as áreas de alimentação e padrões de movimento de animais marinhos (Hobson et al. 2010; Graham et al. 2010). No entanto, embora os isótopos estáveis sejam ferramentas extremamente úteis para estudar a ecologia trófica de animais marinhos, para uma avaliação mais eficaz sobre as variações nos hábitos alimentares a nível intra- e interespecífico, sobreposição ou segregação no uso de recursos por espécies coexistentes usando isótopos estáveis, devemos considerar os gradientes espaço-temporais nos valores isotópicos na base das cadeias alimentares (Graham et al. 2010). Valores de  $\delta^{13}\text{C}$  na base das cadeias alimentares são influenciados diferentemente por gradientes de temperatura, concentração de  $\text{CO}_2$ , composição específica, tamanho e taxas de crescimento dos produtores primários (Laws et al. 1995; Popp et al. 1998; Trueman & Glew 2019). Consequentemente, os valores de  $\delta^{13}\text{C}$  diferem entre organismos que habitam ambientes estuarinos, marinhos ou de água doce (Fry & Sherr 1989; Trueman & Glew 2019), pelágicos ou bentônicos (France 1995), tropicais, temperados ou polares (Cherel & Hobson 2007). Os isótopos estáveis de nitrogênio também variam entre os ecossistemas, dependendo principalmente das concentrações e dos tipos de nutrientes disponíveis (e.g., nitrato ou fixação de  $\text{N}_2$ ). Algumas cianobactérias, como as colônias de *Trichodesmium*, são capazes de assimilar  $\text{N}_2$  em processos de baixíssima discriminação isotópica, resultando em biomassas com razões isotópicas muito semelhantes àquela da fonte  $\text{N}_2$  atmosférico (Capone et al. 2005; Mompean et al. 2013). Assim, menores valores de  $\delta^{15}\text{N}$  são encontrados em águas oligotróficas onde a fixação de  $\text{N}_2$  é a fonte mais importante de nitrogênio para o fitoplâncton (Capone et al. 2005; Mompean et al. 2013), enquanto em águas onde a disponibilidade de nutrientes é maior, como em zonas costeiras ou de ressurgências, o nitrato ( $\text{NO}_3^-$ ) é a principal fonte de nitrogênio para os produtores primários que, consequentemente, irão apresentar valores de  $\delta^{15}\text{N}$  mais altos (Rau et al. 1998; Sigman et al. 2009). Por sua vez, os valores isotópicos dos organismos na base da teia trófica (e.g., zooplâncton) são refletidos nos níveis tróficos superiores. Assim, o desenvolvimento de mapas isotópicos (*isoscapes*) caracterizando os valores de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  na base dos diferentes ecossistemas é essencial ao avaliar os hábitos alimentares de espécies migratórias ou ao comparar diferentes populações de predadores co-específicos para entender sua ecologia, já que as composições de isótopos estáveis dos consumidores refletem aquelas das áreas onde eles têm se alimentado (Graham et al. 2010; McMahon et al. 2013; Rodríguez-Pérez et al. 2018).

Mais recentemente, análises das razões isotópicas em aminoácidos individuais têm sido aplicada como uma ferramenta promissora e poderosa em estudos ecológicos, ajudando a avaliar os níveis tróficos dos consumidores e a determinar o fluxo de energia e nutrientes nos diferentes ecossistemas (Chikaraishi et al. 2009; Matthews & Ferguson 2014; Elliot-Smith et al. 2018). Isso porque os aminoácidos do tipo *fonte* sofrem fracionamento trófico mínimo (McMahon & McCarthy 2016), sendo aplicados para fazer inferências sobre os valores isotópicos da base das teias tróficas onde os animais se alimentam, enquanto aminoácidos do tipo *trófico* sofrem maior fracionamento durante processos metabólicos, sendo usados para avaliar os níveis tróficos dos consumidores (Popp et al. 2007; McMahon & McCarthy 2016; McMahon & Newsome 2019). Assim, a análise de isótopos estáveis em aminoácidos de uma única amostra oferece informações sobre a fonte de alimento, as áreas de forrageio e o nível trófico do animal consumidor.

No Brasil a plataforma continental externa e talude são influenciados por diferentes massas de água que contribuem para diferenças físicas e biogeoquímicas entre as regiões sul (34°S-28°S) e sudeste (28°S-24°S, ver figura 1). A região sudeste é influenciada principalmente pelas águas tropicais que fluem para o sul transportadas pela Corrente do Brasil, juntamente com as ressurgências da Água Central do Atlântico Sul (ACAS) ocorrendo tanto na altura de Cabo Frio no Rio de Janeiro (Acha et al. 2004; Coelho-Souza et al. 2012), e em menor escala no Cabo Santa Marta, em Santa Catarina (Campos et al. 2013). Esse fluxo ascendente de águas frias e ricas em nutrientes fertiliza as camadas superficiais aumentando a produtividade nessas regiões (Guenther et al. 2008; Coelho-Souza et al. 2012; Brandini et al. 2018). Altas densidades de zooplâncton foram observadas na região de Cabo Frio durante o final da primavera e verão, após o aumento da biomassa de produtores primários devido a esses fortes eventos de ressurgência (Valentin 2001; Acha et al. 2004). Além disso, o aumento da abundância de consumidores com maior nível trófico, como lulas e peixes pelágicos, coincide com essas ascensões de nutrientes (Bakun & Parrish 1991; Valentin 2001). Por outro lado, altas concentrações de *Trichodesmium* são encontradas no verão e primavera sob domínio das águas tropicais oligotróficas (Detoni et al. 2015; Lima et al. 2019).

A região sul é sazonalmente influenciada pelas águas tropicais que ocupam a PCE e o talude nos meses mais quentes, enquanto nos períodos mais frios há a prevalência de águas subtropicais, formadas pelo encontro de águas continentais do Rio da Prata e da Lagoa dos Patos, águas tropicais e águas subantárticas transportadas pela Corrente de Malvinas/Falklands (Möller Jr. et al. 2008; Piola et al. 2008), esta última chegando no máximo a 32°S. Por sua vez,

essa dinâmica de massas d'água influencia a disponibilidade de nutrientes e a produtividade da região (Ciotti et al. 1995; Duarte et al. 2014; Gonçalves-Araújo et al. 2018). Tais diferenças na dinâmica das massas d'água entre as regiões sudeste e sul resultam em gradientes físicos que por sua vez influenciam diferentemente os processos biogeoquímicos locais. Espera-se, portanto, que tais padrões físicos criem gradientes sazonais nas composições isotópicas da base da cadeia trófica ao longo dessas duas regiões, que também serão refletidos nas assinaturas isotópicas dos cetáceos.

## HIPÓTESES

Tendo em vista o que foi apresentado acima, as seguintes hipóteses foram testadas neste estudo:

1. Os recursos demersais e costeiros serão mais importantes na dieta das toninhas que os recursos provenientes das redes tróficas pelágicas;
2. Os gradientes físicos latitudinais (sudeste-sul), longitudinais (PCE-talude) e sazonais (outono-primavera) na região oceânica são refletidos na composição isotópica dos produtores e consumidores primários (zooplâncton), descrevendo “*isoscapes*” que caracterizam a região. Portanto, a variação temporal nas razões de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  na base da teia trófica na região sudeste, onde a Água Tropical predomina ao longo do ano, é menor do que na região sul, onde a variabilidade das massas d'água é maior;
3. Dado que as variações isotópicas na base da cadeia devem ser transferidas ao longo desta, esperamos que espécies com distribuição relacionada a águas mais frias, ricas em nutrientes (região sul) ou zonas de ressurgências, apresentam valores de  $\delta^{15}\text{N}$  mais elevados que espécies com ocorrência em águas mais tropicais, quentes e oligotróficas;
4. Espécies de cetáceos que apresentam sobreposição nas áreas de ocorrência apresentarão nichos isotópicos similares, indicando sobreposição na utilização dos recursos;
5. Espécies comumente vistas em associação com outras espécies, formando grupos mistos (e.g., *Tursiops truncatus*) apresentam maior sobreposição de nicho isotópico com as mesmas do que com outras espécies de cetáceos;
6. Os cetáceos generalistas (e.g., *T. truncatus*) apresentam nicho isotópico mais amplo que as espécies especialistas (e.g., *P. macrocephalus*).

## OBJETIVOS

O objetivo principal desta tese foi entender a ecologia alimentar e as relações tróficas das espécies de cetáceos costeiras e oceânicas no sudeste-sul do Brasil. Objetivos específicos:

1. Caracterizar os hábitos alimentares das toninhas (*Pontoporia blainvillei*) e avaliar se há diferenças sexuais e ontogenéticas através da análise de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$ ;
2. Descrever os padrões espaço-temporais nos valores isotópicos da base da rede trófica pelágica (matéria orgânica particulada e organismos zooplancctônicos) nas águas oceânicas do sudeste-sul do Brasil;
3. Determinar a posição trófica relativa dos cetáceos oceânicos, e avaliar se há variações latitudinais (sudeste-sul) nos valores de  $\delta^{15}\text{N}$  e na posição trófica dos mesmos;
4. Estimar a área do nicho isotópico ocupada pelas diferentes espécies de cetáceos oceânicos, e o nível de sobreposição no nicho e na utilização dos recursos entre elas.

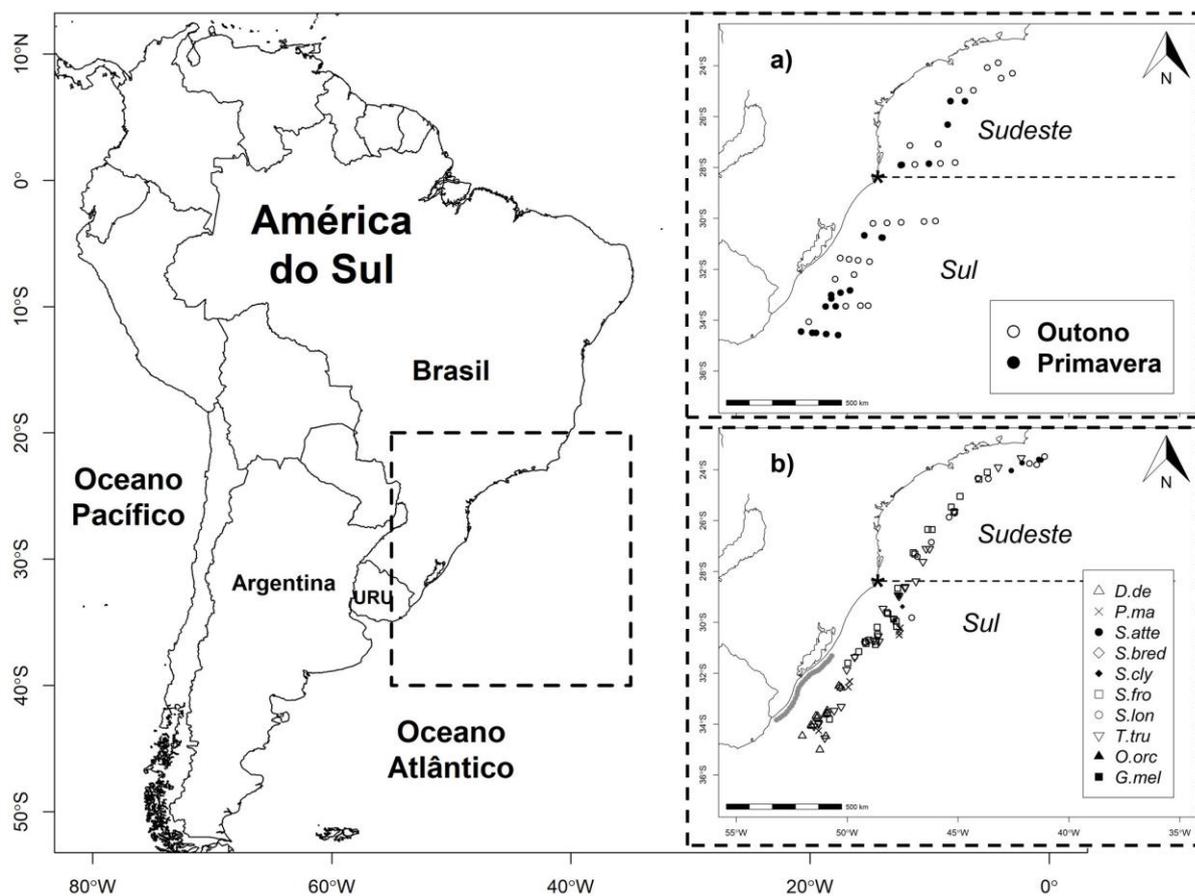


Figura 1 Mapa da área de estudo no oceano Atlântico sul ocidental; Os cruzeiros de pesquisa foram realizados na plataforma continental externa e talude do sudeste-sul do Brasil; a) estações oceanográficas onde as amostras de zooplâncton foram coletadas no outono  $\circ$  e na primavera  $\bullet$ ; b) locais de amostragem de cetáceos: símbolos representam os locais das biópsias de cetáceos oceânicos, linha cinzenta representa a região costeira onde as toninhas foram acidentalmente capturadas no sul do Brasil; a linha pontilhada representa a divisão entre as áreas sudeste e sul, ao norte e ao sul do Cabo de Santa Marta (★),

respectivamente. A tabela 1 contém a lista dos nomes das espécies de cetáceos amostrados neste estudo e suas respectivas abreviações

## **MATERIAL E MÉTODOS**

As espécies de cetáceos costeiros (toninhas, *Pontoporia blainvillei*) foram capturados acidentalmente em redes de pesca em áreas costeiras no Rio Grande do Sul, no sul do Brasil entre 1994 e 2010. As amostras oceânicas foram coletadas durante as expedições do Projeto Talude a bordo do Navio Oceanográfico *Atlântico Sul* da Universidade Federal do Rio Grande (FURG). Os cruzeiros foram realizados entre 2009 e 2015, e a área de estudo inclui a plataforma continental externa e o talude (isóbatas de 150 e 2000 m), entre Cabo Frio (Rio de Janeiro, 22°53'S/41°30'W) e o Chuí (Rio Grande do Sul, 33°44'S/53°16'W, figura 1). Foram realizados cinco cruzeiros no outono e cinco na primavera, permitindo a coleta de dados sazonais e interanuais dos componentes desse ecossistema.

### **Dados costeiros**

#### ***Amostras de toninhas***

Foram obtidas amostras de 125 toninhas capturadas acidentalmente pela frota pesqueira no sul do Brasil. O comprimento total do corpo foi medido como uma linha reta entre a ponta do rosto e a reentrância mediana da nadadeira caudal, e o sexo foi determinado através de exame externo. Os dentes foram coletados e limpos, e armazenados a seco para a estimativa de idade e para a análise de isótopos estáveis. A idade foi estimada através da contagem dos Grupos de Camadas de Crescimento (*Growth Layer Groups*, GLG) na dentina e no cemento dos dentes (Pinedo & Hohn, 2000).

#### ***Amostras das presas***

Espécies de peixes e cefalópodes, que foram registradas no conteúdo estomacal das toninhas em estudos anteriores, foram coletadas pela frota pesqueira na costa sul do Brasil. Essas amostras foram armazenadas a -20°C até a preparação para análise de isótopos estáveis. As amostras de músculo de cada espécime de peixe ou lula foram lavadas com água destilada e secas em estufa a 60°C por 48 horas.

## Dados oceânicos

### *Amostragem nas estações oceanográficas*

Amostras de matéria orgânica particulada (MOP), zooplâncton, temperatura (T) e salinidade (S) foram coletadas em estações oceanográficas pré-determinadas ao longo da área de estudo (Fig. 1). As amostras de MOP foram coletadas através da filtração a vácuo de água superficial em filtros GF/F (0,7  $\mu\text{m}$ ), e usadas como um proxy dos valores isotópicos dos produtores primários (e.g., Hobson et al. 2002). Amostras de zooplâncton foram coletadas usando redes de tipo bongo com malhas 300 e 500  $\mu\text{m}$ , abrangendo todo o perfil vertical de cada estação, coletados desde a superfície até profundidades de 200 m, dependendo da profundidade local. As amostras de ambas as redes foram misturadas e congeladas a  $-20^{\circ}\text{C}$  até processamento. Informações de T e S da água foram registradas em cada estação usando o CTD (*Conductivity-Temperature Depth*, SeaBird CTD/Carrousel 911 + system®). Os valores para S e T apresentados para cada estação foram a média dos 200 m superiores na coluna de água.

### *Amostragem durante os transectos*

Durante os cruzeiros foram feitas transecções em zigue-zague ao longo da área de estudo para a observação e coleta de amostras de cetáceos. As amostras de pele de cetáceos (tabela 1) foram obtidas através de biopsias remotas por meio de balestras equipadas com dardos específicos para coleta de material biológico, diretamente da proa do navio, enquanto os indivíduos praticam *bow-riding* ou a partir do bote inflável quando as condições do mar permitiram (Beaufort < 3). Amostras de pequenos peixes e cefalópodes foram coletados em arrastos com redes de meia água. Além disso, peixes maiores e lulas foram coletadas oportunisticamente com tarrafas e pesca com linha-de-mão. Todas amostras foram mantidas congeladas em  $-20^{\circ}\text{C}$  até processamento. Tecidos metabolicamente ativos como a pele têm taxas de reposição isotópica relativamente rápida, e a meia vida da pele foi estimada em aproximadamente 24 dias ( $\pm 8$ ) para carbono e cerca de 47 dias ( $\pm 19$ ) para isótopos de nitrogênio em golfinhos (Giménez et al. 2016), oferecendo informações sobre a dieta e áreas de forrageio a uma escala sazonal. Esses valores foram considerados semelhantes para as espécies aqui analisadas.

**Tabela 1** Espécies de cetáceos amostradas neste estudo. A tabela inclui os nomes científicos e comum (Português e em Inglês), assim como a abreviação para as espécies amostradas na região oceânica.

Nome científico	Nome comum (Português)	Nome comum (Inglês)	Abreviação
<i>Pontoporia blainvillei</i>	Toninhas	Franciscanas	-
<i>Delphinus delphis</i>	Golfinho-comum	Short-beaked common dolphins	D.de
<i>Physeter macrocephalus</i>	Cachalote	Sperm whales	P.ma
<i>Stenella attenuata</i>	Golfinho-pintado-pantropical	Pantropical spotted dolphins	S.atte
<i>Steno bredanensis</i>	Rough-toothed dolphins	Rough-toothed dolphins	S.bred
<i>Stenella frontalis</i>	Golfinho-pintado-do-Atlântico	Atlantic spotted dolphins	S.fro
<i>Stenella longirostris</i>	Golfinho-rotador	Spinner dolphins	S.lon
<i>Tursiops truncatus</i>	Golfinho-nariz-de-garrafa	Bottlenose dolphins	T.tru
<i>Orcinus orca</i>	Orca	Killer whales	O.orc
<i>Globicephala melas</i>	Baleia-piloto-de-nadadeiras-longas	Long-finned pilot whales	G.mel
<i>Stenella clymene</i>	Golfinho-de-Clymene	Clymene dolphins	S.cly

### Preparação das amostras para análise de isótopos estáveis

Um dente inteiro de cada toninha foi pulverizado, acidificado em ácido clorídrico (HCl, 30%) por meio de defumação em dessecador para remoção do carbono inorgânico, e seco em estufa a 60°C por 3 h (Walker et al. 1999; Knoff et al. 2008). Aproximadamente 2 mg de pó de cada dente foi pesado em cápsulas de estanho para análise de isótopos.

Os filtros contendo a MOP foram secados em estufa a 60°C por 48 h e posteriormente acidificados em HCl (ácido clorídrico, 30%) para remoção do carbono inorgânico. Amostras de zooplâncton foram divididas em duas subamostras: 1) amostra de mistura, na qual foram analisados vários componentes do zooplâncton combinados; 2) amostra com os organismos separados nos principais grupos taxonômicos (copépodos, anfípodos, eufauciáceos, quetognatos e salpas). As amostras de zooplâncton, potenciais presas (peixes e cefalópodes) e de pele de cetáceos foram lavadas com água destilada, secadas em estufa a 60°C por 48 h. Todas as amostras foram maceradas para a obtenção de um pó fino para análise das razões isotópicas.

Os lipídios têm um forte efeito negativo sobre os valores de  $\delta^{13}\text{C}$  (DeNiro & Epstein 1977) e recomenda-se então a remoção para reduzir a variabilidade causada por diferentes conteúdos lipídicos entre organismos ao aplicar isótopos estáveis em estudos sobre hábitos alimentares (Logan et al. 2008). No entanto, a extração química de lipídios tem um forte efeito indesejável nos valores de  $\delta^{15}\text{N}$  (Logan et al. 2008; Ryan et al. 2012). Portanto, recomenda-se a análise de amostras tratadas quimicamente para a interpretação dos valores de carbono, e a de amostras não tratadas para analisar os isótopos de nitrogênio em cada amostra individual

(Ryan et al. 2012). Devido ao nosso grande tamanho da amostra (277 amostras de pele de cetáceos oceânicos) a análise dupla seria inviável. Portanto, foram analisadas duplicatas de 16 amostras de pele de cetáceos, e aplicada uma correção matemática nos valores de  $\delta^{13}\text{C}$  das amostras remanescentes. Os lipídios foram extraídos usando um Soxhlet para enxaguar as amostras com uma solução de clorofórmio:metanol (2:1) por 24 h. Para o desenvolvimento dos *isoscapes* com os valores isotópicos dos organismos zooplânctônicos não foi feita a correção lipídica já que o carbono nos componentes lipídicos da dieta também é usado para sintetizar proteínas quando os animais estão se alimentando de uma dieta rica em lipídios (Wolf et al. 2015). Não foi aplicada correção lipídica aos valores de  $\delta^{13}\text{C}$  nas amostras de presas, uma vez que as mesmas apresentaram baixo conteúdo lipídico (C:N <3,5, Post et al. 2007).

Análises de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  na MOP foram realizadas no laboratório de *Analytical, Environmental and Geo-Chemistry* na Vrije Universiteit Brussel (AMGC, VUB, Bruxelas) com um Analisador Elementar (AE, EuroEA 3000, EuroVector, Milano, Itália) acoplado a um espectrômetro de massa (Isotope Ratio Mass Spectrometer, IRMS, Delta V plus, Thermo Electron Corporation). Aproximadamente 0,5-0,7 mg de cada amostra de zooplâncton, potenciais presas e cetáceos foi pesado em cápsulas de estanho e analisadas com um IRMS (Delta PlusXP, Thermofinnigan) acoplado a um AE (ECS 4010, Costech Analytical, Valência, CA) no *Stable Isotope Core Laboratory*, Washington State University. As composições isotópicas foram relatadas na notação convencional delta ( $\delta$ ) per mil (‰), relativa a Vienna Pee Dee Belemnite ( $\delta^{13}\text{C}$ ) e  $\text{N}_2$  atmosférico ( $\delta^{15}\text{N}$ ). Dois padrões laboratoriais, previamente calibrados para material de referência isotópico distribuído internacionalmente, foram analisados intercalados com as amostras para estimar a precisão do instrumento. O desvio padrão desses padrões foi  $\leq 0,1$  ‰ para  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$ .

### **Extração de aminoácidos e análise de $\delta^{15}\text{N}$**

Amostras de pele de 69 indivíduos das dez espécies de cetáceos oceânicos coletadas nesse estudo foram processadas para análise de  $\delta^{15}\text{N}$  em aminoácidos. O método para a extração de aminoácidos foi descrito em detalhes em Riekenberg et al. (em revisão). Aproximadamente 2-5 mg de cada amostra foi hidrolisada em ácido HCl 6 M aquecendo a amostra a 110°C durante 20 h para decompor as proteínas em aminoácidos individuais. As amostras hidrolisadas foram então derivatizadas para tornar os aminoácidos voláteis através da esterificação isopropílica aquecendo a amostra a 110°C por 2 h em 300  $\mu\text{l}$  de uma mistura de

isopropanol e cloreto de acetila (4:1), seguida da acilação com 300 µl de mistura de cloreto de trimetilacetilo e diclorometano (1:4) durante 2 h a 110°C. Entre cada um dos passos acima, as amostras foram evaporadas até à secura sob um vapor suave de N<sub>2</sub>. As amostras derivatizadas foram então analisadas em um GC-C-IRMS (Thermo Trace 1310) interligada a um Delta V Advantage IRMS através de um forno de combustão GC IsoLink II (Thermo Fisher Scientific), no *Royal Netherlands Institute for Sea Research* (NIOZ, Texel, Holanda).

## **Análise de dados**

Para a correção matemática da influência dos lipídios nas razões de carbono na pele dos cetáceos, foram utilizadas as diferenças entre os valores de  $\delta^{13}\text{C}$  entre as amostras tratadas e não-tratadas, e sua relação com as razões carbono-nitrogênio (C:N) para estimar os coeficientes específicos para a pele dos cetáceos e adaptar ao modelo linear descrito por Post et al. (2007):

$$\delta^{13}\text{C}_{\text{Normalizado}} = \delta^{13}\text{C}_{\text{medido}} + (-12.186 + 3.69 \times \text{C:N})$$

Todas as análises estatísticas e os mapas apresentados neste trabalho foram feitos utilizando a versão 3.6.1 do software livre R (R Core Team 2019).

No capítulo 1 foram testadas as diferenças nos valores médios isotópicos entre fêmeas e machos usando o teste de Welch, que não assume homocedasticidade das variâncias. Modelos aditivos generalizados (GAMs, Hastie e Tibshirani, 1990) foram ajustados para avaliar se o sexo, a idade e o comprimento total do corpo explicavam as mudanças nas razões isotópicas das toninhas. Modelos de mistura isotópicos (*Stable Isotope Analysis in R*, SIAR, Parnell et al. 2010) foram aplicados para estimar as contribuições das espécies de presas para a dieta das toninhas. Diferentes fatores de enriquecimento trófico foram testados e os resultados foram comparados usando o índice de coeficiente de Bhattacharyya para testar as diferenças nos resultados dos modelos e definir o melhor fator para as toninhas.

No capítulo 2 os valores de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  nas amostras de zooplâncton foram utilizados para desenvolver mapas isotópicos caracterizando os padrões espaço-temporais na base da cadeia alimentar ao longo da área de estudo. Diferentes mapas da distribuição do  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  foram criados, com os dados de zooplâncton misto (mistura) e de cada um dos principais grupos taxonômicos zooplânctônicos (anfípodos, quelognotos, copépodos, eufausiáceos e salpas). Os *isoscapes* foram gerados usando o software livre Ocean Data View (ODV) versão 5.1.5

(Schlitzer 2002). Dentro das duas regiões (sudeste e sul), a área foi dividida longitudinalmente entre a quebra de plataforma (*Shelf break* – SB) e a região mais oceânica (*Offshore* – OFF), resultando em quatro subáreas para comparar os padrões espaciais. Os padrões espaço-temporais nos valores isotópicos dos grupos zooplancônicos foram comparados estatisticamente com o Welch two sample t-test, e o Welch ANOVA, que não assume homogeneidade nas variâncias ou normalidade na distribuição dos dados. O teste post-hoc Games-Howell foi aplicado quando houve diferenças estatísticas entre os grupos (Ruxton & Beauchamp 2008). GAMs foram usados para descrever a relação latitudinal não-linear dos valores de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  nos organismos zooplancônicos, e os fatores aditivos incluindo o grupo taxonômico (anfípodos, copépodos, quetognatos, eufausiáceos, salpas, ou mistura), a estação do ano (outono ou primavera), a área (quebra de plataforma ou região mais oceânica), e ano (2012-2015).

No capítulo 3, as variações espaço-temporais nas diferentes espécies de cetáceos foram testadas comparando indivíduos da mesma espécie amostrados na primavera e outono, dentro de cada região (Sul ou Sudeste), ou dentro de cada estação do ano entre as regiões sudeste-sul. Assim, para espécies amostradas em ambas as regiões, mas apenas em uma estação (golfinhos-rotadores na primavera), ou aquelas amostradas em ambas as estações do ano, mas em apenas uma região (golfinhos-comum, cachalotes e golfinhos-de-dentes-rugosos no sul), utilizou-se o teste-*t* de Welch. Para as espécies amostradas em ambas as regiões e estações do ano (golfinhos-pintados-do-Atlântico e golfinhos-nariz-de-garrafa), utilizou-se a ANOVA fatorial. Neste caso, os fatores “estação do ano” e “região” tiveram dois níveis (outono *vs.* primavera e sul *vs.* sudeste, respectivamente), e a ANOVA foi usada para testar a significância de cada um dos fatores e suas interações. Adicionalmente, foi aplicada a análise de agrupamento hierárquico para medir o nível de dissimilaridades nos valores de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  dos cetáceos. Finalmente, o nicho isotópico das diferentes espécies de cetáceos foi estimado usando *Stable Isotope Bayesian Ellypses in R* (SIBER, Jackson et al. 2011) para avaliar o nível de sobreposição ou partição na utilização dos recursos entre elas, e as métricas de Layman a nível populacional foram usadas para se inferir sobre a diversidade de recursos utilizados, os níveis de redundância trófica e espaçamento entre os diferentes indivíduos de cada espécie (Layman et al. 2007; Jackson et al. 2012).

No capítulo 4 foi apresentado uma análise descritiva dos valores isotópicos dos diferentes componentes da cadeia alimentar (MOP, zooplâncton, grupos de presas e cetáceos) e seus padrões sazonais e latitudinais (sudeste-sul). A posição trófica das diferentes espécies

de cetáceos foi estimada usando os valores de  $\delta^{15}\text{N}$  em aminoácidos, e as diferenças entre os aminoácidos do tipo *trófico* e *fonte*. As métricas de comunidades (Layman et al. 2007) foram estimadas para cada região (sudeste-sul) em cada estação (outono-primavera) usando os valores de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  em todos os organismos coletados.

## SÍNTESE DOS RESULTADOS

O primeiro capítulo foi publicado no periódico *Marine Mammal Science*, com o título **Ontogenetic and sexual characterization of the feeding habits of franciscanas, *Pontoporia blainvillei*, based on tooth dentin carbon and nitrogen stable isotopes**. A análise dos dados demonstrou que não houve diferença significativa nos valores de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  entre fêmeas e machos de *P. blainvillei*, indicando que não há diferença na posição trófica e no consumo de presas entre os sexos. Além disso, os valores isotópicos sugeriram que adultos de diferentes faixas etárias consomem espécies de presas semelhantes. Os valores de  $\delta^{15}\text{N}$  nos filhotes e juvenis foram cerca de 2‰ mais altos que nos adultos. Tal diferença foi atribuída à amamentação, pois os animais não desmamados estão se alimentando em um nível trófico relativamente mais alto que fêmeas adultas. Os resultados dos modelos de mistura isotópica diferiram de estudos anteriores com análise de conteúdo estomacal (ACE), e a contribuição das lulas na dieta das toninhas foi menor que a sugerida pela ACE. Modelos de mistura com fatores de enriquecimento trófico relativamente mais altos para carbono (4,8‰-5,4‰) estimaram maior importância de *Cynoscion guatucupa* na alimentação das toninhas adultas, proporcionando resultados mais próximos àqueles com base em ACE. Comparando os resultados dos diferentes modelos de mistura, foi recomendado um fator de enriquecimento trófico entre presas-dentes de 4,8‰-5,4‰ para carbono e 2,0‰-2,4‰ para nitrogênio quando se utilizam isótopos estáveis e modelos de mistura isotópica para estimar a dieta das toninhas. Neste estudo, foi aceita a primeira hipótese desta tese, já que as espécies de presas mais importantes para a dieta foram aquelas relacionadas a ambientes demersais e costeiros.

No capítulo 2, intitulado **Zooplankton-based  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isoscapes from the outer continental shelf and slope in the subtropical western South Atlantic**, foram produzidos mapas isotópicos descrevendo os padrões espaço-temporais na base da teia trófica da PCE e talude no sudeste-sul do Brasil, através dos valores de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  das amostras de zooplâncton coletados ao longo da região. Os grupos zooplanctônicos apresentaram padrões latitudinais

(sudeste-sul), longitudinais (quebra de plataforma-oceano), e sazonais semelhantes. Valores mais altos de  $\delta^{13}\text{C}$  foram observados no outono, enquanto os de  $\delta^{15}\text{N}$  foram mais altos na primavera.  $\delta^{13}\text{C}$  foi relativamente mais baixo na região de PCE e quebra de plataforma, enquanto as águas mais oceânicas foram mais enriquecidas em  $^{13}\text{C}$ . Além disso, houve uma diminuição latitudinal (sudeste-sul) nos valores de  $\delta^{13}\text{C}$  ao longo da região oceânica. No entanto, na região de quebra de plataforma tal padrão não foi observado. Por outro lado,  $\delta^{15}\text{N}$  foi significativamente mais baixo na área oceânica do que na quebra de plataforma, e valores mais altos foram encontrados na região sul em ambas áreas de quebra de plataforma e oceânica. O enriquecimento de  $^{15}\text{N}$  bem como valores deplecionados em  $^{13}\text{C}$  ao longo da quebra de plataforma refletem a ascensão de águas profundas, trazendo nutrientes do tipo  $\text{NO}_3^-$  que é enriquecido em  $^{15}\text{N}$ , assim como carbono inorgânico dissolvido, que é mais enriquecido no carbono leve ( $^{12}\text{C}$ ). Além disso, valores mais altos de  $\delta^{15}\text{N}$  na região sul coincidem com a maior disponibilidade de nutrientes, sobretudo nitrato, contrastando com a região sudeste que, com grandes afloramentos de *Trichodesmium* capazes de assimilar  $\text{N}_2$  em águas oligotróficas, apresentam  $\delta^{15}\text{N}$  mais baixo. Portanto, foi aceita a segunda hipótese desta tese, de que os gradientes físicos e químicos latitudinais, longitudinais e sazonais, que resultam das diferentes massas d'água que influenciam a região, seriam refletidos nas razões isotópicas dos organismos que compõem a base dessas cadeias alimentares.

No capítulo 3, sob o título **The isotopic niche area, overlap and partition amongst odontocete species from the western South Atlantic**, os padrões latitudinais e sazonais observados nas espécies de cetáceos amostradas foram diretamente associados aos padrões isotópicos de suas respectivas regiões de ocorrência. Espécies amostradas na região sul apresentaram valores mais altos de  $\delta^{15}\text{N}$  que as espécies do sudeste. As espécies encontradas somente na região sul apresentaram variações sazonais nos isótopos de carbono e de nitrogênio, enquanto as da região sudeste, com exceção dos golfinhos-rotadores (*Stenella longirostris*), não apresentaram tais gradientes sazonais. Assim, os valores isotópicos dos cetáceos ajudaram a corroborar a hipótese 2, já que os cetáceos da região sudeste não apresentaram variabilidade sazonal nos valores isotópicos, enquanto as espécies da região sul, que é hidrograficamente mais dinâmica, apresentaram tal sazonalidade. Além disso, foi confirmada a hipótese 3 de que as variações espaço-temporais encontradas na base das teias tróficas locais seriam refletidas nas razões isotópicas dos cetáceos, resultando em espécies relacionadas a região sul (com maior disponibilidade de nutrientes) com valores mais altos de  $\delta^{15}\text{N}$  que as da região sudeste (mais oligotrófica).

Os golfinhos-de-dentes-rugosos (*Steno bredanensis*) apresentaram os valores mais altos de  $\delta^{15}\text{N}$  e  $\delta^{13}\text{C}$ , indicando alta posição trófica (no entanto, ver capítulo 4) e ocorrência nas águas neríticas do sul do Brasil. As variações sazonais na amplitude do carbono observada nos cachalotes (*Physeter macrocephalus*) sugerem que há diferença sazonal na amplitude de uso de recursos, mas que os mesmos se alimentam em níveis tróficos similares ao longo do ano. Os golfinhos-rotadores apresentaram os menores valores de  $\delta^{15}\text{N}$  e  $\delta^{13}\text{C}$ , indicando que ocupam uma posição trófica relativamente baixa entre as espécies de cetáceos co-ocorrentes. Além disso, a alta sobreposição de nicho observada entre os golfinhos-rotadores e os golfinhos-pintados-pantropicais (*Stenella attenuata*) na primavera indica que eles competem por recursos similares, e os movimentos sazonais para a região sul observados nos golfinhos-rotadores podem ser uma estratégia para minimizar a competição. O golfinho-comum (*Delphinus delphis*) também apresentou valores altos de  $\delta^{15}\text{N}$  e  $\delta^{13}\text{C}$ , refletindo a sua ocorrência na região sul da área de estudo, que é mais enriquecida no  $^{15}\text{N}$ . Os valores relativamente baixos no  $\delta^{15}\text{N}$  observados nas orcas (*Orcinus orca*) indicam que o ecótipo amostrado no presente estudo se alimenta de níveis tróficos mais baixos. O golfinho-de-Clymene (*Stenela clymene*) apresentou um baixo valor de  $\delta^{15}\text{N}$  indicando que ele ocupa um nível trófico mais baixo, enquanto seu alto  $\delta^{13}\text{C}$  foi relacionado a águas de baixas latitudes em que habita.

Os resultados apresentados neste estudo forneceram suporte isotópico para sugerir a existência de pelo menos duas subpopulações de golfinhos-pintados-do-Atlântico (*Stenella frontalis*), diferindo entre aqueles amostrados na região sudeste e na região sul da área de estudo. A grande sobreposição de nicho isotópico entre os golfinhos-pintados-do-Atlântico da região sul com os golfinhos-comum sugere que indivíduos dessas duas espécies ocupam um nicho ecológico semelhante principalmente nos meses de inverno/primavera, e que, portanto, se sobrepõem na utilização dos recursos. Esse resultado foi de encontro a hipótese 4 desta tese, já que essas espécies, apesar de apresentarem alta sobreposição de nicho isotópico, apresentam alta segregação espacial em suas áreas de ocorrência. Portanto, a segregação espaço-temporal no uso do habitat, observada em estudos anteriores, pode ser uma estratégia para minimizar a competição. No entanto, outras espécies que se sobrepõem nas suas áreas de ocorrência ou que foram frequentemente observadas em grupos mistos apresentaram razões isotópicas semelhantes e alta sobreposição de nicho: os golfinhos-nariz-de-garrafa (*Tursiops truncatus*) com os golfinhos-pintados-do-Atlântico ou com as baleias-piloto (*Globicephala melas*); e os golfinhos-rotadores com os golfinhos-pintados-pantropicais. Portanto, aceitou-se as hipóteses desta tese de que espécies com alta sobreposição espacial (hipótese 4) e as que são comumente

registradas em associações multiespecíficas (hipótese 5) apresentariam maior similaridade e alta sobreposição de nicho isotópico.

Devido a diferença no número amostral entre espécies conhecidamente generalistas (golfinho-nariz-de-garrafa) e as especialistas (cachalotes), não foi possível testar a hipótese 6. No entanto, o amplo nicho isotópico apresentado pelos golfinhos-nariz-de-garrafa corrobora o seu hábito alimentar generalista. Além disso, os golfinhos-comuns e golfinhos-pintados-do-Atlântico da região sul também apresentaram um amplo nicho isotópico, sugerindo que essas espécies também apresentam uma dieta diversificada. Por outro lado, os golfinhos-pintados-pantropicais, apesar do baixo número amostral, apresentaram uma área de nicho isotópica tão ampla quanto a dos golfinhos-rotadores, que tinham um número amostral consideravelmente maior. Além disso, de forma geral, os cetáceos amostrados na primavera apresentaram áreas de nicho isotópico mais amplas que os amostrados nos meses de outono, sugerindo uma sazonalidade na amplitude da utilização dos recursos, que podem estar relacionadas a diferença sazonal na disponibilidade das presas na região.

No capítulo 4, intitulado **From bottom to top: The trophic structure of the South Atlantic pelagic system using bulk and compound specific stable isotopes**, foi observado que ambos  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  aumentaram do zooplâncton aos cetáceos e peixes cartilagosos. O  $\delta^{15}\text{N}$  foi mais alto na MOP do que nos grupos zooplânctônicos, o que provavelmente reflete a diferença na concentração de nutrientes e na taxa de utilização de nitrogênio pelos produtores primários ao longo da coluna de água, já que as amostras de zooplâncton e MOP foram coletadas em diferentes profundidades, e essas variáveis afetam as taxas de fracionamento fitoplanctônico. A grande variabilidade observada em  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  dentro dos diferentes grupos de peixes e cefalópodes reflete os diferentes níveis tróficos e a gama de recursos utilizados pelas diferentes espécies que compuseram cada grupo. Os cachalotes apresentaram os níveis tróficos (NT) mais altos entre as diferentes espécies de cetáceos ( $n = 6$ , NT = 3,5-3,9), enquanto as orcas tiveram uma das posições tróficas mais baixas ( $n = 3$ , NT = 2,8-3,2). Posições tróficas semelhantes (3,2-3,7) foram observadas nos golfinhos-nariz-de-garrafa ( $n = 17$ ), golfinhos-pintados-do-Atlântico ( $n = 16$ ) e golfinhos-comuns ( $n = 10$ ). A análise isotópica em aminoácidos permitiu identificar que os golfinhos-de-dentes-rugosos ( $n = 5$ ) ocorrem nas águas mais enriquecidas em  $^{15}\text{N}$  da plataforma continental no sul do Brasil, e que sua posição trófica (3,1-3,5) não é significativamente maior que a das outras espécies de cetáceos da região sul. Por fim, as métricas para toda a comunidade diferiram entre as regiões sudeste e sul, bem como

sazonalmente em cada região. Os padrões de nicho observados nessas áreas foram associados aos diferentes padrões de água que influenciam cada região.

## CONCLUSÕES

No presente estudo foram analisados os valores isotópicos de carbono e nitrogênio em cetáceos e suas (potenciais) presas para fazer inferências sobre as diferenças sexuais e ontogenéticas nos hábitos alimentares de uma espécie de cetáceo costeiro, e aprender sobre as interações tróficas a nível intra- e interespecífico entre as populações de cetáceos oceânicas no sudeste-sul do Brasil. Foi também apresentado pela primeira vez uma descrição detalhada, com alta resolução espacial e temporal dos padrões dos isótopos estáveis de carbono e nitrogênio para a base das cadeias tróficas nas águas oceânicas do Atlântico Sul Ocidental. Foi demonstrado que informações sobre  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  na base de teias alimentares e seus padrões regionais e sazonais fornecem uma base importante para a interpretação dos valores isotópicos de espécies de consumidores, possibilitando uma avaliação mais detalhada sobre a ecologia e as relações tróficas entre as espécies de cetáceos que ocorrem nesta região. Além disso, através da análise das razões isotópicas nas amostras de pele de cetáceos foi possível, pela primeira vez, fazer inferências sobre os padrões espaço-temporais na ecologia e interações tróficas dessas diferentes espécies que habitam o ambiente oceânico das regiões sudeste e sul do Brasil.

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## **CAPÍTULO 1**

### **Ontogenetic and sexual characterization of the feeding habits of franciscanas, *Pontoporia blainvillei*, based on tooth dentin carbon and nitrogen stable isotopes**

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**Ontogenetic and sexual characterization of the feeding habits of franciscanas, *Pontoporia blainvillei*, based on tooth dentin carbon and nitrogen stable isotopes**

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**Abstract**

We assessed the feeding habits of franciscanas (*Pontoporia blainvillei*) from southern Brazil through carbon and nitrogen stable isotopes measured in the tooth dentin of dolphins from different age classes. Generalized additive models were fitted to assess whether sex, age, and total body length explained changes in franciscanas isotope ratios. Bayesian isotope mixing models were applied to estimate the contributions of prey items, selected based on stomach content analysis (SCA), to franciscanas' diet. Different Trophic Enrichment Factors (TEF) were applied and the results were compared using Bhattachayya's coefficient index to test for differences in model outputs and to define the best TEF for franciscanas. We found different relative importance of the main prey between the methods, and squids' contribution could be lower than suggested by SCA. Since the dentin deposited during nursing seems to influence the isotopic ratios of juveniles, caution is recommended when interpreting dietary estimates based on whole tooth samples. This study is the first to characterize ontogenetic shifts in franciscanas' feeding habits using SIA and testing different TEF values. SIA and SCA complement each other in dietary studies, mitigating potential biases resulting from the use of each method separately.

**Key words:** franciscanas, *Pontoporia blainvillei*, stable isotopes, feeding habits, mixing models.

## INTRODUCTION

The franciscana, *Pontoporia blainvillei*, is a small dolphin endemic to Brazil, Uruguay, and Argentina. Its distribution in shallow coastal waters results in a high spatial overlap with human activities, particularly fisheries (Secchi 2010*a, b*), and as a result entanglement in nets is the main threat to the species (Secchi 2010*b*). In southern Brazil, high fishing related mortality has occurred since the early 1980s (Secchi *et al.* 2003*a*) with several hundreds to a few thousands franciscanas having been killed annually in coastal gillnet entanglements (Prado *et al.* 2013). The abundance of the population inhabiting southern Brazil and Uruguay (Secchi *et al.* 2001, Danilewicz *et al.* 2010) cannot sustain this high mortality (Secchi 2006), and the International Union for the Conservation of Nature has classified the species as Vulnerable in its Red List (Reeves *et al.* 2008).

In southern Brazil, the species' feeding habits have been studied through the analysis of stomach contents from stranded and bycaught animals (Pinedo 1982, Bassoi 2005). Identified food items revealed that diet of franciscana is mainly composed of juvenile demersal fishes and cephalopods, with higher importance of crustaceans for juveniles than for adults (for a review see Danilewicz *et al.* 2002, Rodríguez *et al.* 2002). Nevertheless, information based on stomach content can be biased toward the most recent feeding event, referring to short-term diets and overestimating the importance of slowly digested prey (Pierce and Boyle 1991, Sekiguchi and Best 1997, Bowen and Iverson 2013).

Alternatively, nitrogen and carbon stable isotopes have been used frequently in ecological studies examining diet and trophic level of a wide variety of organisms, including marine mammals (Newsome *et al.* 2010). This is because the isotopic composition of an organism's tissue reflects that of its food source and the environment where it feeds (Graham *et al.* 2010). There is a distinction in  $^{13}\text{C}/^{12}\text{C}$  ratios in organisms inhabiting, for instance, coastal or oceanic, benthic, or pelagic ecosystems, as well as a latitudinal gradient in their  $\delta^{13}\text{C}$  values due to discriminations taking place at the base of the food webs according to several abiotic factors, such as temperature, light, and aqueous  $\text{CO}_2$  concentrations, as well as the phytoplankton size, specific composition, and growth rates (Rau *et al.* 1982, 1992; Laws *et al.* 1995; Popp *et al.* 1998). Moreover, there is a trophic level enrichment in predators'  $\delta^{13}\text{C}$  (ca. 1‰) and  $\delta^{15}\text{N}$  (2-5‰) values in relation to their prey, believed to result from a preferred excretion of the lighter isotope, and consequent accumulation of the heavy, during the conversion of dietary protein into the consumers' tissues (assimilation) and during respiration

(DeNiro and Epstein 1978, 1981; Minagawa and Wada 1984; Peterson and Fry 1987). Therefore, carbon isotopes are largely used to evaluate the basal carbon sources of the food web that supports an organism while nitrogen is used to estimate its trophic position (Peterson and Fry 1987, Post 2002).

