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Ecologia trófica do boto-cinza, *Sotalia guianensis*: padrões temporais, espaciais e individuais

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O presente trabalho em nível de doutorado foi avaliado e aprovado por banca examinadora composta pelos seguintes membros:

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“You must live in the present, launch yourself on every wave, find your eternity in each moment. Fools stand on their island of opportunities and look toward another land. There is no other land; there is no other life but this.”

Henry David Thoreau

RESUMO

O conceito de nicho ecológico é essencial para compreendermos como as espécies utilizam recursos disponíveis no ambiente e como estes recursos estruturam as comunidades ecológicas. A análise de isótopos estáveis tem sido utilizada como uma aproximação para mensurar algumas das *n*-dimensões do nicho de uma espécie. Assim, o nicho isotópico utiliza como coordenadas os valores de $\delta^{13}C$ e $\delta^{15}N$ que registram tanto os recursos consumidos ($\delta^{15}N$) quanto os locais de alimentação ($\delta^{13}C$). Estruturei esta tese em três capítulos, partindo de uma revisão metodológica sobre a seleção, coleta, preservação e preparo dos tecidos de cetáceos comumente utilizados para a análise de isótopos estáveis. Reuni estas informações em um roteiro que poderá orientar futuras pesquisas e facilitar a padronização destes procedimentos em diferentes laboratórios de pesquisa, viabilizando comparações entre espécies de cetáceos. No segundo capítulo, utilizei valores de $\delta^{13}C$ e $\delta^{15}N$ em colágeno ósseo para avaliar a ecologia alimentar e a potencial sobreposição ou partição no nicho isotópico entre o boto-cinza (*Sotalia guianensis*), toninha (*Pontoporia blainvillei*) e o ecótipo costeiro do boto-da-tainha (*Tursiops truncatus gephyreus*) na área de simpatria destas espécies na região sul do Brasil. Utilizei modelos de mistura isotópicas para quantificar a contribuição proporcional das principais presas na dieta de cada espécie e avaliei potenciais variações na composição da dieta destas populações que podem estar relacionadas ao aumento da pressão da pesca nas últimas três décadas. A toninha apresentou valores maiores de $\delta^{15}N$ e baixa sobreposição de nicho isotópico com as espécies de delfinídeos (boto-cinza e o boto-da-tainha). Em contrapartida, o boto-cinza e o boto-da-tainha apresentaram valores similares de $\delta^{13}C$ e $\delta^{15}N$, resultando em uma alta sobreposição isotópica entre si. Esta variação nas posições tróficas e a baixa sobreposição entre a toninha e delfinídeos reflete o consumo de presas provenientes de diferentes tipos de habitat, uma vez que a toninha se alimenta principalmente de presas pelágicas (e.g., anchovas), e os delfinídeos consomem principalmente presas demersais da família Mugilidae (e.g., parati e tainha). Algumas das espécies de presas que contribuem significativamente para a dieta destes golfinhos (e.g., tainha) estão ameaças pela sobreexplotação. Assim, potenciais mudanças na abundância dessas fontes de presas podem influenciar na composição da dieta e seleção de habitat entre essas espécies simpátricas e, consequentemente, impactar a coexistência na região. No capítulo 3, utilizei valores de $\delta^{13}C$ e $\delta^{15}N$ em amostras de dentina obtidas ao longo das camadas de crescimento do boto-cinza para avaliar variação na utilização de recursos alimentares a nível populacional e individual pelas populações da Baía Norte, Baía da Babitonga e Rio Caravelas. Os valores das métricas de nicho trófico (TNW, WIC, BIC e WIC/TNW) para os valores de

$\delta^{15}N$ sugerem que a população de Caravelas possui o maior nicho trófico (TNW) quando comparado as outras populações e é composta por indivíduos especialistas. Este resultado dá suporte a hipótese de variação de nicho, a qual prediz que populações mais generalistas são mais variáveis em termos de utilização de recursos alimentares e apresentam maior grau de especialização individual. Já a população da Baía Norte possui o nicho mais estreito comparado as outras populações e é composta por indivíduos generalistas. Este resultado pode estar associado à coexistência e à alta sobreposição de nicho isotópico com o ecótipo costeiro do boto-da-tainha, ambas também associadas à alta sobreposição de área de vida entre indivíduos da população de boto-cinza devido à organização social caracterizada por um grupo grande e coeso vivendo em uma área pequena e restrita da Baía Norte. Assim, tanto a potencial competição inter- e intraespecífica podem atuar na restrição da expansão do nicho populacional do boto-cinza na Baía Norte, limitando oportunidades de especialização individual. Assim como na Baía Norte, a população de botos-cinza da Baía da Babitonga também possui um nicho estreito, porém, é composta por indivíduos especialistas, provavelmente devido ao menor potencial competição interespecífica com a toninha associada as diferenças no uso de habitat e no consumo das principais presas. Estes resultados auxiliam na compreensão das estratégias de forrageio realizadas pelo boto-cinza, e sugerem que a amplitude do nicho trófico total da população e o grau de especialização individual da dieta podem ser influenciados por diferentes fatores ecológicos (e.g., disponibilidade de recursos, competição intraespecífica e área de vida) agindo de maneira independente ou integrada.

Palavras-chave: isótopos estáveis, nicho isotópico, *Sotalia guianensis*, cetáceos, interações tróficas, ecologia alimentar

ABSTRACT

The concept of niche is essential for understanding how species use resources available in the environment and how these resources structure ecological communities. Stable isotope analysis has been used as a proxy to measure some of the *n*-dimensions of a species' niche in which $\delta^{13}C$ vs. $\delta^{15}N$ are the environmental axes that combine both habitat and resource related components. I structured this dissertation in three chapters, starting with a practical guideline with suitable techniques for sample preparation of biological tissues to be employed that can yield methodological consistency of stable isotope analyses in cetacean species and hence, facilitate interlaboratorial comparisons. In the second chapter, I used $\delta^{13}C$ and $\delta^{15}N$ values in bone collagen to assess the foraging ecology and potential dietary overlap among sympatric Guiana dolphin (*Sotalia guianensis*), franciscana dolphin (*Pontoporia blainvillei*) and the coastal ecotype of the bottlenose dolphin (*Tursiops truncatus gephyreus*) along the southern Brazilian coast. I applied isotopic mixing models to quantify the proportional contribution of primary prey sources to their diet and investigated potential historical dietary shifts that may be related to increased fishing pressure on some of their primary prey species during the last three decades. The franciscana dolphin showed higher values of $\delta^{15}N$ and low isotopic niche overlap with the delphinid species (Guiana and bottlenose dolphins). In contrast, the Guiana and bottlenose dolphins showed similar values of $\delta^{15}N$, with a high degree of isotopic overlap with each other. The different trophic position and the minimal overlap between the franciscana and the delphinid species reflect consumption of prey sources from different habitat types (pelagic vs. demersal), given that franciscana dolphin primarily consumes pelagic prey (e.g., anchovies), whereas the delphinids consume mainly demersal prey species from the Mugilidae family (e.g., parati and mullet). Some of the prey species shown by mixing models to contribute significantly to dolphin diets are considered threatened by overexploitation. Thus, changes in abundance of these prey sources have the potential to impact resource and habitat use among these sympatric dolphin species, and consequently, their coexistence in this region. In chapter 3, I evaluated time series of stable isotope values ($\delta^{15}N$ and $\delta^{13}C$) in sequential growth layer groups within tooth dentin to assess population- and individual-level variation in resource use among Guiana dolphin populations from North Bay, Babitonga Bay and Caravelas River Estuary. The niche metrics (TNW, WIC, BIC and WIC / TNW) for the $\delta^{15}N$ values suggest that the Caravelas population has the widest niche width (TNW) and is comprised mostly of specialist individuals. This result support the Niche Variation Hypothesis, which posits that the degree of individual diet specialization increased with the populations' total niche width. The population from the

Norte Bay, on the other hand, has the narrowest niche compared to the other populations and is comprised of generalist individuals. This result may be associated with the coexistence and the high overlap of the isotopic niche with the bottlenose dolphin, both associated with the high spatial overlap of Guiana dolphin individuals due to their unique social organization characterized by a large, cohesive group living in a small and restricted area of the North Bay. Thus, both potential inter- and intraspecific competition can be acting to restrict the expansion of the population niche in the North Bay, limiting opportunities for individual specialization. As in the Norte Bay, the population from the Babitonga Bay also has a narrow niche, but is mostly comprised of specialist individuals, likely promoted by the lower interspecific competition with a sympatric franciscana dolphin population due to differences in use of core areas and main prey items preferences. These results improve our understanding of the foraging strategies used by the Guiana dolphin, and suggests that the total niche width and degree of diet individual specialization of Guiana dolphin populations can be influenced by different ecological factors (e.g., availability of resources, intraspecific competition. and living area) acting independently or integrated.

Keywords: Stable isotope, isotopic niche, *Sotalia guianensis*, cetaceans, trophic interactions, feeding ecology

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SUMÁRIO

1 INTRODUÇÃO GERAL

O conceito de **nicho ecológico** é essencial para compreendermos como as espécies utilizam recursos disponíveis no ambiente e, consequentemente, como estes recursos estruturam as comunidades ecológicas. A definição moderna proposta por Hutchinson (1957) caracteriza o nicho como um hipervolume *n*-dimensional composto por variáveis ecológicas que refletem as condições e recursos permissivos à existência indefinida de uma espécie. Por questões operacionais, é comum que as variáveis que compõem o nicho sejam divididas em duas classes principais. Uma delas é a classe Grinnelliana (ou nicho Grinnelliano, *sensu* Soberón 2007), composta por variáveis abióticas geralmente associadas a fenômenos ecológicos que variam em ampla escala espacial e temporal. A outra é a classe Eltoniana (ou nicho Eltoniano, *sensu* Soberón 2007), que descreve as interações bióticas entre os organismos e que comumente relaciona-se com fenômenos ecológicos que ocorrem em escalas menores. Hutchinson distinguiu ainda o nicho ecológico de uma espécie em fundamental e realizado, sendo o primeiro representado pelo conjunto de atributos do habitat que permite a presença de uma espécie, enquanto o segundo considera o efeito das interações interespecíficas (e.g., competição, predação) na determinação do nicho de uma espécie (Leibold 1995) (Figura 1).

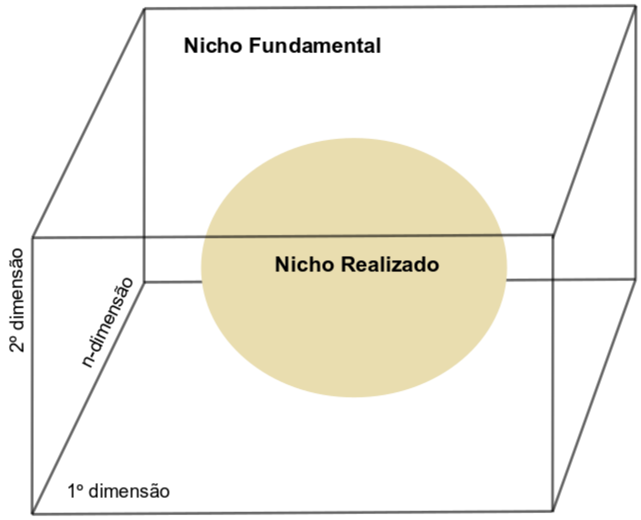

Figura 1. Representação esquemática do nicho fundamental e realizado de uma espécie em um espaço *n*-dimensional (Hutchinson 1957). Cada dimensão ou eixo representa uma condição ou recurso exigido para a sobrevivência de uma determinada espécie.

Compreender esta dinâmica—a interação de espécies entre si, com outras espécies, com seu ambiente e seus recursos—é uma questão central em Ecologia, com importantes implicações para a conservação da biodiversidade. Sabe-se, por exemplo, que a **coexistência** de duas ou mais espécies com requerimentos ecológicos semelhantes pode ocorrer através da diferenciação do nicho em três principais dimensões: uso de habitat, padrão temporal de atividade e recursos alimentares preferenciais. Uma menor sobreposição nestas dimensões pode minimizar os efeitos da competição, facilitando a coexistência (Schoener 1974; Pianka 1980). A dimensão do nicho ecológico na qual estas espécies podem diferir, e então coexistir, é uma particularidade de cada interação, grupo de organismos, habitat e recursos existentes. A maioria dos animais tende a evitar sobreposições, diferindo seus nichos principalmente quanto ao uso de habitat e/ou no uso dos recursos alimentares (Schoener 1974). Neste contexto, a avaliação da amplitude e, consequentemente, da sobreposição ou partição de **nicho trófico** são importantes para quantificar como as espécies se relacionam e utilizam os recursos alimentares em um mesmo local, bem como quais os elementos que garantem uma eventual coexistência.

Em teoria, o nicho ecológico de uma espécie é descrito por meio de uma curva de utilização de recursos baseada na "aproximação do campo médio" (Durret & Levin 1994; Violle et al. 2012). Este é um preceito simplificado utilizado para modelar sistemas e fenômenos altamente complexos, e considera as médias em detrimento das variações entre indivíduos. Esta aproximação, entretanto, tem se mostrado inadequada para descrever a dinâmica do nicho trófico (Bolnick et al. 2003), uma vez que pressupõe que indivíduos de uma mesma população são equivalentes em relação a utilização de recursos (Hutchinson 1957). De acordo com este pressuposto, a distribuição no uso dos recursos dos indivíduos sobrepõe-se amplamente com a da população (Figura 2a). No entanto, diferentes fatores exógenos (e.g., disponibilidade de recursos e sazonalidade; Tebbich et al. 2004) e endógenos (e.g., sexo, idade e polimorfismo; Polis 1983; Skúlason & Smith 1995; Elorriaga-Verplancken et al. 2013) podem gerar variações no nicho trófico entre indivíduos da mesma população (Figura 2b). Em algumas espécies, por exemplo, a variação de nicho trófico entre machos e fêmeas pode ser atribuída ao dimorfismo sexual e aos requerimentos energéticos e fisiológicos associados a reprodução (e.g., Bearhop et al. 2006). Já em relação a idade, as variações podem surgir como uma resposta a mudanças no tamanho corporal e/ou ao período necessário para o aprendizado das habilidades de forrageio (e.g., Troina et al. 2016; De Albernaz et al. 2017).

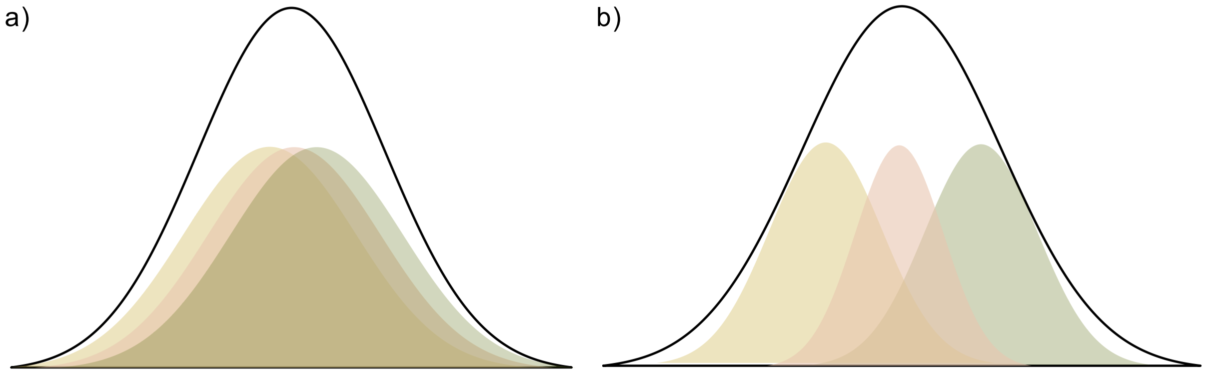

Figura 2. Representação conceitual dos nichos tróficos dos indivíduos (curvas coloridas) dentro do nicho da população (curva delimitada pela linha preta). (a) Cenário sugerido pela teoria clássica de nicho, em que as distribuições individuais no uso de recursos sobrepõem-se com a distribuição de uso de recursos da população; (b) Cenário no qual indivíduos são especializados em determinados subconjuntos de recursos alimentares dentro da distribuição de uso de recursos total observado na população.

Embora a contribuição dos fatores endógenos e exógenos na variação intrapopulacional seja cada vez mais reconhecida na literatura, indivíduos podem também consumir subconjuntos do espectro de recursos da população por razões não atribuídas ao sexo, idade ou polimorfismos (Bolnick & Doebel 2003) (Figura 2b). Esta variação, denominada **especialização individual** ou **variação interindividual** (Bolnick et al. 2003) tem importantes implicações ecológicas podendo modificar a intensidade da competição intraespecífica, bem como as interações e dinâmicas populacionais (Bolnick 2011). A variação intrapopulacional pode ser avaliada por meio de índices de amplitude de nicho e especialização individual (Bolnick et al. 2002, 2003; Araújo et al. 2007) propostos inicialmente por Roughgarden (1972; 1974). Por esta perspectiva, o nicho trófico total da população (TNW – *total niche width*) é dividido nos componentes intraindividual (WIC – *within individual component*), relativo à variância média na diversidade de recursos usados por cada indivíduo, e interindividual (BIC – *between individual component*), relativo à variância média na diversidade de recursos usados por diferentes indivíduos. Assim, quanto menor o valor do componente intraindividual relativo ao nicho trófico total (WIC/TNW), maior será a especialização individual naquela população (Bolnick et al. 2003), e menor será a potencial competição intraespecífica (Bolnick et al. 2003). Esta abordagem pode ser usada para avaliar a prevalência relativa de estratégias especialista vs. generalistas que se reflete no grau de especialização individual em uma população (Figura 3).

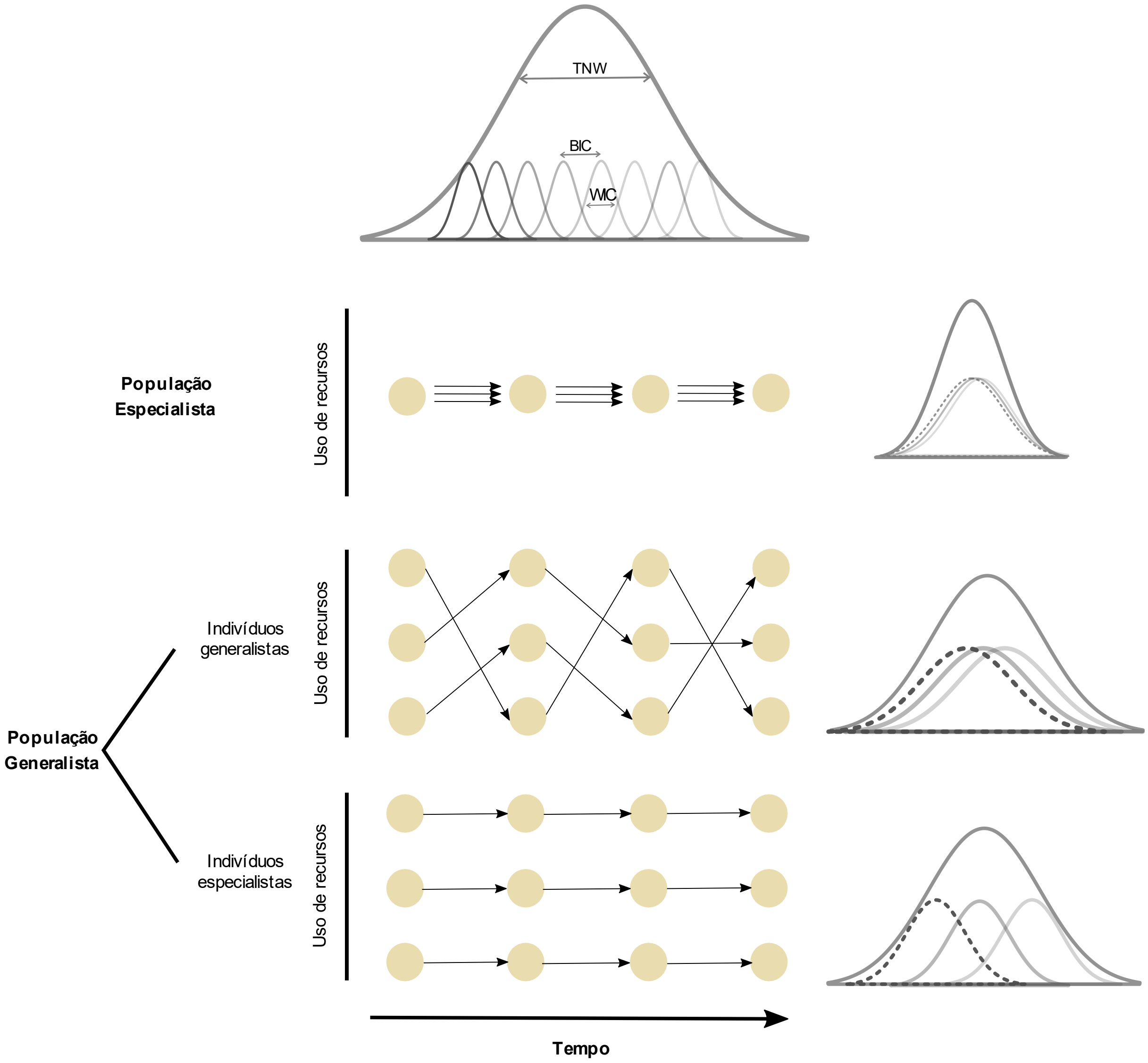

Figura 3. Exemplos de três séries temporais hipotéticas no uso de recursos. A primeira população seria considerada especialista, com indivíduos tambem especialistas, que consomem apenas uma fração do total de recursos disponíveis. Na primeira população generalista, os indivíduos são generalistas e variam amplamente no uso de recursos, resultando em um registro isotópico que varia ao longo do tempo de modo que tanto os indivíduos quanto a população ocupam um amplo nicho. Já na segunda população generalista, os indivíduos são especialistas e mantêm o uso consistente dos recursos dentro de um nicho estreito, mas a variação entre os indivíduos resulta em um nicho amplo da população. Figura adaptada de Vander Zander et al. 2010.

Desta forma, além da compreensão sobre as **interações tróficas** entre espécies coexistentes, se faz necessário avaliar e considerar as variações entre indivíduos que podem influenciar no **tamanho total do nicho trófico** das populações. Porém, mensurar as dimensões do nicho, em especial em nível individual é uma tarefa desafiadora. Alguns autores (Bearhop et al. 2004; Layman et al. 2007; Newsome et al. 2007; Jackson et al. 2011) propuseram a análise de isótopos estáveis como uma aproximação para mensurar algumas das *n*-dimensões do nicho ecológico de uma espécie. No entanto, para entender como os isótopos estáveis podem ser utilizados para inferir a amplitude, a sobreposição ou mesmo a partição de nicho trófico, é necessário definir *a priori* alguns conceitos fundamentais da **análise de isótopos estáveis,** partindo do questionamento mais simples: o que são isótopos estáveis?

1.1 ESTUDO DA ECOLOGIA TRÓFICA ATRAVÉS DE ISÓTOPOS ESTÁVEIS

Isótopos estáveis são átomos de um elemento químico que apresentam o mesmo número de prótons e elétrons, mas diferem em relação ao número de nêutrons no núcleo. Ao contrário dos radioisótopos, os isótopos estáveis persistem sob a mesma forma após serem formados podendo ser encontrados em abundância nos mais variados organismos e ecossistemas. Desde a década de 1970, a análise de isótopos estáveis é aplicada com sucesso em estudos ecológicos (Gannes et al. 1998; Crawford et al. 2008), comumente utilizando isótopos de carbono (^{13}C) e nitrogênio (^{15}N) (Michener & Schell 1994). Nestes estudos, a composição isotópica é expressa pela notação delta (δ), em partes por mil (‰) e obtida através da seguinte equação:

$$\delta X‰ = [(R_{amostra}/R_{padrão}) -1] *1000$$

em que X representa a massa do isótopo pesado (um ou mais nêutrons extras em relação ao isótopo mais leve) de um determinado elemento químico (i.e., ^{13}C e ^{15}N) enquanto $R_{amostra}/R_{padrão}$ corresponde a razão entre os isótopos pesados e leves quantificada na amostra (e.g., $^{13}C/^{12}C$ ou $^{15}N/^{14}N$), expressa em relação àquela encontrada em um padrão internacional estabelecido para cada elemento (e.g., Pee Dee Belemnite – PDB para o carbono e N_2 para o nitrogênio) (Peterson & Fry 1987). Assim, quando a razão entre os isótopos pesado e leve de uma amostra é maior do que aquela encontrada no padrão, dizemos que a amostra possui composição enriquecida (valores positivos de δ), e quando a razão entre os isótopos pesado e leve da amostra é menor do que a razão do padrão, a amostra possui composição empobrecida (valores negativos de δ).

Estudos de ecologia trófica que utilizam a análise de isótopos estáveis como principal ferramenta metodológica estão ancorados no princípio 'você é o que você come'. Isto porque os alimentos são a principal fonte de carbono e nitrogênio nos consumidores e, portanto, é possível determinar as composições isotópicas com base nas fontes alimentares (Peterson & Fry 1987). Porém, trata-se de uma técnica minuciosa que requer o conhecimento das principais fontes de variação isotópica nos consumidores: a composição isotópica das fontes, as discriminações isotópicas entre as fontes e os tecidos do consumidor, e o tempo necessário para que a composição isotópica das fontes seja refletida nos diferentes tecidos do consumidor (taxa de reposição ou *turnover*) (Newsome et al. 2010).

A carga extra de nêutrons encontrada nos isótopos pesados resulta em ligações mais fortes e difíceis de quebrar (Fry 2006). Consequentemente, isótopos pesados reagem de maneira mais lenta em processos físico-químicos quando comparados aos isótopos leves. Este comportamento isotópico distinto, conhecido como **fator de discriminação isotópica (ou trófica)**, faz com que a razão entre isótopos pesados e leves ao final de uma reação (produto) seja diferente da razão inicial entre eles (substrato) (Martínez del Rio et al. 2009). Desta forma, a utilização das composições isotópicas se baseia no fator de discriminação isotópica, de modo que os consumidores apresentam composições enriquecidas em relação as suas fontes alimentares (Tieszen et al. 1983). Esta discriminação observada entre a dieta e os tecidos dos consumidores pode variar também de acordo com as diferentes rotas metabólicas entre tecidos, a taxa de crescimento do organismo, a qualidade nutricional da dieta, e a variação na composição de aminoácidos das proteínas encontrada nos tecidos animais (Newsome et al. 2010).

De maneira geral, a discriminação isotópica do carbono entre a dieta e os tecidos do consumidor é de aproximadamente +0.5 a +1‰ (DeNiro & Epstein 1978). Esta mínima discriminação isotópica entre níveis tróficos é a razão pelo qual o $\delta^{13}C$ é utilizado para inferir a fonte primária de carbono na base da cadeia trófica, demonstrando um gradiente em sistemas aquáticos onde fontes costeiras exibem valores de $\delta^{13}C$ mais enriquecidas em relação a fontes mais oceânicas (McConnaughey & McRoy 1979; Peterson & Fry 1987). Estas diferenças nos valores de $\delta^{13}C$ entre produtores ocorrem principalmente em razão do sistema fotossintético das plantas, em que espécies com ciclo C4 geralmente apresentam composições mais enriquecidas em ^{13}C do que plantas C3 (Peterson & Fry 1987). Já a discriminação isotópica do nitrogênio entre níveis tróficos varia entre +2 a +5‰ (DeNiro & Epstein 1981; Minagawa & Wada 1984; Post 2002) e é atribuída, principalmente, à excreção de ureia e outros compostos nitrogenados empobrecidos em ^{15}N (Minagawa & Wada 1984). Assim, o $\delta^{15}N$ é comumente utilizado como indicador da posição trófica ocupada pelo consumidor na cadeia alimentar (Figura 4) (DeNiro & Epstein 1981; Minagawa & Wada 1984).

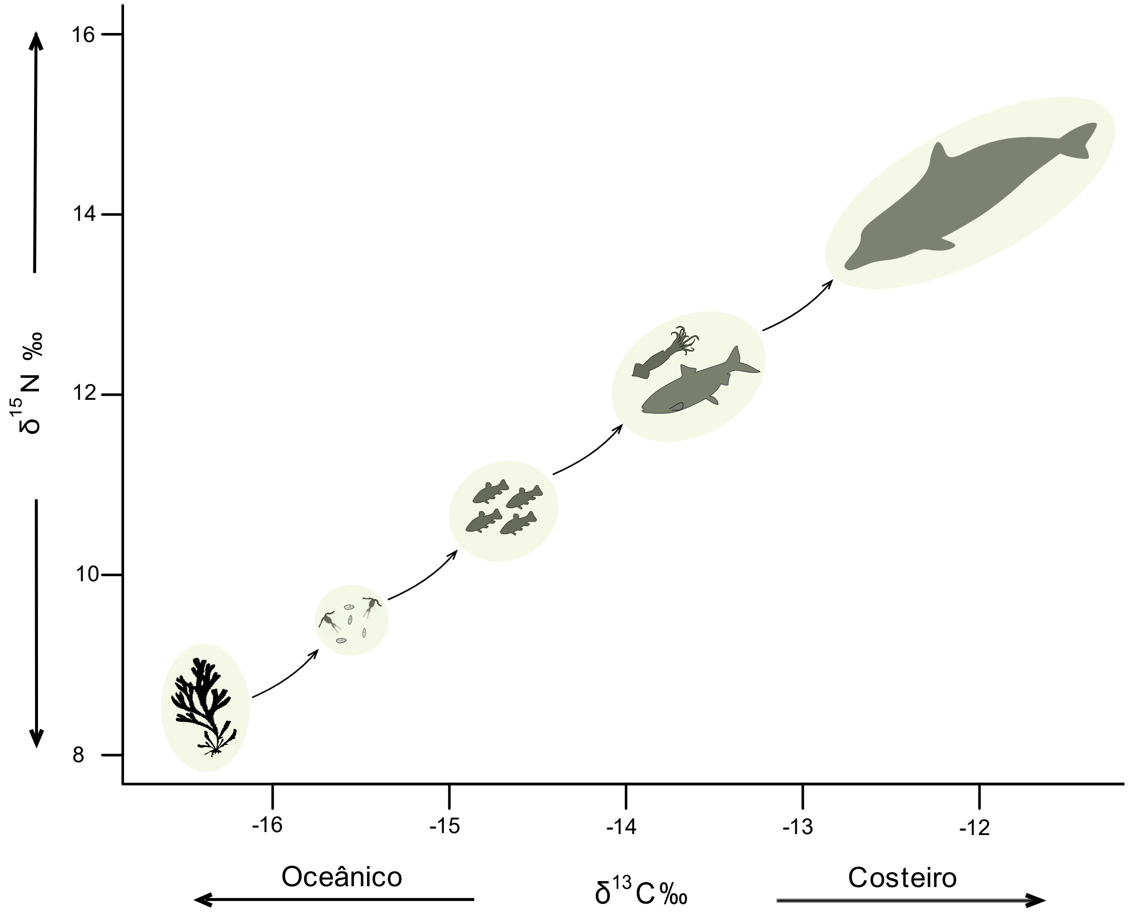

Figura 4. Representação de uma cadeia trófica marinha em que a discriminação isotópica do $\delta^{13}C$ entre níveis tróficos é de aproximadamente +0.5 a +1‰, e é utilizado para inferir a fonte primária de carbono na base da cadeia. Já a discriminação isotópica do $\delta^{15}N$ entre níveis tróficos é de +2 a +5‰, indicando a posição trófica ocupada pelo consumidor na cadeia alimentar.

A **taxa de reposição,** ou ***turnover*** consiste na síntese e renovação de tecido e degradação do tecido pré-existente, a qual varia de acordo com a taxa metabólica do tecido. Tecidos de alta taxa metabólica (e.g., fígado), por exemplo, apresentam taxa de renovação elevada e refletem a dieta recente (Caut et al. 2011). A análise isotópica em tecidos com taxas metabólicas intermediárias (e.g., músculo, pele e hemácias) reflete a dieta do consumidor referente ao período de semanas a meses (Dalerum & Angerbjörn 2005). Já os tecidos de baixa taxa metabólica (e.g., ossos) e tecidos considerados inertes (e.g., vibrissas, barbatanas e dentes), apresentam crescimento contínuo e uma vez formados, não são novamente metabolizados, tendo taxa de renovação isotópica baixa e inexistente, refletindo a dieta do indivíduo a longo prazo (anos) (Knoff et al. 2008). Esta variação nos tipos de tecido é especialmente útil para grandes predadores marinhos, pois um único tecido de baixo metabolismo sequencialmente subamostrado, ou amostras de diferentes tecidos do mesmo indivíduo, permitem avaliar o uso de recursos em diferentes escalas temporais, sem exigir que o mesmo indivíduo seja reamostrado em diferentes eventos (e.g., Matich et al. 2011). Os cetáceos odontocetos, por exemplo, apresentam dentição única (monofiodontes) com dentes compostos por tecidos proteicos e calcificados (Fordyce & Barnes 1994) e deposição contínua de dentina e cimento ao longo da vida, formando camadas de crescimento contrastantes entre si ou GLGs (*Growth Layer groups*; Perrin & Myrick 1980). Assim, a partir da medição das composições isotópicas de carbono e nitrogênio nestas camadas é possível obter informações de forma ordenada e cronológica sobre a dieta do indivíduo (e.g., Rossman et al. 2015). Quando as amostras de dentes são limitadas ou indisponíveis, o colágeno ósseo pode representar uma importante fonte de informações sobre a dieta, uma vez que apresentam taxas de renovação relativamente lentas (Riofrío-Lazo & Aurioles-Gamboa 2013) e, consequentemente, a análise da sua composição isotópica fornece informações sobre a dieta do indivíduo em longa escala temporal (Koch 2007).

Após rever conceitos fundamentais quanto ao nicho ecológico e isótopos estáveis, retorno à questão central: como inferir sobre as interações tróficas entre espécies e considerar as variações intrapopulacionais por meio de isótopos estáveis? Para isso, aplica-se usualmente o conceito de **nicho isotópico**, uma área no espaço isotópico (δ-espaço) que utiliza $\delta^{13}C$ e $\delta^{15}N$ como coordenadas que registram os recursos consumidos e os locais onde os indivíduos se alimentam (Newsome et al. 2007). Desta forma, organismos que apresentam variação na composição da dieta irão refletir tais diferenças na proporção isotópica dos seus tecidos e, consequentemente, na maneira como o nicho isotópico é estruturado neste δ-espaço. O delineamento da amplitude dos nichos isotópicos, bem como das métricas de sobreposição desses nichos podem então fornecer um meio para quantificar nichos tróficos e inferir relações tróficas e sobreposição ou partição de nicho entre indivíduos (e.g., Zhao et al. 2014), populações (e.g., Santos-Carvallo et al. 2015), e espécies coexistentes (e.g., Franco-Trecu et al. 2017; Teixeira et al. 2020).

1.1.1 O boto-cinza como modelo de estudo

Os cetáceos desempenham um papel vital para a estrutura e funcionamento dos ecossistemas. Devido ao grande tamanho corporal e a alta demanda energética, consomem uma variedade de recursos alimentares provenientes de diferentes níveis tróficos (Katona & Whitehead 1988). Por serem predadores de topo de cadeia, atuam no controle da abundância das populações de presas e na regulação "top-down" de cadeias tróficas, no qual sua extinção local pode desencadear uma série de eventos em cascata nos níveis tróficos inferiores (Terborgh & Estes 2013). Atuam também na regulação "bottom-up", ao aumentar a produção primária por meio da disponibilização de nitrogênio e outros nutrientes presentes em seus materiais fecais (Roman & McCarthy 2010; Roman et al. 2016), e ao transferir nutrientes por meio de movimentos horizontais realizados entre microhabitats e/ou movimentos verticais, e.g., quando suas carcaças submergem fornecendo alimento e habitat para comunidades de micro e macro organismos abissais (Jones et al. 1998; Pershing 2010).

As funções dos cetáceos no ecossistema vão além das interações com os níveis tróficos inferiores. Estudos sugerem, por exemplo, que o declínio de algumas espécies pode influenciar na abundância de outros predadores de topo de cadeia (Laws 1977; Springer et al. 2003). Em determinadas situações, os cetáceos podem exibir táticas de forrageio que maximizam a obtenção de recursos alimentares de outras espécies de predadores. Tal interação pode ser observada com aves marinhas, em que os cetáceos conduzem cardumes de peixes para a superfície da água (Anderson & Lovvorn 2008; Veit & Harrison 2017) e até mesmo com humanos, em que os cetáceos arrebanham cardumes de presas em direção a estruturas de pesca artesanais (e.g., cercos fixos) (e.g., Monteiro-Filho 1995; Louzada 2013) ou à áreas mais rasas onde pescadores posicionados aguardam com redes de pesca (e.g., Simões-Lopes et al. 1988; Zappes et al. 2011). Porém, nem todas as interações envolvendo cetáceos e outros predadores são positivas ou cooperativas. Espécies de cetáceos costeiros, por exemplo, são geralmente representadas por populações pequenas que exploram as mesmas presas extraídas, e muitas vezes, sobreexplotadas, pela atividade pesqueira (Trites et al. 1997; Demaster et al. 2001; Teixeira et al. 2020). Assim, esta compreensão sobre o papel dos cetáceos nas cadeias tróficas marinhas e nos ecossistemas é essencial, pois fornece elementos para avaliar as interações entre espécies coexistentes (e.g., sobreposição ou partição de recursos) e suas principais presas consumidas, bem como o potencial impacto da alteração na disponibilidade das presas na estrutura e dinâmica das populações. Em última instância, resolver estas questões pode auxiliar a fomentar a discussão sobre a conservação de espécies de cetáceos, bem como no monitoramento e manejo dos ecossistemas em diferentes âmbitos, incluindo a gestão pesqueira.

Porém, a alimentação críptica de cetáceos implica em muitos casos, na obtenção de dados com resolução limitada para a análise da dieta a nível populacional e individual. Consequentemente, a ecologia trófica de algumas espécies permanece ainda pouco explorada. A maioria dos dados sobre a ecologia alimentar em cetáceos provém da análise de conteúdo estomacal (e.g., Di Beneditto 2001; Barros et al. 2004; Lopes et al. 2012), que consiste na identificação e biometria de partes rígidas (e.g., otólitos de peixes teleósteos e bicos de cefalópodes) recuperadas do estômago de animais mortos encontrados encalhados ou capturados acidentalmente. Nas últimas décadas, porém, a análise de isótopos estáveis tem se mostrado uma ferramenta eficiente para abordar questões complementares referentes ao uso de habitat (Botta et al. 2012; Kiszka et al. 2012), sobreposição ou partição de nicho entre espécies coexistentes (Díaz-Gamboa et al. 2018; Teixeira et al. 2020), padrões migratórios (Mendes et al. 2007; Silva et al. 2019), variações individuais (Rossman et al. 2015; Troina et al. 2016) e populacionais (Yurkowski et al. 2016) na dieta. É válido ressaltar que as análises de conteúdo estomacal e de isótopos estáveis apresentam vantagens e limitações em suas aplicações. Enquanto a primeira técnica fornece uma descrição taxonômica das presas consumidas e sua biomassa em curta escala temporal (Hobson & Wassenaar 1999), a última oferece uma análise da dieta incorporada aos tecidos dos animais em diferentes escalas temporais, porém com uma menor resolução taxonômica (Tieszen et al. 1983; Dalerum & Angerbjörn 2005). Portanto, a combinação destes métodos para a determinação da dieta e seus desdobramentos ecológicos pode ampliar a resolução das interações tróficas (Post 2002) e devem ser considerados complementares sempre que possível.

