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# ECOLOGIA TRÓFICA E MIGRAÇÃO DE *KATSUWONUS PELAMIS* NO OCEANO ATLÂNTICO SUDOESTE

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## LISTA DE ABREVIATURAS

AA aminoácido

ACAS Água Central do Atlântico Sul

ACE Análise de Conteúdo Estomacal

AIE Análise de Isótopos Estáveis

AOTTP Programa de Marcação de Atuns Tropicais do Atlântico

AT Água Tropical

CB Corrente do Brasil

CF Comprimento Furcal

CM Corrente das Malvinas

CPUE Captura por Unidade de Esforço

FE Fenilalanina

GLX Ácido Glutâmico

ICCAT International Commission for the Conservation of Atlantic Tunas

LIS Lisina

PT Posição Trófica

RFMOs Organizações Regionais de Manejo das Pescarias

RJ Rio de Janeiro

RS Rio Grande do Sul

TEF Fator de enriquecimento trófico

TSM Temperatura da Superfície do Mar

ZCST Zona de Convergência Subtropical

#### **RESUMO**

O bonito-listrado (Katsuwonus pelamis) é um peixe pelágico cosmopolita com grande importância na segurança alimentar. É a terceira espécie mais capturada no mundo, com produção média de 2,9 milhões de toneladas/ano entre 2015-18. No Brasil, abastece a indústria do atum enlatado e sustenta a pesca de vara e isca-viva na região Sudeste/Sul. Nesta tese, amostras obtidas em 66 desembarques de bonito-listrado em Rio Grande-RS (45) e Niterói-RJ (21), entre dezembro de 2016 e maio de 2018, foram utilizadas para caracterizar a dieta, a posição trófica, o habitat de forrageio e os movimentos da espécie no Oceano Atlântico Sudoeste. As capturas ocorreram entre as latitudes 20-34°S. De acordo com as características oceanográficas e os padrões de produtividade, foram consideradas duas áreas: norte (20–26°S); sul (30–34°S). As seguintes análises foram utilizadas: conteúdo estomacal (ACE), isótopos estáveis (AIE) de carbono ( $\delta^{13}$ C) e nitrogênio ( $\delta^{15}$ N) no músculo (*bulk*) e AIE de  $\delta^{15}$ N em aminoácidos (AA) trófico (ácido glutâmico, Glx) e fontes (Lisina, Lis; Fenilalanina, Fe). Os modelos de mistura isotópica com prioris informativas da ACE indicaram que na área norte o peixe-lanterna (Maurolicus stehmanni) (mediana: 50%) e krill (Euphausia similis) (31%) foram as principais presas, seguidas por 'peixe e cefalópode' (Carangidae, Ommastrephidae) (14%) e 'pequenos peixes pelágicos' (Clupeidae) (7%). Na área sul, o peixe-lanterna (53%), krill (23%) e 'pequenos peixes pelágicos' (Clupeidae, Engraulidae) (23%) foram as principais fontes alimentares. O  $\delta^{15}$ N foi mais alto nos indivíduos capturados na área sul (bulk:  $12.2 \pm 1.3$  %; Lis:  $6.5 \pm 1.3$  %; Fe:  $10.6 \pm 1.3$  %), em comparação à área norte (bulk: 9,7 ± 1,5 ‰; Lis: 3,4 ± 1,1 ‰; Fe: 8,2 ± 1,2 ‰). A variação latitudinal de  $\delta^{15}$ N foi consistente com o  $\delta^{15}$ N do zooplâncton na região. Indivíduos com  $\delta^{15}$ N distinto do  $\delta^{15}$ N das áreas de captura indicaram movimentos latitudinais, para sul na primavera-verão, e para o norte durante o outono, de acordo com a dinâmica da temperatura da superfície do mar. A área sul apresenta altas densidade de espécies forrageiras, como pequenos peixes pelágicos e krill, sustentando agregações de indivíduos jovens para alimentação e crescimento. O  $\delta^{15}$ N de AA fonte diminuiu com o tamanho do bonito-listrado, indicando forrageio de adultos em baixas latitudes ou áreas offshore. A análise de  $\delta^{15}$ N em AA tróficos e fontes permitiu separar os padrões espaciais no  $\delta^{15}$ N dos padrões ontogenéticos no uso do habitat. O bonito-listrado é um consumidor terciário que aumenta sua posição trófica ( $TP_{Glx-Lis}$ : 3,5–4,0) e se desloca entre áreas subtropicais e tropicais durante seu ciclo de vida no Oceano Atlântico Sudoeste.

**Palavras-chave**: aminoácidos, dieta, habitat, isótopos estáveis, nitrogênio, oceanografia, posição trófica, teias tróficas

#### ABSTRACT

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Skipjack tuna (*Katsuwonus pelamis*) is a cosmopolitan pelagic fish with great importance in food security. It ranks third among marine fish resources harvested by global fisheries, with an average production of 2.9 million tons/year between 2015-18. In Brazil, it supplies the tuna canning industry, being caught by the pole and line fishing. In this study, samples obtained in 66 skipjack landings in Rio Grande-RS (45) and Niterói-RJ (21), between December 2015 and May 2018, were used to characterize skipjack diet composition, trophic position, foraging habitat, and movements in the southwestern Atlantic Ocean. Catches occurred between latitudes 20-34°S. According to the oceanographic characteristics and productivity patterns, two areas were considered: northern (20–26 °S); southern (30–34 °S). The following analyzes were used: stomach content (SCA), stable isotopes (SIA) of carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) in muscle tissue (bulk) and  $\delta^{15}N$  SIA in trophic (glutamic acid, Glx) and source (Lysine, Lys; Phenylalanine, Phe) amino acids (AA). Mixing models with informative priors from SCA indicated that lanternfish (Maurolicus stehmanni) (median: 50%) and krill (Euphausia similis) (31%) were the main prev in the northern area (20–26 °S), followed by "fish and cephalopods" (Carangidae, Ommastrephidae) (14%) and small pelagic fish (Clupeidae) (7%). In the southern area (30–34 °S), lanternfish (53%), krill (23%) and small pelagic fish (Clupeidae, Engraulidae) (23%) were the main food sources. Values of  $\delta^{15}$ N were higher in the southern area (bulk:  $12.2 \pm 1.3$  ‰; Lys:  $6.5 \pm 1.3$  ‰; Phe:  $10.6 \pm 1.3$  ‰) compared to northern area (bulk:  $9.7 \pm 1.5$  %; Lys:  $3.4 \pm 1.1$  %; Phe:  $8.2 \pm 1.2$  %). The latitudinal variation of  $\delta^{15}$ N was consistent with zooplankton  $\delta^{15}$ N isoscapes in the region. Individuals with  $\delta^{15}$ N different from the sampling area  $\delta^{15}$ N baseline indicated latitudinal movements following sea surface temperature dynamics and the Subtropical Convergence Area displacements, southwards in spring-summer, and northwards during the fall. The southern area sustains high forage species densities, such as small pelagic fish and krill, supporting young skipjack feeding aggregations. Source AA  $\delta^{15}$ N values decreased with skipjack size, indicating that adults forage in low latitudes or offshore areas. The  $\delta^{15}$ N analysis of trophic-source AAs allowed us to separate the spatial patterns in  $\delta^{15}$ N baselines from ontogenetic patterns in habitat use. Skipjack is a tertiary consumer that increases its trophic position (TP<sub>Glx-Lis</sub>: 3.5–4.0) and moves between subtropical and tropical areas during its life cycle in the southwestern Atlantic Ocean.

**Keywords**: amino acids, diet, food webs, habitat, nitrogen, oceanography, stable isotopes, trophic position

### Prefácio

*Katsuwonus pelamis*, comumente chamado de bonito-listrado, é um peixe pelágico que ocorre em áreas tropicais e subtropicais dos Oceanos Índico, Pacífico e Atlântico. Desempenha um papel importante na segurança alimentar, sendo a terceira espécie mais capturada no mundo. Entre 2015–18, a produção mundial de bonito-listrado foi de 2,9 milhões de toneladas/ano, em média. No Brasil, é o tunídeo mais abundante, objeto de uma pescaria que abastece a indústria de atum enlatado e o mercado de exportações. Apesar da importância econômica e ecológica, a ecologia trófica e espacial desta espécie ainda é pouco estudada. Esta tese teve como objetivo estudar a ecologia trófica e os movimentos do bonitolistrado no Oceano Atlântico Sudoeste. O trabalho foi feito no âmbito do Projeto Bonito, cujo objetivo principal foi ampliar o conhecimento sobre a bioecologia do bonito-listrado, seu habitat e os aspectos socioeconômicos da pesca no Brasil. Os resultados do Projeto Bonito, produzidos de forma interdisciplinar por pesquisadores de seis instituições, foram compilados em um livro (Madureira & Monteiro-Neto 2020). Este estudo tem por base amostras coletadas em 66 desembarques da frota pesqueira em Rio Grande-RS (45) e Niterói-RJ (21), entre dezembro de 2016 e agosto de 2018.

A tese foi estruturada no modelo com manuscritos em apêndice (ANEXOS 1 e 2). Os anexos são precedidos por uma introdução geral, hipóteses e objetivos. Na sequência são descritas as metodologias e os resultados. Por fim, são apresentadas as conclusões e sugestões para pesquisas futuras.

## 1. INTRODUÇÃO

Cadeias tróficas representam o fluxo de energia e constituem um dos principais atributos da estrutura trófica em comunidades e ecossistemas (Elton 1927). A energia flui entre os níveis tróficos, formados por organismos que obtém sua nutrição de forma similar, desde a base, onde estão os produtores primários, até os consumidores (Polunin & Pinnegar 2002, Garrison 2010, Librelato et al. 2014). Em sistemas oceânicos, o primeiro nível trófico é formado pelo fitoplâncton (e.g. diatomáceas, dinoflagelados), pequenos organismos autótrofos que produzem seu alimento a partir de componentes inorgânicos (nutrientes) e da energia da luz solar (Brandini 2015, Costa & Haimovici 2020). Os produtores primários servem de alimento para herbívoros, consumidores primários no segundo nível trófico (e.g. copépodos, salpas). Pequenos carnívoros do zooplâncton que se alimentam de herbívoros são consumidores secundários (e.g. eufausiáceos, quetognatos), enquanto outros carnívoros maiores (e.g. peixes pequenos), que deles se alimentam são os consumidores terciários. Os mais altos níveis tróficos são ocupados por predadores de topo da cadeia (e.g. tubarões, grandes teleósteos piscívoros, lulas e mamíferos marinhos) (Lalli & Parsons 2008, Pinet 2009, Costa & Haimovici 2020).

A eficiência na transferência de energia entre níveis tróficos é baixa (Pauly & Christensen 1995, Lalli & Parsons 2008, Pinet 2009). Como resultado, em média, apenas 10% da energia dos organismos consumidos em um nível trófico é armazenada na forma de tecido vivo disponível para o nível trófico seguinte (Garrison 2010), limitando o número de níveis tróficos na comunidade (Costa & Haimovici 2020). Cadeias tróficas iniciadas em organismos pequenos geralmente possuem mais níveis tróficos e menor eficiência energética como, por exemplo, as de áreas oceânicas (Lalli & Parsons 2008). As plataformas continentais e áreas de ressurgência, por outro lado, possuem produtores primários maiores e alta concentração de nutrientes, representando ambientes com maior produtividade por unidade de área (Pauly & Christensen 1995). Áreas de ressurgência, por exemplo, são enriquecidas por nutrientes de águas mais profundas e sustentam autótrofos de maior tamanho e formadores de cadeia, os quais servem de alimento para peixes e crustáceos planctívoros, encurtando a cadeia trófica em até dois níveis e resultando em alta biomassa

disponível para predadores de níveis tróficos superiores (Lalli & Parsons 2008, Garrison 2010, Costa & Haimovici 2020).

Os predadores pelágicos possuem hábito alimentar oportunista (Costa & Haimovici 2020), maximizando a ingestão de presas e o ganho energético por meio de uma estratégia de forrageio ótimo (MacArthur & Pianka 1966, Begon et al. 2006, Olson et al. 2016). Os atuns, em particular, possuem adaptações morfológicas e fisiológicas, incluindo o formato do corpo, endotermia regional, grande superfície branquial, alta afinidade por oxigênio e elevada atividade cardíaca (Murua et al. 2017), que lhes permitem suprir seus altos requerimentos energéticos no habitat pelágico/oceânico, onde os nutrientes e, por consequência suas presas, estão distribuídos em manchas (Sund et al. 1981, Olson et al. 2016, Bernal et al. 2017), Duffy et al. 2017).

O bonito-listrado *Katsuwonus pelamis* (Fig. 1) (Linnaeus, 1758) pertence à família Scombridae, a qual compreende 15 gêneros e 49 espécies, entre cavalas, bonitos e atuns (Colette & Nauen 1983). Esta é a única espécie do gênero *Katsuwonus*. O corpo é hidrodinâmico, com formato fusiforme, alongado e arredondado. Não possui escamas, exceto na linha lateral e na porção anterior do corpo, denominada corselete. Possui coloração azulescuro na porção dorsal, e prateada na porção ventral (contra sombreamento), onde estão dispostas 4–7 faixas longitudinais escuras características da espécie (Figueiredo & Menezes 1980, Colette & Nauen 1983). A ausência de bexiga natatória permite a realização de movimentos verticais rápidos para caça e fuga de predadores (Costa & Haimovici 2020) mas, ao mesmo tempo, exige uma velocidade mínima para manter o equilíbrio hidrostático (Colette & Nauen 1983).

A distribuição é cosmopolita, ocorrendo em águas tropicais e subtropicais nos Oceanos Atlântico, Índico e Pacífico (Colette & Nauen 1983). Forma agregações em áreas de convergência, frentes térmicas, áreas de ressurgência e outras descontinuidades. Os cardumes ocorrem também associados com objetos flutuantes, aves e outros organismos, como golfinhos, tartarugas, tubarões e baleias (Colette & Nauen 1983, Matsumoto et al. 1984, Lima et al. 2011).



Figura 1. Palete de bonito-listrado (Katsuwonus pelamis) na empresa Leal Santos, Rio Grande/RS.

O hábitat pelágico desta espécie é delimitado pela concentração elevada de oxigênio dissolvido, entre 3–3,5 mg/L para sobrevivência por longos períodos, e acima de 2,45 mg/L para manter a natação basal (Wild & Hampton 1993), e a temperatura da água entre 15–30°C (Sund et al. 1981). A alta demanda por oxigênio geralmente restringe a espécie à camada de mistura (Wild & Hampton 1993, Mugo et al. 2010), ocorrendo até aproximadamente 260 m de profundidade durante o dia, e próximo à superfície durante a noite (ICCAT 2016). Os adultos podem realizar mergulhos até águas mais profundas, frias e menos oxigenadas abaixo da termoclina (Bernal et al. 2017, Monteiro-Neto et al. 2020).

As características da história de vida indicam a seleção *r*-estrategista (Matsumoto et al. 1984, King 2007, Lalli & Parsons 2008). O bonito-listrado possui tamanho relativamente pequeno, rápido crescimento e ciclo de vida curto (Colette & Nauen 1983, Murua et al. 2017). Pode atingir a primeira maturação entre 1–2 anos de idade, com comprimento furcal (CF) de aproximadamente 45 cm (Colette & Nauen 1983, Matsumoto et al. 1984, Vilela & Castello 1993, Soares et al. 2019, Costa et al. 2020a). A desova é oportunista e ocorre ao longo do ano em águas tropicais, e entre primavera-verão em águas subtropicais (Colette & Nauen 1983, Cayré & Farrugio 1986, Castello & Habiaga 1989, Pinto et al. 2002, Katsuragawa et al. 2020). As larvas são mais abundantes em águas tropicais com temperatura maior que 25°C (Matsumoto et al. 1984, Matsuura 1986, Katsuragawa et al. 2020).

Dentro do grupo dos atuns e afins, o bonito-listrado é a espécie com maior volume de captura. Por nove anos consecutivos, o bonito-listrado está na terceira colocação dentre as espécies marinhas mais pescadas, contribuindo para a segurança alimentar em nível mundial, com 3,2 milhões de toneladas desembarcadas em 2018 (FAO 2020). Cinco estoques de bonito-listrado são considerados para o manejo das pescarias: um no Oceano Índico, dois no Oceano Atlântico (leste e oeste) e dois no Oceano Pacífico (leste e oeste) (Bernal et al. 2017). Como os demais atuns e afins, o bonito-listrado possui distribuição geográfica ampla e é capturado em águas jurisdicionais de diversos países. Desta forma, o ordenamento das pescarias desses recursos é realizado por Organizações Regionais de Manejo das Pescarias (RFMOs, em inglês). No Oceano Atlântico, essa tarefa é realizada pela Comissão Internacional para a Conservação dos Atuns do Atlântico (ICCAT, em inglês).

O estoque de bonito-listrado do Oeste do Atlântico não se encontra sobre-explorado (Cardoso et al. 2020). Entretanto, a produção de 15.355 toneladas em 2019 representa uma queda de ~30% em relação à média das capturas entre 2009–18 (22.440 t) (ICCAT 2021). Essa redução foi relacionada ao menor número de embarcações atuando na pesca com vara e isca-viva em anos recentes (ICCAT 2021). Além disso, estudos indicam a ocorrência de anomalias positivas na temperatura da superfície do mar e baixa produtividade em anos recentes (Schmidt et al. 2019, Madureira et al. 2020), indicando possivelmente um período atípico, de baixa disponibilidade do estoque à pesca (Cardoso et al. 2020).

A principal pescaria de bonito-listrado no Brasil é a pesca de vara e isca-viva realizada na região sudeste/sul (Fig. 2), na qual a espécie representa 90% da captura desembarcada. A frota de vara e isca-viva brasileira possui atualmente 30 embarcações, sediadas em Niterói-RJ (7), Itajaí/Navegantes-SC (17) e Rio Grande-RS (6) (ICCAT 2021), onde estão instaladas indústrias de processamento e exportação de atuns (Zawislak et al. 2020). Nessa modalidade de pesca os peixes são retirados da água um-a-um. Consequentemente, nem todos os peixes dos cardumes são capturados, e não ocorre a captura de tartarugas, tubarões e golfinhos, como pode ocorrer na pesca com rede de cerco (Matsumoto et al. 1984, Lima et al. 2011). Essas características favorecem a atividade do ponto de vista econômico, pois o pescado capturado pelo método seletivo possuiu maior valor agregado (Zawislak et al. 2020).



Figura 2. Pesca de vara e isca-viva de bonito-listrado (*Katsuwonus pelamis*) na região sul do Brasil. Embarcação Katsushio Maru n°5.

O ambiente oceanográfico do bonito-listrado no Sudoeste do Atlântico é caracterizado pela Zona de Convergência Subtropical (ZCST), formada pelo encontro entre a Corrente do Brasil (CB) que flui para o sul transportando Água Tropical (AT), quente e oligotrófica, e a Corrente das Malvinas (CM), que transporta águas subantárticas frias e ricas em nutrientes para o norte (Fig. 3, Garcia 1998, Matsuura & Andrade 2000, Brandini & Pires-Vanin 2020). Em função do contraste na produtividade dessas massas d'água, a costa sudeste/sul do Brasil pode ser dividida em duas áreas (Lopes et al. 2006). A região entre Cabo Frio e Santa Marta (~22°S–28°S) é dominada por AT e enriquecida por ressurgências costeira e de quebra de plataforma, a primeira induzida pelo vento e a segunda pela formação de vórtices frontais da CB (Castro & Miranda 1998, Garcia 1998, Acha et al. 2004, Madureira et al. 2020). Estes mecanismos promovem a fertilização local através da ascensão de Água Central do Atlântico Sul (ACAS), relativamente fria e rica em nutrientes. A região entre Santa Marta e o Chuí (~28°S–34°S), por outro lado, recebe o aporte de águas frias costeiras e dos deságues continentais do Río de la Plata e da Lagoa dos Patos, sendo ainda influenciada pela ZCST durante outono–inverno (Garcia 1998, Möller et al. 2008).



Figura 3. Dinâmica da temperatura da superfície do mar (TSM) e dos cardumes de bonito-listrado, para os períodos de verão (Fevereiro) e inverno (Junho), no Oceano Atlântico Sudoeste. A TSM média mensal de Fevereiro e Junho de 2013 foram obtidas de Coletto (2016). Os três portos de desembarque são destacados. CB: Corrente do Brasil; CM Corrente das Malvinas.

A produtividade biológica da área sul é relativamente alta, sustentando altas densidades de espécies forrageiras como o peixe-lanterna (*Maurolicus stehmanni*) (Madureira et al. 2005) e a anchoita (*Engraulis anchoita*) (Madureira et al. 2009, Costa et al. 2016, Costa et al. 2020b). Essas espécies são as principais vias energéticas conectando os organismos na base da cadeia alimentar até maiores níveis tróficos, sendo consideradas espécies-chave no ecossistema pelágico da região (Schwingel 1998, Velasco & Castello 2005, Gasalla et al. 2007), sustentando diversos predadores, tais como aves (Bugoni et al. 2011, Marques et al. 2018, Costa et al. 2020b), mamíferos (Troina et al. 2020a) e peixes (Castello et al. 1997).

A distribuição e a abundância relativa do bonito-listrado no Oceano Atlântico Sudoeste são influenciadas pela dinâmica da temperatura da superfície do mar (TSM) e da ZCST (Castello & Habiaga 1989, Matsuura & Andrade 2000). Durante a primavera, com o avanço da CB até maiores latitudes, os cardumes deslocam-se até a região sul, onde são obtidas as maiores capturas por unidade de esforço (CPUE) na pescaria. Após o verão, em meados de março–abril, a CB se retrai. Com o avanço de águas frias da CM pelo sul, durante outono/inverno ocorre o deslocamento dos cardumes e da ZCST para o norte, e a CPUE é menor, relativamente ao verão (Castello & Habiaga 1989, Andrade 1996, Andrade & Garcia 1999, Lima et al. 2000, Andrade 2003, Coletto et al. 2019).

Experimentos de marcação realizados pela ICCAT sugerem a realização de movimentos latitudinais pelo bonito-listrado no Oceano Atlântico Sudoeste. Indivíduos marcados na região sul foram recapturados na região sudeste (Luckhurst 2014, Fonteneau 2015, ICCAT 2016). Dados mais recentes do Programa de Marcação de Atuns Tropicais do Oceano Atlântico (AOTTP em inglês), confirmam os movimentos entre as regiões sudeste e sul do Brasil. Mostram ainda movimentos no sentido oeste-leste entre a plataforma continental da região sul e áreas oceânicas, além de uma migração desde a região equatorial até a costa do Rio de Janeiro (AOTTP 2019). Um experimento de marcação instrumentou indivíduos adultos (CF 75–82 cm) com marcas satelitais ao sul de Cabo Frio-RJ, mapeando o deslocamento de sete espécimes seguindo frentes térmicas na quebra da plataforma, e também em mar aberto na porção leste de um vórtice ciclônico da CB (Monteiro-Neto et al. 2020). Além disso, o estudo confirma a realização de mergulhos até águas frias (~14°C) por estes exemplares, até profundidades de ~ 260 m durante o dia, e ascensão à superfície durante a noite (Monteiro-Netto et al. 2020). No Oceano Pacífico, as trajetórias migratórias são também influenciadas pela topografia, e águas com TSM < 18°C atuam como barreiras térmicas, induzindo movimentos latitudinais do bonito-listrado (Kiyofugi et al. 2019).

No Oceano Atlântico Sudoeste, a temperatura de ocorrência do bonito-listrado foi primeiramente reportada entre 17,8–26,2°C, com média de 23,3°C (Castello & Habiaga 1989). A ocorrência foi também associada com a quebra de plataforma, em profundidades variando entre 100–220 m, podendo acontecer em áreas mais costeiras (48 m), quando ocorre a intrusão de águas tropicais sobre a plataforma continental (Castello & Habiaga 1989, Coletto 2016, Coletto et al. 2019, Madureira et al. 2020). Nesta região, agregações desta espécie foram associadas a frentes térmicas e de cor do oceano, à termoclina pronunciada e rasa, a proeminências na quebra da plataforma continental e a intrusões de ACAS, que favorecem as agregações alimentares do bonito-listrado e influenciam sua vulnerabilidade à pesca (Castello & Habiaga 1989, Andrade & Garcia, 1999, Lima et al. 2000, Andrade 2003, Coletto et al. 2019, Madureira et al. 2020).