Furthermore, stable isotope ratios in different tissues from a single individual can provide dietary information over different time-scales, depending on the tissue-specific isotopic rate of turnover (Tieszen *et al.* 1983, Hobson *et al.* 1996). Because dentin in cetaceans is deposited throughout the animals' life, long-term diet can be inferred using the whole tooth (Walker and Macko 1999). Since  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values are homogeneous among different teeth of an individual, a single tooth reflects the dental isotopic signature of a specimen (Walker and Macko 1999). However, whole tooth powder provides isotopic information representing the average signal of a life time. Therefore, the use of the whole tooth isotope values could be complicated by the pattern of deposition of the Growth Layer Groups (GLGs; Perrin and Myrick 1980) in the dentin. When born, odontocetes' teeth are made up of the enamel and one GLG deposited in the dentin during the prenatal period (the prenatal dentin), while the pulp cavity is yet to be fulfilled. Afterwards, there is an annual increment, from the prenatal dentin (outside) filling up the pulp cavity with the next GLGs (Hohn 1990, Rommel 1990). In franciscanas, the first GLG is significantly larger than the subsequent layers, with the latter becoming increasingly narrower with age (Pinedo and Hohn 2000).

Isotope mixing models have often been applied in dietary studies of marine mammals to estimate the relative importance of each prey species in their diet (*e.g.*, Fernández *et al.* 2011, Ryan *et al.* 2014), using information on the consumers and prey isotopic compositions, as well as a factor of enrichment per trophic level, or Trophic Enrichment Factor (TEF), for carbon ( $\Delta^{13}\text{C}$ ) and for nitrogen ( $\Delta^{15}\text{N}$ ) (Parnell *et al.* 2013). Nevertheless,  $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$  can vary considerably depending on the consumers' taxonomic classes, the tissue analyzed, and the isotopic values of the diet (Caut *et al.* 2009, DeMots *et al.* 2010). Moreover, estimates using this method are very sensitive to variations in  $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$  (Bond and Diamond 2011). The use of non-appropriate TEFs significantly affect model outputs and could result in unrealistic estimates of prey importance (Bond and Diamond 2011). Therefore, applying franciscanas-specific diet-to-tissue enrichment factor is crucial for the correct dietary estimations based on stable isotope analysis using isotope mixing models.

The present study aims to assess in franciscana from southern Brazil whether there is resource segregation by sex, body length, or age and whether ontogenetic shifts in their feeding habits occur. Carbon and nitrogen stable isotopes were measured in the teeth of different age classes of franciscanas entangled in fishing nets along the coast of southern Brazil. The isotopic ratios of prey species known to be important food items in the diet of these dolphins were also analyzed. Isotopic mixing models were implemented to estimate the proportional contribution of prey items to each ontogenetic class and models outputs were compared in order to define the most suitable range of  $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$  that can best be applied to franciscana dolphins.

## **METHODS**

### *Sample Collection*

Samples were collected from 125 franciscanas incidentally killed in fishing nets set off Rio Grande do Sul (RS) State, southern Brazil, between 1994 and 2010. This area is part of the Franciscana Management Area-FMA III (*sensu* Secchi *et al.* 2003b), which includes animals from Uruguay. Total body length (TBL) was measured as a straight line from the tip of the rostrum to the fluke notch and sex was determined by external examination. Teeth were collected, cleaned with distilled water, and stored dry for age estimation and for carbon and nitrogen stable isotope measurements.

### *Age estimation*

The age of each individual was estimated by counting the Growth Layer Groups (GLGs; Perrin and Myrick 1980) in the dentin and cementum of thin (25  $\mu\text{m}$ ), mid-longitudinal sections of teeth. The method included the fixation of the whole tooth in 10% formalin and afterward decalcifying the tooth with RDO® (a commercial mixture of acids), sectioning it into thin slices with a freezing microtome, staining with Mayer's hematoxylin, "blued" in ammonia, and mounting the section on microscope slides with 100% glycerin (Pinedo and Hohn 2000). GLGs were counted by two independent readers using an optical microscope (40X and 100X), with no access to the specimens' biological information. Each GLG was considered as representing one year of age based on previous work (Kasuya and Brownell 1979, Pinedo and Hohn 2000).

## *Stable Isotope Analysis*

A clean tooth of each franciscana was crushed into powder, acidified with 30% hydrochloric acid (HCl) to remove the inorganic carbon and dried at 60°C for 3 h (*e.g.*, Walker *et al.* 1999, Knoff *et al.* 2008). Approximately 2 mg of the tooth powder was then put into tin capsules. Isotope analyses were performed at the Stable Isotope Core Laboratory at the Washington State University, with an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA) connected to an Isotope Ratio Mass Spectrometer (Delta PlusXP, Thermofinnigan, Bremen). Stable isotope ratios, expressed in delta ( $\delta$ ) notation and defined as the part per thousand (‰) deviations from the predefined international reference materials, were calculated as:  $\delta_{\text{sample}}(\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}]*1000$ , where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  ratios of the sample and standard, respectively. The standard for carbon is Vienna Pee Dee Belemnite (VPDB) and for nitrogen is atmospheric nitrogen. The within-run analytical precision (standard deviation) of two internal lab standards analyzed after every 12 samples was less than 0.1‰ for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

## *Ontogenetic shifts in diet*

### Statistical analyses

Welch test was performed to test for differences in the mean isotopic values between females and males, not assuming equal variances (Ruxton 2006, Rasch *et al.* 2011). Generalized Additive Models (GAMs) were fitted in order to identify whether there is annual variability in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, and to evaluate whether there is sex-related resource partitioning, and/or ontogenetic shifts in the diet of these dolphins. We first tested for collinearity among the explanatory variables. Age and TBL were correlated ( $r = 0.73$ ,  $P < 0.001$ ), therefore GAMs were performed independently for each one of them. Age, TBL, and year were fitted as continuous variable, whereas sex was fitted as categorical. There is a positive relationship between the mean and variance, therefore a Gamma distribution with log link function was applied to both models with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Since our data include the whole tooth, which represents the average dentin of the specimen's entire life (*e.g.*, from zero to 12 yr in the case of the oldest individual), we initially ran a model with isotopic values as a function of the age, with year and decades (because of the difference in  $n$  across the years, 1994-1999 and 2000-2006, see supplementary material, Table S1) as factors. Given that the model outputs were nonsignificant, and age within the year does not influence the year average isotopic values, we ran two full models for carbon and for nitrogen including all variables (age

or TBL, sex and year). As carbon values are negative and a Gamma family requires positive data values, a transformation was applied to the  $\delta^{13}\text{C}$  values, which were multiplied by -1. GAMs were fitted using backward selection. At each step, the least significant variable was excluded from the model until all remaining variables were significant. Residuals were then checked for normality and to ensure that there were no residual patterns. Model selection was based on the Akaike's information criterion (AIC) and the difference between the  $i^{\text{th}}$  model and the model with the lowest AIC value ( $\Delta\text{AIC}$ ). Finally, significant models with  $\Delta\text{AIC} \leq 2$  were considered suitable to explain the variation in the response variables. All statistical tests and models were performed with a significance level of 0.05, using the free software R v.3.1.2 (R Development Core Team 2014). GAMs were fitted using the *mgcv* package in R (Wood 2011).

In order to understand the change-point in carbon and nitrogen isotope ratios along age categories, the depletion or enrichment in the heavy isotope with age in relation to the mean isotopic values of zero-year-old animals were calculated as the difference of mean values of all specimens comprising the  $i^{\text{th}}$  age and the mean values of zero-year-old individuals:  $\bar{x}\delta^{\text{H}}\text{X}_i - \bar{x}\delta^{\text{H}}\text{X}_{\text{zero}}$ ; where  $\bar{x}\delta^{\text{H}}\text{X}$  is the average delta values for N or C isotope of the  $i^{\text{th}}$  age and zero years old, respectively.

### *Dietary proportions of prey species*

#### Prey samples

The Index of Relative Importance (IRI) of franciscanas' prey identified in previous studies (Basso 2005) was used to select the prey that were considered for stable isotope analysis (see supplementary material, Table S2). Species of fish and squid representing around 95% of the total IRI were obtained from fisheries operating off southern Brazil coast in June 2012 and March 2013. Because there could be a relationship between fish length and their average isotopic values (Jennings *et al.* 2002, Nagata *et al.* 2015), prey samples were collected taking into account the average prey size consumed by the dolphins, according to Basso (2005). Prey samples were stored at  $-20^{\circ}\text{C}$  until preparation for isotope analysis. No lipid correction was applied to  $\delta^{13}\text{C}$  values since prey muscle had low lipid content (C:N < 3.5, Post *et al.* 2007). While preparing the samples, the muscle tissues were first rinsed with distilled water in order to remove any external contamination. The samples were then placed in Petri dishes and dried in an oven at  $60^{\circ}\text{C}$  for 48 h. The dried tissues were ground to a fine powder, weighed inside tin capsules, and their  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were determined.

## Statistical analyses and SIAR

Differences in the isotopic composition of prey species were assessed using nonparametric Kruskal–Wallis ( $H$  test) followed by pairwise comparisons between groups. The relative contribution of each food item to each ontogenetic class was estimated using Stable Isotope Analysis in R (SIAR, Parnell *et al.* 2010). SIAR uses robust probability estimates to assess the distributions of source contributions to the consumer's assimilated diet, accounting for uncertainty in the input parameters, such as isotopic variation of the different dietary sources and trophic discrimination factors (Parnell *et al.* 2010). Ontogenetic classes were defined according to biological information for the population comprising area FMA III (Danilewicz 2003, Danilewicz *et al.* 2004): 1) calves ( $n = 19$ ): zero-year-old and most likely nursing; 2) juveniles ( $n = 33$ ): one- and two-year-old and sexually immature; 3) first adults ( $n = 48$ ): three to five-year-old, sexually mature or in the process of maturation; and 4) adults ( $n = 25$ ): six-years or older and sexually/physically mature individuals. Calves were assumed not to consume solid food and hence were excluded from the SIAR analysis.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  mean and standard deviation of each prey type were used to quantify the dietary proportion in the dolphins' diet. SIARs were run with different Trophic Enrichment Factors (TEF):  $\Delta^{13}\text{C} = 4\text{--}5\text{‰}$  ( $\pm 0.5$  SD) according to reported muscle-to-collagen fractionation (Koch 2007), and  $\Delta^{15}\text{N} = 2\text{--}3.4\text{‰}$  ( $\pm 0.5$  SD) (Minagawa and Wada 1984, Newsome *et al.* 2010). Finally, we calculated the Bhattacharyya's coefficient (BC) for all the iterations of each pairwise comparison in every two models. BC is an index of divergence between two probability distributions (Rauber *et al.* 2008), and was applied to indicate the level of overlap among the models with different TEFs. BC values lie between zero and one, when  $\text{BC} = 0$ , there is complete dissimilarity and when  $\text{BC} = 1$ , complete similarity between distributions (Kailath 1967). Models with  $\text{BC} > 0.6$  were considered to have significant overlap (*e.g.*, Bond and Diamond 2011).

## RESULTS

### *Ontogenetic analysis*

Tooth dentin isotope values ranged from  $-13.1$  to  $-9.6\text{‰}$  for  $\delta^{13}\text{C}$  and from  $16.5$  to  $21.7\text{‰}$  for  $\delta^{15}\text{N}$ . No significant differences between females ( $n = 60$ ) and males ( $n = 65$ ) in  $\delta^{15}\text{N}$  ( $\bar{x}_{\text{females}} = 19.07$ ,  $\text{var}_{\text{females}} = 1.147$ ,  $\bar{x}_{\text{males}} = 19.09$ ,  $\text{var}_{\text{males}} = 1.161$ ,  $t\text{-test} = -0.127$ ,  $df = 122.3$ ,  $P = 0.89$ ) or in  $\delta^{13}\text{C}$  values ( $\bar{x}_{\text{females}} = -10.89$ ,  $\text{var}_{\text{females}} = 0.418$ ,  $\bar{x}_{\text{males}} = -10.92$ ,  $\text{var}_{\text{males}} = 0.484$ ,  $t\text{-test} = 0.2239$ ,  $df = 122.9$ ,  $P = 0.82$ ) were observed. The youngest animals collected were zero-year-old and were encountered within both sexes, while the oldest were 11-year-old

females ( $n = 3$ ) and a single 12-year-old male. Females' TBL ranged from 85 to 171 cm, whereas the males measured between 76 and 147 cm. Details on the number of specimens per ontogenetic class and the average delta values can be seen in Table 1.

#### Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in relation to age and TBL

Backward selection indicated that the GAM model that best explained the variation in  $\delta^{15}\text{N}$  included the effect of TBL (smoother with effective degrees of freedom (edf) = 6.04, adjusted  $R^2 = 0.474$ ,  $P < 0.001$ ) and sex (as factor,  $P < 0.05$ ). The model explained 50.3% of the deviance (Table 2a). The effect of year was nonsignificant and therefore not included in the best model (Table 3). The smoother fitted to describe the effect of TBL suggested that the highest  $\delta^{15}\text{N}$  values are observed in smaller (thus younger) franciscanas (Fig. 1). The model for  $\delta^{15}\text{N}$  with age (instead of TBL) also had a significant smoother for age (edf = 2.77, adjusted  $R^2 = 0.45$ ,  $P < 0.001$  and 46% deviance explained), with its  $\Delta\text{AIC}$  only 1.8 units higher than the model with TBL, being therefore within the best candidate models (Table 3). The best model for  $\delta^{13}\text{C}$  included only the effect of age (smoother with edf = 1.176,  $P < 0.05$ , Table 2b), and more depleted  $^{13}\text{C}$  values were observed in juveniles. Nevertheless, the very low explained deviance (5.07%) and adjusted  $R^2$  (0.04) indicated that overall little variation in  $\delta^{13}\text{C}$  values in franciscanas' teeth was explained by the model. Finally, variables such as TBL, sex, or year were not significant in any of the GAM models (Table 3).

Depletion in  $^{15}\text{N}$  between calves and juveniles were small (approximately 0.5‰). The major shift in  $\delta^{15}\text{N}$  was observed for four-year-old animals (-1.9‰) and nitrogen isotopic ratios were constant in older specimens (Fig. 2a). There was a slight enrichment in  $\delta^{13}\text{C}$  values in all ages in relation to calves, yet these variations were no higher than 1.0‰ (Fig. 2b).

#### *Dietary proportions*

##### Prey sources

Due to the lack of statistical differences in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values among some of the prey species, fish species were grouped according to the similarity in their isotope signals, taking into account their habitat preferences. Averaged  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the prey groups and franciscana dolphins per ontogenetic class are plotted in Fig. 3. Significant differences in  $\delta^{13}\text{C}$  values were observed only between coastal-estuarine fish (CEs— banded croaker, *Paralichthys brasiliensis*) and the other two fish groups: benthopelagic - BP (stripped

weakfish, *Cynoscion guatucupa* and cutlassfish, *Trichiurus lepturus*) ( $P < 0.05$ ) and shelf-pelagic fish - SP (argentine anchovy, *Engraulis anchoita* and marini's anchovy, *Anchoa marinii*) ( $P < 0.001$ ). Shrimp (argentine red shrimp, *Pleoticus muelleri* and argentine stiletto shrimp, *Artemesia longinaris*) also differed from SP fish in  $\delta^{13}\text{C}$  ( $P < 0.001$ ), and was the only group that differed from the others in  $\delta^{15}\text{N}$  values ( $P < 0.05$ ). Although non-significant,  $\delta^{15}\text{N}$  values were slightly higher in squid (common long-finned squid, *Loligo sanpaulensis*) than in the other prey groups.

#### SIAR and Bhattacharyya's Coefficient (BC) for comparison of models outputs

As no differences were found in isotopic compositions between males and females, data were clumped to run the models for both sexes together. SIARs models (1-8) were run with the following trophic enrichment factors (TEF) for carbon ( $\Delta^{13}\text{C}$ ) and nitrogen ( $\Delta^{15}\text{N}$ ), in that order: 4.0‰ and 2.0‰; 4.0‰ and 3.4‰; 5.0‰ and 2.0‰; 5.0‰ and 3.4‰; 5.4‰ and 2.2‰; 5.2‰ and 2.4‰; 4.8‰ and 2.2‰; and lastly 5.0‰ and 2.2‰ (Table 4). Pairwise BC showed the small differences in models output throughout the range of TEF used (Table 4). Considering that there could be differences in TEF and in prey proportions among the age categories, SIAR models, as well as the BC values were calculated for each age category independently: juveniles, first adults, and adults. Models with the lowest  $\Delta^{13}\text{C}$  (models 1 and 2, see Table 4) differed from those with the highest TEF for the same isotope (model 5 and 6,  $\text{BC} < 0.6$  for all pairwise comparisons, Table 4) mainly in the runs to estimate the proportional contribution of prey to the diet of juveniles. Likewise, for first adults, models 1 and 2 outcomes differed significantly only from model 5, the model with the highest  $\Delta^{13}\text{C}$  ( $5.4 \pm 0.5\text{‰}$ ), indicating again that  $\Delta^{13}\text{C}$  affected the models outcome. In adults, BC values from all models comparisons were  $>0.7$ , indicating that differences in TEF did not affect the models outcome. Nevertheless, the estimated proportional contribution of prey, particularly squid and shrimp, slightly varied across the models (Fig. 4).

The mean proportional contributions of each prey group to the diet of franciscanas within each age categories as predicted by the models (1-8) are shown in Fig. 4. Both models 1 and 2, with the lowest  $\Delta^{13}\text{C}$  ( $4.0 \pm 0.5\text{‰}$ ), estimated a large contribution of coastal-estuarine (CEs) fish to the diet of juveniles (82% and 79%, model 1 and model 2, respectively, Fig. 4). The remaining prey had very low proportional contributions estimated (between 2% and 4% among BP, SP fish and shrimp, whereas 9%-13% for squids, Fig. 4). The other models, with

carbon TEF ranging between 4.8‰ and 5.4‰ estimated highest contributions of squid (between 44% and 84%, Fig. 4), followed by CEs fish (between 5% and 33%, Fig. 4), while very low estimated contributions for the other prey types, with small variation among the models (Fig. 4). In the first adults category, models 1 and 2 estimated a large proportional contribution of CEs fish (93% and 82%, model 1 and 2, respectively). In model 1 runs, the remaining prey had considerably low estimated proportional contributions (between 1% and 3%, Fig. 4), whereas in model 2 the second most important prey was shrimp, with an estimated contribution of 14% (Fig. 4). Moreover, models 3-8 estimated highest importance of both CEs fish and squid, while the other prey types presented very low contributions (except shrimp in model 4 outcome, with 28% mean contribution, Fig. 4). Concerning the SIAR runs to estimate the diet of adult franciscanas, in general, the models agreed that CEs fish was a prey type with very high proportional contributions (from 26% to 82%, Fig. 4), followed by squid. However, models 2 and 4, those with high  $\Delta^{15}\text{N}$  ( $3.4 \pm 0.5\text{‰}$ ), estimated the highest proportional contributions to be from shrimp and CEs fish (Fig. 4). Moreover, regarding adults' SIAR estimates, models with  $\Delta^{13}\text{C}$  ranging between 5.2‰-5.4‰ and  $\Delta^{15}\text{N}$  between 2.2‰-2.4‰ had the highest estimated contributions of BP fish (18% and 20%), high estimates of squid (26%-32%), and high estimates for CEs fish (26%-33%). Although these models predicted high contributions of CEs fish, their predictions were the most similar to estimates obtained by SCA.

## DISCUSSION

### *Sex-related foraging habits*

We found no evidence for sex-related resource partitioning based on mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values. This corroborates studies using analysis of stomach content for animals in the same region (Pinedo 1982, Bassoi 2005). Those authors concluded that individuals of both sexes feed upon the same prey species, and the main differences observed were related to prey size and amount consumed by juveniles and adults. In contrast, our models for  $\delta^{15}\text{N}$  suggest an effect of total body length and sex. This species presents sexual dimorphism, with females being larger than males (Secchi 2010a). It is likely that, within the same size range, the models compared females and males from different age categories (*e.g.*, juveniles vs adults) thereby resulting in differences between sex that could actually be attributed to the ontogenetic variations in the nitrogen isotope ratios. According to the models, males are 0.02 units more depleted in  $\delta^{15}\text{N}$  than females. Given that females are larger than males, the model would be

comparing tooth dentin isotope values from older males with younger and relatively larger females, hence younger females will be more enriched in  $\delta^{15}\text{N}$  than the older males (see variation in  $\delta^{15}\text{N}$  along age classes, below), and males will appear to be generally more depleted than females.

### *Ontogenetic variation in diet*

Our study using analysis of the stable isotope compositions for franciscanas has found similar ontogenetic results to prior studies including cetaceans. Calves and juveniles were significantly more enriched in  $^{15}\text{N}$  in relation to the other age classes, a pattern attributed to the nursing period (e.g., Knoff *et al.* 2008, Newsome *et al.* 2009, Riccialdelli *et al.* 2013). Lactating mothers catabolize lipid and protein from their own tissues to produce milk. Therefore, their nursing offspring are feeding at a trophic level higher than their mothers, hence presenting a stepwise  $\delta^{15}\text{N}$  enrichment per trophic level (Fogel *et al.* 1989, Hobson and Sease 1998, Newsome *et al.* 2009).

Franciscana calves start the transition from milk to solid food at very young age (2.5 to 3 mo) and weaning occurs at approximately nine months after birth (Harrison *et al.* 1981, Rodríguez *et al.* 2002). Thus, changes from a milk-based diet to a solid food diet should be isotopically expressed as a significant decrease in  $\delta^{15}\text{N}$  values in the animal's tissues after their first year since birth. However, the amount of dentin deposited during the first year in this species is higher than that deposited later in life, as evidenced by the significantly larger first GLG observed in stained teeth slides compared to older GLGs (Pinedo and Hohn 2000). Hence, as this first GLG comprises a considerable portion of the whole tooth, its isotopic signal will have a proportionally higher influence in the isotopic values of the whole tooth for relatively younger animals. The mean  $\delta^{15}\text{N}$  values in tooth dentin will, therefore, depict the signal of that first year even long after weaning. This might explain the significant shift in  $\delta^{15}\text{N}$  (ca 2‰) only in specimens older than four years in regard to values observed in nursing calves. Whole tooth nitrogen isotope values were biased towards the largest GLG-1 and the mean isotopic values of adults were biased towards that of young dolphins. Our crude change-point analysis indicated that the mean isotopic values of adults stabilized after four years and only then, the adult's isotopic signal dominated that of the juveniles.

With regard to the first adults and adults age classes only (specimens >4 yr old), no age-related variation in  $\delta^{15}\text{N}$  values was observed. Nitrogen isotope values remained relatively

constant, with higher variation only towards the end of the smoothing curve, which was probably due to the small sample size of older individuals. Nitrogen isotope responses to TBL showed a decreasing  $\delta^{15}\text{N}$  trend from shorter to larger animals, followed by a slight increase in the heavy isotope in larger animals (app 150 cm, Fig. 1). However, these differences were very small, and therefore cannot be considered as evidence for changes in trophic level, foraging habitats or prey preferences among adult franciscanas from southern Brazil.

Regarding the patterns of carbon isotope ratios in tooth dentin of franciscana, our models showed that  $\delta^{13}\text{C}$  values vary as a function of age, with more  $^{13}\text{C}$  depleted values observed in younger animals. Such patterns have been reported in previous studies (*e.g.*, Hobson and Sease 1998, Hobson *et al.* 2004) and nursing calves presenting  $\delta^{13}\text{C}$  values lower than adults have been associated with the influence of the high lipid content in the milk (40%-50%, Stewart and Stewart 2009). During the lipid synthesis there is high discrimination against  $^{13}\text{C}$ , resulting in lipids being more  $^{13}\text{C}$ -depleted in comparison to other macromolecular components (DeNiro and Epstein 1977, 1978). Nevertheless, our models indicated that age explained just a small fraction of the patterns in the carbon isotope ratios and differences between older specimens and zero-year-old were never larger than 1‰. Franciscanas occur in coastal waters with only a few records further offshore than the 30 m isobath (Secchi *et al.* 1997, Secchi 2010a) and dolphins of all age classes occur homogeneously throughout the area (Danilewicz *et al.* 2009). Therefore, variation in  $\delta^{13}\text{C}$  should indeed be small and differences in carbon might reflect some small inter-individual variation in dietary preferences along the ages (see discussion on SIAR outcomes below).

#### *Franciscanas' Trophic Enrichment Factor*

There is no estimation of TEF for franciscanas tooth dentin available in the literature. Therefore, the trophic enrichment factors (TEFs) applied in SIAR in the present study were based on values for other marine mammal species and ranged between 4.0‰-5.4‰ for carbon (based on reported muscle-to-collagen fractionation, Koch 2007) and 2.0‰-3.4‰ for nitrogen isotopes (Hobson *et al.* 1996, Caut *et al.* 2011, Borrell *et al.* 2012). Bhattacharyyas' coefficient showed that differences were significant between the models with the lowest and the highest  $\Delta^{13}\text{C}$  ( $\text{BC} < 0.6$ ). On the other hand, since models with higher and lower  $\Delta^{15}\text{N}$  differed only when  $\Delta^{13}\text{C}$  differences were larger, the alternative  $\Delta^{15}\text{N}$  applied seemed to have no significant influence on the models outcomes. Nevertheless, SIAR models with relatively higher  $\Delta^{13}\text{C}$  (between 4.8‰ and 5.4‰) and lower  $\Delta^{15}\text{N}$  (2.0‰ and 2.4‰) seemed to have more realistic

outputs, slightly differing from reported dietary estimates based on SCA (see discussion below).

The variation in  $\Delta^{13}\text{C}$  seemed to have significantly influenced the SIAR estimates especially for juveniles, while in adults, BC index indicated substantial overlap among the different models (all pairwise comparisons  $> 0.6$ ). Thus, small differences in TEF seemed to have no significant effect on the models outcome in adults, indicating that  $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$  in adult franciscanas might fall within the values used in this study. Moreover, models with TEF ranging between 5.2‰-5.4‰ for carbon and 2.2‰-2.4‰ for nitrogen had the highest estimated contributions of BP fish (18% and 20%), high estimates of squid (26%-32%), and CEs fish (26%-33%) in adult franciscanas. Although these models predicted high contributions of CEs fish, their predictions were the most similar to those estimated by SCA.

There is high variability in TEF depending on the consumers' taxonomic classes, the tissue analyzed and the isotopic values of the diet (Caut *et al.* 2009; DeMots *et al.* 2010). Moreover, TEF has been described as having an inverse relationship with  $\delta^{15}\text{N}$  values, indicating that higher predators present smaller isotopic fractionation between their prey and their tissues (Caut *et al.* 2008; Hussey *et al.* 2014), whereas TEF values around 3.4‰ have been described for consumers feeding relatively lower on the food web (Minagawa and Wada 1984), which is not the case of franciscana dolphins. Furthermore, our models with TEF values around 2‰ provided more realistic outcomes, closer to the estimates obtained by analysis of stomach contents. Stable isotope mixing models outputs are very sensitive to variations in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  TEFs and the application of inconsistent  $\Delta^{13}\text{C}$  or  $\Delta^{15}\text{N}$  could result in erroneous estimates of prey proportional contribution in the models outputs (Bond and Diamond 2011). Therefore, applying franciscanas-specific diet-to-tissue enrichment factors is crucial in order to obtain correct and more realistic dietary estimates using stable isotope mixing models. Here, we suggest a TEF of 4.8‰-5.4‰ for carbon and 2.0‰-2.4‰ for nitrogen to be applied in mixing models for dietary estimates, when analyzing the stable isotope composition in tooth dentin from franciscanas in southern Brazil.

#### *Proportional contribution of prey items: Stomach Content (SCA) vs. Stable Isotope Analysis (SIA)*

The majority of our SIAR model estimates (models 3 to 8) suggested that squid was important prey for juveniles followed by coastal-estuarine fish (*Paralonchurus brasiliensis*). However, SCA revealed that squid was more frequent in adults than in juveniles (males  $< 128.2$

cm and females <138.9 cm), while the latter would feed primarily on *Cynoscion guatucupa* (BP fish) and shrimps (Pinedo 1982, Bassoi 2005). The SIAR estimated large contributions of squid and low importance of shrimps to the diet of juveniles was not consistent with those reported based on SCA. Since the digestibility of squid beaks take longer than any other stomach content (Sekiguchi and Best 1997), it is unlike to underestimate the importance of this prey type in SCA. The relatively higher proportional contribution of squid may be an artifact of the prolonged influence of the  $\delta^{15}\text{N}$  in juveniles' whole tooth average isotopes, that parallel to the higher  $\delta^{15}\text{N}$  values observed in squid relative to the other prey groups.

Furthermore, the mean size of prey consumed by juvenile franciscanas is smaller than that consumed by adults (Bassoi 2005). Thus, selecting prey samples for isotopic analysis based on the combined mean size ingested by juveniles and adults could have resulted in dietary estimates biased to opposite directions (*i.e.*, upwards and downwards for juveniles and adults, respectively), since these prey species present length-related shifts in their diet (Santos and Haimovici 1998, Lucena *et al.* 2000, Martins *et al.* 2005). These shifts will most likely put them at different trophic levels, *i.e.*, larger prey sizes would occupy higher trophic positions (Nagata *et al.* 2015), which in turn would result in variations in the  $\delta^{15}\text{N}$  values among different length categories. Therefore, it is worth emphasizing the importance of collecting the prey species within the size range consumed by franciscanas in each age category, hence, the importance of integrating previous information on the species feeding habits instead of relying exclusively on the model's outcome.

The estimated contributions of squids to adults' diet by SIAR are relatively lower than what has been estimated by SCA (Bassoi 2005). The relative importance of squid to fish cannot be accessed through a comparison of IRI values in SCA because these groups have very different digestion rates. Cephalopod beaks take much longer to digest than fish otoliths, leading to an overestimation on the relative importance of the former (Sekiguchi and Best 1997). Therefore, the high importance of squids estimated by SCA could be attributed to their relatively low digestion rates (Sekiguchi and Best 1997). Thus, the lower estimated contribution of squid to the diet of first adults and adult franciscanas estimated in our SIAR analyzes, strongly suggests that the SCA has overestimated the importance of cephalopods in the diet of this species.

The fractional contributions of CE fish (*P. brasiliensis*) were very high for both first adults and adults in all SIAR models. This fish species occurs throughout the year in the study area, being equally abundant along the seasons (Haimovici *et al.* 1996), whereas species

grouped as BP fish (*C. guatucupa* and *T. lepturus*), which are considered the most important according to SCA (Basso 2005), are more frequent during colder and warmer seasons, respectively (Haimovici *et al.* 1996). This seasonality may have influenced their lighter muscle carbon isotope values, while carbon isotopic composition of *P. brasiliensis* resembled those of the other resident components of this coastal-estuarine ecosystem, including franciscanas (*e.g.*, Garcia *et al.* 2007, Botta *et al.* 2012). Consequently, the mixing model has attributed higher importance of coastal-estuarine fish to the dolphins' diet, differing from the results based on SCA. Studies including seasonally important prey as well as soft tissues with faster turnover rates of franciscanas are, therefore, recommended in order to better assess the seasonal diet variation in the species isotopic composition. Moreover, the prey samples collected for the present study were obtained between 2012-13, whereas most of the franciscanas samples analyzed have been from animals bycaught between 1994-2005 (see supplementary table 2). Thus, temporal differences in isotopic composition of prey consumed in the 1990s and early 2000s and those collected for this study were not accounted for, and could have influenced our results. Finally, the high importance of coastal-estuarine fish (*P. brasiliensis*) to adult franciscanas from southern Brazil suggested by isotope mixing models estimates seems to be rather unrealistic since franciscanas spend most of the time in open waters and several studies have reported the importance of benthic-pelagic fish (mainly *C. guatucupa*) to their diet (*e.g.*, Pinedo 1982, Danilewics *et al.* 2002, Basso 2005).

SIAR outcomes for determining proportional contribution of preys groups in the diet of franciscana were potentially affected by the high similarity in isotope signals among prey species, as reported previously by Parnell *et al.* (2010). The data available for prey sources showed that only shrimps were statistically different from the other prey groups in  $\delta^{15}\text{N}$  signatures. Regarding the  $\delta^{13}\text{C}$  values, CEs fish differed from the other fish types, but not from squid and shrimps. Squid did not differ from any of the fish groups, and BP and SP fish did not differ in carbon isotope values. The high similarity in isotopic signatures could be related to their habitat use and feeding behavior. These species comprise a food chain in which they feed upon one another with small ontogenetic and seasonal variations (Haimovici *et al.* 1996, Martins and Haimovici 1997, Santos and Haimovici 1998, Lucena *et al.* 2000, Martins *et al.* 2005). The effect of similarity in the isotopic signal of the prey could be minimized with increased sample size by reducing within source variation (Phillips and Gregg 2001) as the prey types could be clumped according to their relative ontogenetic classes. In addition, the use

of an extra naturally occurring stable isotope, such as sulfur ( $\delta^{34}\text{S}$ ), could enhance the model discrimination power among the food sources (Peterson and Fry 1987, Barros *et al.* 2010).

In the present study we found no isotopic support for differences in trophic position and segregation in prey preferences among adults of different ages. Stable isotopes also confirm the reliance of both females and males upon the same food resources. The tissue chosen for analysis (tooth dentin) and the dentin deposition pattern in this species have probably strongly influenced the observed isotope values, affecting especially the interpretation of the diet of juveniles, due to the potential effect of the wide GLG deposited during the nursing period. Hence, further investigation with high precision sampling of dentin growth layers or by analyzing tissues with faster turnover rates is recommended, especially in young animals, in order to better understand the ontogenetic shifts in their diet. SIAR models with relatively higher trophic enrichment factors for carbon ( $\Delta^{13}\text{C}$  4.8‰-5.4‰) estimated increased importance of *Cynoscion guatucupa* to the diet of adults, providing closer results to those of SCA. Therefore, when using stable isotopes and isotope mixing models to estimate the diet of franciscana dolphins, a diet-to-dentin trophic enrichment factor of 4.8‰-5.4‰ for carbon and 2.0‰-2.4‰ for nitrogen is recommended, as it is impossible to conduct laboratory experiments for logistic and ethic reasons and the values suggested here seem to be a reasonably good *proxy* to the true carbon and nitrogen TEF values for this species. In conclusion, stable isotopes can offer valuable information on the feeding habits of franciscanas, although the results must be interpreted with caution, and analyses should include previous knowledge on dietary preferences with other traditional methods, such as stomach content analysis. Therefore, the use of both SCA and SIA is recommended as the best option for a more detailed assessment of the feeding habits of marine mammals, as it mitigates the bias provided when using each method separately.

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Table 1. Mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values ( $\pm$  SD) and C:N ratios in tooth dentin of franciscana dolphins (*Pontoporia blainvillei*) from southern Brazil. Values are presented for the whole sample ( $n = 125$ ) and for each age category (1–4) including both females and males. Last column includes the Pearson correlation between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values across age categories.

Group	$n$ (♀, ♂)	$\delta^{13}\text{C}$ (‰) (mean $\pm$ SD)	$\delta^{15}\text{N}$ (‰) (mean $\pm$ SD)	C:N	$\delta^{13}\text{C} \sim \delta^{15}\text{N}$ correlation ( $P$ )
Whole sample	125 (60, 65)	$-10.9 \pm 0.7$	$19.1 \pm 1.1$	3.2	
1. Calves	19 (10, 9)	$-11.2 \pm 0.9$	$20.2 \pm 0.8$	3.3	-0.20 (0.40)
2. Juveniles	33 (16, 17)	$-11.0 \pm 0.7$	$19.6 \pm 0.7$	3.1	-0.31 (0.07)
3. First adults	48 (24, 24)	$-10.8 \pm 0.5$	$18.7 \pm 0.9$	3.1	-0.28 (0.05)
4. Adults	25 (10, 15)	$-10.8 \pm 0.6$	$18.3 \pm 0.9$	—	-0.26 (0.21)

Table 2. Results of the best adjusted generalized additive models (GAMs) of the relationship between (a)  $\delta^{15}\text{N}$  and (b)  $\delta^{13}\text{C}$  values and total body length (TBL), age, and sex. The significance levels ( $P$ ),  $F$  statistics ( $F$ ), and estimated degrees of freedom (edf) are shown for each of the continuous explanatory variables (smooth  $s$  of age and TBL), as well as the estimated parameters for the categorical variable (sex), the  $t$ -value, and the significance level ( $\text{Pr}\{>|t|\}$ ). The adjusted  $R^2$ , deviance explained, and generalized cross-validation (GCV) score are shown for each model.

(a)	N $\sim$ $s(\text{TBL}) + \text{sex}$		
Family	Gamma		
Link function	log		
Adjusted $R^2$	0.474		
Deviance explained	50.3%		
GCV score	0.0017		
	edf	$F$	$P$
<i>Explanatory variables</i>			
$s(\text{TBL})$	6.04	15.32	<0.001
	Estimated	$t$	$\text{Pr}\{> t \}$
sex (M)	-0.022	-2.606	<0.05
(b)	C $\sim$ $s(\text{Age})$		
Family	Gamma		
Link function	log		
Adjusted $R^2$	0.04		
Deviance explained	5.07%		
GCV score	0.0036		
	edf	$F$	$P$
<i>Explanatory variables</i>			
$s(\text{Age})$	1.176	3.946	<0.05

Table 3. Generalized additive models with family Gamma and log link function for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  with age or total body length (TBL), sex, and year the specimens were bycaught. The values of adjusted  $R^2$ , deviance explained, model degree of freedom (df), Akaike information criterion (AIC), and  $\Delta\text{AIC}$  are shown. Models are ranked according to AIC values, with significant models highlighted in bold.

Family: gamma link function: log	Adjusted $R^2$	Deviance explained (%)	df	AIC	$\Delta\text{AIC}$
$\delta^{13}\text{C}$					
$\delta^{13}\text{C} \sim s(\text{age})$	0.04	5.07	3.17	248.9	0
$\delta^{13}\text{C} \sim s(\text{age}) + s(\text{year})$	0.037	5.48	4.13	250.4	1.4
$\delta^{13}\text{C} \sim s(\text{TBL})$	0.023	3.2	3.00	251.0	2.1
$\delta^{13}\text{C} \sim s(\text{age}) + \text{sex} + s(\text{year})$	0.030	5.56	5.15	252.3	3.4
$\delta^{13}\text{C} \sim s(\text{TBL}) + s(\text{year})$	0.020	3.73	4.00	252.4	3.5
$\delta^{13}\text{C} \sim s(\text{TBL}) + \text{sex} + s(\text{year})$	0.012	3.78	5.00	254.3	5.4
$\delta^{15}\text{N}$					
$\delta^{15}\text{N} \sim s(\text{TBL}) + \text{sex}$	0.474	50.3	9.04	298.5	0
$\delta^{15}\text{N} \sim s(\text{TBL}) + \text{sex} + s(\text{year})$	0.478	51.5	10.88	299.2	0.7
$\delta^{15}\text{N} \sim s(\text{age})$	0.45	46	4.77	300.3	1.8
$\delta^{15}\text{N} \sim s(\text{age}) + s(\text{year})$	0.45	46.3	5.73	301.4	2.9
$\delta^{15}\text{N} \sim s(\text{age}) + \text{sex} + s(\text{year})$	0.446	46.4	6.70	303.2	4.7
$\delta^{15}\text{N} \sim s(\text{TBL})$	0.448	47.1	7.98	304.1	5.6

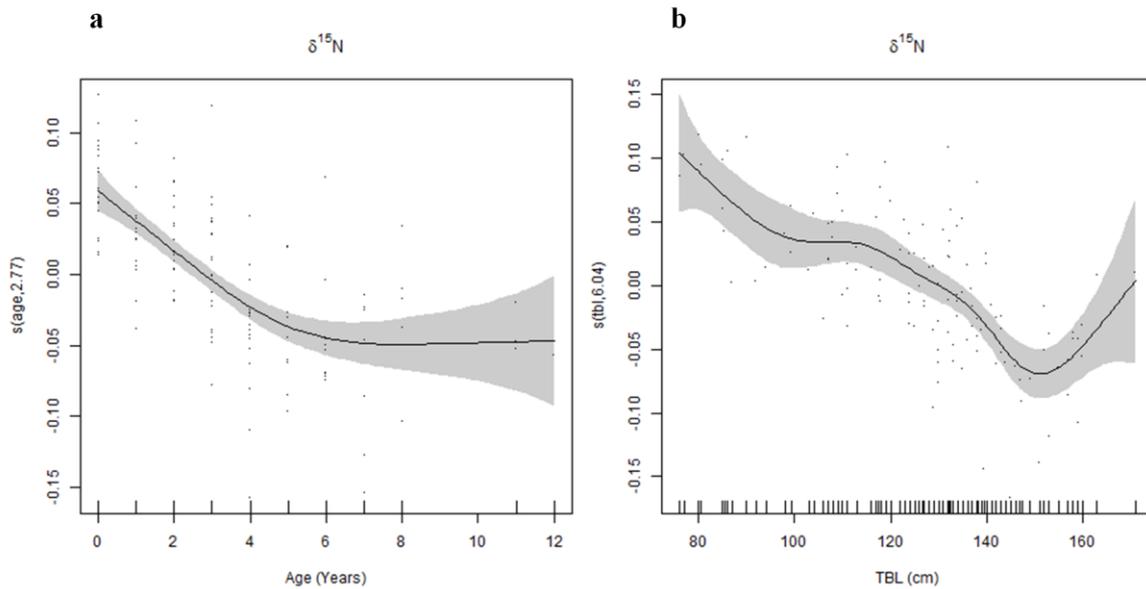
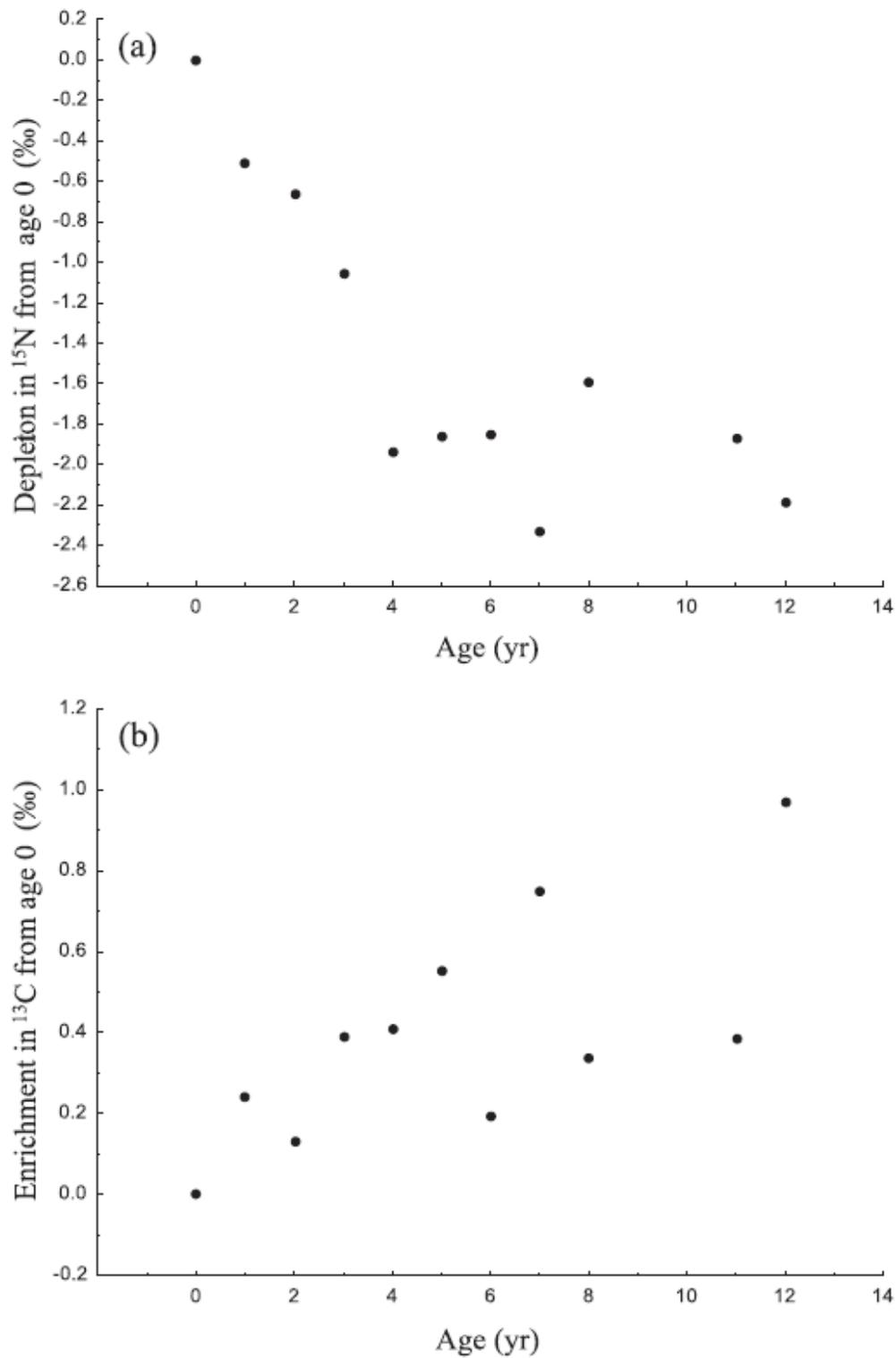
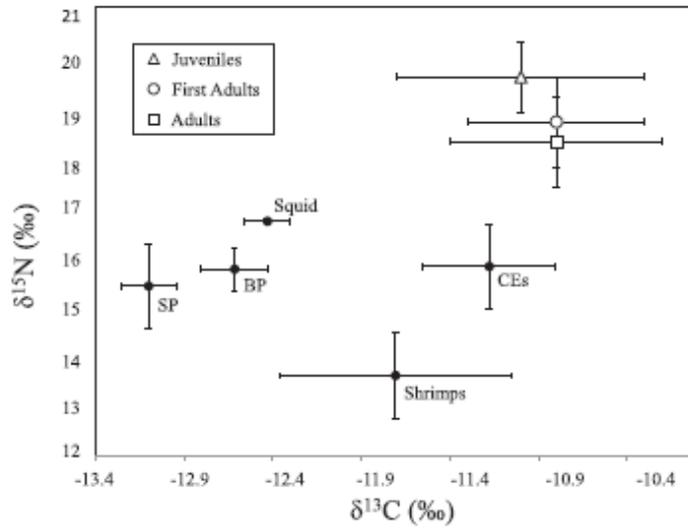


Figure 1: Generalized Additive Models (GAMs) for the effect of age and total body length (TBL) on tooth  $\delta^{15}\text{N}$  values in franciscanas (*Pontoporia blainvillei*). Tick marks in X-axis represent the sampled franciscanas, whereas the Y-axis represents the trend in tooth  $\delta^{15}\text{N}$  in relation to (a) age and (b) TBL. Grey envelopes correspond to the 95% confidence interval.