Entre as espécies de cetáceos que habitam as águas do litoral brasileiro encontra-se o boto-cinza, *Sotalia guianensis,* um delfinídeo com distribuição essencialmente costeira no Atlântico Sul-ocidental (Borobia 1991), desde a Nicarágua (Carr & Bonde 2000) até Santa Catarina, Sul do Brasil (Simões-Lopes 1988). Ao longo da sua distribuição, o boto-cinza apresenta populações que ocupam habitats em alopatria ou simpatria com espécies de cetáceos (Flores & Bazzalo 2004; Hardt et al. 2010). A região da Baía Norte localizada no limite sul da distribuição da espécie, por exemplo, é a única área que se conhece até o momento em que a população residente de boto-cinza ocorre em simpatria com a toninha (*Pontoporia blainvillei*), e o ecótipo costeiro do boto-da-tainha (*Tursiops truncatus gephyreus*). Estes cetáceos simpátricos apresentam diferentes características relacionadas a tamanho corporal, morfologia (e.g., tamanho de crânio, mandíbula e número de dentes; Rosas & Monteiro-Filho 2002; Botta et al. 2012; Cremer et al. 2018; Venuto et al. 2020) e história de vida (e.g., crescimento e maturidade sexual; Rosas & Monteiro-Filho 2002; Barreto & Rosas 2006; Botta et al. 2010; Fruet et al. 2015; Venuto et al. 2020) que provavelmente exigem demandas energéticas distintas e resultam em estratégias de forrageio e capacidade de captura de presas específicas e, consequentemente, influenciam em suas preferências alimentares. Nas últimas décadas, algumas espécies de presas consumidas por esses cetáceos vêm sofrendo um crescente declínio devido à importância econômica para a pesca ou o descarte na pesca acidental (Haimovici & Mendonça 1996), resultando em estoques explotados e/ou em risco de colapso (Haimovici & Cardoso 2017). Assim, informações sobre a ecologia alimentar destas populações de cetáceos simpátricos pode auxiliar na compreensão dos fatores que permitem esta coexistência e como o aumento da pressão de pesca em suas presas pode influenciar nas composições das dietas e, consequentemente, em suas interações tróficas na região.

Outro exemplo de simpatria pode ser observado na região mais ao norte de Santa Catarina, em que a população residente de boto-cinza ocorre com a toninha na Baía da Babitonga (Cremer & Simões-Lopes 2005). Já na região do Banco de abrolhos, Sul da Bahia, há o registro de diferentes espécies de cetáceos (e.g., golfinho de dentes rugosos *Steno bredanensis* e golfinho nariz-de-garrafa *Tursiops truncatus*). No entanto, estas espécies utilizam áreas distintas do Banco de Abrolhos, não ocorrendo no interior do Rio Caravelas e suas áreas costeiras adjacentes que são utilizadas apenas pela população de boto-cinza (Rossi-Santos et al. 2006). Assim, essas populações de boto-cinza estão expostas a diferentes contextos ecológicos, não apenas em termos de presença ou ausência de outras espécies de cetáceos na mesma área (heteroespecíficos), que podem gerar uma potencial competição interespecífica por recursos alimentares, mas também em relação aos padrões de uso de área (alta ou baixa sobreposição espacial entre indivíduos da população), densidade populacional e disponibilidade de recursos. Tais aspectos podem influenciar a dieta e o comportamento de forrageio, favorecendo ou não especializações individuais e refletindo no tamanho de nicho trófico da população. Até o momento, apenas estudos baseados em dados provenientes de conteúdos estomacais descreveram os hábitos alimentares destas populações de boto-cinza, sugerindo uma dieta generalista e oportunista (e.g., Daura-Jorge et al. 2011; Cremer

2012; Rodrigues et al. 2020). A complementação destes estudos por análise de isótopos estáveis pode fornecer dados sobre a composição da dieta assimilada em diferentes escalas temporais, possibilitando uma visão mais ampla e refinada sobre a ecologia alimentar do boto-cinza em diferentes contextos ecológicos.

1.2 OBJETIVOS

1.2.1 Objetivo Geral

Utilizando o nicho ecológico e o uso de recursos como bases conceituais, nesta tese busco compreender o nicho trófico do boto-cinza sob a ótica do espaço de nicho isotópico em diferentes escalas ecológicas: em uma escala ecológica mais ampla, ao comparar a amplitude e sobreposição (ou partição) de nicho trófico do boto-cinza e das espécies de cetáceos simpátricos que compõem a **comunidade** costeira da região da Baía Norte (Capítulo 2); e em uma escala ecológica mais fina, ao avaliar o tamanho do nicho trófico em **populações** do boto-cinza localizadas em diferentes regiões (Baía Norte, Baía da Babitonga e Caravelas) e como a variação individual no forrageio e entre **indivíduos** podem influenciar no nicho trófico destas populações (Capítulo 3). Para responder a estas perguntas, a escolha dos tecidos para realização das análises de isótopos estáveis é um fator determinante. Após uma revisão sobre taxas de crescimento e renovações metabólicas (Capítulo 1), as composições de $\delta^{13}C$ e $\delta^{15}N$ foram avaliadas em colágeno ósseo e ao longo das camadas de deposição contínua de dentina (camadas de crescimento), permitindo que as comparações entre espécies e populações considerassem uma leitura da dieta dos indivíduos em uma ampla escala temporal. Assim, esta tese foi estruturada em três capítulos, partindo de uma revisão metodológica e seguindo com as análises e interpretações sobre o nicho ecológico do boto-cinza em diferentes contextos de interações: entre espécies, populações e indivíduos (Figura 5). Mais especificamente, no capítulo 2 testei a hipótese de que (i) o boto-cinza, a toninha e o ecótipo costeiro do boto-da-tainha apresentariam diferenças em relação a posição trófica e amplitude de nicho isotópico, com uma maior sobreposição de nicho entre as duas espécies mais filogeneticamente relacionadas (boto-cinza e ecótipo costeiro do boto-da-tainha) e (ii) estas espécies apresentam variações temporais na composição da dieta que podem estar relacionadas ao aumento da pressão da pesca em algumas de suas principais espécies de presas durante as três ultimas décadas (1985–2017). No capítulo 3, testei a hipótese de que (i) embora o boto-cinza seja considerada uma espécie generalista, as populações da Baía da Babitonga, Baía Norte e Rio Caravelas apresentam diferenças em relação ao grau de especialização de forrageio individual e, consequentemente, no tamanho de nicho trófico total da população. Tais variações podem indicar uma adaptação aos diferentes contextos ecológicos nos quais estas populações estão inseridas, tais como a presença de heteroespecíficos, densidade populacional, área de vida, padrões espaciais individuais e organização social.

1.2.2 Objetivos Específicos

Capítulo 1: A practical guide on stable isotope analysis: Applications to cetaceans

(i) Gerar um roteiro detalhado que auxilie na seleção, coleta, preservação, e preparo dos tecidos biológicos mais comuns para a análise isotópica em espécies de cetáceos misticetos e odontocetos

(ii) Orientar a interpretação e a análise de dados isotópicos por meio de ferramentas analísticas disponíveis a fim de abordar questões ecológicas específicas.

Capítulo 2: Niche overlap and diet composition of three sympatric coastal dolphin species in the southwest Atlantic Ocean

(i) Caracterizar a ecologia alimentar do boto-cinza, toninha e do ecótipo costeiro do boto-da-tainha através dos valores de $\delta^{13}C$ and $\delta^{15}N$ em amostras de colágeno ósseo

(ii) Estimar a área do nicho isotópico ocupada por estas espécies de cetáceos costeiros, e o nível de sobreposição no nicho e na utilização dos recursos entre elas.

(iii) Quantificar a contribuição proporcional das principais presas para a dieta de cada espécie

(iv) Investigar possíveis variações temporais na dieta das três espécies, através dos valores de $\delta^{13}C$ and $\delta^{15}N$ coletados em indivíduos ao longo de três décadas (1985-2017)

Capítulo 3: Ecologically driven differences in individual diet specialization across three populations of Guiana dolphin

(i) Quantificar e comparar o tamanho de nicho total (TNW), variação intraindividual (WIC), variação interindividual (BIC) e o índice de especialização individual na dieta (WIC/TNW) das populações de botos-cinza da Baía Norte, Baía da Babitonga e Caravelas através dos valores de $\delta^{13}C$ and $\delta^{15}N$ em amostras de dentina obtidas ao longo das camadas de crescimento

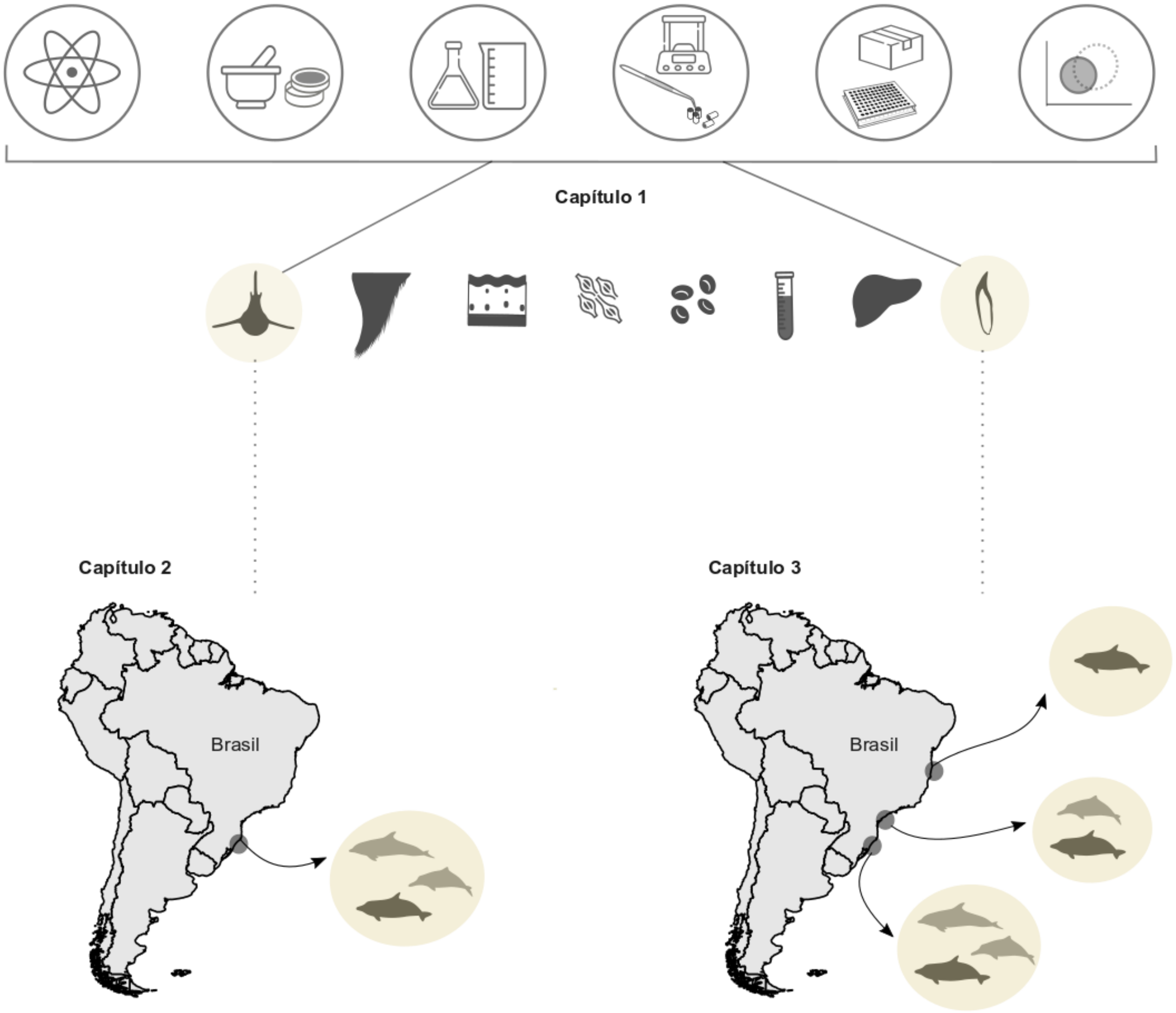

Figura 5. Estruturação da tese partindo de uma revisão metodológica (Capítulo 1), seguindo para as análises e interpretações sobre o nicho ecológico do boto-cinza em diferentes escalas ecológicas e contextos de interações. Em uma escala ecológica mais ampla, ao comparar a amplitude e sobreposição (ou partição) de nicho trófico do boto-cinza e das espécies de cetáceos simpátricos (toninha e ecótipo costeiro do boto-da-tainha) que compõem a **comunidade** costeira da região da Baía Norte (Capítulo 2); e em uma escala ecológica mais fina, ao avaliar o tamanho do nicho trófico em **populações** do boto-cinza localizadas em diferentes regiões (Baía Norte, Baía da Babitonga e Caravelas) e como a variação individual no forrageio e entre **indivíduos** podem influenciar no nicho trófico destas populações (Capítulo 3).

"It is no longer a question of how much food do whales and dolphins need, but whether they are able to get the right kinds of food to survive."

Jerome Spitz

2 CAPÍTULO 1 - A PRACTICAL GUIDE ON STABLE ISOTOPE ANALYSIS: APPLICATIONS TO CETACEANS

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ABSTRACT

1. Trophic ecology information of cetaceans is essential to understand their role in ecosystem processes and dynamics. However, observation of their foraging behavior is usually limited by their elusive nature. The stable isotope analysis is a valuable complementary approach to conventional methods applied to cetaceans because it provides dietary information integrated over different time scales and the potential for using tissues archived in museums and scientific collections.

2. The considerable increase in stable isotope analyses by a growing number of cetacean research groups and the relatively high costs associated with processing and analyzing samples demand the use of proper protocols for tissue collection, sampling and preservation to ensure that accurate isotopic data are obtained.

3. We provide an overview of stable isotope analysis theoretical background and its potential application to cetaceans` trophic ecology. We review the factors that influence isotopic measurements and propose a practical guideline with suitable techniques for sample preparation of biological tissues to be employed by researchers to yield reliability in the interpretation of isotopic data.

4. We provide guidance on how to interpret results and conduct analysis of isotopic data with additional information about the available packages in R that can be used to address different ecological questions with cetacean species.

5. We conclude by providing a synthesis of the main considerations for researchers willing to use isotopic analysis to address ecological questions.

KEYWORDS: odontocetes, baleen whales, sample preservation, lipid extraction, turnover rate, trophic enrichment factors

INTRODUCTION

Cetaceans are widely distributed top predators that regulate prey populations and contribute to the stability of their ecosystems. Because of their large home ranges and high movement capacity, some species can also connect food webs by transporting nutrients across different habitats (Roman et al., 2014). Given the challenges associated with their cryptic nature, conventional methods used to infer their foraging preferences such as direct observation are usually hampered by the fact that these animals are highly mobile, with the ability to feed underwater and to remain submerged for long periods of time. Although stomach content analysis has been widely employed to yield taxonomic inputs of prey species and provided the basis for much of our current understanding, it also presents well-described drawbacks mainly due to differential digestibility rates in prey items and the assessment of diet over a short timescale (Dalerum and Angerbjörn, 2005). The analysis of naturally occurring stable isotope ratios is a useful complementary approach to conventional methods usually applied to cetaceans because it provides time-integrated information on prey assimilated (Tieszen et al., 1983; Hobson, 1999) via analysis of multiple tissues types such as tooth and bone collagen, baleen plates, blood, skin, blubber and muscle collected from live or dead animals (Koch, 2007).

Stable isotope analysis (SIA) relies on the assumption that the isotopic composition of consumers tissues, i.e., the ratio between the heavier and the lighter isotope, reflects the isotopic composition of its consumed and assimilated food sources (DeNiro and Epstein, 1978; Gannes et al., 1997). As prey nutrients are incorporated into the consumer, the isotopic ratio changes by a trophic discrimination factor that reflect isotopic fractionation caused by physiological processes (Martínez del Rio et al., 2009). In general, stable isotope ratios of carbon ($^{13}C/^{12}C$, reported as $\delta^{13}C$ values) increase between 0-1‰ per trophic level and its variation allows to evaluate foraging habitats by reflecting the photosynthetic pathway used by primary producers (i.e., C_3 and C_4). Therefore, $\delta^{13}C$ can show variation between benthic vs. pelagic, nearshore vs. offshore, and between freshwater vs. saltwater consumers and ecosystems (Hobson 1999; Post 2002). By contrast, stable isotope ratios of nitrogen ($^{15}N/^{14}N$, reported as $\delta^{15}N$ values) typically increase 3–5‰ with every trophic level and is used to denote a species' position within the food web (DeNiro & Epstein, 1978; Newsome et al., 2010).

Stable isotope analysis has been successfully applied to answer numerous ecological questions such as resource partitioning (e.g., Ryan et al., 2013; Browning et al., 2014; Costa et al., 2020), habitat use (e.g., Pinela et al., 2010), foraging individual specialization (e.g., Rossman et al., 2015), ontogenetic (e.g., Newsome et al., 2009) and sexual variation in the diet (e.g., Troina et al., 2016) of a wide array of cetacean species. With the improvement of stable isotope measurements and the possibility of complementarity with other data sources (e.g., , fatty acids, molecular identification of prey, compound specific SIA of individual lipids and amino acids, satellite telemetry, hormones, contaminants, DNA markers) more complex questions about migration patterns (e.g., , Watt & Ferguson, 2015), stocks origin of stranded carcasses (e.g., , Hohn et al., 2017), population structure and identification (e.g., Brotons et al., 2019; Genoves et al., 2020; Méndez-Fernandez et al., 2020), temporal dietary shift (e.g., Ning et al., 2020), gestation and lactation (e.g., Clark et al., 2017; Gelippi et al., 2020), weaning age (e.g., Evacitas et al., 2017), and interaction with anthropogenic activities (e.g., Lysiak et al., 2018) are now being addressed.

Several reviews cover the principles underlying stable isotope biogeochemistry (e.g., DeNiro & Epstein, 1978, 1981; Peterson & Fry, 1987; Gannes et al., 1998; Kelly, 2000; Martínez del Rio et al., 2009), and their myriad of applications to marine mammal species (e.g., Crawford et al., 2008; Newsome et al., 2010). Rather than summarizing the theoretical basis or the potential application of SIA in trophic ecology of cetaceans, we provide an accessible protocol to guide new researchers on collection, preservation, and sampling of the most relevant tissues for SIA. Given that these practical steps are not always fully described in papers, a protocol with best practices for SIA can increase reliability in isotopic data and facilitate interlaboratory comparisons across world-wide cetacean species. Although additional isotopes (e.g., sulfur) may have a broad array of applications to trophic ecology, we focus our background information on carbon and nitrogen, the two most frequently used stable isotopes.

SIA METHODS

PLANNING YOUR RESEARCH

When analyzing stable isotope ratios to obtain dietary data, a consistent literature review is essential before tissue selection for an understanding about the physiology of species, the macronutrient composition of tissues (e.g., lipids, protein and carbonate content), tissues` turnover rates (i.e., new tissue synthesis and old tissue replacement using the animal composition present in diet), diet-to-tissue isotope discrimination or enrichment (i.e., difference between isotopic ratios of an animal and its diet), isotopic routing (i.e., differential incorporation of dietary macronutrients between tissues) and how these and other factors (e.g., tissue preservation) can influence isotopic values. Besides tissues information, such literature review will also guide the choice to which individuals (e.g., gender, age classes) and stable isotopes are the most appropriate to answer the chosen ecological question. In some cases, $\delta^{13}C$ and $\delta^{15}N$ do not provide enough discriminatory power to address the question of interest, and the addition of other elements may provide further resolution.

The stable isotope ratios of sulfur ($^{34}S/^{32}S$, reported as $\delta^{34}S$ values), for example, can be combined with $\delta^{13}C$ to help distinguish among primary producers (benthic vs. pelagic producers) in marine food web systems (Connolly et al., 2004), and to identify fine-scale differences in foraging ranges and spatial distribution across populations (Barros et al., 2010; Wilson et al., 2012) and between sexes and ages (Niño-Torres et al., 2006). The stable isotope ratios of oxygen ($^{18}O/^{16}O$, reported as $\delta^{18}O$ values), are linked with differential evaporation rates and precipitation inputs along the hydrological cycle, and is potentially useful to distinguish habitat use (e.g., Endo et al., 2014; Drago et al., 2020) and tracking movements across areas with strong salinity gradients (e.g., Matthews et al., 2016). The stable isotope ratios of strontium ($^{87}Sr/^{86}Sr$ ratios, reported as ^{87}Sr) vary regionally according to oceanographic and biological factors (e.g., riverine inputs, salinity and temperature) that influence the distribution of specific planktonic organisms and affect sea water acidification processes. Consequently, ^{87}Sr concentration in baleen plates of Mysticete species has also been recently used as a potential tracer to investigate movements patterns across oceans (Vighi et al., 2019)

Accounting for potential gender or age- related differences is important to minimize the number of variables that might influence isotope values. Energetic, morphological, and physiological constraints in males and females can result in differences in dietary requirements reflected by distinct isotopic values (Rossman et al., 2015). It is also known that calves usually have elevated $\delta^{15}N$ but lower $\delta^{13}C$ due to milk consumption during the nursing period (Niño-Torres et al., 2006). Unless the research focus is related to ontogenetic patterns, it is best to sample only adult individuals. The researchers should also be aware that the steps used in preparation of tissues for SIA are dependent upon tissue type and preservation methods, and each of these steps will require the use of specific laboratory apparatus with appropriate safety procedures stablished by each research facility.

CHOOSING BIOLOGICAL TISSUES

Because each tissue has a specific biochemical composition and reflects different time periods, isotope values from different tissues are not directly comparable. The choice of tissue will depend largely on the ecological questions, and an interpretation of the tissue growth pattern and turnover rate is required before starting sample collection (Fig. 1). For instance, blood is particularly valuable for SIA because it can be separated into components (e.g., plasma and red blood cells) that illustrate different isotope turnover rates and represent dietary inputs at different periods of time (Kelly, 2000). In cetaceans, plasma provides information about the last three months (approximate time to equilibrium) whereas red blood cells provide long-term information (>175 days) (Caut et al., 2011). Blubber is a highly metabolically active subcutaneous tissue vertically stratified in layers that vary in lipid content (Gómez-Campos et al., 2015). Like blubber, skin is a metabolically active tissue with a turnover time of 2-3 months for $\delta^{13}C$ and 2-6 months for $\delta^{15}N$ (Giménez et al., 2016; Busquets-Vass et al., 2017). For baleen whales, the different skin strata can provide shorter-term ecological resolution as they integrate different time periods (Busquets-Vass et al., 2017; Wild et al., 2018).

In toothed whales (Suborder Odontocete), dentin comprises most of the tooth and is chronologically deposited in layers (i.e., Growth Layer Groups; GLGs; Perrin and Myrick, 1980) filling the pulp cavity (Maas, 2009). Seasonal or ontogenetic changes in diet throughout an animal's life can be recorded by subsampling these different serial layers, while sampling an entire tooth allows to obtain an integrated average of their diet through their lifetime (Walker and Macko, 1999). Instead of teeth, baleen whales (Suborder Mysticete) rely on transversely oriented keratin plates attached to the lateral parts of the upper jaw (Werth, 2018) that allows filter-feed on planktonic organisms and fish (Bannister, 2018). Baleen is a metabolic inert tissue and the variation in isotope values subsampled along its length (i.e., transverse ridges of keratin that constitute the baleen plates) generates multi-years record of individual habitat use, diet and seasonal movement patterns (Matthews and Ferguson, 2015; Busquets-Vass et al., 2017). When tooth (or baleen) samples are limited (e.g., in the case of Ziphiidae, that have only one or two pairs of teeth in the lower jaw) or not available, bone collagen can be an important source of isotopic information. Bone tissues also present relatively slow turnover rates (Riofrío-Lazo and Aurioles-Gamboa, 2013) and isotopic measurements represent dietary information over larger timescales (i.e., years; Koch, 2007). It is recommended, however, the same bone type to be sampled when comparing different individuals within populations, as different bones within individuals may differ in their $\delta^{13}C$ and $\delta^{15}N$ values (Smith et al., 2020).

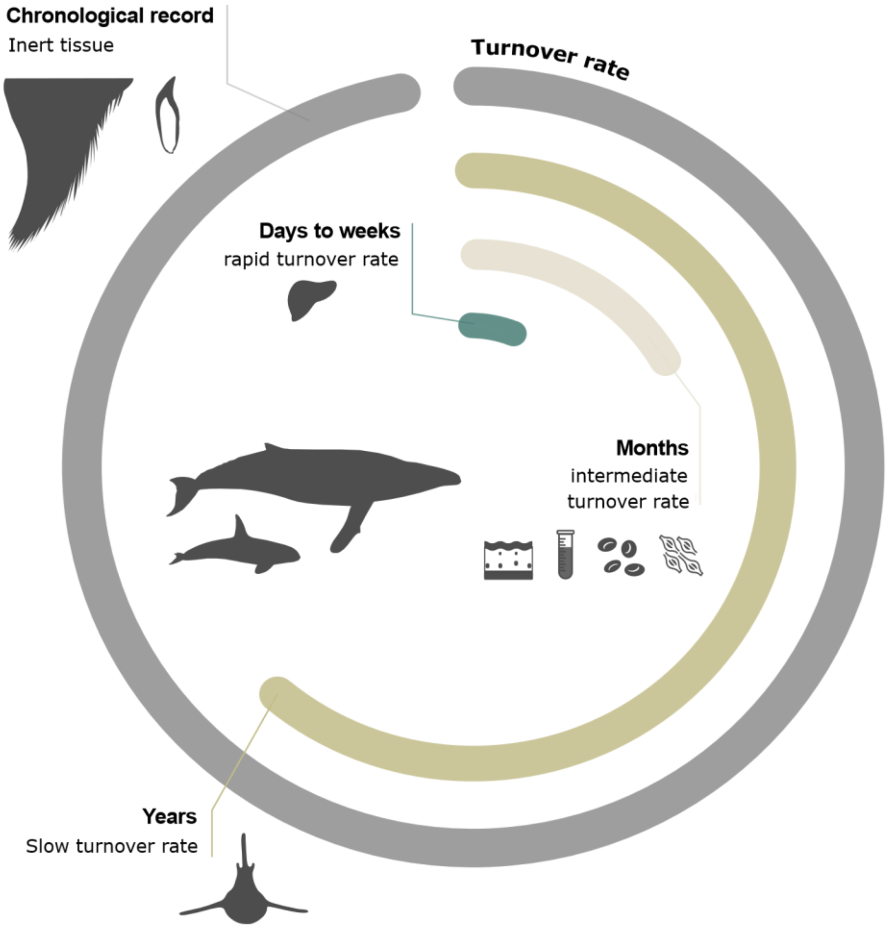


Figure 1. Metabolically active tissues (e.g., liver) have a rapid turnover rate and represent the assimilated diet spanning few days prior to sample collection, whereas less active tissues (e.g., red blood cells, blubber, muscle, skin) indicate dietary integration of several days to months (Caut et al., 2011; Giménez et al., 2016). Bone collagen exhibits a low turnover rate, and its isotopic composition reflects the integration of dietary inputs over the years (Dalerum & Angerbjörn, 2005). Metabolically inert tissues such as tooth enamel or baleen plates are not resorbed after deposition and represent chronological diet information (Koch, 2007). This variation in tissue types is especially useful for marine predators such as cetaceans, as a single low metabolic tissue (e.g., tooth dentin and baleen) sequentially subsampled or samples of different tissues from the same individual allows the assessment of resource use over different timescales, without having to re-sample the same individual on numerous events. These features can also provide dietary information from elusive stages within cetaceans` life history such as length of nursing/lactating period and weaning age.

TISSUE COLLECTION AND PRESERVATION

To preserve sample integrity and obtain reliable measurements of isotope values, potential risk of contamination as a result of handling should be minimized by adopting aseptic techniques during field work and storing biological tissues in sterile containers (e.g., scintillation vials, Eppendorf tubes, vacutainers, whirl pack or grip seal bags) (Jardine et al., 2003). Scientists have developed nonlethal methods for the collection of tissues that can be taken from cetaceans either when they surface to breathe (e.g., skin and blubber) (Noren and Mocklin, 2012) or from animals that are captured and released (e.g., blood) (Norman et al., 2004; Fair, 2006). Although they represent important techniques for obtaining samples for SIA (e.g., Hooker et al., 2001; Bryan et al., 2007; Woshner et al., 2008; Kiszka et al., 2010; Lian et al., 2020), remote biopsies and capture of cetaceans are logistically challenging. Permits and ethical issues should be considered, and the information obtained must counterbalance the potential harm and stress posed to individuals.

Tissues sampled from animals stranded alive (Code 1; Pugliares, 2007), carcasses washed ashore or archived in research collections or museums can also be used for SIA. However, the viability of postmortem tissues will depend on the carcass decomposition condition based on the length of time since death and the ambient temperature of postmortem exposure. Extended exposure and degradation, especially in warm and humid environments, can lead to isotopic enrichment (associated with bacterial metabolism of lighter isotopes) and to reductions in the percent of carbon and nitrogen (Kiljunen et al., 2006). Different tissues (e.g., organs, tooth, baleen plates, bone, skin, blubber, and muscle) can be obtained from well-preserved and moderately decomposed carcasses (Codes 2–3, i.e., little scavenger damage, fresh to mild odor, blubber firm to slightly oily, wrinkled to cracked skin with little to no abrasion, intact organs; Pugliares et al., 2007). In counterpart, the volatile carbon and nitrogen compounds (e.g., carbon dioxide, methane, and ammonia) formed during decomposition decay stages (Stuart, 2013; Keenan & Debruyn, 2019) of advanced decomposed or mummified carcasses (Codes 4-5, i.e., severe scavenger damage, strong odor, blubber and muscle easily falling from bones; liquefied internal organs; Pugliares et al., 2007) likely compromise isotopic measurements of soft tissues, limiting sample options to mineralized and keratinous tissues.

Field samples may require some degree of cleaning to remove any external contamination. As mineralized (tooth and bone) and keratinous (baleen plates) tissues do not easily decompose, dirt or adherent material can be easily removed by cleaning the external part with a solution of 2:1

chloroform:methanol while soft tissues can be rinsed with deionized water. Mineralized (e.g., bone and tooth) and keratinous (e.g., baleen plates) tissues can often be stored under dry conditions, whereas tissues with greater water content (e.g., blood, liver and muscle) are highly susceptible to degradation and must be wrapped in aluminium foil, preserved inside a labeled plastic bag, and dried or frozen at −20°C immediately after collection to avoid potential degradation effects (i.e., decay and mold growth) (Barrow et al., 2008). Freezing, freeze-drying, and oven-drying are the most recommended storage technique due to the minor alteration on isotopic values (Sweeting et al., 2004; Barrow et al., 2008). Whole blood should be collected with neither additives or in heparinized vacuumed tubes to prevent clotting and ensure latter separation of its components (e.g., red blood cells and plasma) (Hobson et al., 1997; Kurle, 2002).

When field collection of biological tissues is not possible, researchers should be aware that archived tissues of cetacean species are usually chemically preserved in formalin, dimethyl sulfoxide (DMSO) buffer, 70% ethanol (EtOH), and formalin-EtOH, that can result in major or minor alterations in $\delta^{13}C$ and $\delta^{15}N$ values (Sweeting et al., 2004; Kiszka et al., 2014). Although the possibility of remotion of these preservative methods remains not fully understood, previous studies have shown that DMSO effect on isotope values of cetacean tissues can be removed with deionized water (Marcoux et al., 2007; Lesage et al., 2010; Foote et al. 2012) or via lipid-extraction methods (see *Lipid Extraction* below) (Lesage et al., 2010; Burrows et al., 2014; Busquets-Vass et al., 2017; Newsome et al., 2018).

TISSUE SAMPLING METHODS

Recent advances in mass spectrometry have resulted in less sample size required for SIA (Michener and Schell, 1994). The exact amount of material depends on the concentration of carbon and nitrogen in the sample, and the sensitivity of the mass spectrometer used for the analysis. Given that some tissue can be lost during preparation, it is ideal to obtain enough sample to perform at least two rounds of analyses.

Whole blood can be directly placed in vials to be dried or freeze-dried directly, or separated into red blood cells (RBC) and plasma components by centrifuge at high velocity for ~5 min. Plasma must be transferred with a pipette to pre-combusted glass fiber filter papers to be decanted (O`Hara et al., 2018), and both components should be dried or freeze dried (Barrow et al., 2008). Skin, blubber, muscle, and liver should be washed with deionized water and directly placed in petri dishes to be dried or vials to be freeze-dried (see *Sample preparation* below).

Whole tooth or bone fragments can be sampled to obtain long-term averaged dietary information. The researcher should first check with the laboratory where samples will be analyzed whether the sample needs to be pulverized or sent in small fragments. Alternatively, chronological life-time information can be acquired by sampling subsets of the GLGs in tooth of odontocetes species or along the surface of baleen plates of mysticetes species at fixed intervals. Tooth must be air-dried and sectioned longitudinally along the buccolingual plane using a water-cooled diamond-bladed saw (Buehler IsoMet®). To optimize the identification of the GLGs, the tooth longitudinal sections can be polished with successive sheets of sandpaper with decreasing grit sizes (e.g., 340, 500, 600, 1200), and the inner surface of each tooth immersed in 25% formic acid (amount of time will vary according to tooth size), rinsed with deionized water and air-dried for 24h or more (Newsome et al., 2006). Dentinal GLGs of odontocetes can be extracted using two methods, i.e., by a micromilling computer-guided system or by manually drilling the layers (Fig. 2). Species with relatively large teeth (e.g., sperm whale, killer whale) can be subsampled using a micro dental drill bit coupled to an abrasive rotatory tool (Dremel®) or with a computer-guided micromilling system (i.e., New Wave™ Research) fitted with a carbide drill bit (e.g., Newsome et al., 2009). Previous studies on delphinid species with smaller teeth manually drilled the entire tooth of individuals from different age groups (e.g., Niño-Torres et al., 2006), the outer and inner dentin to represent early and later life stages, respectively (e.g., Knoff et al., 2008) or combining the older GLGs (Pereira et al., 2020). For subsampling baleen plates**,** keratin powder is sequentially drilled along the L-axis from the proximal part (below the gum line) of the baleen plate to the most distal, i.e., the oldest part of the plate. The distance between sample points depends on the baleen species and the intended temporal resolution (e.g., seasonal or annual) according to the research question, as baleen growth rates vary among species (e.g., Schell et al., 1989a; Mitani et al., 2006; Bentaleb et al., 2011; Aguilar et al., 2014) and may also depend on the age of the individual (Schell et al. 1989b; Best and Schell 1996).

Figure 2. Denting Growth layer groups (GLGs) of the false killer whale (*Pseudorca crassidens*) manually drilled with a micro dental drill bit coupled to an abrasive rotatory tool (Photo: Liane Dias/ EcoMega-IO-FURG).

SAMPLE PREPARATION FOR SIA

Drying and homogenization

Samples must be sufficiently dried to reduce tissue breakdown (changing the ratios of the heavy to light isotopes). Given that high temperatures (e.g., >60°C) can lead to volatilization of the compounds enriched in lighter stable isotopes (De Lecea, Smit, & Fennessy, 2011), samples should be oven-dried at moderate temperatures (50-60°C) for varying periods of time (hours to days) or freeze-dried (Fig. 3) (Jardine et al., 2003; Post et al., 2007). There are no standard protocols when it comes to the decision on whether tissues should be pulverized and homogenized prior to SIA Thus, the researcher should first check with the laboratory for instructions on whether tissue powder or fragments are required. When tissues require homogenization (e.g., , due to the variation in the amino acid or lipid composition of different layers, that can lead to differences in the isotopic values) samples must be pulverized into a fine powder using a mortar and pestle or a ball-mill grinder can ensure isotopic homogeneity and complete combustion. Homogenization is not needed when using powder of metabolically inert tissues (e.g., baleen plates and tooth).

Acidification of mineralized tissues

Bone and tooth dentin are comprised of collagen and hydroxyapatite. Collagen is an organic matrix assimilated from carbon and nitrogen contained in the protein constituents of a consumer's diet. Bioapatite-based carbon is synthesized from a consumer's whole diet (i.e., protein, carbohydrates, and lipids). Thus, collagen and bioapatite experience differential isotopic fractionation and routing of dietary components during the biosynthesis of the tissue and their $\delta^{13}C$ and $\delta^{15}N$ values reflect different portions of a consumer's diet. For the best isotopic data interpretation of these tissues samples, bone and tooth collagen is usually isolated with hydrochloric acid (HCl) (Fig. 3) (DeNiro and Epstein, 1978; Hobson and Welch, 1992). An assumption of this technique is that acids dissolve carbon that forms part of inorganic, non-dietary carbonates associated with organic matrices.

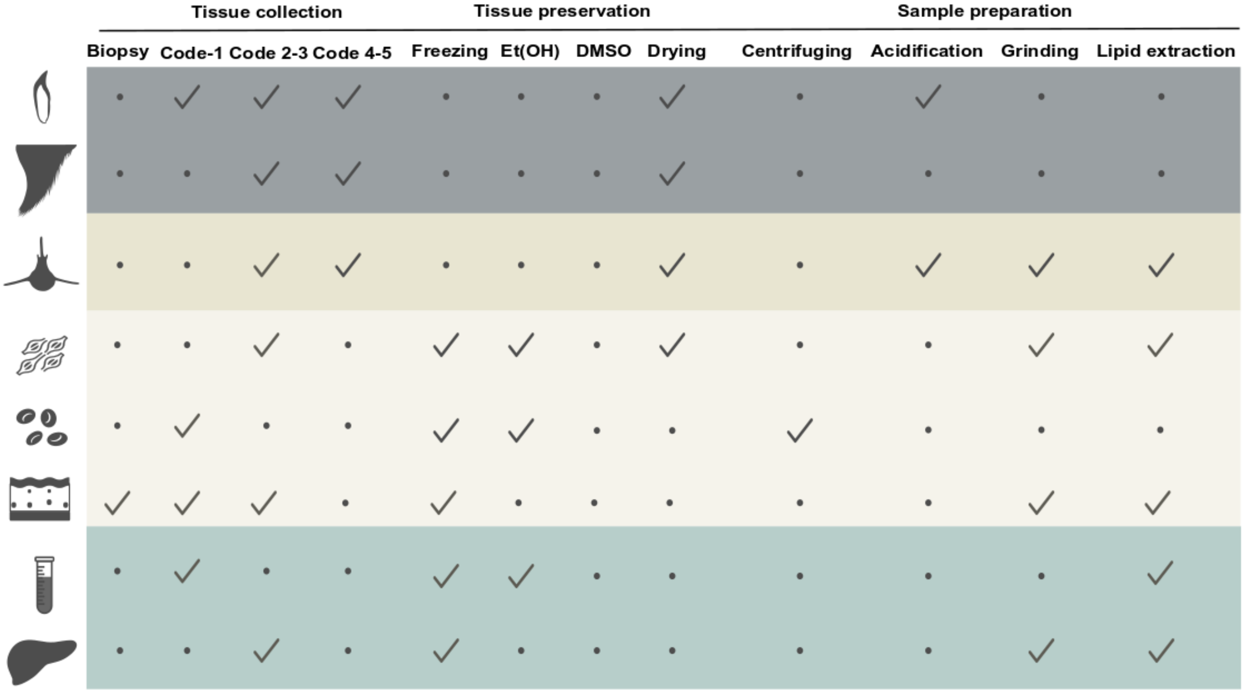
Samples of bone and tooth must be soaked in HCl (0.1 to 0.3 N) at room temperature for ~24 h. This process should be repeated if the acid is still bubbling and samples are still hard by replacing the acid solution every 24 h until bubbling cease (Nelson et al. 2018). Once the samples are flexible, they are completely decalcified. After carefully removing the HCl using a pipette, samples should be washed with successive rinses of deionized water and freeze-dried for +12h. This treatment eliminates traces of inorganic and organic carbon, where the supernatant containing the contaminants is discarded, the residue containing the protein is rinsed to neutrality and the resulting material is collagen (Ambrose, 1990). It remains unclear, however, whether this acid-treatment affects isotopic values from tissues. The simultaneous measurement of $\delta^{13}C$ and $\delta^{15}N$ to estimate trophic position can be problematic given that the acid-treatment used to remove inorganic carbon can also affects $\delta^{15}N$ values (Choy et al., 2016). One alternative is to split tissues into acid-treated and untreated sub-samples for separate analyses of $\delta^{13}C$ and $\delta^{15}N$.