Até recentemente, o conhecimento sobre a dieta do bonito-listrado no Oceano Atlântico Sudoeste tinha sido obtido por meio de análise de conteúdo estomacal (ACE). Zavala-Camin (1982) indicou que a espécie se alimenta de eufausiáceos e pequenos peixes. As principais presas na região sudeste foram o peixe-lanterna e *Euphausia similis* (krill), além de peixes Scombridae, Gempilidae, Trichiuridae e Carangidae (Ankenbrandt 1985). A importância de krill diminuiu e de peixe-lanterna aumentou, respectivamente, com o aumento no comprimento do bonito-listrado (Ankenbrandt 1985). Nas regiões sudeste/sul, a categoria taxonômica majoritária em amostras da frota comercial de vara e isca-viva foi a de peixes, principalmente *Sardinella brasiliensis* (76% volume), peixe-lanterna, e Engraulidae, seguidos por krill (Vilela 1990). Em amostras obtidas em pesca experimental com rede de cerco na região sul, o peixe-lanterna, krill e anchoita foram as principais presas (Vilela 1990, Matsuura & Andrade 2000). No geral foi observada a presença esporádica de lulas na dieta do bonito-listrado (Ankenbrandt 1985, Vilela 1990).

A posição trófica é uma medida integrativa do papel ecológico/funcional de consumidores (Librelato et al. 2014, Quezada-Romegialli et al. 2019), a qual pode ser utilizada para quantificar mudancas na estrutura das cadeias tróficas marinhas (e.g. Pauly et al. 1998, Librelato et al. 2014, Shannon et al. 2014). O nível trófico é um fator importante para determinar a capacidade de explotação de um recurso pesqueiro (Costa & Haimovici 2020). Diversos fatores biológicos, ambientais e a própria atividade pesqueira podem influenciar a estrutura trófica e por consequência a posição trófica de peixes (e.g. Shannon et al. 2014, Houssard et al. 2017, Le-Alvarado et al. 2021). O aumento no tamanho corporal de tunídeos, por exemplo, pode aumentar à tolerância a variações ambientais, aumentando a capacidade de mergulho até maiores profundidades (Bernal et al. 2017) e permitindo a captura de presas de maior tamanho (Graham et al. 2007), resultando no aumento da posição trófica (Pethybridge et al. 2018). A produtividade pode influenciar o tamanho dos produtores primários, e por consequência, o comprimento das cadeias tróficas sustentando estoques pesqueiros (Costa & Haimovici 2020). Dados de ACE (Vilela 1990) indicaram que o bonitolistrado é um consumidor terciário no ecossistema pelágico do Oceano Atlântico Sudoeste (posição trófica = 3,5; Gasalla et al. 2007).

A ACE tem como vantagem a resolução taxonômica, permitindo identificar as presas consumidas pelo predador no ambiente em nível de espécie (Hyslop 1980, Chipps & Garvey 2007). Entretanto, ACE representa um "instantâneo" ou "retrato" das presas ingeridas previamente à captura, em uma escala de tempo frequentemente menor do que 24 h (Magnuson 1969). Neste sentido a ACE pode subestimar presas de digestão rápida, ou superestimar a presença de presas de digestão lenta na dieta (Chipps & Garvey 2007, Olson et al. 2010). Além disso, a elevada frequência de estômagos vazios, preenchidos apenas por iscas, ou com pequenas quantidades de alimento não-identificável (Vooren 1976, Ankenbrandt 1985, Vilela 1990, Roger 1994), indicam a necessidade de utilização de técnicas complementares para estudar a ecologia trófica do bonito-listrado.

Traçadores químicos, como a análise de isótopos estáveis (AIE) de carbono ( $\delta^{13}$ C) e nitrogênio ( $\delta^{15}$ N), por outro lado, oferecem informação sobre os componentes da dieta que foram assimilados nos tecidos dos consumidores em escalas temporais mais amplas (Martinez del Rio et al. 2009, Martinez del Rio & Carleton 2012). Alguns tecidos possuem rápida taxa de incorporação (e.g. fígado, plasma sanguíneo), refletindo a alimentação em escalas entre dias e semanas, enquanto outros (e.g. músculo) integram a dieta assimilada ao longo de meses e até anos (Pinnegar & Polunin 1999, Martinez del Rio et al. 2009, Madigan et al. 2012a).

AIE de  $\delta^{13}$ C e  $\delta^{15}$ N baseia-se na premissa de que consumidores possuem composição isotópica semelhante àquela de suas presas, com um pequeno enriquecimento nos isótopos mais pesados (i.e. aumento do valor de  $\delta$ ) associado à assimilação e à síntese dos tecidos, chamado de discriminação trófica (Fry 2006, Michener & Lajtha 2007). Em geral, o aumento é maior no  $\delta^{15}$ N (3–4‰), e menor no  $\delta^{13}$ C (0–2‰) (DeNiro & Epstein 1978, 1981, Minagawa & Wada 1984). Desta forma, o  $\delta^{15}$ N é utilizado para inferir a posição trófica dos consumidores, enquanto o  $\delta^{13}$ C reflete a composição dos produtores primários das cadeias tróficas (Peterson & Fry 1987).

AIE integra informações sobre o uso de recursos e habitat ao longo de escalas temporais mais amplas que ACE, mas tipicamente não fornece resolução em nível de espécie sobre a composição da dieta. Desta forma, informações mais completas sobre a dieta de um consumidor podem ser obtidas através da combinação entre ACE e AIE (Phillips et al. 2014,

Condini et al. 2015, Swan et al. 2019). Modelos de mistura quantificam a contribuição de diferentes fontes alimentares (presas) na dieta de um consumidor, utilizando os valores isotópicos do consumidor e das presas, corrigidos por meio de um fator de discriminação trófica predador-presa (Boecklen et al. 2011, Phillips et al. 2014). As presas a serem incluídas nos modelos são escolhidas *a priori*, por meio de estudos prévios baseados, por exemplo, em ACE (Moore & Semmens 2008, Madigan et al. 2012b, Franco-Trecu et al. 2013, Phillips et al. 2014, Swan et al. 2019).

Além da dieta e da posição trófica, a AIE tem sido aplicada para estudar os movimentos geográficos de predadores nos ecossistemas marinhos (Graham et al. 2010, Madigan et al. 2014, 2016). Enquanto forrageiam, os consumidores adquirem a composição isotópica das presas de um determinado local. Logo, utilizando a composição isotópica de um predador, é possível rastrear deslocamentos entre ambientes isotopicamente distintos, obtendo informações sobre o tempo de residência e os padrões de movimento (Graham et al. 2010, McMahon & Newsome 2019, Trueman & Glew 2019).

Como a composição isotópica de um organismo depende dos recursos que ele consome e do habitat onde ele vive, o nicho isotópico ou espaço  $\delta$ , é utilizado como *proxy* do nicho ecológico (Bearhop et al. 2004, Newsome et al. 2007). O nicho ecológico pode ser representado por um espaço multidimensional, ou hipervolume, que inclui as características ambientais e os recursos que permitem que uma espécie ocorra (Hutchinson 1957). Desta forma, o nicho isotópico permite quantificar o uso e a partição de recursos entre organismos da população ou comunidade (de Lima et al. 2019, Shipley & Matich 2020). A área de elipse padrão é a métrica mais comumente usada para estimar o nicho isotópico (Jackson et al. 2011). No entanto, sua forma elíptica pode não capturar adequadamente a estrutura multimodal de alguns conjuntos de dados (Franco-Trecu et al. 2014). Por outro lado, uma abordagem a partir de densidades de kernel mostrou-se adequada para aplicação a dados isotópicos que não possuem distribuição normal no espaço bivariado (Eckrich et al. 2019).

Diversos fatores influenciam a distribuição espaço-temporal dos isótopos nos ambientes ao atuarem sobre processos biogeoquímicos, gerando paisagens isotópicas ou *"isoscapes*" (e.g. Graham et al. 2010, McMahon et al. 2013, Troina et al. 2020b). Os valores de  $\delta^{13}$ C variam entre ecossistemas pelágicos/oceânicos (menos enriquecidos em <sup>13</sup>C) ou

bentônicos/costeiros (mais enriquecidos em <sup>13</sup>C), além de haver um gradiente latitudinal negativo no  $\delta^{13}$ C na base das redes tróficas (Rau et al. 1982, France 1995, McMahon et al. 2013, Trueman & Glew 2019). O  $\delta^{13}$ C em produtores primários é influenciado pelos valores de  $\delta^{13}$ C da matriz de carbono inorgânico dissolvido. Assim, a variação espacial de  $\delta^{13}$ C na base da cadeia trófica varia com a temperatura e com a concentração de CO<sub>2</sub> (McMahon et al. 2013). Por outro lado, a distribuição do  $\delta^{15}$ N depende da fonte de nitrogênio inorgânico (e.g. nitrato, amônio, N<sub>2</sub>), suas transformações (e.g. fixação, denitrificação) e do fracionamento associado a assimilação de nutrientes pelo fitoplâncton (Graham et al. 2010).

As características oceanográficas atuantes na região sudeste/sul do Brasil resultam em padrões espaciais do  $\delta^{15}$ N no zooplâncton próximo à base das cadeias tróficas (Troina et al. 2020b). Na área norte (20–28°S), o predomínio de AT oligotrófica da CB em áreas oceânicas favorece a fixação de N<sub>2</sub> por cianobactérias diazotróficas (e.g. *Trichodesmium*) com pouca discriminação isotópica, resultando em valores de  $\delta^{15}$ N mais baixos. Na área sul (28–34°S), o enriquecimento com águas subantárticas frias da CM e das descargas continentais aumenta a disponibilidade de nutrientes enriquecidos em <sup>15</sup>N (e.g. NO<sub>3</sub><sup>-</sup>) para os produtores primários, resultando em valores mais elevados de  $\delta^{15}$ N (Sigman et al. 2009, McMahon et al. 2013, Troina et al. 2020b). Por esta razão, a variabilidade dos valores de  $\delta^{15}$ N na base da cadeia trófica pode dificultar a interpretação dos valores de  $\delta^{15}$ N nos tecidos de um consumidor para inferir sua posição trófica (Whiteman et al. 2019), especialmente quando este se desloca ao longo de amplas áreas geográficas, como é o caso do bonitolistrado no sudoeste do Oceano Atlântico (Matsuura & Andrade 2000).

A AIE em compostos específicos, em particular a análise de  $\delta^{15}$ N em aminoácidos (AA), permite separar os potenciais efeitos da variação do  $\delta^{15}$ N nos organismos da base da cadeia trófica daqueles relacionados à posição trófica do consumidor (Lorrain et al. 2015, Whiteman et al. 2019). Para a análise de  $\delta^{15}$ N, os AA proteicos são comumente divididos em dois grupos (Fig. 4). Os AA que sofrem mínimo enriquecimento isotópico na assimilação das presas são denominados AA "fonte" (e.g. fenilalanina, lisina). Já aqueles que sofrem um enriquecimento significativo a cada transferência trófica são chamados de AA "tróficos" (e.g. ácido glutâmico, prolina) (McClelland & Montoya 2002, Popp et al. 2007, McMahon & Newsome, 2019). Logo, a análise de  $\delta^{15}$ N em AA permite estimar o valor do  $\delta^{15}$ N na base da

cadeia trófica e a posição trófica a partir de uma amostra do tecido do consumidor (Popp et al. 2007, Olson et al. 2010, Lorrain et al. 2015, Houssard et al. 2017, Le-Alvarado et al. 2021).



Figura 4. Valores de  $\delta^{15}$ N idealizados de aminoácidos fonte (e.g. lisina – Lis) e trófico (e.g. ácido glutâmico – Glx) em produtores primários, consumidores primários e consumidores secundários. O valor de  $\Delta^{15}N_{C-D}$  ( $\delta^{15}N_{consumidor} - \delta^{15}N_{dieta}$ ) para a lisina é próximo de zero para os dois consumidores, o que resulta em valores  $\delta^{15}N_{Lis}$  iguais para todos os organismos. Por outro lado, os valores  $\Delta^{15}N_{C-D}$  para o ácido glutâmico, um aminoácido trófico, é positivo para os dois consumidores. Como resultado, os valores de  $\delta^{15}N_{Glx}$  aumentam do produtor a cada nível trófico. Adaptado de Whiteman et al. (2019).

Neste contexto, a presente tese teve como objetivo estudar a ecologia trófica e a migração do bonito-listrado através da utilização de técnicas tradicionais (i.e. análise de conteúdo estomacal, análise de isótopos estáveis de  $\delta^{13}$ C e  $\delta^{15}$ N) e em recente expansão (i.e. análise de isótopos estáveis em aminoácidos específicos). Esta integração permitiu rastrear as principais vias energéticas utilizadas pelo bonito-listrado, inferir sobre os movimentos sazonais, padrões ontogenéticos no hábitat de forrageio e estimar a posição trófica da espécie no ecossistema pelágico do Atlântico Sudoeste. Estas informações em conjunto permitirão um aprimoramento do enfoque ecossistêmico para o manejo sustentável da pesca do bonito-listrado na região.

#### 1.1. Hipóteses

Considerando o exposto, no primeiro manuscrito foram investigadas as hipóteses que seguem:

1) A dieta assimilada pelo bonito-listrado apresenta mudanças espaciais e ontogenéticas;

2) A estratégia de forrageio oportunista e a realização de movimentos sazonais reduz a sobreposição no nicho isotópico entre espécimes de diferentes áreas e classes de tamanho.

No segundo manuscrito foram investigadas as seguintes hipóteses:

3) O padrão ontogenético no  $\delta^{15}$ N do tecido muscular indica mudanças nos habitats de forrageio com o crescimento;

4) A posição trófica do bonito-listrado aumenta com o crescimento.

### 1.2. Objetivos

Objetivo geral:

Estudar a ecologia trófica e os movimentos do bonito-listrado no Oceano Atlântico Sudoeste.

Objetivos específicos:

1) Quantificar a contribuição das fontes alimentares na dieta assimilada, caracterizando variações espaciais e ontogenéticas, a partir de modelos de mistura de  $\delta^{13}$ C e  $\delta^{15}$ N integrados a dados de análise do conteúdo estomacal (Anexo 1);

2) Caracterizar os movimentos sazonais, o uso do habitat e o nicho isotópico através de análise de  $\delta^{13}$ C e  $\delta^{15}$ N no tecido muscular (Anexo 1);

3) Caracterizar mudanças ontogenéticas nos habitats de forrageio através do  $\delta^{15}$ N nos aminoácidos fonte fenilalanina e lisina (Anexo 2);

4) Determinar a posição trófica, caracterizando variações espaciais e ontogenéticas, por meio de análise de conteúdo estomacal e de traçadores isotópicos ( $\delta^{15}$ N no músculo e  $\delta^{15}$ N em aminoácidos trófico e fonte) (Anexo 2).

## 2. MATERIAIS E MÉTODOS

As amostras de bonito-listrado foram obtidas em 66 desembarques da frota de vara e isca-viva em Rio Grande-RS (45) e Niterói-RJ (21), entre dezembro de 2016 e maio de 2018. Em Rio Grande, foram amostrados desembarques da frota sediada neste município, bem como de barcos sediados em Itajaí/Navegantes (12) em desembarques realizados na empresa Leal Santos durante o verão de 2017. As posições de captura foram obtidas através de mapas de bordo e em entrevistas com os mestres das embarcações. Os dados foram subdivididos de acordo com a latitude da captura em área norte (20–28°S) e sul (28–34°S), em função do regime oceanográfico e influência de distintas massas d'água ao norte e ao sul do Cabo de Santa Marta. Na amostragem biológica, o comprimento furcal (CF) de cada espécime foi determinado com ictiômetro (cm) e o peso com balança digital (g). Os indivíduos foram classificados em classes de tamanho de acordo com o CF no qual 50% e 100% dos espécimes atingem a maturação sexual no Oceano Atlântico Sudoeste ( $L_{50} = 46$  cm;  $L_{100} = 63$  cm), determinados por Soares et al. (2019). As classes de CF foram denominadas juvenil (CF < 47 cm), jovem adulto (CF 47–63 cm) e adulto (CF > 64 cm).

As técnicas utilizadas para estudar a ecologia trófica do bonito-listrado foram a análise de conteúdo estomacal (ACE), a análise de isótopos estáveis (AIE) de carbono ( $\delta^{13}$ C) e nitrogênio ( $\delta^{15}$ N), e a AIE de  $\delta^{15}$ N em aminoácidos ( $\delta^{15}$ N AA) trófico (ácido glutâmico, Glx) e fonte (Fenilalanina, Fe; lisina, Lis).

#### 2.1. Análise de conteúdo estomacal

Para ACE, 740 espécimes foram eviscerados para remoção do estômago (norte = 355; sul = 385). O conteúdo estomacal foi fixado em formaldeído 10%, ou mantido congelado até a triagem. Em laboratório, os itens alimentares foram identificados até o menor táxon possível, utilizando guias de identificação de peixes (Figueiredo & Menezes 1980), crustáceos (Gibbons et al. 1999) e cefalópodes (Haimovici et al. 2009). Para cada táxon foram registrados a massa (g) e o número. Os itens alimentares foram classificados quanto ao grau de digestão de acordo com Vaske et al. (2004). Os espécimes das espécies *Engraulis anchoita* e *Sardinella brasiliensis*, classificados nos graus iniciais de digestão (i.e. não-digeridos, digestão inicial) foram desconsiderados da análise, assumindo que se tratavam de isca-viva utilizada na captura do bonito-listrado. Os dados de ACE foram utilizados como *prioris* informativas nos modelos isotópicos de mistura (Anexo 1). A lista taxonômica da dieta, com o número de ocorrências, %FO, a massa e a proporção em massa são disponibilizadas no material suplementar do Anexo 1. A proporção em massa da categoria "peixe-não identificado" foi relativamente alta neste estudo (geral: 21%; área norte 33%; área sul 12%). Considera-se este resultado aceitável e coerente com outros estudos de ACE. Por exemplo, Vilela (1990) quantificou proporções entre 18–33% do volume do conteúdo estomacal para a categoria "restos de peixes". Cabe destacar que o pescado é conservado em gelo nos barcos sediados no Rio de Janeiro, enquanto as frotas sediadas em Santa Catarina e Rio Grande do Sul conservam o pescado em salmoura refrigerada, a temperaturas mais baixas (Martins et al. 2020). Os dados de ACE foram também utilizados no cálculo da posição trófica do bonito-listrado (Anexo 2). A proporção em massa das presas, agrupadas em nível de família, é disponibilizada no material suplementar do Anexo 2.

#### 2.2. Análise de isótopos estáveis

Para AIE, amostras de ~5 g de tecido muscular adjacente à primeira nadadeira dorsal foram removidas de 383 espécimes (norte = 242; sul = 141) com uso de bisturi e congeladas a -20°C. Em laboratório, as amostras de tecido muscular foram descongeladas, lavadas com água destilada, e desidratadas em liofilizador por 24 h ou estufa a 60°C por 48 h, considerando que estes métodos de preparação não produzem diferenças nos valores de  $\delta^{13}$ C e  $\delta^{15}$ N (e.g. de Lecea et al. 2011). As amostras desidratadas foram homogeneizadas com uso de gral e pistilo. Alíquotas de ~0.5 mg do tecido muscular desidratado foram acondicionadas em cápsulas de estanho e pesadas em balança analítica (Metler Toledo XP6) no Centro Integrado de Análises da FURG. Por terem sido coletadas e processadas por dois grupos de pesquisa do Projeto Bonito, as análises de isótopos estáveis foram realizadas em laboratórios distintos, segundo a preferência de cada grupo. As amostras coletadas em Rio Grande foram analisadas no Center for Stable Isotopes da Universidade do Novo México (CSI-UNM); as amostras coletadas em Niterói foram analisadas na Stable Isotope Facility da Universidade da Califórnia, Davis (UCD- SIF). Análises em duplicata foram utilizadas para comparação das medições entre os laboratórios (n = 3). As diferenças entre laboratórios para  $\delta^{13}$ C (0,2) ‰) e  $\delta^{15}$ N (0,1 ‰) não foram significativas, de acordo com o teste não-paramétrico de *Wilcoxon* ( $\delta^{13}$ C: W = 2, p = 0,4;  $\delta^{15}$ N: W = 5; p = 1). As razões isotópicas são reportadas em notação  $\delta$  per mil (‰) através da equação:

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

Onde: X é <sup>13</sup>C ou <sup>15</sup>N e R é a razão <sup>13</sup>C/<sup>12</sup>C ou <sup>15</sup>N/<sup>14</sup>N (Peterson & Fry 1987). Os padrões internacionais para o  $\delta^{13}$ C e  $\delta^{15}$ N foram Vienna Pee Dee Belemnite (V-PDB) e nitrogênio atmosférico (N<sub>2</sub>), respectivamente. Na UNM-CSI as análises foram realizadas através de um Elemental Analyzer (Costech ECS-4010) acoplado ao um Espectrômetro de Massa e Razão Isotópica (Thermo Scientific Delta V Plus) e na UCD-SIF as análises foram realizadas em um Espectrômetro de Massa (Hydra 20-20). A precisão analítica foi estimada através do desvio padrão entre medições de  $\delta^{13}$ C e  $\delta^{15}$ N dos padrões laboratoriais, estimada em ± 0,1 ‰ para  $\delta^{13}$ C e  $\delta^{15}$ N. Devido à baixa razão carbono-nitrogênio (C:N) no tecido muscular do bonito-listrado (C:N > 3,4: n = 6, ~ 1,6% das amostras; média ± desvio padrão: 3,2 ± 0,1), não foram realizados tratamentos químicos para extração de lipídeos previamente às análises (Post et al. 2007).

Uma sub-amostra de 38 espécimes foi processada no Center for Stable Isotopes -UNM, para análise de  $\delta^{15}$ N em aminoácidos. Para obtenção dos aminoácidos constituintes, aproximadamente 10 mg do tecido muscular desidratado foi hidrolisada em 1 mL de ácido clorídrico (HCl-6N) a 110°C por 20 h. A volatização foi obtida via derivatização por meio de esterificação com isopropril N-trifluoroacético de acordo com o protocolo descrito por Whiteman et al. (2019). As amostras foram derivatizadas em bateladas junto de padrões contendo uma mistura de 13 aminoácidos de composição isotópica conhecida. As amostras foram injetadas por um amostrador automático (Thermo Scientific TriPlus RSH) em um Cromatógrafo a Gás (CG, Thermo Scientific Trace 1310) equipado com uma coluna de CG  $60 \text{ m} \times 0.32 \text{ mm}$  ID BPX5  $\times 1.0 \text{ }\mu\text{m}$ . Os aminoácidos derivatizados na forma gasosa foram reduzidos em N2 a 1000°C em um reator de combustão (Thermo Scientific IsoLink II). O gás N<sub>2</sub> foi enviado para um IRMS (Thermo Scientific Delta V Plus) por meio de um Conflo IV para análise isotópica. As amostras foram analisadas em duplicata ou triplicata. A precisão analítica, estimada pelo desvio padrão médio entre rodadas (*within run*) no  $\delta^{15}$ N de ácido glutâmico (Glx), lisina (Lis) e fenilalanina (Fe) foi de 0,3 ‰. O  $\delta^{15}$ N de aminoácidos é reportado em notação δ em relação ao N2 atmosférico.

#### 2.3. Análise de dados

Todas as análises estatísticas foram realizadas no software R (R Core Team 2019). Os mapas foram produzidos em QGIS (Versão 3.4.9).

#### 2.4. Apêndice 1

Os valores de  $\delta^{13}$ C e  $\delta^{15}$ N foram comparados entre áreas (norte e sul) através de análise de variância permutacional multivariada (PERMANOVA), com base na matriz de distância euclidiana. Análises de regressão linear foram utilizadas para testar a relação entre  $\delta^{13}$ C e o CF dos indivíduos e entre  $\delta^{15}$ N do músculo do bonito-listrado com a latitude de captura. O teste não-paramétrico de Mann-Whitney foi utilizado para comparação mensal de  $\delta^{13}$ C e  $\delta^{15}$ N entre as áreas norte e sul e para comparar  $\delta^{13}$ C e  $\delta^{15}$ N entre classes de tamanho das áreas norte e sul.

