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**CARACTERIZAÇÃO DA COMUNIDADE E ANÁLISE DE NICHO ISOTÓPICO  
DE AVES MARINHAS (PROCELLARIIFORMES) ATRAVÉS DE ENCALHES  
NO SUDOESTE DO OCEANO ATLÂNTICO**

Florianópolis  
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**Caracterização da comunidade e análise de nicho isotópico de aves marinhas  
(Procellariiformes) através de encalhes no Sudoeste do Oceano Atlântico**

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

Prof. Dr. Paulo Simões Lopes  
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Certificamos que esta é a **versão original e final** do trabalho de conclusão que foi  
julgado adequado para obtenção do título de mestre em Ecologia.

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Coordenação do Programa de Pós Graduação

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Prof<sup>a</sup> Marta Jussara Cremer  
Orientador(a)

Florianópolis, 2022

Dedico este trabalho  
à minha família que  
sempre me apoiou e  
também a todas as  
mulheres, da terra e  
do mar, que seguem  
na luta pela ciência!

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Escrevendo esses agradecimentos, é impossível não pensar em todos altos e baixos dessa trajetória. Me pego pensando também, que temos a tendência de idealizar conquistas, resultados e o ponto de chegada. A questão é que esquecemos, muitas vezes, que esse ponto de chegada é apenas um ponto representado por um breve momento, uma apresentação, um título ou apenas um papel que afirma uma conquista. Mas no fim das contas, talvez a idealizada conquista seja mais sobre curtir o caminho, sobreviver e aprender com ele. Assim, o ponto de chegada cai do pedestal e vira consequência das rotas que traçamos na vida e que nos fizeram chegar ali. Fazendo uma analogia, diria que é ilusão achar que o crescimento obtido é linear, mesmo que algumas populações biológicas possam apresentar esse tipo de crescimento, outras também crescem com a dinâmica do caos. Eu, particularmente, acredito que uma certa dose de caos pode ser catalizadora de mudanças, ao mesmo tempo que não deve ser vista sob olhares românticos (voltarei a isso mais adiante). Assim, tenho aprendido a reconhecer e agradecer pela não linearidade da vida, aos lugares onde ela leva e a honrar todas as pessoas que fizeram parte dessa jornada (que por mais que seja individual, ela não se faz sozinha).

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Entretanto, assim como os filhotes de Procellariiformes que são contemplados pelo cuidado parental, como agradecer minha força para voar sem agradecer o solo firme que me deu impulso, além de todo cuidado e apoio que tive pra isso? Impossível! Por isso, deixo aqui, eternamente registrado, todo amor e gratidão pela minha família que sempre foi minha base para alçar voo, mesmo durante condições adversas. Mãe (Dolores), pai (Gilberto) e manos (Fred e Nick), amo vocês muito mais do que sou capaz de expressar em palavras, sejam elas escritas ou verbalizadas. Obrigada por todo apoio e incentivo que vocês me deram para poder estudar e chegar onde eu cheguei. Amo muito vocês!

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Desde toda tensão dos estudos e da prova até a nossa aprovação e todos os momentos que compartilhamos morando juntas. Obrigada pela tua alegria e por não desistir de mim. Bárbara, a pessoa que o universo presenteou como o terceiro elemento pra morar comigo e com a Bruna. Obrigada pelas prosas, pelas segundas-feiras de skin care e por ser forever minha parceira de forró e de fritar batata as 7h da manhã. Jô! Obrigada por todas nossas conversas profundas, pelos rangos maravilhosos que tu faz e por ter sido minha parceira no início de toda essa loucura de pandemia.

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“Levantou-se atrás de nós um vento sul de feição  
Seguiu-nos o Albatroz  
E depois dia trás dia, para comer ou para brincar  
Vinha ter com os marinheiros que o chamavam de alta voz.  
[...]  
Adeus, adeus Convidado  
Ainda te quero dizer  
Que apenas sabe rezar, aquele que sabe amar  
Tanto o homem e a ave, como qualquer outro ser.”

“*The Rime of the Ancient Mariner*” - Samuel Taylor Coleridge (1797~1799).

## RESUMO

A ordem Procellariiformes comprehende aves pelágicas que passam a maior parte de suas vidas em alto-mar, recorrendo à terra apenas para reprodução. Esse comportamento dificulta a obtenção de informações sobre a ecologia trófica dessas espécies e o uso de hábitat fora do período reprodutivo. Assim, o registro das carcaças de animais que chegam nas praias pode ser uma ferramenta útil para obter informações ecológicas sobre essas populações de difícil acesso. O presente estudo teve como objetivo geral analisar os registros de Procellariiformes encalhados no sul da Bacia de Santos, sudoeste do Oceano Atlântico, a fim de caracterizar a comunidade que ocorre ao largo da plataforma continental de Santa Catarina e regiões oceânicas adjacentes, além de investigar as relações tróficas e a partição de nicho isotópico nesta comunidade. A dissertação foi estruturada em dois capítulos. No primeiro, busquei caracterizar a comunidade de Procellariiformes através da análise de indivíduos encalhados ao longo do litoral de Santa Catarina. Os dados foram obtidos através do monitoramento realizado pelo Projeto de Monitoramento de Praias da Bacia de Santos no período de janeiro de 2016 a dezembro de 2019. O banco de dados foi explorado para listar a ocorrência de todas as espécies de Procellariiformes registradas e avaliar os seguintes parâmetros individuais: sexo, estágio de desenvolvimento, condição da carcaça, condição corporal e sinais externos de interação antrópica. Os dados também foram utilizados para calcular a frequência numérica e a frequência de ocorrência de cada espécie, ilustrar a distribuição espacial dos encalhes e analisar possíveis influências temporais (mês e estação) na ocorrência das quatro espécies mais abundantes através de modelos generalizados mistos. No total, foram registrados 3.247 indivíduos, representando pelo menos 19 espécies, sendo que destas, cinco estão classificadas com algum nível de ameaça de extinção. A espécie mais frequente foi *Puffinus puffinus*, seguida de *Thalassarche chlororhynchos*, *Procellaria aequinoctialis* e *T. melanophris*. A maioria das carcaças foi classificada em estágio moderado ou avançado de decomposição, o que pode ter influenciado a identificação de parâmetros individuais, como sexo e estágio de desenvolvimento. A maioria dos indivíduos de *Puffinus puffinus* estavam magros ou caquéticos, em relação a condição corporal. Todas as espécies avaliadas por necrópsia apresentaram algum sinal de interação antrópica, sendo que a presença externa de lixo e a interação com petrechos de pesca foram as mais observadas. Os resultados dos modelos indicaram a influência de um padrão mensal na ocorrência de *P. puffinus*, com maior número de registros entre setembro e dezembro, o que corresponde ao período em que a espécie costuma ser mais abundante em águas brasileiras. Os modelos não foram validados para as demais espécies, indicando ausência de um padrão temporal na ocorrência das mesmas. No segundo capítulo, utilizei valores de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$ , obtidos a partir de penas de indivíduos encalhados, para avaliar a potencial sobreposição ou partição no nicho isotópico entre diferentes espécies e entre machos e fêmeas. Oito espécies foram analisadas: *Ardenna gravis*, *Calonectris borealis*, *Macronectes giganteus*, *Oceanites oceanicus*, *P. aequinoctialis*, *P. puffinus*, *T. chlororhynchos* e *T. melanophris*. Os valores de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  foram comparados por espécie e entre machos e fêmeas. A largura do nicho isotópico de cada espécie e entre os sexos foi comparada através de elipses bivariadas. Os valores médios de  $\delta^{13}\text{C}$  diferiram significativamente entre *C. borealis* e *O. oceanicus*, sendo que os valores se mostraram mais enriquecidos para *C. borealis*, sugerindo que a espécie

consome presas mais costeiras. Ambas as espécies diferiram significativamente das demais, apresentando valores menores de  $\delta^{15}\text{N}$ , o que indica o consumo de presas de níveis tróficos inferiores. As demais espécies apresentaram maior sobreposição de nicho isotópico, indicando o consumo de presas de níveis tróficos semelhantes em áreas semelhantes durante o período não reprodutivo. Essa sobreposição pode estar relacionada ao consumo de descarte de pesca, uma vez que essas espécies são reconhecidas por acompanhar embarcações a fim de obter alimento. Não foram observadas diferenças significativas nas médias de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  entre machos e fêmeas, sugerindo que ambos os sexos consumiram recursos de mesmo nível trófico, em áreas semelhantes. Entretanto, os machos de *P. puffinus* e *T. chlororhynchos* apresentaram maior amplitude de nicho isotópico do que as respectivas fêmeas. Os resultados dessa dissertação ressaltam a importância do monitoramento sistemático de encalhes como ferramenta para acessar a ocorrência e a ecologia trófica de populações de aves pelágicas, de ampla distribuição e de interesse conservacionista. Também pontuamos a potencial influência da pesca, tanto em relação a captura accidental quanto a distribuição, forrageio e sobreposição de nicho isotópico entre espécies de Procellariiformes durante o período não reprodutivo.

**Palavras-chave:** Procellariiformes, distribuição, isótopos estáveis, ecologia trófica.



## ABSTRACT

Procellariiformes are pelagic birds that spend most of their lives at sea, coming ashore only for breeding. This behavior leads to a lack of information regarding the species trophic ecology and use of habitat outside the breeding period. Thus, monitoring stranded seabirds can be a useful tool to obtain ecological information about these populations of difficult access. The present study aimed to analyze Procellariiformes strand records in the southwest Atlantic Ocean in order to characterize the community inhabiting off the continental shelf of Santa Catarina and its adjacent oceanic regions. I also investigated the trophic relationship and the isotopic niche partitioning within the community. The study was divided in two chapters. In the first one, I aimed to characterize the Procellariiformes community through the analyze of stranding along the coast of Santa Catarina. Stranded data was obtained through systematic surveys performed by the Santos Basin Beach Monitoring Project, from January/2016 to December/2019. The data base was explored to list the occurrence of all species registered and to evaluate the following parameters: sex, age class, carcass condition, body condition and external signs of interactions with anthropic activities. The survey records were also used to estimate the numeric frequency and frequency of occurrence of each species, illustrate the spatial distribution of strandings and to analyze possible influence of temporal variables (month and season) in the occurrence of the most abundant species through generalized mixed models. A total of 3.247 individuals, belonging to 19 species, were recorded. Five species recorded are threatened of extinction. *Puffinus puffinus*, *Thalassarche chlororhynchos*, *Procellaria aequinoctialis* and *T. melanophris* were the most abundant species. The majority of carcasses were classified as moderated and advanced stages of decomposition, which can influence the identification of individual parameters. Regarding the body condition, the majority of *P. puffinus* was classified as skinny and cachectic, which may be related to the high energetic demand of performing transequatorial migrations. All species analyzed present some sort of sign of interaction with anthropic activities, being the most observed the external presence of garbage and interaction with fishing gears. The results suggest a monthly pattern in the occurrence of *P. puffinus*, with higher numbers of individuals registered between September and December, corresponding to the species non-breeding period. Statistic models were not validated for the remaining species, indicating the absence of a temporal pattern in their occurrence. In the second chapter, I used  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  of feathers from stranded individuals to evaluate the potential isotopic niche partitioning among Procellariiformes species and between males and females. The following species were analyzed: *Ardenna gravis*, *Calonectris borealis*, *Macronectes giganteus*, *Oceanites oceanicus*, *P. aequinoctialis*, *P. puffinus*, *T. chlororhynchos* and *T. melanophris*. The mean values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were compared between species and between males and females. The niche width of each species was estimated through bivariate ellipses. The values of  $\delta^{13}\text{C}$  differed greatly between *C. borealis* and *O. oceanicus*, being more enriched for *C. borealis*, suggesting that the species feed on preys inhabiting more inshore areas. *Calonectris borealis* and *O. oceanicus* presented low values of  $\delta^{15}\text{N}$ , suggesting the consumption of the lower trophic levels within the community, differing significatively from the remaining species. These species presented higher isotopic niche overlap, suggesting the use of similar areas and resources during the non-breeding period. This overlap may be a result of consumption

of fishing discards, since these species are known to follow fishery vessels in order to obtain food. Differences in  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  values between males and females were not significant, indicating that both classes consumed the same trophic level in similar areas. However, males of both *P. puffinus* and *T. chlororhynchos* had larger ellipses area than the respective females. The results pointed here highlight the importance of strand surveys as a tool to access the occurrence and the trophic ecology of pelagic seabirds with wide distribution and of conservation interest. We also highlight the potential influence of fishery activities in the species bycatch, distribution, diet and isotopic niche overlap during the non-breeding period.