Nevertheless, by directly comparing results of raw and decalcified dentin of odontocete species, Brault et al. (2014) and Groom (2018) found no significant differences in the $\delta^{13}C$ and $\delta^{15}N$ values, although C:N ratios slightly differed between treatments. These results suggest that decalcification is not necessary when measuring bulk stable isotopes in dentin of modern odontocetes. The authors attributed their results to potentially low concentration of lipids and carbonates, and to the similarity in $\delta^{13}C$ values of dentin proteins and carbonates (Brault et al., 2014). However, the application of acid-treatment to fossil dentin and modern bone is still recommended (Brault et al., 2014).

Lipid extraction

Lipids are depleted in ^{13}C (i.e., lower $\delta^{13}C$) compared to other biochemical compounds (e.g., proteins or carbohydrates), due to kinetic-isotope effects during the conversion of pyruvate to acetyl coenzymeA in lipid synthesis (DeNiro and Epstein, 1977). This depletion in lipid content can decrease $^{13}C/^{12}C$ in bulk tissues and their respective $\delta^{13}C$ values, which can be wrongly interpreted as dietary or habitat shifts (Logan et al., 2008). Consequently, chemical procedures to correct the influence of lipids on ^{13}C values prior to stable isotope analysis have become common practice. Given that synthesis and storage of lipids vary among tissues types, tissues with high lipid content such as liver, muscle and plasma should be lipid extracted prior to SIA, while the lower lipid proportion in tissues such as dentin, baleen plates and red blood cells do not require lipid extraction (Newsome et al., 2010). One of the most common used in lipid extraction is an adaptation of the Bligh and Dyer's (1959) method, in which lipids are reduced through repeated rinses of chloroform:methanol or petroleum ether solvents sonicated in a fume hood at ambient temperature and rinsed for 12–24 h at higher temperatures using a Soxhlet apparatus or other extraction devices. The amount of time of exposure to solvents will vary according to the lipid content of the tissue being analyzed. The C:N ratios are frequently used as a proxy for determining whether samples have been adequately lipid extracted. Lesage et al. (2010) and Kiszka et al. (2014) suggest that C:N ratio is an efficient lipid removal indicator for cetacean skin when values are <4. Yet, studies have shown that the C:N ratio is not a reliable indicator of lipid content for some cetacean species and tissues (Wilson et al., 2014; Yurkowski et al., 2014). For example, Yurkowski et al. 2014 showed that the C:N ratio was positively correlated with lipid content in liver but negatively correlated with lipid content in muscle tissues of Arctic marine mammal species (e.g., beluga and narwhal).

The use of organic solvents to remove simple lipids may also lead to changes in tissue $\delta^{15}N$ values due to the removal of amino acids (Kiljunen et al., 2006; Sweeting et al., 2006; Logan et al., 2008; Lesage et al., 2010; Choy et al., 2016). This effect varies between –0.15% and +0.30% in cetaceans depending on the taxonomic group (Lesage et al., 2010). An alternative to the $\delta^{15}N$ bias induced by lipid chemical extraction is to analyze duplicate samples (one aliquot lipid extracted, one nonextracted) for a proper estimation of $\delta^{13}C$ and $\delta^{15}N$ values (Logan et al., 2008; Ryan et al., 2012). This duplication of samples is ideal but can also be problematic given the high costs associated and by the fact that some cetaceans' studies rely on small amount of tissues obtained through remoted biopsies. An alternative is to double analyze only a fraction of the total samples and carry out a posterior mathematic correction for the influence of lipids on the $\delta^{13}C$ values of untreated samples (see *Mathematic lipid correction* below).



	Tissue collection				Tissue preservation				Sample preparation			
	Biopsy	Code-1	Code 2-3	Code 4-5	Freezing	Et(OH)	DMSO	Drying	Centrifuging	Acidification	Grinding	Lipid extraction
	•	✓	✓	✓	•	•	•	✓	•	✓	•	•
	•	•	✓	✓	•	•	•	✓	•	•	•	•
	•	•	✓	✓	•	•	•	✓	•	✓	✓	✓
	•	•	✓	•	✓	✓	•	✓	•	•	✓	✓
	•	✓	•	•	✓	✓	•	•	✓	•	•	•
	✓	✓	✓	•	✓	•	•	•	•	•	✓	✓
	•	✓	•	•	✓	✓	•	•	•	•	•	✓
	•	•	✓	•	✓	•	•	•	•	•	✓	✓

Figure 3. Collection of cetacean tissues depending on the carcass decomposition condition (Code 1: animal stranded alive; Code 2-3: well-preserved and moderately decomposed carcasses; Code 4-5: advanced decomposed or mummified carcasses) with specific preservation and preparation methods (in order from top to bottom: tooth, baleen plates, bone, muscle, red blood cells, skin, blubber, plasma, and liver).

Prey samples

Mathematical mixing models are valuable tools to infer the relative contribution of different prey items to consumers' diet. Samples of potential prey sources assessed via stomach contents or visual observation should be obtained from the same region where the animal occurs. Because there could be ontogenetic related changes in the diet of potential prey, consequently affecting their $\delta^{13}C$ and $\delta^{15}N$ (e.g., Nagata et al., 2015), prey samples need to encompass the same length range taken by consumers. Prey samples should also follow the same sampling, cleaning protocols as cetacean species', and stored at -20ºC until preparation for SIA. Potential prey items may include invertebrates (whole body of single or multiple individuals) and tissues of fish, squid and/or even marine birds and mammals. Whole body (in the case of invertebrates) or small amounts of tissue should be washed with deionized water to remove any external contaminants, placed in Petri dishes and dried at 50-60ºC for 12-48h or freeze-dried. One should investigate the need and effectiveness of carrying out acidification to remove carbonates (usually required for invertebrates with calcareous structures, Carabel et al., 2006) and chemical lipid extraction or normalization (see *mathematic lipid correction below*). Giménez et al. (2017a) recommend the use of species-specific linear models to normalize the isotopic composition of prey sources before dietary quantifications through mixing models of specialist consumers and general linear models when dealing with generalist consumers.

Sample submission to the laboratory facility

Samples should be placed into tin (Sn) or silver (Ag) capsules. Tin is an important combustion catalyst and is usually used for $\delta^{13}C$ and $\delta^{15}N$ analyses of tissues, while silver capsules are often used for acid fumed samples because tin decomposes rapidly when exposed to acid (Bosley and Wainright, 1999). Capsules should be weighed using a micro-analytical balance with a readability of 3 decimal places on a milligram (i.e., 0.001mg). If a micro-analytical balance with this readability is not available, some precision may be lost when measuring %C and %N because these values are calculated using the recorded weight of the sample. One possible negative outcome of these weighing errors is the presence of isotopic outliers that can be avoided by sending prepared samples in labelled Eppendorf tubes or glass/plastic vials to be processed and/or weighed at the same laboratory facility that will conduct the analysis for an extra-cost. Weighing pulverized or small pieces of sample tissue can be tricky, especially for tissue powders that are strongly affected by static electricity. Ensure that the microbalance is clean and calibrated, and there is no air flow (e.g., air conditioning fan, open doors) when taring and weighing.

Before weighing out the sample, tare the balance to exclude the initial mass of the pre-weighted capsule. Using sterilized latex gloves during the entire process, carefully handle the capsules with tweezers and move them from the microbalance to a clean metal surface. Sample amount to be loaded into tin capsules is determined by the type of isotopic element needed (i.e., C, N) and the instrumental precision of the laboratory. Thus, before sending samples for analysis is essential to ask about the laboratory`s analytical precision to ensure readability and precision are compatible with the isotope laboratory requirements. Using a spatula or tweezers, transfer a small amount of tissue sample into the capsule. Reweigh capsules containing the sample and continue adding or removing biological material until the target sample weight is obtained. Record the final sample weight and ID in a spreadsheet.

Once the sample has been loaded, tightly seal the capsules by crimping the open end and gently folding in the edges into spherical or cylindrical shape using a pair of clean forceps with blunt tips. Ensure that there are no holes in the capsule that can result in sample leak, and that there are no stray edges of the capsule protruding that can become stuck in the autosampler clogging the equipment. To avoid cross-contamination among samples, always thoroughly wipe supplies and equipment using tissues with a solvent (e.g., ethanol) between samples. Organize and group samples of similar material together in a 96-well untreated polystyrene culture plate to be shipped together with a specific sample submission form with unique sample ID's and weights required by the laboratory (generally available on their website). To secure loaded trays and prevent samples from moving during shipment, close the gap between the 96-well plate and cover, trim an index card(s) between the top lid and bottom tray and secure preferably with elastic bands.

SAMPLE ANALYSIS IN ISOTOPE MASS SPECTROMETRY INSTRUMENTATION

The isotope-ratio mass spectrometry system (IRMS) is usually coupled to an elemental analyzer where $\delta^{13}C$ and $\delta^{15}N$ data can be obtained simultaneously. First, organic tissues wrapped in capsules are converted to pure gases (CO_2, N_2) by using a helium diluter (an inert gas used to carry the sample through the system) prior to transfer into the isotope-ratio mass spectrometer (Jardine et al., 2003; Sulzman, 2007). Samples are focused into the ion source to reach the high precision required to detect natural abundance isotope variations (Kelly et al., 2018). Because the ratio of heavy to light isotope tends to be very small, isotopic abundance is conveniently reported as parts per thousand or per mil (‰), expressed in delta notation (δ) and obtained using the equation:

$$\delta X = [\mathrm{Rsample} \,/\, \mathrm{Rstandard} - 1] * 1000$$

where X is the heavier isotope (e.g., ^{13}C), R_{sample} / $R_{standard}$ is the ratio of the heavy to light isotope (e.g., $^{13}C/^{12}C$) in the sample relative to an internationally accepted standard (Peterson and Fry, 1987). For carbon, the international standard is the Pee dee Belemnite (PDB) marine fossil limestone formation from South Carolina (Craig, 1957), whereas the standard for nitrogen is atmospheric nitrogen (Ehleringer and Rundel, 1989). As international standards are expensive and not always commercially available, each laboratory has internal standards, i.e., compounds with similar physical and chemical features to that of the sample, which are compared against the international standard. A positive δ value means the sample has more of the heavier isotope than the standard, whereas a negative value indicates the sample has less of the heavy isotope than the standard.

DATA ANALYSIS

Raw data interpretation

When results come back from the isotope laboratory, check if the isotopic and elemental data for the analytical standards are consistent throughout the run, and if $\delta^{13}C$, $\delta^{15}N$ and C:N ratios are within the range expected for the system and tissue type. If these conditions are not met, samples should be reanalyzed. Data discrepancy can occur due to sample contamination during collection, preparation, preservation steps, and/or did not run properly during the analysis. If the amount of a sample is too low to repeat analysis, consider excluding the biased data from statistical analyses.

Suess effect correction

Atmospheric CO_2 $\delta^{13}C$ has been declining in the last 200 years, largely due to the release of low $\delta^{13}C$-CO_2 by increased burning of fossil fuel and deforestation (Francey et al., 1999; Gruber et al., 1999; Indermühle et al., 1999; Verburg, 2007). These changes in atmospheric CO_2 $\delta^{13}C$ resulting from long-term anthropogenic influences is referred to as the Suess effect (Keeling, 1979) and needs to be accounted for when interpreting changes in $\delta^{13}C$ in species and their ecosystem. For this reason, studies focusing on historical samples have applied a correction factor of 0.02‰ year, an average of mean values reported by Kortzinger and Quay (2003) and Sonnerup et al. (1999), to samples' $\delta^{13}C$ to account for such a decrease and allow comparison among samples from different time periods (e.g., Riccialdelli et al., 2013; Loizaga de Castro et al., 2016; Teixeira et al., 2020) .

Mathematic lipid correction

When samples have not been lipid extracted, $\delta^{13}C$ values can be *a posteriori* corrected for lipid content by applying mathematical corrections or normalizations (Sweeting et al., 2006; Logan et al., 2008). Despite the time and cost-saving provided by mathematical corrections, they should be based on empiric experiments to obtain more accurate species and tissue-specific models (Logan et al., 2008; Lesage et al., 2010; Yurkowski et al., 2014, Gimenez et al., 2017). Linear and non-linear mathematical models for lipid correction have been fitted for whole body aquatic invertebrates and vertebrates (McConnaughey and McRoy, 1979; Fry et al., 2003; Post et al., 2007), thus researchers should estimate the species- and tissue-specific coefficients to apply to the most appropriate equation. Ideally, a significant number of samples for each species being studied should be double-analyzed, allowing the development of each species' tissue-specific coefficients for the equation that would then be applied to all data for lipid correction. Table 1 presents a compilation of cetacean species for which tissue-specific parameters for mathematical lipid correction have been estimated.

The non-linear model proposed by McConnaughey and McRoy (1979) requires the initial estimation of the lipid content (L) using the carbon-to-nitrogen ratios of the untreated sample ($C{:}N_{Bulk}$):

$$L = \frac{93}{1 + \left((0.246 \times C{:}N_{Bulk}) - 0.775\right)^{-1}}$$

Subsequently, one can apply the estimated L to correct $\delta^{13}C$ values:

$$\delta^{13}C_{Corrected} = \delta^{13}C_{Bulk} + D \times \left[I + \frac{3.9}{\left(1 + \frac{287}{L}\right)} \right]$$

Where D defines the curve slope, indicating the isotopic difference (in ‰) between pure lipid and pure protein. Thus, in other words, $D = \delta^{13}C_{Lipid}$. The equation also includes the constant I.

The mass-balance model developed by Fry et al. (2003) estimates lipid-free $\delta^{13}C$ using C:N ratios from both bulk and lipid-extracted ($C{:}N_{LE}$) samples, as well as D:

$$\delta^{13}C_{Corrected} = \frac{(\delta^{13}C_{Bulk} \times C{:}N_{Bulk}) + [D(C{:}N_{Bulk} - C{:}N_{LE})]}{C{:}N_{Bulk}}$$

The model for mathematical correction proposed by Post et al. (2007) assumes a linear relationship between $C{:}N_{Bulk}$ and $\Delta\delta^{13}C$; the latter is the difference between $\delta^{13}C$ values of chemically treated and non-treated samples. The parameters α (the intercept, $\delta^{13}C$ when C:N equals zero) and β (the slope, the rate of changes in $\delta^{13}C$ for each unit change in C:N) are estimated from a linear regression between $C{:}N_{Bulk}$ and $\Delta\delta^{13}C$, and are then applied in the following equation to estimate lipid-free $\delta^{13}C$:

$$\delta^{13}C_{Corrected} = \delta^{13}C_{Bulk} + (\alpha + \beta \times C{:}N_{Bulk})$$

Tissue- and species-specific mathematical lipid correction is a method frequently used to estimate lipid-free $\delta^{13}C$ values. Nevertheless, as these models assume that C:N ratios are indicative of lipid content, and thus estimate changes in $\delta^{13}C$ based on changes in C:N, potential non-linearity between them may hinder the possibility of such arithmetical corrections (e.g., Wilson et al., 2014). Thus, it is noteworthy emphasizing that, when feasible, duplicate analyses should be carried out, with lipid-extracted samples to obtain $\delta^{13}C$ values and non-treated samples for $\delta^{15}N$ values (Ryan et al., 2012; Cloyed et al., 2020).

Table 1. Cetacean species for which tissue-specific coefficients for mathematical lipid correction have been estimated by analyzing lipid-extracted and non-treated subsets of individual samples.

Species	**Tissue type**	**Reference**
Beaked whales*	Bone	Tatsch et al., 2016
Beluga *Delphinapterus leucas*	Skin, muscle, liver	Lesage et al., 2010; Choy et al., 2016
Bottlenose dolphin *Tursiops truncatus*	Skin	Giménez et al., 2016
Bowhead whale *Balaena mysticetus*	Skin	Lesage et al., 2010
Fin whale *Balaenoptera physalus*	Skin	Ryan et al., 2012
Harbor porpoise *Phocoena phocoena*	Skin	Lesage et al., 2010
Humpback whale *Megaptera novaeangliae*	Skin, blubber	Ryan et al., 2012
Minke whale *Balaenoptera acutorostrata*	Blubber	Ryan et al., 2012
Common dolphin *Delphinus delphis*	Skin	Peters et al., 2020

*data from five species (Cuvier's *Ziphius cavirostris*, Arnoux's *Berardius arnuxii*, Blainville's *Mesoplodon densirostris*, Gray's *M. grayi*, Layard's *M. layardii*, and Hector's *M. hectori*) of beaked whales pooled together to estimate the equation parameters.

Exploratory analysis and hypothesis testing

Stable isotope biplots, also referred to as niche space or isotopic niche, are the most commonly used approach for presenting stable isotope data, while graphical (e.g., scatter plots, error bars) and basic statistical methods provide the framework for interpreting raw isotope data. Mean $\delta^{13}C$ and

$\delta^{15}N$ values between samples can be evaluated using univariate tests, or multivariate models. For example, the parametric ANOVA has been used to assess temporal (Secchi et al., 2016) and ontogenetic dietary shifts in bottlenose dolphins (Knoff et al., 2008) and Commerson's dolphins (Riccialdelli et al., 2013), dietary variation between groups and acoustic clans of sperm whale (Marcoux et al., 2007), as well as potential seasonal dietary shifts and the effect of different areas on isotopic values of short-beaked common dolphin' skin (Loizaga de Castro et al., 2016). The non-parametric Kruskal-Wallis has been used to assess resource use and partitioning among sympatric dolphin species around Mayotte Island (Gross et al., 2009; Kiszka et al., 2011), Morea Island (French Polinesia) (Kiszka et al., 2010), and in Northwest Iberian Peninsula waters (Mendez-Fernandez et al., 2012). Alternatively, generalized linear model (GLM) has been used to assess foraging patterns among narwhal populations (Watt et al., 2013) and trophic relationships in sympatric dolphin species (Teixeira et al., 2020), whereas fitted generalized additive models (GAMs) has been used to assess the temporal, ontogenetic and sex-related variation in the diet of the franciscana dolphin (Troina et al., 2016), and common dolphins (Peters et al., 2020).

Table 2. Summary exemplifying some studies with different tissues` types and the ecological application with cetacean species

Species	**Type of tissue**	**Application**	**Reference**
Dolphins community	Teeth and bone	Trophic interactions	e.g., Costa et al., 2020
Franciscana dolphin *Pontoporia blainvillei*	Liver and muscle	Trophic interactions	e.g., Di Beneditto et al., 2013
Bottlenose dolphin ecotypes *Tursiops truncatus* and Sperm whale *Physeter macrocephalus*	Skin	Trophic interactions	e.g., Díaz-Gamboa et al., 2017
Bottlenose dolphin *Tursiops truncatus*	Teeth	Temporal and sexual variation in diet	e.g., Secchi et al., 2017
Franciscana dolphin *Pontoporia blainvillei*	Teeth	Ontogenetic and sexual variation in diet	e.g., Troina et al., 2016
Short-beaked common dolphins *Delphinus delphis delphis*	Skin	Stock origin of stranded animals	e.g., Elorriaga-Verplancken et al., 2020
Blue whale *Balaenoptera musculus*	Skin	Seasonal foraging and migration pattern	e.g., Busquets-Vass et al., 2020
Fin whale *Balaenoptera physalus*	Baleen plate	Seasonal foraging and migration pattern	e.g., Vighi et al., 2019
Narwhal *Monodon monoceros*	Skin	Geographic variation in diet	e.g., Watt et al., 2013
Indo-Pacific bottlenose dolphins *Tursiops aduncus*	Skin and blubber	Feeding ecology (prey preferences)	e.g., Kiszka et al., 2014
Beluga whales *Delphinapterus leucas*	Teeth	Individual diet specialization	e.g., Yurkowski et al., 2016

Sperm whales *Physeter macrocephalus*	Teeth	Habitat use	e.g., Zupcic-Moore et al., 2017
Bottlenose dolphin *Tursiops truncatus*	Blood and skin	Trophic position	e.g., Woshner et al., 2008
Bottlenose dolphin *Tursiops truncatus*	Teeth	Diet of modern and historic populations	e.g., Walker et al., 1999

Populational trophic structure, habitat use and interspecific interactions

The isotopic niche (Bearhop, Adams, Waldron, Fuller, & MacLeod, 2004; Newsome, Martínez del Rio, Bearhop, & Phillips, 2007) can be delineated in a δ-space in which $\delta^{13}C$ vs. $\delta^{15}N$ are the environmental axes that provide information on the bionomic and scenopoetic components of the ecological niche (Newsome et al. 2007). Isotopic niches can be quantified using metrics (Jackson et al., 2011; Layman et al., 2007) that allow for the assessment of trophic interactions and niche overlap between different groups, populations, or species. Quantitative tools such as Layman`s community-wide metrics of trophic structure can be used to investigate patterns in food web structure and niche with based on mean positions in niche space (Layman et al., 2007). The $\delta^{13}C$ and $\delta^{15}N$ range (CR and NR, respectively), Total area (TA), Mean distance to centroid (CD), Mean nearest neighbor distance (NND), Standard deviation of nearest neighbor distance (SDNND) metrics can be calculated using the package Stable Isotope Analysis in R (SIAR; Parnell et al., 2008; Parnell, Inger, Bearhop, & Jackson, 2010). However, the use of Euclidean methods such as Layman metrics to define the isotopic niche space of a species in a community (Layman et al., 2007) do not account for uncertainty associated with sampling and can be sensitive to sample size (Jackson et al., 2011). Isotopic niche breadth and overlap between cetacean species can be assessed using standard area ellipses estimated by Bayesian inference that incorporates uncertainties such as sampling biases and small sample sizes into niche metrics (Jackson et al., 2011). The package SIBER (Stable isotope Bayesian ellipses in R, Table 3) employs Markov-Chain Monte Carlo simulations to construct ellipses that represent the isotopic niche width (or isotopic niche space) and are produced by estimating the covariance matrix of $\delta^{13}C$ and $\delta^{15}N$, which is the equivalent to the standard deviation for univariate data (Jackson et al., 2011). The trophic overlap can be calculated by estimating the area of the isotopic niche space that intersects between different groups or species (Jackson et al., 2011).

This approach has been applied to assess the trophic relationships, habitat, and resource use of fin whale populations (Ryan et al., 2013; Das et al., 2017), common bottlenose dolphin ecotypes and sperm whales (Díaz-Gamboa et al., 2018), sympatric transient and resident populations of bottlenose dolphin (Santos Carvallo et al., 2015), sympatric coastal (Young et al., 2017; Teixeira et al., 2020) and oceanic dolphin populations (Troina et al., 2020), to name a few. SIBER has also been used to evaluate sexual (Loizaga De Castro et al., 2016), ontogenetic (Pereira et al., 2020) and temporal (Secchi et al., 2016) changes in cetaceans' diet and ecological niches. Additionally, both Layman metrics and SIBER approaches can be combined to help evaluate the trophic diversity and niche partitioning among populations (e.g., Riccialdelli et al., 2017; Brotons et al., 2019) and species (Browning et al., 2014; Giménez et al., 2017b; Troina et al., in press). An alternative approach to estimate isotopic niche area and overlap is the kernel density method (Eckrich, Albeke, Flaherty, Bowyer, & Ben-David, 2020), offering more realistic estimates when data are irregularly distributed within the bivariate space. To our knowledge, this method has not been applied in studies with cetaceans, but it is a promising novel tool.

Lastly, the R package tRophicPosition (Quezada-Romegialli et al., 2018, Table 3) incorporates a Bayesian approach to estimate the trophic position of consumers and allows the use of isotopic information of up to two baselines (e.g., benthic and pelagic primary producers). Knowing the trophic position occupied by cetacean species within their food webs is key to understanding their ecological role in that ecosystem.

Proportional contribution of sources to consumers' diet

Mass-balance mixing models use Bayesian framework to allow the estimation of the proportional contribution of different prey items to the consumers` overall diet based on the isotopic data of consumers (i.e., cetaceans) and potential dietary sources (prey) (Phillips and Gregg, 2003; Parnell et al., 2010). Using the packages MixSIAR (Moore and Semmens, 2008), SIMMR (Parnell, 2016), isotopic mixing models incorporate uncertainty for each parameter and specific diet-to-tissue discrimination factors, also called trophic enrichment factors (i.e., TEFs) (Martínez del Rio et al., 2009, Table 3).

Isotopic mixing models have been applied to estimate the diet of several cetacean species, including baleen whales (e.g., Pomerleau et al., 2012; Witteveen et al., 2012; Ryan et al., 2014) and odontocetes (e.g., Monteiro et al., 2015; Secchi et al., 2016; Troina et al., 2016; Teixeira et al., 2020). The assessment of important prey species is key to quantifying interactions with fishing activities (e.g., Secchi et al., 2016; Wild et al., 2020), being therefore especially useful for conservation purposes. Additionally, isotopic mixing models coupled with isotopic niche metrics estimated with SIBER has helped the assessment of long-term patterns in feeding habits and trophic segregation among sympatric baleen whales, and to differentiate between specialist species and those that have changed prey dominance according to prey availability (e.g., Gavrilchuk et al., 2014).

Nevertheless, the reliability of dietary information through isotopic mixing models relies on several assumptions, such as the use of accurate diet-to-tissue discrimination factors. As models' outputs are sensitive to variations in TEFs (e.g., Caut et al., 2008), the use of inappropriate TEF values may lead to unrealistic dietary estimates. To quantify discrimination factors, consumers are held on a controlled, isotopically constant diet comprising the length of time required for a complete turnover of the tissue of interest. Thus only a few studies have been able to estimate TEFs for cetaceans (e.g., Caut et al., 2011; Browning et al., 2014; Giménez et al., 2016) and average values are usually adapted from closely related species available in the literature. In this case, dietary outputs based on isotopic mixing models using a single discrimination factor need to be considered with caution, as results may be misleading if TEF is incompatible with the target species or tissue analyzed (Caut et al., 2009, Table 3). An alternative to tackle this issue is to test different TEFs and report the range of dietary estimates provided by the different models. For instance, Troina et al. (2016) applied several TEFs for $\delta^{13}C$ and for $\delta^{15}N$ in different mixing models to estimate the proportional contribution of prey items to franciscana dolphins, with Bhattachayya's coefficient index to compare models' outputs. More recently, Bayesian inferences allow to estimate consumers' $\delta^{13}C$ and $\delta^{15}N$ TEFs for the different tissue types, based on their feeding ecology and phylogeny with the SIDER package in R (Healy et al., 2018).

Further, a critical assumption for applying isotopic mixing models is that the consumer's isotopic composition must be within the polygon bounding the isotopic composition of the dietary sources (Phillips & Gregg, 2003), and violations of this assumption can be tested using the 'point-in-polygon' approach (e.g., Benstead et al., 2006). The mixing polygon simulations (Mixing polygons package in R, Table 3) developed by Smith et al. (2013), generate polygons that satisfy the point-in-polygon assumption within a model using Monte Carlo iterations. This approach uses the isotopic distributions of the proposed dietary sources and the trophic enrichment factors to determine if the mixing model designed for cetaceans and their prey sources was appropriate (e.g., Giménez et al., 2017c; Varela et al., 2018; Borrell et al., 2020).

Intra and inter-individual variation in foraging preferences

Ecologists have long recognized that natural populations can be composed of ecologically heterogeneous individuals that feed on different subsets of the available resources (Bolnick et al., 2002; Bearhop et al., 2004). Given that the isotopic niche of a population can be influenced by the different foraging strategies of each consumer, an important step in evaluating population isotopic niche is to examine how differences within vs. between individuals contribute to the observed isotopic variability of that population. Individual specialization is known to be widespread across a diverse set of taxa (Bolnick et al., 2003) including cetaceans (e.g., Rossman et al., 2015; Guerra et al., 2020; Pereira et al., 2020) and refers to the use of a restricted portion of the possible range of available resources.

To assess the level of individual specialization in cetaceans using stable isotopes, tissues with different turnover time (thus reflecting different foraging events through time) or from metabolically inert and continually growing tissues (e.g., tooth or baleen plates) should be sampled. Subsequently, the average intra-individual variance ('within- individual component' of the niche, WIC) can be calculated and divided by the sum of the inter-individual variance ('between-individual component', BIC) and the intra-individual variance (WIC + BIC = TNW, the 'total niche width'). This index (WIC/TNW) varies between 0 and 1, and indicate complete or no individual specialization, respectively (Bolnick et al., 2003). This method has allowed to evaluate whether TNW in a population of beluga whales changed latitudinally in response to ecological opportunity (e.g., availability of prey), and to identify that WIC contributed more to TNC than BIC, indicating a generalist feeding behavior in the species (Yurkowski et al., 2016). Empirical values can be compared with a null model (randomisation of prey items between individuals) and can be implemented in the R package RInSp (Zaccarelli et al., 2013).

Table 3. Key software packages in R and required data to address specific ecological research questions with cetacean species using stable isotope analysis

Research Question	Model	Required data	Reference
Habitat use Interspecific interactions	Layman	Isotopic values of individuals from the focal populations or species	Layman et al., 2007
Habitat use Interspecific interactions niche width and niche overlap	SIBER	Isotopic values of individuals from the focal populations or species	Jackson et al., 2011
Trophic position	tRophicPosition	Isotopic value of consumers; Baseline isotopic values (e.g., primary consumer); Diet-to consumer trophic discrimination factor	Quezada-Romegialli et al., 2018
Intra and inter-individual variation in foraging preferences	RInsp	Multiple samples of each individual encompassing different life-time periods (e.g., different tissues with varying turnover time, dentin GLGs)	Zaccarelli et al., 2013
Proportional contribution of sources to consumers' diet	MixSIAR SIAR SIMMR Mixing polygons	Isotopic values of consumers Mean and standard deviation of isotopic values of sources Trophic discrimination factor	Moore & Semmens, 2008 Parnell et al., 2010 Smith et al., 2013 Parnell, 2016

CONCLUSIONS

The usage of SIA to the study of cetacean species has grown rapidly due to substantial technique advances that allow minimal sample size and rapid processing of a large number of samples. Although SIA provide valuable information on food web interactions and energy flow in various ecosystems, we should also recognize inherent caveats that must be considered when interpreting isotopic data such as the species-specific trophic discrimination factors, overlapping values among prey sources, and temporal or spatial variation in prey and baseline isotopic values that can lead to confounding interpretations of isotopic values of organisms in higher trophic level (Gannes, O'Brien & del Rio, 1997). To minimize such limitations and provide greater resolution on foraging ecology, migration patterns, and population structure of cetacean species, SIA can be coupled with other techniques biochemical tracers such as fatty acids (e.g., Hooker et al., 2001) and compound-specific isotope analysis of amino acids (CSIA-AA) (e.g., Matthews, Ruiz-Cooley, Pomerleau, & Ferguson, 2020).

Based on an overview of existing studies and procedures of SIA applied to cetacean species, we present a practical and accessible guide for the common steps encompassing SIA with best practices for sampling, storing and preparing the most commonly used biological tissues, with the available tools for the treatment and statistical analysis of isotopic data. We also provide a synthesis of the approaches and considerations that are common to most studies (see Box 1). We believe that this guide can yield methodological consistency of SIA in cetacean species and hence, facilitate interlaboratorial comparisons.

Box 1. Basic steps to conduct SIA with cetacean species

- Review the literature to understand the fundamental principles of stable isotopes biochemistry and its application to the focal organism of interest
- Choose the isotope element (C, N, S) and other complementary data sources that are best suitable to answer your ecological question
- Choose the proper biological tissue based on their availability, growth rate, trophic discrimination, and turnover rate
- Contact the laboratory facility to obtain information about the instrumental precision of the mass spectrometer and sample submission instructions (i.e., sample size)
- Apply specific methods of sampling, preservation, and preparation according to the biological tissue used for SIA (e.g., acidification, lipid extraction, centrifugation)
- Check if raw isotopic data are within the range expected for the tissue and the system, evaluating possible discrepancies
- Choose methods for data analysis according to the objective of the study, the structure of the data and the number of variables.

REFERENCES

Aguilar, A., Giménez, J., Gómez–Campos, E., Cardona, L., Borrell, A. 2014. $\delta^{15}N$ value does not reflect fasting in Mysticetes. PLoS ONE, 9, e92288.

Ambrose, S.H., 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. J. Archaeol. Sci. 17, 4311–451.

Ambrose, S.T., Froneman, P.W., Smale, M.J., Cliff, G., Plön, S., 2013. Winter diet shift of long-beaked common dolphins (*Delphinus capensis*) feeding in the sardine run off KwaZulu-Natal, South Africa. Mar. Biol. 160, 15431–1561.

Bannister, J.L. 2018. Baleen whales (Mysticeti), in: Wursig, B., Thewissen, J.G.M., Kovacs K.M. (Eds.), Encyclopedia of marine mammals. 3rd edition, pp. 62–69.

Barros, N.B., Ostrom, P.H., Stricker, C.A., Wells, R.S., 2010. Stable isotopes differentiate bottlenose dolphins off west-central Florida. Mar. Mamm. Sci. 26, 324–336.

Barrow, L.M., Bjorndal, K.A., Reich, K.J., 2008. Effects of preservation method on stable carbon and nitrogen isotope values. Physiol. Biochem. Zool. 81*,* 688–693.

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A., MacLeod, H., 2004. Determining trophic niche width: a novel approach using stable isotope analysis. J. Anim. Ecol. 73, 1007–1012.

Beneditto, A.P.M.D., Rezende, C.E., Camargo, P.B., Kehrig, H.A., 2013. Trophic niche comparison between two predators in northern Rio de Janeiro State, Brazil: a stable isotopes approach. Biota Neotrop. 13, 29–33.

Benstead, J.P., March, J.G., Fry, B., Ewel, K.C., Pringle, C.M., 2006. Testing IsoSource: stable isotope analysis of a tropical fishery with diverse organic matter sources. Ecology 87, 326–333.

Bentaleb, I., Martin, C., Vrac, M., Mate, B., Mayzaud, P., Siret, D., de Stephanis, R., Guinet, C., 2011. Foraging ecology of Mediterranean fin whales in a changing environment elucidated by satellite tracking and baleen plate stable isotopes. Mar. Ecol. Prog. Ser. 438, 285–302.

Best, P.B., Schell, D.M., 1996. Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. Mar. Biol. 124*,* 483–494.

Bligh, E.G., , Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Bolnick, D.I., Yang, L.H., Fordyce, J.A., Davis, J.M., Svanbäck, R., 2002. Measuring individual-level resource specialization. Ecology *83*, 2936–2941.

Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D., Forister, M.L., 2003. The ecology of individuals: incidence and implications of individual specialization. Am. Nat. 161, 1–28.

Borrell, A., Vighi, M., Genov, T., Giovos, I., Gonzalvo, J., 2020. Feeding ecology of the highly threatened common bottlenose dolphin of the Gulf of Ambracia, Greece, through stable isotope analysis. Mar. Mamm. Sci. 1– 13.

Bosley, K.L., Wainright, S.C., 1999. Effects of preservatives and acidification on the stable isotope ratios (^{15}N:^{14}N, ^{13}C:^{12}C) of two species of marine animals. Can. J. Fish. Aquat. Sci. 56, 2181–2185.

Brault, E.K., Koch, P.L., Gier, E., Ruiz-Cooley, R.I., Zupcic, J., Gilbert, K.N., McCarthy, M.D., 2014. Effects of decalcification on bulk and compound-specific nitrogen and carbon isotope analyses of dentin. Rapid Commun. Mass Spectrom. *28*, 2744–2752.

Brotons, J.M., Islas-Villanueva, V., Alomar, C., Tor, A., Fernández, R., Deudero, S., 2019. Genetics and stable isotopes reveal non-obvious population structure of bottlenose dolphins (*Tursiops truncatus*) around the Balearic Islands. Hydrobiologia 842, 233–247.

Browning, N.E., McCulloch, S.D., Bossart, G.D., Worthy, G.A.J., 2014. Fine-scale population structure of estuarine bottlenose dolphins (*Tursiops truncatus*) assessed using stable isotope ratios and fatty acid signature analyses. Mar. Biol. 161, 1307–1317.

Burrows, D.G., Reichert, W. L., Bradley Hanson, M., 2014. Effects of decomposition and storage conditions on the δ^{13}C and δ^{15}N isotope values of killer whale (*Orcinus orca*) skin and blubber tissues. Mar. Mamm. Sci. 30, 747–762.

Busquets-Vass, G., Newsome, S.D., Calambokidis, J., Serra-Valente, G., Jacobsen, J.K., Aguíñiga-García, S., Gendron, D., 2017. Estimating blue whale skin isotopic incorporation rates and baleen growth rates: Implications for assessing diet and movement patterns in mysticetes. PLoS One 12, e0177880.

Busquets-Vass, G., Newsome, S. D., Pardo, M. A., Calambokidis, J., Aguíñiga, S., Páez-Rosas, D., ... & Gendron, D., 2020. Isotope-based inferences of the seasonal foraging and migratory strategies of blue whales in the eastern Pacific Ocean. Mar. Environ. Res. 105201.

Bryan, C. E., Christopher, S.J., Balmer, B.C., Wells, R.S., 2007. Establishing baseline levels of trace elements in blood and skin of bottlenose dolphins in Sarasota Bay, Florida: implications for non-invasive monitoring. Sci. Total Environ. 388, 325–342.

Carabel, S., Godínez-Domínguez, E., Verísimo, P., Fernández, L., Freire, J., 2006. An assessment of sample processing methods for stable isotope analyses of marine food webs. J. Exp. Mar. Biol. Ecol. 336, 254–261.

Caut, S., Ângulo, E., Courchamp, F., 2008. Caution on isotopic model use for analyses of consumer diet. Can. J. Zool. 86, 438–445.

Caut, S., Angulo, E., Courchamp, F., 2009. Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. J. Appl. Ecol. 46, 443–453.

Caut, S., Laran, S., Garcia-Hartmann, E., Das, K., 2011. Stable isotopes of captive cetaceans (*killer whales* and *bottlenose dolphins*). J. Exp. Biol. 214, 538–545.

Choy, E.S., Roth, J.D., Loseto, L.L., 2016. Lipid removal and acidification affect nitrogen and carbon stable isotope ratios of beluga whales (*Delphinapterus leucas*) and their potential prey species in the Beaufort Sea ecosystem. Mar. Biol. 163, 220.

Clark, C.T., Fleming, A.H., Calambokidis, J., Kellar, N.M., Allen, C.D., Catelani, K.N., ... & Harvey, J. T. (2017). Heavy with child? Pregnancy status and stable isotope ratios as determined from biopsies of humpback whales. Conserv. Physiol. *5*(1).

Cloyed, C.S., Da Costa, K.P., Hodanbosi, M.R., Carmichael, R.H., 2020. The effects of lipid extraction on δ^{13}C and δ^{15}N values and use of lipid-correction models across tissues, taxa and trophic groups. Methods Ecol. Evol. 11, 751–762.

Connolly, R.M., Guest, M.A., Melville, A.J., Oakes, J.M., 2004. Sulfur stable isotopes separate producers in marine food-web analysis. Oecologia 138, 161–167.

Costa, A.F., Botta, S., Siciliano, S., Giarrizzo, T., 2020. Resource partitioning among stranded aquatic mammals from Amazon and Northeastern coast of Brazil revealed through Carbon and Nitrogen Stable Isotopes. Sci. Rep. 10, 1–13.

Craig, H., 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim. Cosmochim. Acta 12, 133–149.

Crawford, K., Mcdonald, R. A., Bearhop, S. (2008). Applications of stable isotope techniques to the ecology of mammals. Mammal Rev. 38, 87-107.

Dalerum, F., Angerbjörn, A., 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144, 647–658.

Das, K., Holleville, O., Ryan, C., Berrow, S., Gilles, A., Ody, D., Michel, L.N., 2017. Isotopic niches of fin whales from the Mediterranean Sea and the Celtic Sea (North Atlantic). Mar. Environ. Res. 127, 75–83.

De Castro, R.L., Saporiti, F., Vales, D.G., García, N.A., Cardona, L., Crespo, E.A., 2016. What are you eating? A stable isotope insight into the trophic ecology of short-beaked common dolphins in the Southwestern Atlantic Ocean. Mamm. Biol. 81, 571–578.