A contribuição relativa das fontes alimentares na dieta do bonito-listrado foi determinada através de modelos de mistura isotópicos no pacote SIMMR (Parnell 2019). A dieta foi determinada para cada área e classe de tamanho, exceto para adultos na região sul (n = 7), cujos valores de  $\delta^{15}$ N indicaram a presença de dois grupos distintos na amostra. Dados de  $\delta^{13}$ C e  $\delta^{15}$ N de presas coletadas nos estômagos do bonito-listrado, processadas seguindo a metodologia descrita para as amostras de bonito-listrado, foram utilizadas nos modelos. Além dessas, amostras de espécimes de Carangidae, Engraulidae, Euphausidae, Ommastrephidae e Sternoptychidae coletadas na área de estudo em cruzeiros de pesquisa do Projeto Talude, realizados durante outono e primavera entre 2009–2015 foram utilizadas (Troina 2019, Troina et al. 2020b).

Os modelos foram analisados sem e com *prioris* informativas obtidas via ACE (*Uninformative Prior and Informative Prior*). A proporção em massa das presas foi utilizada como *priori* informativa. Agrupar presas isotopicamente similares é uma forma de incluir várias fontes energéticas e maximizar o poder de discriminação em modelos de mistura (Phillips et al. 2014). As presas foram agrupadas *a priori* por família, similaridade nos valores de  $\delta^{13}$ C e  $\delta^{15}$ N e papel funcional em cada região. Polígonos de mistura (Smith et al. 2013) foram utilizados para testar se os valores de  $\delta^{13}$ C e  $\delta^{15}$ N do bonito-listrado estavam dentro do polígono formado pelos valores isotópicos das presas agrupadas, bem como para testar

três fatores de discriminação trófica estimados para tunídeos (Varela et al. 2011, Madigan et al. 2012a, Varela et al. 2012). Os resultados dos modelos de mistura foram reportados através da mediana e dos intervalos de credibilidade da proporção de cada grupo na dieta do bonitolistrado. As diferenças entre grupos foram comparadas entre as classes de tamanho em cada área com a função "*compare\_groups*" do pacote SIMMR (Parnell 2019).

Lipídeos apresentam depleção em <sup>13</sup>C, de forma que possuem valores de  $\delta^{13}$ C menores que carboidratos e proteínas nos tecidos dos organismos (DeNiro & Epstein 1977, McConnaughey & McRoy 1979, Post et al. 2007). Para remover potenciais efeitos do conteúdo lipídico no  $\delta^{13}$ C das presas, os valores de  $\delta^{13}$ C de amostras com razão C:N > 3,5 foram normalizadas utilizando equações tecido- taxa-específicas (Post et al. 2007, Logan et al. 2008, Hoffman & Sutton 2010).

Para quantificar a área e a sobreposição do nicho isotópico do bonito-listrado os valores de  $\delta^{13}$ C e  $\delta^{15}$ N do tecido muscular foram analisados por meio de densidades de utilização kernel (Eckrich et al. 2019). O tamanho (área) do nicho isotópico e a sobreposição foram estimados entre áreas e classes de tamanho do bonito-listrado para as densidades de kernel de 50% e 75%.

#### 2.5. Apêndice 2

Para determinar a contribuição relativa da base de  $\delta^{15}$ N das áreas norte e sul para as classes de tamanho do bonito-listrado foram utilizados modelos de mistura no pacote SIMMR (Parnell 2019). O  $\delta^{15}$ N de aminoácidos fonte lisina e fenilalanina de juvenis capturados no final do verão (i.e. março) foram utilizados como indicadores do  $\delta^{15}$ N da base de cada área. Esta abordagem assumiu que o bonito-listrado se desloca sazonalmente entre as áreas norte e sul, e isótopos de nitrogênio integram informação sobre o forrageamento de atuns de crescimento rápido por um período de 6–7 meses (Graham 2007, Murua et al. 2017).

Para identificar migrantes recentes com base no  $\delta^{15}N_{Lis}$ , foi utilizado o modelo de mistura de duas fontes descrito por Post (2002) como segue:

$$\alpha = (\delta^{15} N_{Lis-amostra} - \delta^{15} N_{Lis-sul}) / (\delta^{15} N_{Lis-Norte} - \delta^{15} N_{Lis-Sul})$$

Onde:  $\alpha$  é a proporção da base de  $\delta^{15}$ N do norte no tecido do consumidor;  $\delta^{15}$ N<sub>Lis-amostra</sub> são os valores de  $\delta^{15}$ N<sub>Lis</sub> em cada indivíduo, e  $\delta^{15}$ N<sub>Lis-Norte</sub> e  $\delta^{15}$ N<sub>Lis-Sul</sub> são os valores das bases das áreas norte e sul, respectivamente. Indivíduos que tiveram uma proporção superior a 75% de  $\delta^{15}$ N<sub>Lis</sub> de uma base diferente da área de captura foram considerados migrantes recentes.

Modelos lineares foram utilizados para determinar a relação entre os valores de  $\delta^{15}$ N no músculo (*bulk*) e os valores do  $\delta^{15}$ N na base ( $\delta^{15}N_{Fe}$ ,  $\delta^{15}N_{Lis}$ ), e entre o aminoácido trófico e fonte (i.e.  $\delta^{15}N_{Glx}$  vs.  $\delta^{15}N_{Fe}$  e  $\delta^{15}N_{Glx}$  vs.  $\delta^{15}N_{Lis}$ ). A relação do  $\delta^{15}$ N dos aminoácidos base Lis e Fe com o CF do bonito-listrado foi explorada por meio de gráficos de  $\delta^{15}N_{Lis}$  e  $\delta^{15}N_{Fe}$  versus o CF dos espécimes, discriminados pela área de captura.

A posição trófica (PT) foi determinada através da análise de conteúdo estomacal de acordo com Cortés (1999) através do modelo:

$$PT_{ACE} = 1 + \left(\sum_{1}^{n} P_i \times PT_i\right)$$

Onde: *n* é o número de categorias, *Pi* é a proporção em massa da categoria *i*, e *PTi* é a posição trófica da presa da categoria *i*. Doze categorias de presas classificadas em nível de família e pertencendo a três grupos taxonômicos (Crustacea, Mollusca e Teleósteos) foram utilizadas para o cálculo da PT do bonito-listrado, por área e classe de tamanho dentro de cada área. As proporções em massa, os valores de PT das presas e as referências das quais as PTs das presas foram obtidas são indicados no material suplementar do Anexo II.

A PT foi estimada através de  $\delta^{15}$ N no tecido muscular (*bulk*) por área e classe de tamanho em cada área através do pacote tRophicPosition (Quezada-Romegialli et al. 2019). Os valores de  $\delta^{15}$ N de copépodos (i.e. consumidor primário) coletados em estudos prévios na região Sudeste-Sul do Brasil foram utilizados como *proxy* do  $\delta^{15}$ N da base da rede trófica nas áreas norte e sul (Troina et al. 2020b). Em função da maior profundidade de captura de adultos na área norte (62% dos indivíduos capturados em profundidades maiores que 500 m), o valor médio do  $\delta^{15}$ N de copépodos da quebra da plataforma e das áreas oceânicas foi utilizado como *proxy* do  $\delta^{15}$ N da base para determinar a PT de adultos da área norte. A PT de copépodos foi definida como 2,2 assumindo um grau de omnivoría (i.e. 80% pastagem, 20% predação). O fator de discriminação de  $\delta^{15}$ N entre consumidor e dieta pode variar em

função da forma de excreção de nitrogênio, tecido, ambiente, grupo taxonômico e tipo de dieta (Post 2002, McCutchan et al. 2003, Vanderklift & Ponsard 2003, Caut et al. 2009). Foram selecionados dados publicados para tecido muscular de peixes marinhos amoniotélicos ( $\Delta^{15}$ N: 3,7 ± 1,9‰; material suplementar Anexo 2).

Por fim, a PT foi determinada através da diferença do  $\delta^{15}$ N em aminoácidos (AA) trófico e fonte, utilizando a equação (Bradley et al. 2015):

$$PT_{Tr-Sr} = [(\delta^{15}N_{Tr} - \delta^{15}N_{Sr} - \beta)/TEF] + 1$$

Onde:  $\delta^{15}N_{Tr} e \,\delta^{15}N_{Sr}$  são os valores de  $\delta^{15}N$  para os AAs trófico e fonte,  $\beta$  é a diferença entre os valores de  $\delta^{15}N$  dos AAs trófico e fonte selecionados em produtores primários, e TEF é o fator de enriquecimento trófico que representa o aumento nos valores de  $\delta^{15}N$  do AA trófico em relação ao AA fonte por nível trófico. Foram calculadas PT utilizando as combinações entre o  $\delta^{15}N$  em Glx-Lis (PT<sub>Glx-Lis</sub>) e Glx-Fe (PT<sub>Glx-Fe</sub>). Usamos valores médios (± desvio padrão) de  $\beta_{Glx-Lis}$  (3,9 ± 0,5 ‰) e  $\beta_{Glx-Fe}$  (3,6 ± 0,5 ‰), TEF<sub>Glx-Lis</sub> (5,2 ± 0,3 ‰;) e TEF<sub>Glx-Fe</sub> (5,7 ± 0,3 ‰) de uma meta-análise de teleósteos marinhos (Bradley et al. 2015). Os erros foram propagados seguindo as equações disponíveis em Dale et al. (2011) e Sabadel et al. (2020), combinando a incerteza analítica no  $\delta^{15}N$  dos AA com a incerteza nos parâmetros  $\beta$ e TEF.

## 3. SÍNTESE DOS RESULTADOS

Os modelos de mistura indicaram que o peixe-lanterna e krill foram as principais fontes na dieta assimilada pelo bonito-listrado nas áreas norte e sul. Os valores da razão C:N de peixe-lanterna e krill indicaram maior conteúdo lipídico nos tecidos destas presas. Na área sul, pequenos peixes pelágicos (i.e. Clupeidae, Engraulidae) também foram importantes na dieta. No entanto, este grupo teve uma contribuição de apenas 7% na dieta do bonito-listrado na área norte. A baixa contribuição de pequenos peixes pelágicos foi relacionada à baixa disponibilidade de sardinha-verdadeira. Essa espécie apresentou uma queda nas capturas entre 2016–18, em um padrão similar ao observado para o bonito-listrado no mesmo período (Schmidt et al. 2019, Madureira et al. 2020). Esses resultados foram relacionados a anomalias

positivas de TSM, possivelmente devido à predominância de AT sobre a ACAS (Schmidt et al. 2019, Madureira et al. 2020).

As comparações na composição da dieta entre as classes de tamanho indicaram uma relação positiva entre o tamanho das presas com o tamanho do bonito-listrado. A importância de krill foi maior em juvenis e diminuiu com o crescimento do bonito-listrado. As proporções das categorias 'peixe e cefalópode' (Carangidae, Ommastrephidae) e 'pequenos peixes pelágicos' aumentaram nas classes de tamanho jovem adulto e adulto, relativamente à classe juvenil, nas áreas norte e sul, respectivamente. As diferenças na composição da dieta entre áreas indicaram que o bonito-listrado é capaz de adaptar a dieta em função da disponibilidade de presas. O oportunismo na dieta é evidenciado pela ingestão das presas mais abundantes e ricas em energia. As variações espaciais e ontogenéticas na dieta assimilada confirmaram a Hipótese 1.

A variação de  $\delta^{15}$ N na base das teias alimentares explicou a diferença entre as áreas de captura no  $\delta^{15}$ N do bonito-listrado. Na área sul, os valores de  $\delta^{15}$ N foram mais altos no bonito-listrado e nas presas (e.g. krill, peixe-lanterna, pequenos peixes pelágicos), em relação à área norte. Estes resultados foram consistentes com o  $\delta^{15}$ N no zooplâncton ao largo da quebra de plataforma e águas oceânicas da região (Troina et al. 2020b). Para incorporação do sinal isotópico da base das cadeias alimentares, predadores com alta mobilidade devem possuir tempo de residência semelhante ou superior à taxa de renovação isotópica do tecido analisado (Graham et al. 2010), neste caso o músculo. Considerando que a taxa de renovação dos tecidos é positivamente relacionada à taxa de crescimento do organismo (Martinez del Rio et al. 2009), e que o bonito-listrado possui as maiores taxas de crescimento dentre os atuns (Murua et al. 2017), a taxa de incorporação isotópica do músculo do bonito-listrado é provavelmente similar ou maior do que àquela estimada para *Thunnus albacares* (i.e. ~2–4 meses, Graham 2007). Estes resultados indicaram que o bonito-listrado possui um tempo de residência similar ou maior que 2–4 meses nas áreas norte e sul.

A presença de indivíduos com valores de  $\delta^{15}$ N "atípicos" foi interpretada como evidência da movimentação do bonito-listrado entre áreas. Por exemplo, 16 indivíduos capturados entre Abril–Maio na área norte possuíam  $\delta^{15}$ N maior que 12,5 ‰. Esses valores são similares ao valor médio dos indivíduos da área sul (12,2 ± 1,3‰). Na comparação dos valores de  $\delta^{15}$ N ao longo dos meses, as diferenças entre áreas foram consistentes nas duas safras durante o período entre Janeiro–Março, mas entre Abril–Maio os valores de  $\delta^{15}$ N dos indivíduos da área norte aumentaram, e tornaram-se similares ao  $\delta^{15}$ N alto dos indivíduos da área sul. Estes resultados podem ser explicados pelo movimento dos cardumes de bonitolistrado no sentido sul-norte durante o outono, quando ocorre o avanço de águas frias da Corrente das Malvinas pelo sul, e a retração da Corrente do Brasil para menores latitudes (Castello & Habiaga 1989, Andrade & Garcia 1999, Andrade 2003, Coletto et al. 2019).

Três espécimes capturados na área sul apresentaram valores de  $\delta^{15}$ N atípicos ( $\delta^{15}$ N < 9,2 ‰) em relação ao valor médio de  $\delta^{15}$ N da área sul (12,2 ± 1,3‰). Estes indivíduos foram capturados no outono, e possuíam CF relativamente maior (CF 67–73 cm) que a maioria das amostras da área sul. O  $\delta^{15}$ N mais baixo destes indivíduos indicou o uso de áreas com  $\delta^{15}$ N mais baixo no período anterior a captura. No geral e considerando as duas áreas, análises da estrutura em tamanho do bonito-listrado sugerem maior ocorrência de adultos na área norte (Cardoso et al. 2020, Costa et al. 2020a). Além disso, a estrutura em tamanho de espécimes coletados nas áreas norte e sul sugere uma progressão modal, com peixes menores predominando entre primavera–verão, e adultos ocorrendo de forma mais expressiva durante o outono–inverno (e.g. Ankenbrandt 1985, Andrade & Kinas 2004, Soares et al. 2019).

Os padrões espaciais e ontogenéticos de sobreposição no nicho isotópico indicaram uma estratégia que otimiza o crescimento e diminui a competição por recursos (i.e. dieta e habitat). Por exemplo, a sobreposição no nicho isotópico entre áreas foi mínima considerando a densidade de kernel de 50%, e menor que 28% considerando a densidade de kernel de 75%. Este resultado indicou fontes distintas de nitrogênio suprindo as respectivas redes alimentares. Em relação às comparações entre classes de tamanho, as maiores sobreposições de nicho isotópico ocorreram entre juvenis e adultos, cujos valores de  $\delta^{15}$ N foram relativamente baixos, em comparação com jovens adultos. Os jovens adultos tiveram a maior área do nicho isotópico e valores de  $\delta^{15}$ N mais altos, indicando o uso da área sul e maiores proporções de presas de maior posição trófica na dieta (peixe e cefalópodes, pequenos peixes pelágicos). Desta forma, a análise do nicho isotópico indica uma estratégia que diminui a sobreposição no nicho isotópico entre espécimes de diferentes áreas e classes de tamanho, confirmando a Hipótese 2. Os valores de  $\delta^{15}$ N na fenilalanina e lisina foram consistentes com os padrões observados no  $\delta^{15}$ N *bulk*, confirmando que a variação observada no  $\delta^{15}$ N<sub>bulk</sub> é explicada pela variação do  $\delta^{15}$ N na base da cadeia trófica. A ocorrência de valores atípicos de  $\delta^{15}$ N nos AAfonte indicaram a presença de indivíduos que não estavam em equilíbrio com as bases de  $\delta^{15}$ N das áreas de captura. Por exemplo, três indivíduos capturados na área norte durante o outono foram classificados como migrantes recentes. O oposto ocorreu para dois indivíduos na área sul, um juvenil capturado na primavera e um adulto capturado durante o verão.

Os modelos lineares entre o  $\delta^{15}N_{bulk}$  e  $\delta^{15}N$ -AA fonte indicaram que a lisina é um aminoácido fonte aparentemente mais robusto para caracterizar os gradientes de  $\delta^{15}N$  na base da cadeia trófica do bonito-listrado, em comparação com a fenilalanina. Este resultado pode estar relacionado ao menor enriquecimento no  $\delta^{15}N$  entre consumidor-presa para a lisina (-0,3 ‰) em comparação com a fenilalanina (1.5 ‰) observado em uma espécie de tunídeo (Bradley et al. 2014).

Os modelos de mistura de  $\delta^{15}$ N nos AA fonte indicaram mudanças no habitat de forrageio do bonito-listrado ao longo do ciclo de vida. A contribuição das bases isotópicas das áreas norte e sul, conforme indicado pelos valores de  $\delta^{15}N$  em lisina ( $\delta^{15}N_{Lis}$ ) e fenilalanina ( $\delta^{15}N_{Fe}$ ) foram diferentes entre as classes de tamanho. Os valores de  $\delta^{15}N_{Lis}$ indicaram maior contribuição da área sul em juvenis (mediana: 73%, probabilidade = 0.9) e jovens adultos (88%, probabilidade = 1), em relação a área norte. Os adultos tiveram maior contribuição da área norte (89%, probabilidade = 1), em relação a área sul. Da mesma forma, os valores de  $\delta^{15}N_{Fe}$  indicaram maior proporção da base isotópica da área sul em juvenis (75%, probabilidade = 0.9) e jovens adultos (78%, probabilidade = 1), enquanto os adultos apresentaram maior importância da área norte (62%, probabilidade = 0,7), em relação à área sul. Os modelos de mistura regionais indicaram que a área sul, altamente produtiva e com altas densidades de espécies forrageiras é utilizada por juvenis e jovens adultos para engorda e crescimento. Os menores valores de  $\delta^{15}$ N em AA fonte indicaram forrageio de adultos em áreas quentes e oligotróficas, onde predomina a fixação de N2 por cianobactérias diazotróficas com mínimo enriquecimento isotópico (McMahon et al. 2013, Troina et al. 2020b). Estes resultados indicam as mudanças no habitat de forrageio do bonito-listrado ao longo do ciclo de vida, confirmando a Hipótese 3.

A área de desova do bonito-listrado concentra-se na área oceânica das costas norte, nordeste e leste do Brasil (Matsuura 1986, Katsuragawa et al. 2020). Apesar da desova ocorrer ao longo do ano, variações nos índices gônado-somáticos indicam que a desova se concentra entre janeiro e março (Soares et al. 2019). Autores sugerem a realização de uma migração de desova no sentido sul-norte no período de verão (Jablonski et al. 1984, Costa et al. 2020b). Esta hipótese é consistente com os menores valores de  $\delta^{15}$ N em adultos, bem como com a tendência decrescente no  $\delta^{15}$ N em AAs fontes com o crescimento do bonitolistrado acima do CF de 55 cm.

As diferenças na PT estimada com ACE e isótopos estáveis (*bulk* e AA) foram pequenas e possivelmente relacionadas ao tempo de integração de ACE (menor que 24 h) e isótopos de nitrogênio no músculo (entre meses até anos). Na área sul, entretanto, a PT de adultos com base em  $\delta^{15}$ N *bulk* foi menor e distinta da PT calculada com base nos valores de  $\delta^{15}$ N do ácido glutâmico e lisina. Esse resultado foi explicado pela ocorrência de indivíduos com valores de  $\delta^{15}$ N bulk relativamente baixos, os quais não estavam em equilíbrio com a base de  $\delta^{15}$ N da área sul. A estimativa da PT com base em  $\delta^{15}$ N-AA em consumidores que se deslocam ao longo de áreas amplas e gradientes de  $\delta^{15}$ N permite estimar a PT referenciada à base de  $\delta^{15}$ N na qual o indivíduo forrageou previamente (Popp et al. 2007, Le-Alvarado et al. 2021). As estimativas de PT utilizando fenilalanina como aminoácido base subestimaram a PT do bonito-listrado em mais de um nível trófico, relativamente àquelas obtidas via ACE e  $\delta^{15}$ N *bulk*, indicando forte congruência entre as distintas metodologias utilizadas para determinar a PT do bonito-listrado.

A PT estimada para o bonito-listrado indica que esta essa espécie é um consumidor de terceira ordem nas cadeias tróficas pelágicas do Atlântico Sudoeste (PT: 3,5–4,0). Os resultados, em geral, sugerem aumento da PT do bonito-listrado com a ontogenia. Este padrão foi relacionado à redução no consumo de krill e aumento no consumo de presas com maior PT, confirmando a Hipótese 4. Entretanto, o forrageio em áreas oligotróficas oceânicas, sustentadas por produtores primários de menor tamanho e consequentemente maior número de níveis tróficos em comparação com as áreas subtropicais produtivas, pode também contribuir para o aumento na PT dos adultos.

### 4. CONCLUSÕES

Esta tese integrou análises tradicionais e modernas para estudar a ecologia trófica do bonito-listrado no Oceano Atlântico Sudoeste. A dieta foi quantificada através de modelos de mistura de isótopos estáveis de  $\delta^{13}$ C e  $\delta^{15}$ N do predador e das presas, integrados a informações obtidas via análise de conteúdo estomacal. Os elos tróficos que sustentam a população foram caracterizados, destacando as principais fontes energéticas, as mudanças na dieta e na posição trófica com o crescimento do bonito-listrado. As análises de isótopos estáveis de nitrogênio no tecido muscular (*bulk*) e em aminoácidos permitiram rastrear os movimentos sazonais entre áreas e caracterizar os padrões ontogenéticos no habitat de forrageio da espécie.

As principais fontes energéticas utilizadas pelo bonito-listrado são o mesopelágico peixe-lanterna e crustáceos eufausiáceos, comumente denominados de krill. Pequenos peixes pelágicos (Engraulidae e Clupeidae) também são presas importantes na dieta do bonitolistrado na área sul. As mudanças espaciais na dieta, e a menor disponibilidade de bonitolistrado inferida pela redução nas capturas, indicam a capacidade do bonito-listrado de evitar áreas pouco produtivas. Variações ontogenéticas na contribuição das presas indicam maior utilização de krill pelos juvenis. Na área norte o grupo 'peixe e cefalópode' (Carangidae e Ommastrephidae) tem maior importância na dieta com o aumento do tamanho do bonitolistrado. Na área sul a contribuição de pequenos peixes pelágicos (Clupeidae, Engraulidae) na dieta aumenta com o crescimento. O bonito-listrado utiliza espécies forrageiras abundantes e de elevado conteúdo energético para sua sobrevivência e crescimento, apresentando uma estratégia oportunista e aumentando o consumo de presas de maior tamanho ao longo da ontogenia.

Este estudo demonstra o potencial de utilização de isótopos estáveis de nitrogênio para rastrear os movimentos do bonito-listrado. As diferenças observadas entre áreas norte e sul no  $\delta^{15}$ N *bulk* e em aminoácidos fonte (fenilalanina e lisina) foram consistentes com a base de  $\delta^{15}$ N do zooplâncton na região, caracterizada em estudos prévios. A propagação dos padrões espaciais no  $\delta^{15}$ N desde consumidores primários até o bonito-listrado indica um tempo de residência da ordem de 2–4 meses, pelo menos, nas respectivas áreas de alimentação, inferido com base na taxa de incorporação isotópica do nitrogênio estimada para um tunídeo de rápido crescimento. Espécimes com valores de  $\delta^{15}$ N distinto do  $\delta^{15}$ N basal da área na qual foram capturados evidenciaram os movimentos sazonais entre áreas, para o sul durante primavera e verão, e para o norte nas proximidades do outono.