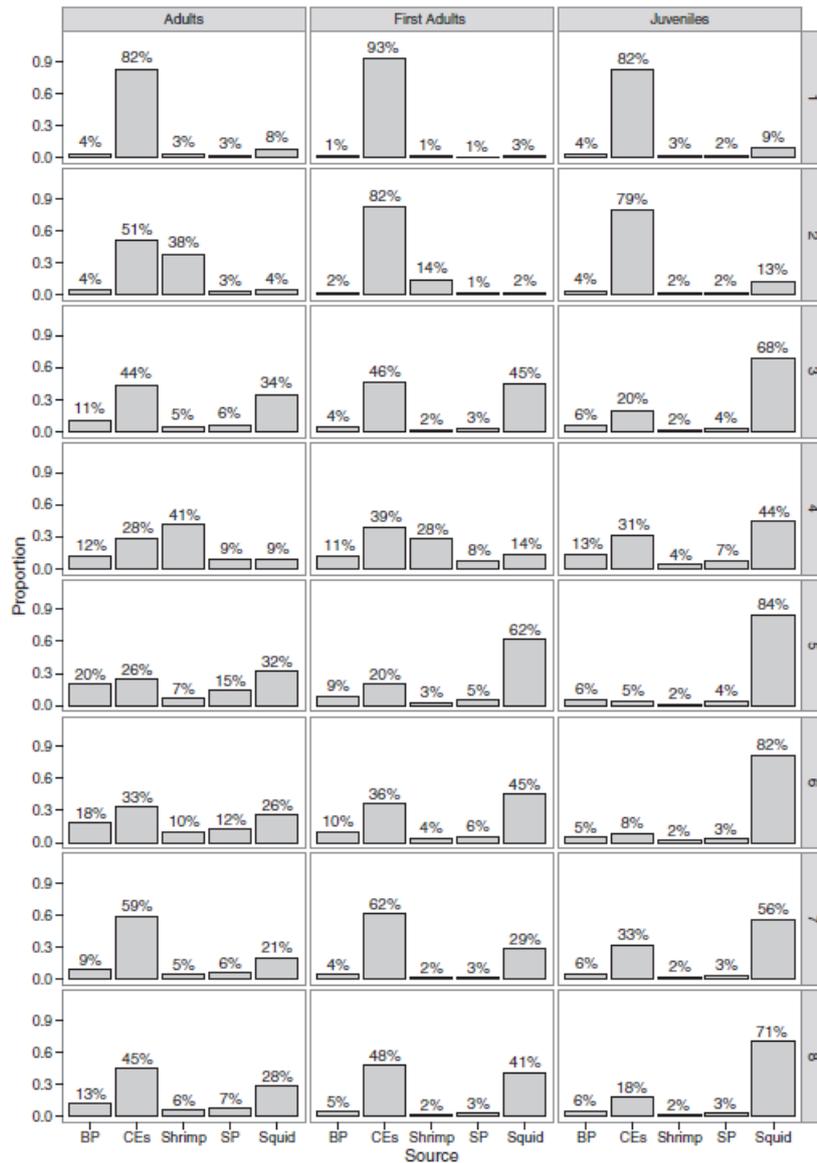
**Figure 2.** (a) Depletion and (b) enrichment (‰) in dentin  $^{15}\text{N}$  and  $^{13}\text{C}$ , respectively, with age in franciscanas (*Pontoporia blainvillei*) from southern Brazil. Values were calculated as a difference in mean  $\delta^{15}\text{N}/\delta^{13}\text{C}$  of zero and  $i^{\text{th}}$  years old animals, and expressed as ‰.



**Figure 3.** Mean ( $\pm$ SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (in ‰) in muscle tissues of prey groups of franciscanas (*Pontoporia blainvillei*) from southern Brazil: Benthopelagic fish (BP), Shelf-pelagic fish (SP), Coastal-estuarine fish (CEs), Squid and Shrimp; and mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values ( $\pm$ SD) in tooth dentin of dolphins per ontogenetic class ( $\Delta$  juveniles;  $\circ$  first adults; and  $\square$  adults). A correction of +4‰ was applied to the prey's muscle carbon isotope values based on reported muscle-to-collagen fractionation.

**Table 4.** Mean Bhattacharyya's Coefficient (BC) for pairwise model comparisons of SIAR mixing-models outputs of Juveniles, First Adult and Adult franciscanas (*Pontoporia blainvillei*) diet. The trophic enrichment factor applied in the models (1-8) range between 4.0‰-5.4‰ for carbon ( $\Delta^{13}\text{C}$ ) and 2.0‰-3.4‰ for nitrogen ( $\Delta^{15}\text{N}$ ).  $0 < \text{BC} < 1$ , when  $\text{BC} = 0$ , there is no similarity and when  $\text{BC} = 1$ , complete similarity between the models. Percentage of similarity is presented below diagonal and BC values are presented above diagonal. Significant differences between models ( $\text{BC} < 0.6$ ) are highlighted in bold.

Juveniles	1	2	3	4	5	6	7	8
1 - $\delta^{13}\text{C}$ 4.0; $\delta^{15}\text{N}$ 2.0		0.9629	0.7013	0.8049	<b>0.5121</b>	<b>0.5719</b>	0.7961	0.6843
2 - $\delta^{13}\text{C}$ 4.0; $\delta^{15}\text{N}$ 3.4	96%		0.7310	0.8270	<b>0.5497</b>	<b>0.6078</b>	0.8207	0.7157
3 - $\delta^{13}\text{C}$ 5.0; $\delta^{15}\text{N}$ 2.0	70%	73%		0.9184	0.9265	0.9414	0.9424	0.9540
4 - $\delta^{13}\text{C}$ 5.0; $\delta^{15}\text{N}$ 3.4	80%	83%	92%		0.8461	0.8699	0.9365	0.9122
5 - $\delta^{13}\text{C}$ 5.4; $\delta^{15}\text{N}$ 2.2	<b>51%</b>	<b>55%</b>	93%	85%		0.9629	0.8786	0.9343
6 - $\delta^{13}\text{C}$ 5.2; $\delta^{15}\text{N}$ 2.4	<b>57%</b>	<b>61%</b>	94%	87%	96%		0.9054	0.9484
7 - $\delta^{13}\text{C}$ 4.8; $\delta^{15}\text{N}$ 2.2	80%	82%	94%	94%	88%	91%		0.9400
8 - $\delta^{13}\text{C}$ 5.0; $\delta^{15}\text{N}$ 2.2	68%	72%	95%	91%	93%	95%	94%	
First Adults	1	2	3	4	5	6	7	8
1 - $\delta^{13}\text{C}$ 4.0; $\delta^{15}\text{N}$ 2.0		0.9482	0.8047	0.7665	<b>0.6080</b>	0.7456	0.8883	0.8139
2 - $\delta^{13}\text{C}$ 4.0; $\delta^{15}\text{N}$ 3.4	95%		0.7703	0.8505	<b>0.5948</b>	0.7302	0.8527	0.7830
3 - $\delta^{13}\text{C}$ 5.0; $\delta^{15}\text{N}$ 2.0	80%	77%		0.8167	0.9236	0.9544	0.9618	0.9736
4 - $\delta^{13}\text{C}$ 5.0; $\delta^{15}\text{N}$ 3.4	77%	85%	82%		0.7715	0.8405	0.8382	0.8299
5 - $\delta^{13}\text{C}$ 5.4; $\delta^{15}\text{N}$ 2.2	<b>61%</b>	<b>59%</b>	92%	77%		0.9377	0.8665	0.9193
6 - $\delta^{13}\text{C}$ 5.2; $\delta^{15}\text{N}$ 2.4	75%	73%	95%	84%	94%		0.9311	0.9555
7 - $\delta^{13}\text{C}$ 4.8; $\delta^{15}\text{N}$ 2.2	89%	85%	96%	84%	87%	93%		0.9635
8 - $\delta^{13}\text{C}$ 5.0; $\delta^{15}\text{N}$ 2.2	81%	78%	97%	83%	92%	96%	96%	
Adults	1	2	3	4	5	6	7	8
1 - $\delta^{13}\text{C}$ 4.0; $\delta^{15}\text{N}$ 2.0		0.8357	0.8652	0.7402	0.7612	0.8102	0.9178	0.8697
2 - $\delta^{13}\text{C}$ 4.0; $\delta^{15}\text{N}$ 3.4	84%		0.7825	0.9142	0.7332	0.8032	0.8336	0.8086
3 - $\delta^{13}\text{C}$ 5.0; $\delta^{15}\text{N}$ 2.0	87%	78%		0.7894	0.9058	0.9133	0.9286	0.9378
4 - $\delta^{13}\text{C}$ 5.0; $\delta^{15}\text{N}$ 3.4	74%	91%	79%		0.8041	0.8472	0.8044	0.8154
5 - $\delta^{13}\text{C}$ 5.4; $\delta^{15}\text{N}$ 2.2	76%	73%	91%	80%		0.9212	0.8707	0.9058
6 - $\delta^{13}\text{C}$ 5.2; $\delta^{15}\text{N}$ 2.4	81%	80%	91%	85%	92%		0.8968	0.9184
7 - $\delta^{13}\text{C}$ 4.8; $\delta^{15}\text{N}$ 2.2	92%	83%	93%	80%	87%	90%		0.9301
8 - $\delta^{13}\text{C}$ 5.0; $\delta^{15}\text{N}$ 2.2	87%	81%	94%	82%	91%	92%	93%	



**Figure 4.** Stable isotope Mixing Models (SIAR) estimates of the mean proportional contribution (%) of each prey group to the diet of Juveniles, First Adult and Adult franciscanas. SIAR models (1-8) with different trophic enrichment factor (TEF) for carbon ( $\Delta^{13}\text{C}$ ) and nitrogen ( $\Delta^{15}\text{N}$ ), in that order are: 4.0‰ and 2.0‰; 4.0‰ and 3.4‰; 5.0‰ and 2.0‰; 5.0‰ and 3.4‰; 5.4‰ and 2.2‰; 5.2‰ and 2.4‰; 4.8‰ and 2.2‰; and lastly 5.0‰ and 2.2‰. Prey groups are: Benthopelagic fish (BP), Shelf-pelagic fish (SP), Coastal-estuarine fish (CEs), Squid and Shrimp.

**Supplementary material:**

Supplementary table 1: Franciscanas (*Pontoporia blainvillei*) by-caught along the coast off Rio Grande, southern Brazil between 1994 and 2010. Number of specimens (*n*) used in this study, their average age ( $\bar{x}$ )  $\pm$  standard deviation (SD), as well as minimum (Min), maximum (Max) and mode ages.

<b>Year</b>	<b><i>n</i></b>	<b>Age (<math>\bar{x} \pm</math> SD)</b>	<b>Min</b>	<b>Max</b>	<b>Mode</b>
1994	40	3.7 $\pm$ 2.6	0	11	3
1995	8	4.4 $\pm$ 4.2	0	11	3
1996	3	1.6 $\pm$ 1.5	0	3	-
1997	4	3.7 $\pm$ 1.7	2	6	-
1999	3	1.3 $\pm$ 2.3	0	4	0
2000	10	3 $\pm$ 2.0	0	6	4
2001	4	3.2 $\pm$ 2.2	0	5	4
2002	16	3.1 $\pm$ 2.4	0	8	2
2003	14	3.6 $\pm$ 3.3	0	12	3
2004	18	2.5 $\pm$ 2.3	0	7	0
2005	2	4 $\pm$ 2.8	2	6	-
2010	1	8	-	-	-

Supplementary table 2: Franciscanas (*Pontoporia blainvillei*) main prey species according to stomach content analysis from Bassoi's (2005) study. Prey species are grouped as Fish, Cephalopod and Crustacean. The Index of Relative Importance (IRI) and the percentage of the total IRI (% Total IRI) are presented within each group for every species. Prey size estimated by Bassoi (2005) are presented as Mean ( $\bar{x}$ ), minimum (Min) and maximum (Max), prey samples collected ( $n$ ) for the present study, as well as the mean size ( $\pm$  SD), and mean ( $\pm$ SD)  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and C:N ratios.

Species	Bassoi 2005			This study			Group	$\delta^{13}\text{C}$ (‰) (Mean $\pm$ SD)	$\delta^{15}\text{N}$ (‰) (Mean $\pm$ SD)	C:N
	IRI	% Total IRI	Length range (cm) $\bar{x}$ Min-Max	N	length $\bar{x}$ (cm)	SD				
<b>Fish (IRI 46.95%)</b>										
<i>Cynoscion guatucupa</i>	1643.1	11.8	5.6 1.2 - 35.6	3	6.2	$\pm 0.9$	BP	-16.5 $\pm 0.2$	15.9 $\pm 0.5$	3.3
<i>Trichiurus lepturus</i>	225.6	1.6	40.2 8.4 - 88.8	2	44.2	$\pm 5.4$	BP	-16.8 $\pm 0.1$	15.5 $\pm 0.4$	3.1
<i>Paralichthys brasiliensis</i>	154.3	1.1	10.2 0.6 - 22.8	5	17.3	$\pm 1.8$	CEs	-15.2 $\pm 0.4$	15.8 $\pm 0.8$	3.3
<i>Anchoa mitchilli</i>	142.8	1.0	6.7 3.1 - 11.2	4	7.7	$\pm 0.1$	SP	-17.1 $\pm 0.1$	16.1 $\pm 0.2$	3.4
<i>Engraulis anchoita</i>	4.8	0.03	10.8 5.4 - 15.11	3	7.9	$\pm 0.1$	SP	-17.1 $\pm 0.3$	14.5 $\pm 0.3$	3.3
<b>Cephalopod (IRI 51.65%)</b>										
<i>Loligo sanpaulensis</i>	11295.8	81.1	10.6 2.3 - 25.0	2	9.2	$\pm 0.3$	Squid	-16.4 $\pm 0.1$	16.7 $\pm 0.03$	3.5
<b>Crustacean (IRI 1.41%)</b>										
<i>Pleoticus muelleri</i>	1.7	0.01	- -	12	-	-	Shrimp	-15.3 $\pm 0.5$	14.1 $\pm 0.4$	3.7
<i>Artemesia longinaris</i>	1.3	0.01	- -	18	-	-	Shrimp	-16.0 $\pm 0.6$	13.3 $\pm 1.0$	3.9

*Note:* IRI was calculated by summing the numerical abundance percentage of a particular prey (%N) and the volumetric percentage (%V), multiplied by the frequency of occurrence percentage (%F):  $\text{IRI} = (\%N + \%V) * \%F$ . The volumetric percentage of prey was calculated after measuring the otoliths and beaks, which enables the estimation of the length and weight of the animal.

Supplementary table 3: Residual error of the Stable Isotope Analysis in R (SIAR) mixing models (1 - 8) with different carbon and nitrogen stable isotopes trophic enrichment factor (TEF,  $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$ ) for each age category (Juveniles, First Adult and Adult).

	Juveniles		First Adult		Adult	
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
1 - $\Delta^{13}\text{C}$ 4.0; $\Delta^{15}\text{N}$ 2.0	0.59	1.82	0.33	0.92	0.74	0.52
2 - $\Delta^{13}\text{C}$ 4.0; $\Delta^{15}\text{N}$ 3.4	0.64	0.27	0.48	0.93	0.94	0.6
3 - $\Delta^{13}\text{C}$ 5.0; $\Delta^{15}\text{N}$ 2.0	0.62	1.43	0.31	0.96	0.57	0.79
4 - $\Delta^{13}\text{C}$ 5.0; $\Delta^{15}\text{N}$ 3.4	0.63	0.58	0.32	0.79	0.51	0.71
5 - $\Delta^{13}\text{C}$ 5.4; $\Delta^{15}\text{N}$ 2.2	0.53	1.09	0.32	0.82	0.59	0.82
6 - $\Delta^{13}\text{C}$ 5.2; $\Delta^{15}\text{N}$ 2.4	0.56	0.93	0.35	0.86	0.58	0.78
7 - $\Delta^{13}\text{C}$ 4.8; $\Delta^{15}\text{N}$ 2.2	0.64	1.33	0.24	0.85	0.53	0.65
8 - $\Delta^{13}\text{C}$ 5.0; $\Delta^{15}\text{N}$ 2.2	0.62	1.21	0.31	0.87	0.57	0.75

## **CAPÍTULO 2**

### **Zooplankton-based $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes from the outer continental shelf and slope in the subtropical western South Atlantic**

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Versão do artigo em revisão no periódico Deep Sea Research Part I

## Zooplankton-based $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes from the outer continental shelf and slope in the subtropical western South Atlantic

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### Abstract

Characterizing the patterns of stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopes at the base of the food webs (baseline) is essential in ecological studies for assessing the feeding habits and migration patterns of marine predators. We analysed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in zooplankton samples collected in spring and autumn (2012-2015), along the south (SCM) and southeast (NCSM) Brazilian oceanic waters. An increase in  $\delta^{13}\text{C}$  from the shelf break towards the offshore was associated with the influence of continental water input and upwellings along the shelf break, which introduce  $^{13}\text{C}$ -depleted inorganic carbon to the surface, where it will be incorporated into the biological system through phytoplankton growth. An opposite trend was observed in  $\delta^{15}\text{N}$ , with higher values along the shelf break related to the intrusion of upwelled deep-water nutrients, contrasting with the oligotrophic offshore waters where  $\text{N}_2$  fixation takes place, resulting in primary and secondary production that is relatively more depleted in  $^{15}\text{N}$ . A latitudinal (north-south) decrease in  $\delta^{13}\text{C}$  along the offshore area coincided with known isotopic patterns in inorganic carbon, which were more  $^{13}\text{C}$ -depleted towards higher latitudes. On the other hand, an increase in  $\delta^{15}\text{N}$  was observed towards the south, reflecting the contrast between the  $\text{N}_2$  fixation in the more oligotrophic waters in the NCSM and the higher nutrient availability in the SCM. Additionally,  $\delta^{13}\text{C}$  was significantly higher in autumn and  $\delta^{15}\text{N}$  was higher in spring. Although inter-annual differences in  $\delta^{15}\text{N}$  were non-significant,  $\delta^{13}\text{C}$  was significantly lower in 2012 than in the later years. This study provides novel information regarding the patterns of baseline  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the southwestern Atlantic Ocean, which will be useful for future investigation of the ecology of higher trophic-level organisms occurring in this area.

**Key words:** Brazil; Stable isotopes; Marine biogeochemistry; Trophic ecology; Shelf break; Slope.

## 1. Introduction

Geochemical methods such as the measurement of bulk carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotopes in plant and animal tissues have been widely used in studies unravelling predators' trophic relationships (Hobson et al., 1994; Newsome et al., 2010), depicting the flow of matter and energy within ecosystems and marine food webs (Botto et al., 2011), and assessing the feeding areas and movement patterns of marine animals (Hobson et al., 2010; Graham et al., 2010). The development of isotopic maps (*isoscapes*) characterizing  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  at the base of the food webs (baseline) provides valuable information to study the ecology of high trophic-level organisms, as the stable isotope compositions of consumers' tissues reflect those of the areas where they have been feeding (Graham et al., 2010; McMahon et al., 2013; Rodríguez-Pérez et al., 2018). While studies at the ocean basin-scale show the broad view of patterns in stable isotopes (McMahon et al., 2013; Magozzi et al., 2017), it is important to characterize baseline isotopic patterns at a finer resolution for ecological studies taking place on a local scale. Baseline  $\delta^{13}\text{C}$  values are influenced differently by gradients in local temperature,  $\text{CO}_2$  concentration, primary producers' specific composition, size and growth rates (Laws et al., 1995; Popp et al., 1998; Trueman and Glew, 2019). Accordingly,  $\delta^{13}\text{C}$  values differ among organisms inhabiting estuarine, marine or freshwater (Fry and Sherr, 1989; Trueman and Glew, 2019), pelagic or benthic ecosystems (France, 1995), tropical, temperate or polar oceans (Cherel and Hobson, 2007). Nitrogen stable isotopes at the base of food webs also vary between ecosystems, depending mainly on the local concentrations and types of nutrient available for microbial growth (e.g., nitrate or  $\text{N}_2$  fixation). Relatively higher  $\delta^{15}\text{N}$  values are observed in regions where nitrate is the main source of N for primary producers, whereas in low nutrient regions, where  $\text{N}_2$  fixation is important, lower  $\delta^{15}\text{N}$  values are observed in primary producers (Sigman et al., 2009). Furthermore, there is a trophic effect between predator-prey stable isotope values, with an increase of approximately 1‰ in  $\delta^{13}\text{C}$  and 2-4‰ in  $\delta^{15}\text{N}$  for each step-wise increase in a consumer's trophic level (DeNiro and Epstein, 1978, 1981; Post, 2002), resulting in baseline (e.g., zooplankton) stable isotope values in an ecosystem being propagated upwards towards higher trophic levels. Therefore, describing the patterns of baseline C and N ratios within and between different areas is important when assessing the feeding habits of migratory species or when comparing different populations of conspecifics to learn about their ecology.

Characterizing the stable isotope composition of zooplankton allows for the assessment of short-term (seasonal) variations in the local  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (El-Sabaawi et al., 2013),

due to their relatively shorter lifespan (e.g., ~20 d for copepods full development, Irvine and Waya, 1999) and faster turnover time in comparison to higher trophic levels (O'Reilly et al., 2002). Seasonal shifts in baseline  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  might affect the isotopic values of predators within that food web, and understanding the patterns in these variations will help make a better assessment of their trophic level and feeding ecology. Zooplankton is one of the key components of pelagic food webs, important in the transfer of carbon and energy from primary producers to higher trophic levels. Phytoplankton, bacteria and protozoa are food sources for copepods and other nano- and micro-zooplankton (Stoecker and Capuzzo, 1990; Guenther et al., 2008; Köster et al., 2011), whereas euphausiids are herbivorous filter-feeders and opportunistic carnivores (Hamner, 1988; Gibbons et al., 1991). Salps include both solitary and aggregate pelagic tunicates that filter-feed on suspended particles, including phytoplankton, detritus and bacteria (Vargas and Madin, 2004; Pakhomov et al., 2019). A large abundance of salps is generally associated with high rates of phytoplankton growth due to upwelling events (Deibel and Paffenhofer, 2009). Some zooplankton groups consist of several commensal or parasitic species, such as the hyperiid amphipods, frequently associating with gelatinous zooplankton (Gasca et al., 2015; Riascos et al., 2015). Among the exclusive carnivores are the chaetognaths, which mainly consume copepods (Sullivan, 1980; Liang and Vega-Pérez, 1995). In turn, these groups are important trophic connections to cartilaginous and bony fish (Brickle et al., 2003; Martins et al., 2005; Padovani et al., 2012), squids (Santos and Haimovici, 1997; Rosas-Luis et al., 2014), seabirds (Bocher et al., 2001), marine turtles (Sampson et al., 2018) and mammals (Santos and Haimovici, 2001).

In the western South Atlantic, the productivity along the Brazilian south/southeastern continental shelf is enhanced by fronts (Acha et al., 2004; Piola et al., 2008a), upwellings and the input from Sub-Antarctic and continental waters (Ciotti et al., 1995; Piola et al., 2008b). These nutrient-rich waters fertilize the upper layers promoting higher primary productivity that sustain large abundance and diversity of consumers (Guenther et al., 2008; Coelho-Souza et al., 2012; Di Tullio et al., 2016). The outer continental shelf and slope in the area is influenced by different water masses that contribute to differing physical and chemical gradients between the southern (34°S–28°S) and southeastern (28°S–24°S) regions. Therefore, in the present work, the latitudinal range of the study region has been divided into two regions, considering as a reference the Cape Santa Marta (28°S, Fig. 1) that separates the southeast and the southern regions: North of Cape Santa Marta (NCSM) and South of Cape Santa Marta (SCM, Fig. 1).

The NCSM region is influenced mainly by the southward flowing tropical waters transported by the Brazil Current (BC) along with upwellings of the South Atlantic Central Water (SACW) occurring both off Cape Frio, in the Rio de Janeiro state (Acha et al., 2004; Coelho-Souza et al., 2012), and at a smaller scale in Cape Santa Marta, in Santa Catarina (Campos et al., 2013). Such upward flow of deep cold water fertilizes the surface layers with deep ocean nutrients, enhancing productivity in these otherwise oligotrophic tropical waters (Guenther et al., 2008; Coelho-Souza et al., 2012; Brandini et al., 2018). High zooplankton densities have been observed in this region during late spring and summer following increased primary producer biomass due to such strong upwelling events (Valentin, 2001; Acha et al., 2004). Additionally, increased abundance of higher trophic level consumers, such as squids and pelagic fish, coincides with those upwellings (Bakun and Parrish, 1991; Valentin, 2001). On the other hand, filter-feeding salps are the most abundant species associated with the oligotrophic tropical waters from the Brazil Current (Lopes et al., 2006).

The southern SCM region is seasonally influenced by the tropical waters that occupy the outer continental shelf and slope mostly during summer, while in autumn and winter the northward flowing Sub-Antarctic Water (SAW) and Plata Plume Water (PPW, freshwater from the La Plata River in Argentina and Uruguay and the Patos Lagoon in Brazil) predominate along the region (Acha et al., 2004; Möller Jr. et al., 2008). PPW dominates in spring, autumn and winter, with increased influence due to high rainfall in years of warm phases of the El Niño Southern Oscillations (ENSO) (Acha et al., 2004; Piola et al., 2008b). Such freshwater input influences the concentration of nutrient and biological productivity in the region (Ciotti et al., 1995; Duarte et al., 2014; Gonçalves-Araujo et al., 2018). Moreover, wind stress is responsible for the seasonal mixing of the surface layers and the upwelling of deeper waters (Lima et al., 1996), contributing to higher productivities in the SCM in comparison to the NCSM region.

Such differences in the dominance of water masses between the SCM and NCSM regions result in physical, chemical and biological gradients, which in turn influence differently their environmental biogeochemical processes. We expect, therefore, that such physical patterns will create seasonal gradients in the isotopic compositions throughout these two regions. The present study aims to characterize the patterns of baseline  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in the pelagic waters along the Brazilian outer continental shelf and slope, and its latitudinal, seasonal and annual trends. We analysed the isotopic compositions in zooplankton samples collected over four years (2012-2015) in contrasting seasons (spring and autumn). The present study provides information on the seasonal patterns in organic  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isoscapes that

will help and complement further studies using these stable isotopes to track the feeding habits, habitat use and trophic interactions of marine animals.

## **2. Material and methods**

### *2.1. Sampling and study area*

Samples were collected during the research cruises of the *Projeto Talude* (Continental Slope Project) on board the RV *Atlântico Sul* during austral spring ( $n = 3$ ) and autumn ( $n = 3$ ) along the outer continental shelf and slope in southern (SCM, 34°S-28°S) and southeastern (NCSM, 28°S-24°S) Brazil (Fig. 1). The study area was divided into these two regions (SCM and NCSM) according to their distinct oceanographic characteristics, as described above. Within each region, we considered separately the results of samples collected at stations along the outer continental shelf and shelf break (*NCSM-SB* and *SCM-SB*,  $\approx 90$ –600 m depth) and those *offshore* (*NCSM-OFF* and *SCM-OFF*) along the continental slope ( $\approx 1100$ –3100 m).

The zooplankton samples as well as environmental information such as sea surface temperature (SST) and salinity (SSS) were collected at fixed (pre-determined) oceanographic stations throughout the study area (Fig. 1). Zooplankton samples were collected using 300 and 500  $\mu\text{m}$  mesh-sized Bongo nets. They are a composite of the whole vertical profile of each station, collected from depths of up to 200 m to the surface, depending on the local bottom depth. Samples from both net were mixed and kept frozen at  $-20^{\circ}\text{C}$  until analysis. Water temperature and salinity at depth were recorded at each station using the conductivity-temperature-depth (CTD) downcast profiles (SeaBird CTD/Carousel 911 + system®). The values for SSS and SST presented for each station were the average of the upper 200 m in the water column.

### *2.2. Stable Isotope Analysis*

After thawing and drying the samples at  $60^{\circ}\text{C}$  a subsample of mixed zooplankton was collected from the main sample and processed for stable isotope analysis. The remaining sample was then sorted into the main taxonomic groups (amphipods, chaetognaths, copepods, euphausiids and salps) for separate isotope analyses, with each taxon subsample including multiple organisms. Samples were then rinsed with distilled water, dried in an oven at  $60^{\circ}\text{C}$  for 48 h, and ground to a fine powder using a mortar and pestle. Acidification with hydrochloric

acid (HCl) of invertebrates with calcareous structures is usually recommended when analysing carbon isotopes for food web studies (Carabel et al., 2006) as it removes the inorganic C that results in higher  $\delta^{13}\text{C}$  values. Nevertheless, such treatment significantly affects the samples'  $\delta^{15}\text{N}$  values (Bunn et al., 1995; Carabel et al., 2006), while it seems not to always affect C isotope ratios (Grey et al., 2001). Therefore, we chose not to treat our samples with HCl.

Lipid extraction was carried out to compare the  $\delta^{13}\text{C}$  values between treated and non-treated samples, by rinsing the samples with chloroform:methanol (2:1) solution for 24 h with a Soxhlet. Due to the effects that chemical lipid extraction have on  $\delta^{15}\text{N}$  it is recommended to double analyze each individual sample separately, allowing for the interpretations of  $\delta^{13}\text{C}$  using the chemically-treated samples, while the non-chemically-treated samples are used for the nitrogen isotopes (Ryan et al., 2012). Nevertheless, double analyses were not always feasible considering our large sample size. Additionally, most individual samples did not have enough material for double analyses. For that reason, only a portion of the samples was double analyzed and mathematical correction was carried out for the samples which chemical lipid extraction was not performed. The mathematical correction was based on the equation adapted from Post et al., (2007), using the difference between  $\delta^{13}\text{C}$  values of treated and non-treated samples, and its relation with the carbon-to-nitrogen ratios (C:N) in a linear model to estimate the zooplankton specific coefficients:  $\delta^{13}\text{C}_{\text{lipid-corrected}} = \delta^{13}\text{C}_{\text{untreated}} + (\alpha + \beta \times \text{C:N})$ , where  $\alpha$  is the value of  $\delta^{13}\text{C}$  when C:N is zero and  $\beta$  is the slope coefficient, or the rate of changes in  $\delta^{13}\text{C}$  for each unit increase in C:N. The slope and the intercept coefficients were estimated separately for the copepods, euphausiids and chaetognaths (see table S1 for each group's coefficients). We did not estimate the parameters for the remaining groups due to small sample size that resulted in low quality of the model fit. Since amphipods' C:N ratios were also significantly different between chemically-treated and untreated samples (paired t-test = -14.2, df = 3,  $p < 0.001$ ), we corrected  $\delta^{13}\text{C}_{\text{amphipods}}$  using the equation for aquatic invertebrates:  $\delta^{13}\text{C}_{\text{lipid-corrected}} = \delta^{13}\text{C}_{\text{untreated}} + 3.388 - ((3.388 \times 3.314)/\text{C:N})$  (Logan et al., 2008). The analysis of stable isotopes in salps does not require any prior chemical treatment or mathematical correction since these organisms do not store large quantities of lipids and their  $\delta^{13}\text{C}$  is not affected (detailed review in Pakhomov et al., 2019). We present the mathematically corrected results of  $\delta^{13}\text{C}$  along with the measured (non-treated) ones for general knowledge and discussion. Nonetheless, the focus of the present study is on documenting the stable isotope composition of organisms representing potential food sources for higher trophic levels. Since dietary lipid carbon is also used to synthesize proteins when animals are feeding on a lipid rich diet (Wolf et al., 2015),

our analysis will focus mainly on the  $\delta^{13}\text{C}$  of samples from which no lipid extraction was performed.

Between 0.5 and 0.8 mg of zooplankton samples were weighed into tin capsules and sent to the Stable Isotope Core Laboratory, Washington State University, for analysis of stable isotope compositions with a continuous flow Isotope Ratio Mass Spectrometer (IRMS, Delta PlusXP, Thermofinnigan) connected to an Elemental Analyser (EA, ECS 4010, Costech Analytical, Valencia, CA). Stable isotopes of carbon and nitrogen are expressed in the delta notation ( $\delta$ ) as the relative per mil (‰) difference between the sample and the standard reference material:  $\delta^{\text{HX}} = [\text{R}_{(\text{SAMPLE})} / \text{R}_{(\text{STANDARD})} - 1] \times 1000$ , where  $\text{HX}$  is the heavy isotope of C or N and R is the ratio of heavy to light isotope ( $^{13}\text{C}:^{12}\text{C}$  or  $^{15}\text{N}:^{14}\text{N}$ ) of the sample or the standard. The international standard for carbon is Vienna Pee Dee Belemnite (VPDB) and for nitrogen is atmospheric  $\text{N}_2$ . Internal lab isotopic reference materials were spread among the samples and analysed for calibration. The within-run analytical precision (standard deviation) of the internal lab standards was 0.05‰ for  $\delta^{13}\text{C}$  and 0.09‰ for  $\delta^{15}\text{N}$ .

### 2.3. Isotopic maps

We used data on organic carbon and nitrogen natural stable isotopic composition to develop seasonal (spring and autumn) isotopic maps (isoscapes) characterizing the zooplankton patterns throughout the study area. The isoscapes included  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data from mixed zooplankton (mixture) and each of the main zooplankton taxonomic groups (amphipods, chaetognaths, copepods, euphausiids and salps). Isoscapes were generated using the freely available software Ocean Data View (ODV) version 5.1.5 (Schlitzer, 2002). The data-interpolating variational analysis (DIVA) gridding method was used for contouring the data, as it produces better interpolation even when data coverage is irregularly spaced (Troupin et al., 2012). The method analyses and interpolates the data taking into account environmental features such as bathymetry, coastlines and currents (Troupin et al., 2012). The latitude and longitude length-scales to estimate the smoothness of the isoscapes were adjusted automatically by DIVA in ODV according to the distribution of the data (the number of samples per zooplankton group in each season). For more details on the method we refer the readers to the Ocean Data View User's Guide Version 5.1.0 and to Troupin et al. (2012). *Statistical analysis*

Shapiro and Levene's test were applied to assess normality and homogeneity of variances, respectively. As the data did not meet the assumptions for parametric statistical analysis, Welch Two Sample t-test was applied to assess whether there were significant

differences in the mean  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , SSS and SST between groups. The isotopic differences among the zooplankton groups, years, and subareas (NCSM-OFF, NCSM-SB, SCM-OFF and SCM-SB) were assessed with the Welch one-way ANOVA, followed by a post-hoc analysis with the Games-Howell Test (Ruxton and Beauchamp, 2008; Shingala and Rajyaguru, 2015). The Welch's test offers solution for comparing populations with unequal variances and has good properties when the normality assumption does not hold (Ruxton, 2006; Rasch et al., 2011). Moreover, the Games-Howell Test is a non-parametric method to compare groups, hence not assuming equal variances or normality (Shingala and Rajyaguru, 2015).

Generalized additive models (GAMs, Hastie and Tibshirani, 1990) were fitted with cubic regression splines to estimate the smoothing curves and model the relationship between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and *latitude* (continuous variable) by *area* (data collected at stations along the shelf break vs offshore), *groups* (mixture, amphipods, chaetognaths, copepods, euphausiids and salps), *season* (spring and autumn) and *year* (2012-2015) as categorical (nominal) variables. As no explanatory variables were collinear ( $r < 0.7$ ), all variables were used in the models. GAMs were fitted with Gaussian family and identity link function. The amount of smoothing ( $\lambda$ ) was not fixed, and the optimal  $\lambda$  was estimated using cross-validation. In the *mgcv* package, *gam* function uses the *estimated degrees of freedom (edf)* to express the output of the cross-validation (or the optimal  $\lambda$ ). We used backward selection with the least significant variable being excluded from the model at each step until all remaining variables were significant. Residuals were checked for normality and for residual patterns (Zuur et al., 2009). The best models were selected using Akaike's information criterion (AIC) and minimized generalized cross-validation (GCV) score. We present the smoothing function (*edf*) showing the relationship of both C and N with latitude per area (offshore and shelf break). Unless stated otherwise, we present  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  average values  $\pm$  standard deviation. All statistical analyses were carried out using the free software R version 3.6.1 (R Core Team, 2019). GAMs were fitted using the *mgcv* package in R (Wood, 2011).

### **3. Results**

#### *3.1. Temperature & Salinity*

The mean SST ( $^{\circ}\text{C}$ ) and SSS in the upper 200 m of the water column are shown in Table S2 as the overall mean ( $\pm\text{SD}$ ) for each region (NCSM and SCM), area (shelf break and offshore), season (autumn and spring) and year (2012-2015). During both spring and autumn cruises, SSS had the highest values in the NCSM-OFF ( $37\pm 0.2$  in spring and  $37\pm 0.3$  in autumn)

and the lowest in the SCM–SB ( $35\pm 0.8$  and  $36\pm 0.9$ , see S2 and S3). There were no seasonal differences in SSS, but it was significantly lower in the SCM ( $t$ -test =  $-4.05$ ,  $df = 55.68$ ,  $p < 0.001$ ) and along the shelf break ( $t$ -test =  $-2.14$ ,  $df = 56.45$ ,  $p < 0.05$ ). SST was lower in spring ( $19.9\pm 1.9^\circ\text{C}$ ) than in autumn ( $20.9\pm 1.3^\circ\text{C}$ ,  $t$ -test =  $2.15$ ,  $df = 26.93$ ,  $p < 0.05$ ) and higher in the NCSM ( $21.3\pm 1.8^\circ\text{C}$ ) than in the SCM ( $20.2\pm 1.3^\circ\text{C}$ ,  $t$ -test =  $-2.64$ ,  $df = 39.53$ ,  $p < 0.05$ ). The mean SST in each subarea per year is shown in Fig. 2 along with the results of the statistical tests.

### 3.2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the main zooplankton groups

Seventy-three samples of zooplankton were collected throughout the six cruises in the austral spring and autumn between 2012 and 2015 for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analyses. The isotopic compositions and number of samples for each zooplankton group are presented in Table 1. Details on the number of stations and samples of each group per cruise are provided in Table S4. Mean  $\delta^{13}\text{C}$  values were significantly different among the zooplankton groups (Welch ANOVA  $F(5,114) = 7.53$ ,  $p < 0.0001$ ). Highest mean  $\delta^{13}\text{C}$  values were usually observed in mixed zooplankton (Table 1, Fig. 3 and 4). The copepods were the most  $^{13}\text{C}$ -depleted group (Fig. 3 and 4), significantly differing from amphipods ( $p < 0.05$ ), mixture ( $p < 0.001$ ) and chaetognaths ( $p < 0.05$ ). Mathematically corrected  $\delta^{13}\text{C}$  was significantly higher than the measured  $\delta^{13}\text{C}$  in copepods ( $t$ -test =  $8.13$ ,  $df = 149.97$ ,  $p < 0.001$ ), euphausiids ( $t$ -test =  $12.18$ ,  $df = 126.6$ ,  $p < 0.001$ ) and chaetognaths ( $t$ -test =  $11.42$ ,  $df = 100.23$ ,  $p < 0.001$ ). Differences in  $\delta^{15}\text{N}$  values were also significant ( $F(5,118) = 3.78$ ,  $p < 0.005$ ). Chaetognaths had the highest mean  $\delta^{15}\text{N}$  in all areas, usually followed by euphausiids (Fig. 3 and 4). They were significantly more  $^{15}\text{N}$ -enriched than copepods ( $p < 0.005$ ), amphipods ( $p < 0.05$ ), and mixture ( $p < 0.05$ ). Amphipods and copepods were frequently the two most  $^{15}\text{N}$ -depleted zooplankton groups (Fig. 3 and 4).

### 3.3. Spatiotemporal patterns in zooplankton $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

We found significant differences in mean  $\delta^{15}\text{N}$  (Welch ANOVA  $F(3,150) = 45.88$ ,  $p < 0.001$ ) and  $\delta^{13}\text{C}$  ( $F(3,142) = 7.512$ ,  $p < 0.001$ ) among the four subareas. The spatial patterns in zooplankton  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were similar in both spring and autumn seasons: the highest  $\delta^{15}\text{N}$  values were observed in the SCM-SB, followed by SCM-OFF and NCSM-SB, while the NCSM-OFF had the lowest  $\delta^{15}\text{N}$  values (Fig. 5a). The post-hoc Games-Howell was significant for all pair-wise comparisons in  $\delta^{15}\text{N}$  ( $p < 0.001$ ), except between the NCSM-SB and SCM-OFF subareas ( $p = 0.99$ ). Conversely, the NCSM-OFF had the highest  $\delta^{13}\text{C}$  values,

significantly more  $^{13}\text{C}$ -enriched than every other subarea ( $p < 0.001$ ). Intermediate  $\delta^{13}\text{C}$  values were observed in the SCM-OFF and SCM-SB, followed by the most  $^{13}\text{C}$ -depleted NCSM-SB (Fig. 5b), though differences in  $\delta^{13}\text{C}$  among the latter three areas were not significant ( $p > 0.05$ ). The isotopic maps for spring (Fig. 6) and in autumn (Fig. 7) seasons display the patterns in organic  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  along the study area. There was a shelf break-to-offshore enrichment in  $^{13}\text{C}$  ( $t$ -test = -3.22,  $df = 257$ ,  $p < 0.01$ ), while an opposite coast-ocean trend was evident in  $\delta^{15}\text{N}$  values, which were more  $^{15}\text{N}$ -enriched along the shelf break ( $t$ -test = 8.22,  $df = 264.7$ ,  $p < 0.001$ ). Additionally, significant seasonal differences were found in both stable isotopes:  $\delta^{13}\text{C}$  was significantly higher in autumn ( $t$ -test = 3.18,  $df = 231.8$ ,  $p < 0.01$ ), whereas higher  $\delta^{15}\text{N}$  values were observed in spring ( $t$ -test = -4.17,  $df = 237.5$ ,  $p < 0.001$ ). There were no significant differences in  $\delta^{15}\text{N}$  between the years 2012 and 2015 (Welch ANOVA  $F(3,94)=2.25$ ,  $p = 0.08$ ). However,  $\delta^{13}\text{C}$  was significantly lower in 2012 than in the later years ( $F(3,87)=35.4$ ,  $p < 0.001$ ).

The best GAM to describe  $\delta^{13}\text{C}$  patterns included latitude by area (*shelf break* and *offshore*), taxonomic groups and year (Table 2). Smoother for the shelf break area was significant for  $p < 0.05$  ( $edf = 8.64$ ), showing high oscillations in  $\delta^{13}\text{C}$  along the shelf break (Fig. 8a). The significant smoother for the offshore area indicated a negative relationship with latitude ( $edf = 6.26$ ,  $p < 0.001$ ), with lower  $\delta^{13}\text{C}$  values towards the south of the study area (Fig. 8b). The GAM that best described the patterns in  $\delta^{15}\text{N}$  was significant to all explanatory variables (latitude, area, group, season and year, Table 2). Shelf break was 2‰ more enriched in  $^{15}\text{N}$  than offshore, and  $\delta^{15}\text{N}$  in spring was estimated to be approximately 1‰ higher than in autumn. The curves showed a southward increasing trend in  $\delta^{15}\text{N}$  (Figs. 9a and b).

#### 4. Discussion

Plankton-based isoscapes on the ocean basin-scale display the spatio-temporal patterns in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Graham et al., 2010; McMahon et al., 2013; Magozzi et al., 2017), that help the interpretation of studies using stable isotopes to understand the trophic ecology and dynamics of marine organisms. However, characterizing the baseline isotopic variations at a finer spatial scale is essential for better assessments of the foraging ecology and habitat use in local species and communities. In the present work we describe the patterns of baseline carbon and nitrogen stable isotope composition along the shelf break and continental slope in the subtropical western South Atlantic Ocean. Although assessing the trophic relationships within

the zooplankton community is beyond the scope of this study, we will briefly discuss the overall differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among the different taxonomic groups, taking into account what is known about such relationships. This is because identified isotopic differences result from relevant ecological (e.g., trophic level) and physiological (e.g., tissue type and macromolecular components) characteristics that ought to be taken into account prior to considering the isotopic variations in relation to regional oceanographic processes. Subsequently, we shall discuss the overall isoscape patterns observed throughout the study area that were consistent in all zooplankton groups.

#### *4.1. Taxonomic differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$*

Copepods were the most  $^{13}\text{C}$ -depleted group in all areas, with the exception of the NCSM-SB, where euphausiids had the lowest  $\delta^{13}\text{C}$  values. Both copepods and euphausiids showed differences between lipid-corrected and measured (untreated)  $\delta^{13}\text{C}$ , with the latter being significantly more  $^{13}\text{C}$ -depleted. Due to the high discrimination against  $^{13}\text{C}$  during their synthesis (DeNiro and Epstein, 1977, 1978), lipids are relatively more depleted in the heavy carbon isotopes than the other macromolecular components, including proteins. Even after lipid correction copepods had the lowest  $\delta^{13}\text{C}$ , whereas euphausiids showed higher  $\delta^{13}\text{C}$  values along with the chaetognaths (see S5). The significant differences between corrected and measured  $\delta^{13}\text{C}$  suggests that the high lipid concentration in their tissues were influencing their carbon isotopes, but differences between groups were due to their relative trophic positions (DeNiro and Epstein, 1978; Post, 2002). Furthermore, chaetognaths and euphausiids were significantly more enriched in  $^{15}\text{N}$  than the other zooplankton groups. The higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values observed in chaetognaths coincide with their higher trophic position within the zooplankton community, as this group includes exclusively carnivorous species, with copepods as the main item in their diet (Sullivan, 1980; Liang and Vega-Pérez, 1995). Chaetognaths and euphausiids were between 0.8 and 2.3% more enriched in  $^{15}\text{N}$  than copepods, depending on the region. Such  $^{15}\text{N}$ -enrichment could indicate that euphausiids occurring in the shelf break and continental slope of the subtropical western South Atlantic prey on copepods, as has been reported elsewhere (Gibbons et al., 1991), especially when phytoplankton abundance is low. Copepods and salps had the lowest  $\delta^{15}\text{N}$  values. Decreasing phytoplankton abundances have been associated with high densities of copepods, and regarded as a result of the grazing pressure by the latter (Brandini et al., 2014; Duarte et al., 2014). Salps have a filter-feeding behaviour, and the size range of preyed particles indicates that they feed mostly on phytoplankton (Harbison and McAlister, 1979; Vargas and Madin, 2004). Thus, the lowest  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$

values observed in salps and copepods support their lower trophic positions relative to the other zooplankton groups, as well as their role as primary consumers in the food web of this region. On the other hand, chaetognaths and euphausiids seem to represent the top of the zooplankton food chain throughout the study area.