De Lecea A.M., Smit A.J., Fennessy, S.T., 2011. The effects of freeze/thaw periods and drying methods on isotopic and elemental carbon and nitrogen in marine organisms, raising questions on sample preparation. Rapid Commun. Mass Spectrom. 25, 3640–3649.

DeNiro, M.J., Epstein, S., 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science, 197, 261–263.

DeNiro, M. J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et cosmochimica acta, 42, 495–506.

DeNiro, M. J., Epstein, S., 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta 45, 341–351.

Díaz-Gamboa, R.E., Gendron, D., Busquets-Vass, G., 2018. Isotopic niche width differentiation between common bottlenose dolphin ecotypes and sperm whales in the Gulf of California. Mar. Mamm. Sci., 34, 440–457.

Drago, M., Valdivia, M., Bragg, D., González, E.M., Aguilar, A., Cardona, L., 2020. Stable oxygen isotopes reveal habitat use by marine mammals in the Río de la Plata estuary and adjoining Atlantic Ocean. Estuar. Coast. Shelf Sci.106708.

Eckrich, C.A., Albeke, S.E., Flaherty, E.A., Bowyer, R.T., Ben-David, M., 2020. rKIN: Kernel-based method for estimating isotopic niche size and overlap. J. Anim. Ecol. 89, 757-771.

Endo, T., Kimura, O., Sato, R., Kobayashi, M., Matsuda, A., Matsuishi, T., Haraguchi, K., 2014. Stable isotope ratios of carbon, nitrogen, and oxygen in killer whales (*Orcinus orca*) stranded on the coast of Hokkaido, Japan. Mar. Poll. Bull. 86, 238–243.

Ehleringer, J.R., Rundel, P.W. 1989. Stable isotopes: history, units, and instrumentation, in: Stable isotopes in ecological research, Springer, New York, NY, pp. 1-15.

Elorriaga-Verplancken, F.R., Paniagua-Mendoza, A., Blanco-Jarvio, A., Carone, E., Robles-Hernández, R., Ballínez-Ambriz, C., Rosales-Nanduca, H., 2020. Stable isotope assessment of a mass stranding of short-beaked common dolphins (*Delphinus delphis delphis*) reveals their provenance: Integrating knowledge of a little-known odontocete in the Gulf of California. Reg. Stud. Mar. Sci. 40, 101503.

Evacitas, F.C., Kao, W.Y., Worthy, G.A., Chou, L.S., 2017. Annual variability in dentin $\delta^{15}N$ and $\delta^{13}C$ reveal sex differences in weaning age and feeding habits in Risso's dolphins (*Grampus griseus*). Mar. Mamm. Sci. 33, 748–770.

Norman, S.A., Hobbs, R.C., Foster, J., Schroeder, J.P., Townsend, F.I., 2004. A review of animal and human health concerns during capture-release, handling and tagging of odontocetes. J. Cetacean Res. Manag. 6, 53–62.

Fair, P.A., 2006. Protocols for conducting dolphin capture-release health assessment studies.

Francey, R.J., Allison, C.E., Etheridge, D.M., Trudinger, C.M., Enting, I.G., Leuenberger, M., ... & Steele, L. P., 1999. A 1000-year high precision record of $\delta^{13}C$ in atmospheric CO_2. Tellus B Chem. Phys. Meteorol. 51, 170–193.

Fry, B., Baltz, D.M., Benfield, M.C. Fleeger, J.W., Gace, A., Haas, H.L., Quiñones-Rivera, Z.J., 2003. Stable isotope indicators of movement and residency for brown shrimp (*Farfantepenaeus aztecus*) in coastal Louisiana marsh capes. Estuaries 26, 82–97.

Gannes, L. Z., O'Brien, D.M., Del Rio, C. M., 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78, 1271–1276.

Gannes, L.Z., Martínez del Rio, C., Koch, P.L., 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. Comp. Biochem. Physiol. 119, 725–757.

Gavrilchuk, K., Lesage, V., Ramp, C., Sears, R., Bérubé, M., Bearhop, S., Beauplet, G., 2014. Trophic niche partitioning among sympatric baleen whale species following the collapse of groundfish stocks in the Northwest Atlantic. Mar. Ecol. Prog. Ser. 497, 285–301.

Gelippi, M., Popp, B., Gauger, M. F., Caraveo-Patiño, J., 2020. Tracing gestation and lactation in free ranging gray whales using the stable isotopic composition of epidermis layers. Plos one, 15, e0240171.

Genoves, R.C., Fruet, P.F., Botta, S., Beheregaray, L.B., Möller, L.M., Secchi, E.R., 2020. Fine-scale genetic structure in Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) is associated with social structure and feeding ecology. Mar. Biol. 167, 34.

Giménez, J., Ramírez, F., Almunia, J., Forero, M.G., de Stephanis, R., 2016. From the pool to the sea: Applicable isotope turnover rates and diet to skin discrimination factors for bottlenose dolphins (*Tursiops truncatus*). J. Exp. Mar. Biol. Ecol. 475, 54–61.

Giménez, J., Ramírez, F., Forero, M.G., Almunia, J., de Stephanis, R., Navarro, J., 2017a. Lipid effects on isotopic values in bottlenose dolphins (*Tursiops truncatus*) and their prey with implications for diet assessment. Mar. Biol. 164, 122.

Giménez, J., Cañadas, A., Ramírez, F., Afán, I., García-Tiscar, S., Fernández-Maldonado, C., ... & de Stephanis, R., 2017b. Intra-and interspecific niche partitioning in striped and common dolphins inhabiting the southwestern Mediterranean Sea. Mar. Ecol. Prog. Ser. 567, 199–210.

Giménez, J., Marçalo, A., Ramírez, F., Verborgh, P., Gauffier, P., Esteban, R., ... & Vingada, J., 2017c. Diet of bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Cadiz: Insights from stomach content and stable isotope analyses. Plos One, 12, e0184673.

Giménez, J., Cañadas, A., Ramírez, F., Afán, I., García-Tiscar, S., Fernández-Maldonado, C., ... & de Stephanis, R., 2018. Living apart together: Niche partitioning among Alboran Sea cetaceans. Ecol. Indic. 95, 32–40.

Gómez-Campos, E., Borrell, A., Correas, J., Aguilar, A., 2015. Topographical variation in lipid content and morphological structure of the blubber in the striped dolphin. Sci. Mar. 79, 189–197.

Groom, B., 2018. Effects of decalcification, species, and mammalian order on bulk stable isotope values from marine mammal teeth. University of California, San Diego.

Gross, A., Kiszka, J., Van Canneyt, O., Richard, P., Ridoux, V., 2009. A preliminary study of habitat and resource partitioning among co-occurring tropical dolphins around Mayotte, southwest Indian Ocean. Estuar. Coast. Shelf Sci. 84, 367–374.

Gruber, N., Keeling, C.D., Bacastow, R.B., Guenther, P.R., Lueker, T. J., Wahlen, M., ... & Stocker, T.F., 1999. Spatiotemporal patterns of carbon-13 in the global surface oceans and the oceanic Suess effect. Global Biogeochem. Cycles 13, 307–335.

Guerra, M., Wing, L., Dawson, S., Rayment, W., 2020. Stable isotope analyses reveal seasonal and inter-individual variation in the foraging ecology of sperm whales. Mar. Ecol. Prog. Ser. 638, 207–219.

Healy, K., Guillerme, T., Kelly, S.B.A., Inger, R., Bearhop, S. Jackson, A.L., 2018. SIDER: a R package for predicting trophic discrimination factors of consumers based on their ecology and phylogenetic relatedness. Ecography 41, 1393–1400.

Hobson, K.A., Welch, H.E., 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar. Ecol. Prog. Ser. 9–18.

Hobson, K.A., Schell, D.M., Renouf, D., Noseworthy, E., 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Can. J. Fish. Aquat. Sci., 53, 528–533.

Hobson, K.A., 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120, 314–326.

Hohn, A.A., Thomas, L., Carmichael, R.H., Litz, J., Clemons-Chevis, C., Shippee, S. F., ... & Zolman, E.S., 2017. Assigning stranded bottlenose dolphins to source stocks using stable isotope ratios following the Deepwater Horizon oil spill. Endanger. Species Res. 33, 235–252.

Hooker, S.K., Iverson, S.J., Ostrom, P., Smith, S.C., 2001. Diet of northern bottlenose whales inferred from fatty-acid and stable-isotope analyses of biopsy samples. Can. J. Zool. 79, 1442–1454.

Indermühle, A., Stocker, T.F., Joos, F., Fischer, H., Smith, H.J., Wahlen, M., ... & Meyer, R., 1999. Holocene carbon-cycle dynamics based on CO_2 trapped in ice at Taylor Dome, Antarctica. Nature, 398, 121−26.

Iverson, S.J., 2009. Blubber, in: W. F. Perrin, B. Würsig, J. G. M. Thewissen (Eds.), Encyclopedia of marine mammals (Second ed.). San Diego, CA: Academic Press.

Jackson, A.L., Inger, R., Parnell, A.C., Bearhop, S., 2011. Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. J. Anim. Ecol. 80, 595–602.

Jardine, T.D., McGeachy, S.A., Paton, C.M., Savoie, M., Cunjak, R.A., 2003. Stable isotopes in aquatic systems: sample preparation, analysis and interpretation (Vol. 2656). [Fredericton, NB]: Fisheries and Oceans.

Keeling, C.D., MOOK, W.G., Tans, P.P., 1979. Recent trends in the $^{13}C/^{12}C$ ratio of atmospheric carbon dioxide. Nature, 277, 121–123.

Keenan, S.W., DeBruyn, J.M., 2019. Changes to vertebrate tissue stable isotope ($\delta^{15}N$) composition during decomposition. Sci. Rep. 9, 1–12.

Kelly, J.F., 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can. J. Zool. 78, 1–27.

Kelly, S., Brodie, C., Hilkert, A., 2018. Isotopic-Spectroscopic Technique: Stable Isotope-Ratio Mass Spectrometry (IRMS), in: Modern techniques for food authentication, Academic Press, pp. 349-413.

Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., Jones, R.I., 2006. A revised model for lipid-normalizing $\delta^{13}C$ values from aquatic organisms, with implications for isotope mixing models. J. Appl. Ecol. 43*,* 1213–1222.

Kiszka, J., Oremus, M., Richard, P., Poole, M., Ridoux, V., 2010. The use of stable isotope analyses from skin biopsy samples to assess trophic relationships of sympatric delphinids off Moorea (French Polynesia). J. Exp. Mar. Biol. Ecol. 395, 48–54.

Kiszka, J., Simon-Bouhet, B., Martinez, L., Pusineri, C., Richard, P., Ridoux, V., 2011. Ecological niche segregation within a community of sympatric dolphins around a tropical island. Mar. Ecol. Prog. Ser. 433, 273–288.

Kiszka, J.J., Méndez-Fernandez, P., Heithaus, M.R., Ridoux, V., 2014. The foraging ecology of coastal bottlenose dolphins based on stable isotope mixing models and behavioral sampling. Mar. Biol.161, 953–961.

Knoff, A., Hohn, A., Macko, S., 2008. Ontogenetic diet changes in bottlenose dolphins (*Tursiops truncatus*) reflected through stable isotopes. Mar. Mamm. Sci. 24, 128–137.

Koch, P.L., 2007. Isotopic Study of the Biology of Modern and Fossil Vertebrates, in: Michener, R., Lajtha, K. (Eds.), Stable Isotopes in Ecology and Environmental Science, Blackwell Publishing, Boston, pp. 99–154.

Kurle, C.M., 2002. Stable-isotope ratios of blood components from captive northern fur seals (*Callorhinus ursinus*) and their diet: applications for studying the foraging ecology of wild otariids. Can. J. Zool. 80, 902–909.

Layman, C.A., Arrington, D.A., Montaña, C.G., Post, D.M., 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88, 42–48.

Lesage, V., Morin, Y., Rioux, È., Pomerleau, C., Ferguson, S.H., Pelletier, É., 2010. Stable isotopes and trace elements as indicators of diet and habitat use in cetaceans: predicting errors related to preservation, lipid extraction, and lipid normalization. Mar. Ecol. Prog. Ser. 419, 249–265.

Lian, M., Castellini, J.M., Miller, D., Griff, B., Vertyankin, V.V., Dupont, J., ... & O'Hara, T.M., 2020. Assessing δ^{13}C, δ^{15}N and total mercury measures in epidermal biopsies from Gray Whales. Front. Mar. Sci. 7, 133.

Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A., Lutcavage, M.E., 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. J. Anim. Ecol. 838–846.

Loizaga De Castro, R., Saporiti, F., Vales, D.G., García, N.A., Cardona, L., Crespo, E.A., 2016. Feeding ecology of dusky dolphins *Lagenorhynchus obscurus*: evidence from stable isotopes. J. Mammal. 97, 310–320.

Lysiak, N.S., Trumble, S.J., Knowlton, A.R., Moore, M.J., 2018. Characterizing the duration and severity of fishing gear entanglement on a North Atlantic right whale (Eubalaena glacialis) using stable isotopes, steroid and thyroid hormones in baleen. Front. Mar. Sci. 5, 168.

Maas, M.C., 2009. Bones and teeth, histology of, in: Encyclopedia of marine mammals, Academic Press, pp. 124-129.

Marcoux, M., McMeans, B.C., Fisk A.T., Ferguson S. H., 2012. Composition and temporal variation in the diet of beluga whales, derived from stable isotopes. Mar. Ecol. Prog. Ser. 471, 283–291.

Martínez del Rio, C., Wolf, N., Carleton, S.A., Gannes, L.Z., 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biol. Rev. 84, 91–111.

Matthews, C.J., Ferguson, S.H., 2015. Weaning age variation in beluga whales (*Delphinapterus leucas*). J. Mammal. 96, 425-437.

Matthews, C.J., Ruiz-Cooley, R.I., Pomerleau, C., Ferguson, S.H., 2020. Amino acid δ^{15}N underestimation of cetacean trophic positions highlights limited understanding of isotopic fractionation in higher marine consumers. Ecol. Evol. 10, 3450–3462.

Matthews, C. J., Longstaffe, F. J., Ferguson, S. H., 2016. Dentine oxygen isotopes ($\delta^{18}O$) as a proxy for odontocete distributions and movements. Ecol. Evol. 6, 4643–4653.

McConnaughey, T., McRoy, C.P., 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Mar. Biol. 53, 257–262.

Mendes, S., Newton, J., Reid, R.J., Zuur, A.F., Pierce, G.J., 2007. Stable carbon and nitrogen isotope ratio profiling of sperm whale teeth reveals ontogenetic movements and trophic ecology. Oecologia, 151, 605–615.

Mèndez-Fernandez, P., Bustamante, P., Bode, A., Chouvelon, T., Ferreira, M., Lopez, A., ... & Caurant, F., 2012. Foraging ecology of five toothed whale species in the Northwest Iberian Peninsula, inferred using carbon and nitrogen isotope ratios. J. Exp. Mar. Biol. Ecol. 413, 150–158.

Mitani, Y., Bando, T., Takai, N., Sakamoto, W., 2006. Patterns of stable carbon and nitrogen isotopes in the baleen of common minke whale *Balaenoptera acutorostrata* from the western North Pacific. Fish. Sci. 72*,* 69–76.

Monteiro, S., Ferreira, M., Vingada, J.V., López, A., Brownlow, A., Méndez-Fernandez, P., 2015. Application of stable isotopes to assess the feeding ecology of long-finned pilot whale (*Globicephala melas*) in the Northeast Atlantic Ocean. J. Exp. Mar. Biol. Ecol. 465, 56–63.

Moore, J.W., Semmens, B.X., 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecol. Lett., 11, 470–480.

Nagata, R.M., Moreira, M.Z., Pimentel, C.R., Morandini, A.C., 2015. Food web characterization based on δ15N and δ13C reveals isotopic niche partitioning between fish and jellyfish in a relatively pristine ecosystem. Mar. Ecol. Prog. Ser. 519, 13–27.

Nelson, M.A., Quakenbush, L.T., Mahoney, B.A., Taras, B.D., Wooller, M.J., 2018. Fifty years of Cook Inlet beluga whale feeding ecology from isotopes in bone and teeth. Endang. Species Res. 36, 77–87.

Newsome S.D., Koch P.L., Etnier M.A., Aurioles-Gamboa D., 2006. Using carbon and nitrogen isotope values to investigate maternal strategies in northeast Pacific otariids. Mar. Mamm. Sci. 22, 556–572.

Newsome, S.D., Martinez del Rio, C., Bearhop, S., Phillips, D.L., 2007. A niche for isotopic ecology. Front Ecol Environ. 5, 429–436.

Newsome, S.D., Etnier, M.A., Monson, D.H., Fogel, M.L., 2009. Retrospective characterization of ontogenetic shifts in killer whale diets via $\delta^{13}C$ and $\delta^{15}N$ analysis of teeth. Mar. Ecol. Prog Ser. 374, 229–242.

Newsome, S.D., Clementz, M.T., Koch, P.L., 2010. Using stable isotope biogeochemistry to study marine mammal ecology. Mar. Mamm. Sci. 26, 509–572.

Newsome, S.D., Chivers, S.J., Berman Kowalewski, M., 2018. The influence of lipid-extraction and long-term DMSO preservation on carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values in cetacean skin. Mar. Mamm. Sci. 34, 277–293.

Ning, X., Gui, D., He, X., Wu, Y., 2020. Diet Shifts Explain Temporal Trends of Pollutant Levels in Indo-Pacific Humpback Dolphins (Sousa chinensis) from the Pearl River Estuary, China. Environ. Sci. Technol.

Niño-Torres, C.A., Gallo-Reynoso, J.P., Galván-Magaña F., Escobar-Briones, E., Macko, S.A., 2006. Isotopic analysis of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ "A feeding tale" in teeth of the longbeaked common dolphin, *Delphinus capensis*. Mar. Mamm. Sci. 22, 831–846.

Noren, D.P., Mocklin, J.A., 2012. Review of cetacean biopsy techniques: Factors contributing to successful sample collection and physiological and behavioral impacts. Mar. Mamm. Sci. 28, 154–199.

O'Hara, T.M., Templeton, M., Castellini, J. M., Wells, R., Beckmen, K., Berner, J., 2018. Use of blood-soaked cellulose filter paper for measuring carbon and nitrogen stable isotopes. J. Wildl. Dis. 54, 375–379.

Parnell, A., 2008. SIAR: stable isotope analysis in R. *http://cran. r-project. org/web/packages/siar/index. html.*

Parnell, A.C., Inger, R., Bearhop, S., Jackson, A.L., 2010. Source partitioning using stable isotopes: coping with too much variation. PLoS ONE 5, e9672.

Parnell, A.C., 2016. simmr: A Stable Isotope Mixing Model. Version 0.3. https://cran.r-project.org/web/packages/simmr/

Pereira, L.B., Botta, S., Teixeira, C.R., Fruet, P., Simões-Lopes, P.C., Daura-Jorge, F.G., 2020. Feeding ecology of two subspecies of bottlenose dolphin: a tooth tale. Aquat. Ecol. 1–15.

Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Evol. Syst. 18, 293–320.

Phillips, D.L., Gregg, J.W., 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia *136*, 261–269.

Pinela, A.M., Borrell, A., Cardona, L., Aguilar, A., 2010. Stable isotope analysis reveals habitat partitioning among marine mammals off the NW African coast and unique trophic niches for two globally threatened species. Mar. Ecol. Prog. Ser. 416, 295–306.

Pomerleau, C., Lesage, V., Ferguson, S.H., Winkler, G., Petersen, S.D., Higdon, J.W., 2012. Prey assemblage isotopic variability as a tool for assessing diet and the spatial distribution of bowhead whale *Balaena mysticetus* foraging in the Canadian eastern Arctic. Mar. Ecol. Prog. Ser. 469, 161–174.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montana, C.G., 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152, 1791–189.

Pugliares, K.R., Bogomolni, A., Touhey, K.M., Herzig, S.M., Harry, C.T., Moore, M.J., 2007. Marine mammal necropsy: an introductory guide for stranding responders and field biologists. Woods Hole Oceanographic Institution Technical Document, *6*, 117.

Quezada-Romegialli, C., Jackson, A.L., Hayden, B., Kahilainen, K.K., Lopes, C., Harrod, C., 2018. tRophicPosition, an R package for the Bayesian estimation of trophic position from consumer stable isotope ratios. Methods Ecol. Evol. 9, 1592–1599.

Riccialdelli, L., Newsome, S.D., Dellabianca, N.A., Bastida, R., Fogel, M.L., Goodall, R.N.P., 2013. Ontogenetic diet shift in Commerson's dolphin (*Cephalorhynchus commersonii commersonii*) off Tierra del Fuego. Polar Biol. 36, 6171–627.

Riccialdelli, L., Viola, M.N.P., Panarello, H.O., Goodall, R.N.P., 2017. Evaluating the isotopic niche of beaked whales from the southwestern South Atlantic and Southern Oceans. Mar. Ecol. Prog. Ser. 581, 1831–198.

Riofrío-Lazo, M., Aurioles-Gamboa, D., 2013. Timing of isotopic integration in marine mammal skull: comparative study between calcified tissues. Rapid Commun. Mass Spectrom. 27, 10761–1082.

Roman, J., Estes, J.A., Morissette, L., Smith, C., Costa, D., McCarthy, J., ... & Smetacek, V., 2014. Whales as marine ecosystem engineers. Front. Ecol. Environ. 12, 3771–385.

Rossman, S., Ostrom, P.H., Stolen, M., Barros, N.B., Gandhi, H., Stricker, C.A., Wells, R.S., 2015. Individual specialization in the foraging habits of female bottlenose dolphins living in a trophically diverse and habitat rich estuary. Oecologia 178, 415–425.

Ryan, C., McHugh, B., Trueman, C.N, Harrod, C., Berrow, S.D, O'Connor, I., 2012. Accounting for the effects of lipids in stable isotope ($\delta^{13}C$ and $\delta^{15}N$ values) analysis of skin and blubber of balaenopterid whales. Rapid Commun. Mass Spectrom. 26, 2745–2754.

Ryan, C., McHugh, B., Trueman, C.N., Sabin, R., Deaville, R., Harrod, C., ... & Ian, O., 2013. Stable isotope analysis of baleen reveals resource partitioning among sympatric rorquals and population structure in fin whales. Mar. Ecol. Prog. Ser. 479, 251–261.

Ryan, C., Berrow, S.D., McHugh, B., O'Donnell, C., Trueman, C.N., O'Connor, I., 2014. Prey preferences of sympatric fin (Balaenoptera physalus) and humpback (Megaptera novaeangliae) whales revealed by stable isotope mixing models. Mar. Mamm. Sci. 30, 242–258.

Santos Carvallo, M., Pérez Alvarez, M.J., Muniain, V., Moraga, R., Oliva, D., Sepúlveda, M., 2015. Trophic niche overlap between sympatric resident and transient populations of bottlenose dolphins in the Humboldt Current System off north-central Chile.

Schell, D.M., Saupe, S.M., Haubenstock, N., 1989a. Natural Isotope Abundances in Bowhead Whale (*Balaena mysticetus*) Baleen: Markers of Aging and Habitat Usage, in: Rundel, P. W., Ehleringer, J.R., Nagy, K.A. (Eds), Stable Isotopes in Ecological Research. Ecological Studies (Analysis and Synthesis), vol 68. Springer, New York, NY.

Schell, D.M., Saupe, S.M., Haubenstock, N., 1989b. Bowhead whale (*Balaena mysticetus*) growth and feeding as estimated by $\delta^{13}C$ techniques. Mar. Biol. 103*,* 433–443.

Secchi, E.R., Botta, S., Wiegand, M.M., Lopez, L.A., Fruet, P.F., Genoves, R.C., Di Tullio, J.C., 2016. Long-term and gender-related variation in the feeding ecology of common bottlenose dolphins inhabiting a subtropical estuary and the adjacent marine coast in the western South Atlantic. Mar. Biol. Res. 13, 121–134.

Silva, M.A., Borrell, A., Prieto, R., Gauffier, P., Bérubé, M., Palsbøl, P.J., Colaço, A., 2019. Stable isotopes reveal winter feeding in different habitats in blue, fin and sei whales migrating through the Azores. R. Soc. Open Sci. 6, 181800.

Smith, J.A., Mazumder, D., Suthers, I.M., Taylor, M.D., 2013. To fit or not to fit: evaluating stable isotope mixing models using simulated mixing polygons. Methods Ecol. Evol. 4, 612–618.

Smith, K.J., Sparks, J.P., Timmons, Z.L., Peterson, M.J., 2020. Cetacean skeletons demonstrate ecologically relevant variation in intraskeletal stable isotopic values. Front. Mar. Sci. 7, 388.

Stuart, B., 2013. Decomposition chemistry: overview, analysis, and interpretation.

Sulzman, E.W., 2007. Stable isotope chemistry and measurement: a primer. Stable isotopes in ecology and environmental science, 1.

Sweeting, C.J., Polunin, N.V., Jennings, S., 2004. Tissue and fixative dependent shifts of $\delta^{13}C$ and $\delta^{15}N$ in preserved ecological material. Rapid Commun. Mass Spectrom, 18, 2587-2592.

Sweeting, C.J., Polunin, N.V.C, Jennings, S., 2006. Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. Rapid Commun. Mass Spectrom. 20, 595– 601.

Teixeira, C.R., Botta, S., Daura-Jorge, F.G., Pereira, L.B., Newsome, S.D., Simões-Lopes, P.C., 2020. Niche overlap and diet composition of three sympatric coastal dolphin species in the southwest Atlantic Ocean. Mar. Mamm. Sci.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., Slade, N.A., 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for δ^{13}C analysis of diet. Oecologia 57, 32–37.

Troina, G., Botta, S., Secchi, E.R., Dehairs, F., 2016. Ontogenetic and sexual characterization of the feeding habits of franciscanas, *Pontoporia blainvillei*, based on tooth dentin carbon and nitrogen stable isotopes. Mar. Mamm. Sci. 32, 1115–1137.

Troina G.C., Botta S., Dehairs F., Di Tullio J.C., Elskens M., Secchi E.R., 2020 Skin δ^{13}C and δ^{15}N reveal spatial and temporal patterns of habitat and resource use by free-ranging odontocetes from the southwestern Atlantic Ocean. Mar. Biol. 167, 1–19.

Varela, J.L., Rojo-Nieto, E., Sorell, J.M., Medina, A., 2018. Using stable isotope analysis to assess trophic relationships between Atlantic bluefin tuna (*Thunnus thynnus*) and striped dolphin (*Stenella coeruleoalba*) in the Strait of Gibraltar. Mar. Environ. Res. 139, 57–63.

Verburg, P., 2007. The need to correct for the Suess effect in the application of δ^{13}C in sediment of autotrophic Lake Tanganyika, as a productivity proxy in the Anthropocene. J. Paleolimnol. 37, 591–602.

Vighi, M., Borrell, A., Víkingsson, G., Gunnlaugsson, T., Aguilar, A., 2019. Strontium in fin whale baleen: A potential tracer of mysticete movements across the oceans? Sci. Total Environ. 650, 1224–1230.

Walker, J.L., Potter, C.W., Macko, S.A., 1999. The diets of modern and historic bottlenose dolphin populations reflected through stable isotopes. Mar. Mamm. Sci. 15, 335–350.

Walker, J.L., Macko, S.A., 1999. Dietary studies of marine mammals using stable carbon and nitrogen isotopic ratios of teeth. Mar. Mamm. Sci. 15, 314–334.

Watt, C.A., Heide-Jørgensen, M.P., Ferguson, S.H., 2013. How adaptable are narwhal? A comparison of foraging patterns among the world's three narwhal populations. Ecosphere 4, 1–15.

Watt, C.A., Ferguson, S.H., 2015. Fatty acids and stable isotopes (δ^{13}C and δ^{15}N) reveal temporal changes in narwhal (*Monodon monoceros*) diet linked to migration patterns. Mar. Mamm. Sci. 31, 21-44.

Werth, A.J., 2018. Baleen, in: Wursig, B., Thewissen, J.G.M., Kovacs, K.M. (Eds.). Encyclopedia of marine mammals. 3rd edition, p 60-61.

Wild, L.A., Chenoweth, E.M., Mueter, F.J., Straley, J.M., 2018. Evidence for dietary time series in layers of cetacean skin using stable carbon and nitrogen isotope ratios. Rapid Commun. Mass Spectrom. 32, 1425–1438.

Wild, L.A., Mueter, F., Witteveen, B., Straley, J.M., 2020. Exploring variability in the diet of depredating sperm whales in the Gulf of Alaska through stable isotope analysis. R. Soc. Open Sci. 7, 191110.

Wilson, R.M., Kucklick, J.R., Balmer, B.C., Wells, R.S., Chanton, J.P., Nowacek, D.P., 2012. Spatial distribution of bottlenose dolphins (Tursiops truncatus) inferred from stable isotopes and priority organic pollutants. Sci. Total Environ. 425, 223–230.

Wilson R.M., Chanton J.P., Balmer B.C., Nowacek D.P., 2014. An evaluation of lipid extraction techniques for interpretation of carbon and nitrogen isotope values in bottlenose dolphin (*Tursiops truncatus*) skin tissue. Mar. Mamm. Sci. 30, 85–103.

Witteveen, B.H., Worthy, G.A., Foy, R.J., Wynne, K.M., 2012. Modeling the diet of humpback whales: An approach using stable carbon and nitrogen isotopes in a Bayesian mixing model. Mar. Mamm. Sci. 28, E233–E250.

Woshner, V., Knott, K., Wells, R., Willetto, C., Swor, R., O'Hara, T., 2008. Mercury and selenium in blood and epidermis of bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay, FL: interaction and relevance to life history and hematologic parameters. EcoHealth 5, 360–370.

Young, H., Nigro, K., McCauley, D.J., Ballance, L.T., Oleson, E.M., Baumann-Pickering, S., 2017. Limited trophic partitioning among sympatric delphinids off a tropical oceanic atoll. PloS one 12, e0181526.

Yurkowski, D.J, Hussey, N.E., Semeniuk, C., Ferguson, S.H., Fisk, A.T., 2014. Effects of lipid extraction and the utility of lipid normalization models on $\delta^{13}C$ and $\delta^{15}N$ values in Arctic marine mammal tissues. Polar Biol. 38, 131–143.

Yurkowski, D.J., Ferguson, S., Choy, E.S., Loseto, L.L., Brown, T.M., Muir, D.C., ... & Fisk, A.T., 2016. Latitudinal variation in ecological opportunity and intraspecific competition indicates differences in niche variability and diet specialization of Arctic marine predators. Ecol. Evol. 6, 1666–1678.

Zaccarelli, N., Bolnick, D.I., Mancinelli, G., 2013. RInSp: an r package for the analysis of individual specialization in resource use. Methods Ecol. Evol. 4, 1018–10.

Zupcic-Moore, J.R., Ruiz-Cooley, R.I., Paliza, O., Koch, P.L., McCarthy, M.D., 2017 Using stable isotopes to investigate foraging variation and habitat use of sperm whales from northern Peru. Mar. Ecol. Prog. Ser. 579, 201–212.

3 CAPÍTULO 2 - NICHE OVERLAP AND DIET COMPOSITION OF THREE SYMPATRIC COASTAL DOLPHIN SPECIES IN THE SOUTHWEST ATLANTIC OCEAN

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ABSTRACT

Sympatric species are expected to differ in ecological requirements to minimize niche overlap and avoid competition. Here we assess the trophic interactions among three coexisting dolphin species from southern Brazil: the franciscana dolphin (*Pontoporia blainvillei*), the Guiana dolphin (*Sotalia guianensis*), and the Lahille's bottlenose dolphin (*Tursiops truncatus gephyreus*). We evaluated temporal variation in carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values of bone collagen to examine potential dietary shifts resulting from increased fishing activity over the past three decades. We estimated the degree of niche overlap among these species and the contribution of potential prey sources to their diet. $\delta^{15}N$ values were consistent among species and across years, while $\delta^{13}C$ values increased for Guiana dolphins and decreased for bottlenose dolphins, suggesting changes in diet and/or foraging habitats through time. The similar $\delta^{13}C$ and $\delta^{15}N$ values and the high niche overlap between Guiana and bottlenose dolphins indicate that these species are primarily feeding on demersal prey. The franciscana diet is primarily composed of pelagic prey, resulting in a lower niche overlap in comparison with the other dolphin species. Our study provides further information about the foraging ecology of this unique dolphin community in southern Brazil with implications for its management and conservation.

Keywords: isotopic niche, *Pontoporia blainvillei*, *Sotalia guianensis*, stable isotopes, trophic position, *Tursiops truncatus gephyreus*

INTRODUCTION

The concept of niche is essential for understanding how biotic and abiotic variables regulate resource use and competition among species. Niche theory predicts that sympatric species can partition resources through variation in foraging strategies or through variation in when and/or where resources are exploited (Pianka, 1980; Schoener, 1974). Therefore, quantifying niche overlap of sympatric species can provide insights into the underlying mechanisms that enable coexistence, such as how species share or compete for resources. This information may eventually be used to predict community responses to natural or anthropogenic ecosystem change, especially those related to prey distribution and abundance (Chase & Leibold, 2003; Geange, Pledger, Burns, & Shima, 2011).

Resource partitioning through variation in foraging strategies can be attributed to morphological differences among coexisting species (Schoener, 1974). This is mainly due to the influence of an organism's morphology on its foraging performance, which can limit the range of prey that can be efficiently captured (Taylor, 1987). Off the coast of Santa Catarina, Brazil in the Southwest Atlantic Ocean (SWAO), a direct sympatry (sensu Bearzi, 2005) among three dolphin species with marked differences in morphological traits—the franciscana dolphin (*Pontoporia blainvillei*), the Guiana dolphin (*Sotalia guianensis*), and the coastal ecotype of the bottlenose dolphin (*Tursiops truncatus gephyreus*)—provides an interesting opportunity to evaluate resource partitioning (Figure 1). The franciscana dolphin (Pontoporidae) is usually grouped within the so-called river dolphins, that consist of relict dolphin lineages (Cassens et al., 2000). These species have a unique combination of morphological features such as a flexible neck, elongated skull, narrow jaw, and rostrum (Cassens et al., 2000) with a relatively large number (up to 250) of small needle-like teeth (Werth, 2006). The Guiana and bottlenose dolphins are grouped within the more diverse delphinid family characterized by blunt heads, wider jaws, and a lower number of robust and conical teeth (up to 100) in comparison to river dolphins (Werth, 2006). These three species also have different body dimensions (Botta, Muelbert, & Secchi, 2012; Cremer et al., 2018; Rosas & Monteiro-Filho, 2002; Venuto, Botta, Barreto, Secchi, & Fruet, 2020) and life history traits (i.e., growth rates and age at maturity; Barreto & Rosas, 2006; Botta et al., 2010; Fruet, Daura-Jorge, Möller, Genoves, & Secchi, 2015; Rosas & Monteiro-Filho, 2002; Venuto et al., 2020) that likely result in contrasting energetic demands, which in turn can affect their behavior and ability to catch prey, and by extension their dietary preferences.

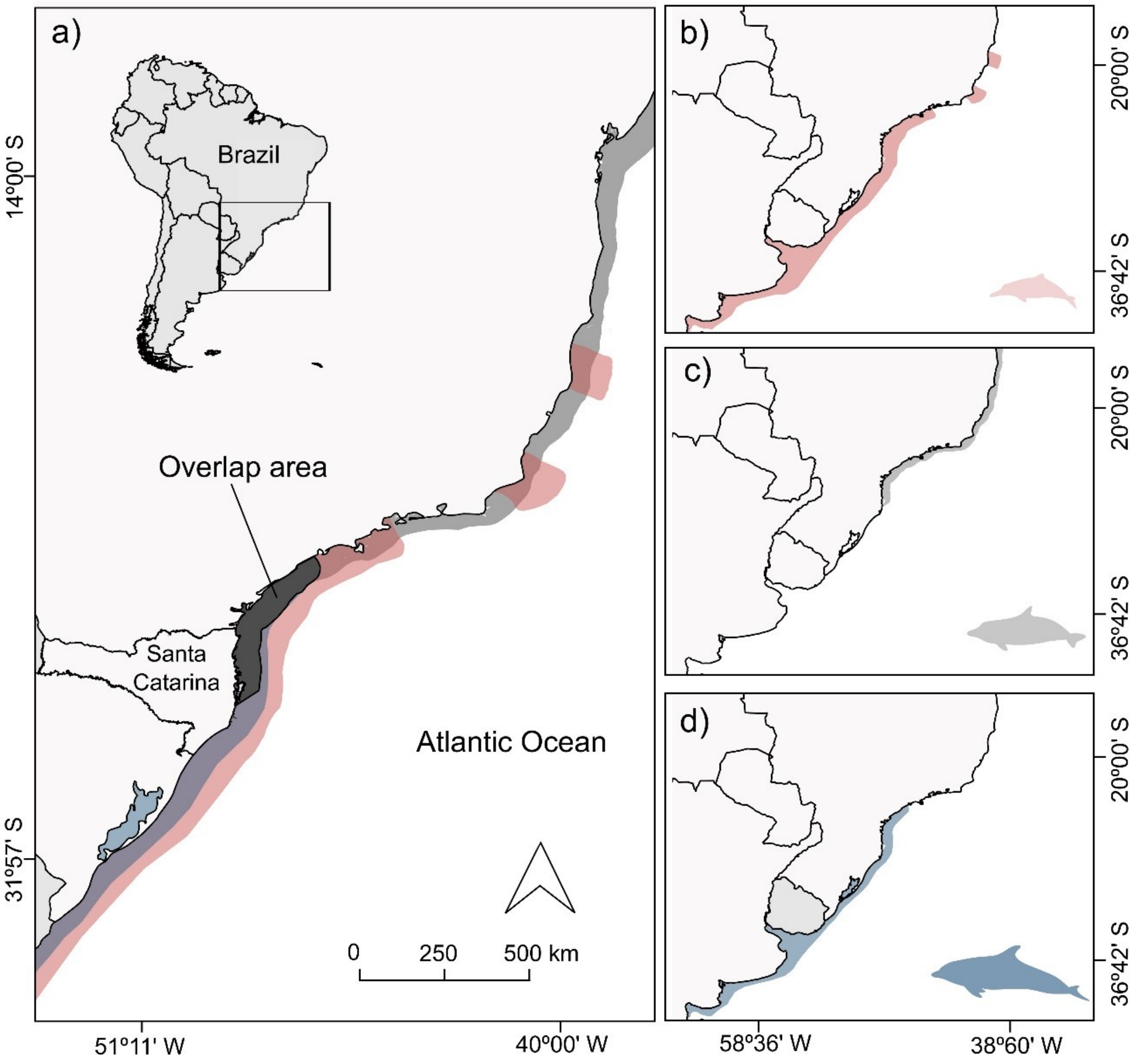

Figure 1. (a) Overlap (dark gray) in the distribution of the franciscana (*Pontoporia blainvillei*) (red), Guiana (*Sotalia guianensis*) (gray) and Lahille's bottlenose (*Tursiops truncatus gephyreus*) (blue) dolphins off the Santa Catarina coast in southern Brazil. Insets (b), (c), and (d) represent the distribution of each species independently

The diet of these three sympatric dolphin species was previously described via stomach content analysis (Bassoi, 2005; Daura-Jorge, Wedekin, & Simões-Lopes, 2011; Milmann, Danilewicz, Machado, Santos, & Ott, 2016), which revealed that the most important prey species for both Guiana and bottlenose dolphins is the pelagic *Trichiurus lepturus*, while the demersal *Stellifer rastrifer* is the preferred prey for the frasciscana dolphin. Studies suggest that the franciscana dolphin feeds mainly on small size prey (Bittar & di Beneditto, 2009; Cremer, Pinheiro, & Simões-Lopes, 2012), which might be related to its rather unique skull morphology. In contrast, Guiana and bottlenose dolphins exhibit a high degree of dietary plasticity with respect to prey size (Daura-Jorge et al., 2011; Milmann et al., 2016).