**Key-words:** Procellariiformes, distribution, stable isotopes, trophic ecology.

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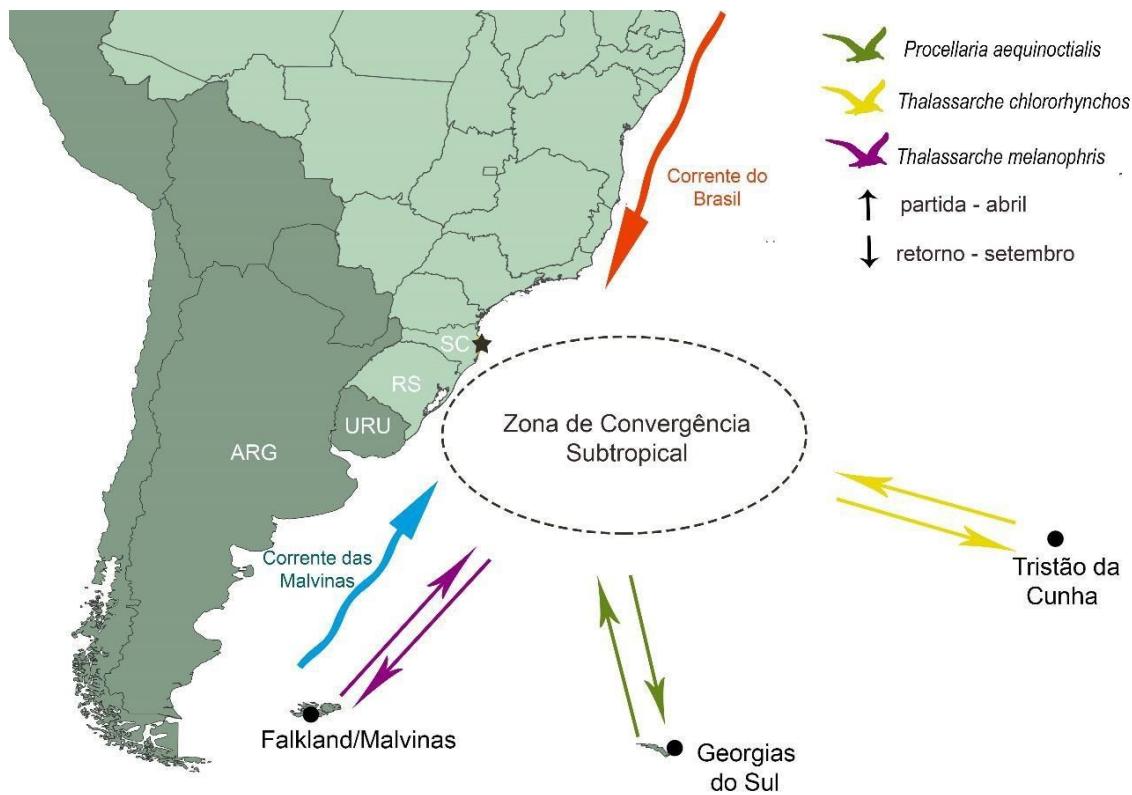
# **1 INTRODUÇÃO GERAL**

## **1.1 Os Procellariiformes**

A ordem Procellariiformes compreende aves pelágicas, como albatrozes e petréis, que passam a maior parte de suas vidas em alto-mar, recorrendo à terra apenas para reprodução (WARHAM 1990). São aves de grande longevidade, que atingem a maturidade sexual tardiamente e apresentam baixas taxas de reprodução, produzindo em geral um ovo por temporada reprodutiva (OLMOS et al. 2001). A característica diagnóstica do grupo é a presença de narinas tubulares, as quais estão associadas a um significativo desenvolvimento do sistema olfatório (PRINCE 1987). Estudos indicam que diversas espécies de Procellariiformes são capazes de identificar diferentes compostos aromáticos associados às suas presas primárias, podendo utilizar essas pistas olfativas para navegação e localização das áreas de alimentação (HUTCHISON & WENZEL 1980; NEVITT 1999; NEVITT & VEIT 1999).

Os Procellariiformes se distribuem amplamente pelos oceanos ao redor do mundo, apresentando maior riqueza e abundância nos oceanos do Hemisfério Sul (SCHREIBER & BURGUER 2002). No Brasil, há registros de 45 espécies de Procellariiformes (PACHECO et al. 2021), das quais 12 estão classificadas em alguma categoria de ameaça de extinção, segundo o Livro vermelho da Fauna Brasileira Ameaçada de Extinção (MMA 2018). As regiões sul e sudeste do Brasil são importantes áreas de alimentação para as espécies de aves marinhas provenientes do Atlântico Sul e central, da região subantártica, da Nova Zelândia e do Hemisfério Norte (NEVES et al. 2006a). Nessa região está situada a Zona de Convergência Subtropical, onde as águas provenientes do Norte, trazidas pela corrente do Brasil, se encontram com as águas subantárticas ricas em nutrientes trazidas pela corrente das Malvinas (CASTRO FILHO 1990). A alta produtividade da região, especialmente no inverno, resulta no aumento da disponibilidade de recursos utilizados pelas aves marinhas e coincide com a dispersão pós-reprodutiva de algumas espécies como *Procellaria aequinoctialis*, *Thalassarche chlororhynchos* e *T. melanophris* (Figura 1) (NEVES et al. 2006a). A região também é utilizada por espécies de Procellariiformes ao longo do ano, tanto por adultos reprodutivos, quanto por juvenis. *Thalassarche chlororhynchos*, por exemplo, ocorre no Sul e Sudeste do Brasil ao longo

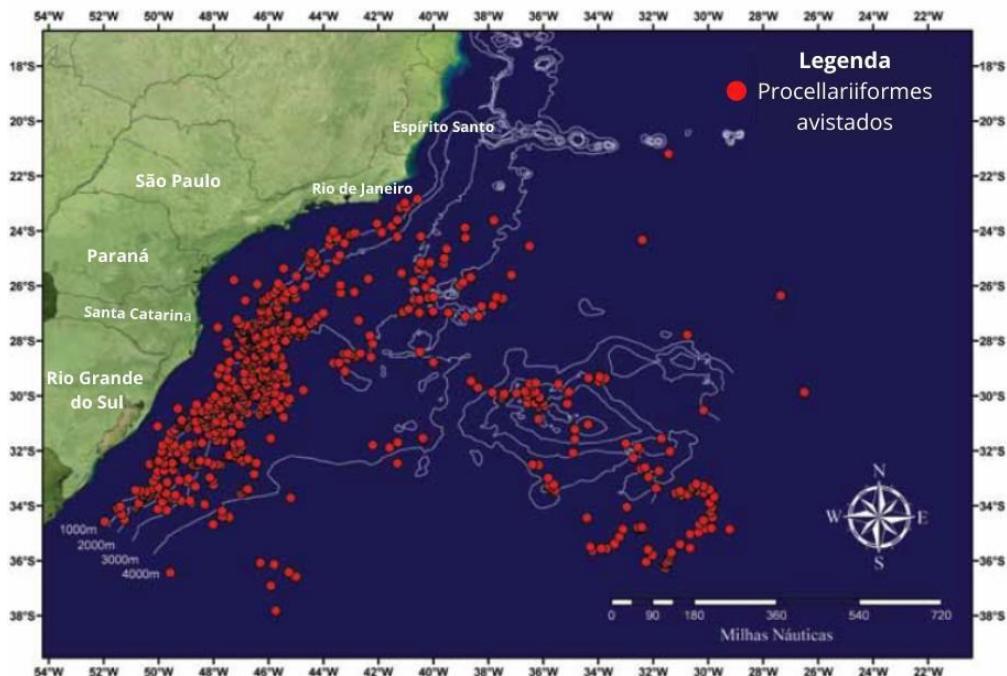
do ano, com predomínio no inverno (NEVES et al. 2006b), sendo também uma das espécies mais comuns



**Figura 1** - Mapa ilustrativo indicando as áreas de reprodução de *Procellaria aequinoctialis* (Ilhas Georgias do Sul), *Thalassarche chlororhynchos* (Falkland/Malvinas) e *T. melanophris* (Tristão da Cunha). As setas coloridas indicam e o período de partida (abril) para as áreas de alimentação na região sul do Brasil e o retorno (setembro) para as respectivas colônias reprodutivas.

Essas características locais e sazonais que resultam em abundância de presas, fazem com que as áreas de ocorrência dos Procellariiformes coincidam com a principal área de interesse e operação das frotas pelágicas no Brasil, que ocorre do Espírito Santo até a fronteira com o Uruguai (NEVES et al. 2006b) (Figura 2). Os Procellariiformes dependem diretamente de recursos marinhos como fonte de alimento ao longo de todo seu ciclo de vida. Além de predarem peixes, cefalópodes e outros invertebrados planctônicos, também capturam presas debilitadas ou mortas na superfície do mar (SCHREIBER & BURGUER 2001). Esse comportamento os torna vulneráveis às interações com atividades pesqueiras, complementando a dieta com descartes e capturando iscas em anzóis, muitas vezes

resultando na captura incidental pela pesca (NEVES et al. 2006a). A identificação dessas áreas é fundamental para a conservação das aves marinhas, pois elas necessitam de áreas chave para realizarem a muda e adquirirem reservas energéticas ao longo do seu ciclo anual (SICK 1997; VOOREN & BRUSQUE 1999; SERAFINI & LUGARINI 2014).



**Figura 2** - Área de ocorrência de Procellariiformes que interagem com barcos de pesca brasileiros (Adaptado de: Projeto Albatroz/Neves et al., 2006).

Entre as aves, os Procellariiformes representam a ordem com maior número de espécies ameaçadas de extinção com evidência de maior declínio populacional em longo prazo, especialmente para as populações do Atlântico Sul (PONCET et al. 2006; DELORD et al. 2008; CROXALL et al. 2012). A captura incidental pela pesca de espinhel é a maior ameaça global à sobrevivência da maioria das populações de albatrozes e petréis (GALES 1998; WEIMERSKIRCH et al. 1999). Essa arte de pesca consiste em uma linha principal com cerca de 80km de comprimento, onde são fixados entre 800 e 1.200 anzóis iscados, visando a captura de espécies como atuns, espadarte e tubarões (SILVEIRA & STRAUBE 2008; NEVES & MANCINI 2009). Espécies das famílias Diomedeidae e Procellariidae são diretamente afetadas por esse tipo de pesca, uma vez que lulas representam um

importante item na dieta de várias espécies e são utilizadas como iscas na pesca de espinhel (OLMOS et al. 2006).

Assim, em 2001 foi firmado o Acordo para a Conservação de Albatrozes e Petréis (ACAP), um tratado internacional para mitigar as ameaças a essas populações, incluindo medidas para reduzir a captura incidental pela pesca (NEVES et al. 2006a). No Brasil, o Plano de Ação Nacional para Conservação de Albatrozes e Petréis (PLANACAP) foi elaborado visando proteger tanto as espécies residentes quanto as migratórias que ocorrem em águas brasileiras, tendo sua versão original publicada em 2006, recomendando a entrada do país no ACAP (HURTADO et al. 2020). O acordo foi ratificado em 2008, entrando em vigor no mesmo ano.