Salps and amphipods had very similar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, showing no evidence for trophic level distinctions between these two groups. This could indicate an overlap in the use of resources, with amphipods feeding on the particles filtered by the salps, which is a feeding strategy observed in some amphipod species (Madin and Harbison, 1977). However, amphipods collected in the NCSM-OFF area were on average 1.5‰ more enriched in  $^{15}\text{N}$  than the salps in that same region. It is known that some hyperiid-amphipod species feed on their host's tissues (Riascos et al., 2015). As oceanographic patterns along the study area reflect prevalence of oligotrophic conditions with very low phytoplankton biomass, this could help explain the apparent amphipods' predation on their host's tissues only in the offshore area in the NCSM. As previously mentioned, throughout our study area the influence of distinct water masses results in differences in the amount of suspended particles in the water column between the NCSM and the SCM regions. Subantarctic waters, upwellings and continental water input influence the SCM region (Ciotti et al., 1995; Lima et al., 1996; Piola et al., 2008a), while seasonal wind induced upwelling of SACW along the shelf break plays a role in the NCSM region (Coelho-Souza et al., 2012) by increasing nutrient concentrations that enhance primary productivity, consequently mounting food availability for herbivorous zooplankton (Odebrecht and Garcia, 1997; Guenther et al., 2008; Duarte et al., 2014). In contrast, the NCSM-OFF area is mainly influenced by warm, nutrient-depleted tropical waters carried by the Brazil Current (Castro et al., 2006; Braga and Niencheski, 2006), resulting in lower concentrations of suspended particles in the water column (Valentin and Monteiro-Ribas, 1993). Reduced availability of food for herbivorous zooplankton could result in the prevalence of carnivorous amphipod species in the offshore community, with possible increased consumption upon gelatinous zooplankton tissues, unlike the other areas, where filtering of suspended matter seems to support the feeding requirements of herbivorous amphipods.

Mixed samples had the highest  $\delta^{13}\text{C}$  values relative to the other zooplankton groups. This could be due to the presence of pteropods, holoplanktonic gastropods with aragonite calcium carbonate shells (Van der Spoel and Dadon, 1999). Because of their small size and relatively low abundances within our samples, we were unable to obtain sufficient material to analyse pteropods for stable isotopes separately. However, one of the oceanographic stations had a

relatively large abundance of pteropods, and we had enough material to analyse them for carbon and nitrogen stable isotopes. In that station, the  $\delta^{13}\text{C}$  of mixed zooplankton was the highest of all mixture samples (-15.9‰). Additionally, while the  $\delta^{13}\text{C}$  of pteropods was -12.1‰, the remaining zooplankton groups were considerably more depleted in  $^{13}\text{C}$  (-21.9‰ in amphipods and -20.5‰ in copepods). As dissolved inorganic carbon (which is enriched in  $^{13}\text{C}$  relative to organic carbon) plays a greater role in determining the shell carbonates  $\delta^{13}\text{C}$  of aquatic invertebrates (Keith et al., 1964; Mook, 1971) in comparison to dietary carbon (DeNiro and Epstein, 1978), calcareous shells have significantly higher  $\delta^{13}\text{C}$  values than organic carbon, contributing to whole-body enrichment in  $^{13}\text{C}$ . Despite the lack of replicates for statistical inferences on the influence of pteropods on whole mixture  $\delta^{13}\text{C}$  in the other oceanographic stations, it seems that carbonates in their shells have contributed to the observed  $^{13}\text{C}$ -enrichment. As stated in the methods, we did not treat the samples with HCl prior to stable isotope analysis. Thus, while the presence of inorganic carbon may not have influenced the  $\delta^{13}\text{C}$  values on the other zooplankton groups (e.g., amphipods and copepods), it most likely affected the C isotopes in mixture samples.

#### *4.2. Latitudinal and seasonal patterns in zooplankton isoscapes*

Values of  $\delta^{13}\text{C}$  along the study area showed  $^{13}\text{C}$ -depletion towards the south, with the offshore area being  $^{13}\text{C}$ -enriched in comparison to the waters along the shelf break (Fig. 8). Such patterns were evident in all zooplankton groups and were apparent in the isotopic maps (Figs. 6 and 7). Factors responsible for variations in  $^{13}\text{C}:^{12}\text{C}$  ratios at the base of the food webs include aqueous  $\text{CO}_2$  concentrations (depending on SST), phytoplankton assemblage and specific growth rates (Laws et al., 1995; Popp et al., 1998; Magozzi et al., 2017). Lower phytoplankton  $\delta^{13}\text{C}$  values are associated with higher concentrations of aqueous  $\text{CO}_2$  (at lower SST) and/or low phytoplankton growth rates (Laws et al., 1995; Rau et al., 1996). Zooplankton  $^{13}\text{C}$ -depleted values along the shelf break could be due to the influence of continental water input from the Patos Lagoon and La Plata River (the Plata Plume Water, PPW), as  $\delta^{13}\text{C}$  values for phytoplankton and zooplankton are more negative in marine regions under the influence of riverine/terrestrial waters (Schell et al., 1998; Graham et al., 2010). Our temperature and salinity data showed the influence of continental waters along the shelf break, especially in the SCM region, where temperature (Fig. 2) and salinity (see S3) were lower than in the other areas. Lima et al. (2019), in a study on the phytoplankton communities along the same study area and period, have identified the presence of five water masses: PPW was present along the shelf break in the SCM region, Subtropical Shelf Water and Shelf Water occurred along the

shelf break from NCSM to SCM, while Tropical Water was observed along the offshore area. PPW dominates along the continental shelf during autumn and winter in the SCM region in southern Brazil (Piola et al., 2008b; Möller Jr. et al., 2008), and low  $\delta^{13}\text{C}$  values have been reported for sedimentary and suspended particulate organic matter (-23.5‰ to -18.9‰) sampled in the Patos Lagoon (Mont'Alverne et al., 2016). Zooplankton  $\delta^{13}\text{C}$  values vary according to the C isotopic composition of primary producers that sustain the base of their food web, which in turn will be strongly influenced by the  $\delta^{13}\text{C}$  values of the ambient dissolved inorganic carbon (DIC) pool (McMahon et al., 2013).  $\delta^{13}\text{C}_{\text{DIC}}$  usually ranges between -11 and -16‰ in freshwater, although it can be as low as -24‰ (Amazon River) (Mook and Tan, 1991). In contrast,  $\delta^{13}\text{C}_{\text{DIC}}$  is higher in oceanic waters, often between -1 to +2‰ (Sackett and Moore, 1966). Therefore, phytoplankton growing under the influence of freshwater DIC will have lower  $\delta^{13}\text{C}$  values (Fry and Sherr, 1989; De Brabandere et al., 2002), and so will its zooplanktonic consumers. The PPW plays an important role in increasing nutrient concentrations and, consequently, phytoplankton growth in southern Brazilian coastal waters (Ciotti et al., 2010; Gonçalves-Araujo et al., 2018), which will be available for consumption and sustaining large zooplankton biomasses (Duarte et al., 2014). Furthermore, upwelling of SACW along the shelf break (Palma and Matano, 2009; Coelho-Souza et al., 2012) could explain the relatively lower values of zooplankton  $\delta^{13}\text{C}$  in comparison to the offshore area. Our CTD data showed lower SST along the shelf break and in spring (see section 3.1 and S2). Additionally, our water temperature- and salinity-depth profiles recorded the presence of SACW (SST 15-20°C, SSS 35-36) (Castro et al., 2006; this work) reaching the surface (~25 m deep) in the NCSM-SB during spring months (data not shown). While in surface water  $\delta^{13}\text{C}_{\text{DIC}}$  is more  $^{13}\text{C}$ -enriched because of the preferential removal of the light carbon isotope by phytoplankton (Gruber et al., 1999), lower  $\delta^{13}\text{C}$  values are observed in deep-water DIC, resulting from bacterial respiration on sinking isotopically light phytoplankton detritus (Kroopnick, 1985). Accordingly, upwelling events bring  $^{13}\text{C}$ -depleted DIC from deep waters (Gruber et al., 1999; McMahon et al., 2013), which will be available for phytoplankton growth in the photic zone. Thus, the lower  $\delta^{13}\text{C}$  values along the shelf break, especially off the region of Cape Frio in Rio de Janeiro (NCSM-SB, Fig. 5), are likely a consequence of the input of  $^{13}\text{C}$ -depleted upwelled DIC used by phytoplankton for growth.

The highest  $\delta^{13}\text{C}$  values were observed in the NCSM-OFF subarea. Published data on the surface-water  $\delta^{13}\text{C}_{\text{DIC}}$  for the western South Atlantic indicate a slight trend of south-to-north increasing values between 35°S and 10°S, a latitudinal zone which covers the area studied

here (Gruber et al., 1999; Quay et al., 2003). Higher  $\delta^{13}\text{C}_{\text{DIC}}$  values in NCSM-OFF waters will result in phytoplankton being more enriched in  $^{13}\text{C}$  and, consequently, in higher zooplankton  $\delta^{13}\text{C}$  values. The latitudinal gradient in offshore zooplankton  $\delta^{13}\text{C}$  with more  $^{13}\text{C}$ -depleted values southwards (Fig. 8b) fits with the latitudinal  $\delta^{13}\text{C}_{\text{DIC}}$  gradient reported in the literature. The shelf-break-to-offshore enrichment gradient in  $^{13}\text{C}$  is most likely due to the contrast between the influence of  $^{13}\text{C}$ -depleted continental water and the deep-water DIC available for primary producers' growth along the shelf break, while in the offshore waters a north-to-south trend of decreasing  $\delta^{13}\text{C}_{\text{DIC}}$  will result in lower  $\delta^{13}\text{C}$  values at the base of the food web going southward. The zooplankton  $\delta^{13}\text{C}$  values along the shelf break did not show such a latitudinal trend (Fig. 8a). This might be a consequence of the influence of either terrestrial water on the continental shelf reaching the shelf break or due to upwelling events along the shelf break, as discussed above.

Moreover, zooplankton  $\delta^{15}\text{N}$  was higher along the shelf break ( $\approx +2\text{‰}$ ), in spring ( $\approx +1\text{‰}$ , Table 2) and increased towards the south of the study area (Fig. 9). Depletion in the heavy N isotopes observed in zooplankton sampled in the NCSM-OFF subarea (Table 1, Fig. 5) suggests the incorporation of N from a relatively more  $^{15}\text{N}$ -depleted food source. The NCSM-OFF area had the highest values for temperature and salinity, being strongly influenced by the oligotrophic tropical water mass during the study period (Lima et al., 2019). Some diazotrophic cyanobacteria, such as those of the genus *Trichodesmium*, assimilate  $\text{N}_2$  with very low isotope discrimination, resulting in biomasses with low isotopic ratios close to that of the atmospheric  $\text{N}_2$  source and lower than for fixed-N species dissolved in surface water (Capone et al., 2005; Mompean et al., 2013). Therefore, lower  $\delta^{15}\text{N}$  values are often found in oligotrophic waters, where  $\text{N}_2$  fixation can be a major source of nitrogen for phytoplankton. In Brazil, a large abundance of *Trichodesmium* has been observed in areas under the influence of tropical water, especially in the region north of Cape Santa Marta (Detoni et al., 2015; Lima et al., 2019). A sample of *Trichodesmium* collected in the NCSM in the spring of 2014 had high  $\delta^{13}\text{C}$  values ( $-18.4\text{‰}$ ) and very low  $\delta^{15}\text{N}$  values ( $-0.4\text{‰}$ , unpublished data). The same patterns of  $^{13}\text{C}$ -enrichment and  $^{15}\text{N}$ -depletion observed in the NCSM-OFF have been associated with the incorporation of fixed- $\text{N}_2$  into marine food webs, mainly in oligotrophic waters from tropical and subtropical regions (Carpenter et al., 1997; Mompean et al., 2013). Thus, organic carbon and nitrogen fixed by *Trichodesmium* could be assimilated by the zooplankton and expressed as both higher  $\delta^{13}\text{C}$  and lower  $\delta^{15}\text{N}$  values in the NCSM-OFF area. Additionally, in the same period as our sampling, higher concentration of dissolved inorganic nitrogen (DIN,

sum of the concentrations of nitrate, nitrite and ammonium) were observed towards the south of our study area, in the SCM region (Lima et al., 2019). The south-to-north decrease in zooplankton  $\delta^{15}\text{N}$  values along with increasing  $\delta^{13}\text{C}$  values observed in the offshore area could thus be the result of a higher contribution of *Trichodesmium* nitrogen fixation due to lower N-nutrient concentrations and higher  $\delta^{13}\text{C}$ -DIC values in the warmer oligotrophic NCSM waters. In contrast, towards the south, the increased influence of nutrient-rich waters providing other sources of nitrogen for phytoplankton growth resulted in relatively higher  $\delta^{15}\text{N}$  values; while lower  $\delta^{13}\text{C}$ -DIC (Gruber et al., 1999) led to lower  $\delta^{13}\text{C}$  values in primary producers, which is a pattern reflected in the zooplankton isotopic composition. Similar  $\delta^{15}\text{N}$  trends have been reported for the North Pacific, where copepods and chaetognaths sampled in the southern California Bight had higher  $\delta^{15}\text{N}$  values than those from the North Pacific Central Gyre (NPCG) (Mullin et al., 1984). The authors associated these results with the importance of  $\text{N}_2$  fixation in the NPCG, contrasting with the California Bight, where nitrate ( $\text{NO}_3^-$ ) was the main source of nitrogen for phytoplankton. In waters with higher nutrient concentrations, such as in coastal or upwelling zones,  $\text{NO}_3^-$  is the main source of nitrogen for primary producers. Deep-water  $\text{NO}_3^-$   $\delta^{15}\text{N}$  values tend to be constant around +5‰ (Sigman et al., 2009), while the surface-water  $\text{NO}_3^-$  pool becomes more enriched in  $^{15}\text{N}$  as growing phytoplankton discriminate against the heavy isotope (Altabet and Francois, 1994). Consequently, in waters where  $\text{NO}_3^-$  is the main nutrient source for primary producers, plankton will be characterized by relatively higher  $\delta^{15}\text{N}$  values in comparison with nutrient-poor areas where  $\text{N}_2$  fixation is the main source of fixed organic carbon and nitrogen (Montoya et al., 2002; Sigman et al., 2009). Such patterns were seen in our study with the latitudinal increase, as well as the shelf-break-to-offshore decrease in  $\delta^{15}\text{N}$  values: while the higher  $\text{N}_2$ -fixation offshore introduced isotopically light N to the system, along the shelf break and towards the south the higher DIN availability (Lima et al., 2019) resulted in relatively higher plankton  $\delta^{15}\text{N}$  values. Further, our environmental data provided support to the seasonal influence of upwelled SACW along the shelf break, where lower SST was registered in spring. Accordingly, the seasonal differences in the nitrogen isotope ratios, with higher mean zooplankton  $\delta^{15}\text{N}$  values in spring and along the shelf break, agree with our hypothesis that upwelling waters contributed to  $^{15}\text{N}$ -enriched deep-water nutrients into the photic zone.

It is widely recognized that understanding the spatio-temporal patterns in baseline  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  is crucial for the interpretation of animal stable isotopes in the context of migration and trophic ecological studies. Baseline  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isoscapes have been constructed in large

spatial scales in the Atlantic Ocean using available zooplankton stable isotope data (Graham et al., 2010; McMahon et al., 2013). Global-scale variations in phytoplankton  $\delta^{13}\text{C}$  have been predicted by models based on environmental variables such as  $\text{CO}_2$  concentrations and sea surface temperatures (Magozzi et al., 2017). Nevertheless, baseline isotopic values at a regional-scale are harder to predict, as they depend on information about the local conditions and variables that are not always available (Trueman and Glew, 2019). Although the isoscape patterns observed here were generally in line with predicted values for baseline  $\delta^{13}\text{C}$  in the western South Atlantic Ocean (-20 to -22‰),  $\delta^{15}\text{N}$  values were highly variable in comparison to previous studies (Graham et al., 2010; McMahon et al., 2013; Magozzi et al., 2017). Additionally, in the present study we were able to detect the latitudinal and longitudinal patterns at a finer scale of resolution, as well as the seasonal variations in baseline isotope values.

#### 4.3. Annual (2012-2015) changes in organic $\delta^{13}\text{C}$

Organic  $\delta^{13}\text{C}$  was significantly lower in 2012 than in the years 2013-2015 (see results for Welch ANOVA under section 3.3). Such pattern was evident in each of the individual zooplankton groups. The estimated increase in  $\delta^{13}\text{C}$  was 1.2 to 1.7‰ in 2013-2015 in comparison to 2012 (Table 2). The El Niño Southern Oscillation (ENSO) is characterized by warm/rainy (El Niño) and cold/dry (La Niña) phases. The effects of the strong La Niña that took place between 2010 and 2012 (Climate Prediction Center/NOAA, 2017) could have resulted in the lower  $\delta^{13}\text{C}$  values observed in 2012. Both phases of ENSO affect differently the wind intensity and patterns in the South Atlantic Ocean (Colberg et al., 2004). This climate abnormality could have caused wind stresses that intensified the advection of the colder deep SACW along the shelf break. Indeed, our CTD data showed that SST was lower along the shelf break in 2012 than it was in the same area in the following years (Fig. 2). There was only one cruise in 2012, taking place during spring, a period of more intense upwelling of the SACW (Acha et al., 2004). Regarding the annual  $\delta^{13}\text{C}$  and SST data within each season (table S6), the main differences between years were along the shelf break. The offshore area had similar values for SST and  $\delta^{13}\text{C}$  between years in spring cruises. Stronger upwelling due to enhanced wind stress explains the decreased SST along the shelf break in 2012. Furthermore, the ascension of deep SACW brings deep-water  $^{13}\text{C}$ -depleted DIC (as discussed in section 4.2), making it available for photosynthesis and subsequently to the zooplankton community. Additionally, deep waters have higher  $\text{CO}_2$  concentrations, which would result in greater apparent isotopic discrimination by the phytoplankton and in lower  $\delta^{13}\text{C}$  values of the grazing

zooplankton in 2012. The normal climate conditions in the later years contrasted with 2012, and the less intense advection of deep SACW, especially in autumn months, was evident in the higher SST and zooplankton  $\delta^{13}\text{C}$  values.

### 5. Conclusions

In the present work we provide a fine-scale description of the spatial and temporal patterns of zooplankton carbon and nitrogen stable isotope compositions in the subtropical western South Atlantic. Data on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  at the base of food webs and their regional and seasonal patterns provide a basis for further detailed assessment of the ecology and trophic relationships among pelagic animals occurring in the area. Zooplankton stable isotopes in south-southeast Brazil showed an enrichment in  $^{13}\text{C}$  from the shelf break to offshore waters, reflecting the upwelling advection of isotopically light DIC to the surface and the influence of continental waters along the continental shelf break, which contrasts with more  $^{13}\text{C}$ -enriched values offshore. On the other hand, the offshore area had significantly lower  $\delta^{15}\text{N}$  values than the shelf break did, especially in the region NCSM, indicating a larger contribution of fixed- $\text{N}_2$  to organic nitrogen; in contrast, the  $^{15}\text{N}$ -enrichment along the shelf break suggested the input of relatively more  $^{15}\text{N}$ -enriched nutrients from the upwelling of the SACW. Additionally, higher  $\delta^{15}\text{N}$  values were observed in zooplankton to the south of the study area, along both the offshore and shelf break areas, reflecting the input of nutrient rich waters in the south, in contrast with the oligotrophic waters from the Brazil current in the NCSM. Finally, lower  $\delta^{13}\text{C}$  values were observed towards the south in the offshore area. Nevertheless, in the continental shelf break no such pattern was observed most likely owing to the influence of continental waters and upwellings in this area, especially in the NCSM region, which in turn affected the organic carbon values concealing the latitudinal effect. The results presented here can contribute to future studies on the assessment of the feeding habits and trophic interactions, as well as the movement patterns of marine predators that occur in the marine ecosystems of the outer continental shelves and slopes in the subtropical western South Atlantic.

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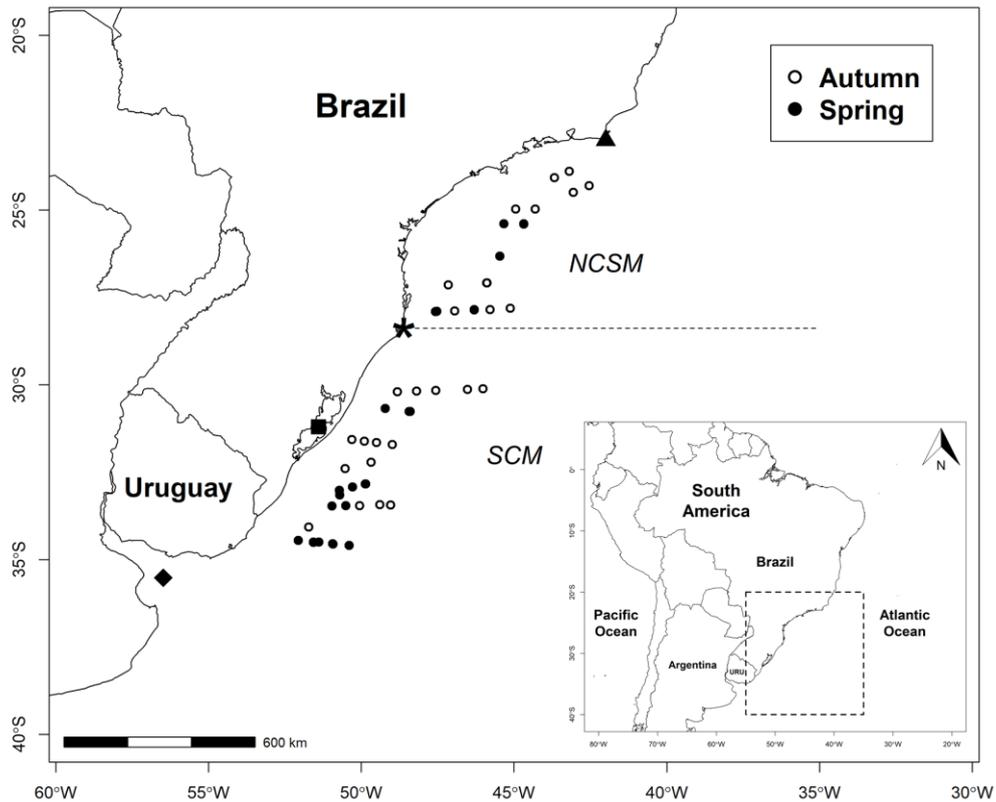
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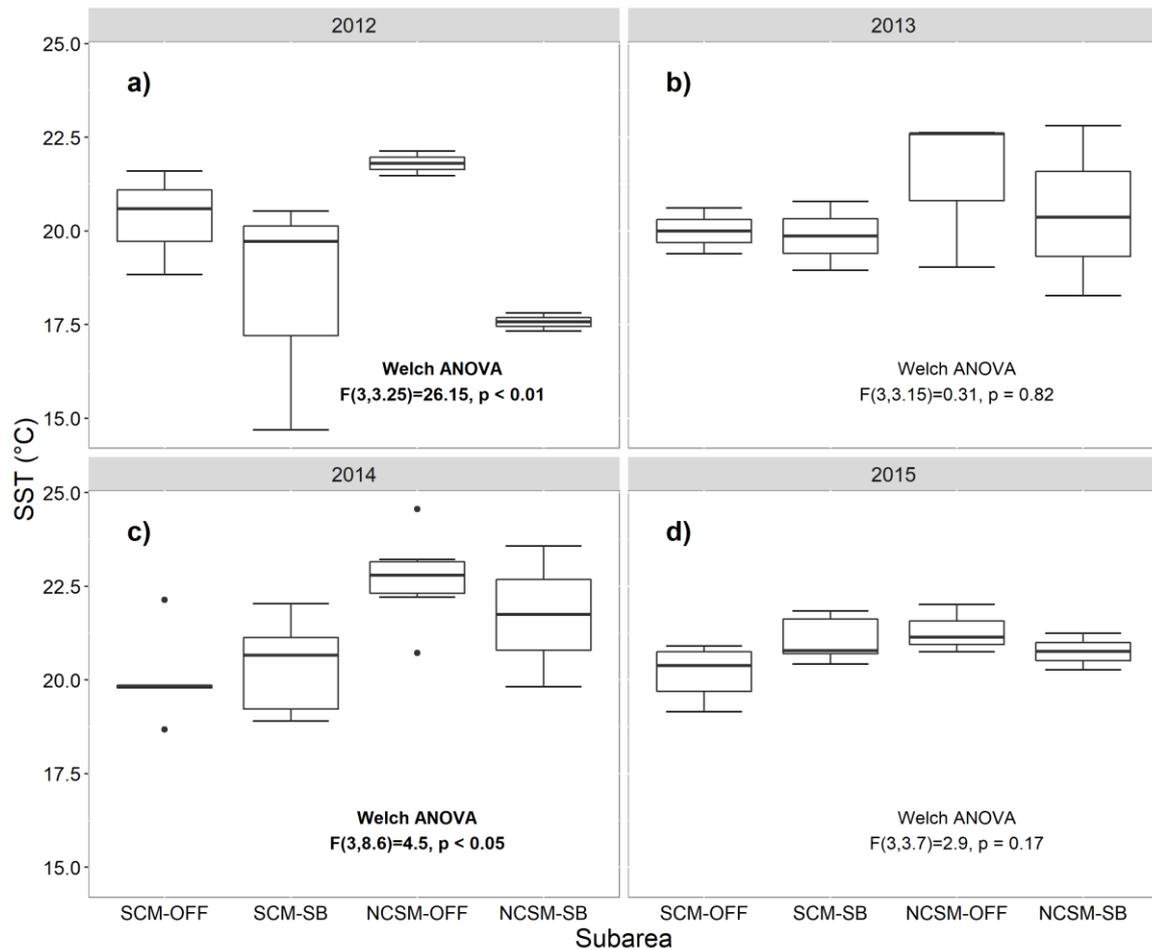
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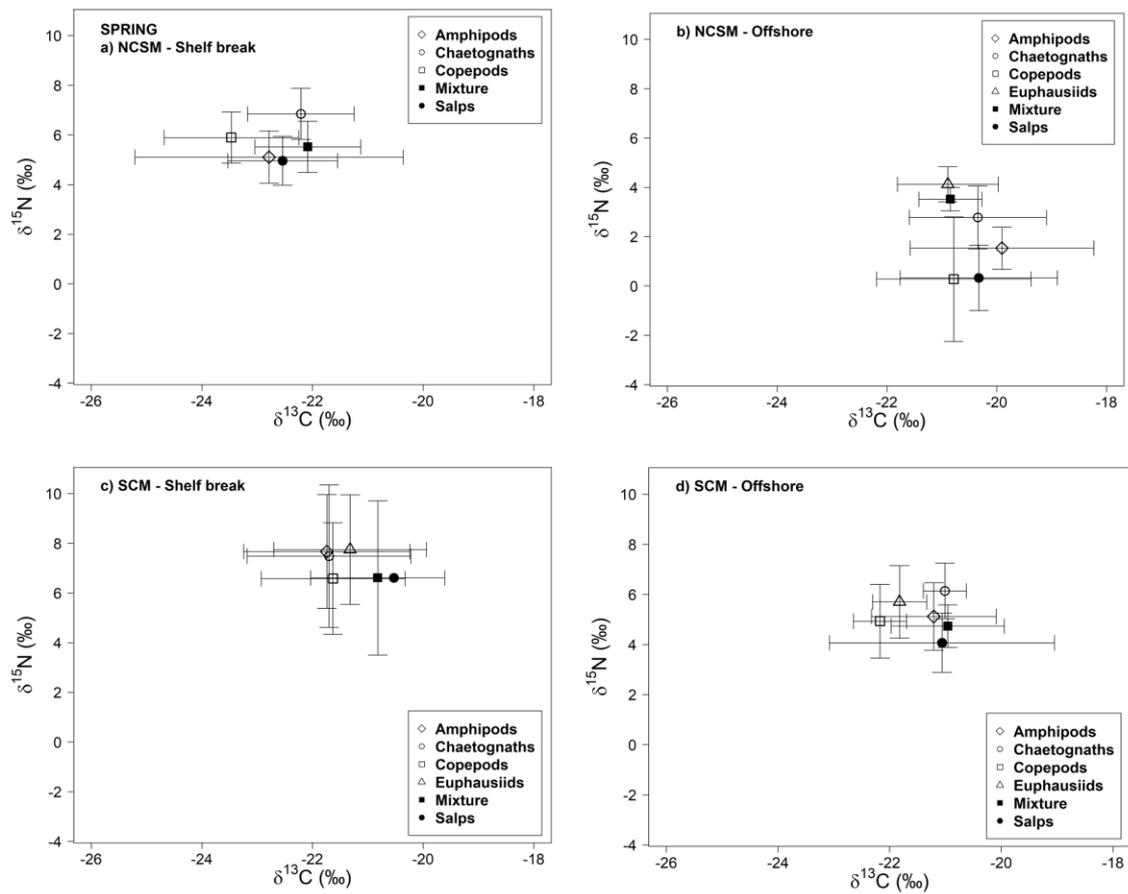
**Figure 1** The study area: the outer continental shelf and slope in south-southeast Brazil. Open and closed circles represent the location of the sampling stations in autumn and spring, respectively; Cape Santa Marta is indicated with ★; the dashed line delimitates the regions north (NCSM) and south (SCM) of Cape Santa Marta; ▲ indicates de position of Cape Frio (Rio de Janeiro); ■ the Patos Lagoon, and ◆ La Plata River.



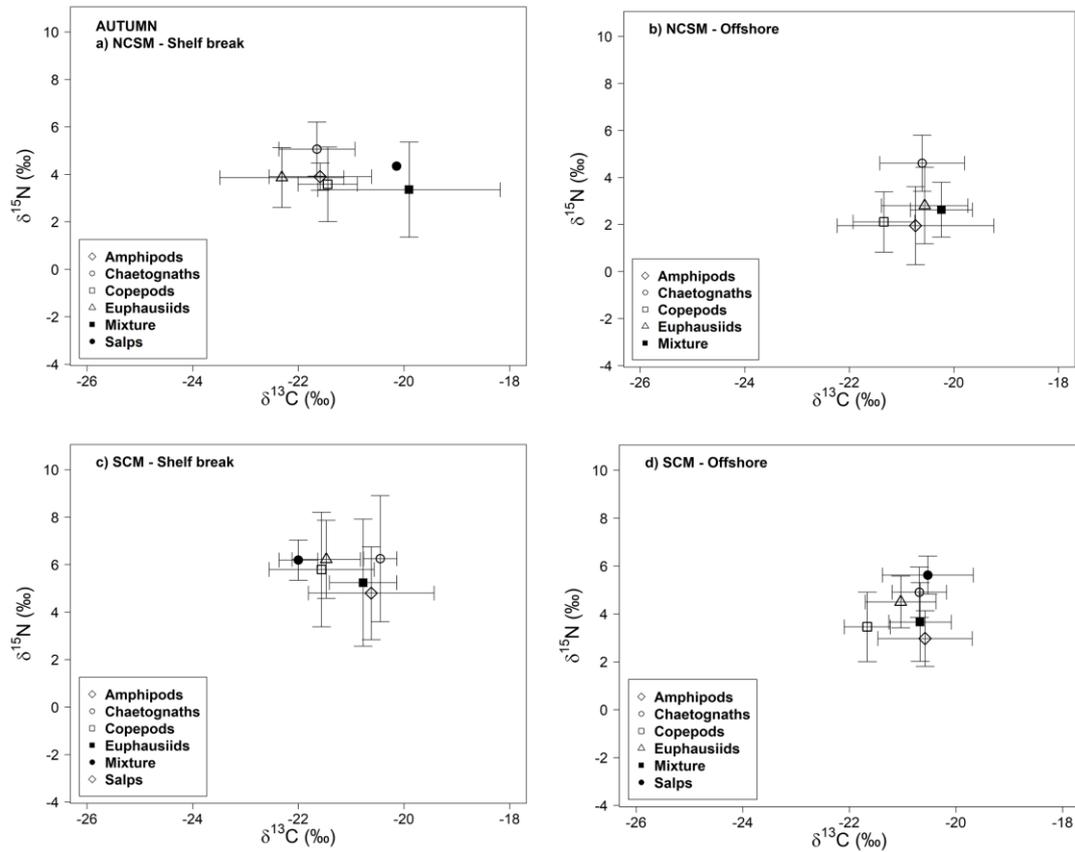
**Figure 2 Annual Sea Surface Temperature (SST, °C) in each subarea: North of Cape Santa Marta shelf break (NCSM-SB) and offshore (NCSM-OFF), and South of Cape Santa Marta shelf break (SCM-SB) and offshore (SCM-OFF). SST representing each oceanographic station is the average of the upper 200 m. Welch ANOVA tests showing significant differences in SST among the subareas are highlighted in bold.**

**Table 2** Number of samples (N), mean measured  $\delta^{13}\text{C}$  and lipid corrected ( $\delta^{13}\text{C}_{\text{Norm.}}$ ),  $\delta^{15}\text{N}$  and carbon-to-nitrogen (C:N) ratio ( $\pm$  Standard Deviation) in each of the main zooplankton taxonomic groups (Mixture, Amphipods, Chaetognaths, Copepods, Euphausiids and Salps) collected in each of the four oceanographic regions: North of Cape Santa Marta-shelf break (NCSM-SB ) and offshore (NCSM-OFF), and South of Cape Santa Marta-shelf break (SCM-SB) and offshore (SCM-OFF) along the Brazilian outer continental shelf and slope. C:N weight ratio was calculated from the percentage dry weight (%C and %N) in each sample.

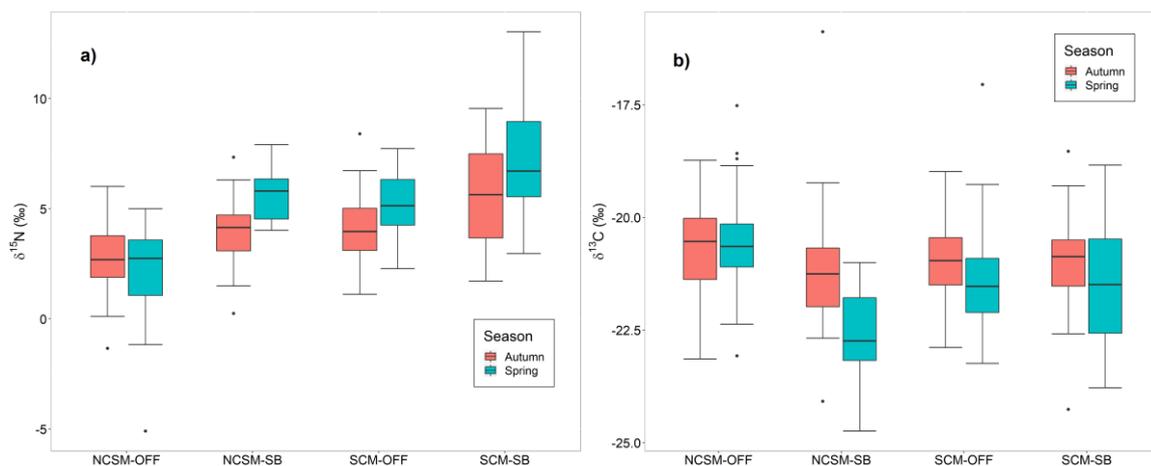
<b>Group</b>	<b>N</b>	<b><math>\delta^{13}\text{C}</math> (‰)</b>	<b><math>\delta^{13}\text{C}_{\text{NORM.}}</math> (‰)</b>	<b><math>\delta^{15}\text{N}</math> (‰)</b>	<b>C:N</b>
<b>NCSM-SB</b>					
<i>Mixture</i>	11	$-20.5 \pm 1.8$	-	$3.9 \pm 2.0$	$5.6 \pm 1.4$
<i>Amphipods</i>	8	$-21.9 \pm 1.3$	$-20.4 \pm 1.1$	$4.2 \pm 0.8$	$6.1 \pm 1.0$
<i>Chaetognaths</i>	9	$-21.8 \pm 0.8$	$-19.4 \pm 0.6$	$5.6 \pm 1.3$	$6.0 \pm 1.8$
<i>Copepods</i>	12	$-22.1 \pm 1.2$	$-20.4 \pm 1.1$	$4.3 \pm 1.7$	$6.4 \pm 2.9$
<i>Euphausiids</i>	5	$-22.3 \pm 1.1$	$-19.6 \pm 0.5$	$3.8 \pm 1.2$	$6.4 \pm 2.2$
<i>Salps</i>	5	$-22.0 \pm 1.4$	-	$4.8 \pm 0.9$	$6.1 \pm 1.5$
<b>NCSM-OFF</b>					
<i>Mixture</i>	14	$-20.4 \pm 0.6$	-	$2.9 \pm 1.0$	$5.0 \pm 0.4$
<i>Amphipods</i>	11	$-20.4 \pm 1.5$	$-18.9 \pm 1.4$	$1.8 \pm 1.3$	$6.2 \pm 1.0$
<i>Chaetognaths</i>	9	$-20.5 \pm 1.0$	$-18.5 \pm 1.1$	$3.6 \pm 1.5$	$5.2 \pm 0.9$
<i>Copepods</i>	16	$-21.1 \pm 1.0$	$-19.8 \pm 1.1$	$1.3 \pm 2.0$	$5.7 \pm 1.2$
<i>Euphausiids</i>	17	$-20.7 \pm 0.8$	$-19.1 \pm 0.6$	$3.2 \pm 1.5$	$4.7 \pm 1.0$
<i>Salps</i>	3	$-20.3 \pm 1.4$	-	$0.3 \pm 1.3$	$7.3 \pm 0.7$
<b>SCM-SB</b>					
<i>Mixture</i>	21	$-20.8 \pm 0.9$	-	$5.8 \pm 2.8$	$5.6 \pm 0.9$
<i>Amphipods</i>	19	$-21.1 \pm 1.4$	$-19.6 \pm 1.3$	$6.0 \pm 2.5$	$5.9 \pm 0.8$
<i>Chaetognaths</i>	16	$-21.2 \pm 1.3$	$-19 \pm 1.0$	$6.9 \pm 2.7$	$5.4 \pm 1.0$
<i>Copepods</i>	21	$-21.6 \pm 1.1$	$-20.2 \pm 1.1$	$6.1 \pm 2.3$	$5.7 \pm 1.5$
<i>Euphausiids</i>	15	$-21.4 \pm 1.0$	$-19.5 \pm 1.0$	$6.9 \pm 2.0$	$5.2 \pm 1.1$
<i>Salps</i>	3	$-21.5 \pm 0.8$	-	$6.3 \pm 0.6$	$4.9 \pm 1.0$
<b>SCM-OFF</b>					
<i>Mixture</i>	25	$-20.7 \pm 0.7$	-	$4.0 \pm 1.5$	$5.3 \pm 0.9$
<i>Amphipods</i>	20	$-20.8 \pm 0.9$	$-19.4 \pm 0.9$	$3.7 \pm 1.6$	$5.7 \pm 0.6$
<i>Chaetognaths</i>	19	$-20.8 \pm 0.5$	$-19 \pm 0.5$	$5.3 \pm 1.2$	$4.8 \pm 0.5$
<i>Copepods</i>	27	$-21.8 \pm 0.5$	$-20.8 \pm 0.4$	$3.9 \pm 1.6$	$5.2 \pm 0.6$
<i>Euphausiids</i>	30	$-21.3 \pm 0.7$	$-19.6 \pm 0.7$	$4.9 \pm 1.3$	$4.8 \pm 0.8$
<i>Salps</i>	9	$-20.8 \pm 1.6$	-	$4.6 \pm 1.2$	$5.8 \pm 0.8$



**Figure 3 Zooplankton mean ( $\pm$ SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in spring months between 2012 and 2015, within each region: North of Cape Santa Marta (NCSM) shelf break (a) and offshore (b), and South of Cape Santa Marta (SCM) shelf break (c) and offshore (d).**



**Figure 4** Zooplankton mean ( $\pm$ SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in autumn months between 2012 and 2015, within each region: North of Cape Santa Marta (NCSM) shelf break (a) and offshore (b), and South of Cape Santa Marta (SCM) shelf break (c) and offshore (d).



**Figure 5** Boxplot with seasonal (spring and autumn) median  $\delta^{15}\text{N}$  (a) and  $\delta^{13}\text{C}$  (b) for zooplankton samples (all groups together), with 50% of data points within the inter quartile range, minimum and maximum values in each area: North of Cape Santa Marta offshore (NCSM-OFF) and shelf break (NCSM-SB), and South of Cape Santa Marta offshore (SCM-OFF) and shelf break (SCM-SB).

**Table 3 Generalized Additive Models of the relationship between (a)  $\delta^{13}\text{C}$  and (b)  $\delta^{15}\text{N}$  values and Latitude (Lat, continuous variable) by area (data collected for the stations along the shelf break vs offshore), zooplankton taxonomic groups (Group), Season (spring and autumn) and Year (2012-2015) as categorical variables. Adjusted  $R^2$  ( $R^2$  adj.), deviance explained, Generalized Cross-Validation (GCV) score and number of samples are shown for each ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) model. The significance levels (p-Value), F statistics (F) and estimated degrees of freedom (edf) are shown for the continuous explanatory variable, as well as the estimated parameters for the categorical variables, the t-Value and the p-Value. Significant values are highlighted in bold.**

<b>a) <math>\delta^{13}\text{C} = \alpha + s(\text{Lat, by area}) + \text{Group} + \text{Year} + \varepsilon</math></b>				
$R^2$ adj = 0.39	Deviance explained = 43.1%			
GCV = 0.8232	n = 342			
	Estimate	Std. err.	t-value	p-value
<b>Intercept</b>			-	
(amphipods)	-22.21	0.17	130.38	< <b>0.001</b>
<b>Factor(Group)</b>				
<i>Chaetognaths</i>	-0.05	0.17	-0.32	0.74
<i>Copepods</i>	-0.7	0.15	-4.5	< <b>0.001</b>
<i>Euphausiids</i>	-0.41	0.16	-2.6	< <b>0.01</b>
<i>Mixture</i>	0.3	0.15	1.9	0.05
<i>Salps</i>	0.05	0.23	0.21	0.83
<b>Factor(Year)</b>				
2013	1.4	0.22	6.05	< <b>0.001</b>
2014	1.2	0.16	7.23	< <b>0.001</b>
2015	1.7	0.16	10.7	< <b>0.001</b>
<b>Approximate significance of smooth terms:</b>				
	edf	Ref.df	F	p-value
<b>s(Lat):</b>				
<b>Offshore</b>	6.78	7.57	5.99	< <b>0.001</b>
<b>s(Lat): Shelf break</b>	8.72	8.94	2.44	< <b>0.01</b>
<b>b) <math>\delta^{15}\text{N} = \alpha + s(\text{Lat, by area}) + \text{Group} + \text{Area} + \text{Season} + \text{Year} + \varepsilon</math></b>				
$R^2$ adj = 0.55	Deviance explained = 58.3%			
GCV = 2.4127	n = 342			
	Estimate	Std. err.	t-value	p-value
<b>Intercept</b>				
(amphipods)	2.7	0.37	7.18	< <b>0.001</b>
<b>Factor(Group)</b>				
<i>Chaetognaths</i>	1.45	0.29	5.01	< <b>0.001</b>
<i>Copepods</i>	0.01	0.26	0.04	0.96

<i>Euphausiids</i>	1.06	0.27	3.85	< <b>0.001</b>
<i>Mixture</i>	0.15	0.27	0.57	0.56
<i>Salps</i>	0.05	0.4	0.13	0.89
<b>Factor(Area)</b>				
<i>Shelf break</i>	2.04	0.17	11.57	< <b>0.001</b>
<b>Factor(Season)</b>				
<i>Spring</i>	0.99	0.24	4.06	< <b>0.001</b>
<b>Factor(Year)</b>				
2013	1.3	0.43	3.03	< <b>0.01</b>
2014	-0.1	0.29	-0.31	0.75
2015	0.1	0.34	0.34	0.73

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**Approximate significance of smooth terms:**

	<b>edf</b>	<b>Ref.df</b>	<b>F</b>	<b>p-value</b>
<b>s(Lat):</b>				
<b>Offshore</b>	6.73	7.53	13.86	< <b>0.001</b>
<b>s(Lat): Shelf</b>				
<b>break</b>	5.99	6.87	13.95	< <b>0.001</b>

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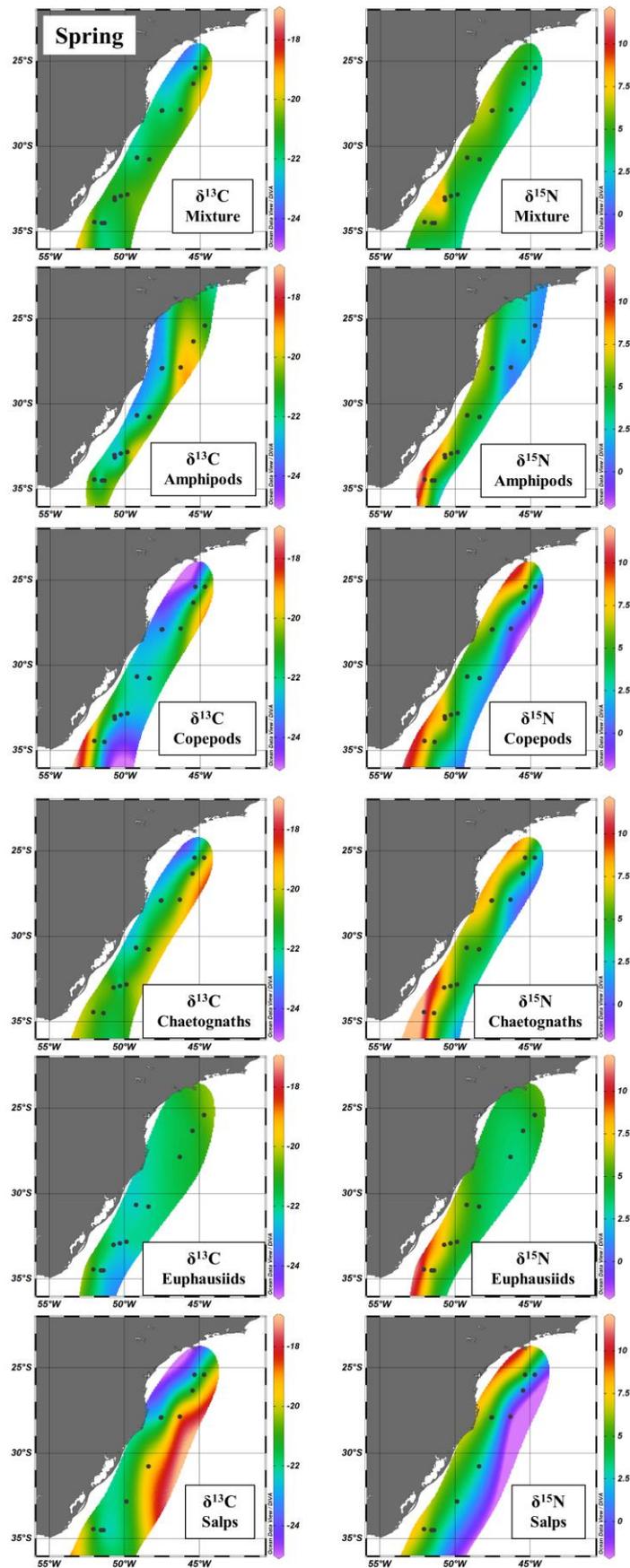


Figure 6 Spring isoscapes of  $\delta^{13}\text{C}$  (left) and  $\delta^{15}\text{N}$  (right) between 2012-2015 in the different zooplankton groups: mixture, amphipods, copepods, chaetognaths, euphausiids and salps. Black circles represent the location of the sampling stations.