Although *T. lepturus* is not considered an important resource for fisheries in southern Brazil, the high bycatch mortality of this pelagic species by the bottom trawling fleet (Haimovici & Mendonça, 1996; Haimovici & Fischer, 2007) may negatively impact *T. lepturus* stocks and by extension influence the diet of Guiana and bottlenose dolphins. Other important prey consumed by these three sympatric dolphins are demersal species (e.g., , *Mugil liza*, *Micropogonias furnieri*, *Macrodon atricauda*, *Umbrina canosai*, and *Cynocion guatucupa*), which have decreased in abundance over time, with stocks now considered fully exploited and/or at risk of collapse (Haimovici & Cardoso, 2017). Together, these demersal fish species represent more than half of local marine fish landings in southern Brazil. The ecological impact of overfishing has been monitored for decades along the southern Brazilian coast (Castello, Sunyé, Haimovici, & Hellebrandt, 2009; Haimovici & Cardoso, 2017; Vasconcellos & Gasalla, 2001) and has likely influenced the foraging behavior and diet composition of these dolphin species and possibly other coastal marine top consumers in this region. During the last decade, franciscana and bottlenose dolphins from Rio Grande in southern Brazil showed a temporal change in diet composition that likely corresponds to increased fishing pressure in this region, which impacted the availability of their primary prey sources (e.g., , *M. furnieri* and *Menticirrhus* sp.; Secchi, Ott, & Danilewicz, 2003; Secchi et al., 2017). Thus, detailed information on feeding ecology can help elucidate how increasing fishing activities in this region can influence diet composition and trophic relationships among these top predators, supporting more effective management for the conservation of coastal marine mammal populations and the ecosystems that support them.

Stable isotope analysis has emerged as a useful tool to assess trophic interactions and resource partitioning among marine mammals and has been used extensively to study sympatric dolphin species (e.g., , Kiszka et al., 2011; Loizaga de Castro, Saporiti, Vales, Cardona, & Crespo, 2017). As applied to marine top consumers, this approach commonly utilizes carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis to characterize foraging habitats and trophic level (DeNiro & Epstein, 1978; Fry, 2006; Newsome, Clementz, & Koch, 2010). The ecological niche of a population or species can thus be depicted by its isotopic composition or isotopic niche (Bearhop, Adams, Waldron, Fuller, & MacLeod, 2004; Newsome, Martinez del Rio, Bearhop, & Phillips, 2007) in which $\delta^{13}C$ vs. $\delta^{15}N$ are the environmental axes that combine both habitat and resource related components. Isotopic niches can be quantified using metrics (Jackson, Inger, Parnell, & Bearhop, 2011; Layman, Arrington, Montanä, & Post, 2007) that allow for the assessment of trophic relationships and niche overlap within and among individuals (e.g., , Zhao, Villéger, Lek, & Cucherousset, 2014), populations (e.g., , Santos-

Carvallo et al., 2015), and even species (e.g., , Franco-Trecu, Drago, Costa, Dimitriadis, & Passadore, 2017).

Here, we used $\delta^{13}C$ and $\delta^{15}N$ analysis to assess the foraging ecology and potential dietary overlap among sympatric franciscana, Guiana, and bottlenose dolphins along the southern Brazilian coast. Specifically, we compared the width and degree of overlap in the isotopic niche among these species and used a Bayesian mixing model to quantify the proportional contribution of primary prey sources to their diet. Based on differences in morphology, we hypothesized that we would observe significant differences in their isotopic niche, with larger niche overlap among the two most closely related species, Guiana and bottlenose dolphins. Second, we analyzed $\delta^{13}C$ and $\delta^{15}N$ values of individuals collected across the past three decades (1985–2017) to investigate historical dietary shifts that may be related to increased fishing pressure on some of their primary prey species during this period.

MATERIAL AND METHODS

Study area

The coast of Santa Catarina in southern Brazil is composed of a mosaic of bays, rocky coastal islands, sandy coastline, estuaries, lagoons, and mangrove forests. This region is influenced by Subantarctic Shelf Water transported northward by the Malvinas/Falkland Current and Tropical Water and South Atlantic Central Water carried southward by the Brazil Current, characterizing the Subtropical Convergence (Ciotti, Odebrecht, Fillmann, & Möller, 1995; Möller, Piola, Freitas, & Campos, 2008). This system generates high nutrient concentration and biological productivity (Muelbert et al., 2008). In addition to marine currents, discharge of freshwater from the La Plata River and, to a lesser extent, the Patos Lagoon Estuary also influences nutrient dynamics in the coastal zone (Muelbert et al., 2008).

Sample collection

We subsampled vertebral epiphysis from 75 stranded carcasses ($n^{\text{franciscana dolphin}} = 34$; $n^{\text{Guiana dolphin}} = 23$; $n^{\text{bottlenose dolphin}} = 18$) found along the Santa Catarina coast from Itajaí (26°91S, 48°67`W) to Laguna (28°30’S, 48°50’W) between 1985 and 2017 (see Supplementary Material Figure S1). The specimens are archived in the scientific collection of the Aquatic Mammals Laboratory (LAMAQ) at the Universidade Federal de Santa Catarina (UFSC). We selected potential prey species based on the percent frequency of occurrence (%FO) from previous stomach content analyses for local and adjacent populations of franciscana (Bassoi, 2005), Guiana (Daura-Jorge et al., 2011) and bottlenose dolphins (Milmann et al., 2016). We focused on species with %FO > 10% based on stomach content analysis. Given that stable isotope values may vary geographically, we attempted to obtain prey with artisanal fishers of the southern Brazilian coast from the same regions where the stranded carcasses were found. We could not collect five fish species (*M. atricauda*, *U. canosai*, *Engraulis anchoita* and *Urophycis brasiliensis*), and hence, we relied on isotope values for these species collected in the southern Brazil and previously reported in the literature (Bugoni, McGill, & Furness, 2010; Zenteno et al., 2015). Due to the potential correlation between fish length/size and isotopic composition (Nagata, Moreira, Pimentel, & Morandini, 2015), we also considered the average size of prey consumed by each dolphin species when selecting samples for analysis. We analyzed stable isotope values of a total of 92 prey specimens from 17 fish and one cephalopod species (see Supplementary Material Table S2).

Stable isotope analysis

We extracted bone collagen via demineralization in 0.2 N hydrochloric acid (HCl) for ~96 hr at room temperature with replacement of the acid solution every ~24 hr. Each sample was washed with successive rinses of deionized (DI) water and lipids were extracted by repeated soaks in a 2:1 chloroform:methanol solvent solution, rinsed with DI water, and dried for ~15 hr in a freeze-dryer. White dorsal muscle of fishes and the mantle of cephalopods were stored at −20°C until preparation for isotope analyses. We then collected a section of muscle tissue from each fish from the region between the lateral line and the dorsal fin. We rinsed samples with deionized water to remove external contaminants and dried them at 60°C for 48 hr. We then ground the dried tissues to a fine powder using a mortar and pestle. No lipid corrections were applied to prey $\delta^{13}C$ values because muscle samples had weight percent C:N ratios (<3.5), indicating negligible lipid contents (Post et al., 2007).

We weighed dried collagen or muscle/mantle tissue (~0.6 mg) into tin capsules and measured $\delta^{13}C$ and $\delta^{15}N$ values using a Costech 4010 Elemental Analyzer (Valencia, CA) coupled to a Thermo Scientific Delta V Plus (Bremen, Germany) isotope ratio mass spectrometer at the University of New Mexico Center for Stable Isotopes (Albuquerque, NM). Results are expressed in parts per thousand (‰) and delta notation (δ) using the equation: $\delta_{sample} = [R_{sample}/R_{standard} - 1] \times 1.000$, where Rsample and Rstandard are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the sample and standard, respectively (Peterson & Fry, 1987). The standards are Vienna Pee Dee Belemnite limestone (V-PDB) for $\delta^{13}C$ and atmospheric N^2 for $\delta^{15}N$. The analytical precision based on the within-run standard deviation of protein reference materials (casein and acetanilide) was ≤0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. To directly compare the carbon isotope composition of historical and modern specimens, we applied a correction factor of 0.022%/year to $\delta^{13}C$ values of historical specimens to account for the Suess effect, or the anthropogenic decrease in the $\delta^{13}C$ values of atmospheric CO2 caused by burning of fossil fuels (Francey et al., 1999; Indermühle et al., 1999). We also measured the C:N ratios of each sample, which were in the expected range (2.8–3.5) for pure protein (Ambrose, 1990; see Supplementary Material Table S1).

Data analysis

$\delta^{13}C$ and $\delta^{15}N$ variation among species and years

We fitted Gamma generalized linear models (GLM) with an inverse link function (Zuur, Ieno, Walker, Saveliev, & Smith, 2009) to model $\delta^{13}C$ and $\delta^{15}N$ values (response variables) as a function of species and stranding year (explanatory variables). As $\delta^{13}C$ values are negative and Gamma family requires positive data values, we used $\delta^{13}C$ absolute values. We ran models separately for $\delta^{13}C$ and $\delta^{15}N$ considering additive, interactive, and isolated relations between response and explanatory variables. Model selection was performed through a stepwise backward procedure, and the best model was identified by adjusted Akaike's information criterion (AICc) and Akaike weights (Burnham & Anderson, 2002). Significant models with delta AICc ≤2 were considered suitable to explain the variation in $\delta^{13}C$ and $\delta^{15}N$ values and residuals were checked to ensure normality.

Isotopic niche width and overlap

We compared the isotopic niche width of each species by generating bivariate ellipses in SIBER (Stable Isotope Bayesian Ellipses in R; Jackson et al., 2011), which employs Markov-Chain Monte Carlo (MCMC) simulations to construct parameters of ellipses based on sampling points. We estimated the standard ellipse area corrected for small sample sizes (SEAc, expressed as ‰^{2}), which represents the mean core area of each species' isotopic niche (Jackson et al., 2011; Layman et al., 2007). We used the SEAc to estimate the proportional niche width overlap among species. Additionally, we calculated the Bayesian standard ellipse area (SEA_B) to obtain unbiased estimates of the isotopic niche widths with credibility intervals (Jackson et al., 2011). To test for significant differences, we ran 20,000 MCMC iterations and constructed 95% credible intervals around the mean of each species.

Stable isotope mixing models

We estimated the relative contribution of prey species from different trophic levels to the diet of the three dolphin species using the Stable Isotope Mixing Models in R (simmr) package (Parnell, 2016). This Bayesian framework uses robust probability estimates to assess the distribution of sources contribution to the assimilated diet of consumers (Parnell et al., 2010) and incorporates $\delta^{13}C$ and $\delta^{15}N$ of consumers, $\delta^{13}C$ and $\delta^{15}N$ means and standard deviation of both the sources of prey (see Supplementary Material Table S2) and trophic discrimination factors (TDFs) defined as the difference in isotope values between consumers and their diet. We ran mixing models separately for each dolphin species with prey sources identified from previous stomach content analysis of individuals stranded in our study area and adjacent areas (Bassoi, 2005; Daura-Jorge et al., 2011; Milmann et al., 2016); note that the suite of prey differed among models for each dolphin species (Table S2). This approach enabled us to compare the long-term contribution of prey from different trophic levels to the diet of each dolphin species.

As there are no estimates of $\delta^{13}C$ and $\delta^{15}N$ TDFs values for dolphin bone collagen, we used the published mean (± *SD*) value of diet-to-skin TDF estimates for the bottlenose dolphin of 1.0‰ ± 0.4‰ for $\delta^{13}C$ and 1.6‰ ± 0.5‰ for $\delta^{15}N$ (Gimenez, Ramirez, Almunia, Forero, & de Stephanis, 2016), and adjusted them using $\delta^{13}C$ and $\delta^{15}N$ values of paired skin and bone collagen of franciscana dolphin ($n = 7$) (S.B., unpublished data). The mean difference between paired skin and collagen δ13C values was +2.6‰ and + 0.5‰ for $\delta^{13}C$ and $\delta^{15}N$ values, respectively. These mean values were added to the reported TDFs values for bottlenose dolphin (Gimenez et al., 2016) to yield final values of $\Delta^{13}C = 3.6‰ \pm 0.4‰$ and $\Delta^{15}N = 2.1‰ \pm 0.5‰$. Mean $\delta^{13}C$ and $\delta^{15}N$ values of prey for each dolphin species were analyzed with a cluster analysis (Ward's minimum variance method) to group them for running the mixing models. Ecological groups were defined considering the clusters, the prey habitat type (pelagic vs. demersal) and trophic levels, including high and low trophic level pelagic fish, high and low trophic level demersal fish, and cephalopods (see Supplementary Material Figures S2, S3, and S4, Table S2). Results are reported as mean ± standard deviation (*SD*) unless otherwise stated.

RESULTS

$\delta^{13}C$ and $\delta^{15}N$ variation among species

$\delta^{13}C$ values were similar among the three dolphin species varying from −13.4‰ ± 0.4‰ for franciscana, −12.9‰ ± 0.4‰ for Guiana, and − 12.7‰ ± 0.7‰ for the bottlenose dolphin. In contrast, franciscana dolphin had the highest mean δ^{15}N values (16.9‰ ± 0.8‰), followed by Guiana (16.1‰ ± 0.6‰) and the bottlenose dolphin (15.5‰ ± 1.6‰) (Figure 2a).

The most parsimonious models in our two candidate set had contrasting δ^{13}C and δ^{15}N values for species (Table 1), with δ^{13}C values significantly lower ($t = -5.170$, $p < .001$) and δ^{15}N values significantly higher ($t = -4.734$, $p < .001$) for franciscana in relation to Guiana and the bottlenose dolphin. No residual patterns were evident in the validation of these models. The model that included the dolphin species and year of collection was also one of the best candidate models, suggesting a temporal effect in δ^{13}C values dependent of species, the AIC value of this model was 1.2 units higher than the model that only included species as an explanatory variable. δ^{13}C decreased for the bottlenose dolphin but increased for Guiana across years; there was no significant temporal trend in franciscana (Figure 2b). The fitted model for δ^{15}N values suggests variation among species with no temporal effect within each dolphin species (Figure 2c).

Table 1 Generalized linear models with Gamma family and inverse link function for δ^{13}C and δ^{15}N values with species, year, and interaction terms as explanatory variables. Models are ranked by the adjusted Akaike information criterion (AICc).

	Models	*df*	logLink	AICc	ΔAICc	w_i	pseudo-R^2
δ^{13}C	*Species*	4	- 49.49	107.6	0.0	0.515	0.38
δ^{13}C	*species * year*	7	- 46.56	108.8	1.2	0.278	0.45
δ^{13}C	*species + year*	5	- 49.25	109.4	1.8	0.207	0.39
δ^{13}C	*Year*	3	- 63.41	133.2	25.5	0.000	0.01
δ^{15}N	*Species*	4	-107.20	223.0	0.0	0.667	0.26
δ^{15}N	*species + year*	5	-106.98	223.9	1.8	0.268	0.27
δ^{15}N	*species * year*	7	-106.18	228.0	5.0	0.054	0.28
δ^{15}N	*Year*	3	-117.88	242.0	19.1	0.000	0.01

*indicates an interaction; AICc is the difference between the AICc of the model and the minimum AICc; wi indicates the AIC weights.

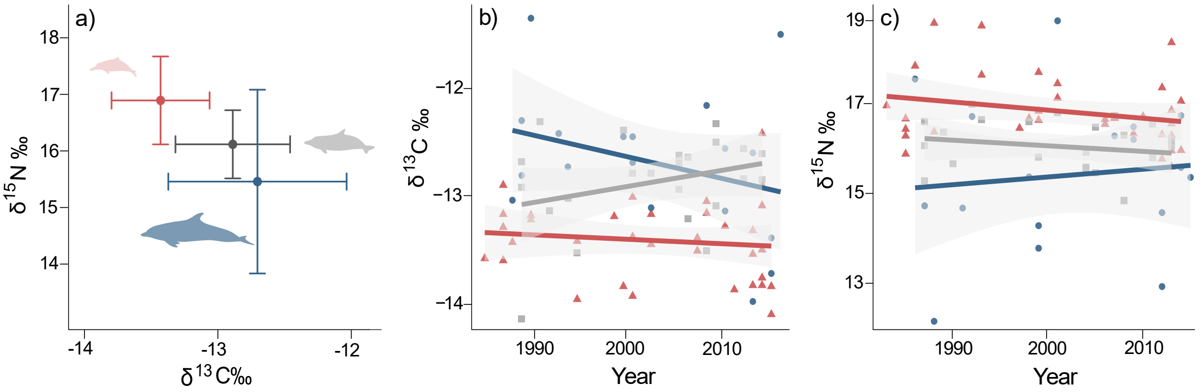

Figure 2 a) Bone collagen δ^{13}C and δ^{15}N mean values for the franciscana (*Pontoporia blainvillei*), Guiana (*Sotalia guianensis*), and Lahille's bottlenose (*Tursiops truncatus gephyreus*) dolphins. b) Temporal trends in bone collagen δ^{13}C and c) δ^{15}N values of franciscana (red triangles) Guiana (gray triangles), and bottlenose (blue triangles) dolphins collected from 1985 to 2017

Isotopic niche width and overlap

The bottlenose dolphin had the largest standard ellipse area (SEAc; 3.5‰^{2} and SEA$_B$: 3.2‰^{2}; CI: 1.9‰2–5.1‰^{2}) while Guiana (SEAc: 0.8‰2 and SEA$_B$: 0.8‰^{2}; 95% CI: 0.5‰2–1.2‰^{2}) and franciscana (SEAc: 0.9‰^{2} and SEAB: 0.9‰^{2}; 95% CI: 0.6‰^{2}–1.2‰^{2}) had much smaller areas. Based on SEAc estimates, Guiana (0.1‰2) and the bottlenose dolphin (0.2‰^{2}) had minimal overlap with franciscana (Figure 3). In contrast, the SEAc ellipses of Guiana and the bottlenose dolphin overlapped by 0.9‰^{2}, which represent 100% of the former and 24.4% of the latter species isotopic niche width.

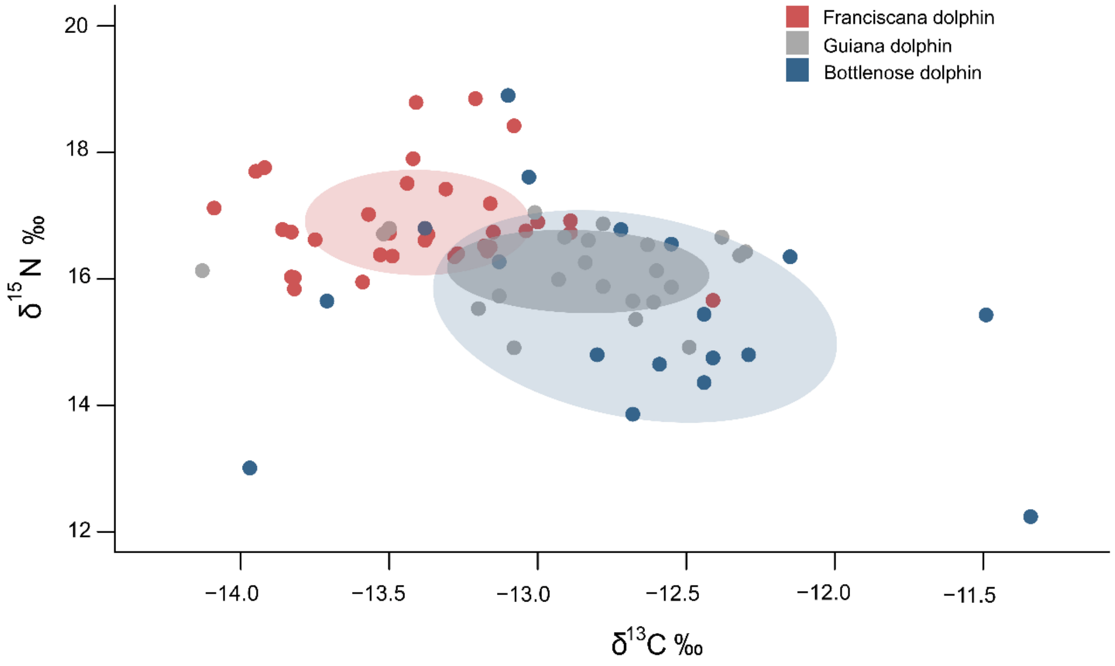

Figure 3 $\delta^{13}C$ and $\delta^{15}N$ biplot illustrating the isotopic niche of franciscana (*Pontoporia blainvillei*), Guiana (*Sotalia guianensis*) and Lahille’s bottlenose (*Tursiops truncatus gephyreus*) dolphins off the Santa Catarina coast in southern Brazil. Each point represents an individual and solid ellipses represent the standard ellipse area corrected for small sample sizes (SEAc)

Stable isotope mixing models

Stable isotope composition of potential prey items varied from −18.1‰ ± 0.4‰ to −14.2‰ ± 0.5‰ for $\delta^{13}C$ and from 13.3‰ ± 0.5‰ to 17.0‰ ± 0.3‰ for $\delta^{15}N$. Bayesian mixing model-based estimates of trophic levels revealed interspecific differences among the three species (Table 2). Low trophic level pelagic fish contributed a median of 51% (CI: 0.14%–0.80%) to the diet of franciscana, while low trophic level demersal fish contributed a median of 46% (CI: 0.25%–0.58%) to the diet of Guiana. Low trophic level demersal fish were also important for the bottlenose dolphin and contributed a mean of 72% (CI: 0.45%–0.94%) to this species diet (Figure 4, Table 2).

Table 2 Stable isotope mixing model results with predicted diet proportions (2.5% and 97.5% quantiles) of each prey group to the diet of the franciscana (*Pontoporia blainvillei*), Guiana (*Sotalia guianensis*) and Lahille's bottlenose (*Tursiops truncatus gephyreus*) dolphin off the coast of Santa Catarina, southern Brazil. Mean values are in parentheses and bold values represent the highest prey item contribution

Species	Pel HL	Pel LL	Dem HL	Dem LL	Dem	Ceph

Franciscana dolphin	0.02-0.39 (0.16)	0.14-0.80 **(0.51)**	-	-	0.02-0.47 (0.18)	0.03-0.30 (0.15)
Guiana dolphin	0.01-0.20 (0.06)	0.01-0.57 (0.15)	0.05-0.42 (0.27)	0.25-0.58 **(0.46)**	-	0.01-0.12 (0.04)
Bottlenose dolphin	0.01-0.23 (0.07)	-	0.01-0.41 (0.14)	0.45-0.94 **(0.72)**	-	0.01-0.06 (0.07)

Pel HL = Pelagic fish from high trophic levels, Pel LL = Pelagic fish from low trophic levels, Dem = Demersal fish, Dem HL = Demersal fish from high trophic levels, Dem LL = Demersal fish from low trophic levels, Ceph = cephalopods. See Electronic Supplementary Material; Table 2 for prey species included in each ecological group as they may not include the same items for each dolphin species

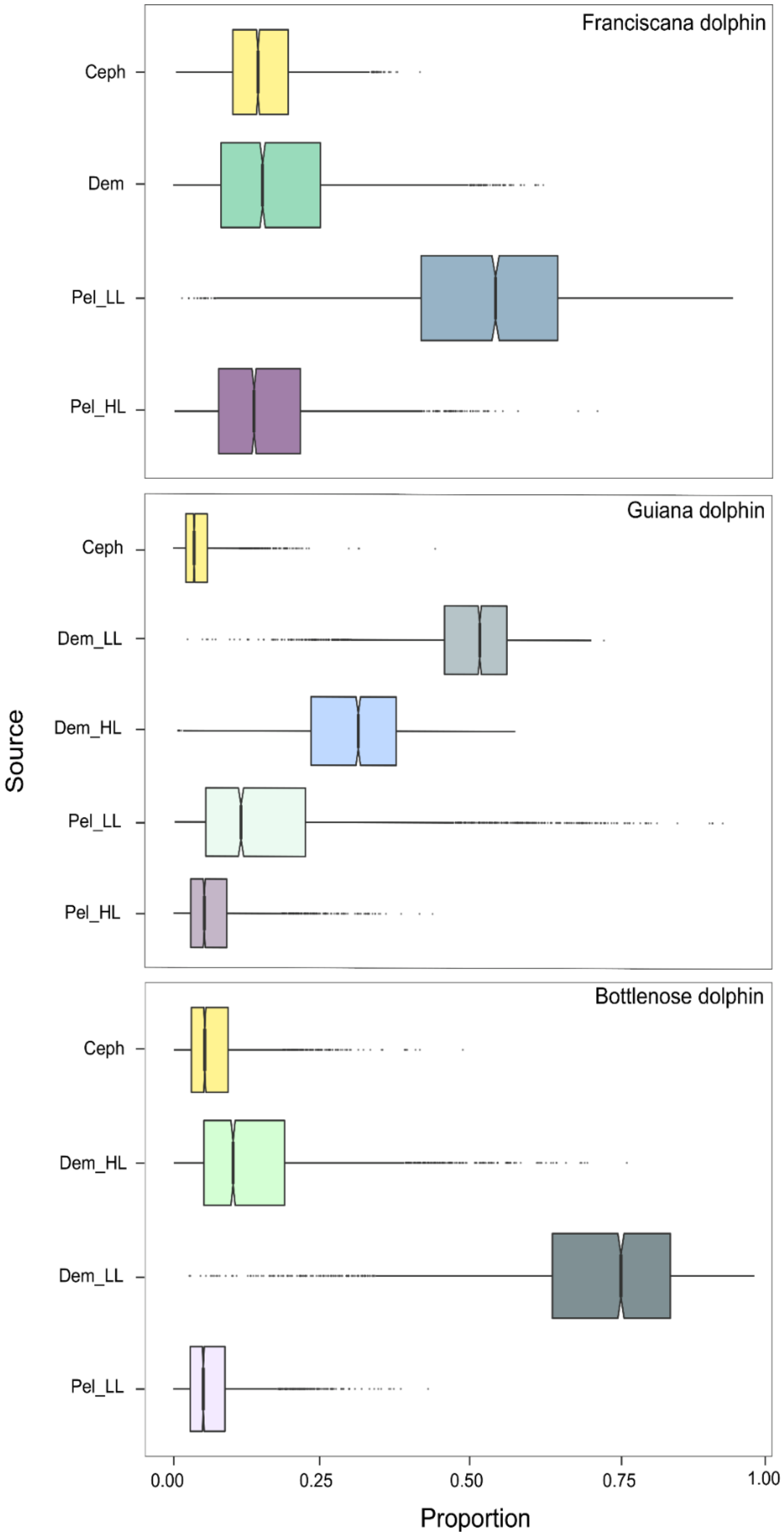

Figure 4 Bayesian mixing model based on estimates of trophic levels (mean, 25% and 75% percentiles) for the franciscana (*Pontoporia blainvillei*), Guiana (*Sotalia guianensis*) and Lahille's bottlenose (*Tursiops truncatus gephyreus*) dolphins off the Santa Catarina coast in southern Brazil. Pel HL = Pelagic fish from high trophic levels, Pel LL = Pelagic fish from low trophic levels, Dem = Demersal fish, Dem HL = Demersal fish from high trophic levels, Dem LL = Demersal fish from low trophic levels, Ceph = cephalopods.

DISCUSSION

Our study is the first to assess resource partitioning among franciscana, Guiana, and Lahille's bottlenose dolphins within the unique area where these three species coexist. Our main findings suggest some degree of spatial segregation in foraging behavior and prey preferences for these species along the southern Brazilian coast. Stable isotope analysis showed that franciscana primarily consumes low trophic level pelagic prey like *E. anchoita*, while Guiana and the bottlenose dolphin tend to consume demersal fish species such as *Mugil curema* and *M. liza,* respectively. Relative to the size of the distribution of each species, their sympatry along the southern Brazilian coast seems to be restricted to a rather small latitudinal band (Figure 1). Locally, franciscana uses open waters with mean depth of 30 m (Danilewicz et al., 2009), which may favor selection of pelagic prey in the water column or near the surface. In contrast, the Guiana and the coastal ecotype of the bottlenose dolphin along the southern Brazilian coast use shallower waters with a mean depth of 3 m (Di Tullio, Fruet, & Secchi, 2015; Simões-Lopes et al., 2019; Wedekin, Daura-Jorge, & Simões-Lopes 2010), which favors selection of demersal prey captured on or near the bottom. Indeed, the minimal isotopic overlap observed between franciscana and the delphinid species can be associated with this broader distribution of franciscana in open waters that likely results in low $\delta^{13}C$ values.

The different trophic position occupied by the two delphinid species was a result of low $\delta^{15}N$ values of Guiana and the bottlenose dolphin compared to franciscana, which likely reflect consumption of prey sources from different habitat types (pelagic vs. demersal). The $\delta^{13}C$ and $\delta^{15}N$ mean values and the high degree of isotopic niche overlap between Guiana and the bottlenose dolphin suggest similar foraging areas and/or prey preferences with both species occupying equivalent trophic positions. This high degree of overlap can be related to the important contribution of demersal fish species to both Guiana and the bottlenose dolphin's diet. Although prey sources used for mixing models differ among delphinid species, it is important to note that both Guiana and the bottlenose dolphin are primarily consuming mullet species along the southern Brazilian coast (Daura-Jorge et al., 2011; Milmann et al., 2016). *Mugil curema* and *M. liza* are common dolphin prey that consume a mixed diet of macroalgae, microphytobenthos, and small benthic invertebrates and thus likely occupy a lower trophic level than more pelagic prey (Cardona, 2001; Garcia et al., 2018; Drake, Arias, & Gállego, 1984). Consumption of these omnivorous prey could contribute to the similar and lower δ15N values of Guiana and the bottlenose dolphin relative to franciscana. In contrast, pelagic fish assemblages are usually planktophagous or piscivorous, which may explain the relatively high

$\delta^{15}N$ values and by extension higher trophic level of franciscana dolphin compared to the other species.

Bottlenose dolphins are known to exhibit behavioral flexibility with a wide variety of foraging strategies and prey selection via adaptation to local ecological conditions (Sargeant, Wirsing, Heithaus, & Mann, 2007). Patterns in SEA_C and SEA_B estimates among species show that bottlenose dolphins consume a more diverse set of prey than franciscana and Guiana dolphins. This pattern may be driven in part by the possibility that our sample of bottlenose dolphin represent a mixture of resident and transient individuals that use different foraging strategies (Fruet et al., 2014). A subset of the small and highly resident bottlenose dolphin population of Laguna in southern Brazil seasonally consumes a high proportion of M. liza in cooperation with artisanal fishermen, which is a unique and specialized foraging tactic (Daura-Jorge, Cantor, Ingram, Lusseau, & Simões-Lopes, 2012; Simões-Lopes, Fabian, & Menegheti, 1998). In contrast, coastal transient individuals forage over a broader geographical area in a variety of habitats and, by extension, likely have more diverse diets. The broader geographic range and the wide variety of foraging tactics showed by the bottlenose dolphin may explain why some individuals plot outside of the mixing polygon produced from analysis of the limited prey types analyzed here (see Supplementary Material Figure S5). The similarity between SEA_C and SEA_B observed for franciscana and Guiana has been previously observed for populations off the southeastern Brazilian coast (Di Beneditto & Monteiro, 2016). The narrowness of SEA_C and SEA_B of Guiana is consistent with the restricted home range and site fidelity of the resident population that inhabits the northern bay of Santa Catarina (Flores, 1999; Wedekin, Daura-Jorge, Piacentini, & Simões-Lopes, 2007). In addition, this resident population seems to avoid productive areas near mangroves or the mouths of estuaries that are close to urban areas, thereby reducing their access to a more diverse array of prey types (Wedekin et al., 2007).

Guiana and the bottlenose dolphin had similar $\delta^{13}C$ values, suggesting that they tend to forage in similar habitats. Observational data, however, suggest a fine-scale segregation in habitat use between these two species, where the resident Guiana population is restricted to the western section of the northern bay of Santa Catarina and bottlenose dolphin occurs mostly in the eastern section of the bay (Flores & Fontoura, 2006; Wedekin et al., 2007). These habitat preferences likely reduce either interference or exploitative competition (Wedekin, Daura-Jorge, & Simões-Lopes, 2004). Interestingly, these two species showed opposite patterns in $\delta^{13}C$ values over time, and we hypothesize that the observed temporal shifts in $\delta^{13}C$ values through time may reflect changes in resource availability. Specifically, the increases in $\delta^{13}C$ values observed in Guiana through time is consistent with a recent shift in habitat use of this resident population, which moved from a small and open inlet to a wider and more protected inlet likely due to changes in prey abundance (Wedekin et al., 2007). It is important to note that we cannot rule out the possibility that the $\delta^{13}C$ values of potential prey has changed over time, which may contribute to the observed temporal variation in dolphin carbon isotope composition.

As mentioned above, the apparent interaction between foraging ecology and commercial fishing activities has been previously noted for franciscana and the bottlenose dolphin (Secchi et al., 2003, 2016) from adjacent waters in southern Brazil. Additionally, the mullet species (*M. liza*) consumed by bottlenose dolphins are increasingly over- exploited in southern Brazil (Chao et al., 2015; de Abreu-Mota et al., 2018; Haimovici, 1998; Haimovici & Cardoso, 2017). Not only are current landings of *M. liza* unsustainable (Sant'Ana & Kinas, 2018), Santa Catarina also has produced the largest proportion (45%) of total mullet (Mugil) landings for any region in southern Brazil over the past 30 years (de Abreu-Mota et al., 2018). The increased local fishing pressure on mullet and its high contribution to bottlenose dolphin diet raise concerns about resource competition with fisheries, which could lead to changes in diet composition, and by extension, influence how it coexists with the two other dolphin species in this region.

These dolphin species are all considered to be threatened to various degrees primarily by high bycatch-related mortality (Fruet et al., 2012; Secchi, 2010). Our isotope-based approach suggests that franciscana, Guiana, and the bottlenose dolphin may be threatened by resource competition with fisheries, which impacts how they partition prey sources and foraging habitats along the southern Brazilian coast. Some of the prey species shown by mixing models and previous stomach content analysis (Bassoi, 2005; Daura-Jorge et al., 2011; Milmann et al., 2016) to contribute significantly to dolphin diets are considered threatened by overexploitation. For example, *M. atricauda* has suffered a marked decrease in biomass in recent decades, indicating a decline of at least 50% in commercial stocks of this species (Carvalho-Filho, Santos, & Sampaio, 2010). Similarly, local artisanal fishers have reported a severe decline in catches of *M. furnieri* and *M. liza* (Martins, Medeiros, Di Domenico, & Hanazaki, 2018). Thus, changes in abundance of these prey sources—whether due to environmental effects or unsustainable fishing pressure—has the potential to impact resource and habitat use among these sympatric dolphin species and should be closely monitored. If food resources become limited, interspecific competition among these three sympatric species will increase, which may have a variety of ecological implications. For example, both inter- and intraspecific competition influence both the total niche width and prevalence of individual specialization (Araújo et al., 2010). Therefore, future studies on within and between individual diet variation could strengthen our understanding of how these three species coexist and are impacts by future changes in resource availability. By providing both population- and individual-level estimates of dietary niche, isotope analysis could be an effective tool to better understand franciscana, Guiana, and Lahille's bottlenose dolphin population dynamics and contribute with species management and conservation plans.

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AUTHOR CONTRIBUTIONS

Clarissa Teixeira: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing-original draft; writing- review and editing. Silvina Botta: Data curation; formal analysis; funding acquisition; methodology; resources; software; supervision; validation; visualization; writing-original draft; writing-review and editing. Fabio Daura-Jorge: Conceptualization; data curation; formal analysis; funding acquisition; investigation; project administration; resources; software; supervision; validation; visualization; writing-original draft; writing-review and editing. Luiza Pereira: Conceptualization; formal analysis; investigation; visualization; writing-original draft; writing-review and editing. Seth Newsome: Data curation; formal analysis; funding acquisition; investigation; methodology; validation; visualization; writing-original draft; writing-review and editing. P. C. Simões Lopes: Conceptualization; funding acquisition; investigation; project administration; resources; supervision; validation; visualization; writing-original draft; writing-review and editing.

REFERENCES

Ambrose, S. H. (1990). Preparation and characterization of bone and tooth collagen for isotopic analysis. *Journal of Archaeological Science, 17,* 431–451.

Araújo, M. S., Martins, E. G., Cruz, L. D., Fernandes, F. R., Linhares, A. X., Dos Reis, S. F., & Guimaraes Jr, P. R. (2010). Nested diets: a novel pattern of individual-level resource use. *Oikos, 119*, 81–88.

Barreto, A. S., & Rosas, F. C. (2006). Comparative growth analysis of two populations of *Pontoporia blainvillei* on the Brazilian coast. *Marine Mammal Science*, *22*, 644–653.

Bassoi, M. (2005). *Feeding ecology of franciscana dolphin, Pontoporia blainvillei (Cetacea: Pontoporiidae), and oceanographic processes on the southern Brazilian coast.* Ph.D. thesis, University of Southampton, Southampton, England.

Bearhop, S., Adams, C. E., Waldron, S., Fuller, R. A., & MacLeod, H. (2004). Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology, 73,* 1007–1012.

Bearzi, M. (2005). Dolphin sympatric ecology. *Marine Biology Research, 1,* 165–175.

Bittar, V. T., & Di Beneditto, A. P. M. (2009). Diet and potential feeding overlap between *Trichiurus lepturus* (Osteichthyes: Perciformes) and *Pontoporia blainvillei* (Mammalia: Cetacea) in northern Rio de Janeiro, Brazil. *Zoologia, 26*.

Botta, S., Secchi, E. R., Muelbert, M. M., Danilewicz, D., Negri, M. F., Cappozzo, H. L., & Hohn, A. A. (2010). Age and growth of franciscana dolphins, *Pontoporia blainvillei* (Cetacea: Pontoporiidae) incidentally caught off southern Brazil and northern Argentina. *Journal of the Marine Biological Association of the United Kingdom*, *90*, 1493–1500.

Botta, S., Muelbert, M. M. C., & Secchi, E. R. (2012). Morphometric relationship of Franciscana dolphin, *Pontoporia blainvillei* (Cetacea), off Rio Grande do Sul coast, Southern Brazil. *Latin American Journal of Aquatic Mammals, 5,* 117–123.

Bugoni, L., McGill, R., & Furness, R. W. (2010). The importance of pelagic longline fishery discards for a seabird community determined through stable isotope analysis. *Journal of Experimental Marine Biology and Ecology, 391*, 190–200.

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: a practical information-theoretic approach.* Springer-Verlag, New York.

Cardona, L. (2001). Non-competitive coexistence between Mediterranean grey mullet: evidence from seasonal changes in food availability, niche breath and trophic overlap. *Journal of Fish Biology*, *59*, 729–744.

Carvalho-Filho, A., Santos, S., & Sampaio, I. (2010). *Macrodon atricauda* (Günther, 1880) (Perciformes: Sciaenidae), a valid species from the southwestern Atlantic, with comments on its conservation. *Zootaxa*, *2519*, 48–58.

Cassens, I., Vicario, S., Waddell, V. G., Balchowsky, H., Van Belle, D., Ding, W., ... & Meyer, A. (2000). Independent adaptation to riverine habitats allowed survival of ancient cetacean lineages. *Proceedings of the National Academy of Sciences*, *97*, 11343–11347.

Castello, J. P., Sunyé, P. S., Haimovici, M., & Hellebrandt, D. (2009). Fisheries in southern Brazil: a comparison of their management and sustainability. *Journal of Applied Ichthyology*, *25*, 287–293.

Chao, N. L., Frédou, F. L., Haimovici, M., Peres, M. B., Polidoro, B., Raseira, M., ... & Carpenter, K. (2015). A popular and potentially sustainable fishery resource under pressure–extinction risk and conservation of Brazilian Sciaenidae (Teleostei: Perciformes). *Global Ecology and Conservation, 4,*117–126.

Chase, J. M., & Leibold, M. A. (2003). *Ecological niches: linking classical and contemporary approaches.* University of Chicago Press.

Ciotti, Á. M., Odebrecht, C., Fillmann, G., & Moller Jr, O. O. (1995). Freshwater outflow and Subtropical Convergence influence on phytoplankton biomass on the southern Brazilian continental shelf. *Continental Shelf Research, 15*, 1737–1756.