A capacidade de uso de áreas e recursos distintos, através do movimento e do oportunismo na alimentação, indica baixa competição intraespecífica, e contribui para maximização do crescimento para esta espécie completar seu ciclo de vida no Oceano Atlântico Sudoeste. A alta densidade de espécies forrageiras na região sul sustenta densas agregações de indivíduos jovens, que a utilizam para alimentação e crescimento. O  $\delta^{15}N$  dos aminoácidos fonte indicou que a diminuição do  $\delta^{15}N_{bulk}$  observada em adultos se deve ao forrageio em áreas quentes e oligotróficas, cuja base possui  $\delta^{15}N$  relativamente mais baixo. A literatura indica que o uso de áreas tropicais esteja relacionado à reprodução da espécie, a qual ocorre em menores latitudes e/ou áreas offshore. A produção primária das áreas oligotróficas seria insuficiente para sustentar altas densidades de presas epipelágicas. Assim, a mudança do habitat subtropical para o habitat tropical exigiria uma mudança na estratégia de forrageio, possivelmente via realização de mergulhos em águas mais frias, abaixo da termoclina, e o consumo de presas com maior tamanho e PT. O bonito-listrado é um consumidor de terceira ordem nas cadeias tróficas pelágicas do Atlântico Sudoeste, que aumenta a sua posição trófica ao longo da ontogenia (PT 3,5–4,0).

O presente estudo agrega novas informações sobre a ecologia trófica e os movimentos do bonito-listrado no Atlântico Sudoeste. As alterações na dieta maximizam o crescimento e o sucesso desta espécie, enquanto se desloca entre áreas tropicais e subtropicais, e preda sobre presas ricas em energia, adapta a dieta e aumenta sua posição trófica ao longo da ontogenia.

### 5. RECOMENDAÇÕES

Considerando: (1) o potencial para o uso de traçadores isotópicos para estudar a migração do bonito-listrado, e; (2) que durante o presente estudo (2016–18) o Oceano Atlântico Sudoeste foi influenciado por anomalias na temperatura da superfície do mar, sugere-se:

• Investigar a taxa de incorporação de isótopos de  $\delta^{15}$ N no músculo e aminoácidos fonte do bonito-listrado, relacionando o aumento no  $\delta^{15}$ N com o aumento do comprimento furcal em períodos de tempo específicos, determinados através da curva de crescimento da espécie nesta região;

• Investigar a conectividade do estoque por meio de análise de isótopos estáveis de nitrogênio *bulk* e em aminoácidos fonte, de indivíduos coletados na região sudeste/sul e nas costas leste, nordeste e norte do Brasil;

• Realizar monitoramento dos hábitos alimentares do bonito-listrado em longo prazo.

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# **APÊNDICE 1**

Isotope-based inferences of skipjack tuna feeding ecology and movement in the southwestern Atlantic Ocean

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## ABSTRACT

Skipjack tuna (Katsuwonus pelamis) sustain a large-scale fishery in the southwest Atlantic Ocean (SWA), but information about its foraging ecology in this region is still limited. Here we use carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) stable isotope analysis of muscle from individuals collected in 2017–2018 (n = 383) to quantify diet composition and characterize movement patterns. We found a relatively small degree of variation in  $\delta^{13}$ C (range: -18.9 to -16.5%) in comparison to  $\delta^{15}$ N values (6.7 to 14.7%). At higher latitudes in the southern area (30–34°S), individuals had higher mean ( $\pm$ SD)  $\delta^{15}$ N values (12.2  $\pm$  1.3‰) in comparison to those collected in the northern area  $(9.7 \pm 1.5\%)$  between 20–26°S. At the northern area, isotope mixing models with informative priors showed that lanternfish (median: 50%) and krill (31%) were the primary foods. In the southern area, lanternfish (53%), krill (23%) and small pelagic fish (23%) were the primary food sources. Spatial shifts in diet composition were related to warming events that likely resulted in low abundance of sardines in the northern area. The latitudinal pattern in skipjack and krill  $\delta^{15}$ N values mirrored that of regional zooplankton isoscapes, suggesting residency at the timescale of isotopic turnover for muscle  $(\sim 2-4 \text{ months})$ , and that geographical variation in the baseline isotopic composition can be exploited to characterize seasonal movements of skipjack and other top marine consumers in this region.

**Keywords**:  $\delta^{13}$ C and  $\delta^{15}$ N, ecosystem-based management, fisheries, food webs, mixing models, *Katsuwonus pelamis*, tracers

## **INTRODUCTION**

Understanding trophic links and energy flux in exploited ecosystems is a primary component of ecosystem-based fisheries management, whose primary objective is to sustain healthy marine ecosystems and the fisheries they support (Cury et al., 2008; Hilborn et al., 2020). Tunas (family Scombridae) support extensive fisheries worldwide in terms of landings and economic value (Brill and Hobday, 2017; FAO, 2018). Like marine mammals and seabirds, tunas evolved to take advantage of places and times that are best-suited to different phases of their life cycle, often migrating long distances for breeding and feeding (Dufour et al., 2010). Some tuna species reach sexual maturity quickly and have the fastest growth rates among fishes (Murua et al., 2017) as a consequence of their anatomical, biochemical, and physiological adaptations for a high energy expenditure lifestyle that is fueled by generalist foraging strategies (Olson et al., 2016).

Skipjack tuna (*Katsuwonus pelamis*) have a global distribution in tropical and subtropical oceans (Wild and Hampton, 1993) and have been consistently placed in the top three marine species that contribute to food security worldwide (FAO, 2018). In the southwestern Atlantic Ocean (SWA), the species has a high social and economic relevance for fishers, producers, and markets that sustain the tuna canning industry in Brazil (Schmidt et al., 2019). Brazilian catches averaged 23,566 t/year from 2000–2018, reaching a peak of 32,438 t in 2013 followed by a 56% decrease to 18,133 t two years later (ICCAT, 2019). This decrease in catches was not related to overfishing, but rather to sea surface temperature (SST) anomalies, specifically an extreme heating episode in the SWA (Manta et al., 2018) that also impacted the sardine fishery in Brazil (Schmidt et al., 2019).

The annual lifecycle of skipjack in the SWA involves movements between spawning and feeding grounds located at tropical and subtropical areas, respectively (Matsuura 1986; Castello and Habiaga, 1989; Matsuura and Andrade, 2000). Spawning occurs in waters with SST >24°C and can occur year-round in the Guiana Current off the northeast coast of Brazil, and seasonally in the Brazil Current (BC) off the southwest coast of Brazil (Matsuura, 1986). Skipjack feeding grounds are located near the Subtropical Convergence ( $30-36^{\circ}$ S) formed by the confluence of the warm and oligotrophic waters carried by the BC and the nutrientrich and cold subantarctic waters of the Malvinas Current (Garcia, 1997). Over the continental shelf, primary production in this region is also boosted by the Rio de la Plata and Patos Lagoon drainages (Möller et al., 2008). Consequently, this region sustains a large biomass of pelagic forage fish like Argentine anchovy (*Engraulis anchoita*) and lanternfish (*Maurolicus stehmanni*) (Matsuura and Andrade, 2000; Madureira et al., 2009), which represent important food resources for numerous top predators such as pelagic and demersal fish, seabirds, and marine mammals (Castello, 1997; Velasco and Castello, 2005; Marques et al., 2018).

The fishing season for skipjack in the SWA starts during the austral spring (October– December) when schools are swimming southwards following the BC. The peak of the fishing season and relative abundance of the species in the region occurs during the summer (January–March) when SST reaches its maximum. During the fall (April–June), the schools move northwards again as the SST decreases with the advance of the Malvinas Current from the south (Andrade and Garcia, 1999; Lima et al., 2000; Coletto et al., 2019).

Several studies based on stomach content analysis (SCA) have shown that skipjack diet is affected by prey distribution, migration, and oceanographic conditions (e.g. Roger, 1994; Potier et al., 2002). Euphausiids (i.e. krill) and small pelagic fish are the primary prey of skipjack in the eastern Pacific Ocean (Alverson, 1963). Krill is the primary prey of skipjack in coastal Baja California, while flying-fishes are important prey in offshore areas (Alatorre-Ramíres et al., 2017). The lightfish (*Vinciguerria nimbari*) can be an important prey item for skipjack in the eastern tropical Atlantic Ocean (Dragovich and Potthoff, 1972). A recent study found that krill (*Meganyctiphanes norvegica*), flying fish (*Exocoetus volitans*), and the anchovy (*Engraulis encrasicolus*) were the primary prey of skipjack in the Balearic Sea, Alboran Sea, and Gulf of Cadiz respectively in the Mediterranean Sea (Varela et al., 2019). In the SWA off Brazil, the primary prey of skipjack in the 1990's were lanternfish (*M. stehmanni*) and krill (*Euphausia similis*), and the importance of the latter prey type decreased with increasing skipjack size (Ankenbrandt, 1985). In the southern region of the SWA, the Argentine anchovy (*Engraulis anchoita*) has also been noted as an important source of prey (Vilela, 1990).

Conventional dietary techniques such as SCA, however, can only provide a snapshot of ingested foods and likely underestimate diet items that are quickly assimilated by the consumer (Chipps and Garvey, 2007). Additionally, the high frequency of empty stomachs of species like skipjack, or those filled with bait species, are also limitations of using SCA to study the diet of commercially exploited species like tunas over large spatial and temporal scales (Vooren 1976; Ankenbrandt, 1985; Vilela, 1990, Roger, 1994; Varela et al., 2013). Given these limitations and the overall paucity of information on the diet of skipjack in the SWA, additional proxies are needed to better characterize dietary shifts across a range of latitudes and ontogeny to understand the energy pathways that sustain large-scale fisheries in this region.

Carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) stable isotopes are widely used as intrinsic biochemical tracers of animal diet composition and movement (Phillips et al., 2014; Hobson and Wassenaar, 2019). The main premise of stable isotope analysis (SIA) is that the  $\delta^{13}$ C and  $\delta^{15}$ N values of consumer tissues mirror those of its food, but are slightly offset due to physiologically-mediated processes associated with assimilation and tissue synthesis, often called trophic discrimination. The systematic enrichment in the heavier isotope with increasing trophic level is greater for  $\delta^{15}N$  (2–4‰) than for  $\delta^{13}C$  (0–2‰) (DeNiro and Epstein, 1978; 1981) and as such nitrogen isotopes are often used to estimate trophic position (Post, 2002), while carbon isotopes are used to assess the relative influence of different primary producers in fueling the food webs utilized by the consumer (Peterson and Fry, 1987). One of the primary advantages of SIA over conventional dietary proxies like SCA is that it can provide a time-integrated measure of dietary inputs depending on the tissue analyzed (Martinez del Rio et al., 2009). Some tissues (e.g. liver) have rapid isotopic incorporation rates that reflect information about the ecology and physiology of organisms from days to weeks, while others like muscle or bone collagen reflect ecological information integrated over month to year timescales (Pinnegar and Polunin, 1999; Martinez del Rio et al., 2009; Martinez del Rio and Carleton, 2012).

Often the most complete dietary information is obtained when SCA and SIA are combined because the advantages of one technique offset the disadvantages of the other (Phillips et al., 2014; Swan et al., 2019). Specifically, SIA integrates information on resource and habitat use over multiple timescales but does not typically provide taxon-specific data on diet composition, which is an advantage of SCA. As such, SCA and SIA can be combined

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to estimate the contribution of different sources (prey) to a mixture (consumer) via isotopic mixing models (Phillips et al., 2014) that use informative priors based on SCA identification of prey species consumed by the population (Moore and Semmens, 2008; Franco-Trecu et al., 2013; Swan et al., 2019).

Studies focused on niche partitioning and resource use often quantify isotopic niches of individuals and/or populations, which provide a proxy for resource and/or habitat use (Newsome et al., 2007). The most common metric used to estimate isotopic niches is the standard ellipse area calculated by means of frequentist or Bayesian methods (Jackson et al., 2011). However, the elliptical nature of SEAs does not adequately capture the multi-modal structure of some datasets, which can lead to inaccurate estimates of the isotopic niche (Franco-Trecu et al., 2014). More recently, an approach based on kernel densities has been applied to isotopic data as it is less sensitive to extreme (outlier) values and performs well with more structured multi-modal datasets (Eckrich et al., 2019).

In this study, we aimed to quantify skipjack diet composition and characterize movement patterns in the SWA. We combined  $\delta^{13}$ C and  $\delta^{15}$ N values of skipjack muscle and their primary prey with data from SCA to generate quantitative estimates of the assimilated diet in the primary feeding grounds of the species in this region. Lastly, we used kernel isotopic niche estimates to explore habitat use and seasonal movement patterns across ontogeny along the range of latitudes (20–34°S).

## MATERIAL AND METHODS

### **Sample collection**

Muscle tissue samples were obtained from skipjack collected during catch landings at Niterói (Rio de Janeiro –RJ) and Rio Grande (Rio Grande do Sul–RS), Brazil between January 2017 and December 2018 as part of the Bonito Project. Fishing positions were plotted using QGIS software (Version 3.4.9). Catches occurred between 20–34°S, and skipjack and prey samples were divided into two groups hereafter referred to as northern (20–28°S) and southern (28–34°S) areas (Fig 1). We chose these areas because they are characterized by distinct oceanographic conditions. The southern area is heavily influenced

by the cold waters of Malvinas Current, especially during fall and winter (Garcia, 1997; Lopes et al., 2006) and continental water runoff from Rio de la Plata and Patos Lagoon (Möller et al., 2008). The northern area is dominated by tropical water and coastal and shelfbreak upwellings of South Atlantic Central Water driven by the Brazil Current (Campos et al., 1995; Campos et al., 2000; Acha et al., 2004; Lorenzetti et al., 2009). The northern and the southern areas are 1,000–1,200 km distant from each other. The oceanographic processes driving nutrient availability and distribution were found to be responsible for the latitudinal patterns in zooplankton  $\delta^{13}$ C and  $\delta^{15}$ N isoscapes (Troina et al., 2020).

All individuals (n = 383) were measured for straight fork length (SFL), weighed to the nearest gram, and sexed by macroscopic identification of ovaries and testes. Individuals were categorized into size groups according to the SFL that 50% ( $L_{50} = 46$  cm) and 100% ( $L_{100}=63$  cm) of the specimens are likely to have reached sexual maturity in the SWA (Soares et al., 2019). Size groups were defined as juveniles (SFL < 47cm), young adults (SFL 47–63 cm), and adults (SFL > 64 cm).

#### Stable isotope analysis

We used a scalpel to remove ~5 g of white muscle from the region adjacent to the second dorsal fin of each individual, which was kept frozen (-20°C) until processing in the laboratory. For isotope analysis, samples were rinsed with distilled water, lyophilized or oven-dried at 60°C for 48 h, and homogenized with a mortar and pestle (Sulzman, 2007). Aliquots of ~0.5 mg of the resulting powder were weighed into tin capsules and  $\delta^{13}$ C and  $\delta^{15}$ N values were measured at the University of New Mexico (UNM) Center for Stable Isotopes (CSI) in Albuquerque, NM or the University of California Davis (UCD) Stable Isotope Facility (SIF) in Davis, CA. Analyses at UNM-CSI were performed with a Costech ECS-4010 elemental analyzer (Valencia, CA) coupled to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer (Bremen, Germany), while analyses at UCD-SIF were performed with a Europa Hydra 20-20 mass spectrometer. Isotope values are reported in  $\delta$  notation using the equation:  $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$ , where *X* is <sup>13</sup>C and <sup>15</sup>N and R is the ratio <sup>13</sup>C/<sup>12</sup>C or <sup>15</sup>N/<sup>14</sup>N (Peterson and Fry, 1987). Internationally accepted standards for  $\delta^{13}$ C and  $\delta^{15}$ N are Vienna Pee Dee Belemnite (V-PDB) and atmospheric N<sub>2</sub>, respectively. Within-run analytical precision (±SD) was estimated via analysis of proteinaceous internal

reference materials and estimated to be  $\pm 0.1\%$  for both  $\delta^{13}$ C and  $\delta^{15}$ N. We compared interlaboratory measurements by analyzing replicates of skipjack muscle (n=3) measured in both isotope laboratories. The mean difference for  $\delta^{13}$ C (0.2‰, paired t-test,  $t_2 = -8.41$ , p = 0.01) and for  $\delta^{15}$ N (0.1‰, paired t-test,  $t_2 = 1.37$ , p = 0.15) was small and the slope for the linear regression among replicates did not differ from zero for  $\delta^{13}$ C (r<sup>2</sup> = 0.98, F<sub>1,1</sub> = 56.86, p = 0.08). Owing to low mean (±SD) weight percent [C]:[N] ratios of skipjack white muscle (3.2  $\pm$  0.1) that is indicative of pure protein, we did not lipid-extract samples prior to isotope analysis (Post et al., 2007).

## Skipjack potential prey

Ankenbrandt (1985) analyzed skipjack stomachs collected from the commercial pole and line fishery in Brazil between 1981–82 and reported krill (*Euphausia similis*) and lanternfish as the primary prey between 22–28°S. Vilela (1990) analyzed samples from the commercial pole and line fleet between 1986–89, as well as samples from exploratory purseseine fishing (1983–86). Sardines were the primary prey found in stomachs collected from the commercial pole and line fleet samples, while lanternfish, krill and anchovy were the primary prey of tuna caught in the exploratory purse-seine fishery (Vilela, 1990). Based on this previous work, we selected potential prey items consumed by skipjack in the study area. Isotopic data for these prey species were obtained from published studies conducted in the same area (Troina, 2019; Troina et al., 2020) as well as from samples we collected throughout the study (n=19) and one sardine purse-seine landing at Rio Grande in May 2019 (n=12). Prey samples were processed and analyzed following the same protocol we used for skipjack muscle.

### Data analysis

Permutational multivariate analysis of variance (PERMANOVA) was used to test for differences in isotope values among skipjack collected in the two areas (northern and southern) and between sex. Permanova was based on Euclidian distance matrix of  $\delta^{13}$ C and  $\delta^{15}$ N values. The homogeneity of multivariate dispersion was tested with 'betadisper' function from 'vegan' package (Oksanen et al., 2020). Linear regression analyses were applied to test the relationship between skipjack  $\delta^{13}$ C values and SFL, and between  $\delta^{15}$ N values of skipjack and euphausiids latitude of collection. Mann-Whitney rank sum tests were used to verify the differences in skipjack muscle  $\delta^{13}$ C and  $\delta^{15}$ N values for the monthly comparisons between areas during the 2017 and 2018 fishing seasons and for comparisons among size classes from each area. Statistical analyses and graphical outputs were performed in R 3.6.0 (R Core Team, 2019).

Because lipids are depleted in <sup>13</sup>C, we used tissue- and taxa-specific equations to normalize  $\delta^{13}$ C values of prey samples that had C:N > 3.5. We used Eqn. 1 and Eqn.2 from Logan et al. (2008) for fish samples (except lanternfish) and for Euphausiids, respectively. Lanternfish  $\delta^{13}$ C values were normalized following Hoffman et al. (2011). Lipid normalization for carbon isotope values of cephalopod muscle was performed following Logan and Lutcavage (2013), that applied a general equation for fish muscle reported in Logan et al. (2008). Food web studies often use hydrochloric acid (HCl) to remove the inorganic C, as calcareous structures may bias  $\delta^{13}$ C values (Carabel et al., 2006). We choose not to treat prey samples with HCl because studies show that this pretreatment can influence  $\delta^{15}$ N values (Bunn et al., 1995; Carabel et al., 2006).

To reduce the number of sources for ensuring discriminatory power in our mixing models (Phillips et al., 2014), we grouped sources *a priori* by family and region (northern and southern) and compared their mean  $\delta^{13}$ C and  $\delta^{15}$ N values (Mann-Whitney rank sum test). We then grouped families based on their statistical similarity (p > 0.05 for  $\delta^{13}$ C and  $\delta^{15}$ N) and considering their ecological function within each region (Phillips et al., 2005). Using mean isotope values for prey groups sources, we simulated mixing polygons through a Bayesian statistical framework with the *sp* and *splancs* packages in R (Bivand et al., 2013; Rowlingson and Diggle, 2017). This is a quantitative method based on a point-in-polygon premise for assessing whether a proposed mixing model is likely to explain the isotope values of consumers and the accuracy of trophic discrimination factors (TDF; Smith et al., 2013). Mixing polygons were run with 1500 iterations.

The mixing polygon for skipjack collected in the northern area was simulated with the following prey groups: (1) krill (Euphausiidae); (2) cephalopods and fish (Carangidae, Ommastrephidae); (3) lanternfish (*Maurolicus stehmanni*); and; (4) small pelagic fish (Clupeidae). The mixing polygon for skipjack collected in the southern area was simulated with the following prey groups: (1) krill (Euphausiidae); (2) cephalopods (Ommastrephidae);

(3) lanternfish (*Maurolicus stehmanni*) and; (4) small pelagic fish (Clupeidae, Engraulidae). For each polygon simulation, we used three sets of trophic discrimination factors (TDF) to correct for isotopic offset between skipjack and prey sources: (1) *Thunnus orientalis* reported in Madigan et al. (2012) ( $\Delta^{13}$ C 1.8 ± 0.4 ‰,  $\Delta^{15}$ N = 1.9 ± 0.4 ‰); (2) adult *Thunnus thynuus* ( $\Delta^{13}$ C -0.2 ± 0.6‰;  $\Delta^{15}$ N = 1.6 ± 0.2‰ reported in Varela et al. 2011) and; (3) juvenile *Thunnus thynuus* ( $\Delta^{13}$ C 0.3 ± 0.1‰;  $\Delta^{15}$ N = 1.5 ± 0.1‰ reported in Varela et al. 2012) (Appendix 1, Fig. A1). We chose to use the TDF estimates reported in Madigan et al. (2012)

The relative contribution of prey groups to skipjack diet was estimated with Stable Isotope Mixing Models in R (SIMMR; Parnell, 2019). SIMMR were run with uninformative priors (SIMM-UP) and with informative priors (SIMM-IP) for each site and size class. We used the previously mentioned Bonito Project SCA dataset to construct informative priors for the SIMM-IP. We specified priors means and standard deviations and generated prior distributions through "simmr elicit" function in the SIMMR package. We used prey mass as priors because it is considered the best measure of relative importance of prey in animal diet (Swan et al., 2019). When estimating total biomass for small pelagic fish, we removed anchovies and sardines that had a low degree of digestion and assumed they were ingested as bait. Mean (±SD) isotope values for prey groups and informative priors used in mixing models are shown in Table 1. Models were fitted using 10,000 iterations, 1000 burn-in, with a thinning interval of 10 and four Markov Chain Monte Carlo (MCMC) chains. Convergence was first checked before further consideration. Results are reported as the median and (2.5-97.5%) credible interval for the contribution of each prey group to skipjack diet. Differences in prey contribution between size groups were compared through "compare\_groups" function in SIMMR and reported with probabilities.

Finally, we used the rKIN package (Eckrich et al., 2019) to estimate a Kernel Utilization Density (KUD) isotopic niche for skipjack from each area (northern and southern) or ontogenetic group (juvenile, young adults, adults). Similar to standard ellipse analysis, we assumed that the overlap of KUD provides an estimate for isotopic niche overlap between area and ontogenetic groups. Niche size and overlap are reported for 50%, and 75% KUD contours.

## RESULTS

## Skipjack $\delta^{13}$ C and $\delta^{15}$ N Values

Mean  $\delta^{13}$ C and  $\delta^{15}$ N (±SD) values and sample sizes for each area and ontogenetic group are reported in Table 2. Straight fork length (SFL) for all individuals ranged from 37– 80 cm (mean ± SD: 51.6 ± 8.4 cm) and total body mass ranged from 0.9–12.0 kg (mean ± SD: 3.3 ± 2.0 kg). No differences were detected between sexes with PERMANOVA (p = 0.61). Isotopic values differed between areas (p < 0.001), and multivariate dispersion did not differ between areas (p > 0.05), suggesting that differences were not due to multivariate dispersion.  $\delta^{13}$ C values increase with skipjack SFL (F<sub>1,381</sub> = 57.54, p < 0.001; supplementary Figure A1a).  $\delta^{15}$ N values increased towards higher latitudes in both skipjack muscle (F<sub>1,381</sub> = 308.4, p < 0.001) and euphausiids (F<sub>1,41</sub>= 43.3, p < 0.001; supplementary Figure A1b).  $\delta^{15}$ N values differed among size groups within each area, with higher values observed in young adults. Adults from the southern area, however, did not differ from any other size group in either areas for both  $\delta^{13}$ C and  $\delta^{15}$ N isotopes (Table 2, Appendix 1, Table A2).

No differences in  $\delta^{13}$ C values were observed in the monthly comparison (except on March 2017), although a slight increase occurred over time in both fishing seasons (Fig. 2).  $\delta^{15}$ N values differed consistently across areas, but became similar after March–April (Fig. 2, Appendix 1, Table A3).