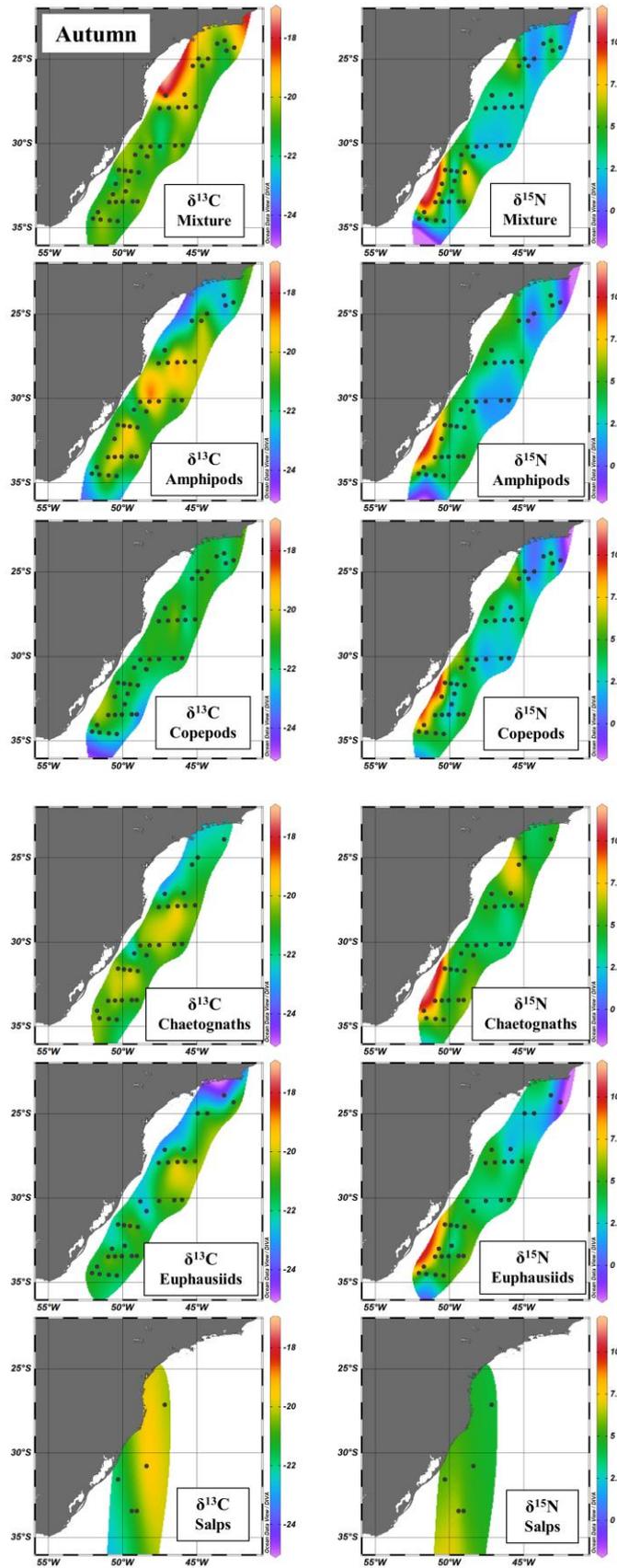
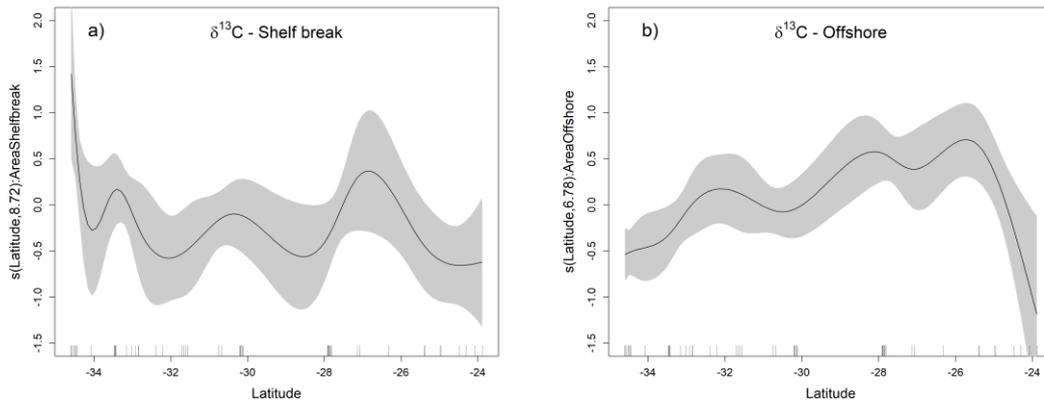
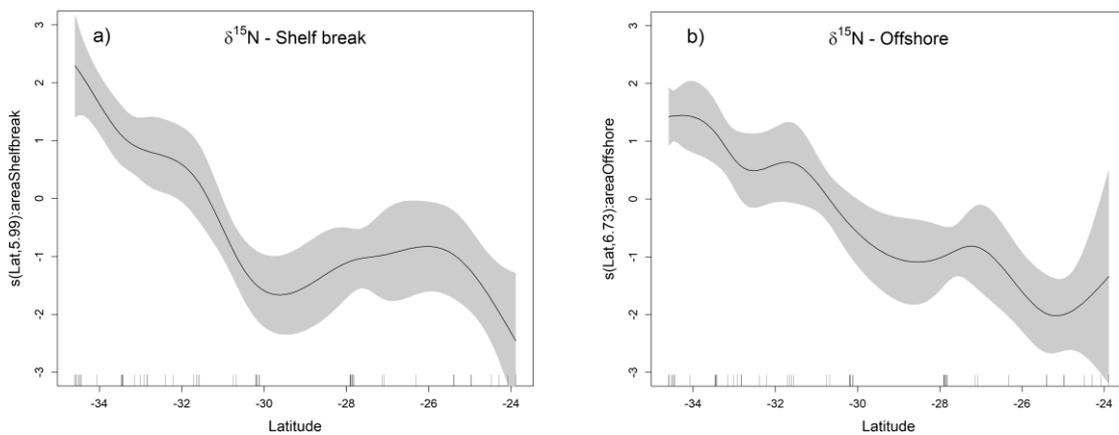


Figure 7 Autumn isoscapes of  $\delta^{13}\text{C}$  (left) and  $\delta^{15}\text{N}$  (right) between 2012-2015 in the different zooplankton groups: mixture, amphipods, copepods, chaetognaths, euphausiids and salps. Black circles represent the location of the sampling stations.



**Figure 8** Generalized additive model estimated smoothing curves ( $s$ , estimated degrees of freedom, edf) for the effect of latitude on zooplankton  $\delta^{13}\text{C}$  along the shelf break (a) and offshore (b) areas.



**Figure 9** Generalized additive model estimated smoothing curve ( $s$ , estimated degrees of freedom, edf) for the effect of latitude on zooplankton  $\delta^{15}\text{N}$  along the shelf break (a) and offshore (b).

## Supplementary material

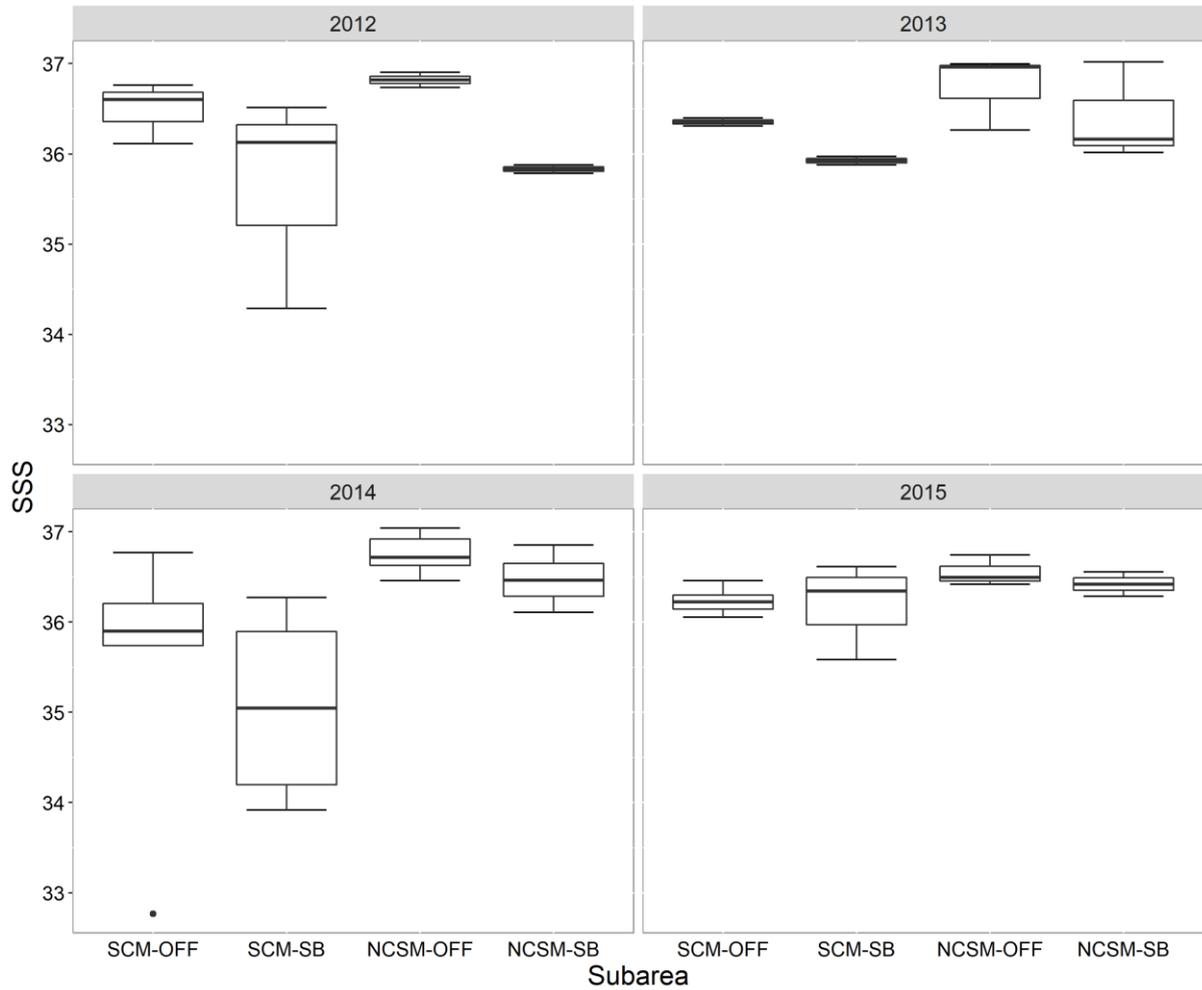
**Supplementary Material Table S1** The equations for  $\delta^{13}\text{C}$  mathematical lipid correction ( $\delta^{13}\text{C}_{\text{lipid-corrected}}$ ) based on Post et al. (2007). The specific coefficients (intercept and slope) for each zooplankton group were estimated using the differences between  $\delta^{13}\text{C}$  values of chemically-treated and non-treated ( $\delta^{13}\text{C}_{\text{untreated}}$ ) samples, and their relationship with C:N ratios in a linear model. The number of individuals within each group with double analysis (chemically-treated and non-treated) is denoted with N.  $R^2$  is the measure of quality of fit for each model.

<b>Group</b>	<b>N</b>	<b>Equation for mathematical lipid correction</b>	<b><math>R^2</math></b>
Chaetognaths	7	$\delta^{13}\text{C}_{\text{lipid-corrected}} = \delta^{13}\text{C}_{\text{untreated}} - 0.54 + 0.49 \times \text{C:N}$	0.52
Copepods	12	$\delta^{13}\text{C}_{\text{lipid-corrected}} = \delta^{13}\text{C}_{\text{untreated}} - 1.54 + 0.5 \times \text{C:N}$	0.82
Euphausiids	14	$\delta^{13}\text{C}_{\text{lipid-corrected}} = \delta^{13}\text{C}_{\text{untreated}} - 1.55 + 0.67 \times \text{C:N}$	0.68

**Supplementary Material Table S2** Upper 200m average ( $\pm$ SD) temperature (T, °C) and salinity (SSS) between regions (southeast - NCSM and south - SCM), area (shelf break - SB and offshore - OFF), season (autumn and spring), year (2012-2015) and within each season for each of the four sections of the study area (NCSM–SB, NCSM–OFF, SCM–SB and SCM–OFF).

	<b>T (°C)</b>	<b>SSS</b>
<b>Region</b>		
NCSM	21.3 ( $\pm$ 1.8)	36.5 ( $\pm$ 0.3)
SCM	20.2 ( $\pm$ 1.3)	35.9 ( $\pm$ 0.8)
<b>Area</b>		
SB	20.3 ( $\pm$ 1.8)	35.9 ( $\pm$ 0.8)
OFF	20.9 ( $\pm$ 1.4)	36.3 ( $\pm$ 0.7)
<b>Season</b>		
Spring	19.9 ( $\pm$ 1.9)	36.1 ( $\pm$ 0.7)
Autumn	20.9 ( $\pm$ 1.3)	35.9 ( $\pm$ 0.8)
<b>Year</b>		
2012	19.5 ( $\pm$ 2.3)	36.2 ( $\pm$ 0.7)
2013	20.5 ( $\pm$ 1.6)	36.4 ( $\pm$ 0.4)
2014	21.2 ( $\pm$ 1.6)	35.9 ( $\pm$ 1.1)
2015	20.7 ( $\pm$ 0.7)	36.3 ( $\pm$ 0.3)
<b>Spring</b>		
NCSM-SB	18 ( $\pm$ 1.3)	36 ( $\pm$ 0.1)
NCSM-OFF	22 ( $\pm$ 0.8)	37 ( $\pm$ 0.2)
SCM-SB	19 ( $\pm$ 2.5)	35 ( $\pm$ 0.8)
SCM-OFF	20 ( $\pm$ 1.1)	36 ( $\pm$ 0.4)
<b>Autumn</b>		
NCSM-SB	21 ( $\pm$ 1.7)	36 ( $\pm$ 0.3)
NCSM-OFF	22 ( $\pm$ 1.6)	37 ( $\pm$ 0.3)
SCM-SB	21 ( $\pm$ 0.8)	36 ( $\pm$ 0.9)
SCM-OFF	20 ( $\pm$ 0.8)	36 ( $\pm$ 0.9)

**Supplementary Material Fig S3** Annual Sea Surface Salinity (SSS) in each subarea: North of Cape Santa Marta shelf break (NCSM-SB) and offshore (NCSM-OFF), and South of Cape Santa Marta shelf break (SCM-SB) and offshore (SCM-OFF). SSS representing each oceanographic station is the average of the upper 200 m.

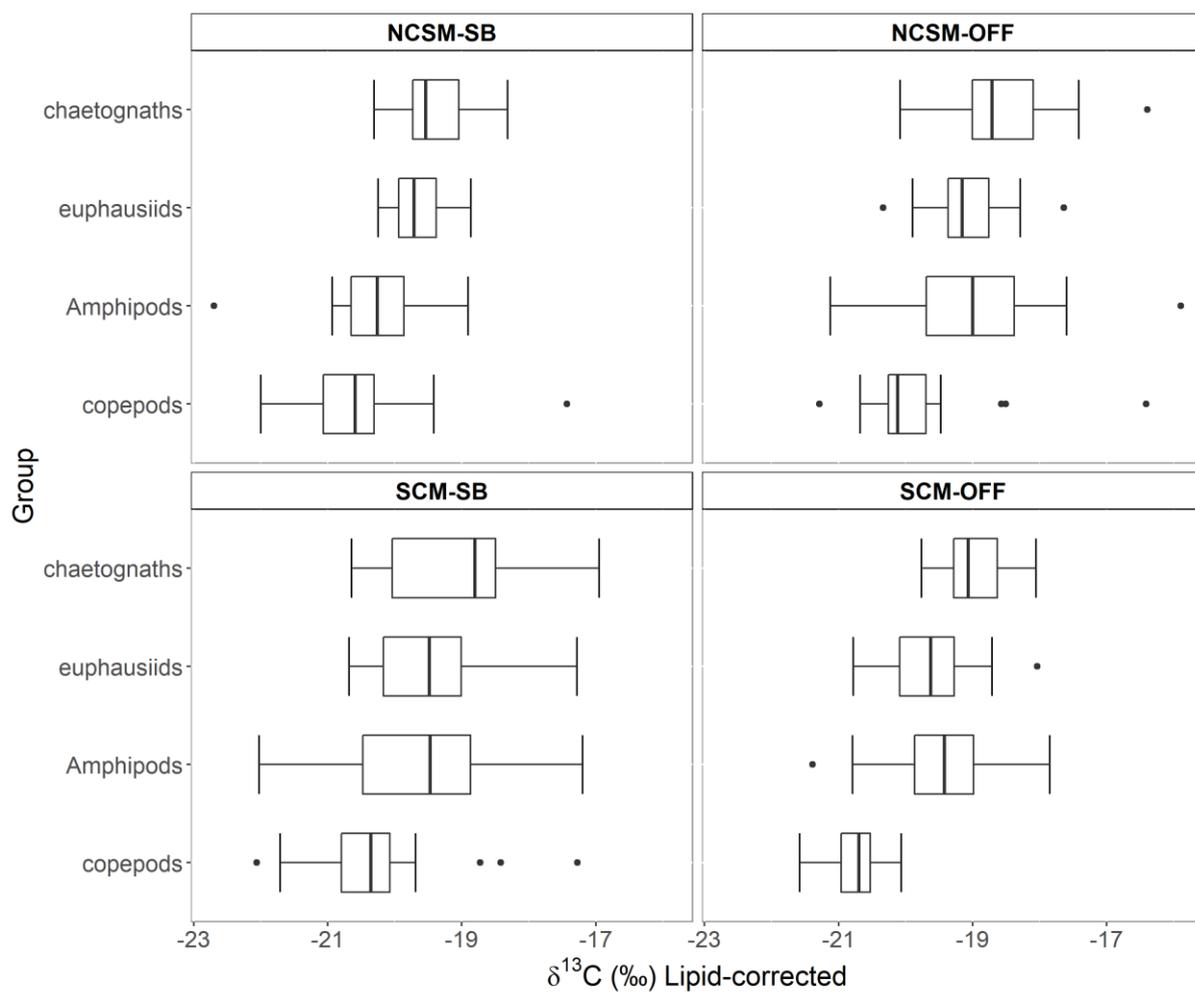


**Supplementary Material Table S4** total number of stations in each research cruise between 2012 and 2015 where zooplankton samples were collected, and the number of samples for each zooplankton group: mixture, amphipods, chaetognaths, copepods, euphausiids and salps.

<b>Cruise</b>	<b>Year</b>	<b>Season</b>	<b>Stations</b>	<b>Mixture</b>	<b>Amphipods</b>	<b>Chaetognaths</b>	<b>Copepods</b>	<b>Euphausiids</b>	<b>Salps</b>
1	2012	Spring	11	10	9	8	9	5	4
2	2013	Autumn	10	10	6	1	10	1	-
3	2014	Autumn	12	12	8	6	12	12	2
4	2014	Spring	11	10	10	10	13*	12*	9
5	2015	Autumn	23	23	23	23	26*	31*	4
6	2015	Spring	6	6	2	5	6	6	1
<b>Total</b>			73	71	58	53	76	67	20

\*organisms were sorted into size classes and analysed separately; therefore number of copepod and euphausiid samples exceed number of stations

**Supplementary Material Fig S5** Mathematically lipid-corrected  $\delta^{13}\text{C}$  in chaetognaths, euphausiids, amphipods and copepods in each subarea: North of Cape Santa Marta shelf break (NCSM-SB) and offshore (NCSM-OFF), and South of Cape Santa Marta shelf break (SCM-SB) and offshore (SCM-OFF). Equations for lipid correction are presented in Table S1. Amphipods were lipid-corrected using the equation for aquatic invertebrates described in Logan et al., (2008).



**Supplementary Material Table S6** Mean ( $\pm$  standard deviation) of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (composite of all species), Sea Surface Temperature (SST,  $^{\circ}\text{C}$ , upper 200 m) and Sea Surface Salinity (SSS, upper 200 m) separated by season (autumn and spring). Values are presented by year within each subarea: North of Cape Santa Marta shelf break (NCSM-SB) and offshore (NCSM-OFF), and South of Cape Santa Marta shelf break (SCM-SB) and offshore (SCM-OFF)

<b>Autumn</b>				
<b>Subarea/Year</b>	<b><math>\delta^{13}\text{C} \pm \text{SD}</math></b>	<b><math>\delta^{15}\text{N} \pm \text{SD}</math></b>	<b>SST (200m) <math>\pm</math> SD</b>	<b>SSS (200m) <math>\pm</math> SD</b>
<b>NCSM-SB</b>				
2013	-20.7 $\pm$ 2.1	4.8 $\pm$ 2	20.5 $\pm$ 2.3	36.4 $\pm$ 0.5
2014	-21.8 $\pm$ 1.1	3.4 $\pm$ 1.2	22.4 $\pm$ 1.2	36.6 $\pm$ 0.3
2015	-21.0 $\pm$ 0.5	4.0 $\pm$ 0.8	20.8 $\pm$ 0.7	36.4 $\pm$ 0.2
<b>NCSM-OFF</b>				
2013	-21.2 $\pm$ 1	3.0 $\pm$ 1.7	21.4 $\pm$ 2.1	36.7 $\pm$ 0.4
2014	-21.0 $\pm$ 0.8	1.6 $\pm$ 1.8	23.5 $\pm$ 1	36.8 $\pm$ 0.2
2015	-20.2 $\pm$ 0.8	3.2 $\pm$ 0.7	21.3 $\pm$ 0.6	36.6 $\pm$ 0.2
<b>SCM-SB</b>				
2013	-21.3 $\pm$ 0.7	6.6 $\pm$ 1.5	19.9 $\pm$ 1.3	35.9 $\pm$ 0.1
2014	-21.8 $\pm$ 1.2	5.8 $\pm$ 2.2	20.8 $\pm$ 0.6	34.7 $\pm$ 1.4
2015	-20.8 $\pm$ 0.8	5.4 $\pm$ 2.4	21.1 $\pm$ 0.6	36.2 $\pm$ 0.4
<b>SCM-OFF</b>				
2013	-21.2 $\pm$ 0.4	2.7 $\pm$ 1.2	20.0 $\pm$ 0.9	36.4 $\pm$ 0.1
2014	-21.2 $\pm$ 1	3.8 $\pm$ 1.3	21.0 $\pm$ 1.6	34.8 $\pm$ 2.8
2015	-20.9 $\pm$ 0.7	4.1 $\pm$ 1.5	20.2 $\pm$ 0.7	36.2 $\pm$ 0.1
<b>Spring</b>				
<b>Subarea/Year</b>	<b><math>\delta^{13}\text{C} \pm \text{SD}</math></b>	<b><math>\delta^{15}\text{N} \pm \text{SD}</math></b>	<b>SST (200m) <math>\pm</math> SD</b>	<b>SSS (200m) <math>\pm</math> SD</b>
<b>NCSM-SB</b>				
2012	-23.0 $\pm$ 0.8	5.5 $\pm$ 0.9	17.6 $\pm$ 0.3	35.8 $\pm$ 0.1
2014	-22.2 $\pm$ 1.5	5.9 $\pm$ 1.4	19.8 $\pm$ NA	36.1 $\pm$ NA
<b>NCSM-OFF</b>				
2012	-21.7 $\pm$ 0.8	2.7 $\pm$ 1	21.8 $\pm$ 0.5	36.8 $\pm$ 0.1
2014	-20.2 $\pm$ 1	1.9 $\pm$ 2.3	22.0 $\pm$ 1.2	36.7 $\pm$ 0.3
2015	-19.7 $\pm$ NA	4.6 $\pm$ NA	NA	NA
<b>SCM-SB</b>				
2012	-22.8 $\pm$ 0.7	6.3 $\pm$ 2.1	18.3 $\pm$ 3.2	35.6 $\pm$ 1.2
2014	-21.1 $\pm$ 0.8	7.1 $\pm$ 2.3	19.9 $\pm$ 1.8	35.4 $\pm$ 0.7
2015	-20.3 $\pm$ 1.3	8.3 $\pm$ 2.9	NA	NA
<b>SCM-OFF</b>				
2012	-22.0 $\pm$ 0.9	4.8 $\pm$ 0.9	20.3 $\pm$ 1.4	36.5 $\pm$ 0.3
2014	-21.2 $\pm$ 1.2	4.9 $\pm$ 1.4	19.4 $\pm$ 0.7	35.9 $\pm$ 0.2
2015	-21.2 $\pm$ 0.8	5.9 $\pm$ 1.5	NA	NA

## **CAPÍTULO 3**

### **The isotopic niche area, overlap and partition amongst odontocete species from the western South Atlantic**

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Versão do artigo submetido ao periódico Marine Biology

## **The isotopic niche area, overlap and partition amongst odontocete species from the Southwestern Atlantic Ocean**

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## Abstract

Large diversity and abundance of cetacean species occur along the Brazilian outer continental shelf and slope waters. In the present study we analysed carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotopes in skin biopsies of ten odontocete species (Delphinidae and Physteridae) to assess the intra- and interspecific patterns in the use of resources along the region. Our isotopic analysis allowed us to identify two potential subpopulations of *Stenella frontalis*. High core isotopic niche overlap between *S. frontalis* from the southern range of the study area and *Delphinus delphis*, especially in spring, suggest that they share similar resources and rely on spatiotemporal segregation to achieve niche differentiation and to minimize competition. Our isotopic data also pointed to high similarity of *Tursiops truncatus*, *S. frontalis* and *Globicephala melas* in the use of resources. *Steno bredanensis* had the highest  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , indicating its relatively high trophic position and occurrence in neritic environments. *S. longirostris* showed consistently low  $\delta^{15}\text{N}$  values, indicating that they feed at relatively lower trophic levels, and lack of niche overlap with most delphinid species, except *S. attenuata*. *Orcinus orca* and *Globicephala melas* had very similar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, consistent with feeding in offshore waters.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in *Physeter macrocephalus* suggested that individuals feed on similar trophic levels, but over a wide spatial range. The data presented here provides a first insight into the trophic interactions and ecological niches of cetaceans in oceanic waters of the subtropical western South Atlantic.

**Keywords:** cetaceans, stable isotopes, ecological niche, trophic ecology, Western South Atlantic Ocean, dolphins

## Introduction

A species niche is shaped by the balance between the environmental conditions it can endure and those required for its survival, as well as by the effect it has on such environment (Chase and Leibold 2003). It can be limited by physical factors such as water temperature, salinity or ocean bottom topography, as well as by biological interactions including the food resources available, predation and inter-specific competition (Jaksić and Braker 1983; Taniguchi and Nakano 2000; Miyasaka et al. 2003). The competitive exclusion principle posits that two species with identical ecological niches cannot coexist (Hardin 1960). Therefore, ecological equivalent species present spatial or temporal segregation in their occurrences (Taniguchi and Nakano 2000; Jorgensen et al. 2019), and sympatric species should have distinct niches to avoid competition (Giménez et al. 2017). Often competing species coexist at a large spatial scale, although at a finer scale they are shown to segregate in their distributions to minimize competition (Taniguchi and Nakano 2000; Miyasaka et al. 2003). Nevertheless, competing species may not always exclude one another, but rather control the abundances of their competing counterparts (Inger and Greenberg 1966; Brown et al. 1979). Information on the feeding habits of sympatric species allows for the evaluation of the magnitude of intra- and inter-specific overlap in the use of resources, and the effects that their interactions will have on the ecosystems they inhabit. Moreover, knowledge of the species niche enables the assessment of population and community ecological amplitude and plasticity, the level of specialization and the ability to respond to environmental changes. Specialist species can have more advantage over generalists when resources are abundant (Terraube et al. 2011). However, when the availability of resources changes, generalist species have greater capacity to adapt and exploit other available resources (Secchi et al. 2016), while specialist species may face considerable decline in their abundances and potentially extinction if the over-exploited resource is their main prey type (Bearzi et al. 2006; Lacy et al. 2017). This is particularly relevant in the case of marine mammals, often competing with fisheries that target their main prey species (Bearzi et al. 2006; Secchi et al. 2016; Lacy et al. 2017; Díaz López et al. 2019).

For marine organisms such as cetaceans the assessment of their ecological interactions is particularly difficult due to the limited access to information on these populations, hampering the assessment of their feeding habits. A large diversity of cetacean species occurs along the Brazilian outer continental shelf and slope (Moreno et al. 2005; Di Tullio et al. 2016; Bastida et al. 2018). Amongst the species that inhabit these offshore waters are the short-beaked common dolphins (*Delphinus delphis*, Linnaeus 1758), whose distributional range

encompasses cold waters in temperate regions and waters associated with upwelling systems (Jefferson et al. 2009; Tavares et al. 2010). The genus *Stenella* comprises several species that occur throughout large latitudinal and depth ranges in the Brazilian tropical and sub-tropical waters (Moreno et al. 2005; Amaral et al. 2015; Di Tullio et al. 2016). The Atlantic spotted dolphin (*Stenella frontalis*, Cuvier 1829) occurs from nearshore to oceanic waters, from warmer tropical regions to colder upwelling areas (Moreno et al. 2005; Di Tullio et al. 2016). The spinner dolphin (*S. longirostris*, Gray 1828), the pantropical spotted dolphin (*S. attenuata*, Gray 1846), and the Clymene dolphin (*S. clymene*, Gray 1850) prefer warmer oceanic waters (Di Tullio et al. 2016) and show large overlap in their distribution ranges (Moreno et al. 2005). Mixed-species associations including pantropical spotted and spinner dolphins have been reported along this region (Di Tullio et al. 2016). Killer whales (*Orcinus orca*, Linnaeus 1758) have been recorded in coastal to oceanic waters off Brazil (Santos and Netto 2005; Dalla Rosa and Secchi 2007; Di Tullio et al. 2016), and distinct ecotypes have been suggested by the analysis of stable isotopes in teeth of stranded specimens (Botta et al. 2012; Tatsch 2015). The bottlenose dolphins (*Tursiops truncatus*) in the southwestern Atlantic (SWA) are found in both coastal and oceanic waters and are regarded as different ecotypes (Costa et al. 2015; 2016; Fruet et al. 2017), though the coastal populations were recently considered as a new subspecies (Lahille's bottlenose dolphins *Tursiops truncatus gephyreus*) by the Committee on Taxonomy (2017). Nonetheless, the oceanic ecotype is frequently found in association with other Delphinidae species (Di Tullio et al. 2016). There is little information about the distribution range of the rough-toothed dolphin (*Steno bredanensis*) in the SWA, although they have been registered in the inner continental shelf waters off Brazil (Ott and Danilewicz 1996; Santos et al. 2017; 2019). In offshore waters they were only reported in the southern region, in both autumn and spring months (Di Tullio et al. 2016; this study). Finally, the sperm whales (*Physeter macrocephalus*) were the most frequently sighted cetacean species by Di Tullio and colleagues (2016) in a long-term survey along the study area. The species showed seasonal variability in their distribution patchiness, being more aggregated in large group sizes in autumn, while sparser and smaller groups were observed in spring (Di Tullio et al. 2016).

Such richness in cetacean species requires distinct feeding behaviour, represented by generalists or specialists that might prey upon distinct species, trophic levels or target different size ranges. The sperm whales, for instance, prey on deep-water squid species (Clarke et al. 1980; Santos and Haimovici 2000). Killer whales are known apex predators, although a generalist species on a global scale, different ecotypes display remarkable specialization in

terms of feeding habits and target prey species (Ford et al. 1998; Secchi and Vaske Jr. 1998; Pitman and Ensor 2003; Passadore et al. 2015; Samarra et al. 2018). A large variety of small meso-to-epipelagic fish and squid comprises the diet of several small delphinids, such as the common dolphins or those species of the genus *Stenella* (Di Benedetto et al. 2001; Santos and Haimovici 2002; Melo et al. 2010; Lopes et al. 2012). Nevertheless, due to their occurrence at long distances from the coast, little is known about the intra and inter-specific trophic ecology and level of interactions among the cetacean populations inhabiting the Brazilian offshore waters. In the rare events of specimens from offshore populations stranding along the coast (Prado et al. 2016), information gathered from stomach contents most likely does not reflect the diet of healthy animals, as it may include coastal prey consumed prior to death as the animal was out of its natural range. In this context, stable isotopes have been increasingly applied to infer the feeding habits of marine mammals (Newsome et al. 2010), as the carbon and nitrogen isotope ratios of the assimilated food are reflected in the consumers' tissues (DeNiro and Epstein 1978; 1981; Tieszen et al. 1983). There is a prey-to-predator trophic enrichment factor (TEF) in both carbon and nitrogen isotopes, and in cetaceans' skin TEF has been estimated to be  $\sim 1.0\text{‰}$  ( $\pm 0.4$ ) for  $\delta^{13}\text{C}$  and  $\sim 1.6\text{‰}$  ( $\pm 0.5$ ) for  $\delta^{15}\text{N}$  (Giménez et al. 2016). The isotopic values of individuals within a population represent their feeding areas and resources used ( $\delta^{13}\text{C}$ ), as well as their relative trophic positions ( $\delta^{15}\text{N}$ ) (Bearhop et al. 2004; Layman et al. 2012). Moreover, stable isotopes can be used to estimate the area of the isotopic niche of species or populations (Jackson et al. 2011), which allows for quantitative approximations of their ecological niche (Bearhop et al. 2004; Layman et al. 2012). For instance, the isotopic niche can be applied to infer the level of spatial or trophic overlap or partition between different species within communities (Jackson et al. 2011). Additionally, the distributions of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  within the  $\delta$  space provide insights into the extent of species spacing, offering information on the relative position of individuals to one another within the niche space, and being therefore used to infer the extend of trophic redundancy within populations or communities (Layman et al. 2007; Jackson et al. 2012).

Essentially, stable isotopes have proven to be effective tools to study the trophic ecology of marine predators (Newsome et al. 2010; Seyboth et al. 2018). Nevertheless, for effective assessments of intra and inter-specific dietary variations, overlap or segregation in the use of resources by coexisting species using stable isotopes, we must consider the spatiotemporal gradients in the isotopic values at the base of the local food webs (Graham et al. 2010; McMahon et al. 2013). In the SWA, distinct oceanographic conditions result in biogeochemical

gradients along the outer continental shelf and slope off Brazil (see study area below), that are reflected in the carbon and nitrogen stable isotope ratios at the base of the local food webs (Troina et al. in review). As a result, a north-to-south increase in  $\delta^{15}\text{N}$  is observed along the area, and  $\delta^{15}\text{N}$  values are also higher in the shelf break than further offshore. Additionally, a  $^{15}\text{N}$ -enrichment is reported for zooplankton  $\delta^{15}\text{N}$  in spring in comparison to autumn months (Troina et al. in review). Regarding the carbon isotopes, there is a shelf break-to-offshore enrichment in  $^{13}\text{C}$ , and a latitudinal decrease in  $\delta^{13}\text{C}$  observed in the offshore area but not along the shelf break (Troina et al. in review).

In the present study we assessed the spatiotemporal patterns in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of co-occurring cetacean species in the offshore waters of the southeast and southern Brazil. We used stable isotopes to estimate the isotopic niche area and the level of overlap or partition in niche space and resource utilization among the different species. We hypothesize that the baseline offset in  $\delta^{15}\text{N}$  values will be reflected in the cetacean's isotopic values, which will be higher in the southern species. Furthermore, species occurring in the south-eastern area, which is oceanographically more stable (see study area below) will have less seasonal variation in their isotopic values than those species occurring in the southern area, which is hydrographically more dynamic and more subject to seasonal variation. Finally, by controlling these baseline patterns in isotopic values, we shed some new light on the trophic ecology and interactions among the cetacean species that inhabit these offshore waters in the subtropical southwestern Atlantic Ocean.

## **Materials and Methods**

### **Study area**

The study area was surveyed onboard the RV *Atlântico Sul* from the Federal University of Rio Grande (FURG) and encompasses the outer continental shelf (~150 m isobath) and slope (~1,500 m isobath) off south-east and southern Brazil (Fig. 1). The south-eastern (SE) region between 24°S–28°S is mainly influenced by tropical waters of the Brazil current and the seasonal wind generated ascension of the South Atlantic Central Water (Acha et al. 2004; Brandini et al. 2018). In contrast, the southern (S) area between 28°S–34°S is seasonally influenced by the tropical waters of the Brazil current that dominate the region in warmer months, while in colder periods there is the prevalence of the subtropical shelf water, formed by the encounter of continental waters from the La Plata river and Patos Lagoon, tropical waters

and the Subantarctic shelf water transported by the northward flowing Patagonian current (Möller Jr. et al. 2008; Piola et al. 2008), the latter reaching up to 32°S. In turn, the physical gradients throughout the area result in baseline shifts in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, previously characterized by the isotopic values of zooplankton sampled in the region (Troina et al. in review). The resulting offset in baseline isotopic values differentiate the SE and S regions, hence analysis of cetacean's isotopic values will consider the location where they were sampled.

## Sampling

Samples from bow-riding cetaceans were collected from the bow of the RV or from a small boat deployed from the ship. Skin samples were obtained using a 120-lb draw weight crossbow with modified darts specifically designed for sampling, with different size tips depending on the target species. The surveys were carried out during austral spring ( $n = 5$ ) and autumn ( $n = 5$ ) between 2009 and 2015. An overview of the total number of samples obtained for each species is presented in Table 1. All samples were then kept frozen at  $-20^\circ\text{C}$  till analysis. Metabolic active tissues such as skin have relatively faster isotopic turnover time, and skin half-life was estimated to be of approximately 24 days ( $\pm 8$ ) for carbon and about 47 days ( $\pm 19$ ) for nitrogen isotopes in bottlenose dolphins (Giménez et al. 2016) and these values were assumed to be similar for the species analysed here.

## Stable isotopes

Lipids have a strong negative effect on  $\delta^{13}\text{C}$  values (DeNiro and Epstein 1977) and should hence be removed in order to reduce the variability caused by different lipid contents among organisms when applying stable isotopes in dietary studies (Logan et al. 2008). Nevertheless, chemical lipid extraction has a strong undesirable effect on  $\delta^{15}\text{N}$  (Logan et al. 2008; Ryan et al. 2012). Therefore, it is recommended the analysis of chemically-treated samples for carbon and non-chemically-treated samples for nitrogen isotopes in each individual sample (Ryan et al. 2012). Because of our large sample size ( $n = 277$ , Table 1), double analysis would be time intensive and expensive. We have therefore double analysed a portion of the cetacean samples ( $n = 16$ ) and performed mathematical corrections on the remaining  $\delta^{13}\text{C}$  values. We used the differences between  $\delta^{13}\text{C}$  values of treated and non-treated samples, and their relation with the carbon-to-nitrogen ratios (C:N) in a linear model to estimate the cetaceans' skin specific coefficients for the following equation adapted from Post et al. (2007):

$$\delta^{13}\text{C}_{\text{Normalized}} = \delta^{13}\text{C}_{\text{untreated}} + (-12.186 + 3.69 \times \text{C:N})$$

Lipids were extracted using a Soxhlet to rinse the samples with a chloroform:methanol (2:1) solution for 24 h. All skin samples were dried at 60°C for 48 h and powdered with a mortar and pestle. About 0.5-0.7 mg of each sample was weighed into tin capsules and analysed with an Isotope Ratio Mass Spectrometer (IRMS, Delta PlusXP, Thermofinnigan) connected to an Elemental Analyser (EA, ECS 4010, Costech Analytical, Valencia, CA) at the Stable Isotope Core Laboratory, Washington State University. The isotopic compositions were reported in the conventional delta ( $\delta$ ) per mil (‰) notation, relative to Vienna Pee Dee Belemnite ( $\delta^{13}\text{C}$ ) and atmospheric  $\text{N}_2$  ( $\delta^{15}\text{N}$ ). Two lab standards, which had been previously calibrated to internationally distributed isotopic reference material, were analyzed interspersed with the samples to estimate instrument precision. The standard deviation of these standards was  $\leq 0.1\%$  for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

### Data analysis

To test for seasonal differences, we compared the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in individuals of the same species sampled in spring and autumn, within each region (South - S or Southeast - SE). The spatial (latitudinal) patterns of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were assessed seasonally, for specimens sampled within each region separately. For species sampled in both regions but only in one season (spinner dolphins in spring), or those sampled in both seasons, but only in one region (short-beaked common dolphins, sperm whales and rough-toothed dolphins in the S), we used the Welch two sample *t*-test. For species sampled in both regions and seasons (Atlantic spotted and bottlenose dolphins) we used a Two-way factorial design with  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  as response variables. The factors of season and region each had two levels (autumn vs. spring and S vs. SE, respectively), and the ANOVA was used to test the significance of each of the main factors and their interactions. Additionally, we applied hierarchical cluster analysis to measure the level of dissimilarities in cetacean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Since the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  means are largely different, we first standardized the data using the *scale()* function in R version 3.6.0 (R Core Team 2019). We then calculated the pairwise Euclidean distance matrix (complete-linkage) with the function *dist()*, and produced the hierarchical tree with *hclust()* to identify the cetacean groups.

Inter-specific patterns in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, their isotopic niche width, level of overlap or segregation in niche areas, regional and seasonal patterns were evaluated through population (Jackson et al. 2012) and community (Layman et al. 2007) metrics, as well as the Isotope Niche Area using the Stable Isotope Bayesian Ellipses in R (SIBER; Jackson et al.

2011). SIBER allows the estimation of the Standard Ellipse Areas (SEA), which is a measure of the mean population isotopic niche. The standard ellipse is calculated from the variance and covariance of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, containing approximately 40% of the data. The Total Area (TA) encompassing the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values also gives an estimate of the population trophic niche area, representing the extent of trophic diversity within that population. We will present the SEA corrected for small sample size ( $\text{SEA}_C$ ,  $\%{}^2$ ), the SEA based on the posterior distribution of the covariance matrix for each group (the Bayesian SEA, denoted as  $\text{SEA}_B$ ) and the TA. The community-wide metrics proposed by Layman et al. (2007), later adapted for population-wide applications (Jackson et al. 2012), offer a quantitative approach to obtain information on important aspects of the trophic structure and dynamics. Such metrics include the range of carbon (CR) and nitrogen (NR), calculated as the distance between the individuals with the lowest and the highest  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, respectively, within a population (Jackson et al. 2012). NR represents the vertical structure (trophic length), whereas CR provides information regarding the diversity of food sources explored by the different individuals within a specific population. The mean distance to the centroid (CD) offers additional information on the degree of trophic diversity within the populations, with high values indicating more isotopic dispersion, implying that a wide variety of trophic niches is explored by the different individuals, whereas low CD suggests limited diversity of niches. The mean nearest neighbour distance (MNND) provides a measure of the degree of individuals spacing, while the standard deviation of the nearest neighbour distance (SDNND) provides a measure of population trophic evenness. They both reflect the relative position of individuals to one another within the niche space, providing useful information on the extent of trophic redundancy within the population. High MNND values indicate wider spread within bi-plot space, while low values suggest higher density of individuals within the trophic niche space and trophic redundancy. High values of SDNND, on the other hand, suggest skewed spreads of isotopic niche (for instance towards higher trophic levels), while low values suggest more evenly spread niches (Layman et al. 2007). The isotopic niche area and population metrics were calculated for all cetacean species whose number of individual samples obtained in each region-within-season combination (autumn-S, autumn-SE, spring-S and spring-SE) allowed for such statistical inference ( $n > 6$ ). Finally, the percentage overlap or segregation in core isotopic niche area was calculated for every pair of cetacean species. All metrics were calculated with the R package SIBER version 2.1.4 (Jackson et al. 2011) and the statistical analyses were carried out in R version 3.6.0 (R Core Team 2019).