Cremer, M. J., Pinheiro, P. C., & Simões-Lopes, P. C. (2012). Presas consumidas pelo boto-cinza *Sotalia guianensis* (Cetacea, Delphinidae) e pela toninha *Pontoporia blainvillei* (Cetacea, Pontoporiidae) em ambiente estuarino no sul do Brasil.

Iheringia. Série Zoologia,_102, 131–137.

Cremer, M. J., Holz, A. C., Sartori, C. M., Schulze, B., Paitach, R. L., & Simões-Lopes, P. C. (2018). Behavior and Ecology of Endangered Species Living Together: Long-Term Monitoring of Resident Sympatric Dolphin Populations. In M. R. Rossi-Santos & C. W. Finkl (Eds), *Advances in Marine Vertebrate Research in Latin America* (pp. 477–508). Springer Publishing.

Danilewicz, D., Secchi, E. R., Ott, P. H., Moreno, I. B., Bassoi, M., & Borges-Martins, M. (2009). Habitat use patterns of franciscana dolphins (*Pontoporia blainvillei*) off southern Brazil in relation to water depth. *Journal of the Marine Biological Association of the United Kingdom, 89,* 943–949.

Daura-Jorge, F. G., Wedekin, L. L., & Simões-Lopes, P. C. (2011). Feeding habits of the Guiana dolphin, *Sotalia guianensis* (Cetacea: Delphinidae), in Norte Bay, southern Brazil. *Scientia Marina, 75,* 163–169.

Daura-Jorge, F. G., Cantor, M., Ingram, S. N., Lusseau, D., & Simões-Lopes, P. C. (2012). The structure of a bottlenose dolphin society is coupled to a unique foraging cooperation with artisanal fishermen. *Biology Letters,* 1–4.

de Abreu-Mota, M. A., Medeiros, R. P., & Noernberg, M. A. (2018). Resilience thinking applied to fisheries management: perspectives for the mullet fishery in Southern-Southeastern Brazil. *Regional Environmental Change,* 1–12.

DeNiro, M. J., & Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta, 42,* 495–506.

Di Beneditto, A. P. M., & Monteiro, L. R. (2016). Isotopic niche of two coastal dolphins in a tropical marine area: specific and age class comparisons. *Journal of the Marine Biological Association of the United Kingdom, 96,* 853–858.

Di Tullio, J. C., Fruet, P. F., & Secchi, E. R. (2015). Identifying critical areas to reduce bycatch of coastal common bottlenose dolphins Tursiops truncatus in artisanal fisheries of the subtropical western South Atlantic. *Endangered Species Research, 29,* 35–50.

Drake, P., Arias, A. M., & Gállego, L. (1984). La biología de los mugílideos (Osteichthyes, Mugilidae) en los esteros de las salinas de San Fernando (Cádiz). III. Hábitos alimentarios y su relación con la morfometría del aparato digestivo. *Investigaciones Pesqueras, 48,* 337–367.

Flores, P. A. C. (1999). Preliminary results of a photo identification study of the marine tucuxi, *Sotalia fluviatilis*, in Southern Brazil. *Marine Mammal Science, 15,* 840–847.

Flores, P. A. C., & Fontoura, N. F. (2006). Ecology of marine tucuxi and bottlenose dolphins in Baía Norte, Santa Catarina State, southern Brazil. *Latin American Journal of Aquatic Mammals, 5,* 105–115.

Franco-Trecu, V., Drago, M., Costa, P., Dimitriadis, C., & Passadore, C. (2017). Trophic relationships in apex predators in an estuary system: A multiple-method approximation. *Journal of Experimental Marine Biology and Ecology, 486,* 230–236.

Francey, R. J., Allison, C. E., Etheridge, D. M., Trudinger, C. M., Enting, I. G., Leuenberger, M., ... & Steele, L. P. (1999). A 1000-year high precision record of δ13C in atmospheric CO2. *Tellus B: Chemical and Physical Meteorology,* 51, 170–193.

Fruet, P. F., Kinas, P. G., da Silva, K. G., Di Tullio, J. C., Monteiro, D. S., Dalla Rosa, L., ... & Secchi, E. R. (2012). Temporal trends in mortality and effects of by-catch on common bottlenose dolphins, *Tursiops truncatus*, in southern Brazil. *Journal of Marine Biological Association of United Kingdom, 92,* 1865−1876.

Fruet, P. F., Secchi, E. R., Daura-Jorge, F., Vermeulen, E., Flores, P. A., Simões-Lopes, P. C., ... & Dalla Rosa, L. (2014). Remarkably low genetic diversity and strong population structure in common bottlenose dolphins (*Tursiops truncatus*) from coastal waters of the Southwestern Atlantic Ocean. *Conservation Genetics*, *15*, 879−895.

Fruet, P. F., Daura-Jorge, F. G., Möller, L. M., Genoves, R. C., & Secchi, E. R. (2015). Abundance and demography of bottlenose dolphins inhabiting a subtropical estuary in the Southwestern Atlantic Ocean. *Journal of Mammalogy*, *96*, 332−343.

Fry, B. (2006). *Stable isotope ecology*. Springer, New York, NY.

Garcia, A. F. S., Garcia, A. M., Vollrath, S. R., Schneck, F., Silva, C. F. M., Marchetti, Í. J., & Vieira, J. P. (2018). Spatial diet overlap and food resource in two congeneric mullet species revealed by stable isotopes and stomach content analyses. *Community Ecology, 19,* 116−124.

Geange, S. W., Pledger, S., Burns K. C., & Shima, J. S. (2011). A unified analysis of niche overlap incorporating data of different types. *Methods in Ecology and Evolution, 2,* 175−184.

Gimenez, J., Ramirez, F., Almunia, J., Forero, M. G., & de Stephanis, R. (2016). From the pool to the sea: applicable isotope turnover rates and diet to skin discrimination factors for bottlenose dolphins (*Tursiops truncatus*). *Journal of Experimental Marine Biology and Ecology, 475,* 54–61.

Haimovici, M., Martins, A. S., & Vieira, P. C. (1996). Distribuição e abundância de peixes teleósteos demersais sobre a plataforma continental do sul do Brasil. *Revista Brasileira de Biologia, 56,* 27−50.

Haimovici, M., Mendonça, J. T. (1996) Descartes da fauna acompanhante na pesca de arrasto e tangones dirigida a linguados e camarões na plataforma continental do sul do Brasil. *Atlântida*, *18*, 161−177.

Haimovici, M. (1998). Present state and perspectives for the Southern Brazil shelf demersal fisheries. *Fisheries Management and Ecology, 5,* 277–289.

Haimovici, M., Fischer, L. G. (2007) Avaliação de descartes na pesca de arrasto. In M. Haimovici (Ed.), *A prospecção pesqueira e abundância de estoques marinhos no Brasil nas décadas de 1960 a 1990: levantamento de dados e avaliação crítica* (pp 165−183). MMA/SMCQ, Brasília.

Haimovici, M., & Cardoso, L. G. (2017). Long-term changes in the fisheries in the Patos Lagoon estuary and adjacent coastal waters in Southern Brazil. *Marine Biology Research, 13,* 135–150.

Indermühle, A., Stocker, T. F., Joos, F., Fischer, H., Smith, H. J., Wahlen, M., ... & Meyer, R. (1999). Holocene carbon-cycle dynamics based on CO_2 trapped in ice at Taylor Dome, Antarctica. *Nature, 398,* 121−26.

Jackson, A.L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses. *Journal of Animal Ecology, 80,* 595−602.

Kaiya, Z. (1982). Classification and phylogeny of the Superfamily Platanistoidea, with notes on evidence of the monophyly of the Cetacea. *The Scientific Reports of the Whales Research institute***,** *34,* 93−108.

Kiszka, J., Simon-Bouhet, B., Martinez, L., Pusineri, C., Richard, P., & Ridoux, V. (2011). Ecological niche segregation within a community of sympatric dolphins around a tropical island. *Marine Ecology Progress Series, 433,* 273−288.

Layman, C. A., Arrington, D. A., Montanä, C. G., & Post, D. M. (2007). Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology, 88,* 42−48.

Loizaga de Castro, R., Saporiti, F., Vales, D. G., Cardona, L., & Crespo, E. A. (2017). Using stable isotopes to assess whether two sympatric dolphin species share trophic resources. *Marine Mammal Science, 33,* 1235−1244.

Martins, I. M., Medeiros, R. P., Di Domenico, M., & Hanazaki, N. (2018). What fishers' local ecological knowledge can reveal about the changes in exploited fish catches. *Fisheries research*, *198*, 109−116.

Milmann, L., Danilewicz, D., Machado, R., Santos, R. A. D., & Ott, P. H. (2016). Feeding ecology of the common bottlenose dolphin, *Tursiops truncatus*, in southern Brazil: analyzing its prey and the potential overlap with fisheries. *Brazilian Journal of Oceanography, 64,* 415−422.

Möller Jr, O. O., Piola, A. R., Freitas, A. C., & Campos, E. J. D. (2008). The effects of river discharge and seasonal winds on the shelf off Southeastern South America. *Continental Shelf Research, 28,* 1607–1624.

Moreno, I. B., Tavares, M., Danilewicz, D., Ott, P. H., & Machado, R. (2009). Descrição da pesca costeira de média escala no litoral norte do Rio Grande do Sul: comunidades pesqueiras de Imbé/Tramandaí e Passo de Torres/Torres. *Boletim do Instituto de Pesca, 35,* 129−140.

Muelbert, J. H., Acha, M., Mianzan, H., Guerrero, R., Reta, R., Braga, E. S., ... & Ramírez, F. (2008). Biological, physical and chemical properties at the Subtropical Shelf Front Zone in the SW Atlantic Continental Shelf. *Continental Shelf Research, 28,* 1662−1673.

Nagata, R. M., Moreira, M. Z., Pimentel, C. R., & Morandini, A. C. (2015). Food web characterization based on d^{15}N and d^{13}C reveals isotopic niche partitioning between fish and jellyfish in a relatively pristine ecosystem. *Marine Ecology Progress Series, 519,* 13–27.

Newsome, S. D., Martinez del Rio, C., Bearhop, S., & Phillips, D. L. (2007). A niche for isotope ecology. *Frontiers in Ecology and the Environment, 5,* 429−436.

Newsome, S. D., Clementz, M. T., & Koch, P. L. (2010). Using stable isotope biogeochemistry to study marine mammal ecology. *Marine Mammal Science, 26,* 509−572.

Parnell, A. C., Inger, R., Bearhop, S., & Jackson, A. L. (2010). Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE, 5,* e9672.

Parnell, A. C. (2016). simmr-package A Stable Isotope Mixing Model. Version 0.3. ⟨*https://cran.r-project.org/web/packages/simmr/*⟩.

Peters, K. J., Bury, S. J., Betty, E. L., Parra, G. J., Tezanos-Pinto, G., & Stockin, K. A. (2020). Foraging ecology of the common dolphin *Delphinus delphis* revealed by stable isotope analysis. *Marine Ecology Progress Series*, *652*, 173−186.

Pianka, E. R. (1980). Guild structure in desert lizards. *Oikos,* 194−201.

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi J., & Montana, C. G. (2007). Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia, 152,* 179–189.

Rosas, F. C. W., & Monteiro-Filho, E. L. A. (2002). Reproduction of the estuarine dolphin (Sotalia guianensis) on the coast of Paraná, southern Brazil. *Journal of Mammalogy, 83,* 507–515.

Sant'Ana, R., & Kinas, P. G. (2018). Avaliação do Estoque de Tainha (*Mugil liza*): ampliação dos modelos Bayesianos de Dinâmica de Biomassa para múltiplas séries de CPUE, com adição de temperatura superficial do mar e capturabilidade autocorrelacionada. Oceana. http://brasil.oceana.org/sites/default/files/avaliacao_de_estoque_tainha_oceana__integra.pdf . Accessed 19 June 2019.

Santos-Carvallo, M., Pérez-Alvarez, M. J., Muniain, V., Moraga, R., Oliva, D., & Sepúlveda, M. (2015). Trophic niche overlap between sympatric resident and transient populations of bottlenose dolphins in the Humboldt Current System off North-Central Chile. *Marine Mammal Science, 31*, 790−799.

Sargeant, B. L., Wirsing, A. J., Heithaus, M. R., & Mann, J. (2007). Can environmental heterogeneity explain individual foraging variation in wild bottlenose dolphins (Tursiops sp.)? *Behavioral Ecology and Sociobiology, 61,* 679−688.

Schoener, T. W. (1974). Resource partitioning in ecological communities. *Science*, 27−39.

Secchi, E. R., Ott, P. H., & Danilewicz, D. (2003). Effects of fishing by-catch and conservation status of the franciscana dolphin, *Pontoporia blainvillei*. In N. Gales, M. Hindell, & K. Kirkwood, (Eds.), *Marine Mammals: Fisheries, Tourism and Management Issues* (pp. 174−191). Collinwoop: CSIRO Publishing.

Secchi, E. R. (2010). Review on the threats and conservation status of franciscana, Pontoporia blainvillei (Cetacea, Pontoporiidae). In M. Ruiz-García, & J. Shostel (Eds.), *Biology, evolution, and conservation of river dolphins within South America and Asia* (pp. 301–322). Wildlife Protection, Destruction and Extinction Series, Nova Science Publishers, New York.

Secchi, E. R., Botta, S., Wiegand, M. M., Lopez, L. A., Fruet, P. F., Genoves, R. C., Di Tullio, J. C. (2017). Long-term and gender-related variation in the feeding ecology of common bottlenose dolphins inhabiting a subtropical estuary and the adjacent marine coast in the western South Atlantic. *Marine Biology Research, 13,* 121–134.

Simões-Lopes, P. C., Fabian, M. E., & Menegheti, J. O. (1998). Dolphin interactions with the mullet artisanal fishing on Southern Brazil: a qualitative and quantitative approach. *Revista Brasileira de Zoologia, 15,* 709–726.

Simões-Lopes, P. C., Daura-Jorge, F. G., Lodi, L., Bezamat, C., Costa, A. P. B., & Wedekin, L. L. (2019). Bottlenose dolphin ecotypes of the western South Atlantic: the puzzle of habitats, coloration patterns and dorsal fin shapes. *Aquatic Biology, 28,* 101–111.

Taylor, M. A. (1987). How tetrapods feed in water: a functional analysis by paradigm. *Zoological Journal of the Linnean Society, 91,* 171–195.

Vasconcellos, M., & Gasalla, M. A. (2001). Fisheries catches and the carrying capacity of marine ecosystems in southern Brazil. *Fisheries Research*, *50*, 279–295.

Venuto, R., Botta, S., Barreto, A. S., Secchi, E. R., & Fruet, P. F. (2020). Age structure of strandings and growth of Lahille's bottlenose dolphin (*Tursiops truncatus gephyreus*). *Marine Mammal Science*, 1–15.

Wedekin, L. L., Daura-Jorge, F. G., & Simões-Lopes, P. C. (2004). An aggressive interaction between bottlenose dolphins (*Tursiops truncatus*) and estuarine dolphins (*Sotalia guianensis*) in southern Brazil. *Aquatic mammals, 30,* 391–397.

Wedekin, L. L., Daura-Jorge, F. G., Piacentini, V. Q., & Simões-Lopes, P. C. (2007). Seasonal variations in spatial usage by the estuarine dolphin, *Sotalia guianensis* (van Bénéden, 1864) (Cetacea; Delphinidae) at its southern limit of distribution. *Brazilian Journal of Biology, 67,* 1–8.

Wedekin, L. L., Daura-Jorge, F. G., & Simões-Lopes, P. C. (2010). Habitat preferences of guiana dolphins, *Sotalia guianensis* (Cetacea: Delphinidae), in Norte bay, southern Brazil. *Journal of the Marine Biological Association of the United Kingdom*, *90*, 1561–1570.

Werth, A. J. (2006). Mandibular and dental variation and the evolution of suction feeding in Odontoceti. *Journal of Mammalogy, 87,* 579−588.

Zenteno, L., Crespo, E., Vales, D., Silva, L., Saporiti, F., Oliveira, L. R., ... Cardona, L. (2014). Dietary consistency of male South American sea lions (*Otaria flavescens*) in southern Brazil during three decades inferred from stable isotope analysis. *Marine Biology, 162,* 275−289.

Zhao, T., Villéger, S., Lek, S., & Cucherousset, J. (2014). High intraspecific variability in the functional niche of a predator is associated with ontogenetic shift and individual specialization. *Ecology and Evolution, 4,* 4649−4657.

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). Mixed effects models and extensions in ecology with R. Springer Science & Business Media.

Electronic Supplementary Material

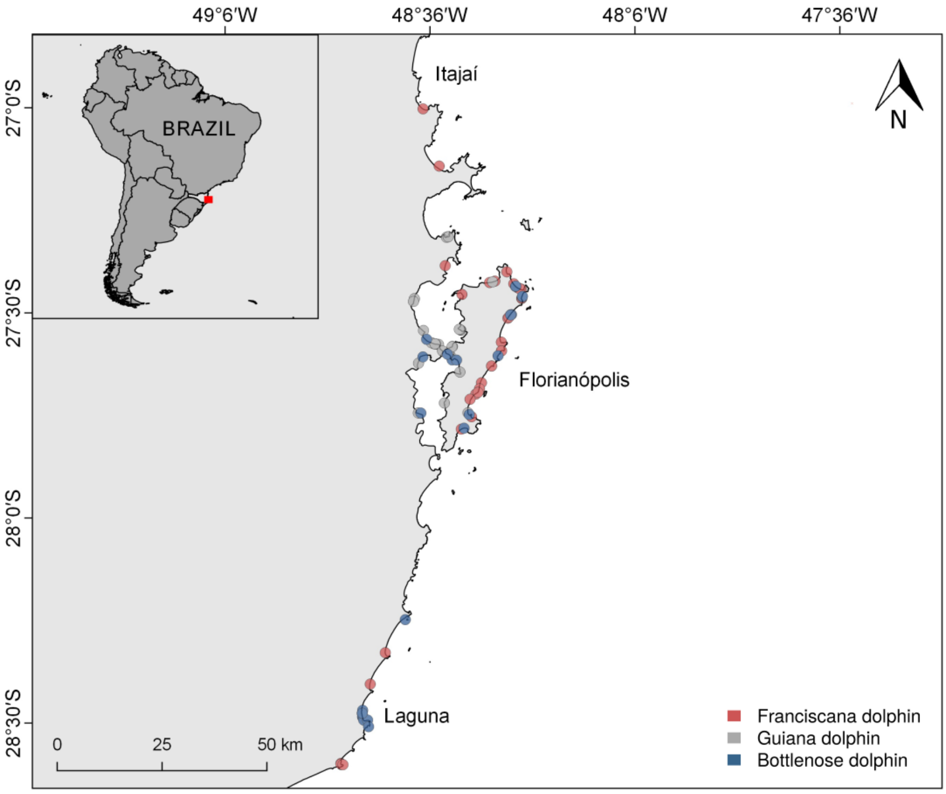


Figure 1 Franciscana (*Pontoporia blainvillei*), Guiana (*Sotalia guianensis*) and Lahille`s bottlenose (*Tursiops truncatus gephyreus*) dolphins bycaught along the Santa Catarina coast in southern Brazil, between 1985 to 2017.

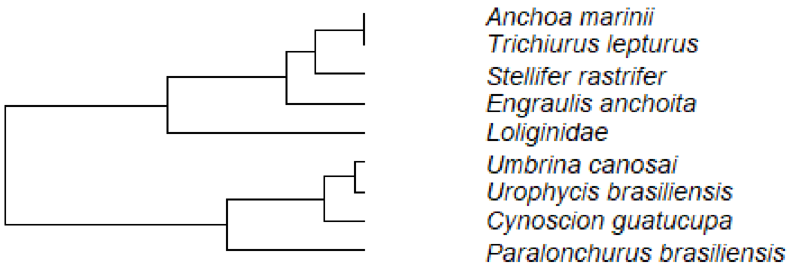


Figure 2 Results of the cluster analysis (Ward's methods) based on $\delta^{13}C$ and $\delta^{15}N$ in prey items with %FO >10% in franciscana dolphin (*Pontoporia blainvillei*) obtained from literature.

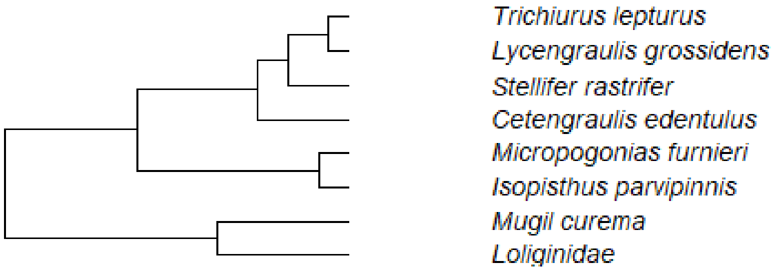


Figure 3 Results of the cluster analysis (Ward's methods) based on δ^{13}C and δ^{15}N in prey items with %FO >10% in Guiana dolphin (*Sotalia guianensis*) obtained from literature.

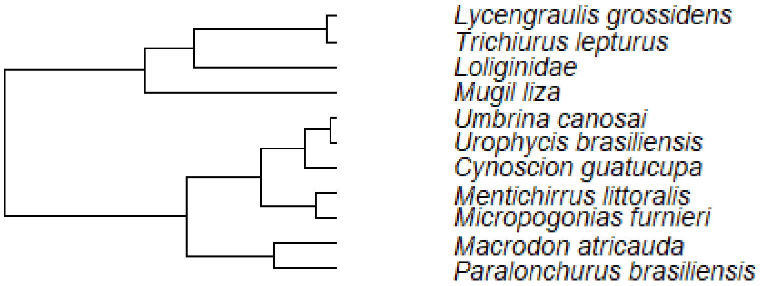
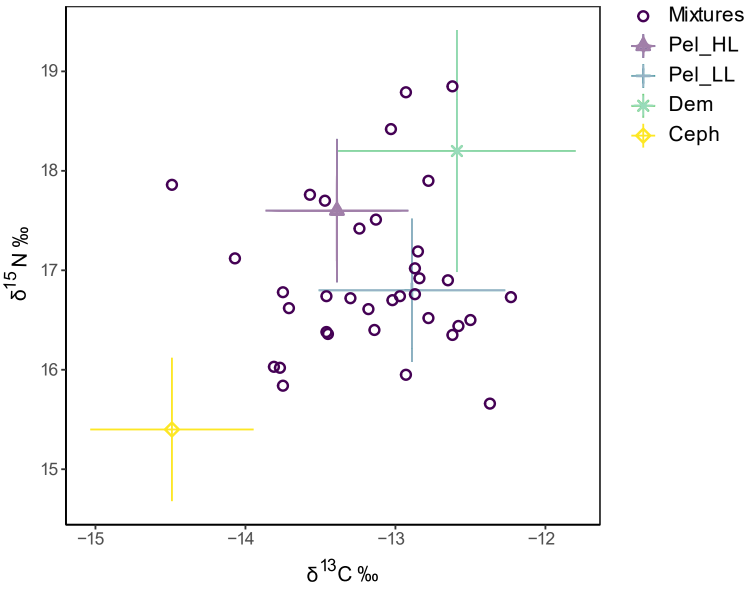


Figure 4 Results of the cluster analysis (Ward's methods) based on δ^{13}C and δ^{15}N in prey items with %FO >10% in the Lahille`s bottlenose dolphin (*Tursiops truncatus gephyreus*) obtained from literature.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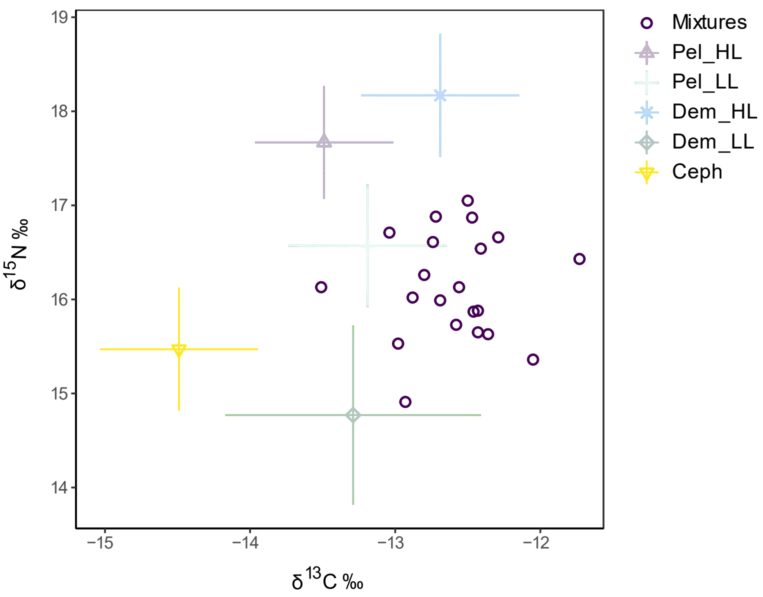
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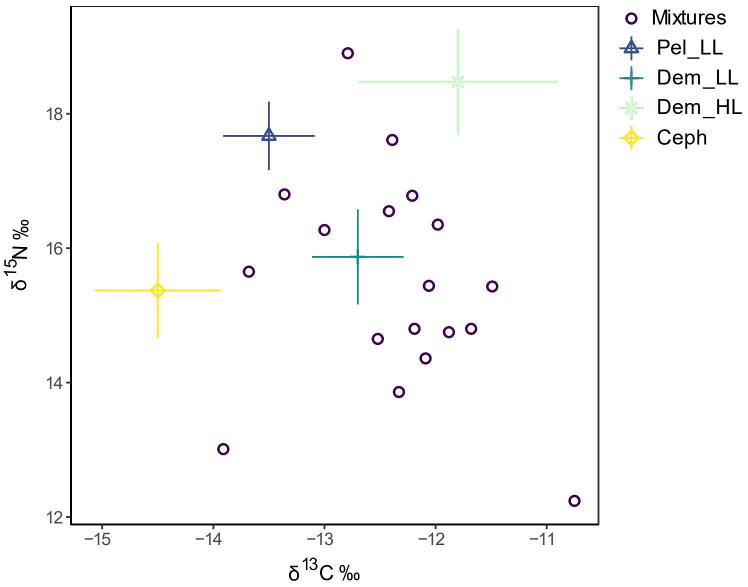
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δ 13 C ‰

Figure 5 Individual $\delta^{13}C$ and $\delta^{15}N$ values for bone collagen samples of franciscana (*Pontoporia blainvillei*), Guiana (*Sotalia guianensis*) and Lahille`s bottlenose (*Tursiops truncatus gephyreus*) dolphins off the Santa Catarina coast in southern Brazil. $\delta^{13}C$ and $\delta^{15}N$ values (mean ± *SD*) of their main prey sources corrected for trophic discrimination factors.

Table 1 $\delta^{13}C$ and $\delta^{15}N$ values (mean ± *SD* in ‰), carbon to nitrogen ratio (C:N) for each sample and year of stranding of the franciscana (*Pontoporia blainvillei*), Guiana (*Sotalia guianensis*) and Lahille`s bottlenose (*Tursiops truncatus gephyreus*) dolphins bone collagen samples along the Santa Catarina coast in southern Brazil.

Sample ID	Year	$\delta^{13}C$	$\delta^{15}N$	C:N
		Franciscana dolphin		
UFSC - 1435	2015	-13.1	18.4	2.9
UFSC - 1071	1988	-13.4	17.9	3.0
UFSC - 1439	2016	-13.8	16.0	2.9
UFSC - 1314	2003	-13.4	17.5	3.0
UFSC - 1037	1987	-13.3	16.4	2.9
UFSC - 1440	2015	-13.8	16.0	2.8
UFSC - 1437	2015	-12.9	16.9	2.9
UFSC - 1371	2009	-13.0	16.8	2.9
UFSC - 1411	2014	-13.3	17.4	2.9
UFSC - 1284	2000	-13.9	16.7	2.9
UFSC - 1290	2001	-13.4	16.7	3.4
UFSC - 1429	2015	-13.5	16.4	3.1
UFSC - 1216	1995	-13.9	17.7	3.4
UFSC - 1438	2015	-13.8	16.6	3.2
UFSC - 1215	1995	-13.4	18.8	3.4
UFSC - 1046	1987	-13.2	16.5	3.2
UFSC - 1092	1990	-13.2	18.9	3.1
UFSC - 1358	2008	-13.5	16.7	3.5
UFSC - 1058	1987	-12.9	16.7	3.1
UFSC - 1262	1999	-13.2	16.5	3.1
UFSC - 1091	1990	-13.2	16.4	3.2
UFSC - 1054	1987	-13.6	15.9	3.2
UFSC - 1369	2009	-13.2	16.8	3.2
UFSC - 1441	2016	-14.1	17.1	3.3
UFSC - 1357	2008	-13.4	16.6	3.2
UFSC - 1301	2001	-13.9	17.8	3.2
UFSC - 1434	2015	-12.4	15.7	3.1
UFSC - 1419	2014	-13.5	16.4	3.1
UFSC - 1288	2001	-13.0	16.9	3.2
UFSC - 1397	2012	-13.9	16.8	3.1
UFSC - 1007	1985	-13.6	17.0	3.4
UFSC - 1413	2014	-13.8	15.8	3.1
UFSC - 1390	2011	-13.3	16.4	3.2
UFSC - 1310	2003	-13.2	17.2	3.2
		Guiana dolphin		

UFSC - 1333	2006	-12.6	15.6	2.8
UFSC - 1083	1989	-14.1	16.1	3.1
UFSC - 1410	2013	-12.5	15.9	2.8
UFSC - 1436	2015	-12.6	16.1	2.8
UFSC - 1082	1989	-12.9	16.7	2.9
UFSC - 1312	2003	-12.8	16.9	2.9
UFSC - 1332	2006	-12.7	15.7	2.8
UFSC - 1377	2010	-12.3	16.4	2.8
UFSC - 1373	2009	-13.5	16.8	3.0
UFSC - 1386	2010	-12.5	14.9	2.8
UFSC - 1268	2000	-12.4	16.7	2.8
UFSC - 1130	1994	-13.0	17.1	2.9
UFSC - 1421	2015	-12.8	16.3	3.1
UFSC - 1079	1989	-12.7	15.4	3.1
UFSC - 1386	2010	-13.1	14.9	3.2
UFSC - 1104	1991	-12.3	16.4	3.0
UFSC - 1408	2013	-12.9	16.6	3.1
UFSC - 1108	1992	-13.1	15.8	3.1
UFSC - 1354	2007	-13.2	15.5	3.2
UFSC - 1218	1995	-13.5	16.7	3.4
UFSC - 1352	2007	-12.6	16.5	3.0
UFSC - 1291	2001	-12.8	15.9	3.1
UFSC - 1336	2006	-12.9	15.9	3.1

Lahille`s Bottlenose dolphin

UFSC - 1072	1988	-13.0	17.6	3.0
UFSC - 1477	2016	-13.7	15.6	2.8
UFSC - 1116	1993	-12.4	14.7	2.9
UFSC - 1209	1994	-12.7	16.8	2.8
UFSC - 1398	2011	-13.1	16.3	2.8
UFSC - 1081	1989	-12.8	14.8	2.9
UFSC - 1285	2001	-12.4	14.4	2.9
UFSC - 1415	2014	-13.9	13.0	2.8
UFSC - 1089	1990	-11.3	12.2	2.8
UFSC - 1349	2009	-12.1	16.4	2.9
UFSC - 1395	2011	-12.5	16.5	2.8
UFSC - 1077	1989	-12.3	14.8	2.8
UFSC - 1443	2016	-13.4	16.8	2.8
UFSC - 1281	2000	-12.4	15.4	2.9
UFSC - 1317	2003	-13.1	18.9	3.1
UFSC - 1420	2014	-12.6	14.6	3.1
UFSC - 1285	2001	-12.7	13.9	3.1
UFSC - 1289	2017	-11.5	15.4	3.0

Table 2 $\delta^{13}C$ and $\delta^{15}N$ values (mean ± *SD* in ‰) and sample sizes (*n*) of the potential prey species of the franciscana (*Pontoporia blainvillei*), Guiana (*Sotalia guianensis*) and Lahille`s bottlenose (*Tursiops truncatus gephyreus*) dolphins off the Santa Catarina coast in southern Brazil.

Species	Common name	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Franciscana dolphin				
Pelagic HL				
Trichiurus lepturus	Cutlass fish	7	-17.0 ± 0.3	15.5 ± 0.5
Anchoa marinii	Anchovy	5	-17.0 ± 0.2	15.5 ± 0.3
Pelagic LL				
*Engraulis anchoita**	Argentine anchovy	14	-16.5 ± 0.5	14.7 ± 0.5
Demersal				
Stellifer rastrifer	Rake stardrum	3	-16.7 ± 0.2	16.1 ± 0.5
Paralonchurus brasiliensis	Banded croacker	3	-14.2 ± 0.5	15.6 ± 0.6
Cynoscion guatucupa	weakfish	5	-15.7 ± 0.3	17.1 ± 0.3
*Urophycis brasiliensis**	Brazilian codling	11	-15.6 ± 0.5	16.5 ± 0.7
*Umbrina canosai**	Argentine croacker	10	-15.5 ± 0.3	16.6 ± 0.8
Cephalopods				
Loliginidae	squid	5	-18.1 ± 0.4	13.3 ± 0.5
Guiana dolphin				
Pelagic HL				
Trichiurus lepturus	Cutlass fish	7	-17.0 ± 0.3	15.5 ± 0.5
Lycengraulis grossidens	Atlantic sabretooth anchovy	4	-17.2 ± 0.3	15.6 ± 0.5
Pelagic LL				
Cetengraulis edentulus	Atlantic anchoveta	5	-16.8 ± 0.4	14.4 ± 0.3
Demersal HL				
Stellifer rastrifer	Rake stardrum	3	-16.7 ± 0.2	16.1 ± 0.5
Micropogonias furnieri	White croaker	5	-16.1 ± 0.1	15.9 ± 0.1
Isopisthus parvipinnis	Bigtooth corvina	1	-15.9 ± 0.5	16.3 ± 0.5
Demersal LL				
Mugil curema	White mullet	3	-16.8 ± 0.8	12.6 ± 0.9
Cephalopods				
Loliginidae	squid	5	-18.1 ± 0.4	13.3 ± 0.5
Lahille`s Bottlenose dolphin				

Pelagic HL				
Trichiurus lepturus	Cutlass fish	7	-17.0 ± 0.3	15.5 ± 0.5
Lycengraulis grossidens	Atlantic sabretooth anchovy	4	-17.2 ± 0.3	15.6 ± 0.5
Demersal HL				
*Macrodon atricauda***	King weakfish	5	-14.5 ± 0.7	17.0 ± 0.3
Mentichirrus littoralis	Gulf kingcroaker	3	-16.3 ± 0.7	16.3 ± 0.4
*Urophycis brasiliensis**	Brazilian codling	11	-15.6 ± 0.5	16.5 ± 0.7
*Umbrina canosai**	Argentine croacker	10	-15.5 ± 0.3	16.6 ± 0.8
Paralonchurus brasiliensis	Banded croacker	3	-14.2 ± 0.5	15.6 ± 0.6
Micropogonias furnieri	White croaker	5	-16.1 ± 0.1	15.9 ± 0.1
Cynoscion guatucupa	weakfish	5	-15.7 ± 0.3	17.1 ± 0.3
Demersal LT				
Mugil liza	Lebranche mullet	3	-16.3 ± 0.1	12.8 ± 1.3
Cephalopods				
Loliginidae	squid	5	-18.1 ± 0.4	13.3 ± 0.5

Reference:
* Bugoni et al. 2010;
** Zenteno et al. 2015

Table 3 Estimated regression parameters, standard errors (S.E.), *t*-values and *P*-values for the two Gamma GLM models with inverse link function showed in Table 1.

Model:	Estimate	S.E.	*t* value	*p*
(Intercept)	0.32491	0.12917	2.51527	0.01423
Year	-0.00012	0.00006	-1.90488	0.06096
GroupFranciscana_dolphin	-0.20518	0.15505	-1.32329	0.19011
GroupGuiana_dolphin	-0.42374	0.18541	-2.28549	0.02536
Year: GroupFranciscana_dolphin	0.0001	0.00008	1.29594	0.19932
Year: GroupGuiana_dolphin	0.00021	0.00009	2.2794	0.02574

Model:	Estimate	S.E.	*t* value	*p*
(Intercept)	0.00656	0.0892	0.07358	0.94155
Year	0.00003	0.00004	0.6517	0.5167
GroupFranciscana_dolphin	-0.0055	0.00116	-4.73487	0.00001
GroupGuiana_dolphin	-0.00264	0.00127	-2.08203	0.04095

4 CAPÍTULO 3 - ECOLOGICALLY DRIVEN DIFFERENCES IN INDIVIDUAL DIET SPECIALIZATION ACROSS THREE POPULATIONS OF GUIANA DOLPHIN

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ABSTRACT

Populations usually considered foraging generalists may include specialized individuals feeding on a restricted subset of the populations` total prey spectrum. By analyzing time series of stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) in sequential growth layer groups within tooth dentin, we measured population- and individual-level variation in resource use of three populations of Guiana dolphin from the western South Atlantic—Caravelas, Norte Bay and Babitonga Bay. The niche metrics (TNW, WIC, BIC and WIC / TNW) for the $\delta^{15}N$ values suggest that the Caravelas population has the widest niche width and is comprised mostly of specialist individuals. This result support the Niche Variation Hypothesis, which posits that the degree of individual diet specialization increased with the populations' total niche width. The population from the Norte Bay, on the other hand, has the narrowest niche compared to the other populations and is comprised of generalist individuals. This result may be associated with the coexistence and the high overlap of the isotopic niche with the bottlenose dolphin, both associated with the high spatial overlap of Guiana dolphin individuals due to their unique social organization characterized by a large, cohesive group living in a small and restricted area of the North Bay. Thus, both potential inter- and intraspecific competition can be acting to restrict the expansion of the population niche in the North Bay, limiting opportunities for individual specialization. The Guiana dolphin population from the Babitonga Bay also has a narrow niche, but mostly comprised of specialist individuals, likely promoted by the lower interspecific competition with a sympatric franciscana dolphin population due to differences in use of core areas and main prey items preferences. These variations in niche metrics suggest that although Guiana dolphin can be considered generalist at the population level, variability in resource use at the individual level may result from different ecological contexts in terms of presence of heterospecifics, populational and individuals' spatial patterns and resource availability. Understanding the different foraging strategies exhibited by the Guiana dolphin along its distribution is relevant in the context of the anthropogenic pressures that threaten their persistence.

KEYWORDS: Niche width; intraspecific variation; stable isotopes; foraging ecology; competition

INTRODUCTION

Natural populations can be composed of ecologically heterogeneous individuals that adopt different foraging strategies and feed on different prey types (Bolnick et al. 2002; Bearhop et al. 2004). While variation between sexes (Elorriaga-Verplancken et al. 2013), ages (e.g., Polis 1984), and discrete polymorphisms (Skúlason and Smith 1995) has long been recognized, individual diet specialization is defined as organisms that have narrower dietary niches than their population's irrespective of age, sex, or morphology (Bolnick et al. 2003), as a result of multiple ecological drivers acting individually or in concert (Araújo et al. 2011; Costa-Pereira et al. 2018). Although individual diet specialization has received attention for its consequences on population dynamics, species interactions and coexistence (Bolnick et al. 2011), many fundamental questions remain unclear, such as how populational and ecological traits can direct or indirectly influence the degree of individual diet specialization.

It has been suggested that the niche width of a population and the degree of individual diet specialization reflect a balance between intra and interspecific competition (Bolnick et al. 2003; Araujo et al. 2011). The niche variation hypothesis (NVH), for example, predicts that a release from or reduction in interspecific competition results in an expansion of population niche width (Van Valen 1965). Subsequent theoretical work suggested that the total niche width (TNW) of populations can expand in two ways (Roughgarden 1972). The first way is via minimization in resource use overlap among conspecifics which reduces individual niche width relative to TNW. In a second way, all individuals become more generalist and use the full set of resources available. Interspecific competition constrains population niche width such that conspecifics forage on similar prey types, dampening the degree of individual diet specialization (Bolnick et al. 2003). In contrast, intraspecific competition frequently decreases individual niche width, and enhance prevalence of individual diet specialization (Svanbäck and Bolnick 2005).