## **Isotope mixing models**

The highest proportion of individuals inside the 95% probability contours in both the northern and southern mixing polygons were obtained with TDF values reported by Madigan et al. (2012) (supplementary Figure A2). Median estimates and credible intervals from mixing models with uninformative (SIMM-UP) and informative (SIMM-IP) priors for both areas and all size classes are reported in Table 3. In the northern area, SIMM-UP indicated that diet was composed of lanternfish (median: 50%) and krill (31%), followed by cephalopods and fish (12%), and lastly small pelagic fish (7%). SIMM-IP estimates were very similar for this area, indicating that diet was composed of lanternfish (50%) and krill (29%), cephalopods and fish (14%) and small pelagic fish (7%) (Fig. 3). In the southern area, SIMM-UP indicated lanternfish as the main prey (64%), followed by similar proportions of

small pelagic fish (14%), cephalopods (13%) and krill (9%). SIMM-IP estimates slightly differed for this area and indicated lanternfish as the main prey (53%), followed by krill (23%), small pelagic fish (23%) and cephalopods (1%) (Fig. 3).

Ontogenetic diet shifts were consistent between SIMM-UP and SIMM-IP model types in both areas (Table 3; Fig 4). In both areas, the contribution of krill decreased from juveniles to young adults (Probability > 0.98). In the northern area, the proportion of cephalopods and fish and small pelagic fish increased in young adults and adults, in comparison to juveniles (P > 0.94). In the southern area, the importance of small pelagic fish increased with skipjack size (P > 0.97). Lanternfish importance decreased with skipjack size in the northern area (P > 0.75), while no ontogenetic shifts in lanternfish consumption were evident in the south. No shift in the proportion of cephalopods occurred among size classes in the southern area. Due to small sample size (n = 7), we were not able to estimate diet composition of adult skipjack captured in the southern area.

## Isotopic niche width

Isotopic niche estimates were larger for skipjack collected in the northern area, in comparison to the south (Fig. 5a), and individuals (n = 26) collected in the northern area were observed inside the KUD contours defined by skipjack captured in the southern area and vice-versa (n = 12). Isotopic niche overlaps between areas varied from 0% to 64.7% depending on the KUD of inference (Table 4).

KUD estimates for ontogenetic groups showed that young adults have larger isotopic niches in comparison to juveniles and adults (Fig. 5b). Contours for KUD showed differences in the isotopic niche among skipjack size classes. The core area for juveniles, denoted by 50% contour, had the lowest  $\delta^{13}$ C and  $\delta^{15}$ N values, whereas young adults were distributed across the entire isotopic space and appeared to have two core areas: a larger one defined by relatively high  $\delta^{15}$ N values and a smaller one with intermediate to low  $\delta^{15}$ N values (Fig. 5b).

Finally, the core area of adults had similar (low)  $\delta^{15}$ N values to juveniles, while adult  $\delta^{13}$ C values were higher than other size classes. Overlap estimates for 50% contours were greatest among adults and young adults (36.3%), adults and juveniles (35.7%) and juveniles and adults (33.4%) (Table 4).

## DISCUSSION

We applied complementary approaches to study skipjack tuna foraging ecology and movement in the Southwest Atlantic Ocean. Mixing models showed that the primary prey for skipjack is lanternfish in both the northern and southern feeding grounds. Krill was also important prey in both areas, while cephalopods and fish and small pelagic fish (e.g. anchovy and sardine) were the secondary prey in the northern and southern areas, respectively. In regards to ontogenetic dietary patterns, the importance of krill and lanternfish decreased, while the contributions from cephalopods and fish, and small pelagic fish increased with increasing skipjack size. Latitudinal trends in skipjack muscle tissue were consistent with those of regional isoscapes of zooplankton (Troina et al., 2020), suggesting a degree of residency similar in timescale to the isotopic incorporation of tuna muscle (2–4 months) (Graham, 2007), and that baseline  $\delta^{15}$ N gradients may be useful for tracking movement of skipjack and other top marine consumers in this region. Lastly, the isotope-based ontogenetic patterns in skipjack seasonal movements agree with data on size structure dynamics gleaned from fishery landings.

## Skipjack diet composition

Our results show that three primary prey types sustain the skipjack population in SWA feeding grounds: lanternfish, and krill in the northern area, and lanternfish, krill and small pelagic fish in the southern area. Skipjack forages mostly on fish and crustacean prey in the Atlantic Ocean (Olson et al., 2016). The lightfish (*Vinciguerria nimbari*) have been identified as an abundant food source in eastern tropical Atlantic (Dragovich and Potthoff, 1972). In the area between 22–28°S corresponding to the northern area in our study, Ankenbrandt (1985) found that the main prey consumed in the 1981–82 fishing season were lanternfish (*Maurolicus stehmanni*) and krill (*Euphausia similis*) that represented 26.7% and 22.2% of stomach content by volume (V), respectively. Vilela (1990) also found that lanternfish was the primary prey for skipjack (45% V) between 30–34°S from 1983–86, followed by krill (12% V), and anchovy (*Engraulis anchoita;* 8% V). Hydroacoustic cruises conducted between 1995–1997 indicated that lanternfish occurs between 22–34°S in the SWA, and the highest abundances for the species were found near the shelf break and slope areas, where the South Atlantic Central Water meets the Tropical Water from Brazil Current

(Madureira et al., 2005). During winter, the greatest lanternfish abundance was found in the northern area off Rio de Janeiro, while during the fall and spring-summer surveys, the highest abundance occurred in the southern area (Madureira et al., 2005). Overall, these patterns suggest that there is a strong trophic link between skipjack and lanternfish in the SWA.

Skipjack was also reported to consume krill in the Pacific and Indian Oceans (Olson et al., 2016; Vooren, 1976), while north Atlantic krill (*Meganyctiphanes norvegica*) was the primary food source in the Balearic Sea (Varela et al., 2019). Dense patches of euphausiids are common in oceanographic fronts between oligotrophic and cold nutrient-rich waters in the Southern California Bight (Fiedler and Bernard, 1987). These conditions are similar to those associated with skipjack foraging behavior and fishing activity in the SWA (Andrade, 2003; Coletto et al., 2019) where *Euphausia similis* occurs in the convergence of subtropical and subantartic waters masses that generate favorable conditions for krill growth, providing large nutrient-rich patches of forage for pelagic top consumers (Antezana and Brinton, 1981; Lopes et al., 2006).

Mixing models show higher proportion of small pelagic fish in skipjack diet in the southern area in comparison to the northern area. Also, the ontogenetic shift in the importance of small pelagic fish with increasing size was more pronounced in the southern area, suggesting a greater availability of this prey at higher latitudes. The reduced contribution of small pelagic fish in the northern area may be related to the low abundance of the Brazilian sardine (*Sardinella brasiliensis*), as indicated by the lowest ever catch recorded by the Brazilian industry in 2017 (Schmidt et al., 2019). Several impacts of climate change have been documented on marine fisheries in the SWA, with evidences that sardine is moving to the south of its traditional range (23–29°S) (Franco et al., 2020), which appear to be related to positive SST anomalies and the southward influence of the Brazil Current (Gianelli et al., 2019; Schmidt et al., 2019; Franco et al., 2020). It is likely that the spatial and ontogenetic variation on skipjack diet observed here are further elucidating effects of the extreme warming events recorded during recent years in the SWA (Manta et al., 2018).

Our mixing models indicate ontogenetic shifts in skipjack diet. Lanternfish and krill importance decreased with skipjack size, while the proportion of cephalopods and fish and small pelagic fish increased. Ankenbrandt (1985) found that smaller skipjack relied more on krill, while the importance of lanternfish and fish in general increased with skipjack size.

Vilela (1990), however, found no shifts in diet with respect to skipjack size. Graham et al. (2007) found that a critical size threshold of ~45 cm for yellowfin tuna (*Thunnus albacares*) was likely associated with an endothermic capability to access larger prey in deeper, colder waters. Likewise, the average size of prey increased with the size of bigeye tuna (*Thunnus obesus*) and yellowfin tuna from the Indian Ocean (Ménard et al., 2006) and the eastern Pacific off Ecuador (Varela et al., 2017). The asymmetric shape of prey size distribution, however, suggests that tunas may continue to feed on small prey when they reach larger body sizes (Young et al., 2010), which is consistent with the more even proportions of prey groups in adult skipjack diet observed in the northern area of the SWA (Table 3 and Fig. 4).

The relatively small but notable occurrence of skipjack with outlying  $\delta^{15}$ N values at both areas indicates that a few individuals were not in equilibrium with the isotopic composition of local prey. The inclusion of these individuals in our mixing models may bias the estimates of dietary composition to some degree, however, these outliers represent a low proportion (~11%) of the individuals we sampled. Future isotope-based studies could address this potential uncertainty by focusing on tissues (e.g. liver or blood plasma) with faster isotopic incorporation rates.

The posteriors for cephalopods contribution in our SIMM-IP in the southern area mirrored the informative priors. Since only hard parts such as beaks accumulate in predator stomachs with minimal digestion (Clarke, 1986), biomass estimates may underestimate cephalopod contribution to skipjack diet. The importance of cephalopods is minimal regardless of the index we used as priors in mixing models; i.e. biomass <2%; frequency of occurrence, FO 2–3% (Supplementary Table A1). Ankenbrandt (1985) reports Ommastrephidae as the most frequent cephalopod family in skipjack stomach contents (1.7% FO), followed by Argonautidae (1% FO). Data from Vilela (1990) shows that Teutoidea contributed only 1.3% of stomach content volume and had 6% FO in experimental purse seining, while only representing of 0.13% volume and 0.75% FO in commercial fishing. Santos and Haimovici (2002) reviewed the role of cephalopods in SWA food webs, and classified skipjack as an occasional predator (i.e. < 10% FO) for Ommastrephidae and Argonautidae. Overall, these patterns generally agree with our mixing model results, however, it is worth noting that dietary data based on SCA may be biased because some

Ommastrephidae are diel migrants and pole and line fishing depends on visual sighting of tuna at the surface during the day (Lima et al., 2000).

In the southern area, estimates of diet composition based on SIMM-IP were considered more realistic in comparison to SIMM-UP. The effect of priors on mixing model posteriors is greater when the isotopic variation among sources is limited (Franco-Trecu et al., 2013; Swan et al., 2019), and the discriminatory power of mixing models is directly related to the degree of isotopic differences among sources of prey that defines the geometry of the mixing space (Phillips et al., 2014). The incorporation of priors into mixing models has the advantage of setting the range of feasible solutions for each source in a given model (Moore and Semmens, 2008), but caution should be taken with this approach as the priors can transfer biases from other methods (e.g. SCA) into dietary estimates derived from mixing models (Franco-Trecu et al., 2013; Swan et al., 2019).

### Skipjack habitat use

Skipjack caught in the southern area had higher mean  $\delta^{15}$ N values than fish captured in the northern area. This pattern could be related to diet (i.e. trophic level) as krill had a greater importance for skipjack in the northern area in comparison to the southern area. More importantly, the trend of increasing  $\delta^{15}$ N values with latitude was clear in  $\delta^{15}$ N values of prev collected from the two regions: e.g. krill  $\delta^{15}$ N values on average were ~1.9% higher in the south in comparison to the north. Also,  $\delta^{15}$ N values for both skipjack and krill increased with latitude, suggesting that baseline patterns were likely the cause of differences on both prey and skipjack  $\delta^{15}$ N values between areas. Published basin-scale isoscapes show that zooplankton  $\delta^{15}$ N values are ~4‰ near the upper margin of the northern area at Abrolhos Bank (Fig. 1), and increase to  $\sim 6\%$  near the southern area of our study (McMahon et al., 2013), which is likely driven by the greater influence of  $N_2$  fixation by phytoplankton in the oligotrophic waters of the northern area versus higher nutrient availability and primary production in the southern area (Troina et al., 2020). The latitudinal trend in skipjack and krill  $\delta^{15}$ N composition is consistent with previous work with pelagic predators from other ocean basins. Yellowfin tuna and mesozooplankton  $\delta^{15}$ N values varied consistently with latitude in the Eastern Tropical Pacific (Popp et al., 2007). Swordfish (Xiphias gladius) and vellowfin tuna also reflected spatial variation in baseline  $\delta^{15}$ N values in the Indian Ocean

(Ménard et al., 2007), while the  $\delta^{15}$ N values varied with sampling location for dolphinfish (*Coryphaena hippurus*) and yellowfin tuna in the North Atlantic Ocean (Logan and Lutcavage, 2013).  $\delta^{13}$ C can also vary with latitude of sampling locations as shown for bigeye (*Thunnus obesus*), albacore (*Thunnus alalunga*) and yellowfin tunas (Logan et al., 2020). These patterns suggest that the baseline variation in  $\delta^{15}$ N is likely the primary driver of the latitudinal trend in skipjack muscle  $\delta^{15}$ N values in the SWA.

To reflect gradients in baseline isotope composition, mobile predators should have a degree of residency that is similar to or greater than the isotopic incorporation rates of their tissues (Graham et al., 2010). Fisheries data show that skipjack occurs in the southern foraging grounds of the SWA from October–September until May–June of the following year (Coletto et al., 2019). It is important to note that our opportunistic sampling effort occurred for a period of 6–7 months during two years (2017 and 2018) in both the northern and southern areas. Isotopic incorporation is directly related to growth rates (Martinez del Rio et al., 2009), and since skipjack have the fastest growth rates among tunas (Murua et al., 2017), incorporation rates should be higher than those estimated for Pacific bluefin tuna (~ 5.5 months; Madigan et al., 2012), but are likely more similar to those estimated for juvenile yellowfin tuna (~2–4 months; Graham, 2007). Therefore, the increase in skipjack muscle  $\delta^{15}$ N values observed at higher latitudes suggests a degree of residency on the timescale of a few months in the productive southern foraging grounds.

In addition to nitrogen isotope difference between the northern and southern areas, we observed temporal patterns in skipjack  $\delta^{15}$ N that are likely the product of seasonal latitudinal movements. Some skipjacks captured in the northern area had higher  $\delta^{15}$ N values (>12.5‰) than expected based on the 50% KUD niche estimates that were more similar to mean  $\delta^{15}$ N values of skipjack captured in the southern area. Most of these outlier individuals (n = 16 or 76%) were caught during April and May. When comparing skipjack isotope composition between areas over time, we found that  $\delta^{15}$ N values were different during summer months (January–March), but during April–May  $\delta^{15}$ N values for individuals captured in the northern area increase and become similar to those for individuals captured in the southern area, which can be explained by the seasonal movement of skipjack from south to north in the fall. Skipjack latitudinal movements in the SWA are driven by seasonal displacements of the Subtropical Convergence Area and the warm waters of the BC that determine the seasonal distribution of SST across this region (Castello and Habiaga, 1989; Vilela and Castello, 1993; Andrade, 2003; Coletto et al., 2019). Skipjack seasonal migration following the displacements of BC in the area were observed through fishing effort and catch dynamics (Andrade 2003; Coletto et al., 2019), and also by tag and recovery experiments, as individuals released in the southern area were subsequently recaptured in the northern area (Luckhurst, 2014; Fonteneau, 2015; ICCAT, 2016).

We also observed a smaller number of individuals (n = 3) captured in the southern area that had surprisingly low  $\delta^{15}$ N values (<9.2‰) similar to those of skipjack captured in the northern area. All three individuals were adults, significantly larger (SFL=67-73) than most fish caught in the southern area. This pattern suggests that adult skipjack primarily forage at lower latitudes, but move towards the productive foraging areas at higher latitudes during the fall. Length frequency analysis shows a seasonal modal progression, with smaller sized individuals occurring during spring and larger individuals in the fall (Ankenbrandt, 1985; Andrade and Kinas, 2004; Soares et al., 2019). Larvae/egg distribution and size at sexual maturity suggest that spawning rarely takes place in the southern area, but instead during the summer months near Abrolhos Bank (~18°S) near the northern margin of the northern area (Matsuura, 1986; Vilela and Castello, 1993; Matsuura and Andrade, 2000; Soares et al., 2019). The higher occurrence of larger individuals during the fall could explain the increase in  $\delta^{13}$ C values observed at both sites towards the end of the fishing seasons, as  $\delta^{13}$ C increase with skipjack size. Patterns in the isotopic niche of different size groups thus appear to be linked to skipjack lifecycle. Specifically, there was a large overlap in the 50% KUD contours between juveniles and adults, whose  $\delta^{15}$ N values are lower in comparison to young adults. In contrast, young adults have higher  $\delta^{15}$ N values because they primarily forage in the most productive southern foraging grounds at higher latitudes near the subtropical convergence, where baselines have higher  $\delta^{15}$ N values, and they also consume higher trophic level prey (e.g. cephalopods and fish, and small pelagic fish), while juveniles primarily consume lanternfish and krill.

### CONCLUSION

Understanding trophic links and energy flux in exploited ecosystems is imperative for fisheries management. As opportunistic predators, tuna foraging strategies evolved to satisfy their high energetic demands by maximizing intake of energy-rich foods (Olson et al., 2016). The opportunistic strategy for skipjack includes the consumption of energy-rich and abundant prey such as lanternfish and krill, which form strong trophic links in the pelagic food webs of the SWA. Lanternfish and krill were the main prey regardless of skipjack size, and young adults and adults also consume cephalopods and fish (i.e. Omastrephidae, Carangidae) in the northern area, and small pelagic fish (i.e. Clupeidae and Engraulidae) in the southern area. We also show that the observed differences in skipjack  $\delta^{15}$ N values between the northern and southern areas reflect baseline trends in the nitrogen isotopic composition of zooplankton near the base of the pelagic food webs. Since this pattern was conserved from primary consumers to a higher trophic level pelagic predator, skipjack may have a degree of residency in the order of  $\sim$ 2–4 months in the northern or southern SWA foraging grounds based on isotopic incorporation rates for similar sized yellowfin tuna. Finally, we recommend that the foraging patterns of the SWA skipjack population be continuously monitored to better understand how oceanographic conditions influence skipjack distribution patterns in this highly dynamic pelagic environment.

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	models for skipjack tuna collected in the northern and southern areas of Southwest Atlantic Ocean.												
		Informative Prior											
Area	Prey Group	n	$\delta^{13}C \pm SD$	$\delta^{13}C_{norm} \pm SD$	$\delta^{15}N\pm SD$	$C{:}N\pm SD$	Biomass proportion $\pm$ SD						
North	krill	20	$-21.0 \pm 1.2$	$-19.1 \pm 0.5$	$3.7 \pm 1.1$	$4.9\pm1.4$	$0.065 \pm 0.052$						
	cephalopods and fish	15	$-18.6\pm0.4$	$-18.0\pm0.5$	$6.7 \pm 1.4$	$3.4\pm0.2$	$0.148\pm0.073$						
	lanternfish	27	$-20.3 \pm 0.4$	$-20.3 \pm 0.4$	$10.0\pm1.0$	$3.8\pm0.2$	$0.057 \pm 0.054$						
	small pelagic fish	6	$-17.0 \pm 1.3$	$-17.0 \pm 1.3$	$11.7\pm0.8$	$3.3\pm0.1$	$0.729 \pm 0.132$						
South	krill	23	$-21.8 \pm 0.7$	$-19.8\pm0.5$	$5.6 \pm 1.2$	$4.8\pm0.7$	$0.855 \pm 0.060$						
	cephalopods	23	$-19.6 \pm 0.6$	$-18.1 \pm 0.5$	$8.5\pm1.7$	$3.6\pm0.2$	$0.046 \pm 0.039$						
	lanternfish	41	$-20.5 \pm 0.9$	$-20.1 \pm 0.4$	$10.6 \pm 1.4$	$4.2\pm0.9$	$0.047 \pm 0.039$						
	small pelagic fish	15	$-18.2 \pm 0.5$	$-17.8 \pm 0.5$	$13.6 \pm 0.6$	$3.4 \pm 0.2$	$0.053 \pm 0.037$						

**Table 1**. Mean measured  $\delta^{13}$ C,  $\delta^{13}$ C normalized for lipid contents ( $\delta^{13}$ C<sub>norm</sub>),  $\delta^{15}$ N values (‰), weight percent carbon-to-nitrogen (C:N) ratios, and informative priors of four prey categories used in mixing models for skipjack tuna collected in the northern and southern areas of Southwest Atlantic Ocean.

**Table 2** Mean ( $\pm$  SD)  $\delta^{13}$ C and  $\delta^{15}$ N values (‰) for areas (northern and southern) and size classes of skipjack collected in the Southwest Atlantic Ocean (n = 383). Size groups not connected by the same superscript letter are significantly different (p < 0.05).

		n	$\delta^{13}$ C mean ± SD	$\delta^{13}$ C Range	$\delta^{15}$ N mean ± SD	$\delta^{15}$ N Range
Northern		242	$-17.6\pm0.4$	-18.9 to -16.5	$9.7\pm1.5$	6.7 to 13.9
	Juveniles	78	$-17.9\pm0.2^{a}$	-18.5 to -17.3	$9.0 \pm 1.2^{a}$	6.7 to 13.3
	Young Adults	130	$-17.5\pm0.5^{b}$	-18.9 to -16.5	$10.2 \pm 1.6^{b}$	7.1 to 13.9
	Adults	34	$-17.4\pm0.4^{bc}$	-18.2 to -16.7	$9.5 \pm 1.0^{\circ}$	8.0 to 13.1
Southern		141	$-17.7\pm0.5$	-18.6 to -16.6	$12.2\pm1.3$	9.0 to 14.7
	Juveniles	26	$\textbf{-17.9} \pm 0.6^{ad}$	-18.6 to -16.6	$11.2 \pm 1.2^{\rm d}$	9.5 to 13.5
	Young Adults	108	$-17.6\pm0.4^{e}$	-18.9 to -16.7	$12.5\pm1.1^{e}$	10.1 to 14.7
	Adults	7	$\text{-}17.7\pm0.3^{abcde}$	-18.1 to -17.1	$10.9\pm2.2^{bcde}$	9.0 to 13.9

**Table 3.** Estimates of diet proportions (medians and 95% credible intervals in parentheses) based on stable isotope mixing models with uninformative (SIMM-UP) and informative (SIMM-IP) priors for skipjack captured in the northern and southern areas of the Southwest Atlantic Ocean and of various sizes. Juveniles (JUV), young adults (YAD), adults (AD).

		SIMM-UP	SIMM-IP	JUV-UP	JUV-IP	YAD-UP	YAD-IP	AD-UP	AD-IP
Northern	krill	0.31	0.29	0.42	0.38	0.21	0.20	0.31	0.30
		(0.21–0.38)	(0.23–0.33)	(0.37–0.46)	(0.33–0.43)	(0.07–0.32)	(0.13–0.27)	(0.15–0.43)	(0.20–0.38)
	cephalopods/fish	0.12	0.14	0.04	0.07	0.18	0.19	0.18	0.19
		(0.02–0.26)	(0.09–0.21)	(0.01–0.08)	(0.04–0.10)	(0.03–0.38)	(0.11–0.28)	(0.03–0.41)	(0.11–0.31)
	lanternfish	0.50	0.50	0.53	0.52	0.51	0.50	0.40	0.39
		(0.47–0.53)	(0.47–0.53)	(0.49–0.56)	(0.49–0.56)	(0.46–0.55)	(0.47–0.55)	(0.34–0.46)	(0.33–0.45)
	small pelagic fish	0.07	0.07	0.02	0.02	0.10	0.11	0.10	0.12
		(0.02–0.12)	(0.04–0.10)	(0.00–0.04)	(0.01–0.04)	(0.02–0.18)	(0.07–0.15)	(0.02–0.18)	(0.07–0.17)
Southern	krill	0.09	0.23	0.24	0.35	0.08	0.21		
		(0.01–0.19)	(0.19–0.27)	(0.12–0.36)	(0.26–0.49)	(0.02–0.17)	(0.17–0.26)		
	cephalopods	0.13	0.01	0.08	0.01	0.11	0.01		
		(0.02–0.23)	(0.00–0.03)	(0.02–0.22)	(0.00–0.07)	(0.02–0.20)	(0.00–0.03)		
	lanternfish	0.64	0.53	0.60	0.57	0.63	0.52		
		(0.55–0.72)	(0.47–0.59)	(0.44–0.73)	(0.35–0.71)	(0.55–0.70)	(0.46–0.59)		
	small pelagic fish	0.14	0.23	0.06	0.05	0.18	0.26		
		(0.05–0.22)	(0.20–0.26)	(0.01–0.16)	(0.01–0.18)	(0.10–0.25)	(0.22–0.29)		

	Groups	50%	75%
Site	North x South	0	22.7
	South x North	0.1	27.6
Size Groups	Juveniles x Young Adults	15.7	42.8
	Juveniles x Adults	33.4	36.9
	Young Adults x Juveniles	7.5	24.2
	Young Adults x Adults	16.2	37.2
	Adults x Juveniles	35.7	40.6
	Adults x Young Adults	36.3	72.2

 Table 4. Estimates of isotopic niche overlap for skipjack by area and ontogenetic group according to kernel utilization densities at 50% and 75% contours. Overlaps are percentage of the first group area over the second.