Uma série de medidas mitigatórias vem sendo desenvolvidas para minimizar a captura incidental de aves marinhas por diferentes artes de pesca. O *toriline*, por exemplo, consiste em um cabo longo com fitas coloridas que ficam pendentes de forma a afastar as aves das embarcações (NEVES et al. 2006a). Estudos realizados pelo Projeto Albatroz indicam que o uso do *toriline* diminuiu em 67% o número de tentativas de captura de iscas pelas aves. Essa medida se mostrou a mais significativa nos primeiros 50 metros atrás do barco, reduzindo 97% das tentativas de captura, uma vez que essa é uma distância crítica, onde as iscas estão mais próximas da superfície e, consequentemente, mais acessíveis para as aves (PROJETO ALBATROZ 2013a). Assim como o *toriline*, a largada noturna dos espinheis pode ser aplicada tanto em pescarias de espinhel pelágico como de fundo. Nessa prática, os espinheis são lançados e recolhidos entre o anoitecer e o amanhecer, evitando os períodos de maior atividade de forrageio das aves (BIRDLIFE INTERNATIONAL 2009; PROJETO ALBATROZ 2013a). A partir de 2013, uma técnica chamada “hook pod” começou a ser testada, a qual consiste em uma cápsula que envolve a ponta do anzol e abre automaticamente com pressão d’água a partir de 20 metros de profundidade, evitando que as aves sejam fisgadas tentando capturar as iscas (PROJETO ALBATROZ 2013b). O desenvolvimento e aplicabilidade dessas medidas são de extrema importância para diminuir a captura incidental de aves marinhas, bem como para desenvolver atividades de pesca mais sustentáveis em conjunto com a comunidade pesqueira.

### **1.1.1 Registro de encalhes e análises isotópicas como ferramenta de estudo**

Estudar espécies de hábitos pelágicos e de ampla distribuição pode apresentar diversos desafios devido às dificuldades de acessar essas espécies em seu habitat natural, além de demandar elevados recursos financeiros e logísticos. Assim, o registro das carcaças de animais que chegam nas praias pode ser uma ferramenta útil para acessar essas populações (BRUSIUS et al. 2020). Encalhe é o termo utilizado para se referir a um animal marinho encontrado morto ou debilitado em terra e sem a capacidade de voltar para o mar (BRANCO et al. 2015) ou continuar seu voo sobre ele (GERACI & LOUNSBURY 2005). A dinâmica de encalhe de uma carcaça envolve três principais fatores: a presença da espécie na região, a morte ou debilidade do indivíduo e a condição de deriva da carcaça (BRUSIUS et al. 2020). Além disso, as características ambientais e a ocorrência de eventos climáticos como vento intenso, dinâmica das correntes oceânicas e a altura e direção das ondas tendem a afetar a deriva das carcaças e a probabilidade das mesmas chegarem à praia (PERRIN & GERACI 2009; TAVARES et al. 2016; MELO 2017). O registro de encalhes pode ter custos mais reduzidos que outras abordagens, além de representar um método não invasivo que permite a identificação, coleta e análise minuciosa dos indivíduos sem causar distúrbios populacionais, sendo pertinente para estudos ecológicos de espécies de interesse conservacionista (BARRET et al. 2007; PELTIER & RIDOUX 2015; TAVARES et al. 2016).

Para os Procellariiformes, há uma grande escassez de informações sobre a ecologia trófica e uso de habitat fora do período reprodutivo (CHEREL et al. 2006). Entretanto, é durante o período não-reprodutivo que as aves estão mais suscetíveis à influência de fatores ambientais (CHASTEL et al. 1995; GUINET et al. 1998) e à interação com a pesca (ROBERTSON & GALES 1998), e são estimadas as maiores taxas anuais de mortalidade do grupo (BARBRAUD & WEIMERSKIRCH 2003; SANDVIK et al. 2005). Embora o número de carcaças encontradas nas praias não seja uma representação real do número total de mortes (PERRIN & GERACI 2009), esses dados representam uma amostra da população viva (TEM DOESCHAT et al. 2018), permitindo caracterizar aspectos ecológicos desses indivíduos e/ou populações. Os registros de Procellariiformes encalhados na costa brasileira já foram utilizados para investigar a exposição à poluentes (SALVAGNI 2013), ingestão de resíduos sólidos (COLABUONO 2011; TAVARES et al. 2017), possíveis causas de mortalidade (MARIANI 2019), hábitos

alimentares (PETRY et al 2008; PEREZ 2012), influência de processos oceanográficos (TAVARES 2016) e a influência de condições climáticas adversas (BUGONI et al. 2007) nos eventos de encalhe.

As carcaças de aves marinhas também podem contribuir para o entendimento da distribuição geográfica de espécies e fornecer amostras biológicas (i.e, tecidos e órgãos) que permitem estudos sobre a ecologia desses organismos sem causar danos aos mesmos (HALL et al. 2010). Por exemplo, o primeiro registro do albatroz-de-Tristão (*Diomedea dabbenena*) em território brasileiro foi documentado por meio de uma carcaça encontrada na praia de Santos, São Paulo, em 1984 (GRANTSU 1995). Já a ocorrência de *Calonectris diomedea* no sudoeste do Oceano Atlântico foi determinada a partir da carcaça de um indivíduo encalhado no litoral do Rio Grande do Sul, a qual permitiu a obtenção de material biológico para estudos morfológicos e análise de isótopos estáveis (OLIVEIRA et al. 2019). A análise de isótopos estáveis nos tecidos de aves marinhas tem sido utilizada para investigar e ecologia trófica e o uso do habitat, especialmente durante o período não-reprodutivo (CHEREL et al. 2000, 2006; HEDD & MONTEVECCHI 2006; WILEY et al. 2012) e em escala de comunidade (HOBSON et al. 1994; FORERO et al. 2004; PHILLIPS et al. 2009, 2011; JACKSON et al. 2011).

O conceito de nicho isotópico se refere a uma área no espaço isotópico ( $\delta$ -espaço) que utiliza  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  como coordenadas que representam duas dimensões do nicho: os recursos consumidos e as áreas onde os indivíduos obtiveram esses alimentos (HOBSON et al. 1994; NEWSOME et al. 2007). Assim, a variação na composição da dieta irá refletir as diferenças na proporção isotópica dos seus tecidos e, consequentemente, na maneira como o nicho isotópico é estruturado neste  $\delta$ -espaço. No ambiente marinho, os valores de  $\delta^{13}\text{C}$  podem atuar como potenciais identificadores geográficos (QUILLFELDT et al. 2005), uma vez que os recursos litorâneos (*inshore*) são mais enriquecidos em  $\delta^{13}\text{C}$  do que os recursos em alto mar (*offshore*) (PETERSON & FRY 1987; KELLY 2000). Os valores de  $\delta^{13}\text{C}$  também variam conforme o gradiente latitudinal, apresentando valores mais enriquecidos em águas subtropicais (baixas latitudes) e menos enriquecidos em águas Antárticas (altas latitudes), sendo aplicado na investigação das áreas de forrageio de predadores marinhos (QUILFELDT et al. 2005; CHEREL & HOBSON 2007). Os valores de  $\delta^{15}\text{N}$  indicam o nível trófico da presa consumida, pois esses valores aumentam previsivelmente com o aumento do nível trófico, uma vez que  $^{14}\text{N}$  é preferencialmente

excretado em forma de resíduo nitrogenado (STEELE & DANIEL 1978; MINAGAWA & WADA 1984; KELLY 2000).

O nicho isotópico também pode ser utilizado como um *proxy* para investigar o nicho ecológico e descrever comportamentos alimentares especialistas ou generalistas de indivíduos dentro de uma população, ou para inferir o nível de sobreposição ou partição espacial entre diferentes espécies de uma comunidade (JACKSON et al. 2011). A análise de nicho isotópico pode indicar se espécies simpátricas segregam ou não o uso de recursos para minimizar ou evitar competição (e.g. GIMENEZ et al. 2017). Entretanto, para acessar de forma efetiva as variações intra e inter-específicas na dieta, sobreposição e segregação no uso de recursos por espécies coexistentes, é necessário considerar os gradientes espaço-temporais nos valores isotópicos na base da teia alimentar local (GRAHAM et al. 2010; MCMAHON et al. 2013). Na região sudoeste do Oceano Atlântico, as águas tropicais provenientes da corrente do Brasil predominam nos meses mais quentes, enquanto no período mais frio ocorre a predominância das águas subantárticas ricas em nutrientes, formando um gradiente físico que resulta em diferenças latitudinais (norte-sul) e longitudinal (*inshore – offshore*) nos valores base de  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  (TROIÑA et al. 2020).

Amostras de diferentes tecidos podem ser utilizadas para análises de isótopos estáveis, uma vez que esses tecidos refletem as informações isotópicas das presas consumidas durante o período de sua formação (BEAROHP et al. 2002; PEARSON et al. 2003; CHEREL et al. 2005, BECKER et al. 2007; HERRERA & REYNA 2007).

Entretanto, cada tipo de tecido apresenta uma taxa de renovação diferente, integrando essas informações em diferentes escalas temporais e espaciais. Tecidos com alta taxa de renovação isotópica refletem a dieta recente do consumidor, enquanto tecidos com baixa taxa de renovação podem refletir a média dos itens consumidos em longo prazo (HOBSON et al. 1992). As penas das aves representam uma estrutura metabolicamente inerte, apresentando uma composição isotópica que reflete o habitat ocupado e as presas consumidas pelo indivíduo durante o intervalo entre mudas (BEARHOP et al. 2002; PEARSON et al. 2003; INGER & BEARHOP 2008). Para os Procellariiformes, o padrão de muda pode variar entre as espécies, com o tipo de pena (BRIDGE 2006; BUGONI 2008), e entre populações (CATRY et. al 2013). Entretanto, a maioria dos Procellariiformes no Hemisfério Sul realiza o processo de muda no mar e o valor

isotópico das penas tendem a refletir a dieta e o habitat ocupado pelos indivíduos durante o período não-reprodutivo, que é a fase menos conhecida do seu ciclo anual (WARHAM 1990, 1996; BRIDGE 2006).

Diante da atual situação de vulnerabilidade das aves marinhas, o acesso às carcaças e animais debilitados que chegam nas praias permite obter informações sobre a ocorrência das espécies e características da comunidade, além de fornecer material biológico para análises minuciosas sobre a ecologia trófica e o hábitat utilizado por essas espécies ao longo do seu ciclo de vida. Essas informações são essenciais para subsidiar ações de conservação mais efetivas e propriamente direcionadas às áreas de reprodução, *stopover* e invernada (HOBSON 1999).

## 1.2 OBJETIVO GERAL

Buscamos analisar os registros de Procellariiformes encalhados no sul da Bacia de Santos a fim de caracterizar a comunidade que ocorre ao largo da plataforma continental de Santa Catarina e regiões oceanográficas adjacentes, além de investigar as relações tróficas e a partição de nicho isotópico nesta comunidade. No primeiro capítulo testamos a hipótese de que há uma estruturação temporal na ocorrência de encalhes, regida pela dinâmica reprodutiva e migratória de cada espécie. No segundo capítulo, esperamos observar maior sobreposição de nicho isotópico entre as espécies (*P. aequinoctialis*, *T. chlororhynchos* e *T. melanophris*), conhecidas por apresentar maior incidência de interação com a pesca.

### 1.2.1 Objetivos Específicos

#### **Capítulo 1: Occurrence of seabirds (Procellariiformes) in Southwest Atlantic Ocean during non-breeding period through the analysis of stranding**

- (i) caracterizar a comunidade de Procellariiformes que ocorre em regiões oceânicas adjacentes a Santa Catarina e os seguintes parâmetros de cada espécie: sexo e estágio de desenvolvimento, condição da carcaça, condição corporal e frequências de ocorrência;
- (ii) investigar a ocorrência de interações antrópicas;
- (iii) analisar a ocorrência das espécies em função de variáveis temporais (estação

- e mês);
- (iv) ilustrar a distribuição espacial e áreas de concentração dos encalhes de Procellariiformes no litoral de Santa Catarina.

**Capítulo 2: Isotopic niche overlap among seabirds species (Procellariiformes) during the non-breeding period in the southwest Atlantic Ocean**

- (i) Investigar as relações tróficas da comunidade de Procellariiformes durante o período não reprodutivo através da análise de isótopos estáveis de carbono ( $\delta^{13}\text{C}$ ) e nitrogênio ( $\delta^{15}\text{N}$ ) em penas.
- (ii) Analisar a partição de nicho isotópico entre fêmeas e machos durante o período não reprodutivo.
- (iii) Estimar a área do nicho isotópico ocupada pelas diferentes espécies de Procellariiformes e entre machos e fêmeas, além do nível de sobreposição no nicho.