Inter and intraspecific interactions can also affect ecological opportunity (i.e., resource availability). In this sense, the optimal foraging theory (OFT) posits that individuals will select their prey sources aiming to maximize their rate of energy intake, allowing dietary niches to diverge. As the abundance of preferred prey decreases (e.g., increased competition), individuals may choose to explore other available resources, widening their total niche and leading to an increase in individual diet specialization (Stephens and Krebs 1986; Bolnick et al. 2010). Thus, factors that regulate intra- and interspecific competition such as population density, social organization (e.g., group size and cohesion), spatial patterns (e.g., high or low individual spatial overlap), and resource availability can have a strong influence on the niche width of a population and the degree of individual diet specialization (Krause and Ruxton 2002, Sheppard et al. 2018).

The Guiana dolphin, *Sotalia guianensis*, provides a good model to evaluate the implications of individual-level variation in population niche width and individual dietary specialization. This species is distributed in shallow areas of estuaries and coastal waters along the western Atlantic Ocean (Borobia et al. 1991), in discrete populations of mostly resident individuals that occupy similar habitats in allopatry, or in sympatry with other species (Flores and Bazzalo 2004; Hardt et al. 2010). Although multiple dolphin species occur along the tropical Brazilian coast, they are mostly observed using areas distant from coast and river mouths, while the Guiana dolphin is the only species using coastal waters near and within the Caravelas River Estuary (Rossi-Santos et al. 2006). Farther south along the subtropical Brazilian coast, however, the Guiana dolphin population in Norte Bay occurs with the franciscana dolphin (*Pontoporia blainvillei*) and the Lahille's bottlenose dolphin (*Tursiops truncatus gephyreus*), while in the Babitonga Bay the Guiana dolphin population only co-occurs with the franciscana dolphin (Cremer and Simões-Lopes 2005). These different co-occurrence patterns with other dolphin species in distinct habitats can change the potential inter and intra-competition pressures which, in turn, can influence their total trophic niche width and levels of individual diet specialization.

Stomach content analysis revealed that these populations are opportunistic and generalist foragers at the population level with a diet consisting mostly of demersal fish species and cephalopods (Daura-Jorge et al. 2011; Cremer et al. 2012; Rodrigues et al. 2020). Recent studies have also explored the trophic interactions between Guiana dolphin and other sympatric species, showing an overlap in diet between Norte and Babitonga Bay populations with the Lahille’s bottlenose (Teixeira et al. 2020) and franciscana dolphin (Hardt et al. 2013), respectively. Despite these advances, no study has evaluated individual-level dietary variation within and across Guiana dolphin populations likely because longitudinal foraging records required for this evaluation are particularly challenging to obtain for large mobile predators living in marine environments.

Stable isotope analysis has become a reliable tool to construct ecological records at the individual level that reflect a combination of foraging and movement information (e.g., Rossman et al. 2015; Yurkowski et al. 2016). In general, variation in carbon isotope ($\delta^{13}C$) values among consumers reflect the sources of primary production (e.g., phytoplankton vs macroalgae) in the habitats where they forage (e.g., oceanic vs nearshore), whereas nitrogen isotope (δ^{15}N) values are commonly used as proxies for consumer trophic position (DeNiro and Epstein 1978, 1981; Newsome et al. 2010). Furthermore, the isotopic composition of metabolically inert but continuously growing tissues (e.g., baleen plates and tooth dentin) provides a sequential archive of ecological information over different timescales (Walker and Macko 1999) that can be used to measure a population's total niche width (TNW), as well as the within- and between individual components of the niche (WIC and BIC respectively) (e.g., Newsome et al. 2009). When other confounding factors that influence consumer isotope values can be constrained (e.g., resident populations), such as movement across baseline isotopic gradients (Graham et al. 2010; Troina et al. 2020), this approach can be used to evaluate the relative prevalence of specialist vs. generalist foraging strategies that is reflected in the degree of individual diet specialization in a population (Newsome et al. 2009; Vander Zanden et al. 2013).

Despite the growing interest in the study of individual specialization, few studies have focused on the relationship between individual specialization, spatial behavior, and population traits such as social organization, home range and population density. Here we generated a δ^{13}C and δ^{15}N dataset from serially sampled teeth to quantify individual- and population-level niche components among three Guiana dolphin populations living in different ecological contexts, in terms of presence of other species, individuals' spatial patterns (e.g., high or low spatial overlap within conspecifics) and resource availability that likely vary along the Brazilian coast and across the habitats where these populations occur. Our aim was to investigate whether total niche width and degree of individual specialization of these populations can be an outcome of the ecological contexts in which they are inserted in. If so, we expect that changes in these populational traits—home-range, group size, social organization, population density—mirror the variation in total niche width and degree of individual specialization.

MATERIAL AND METHODS

Sample site and collection

Teeth were collected from stranded carcasses found along the Caravelas River Estuary (n = 9) in the eastern Brazilian coast, the Babitonga (n =11) and Norte Bay (n = 16), in the southern Brazilian coast (Fig. 1a). The specimens are archived in the scientific collections of the Instituto Baleia Jubarte (IBJ), the Acervo Biológico Iperoba, from the Universidade da Região de Joinville (Univille), and the Aquatic Mammals Laboratory (LAMAQ) from the Universidade Federal de Santa Catarina (UFSC), Brazil.

The Caravelas River Estuary (17°30'S, 39°30'W) is adjacent to the Abrolhos bank, an extension of the continental shelf influenced by a large estuarine-mangrove complex (Herz 1991). The Caravelas River Estuary system is the second largest mangrove forest of Brazil's northeast region, with an area of approximately 66 km^2 (Herz 1991). This region is characterized by a great diversity of habitats such as open waters protected by the Abrolhos coral reef, mangrove forests with channels, sandy beaches, and banks of shallow waters (Rossi-Santos et al. 2007). The Guiana dolphin population from the Caravelas River is composed of ~57-124 resident and transient individuals (Rossi-Santos et al. 2007; Cantor et al. 2008) in small groups (3-4 individuals) using a large home-range within the river and adjacent coastal areas (Rossi-Santos et al. 2006, 2007).

The Babitonga and Norte Bay are both located in Southern Brazil. The Babitonga Bay (26°28'S, 48°50'W) comprises 160 km^2 characterized by islands, mangroves, rocky shores, and muddy-sand beaches. The Guiana dolphin population from the Babitonga bay is estimated at ~208 individuals (Cremer et al. 2011) organized in small groups (5-6 individuals) that use mainly the innermost area of the bay (Cremer et al. 2011, 2018). The Norte Bay (27°30'S, 48°32'W) is located between the Santa Catarina island and the mainland, and encompasses a protected area of approximately 250 km^2 delimited by rocky shores, sandy beaches, and mangrove forests with oceanographic features greatly influenced by the adjacent open seawaters (Cerutti 1996). The Guiana dolphin population from the Norte Bay has ~130 individuals (L. Wedekin, unpublished data) that overlap their home range in a small and restricted area (Flores and Bazzalo 2004).

Sample preparation

In specimens with more than one tooth available (*n* =29), we used the method for age estimation in tooth dentin to guide the milling process through the Growth Layer Groups (GLGs; Perrin and Myrick 1980). The method included the longitudinal section of the tooth to obtain the central portion. We then fixed this thick section in 10% formalin, decalcified in a commercial mixture of acids (RDO®) for 7-17h, and cut in 25 µm-thin sections using a freezing microtome. After thin sectioning, we stained the sections in Mayer's hematoxylin, "blued" in ammonia, and mounted on microscope slides with 100% glycerin (Hohn et al. 1989). We restricted our samples to mature individuals only, considering mature males ≥6 years and/or with a body length greater than or equal to 170 cm, and females ≥5 years and/or with body length greater than or equal to 164 cm (Ramos et al. 2000; Rosas & Monteiro-Filho 2003).

To obtain individual dentine GLG samples from each tooth, we used a water-cooled diamond-bladed saw (Buehler IsoMet®) to cut Guiana dolphin teeth in two longitudinal sections. To optimize the identification of the growth layers, we polished the longitudinal sections with successive sheets of sandpaper with decreasing grit sizes (340, 500, 600, 1200 and 12000). The inner surface of each tooth was immersed in 25% formic acid for 1-1.5h, rinsed with deionized water and air-dried for 24h (Newsome et al. 2006). We assume that such treatment did not influence the isotopic values because the surface portion of the tooth represents only a small fraction of the total sample. We mounted the polished tooth on glass slide, and subsampled ~1 mg of raw bulk dentin using a computer-guided micromill (Merchantek®) fitted with a 300 µm-diameter carbide drill bit at a depth of 250 µm. We milled five tracks from each tooth and due to the small size of Guiana dolphin teeth, tracks reflecting older portions of an individual lifetime (closer to the pulp cavity) contained more GLGs than tracks near the neonate line. We collected a total of 180 dentin powder samples (~1mg each) placed directly into tin capsules for stable isotope analysis.

Despite the high precision in microsampling provided by the micromill system, the tooth size, and the conical arrangement of the dentin layers in Guiana dolphin constrain the collection of adequate amounts of sample from each GLG for the demineralization process. As decalcification may not be required to measure bulk isotope values in dentin from modern odontocetes, likely due to the low concentration of lipids and carbonates, and the similarity in $\delta^{13}C$ values of dentin protein and carbonate (Brault et al. 2014), here we chose to avoid sample loss associated with the demineralization process by using raw dentin samples.

We determined $\delta^{13}C$ and $\delta^{15}N$ values using a Costech 4010 Elemental Analyzer (Valencia, CA, USA) coupled to a Thermo Scientific Delta V Plus (Bremen, Germany) isotope ratio mass spectrometer at the University of New Mexico Center for Stable Isotopes (Albuquerque, NM). Results are expressed in parts per thousand (‰) and delta notation (δ) using the equation: $\delta_{sample} = [R_{sample} / R_{standard} - 1] * 1000$, where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the sample and standard, respectively (Peterson and Fry 1987). The standards are Vienna Pee Dee Belemnite limestone (VPDB) for $\delta^{13}C$ and atmospheric N^2 for $\delta^{15}N$. The analytical precision based on the standard deviation of the standard laboratory replicas (Acetanilide) was ≤ 0.1 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. The C:N ratios of each sample were in the expected range (2.8–3.5) for pure protein (Ambrose 1990).

Data analysis

As our samples were restricted to the last two decades, we did not apply the Suess effect correction of 0.02% in $\delta^{13}C$ values and excluded from the analysis the $\delta^{13}C$ and $\delta^{15}N$ values of the first GLG that can be associated with mother-to-offspring transfer of nutrients during lactation and weaning. We first investigated the $\delta^{13}C$ and $\delta^{15}N$ values variation among populations by adjusting Gaussian generalized linear mixed model (GLMM) with log link function to model GLG isotopic values as a function of populations. Additionally, we included sex as a covariate and individuals as a random effect. Given that our data exploration suggested a lack of interaction between sex and population, we did not include interactive terms between these two factors to avoid overparameterization of the models. The model selection procedure was based on Akaike's information criterion (AIC) and Akaike weight (Burnham and Anderson 2002), using the R package 'MuMIn' (Bartoń 2019). We checked for scaled residuals using DHARMa package (Hartig 2018). The threshold significance level in all statistical tests was 95% ($p < 0.05$).

We measured the degree of individual specialization of each population following the Roughgarden`s (1979) framework, which partitioned the total niche width of a population (TNW) in two components: the within-individual component (WIC) that reflects the average of individual niches width, and the between-individual component (BIC) that represents the variance among individuals' niche. We estimated BIC as the total standard deviation in $\delta^{13}C$ and $\delta^{15}N$ values of sampled individuals (Bolnick et al. 2003), and WIC as the mean standard deviation in $\delta^{13}C$ and $\delta^{15}N$ values obtained along the longitudinal growth layers within individuals. Therefore, we used the mean variability within individuals (WIC) as a measure of temporal consistency and the degree of individual specialization as the percent of within-individual component of total niche width (WIC/TNW), where low values (close to zero) indicate higher specialization and high values (close to 1) indicate lower individual specialization (Bolnick et al. 2003).

We evaluated the differences in TNW, WIC, BIC and WIC/TNW among populations with a Monte-Carlo permutation procedure using the R package 'RInSp' (Zaccarelli et al. 2013). To obtain *p*-values for WIC/TNW within each population against a null model (a population composed of generalist individuals that sample randomly from the population's TNW), we used a nonparametric Monte Carlo procedure of 10000 replicates (Bolnick et al. 2002).

RESULTS

We found no influence of the variable sex for both mean tooth GLG δ^{13}C (*df* = 33, t-value = 0.557, $p = 0.58$) and δ^{15}N values (*df* = 33, t-value = -1.028, $p = 0.31$). The GLMMs also showed significantly lower δ^{13}C values in Norte Bay compared to Caravelas River (estimate=0.06775, se: 0.01325, z-value: 5.11, $p < 0.001$) and Babitonga Bay (estimate=0.078880, se: 0.012147, z-value: 6.49, $p<0.001$), whereas δ^{15}N values are significantly higher in Babitonga (estimate=0.28213, se: 0.02291, z-value: 12.32, $p<0.001$) and Norte Bay (estimate=0.30046, se: 0.02170, z-value: 13.85, $p<0.001$) compared to Caravelas River population. The GLG δ^{13}C values ranged from -15.4‰ to -13.2‰ (mean ± *SD*: -14.0 ± 0.5‰) for the Norte Bay population; from -14.2‰ to -12.3‰ (mean ± *SD*: -13.1 ± 0.4‰) for the Caravelas River population, and from -13.8‰ to -12.0‰ (mean ± *SD*: -12.9 ± 0.4‰) for the Babitonga Bay population (Fig. 1b). The GLG δ^{15}N values ranged from 9.4‰ to 14.5‰ (mean ± *SD*: 11.6 ± 1.2‰) for the Caravelas River population, from 13.8‰ to 16.6‰ (mean ± *SD*: 15.5 ± 0.7‰) for the Babitonga Bay population, and from 14.5‰ to 16.8‰ for the Norte Bay population (mean ± *SD*: 15.8 ± 0.5‰) (Fig. 1c).

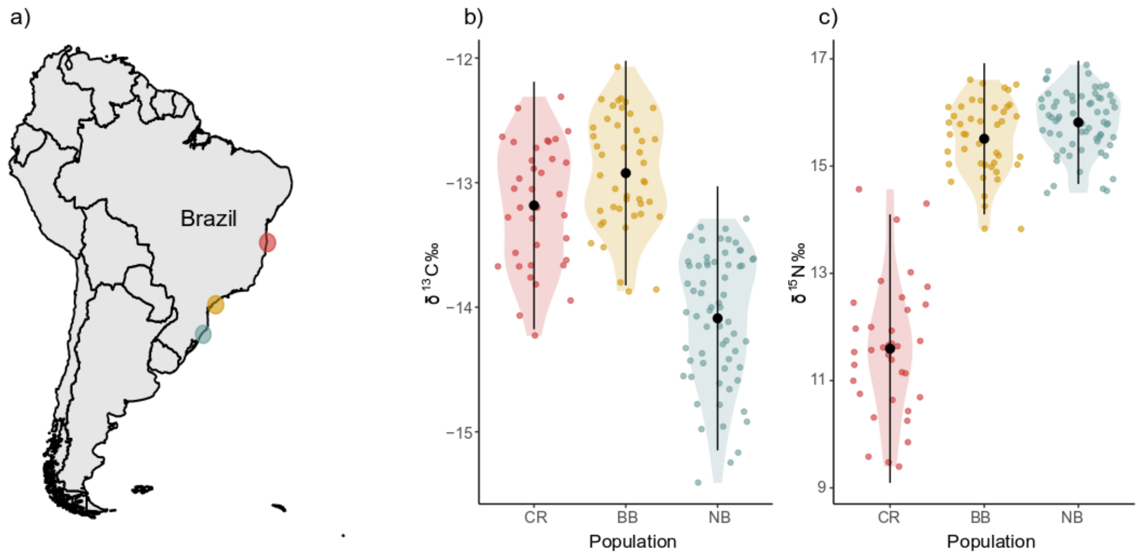

Figure 1. a) Geographical location of the Caravelas River (red), Babitonga (yellow) and Norte Bay along the Brazilian coast **b)** Violin plots of the GLG δ^{13}C mean values and **c)** GLG δ^{15}N mean values for the Guiana dolphin, *Sotalia guianensis* populations from the Caravelas River (CR), Babitonga (BB) and Norte Bay (NB). Points indicate mean values of δ^{13}C and δ^{15}N values and the lines indicate standard deviation.

TNW, WIC, BIC and the WIC/TNW values of Guiana dolphin populations from the Caravelas River, Babitonga and Norte Bay are reported in Table 1 and Fig. (b) and (c). The WIC for $\delta^{13}C$ values was similar among all Guiana dolphin populations, while the WIC for $\delta^{15}N$ values was higher for the Caravelas River and Norte Bay populations. The BIC for $\delta^{13}C$ values was similar among all populations, while the BIC for $\delta^{15}N$ values was significantly higher for the Caravelas River population. TNW for $\delta^{13}C$ values was similar among all populations, while the TNW for $\delta^{15}N$ values significantly varied among populations, with the highest mean value for the Caravelas River and the lowest mean value for the Babitonga Bay. Although the three Guiana dolphin populations showed similar values of WIC/TNW for $\delta^{13}C$, the Monte Carlo resampling procedure used to evaluate the individual specialization relative to a null model (population of generalist individuals) revealed lower WIC/TNW for $\delta^{15}N$ values for the Caravelas River and Babitonga Bay ($p < 0.01$) compared to the Norte Bay population ($p = 0.18$), suggesting that the Norte Bay population is comprised of more generalist individuals while Caravelas and Babitonga Bay populations are comprised of more specialist individuals (Fig. 2b).

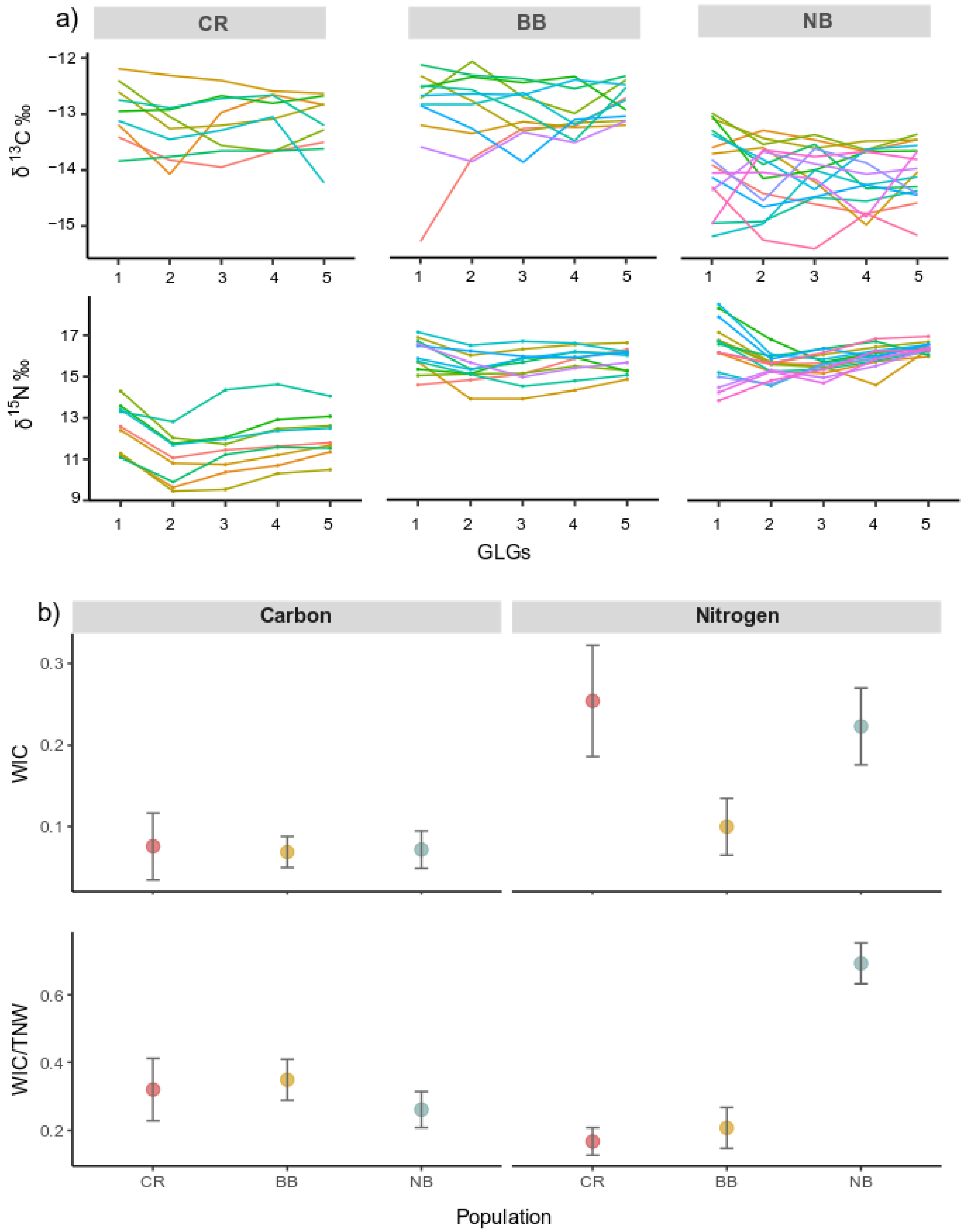

Figure 2 a) $\delta^{13}C$ and $\delta^{15}N$ values in sequential growth layer groups within tooth dentin in Guiana dolphin populations of Caravelas River (CR), Babitonga Bay (BB) and Norte Bay (NB) and b) Within individual variation (WIC) and degree of individual specialization (WIC/TNW) for $\delta^{13}C$ and $\delta^{15}N$ values of Guiana dolphin populations from CR, BB and NB. WIC/TNW range from 0 (all individuals are specialists) to 1 (all individuals are generalists)

Table 1. Within individual component (WIC), between individual component (BIC), total isotopic niche width (TNW) and individual specialization index (WIC/TNW) for $\delta^{13}C$ and $\delta^{15}N$ values of Guiana dolphin, *Sotalia guianensis* populations from the Caravelas River (CR), Babitonga (BB) and Norte Bay (NB) in ‰.

Population	WIC		BIC		TNW		WIC/TNW	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
CR	0.076	0.254	0.162	1.266	0.239	1.521	0.320	0.167
BB	0.069	0.100	0.128	0.384	0.198	0.485	0.349	0.207
NB	0.072	0.223	0.204	0.098	0.276	0.321	0.261	0.693

DISCUSSION

We found similar TNW, WIC, BIC, and WIC/TNW values for $\delta^{13}C$ across the three Guiana dolphin populations, suggesting no differences in foraging areas in terms of source of primary production among individuals. However, we found differences in TNW, WIC, BIC, and WIC/TNW values for $\delta^{15}N$ across populations, suggesting variability in resource use among individuals and different levels of individual diet specialization. Although Guiana dolphin is a species typically considered to be a dietary generalist and opportunistic, our results indicate that variability at the individual level may arise as an outcome from different ecological contexts in which these populations are exposed.

Both niche components (WIC and BIC) can contribute to the population-level patterns. Our results indicate that Guiana dolphin population from the Caravelas has the broadest TNW, largely driven by variation among individuals (higher BIC value). This population also has the lowest WIC/TNW for $\delta^{15}N$ values of the three populations. The combination of a low WIC/TNW ratio and a high TNW values suggest a generalist population comprised of more specialized individuals. This result is supported by the niche variation hypothesis (Van Valen 1965), where the release from interspecific competition promotes the niche expansion via greater inter- individual variation. As no other dolphin species occurs within the Caravelas River Estuary – the Guiana dolphin has the opportunity to forage upon more prey types and functional groups by increasing their niche size and interindividual variation. In addition, the Guiana dolphin from Caravelas is known to use a wide variety of habitats in adjacent coastal areas (Rossi-Santos et al. 2007), foraging as solitary individuals or in small groups (Rossi-Santos et al. 2010) and likely structuring its population with both resident and transient individuals (Rossi-Santos et al. 2007; Cantor et al. 2012). Therefore, the ecological opportunity for this population to broaden their diet and expand their niche through specialized individuals is likely promoted by a lower interspecific competition (due to the absence of other sympatric dolphin species), combined with the avoidance of intraspecific competition (i.e., small foraging groups within a small population structured in resident and transient individuals) within a patchy and dynamic environment.

The Guiana dolphin population from the Norte Bay, on the other hand, has the narrowest populational niche (lowest TNW for $\delta^{15}N$ values), comprised of generalist individuals (lowest BIC and highest WIC/TNW for $\delta^{15}N$ values). This population resides in the southern limit of the species distribution in one of the areas with the highest abundance of fish species along the Brazilian coast (Ribeiro et al. 2014), mainly due to the high primary productivity promoted by the influenced by the Subtropical Convergence between the Brazil and Malvinas/Falkland currents (Ciotti et al. 1995). However, in a fine scale, this population do not use the most productive areas within the Norte Bay (e.g., near mangroves or the mouths of estuaries) (Wedekin et al. 2007), restricting their spatial distribution to the western margin of the bay (Flores and Fontoura 2006; Wedekin et al. 2007). This reduced home range imposes limits regarding foraging options, forcing individuals to prey on the same prey sources within this restricted area and behave more opportunistic in response to the random fluctuation of resource availability within their habitat.

The reduced home range of Guiana dolphins in Norte Bay can be a reaction to the co-occurrence with the bottlenose dolphins in the area. The presence of bottlenose dolphins is also one of the explanations to the distinctive social organization of this Guiana dolphin population, characterized by a large, stable and cohesive social group. An aggressive interaction between the Guiana and the bottlenose dolphins was previously reported in the area (Wedekin et al. 2007), and recent evidence from isotope-based analyses show that these populations have similar foraging areas and prey preferences, leading to a high isotopic niche overlap caused by shared consumption of demersal mullet species (Teixeira et al. 2020). Therefore, the generalist and opportunistic feeding strategies observed in Guiana dolphin individuals from Norte Bay may reflect not only the potential interspecific competition (high niche overlap) with the bottlenose dolphin, but also the effects of the extensive individual home range overlap generated by their cohesive social organization in a large group within a limited area – which could be a defensive behavior motivated by the presence of bottlenose dolphins, or by ecological stressful conditions (see Lesica and Allendori 1995) imposed by the environmental conditions of the southern limit of their distribution.

Like the Norte Bay, the Guiana dolphin population from the Babitonga Bay also has a narrow niche (intermediate TNW value for δ^{15}N values) but is mostly comprised of specialist individuals (low WIC/TNW for δ^{15}N values) highly consistent in resource use through time (lowest WIC for δ^{15}N values). Although the Guiana dolphin population from Babitonga Bay also feeds in adjacent areas of the Bay, individuals use predominantly the innermost area of the Bay likely due to a higher ichthyofaunal abundance (Cremer 2018). Despite its direct sympatry with the franciscana dolphin, both populations use distinct core areas (Cremer et al. 2018) and vary in main prey items preferences (Cremer et al. 2012; Hardt et al. 2013). These differences in habitat use and dietary preferences with the frasciscana dolphin leads to less potential interspecific competition. In addition, this Guiana dolphin population is socially organized in small groups foraging within a complex habitat. This behavioral trait combined with the individual diet specialization may reflect a strategy to reduce intraspecific competition and to release from interspecific competition with the sympatric frasciscana dolphin population.

Our study provides novel insight into the ecological context under which individual dietary specialization manifests in Guiana dolphin populations. Previous studies have pictured Guiana dolphins as generalist foragers (Daura-Jorge et al. 2011; Cremer et al. 2012; Rodrigues et al. 2020), but the evidence comes mostly from conventional methods (e.g., stomach content analysis) that represent the immediate prey consumption. Our findings improve our understanding of the complex foraging strategies of a cryptic coastal species and suggests that the total niche width and degree of diet individual specialization of Guiana dolphin populations can be an outcome of independently or integrated ecological drivers such as resource availability, inter- and intraspecific competition (i.e., consumers density) and individual spatial home range. Nevertheless, a note of caution is necessary since our study did not evaluated the isotopic composition nor the diversity and abundance of prey sources within these different areas. Given that ecological opportunity is also considered an important ecological driver of individual specialization, future studies should further explore resource availability (e.g., information on spatial or temporal variation in prey community composition and abundance) to disentangle the individual specialization in Guiana dolphin populations. Such studies are needed to identify additional mechanisms that promote and maintain individual specialization that can be particularly important for species coexistence and population viability.

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REFERENCES

Araújo MS, Bolnick DI, Layman CA (2011) The ecological causes of individual specialisation. Ecology letters, 14: 948-958.

Bartoń K (2019) MuMIn: Multi-modal inference. Model selection and model averaging based on information criteria (AICc and alike).

Bolnick DI, Yang LH, Fordyce JA, Davis JM, Svanbäck R (2002) Measuring individual-level resource specialization. Ecology 83:2936-2941.

Bolnick DI, Svanback R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML (2003) The ecology of individuals: incidence and implications of individual specialization. American Naturalist 161:1–28.

Bolnick DI, Ingram T, Stutz WE, Snowberg LK, Lau OL, Paull JS (2010) Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. Proceedings of the Royal Society B: Biological Sciences, 277:1789-1797.

Bolnick DI, Amarasekare P, Araújo MS, Bürger R, Levine JM, Novak M, Rudolf VHW, Schreiber SJ, Urban MC, Vasseur DA (2011) Why intraspecific trait variation matters in community ecology. Trends in ecology & evolution 26:183-192.

Borobia M, Siciliano S, Lodi L, Hoek W (1991) Distribution of the South American dolphin Sotalia fluviatilis. Canadian Journal of Zoology 69: 1025-1039.

Brault EK, Koch PL, Gier E, Ruiz-Cooley RI, Zupcic J, Gilbert KN, McCarthy MD (2014) Effects of decalcification on bulk and compound-specific nitrogen and carbon isotope analyses of dentin. Rapid Communications in Mass Spectrometry 28:2744-2752.

Burnham KP, Anderson DR (2002) A practical information-theoretic approach. In: Model selection and multimodel inference, 2nd ed. Springer, New York, 2.

Cantor M, Wedekin LL, Daura-Jorge FG, Rossi-Santos MR, Simões-Lopes PC (2012) Assessing population parameters and trends of Guiana dolphins (*Sotalia guianensis*): An eight-year mark-recapture study. Marine Mammal Science 28:63-83.

Cerutti R.L (1996) Contribuição ao conhecimento da poluição doméstica na Baía Norte, área da Grande Florianópolis, SC. MSc. thesis. Universidade Federal de Santa Catarina.

Chase JM, Leibold MA (2003) Ecological niches: linking classical and contemporary approaches. University of Chicago Press.

Ciotti ÁM, Odebrecht C, Fillmann G, Moller JrOO (1995) Freshwater outflow and Subtropical Convergence influence on phytoplankton biomass on the southern Brazilian continental shelf. Continental Shelf Research 15:1737-1756.

Cremer MJ, Simões-Lopes PC (2005) The occurrence of Pontoporia blainvillei (Gervais & d'Orbigny) (Cetacea, Pontoporiidae) in an estuarine area in southern Brazil. Revista Brasileira de Zoologia 22:717-723.

Cremer MJ, Simões-Lopes PC (2008) Distribution, abundance, and density estimates of franciscanas, *Pontoporia blainvillei* (Cetacea: Pontoporiidae), in Babitonga bay, southern Brazil. Revista Brasileira de Zoologia 25:397-402.

Cremer MJ, Hardt FA, Tonello-Júnior AJ, Simões-Lopes, PC (2011) Distribution and status of the Guiana dolphin *Sotalia guianensis* (Cetacea, Delphinidae) population in Babitonga bay, Southern Brazil. Zoological Studies 50:327-337.

Cremer MJ, Pinheiro PC, Simões-Lopes PC (2012) Prey consumed by Guiana dolphin *Sotalia guianensis* (Cetacea, Delphinidae) and franciscana dolphin *Pontoporia blainvillei* (Cetacea, Pontoporiidae) in an estuarine environment in southern Brazil. Iheringia. Série Zoologia 102:131-137

Cremer MJ, Holz AC, Sartori CM, Schulze B, Paitach RL, Simões-Lopes PC (2018) Behavior and ecology of endangered species living together: Long-term monitoring of resident sympatric dolphin populations. In: Rossi-Santos MR, Finkl CW (Eds) Advances in marine vertebrate research in Latin America. Springer, Cham, pp 477-508.

Daura-Jorge FG, Wedekin LL, Simões-Lopes PC (2011) Feeding habits of the Guiana dolphin, *Sotalia guianensis* (Cetacea: Delphinidae), in Norte Bay, southern Brazil. Scientia Marina 75:163-169.

de Castilho PV, Lopes PCS (2008) Sea mammals in archaeological sites on the southern coast of Brazil. Revista do Museu de Arqueologia e Etnologia 18:101-113.

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochimica et cosmochimica acta 42:495-506.

DeNiro MJ, Epstein S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et cosmochimica acta 45:341-351.

Ekau W (1999) Topographical and hydrographical impacts on zooplankton community structure in the Abrolhos Bank region, East Brazil. Archive of Fishery and Marine Research 47:307-320.

Elorriaga-Verplancken F, Aurioles-Gamboa D, Newsome SD; Martinez-Diaz SF (2013) d^{15}N and d^{13}C values in dental collagen as a proxy for age and sex related variation in foraging strategies of California sea lions. Marine Biology 160:641-652.

Flores PAC, Bazzalo M (2004) Home ranges and movements patterns of the marine tucuxi *Sotalia fluviatilis* in Baía Norte, southern Brazil. The Latin American Journal of Aquatic Mammals 3:37-52.

Flores PAC, Fontoura NF (2006) Ecology of marine tucuxi and bottlenose dolphins in Baía Norte, Santa Catarina State, southern Brazil. Latin American Journal of Aquatic Mammals 5:105-115.

Graham BS, Koch PL, Newsome SD, McMahon KW, Aurioles D (2010) Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. In: Isoscapes (pp. 299-318). Springer, Dordrecht.

Hardt FAS, Cremer MJ, Tonello JrAJ, Simões-Lopes PCA (2010) Residence patterns of the Guiana dolphin Sotalia guianensis in Babitonga Bay, south coast of Brazil. Latin American Journal of Aquatic Mammals 8:117-121.

Hardt FAS, Cremer MJ, Tonello Junior AJ, Bellante A, Buffa G, Buscaino G, Mazzola S, Barreto AS, Martinelli LA, Zuppi GM (2013) Use of carbon and nitrogen stable isotopes to study the feeding ecology of small coastal cetacean populations in southern Brazil. Biota Neotropica 13:90-98.

Hartig F (2018) DHARMa: Residual Diagnostics for Hierarchical Multi-Level. Mixed Regression Models.

Herz R (1991) Manguezais do Brasil. USP.

Hohn AA, Scott MD, Wells RS, Sweeney JC, Irvine AB (1989) Growth layers in teeth from known-age, free-ranging bottlenose dolphins. Marine Mammal Science 5:315–342.

Hutchinson GE (1957) The multivariate niche. In Cold Spring Harbor Symposia on Quantitative Biology 22:415-421.

Krause J, Ruxton GD, Ruxton G, Ruxton IG (2002) Living in groups. Oxford University Press.

Lesica P, Allendorf FW (1995) When are peripheral populations valuable for conservation? Conservation biology 9:753-760.

Muelbert JH, Acha M, Mianzan H, Guerrero R, Reta R, Braga ES, Garcia VMT, Berasetegui A, Gomez-Erache M, Ramírez F (2008) Biological, physical and chemical properties at the Subtropical Shelf Front Zone in the SW Atlantic Continental Shelf. Continental Shelf Research 28:1662-1673.

Newsome SD, Koch PL, Etnier MA, Aurioles-Gamboa D (2006) Using carbon and nitrogen isotope values to investigate maternal strategies in northeast Pacific otariids. Marine Mammal Science 22:556-572.

Newsome SD, Tinker MT, Monson DH, Oftedal OT, Ralls K, Staedler MM, Fogel ML, Estes JA (2009) Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). Ecology 90:961-974.

Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study marine mammal ecology. Marine Mammal Science 26:509-572.

Perrin WF, Myrick CA (1980) Report of the workshop on growth of odontocetes and sirenians. In Age determination of toothed whales and sirenians. International Whaling Commission, Cambridge: 229.

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annual review of ecology and systematics 18:293-320.

Polis GA (1984) Age structure component of niche width and intraspecific resource partitioning: can age groups function as ecological species? The American Naturalist 123: 541-564.

Ramos RMA, Di Beneditto AP, Lima NRW (2000) Growth parameters of *Pontoporia blainvillei* and *Sotalia fluviatilis* (Cetacea) in northern Rio de Janeiro, Brazil. Aquatic Mammals 26:65-75.

Ribeiro GC, Soeth M, Andrade VK, Spach HL, Cattani AP (2014) Nycthemeral and monthly occupation of the fish assemblage on a sheltered beach of Baía Norte, Florianópolis, Santa Catarina State, Brazil. Brazilian Journal of Oceanography 62:209-223.

Rodrigues VLA, Wedekin LL, Marcondes MCC, Barbosa L, Farro APC (2020) Diet and foraging opportunism of the Guiana Dolphin (*Sotalia guianensis*) in the Abrolhos Bank, Brazil. Marine Mammal Science 36:436-450.

Rosas FCW, Barreto AS, Monteiro-Filho ELA (2003) Age and growth of the estuarine dolphin (*Sotalia guianensis*) (Cetacea, Delphinidae) on the Paraná coast, southern Brazil. Fishery Bulletin 101:377-383.

Rossi-Santos MR, Wedekin LL, Sousa-Lima RS (2006). Distribution and habitat use of small cetaceans in the coast of the Abrolhos Bank, eastern Brazil. Latin American Journal of Aquatic Mammals 5:23-28.

Rossi-Santos MR, Wedekin LL, Monteiro-Filho ELA (2007) Residence and site fidelity of *Sotalia guianensis* in the Caravelas River Estuary, eastern Brazil. Journal of Marine Biological Association of United Kingdom 87:207-212.

Rossi-Santos MR, Wedekin L, Monteiro-Filho ELA (2010) Habitat use of the Guiana dolphin, *Sotalia guianensis* (Cetacea, Delphinidae), in the Caravelas River estuary, eastern Brazil. Latin American Journal of Aquatic Mammals 8:111-116.

Rossman S, Ostrom PH, Stolen M, Barros NB, Gandhi H, Stricker CA, Wells RS (2015) Individual specialization in the foraging habits of female bottlenose dolphins living in a trophically diverse and habitat rich estuary. Oecologia,178:415-425.

Roughgarden J (1972) Evolution of niche width. American Naturalist 106:683-718.

Roughgarden J (1979) Theory of population genetics and evolutionary ecology: an introduction. Macmillan, New York

Schaeffer-Novelli Y, Cintrón-Molero G, Adaime RR, de Camargo TM (1990) Variability of mangrove ecosystems along the Brazilian coast. Estuaries 13:204-218.

Sheppard CE, Inger R, McDonald RA, Barker S, Jackson AL, Thompson FJ, Vitikainen EIK, Cant MA, Marshall HH (2018) Intragroup competition predicts individual foraging specialisation in a group-living mammal. Ecology letters 21:665-673.

Simões-Lopes PC (1988) Ocorrência de uma população de Sotalia fluviatilis (Gervais, 1853) (Cetacea, Delphinidae) no limite sul de sua distribuição, Santa Catarina, Brasil. Biotemas 1:57-62.

Skúlason S, Smith TB (1995) The ecology of resource polymorphism in vertebrates: Reply. Trends in Ecology and Evolution 11:25-25.

Stephens DW, Krebs, JR (1986) Foraging theory (Vol. 1). Princeton University Press.