**Fig. 1. Seasonal distribution for skipjack tuna in the Southwest Atlantic Ocean.** Size classes are denoted by symbol size: juvenile (JUV), young adult (YAD), and adult (AD). Seasons are indicated by symbol shapes and colors: summer (red, Jan–Mar); fall (blue, Apr–Jun); spring (orange, Oct–Dec). No fishing activities occur during the winter (Jul–Sep). The limit (dashed line) between northern and southern areas was defined in Santa Marta Cape (~28°S). Points from the same sampling event are shown as a square grid around the central catch position.



Fig. 2. Monthly variation in mean  $\delta^{13}C$  and  $\delta^{15}N$  values of skipjack in the northern and southern areas of the Southwest Atlantic Ocean during 2017 and 2018 fishing seasons. Error bars represent SD. Asterisks denote statistical significance of monthly comparison on isotope values between areas. \*\*\* p < 0.001, \*\* p < 0.05 (see Table A3 for more details).



**Fig. 3. Posterior mixing models estimates for skipjack diet composition.** Contribution of prey sources to skipjack diet in the northern (top plots) and southern (bottom plots) areas of Southwest Atlantic Ocean estimated with mixing models using uninformative (SIMM-UP) and informative (SIMM-IP) priors. Table 3 reports medians and 95% credible intervals for each prey type: krill (KRL), cephalopods and fish (CEPH+FISH), lanternfish (LNT), and small pelagic fish (SPF).



Fig. 4. Ontogenetic shifts in prey proportion on skipjack diet estimated by mixing models with uninformative (UP) and informative (IP) priors. The proportion of krill and lanternfish decreased, while the contribution from cephalopods and fish and small pelagic fish increased with skipjack size, respectively. Colors denotes size groups: JUV (juvenile); YAD (young adult); AD (adult).



Fig. 5.  $\delta^{13}$ C and  $\delta^{15}$ N isotopic niche widths for skipjack tuna (Katsuwonus pelamis) in the Southwest Atlantic Ocean. Bi-plots for the (a) northern and southern areas, and (b) ontogenetic groups are shown. Kernel utilization density and niche width (insets) were generated for 50% (darker colors), and 75% (lighter colors) contour levels.

# Material suplementar Apêndice 1

Table A1. Number and frequency of occurrence (FO), and mass of food items found on skipjack tuna (*Katsuwonus pelamis*) stomach content analysis between December 2016 and December-2018 in the northern and southern areas of Southwest Atlantic Ocean. Prey categories not included in the mixing models are indicated by n/a.

			North				South				all			
			FO		Total bio	omass	FO		Total bio	mass	FO		Total bio	mass
Order	Prey category	Species	n	%	g	%	n	%	g	%	n	%	g	%
Clupeiformes	small pelagic fish	Clupeidae NI	61	15	2,227	17		0		0	61	8	2,227	8
	small pelagic fish	Engraulidae NI	5	1	57	0		0		0	5	1	57	0
	small pelagic fish	Engraulis anchoita	5	1	44	0	57	14	915	5	62	8	959	3
	n/a	Harengula clupeola	2	0	59	0		0		0	2	0	59	0
	small pelagic fish	Sardinella brasiliensis	74	18	3,467	27	10	3	167	1	84	10	3,634	12
Decapoda	n/a	Crab NI	7	2	24	0		0		0	7	1	24	0
	n/a	Decapoda		0		0	2	1	4	0	2	0	4	0
	n/a	Dendrobranchiata	4	1	2	0		0		0	4	0	2	0
Euphausiacea	krill	Euphausia similis	9	2	796	6	166	42	11,905	70	175	22	12,701	43
			2	0	3	0		0		0	2	0	3	0
Isopoda	n/a	Isopoda NI	2	0	3	0		0		0	2	0	3	0
Octopoda	n/a	Argonauta nodosa	2	0	17	0	5	1	104	1	7	1	121	0
	n/a	Octopoda		0		0	1	0	1	0	1	0	1	0
Oegopsida	cephalopods	Illex argentinus		0		0	2	1	3	0	2	0	3	0
	cephalopods	Ommastrephidae		0		0	7	2	158	1	7	1	158	1
	cephalopods	Ornithoteuthis antillarum		0		0	3	1	72	0	3	0	72	0
	cephalopods + fish	Teuthoidea	10	2	120	1		0		0	10	1	120	0
Perciformes	cephalopods + fish	Carangidae	4	1	286	2		0		0	4	0	286	1
	cephalopods + fish	Selar crumenophthalmus	8	2	980	8		0		0	8	1	980	3
	cephalopods + fish	Trachurus lathami		0		0	1	0	5	0	1	0	5	0

Table A1 Continued			North		South				all					
			FO		Total biomass		FO		Total biomass		FO		Total bi	omass
Order	Prey category	Species	n	%	g	%	n	%	g	%	n	%	g	
Scorpaeniformes	n/a	Dactylopterus volitans	19	5	237	2	2	1	125	1	21	3	362	1
Stomiiformes	lanternfish	Maurolicus stehmanni	3	1	30	0	19	5	1,092	6	22	3	1,122	4
Tetraodontiformes	n/a	Tetraodontiformes	1	0	0	0		0		0	1	0	0	0
Thecosomata	n/a	Cavolinia sp.	19	5	12	0		0		0	19	2	12	0
	n/a	Cavolinia uncinata	1	0	1	0		0		0	1	0	1	0
	n/a	Cavoliniidae	2	0	4	0	1	0	2	0	3	0	6	0
	n/a	Diacavolinia atlantica	1	0	2	0		0		0	1	0	2	0
	n/a	Diacavolinia bicornis	1	0	1	0		0		0	1	0	1	0
	n/a	Diacavolinia elegans	1	0	3	0		0		0	1	0	3	0
	n/a	Diacavolinia sp.	1	0	1	0		0		0	1	0	1	0
Other	n/a	Crustacea NI	1	0	1	0		0		0	1	0	1	0
	n/a	Gastropoda	1	0	1	0		0		0	1	0	1	0
	n/a	Fish larvae	3	1	52	0		0		0	3	0	52	0
	n/a	Organism NI	8	2	75	1	12	3	294	2	20	2	370	1
	n/a	Egg NI	1	0	0	0		0		0	1	0	0	0
	n/a	Parasite	41	10	11	0		0		0	41	5	11	0
	n/a	Plastic	3	1	15	0	2	1	10	0	5	1	25	0
	n/a	Other	1	0	2	0		0		0	1	0	2	0
	n/a	Unidentified fish	112	27	4,213	33	106	27	2,037	12	218	27	6,251	21
	n/a	Zooplankton	1	0	1	0		0		0	1	0	1	0
		Total	414	100	12,745	100	396	100	16,894	100	810	100	29,640	100
		Total in 4 prey groups	184	44	8,066	63	264	67	14,249	84	448	55	22,316	75





 $\begin{array}{l} \mbox{Regression between $\delta^{13}$C$ values and straight fork length (a), and between latitude and $\delta^{15}$N$ values for skipjack tuna and krill in the Southwestern Atlantic (b). Equations describing these lines are: $\delta^{13}$C$ = $-18.64$ (\pm0.13) + SFL x 0.02$ (\pm0.002); $\delta^{15}$N_{skipjack}$ = $0.28$ (\pm0.01)*Latitude + $2.8$ (\pm0.45) (p < 0.001); $\delta^{15}$N_{krill}$ = $0.31$ (\pm0.04)*Latitude - $4.8$ (\pm1.45) (p < 0.001). The slopes in b are not different (F = $0.18$, p-value = $0.66$). } \end{array}$ 



## Fig. A2.

Simulated mixing polygons for skipjack tuna (*Katsuwonus pelamis*) from northern and southern areas of Southwestern Atlantic Ocean. Trophic discrimination factors (TDF) for *Thunnus orientalis* ( $\Delta^{13}$ C 1.8 ‰ ± 0.4;  $\Delta^{15}$ N = 1.9 ‰ ± 0.4, Madigan et al. 2012), for adult ( $\Delta^{13}$ C -0.16 ± 0.6‰;  $\Delta^{15}$ N = 1.6 ± 0.2‰, Varela et al. 2011) and juvenile *Thunnus thynuus* ( $\Delta^{13}$ C 0.32 ± 0.04‰;  $\Delta^{15}$ N = 1.46 ± 0.06‰, Varela et al. 2012) were tested to correct the offset between skipjack (open circles) and prey sources (white triangles). Colors denotes the probabilities contours (black lines) at every 10% level. TDF from Madigan et al. (2012) were applied in the mixing models in this study.

## Table A2

Mann-Whitney rank sum test comparing  $\delta^{13}$ C and  $\delta^{15}$ N values between skipjack tuna (*Katsuwonus pelamis*) size classes (JUV juveniles, YAD young adults, AD adults) from each area (north and south) of the Southwest Atlantic Ocean. Sample sizes for each group are shown in Table 2. Significant p-values are in bold (p < 0.05).

	Mann-Whitney rank sum test										
		$\delta^{13}$ C	•	$\delta^{15} \mathrm{N}$							
Group co	mparison	U	p-value	U	p-value						
JUV-North	YAD-North	8065	< 0.001	7278	< 0.001						
JUV-North	AD-South	356	0.187	422	0.018						
JUV-South	JUV-North	1194	0.178	136	< 0.001						
JUV-North	YAD-South	5996	< 0.001	8202	< 0.001						
JUV-North	AD-North	2270	< 0.001	1693	0.020						
YAD-North	AD-North	2547	0.172	1631	0.019						
JUV-South	YAD-North	2468	< 0.001	968	0.001						
YAD-South	YAD-North	8351	0.012	1815	< 0.001						
YAD-North	AD-South	288	0.104	522	0.516						
JUV-South	AD-North	695	< 0.001	93	<0.001						
AD-South	AD-North	181	0.031	82	0.211						
JUV-South	YAD-South	1889	0.006	2178	< 0.001						
JUV-South	AD-South	119	0.232	93	< 0.001						
YAD-South	AD-North	2468	0.003	73	0.450						
YAD-South	AD-South	310	0.430	228	0.080						

areas or s																
												Mann-Whitney rank sum test				
		Sample	size	North		South				$\delta^{13}$ C		$\delta^{15}$ N				
Year	Month	North	South	$\delta^{13}$ C (±	SD)	$\delta^{15}$ N (:	± SD)	$\delta^{13}$ C (±	ESD)	$\delta^{15}$ N (:	± SD)	U	p - value	U	p - value	
2017	Jan	11	8	17.8	0.3	9.9	0.7	17.7	0.3	12.1	1.1	39	0.717	2	<0.001	
	Feb		15					17.4	0.3	12.7	0.8					
	Mar	84	9	17.5	0.5	9.0	0.8	17.1	0.3	12.4	0.8	175	0.009	0	<0.001	
	Apr	15		17.0	0.2	9.5	1.0									
	May	37	2	17.6	0.4	10.2	1.6	17.0	0.2	12.2	1.6	10	0.097	12	0.132	
	Jun	9		17.3	0.3	10.0	0.7									
	Nov	38	18	17.9	0.4	9.6	1.8	17.9	0.5	12.0	1.3	327	0.801	95	<0.001	
	Dec	12	15	17.8	0.5	10.4	1.7	18.1	0.5	11.7	1.3	224	0.518	86	0.003	
2018	Jan	10	10	17.8	0.2	9.2	0.6	17.7	0.6	12.3	1.1	48	0.912	0	<0.001	
	Feb		12					17.7	0.4	11.6	1.4					
	Mar	5	16	17.7	0.4	9.6	0.7	17.7	0.4	11.7	1.3	42	0.905	4	0.001	
	Apr	11	3	17.6	0.3	11.0	2.6	17.4	0.3	13.4	0.7	13	0.659	7	0.170	
	May	10	15	17.6	0.3	12.3	1.1	17.5	0.4	12.2	1.9	54	0.261	74	0.978	
	Dec		18					17.8	0.3	12.5	1.2					

Sample size, mean ( $\pm$  SD)  $\delta^{13}$ C and  $\delta^{15}$ N values for skipjack tuna (*Katsuwonus pelamis*) used in the comparison between the southern and northern areas of Southwest Atlantic Ocean. Significant p-values are in bold (p < 0.05).

# **APÊNDICE 2**

Segundo manuscrito da tese, submetido à Marine Ecology Progress Series no dia 30/07/2021.

Multi-proxy approach for studying the foraging habitat and trophic position of a migratory marine consumer in the southwestern Atlantic Ocean

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### Abstract

Skipjack tuna (Katsuwonus pelamis) sustain the largest catches among tuna species. Despite its relevance for global food security and tuna canning industry, information about this species foraging ecology in the southwestern Atlantic Ocean (SWAO) is limited. We combined amino acid (AA) and bulk tissue nitrogen isotope ( $\delta^{15}N$ ) with stomach content analysis (SCA) to study skipjack foraging habitat and trophic position (TP) in the SWAO. Mean ( $\pm$  SD)  $\delta^{15}$ N values of source AAs lysine (Lys: 6.5  $\pm$  1.3‰) and phenylalanine (Phe:  $10.6 \pm 1.3\%$ ) were higher at higher latitudes (30–34°S) of the southern area relative to the northern area between 20–26°S (Lys:  $3.4 \pm 1.1\%$ , Phe:  $8.2 \pm 1.2\%$ ). Correlations between bulk tissue  $\delta^{15}N(\delta^{15}N_{bulk})$  and source AA  $\delta^{15}N$  show that Lys is a more robust indicator of  $\delta^{15}$ N baseline variation in this region than Phe. The southern area sustains a high abundance of forage that is used by smaller skipjack to fuel growth.  $\delta^{15}N_{Lvs}$  and  $\delta^{15}N_{Phe}$  decreased with skipjack size, indicating that adults forage at lower latitudes or in offshore areas. Estimates of TP based on SCA ( $TP_{sca}$ ) indicated high consumption of krill in the southern area ( $TP_{sca}$ ) 3.6) and consumption of teleosts in the northern area ( $TP_{sca} = 3.8$ ). TP estimates based on the trophic (glutamic acid, Glx)-source AAs pair Glx-Lys were in much better agreement with TP<sub>sca</sub> or estimates of TP based on bulk tissue analysis (TP<sub>bulk</sub>) than those based on Glx-Phe. Our results indicate that skipjack is a tertiary consumer that forages across distinct food webs throughout its lifecycle in the SWAO and whose TP increases with ontogeny (TP<sub>Glx-Lys</sub>: 3.5– 4.0).

**Keywords**: foraging ecology, movement, AA-CSIA, stable isotopes, nitrogen, amino acids, skipjack tuna, *Katsuwonus pelamis* 

#### INTRODUCTION

Skipjack tuna (*Katsuwonus pelamis*, Scombridae) sustains the largest commercial catches among tuna species, ranking third among marine fish resources harvested by global fisheries (FAO 2020). The species forms large schools in tropical and subtropical areas of the Atlantic, Indian, and Pacific Oceans (Colette & Nauen 1983). Life-history characteristics such as rapid growth, early age-at-maturity, opportunistic spawning, and short lifespan justify its high exploitation capacity (Murua et al. 2017). Management strategies of migratory marine resources requires knowledge of movement dynamics and trophic relationships (King 2007). Despite its global distribution and great economic relevance, skipjack movement patterns are poorly resolved due to its lower monetary value and relatively small size in comparison to other tunas, which, for example, limits the use of satellite tags to study skipjack migration and stock structure. In addition, tagging does not resolve foraging habits, necessitating the use of alternative and complementary techniques like stomach content analysis (SCA) and stable isotope analysis (SIA) to study ontogenetic patterns in foraging habitat and trophic position (TP) of top predators in pelagic food webs (Popp et al. 2007, Lorrain et al. 2015, Pethybridge et al. 2018, Le-Alvarado et al. 2021, Troina et al. 2021).

TP is an integrative measure of the role of consumers in a food chain that is useful for quantifying changes in the structure of marine food webs (e.g. Pauly et al. 1998, Librelato et al. 2014, Shannon et al. 2014, Pethybridge et al. 2018). Nitrogen isotope ( $\delta^{15}$ N) analysis is often used to estimate TP and food chain length due to systematic increases ~2–4‰ in  $\delta^{15}$ N values between consumers and their prey (DeNiro & Epstein 1981, Minagawa & Wada 1984, Peterson & Fry 1987, Post 2002). An often complicating factor in interpreting nitrogen isotope data of top consumers is that  $\delta^{15}$ N values of primary producers at the base of food webs can vary spatially as a function of taxonomy, inorganic nitrogen source (e.g. N<sub>2</sub>, nitrate, ammonia), and the efficiency with which nitrogen sources are utilized by primary producers (Sigman et al. 2009, Graham et al. 2010, McMahon et al. 2013, Trueman & Glew 2019). Thus, interpreting bulk tissue  $\delta^{15}$ N values ( $\delta^{15}$ N<sub>bulk</sub>) is complicated by the potential effects of changes in TP and variations in the isotope values of primary producers that fuel pelagic marine food webs (Graham et al. 2010, McMahon & Newsome 2019). Compound-specific stable isotope analysis (CSIA) of individual amino acids (AA) allows for the separation of the effects due to variation in baseline  $\delta^{15}$ N values from those related to changes in consumer TP (Popp et al. 2007). Trophic AA (e.g. glutamic acid, proline) undergo significant <sup>15</sup>N-enrichment during trophic transfer, while source AAs (e.g. phenylalanine, lysine) experience minimal isotopic alteration as they are passed from prey to consumer (McClelland & Montoya 2002, Popp et al. 2007, McMahon & Newsome 2019). Therefore, the primary advantage of CSIA is that the  $\delta^{15}$ N values at the base of the food web and the TP of the consumer can be estimated via analysis of a single consumer tissue sample. This method can be particularly useful for studying the trophic ecology of migratory tunas, that may shift their diet (i.e. TP) and/or foraging areas with ontogeny (e.g. Popp et al. 2007, Graham et al. 2010, Madigan et al. 2014, 2016, Le-Alvarado et al. 2021).

Phenylalanine (Phe) has been the preferred source AA and proxy for baseline  $\delta^{15}N$ values (Lorrain et al. 2015, Le-Alvarado et al. 2021). For example,  $\delta^{15}N_{phe}$  values of vellowfin tuna (*Thunnus albacares*) muscle were correlated with  $\delta^{15}N$  values in primary producers (i.e. particulate organic matter) and primary consumers (e.g. copepods, barnacles) in the Indian and Pacific Oceans (Popp et al. 2007, Lorrain et al. 2015) and the Gulf of Mexico (Le-Alvarado et al. 2021). However, TP estimates using Phe as the source AA were unrealistic based on stomach content data for common dolphin Delphinus delphis (Ruiz-Cooley et al. 2021), and a full trophic level lower than expected for yellowfin tuna (Bradley et al. 2015, Lorrain et al. 2015). In addition, controlled feeding experiments show that Phe may undergo substantial nitrogen isotope fractionation during trophic transfer (Bradley et al. 2014, McMahon & McCarthy 2016), and can also be influenced by nitrogen balance associated with fasting or nutritional stress (Lübcker et al. 2020, O. Shipley unpubl. data). For example, a feeding experiment on captive Pacific bluefin tuna (Thunnus orientalis) by Bradley et al. (2014) reported significant consumer-diet  $\delta^{15}N$  offsets for Phe (1.5 ± 0.3‰), but negligible offset for Lysine (Lys:  $-0.3 \pm 0.4\%$ ). This suggests that Lys may be a promising, but underutilized source-AA for studying  $\delta^{15}$ N baseline patterns and the trophic ecology of tuna and other top marine consumers (Bradley et al. 2014, McMahon & Newsome 2019).

The distinct oceanographic regimes between the northern (20–28°S) and southern (28–34°S) areas of skipjack feeding grounds in the southwestern Atlantic Ocean (SWAO) result in spatial patterns in the  $\delta^{15}$ N values of zooplankton near the base of the food web (Troina et al. 2020a). Lower baseline  $\delta^{15}$ N values in the northern area are likely the result of a substantial contribution of a <sup>15</sup>N-depleted inorganic nitrogen source in the tropical oligotrophic waters of the Brazil Current, in which diazotrophic cyanobacteria (e.g. *Trichodesmium*) perform N<sub>2</sub> fixation with characteristically low  $\delta^{15}$ N values (Sigman et al. 2009, McMahon et al. 2013, Troina et al. 2020a). In the southern area, higher  $\delta^{15}$ N values result from a significant input of <sup>15</sup>N-enriched nitrogen sources (e.g. NO<sub>3</sub><sup>-</sup>) derived from freshwater discharge (e.g. Río de La Plata), and/or the cold nutrient-rich subantarctic waters of the Malvinas Current (Garcia 1997, Matsuura & Andrade 2000, Möller et al. 2008, McMahon et al. 2013, Troina et al. 2020a).

Fishery and tag-recapture data indicate that skipjack distribution in the SWAO is influenced by seasonal changes in sea surface temperature. During the austral spring-summer (September–February), skipjack schools move southwards following the warm waters of the Brazil Current (BC), making the southern area the most productive skipjack fishing grounds during this period. During the austral fall-winter (March–August) the Malvinas Current advances from the south and schools return northwards as the BC retracts to lower latitudes. Accordingly, the major skipjack fishing grounds are located in the northern area during the austral fall-winter (Castello & Habiaga 1989, Andrade & Garcia 1999, Lima et al. 2000,Matsuura & Andrade 2000, Andrade 2003, Luckhurst 2014, Coletto et al. 2019).

Higher primary productivity in the southern area sustains large biomasses of forage fish, including the Argentinean anchovy (*Engraulis anchoita*) (Madureira et al. 2009, Costa et al. 2016), and the lanternfish (*Maurolicus stehmanni*) (Madureira et al. 2005). These species are key components of pelagic nektonic food webs in the SWAO (Velasco & Castello 2005, Gasalla et al. 2007), providing the forage base for several predators, including seabirds (Bugoni et al. 2010, Marques et al. 2018, Costa et al. 2020a), marine mammals (Troina et al. 2020b), and large pelagic fish like tunas (Castello et al. 1997). Anchovy and lanternfish together with euphausiid crustaceans (*Euphausia similis*) are the key food items for skipjack in the SWAO (Zavala-Camim 1982, Ankenbrandt 1985, Vilela 1990, Castello et al. 1997,

Coletto et al. 2020, 2021). SCA indicates that skipjack is a tertiary consumer with a TP of 3.5 in this region (Vilela 1990, Gasalla et al. 2007). SCA and mixing models of bulk tissue carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) isotope data suggest a positive relationship between skipjack size and TP (Ankenbrandt 1985, Coletto et al. 2021), but ontogenetic patterns in skipjack TP have not been thoroughly explored to date. Conversely, adult skipjack had lower  $\delta^{15}$ N<sub>bulk</sub> values than those of young adults, suggesting that adults have lower TP or forage in areas with lower baselines  $\delta^{15}$ N values (Troina et al. 2020a). These results indicate that additional proxies, like AA  $\delta^{15}$ N analysis are needed to better understand ontogenetic patterns in the foraging habitat and TP of skipjack in the SWAO.

Here, we used a multi-proxy approach to study skipjack foraging habitat and trophic position in the SWAO. Specifically, we used (1) AA  $\delta^{15}N$  analysis to characterize ontogenetic shifts in skipjack foraging and movement patterns; (2) SCA along with bulk tissue and AA  $\delta^{15}N$  analysis to characterize spatial and ontogenetic variation in skipjack TP in the SWAO. Our findings on the seasonal movement and ecological role of skipjack contributes valuable information for fisheries management in the SWAO. In addition, our results have methodological implications for how amino acid  $\delta^{15}N$  data are used to evaluate spatial variation in baseline nitrogen isotope composition and trophic level in top marine consumers.