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**CAPÍTULO 1 - OCCURRENCE OF SEABIRDS  
(PROCELLARIIFORMES) IN SOUTHWEST ATLANTIC OCEAN DURING  
NON-BREEDING PERIOD THROUGH THE ANALYSIS OF STRANDINGS**

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## ABSTRACT

Monitoring stranded seabirds can be an useful tool to investigate the distribution and the ecology of wide distributed and pelagic populations. The present study aimed to analyze Procellariiformes strandings in order to characterize the community inhabiting off the Continental shelf of Santa Catarina, in the Southwest Atlantic Ocean. Stranding data were obtained through systematic surveys performed by the Santos Basin Beach Monitoring Project from January/2016 to December/2019. The data base was explored to list the occurrence of all species registered and to evaluate the following parameters: sex, age class, carcass condition, body condition and external signs of interactions with anthropic activities. The survey records were also used to calculate the numeric frequency and frequency of occurrence of each species, to illustrate the spatial distribution of strandings and to analyze a possible influence of temporal variables (month and season) in the occurrence of the most abundant species through generalized mixed models. A total of 3.247 individuals and 19 species, was recorded. Five species registered are classified as threatened of extinction. *Puffinus puffinus*, *Thalassarche chlororhynchos*, *Procellaria aequinoctialis* and *T. melanophris* were the most abundant species. The majority of carcasses was classified as in moderated and advanced stages of decomposition, which influenced the identification of some biological parameters. Regarding the body condition, the majority of *P. puffinus* was classified as skinny and cachectic, which may be a condition related to the energetic demand of performing transequatorial migrations. All species analyzed presented signs of interaction with anthropic activities, being the external presence of garbage and interaction with fishing gears the most frequent. The results suggest a monthly pattern in the occurrence of *P. puffinus*, with higher numbers of individuals registered between September and December, corresponding to the species non-breeding period. Although the models were not validated for the remaining species, indicating an absence of a temporal pattern in their occurrence, the results reaffirmed the presence of *T. chlororhynchos* in the Southwest Atlantic Ocean in both breeding and non-breeding period.

**Key-words:** Procellariiformes, distribution, mortality, *Puffinus puffinus*.

## INTRODUCTION

Procellariiformes are pelagic birds that spend most of their lives at sea, inhabiting land only for reproduction (Warham 1990). As a result, the species are more accessible for biological investigations only part of the year, making our knowledge about their ecology restricted to the reproductive period (Prince 1987, Cherel and Klages 1998). The lack of information about the non-breeding period is of particular concern for Procellariiformes, since their highest rates of mortality are estimated for this part of the annual cycle (Barbraud and Weimerskirch 2003, Sandvik et al. 2005). The high mortality during the non-breeding period is particularly related to environmental changes at sea which can affect individual's survival (Chastel et al. 1995, Guinet et al. 1998) and the overlap between wintering grounds and the areas explored by industrial fishery (Robertson & Gales 1998). Among birds, Procellariiformes represent the order containing the greatest number of species classified in some level of extinction threat (Croxall et al. 2012). In Brazil, 42 species of Procellariiformes have been recorded, of which 12 are classified in some category of extinction threat according to the criteria of the International Union for the Conservation of Nature (IUCN 2018).

The continental shelf on the Southern and Southeastern region of Brazil is an important feeding area for Procellariiformes from the South and Central Atlantic, the sub-Antarctic region, New Zealand and the Northern Hemisphere (Neves et al. 2006a). The regions are under the influence of the Subtropical Convergence Zone, a high productive area, especially in winter, which increases the availability of resources used by seabirds and matches the period in which some species, such as *Procellaria aequinoctialis*, *Thalassarche chlororhynchos* e *T. melanophris* depart from the breeding colonies (Neves et al. 2006). These species are also commonly registered though strand surveys on Brazilian beaches (Farias et al. 2014). The pelagic seabird community occurring off the Brazilian coast can vary through time, since seasonal variations in species abundance can be related to their specific reproductive phylogeny, migratory pattern and the seasonal dynamic of Brazil – Malvinas currents (Jiménez et al. 2011). The community of Procellariiformes off Brazilian waters is expected to be composed of species breeding in the North or South Hemisphere that migrate to the area after the breeding period (Onley and Scofield 2007; Neves et al. 2006a), species reaching the area when performing long foraging trips during the breeding period (Bugoni and Furnes 2009; Gabani 2020); and

mainly immature individuals (Neves et al. 2006).

Studying pelagic, migratory and widely distributed species can present several challenges due to the difficulties of accessing these species in their habitat (Brusius et al. 2020), in addition to demand high financial and logistical costs (Branco et al, 2010). However, accessing stranded animals involves relatively lower costs and offers a non-invasive method to access populations without disturbances (Barrett et al. 2007, Peltier and Ridoux 2015, Tavares et al. 2016). The record of beached carcasses can also serve as complementary information about the geographic distribution and mortality of different species, seasonality of events, occurrence of diseases, and the relationship with anthropic and environmental variables (Brusius et al. 2020).

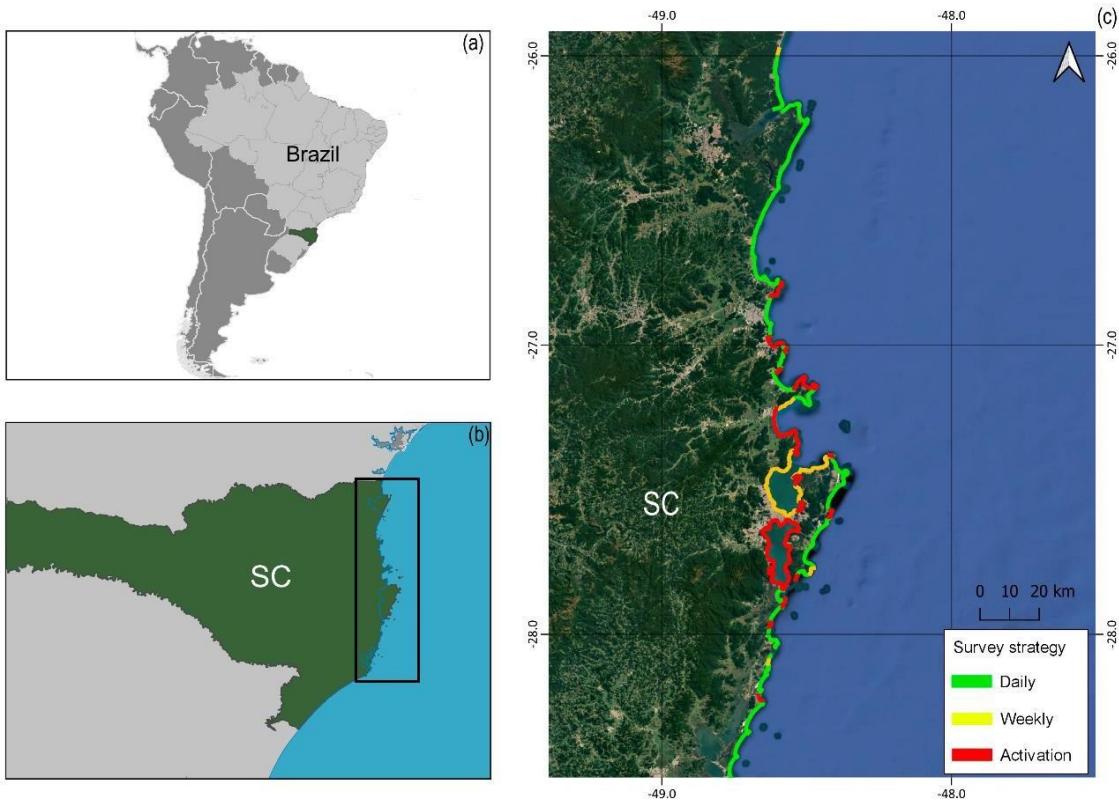
In Brazil, the first record of Tristan Albatross (*Diomedea dabbenena*) (Grantsau 1995) and the occurrence of *Calonectris diomedea* in the southwestern Atlantic Ocean (Oliveira et al. 2019) were confirmed through evaluation of stranded carcasses. Although the number of beached carcasses does not represent the whole community, neither the total number of deaths (Perrin and Geraci 2009), these data is indeed a representative sample of the living population and can provide relevant knowledge about the ecology and distribution of Procellariiformes across the annual cycle, especially during the non-breeding period (ten Doeschat et al. 2018).

This work aimed to identify and characterize the Procellariiformes community through stranding registers in the Southwest Atlantic Ocean, evaluating species and individual attributes, in addition to the occurrence of anthropic interactions. We also analyzed the most abundant species considering temporal variables, expecting to observe some structure in the species occurrence shaped by their reproductive and migratory dynamics. We look forward to contribute with knowledge regarding the occurrence of Procellariiformes in the south of Santos Basin, for which information is still scarce.

## METHODS

### Study area

We analyzed the data of Procellariiformes stranded in the beaches of Santa Catarina State, from Itapoá ( $26^{\circ}03'47''S$ ;  $48^{\circ}37'05''W$ ) to Laguna ( $28^{\circ}29'42''S$ ;  $48^{\circ}45'23''W$ ), covering a total of 522km (Figure 1). This region is part of the Santos Basin (between  $23^{\circ}S$  and  $28^{\circ}S$ ), which includes the coast of Rio de Janeiro, São Paulo, Paraná and Santa Catarina states and occupies a large area offshore Southern and South Brazil (Williams and Hubbard 1984). The inner continental shelf of Santa Catarina is characterized by coastal waters that are vertically homogeneous in terms of temperature and salinity as a result of wind and tide dynamics (Castro Filho 1990). The outer continental shelf is under the influence of the Subtropical Convergence between the southward and northward flowing of Brazil and Malvinas Currents, respectively (Möller et al. 2008). The western boundary of the Subtropical Convergence is mainly located along the shelf break, and shows remarkable seasonal migration. The northern limit fluctuates between  $33^{\circ}S$  in winter and  $38^{\circ}S$  in summer (Gordon 1984). In addition to marine currents, discharge of freshwater from the La Plata River and the Patos Lagoon estuary also influences the physical and chemical conditions for high biological production on the shelf (Muelbert et al. 2008).



**Figure 1:** Location of the study area in (a) Southern Brazil. The black square indicates the (b) area sampled and (c) the colored lines represent the survey system (green: daily; yellow: weekly; red: activation) in each monitored section of Santa Catarina (SC) coastline (adapted from PETROBRAS 2017).

## Data sample

In this work we analyzed data recorded from January 2016 to December 2019 (four years) by the Santos Basin Beach Monitoring Project (PMP-BS) team, with the database available from the Aquatic Biota Monitoring Information System (SIMBA) ([www.simba.petrobras.com.br](http://www.simba.petrobras.com.br)). The licenses and research permits for this monitoring program and the biological sampling were issued by the Brazilian government (IBAMA-ABIO 640/2015). The PMP-BS was developed as a requirement set by Brazilian Institute of the Environment (IBAMA) to evaluate possible impacts on marine tetrapods resulted from oil and gas production and transport by Petrobras at the pre-salt province. The surveys were systematically performed by a trained team and the area was monitored by car, four-wheel ATVs, foot, and boats, depending on the beach and coast characteristics. The surveys occurred either daily (50.7% of the area) or weekly (18.4% of the area); however, in areas of difficult access, carcasses were only collected by the field teams

when called by general public and institutions (i.e, activation) (30.8% of the area). All the carcasses of marine tetrapods detected from waterline to foredunes were recorded, counted, identified and also removed from the beach or marked to avoid recounting. In this work we analyzed only the information related to Procellariiformes. In the field, the survey team identified the species (or the lowest taxonomic level), assigned individual identification code, recorded geographical coordinates, carcass condition and suggestive signs of anthropic interactions. Animals found alive were transferred to rehabilitation centers for potential posterior reintroduction; those found dead, whenever possible, were taken to laboratories to be necropsied, where the information collected at field (e.g species identification) was confirmed. Sex and age class were determined, whenever possible and depending on the condition of the carcass, through macroscopic analyses of gonads during necropsy.