## Results

### Seasonal and spatial patterns in cetaceans $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values

A total of 277 samples were obtained from ten cetacean species of the families Delphinidae and Physeteridae (Table 1). In both seasons, cetaceans sampled in the S were generally more  $^{15}\text{N}$ -enriched than those sampled in the SE region (Table 2, Fig. 2). Rough-toothed (RTD) and short-beaked common dolphins (SBCD) had the highest  $\delta^{15}\text{N}$  values, followed by sperm whales (SW) (Table 2, Fig. 2). Atlantic spotted (ASD) and bottlenose dolphins (BND) showed consistently similar mean  $\delta^{15}\text{N}$  values across both regions and seasons (Fig. 2). Relatively lower  $\delta^{15}\text{N}$  values were observed in killer whales (KW), pantropical spotted (PTSD), spinner (SPD) and Clymene dolphins (CLYD) (Table 2, Fig. 2). Regarding the carbon isotopic composition, the same pattern was observed, with the highest mean  $\delta^{13}\text{C}$  values observed in specimens sampled in the S (spring), followed by S (autumn), then SE (spring), and the lowest in the SE in autumn (Table 2). The single CLYD sampled in the S (spring) had the most  $^{13}\text{C}$ -enriched values. SBCD and RTD consistently showed the highest  $\delta^{13}\text{C}$  values, followed by Atlantic spotted and bottlenose dolphins. The lowest  $\delta^{13}\text{C}$  values were observed in SPD, KW, long-finned pilot whales (LFPW, *Globicephala melas*) and PTSD.

ASD, SPD and BND were sampled in both SE and S regions in spring, allowing for the assessment of their carbon and nitrogen latitudinal pattern. BND and ASD showed the same patterns in  $\delta^{15}\text{N}$  values, with significant latitudinal differences in spring ( $p < 0.001$ , Table 3), but not in autumn. These two species had lower mean  $\delta^{13}\text{C}$  values in the SE than in the S regions (in both seasons), although the differences were only statistically significant for ASD ( $p < 0.001$ , Table 3). SPD, on the other hand, sampled in the S region only in spring, showed no statistical difference in either  $\delta^{13}\text{C}$  ( $p = 0.12$ ) or  $\delta^{15}\text{N}$  values between these two regions ( $p = 0.87$ , Table 3).

SW, RTD, ASD, BND and SBCD were sampled in both seasons in the S region (Table 2). SW showed higher mean  $\delta^{13}\text{C}$  in spring than in autumn. This was the only species showing significant seasonal differences for this isotope in the S (Table 3). For  $\delta^{15}\text{N}$  values, all species but the RTD presented significantly higher values in spring than in autumn (Table 2). Among the species sampled in the SE region, only ASD, SPD, BND and PTSD were sampled in both seasons. SPD showed significant seasonal differences in both carbon and nitrogen stable isotopes. On the other hand, there were no significant seasonal differences in the mean  $\delta^{13}\text{C}$  and

$\delta^{15}\text{N}$  values for ASD or BND sampled in the SE. Only one sample was obtained from PTSD in autumn hindering any comparison (Table 2).

In autumn, our hierarchical cluster analysis placed the RTD in a separated branch representing the inner continental shelf, isotopically distinct from the outer continental shelf and oceanic species (node 1, Fig. 3a). Two main groups were split mainly by their  $\delta^{13}\text{C}$  values for the oceanic species cluster (Fig. 3b). There was a large dissimilarity between the ASD sampled in the SE and in the S regions, resulting in these two populations being clustered in distinct branches (Fig. 3a). The ASD sampled in the SE region were isotopically more similar to the KW and to the PTSD than to their southern conspecifics. The BND were largely similar to the ASD within the branch representing mainly the southern species. In spring, the CLYD showed large dissimilarity with the other cetacean species (node 1, Fig. 3c), while the remaining species were divided in two main groups: one containing the specimens sampled in the SE region, and another with those sampled in the S region. These two clusters seem to differ largely in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Fig. 3d), with the most  $^{15}\text{N}$ -enriched group being composed exclusively of those specimens sampled in the S region. In the most  $^{13}\text{C}$ - and  $^{15}\text{N}$ -depleted group, the BND showed high similarity with the ASD, separated from the PTSD and SPD (nodes 6 and 7, Fig. 3c).

### **Isotopic niche: specific, spatial and seasonal patterns**

The population isotopic metrics are presented in Table 4 for samples collected within each region-within-season combination. In spring, SBCD had the largest isotopic niche area (TA,  $\text{SEA}_\text{C}$  and  $\text{SEA}_\text{B}$ ), followed by BND and ASD (Fig. 4). When comparing between SE and S species, the largest values of niche area and NR were observed in those sampled in the S region, both in spring and in autumn (Table 4). Species sampled in autumn had generally smaller estimated isotopic niche areas in comparison to those sampled in spring (Table 4). Species' CR was larger in spring, regardless of the region, than in autumn. CD, MNND and SDNND were slightly higher in the S region in spring than in the remaining region-within-season combinations (Table 4).

In both seasons, BND showed the highest percentage overlap with other species, particularly with ASD in the SE region, and in the S with SBCD and ASD (Table 5, Fig. 4). In the SE, there was a large overlap between SPD and PTSD in spring. In the S region (spring) the isotopic niche area of SPD seemed to be more isolated than that of the other cetacean species, occupying relatively lower trophic position. SBCD and ASD showed large overlap in their

isotopic niche area in spring. SW showed large isotopic niche area and CR (Table 4), overlapping with SBCD, BND and ASD.

The within species latitudinal comparison of isotopic niche overlap (Table 5) showed that in spring, ~75% of the isotopic niche of ASD occurring in the SE was occupied by those sampled in the S; ~84% of the isotopic niche of SPD sampled in the S was occupied by those sampled in the SE; and ~64% of the isotopic niche of BND sampled in the S was occupied by those sampled in the SE (Table 5). It is noteworthy that the ASD and BND sampled in the S had higher  $\delta^{15}\text{N}$  values (higher vertical position of the standard ellipses) than those in the SE (Fig. 5). In autumn, there was approximately 50% of isotopic niche area overlap in ASD from SE and S; and ~68% of the area of BND sampled in the SE was occupied by those sampled in the S (Table 5).

## **Discussion**

In the present study we provide insights on the trophic ecology and interactions of free-ranging odontocetes in the offshore waters of the western South Atlantic through the analysis of skin  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. We demonstrate that patterns in isotopic compositions of cetaceans between the SE and S regions are related to local seasonal gradients in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and provide original information on their intra- and inter-specific trophic patterns.

### **Seasonal and latitudinal patterns in cetacean isotopic values**

Cetacean  $\delta^{15}\text{N}$  values showed some consistent seasonal and latitudinal patterns, being higher in species sampled in spring than in autumn and in those sampled in the S compared to the SE region. These seasonal and latitudinal patterns follow the isotopic compositions at the base of the food web along the region (Troina et al. in review). These authors demonstrated that zooplankton  $\delta^{15}\text{N}$  shows latitudinal, longitudinal and seasonal gradients throughout this area, with values significantly higher in the S than in the SE, along the shelf break than in deeper offshore waters, and in spring than in autumn. Odontocetes sampled in the SE had generally lower  $\delta^{15}\text{N}$  values than their southern counterparts. Such patterns also persisted in the intra-specific level, as the BND and the ASD sampled in the S region had significantly more  $^{15}\text{N}$ -enriched  $\delta^{15}\text{N}$  values than their SE conspecifics.

The cluster analysis separated the cetacean species into two main groups based on their  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, mainly corresponding to the SE and S regions (Fig. 3). Firstly, when analysing these patterns, it is important to consider the turnover time in cetacean skin tissues, that results in the isotopic signals probably representing the resources consumed in the previous months (Giménez et al. 2016). Hence, the isotopic signal of cetaceans sampled in autumn and spring months refer to their diet and foraging areas during the austral summer and winter, respectively. The autumn cluster split the outer shelf, shelf break and oceanic species in two main groups differing mainly in their  $\delta^{13}\text{C}$  values (Fig. 3b). The group with the most  $^{13}\text{C}$ -depleted values included most of the species from the SE region, where the upwelling of deep-water results in lower surface baseline  $\delta^{13}\text{C}$  values (Troina et al. in review). The second cluster basically included species from the S region, showing higher  $\delta^{13}\text{C}$  values. RTD, only sampled in the S, was clustered alone probably due to its distribution in coastal-to-inner continental shelf waters in Brazil (Ott and Danilewicz 1996; Santos et al. 2017; 2019).

Samples taken in spring months, representing the winter feeding of the cetaceans, showed a clear latitudinal gradient, where the southern cluster was separated from the southeastern (node 2 in Fig. 3c). These two clusters identified differences in cetaceans  $\delta^{13}\text{C}$  (more  $^{13}\text{C}$ -depleted values observed in species from the SE region) and  $\delta^{15}\text{N}$  values (higher in the S region, Fig. 3d). Indeed, there is a clear gradient in water masses between the SE and S regions in winter: the Tropical Waters carried by the Brazil current predominate beyond the continental slope in the SE (Brandini 1990), while the S region is influenced by Subantarctic Shelf Waters, and the mixing of continental and tropical waters (Möller Jr. et al. 2008). Accordingly, the seasonal dynamics in the S region water masses are reflected in the baseline  $\delta^{15}\text{N}$  values, which are more  $^{15}\text{N}$ -enriched in spring than in autumn (Troina et al. in review). Likewise, the seasonal gradient in  $\delta^{15}\text{N}$  observed in the odontocetes sampled in the S region in contrast to the lack of seasonal differences in those sampled in the SE validates our hypothesis that the local oceanographic dynamics would be reflected in the higher trophic levels in a predictable fashion.

It is widely acknowledged that the nitrogen isotopes reflect the trophic position of organisms within their food chain (DeNiro and Epstein 1981; Minagawa and Wada 1984; Post 2002). Nevertheless, it is essential to understand the patterns in isotopic variations at the base of the local food webs for better assessments of the trophic dynamics in higher trophic level organisms, especially when comparing species or populations from areas with such distinct hydrographic characteristics. The pattern of increased  $\delta^{15}\text{N}$  values in the southern cetacean

species in comparison to the SE groups is consistent with baseline  $\delta^{15}\text{N}$  values (Troina et al. in review), unlikely representing distinct trophic positions between S and SE conspecifics, but rather the result of shifted baseline  $\delta^{15}\text{N}$  values which are being propagated up the food web.

### **Species-specific patterns in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$**

#### *Atlantic spotted dolphins*

ASD showed significant latitudinal differences in their isotopic values, with the Euclidean distances between SE and S conspecifics being consistently large in both seasons (Fig. 3). The existence of at least two populations had already been suggested as a result of the hiatus in their distribution, and likely reproductive isolation in the SWA (Moreno et al. 2005). The specimens sampled here comprise the southern range of their known distribution in the SWA. However, our isotopic data suggests that the sampled individuals are not part of one single population, but rather belong to two separate populations with two distinct isotopic niche areas: the one in the south with more enriched  $^{13}\text{C}$  and  $^{15}\text{N}$ , and the other in the SE with lower  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. As these two regions show such a strong gradient in their water masses that are reflected in their carbon and nitrogen stable isotopes (Troina et al. in review), it is expected that populations that occupy each of these two regions have distinct isotopic signatures. The relative depletion in  $^{13}\text{C}$  observed in the ASD from the SE in comparison to those from the S region provides further support for the existence of two distinct populations. The species shows a distribution highly associated to the outer continental shelf and shelf break waters, at approximately the 250 m isobath (Amaral et al. 2015; Di Tullio et al. 2016). The baseline (zooplankton)  $\delta^{13}\text{C}$  values along the shelf break in this region are significantly more depleted in the heavy isotopes than farther offshore (Troina et al. in review). On the opposite to the pattern of a latitudinal depletion in baseline  $\delta^{13}\text{C}$  values observed in offshore waters, along the shelf break the  $\delta^{13}\text{C}$  is more  $^{13}\text{C}$ -depleted in the SE than in the S region (Troina et al. in review). Additionally, specimens sampled in the SE region did not show seasonal differences in either  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values, whereas those sampled in the S region did.  $\delta^{15}\text{N}$  was significantly higher in spring than in autumn for the southern ASD, which is consistent with seasonal variations in baseline  $\delta^{15}\text{N}$  in the region (Troina et al. in review). Although additional studies using other techniques such as mitochondrial DNA can help support or refute this hypothesis, for the sake of discussing the results observed here, we will refer to the south-eastern and

southern Atlantic spotted dolphins (ASD-SE and ASD-S, respectively) as two distinct populations.

#### *Bottlenose dolphins*

BND sampled in the SE and S regions showed significant differences in  $\delta^{15}\text{N}$  values in spring but not in autumn, while  $\delta^{13}\text{C}$  values were equal between S and SE conspecifics in both seasons. Likewise the overall pattern in cetaceans from these two areas, the  $\delta^{15}\text{N}$  of BND sampled in the S region were higher than those from the SE. Nonetheless, despite the latitudinal differences in  $\delta^{15}\text{N}$ , SE and S specimens were grouped in the same branch in both seasons in our cluster analysis (Fig. 3). Moreover, our cluster analysis showed that the isotopic values of BND were more similar to the remaining S species in autumn (Fig. 3a and b), while in spring they had higher similarity with the SE species (Fig. 3c and d). The BND isotopic values were consistent with their distribution patterns, which peaked in the SE region in spring, whereas the highest peaks were observed between 28 and 30°S (S region) in autumn (Di Tullio et al. 2016). The high level of genetic variability in the offshore BND ecotype suggests that they form a large population (Fruet et al. 2017) that occurs in both SE and S regions (Oliveira et al. 2019). Thus, it is possible that some individuals of this population perform seasonal movements between these two areas throughout the year. Along the study area, cetacean density and species richness were the highest in the S region in spring (Di Tullio et al. 2016). Therefore, it is likely that some BND avoid the S region at this time of the year to minimize inter-specific competition.

#### *Spinner dolphins*

SPD did not show latitudinal gradients in either  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values. However, differently from the ASD and BND in the SE region, the SPD showed significant seasonal differences in both isotopes. This species was observed in the S region only in spring (Di Tullio et al. 2016), apparently exploring farther south only when the tropical waters from the Brazil current reach the region (e.g. Secchi and Siciliano 1995). Moreover, the SPD have higher distribution densities in oceanic waters (500 – 1000 m isobaths) (Moreno et al. 2005; Di Tullio et al. 2016), where there is a latitudinal decrease in  $\delta^{13}\text{C}$  values southwards (Troina et al. in review). The isotopic signal of the prey consumed during this latitudinal movement will be reflected later as it takes about two months for the turnover of assimilated prey in skin tissues (Giménez et al. 2016). The seasonal differences in the isotope values of SPD sampled in the SE region raises a hypothesis that in autumn it reflected the more  $^{13}\text{C}$ -depleted isotopic values from

the S region acquired in the previous (summer) months. Furthermore, their lower  $\delta^{15}\text{N}$  values, even in autumn when they would be reflecting the more  $^{15}\text{N}$ -enriched values from the S region (Troina et al. in review), and their lower  $\delta^{13}\text{C}$  values, despite their occurrence in offshore waters where baseline  $\delta^{13}\text{C}$  values are slightly higher than along the shelf break (Troina et al. in review), provide further support for this species relatively lower trophic position in comparison to the other cetacean species in the western south Atlantic. This agrees with data on stomach contents of SPD in offshore islands off northeastern Brazil, which showed large proportion of small epi- and mesopelagic fish as their main prey items (Silva-Jr. et al. 2007).

### *Short-beaked common dolphins*

There are two stocks of common dolphins south of  $20^{\circ}\text{S}$  in the SWA, the long-beaked morphotype in the S and SE region, which is restricted to coastal waters (Jefferson et al. 2009; Tavares et al. 2010; Cunha et al. 2015), thus occurring out of the range of the present study, and the short-beaked (SBCD) morphotype in the S region, that is distributed predominantly near the outer continental shelf and shelf break (Di Tullio et al. 2016). Consequently, all common dolphins sampled in the present study are those restricted to the S region, which has higher  $\delta^{15}\text{N}$  values (Troina et al. in review). Amongst the species sampled in the S region, SBCD had the second highest mean  $\delta^{15}\text{N}$  values, only lower than the RTD. The relatively high  $\delta^{15}\text{N}$  values could be due to their occurrence (hence feeding) along the outer continental shelf and shelf break (Di Tullio et al. 2016), where there is an offset in baseline  $\delta^{15}\text{N}$  due to the upwelling of nutrient rich SACW (Troina et al. in review).

### *Sperm whales*

Sperm whales were sampled only in the S region, and most of the samples are from spring months. Their CR was considerably higher in spring (4.7‰) than in autumn (1.6‰) and their NR was the smallest amongst the cetacean species in the S region. These results suggest that sperm whales sampled here feed upon prey of similar trophic levels in both seasons, though over a wide spatial range, especially in spring. This is expected for a migratory species. The short-finned squid *Illex argentinus* is an important prey item for sperm whales in the SWA (Clarke et al. 1980; Santos and Haimovici 2000) and the most abundant demersal squid in southern Brazil (Haimovici et al. 2009). They have a patchy distribution in the S region along the outer continental shelf and slope in summer/autumn, and present a wider distribution in winter/spring (Haimovici et al. 2014). Thus, our isotopic data corroborates the hypothesis of Di Tullio et al. (2016) that sperm whales' seasonal variations in distribution follow the seasonality

in the distribution patterns of this prey. Sperm whales form fewer but larger group aggregations in autumn, whereas in spring they are more evenly distributed throughout the S region (Di Tullio et al. 2016). Accordingly, if they fed on the short fin squid in winter/spring, their isotopic values reflected this prey signal by the time of our sampling showing the observed large variability in  $\delta^{13}\text{C}$  values.

#### *Other species*

The RTD in the SWA occur along the continental shelf in coastal waters (Ott and Danilewicz 1996; Santos et al. 2017; 2019). Despite the long-term survey effort carried out throughout both the SE and S regions, in deeper offshore waters (~ 200-400 m isobaths) the species was only registered in the S region (Di Tullio et al. 2016; this study). This species had the highest  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, suggesting nearshore feeding and higher trophic position than the remaining cetacean species observed in the S region. There is little information on their feeding habits. However, the cutlassfish *Trichiurus lepturus*, and cephalopods were found in stomach contents of RTD stranded along the coast or accidentally caught in the SE region (Di Benedetto et al. 2001; Melo et al. 2010). The cutlassfish are fierce predators that feed upon a large variety of fish and squids (Martins et al. 2005). They are very abundant demersal bony fish in southern Brazil continental shelf and shelf break (Martins and Haimovici 1997), making them potential prey for the RTD inhabiting the S region.

The isotopic values of KW in the present study were amongst the lowest of all species sampled in autumn in the S region (Fig. 2). Analysis of isotopic composition in teeth of KW along southern Brazil identified three distinct ecotypes (Tatsch 2015). The highest  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were observed in the coastal ecotype, while the oceanic had the lowest values (Botta et al. 2012; Tatsch 2015). The low  $\delta^{15}\text{N}$  in KW sampled here suggest feeding at lower trophic levels. Indeed, on the event of the sampling of these KW they were observed preying upon a minke whale (*Balaenoptera* spp.) (Di Tullio pers. obs.). Minke whales feed mainly on invertebrates (euphausiids and copepods) or on small pelagic schooling fish (Secchi et al. 2003; Bastida et al. 2018). Their  $\delta^{15}\text{N}$  values should therefore represent that of a relatively lower trophic level consumer.

Our cluster analysis placed the LFPW within the same branch as the BND (Fig. 3a). These odontocete species had very similar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Fig. 2), and the LFPW  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot fell within the BND isotopic niche area (Fig. 4). Individuals of these two species were frequently reported forming mixed-species associations and show similar distribution

patterns (Di Tullio et al. 2016). Their similarity in isotope compositions suggests that these associations could be established to search for food and improve prey detection.

The PTSD showed similar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in both seasons. Although the single sample in autumn did not allow statistical analysis, the similar isotope values between seasons is consistent with the other cetacean species that occurred in the SE region (see discussion above). Their relatively lower  $\delta^{15}\text{N}$  values suggest lower trophic position, which is in line with their reported dietary preferences on small epi- and mesopelagic fish and squid (Robertson and Chivers 1997; Wang et al. 2003; 2012).

Lastly, the only CLYD sampled had the most distinct isotopic signal, with considerably lower  $\delta^{15}\text{N}$  in comparison to the other cetacean species in the same region (Fig. 2), and very high  $\delta^{13}\text{C}$  value. This species occurs in oceanic waters beyond the continental shelf, showing preference for warmer waters in low latitudes (Moreno et al. 2005; Amaral et al. 2015). Although the CLYD was sampled in the S region, its low  $\delta^{15}\text{N}$  and high  $\delta^{13}\text{C}$  values are consistent with high temperature, oligotrophic waters of the Brazil current (Troina et al. in review). Indeed, this CLYD was sampled in the northernmost portion of the S region ( $\sim 29^\circ\text{S}$ ) at high depths (1467 m isobath) during a spring cruise, when the warm waters of the Brazil current predominate along the region (Möller Jr. et al. 2008). Moreover, their high  $\delta^{13}\text{C}$  are in agreement with feeding at lower latitudes (Rau et al. 1982; Magozzi et al. 2017).

### **Isotopic niche overlap and partition**

The ASD and BND showed a high overlap in their isotopic niche areas along both regions and seasons. These two species had the highest  $\delta^{15}\text{N}$  values in the SE, indicating relatively high trophic positions. Additionally, they were frequently recorded forming mixed-species associations in the SWA (Di Tullio et al. 2016). Elsewhere, mixed-species association including these two species involved mainly affiliative and aggressive behaviour (Herzing and Johnson 1997). Nevertheless, the isotopic similarity observed in the present study suggests that in the SWA these social interactions might be a strategy to search for food and improve prey detection.

The ASD-SE had one of the smallest isotopic niche areas estimated in the SE region. Additionally, the relatively low NR, CR and CD suggest high trophic redundancy and limited diversity of niches. Stomach contents of ASD sampled in the SE region included pelagic and demersal fish and squid (Melo et al. 2010; Lopes et al. 2012), although a predominantly teuthophagous feeding habit has been suggested (Di Benedetto et al. 2001; Lopes et al. 2012).

Thus, selectivity in their prey preferences would explain their relatively narrower isotopic ranges and small niche areas observed here.

The Atlantic spotted dolphins sampled in the S region (ASD-S) showed remarkable seasonal differences in isotopic niche area and NR. In spring, their isotopic area and NR were the largest amongst all species in the S region, which suggests feeding at different trophic levels and high degree of trophic diversity (Layman et al. 2007). In autumn, in contrast, the population metrics estimated suggested high trophic redundancy, with individuals using rather similar prey resources. Although there are no data on the stomach contents of the ASD from southern Brazil, the species is known to feed on a wide variety of prey items, including epi- and mesopelagic fish and squids (Herzing and Elliser 2014; Herzing and Perrin 2018). However, there is seasonality in the density and diversity of potential demersal prey species in the S region, with high catches and species richness in winter and spring months (Haimovici et al. 1994) which would result in larger isotopic niche of specimens in this region in spring.

Moreover, a significantly large core isotopic niche overlap between the ASD-S and the SBCD was evidenced in spring and confirmed by the cluster analysis (Fig. 3a and c). Analysis of larger temporal scale (tooth samples) also showed similar mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Botta et al. 2012) and large isotopic niche overlap for these two species (Tatsch 2015). Both species feed on similar prey types (Santos and Haimovici 2001; Melo et al. 2010; Herzing and Perrin 2018; Perrin 2018) and are strongly associated with the outer continental shelf and shelf break waters (~250 m depths), but occur in parapatry with decreasing abundance of one species as the other begins to appear (Di Tullio et al. 2016). As they rely on similar food resources, potentially occupying the same ecological niche and competing for the same resources, the spatiotemporal segregation in the areas of occurrence observed for these two species seems to be a means to avoid competition.

SBCD showed seasonal difference in their isotopic niche areas, being considerably larger in spring than in autumn. The large NR, which was rather similar in both seasons (Table 4), suggests feeding on a wide range of trophic levels. This pattern provides evidence for high individual specialization in resource use within the population, with individuals feeding at different trophic levels. However, their isotopic niche area expands (spring) and shrinks (autumn) seasonally, which probably relates to the seasonal variation in prey abundances and distributions. Their prey includes a large variety of small meso- and epipelagic fish and squid species (Young and Cockcroft 1994; Melo et al. 2010; Giménez et al. 2018; Marçalo et al.

2018). The most important prey species found in the stomach content of SBCD from the north of Patagonia was the argentine anchovy *Engraulis anchoita* (Romero et al. 2012). The distribution and abundance of anchovies in southern Brazil is highly seasonal, and biomasses are the highest in winter and early spring (Lima and Castello 1995). In this period this fish species is widely distributed throughout the whole extension of the southern continental shelf, while in summer they have patchy distribution and extremely low abundance (Lima and Castello 1995). This is consistent with the seasonal differences in SBCD' isotopic niche areas, which is considerably larger in spring than in autumn.

The isotopic niche area for sperm whales was estimated only for the spring period. They had complete isotopic segregation with the SPD, and had high isotopic overlap with the SBCD, ASD and BND (table 5). Their niche differentiation with SPD is expected as these two species densities peak at different latitudinal and depth ranges (Di Tullio et al. 2016). In contrast, the isotopic niche similarity with the other species suggests that they share resources along the shelf break in the S region, area commonly used by these odontocetes (Di Tullio et al. 2016).

Our isotopic data showed apparent niche partitioning between the SPD and the other cetacean species in both seasons, except for the PTSD in the SE (Fig. 4 and 5). Their occurrence in offshore waters is considerably different from the other cetacean species sampled in the S region that prefer shelf break and upper slope waters, with the exception of the sperm whales (Di Tullio et al. 2016). In the SE region the ASD and BND have their densities peak at 250 and 500 m depth, respectively. Their distribution contrasts with that of the SPD and the PTSD, the latter being the only species with whom SPD overlapped in isotopic niche space. As there is a shelf break-to-slope gradient in  $\delta^{13}\text{C}$  values (Troina et al. in review), species with preference for the outer continental shelf and shelf break waters will have different isotopic compositions from those with further offshore distributions, which was evident in our analysis.

The large overlap and isotopic similarity between the SPD and the PTSD in spring suggests that they share similar resources. These two species have similar distribution patterns, with preference for deeper, warm waters of the Brazil current (Moreno et al. 2005; Di Tullio et al. 2016). Likewise the SPD, the PTSD feed on epi- and mesopelagic fish and squid, including fish of the families Myctophidae and Exocoetidae, and Ommastrephidae squids (Bernard and Hohn 1989; Robertson and Chivers 1997; Wang et al. 2003; 2012). Moreover, nocturnal feeding on vertical migrating fish prey has been observed for both species (Baird et al. 2001; Dolar et al. 2003). Thus, as these two species show preference for similar habitats and prey type, they

might be competing for the same food resources. Hence, eventual seasonal movements of SPD towards southern waters (Di Tullio et al. 2016) might be a strategy to reduce interspecific competition.

## **Conclusion**

In the present study, we used for the first time carbon and nitrogen stable isotopes in skin biopsies to assess the trophic ecology and structure of coexisting cetaceans in the oceanic waters of the western south Atlantic. Consistent latitudinal and seasonal patterns in cetaceans isotopic values that are directly associated with the isoscapes of their respective region of occurrence were observed. The rough-toothed dolphins had the highest  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values, which supports the species relatively high trophic position, and occurrence in the neritic waters off southern Brazil. The sperm whales showed seasonal variation in their isotopic niche areas, indicating the use of a wide range of resources in winter and spring months, but feeding at similar trophic levels throughout the year. The bottlenose dolphins had consistently high similarity in their isotopic compositions with the Atlantic spotted dolphins and the pilot whales, suggesting that the observed mixed-species assemblages including these species, reported in previous studies, are due to similar foraging activities. The spinner dolphins had the lowest  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values, indicating they occupy a relatively lower trophic position among the co-occurring cetacean species. Additionally, the high niche overlap observed between spinner dolphins and the pantropical spotted dolphins in spring indicate that they compete for similar resources, and the seasonal movements of the former to southern areas might be a strategy to minimize competition. Moreover, our data provided isotopic-based support for the existence of two subpopulations of the Atlantic spotted dolphins along the study area. Finally, the large isotopic niche overlap between the Atlantic spotted dolphins from the southern region with the common dolphins suggested that they occupy a similar ecological niche, and the spatiotemporal segregation in habitat use, observed in previous studies, may be a strategy to minimize competition.

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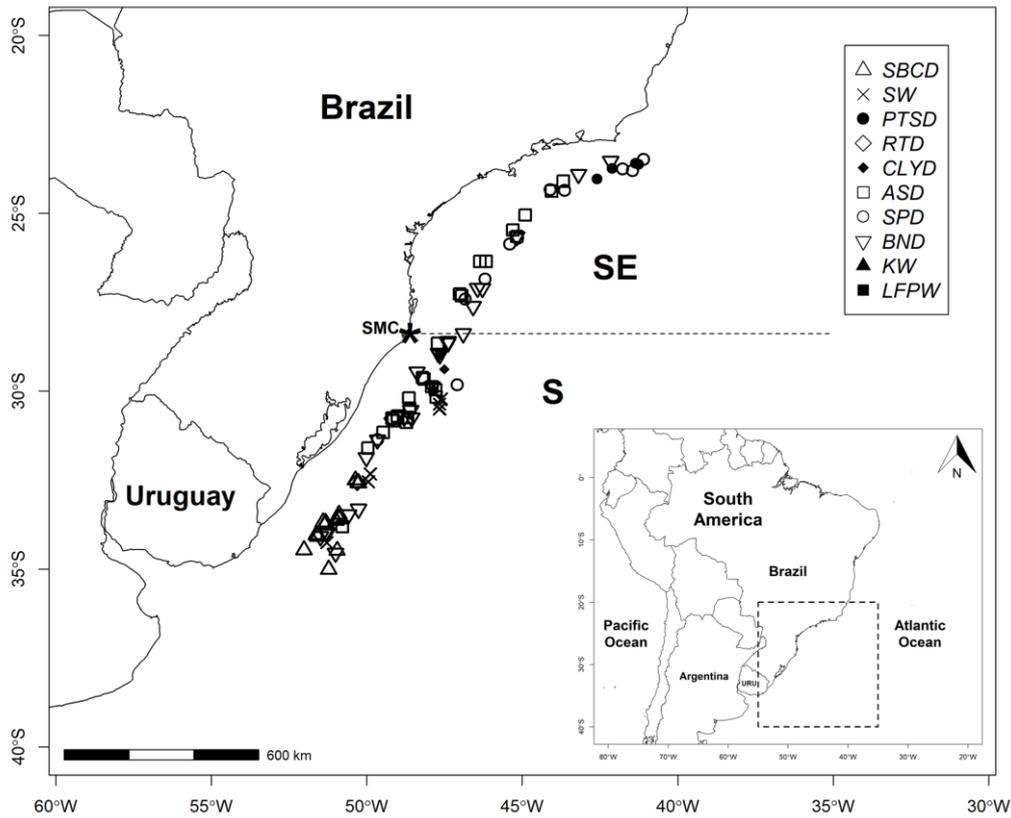
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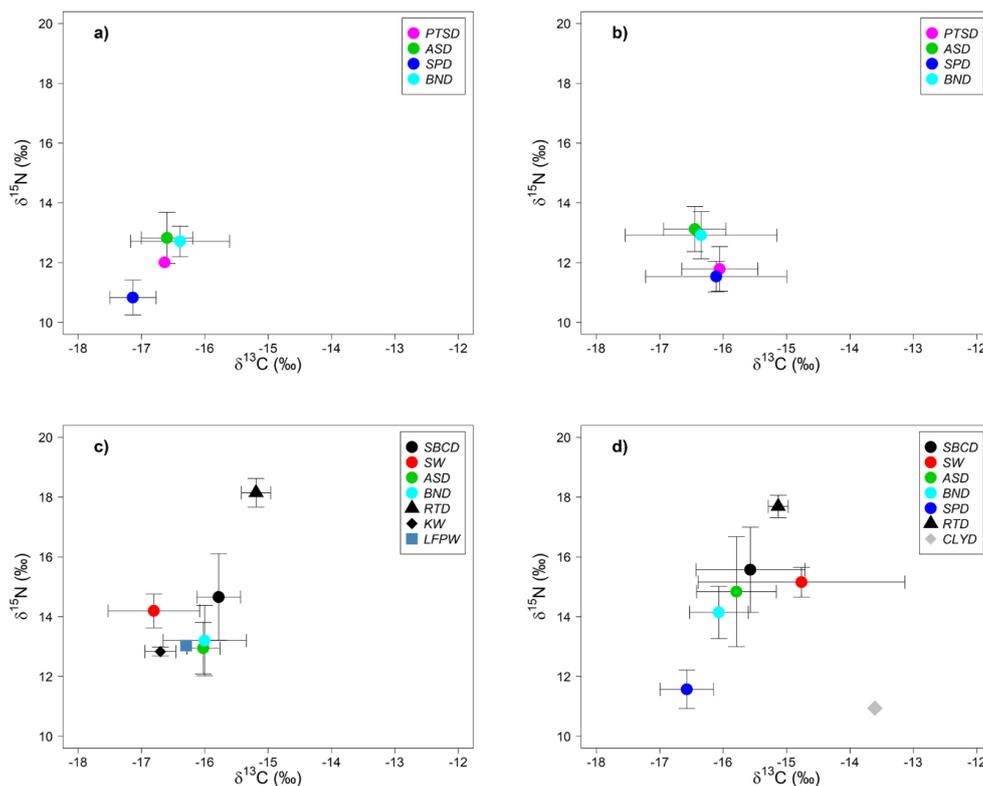
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**Fig. 1 Study area and cetaceans sampling locations. The Santa Marta Cape (SMC) is indicated with a \*; the dotted line separates the southeast (SE) and the southern (S) regions. Species code: ASD: Atlantic spotted dolphins (*Stenella frontalis*); BND: bottlenose dolphins (*Tursiops truncatus*); CLYD: Clymene dolphins (*S. clymene*); KW: killer whales (*Orcinus orca*); LFPW: long-finned pilot whale (*Globicephala melas*); PTSD: pantropical spotted dolphins (*S. attenuata*); RTD: rough-toothed dolphins (*Steno bredanensis*); SBCD: short-beaked common dolphins (*Delphinus delphis*); SPD: spinner dolphins (*S. longirostris*); and SW: sperm whales (*Physeter macrocephalus*)**

**Table 1** the total number of samples (n) for each cetacean species sampled in this study. The scientific and common name, as well as the specific code used in the plots are presented

Species	Common name	Code	n
<i>Delphinus delphis</i>	Short-beaked common dolphins	SBCD	54
<i>Physeter macrocephalus</i>	Sperm whales	SW	10
<i>Stenella attenuata</i>	Pantropical spotted dolphins	PTSD	7
<i>Steno bredanensis</i>	Rough-toothed dolphins	RTD	7
<i>Stenella frontalis</i>	Atlantic spotted dolphins	ASD	74
<i>Stenella longirostris</i>	Spinner dolphins	SPD	44
<i>Tursiops truncatus</i>	Bottlenose dolphins	BND	76
<i>Orcinus orca</i>	Killer whales	KW	3
<i>Globicephala melas</i>	Long-finned pilot whales	LFPW	1
<i>Stenella clymene</i>	Clymene dolphins	CLYD	1



**Fig. 2** Biplot with the mean ( $\pm$ SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for each cetacean species in a) autumn-southeast; b) spring-southeast; c) autumn-south; and d) spring-south. Species names and respective abbreviations are displayed in table 1

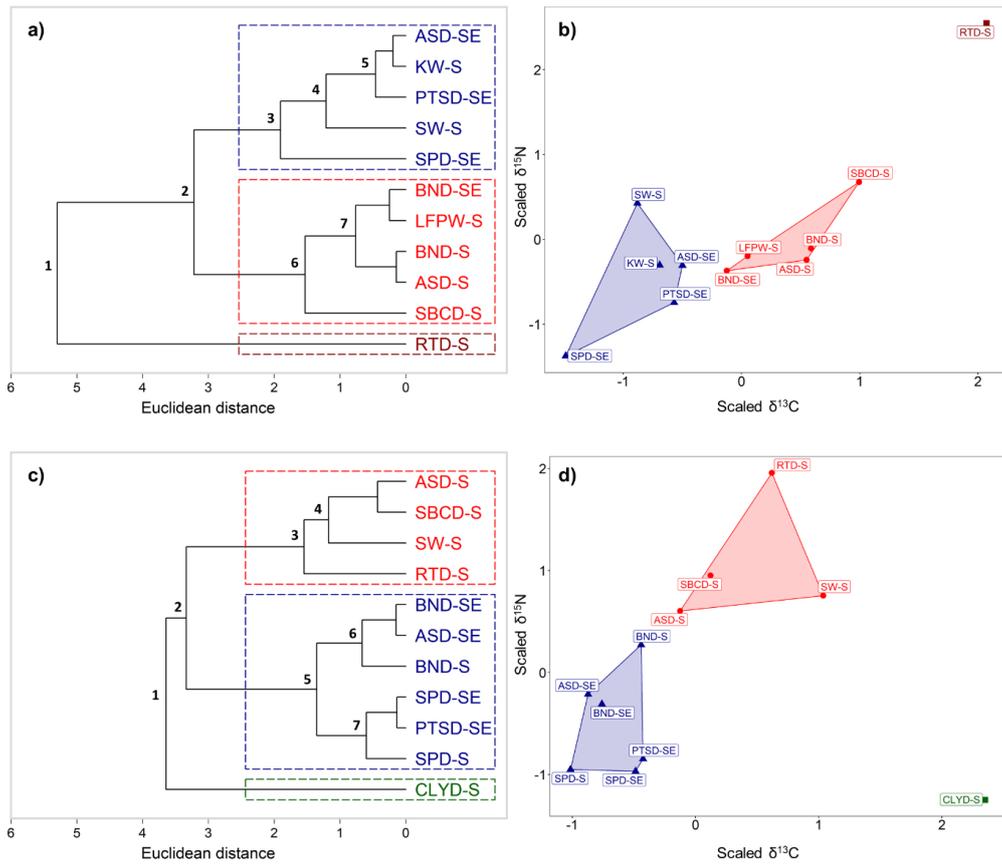
**Table 2** Total number of samples (n); mean ( $\pm$  standard deviation, SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (‰);  $\delta^{13}\text{C}$  corrected for lipid ( $\delta^{13}\text{C}_{\text{Norm.}}$ ); and carbon-to-nitrogen (C:N) ratio <sup>a</sup> for cetacean species sampled within each season (spring and autumn) in each region (southeast and south). Species names and respective abbreviations are displayed in table 1

Species	n	$\delta^{13}\text{C}$ (‰) mean $\pm$ SD	$\delta^{13}\text{C}_{\text{Norm.}}$ (‰) mean $\pm$ SD	$\delta^{15}\text{N}$ (‰) mean $\pm$ SD	C:N mean $\pm$ SD
<b>Spring</b>					
<i>Southeast</i>					
PTSD	6	-18.1 $\pm$ 0.4	-16.1 $\pm$ 0.6	11.8 $\pm$ 0.7	3.8 $\pm$ 0.2
ASD	21	-17.9 $\pm$ 0.3	-16.4 $\pm$ 0.5	13.1 $\pm$ 0.8	3.7 $\pm$ 0.2
SPD	18	-18.5 $\pm$ 0.5	-16.1 $\pm$ 1.1	11.5 $\pm$ 0.5	4 $\pm$ 0.4
BND	23	-17.6 $\pm$ 0.5	-16.3 $\pm$ 1.2	12.9 $\pm$ 0.8	3.6 $\pm$ 0.3
<i>South</i>					
SBCD	39	-16.8 $\pm$ 0.7	-15.6 $\pm$ 0.9	15.6 $\pm$ 1.4	3.6 $\pm$ 0.2
SW	6	-18.4 $\pm$ 0.5	-14.8 $\pm$ 1.6	15.2 $\pm$ 0.5	4.3 $\pm$ 0.6
RTD	3	-16.1 $\pm$ 0.1	-15.1 $\pm$ 0.2	17.7 $\pm$ 0.4	3.6 $\pm$ 0.1
ASD	22	-17.2 $\pm$ 0.7	-15.8 $\pm$ 0.6	14.8 $\pm$ 1.8	3.7 $\pm$ 0.2
SPD	10	-18.6 $\pm$ 0.4	-16.6 $\pm$ 0.4	11.6 $\pm$ 0.6	3.8 $\pm$ 0.2
BND	13	-17.6 $\pm$ 0.4	-16.1 $\pm$ 0.5	14.1 $\pm$ 0.9	3.7 $\pm$ 0.1
CLYD	1	-18.0	-13.6	10.9	4.5
<b>Autumn</b>					
<i>Southeast</i>					
PTSD	1	-17.5	-16.6	12.0	3.5
ASD	15	-17.6 $\pm$ 0.5	-16.6 $\pm$ 0.4	12.8 $\pm$ 0.9	3.6 $\pm$ 0.1
SPD	16	-18.4 $\pm$ 0.2	-17.1 $\pm$ 0.4	10.8 $\pm$ 0.6	3.7 $\pm$ 0.1
BND	9	-17.3 $\pm$ 0.2	-16.4 $\pm$ 0.8	12.7 $\pm$ 0.5	3.5 $\pm$ 0.2
<i>South</i>					
SBCD	15	-16.9 $\pm$ 0.8	-15.8 $\pm$ 0.3	14.7 $\pm$ 1.4	3.6 $\pm$ 0.2
SW	4	-17.9 $\pm$ 0.2	-16.8 $\pm$ 0.7	14.2 $\pm$ 0.6	3.6 $\pm$ 0.1
RTD	4	-16.2 $\pm$ 0.2	-15.2 $\pm$ 0.2	18.1 $\pm$ 0.5	3.6 $\pm$ 0.1
ASD	16	-17.4 $\pm$ 0.3	-16 $\pm$ 0.3	12.9 $\pm$ 0.9	3.7 $\pm$ 0.1
BND	31	-17.5 $\pm$ 0.4	-16 $\pm$ 0.7	13.2 $\pm$ 1.2	3.7 $\pm$ 0.2
KW	3	-17.6 $\pm$ 0.2	-16.7 $\pm$ 0.2	12.8 $\pm$ 0.2	3.5 $\pm$ 0
LFPW	1	-18.7	-16.3	13.0	4.0

<sup>a</sup> C:N weight ratio calculated from the percentage dry weight (%C and %N) in the sample

**Table 3 results of the statistical tests to compare cetaceans seasonal and latitudinal differences in mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Species names and respective abbreviations are displayed in table 1**

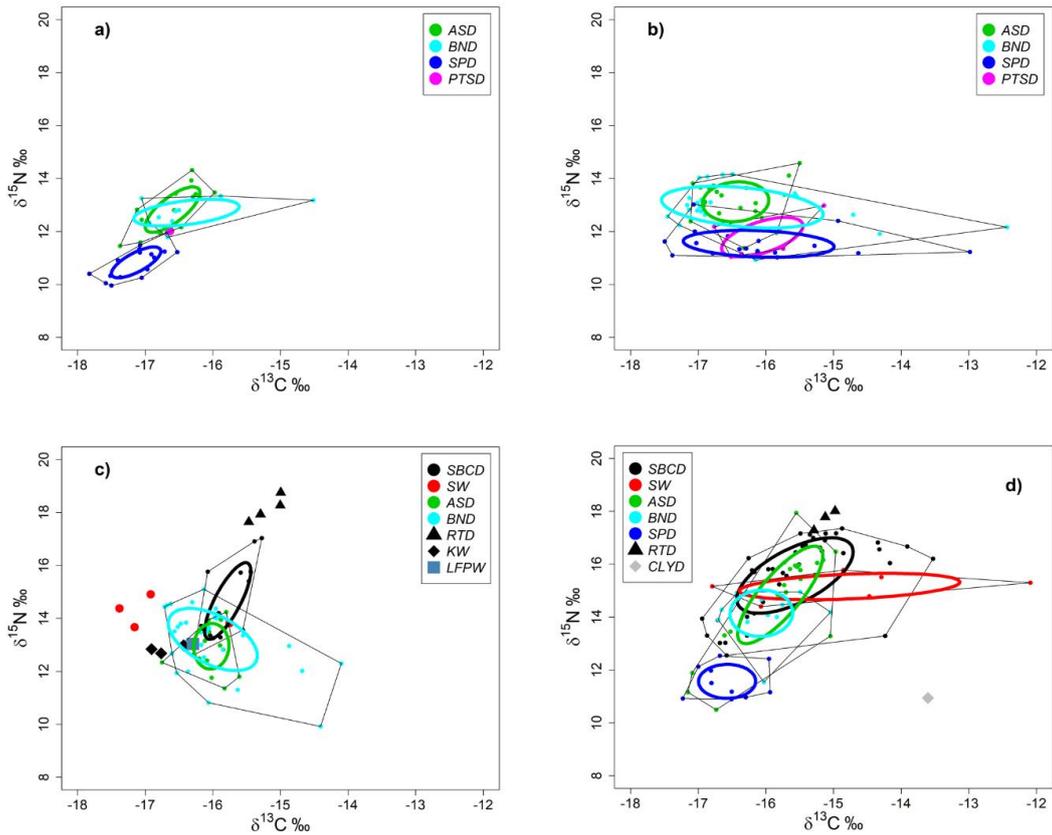
<b>Welch Two Sample t-test:</b>		
<b>Species</b>	<b><math>\delta^{13}\text{C}</math></b>	<b><math>\delta^{15}\text{N}</math></b>
	<b>Seasonal effect (Autumn vs. Spring in the S)</b>	
SBCD	t = 1.29, df = 51.8, p = 0.2	<b>t = 2.08, df = 25.1, p &lt; 0.05</b>
SW	<b>t = 2.69, df = 7.3, p &lt; 0.05</b>	<b>t = 2.76, df = 5.9, p &lt; 0.05</b>
RTD	t = 0.41, df = 4.9, p = 0.69	t = -1.42, df = 4.9, p = 0.21
SPD	<b>Seasonal effect (Autumn vs. Spring in the SE)</b>	
	<b>t = 3.69, df = 21, p &lt; 0.01</b>	<b>t = 3.7, df = 30.1, p &lt; 0.001</b>
	<b>Latitudinal effect (SE vs. S in spring)</b>	
	t = -1.58, df = 23.9, p = 0.12	t = 0.16, df = 15.4, p = 0.87
<b>Factorial ANOVA:</b>		
<b>Species</b>	<b><math>\delta^{13}\text{C}</math></b>	<b><math>\delta^{15}\text{N}</math></b>
ASD		
Region	<b>F(1, 70) = 30.52, p &lt; 0.001</b>	<b>F(1, 70) = 13.67, p &lt; 0.001</b>
Season	F(1, 70) = 2.87, p = 0.09	<b>F(1, 70) = 15.11, p &lt; 0.001</b>
Region:season	F(1, 70) = 0.14, p = 0.71	<b>F(1, 70) = 7.7, p &lt; 0.01</b>
<b>Post hoc <math>\delta^{13}\text{C}</math></b>		
Seasonal S	p = 0.45	
Seasonal SE	p = 0.797	
Latitudinal Spring	<b>p &lt; 0.001</b>	
Latitudinal Autumn	<b>p &lt; 0.001</b>	
<b>Post hoc <math>\delta^{15}\text{N}</math></b>		
Seasonal S		<b>p &lt; 0.001</b>
Seasonal SE		p = 0.88
Latitudinal Spring		<b>p &lt; 0.001</b>
Latitudinal Autumn		p = 0.99
BND		
Region	F(1, 72) = 2.32, p = 0.13	<b>F(1, 72) = 13.12, p &lt; 0.001</b>
Season	F(1, 72) = 0.009, p = 0.92	<b>F(1, 72) = 6.87, p &lt; 0.05</b>
Region:season	F(1, 72) = 0.06, p = 0.8	F(1, 72) = 2.2, p = 0.14
<b>Post hoc <math>\delta^{15}\text{N}</math></b>		
Seasonal S		p = 0.02
Seasonal SE		p = 0.946
Latitudinal Spring		<b>p &lt; 0.01</b>
Latitudinal Autumn		p = 0.53