#### **MATERIALS & METHODS**

#### 2.1. Sampling

Skipjack muscle samples were collected at fishing fleet landings during the Bonito Project from 2016–2018 at Niterói and Rio Grande, Brazil (Fig. 1). Catch positions were obtained from logbooks and plotted using QGIS software (Version 3.4.9). Samples were classified by depth strata using QGIS as follows: inner shelf (<100m), outer shelf (100–200m), shelf break and slope (200–500m), and offshore (>500m). Specimens were weighed (g) and measured for straight fork length (SFL) to the nearest centimeter. Size groups were defined according to the SFL at first maturity ( $L_{50} = 46$  cm), and the SFL at which all

individuals are mature ( $L_{100} = 63$  cm) in the SWAO (Soares et al. 2019). Size groups were defined as juvenile (SFL < 47 cm), young adult (SFL 47–63 cm), and adult (SFL > 64 cm).

## **2.2.** Amino acid (AA) $\delta^{15}$ N analysis

For AA  $\delta^{15}$ N analysis at the University of New Mexico Center for Stable Isotopes, skipjack tuna white muscle tissue samples (n = 38) were selected according to location of capture (northern and southern areas) and SFL. Due to the low C:N ratios  $(3.2 \pm 0.1)$  observed in the bulk tissue dataset, we did not extract lipids from muscle tissues prior to AA  $\delta^{15}N$ analysis. Approximately 10 mg of muscle tissue was hydrolyzed to constituent amino acids in 1 mL of 6 N hydrochloric acid (HCL) at 110° C for 20 h; tubes were flushed with N<sub>2</sub> to prevent oxidation during hydrolysis. Acid hydrolysis converts glutamine into glutamic acid (hereafter referred to as Glx). Amino acids were then derivatized to N-trifluoroacetic acid isopropyl esters following established protocols (Silfer et al. 1991, Whiteman et al. 2019). Samples were derivatized in batches alongside an in-house AA reference material containing a mixture of 13 amino acids of known isotopic composition. Samples were injected by a Thermo Scientific TriPlus RSH Autosampler into a Thermo Scientific Trace 1310 Gas Chromatograph (GC) outfitted with a 60 m  $\times$  0.32 mm ID BPX5  $\times$  1.0 µm GC column (inlet temperature: 250°C). The GC oven ramped from 70°C to 300°C (70°C hold for 1 minute, increase to 120°C at a rate of 15°C per minute, increase to 195°C at a rate of 4°C per minute, increase to 235°C at a rate of 5°C per minute, increase to 300°C at a rate of 15°C per minutes, hold at 300°C for 8 minutes) and separated, gaseous derivatized AA were reduced into N2 at 1000°C in a Thermo Scientific IsoLink II combustion reactor. The N<sub>2</sub> gas was sent to a Thermo Scientific Delta V Plus IRMS via a Conflo IV for isotopic analysis. Samples were analyzed in duplicate or triplicate and bracketed by injections of the in-house AA reference material. Analytical precision, measured as the mean within-run standard deviation of glutamic acid/glutamine (Glx), lysine (Lys), and phenylalanine (Phe) was 0.3‰. All bulk tissue and AA  $\delta^{15}$ N data are reported in  $\delta$ -notation calibrated to the internationally accepted standard of atmospheric N<sub>2</sub> (AIR).

## 2.3. AA data analyses

Student *t-tests* were used to compare  $\delta^{15}N_{Glx}$  and  $\delta^{15}N_{Lys}$  values between areas (northern vs southern). Non-parametric Wilcoxon test were used to compare  $\delta^{15}N_{Phe}$  values

between areas. Pairwise Wilcoxon tests with Bonferroni adjusted p-values for multiple comparisons were used to compare  $\delta^{15}N_{Glx}$ ,  $\delta^{15}N_{Lys}$  and  $\delta^{15}N_{Phe}$  values among size classes (juvenile, young adult, adult) within areas. Linear models were used to evaluate the relationships between  $\delta^{15}N_{Glx}$  vs.  $\delta^{15}N_{Lys}$ , and  $\delta^{15}N_{Glx}$  vs.  $\delta^{15}N_{Phe}$ . Linear models were also used to evaluate the relationships between  $\delta^{15}N_{Lys}$ , and  $\delta^{15}N_{Glx}$  vs.  $\delta^{15}N_{Phe}$ . Linear models were also used to evaluate the relationships between  $\delta^{15}N_{bulk}$  vs.  $\delta^{15}N_{Lys}$  values, and  $\delta^{15}N_{bulk}$  vs.  $\delta^{15}N_{Phe}$  values.

### 2.4. Baseline mixing model

To determine the relative contribution of the northern and southern areas for skipjack size classes, we used Stable Isotope Mixing Models in R (SIMMR; Parnell 2019). We used  $\delta^{15}$ N of Lys and Phe for juveniles caught in late summer (March) as end-members for each area in the mixing model; northern  $\delta^{15}N_{Lys}$  = 3.5  $\pm$  0.1‰ (n = 3) and southern  $\delta^{15}N_{Lys}$  = 6.1  $\pm 0.8\%$  (n = 4); northern  $\delta^{15}N_{Phe} = 7.8 \pm 1.4\%$  (n = 3) and southern  $\delta^{15}N_{Phe} = 10.5 \pm 0.8\%$ (n = 4). This approach assumes that skipjack move seasonally between northern and southern areas in the SWAO and muscle  $\delta^{15}N_{Lys}$  and  $\delta^{15}N_{Phe}$  values integrate ~ 6–7 months of foraging history in fast growing tunas (Graham 2007, Murua et al. 2017). Results are reported as the median and 95% credible intervals for the proportion of each baseline reflected in skipjack size classes. To identify recent migrants that had anomalous (outlier)  $\delta^{15}N_{Lys}$  values within areas we used a two-end-member mixing model described by Post (2002) as follows:  $\alpha =$  $(\delta^{15}N_{Lys-cons} - \delta^{15}N_{Lys-South}) / (\delta^{15}N_{Lys-North} - \delta^{15}N_{Lys-South});$  where  $\alpha$  is the proportion of the northern baseline in consumer tissue;  $\delta^{15}N_{Lys-cons}$  are the measured  $\delta^{15}N_{Lys}$ values on each sample, and  $\delta^{15}N_{Lvs-North}$  and  $\delta^{15}N_{Lvs-South}$  are the baseline end-members for the northern and southern areas, respectively. Individuals that had a proportion greater than 75% of different baseline from the regional baseline they were captured were considered recent migrants.

## 2.5. Trophic position via AA $\delta^{15}$ N analysis

The TP for skipjack with AA  $\delta^{15}$ N data (n=38) were calculated using the equation: TP<sub>Tr-Sr</sub> = [( $\delta^{15}$ N<sub>Tr</sub> -  $\delta^{15}$ N<sub>Sr</sub> -  $\beta$ )/TDF<sub>AA</sub>]+ 1, where  $\delta^{15}$ N<sub>Tr</sub> and  $\delta^{15}$ N<sub>Sr</sub> are the  $\delta^{15}$ N values for trophic and source AAs,  $\beta$  is the difference between the  $\delta^{15}$ N values of the selected trophic and source AAs in primary producers, and *TDF<sub>AA</sub>* is the trophic discrimination factor representing the increase in the  $\delta^{15}$ N values of the trophic relative to the source AA per trophic level. We calculated two TP estimates: one using Glx-Lys  $\delta^{15}$ N values (TP<sub>Glx-Lys</sub>) and one using Glx-Phe  $\delta^{15}$ N values (TP<sub>Glx-Phe</sub>). We used mean (± SD)  $\beta_{Glx-Lys}$  (3.9 ± 0.5‰) and  $\beta_{Glx-Phe}$  (3.6 ± 0.5‰),  $TDF_{Glx-Lys}$  (5.2 ± 0.3‰;), and  $TDF_{Glx-Phe}$  (5.7 ± 0.3‰) values derived from a meta-analysis of marine teleosts (Bradley et al. 2015). Errors were propagated following equations available in Dale et al. (2011) and Sabadel et al. (2020), by combining the analytical uncertainty in AA  $\delta^{15}$ N values with the uncertainty in  $\beta_{Tr-Sr}$  and  $TDF_{Tr-Sr}$ . TP<sub>Glx-Lys</sub> and TP<sub>Glx-Phe</sub> were compared between areas (northern vs southern) using Student *t*-tests. Pairwise Wilcoxon tests with Bonferroni adjusted p-values for multiple comparisons were used to compare TP<sub>Glx-Lys</sub> and TP<sub>Glx-Phe</sub> among size classes (juvenile, young adult, adult) within areas.

#### **2.6.** Trophic position via stomach content analysis

We used a dataset (n = 740) collected during the Bonito Project for calculating trophic position based on stomach content analysis ( $TP_{sca}$ ). Food items were sorted and identified to the lowest taxon possible using identification keys for Crustacea, Mollusca, and Teleostei (e.g. Figueiredo & Menezes 1980, Gibbons et al. 1999, Haimovici et al. 2009). For each individual stomach, prey items from each category were weighed (g) and counted. The degrees of digestion were determined using criteria developed by Vaske et al. (2004). Bait species (*Sardinella brasiliensis, Engraulis anchoita*) showing initial stages of digestion (i.e. non-digested, starting digestion) were removed from analysis.

TP<sub>sca</sub> was calculated as: TP<sub>sca</sub> = 1 + ( $\sum_{i=1}^{n} P_i \times TP_i$ ), where *n* is the number of prey categories,  $P_i$  is the mass proportion of prey category *i*, and  $TP_i$  is the trophic position of prey category *i* (Cortés 1999). Twelve prey categories belonging to three taxonomic groups (i.e. Crustacea, Mollusca, Teleostei) were used to calculate TP<sub>sca</sub> for skipjack. TP's of prey categories reported in the literature, and their mass percentages are provided in Table S1. TP for the unidentified Teleostei was calculated through the weighted mean of mass of fish prey categories. The average between two independent TP estimates was used as euphausiids' *Euphausia pacifica* (TP = 2.5, Sogawa et al. 2017) and *Thysanopoda* spp. (TP = 2.3, Hannides et al. 2009).

# 2.7. Trophic position via bulk tissue $\delta^{15}$ N analysis

We estimated bulk tissue trophic position (TP<sub>bulk</sub>) with  $\delta^{15}$ N data for 383 skipjack published in Coletto et al. (2021). The Bayesian package tRophicPosition (Quezada-Romegialli et al. 2019) in R (R Core Team 2019) was used to estimate TP<sub>bulk</sub> by area (northern and southern) and size class (juvenile, young adult, adult) within area. This approach includes sources of variation for  $\delta^{15}$ N baseline estimates and consumer-diet trophic discrimination factors (TDF) to provide robust estimates of TP. We used the Onebaseline model to account for the difference in  $\delta^{15}$ N values in primary consumers and skipjack bulk tissue between areas in the SWAO (Troina et al. 2020a, Coletto et al. 2021). Published  $\delta^{15}N$ values for copepods sampled along the shelf break in the SWAO were used as isotopic baseline proxies (Troina et al. 2020a). Because the majority of adult skipjack (62%) sampled in the northern area were caught in offshore waters (i.e. depth >500 m, Fig. S1, S2), we used the average for copepods sampled in shelf break and offshore waters (Troina et al. 2020a) as an isotopic baseline to estimate adult TPs in this area. The baseline TP for copepods was set to 2.2 assuming a low degree of omnivory (i.e. 80% grazing, 20% predation), which is in the lower range of TP estimates for copepod species in the North Pacific (Hannides et al. 2009). Studies show that TDFs for  $\delta^{15}$ N can vary with form of nitrogen excretion, tissue type, environment, taxa, and diet quality (Post 2002, McCutchan et al. 2003, Vanderklift & Ponsard 2003, Caut et al. 2009). We selected published TDF values for muscle in marine ammoniotelic fish ( $\Delta^{15}$ N: 3.7 ± 1.9‰, n = 302, Table S2) and used the function *simulateTDF* to include TDF values and their variability in the models. Simulations were run with 4 chains and 20,000 adaptations.

## RESULTS

The overall range of straight fork length (SFL) for skipjack included in SCA was 36.5-83.0 cm (n = 740), 37.0-80.0 cm for bulk-tissue  $\delta^{15}$ N analysis (n = 383), and 39.0-80.0 cm for AA  $\delta^{15}$ N (n = 38). Sample sizes were consistent across areas and size classes, except for adult in the southern area that had a small sample size (Table 1).

## 3.1. Glx, Lys and Phe $\delta^{15}$ N values

Skipjack collected in the southern area had higher  $\delta^{15}N_{Glx}$  (*t-test*, *t* = -3.16, df = 32.4, p < 0.05),  $\delta^{15}N_{Lys}$  (*t-test*, *t* = -4.48, df = 31.7, p < 0.001), and  $\delta^{15}N_{Phe}$  (Wilcoxon test, W = 80, p < 0.05) values than in the northern area (Table 2). There was a significant positive correlation between skipjack muscle  $\delta^{15}N_{Glx}$  and  $\delta^{15}N_{Lys}$  values (Fig. 2a):  $\delta^{15}N_{Glx} = 0.56$  (± 0.1)\*  $\delta^{15}N_{Lys}$  -20.11 (± 0.5); ( $F_{1,36} = 32.59$ ; p < 0.001;  $R^2 = 0.46$ ); as well as between  $\delta^{15}N_{Glx}$  and  $\delta^{15}N_{Phe}$  values (Fig. 2b):  $\delta^{15}N_{Glx} = 0.40$  (± 0.1)\*  $\delta^{15}N_{Phe} - 19.13$  (± 1.3); ( $F_{1,36} = 9.68$ ; p < 0.05;  $R^2 = 0.19$ ). There was a stronger relationship between  $\delta^{15}N_{bulk}$  and  $\delta^{15}N_{Lys}$  values (Fig. 3a,  $F_{1,36} = 174.2$ , p < 0.001;  $R^2 = 0.30$ ).  $\delta^{15}N_{Glx}$ ,  $\delta^{15}N_{Lys}$  and  $\delta^{15}N_{Phe}$  values did not differ among size groups in either area (pairwise Wilcoxon test, p > 0.05) (Table 2). In the southern area,  $\delta^{15}N_{Lys}$  values increased sharply with skipjack size up to ~ 54 cm SFL, and then decreased in skipjack larger than ~ 55 cm SFL (Fig. 4a). A similar pattern was observed in skipjack collected from the northern area.  $\delta^{15}N_{Phe}$  values also increased with skipjack size up to ~ 60 cm SFL in both areas, and decreased in large skipjack in the northern area (Fig. 4b).

#### **3.2. Baseline mixing model**

The relative contribution of the regional isotopic baselines, as indicated by  $\delta^{15}N_{Lys}$ and  $\delta^{15}N_{Phe}$  values, was different among size classes (Table 3, Fig. 5). The median of the proportion of the southern baseline based on  $\delta^{15}N_{Lys}$  was higher in juveniles (73%, probability = 0.9) and young adults (88%, probability = 1), while adults had  $\delta^{15}N_{Lys}$  values indicating a strong influence of the northern area (89%, probability = 1). The median proportion of the southern baseline based on  $\delta^{15}N_{Phe}$  was higher in juveniles (75%, probability = 0.9) and young adults (78%, probability = 1), while adults had  $\delta^{15}N_{Phe}$  values indicating a greater importance of the northern area (62%, probability = 0.7).

Mixing models using  $\delta^{15}N_{Lys}$  values to quantify the relative proportion of baselines between areas for individual skipjack showed that three individuals caught in the northern area had high  $\delta^{15}N_{Lys}$  values, indicating that they had recently moved from the southern area. Likewise, two individuals sampled in the southern area had  $\delta^{15}N_{Lys}$  values indicative of recent migration from the northern area (Fig. S3).

### 3.3. TPsca, TPbulk, TPGlx-Lys and TPGlx-Phe

In the northern area, Teleostei was the most important taxonomic group identified in SCA, while euphausiids were the dominant prey consumed by skipjack in the southern area (Table S1). Mean ( $\pm$  SD) TP<sub>sca</sub> for all skipjack in the SWAO was  $3.7 \pm 0.2$ . TP<sub>sca</sub> was slightly but not significantly higher for skipjack caught in the northern (3.8) than the southern area (3.6) (Table 4). In the northern area, there were subtle shifts in proportions of prey types consumed among size classes. In the southern area, the importance of euphausiids decreased with ontogeny. TP<sub>sca</sub> increased slightly with skipjack size in the northern (3.8–3.9) in comparison to the southern area (3.5–3.9) (Table 4, Fig. 6).

TP<sub>bulk</sub> was similar between the northern (3.7) and southern (3.8) areas (Table 4). In the northern area, adults had higher TP<sub>bulk</sub> (4.0) in comparison to young adults (3.8, probability = 0.9) and juveniles (3.5; probability = 1) (Fig. 6). In the southern area, young adults had higher TP<sub>bulk</sub> (3.9) in comparison to juveniles (3.6; probability = 0.9) (Fig. 6). TP<sub>bulk</sub> estimates for adults in the southern area were similar to that of juveniles, and highly variable (Table 4).

TP<sub>Glx-Lys</sub> varied from 2.9–4.1 with a mean ( $\pm$  SD) of 3.7  $\pm$  0.3. The mean TP<sub>Glx-Lys</sub> for skipjack was similar between the northern (3.8  $\pm$  0.2) and southern areas (3.6  $\pm$  0.3) (*t-test*, t = 1.9, df = 35.4, p = 0.06). In the northern area, TP<sub>Glx-Lys</sub> was higher in adult skipjack (3.9  $\pm$  0.1) in comparison to juveniles (3.5  $\pm$  0.2) (pairwise Wilcoxon test, p < 0.05, Fig. 6). TP<sub>Glx-Lys</sub> were not different among size groups in the southern area (pairwise Wilcoxon test, p > 0.05).

TP<sub>Glx-Phe</sub> varied from 2.1–3.3 with a mean ( $\pm$  SD) of 2.7  $\pm$  0.3, and was similar between the northern (2.7  $\pm$  0.3) and southern areas (2.7  $\pm$  0.3) (*t-test*, t = 0.4, df = 35.3, p = 0.7). There were no differences in TP<sub>Glx-Phe</sub> among size classes in either the northern and southern areas (pairwise Wilcoxon test, p > 0.05, Fig. 6).

## DISCUSSION

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We applied multiple techniques to study the foraging habitat and TP of a widely distributed pelagic consumer that plays an important role in food security in the SWAO and worldwide. Our study shows that the SWAO skipjack population uses habitat with distinct  $\delta^{15}$ N baselines over its lifetime.  $\delta^{15}$ N analysis of the source AAs Lys and Phe demonstrates that previously observed variation in bulk tissue  $\delta^{15}$ N values is driven by latitudinal gradients in baseline  $\delta^{15}$ N. Regional baseline mixing models indicated that the productive southern area is used by juveniles and young adults to fuel growth. The decrease in  $\delta^{15}N_{Lvs}$  and  $\delta^{15}N_{Phe}$ values with skipjack size showed that adults forage in warmer, oligotrophic areas with lower  $\delta^{15}$ N baselines. By disentangling baseline and ontogenetic patterns in  $\delta^{15}$ N values with trophic (Glx) and source (Lys) amino acids, our results showed that skipjack is a tertiary consumer that increase its TP over ontogeny (TP<sub>Glx-Lys</sub>: 3.5–4.0). Lastly, TP estimates using Phe as the canonical source AA consistently underestimated TP across size classes, while estimates using Lys agreed with those based on SCA and bulk tissue  $\delta^{15}$ N. Our multi-proxy approach provides novel insights into the foraging habitat, movement dynamics, and the TP of skipjack in pelagic food webs of the SWAO, which will better inform ecosystem models and management strategies for the sustainable use of this fishery resource in this region.

## 4.1. Foraging habitats and movement dynamics

Skipjack captured in the southern area had higher  $\delta^{15}N_{Lys}$  and  $\delta^{15}N_{Phe}$  values than those captured in the northern area (Table 2). These results were consistent with fine-scale zooplankton (e.g. copepods, euphausiids)  $\delta^{15}N$  isoscapes characterized by a positive correlation between baselines  $\delta^{15}N$  values and latitude, and a decrease in baseline  $\delta^{15}N$ offshore in the SWAO (Troina et al. 2020a). Bulk tissue and AA  $\delta^{15}N$  values in cetaceans foraging along the SWAO showed similar trends mirroring  $\delta^{15}N$  baselines (Troina et al. 2020b, Troina et al. 2021). Coletto et al. (2021) reported a trend of increasing skipjack bulk muscle tissue  $\delta^{15}N$  values with increasing latitude. This result was likely driven by spatial variation in baseline  $\delta^{15}N_{bulk}$  and  $\delta^{15}N_{Lys}(R^2 = 0.82)$  or  $\delta^{15}N_{Phe}(R^2 = 0.30)$  values confirmed that most of the variation in skipjack  $\delta^{15}N$  values is explained by  $\delta^{15}N$  variation at the base of the food web in the SWAO (Fig. 3). Note that the goodness-of-fit between  $\delta^{15}N_{bulk}$  and  $δ^{15}N_{Lys}$  was much higher than  $δ^{15}N_{bulk}$  and  $δ^{15}N_{Phe}$ . This result indicate that Lys may be a more reliable proxy for baseline  $δ^{15}N$  values for skipjack, as Lys undergo less isotope fractionation between consumer-diet in tunas, in comparison to Phe (Bradley et al. 2014). Overall, these findings are consistent with studies on pelagic predators in other ocean basins. The latitudinal pattern in  $δ^{15}N$  values of yellowfin and swordfish (*Xiphias gladius*) was explained by spatial variation in  $δ^{15}N$  baselines propagating up the food chain in the western Indian Ocean (Ménard et al. 2007). Off eastern Australia, several top predators had  $δ^{15}N_{bulk}$ values that were consistent with a shift from lower baseline  $δ^{15}N$  values in the oligotrophic Coral Sea to higher values in nutrient-rich waters of the Tasman Sea (Revill et al. 2009). In the eastern Tropical Pacific, the large range in yellowfin tuna  $δ^{15}N_{bulk}$  values (11‰) was explained by spatial variation in  $δ^{15}N$  baselines (Lorrain et al. 2015). Similarly,  $δ^{15}N_{bulk}$ values in yellowfin and bigeye (*Thunnus obesus*) tunas were also related to nutrient dynamics in the western central Pacific Ocean, which induce important variability in the  $δ^{15}N$  values at the base of the food web (Popp et al. 2007, Graham et al. 2010, Olson et al. 2010).

In general, skipjack caught in either the southern or northern areas showed  $\delta^{15}N_{Lys}$ and  $\delta^{15}N_{Phe}$  values consistent with the foraging ground where they were sampled, suggesting they were in nitrogen isotopic equilibrium with the local baseline. However, some individuals appear as "outliers" (Fig. 2a, 2b) and showed a mismatch between their  $\delta^{15}N$  values and baseline  $\delta^{15}$ N values in their area of capture, which likely reflects recent long-distance movements between these two isotopically distinct areas. Specifically, two individuals caught in the southern area had  $\delta^{15}N_{Lys}$  nearly identical to the northern  $\delta^{15}N_{Lys}$  baseline (Fig. 2a, Fig. S3). One was an adult captured during summer, and the other a juvenile captured during spring. These individuals likely followed the known skipjack migration pattern, in which schools move southwards with seasonal shifts in the Brazil Current (BC) during spring and summer (Matsuura & Andrade 2000). Three additional individuals captured in the northern area had  $\delta^{15}N_{Lys}$  values 2.2–4.1‰ higher than mean  $\delta^{15}N_{Lys}$  values for this area (Fig. 2a, Fig. S3). Cold waters may act as thermal barriers for skipjack, inducing latitudinal movements to avoid waters with sea surface temperature (SST) lower than 18°C (Kiyofugi et al. 2019). Accordingly, these individuals were caught during the fall, when skipjack schools foraging in the southern area return to lower latitudes as the cold water of the Malvinas Current advances from the south and the BC retracts northwards (Castello & Habiaga 1989, Andrade & Garcia 1999, Andrade 2003, Coletto et al. 2019, Coletto et al. 2021). Our AA  $\delta^{15}$ N results thus corroborate previous studies that demonstrated skipjack move seasonally following SST dynamics in the SWAO (Castello & Habiaga 1989, Matsuura & Andrade 2000) based on spatio-temporal patterns in catch statistics (e.g. Andrade 2003, Coletto et al. 2019), tagging and recapture (e.g. Luckhurst 2014), and intrinsic isotopic markers in bulk tissues (i.e.  $\delta^{15}$ N<sub>bulk</sub>) (Coletto et al. 2021). Similarly, source AA  $\delta^{15}$ N analysis was used to distinguish recent Pacific bluefin tuna migrants from eastern Pacific Ocean residents (Madigan et al. 2014). Isotopically distinct ecoregions in the western Pacific Ocean were used to infer previous foraging areas of PBFT in Taiwan spawning grounds, with two outlier individuals being identified as potential transoceanic migrators (Madigan et al. 2016).