## **Data exploitation**

We considered all Procellariiformes registered throughout the four years of survey to elaborate the species list. Species status of occurrence was based on the commented list of Brazilian birds by the Brazilian Committee Ornithological Records (Pacheco et al. 2021) and the level of extinction threat followed the criteria established by the Union for the Conservation of Nature (IUCN, 2018). We only considered the data confirmed through necropsy to evaluate the following aspects of Procellariiformes community: (i) biological parameters; (ii) carcass condition; (iii) body condition; (iv) interaction with anthropic activities. The biological parameters (i) considered were sex (male/female) and age class (adult/juvenile). The carcass condition (ii) was analyzed for Procellariiformes families and classified in five levels: code 5 (mummified or skeleton only); code 4 (severely decomposed); code 3 (moderately decomposed); and code 2 (freshly dead); code 1 was assigned to individuals found alive (Geraci and Lounsbur, 2005). The body condition (iii) was classified as great, good, skinny or cachectic (Sanches, 2008). Interaction with anthropic activities (iv) was based on external evidence of fishery line, hooks and net, suggesting the interaction with fishing gears; external presence of garbage; signs of human aggression; and the presence of oil, following the protocols established by PETROBRAS (2019).

## Data Analysis

In order to avoid methodological bias, we only considered for the following estimative and analyzes the data obtained through daily surveys. We estimated the numeric frequency (NF) and the frequency of occurrence (FO) of each species according to the following equations:

$$NF = \frac{N \text{ individuals sp 1}}{\text{Total } N \text{ of individuals}} \times 100$$

$$FO = \frac{N \text{ months sp 1}}{\text{total } N \text{ of months sampled}}$$

$$NF =$$

$$\frac{N \text{ individuals sp 1}}{\text{Total } N \text{ of individuals}}$$

$$\times 100$$

$$FO =$$

$$\frac{N \text{ months sp 1}}{\text{total } N \text{ of}}$$

$$\times 10$$

The species were classified in three categories according to their numeric frequency: 1) frequent species ( $NF \geq 11.0$ ), 2) occasional species ( $NF$  between 5.6 and 10.9), and 3) rare species ( $NF \leq 5.5$ ). The frequency intervals were stipulated by dividing the second highest value of  $NF$  by 3, which is the number of frequency categories. The geographic coordinates of each individual stranded were used to elaborate a Kernel density map, with a fixed density factor, in order to visualize the spatial distribution and concentration areas of all registers obtained through daily surveys (QGIS 3.16.5). In addition, we used generalized mixed models (GLMM) to test the influence of temporal variables in the occurrence of the most abundant species, which presented a minimum of 200 individuals registered along the four years of survey. Season and month were considered the predictor variables and the number of strandings of each species the response variable. Seasons were classified as: summer (January-March), autumn (April – Jun), winter (July – September) and spring (October-December). We considered year as the random effect and week as the sample unit. We used a log-link function and Negative Binomial error distribution to account for over-dispersion. The performance of three alternative models was assessed for four species ( $n > 200$ ): *Procellaria aequinoctialis*, *Puffinus puffinus*, *Thalassarche chlororhynchos* and *T. melanophris*. Model 1 and Model 2 were compared to the Null model, that proposes the absence of temporal influence in the species occurrence.

**Null model:** glmmTMB(stranding ~ 1 + (1|year), family = "nbinom2")

**Model 1:** glmmTMB(stranding ~ month - 1 + (1|year), family = "nbinom2")

**Model 2:** glmmTMB(stranding ~ season - 1 + (1|year), family = "nbinom2")

The best model was selected with the Akaike Information Criterion (AIC) and was validated using diagnostic plots through the package DHARMA (Hartig 2018). When the level of support for the models was  $(\Delta AIC) < 5$ , we considered the model insufficient to explain the data variation. All statistical analyzes and graphics were performed using the software R Studio, version 4.05 (R.Studio 2017). Maps were made using QGIS program, version 3.16.5.

## RESULTS

Through the four years of beach surveys analyzed, a total of 3.247 individuals were recorded and at least 19 species were identified, being one identified only at genus level (*Calonectris* spp.) Five of them were classified in some category of extinction threat (Table 1). The family Procellariidae (NF = 69.1%) had the largest number of individuals found stranded on the beaches, followed by Diomedeidae (NF= 28.6%). *Puffinus puffinus* was the most frequent species (NF = 44.4%), presenting an unusual high mortality recorded between October and November of 2016, when 858 animals were counted. *Procellaria aequinoctialis* (NF = 11.1) and *Thalassarche chlororhynchos* (NF=16.4%) were also classified as frequent species. Species (i.e *Halobaena caerulea*, *Macronectes halli* and *Procellaria conspicillata*) lacking information regarding the numeric frequency and frequency of occurrence were not found through daily surveys and were excluded from these analyzes. The identification at species level was not possible to achieve for many individuals, which were classified until the family or genus level.

**Table 1:** List of Procellariiformes species registered through stranding surveys in the coast of Santa Catarina, including the occurrence (CBRO) and conservation (IUCN) status of each species, the total number of individuals recorded (N ind), numeric frequency (NF), NF classification (frequent, occasional and rare), frequency of occurrence (FO), proportion of males and females (M/F), proportion of adults and juveniles (A/J). NF, FO, M/F and A/J are expressed in percentage (%). CBRO: VI(S) = not breeding seasonal visitor from South; VI(E) = not breeding seasonal visitor from East; VI # = not breeding seasonal visitor which occurrence status is not confirmed. IUCN: VU = vulnerable; EN = endangered; LC = least concern; NT = near threatened.

Species	CRBO	IUCN	N ind	NF	Classification	FO	M / F	A/J
Procellariiformes NI			57	1.8		43.8	--	--
<b>Diomedeidae</b>			902	28.6	Frequent	89.6	46/54	57/43
Diomedeidae NI			16	0.5		31.3	0/100	100/0
<i>Diomedea epomophora</i>	VI #	VU	2	0.1	Rare	4.2	50/50	100/0
<i>Thalassarche chlororhynchos</i>	VI (S)	EN	519	16.4	Frequent	87.5	43/47	65/35
<i>Thalassarche melanophris</i>	VI (S)		272	8.6	Occasional	64.6	54/46	32/68
<i>Thalassarche</i> sp.			93	2.9		50.0	50/50	100/0
<b>Oceanitidae</b>			22	0.6	Rare	22.9	90/10	80/20
<i>Oceanites oceanicus</i>	VI(S)	LC	21	0.6	Rare	22.9	83/17	78/22
<i>Oceanites</i> sp.			1	--		2.1	100/0	100/0

<b>Procellariidae</b>			2266	69.1	Frequent	97.9	51/49	54/46
Procellariidae NI			16	0.5		18.8	100/0	50/50
<i>Ardenna gravis</i>	VI (S)	LC	40	1.2	Rare	41.7	52/48	50/50
<i>Ardenna grisea</i>	VI (S)	NT	26	0.8	Rare	35.4	37/63	62/38
<i>Calonectris</i> sp.	VI (E)		198	6.1	Occasional	45.8	55/45	51/49
<i>Daption capense</i>	VI (S)		12	0.4	Rare	12.5	28/72	75/25
<i>Fulmarus glacialisoides</i>	VI (S)	LC	5	0.2	Rare	8.3	100/0	100/0
<i>Halobaena caerulea</i>	VI (S)	LC	1	--	--	2.1	100/0	100/0
<i>Macronectes giganteus</i>	VI (S)	LC	58	1.3	Rare	29.2	61/39	30/70
<i>Macronectes halli</i>	VI (S)	LC	2	--	--	--	50/50	0/100
<i>Macronectes</i> sp.			12	0.4		12.5	100/0	50/50
<i>Pachyptila belcheri</i>	VI (S)	LC	5	0.2	Rare	4.2	100/0	100/0
<i>Pachyptila desolata</i>	VI (S)	LC	8	0.2	Rare	8.3	25/75	67/37
<i>Pachyptila</i> sp.			3	0.1		4.2	0/100	50/50
<i>Procellaria aequinoctialis</i>	VI (S)	VU	354	11.1	Frequent	77.1	37/67	76/24
<i>Procellaria conspicillata</i>	VI (S)	VU	1	--	--	--	0/100	100/0
<i>Procellaria</i> sp.			6	0.2		10.4	100/0	50/50
<i>Pterodroma incerta</i>	VI (S)	EN	7	0.2	Rare	12.5	0/100	100/0
<i>Pterodroma mollis</i>	VI (S)	LC	5	0.2	Rare	10.4	50/50	37/67
<i>Pterodroma</i> sp.			4	0.1		8.3	--	--
<i>Puffinus puffinus</i>	VI (E)	LC	1453	44.4	Frequent	68.8	53/47	51/49
<i>Puffinus</i> sp.			50	1.6		39.6	100/0	67/37

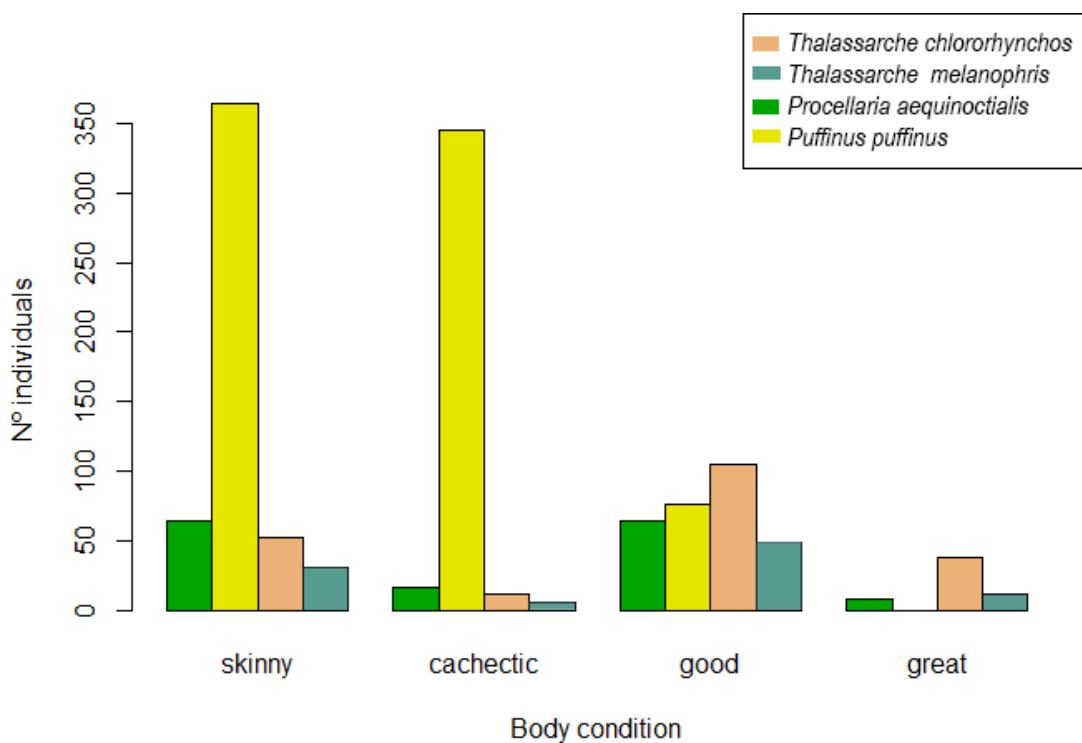
A total of 2.232 individuals, 68.7% of all registers, were necropsied. The proportion of male and females, adult and juveniles for the most abundant species was not discrepant. However, only males were identified for *Puffinus* sp., *Procellaria* sp., *Macronectes* sp., *F. glacialisoides*, *H. caerulea* and *P. belcheri*; whereas the few individuals registered for *P. conspicillata* and *P. incerta* were all females. In addition, only adults were registered for *D. epomophora*, *F. glacialisoides*, *H. caerulea* and *P. incerta*.