Svanbäck R, Bolnick DI (2005) Intraspecific competition affects the strength of individual specialization: an optimal diet theory method. Evolutionary Ecology Research 7:993-1012.

Teixeira CR, Botta S, Daura-Jorge FG, Pereira LB, Newsome SD, Simões-Lopes PC (2020) Niche overlap and diet composition of three sympatric coastal dolphin species in the southwest Atlantic Ocean. Marine Mammal Science.

Van Valen L (1965) Morphological variation and width of ecological niche. The American Naturalist 99:377-390.

Vander Zanden HB, Bjorndal KA, Bolten AB (2013) Temporal consistency and individual specialization in resource use by green turtles in successive life stages. Oecologia 173:767-777.

Walker JL, Potter CW, Macko SA (1999) The diets of modern and historic bottlenose dolphin populations reflected through stable isotopes. Marine Mammal Science 15:335-350.

Wedekin LL, Daura-Jorge FG, Piacentini VQ, Simões-Lopes PC (2007) Seasonal variations in spatial usage by the estuarine dolphin, *Sotalia guianensis* (van Bénéden, 1864) (Cetacea; Delphinidae) at its southern limit of distribution. Brazilian Journal of Biology 67:1-8.

Yurkowski DJ, Ferguson S, Choy ES, Loseto LL, Brown TM, Muir DC. Semeniuk CAD, Fisk AT (2016) Latitudinal variation in ecological opportunity and intraspecific competition indicates differences in niche variability and diet specialization of Arctic marine predators. Ecology and evolution 6:1666-1678.

Zaccarelli N, Bolnick DI, Mancinelli G (2013) RInSp: an r package for the analysis of individual specialization in resource use. Methods in Ecology and Evolution 4:1018-10.

Supplementary Material

Table S1 δ^{13}C values from milled tracks (1 to 5) in each Guiana dolphin (*Sotalia guianensis*) tooth dentin. Tracks reflecting older portions (3 to 5) of an individual lifetime (closer to the pulp cavity) contained more GLGs than tracks near the neonate line (1 to 2).

				δ^{13C} values				
ID	**Year**	**Location**	**Age**	**1**	**2**	**3**	**4**	**5**
179	2013	CR	-	-13.4	-13.8	-13.9	-13.7	-13.5
506	2017	CR	10	-13.2	-14.1	-12.9	-12.6	-12.8
122	2010	CR	16	-12.2	-12.3	-12.4	-12.6	-12.6
120	2010	CR	21	-12.6	-13.3	-13.2	-13.1	-12.8
312	2014	CR	6	-12.4	-13.0	-13.6	-13.7	-13.1
311	2014	CR	23	-12.9	-12.9	-12.7	-12.8	-12.7
313	2014	CR	10	-13.8	-13.8	-13.6	-13.7	-13.6
476	2016	CR	31	-12.7	-12.9	-12.7	-12.7	-13.2
503	2017	CR	12	-13.1	-13.4	-13.3	-13.1	-14.2
868	2016	BB	7	-15.3	-13.0	-13.3	-13.2	-12.7
898	2016	BB	12	-13.2	-13.3	-13.1	-13.2	-13.2
161	2007	BB	-	-12.3	-12.7	-13.3	-13.2	-13.1
1138	2017	BB	25	-12.7	-12.0	-12.7	-13.0	-12.4
1136	2017	BB	25	-12.5	-12.3	-12.4	-12.3	-12.9
220	2008	BB	-	-12.1	-12.3	-12.4	-12.6	-12.3
55	2006	BB	-	-12.5	-12.6	-12.9	-13.5	-12.5
415	2013	BB	-	-12.8	-12.9	-12.6	-13.2	-12.8
33	2005	BB	-	-12.7	-12.6	-12.7	-12.4	-12.5
212	2008	BB	-	-12.9	-13.3	-13.9	-13.1	-13.0
681	2015	BB	-	-13.6	-13.9	-13.3	-13.5	-13.1
1104	1991	NB	10	-13.9	-14.4	-14.6	-14.7	-14.5
1180	1994	NB	23	-13.6	-13.2	-13.4	-13.6	-13.4
1218	1995	NB	23	-13.7	-13.6	-14.2	-14.9	-14.0
1108	1992	NB	11	-13.0	-13.4	-13.6	-13.4	-13.4
1373	2009	NB	7	-12.9	-13.5	-13.3	-13.6	-13.7
1354	2007	NB	10	-13.0	-14.1	-14.0	-13.6	-13.6
1410	2013	NB	22	-13.2	-13.9	-13.5	-14.3	-14.3

1352	2007	NB	13	-14.9	-14.9	-14.4	-14.5	-14.3
1329	2005	NB	8	-15.1	-14.9	-14.0	-14.2	-14.1
1377	2010	NB	7	-13.3	-13.8	-14.3	-13.6	-13.5
1336	2006	NB	8	-14.1	-14.6	-14.4	-14.2	-14.4
1208	1995	NB	21	-13.8	-14.5	-13.6	-13.8	-14.4
1436	2015	NB	7	-14.3	-13.6	-13.8	-14.0	-13.9
1289	2001	NB	19	-14.0	-14.0	-14.1	-14.8	-13.6
1291	2001	NB	30	-14.9	-13.6	-13.7	-13.6	-13.8
1203	1995	NB	19	-14.3	-15.2	-15.4	-14.7	-15.1

Table S2 δ^{15}N values from milled tracks (1 to 5) from each Guiana dolphin (*Sotalia guianensis*) tooth dentin. Tracks reflecting older portions (3 to 5) of an individual lifetime (closer to the pulp cavity) contained more GLGs than tracks near the neonate line (1 to 2).

δ^{15}N values								
ID	**Year**	**Location**	**Age**	**1**	**2**	**3**	**4**	**5**
179	2013	CR	-	12.5	11.0	11.3	11.5	11.7
506	2017	CR	10	11.1	9.5	10.3	10.6	11.2
122	2010	CR	16	12.3	10.7	10.6	11.1	11.6
120	2010	CR	21	11.2	9.4	9.4	10.2	10.4
312	2014	CR	6	14.2	11.9	11.6	12.4	12.5
311	2014	CR	23	13.5	11.6	12.0	12.8	13.0
313	2014	CR	10	11.0	9.8	11.1	11.5	11.4
476	2016	CR	31	13.2	12.7	14.3	14.5	14.0
503	2017	CR	12	13.3	11.6	11.9	12.3	12.4
868	2016	BB	7	14.5	14.7	15.0	15.7	16.2
898	2016	BB	12	15.6	13.8	13.8	14.2	14.7
161	2007	BB	-	16.8	15.9	16.2	16.4	16.5
1138	2017	BB	25	14.9	15.0	15.0	15.4	15.2
1136	2017	BB	25	15.2	15.0	15.8	15.8	15.1
220	2008	BB	-	16.6	15.2	15.6	16.1	16.0
55	2006	BB	-	15.6	15.0	14.4	14.7	14.9
415	2013	BB	-	17.0	16.4	16.6	16.5	16.1

33	2005	BB	-	15.7	15.2	15.7	16.1	15.9
212	2008	BB	-	16.4	16.1	15.8	15.8	16.0
681	2015	BB	-	16.4	15.5	14.8	15.3	15.5
1104	1991	NB	10	16.1	15.5	15.6	16.1	16.4
1180	1994	NB	23	16.0	15.1	15.1	15.6	15.9
1218	1995	NB	23	16.6	15.6	15.4	14.5	16.2
1108	1992	NB	11	17.0	15.6	16.0	16.3	16.6
1373	2009	NB	7	16.6	15.1	15.3	15.8	15.8
1354	2007	NB	10	18.2	16.7	15.6	16.1	16.3
1410	2013	NB	22	16.5	15.9	16.3	16.6	15.9
1352	2007	NB	13	16.6	15.1	15.3	15.7	16.6
1329	2005	NB	8	15.1	14.5	15.5	15.9	16.4
1377	2010	NB	7	18.4	16.0	15.7	16.2	16.4
1336	2006	NB	8	17.8	15.7	16.3	15.8	16.5
1208	1995	NB	21	14.9	14.5	15.5	15.8	16.2
1436	2015	NB	7	14.4	15.2	14.8	15.4	16.2
1289	2001	NB	19	14.1	15.1	14.6	15.7	16.3
1291	2001	NB	30	13.7	14.7	15.2	16.1	16.2
1203	1995	NB	19	16.1	15.5	16.1	16.7	16.8

5 CONCLUSÃO GERAL

A análise de isótopos estáveis vem sendo cada vez mais reconhecida como uma importante ferramenta para estudos tróficos de diferentes organismos marinhos, incluindo pequenos cetáceos. Porém, é importante reconhecermos que sua aplicabilidade está vinculada a diferentes fatores tais como a escolha de tecidos biológicos e, consequentemente, a variação temporal na integração da informação sobre o uso de recursos pelos indivíduos amostrados. Por meio do conhecimento empírico, somado a uma extensa revisão bibliográfica, gerei um protocolo acessível para pesquisadores sobre a seleção, coleta, preservação e preparo dos tecidos de cetáceos comumente utilizados para a análise de isótopos estáveis. Além disso, demonstrei como cada uma destas etapas pode influenciar nos dados isotópicos e como estes efeitos podem ser minimizados através de correções matemáticas. Reuni estas informações em um roteiro simples, porém detalhado— que envolve desde o delineamento do estudo (e.g., escolha do tecido e indivíduos, escolha do laboratório para análise, envio de material biológico) até as ferramentas disponíveis para conduzir análises e interpretar os dados gerados— que poderá orientar futuras pesquisas e facilitar a padronização destes procedimentos em diferentes laboratórios de pesquisa, viabilizando comparações entre espécies de cetáceos.

O Capítulo 2 possibilitou uma avaliação mais detalhada sobre a ecologia e as interações tróficas entre espécies de cetáceos simpátricos na região de Santa Catarina. Ao avaliar as composições de $\delta^{13}C$ e $\delta^{15}N$ em amostras de colágeno ósseo de boto-cinza, toninha e do ecótipo costeiro do boto-da-tainha, demonstrei que a toninha apresenta valores maiores de $\delta^{15}N$ em relação as espécies de delfinídeos (boto-cinza e ecótipo costeiro do boto-da-tainha), refletindo em uma posição trófica superior e baixa sobreposição de nicho isotópico com estas espécies. Em contrapartida, o boto-cinza e o boto-da-tainha apresentaram valores similares de $\delta^{13}C$ e $\delta^{15}N$, refletindo em alta sobreposição isotópica entre si. Este resultado corroborou nossa hipótese inicial, de que o grau de sobreposição entre as espécies de delfinídeos seria maior em relação a toninha. Para compreender melhor estes resultados, gerei modelos de mistura isotópicas adaptando fatores de discriminação trófica já existentes na literatura, além da composição isotópica das principais presas e para cada espécie. Tais modelos demonstraram que a variação nos níveis tróficos e a baixa sobreposição entre a toninha e delfinídeos provavelmente refletem o consumo de presas provenientes de diferentes tipos de habitat (pelágico vs. demersal), uma vez que a toninha se alimenta principalmente de presas pelágicas (e.g., anchovas), enquanto o boto-cinza e o ecótipo costeiro do golfinho-nariz-de garrafa consomem principalmente presas demersais da família Mugilidae (e.g., parati e tainha). Esta diferença no consumo de presas pode estar relacionada a distribuição destas espécies na região. A utilização de águas menos costeiras e de maior profundidade pela toninha pode favorecer a seleção de presas pelágicas próximas a superfície da água, enquanto a utilização de águas costeiras mais rasas pelas espécies de delfinídeos pode, por sua vez, favorecer a seleção de presas demersais próximas ao fundo.

Embora os valores de $\delta^{13}C$ entre o boto-cinza e o ecótipo costeiro do boto-da-tainha sugiram similaridade nas áreas de forrageio, estas espécies apresentam uma segregação no uso do habitat dentro da região da Baía Norte, em que o boto-cinza restringe-se à seção oeste da baía norte de Santa Catarina e o boto-da-tainha ocorre principalmente na seção leste da baía (Flores & Fontoura 2006; Wedekin et al. 2007), provavelmente reduzindo a competição por interferência ou exploração de recursos (Wedekin et al. 2004). O interessante é que estas espécies apresentaram padrões opostos em relação aos valores de $\delta^{13}C$ ao longo do tempo, e tais variações temporais podem refletir mudanças na disponibilidade de presas na região. O aumento nos valores de $\delta^{13}C$ observados no boto-cinza, por exemplo, são consistentes com uma recente mudança no uso do habitat desta população, provavelmente devido a alterações locais na abundância de presas (Wedekin et al. 2007). Porém, não podemos descartar a possibilidade de variação temporal nos valores de $\delta^{13}C$ das presas, o que pode ter contribuído para a variação temporal observada na composição isotópica do boto-cinza.

Algumas das espécies de presas que contribuem significativamente para a dieta destas espécies simpátricas (e.g., *M. atricauda, M. furnieri* e *M. liza*) vem sofrendo uma queda acentuada na biomassa e abundância devido a crescente atividade pesqueira na região. A alta contribuição da tainha na dieta do ecótipo costeiro do boto-da-tainha, por exemplo, sugere uma competição de recursos com a atividade pesqueira. Tal competição pode, em teoria, alterar a composição da dieta do boto-da-tainha e, consequentemente, influenciar sua coexistência com as demais espécies de cetáceos simpátricos na região. Considerando o atual cenário de sobreexplotação da tainha e outras presas consumidas pela toninha, boto-cinza e boto-da-tainha, estes resultados sugerem que potenciais mudanças na abundância dessas fontes de presas podem influenciar no uso de recursos e seleção de habitat entre essas espécies e devem ser monitadas e consideradas em planos de gestão e manejo da região.

No terceiro capítulo, o perfil cronológico de $\delta^{13}C$ e $\delta^{15}N$ amostrados da dentina ao longo das camadas de crescimento de indivíduos de boto-cinza demonstrou variações na largura total do nicho populacional e no grau de especialização individual em diferentes populações ao longo da costa brasileira (Caravelas, Babitonga e Baía Norte), corroborando nossa hipótese inicial. Os valores das métricas de nicho trófico (TNW, WIC, BIC e WIC/TNW) para os valores de $\delta^{15}N$ demonstraram que a população de Caravelas possui o nicho populacional mais amplo em relação as outras espécies e é composta por indivíduos especialistas, e tal expansão de nicho pode estar relacionada a liberação competitiva. Assim, este resultado dá suporte a hipótese da variação de nicho (Van Valen 1965), a qual prediz que populações mais generalistas (isto é, com nicho populacional mais amplo) são mais variáveis em termos de utilização de recursos alimentares e apresentam maior grau de variação interindividual ou especialização individual. Já a população da Baía Norte possui o nicho mais estreito comparado as outras populações e é composta por indivíduos generalistas. Este resultado pode estar associado à coexistência e à alta sobreposição de nicho isotópico com o ecótipo costeiro do boto-da-tainha, ambas associadas à alta sobreposição de área de vida entre indivíduos da população de boto-cinza devido à sua organização social caracterizada por um grande e coeso grupo em uma área pequena e restrita da Baía Norte. Assim, tanto a potencial competição inter- e intraespecífica potencialmente restringem a expansão do nicho populacional na Baía Norte, limitando oportunidades de especialização individual. Assim como a população da Baía Norte, a população de botos-cinza da Baía da Babitonga apresentou um nicho populacional estreito, porém composta por indivíduos especialistas, provavelmente devido a menor competição interespecífica com a toninha associada as diferenças no uso de habitat e no consumo das principais presas. Tais diferenças sugerem que, embora o boto-cinza possa ser considerado uma espécie generalista e oportunista em nível populacional, variações em nível individual podem surgir como resultado de diferentes contextos ecológicos aos quais essas populações estão expostas (e.g., área de vida individual e populacional, tamanho de grupo, densidade populacional).

Os resultados obtidos no Capítulo 3 fornecem uma nova visão do contexto ecológico sob o qual a especialização alimentar individual pode se manifestar em diferentes populações de boto-cinza. Estudos prévios baseados em dados provenientes de conteúdos estomacais descreveram os hábitos alimentares destas populações de boto-cinza e sugerem uma dieta generalista e oportunista (Daura-Jorge et al. 2011; Cremer et al. 2012; Rodrigues et al. 2020). Assim, os resultados deste estudo auxiliam na compreensão das estratégias de alimentação realizadas pelo boto-cinza, e demonstram que tanto a amplitude do nicho total da população quanto o grau de especialização individual da dieta podem ser um resultado de diferentes fatores ecológicos agindo de maneira independente ou integrada, tais como a disponibilidade de recursos, competição intraespecífica e área de uso espacial individual. Porém, a interpretação destes dados também exige certa cautela, uma vez neste estudo não avaliei a composição isotópica, diversidade e abundância de fontes de presas nessas diferentes áreas de estudo. Dado que a oportunidade ecológica também é um importante fator a ser considerado quando falamos sobre mecanismos que atuam na especialização individual, estudos futuros devem explorar a disponibilidade de recursos, considerando a variação espacial ou temporal na composição e abundância da comunidade de presas nestas regiões. Tais estudos são necessários para identificar mecanismos adicionais que promovem e mantém a especialização individual, e que podem ser particularmente importante para a coexistência de espécies e viabilidade populacionais.

6 REFERÊNCIAS

ANDERSON, E. M., LOVVORN, J. R. Gray whales may increase feeding opportunities for avian benthivores. **Marine Ecology Progress Series**, v. 360, n. 291-296, 2008.

BARRETO, A. S., ROSAS, F. C. Comparative growth analysis of two populations of *Pontoporia blainvillei* on the Brazilian coast. **Marine Mammal Science**, v. 22, p. 644–653, 2006.

BARROS, N. B., PARSONS, E. C. M., JEFFERSON, T. A. Prey of offshore bottlenose dolphins from the South China Sea. **Aquatic Mammals**, v. 26, n. 1, p. 2-6, 2000.

BEARHOP, S. ADAMS, C. E., WALDRON, S., FULLER, R. A., MACLEOD, H. Determining trophic niche width: a novel approach using stable isotope analysis. **Journal of animal ecology**, v. 73, n. 5, p. 1007-1012, 2004.

BISI, T. L., LEPOINT, G., DE FREITAS AZEVEDO, A., DORNELES, P. R., FLACH, L., DAS, K., MALM, O., LAILSON-BRITO, J. Trophic relationships and mercury biomagnification in Brazilian tropical coastal food webs. **Ecological Indicators**, v. 18, p. 291-302, 2012.

BOLNICK, D. I.; DOEBELI, M. Sexual dimorphism and adaptive speciation: two sides of the same ecological coin. **Evolution**, v. 57, n. 11, p. 2433-2449, 2003.

BOLNICK, D. AMARASEKARE, P., ARAÚJO, M. S., BURGER, R., LEVINE, J. M., NOVAK, M., RUDOLF V. H. W., SCHREIBER, S. J., URBAN, M. C., VASSEUR, D. A. Why intraspecific trait variation matters in community ecology. **Trends in ecology & evolution**, v. 26, n. 4, p. 183-192, 2011.

BOROBIA, M., SICILIANO, S., LODI, L., HOEK, W. Distribution of the South American dolphin *Sotalia fluviatilis*. **Canadian Journal of Zoology**, v. 69, n. 4, p. 1025-1039, 1991.

BOTTA, S., SECCHI, E. R., MUELBERT, M. M., DANILEWICZ, D., NEGRI, M. F., CAPPOZZO, H. L., HOHN, A. A. Age and growth of franciscana dolphins, *Pontoporia blainvillei* (Cetacea: Pontoporiidae) incidentally caught off southern Brazil and northern Argentina. **Journal of the Marine Biological Association of the United Kingdom**, v. 90, p. 1493–1500, 2010.

BOTTA, S., HOHN, A. A., MACKO, S. A., SECCHI, E. R. Isotopic variation in delphinids from the subtropical western South Atlantic. **Journal of the Marine Biological Association of the United Kingdom**, v. 92, n. 8, 1689, 2012.

CARR, T., BONDE, R. K. Tucuxi (*Sotalia fluviatilis*) occurs in Nicaragua, 800 km north of its previously known range. **Marine Mammal Science**, v. 16, n. 2, p. 447-452, 2000.

CAUT, S, LARAN, S., GARCIA-HARTMANN, E., DAS, K. Stable isotopes of captive cetaceans (*killer whales* and *bottlenose dolphins*). **Journal of Experimental Biology**, v. 214, p. 538-545, 2011.

CHASE, J. M., LEIBOLD, M. A. **Ecological niches: linking classical and contemporary approaches**. University of Chicago Press, 2003.

CREMER, M. J., SIMÕES-LOPES, P. C. The occurrence of *Pontoporia blainvillei* (Gervais & d'Orbigny) (Cetacea, Pontoporiidae) in an estuarine area in southern Brazil. **Revista Brasileira de Zoologia,** v. 22, p. 717-723, 2005.

CREMER, M. J., PINHEIRO, P. C, SIMÕES-LOPES, P. C. Prey consumed by Guiana dolphin *Sotalia guianensis* (Cetacea, Delphinidae) and franciscana dolphin *Pontoporia blainvillei* (Cetacea, Pontoporiidae) in an estuarine environment in southern Brazil. **Iheringia. Série Zoologia**, v. 102, p. 131-137, 2012.

CREMER, M. J., HOLZ, A. C., SARTORI, C. M., SCHULZE, B., PAITACH, R. L., SIMÕES-LOPES, P. C. Behavior and Ecology of Endangered Species Living Together: Long-Term Monitoring of Resident Sympatric Dolphin Populations. Em: Rossi-Santos, M. R., Finkl, C. W. (Eds), **Advances in Marine Vertebrate Research in Latin America** (pp. 477–508). Springer Publishing, 2018.

CRAWFORD, K., MCDONALD, R. A., Bearhop, S. Applications of stable isotope techniques to the ecology of mammals. **Mammal Review**, v. 38, n 1, p. 87-107.

DALERUM, F., ANGERBJORN, A. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. **Oecologia**, v. 144, n. 4, p. 647-658, 2005.

DE ALBERNAZ, T. L., SECCHI, E. R., DE OLIVEIRA, L. R., Botta, S. Ontogenetic and gender-related variation in the isotopic niche within and between three species of fur seals (genus Arctocephalus). **Hydrobiologia**, v. 787, n. 1, p. 123-139, 2017.

DEMASTER, D. P. FOWLER, C. W., PERRY, S. L., RICHLEN, M. F. Predation and competition: the impact of fisheries on marine-mammal populations over the next one hundred years. **Journal of Mammalogy**, v. 82, n. 3, p. 641-651, 2001.

DENIRO, M. J., EPSTEIN, S. Influence of diet on the distribution of nitrogen isotopes in animals. **Geochimica et cosmochimica acta**, v. 45, n. 3, p. 341-351, 1981.

DI BENEDITTO, A. P. M., RAMOS, R. M. A. Stomach contents of delphinids from Rio de Janeiro, southeastern Brazil. **Atlantic**, v. 2, n. 2, 2001.

DÍAZ-GAMBOA, R. E., GENDRON, D., BUSQUETS-VASS, G. Isotopic niche width differentiation between common bottlenose dolphin ecotypes and sperm whales in the Gulf of California. **Marine Mammal Science**, v. 34, n. 2, p. 440-457, 2018.

DURRETT, R., LEVIN, S. The importance of being discrete (and spatial). **Theoretical population biology**, v. 46, n. 3, p. 363-394, 1994.

ELORRIAGA-VERPLANCKEN, F., AURIOLES-GAMBOA, D., NEWSOME, S. D., MARTÍNEZ-DÍAS, S. F. $\delta^{15}N$ and $\delta^{13}C$ values in dental collagen as a proxy for age-and sex-related variation in foraging strategies of California sea lions. **Marine biology**, v. 160, n. 3, p. 641-652, 2013.

FLORES P. A. C., BAZZALO, M. Home ranges and movements patterns of the marine tucuxi *Sotalia fluviatilis* in Baía Norte, southern Brazil. **The Latin American Journal of Aquatic Mammals**, v. 3, p. 37-52, 2004.

FLORES P. A. C., FONTOURA N. F. Ecology of marine tucuxi and bottlenose dolphins in Baía Norte, Santa Catarina State, southern Brazil. **Latin American Journal of Aquatic Mammals**, 5:105-115, 2006.

FORDYCE, R. E.; BARNES, L. G. The evolutionary history of whales and dolphins. **Annual Review of Earth and Planetary Sciences**, v. 22, n. 1, p. 419-455, 1994.

FRANCO-TRECU, V., DRAGO, M., COSTA, P., DIMITRIADIS, C., PASSADORE, C. Trophic relationships in apex predators in an estuary system: a multiple-method approximation. **Journal of Experimental Marine Biology and Ecology**, v. 486, p. 230-236, 2017.

FRUET, P. F., DAURA-JORGE, F. G., MOLLER, L. M., GENOVES, R. C., SECCHI, E. R. Abundance and demography of bottlenose dolphins inhabiting a subtropical estuary in the Southwestern Atlantic Ocean. **Journal of Mammalogy**, v. 96, n. 332-343, 2015

FRY, Brian. **Stable isotope ecology**. New York: Springer, 2006.

GANNES, L. Z., DEL RIO, C. M., KOCH, P. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. **Comparative biochemistry and physiology Part A: Molecular & integrative physiology**, vol. 119, n. 3, p. 725-737, 1998.

HAIMOVICI, M., MENDONÇA, J. T. Descartes da fauna acompanhante na pesca de arrasto e tangones dirigida a linguados e camarões na plataforma continental do sul do Brasil. **Atlântida,** v. 18, 161–177, 1996.

HAIMOVICI, M., CARDOSO, L. G. Long-term changes in the fisheries in the Patos Lagoon estuary and adjacent coastal waters in Southern Brazil. **Marine Biology Research**, v. 13, n. 135-150, 2017.

HARDT, F. A. S, CREMER, M. J., TONELLO JRAJ, SIMÕES-LOPES P. C. A. Residence patterns of the Guiana dolphin *Sotalia guianensis* in Babitonga Bay, south coast of Brazil. **Latin American Journal of Aquatic Mammals,** v. 8, p. 117-121, 2010.

HOBSON, K. A., WASSENAAR, L. I. Stable isotope ecology: an introduction. **Oecologia**, v. 120, n. 3, 312-313, 1999.

HUTCHINSON, G. E. Population studies-animal ecology and demography-concluding remarks. In: **Cold Spring Harbor Symposia on Quantitative Biology**. 1 BUNGTOWN RD, PLAINVIEW, NY 11724: COLD SPRING HARBOR LAB PRESS, p. 415-427, 1957.

JACKSON, A. L., INGER, R., PARNELL, A. C., BEARHOP, S. Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. **Journal of Animal Ecology**, v. 80, n. 3, p. 595-602, 2011.

JONES, E. G., COLLINS M. A., BAGLEY P.M., ADDISON, S., PRIEDE, I. G. The fate of cetacean carcasses in the deep sea: observations on consumption rates and succession of scavenging species in the abyssal north-east Atlantic Ocean. **Proceedings of The Royal Society B**, v. 265, p. 1119-1127, 1998.

KATONA, S., WHITEHEAD, H. Are cetacea ecologically important. **Oceanography and marine biology annual review***,* v. *26*, p. 553-568, 1988.

KNOFF, A., HOHN, A, MACKO, S. Ontogenetic diet changes in bottlenose dolphins (*Tursiops truncatus*) reflected through stable isotopes. **Marine Mammal Science**, v. 24, n. 1, p. 128-137, 2008.

KOCH, P. L. **Isotopic Study of the Biology of Modern and Fossil Vertebrates**. In Michener, R., Lajtha, K (Eds), Stable Isotopes in Ecology and Environmental Science (pp. 99–154). Blackwell Publishing, Boston, 2007.

LAWS, R. M. Seals and whales of the Southern Ocean. **Philosophical Transactions of the Royal Society of London. B, Biological Sciences**, v. 279, n. 963, p 81-96, 1977.

LAYMAN, C. A., ARRINGTON, D. A., MONTANA, C. G., POST, D. M. Can stable isotope ratios provide for community-wide measures of trophic structure? **Ecology**, v. 88, n. 1, p. 42-48, 2007.

LEIBOLD, M. A. The niche concept revisited: mechanistic models and community context. **Ecology**, v. 76, n. 5, p. 1371-1382, 1995.

LOPES, X. M., DA SILVA, E., BASSOI, M., DOS SANTOS, R. A., DE OLIVEIRA SANTOS, M. C. Feeding habits of Guiana dolphins, Sotalia guianensis, from south-eastern Brazil: new items and a knowledge review. **Journal of the Marine Biological Association of the United Kingdom**, p. 1723-1733, 2012.

LOUZADA, C. N. How do Guiana dolphin (*Sotalia guianensis*), from the Cananéia estuary in State of São Paulo, use cerco-fixo fish traps in their fishing activities? **Revista de Etologia**, v. 12, n. 1-2, p.18-24, 2013.

MARTÍNEZ DEL RIO, C., WOLF, N., CARLETON, S. A., GANNES, L. Z. Isotopic ecology ten years after a call for more laboratory experiments. **Biological Reviews**, 84, p. 91-111, 2009.

MATICH, P., HEITHAUS, M. R., LAYMAN, C. A. Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. **Journal of Animal Ecology**, v. 80, n. 1, p. 294-305, 2011.

MCCONNAUGHEY, T.; MCROY, C. P. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. **Marine Biology**, 53.3, p. 257-262, 1979.

MENDES, S., NEWTON, J., REID, R. J., ZUUR, A. F., PIERCE, G. J. Stable carbon and nitrogen isotope ratio profiling of sperm whale teeth reveals ontogenetic movements and trophic ecology. **Oecologia**, v. 151, p. 605-615, 2007.

MICHENER, R. H., SCHELL, D. M. Stable isotope ratios as tracers in marine aquatic food webs. Em: **Stable isotopes in ecology and environmental science**. Capítulo 7. Vol 1 (eds LAJTHA, K. & MICHENER, R. H.) Blackwell scientific publications, 1994.

MINAGAWA, M., WADA, E. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between δ 15 N and animal age. **Geochimica et cosmochimica acta**, v. 48, n. 5, p. 1135-1140, 1984.

NEWSOME, S. D., MARTINEZ DEL RIO, C., BEARHOP, S., Phillips, D. L. A niche for isotopic ecology. **Frontiers in Ecology and the Environment**, v. 5, n. 8, p. 429-436, 2007.

NEWSOME, S. D.; CLEMENTZ, M. T., KOCK, P. L. Using stable isotope biogeochemistry to study marine mammal ecology. **Marine Mammal Science***,* v. 26*,* p. 509-572, 2010.

PERRIN, W. F., MYRICK, A. C. (Eds.). **Age determination of toothed whales and sirenians** (No. 3). International Whaling Commission, 1980.

PERSHING, A. J., CHRISTENSEN, L. B., RECORD, N. R., SHERWOOD, G. D., STETSON, P. B. **The impact of whaling on the ocean carbon cycle: why bigger was better**. PloS one, v. 5*,* n. 8, e12444, 2010.

PETERSON, B. J.; FRY, B. Stable isotopes in ecosystem studies. **Annual review of ecology and systematics**, v. 18, n. 1, p. 293-320, 1987.

PIANKA, E. R. Guild structure in desert lizards. **Oikos**, v. 35, p. 194–201, 1980.

POLIS, G. A. Age structure component of niche width and intraspecific resource partitioning: can age groups function as ecological species? **The American Naturalist**, v. 123, n. 4, p. 541-564, 1984.

POST, D. M. Using stable isotopes to estimate trophic position: models, methods, and assumptions. **Ecology**, v. 83, n. 3, p. 703-718, 2002.

RIDGWAY, S. H.; HARRISON, S. R. (eds.). **Handbook of marine mammals.** London, Academic Press, p. 416, 1994.

RIOFRÍO-LAZO, M., AURIOLES-GAMBOA, D. Timing of isotopic integration in marine mammal skull: comparative study between calcified tissues. **Rapid Communications in Mass Spectrometry**, v. 27, n. 9, p. 1076-1082, 2013.

RODRIGUES, V. L. A., WEDEKIN, L. L., MARCONDES, M. C. C., BARBOSA, L., FARRO, A. P. C. Diet and foraging opportunism of the Guiana Dolphin (*Sotalia guianensis*) in the Abrolhos Bank, Brazil. **Marine Mammal Science** 36:436-450, 2020.

ROMAN, J., MCCARTHY, J. J. The whale pump: marine mammals enhance primary productivity in a coastal basin. **PloS one**, v. 5, n. 10, e13255, 2010.

ROMAN, J., NEVINS, J., ALTABET, M., KOOPMAN, H., MCCARTHY, J. Endangered right whales enhance primary productivity in the Bay of Fundy. **PLoS One**, v. 11, n. 6, e0156553, 2016.

ROSAS, F. C. W., MONTEIRO-FILHO, E. L. A. Reproduction of the estuarine dolphin (*Sotalia guianensis*) on the coast of Paraná, southern Brazil. **Journal of Mammalogy**, v. 83*,* p. 507–515, 2002.

ROSSI-SANTOS, M. R., WEDEKIN, L. L., SOUSA-LIMA, R. S. Distribution and habitat use of small cetaceans in the coast of the Abrolhos Bank, eastern Brazil. **Latin American Journal of Aquatic Mammals,** v. 5, p. 23-28, 2006.

ROSSMAN, S., OSTROM, P. H., STOLEN, M., BARROS, N. B., GANDHI, H., STRICKER, C. A., WELLS, R. S. Individual specialization in the foraging habits of female bottlenose dolphins living in a trophically diverse and habitat rich estuary. **Oecologia**, v. 178, n. 2, p. 415-425, 2015.

ROUGHGARDEN, J. Evolution of niche width. **The American Naturalist**, 106.952, p. 683-718, 1972.

ROUGHGARDEN, J. Niche width: biogeographic patterns among Anolis lizard populations. **The American Naturalist,** 108.962, p. 429-442, 1974.

SANTOS CARVALHO, M., PÉREZ ALVAREZ, M. J., MUNIAIN, V., MORAGA, R., OLIVA, D., SEPÚLVEDA, M. Trophic niche overlap between sympatric resident and transient populations of bottlenose dolphins in the Humboldt Current System off north-central Chile, 2015.

SCHOENER, T. W. Resource partitioning in ecological communities. **Science,** v. 185, n. 4145, p. 27-39, 1974.

SILVA, M. A., BORRE, A., PRIETO, R., GAUFFIER, P., BÉRUBÉ, M., PALSBOL, P. J., COLAÇO, A. Stable isotopes reveal winter feeding in different habitats in blue, fin and sei whales migrating through the Azores. **Royal Society open science**, v. 6, 181800, 2019.

SIMÕES-LOPES, P. C. Ocorrência de uma população de *Sotalia fluviatilis* (Gervais, 1853) (Cetacea, Delphinidae) no limite sul de sua distribuição, Santa Catarina, Brasil. **Biotemas**, v. 1, n. 1, p. 57-62, 1988.

SKÚLASON, S., SMITH, T. B. Resource polymorphisms in vertebrates. **Trends in ecology & evolution**, v. 10, n. 9, p. 366-370, 1995.

SOBERÓN, J. Grinnellian and Eltonian niches and geographic distributions of species. **Ecology letters**, v. 10, n. 12, p. 1115-1123, 2007.

SPRINGER, A. M., ESTES, J. A., VAN VLIET, G. B., WILLIAMS, T. M., DOAK, D. F., DANNER, E. M., FORNEY, K. A., PFISTER, B. Sequential megafaunal collapse in the North Pacific Ocean: An ongoing legacy of industrial whaling? **Proceedings of the National Academy of Sciences**, v. 100, n. 21, p. 12223-12228, 2003.

TEBBICH, S., TABORSKY, M., FESSL, B., DVORAK, M., WNKLER, H. Feeding behavior of four arboreal Darwin's finches: adaptations to spatial and seasonal variability. **The Condor**, v. 106, n. 1, 95-105, 2004.

TEIXEIRA, C. R., BOTTA, S., DAURA-JORGE, F. G., PEREIRA, L. B., NEWSOME, S. D., SIMÕES-LOPES, P. C. Niche overlap and diet composition of three sympatric coastal dolphin species in the southwest Atlantic Ocean. **Marine Mammal Science**, 2020.

TERBORGH, J., ESTES, J. A. (Eds.). **Trophic cascades: predators, prey, and the changing dynamics of nature.** Island press. 2013.

TIESZEN, L. L., BOUTTON, T. W., TESDAHL, K. G., SLADE, N. A. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for δ 13 C analysis of diet. **Oecologia**, v. 57, n. 1-2, p. 32-37, 1983.

TRITES, A. W., CHRISTENSEN, V., PAULY, D. Competition between fisheries and marine mammals for prey and primary production in the Pacific Ocean. **Journal of Northwest Atlantic Fishery Science**, 1997.

TROINA, G., BOTTA, S., SECCHI, E. R., DEHAIRS, F. Ontogenetic and sexual characterization of the feeding habits of franciscanas, *Pontoporia blainvillei*, based on tooth dentin carbon and nitrogen stable isotopes. **Marine Mammal Science**, v. 32, p. 1115–1137, 2016.

VAN VALEN, L. Morphological variation, and width of ecological niche. **The American Naturalist**, v. 99, n. 908, p. 377-390, 1965.

VEIT, R. R., HARRISON, N. M. Positive interactions among foraging seabirds, marine mammals and fishes and implications for their conservation. **Frontiers in Ecology and Evolution**, v. 5, p. 121, 2017.

VENUTO, R., BOTTA, S., BARRETO, A. S., SECCHI, E. R., Fruet, P. F. Age structure of strandings and growth of Lahille's bottlenose dolphin (*Tursiops truncatus gephyreus*). **Marine Mammal Science**, v. 36, p. 802–812, 2020.

VIOLLE, C., ENQUIST, B. J., MCGILL, B. J., JIANG, L. I. N., ALBERT, C. H., HULSHOF, C., JUNG, V., MESSIER, J. The return of the variance: intraspecific variability in community ecology. **Trends in ecology & evolution**, v. 27, n. 4, p. 244-252, 2012.

WALKER, J. L., MACKO, S. A. Dietary studies of marine mammals using stable carbon and nitrogen isotopic ratios of teeth. **Marine Mammal Science**, v. 15, n. 2, p. 314-334, 1999.

WEDEKIN, L. L., DAURA-JORGE, F. G., SIMÕES-LOPES, P. C. An aggressive interaction between bottlenose dolphins (*Tursiops truncatus*) and estuarine dolphins (*Sotalia guianensis*) in southern Brazil. **Aquatic mammals***,* v. 30*,* p. 391-397, 2004.

WEDEKIN, L. L., DAURA-JORGE, F. G., PIACENTINI, V. Q., SIMÕES-LOPES, P. C. Seasonal variations in spatial usage by the estuarine dolphin, *Sotalia guianensis* (van Bénéden, 1864) (Cetacea; Delphinidae) at its southern limit of distribution. **Brazilian Journal of Biology***,* v. 67*,* p. 1-8, 2007.

YURKOWSKI, D. J., FERGUSON, S., CHOY, E. S., LOSETO, L. L., BROWN, T. M., MUIR, D. C., SEMENIUK A. C., FISK, A. T. Latitudinal variation in ecological opportunity and intraspecific competition indicates differences in niche variability and diet specialization of Arctic marine predators. **Ecology and evolution**, v. 6, p. 1666–1678, 2016.

ZAPPES, C. A., ANDRIOLO, A., SIMÕES-LOPES, P. C., DI BENEDITTO, A. P. M. 'Human-dolphin (*Tursiops truncatus* Montagu, 1821) cooperative fishery' and its influence on cast net fishing activities in Barra de Imbé/Tramandaí, Southern Brazil. Ocean & Coastal Management, v. 54, n. 5, p. 427-432, 2011.

ZHAO, T., VILLÉGER, S., LEK, S., CUCHEROUSSET, J. High intraspecific variability in the functional niche of a predator is associated with ontogenetic shift and individual specialization. **Ecology and Evolution***,* v. 4, p. 4649-4657, 2014.