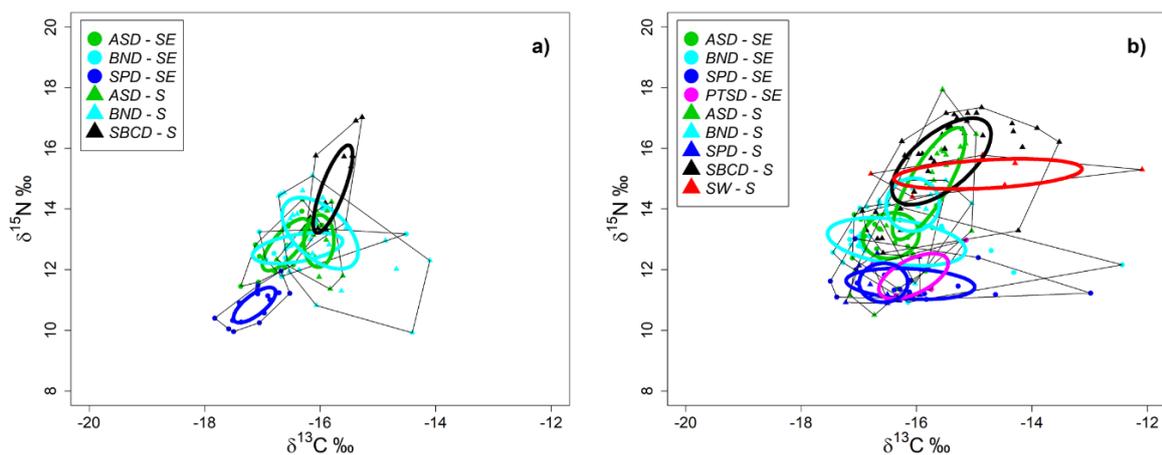
**Fig. 3** Dendrograms and cluster plots resulting from the hierarchical cluster analysis (complete linkage) based on skin  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of cetaceans from the Brazilian outer continental shelf and slope in autumn (a-b) and spring (c-d). Species names and respective abbreviations are displayed in table 1

**Table 4 Isotopic niche and population metrics for cetacean species sampled in each region (southeast and south) and season (spring and autumn): Total area (TA) of the convex hull encompassing the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values; Standard ellipse area corrected for sample size ( $\text{SEAC}$ ); Bayesian standard ellipse area ( $\text{SEAB}$ ) with the 95% confidence interval (CI);  $\delta^{15}\text{N}$  range (NR);  $\delta^{13}\text{C}$  range (CR); Mean distance to centroid (CD); Nearest neighbour distance (NND); and Standard deviation of the nearest neighbour distance (SDNND). Species names and respective abbreviations are displayed in table 1**

Species	TA	$\text{SEAC}$	$\text{SEAB}$ (95% CI)	NR	CR	CD	NND	SDNND
<b>Spring</b>								
<i>Southeast</i>								
PTSD	1.56	1.45	1.04 (0.46 - 2.92)	1.94	1.62	0.80	0.64	0.40
ASD	3.34	1.21	1.13 (0.7 - 1.76)	3.21	1.61	0.77	0.31	0.19
SPD	5.31	1.86	1.62 (1.04 - 2.77)	2.00	4.51	0.95	0.49	0.41
BND	8.44	2.92	2.76 (1.77 - 4.16)	3.23	5.02	1.19	0.37	0.47
<i>South</i>								
SBCD	10.98	3.09	3.02 (2.17 - 4.13)	4.80	3.41	1.46	0.29	0.33
SW	3.16	3.00	2.01 (0.94 - 5.69)	1.38	4.70	1.32	1.05	0.60
ASD	7.93	2.28	2.16 (1.46 - 3.61)	7.44	2.18	1.51	0.37	0.44
SPD	1.69	0.96	0.78 (0.4 - 1.61)	1.65	1.29	0.69	0.40	0.20
BND	3.26	1.39	1.19 (0.67 - 2.17)	3.41	1.66	0.74	0.49	0.57
<b>Autumn</b>								
<i>Southeast</i>								
ASD	1.77	0.79	0.76 (0.43 - 1.3)	2.87	1.40	0.83	0.28	0.13
SPD	1.14	0.49	0.47 (0.27 - 0.79)	2.00	1.30	0.61	0.23	0.14
BND	2.11	1.27	1.05 (0.53 - 2.21)	1.56	2.54	0.71	0.49	0.43
<i>South</i>								
SBCD	2.28	1.04	0.96 (0.59 - 1.74)	4.42	1.02	1.34	0.25	0.21
ASD	1.76	0.76	0.68 (0.4 - 1.13)	2.88	1.15	0.76	0.25	0.14
BND	8.15	2.15	2.02 (1.46 - 3)	5.18	2.62	1.10	0.33	0.37



**Fig. 4** Standard Ellipse Area corrected for sample size ( $SEAc$ ) for species with number of samples ( $n$ ) > 6 in each region within season: Autumn – southeast (a); Spring – southeast (b); Autumn – south (c); Spring – south (d); and  $\delta^{13}C$  and  $\delta^{15}N$  biplots of individual cetaceans from which  $n < 6$ . Polygons represent the Total Area (TA) encompassing the  $\delta^{13}C$  and  $\delta^{15}N$  values. Species names and respective abbreviations are displayed in table 1



**Fig. 5** Latitudinal (Southeast to south) comparison of Standard Ellipse Area corrected for sample size ( $SEAc$ ) for those cetacean species sampled in both regions in a) autumn and b) spring. Polygons represent the Total Area (TA) encompassing the  $\delta^{13}C$  and  $\delta^{15}N$  values. Species names and respective abbreviations are displayed in table 1

**Table 5** The percentage of overlap (%) in the isotopic niche area, measured as the Standard ellipse area corrected for sample size (SEAc), between every two cetacean species within each region (southeast - SE and south - S); and for the same species with individuals sampled in both regions (SE x S); in spring and autumn. Species names and respective abbreviations are displayed in table 1

Species 1	Species 2	% species 1 area	% species 2 area
<b>Spring</b>			
<i>Southeast</i>			
PTSD	ASD	38.85	46.40
PTSD	SPD	67.14	52.33
PTSD	BND	70.51	35.08
ASD	SPD	38.48	25.11
ASD	BND	96.84	40.34
SPD	BND	60.12	38.37
<i>South</i>			
SBCD	SW	43.93	45.23
SBCD	ASD	60.12	81.67
SBCD	SPD	7.79	25.12
SBCD	BND	40.91	90.95
SW	ASD	26.24	34.62
SW	SPD	0.00	0.00
SW	BND	27.06	58.44
ASD	SPD	25.65	60.91
ASD	BND	48.51	79.40
SPD	BND	24.12	16.62
<i>SE x S</i>			
ASD (SE)	ASD (S)	75.39	40.26
BND (SE)	BND (S)	30.61	64.23
SPD (SE)	SPD (S)	43.33	84.20
<b>Autumn</b>			
<i>Southeast</i>			
ASD	SPD	25.33	40.51
ASD	BND	68.64	42.87
SPD	BND	24.53	9.58
<i>South</i>			
SBCD	ASD	50.17	68.76
SBCD	BND	60.02	28.95
ASD	BND	99.53	35.02
<i>SE x S</i>			
ASD (SE)	ASD (S)	51.81	54.23
BND (SE)	BND (S)	68.73	40.54

## **CAPÍTULO 4**

**From bottom to top: The trophic structure of the South Atlantic pelagic system using bulk and compound specific stable isotopes**

## From bottom to top: The trophic structure of the South Atlantic pelagic system using bulk and compound specific stable isotopes

### Abstract

In this study we provide a qualitative analysis of the carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotopes in the different organisms comprising the pelagic compartment of the outer continental shelf and slope in the southeast and southern Brazil. We analysed bulk  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in Particulate Organic Matter (POM), zooplankton, squids, bony and cartilaginous fish and cetacean species. Additionally, we carried out compound-specific isotope analysis of individual amino acids in 69 individuals of the 10 cetacean species sampled to complement the analysis of baseline patterns in nitrogen values, and to estimate their relative trophic positions. Both bulk  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  increased from zooplankton to cetacean and cartilaginous fish.  $\delta^{15}\text{N}$  was higher in POM than in the zooplankton groups, which likely reflects the difference in the concentration of nutrients and in the rate of nitrogen uptake by primary producers through the water column, as the zooplankton and POM samples were collected at different depth ranges. The large variability in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  within the different fish groups most likely reflects the range of trophic levels and resource used by the different species. The highest trophic levels in cetacean species were observed in *Physeter macrocephalus* (n = 6, TL = 3.5-3.9), while *Orcinus orca* had one of the lowest trophic positions (n = 3, TL = 2.8-3.2). Similar trophic positions (3.2-3.7) were identified among *Tursiops truncatus* (n = 17), *Stenella frontalis* (n = 16) and *Delphinus delphis* (n = 10). Compound-specific isotope analysis allowed us to identify that *Steno bredanensis* (n = 5) occur along the more  $^{15}\text{N}$ -enriched waters of the continental shelf in southern Brazil, and that their trophic position (3.1-3.5) is not significantly higher than that of the remaining cetacean species. Finally, the community-wide metrics differed between the southeast and southern regions, as well as seasonally within each region. The niche patterns observed in these areas were associated with the different water patterns influencing each region.

**Key words:** cetaceans, food web, stable isotopes, amino acids

## Introduction

The structure and dynamics of food-webs are influenced by the number of feeding links between primary producers and top predators (food-chain length), the amount of food resources available, the level of omnivory and species interactions (Post 2002; Vandermeer 2006; Namba et al. 2008; Long et al. 2011). Food-chain length can affect the fate of trophic cascades (Estes et al. 1998), and longer food-webs are less stable and more easily affected by environmental disturbances (Pimm and Kitching 1987; Long et al. 2011). Resource availability determines the level of specialism or generalism in an ecosystem (Terraube et al. 2011). Therefore, interactions between predators and their prey shape natural populations, communities and ecosystems. As such, marine mammals play key roles in the structure and dynamics of the ecosystems they inhabit (Bowen 1997; Estes et al. 1998; Roman et al. 2014, 2016), exerting top-down control on the abundances of intermediate trophic levels (Estes et al. 1998; Springer et al. 2003). In addition, by competing for similar resources (e.g., krill), cetaceans have significant impact on the abundance of co-occurring species (Laws 1985; Ballance et al. 2006). They also affect bottom-up processes by enhancing primary productivity with nutrients from their excremental materials (Nicol et al. 2010; Roman and McCarthy 2010; Lavery et al. 2014; Roman et al. 2016), by transferring energy from surface waters through vertical movements in search for food, or when their carcasses sink serving as food or shelter for organisms at high depths (Bennett et al. 1994; Jones et al. 1998). Therefore, an ecosystem approach for understanding their trophic ecology and ecological role is crucial, and implies the assessment of their interactions with competing co-occurring species, potential prey and predators.

Marine mammals feeding habits have been successfully studied through the analysis of bulk carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotopes in their tissues and in their prey (Newsome et al. 2010; Troina et al. 2016; Seyboth et al. 2018). The isotopic values in consumers resemble that of their food sources with a small difference between trophic levels (DeNiro and Epstein 1978; 1981; Tieszen et al. 1983). In cetaceans skin this prey-to-predator trophic difference is approximately +1.0‰ for  $\delta^{13}\text{C}$  and +1.6‰ for  $\delta^{15}\text{N}$  (Giménez et al. 2016). More recently, compound-specific isotope analyses of individual amino acids have been applied as a promising and powerful tool in ecological studies, helping to assess consumers trophic levels and trace energy and nutrient flow within and among ecosystems (Chikaraishi et al. 2009; Matthews and Ferguson 2014; Elliot-Smith et al. 2018). That is because some (*source*) amino acids undergo minimal trophic fractionations (McMahon and McCarthy 2016), being therefore applied to make inferences about the baseline isotopic values where the animals feed,

while other (*trophic*) amino acids undergo large fractionation during metabolic processes and are used to assess consumers trophic levels (Popp et al. 2007; McMahon and McCarthy 2016; McMahon and Newsome 2019).

The main objective of this study is to provide a descriptive (qualitative) analysis of the food-web in the pelagic ecosystem in the subtropical Southwestern Atlantic Ocean, through the analysis of the stable carbon and nitrogen isotopes in the tissues of the different organisms that comprise this food chain, and to estimate the relative trophic position of the different cetacean species.

## **Materials and methods**

### **Study area**

The study area encompasses the outer continental shelf (~150 m isobath) and slope (~1.500 m isobath) off south-east and southern Brazil (Fig. 1). Tropical waters of the Brazil current and the seasonal wind generated upwelling of the South Atlantic Central Water are the major water masses affecting the south-eastern region (SE, 24°S–28°S) (Acha et al. 2004; Brandini et al. 2018). The southern region (S, 28°S–34°S) is seasonally influenced by the tropical waters that dominate in warmer months, while in winter there is the prevalence of the subtropical shelf water, formed by the encounter of continental, tropical and Subantarctic shelf water (Möller Jr. et al. 2008; Piola et al. 2008). In turn, the physical gradients throughout the area result in an offset in baseline  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values that differentiate between the SE and S regions (Troina et al. in review – chapter 2). Therefore, the pelagic compartment will be assessed separately for each region, within each season.

### **Sample collection and preparation**

The samples were collected during ten research cruises in the scope of the *Projeto Talude* (Continental Slope Project) on board the RV *Atlântico Sul* (Federal University of Rio Grande, FURG). The cruises took place between 2009 and 2015 during austral spring (n = 5) and autumn (n = 5). Particulate Organic Matter (POM) and zooplankton samples were collected at pre-determined oceanographic stations. Samples of potential prey (fish and squids) for cetaceans were collected using a midwater trawling when the vessel's echosounder detected

strong fishing school signal. Cetacean skin samples were collected from bow-riding animals from the bow of the RV or from a small boat deployed from the ship. Skin samples were obtained using a 120-lb draw weight crossbow with modified darts specifically designed for sampling, with different size tips depending on the target species.

Surface water was collected to analyse the POM as a proxy for the isotopic values of primary producers (Hobson et al. 2002). These samples were pre-filtered with a 200  $\mu\text{m}$  mesh to separate the larger organisms and particles before vacuum filtration on Whatman GF/F (0.7  $\mu\text{m}$ ) glass filters. Zooplankton samples are a composite of the whole vertical profile of each station, collected between zero and up to 200 m, depending on the local bottom depth, using 300- and 500- $\mu\text{m}$ -mesh-size Bongo nets. All samples (POM, zooplankton, prey and cetacean) were immediately stored at  $-20^{\circ}\text{C}$  without any chemical treatment until processing.

POM filters were dried in oven at  $60^{\circ}\text{C}$  for 48h and acidified by HCl (30% hydrochloric acid) fumigation to remove inorganic carbon. A subsample of mixed zooplankton was collected from the main sample while the remaining sample was then sorted into the main taxonomic groups (amphipods, chaetognaths, copepods, euphausiids and salps) for separate isotope analyses, with each taxon subsample including multiple organisms. Samples of cetacean skin, as well as fish and squid muscles were then rinsed with distilled water, dried in an oven at  $60^{\circ}\text{C}$  for 48 h, and ground to a fine powder using a mortar and pestle. Acidification with HCl was not carried out in the zooplankton groups, as their effect on invertebrates  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values is uncertain (Bunn et al. 1995; Grey et al. 2001; Carabel et al. 2006).

### **Stable isotopes**

Analyses of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in POM were carried out at the land-based laboratory Analytical, Environmental and Geo-Chemistry at the Vrije Universiteit Brussel (AMGC, VUB, Brussels) with an Elemental Analyser (EA, EuroEA 3000, EuroVector, Milano, Italy) coupled to an Isotope Ratio Mass Spectrometer (IRMS, Delta V plus, Thermo Electron Corporation). Zooplankton, prey and cetacean each sample were analysed at the Stable Isotope Core Laboratory, Washington State University, with an IRMS (Delta PlusXP, Thermofinnigan) connected to an EA (ECS 4010, Costech Analytical, Valencia, CA). The isotopic compositions were reported in the conventional delta ( $\delta$ ) per mil notation (‰), relative to Vienna Pee Dee Belemnite ( $\delta^{13}\text{C}$ ) and atmospheric  $\text{N}_2$  ( $\delta^{15}\text{N}$ ). Internal lab standards were spread among the samples and analysed to estimate instrument precision. The within-run analytical precision of these internal standards was  $\leq 0.1\%$  for both isotopes.

## Compound specific isotope analysis

About 2-5 mg of 69 cetacean skin samples were processed for analysis of  $\delta^{15}\text{N}$  in amino acids. The method of amino acid extraction is described in detail by Riekenberg et al. (in review). It includes acid hydrolysis to break down the proteins into individual amino acids by heating the sample at 110°C in 6 M HCl for 20 h; the derivatization to make the amino acids volatile which includes two steps: isopropyl esterification by heating the sample at 110°C for 2 h in 300  $\mu\text{l}$  of a mixture of isopropanol and acetyl chloride (4:1), followed by the acylation with 300  $\mu\text{l}$  of a trimethylacetyl chloride and dichloromethane (1:4) mixture for 2 h at 110°C. Between each of the abovementioned steps, the samples were evaporated to dryness under a gentle stream of  $\text{N}_2$ . The derivatized samples were then analysed in a gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS, Thermo Trace 1310) interfaced to a Delta V Advantage IRMS through a GC IsoLink II combustion furnace (Thermo Fisher Scientific), at the Royal Netherlands Institute for Sea Research (NIOZ, Texel, The Netherlands).

## Data analysis

### *Grouping prey species*

Prey species were grouped into trophic guilds according to their taxonomy and habitat preferences consulting specific literature (Seeliger et al. 1997; Hastings et al. 2014; Priede 2017). The species composition of each prey group and total number of samples are displayed in Table 1.

### *Mathematical lipid correction*

Cetaceans  $\delta^{13}\text{C}$  values were mathematically lipid corrected using the coefficients estimated by Troina et al. (in review – chapter 3) in the equation adapted from Post et al. (2007):

$$\delta^{13}\text{C}_{\text{Normalized}} = \delta^{13}\text{C}_{\text{untreated}} + (-12.186 + 3.69 \times C:N) \quad \text{Eq. 1}$$

### *Cetacean trophic position*

The trophic position of cetacean species was estimated using two Trophic Enrichment Factors (TEF) for trophic (glutamic acid, Glu) and the source (glycine, Gly or phenylalanine, Phe) amino acids, in different published equations for comparisons:

Equation 2 uses a TEF of 7‰ between Glu and Gly (Popp et al. 2007):

$$TL = 1 + \left( \frac{\delta^{15}N_{Glu} - \delta^{15}N_{Gly}}{7} \right) \quad \text{Eq. 2}$$

Equations 3-5 considers the TEF between Glu and Phe, as well as the difference between trophic and source amino acids in the organisms at the base of the food web ( $\beta$ ), estimated to be 3.4‰ (Chikaraishi et al. 2009), 1.2‰ (McCarthy et al. 2013), and 3.6‰ (Bradley et al. 2015). We presented the TL of cetaceans based on estimations using these three estimated  $\beta$  values, with the TEF of 5.7‰ suggested by Bradley and colleagues (2015):

$$TL = 1 + \left( \frac{\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - \beta}{5.7} \right) \quad \text{Eq. 3 to 5}$$

### *Layman community metrics*

Six community metrics based on the  $\delta^{13}C$  and  $\delta^{15}N$  of all group members were used to assess the aspects of the trophic structure in each region (Layman et al. 2007). The metrics were calculated based on the sample mean of each group (zooplankton to cetaceans) within the community, using a Bayesian probabilistic method (bootstrapping 10000), which allows for the estimation of the uncertainty of sample mean (Jackson et al. 2011). The range of carbon (CR) and nitrogen (NR) were calculated as the distance between the individuals with the lowest and the highest  $\delta^{13}C$  and  $\delta^{15}N$  values, respectively. NR represents the vertical structure (trophic length), whereas CR provides information regarding the diversity of basal sources explored by the different individuals. The Total Area (TA) encompasses the  $\delta^{13}C$  and  $\delta^{15}N$  values, and represents the total niche area and the extent of trophic diversity within that community. The mean distance to the centroid (CD) offers additional information on the degree of trophic diversity. High CD indicates more isotopic dispersion, implying that a wide variety of trophic niches is explored by the different groups, whereas low CD suggests limited diversity of niches. The mean nearest neighbour distance (MNND) provides a measure of the degree of individuals spacing, while the standard deviation of the nearest neighbour distance (SDNND) provides a measure of trophic evenness. They both reflect the relative position of individuals to one another within the niche space, providing useful information on the extent of trophic redundancy. High MNND indicates wider spread within bi-plot space, while low values suggest higher density of individuals within the trophic niche space and trophic redundancy. High values of SDNND, on the other hand, suggest skewed spreads of isotopic niche (for instance towards higher trophic levels), while low values suggest more evenly spread niches (Layman et al. 2007). These community metrics were calculated for each region-within-season

combination (autumn and spring, south and southeast) in R version 3.6.0 (R Core Team 2019) using the Stable Isotope Bayesian Ellipse in R (SIBER) package version 2.1.4 (Jackson et al. 2011).

## Results

### *Groups' stable isotope values*

A total of 1189 samples including POM, zooplankton, fish, squids and cetaceans were analyzed for bulk  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in the present study (Table 1). We provide detailed information on the number of samples and mean ( $\pm$ SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of each group in autumn in the SE (Table 2) and S regions (Table 3), and in spring in the SE (Table 4) and S regions (Table 5). Both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values increased from baseline planktonic organisms (POM and zooplankton) to fish, squids, and were highest among the cartilaginous fish and the cetacean species (Figs. 1-5).  $\delta^{15}\text{N}$  was higher in POM than in zooplankton, especially in the SE region.  $\delta^{13}\text{C}$  values were the lowest in POM, increasing through the zooplankton groups up to the cetaceans. There was a large variability in the  $\delta^{15}\text{N}$  in the prey groups, especially within epipelagic squids, and benthic- and mesopelagic fish, demersal and pelagic neritic fish groups.  $\delta^{13}\text{C}$  was less variable within the fish and squid groups. The largest variability was observed in the benthic- and epipelagic fish groups. The cartilaginous fish had, overall, lower  $\delta^{13}\text{C}$  and higher  $\delta^{15}\text{N}$  than the cetacean species.

### *Cetacean source amino acids*

The seasonal (autumn-spring) and latitudinal (southeast-south) patterns in cetacean source amino acids were similar to that of bulk  $\delta^{15}\text{N}$  in zooplankton presented in Troina et al. (in review - chapter 2):  $\delta^{15}\text{N}$  was higher in the south (both seasons) than in the SE, and relatively higher in spring than in autumn (Fig. 6).

### *Cetacean trophic levels*

The difference between trophic and source amino acids are shown in Fig. 7 for each species within each region and season. Equation 2 resulted in relatively higher TLs than equations 3-to-5 (Table 6, Fig. 8), the latter three equations had fairly similar TL estimates (Fig.

9). The difference in the estimated TL between the equations can be attributed to the different source amino acid used. Equation 2 (Popp et al. 2007) uses glycine, which had highly variable  $\delta^{15}\text{N}$  values, resulting in estimated TL differences of up to 2. The equations 3-5 used phenylalanine as source amino acid, which were considerably more homogeneous (Fig. 10). Perhaps that is the reason TEF for glutamic acid and phenylalanine has been consistently used for trophic level estimates (McClelland and Montoya 2002; Chikaraishi et al. 2009; Germain et al. 2013; McMahan and McCarthy 2016). We will therefore focus our discussion on the TL estimated based on equations 3-5.

Sperm whale was the species with the highest TL (Fig. 9), estimated between 3.5 and 3.9 ( $\pm 0.4$ , Table 6). The TL in pantropical spotted dolphin (3.5-3.9) and long-finned pilot whale (3.4-3.8) was also high, although amino acid analysis was carried out in only one individual of each species (Table 6). Bottlenose, short-beaked common and Atlantic spotted dolphins had similar TL, ranging between 3.2 and 3.7. The lowest TL was recorded in the Clymene dolphin (2.7-3.2) and the killer whale (2.8-3.2). The rough-toothed and spinner dolphins had the second lowest and similar TL, which was estimated between 3.1-3.5. SBCD

#### *Community Layman metrics*

In spring, the southeast region had higher TA and MNND; NR and CD were similar in both regions, but CR and SDNND were higher in the south (Fig. 11). In autumn, the southern region had much higher TA, NR, CR and CD, and lower MNND and SDNND than the southeast (Fig. 11).

## **Discussion**

#### *Food chain*

There was a large variability in POM  $\delta^{15}\text{N}$  in both seasons. POM samples comprise a mixture of particles that include not only phytoplankton, but also detritus of any origin, bacteria and even small zooplankton. Thus, a single POM sample contains organisms of a variety of trophic levels. Additionally, the different phytoplankton species that comprise the POM samples (Lima et al. 2019) provide a great source of isotopic variation on their own, along with phytoplankton size, growth rates and the nutrients available that also play a major role in the high variations in primary producer's isotopic composition (Montoya and McCarthy 1995;

Pennock et al. 1996). Furthermore, POM was similar to (in the southern region) or higher than (in the southeast) the zooplankton in both seasons. The high  $\delta^{15}\text{N}$  in POM in comparison to the zooplankton can be explained by the water depth where POM (surface water, ~1 m) and zooplankton (0-200 m) samples were collected and the gradient in the concentrations of the nutrient available. It has been demonstrated that isotopic fractionations in particulate nitrogen is strongly dependent on the nutrient concentrations (Pennock et al. 1996). In the south-eastern region, in relatively deeper (0-200 m) and mixed compartments of the water column, nutrient is less limiting than light, and fractionation against the heavy  $^{15}\text{N}$  results in lower  $\delta^{15}\text{N}$  in POM (not measured) and consequently lower  $\delta^{15}\text{N}$  in zooplankton. In the shallower (0-1 m) compartment, nutrient might be the limiting factor for phytoplankton growth while light is plentiful, thus less apparent discrimination occurs resulting in relatively higher  $\delta^{15}\text{N}$  in the primary producers. In the southern region on the other hand, wind stress causing mixture of the upper layers may results in more nutrients available (Lima et al. 1996). Thus, little differences would be found between 0-200 m deep primary producers, resulting in similar  $\delta^{15}\text{N}$  values in both POM and zooplankton.

Apart from  $\delta^{15}\text{N}$  in POM and zooplankton, both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values showed a step-wise enrichment in the heavy isotopes from POM to zooplankton, squids, fish, cetaceans and cartilaginous fish. Most fish and squids had lower  $\delta^{15}\text{N}$  than the cetacean species, suggesting they occupy a relatively lower trophic position. Some benthopelagic fish, mainly *Trichiurus lepturus*, had  $\delta^{15}\text{N}$  values comparable to those of some cetacean species, thus showing relatively higher trophic positions. Indeed, *Trichiurus lepturus* is a fierce predator, with adults reaching up to 160 cm and feeding on a large variety of fish and squids (Martins et al. 2005).  $\delta^{15}\text{N}$  values in cetaceans were similar or lower than those in the cartilaginous fish. On the other hand, their  $\delta^{13}\text{C}$  values were considerably higher, especially in spring (Fig. 4). Higher  $\delta^{15}\text{N}$  in the cartilaginous fish might indicate they prey upon the cetacean species or other near-top predators. Their relatively lower  $\delta^{13}\text{C}$  in comparison to the cetacean species could indicate spatial segregation might occur, and that they might be feeding in more  $^{13}\text{C}$ -depleted areas.

The large variability within the epi-pelagic squid group is due to the difference in species compositions, as it includes two *Argonauta spp.* with considerably higher  $\delta^{15}\text{N}$  in autumn (~15‰, data not shown), whereas the remaining squid species had much lower  $\delta^{15}\text{N}$  (7-9‰). The large variability in the small mesopelagic fish in autumn is mainly due to the large range in  $\delta^{15}\text{N}$  values of the myctophidae fish that varied in almost 5‰ in both southeast and southern regions (data not shown). This suggests that our sampled myctophidae fish represent

at least two different trophic levels. The demersal fish group included species with high (*Saurida caribbaea*, 10-13‰) and low (*Arioma bondi*, 4-8‰)  $\delta^{15}\text{N}$  values, thus, probably occupying more than one trophic level. The pelagic-neritic fish group included only one fish species (*Coryphaena hippurus*, Table 1). The  $\delta^{15}\text{N}$  averaged  $\sim 11\text{‰}$  in both seasons, but variability was larger in autumn ( $\pm 2.7\text{‰}$ ) than in spring ( $\pm 1\text{‰}$ ). Since this difference was not due to location (both southeast and southern regions had similar mean  $\pm$  SD within each season), this large  $\delta^{15}\text{N}$  variability is certainly due to intra-specific differences in trophic levels, that might be related to age categories. Additional analysis considering the total length of the specimens collected might help understand these results.

Likewise, the large variability in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  within the benthopelagic and epi-pelagic fish groups can be explained by the different number of species they included. Further analysis to investigate the degree of isotopic variations within and among species will allow us to identify the level of trophic diversity within each of these groups.

#### *Cetacean bulk vs. amino acid $\delta^{15}\text{N}$*

The seasonal and spatial patterns in  $\delta^{15}\text{N}$  in cetacean source amino acids coincided with that of bulk  $\delta^{15}\text{N}$  in zooplankton (Troina et al. in review – chapter 2). This provided further support for the offset in baseline  $\delta^{15}\text{N}$  values based on location, that results from different oceanographic characteristics along the study area, as discussed in detail by Troina et al. (in review – chapter 2).

The high TL estimated for sperm whales based on trophic-to-source amino acids differences in  $\delta^{15}\text{N}$  were expected as these animals feed upon large, thus presumably high trophic level squids at high depths (Clarke et al. 1980; Santos and Haimovici 2000). The bulk  $\delta^{15}\text{N}$  of an important prey item, *Illex argentinus* (Clarke et al. 1980; Santos and Haimovici 2000), grouped among the demersal-pelagic squids (Table 1) ranged between 9 and 13‰, which supports a relatively high trophic position of this prey in these oceanic waters.

The low TL of spinner dolphins agrees with bulk SIA, showing that their low bulk  $\delta^{15}\text{N}$  is not only due to their occurrence in waters with low baseline  $\delta^{15}\text{N}$  (Troina et al. in review – Chapter 3), but also because they occupy relatively lower trophic positions. Indeed, bulk  $\delta^{15}\text{N}$  in epi- and mesopelagic fish, important prey items (Silva-Jr. et al. 2007), was relatively lower, roughly between 8-10‰, while spinner dolphin bulk  $\delta^{15}\text{N}$  was  $\sim 11.6\text{‰}$  ( $\pm 0.6$ , Tables 2-5). This

is just about the  $\sim 1.6\text{‰}$  ( $\pm 0.5$ ) trophic enrichment factor estimated for cetacean skin  $\delta^{15}\text{N}$  (Giménez et al. 2016).

The single pantropical spotted dolphins analysed for amino acid  $\delta^{15}\text{N}$  had relatively high TL in comparison to the other species in the SE region. Amino acid analysis provided further resolution that allowed us to perceive the trophic level difference between pantropical spotted and spinner dolphins. Perhaps their similarity and niche overlap estimated using bulk stable isotopes (Troina et al. in review - chapter 3) refers to similar resources, but differences in prey size or trophic levels. This hypothesis seems reasonable given the similar  $\delta^{15}\text{N}$  values observed in the source amino acids of these two species (Fig. 7). Nevertheless, the amino acid data for pantropical spotted dolphins included only one individual, while sample size for bulk isotope analysis was larger. Therefore, partition in resource use (prey size or trophic level) between them should not be assumed without further studies with increased sample size.

The relatively lower TL in rough-toothed dolphins estimated by  $\delta^{15}\text{N}_{\text{amino acids}}$  in comparison with the highest  $\delta^{15}\text{N}_{\text{bulk}}$  observed in the Troina et al. (in review - Chapter 3) suggests that they forage in waters where baseline N is relatively  $^{15}\text{N}$ -enriched, which supports their occurrence in inner shelf waters (Ott and Danilewicz 1996; Santos et al. 2017; 2019), as suggested in Troina et al. (in review – Chapter 3). This was indeed confirmed by their source  $\delta^{15}\text{N}$ , which was higher than that of the remaining species. Only short-beaked common dolphins had similar enrichment in  $^{15}\text{N}$  in their source amino acids, and they had the second highest bulk  $\delta^{15}\text{N}$  values. This strongly suggests that they occur along the continental shelf area, which was not sampled for baseline isotope measurements in the present study. Indeed, cetacean species occurring in southern Brazilian coastal regions have much more enriched  $^{13}\text{C}$  and  $^{15}\text{N}$  values (Botta et al. 2012; Secchi et al. 2016; Troina et al. 2016), which was the same pattern observed in rough-toothed dolphin bulk stable isotopes. Therefore, rough-toothed dolphin occurrence along the continental waters would explain their relatively higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values.

The similar TL observed in bottlenose, Atlantic spotted and short-beak common dolphins provides further support for these species' similarity in the use of resources. The amino acid data indicated that killer whales had the lowest TL, suggesting that these individuals were feeding at lower trophic levels, as suggested by Troina et al. (in review – Chapter 3). While  $\delta^{15}\text{N}_{\text{bulk}}$  was very similar between killer and long-finned pilot whales,  $\delta^{15}\text{N}_{\text{amino acids}}$  showed that the latter is at least 1 TL higher than the former. Thus, the baseline  $\delta^{15}\text{N}$  where this individual had been feeding has an offset that was masked by the bulk  $\delta^{15}\text{N}$ . Indeed, the source amino

acids in killer whales were relatively more  $^{15}\text{N}$ -enriched than those of the long-finned pilot whale (Fig. 7). Both bulk  $\delta^{15}\text{N}$  (10.9‰) and amino acid TL estimates (2.7-3.2) suggested relatively low TL for the Clymene dolphin sampled here. There is still little information about their trophic ecology (Jefferson 2018), but mesopelagic fish and squids may be consumed (Fertl et al. 1997; Sakyi et al. 2018).

It is important to highlight that the analyses presented here are preliminary, and models with different  $\text{TEF}_{\text{Glu-Phe}}$  shall be tested in order to evaluate the best and most realistic values for cetaceans.  $\text{TEF}_{\text{Glu-Phe}}$  can be highly variable and depends on the quality of dietary protein and the type of nitrogen excretion (McMahon et al. 2015; McMahon and McCarthy 2016). Higher class consumers that excrete urea show lower TEF between glutamic acid and phenylalanine (McMahon and McCarthy 2016), and perhaps the values applied in these preliminary analyses were not the most suitable for cetacean species, and further consideration should be given to this matter.

#### *Community structures: comparative analysis of niche metrics*

There were large latitudinal differences (southeast vs. south regions) in the community-wide metrics in both seasons. In autumn, the southern region had considerably larger TA, NR, CR and CD, while MNND and SDNND were higher in the southeast. In spring, TA and MNND were higher in the southeast and both regions had similar NR and CR, whereas CD and SDNND were higher in the south. These differences seem to be associated with the seasonal patterns in the water masses. The southern region is highly dynamic in colder months, influenced by continental, tropical and Subantarctic shelf water masses (Möller Jr. et al. 2008; Piola et al. 2008). The input of these waters provides different source of nutrients to the region, likely reflecting the larger variability in the carbon isotopes (high CR). Additionally, this increased production leads to high density of prey (Haimovici et al. 1994; 2014). The increase in prey available will attract more consumers to forage in the area, hence increasing the length of the food-chain (NR) and the total area of isotopic niche. The high CD in the southern region indicates that groups are more disperse in their niche, exploring different resources. On the other hand, the low MNND suggests that there is relatively high trophic redundancy within groups, thus groups could be more specialists in the exploitation of resources in the southern region. Specialist feeding habits can be advantageous in systems where resources are plentiful (Terraube et al. 2011), what is the case of the S region in colder periods (Haimovici et al. 1994).

Accordingly, if there are more resources, individual populations can exploit different food types (large CD), but showing high trophic redundancy within populations (low MNND and SDNND).

The higher TA in the southeast in spring could be due to the intrusion of new nutrient from the upwellings of the SACW, and the consequent increased productivity in the region (Guenther et al., 2008; Coelho-Souza et al., 2012; Brandini et al., 2018), in comparison with the southern region where warm tropical waters predominate in warmer months (Möller Jr. et al. 2008). This could explain the lower TA, NR, CR and CD in spring than in autumn in the southern region. The higher MNND observed in the southeast in comparison to the southern region suggests high variability in the use of resources within groups, thus less trophic redundancy. Additionally, within the southeast region higher TA, NR, CR, CD and MNND were observed in spring, supporting the influence of the upwellings on the region and how it seasonally affects the isotopic values of the organisms in the region.

The community-wide metrics can offer important information on food-web structure between different locations and time periods. However, better interpretation of these isotopic metrics can be achieved if assessed along with information on species density and diversity. This would allow for the assessment of how species movements, diversity and abundances would affect the food-web isotopic niche patterns in the intra and interspecific levels.

#### *Limitations and recommendations for future works*

It is essential to measure environmental parameters to help understand the baseline gradients in stable isotopes values. For future works, we strongly recommend to collect samples for the analysis of the Dissolved Inorganic Carbon (DIC) at different depth ranges of the water column in order to measure its concentration and  $\delta^{13}\text{C}_{\text{DIC}}$ . Also, it would be important to analyse nutrient concentrations (nitrate, nitrite and ammonium), along with collecting POM samples for stable isotope analysis. Such environmental information will improve the interpretations of the patterns in baseline stable isotope compositions.

Because it is an offshore region where the main primary producer is phytoplankton, similar  $\delta^{13}\text{C}$  values in the potential prey groups were found. It was therefore difficult to detect differentiation among prey types and apply models to estimate their proportional contribution to the cetacean species. We suggest the use of an additional stable isotope for a three-

dimensional analysis of isotopic niche and assess whether resolution can be improved (e.g., Rossman et al. 2016). For instance, Sulphur ( $\delta^{34}\text{S}$ ) stable isotopes differ between neritic and oceanic waters (Barros et al. 2010). Although  $\delta^{34}\text{S}$  values are rather constant around +21‰ in oceanic waters, they are significantly lower in benthic environments than in pelagic waters (Trueman and Glew 2019). Therefore, they might help to identify if prey and cetacean species are feeding at high depths or upon epi-to-mesopelagic prey, or to differentiate between those that feed towards neritic waters (e.g., *Steno bredanensis*) and those that exploit oceanic regions.

Additionally, the compound-specific analysis of  $\delta^{15}\text{N}$  in individual amino acids represented a great source of information to this study, allowing to assess the spatial differences in cetacean habitat use and their respective trophic levels. For future studies though, we suggest the analysis of  $\delta^{15}\text{N}$  in the amino acids of the baseline components of the food-web (zooplankton or POM) to allow for the estimation of  $\beta$ , the difference between baseline trophic and source amino acids, for the region where the predators being studied occur, and not to rely on values from the literature, as they might differ.

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**Table 1** Group, identification code and number of samples obtained from baseline (Particulate Organic Matter and zooplankton) to prey (fish and squids), cartilaginous fish and cetaceans in each region (southeast – SE and south – S) within each season (spring and autumn).

Group ( <i>Species/family</i> )	Code	Spring		Autumn		Total
		SE	S	SE	S	
<b>Particulate Organic Matter</b>	POM	12	12	18	33	75
<b>Zooplankton</b>						
Amphipods	AMPH	6	15	13	24	58
Chaetognaths	CHAET	8	16	10	19	53
Copepods	COP	11	18	17	30	76
Euphausiids	EUPH	6	18	16	27	67
Misxute	MIX	8	18	17	28	71
Salps	SALP	7	7	1	5	20
<b>Fish</b>						
<b>Bathy-demersal fish</b>	B-DEM-FSH					
<i>Chlorophthalmus agassizi</i>		-	7	-	-	7
<i>Lophius gastrophysus</i>		-	1	-	-	1
<b>Bathy-pelagic fish</b>	B-PEL-FSH					
<i>Argentina striata</i>		-	6	-	-	6
<b>Benthopelagic fish</b>	BP-FSH					
<i>Aluterus monoceros</i>		-	6	1	-	7
<i>Benthodesmus elongatus</i>		-	1	-	-	1
<i>Benthodesmus sp</i>		-	-	-	4	4
<i>Beryx splendens</i>		-	-	-	2	2
<i>Coelorinchus marinii</i>		-	1	-	-	1
<i>Evoxymetopon taeniatus</i>		-	4	-	-	4
<i>Lepidopus sp</i>		-	8	-	-	8
<i>Merluccius hubbsi</i>		-	7	-	-	7
<i>Symphurus sp</i>		-	1	-	-	1
<i>Synagrops bellus</i>		-	4	-	-	4
<i>Synagrops spinosus</i>		-	6	9	-	15
<i>Thyrsitops lepidopoides</i>		-	1	-	-	1
<i>Trichiurus lepturus</i>		-	12	2	10	24
<b>Demersal fish</b>	DEM-FSH					
<i>Antigonia capros</i>		-	-	-	2	2
<i>Ariomma bondi</i>		5	2	3	7	17
<i>Bellator brachyichir</i>		-	4	-	-	4
<i>Bembrops heterurus</i>		-	3	-	-	3
<i>Saurida caribbaea</i>		-	8	5	6	19
<b>Epipelagic fish</b>	EPIPEL-FSH					
<i>Exocoetidae</i>		2	6	4	-	12
<i>Exocoetus volitans</i>		-	3	1	-	4
<i>Scomberesox saurus</i>		-	-	-	2	2
<i>Seriola fasciata</i>		-	4	3	-	7
<b>Epipelagic neritic fish</b>	EPIPEL-NER-FSH					
<i>Engraulis anchoita</i>		-	3	-	-	3
<b>Large Mesopelagic fish</b>	L-MESPEL-FSH					
<i>Zenopsis conchifera</i>		-	5	-	1	6

<b>Large Pelagic oceanic fish</b>	L-PEL-OC-FSH					
<i>Xiphias gladius</i>		-	-	-	1	1
<b>Maurolicus stehmanni</b>	M.STEH					
<i>Maurolicus stehmanni</i>		22	44	19	12	97
<b>Medium Mesopelagic fish</b>	M-MESPEL-FSH					
<i>Nealotus tripes</i>		-	-	-	4	4
<i>Paralepididae</i>		-	-	-	10	10
<b>Pelagic fish</b>	PEL-FSH					
<i>Caranx sp</i>		-	-	2	-	2
<b>Pelagic neritic fish</b>	PELNER-FSH					
<i>Coryphaena hippurus</i>		8	15	5	13	41
<b>Reef associated fish</b>	REEF-FSH					
<i>Balistes capriscus</i>		-	-	-	3	3
<i>Kyphosus sp</i>		-	-	3	-	3
<b>Small Mesopelagic fish</b>	S-MESPEL-FSH					
<i>Diaphus dumerilii</i>		-	-	-	4	4
<i>Gonostomatidae</i>		-	-	-	1	1
<i>Hygophum taaningi</i>		-	-	-	2	2
<i>Myctophidae</i>		3	2	5	18	28
<i>Stomiidae</i>		-	-	-	3	3
<i>Symbolophorus barnardi</i>		-	-	-	4	4
<b>Small Pelagic oceanic fish</b>	S-PEL-OC-FSH					
<i>Bregmaceros atlanticus</i>		-	-	3	2	5
<hr/>						
<b>Cartilaginous fish</b>						
<b>Benthopelagic Cartilaginous fish</b>	BP-CART					
<i>Galeorhinus galeus</i>		1	-	-	-	1
<b>Pelagic Oceanic Cartilaginous fish</b>	PL-OC-CART					
<i>Isurus</i>		-	1	-	3	4
<i>Prionace glauca</i>		-	1	-	1	2
<i>Sphyrna</i>		-	-	-	2	2
<hr/>						
<b>Squids</b>						
<b>Demersal Pelagic squid</b>	DEMPEL-SQD					
<i>Illex argentinus</i>		-	6	4	-	10
<i>Loligo plei</i>		-	-	1	5	6
<i>Loligo sanpaulensis</i>		-	1	-	-	1
<b>Epipelagic squid</b>	EPIPEL-SQD					
<i>Argonauta argo</i>		-	-	-	1	1
<i>Argonauta nodosa</i>		-	1	1	-	2
<i>Ommastrephes bartramii</i>		4	29	3	20	56
<i>Ornithoteuthis</i>		-	-	-	2	2
<i>Thysanoteuthis rhombus</i>		1	-	2	-	3
<b>Meso-to-bathypelagic squid</b>	MESO-BATH-SQD					
<i>Abralia redfieldi</i>		-	-	-	5	5
<i>Abralia sp</i>		-	6	-	-	6
<i>Abralia veranyi</i>		-	-	-	3	3
<i>Abraliopsis</i>		-	-	-	4	4

<b>Mesopelagic squid</b>		<b>MESOPEL-SQD</b>				
<i>Heteroteuthis</i>		-	-	4	-	4
<b>Cetaceans</b>						
Short-beaked common dolphins	SBCD	-	39	-	15	54
<i>Delphinus delphis</i>						
Sperm whales	SW	-	6	-	4	10
<i>Physeter macrocephalus</i>						
Pantropical spotted dolphins	PTSD	6	-	1	-	7
<i>Stenella attenuata</i>						
Rough-toothed dolphins	RTD	-	3	-	4	7
<i>Steno bredanensis</i>						
Atlantic spotted dolphins	ASD	21	22	15	16	74
<i>Stenella frontalis</i>						
Spinner dolphins	SPD	18	10	16	-	44
<i>Stenella longirostris</i>						
Bottlenose dolphins	BND	23	13	9	31	76
<i>Tursiops truncatus</i>						
Killer whales	KW	-	-	-	3	3
<i>Orcinus orca</i>						
Long-finned pilot whales	LFPW	-	-	-	1	1
<i>Globicephala melas</i>						
Clymene dolphins	CLYD	-	1	-	-	1
<i>Stenella clymene</i>						

**Table 2** Number of samples (n) and mean ( $\pm$  Standard Deviation - SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for each group (baseline to fish, squids and cetaceans, see Table 1) sampled in autumn in the southeast region.