Our results also showed a decrease in  $\delta^{15}N_{Lvs}$  and  $\delta^{15}N_{Phe}$  values with increasing skipjack size, indicating that adults forage more often in the northern area, which is characterized by lower baselines  $\delta^{15}$ N. The spatial distribution of skipjack size-classes by depth (Fig. S1) showed that adults were caught more often in the northern area and further offshore in deeper waters, where zooplankton  $\delta^{15}$ N values were lower than in higher latitudes and at the continental shelf break (Troina et al. 2020a). Previous studies on this population confirm that adults are more frequently caught in the northern area relative to the southern area (e.g. Ankenbrandt 1985, Andrade & Kinas 2004, Soares et al. 2019, Costa et al. 2020b). The higher contribution of the northern isotopic baseline to adult skipjack  $\delta^{15}$ N values appears to be linked to foraging and reproduction in warm and permanently stratified tropical waters found in lower latitudes. Although spawning seems to be opportunistic (Cayré & Farrugio 1986, Castello & Habiaga 1989, Vilela & Castello 1993) and distributed throughout the year in areas with SST higher than 24°C in the western Atlantic, larval density is higher during the summer near the Abrolhos Bank and the North Brazil Current (Fig. 1; Jablonski et al. 1984, Matsuura 1986, Matsuura & Andrade 2000, Pinto et al. 2002, Katsuragawa et al. 2020).

In contrast to patterns for adults, mixing models indicated a higher contribution of the southern isotopic baseline to juveniles and young adults. Higher feeding frequency, reflected as an overall lower frequency of empty stomachs, was observed in the southern area for every size class, notably for juveniles (Coletto et al. 2020). Previous work also shows that skipjack

from the southern area have the highest growth rates, which supports the idea that this area is the primary foraging ground for juveniles and young adults (Costa et al. 2020b). Finally, this conclusion is also supported by the high abundance of forage found in the productive waters of the southern area, such as small pelagic fish like anchovy and lanternfish (Matsuura & Andrade 2000, Madureira et al. 2005, 2009) and krill (i.e. *Euphausia similis*) that collectively form the resource base for skipjack in the SWAO (Zavala-Camim 1982, Ankenbrandt 1985, Vilela 1990, Coletto et al. 2021).

#### 4.2. Skipjack foraging ecology and trophic position

Our multi-proxy estimates of skipjack TP show this species is a tertiary consumer in the pelagic food webs of the SWAO (overall TP range: 3.5–4.0) which is consistent with previous reports of skipjack TP derived from ecotrophic models in this region (TP = 3.5, Gasalla et al. 2007) and the central Pacific (TP = 3.9, Kitchell et al. 2002). In contrast, TP estimates reported here are lower than those based on bulk tissue  $\delta^{15}$ N analysis of a limited number of skipjack (n = 3) in the SWAO (TP<sub>bulk</sub> = 4.2 ± 0.4, Bugoni et al. 2010) and those based on AA  $\delta^{15}$ N analysis of skipjack in the North Pacific subtropical gyre (TP = 4.2 ± 0.4, Choy et al. 2015).

Considering all size classes, TP<sub>sca</sub> was slightly higher in the northern area (3.8) than in the southern area (3.6). Previous studies showed that nearly 75% of stomach contents by volume (V) in the northern area were lanternfish (*Maurolicus stehmanni*) and other teleosts (Ankenbrandt 1985), indicating that teleosts (TP range 2.8–3.0) are an important resource for skipjack in this area (Coletto et al. 2020). Lanternfish distribution is linked to the limit between the South Atlantic Central Water (SACW) and warm tropical waters (Madureira et al. 2005), indicating spatial overlap between skipjack and lanternfish in shelf break/slope habitats in the SWAO (Monteiro-Neto et al. 2020). Ecotrophic models pointed out lanternfish and anchovy are the main trophic links between basal and upper trophic levels in pelagic food webs in the SWAO (Velasco & Castello 2005, Gasalla et al. 2007). In contrast, lower TP<sub>sca</sub> estimates in the southern area are likely associated with heavy foraging activity on dense patches of krill, which occupy a lower TP than lanternfish. Andrade (2003) suggests that peaks of skipjack fishing activity in the southern area might be related to availability of *E. similis*, as krill is more abundant in summer over the shelf (Gorri 1995). This is supported
by our observations of dense skipjack feeding aggregations in neritic waters in the southern area, where the main prey found in stomach contents was krill (Coletto et al. 2020, Table S1).

In general, skipjack TP increased through ontogeny, which is likely driven by smaller sized individuals consuming krill and larger skipjack consuming teleosts (Ankenbrand 1985, Table S1). Our published isotope-based estimates of diet composition indicate a higher importance of krill for juveniles, while higher TP prey such as small pelagic fish, Carangidae, and Ommastrephidae increased in importance for adults (Coletto et al. 2021). These results agree with ontogenetic patterns in vertical habitat use for skipjack. Smaller-sized skipjack are epipelagic and forage above the thermocline, while adults can perform short dives into deeper, colder, and less oxygenated waters (Bernal et al. 2017, Monteiro-Neto et al. 2020). Increased diving capability enhances access to larger, deep-dwelling prey (Graham et al. 2007). For example, vertical movements of large skipjack tagged in the SWAO were strongly correlated with lanternfish nictemeral behavior (Madureira et al. 2005, Monteiro-Neto et al. 2020). Furthermore, global compilations show that deeper foraging tuna species have higher TP (Pethybridge et al. 2018). TP patterns may also result from differences in the assemblages of primary producers in highly productive versus oligotrophic waters. In the eastern tropical Pacific, an inshore-offshore increase in yellowfin TP occurred due to greater food chain length resulting from smaller phytoplankton dominating in oligotrophic waters, while larger primary producers generally dominate nutrient-rich regions (Olson et al. 2010), which may shorten food chains and by extension decrease the TP of top consumers.

Exceptions to the trend of increasing TP with ontogeny were estimates of  $TP_{sca}$  in the northern area and  $TP_{bulk}$  for adults in the southern area.  $TP_{sca}$  depends on the relative mass proportions of prey categories and their respective TP (Cortés 1999). The dominance of teleost prey with a narrow range in TP may have flattened ontogenetic  $TP_{sca}$  pattern in the northern area. Observed differences in  $TP_{sca}$  and  $TP_{bulk}$  were expected as SCA may identify food items ingested by skipjack only up to 12 h prior to capture (Magnuson 1969), while complete isotopic incorporation for tuna muscle may vary from 6 months in fast-growing juvenile yellowfin to 2 years in slow-growing adult Pacific bluefin tuna (Graham 2007, Madigan et al. 2012, Bradley et al. 2014). The lower and highly variable  $TP_{bulk}$  observed in

adults from the southern area diverged from  $TP_{Glx-Lys}$ , likely due the presence of individuals with low  $\delta^{15}N_{bulk}$  values in this group that were not in equilibrium with the southern  $\delta^{15}N$ baseline (Coletto et al. 2021).

TP<sub>Glx-Phe</sub> underestimated skipjack TP by nearly one trophic level relative to TP<sub>sca</sub>, TP<sub>bulk</sub> and TP<sub>Glx-Lys</sub>. A similar result was found for yellowfin tuna by Lorrain et al. (2015), who suggested that a TDF<sub>Glx-Phe</sub> for the pairing Glx-Phe of ~7.6‰ may be too large. Nuche-Pascual et al. (2021) indicated that TDF<sub>Glx-Phe</sub> varies with diet protein content and feeding regime in teleosts, and suggested the use of taxon-specific trophic-source TDF values. We used a lower TDF<sub>Glx-Phe</sub> of 5.7‰ to estimate TP<sub>Glx-Phe</sub> in skipjack based on a large compilation of marine teleosts (Bradley et al. 2015). If we assume estimates of  $\beta_{Glx-Phe}$  (3.6 ± 0.5‰) are robust for phytoplankton-fueled pelagic food webs in the SWAO, then TDF<sub>Glx-Phe</sub> must be even lower than 5.7‰. Similar to patterns in bulk tissue  $\delta^{15}$ N discrimination (Trueman et al. 2005), Glx may experience less nitrogen isotope fractionation in rapidly growing skipjack, or alternatively there could be significant trophic-relative fractionation of Phe, which previous work estimates might be as large as 1.5‰ in bluefin tuna (Bradley et al. 2014).

#### CONCLUSION

This study provides new information on skipjack foraging and movement ecology in the SWAO. The productive southern area supports a high abundance of forage species and large feeding aggregations of young skipjack. AA  $\delta^{15}N$  analysis allowed us to separate the effects of foraging across distinct isotopic baselines from changes in TP with ontogeny. The decrease in  $\delta^{15}N_{Lys}$  and  $\delta^{15}N_{Phe}$  values with increasing skipjack size indicate that fully mature individuals forage in lower latitudes and further offshore areas. We found that Lys  $\delta^{15}N$ values were a more faithful proxy for spatial variation in baseline  $\delta^{15}N$  than Phe  $\delta^{15}N$  values. We also found that TP estimates based on differences in  $\delta^{15}N$  values between Glx (trophic) and Lys (source) were more accurate than those derived from comparison of Glx and Phe. Stock management strategies should consider that the southern region supports dense juvenile aggregations, which use these areas seasonally to enhance growth. If non-selective fishing gear (e.g. purse seining) are used in the future due to economic pressure, monitoring will be critical to control the catch of a minimum size above the size of first-maturity. Finally, we encourage future studies applying bulk tissue and amino acid  $\delta^{15}$ N analysis to cover the entire latitudinal distribution of the species in the SWAO to provide enhanced insights of skipjack movement and stock connectivity in this region.

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## Tables

Table 1. Sample size (n) and mean ( $\pm$  SD) straight fork length (SFL, cm) for specimens used to estimate skipjack trophic position via stomach content analysis (TP<sub>SCA</sub>), bulk tissue  $\delta^{15}$ N (TP<sub>bulk</sub>), and amino acid (TP<sub>AA</sub>)  $\delta^{15}$ N analysis grouped by size class and area of capture in the southwestern Atlantic Ocean.

			TP <sub>SCA</sub>	]	ΓP <sub>bulk</sub>		$TP_{AA}$
Area	Size class	n	$SFL \pm SD$	n	$SFL \pm SD$	n	$SFL \pm SD$
Northern		355	$53.0\pm10.2$	242	$51.3\pm9.3$	18	$58.2 \pm 14.2$
	Juvenile	112	$42.8\pm2.8$	78	$41.8\pm2.1$	5	$40.9\pm3.0$
	Young adult	187	$53.7\pm4.6$	130	$52.5\pm4.6$	5	$53.7\pm5.3$
	Adult	56	$71.2\pm5.5$	34	$68.4\pm4.6$	8	$71.8\pm5.3$
Southern		385	$51.2\pm5.5$	141	$52.3\pm6.4$	20	$49.9\pm5.7$
	Juvenile	99	$45.0\pm1.9$	26	$43.9\pm2.3$	7	$44.7\pm1.7$
	Young adult	278	$53.0\pm4.0$	108	$53.4\pm4.4$	12	$51.7\pm3.9$
	Adult	8	$66.4 \pm 3.4$	7	$66.3\pm3.9$	1	64.0
	Total	740		383		38	

Table 2. Mean ( $\pm$  SD) and range of  $\delta^{15}$ N values for glutamic acid (Glx), lysine (Lys), and phenylalanine (Phe) of skipjack size classes for each area of the southwestern Atlantic Ocean. Corrected (corr) mean ( $\pm$  SD)  $\delta^{15}$ N values used in mixing models do not include outliers (n = 5) that had  $\delta^{15}$ N values indicative of recent migration from another area.

Area	Size class	n	$\delta^{15}N_{Glx}$ (‰)	$\delta^{15}N_{Glx\;corr}(\%)$	$\delta^{15}N_{Lys}(\rlap{m})$	$\delta^{15}N_{Lys\;corr}(\text{\%})$	$\delta^{15} \mathrm{N}_{\mathrm{Phe}}$ (%)	$\delta^{15}N_{Phe\ corr}(\%)$
Northern		18	$22.3 \pm 1.6  (20.1 - 25.7)$	$21.8 \pm 1.1$	$4.1\pm 1.8(1.6-8.2)$	$3.4 \pm 1.1$	$8.9\pm 1.9(6.7-12.8)$	$8.2\pm1.2$
	Juvenile	5	$22.0\pm2.3\;(20.1-25.6)$	$20.6\pm0.6$	$5.1\pm 2.3\;(3.4-8.2)$	$3.5\pm0.1$	$9.7\pm 2.9\ (6.7-12.8)$	$7.8 \pm 1.4$
	Young adult	5	$22.8 \pm 1.8 \; (20.9 - 25.7)$	$22.1\pm0.9$	$4.6 \pm 1.3 \ (3.2 - 6.3)$	$4.2 \pm 1.0$	$8.7\pm 1.8\ (6.9-11.3)$	$8.0 \pm 1.3$
	Adult	8	$22.2 \pm 1.0 \; (20.9 - 23.5)$	$22.2\pm1.0$	$3.0\pm 1.2\ (1.6-5.0)$	$3.0 \pm 1.2$	$8.5 \pm 1.2 \ (7.3 - 11.2)$	$8.5 \pm 1.2$
Southern		20	$23.8 \pm 1.2  (20.8 - 25.6)$	$23.9\pm1.2$	$6.3 \pm 1.3(3.9 - 8.4)$	$6.5\pm1.3$	$10.6 \pm 1.3  (8.6 - 13.0)$	$10.6 \pm 1.3$
	Juvenile	7	$23.2 \pm 1.7 \; (20.8 - 25.6)$	$23.5\pm1.7$	$5.8 \pm 1.1 \; (4.1 - 7.3)$	$6.1\pm0.8$	$10.3\pm0.8\;(9.1-11.2)$	$10.5\pm0.8$
	Young adult	12	$24.1 \pm 0.9 \; (22.5 - 23.6)$	$24.1\pm0.9$	$6.9\pm 1.2\ (4.5-8.4)$	$6.9 \pm 1.2$	$10.6 \pm 1.5 \; (8.6 - 13.0)$	$10.6 \pm 1.5$
	Adult	1	$23.6\pm0.2$	$23.6\pm0.2$	$3.9\pm0.3$	$3.9\pm0.3$	$11.4 \pm 0.4$	$11.4 \pm 0.4$

Table 3. Bayesian estimates (medians with 95 credible intervals in parentheses) for the contribution of the northern and southern regional baselines to skipjack tissue by size class, in the southwestern Atlantic Ocean. Mixing models included  $\delta^{15}$ N data for the source amino acids lysine (Lys) and phenylalanine (Phe).

		Juvenile	Young Adult	Adult
Lys	Northern	0.27 (0.05 - 0.65)	0.12 (0.02 – 0.37)	0.88 (0.55 - 0.98)
	Southern	0.73 (0.35 - 0.95)	0.88 (0.63 - 0.98)	0.12 (0.02 - 0.45)
Phe	Northern	0.25 (0.04 - 0.68)	0.22 (0.04 - 0.55)	0.62 (0.21 - 0.92)
	Southern	0.75 (0.32 - 0.96)	0.78 (0.45 - 0.96)	0.38 (0.08 - 0.79)

Table 4. Trophic position (TP) estimates for skipjack in the southwestern Atlantic Ocean grouped by area of capture, size class, and method of estimation. TP was estimated using stomach content analysis (TP<sub>sca</sub>); bulk tissue  $\delta^{15}$ N analysis (TP<sub>bulk</sub>); glutamic acid-lysine  $\delta^{15}$ N analysis (TP<sub>Glx-Lys</sub>), and glutamic acid-phenylalanine (TP<sub>Glx-Phe</sub>). Estimates for TP<sub>bulk</sub> are reported as median and 95 credible intervals, while estimates for TP<sub>Glx-Lys</sub> and TP<sub>Glx-Phe</sub> are reported as mean ±SD.

Area	Size Class	TP <sub>sca</sub>	$\mathrm{TP}_{\mathrm{bulk}}$	$TP_{Glx-Lys}$	$TP_{Glx-Phe}$
North		3.8	3.7 (3.3 – 4.0)	$3.8 \pm 0.2$	$2.7\pm0.3$
	Juvenile	3.8	3.5 (3.2 - 3.8)	$3.5\pm0.2$	$2.5\pm0.3$
	Young Adult	3.8	3.8 (3.5 – 4.2)	$3.7\pm0.2$	$2.9\pm0.3$
	Adult	3.9	4.0 (3.8 – 4.2)	$3.9\pm0.1$	$2.8\pm0.2$
South		3.6	3.8 (3.5 – 4.1)	$3.6\pm0.3$	$2.7\pm0.3$
	Juvenile	3.5	3.6 (3.3 – 3.9)	$3.6\pm0.3$	$2.6\pm0.4$
	Young Adult	3.6	3.9 (3.6 – 4.3)	$3.6\pm0.3$	$2.7\pm0.3$
	Adult	3.8	3.5 (2.8 – 4.2)	$4.0\pm0.2$	$2.5\pm0.2$

### **Figures**



Fig. 4. Distribution of skipjack tuna samples collected in the southwestern Atlantic Ocean. Symbols in the northern (red) and southern (green) areas indicate techniques used to estimate trophic position: stomach content analysis (SCA, triangle); bulk tissue  $\delta^{15}$ N analysis (bulk- $\delta^{15}$ N, medium circle); and amino acid nitrogen analysis (AA- $\delta^{15}$ N, large circle). Inset shows the Subtropical Convergence Area and major currents in the region: North Brazil Current (NBC), Brazil Current (BC), Malvinas Current (MC).



Fig. 5. Relationship between  $\delta^{15}$ N values for (A) glutamic acid (Glx) and lysine (Lys) values, and (B) Glx and phenylalanine (Phe). Circle size denote size class and symbols represent mean values for size classes for each area: juvenile (JUV, circle), young adult (YAD, triangle), adult (AD, square); error bars represent one standard deviation.



Fig. 6. Relationship between (A)  $\delta^{15}N_{bulk}$  and  $\delta^{15}N_{Lys}$  values, and (B)  $\delta^{15}N_{bulk}$  and  $\delta^{15}N_{Phe}$  values for skipjack in the southwestern Atlantic Ocean.



Fig. 7. Relationship between skipjack straight fork length and (A)  $\delta^{15}N_{Lys}$  and (B)  $\delta^{15}N_{Phe}$  values in the southwestern Atlantic Ocean.



Fig. 8. Estimated mean ( $\pm$  SD) contribution of the northern (red) and southern (green) regional nitrogen isotopic baselines to skipjack muscle tissue in the southwestern Atlantic Ocean, inferred from source amino acids (A) lysine, and (B) phenylalanine. JUV = juvenile; YAD = young adult; AD = adult. See Table 3 for credible intervals.



Fig. 9. Trophic position (TP) estimates for skipjack in the southwestern Atlantic Ocean by size class (JUV = juvenile; YAD = young adult; AD = adult), area (northern: upper panels; southern: lower panels), and method of estimation. TP was estimated using stomach content analysis ( $TP_{SCA}$ ), bulk tissue  $\delta^{15}N$  analysis( $TP_{bulk}$ ), glutamic acid–lysine  $\delta^{15}N$  analysis ( $TP_{Glx-Lys}$ ), and glutamic acid–phenylalanine  $\delta^{15}N$  analysis ( $TP_{Glx-Phe}$ ). Bayesian  $TP_{bulk}$  estimates (medians with 95% credible intervals) are shown in Table 2.

	Northern	l			Southe	ern					
Class/family	JUV	YAD	AD	Total	JUV	YAD	AD	Total	Grand Total	TP	Reference
	(112)	(187)	(56)	(355)	(99)	(278)	(8)	(385)	(740)		
Crustacea	15	13	0	8	75	71	59	71	43		
Euphausiidae	14	13	0	8	75	71	59	71	43	2.4	Sogawa et al. 2017, Hannides et al. 2009
Crustacean NI	1	0	0	0	0	0	0	0	0	2.1	Gasalla et al. 2007
Mollusca	5	1	0	1	0	1	41	2	2		
Argonautidae	0	0	0	0	0	0	24	1	0	3.2	Pauly & Christensen 1995
Cavoliniidae	0	0	0	0	0	0	0	0	0	2.0	Gilmer 1974
Gastropod NI	5	0	0	1	0	1	16	1	1	2.0	Gilmer 1974
Ommastrephidae	0	0	0	0	0	0	0	0	0	4.0	Gasalla et al. 2007
Teleostei	80	86	100	91	25	28	0	26	55		
Carangidae	0	3	20	10	0	0	0	0	4	3.0	Pauly & Christensen 1995
Clupeidae											Pauly & Christensen 1995, Schneider & Schwingel 1999,
	55	49	38	46	1	1	0	1	21	2.8	Yang et al. 2020
Dactylopteridae	1	4	0	2	0	1	0	1	1	3.7	Randall 1967, Froese & Pauly 2021
Engraulidae	1	1	0	1	6	6	0	6	3	3.0	Gasalla et al. 2007
Sternoptychidae	0	1	0	0	5	7	0	7	4	3.1	Gasalla et al. 2007
Teleostei NI	22	28	42	33	13	13	0	12	21	2.9	Average TP from fish families weighted by mass

# Material suplementar Apêndice 2

Table S2. Taxonomic list with prey mass percentage quantified in skipjack stomach content analysis (SCA) by size class and area. Trophic position (TP) values and references used to calculate  $TP_{sca}$ . JUV (juvenile); YAD (young adult); AD (adult). Sample sizes are shown in parenthesis.

Table S3. Studies used to derive nitrogen trophic discrimination factors (TDF, $\Delta^{15}$ N) for muscle
tissue in marine ammoniotelic fish. The mean TDF and standard deviation was used in bulk tissue $\delta^{15}N$
trophic position estimates. Standard deviation was obtained by subtracting the higher from the minimum
value divided by two.

Publication	Common name	Scientific name	$\Delta^{15}N$ (‰)	$\pm$ SD	n
Dempson & Power 2004	Atlantic salmon	Salmo salar	5.0	0.2	10
Gaston & Suthers 2004	Australian mado	Atypichthys strigatus	5.6	0.1	18
Madigan et al. 2012	Bluefin tuna	Thunnus orientalis	1.9	0.3	10
Miller 2000	Pacific herring	Clupea pallasi	4.5	-	32
Suzuki et al. 2005	Japanese bass	Lateolabrax japonicus	3.1	0.3	32
Sweeting et al. 2007	Sea bass	Dicentrarchus labrax	4.0	0.4	75
Sweeting et al. 2007	Sea bass	Dicentrarchus labrax	3.8	0.3	75
Trueman et al. 2005	Atlantic salmon	Salmo salar	2.7	0.3	25
Trueman et al. 2005	Atlantic salmon	Salmo salar	2.3	0.3	25
-			3.7	1.9	302



Fig. S10. Distribution of skipjack tuna samples used to estimate trophic position (TP) in the southwestern Atlantic Ocean. Size-classes are indicated in columns, methods used to estimate TP are indicated in rows. Stomach content analysis (SCA); bulk tissue  $\delta^{15}$ N analysis (bulk  $\delta^{15}$ N); and amino acid (AA) nitrogen analysis (AA  $\delta^{15}$ N). The Santa Marta Cape (SM) is indicated as the limit between the northern and southern areas. The 200m isobath indicative of shelf break position is plotted.



Fig. S2. Proportion of samples analyzed for SCA in relation to depth strata, by size class, for the (A) northern and (B) southern areas of the southwestern Atlantic Ocean.



Fig. S3. Relative contribution of the northern (white) and southern (black) regional  $\delta^{15}N_{Lys}$  baselines to individual skipjack tissue (n = 38). Asterisks indicate individuals that had a proportion greater than 75% of different baseline from the regional baseline they were captured. Area of sample collection is indicated on the top, size class is indicated on the bottom.

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