The majority of the carcasses found were classified into cod 4 (51.6%) and cod 3 (13.6%), respectively. A total of 201 individuals (8.7%) were found alive (Table 2). The body condition was estimated for the most abundant species (Figure 2). *Puffinus puffinus* has the majority of individuals classified as skinny and cachectic, and no individual was classified as great. Evidence of anthropic interactions were identified for 12% of the individuals analyzed and all species presented some sort of human interaction (Table 3).

External presence of garbage (67.6%) and interaction with fishing gears (22%) were, respectively, the main anthropic interactions registered.

**Table 2** - Evaluation of carcass condition (code 1: alive; code 2: freshly dead; code 3: moderately decomposed; code 4: severely decomposed; code 5: mummified or skeleton only) for Procellariiformes families stranded in the coast of Santa Catarina, Southern Brazil. Values are presented in percentage (%).

Family	code 1	code 2	code 3	code 4	code 5	Total
Diomedeidae	2.8	3.6	6.8	78.9	8.1	26.9
Oceanitidae	27.8	22.3	16.7	33.4	0	0.9
Procellariidae	10.8	9.5	16.2	55.6	8.1	72.3
<b>Total</b>	<b>8.7</b>	<b>7.9</b>	<b>13.7</b>	<b>61.6</b>	<b>8.1</b>	<b>100</b>

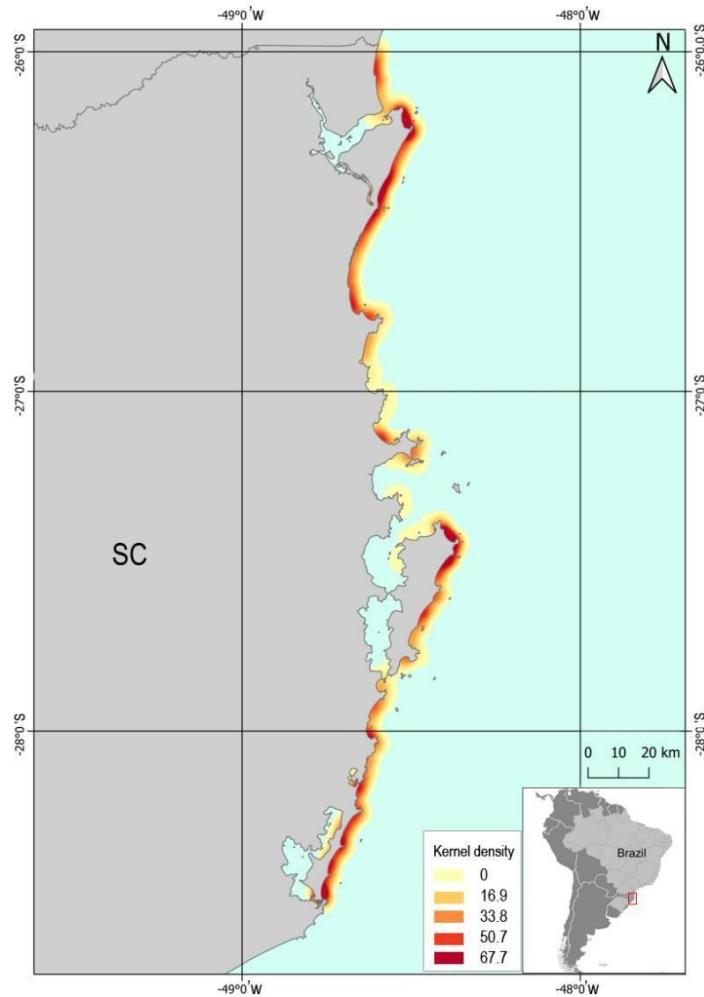


**Figure 2:** Body condition categories (great, good, skinny, cachectic) for the most abundant Procellariiformes species (*Procellaria aequinoctialis*, *Puffinus puffinus*, *Thalassarche chlororhynchos* and *Thalassarche melanophris*) registered from 2016 to 2019 in the coast of Santa Catarina, Southern Brazil.

**Table 3:** Anthropic interactions identified for Procellariiformes species: human aggression (aggression); interaction with fishery gears (fishery), signs of oil stain (oil) and external presence of waste material (garbage). ‘N ind’ represents the number of individuals of each species that were necropsied and ‘%’ the respective proportion of individuals with evidence of anthropic interactions. NI = not identified.

Species	N ind	%	aggression	fishery	garbage	oil
<b>Diomedeidae</b>	615	7.0	4	30	40	2
Diomedeidae NI	5	0	2	15	20	1
<i>Diomedea epomophora</i>	2	100	0	0	2	0
<i>Thalassarche chlororhynchos</i>	372	3.7	1	9	9	1
<i>Thalassarche melanophris</i>	198	8.0	1	6	8	0
<i>Thalassarche</i> sp.	38	2.6	0	0	1	0
<b>Oceanitidae</b>	16	12.5	0	0	2	0
<i>Oceanites oceanicus</i>	14	14.3	0	0	2	0
<b>Procellariidae</b>	1599	14.5	8	79	294	38
Procellariidae NI	4	0	4	0	149	19
<i>Ardenna gravis</i>	32	9.3	0	0	3	0
<i>Ardenna grisea</i>	17	17.6	0	0	3	0
<i>Calonectris</i> sp.	143	14.0	0	0	16	2
<i>Daption capense</i>	12	25	0	0	3	0
<i>Fulmarus glacialisoides</i>	3	33.3	0	0	1	0
<i>Halobaena caerulea</i>	1	100	0	1	1	0
<i>Macronectes giganteus</i>	38	28.9	0	0	10	0
<i>Macronectes halli</i>	2	100	1	2	0	0
<i>Macronectes</i> sp.	8	0	0	0	0	0
<i>Procellaria aequinoctialis</i>	233	16.7	0	12	25	2
<i>Procellaria conspicilata</i>	1	0	0	0	0	0
<i>Procellaria</i> sp.	3	0	0	0	0	0
<i>Pachyptila belcheri</i>	2	0	0	0	0	0
<i>Pachyptila desolata</i>	5	60	1	0	2	0
<i>Pachyptila</i> sp.	3	33.3	0	0	0	0
<i>Pterodroma incerta</i>	4	0	0	0	0	0
<i>Pterodroma mollis</i>	5	20	0	0	1	0
<i>Pterodroma</i> sp.	4	0	0	0	0	0
<i>Puffinus puffinus</i>	1069	13	2	65	80	14
<i>Puffinus</i> sp.	12	25	0	0	2	1
<b>N individuals</b>	<b>2232</b>	<b>22.3</b>	<b>12</b>	<b>109</b>	<b>336</b>	<b>40</b>

The distribution of Procellariiformes stranded along the coast of Santa Catarina is represented in figure 3. The heat map indicated higher stranding concentration around the north coast of Santa Catarina state, the northeast of Florianópolis island and in the south. Areas represented by a brighter color, indicating zero density of strandings, are a result from the exclusion of data obtained through activation and weekly surveys.

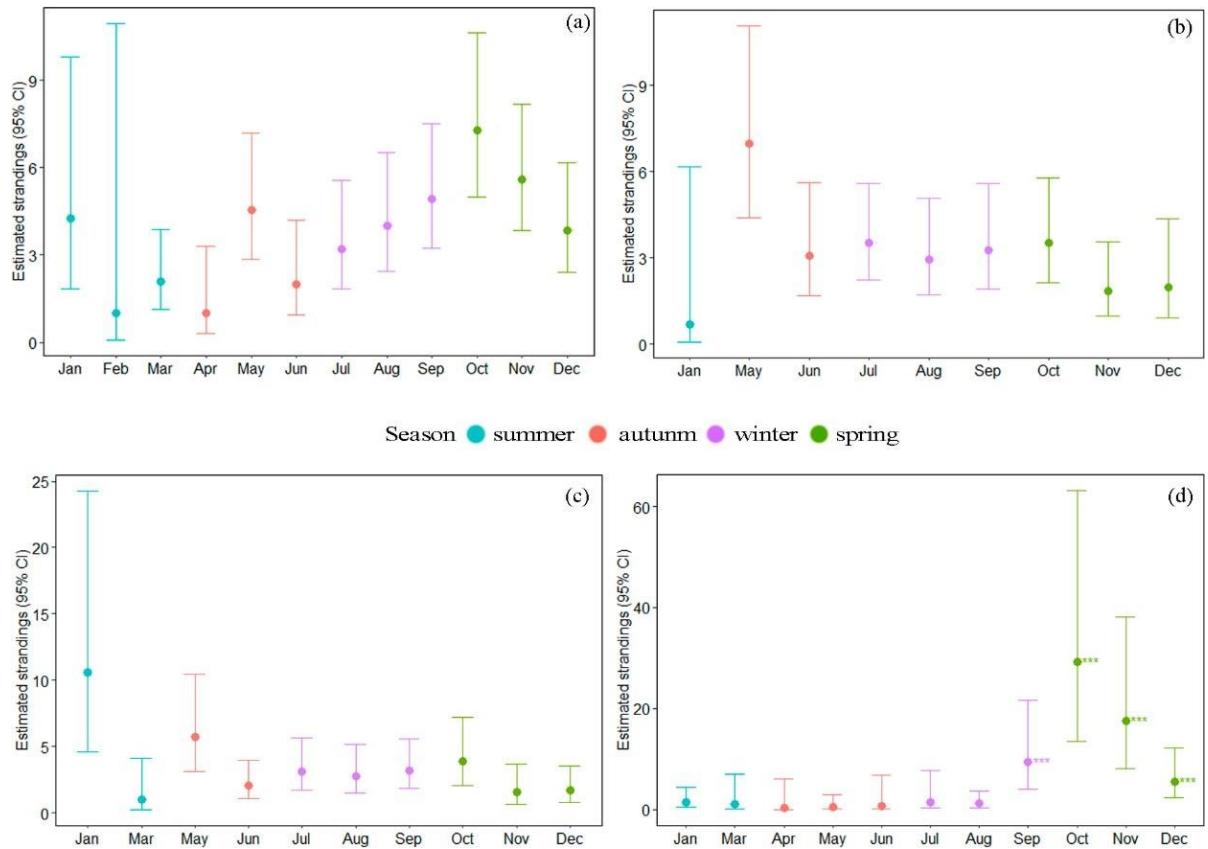


**Figure 3:** Spatial distribution of Procellariiformes stranded along the study area in southern Brazil. Color code indicates kernel density for all strands registered through daily surveys from January 2016 to December 2019 in the coast of Santa Catarina.

GLMM results indicate a temporal trend in the occurrence of *P. puffinus*, where the variable month (Model 1: AIC= 663.5) explained 75% of the strandings (conditional R<sup>2</sup>= 0.75). Although *P. puffinus* was registered along the whole year, except for February, the results pointed significant values in the species occurrence from September to December (Figure 4). Even though the Model 1 had the best fit value (lowest AIC) for *P. aequinoctialis*, and the Model 2 for *T. chlororhynchos* and *T. melanophris*, these models also had  $\Delta$  AICc<5 when compared to the second ranked model (Table 4). As a consequence, we considered that these models were insufficient to explain data distribution.

**Table 4:** Generalized linear mixed models (GLMM) with Negative Binomial family and loglink function for the most abundant Procellariiformes species, with month and season as explanatory variables, and year as the random effect. Models are ranked by the adjusted Akaike information criterion (AICc).

Species	Null model				Model 1				Model 2			
	df	logLik	AICc	$\Delta$ AICc	df	logLik	AICc	$\Delta$ AICc	df	logLik	AICc	$\Delta$ AICc
<i>Thalassarche chlororhynchos</i>	13	-316,32	638,9	4,43	14	-303,15	638,2	3,74	6	-310,85	634,4	0
<i>Thalassarche melanophris</i>	13	-177,1	360,5	4,73	11	-166,32	358,8	3,03	6	-171,28	355,8	0
<i>Procellaria aequinoctialis</i>	6	-212,61	438,2	5,01	12	-202,58	433,2	0	3	-215,28	436,8	3,62
<i>Puffinus puffinus</i>	3	-354,83	715,9	52,46	13	-316,61	663,5	0	6	-322,89	678,7	15,23



**Figure 4:** Monthly occurrence of the most abundant Procellariiformes species ( $n > 200$ ) registered along the coast of Santa Catarina, Southern Brazil. (a) *Thalassarche chlororhynchos*; (b) *Thalassarche melanophris*; (c) *Procellaria aequinoctialis* and (d) *Puffinus puffinus*.