Autumn - South-eastern region Group	n	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		Mean	$\pm$ SD	Mean	$\pm$ SD
POM	18	-21.3	1.4	5.6	3.1
AMPH	13	-21.1	1.3	2.9	1.6
CHAET	10	-21.2	0.9	4.9	1.1
COP	17	-21.4	0.6	2.8	1.6
EUPH	16	-21.1	1.2	3.1	1.6
SALP	1	-20.1	NA	4.4	NA
MIX	17	-20.1	1.2	3.0	1.6
BP-FSH	12	-20.2	1.6	10.7	2.3
DEM-FSH	8	-19.6	0.4	9.4	1.1
DEMPPEL-SQD	5	-19.3	0.5	6.4	2.3
EPIPEL-FSH	8	-18.7	0.5	8.1	1.9
EPIPEL-SQD	6	-18.9	0.7	10.5	2.9
M.STEH	19	-20.9	0.5	7.2	2.3
MESOPEL-SQD	4	-20.3	0.1	5.8	0.2
PEL-FSH	2	-18.1	0.2	6.2	0.4
PELNER-FSH	5	-18.4	0.5	11.3	2.7
REEF-FSH	3	-18.8	0.3	9.2	1.0
S-MESOPEL-FSH	5	-20.1	0.9	10.2	1.9
S-PEL-OC-FSH	3	-19.0	0.3	7.1	0.6
ASD	15	-16.6	0.4	12.8	0.9
BND	9	-16.4	0.8	12.7	0.5
PTSD	1	-16.6	NA	12.0	NA
SPD	16	-17.1	0.4	10.8	0.6

**Table 3** Number of samples (n) and mean ( $\pm$  Standard Deviation - SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for each group (baseline to fish, squids and cetaceans, see Table 1) sampled in autumn in the south region.

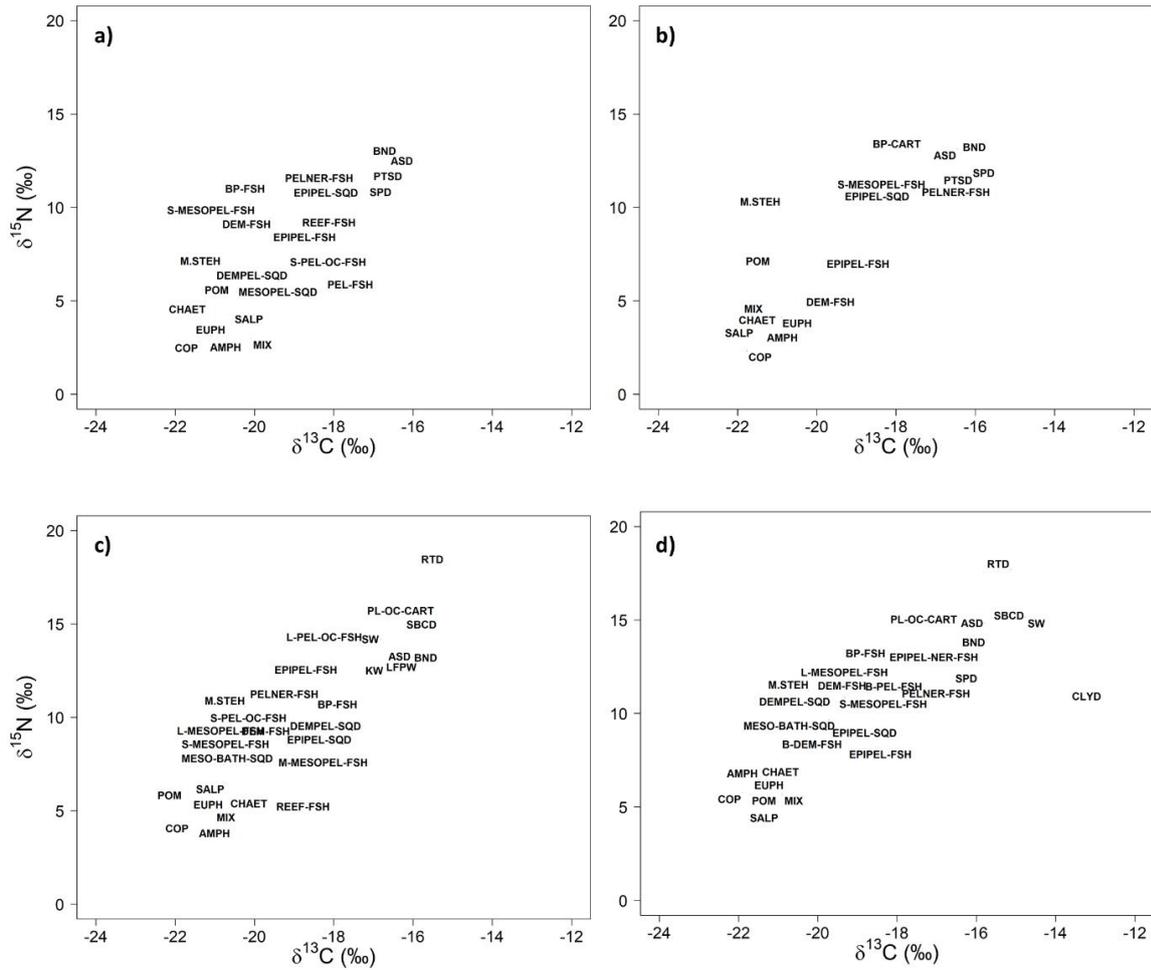
Autumn - Southern region Group	n	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		Mean	$\pm$ SD	Mean	$\pm$ SD
POM	33	-22.1	1.4	6.2	2.4
AMPH	24	-20.6	1.0	3.8	1.8
CHAET	19	-20.6	0.5	5.4	1.9
COP	30	-21.6	0.7	4.4	2.2
EUPH	27	-21.2	0.7	5.0	1.5
SALP	5	-21.1	1.0	5.8	0.8
MIX	28	-20.7	0.6	4.3	2.2
BP-FSH	16	-18.4	0.7	10.7	3.9
DEM-FSH	15	-19.1	0.5	9.0	2.8
DEMPPEL-SQD	5	-19.0	0.3	9.2	0.8
EPIPEL-FSH	2	-18.0	0.4	12.2	1.7
EPIPEL-SQD	23	-19.1	0.5	8.8	2.5
L-MESPEL-FSH	1	-19.9	NA	9.3	NA
L-PEL-OC-FSH	1	-18.2	NA	14.0	NA
M-MESPEL-FSH	14	-19.3	0.4	7.9	1.5
M.STEH	12	-20.2	0.2	10.6	0.9
MESO-BATH-SQD	12	-19.7	0.4	8.1	0.8
PELNER-FSH	13	-18.5	0.6	11.3	2.4
PL-OC-CART	6	-17.1	0.4	15.7	1.2
REEF-FSH	3	-18.8	0.1	5.6	0.3
S-MESPEL-FSH	32	-19.8	1.1	8.9	2.3
S-PEL-OC-FSH	2	-19.3	0.1	10.0	0.0
ASD	16	-16.0	0.3	12.9	0.9
BND	31	-16.0	0.7	13.2	1.2
KW	3	-16.7	0.2	12.8	0.2
LFPW	1	-16.3	NA	13.0	NA
RTD	4	-15.2	0.2	18.1	0.5
SBCD	15	-15.8	0.3	14.7	1.4
SW	4	-16.8	0.7	14.2	0.6

**Table 4** Number of samples (n) and mean ( $\pm$  Standard Deviation - SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for each group (baseline to fish, squids and cetaceans, see Table 1) sampled in spring in the southeast region.

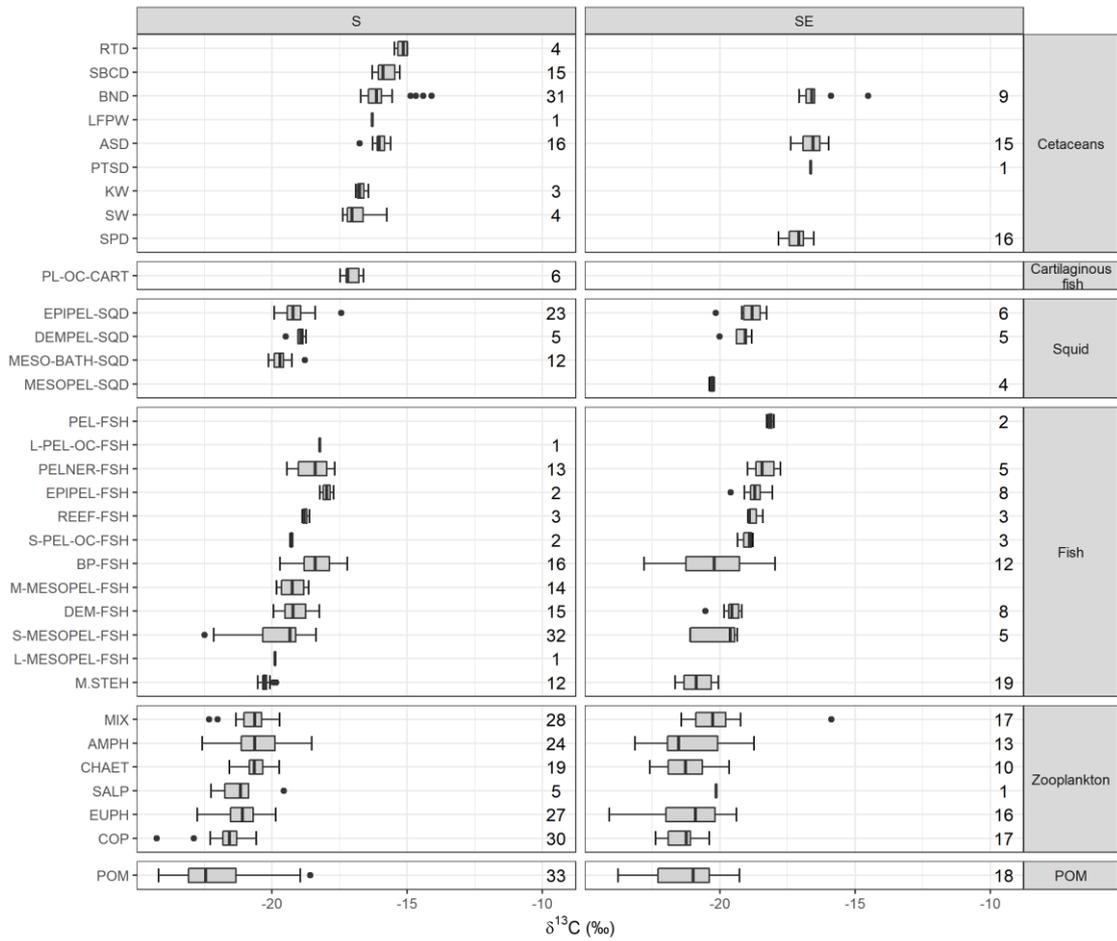
Spring - South-eastern region Group	n	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
POM	12	-21.1	1.3	6.8	2.6
AMPH	6	-20.9	2.3	2.7	2.0
CHAET	8	-21.0	1.4	4.3	2.4
COP	11	-21.8	1.9	2.3	3.5
EUPH	6	-20.9	0.9	4.1	0.7
SALP	7	-21.6	1.6	3.0	2.7
MIX	8	-21.3	0.9	4.3	1.2
BP-CART	1	-17.4	NA	13.7	NA
DEM-FSH	5	-20.2	0.9	4.6	1.5
EPIPEL-FSH	2	-19.0	1.0	7.3	0.3
EPIPEL-SQD	5	-19.2	0.7	10.9	1.3
M.STEH	22	-20.9	1.5	10.0	1.7
PELNER-FSH	8	-17.3	0.6	11.2	0.9
S-MESOPHEL-FSH	3	-19.3	0.5	10.9	0.1
ASD	21	-16.4	0.5	13.1	0.8
BND	23	-16.3	1.2	12.9	0.8
PTSD	6	-16.1	0.6	11.8	0.7
SPD	18	-16.1	1.1	11.5	0.5

**Table 5** Number of samples (n) and mean ( $\pm$  Standard Deviation - SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for each group (baseline to fish, squids and cetaceans, see Table 1) sampled in spring in the south region.

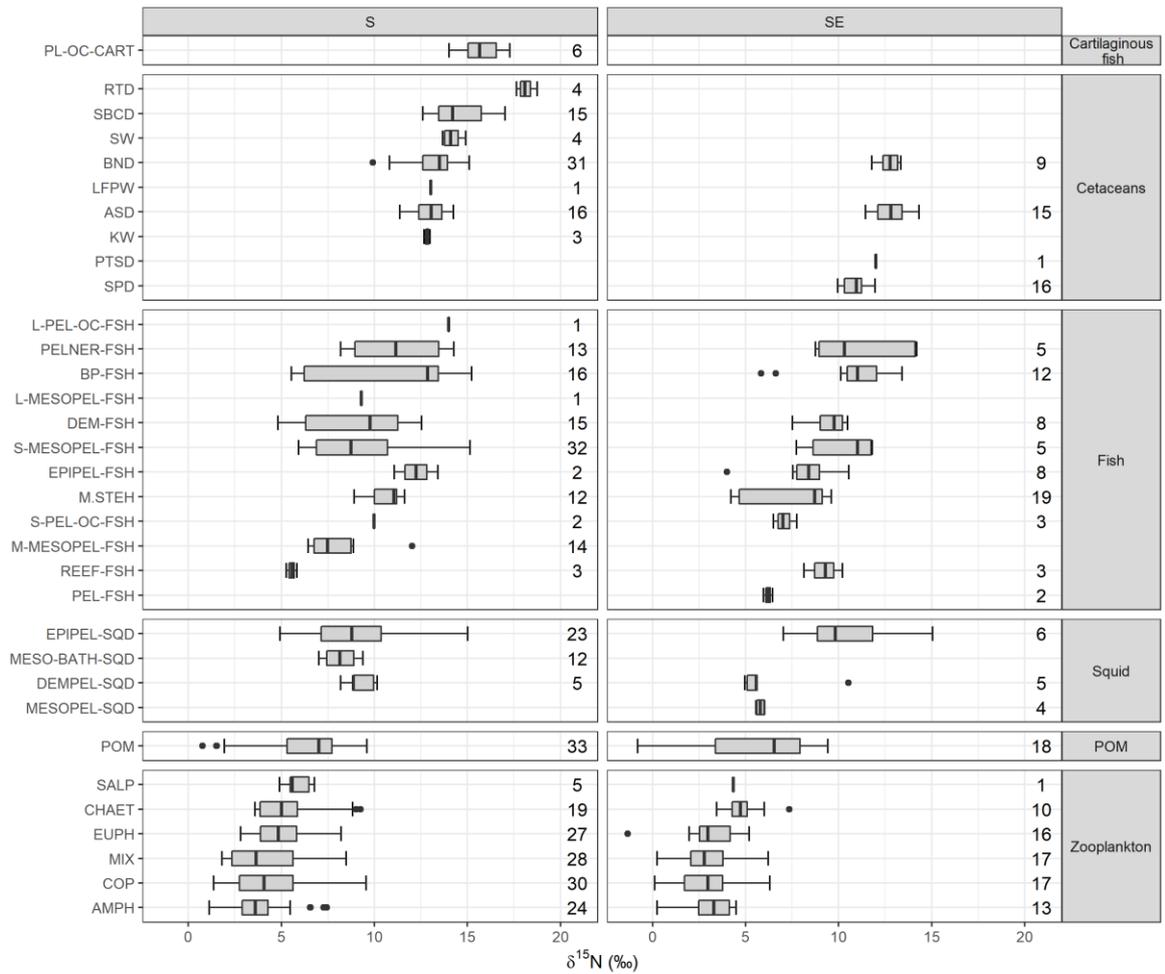
Spring - Southern region Group	n	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		Mean	$\pm$ SD	Mean	$\pm$ SD
POM	12	-21.7	1.3	5.7	2.9
AMPH	15	-21.5	1.3	6.5	2.3
CHAET	16	-21.4	1.2	6.9	2.3
COP	18	-21.9	1.0	5.8	2.0
EUPH	18	-21.6	0.9	6.5	2.0
SALP	7	-21.0	1.9	4.4	1.4
MIX	18	-20.9	1.1	5.7	2.4
B-DEM-FSH	8	-19.5	0.6	8.7	1.0
B-PEL-FSH	6	-18.7	0.6	11.1	1.1
BP-FSH	51	-18.3	1.1	12.9	3.0
DEM-FSH	17	-18.8	0.7	11.5	1.7
DEMPPEL-SQD	7	-19.8	0.9	11.0	1.9
EPIPEL-FSH	13	-19.1	1.0	8.2	1.9
EPIPEL-NER-FSH	3	-18.1	0.3	13.3	0.6
EPIPEL-SQD	30	-19.6	0.7	8.6	2.1
L-MESPEL-FSH	5	-18.4	0.8	12.5	0.9
M.STEH	44	-20.2	1.1	11.2	1.8
MESO-BATH-SQD	6	-19.7	0.3	9.7	0.5
PELNER-FSH	15	-17.8	0.6	10.8	1.1
PL-OC-CART	2	-18.1	2.2	15.4	3.7
S-MESPEL-FSH	2	-19.3	0.3	10.8	0.1
ASD	22	-15.8	0.6	14.8	1.8
BND	13	-16.1	0.5	14.1	0.9
CLYD	1	-13.6	NA	10.9	NA
RTD	3	-15.1	0.2	17.7	0.4
SBCD	39	-15.6	0.9	15.6	1.4
SPD	10	-16.6	0.4	11.6	0.6
SW	6	-14.8	1.6	15.2	0.5



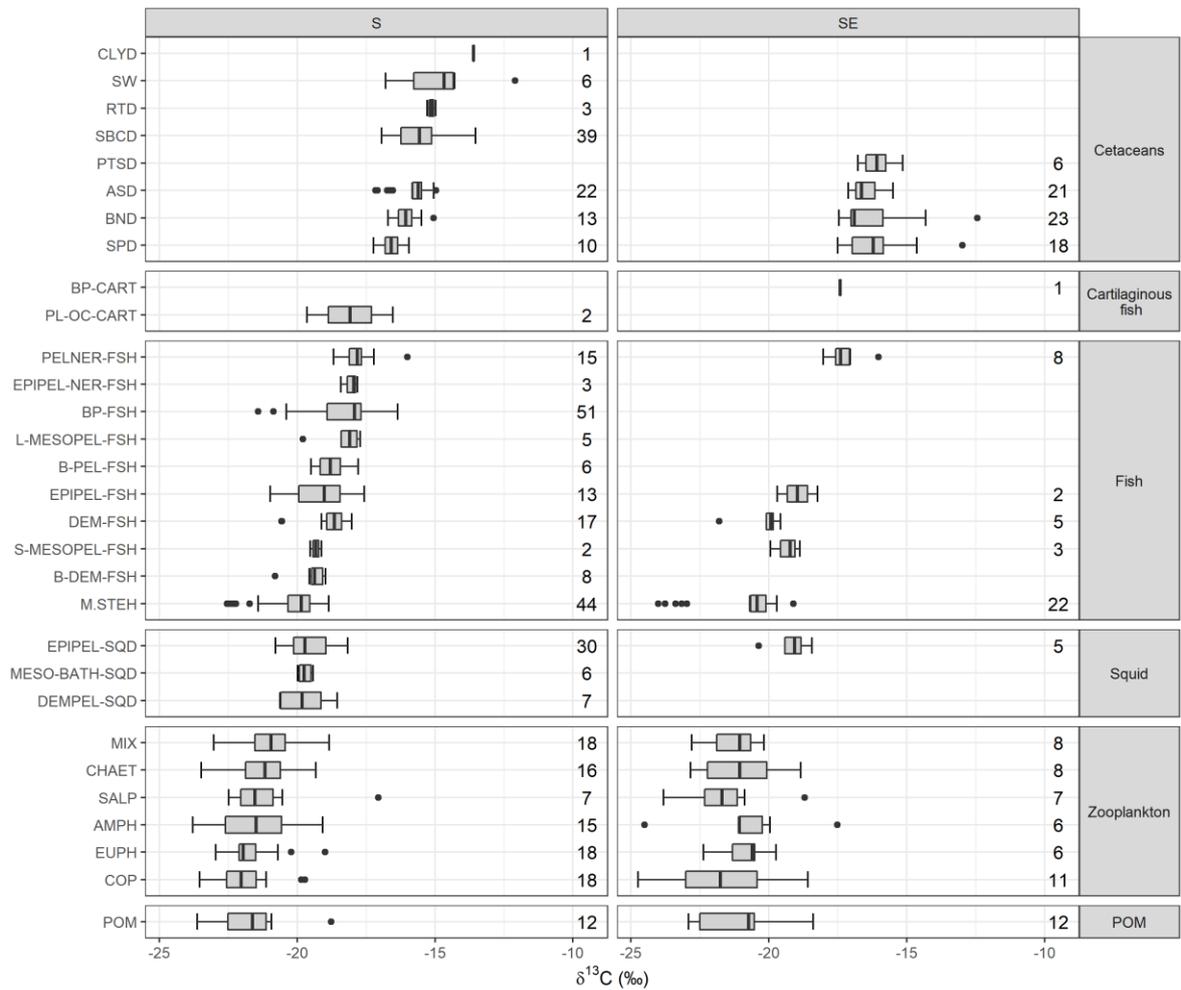
**Figure 1** Mean  $\delta^{13}\text{C}$ -  $\delta^{15}\text{N}$  biplots for the different groups (see table 1 for groups codes) in the southeast region in autumn (a) and spring (b) and south in autumn (c) and spring (d).



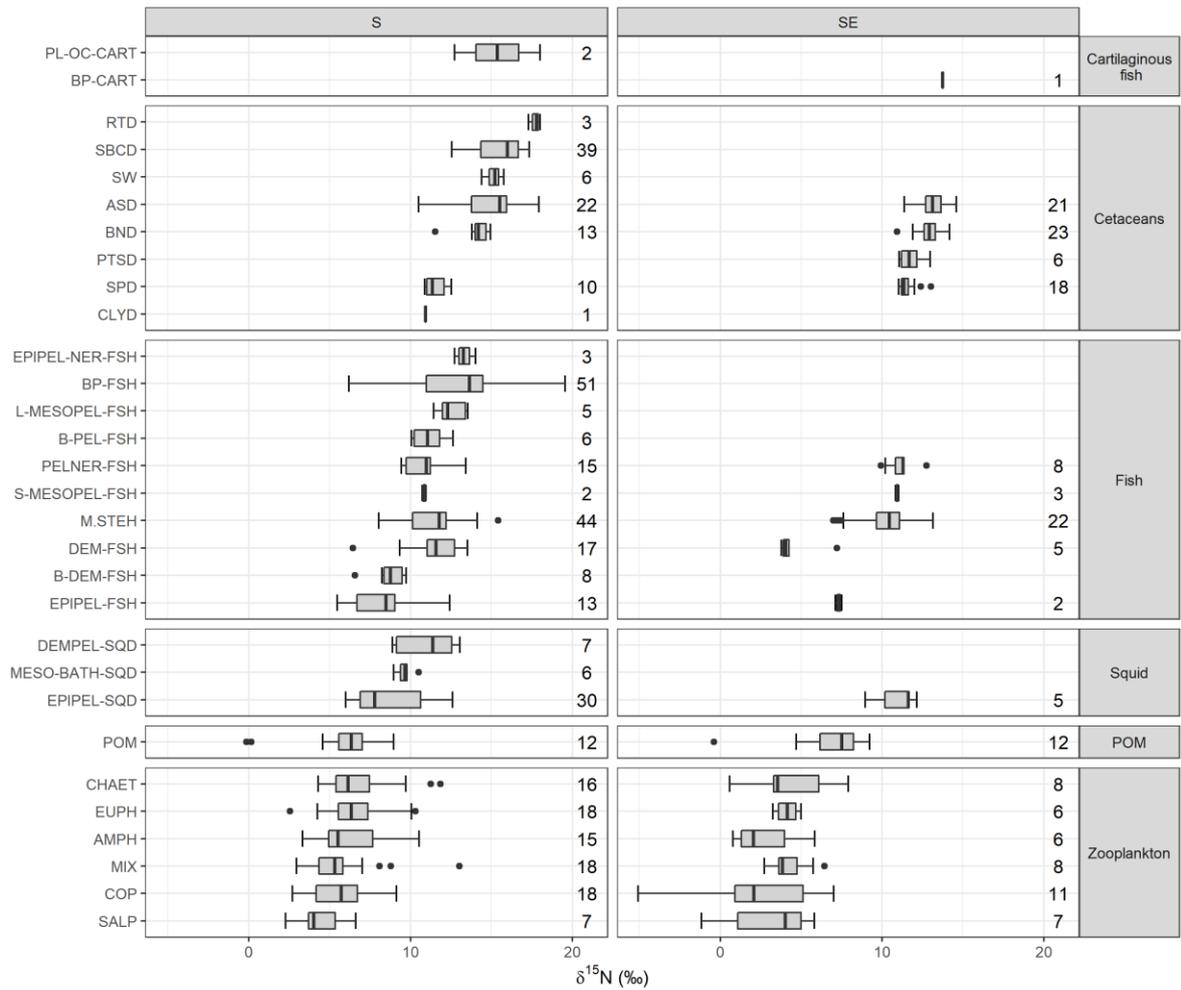
**Figure 2** Boxplot of  $\delta^{13}\text{C}$  values for each group (Particulate Organic Matter – POM, zooplankton, fish, squids, cartilaginous fish and cetaceans) in the south (S) and southeast (SE) regions in autumn. For groups code see Table 1.



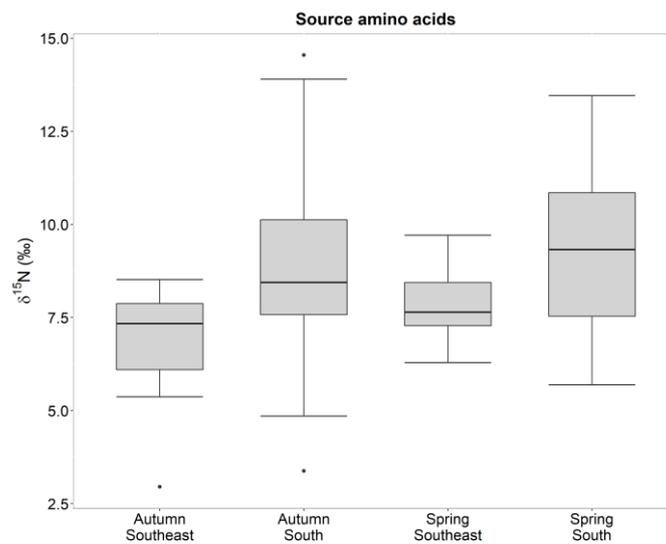
**Figure 3** Boxplot of  $\delta^{15}\text{N}$  values for each group (Particulate Organic Matter – POM, zooplankton, fish, squids, cartilaginous fish and cetaceans) in the south (S) and southeast (SE) regions in autumn. For groups code see Table 1.



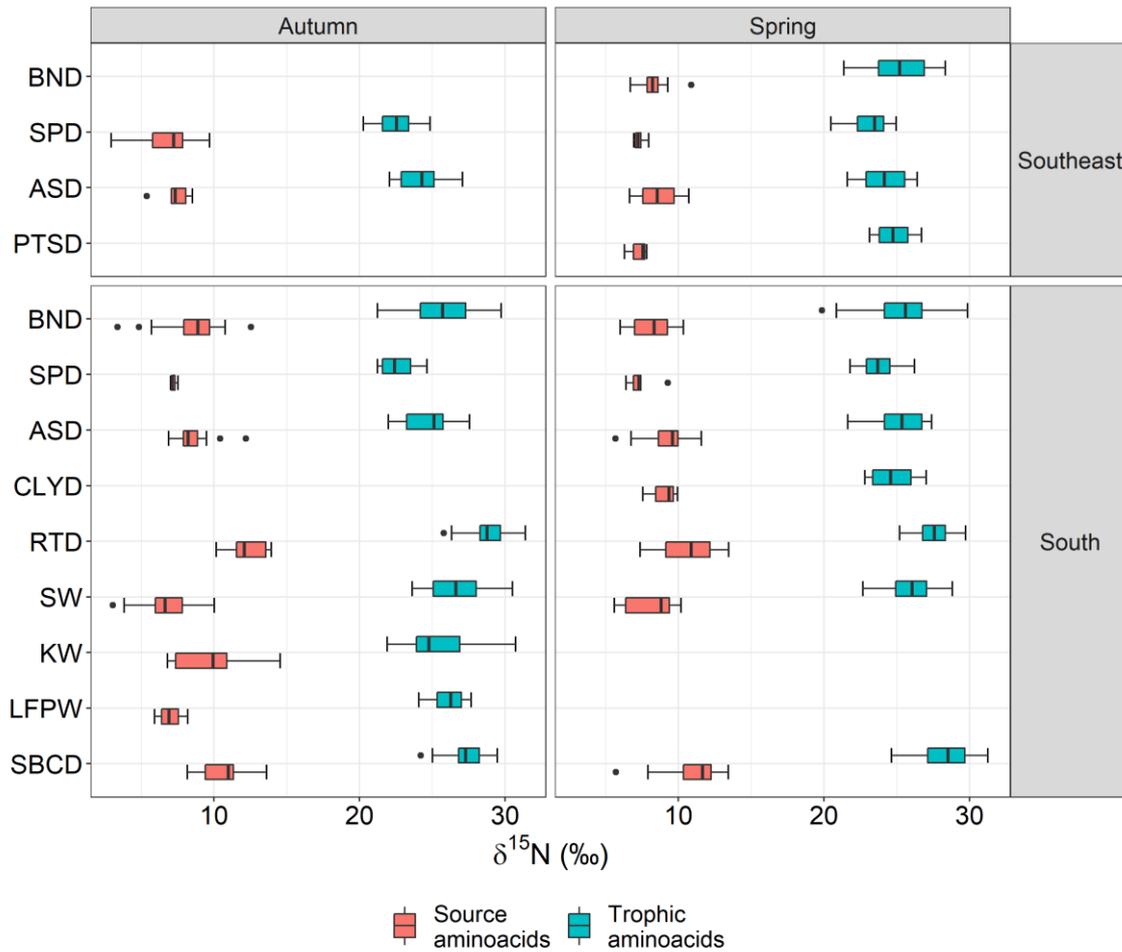
**Figure 4** Boxplot of  $\delta^{13}\text{C}$  values for each group (Particulate Organic Matter – POM, zooplankton, fish, squids, cartilaginous fish and cetaceans) in the south (S) and southeast (SE) regions in spring. For groups code see Table 1.



**Figure 5** Boxplot of  $\delta^{15}\text{N}$  values for each group (Particulate Organic Matter – POM, zooplankton, fish, squids, cartilaginous fish and cetaceans) in the south (S) and southeast (SE) regions in spring. For groups code see Table 1.



**Figure 6** A composite of the source amino acid (Lysine, Phenylalanine, Serine and Tyrosine)  $\delta^{15}\text{N}$  values in all (n = 69) cetacean species within each region/season.



**Figure 7**  $\delta^{15}\text{N}$  in source and trophic amino acids for each species shown per region and season. Species abbreviation code is displayed in Table 1.

**Table 6** Mean  $\pm$  Standard Deviation (SD) estimated trophic level (TL) for cetacean species with different equations (2-5). Number of samples per species (N) for which compound specific  $\delta^{15}\text{N}$  were analyzed. Species abbreviation code is displayed in Table 1.

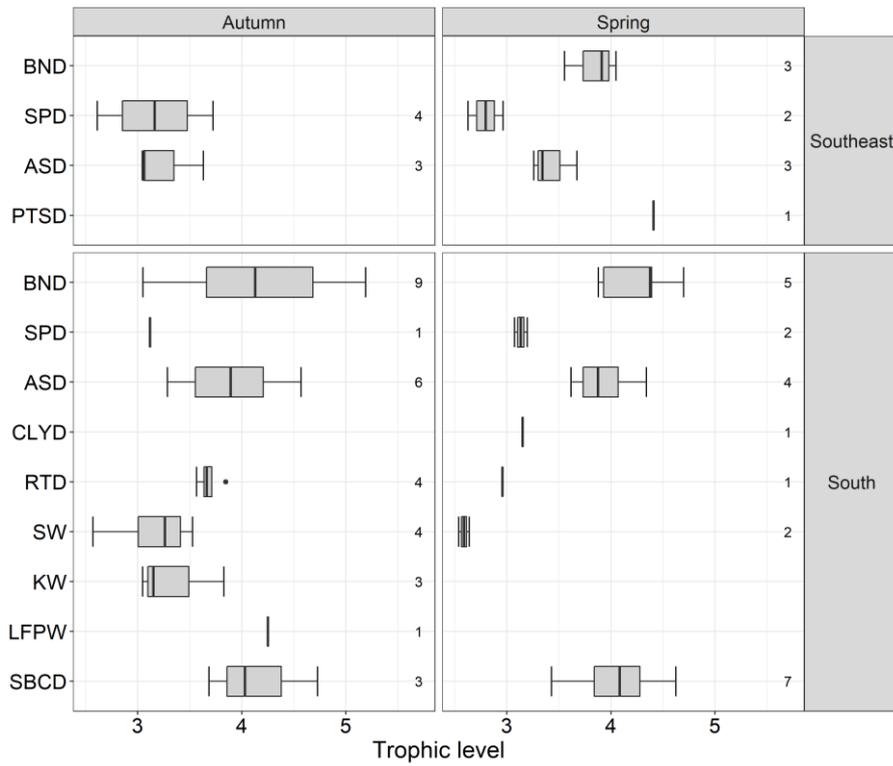
Species	N	TL Eq. 2	TL Eq. 3	TL Eq. 4	TL Eq. 5
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
SBCD	10	4.1 $\pm$ 0.4	3.3 $\pm$ 0.2	3.7 $\pm$ 0.2	3.3 $\pm$ 0.2
LFPW	1	4.2	3.4	3.8	3.4
KW	3	3.4 $\pm$ 0.4	2.8 $\pm$ 0.1	3.2 $\pm$ 0.1	2.8 $\pm$ 0.1
SW	6	3.0 $\pm$ 0.4	3.5 $\pm$ 0.4	3.9 $\pm$ 0.4	3.6 $\pm$ 0.4
PTSD	1	4.4	3.5	3.9	3.5
RTD	5	3.5 $\pm$ 0.3	3.1 $\pm$ 0.3	3.5 $\pm$ 0.3	3.1 $\pm$ 0.3
CLYD	1	3.1	2.8	3.2	2.7
ASD	16	3.7 $\pm$ 0.4	3.2 $\pm$ 0.1	3.6 $\pm$ 0.1	3.2 $\pm$ 0.1
SPD	9	3.1 $\pm$ 0.3	3.1 $\pm$ 0.2	3.5 $\pm$ 0.2	3.1 $\pm$ 0.2
BND	17	4.1 $\pm$ 0.6	3.3 $\pm$ 0.3	3.6 $\pm$ 0.2	3.2 $\pm$ 0.2

Eq. 2: TEF = 7‰, Popp et al. (2007)

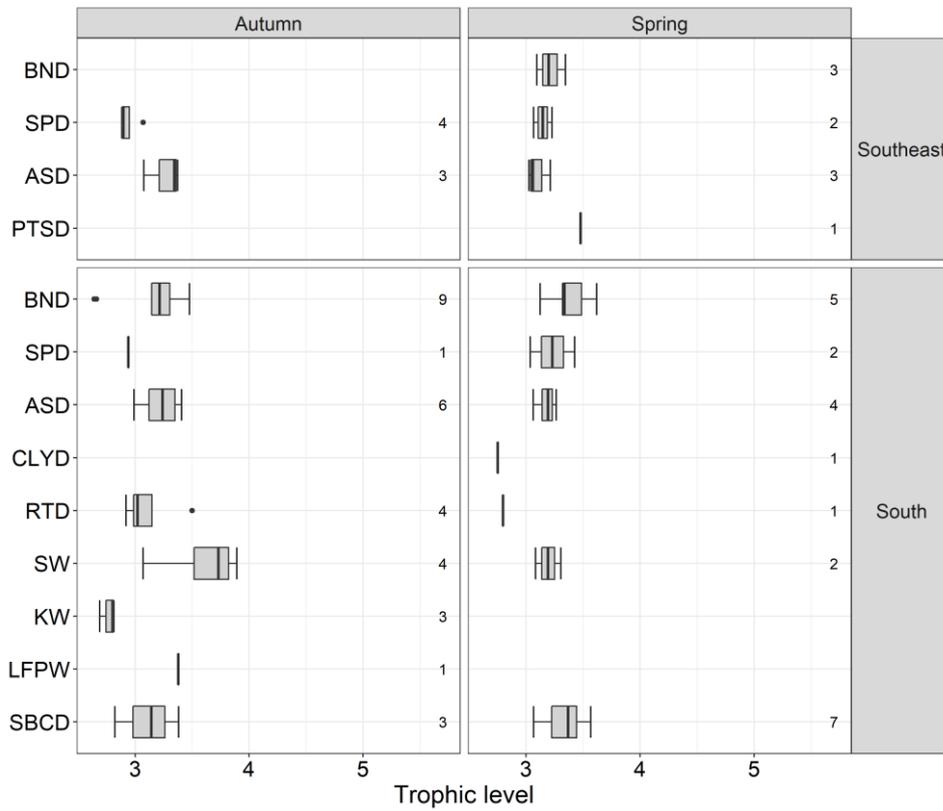
Eq. 3: TEF = 5.7‰,  $\beta$  = 3.4‰,  $\beta$  from Chikaraishi et al. (2009); TEF from Bradley et al. (2015)

Eq. 4: TEF = 5.7‰,  $\beta$  = 1.2‰,  $\beta$  from McCarthy et al. (2013); TEF from Bradley et al. (2015)

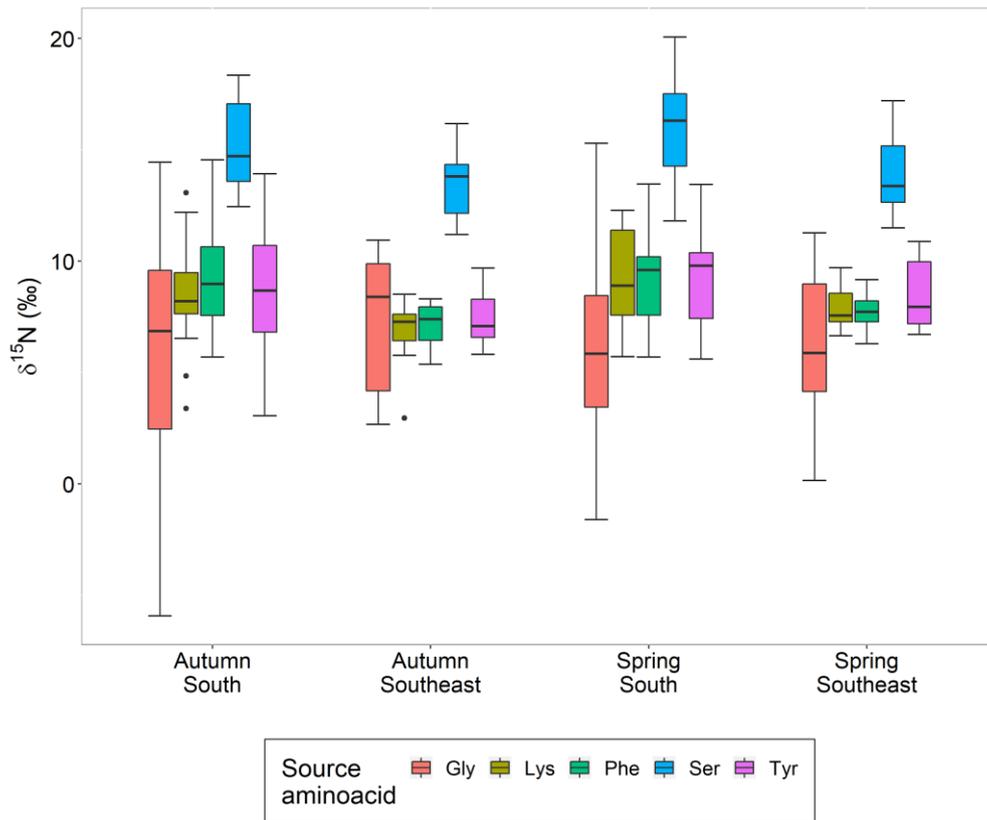
Eq. 5: TEF = 5.7‰,  $\beta$  = 3.6‰,  $\beta$  and TEF from Bradley et al. (2015)



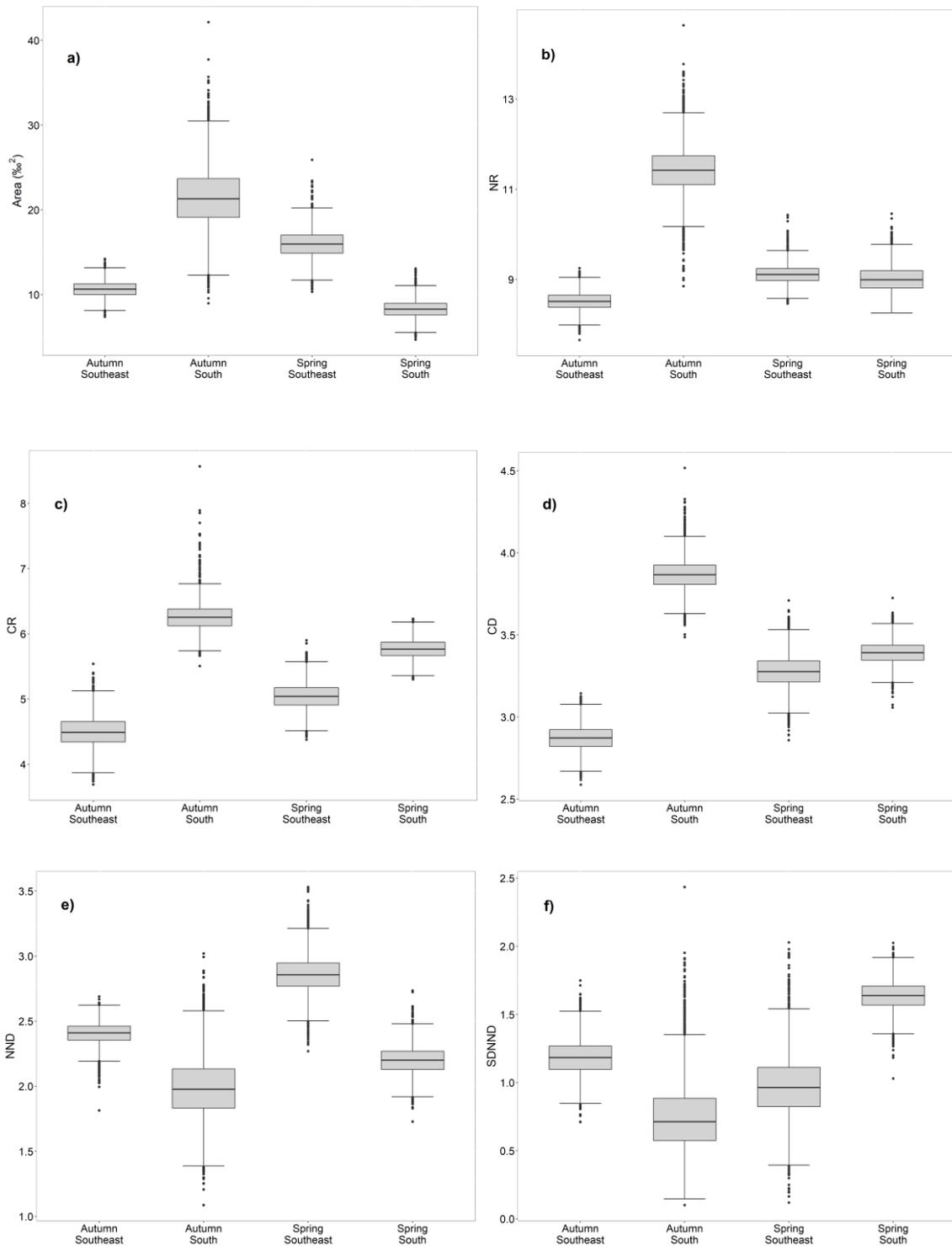
**Figure 8 Cetacean trophic level as estimated by Equation 2 (Popp et al. 2007) with a Trophic Enrichment Factor of 7%. Estimated TL is shown for each season (autumn and spring) and region (southeast and south) the cetaceans were sampled. Cetacean species abbreviation is shown in table 1.**



**Figure 9** Cetacean trophic level (TL) as estimated by equations 3-to-5, based on difference between glutamine and phenylalanine amino acids, Trophic Enrichment Factor of 5.7‰ (Bradley et al. 2015), and the difference between  $\delta^{15}\text{N}$  values of trophic and source amino acids in primary producers  $\beta = 3.4\text{‰}$  (Chikaraishi et al. 2009),  $\beta = 1.2\text{‰}$  (McCarthy et al. 2013) and  $\beta = 3.6\text{‰}$  (Bradley et al. 2015). Estimated TL is shown for each season (autumn and spring) and region (southeast and south) the cetaceans were sampled. Cetacean species abbreviation is shown in table 1.



**Figure 10**  $\delta^{15}\text{N}$  in cetacean source amino acids within each season and region: Gly = glycine; Lys = lysine; Phe = phenylalanine; Ser = serine and Tyr = tyrosine.



**Figure 11** Boxplot with Bayesian estimated community metrics for each region (southeast and south) within season (spring and autumn): a) Total area (TA); b)  $\delta^{15}\text{N}$  range (NR); c)  $\delta^{13}\text{C}$  range (CR); d) Mean distance to centroid (CD); e) Nearest neighbour distance (NND); and f) Standard deviation of the nearest neighbour distance (SDNND).