## DISCUSSION

We describe some relevant aspects of Procellariiformes community inhabiting waters off the Southern Brazilian coast, highlighting the relevance of stranding surveys to obtain ecological information of pelagic seabirds, including their potential occurrence and threats. To our concern, this is the first study to approach the occurrence of Procellariiformes off the coast of Santa Catarina through the analysis of strandings. Although caution is needed when interpreting stranding data (Peltier and Rudoux 2015), the results found here represent a sample of the living populations and therefore, contribute with information about the species inhabiting this sector of the subtropical Western South Atlantic during their annual cycle.

The species recorded through the four years of beach survey along Santa Catarina state represent 40% of the total Procellariiformes species expected for Brazilian waters, including four species (*A. gravis*, *A. grisea*, *O. oceanicus* and *P. puffinus*) and one genus (*Calonectris* spp.) that perform transequatorial migration between the breeding and feeding areas. The Procellariiformes community also included 16 species of seasonal visitors from South and one seasonal visitor (*D. epomophora*) whose occurrence status is not confirmed (Pacheco et al. 2021). The composition of Procellariiformes community off the Brazilian coast was described by Bugoni et al. (2009) through cruises on long-line fishing vessels operating in deep waters over the continental shelf and offshore waters in southern Brazil. With exception to *D. dabbenena* and *D. exulans*, all species registered in this study were also found stranded along the coast of Santa Catarina. In addition to these species, we also registered the occurrence of *D. epomophora*, *Halobaena caerulea*, *Pachyptila belcheri*, *P. desolata*, *Pterodroma incerta* and *P. mollis*, although they were all classified as rare species.

*Diomedea epomophora* was the only biannually breeder registered in this study and was classified as a rare species with the lower NF among the Procellariiformes found through daily surveys. The species attend the colonies in New Zealand islands and perform circumpolar migration until the feeding areas off southern Chile, Argentina and Falkland Islands (Robertson and Kinski 1972; Imber 1999). *Ardenna gravis*, *A. grisea* and *O. oceanicus* migrate from breeding colonies in the South Atlantic to the feeding areas in the

North Hemisphere (Dabbene 1922; Roberts 1940; Brown et al 1980; Warham 1996). Although they were also classified as rare species, it does not confirm their low abundance in the southwest Atlantic Ocean, since the carcasses of small individuals, such as *O. oceanicus*, are rapidly predated by scavengers, decreasing their chances of being found at the beach (Seys *et al.* 2002).

Closely related taxa of the genus *Calonectris* (*C. diomedea*, *C. borealis* and *C. edwardsii*) were considered a single species, until recently (Roscales *et al.* 2010). *Calonectris diomedea* comprises Atlantic (*C. d. borealis*) and Mediterranean (*C. d. diomedea*) subspecies whose specific classification still remain under taxonomic debate (Heidrich *et al.* 1998, Camphuysen & Van Der Meer 2001). Both sub-species can be found along the coast of Rio Grande do Sul, southern Brazil, from December to May (Vooren and Brusque 1999; González-Solís *et al.* 2007). Although *C. borealis* is listed as common northern visit in Brazilian waters (Piacentini *et al.* 2015) the visual classification of both species is difficult to perform at field, demanding more specific approaches (i.e genetic analyzes). To avoid confusion, we hereafter consider all these individuals as *Calonectris* sp., for which occurrence off the coast of Santa Catarina was classified as occasional.

The stranding of a carcass on specific beaches relies on local meteorological and oceanographic variables combined to the species abundance at sea, mortality rate, drift dynamic, carcass buoyancy, finding and reporting of the carcass (Peltier and Ridoux 2015). In the southern Brazilian coast, the environmental conditions, the peculiar characteristics of the wide continental shelf and the upwelling of the South Atlantic Central Water are pointed as the main causes of the high occurrence of strandings in the area (Barbieri *et al* 2019; Brusius *et al.* 2020). We estimated the distribution of strandings in the coast of Santa Catarina in order to visualize potential areas of concentration. The register of carcasses through activation can be biased since reporting a beached carcasses are more likely to occur in more populated areas and during summer months, when the people flow is more intense and the chances of reporting a strand individual increase. As a result, we excluded from this analysis the data obtained by weekly and activation surveys, which also influenced the representation of the strands distribution in the whole surveyed area. Because our focus was to analyze the stranding data to access the species occurrence and to characterize the community, we did not investigate the environmental and oceanic variables which could explain the carcass deposition in certain areas.

Although the dynamic of carcass buoyancy and drift at sea is not well documented for seabirds, the drift dynamic is the main factor influencing the stranding data series and it is mainly affected by wind and tidal currents (Peltier and Ridoux 2015). Therefore, environmental factors and the duration of drifting can determine the stages of decomposition by which a carcass strand. Here, most of Procellariiformes carcasses were classified as code 3 and 4, corresponding to moderate and advanced stages of decomposition. The carcass condition can restrain the identification of attributes for many individuals due to the advanced decomposition of biological tissues and thus, carcasses in advanced stage of decomposition (code 4) and mummified (code 5) were rarely submitted to necropsy .

The general proportion of sex and age class for each species analyzed here suggests that individuals of different classes (i.e male and females; adults and juveniles) are occurring in the same habitat and thus, are potentially vulnerable to the same impacts. These findings are supported by stable isotope analyses of  $\delta^{13}\text{C}$ , in which males and females of different Procellariiformes species did not differ in terms of their isotope signatures, suggesting no difference in their use of habitat during the non-breeding period (Vanin, unpublished data). However, considering Procellariiformes attending fishing vessels in the southwest Atlantic, Bugoni and Furness (2009) found a predominance of juveniles of some species such as *T. melanophris*, *M. giganteus* and *F. glacialisoides*; whereas for *P. aequinoctialis*, *A. gravis* and *T. chlororhynchos*, a mix of immatures and adults was found.

Changes in adult mortality have greater impact on population trajectories than variation in other demographic parameters such as breeding success, proportion of deferring breeders, juvenile survival and recruitment (Croxall and Rothery 1991, Arnold et al. 2006, Véran et al. 2007). As a result, population recovery from significant adult mortality tends to be slow (Russell 1999), affecting reproductive success in subsequent years (Wooller et al. 1989). For Procellariiformes, the discrepancies of adult males and females mortality, resultant from bycatch, can include differences at sea distributions and differential access to feeding resources due to sexual size dimorphism (Bugoni et al. 2011). However, for the majority of species recorded here, the high number of individuals with undetermined sex and age class, due to the carcass decomposition stage, precluded the application of more specific analyzes to infer an accurate proportion of these parameters within the

community.

Environmental conditions, such as wind direction and intensity, can both increase or decrease the energetic demand during flight (Weimerskirch et al. 2012) and thus, interfere in the individual body condition. In turn, the body condition influences the individual breeding cycle, leading to reproductive success or breeding skipping (Chastel et al 1995). *Puffinus puffinus* presented the highest number of individuals with skinny and cachectic body conditions and none classified as great, suggesting that stranding incidence was more common for individuals with a lower body mass. Migration is a period of high energetic demand (Berthold 1975, Blem 1980) and birds can face a set of challenges when migrating, including adverse weather (Gauthreaux 1971, Alerstam 1990), changes in the availability and quality of food (Bibby and Green 1980, Moore et al. 1995) and competition for resources (Moore and Yong 1991). Moreover, beginning migration in poor nutritional condition can increase the risk of suppressed immune function during the migratory route (Owen 2004). Migratory seabirds can also experience exhaustion and starvation when facing stormy conditions (Jones et al. 2008). Therefore, the poor body condition found for the majority of *P. puffinus* may be a result of the high energetic demand of performing transequatorial migration and the potential occurrence of storms along the migratory route (Lincoln 1979). The remaining species analyzed presented individuals in all categories of body condition, with higher numbers of individuals classified as ‘good’. Fishing discards from pelagic fishery in Brazil is a significant food source for Procellariiformes during the non-breeding period (Bugoni et al 2008). Because *P. aequinoctialis*, *T. chlororhynchos* and *T. melanophris* are frequently interacting with fishing vessels in order to obtain food (Bugoni et al. 2008, Jiménez et al 2017), their better body condition may be a result of the consumption of prey provided by fishery wastes.

Six species recorded in this study are accomplished on the Agreement on the Conservation of Albatrosses and Petrels (ACAP), an international treaty to mitigate threats to these populations, including measures to reduce bycatch in fisheries (Neves et al. 2006a). Our findings indicated suggestive signs of anthropic interaction for all species analyzed, highlighting the external presence of garbage and interaction with fishing gears. Procellariiformes represent the most threatened order among birds, accounting for 70% of worldwide seabird longline fishery bycatch (Brothers et al. 1999). In Brazil, 12 species are classified as threatened of extinction (MMA 2018), and five of them were found in

our study. About 300,000 seabirds, among which 100,000 are albatrosses, die each year captured by fishing hooks worldwide (Anderson et al. 2011). For the Brazilian longline fleet, the overall seabird capture rates were estimated as 0.229 birds per 1,000 hooks, varying from 0 to 0.542, according to season (Bugoni et al. 2008). The cold season (June–November) was pointed as the period of highest intensity of fishing activities, especially in Southern Brazil (Bugoni et al. 2008). Considering the high amount of fishing hooks daily thrown in the sea by the longline fleet, it is estimated that around 4,000 albatrosses die each year in Brazil by incidental capture in longline fishing (Neves et al., 2006b). This information is particularly relevant since, for many species approached (e.g *P. puffinus*, *P. aequinoctialis*, *T. chlororhynchos* and *T. melanophris*), the period of higher intensity of fishery activities englobes the months with high incidence of stranding registered here.

In addition to bycatch, oil spills (Ford et al. 2001) and the presence of large amount of plastic in the ocean, which can be accidentally ingested (Robards et al. 1995) or result in entanglement (Moore et al. 2009), are also important factors influencing the mortality and stranding of seabirds. It is estimated that 99% of seabirds will be affected by plastic ingestion until 2050 (Wilcox et al. 2015). The higher number of Procellariiformes stranding in Brazil also suggest that the Brazilian coast may face environmental instabilities that are not completely understood (Hurtado et al. 2020). Since only a fraction of animals that die at sea arrive at the beach, the number of individuals stranded is not a precise representation of death and thus, should be carefully interpreted . However, the analysis of carcasses also represents a relevant method to investigate potential threats and causes of death, subsidizing conservation strategies.

The abundance, richness and biomass of Procellariiformes occurring off the Brazilian coast can vary through time as a result of the species reproductive phylogeny, migratory pattern and the seasonal dynamic influence of Brazil – Malvinas Currents (Jiménez et al. 2011). *Puffinus puffinus* breeds in the northern hemisphere (Onley and Scofield 2007) and migrates to the South Atlantic Ocean after breeding, from September to March (Guilford et al. 2009, Freeman et al. 2013). The species was the most frequent along the four years of survey, with the highest number of records from September to December, corresponding to the species non-breeding period (Guilforft et al. 2009). A mass stranding was recorded for the species in 2016, when 59% of all *P. puffinus* registered stranded, including the highest number of individuals stranded alive. The mortality of *P. puffinus*

is mainly associated with the intensification of large-scale oceanographic processes, such as the El Niño–Southern Oscillation (Tavares et al. 2016). Even considering the environmental variables influencing the carcass drift (Perrin and Geraci 2009), the high number of *P. puffinus* observed during beach monitoring represents a very high mortality of the species during the non-breeding period in the Southwest Atlantic Ocean. Mass strandings were previously recorded in Southern Brazil for *T. chlororhynchos*, *T. melanophris*, *A. gravis*, *C. diomedea*, *P. aequinoctialis* and *P. puffinus*, although the causes of these events remain poorly known (Farias et al. 2014).

In addition to *P. puffinus*, *T. chlororhynchos*, *T. melanophris* and *P. aequinoctialis* also had a high number of stranded individuals. These species depart from colonies to the feeding areas around April, inhabiting waters of Southern Brazil during the non-breeding period (Neves et al. 2006a). *Thalassarche chlororhynchos* and *T. melanophris* are amongst the most abundant Procellariiformes species in Southern Brazil. *Thalassarche chlororhynchos* is expected to occur in Brazilian waters throughout the whole year, predominating from April to August; whereas *T. melanophris*, especially juveniles, is more abundant during winter (June – August) (Neves et al. 2006a). These three species are also recognized as the main ones captured by the longline fleet in Brazil (Neves et al. 2007, Bugoni et al. 2008), since the period of higher fishery activities matches these species non-breeding period (Neves et al. 2006a).

Our initial hypothesis considered that the species occurrence was structured by a temporal pattern regarding the reproductive and migratory cycle of each species. The models applied were only validated for *P. puffinus*, indicating a monthly pattern in the species occurrence, with a higher number of registers from September to December, corresponding to the species non-breeding period. Although the models indicated no temporal pattern in the occurrence of *T. chlororhynchos*, the species was the only one continuously registered along the year, supporting what was expected even during the breeding period, flying long distances from the breeding colonies. Individuals tagged with satellite transmitters at Tristan da Cunha archipelago traveled, to forage in the South and Southeast of Brazil, Uruguay and Argentina (Gabani 2020). Therefore, our result reaffirmed the species presence in the southwest Atlantic Ocean along the year, including the breeding period.

The models were also not validated for *P. aequinoctialis* and *T. melanophris*, refuting our initial hypothesis and suggesting an absence of temporal patterns in their occurrence in this sector of the southwest Atlantic. However, all models considering the influence of temporal variables showed lower AIC values in comparison to the null-model, suggesting that despite the absence of a temporal pattern, the strandings did not occur randomly in terms of time. Since we analyzed four years of beach surveys, it might be necessary to include longer term data to accurately infer temporal patterns in the species occurrence and delineate potential factors of influence.

In conclusion, our study highlights the importance of long-term beach surveys as a non-invasive method to investigate the occurrence of species of difficult access, especially those under threat of extinction. We also emphasize the relevance of this method to access biological parameters of species and turn a light about the community aspects and potential threats. The results pointed here reaffirm the importance of the southwest sector of the Atlantic Ocean as an important feeding area for Procellariiformes species during the non-breeding period. However, to better understand the potential causes of strand events, we suggest for future studies to access local environmental variables (e.g climatic events and oceanographic conditions) that can influence the carcass drift and the stranding dynamics.

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### *Conflicts of interest*

The authors declare no competing interests and that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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**CAPÍTULO 2 – ISOTOPIC NICHE OVERLAPP AMONG SEABIRDS SPECIES  
(PROCELLARIIFORMES) DURING THE NON-BREEDING PERIOD IN THE  
SOUTHWEST ATLANTIC OCEAN**

To be submitted to *Journal of Marine Biology*.

## ABSTRACT

Procellariiformes are pelagic birds that spend most of their life at sea, resulting in a gap of knowledge about their trophic ecology and the use of habitat outside the breeding period. However, to acquire ecological information about these species, we can use a proxy named isotopic niche. Here, we used  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  from feathers obtained through stranded individuals in order to evaluate the isotopic niche partitioning among Procellariiformes species and between males and females. The following species were analyzed: *Ardenna gravis*, *Calonectris borealis*, *Macronectes giganteus*, *Oceanites oceanicus*, *Procellaria aequinoctialis*, *Puffinus puffinus*, *Thalassarche chlororhynchos* and *T. melanophris*. The mean values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were compared between species and between males and females. The niche width of each species was estimated through bivariate ellipses. The values of  $\delta^{13}\text{C}$  differed greatly between *C. borealis* and *O. oceanicus*, being more enriched for *C. borealis*, suggesting that the species consume preys inhabiting less oceanic areas. *Calonectris borealis* and *O. oceanicus* presented low values of  $\delta^{15}\text{N}$ , suggesting the consumption of lower trophic levels regarding other species. Based on SEAc estimates, *T. melanophris* has the highest ellipses overlap with *P. aequinoctialis* and *M. giganteus*, while the last species also overlapped with *P. aequinoctialis*. In contrast, no overlap was found between *P. aequinoctialis* and *A. gravis*, and between *O. oceanicus* and *C. borealis*, suggesting that these species differ in terms of both feeding areas and the trophic level consumed. Although the isotopic signature of the preys consumed was not accessed here, the results suggest that the majority of species analyzed used similar areas and resources during the non-breeding period. This overlap may also be a result of consuming fishing discards, since these species are known to follow fishery vessels in order to obtain food. Differences in  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  values between males and females were not significant, indicating that even species that present sexual segregation in the consume of resources are foraging in the same trophic level and in similar inshore areas. However, both males of *T. chlororhynchos* and *P. puffinus* presented larger niche width than the respective females. This study highlights the importance of stable isotope analysis as a relevant method to access the trophic relationship and to identify the potential areas inhabited by pelagic seabirds during the non-breeding period.

## KEY-WORDS

Stable isotopes, trophic ecology, fishery, Procellariiformes

## INTRODUCTION

The concept of ecological niche is essential to understand species distribution (Pianka 2000; Chase and Leibold 2003). Niche is described as a n-dimensional space including the interaction of conditions and resources necessary for the species occurrence and survival (Hutchinson 1957). Knowledge of the habitat requirements of a species is a crucial information to direct conservation and management strategies, especially for widely distributed organisms, such as seabirds (Martin and Finch 1995). Procellariiformes are pelagic birds that spend most of their life at sea, inhabiting land only for reproduction (Warham 1990). This behavior results in a gap of knowledge about the trophic ecology and the use of habitat by these during the non-breeding period (Prince and Morgan 1987; Cherel and Klages 1998). Seabirds are central-place foraging when inhabiting the breeding colonies, where intense competition among sympatric species is expected (Phillips et al 2009). Although competition is reduced when many individuals disperse from the colonies to the feeding areas (Bodey et al 2014), it does not exclude the potential niche overlap among closely related species during the non-breeding period (Phillips et al. 2008; Quillfeldt et al. 2013).

Procellariiformes species directly depend on marine resources throughout their life-cycles. Their diet is generally composed of fishes, cephalopods and planktonic crustaceans, which preference can vary according to the species (Sick 1997; Olmos et al. 2001). Procellariiformes also supplement their diet through the consumption of fishery wastes and capturing baits on hooks of longline fishery (Neves et al. 2006a). In high productive areas, such as the Brazil-Falkland Confluence (Huang 2011; Jiménez et al. 2016), fishery activities tend to be more intense and it is recognized to influence the seabirds distribution, diet and feeding behavior (Ryan and Moloney 1988; Garthe 1997). Moreover, fishery discards can influence the structure of seabirds' community by supplementing the diet with reliable resources of easier capture and that probably would not be naturally accessible (Garnadeiro et al. 2013).

The coexistence of species with similar ecological requirements is expected to occur through niche divergence, such as differences in the use of habitat, preferential feeding resources and temporal pattern of activity (Hutchinson 1957). Areas of high productivity and environmental heterogeneity can also facilitate the coexistence of closely related species if they exhibit some degree of niche differentiation (Waugh and Weimerskirch 2003), reducing the effects of competition (Schoener 1974; Pianka 1980). However, niche overlap can occur if natural resources are highly abundant, whereas for seabirds that follow fishing vessels, the predictability of fishery discards can result in a spatial-temporal overlap between species that otherwise segregate at sea (Jiménez et al. 2007).

In turn, the evaluation of trophic niche partitioning is important to understand the trophic relationship among species and to investigate which elements may facilitate coexistence. However, mapping the distribution and the resources consumed by pelagic birds can be limited by the logistical and financial costs of accessing these species in their natural habitat (Branco et al. 2010; Brusius et al. 2020). Therefore, to acquire ecological information about species of difficult access, we can use a proxy named isotopic niche (Newsome et al. 2010). Isotopic values, when plotted in a  $\delta$ -space, can be compared to the n-dimensional space of the ecological niche (Bearhop et al. 2004; Newsome et al. 2007). Stable isotope analysis (SIA) assumes that the isotopic composition of consumer tissues reflects the isotopic composition of its food sources (DeNiro and Epstein 1978; Gannes et al. 1997).

In general, carbon ( $\delta^{13}\text{C}$ ) values increase 0-1‰ per trophic level, and its variation is used to evaluate foraging habitats (Quillfeldt et al. 2005). In the marine environment,  $\delta^{13}\text{C}$  values change between inshore and offshore habitats, where inshore resources are more enriched in  $\delta^{13}\text{C}$  than those offshore (Hobson 1999; Kelly 2000; Post 2002). Moreover,  $\delta^{13}\text{C}$  values are also applied to investigate the foraging areas of marine predators through a latitudinal gradient, since  $\delta^{13}\text{C}$  is more enriched in subtropical water (low latitudes) and less enriched in Antarctic waters (high latitudes). On the other hand, nitrogen ( $\delta^{15}\text{N}$ ) is used to estimate trophic positions (Post 2002; Vanderklift and Ponsard 2003) and trophic relationships (Secchi et al. 2016), because  $^{14}\text{N}$  is preferentially excreted as nitrogenated waste. The values of nitrogen ( $\delta^{15}\text{N}$ ) increase 3–5‰ within each trophic level along the food chain (DeNiro and Epstein 1978; Newsome et al. 2010).

The period over which a specific animal tissue reflects the isotopic signature of the consumer diet depends on the isotopic turnover rate in the tissue analyzed (Hobson and Clark 1992). Tissues with fast isotopic turnover will reflect the recent diet (Caut et al. 2011) while those with slow turnover will reflect the long-term (Knoff et al. 2008). Feather keratin is metabolic inert, and therefore its isotopic composition can reflect the diet and habitat occupied during the moult process (Bearhop et al. 2002; Pearson et al. 2003; Inger and Bearhop 2008). Since the majority of Procellariiformes in the South Hemisphere moult at sea after breeding (Warham 1990, 1996; Bridge 2006), the isotopic values of feathers reflect the resources consumed by these species during the non-breeding period (Cherel et al. 2000, Phillips et al. 2009, Jaeger et al. 2010).

The isotopic niche can be quantified using metrics (Jackson et al. 2011; Layman et al. 2007) that allow the assessment of trophic ecology and niche overlap between different groups. For Procellariiformes, SIA has been used to infer diet and trophic relationships, foraging ecology, population management (Inger and Bearhop 2008), habitat preference during the non-breeding period (Charel et al. 2013), identification of potential breeding and moult origins (Hobson 1999; Kelly et al. 2002; Rubenstein and Hobson 2004), resource segregation between sympatric species (Jiménez et al. 2017) and the importance of long-line fishing discards for seabirds (Bugoni et al. 2010). The last one is of particular concern since the incidental capture by long-line fishery is the main factor threatening Procellariiformes populations (Neves et al. 2006a).

To investigate the intra and inter specific variations in the diet composition of coexistent species, it is also necessary to consider the spatiotemporal gradients of isotopic values in the base of local food web (Graham et al 2010; McMahon et al 2013). The Southwest Atlantic Ocean is under the seasonal influence of marine currents, in which the subantarctic waters from the Malvinas Current predominate during winter and the tropical waters of Brazil Current prevail in warmer months (Piola et al. 2004). The dynamic of marine currents also results in latitudinal (north-south) and longitudinal (inshore-offshore) gradients in the base values of  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  (Troina et al. 2020). Due to its high productivity, especially in winter, the southwest Atlantic Ocean is also an important feeding site for Procellariiformes species from South Atlantic, subantarctic zone, New Zealand and the North Hemisphere (Neves et al. 2006). However, the characteristic of this area is especially attractive for pelagic fisheries operating in Brazil, which end up

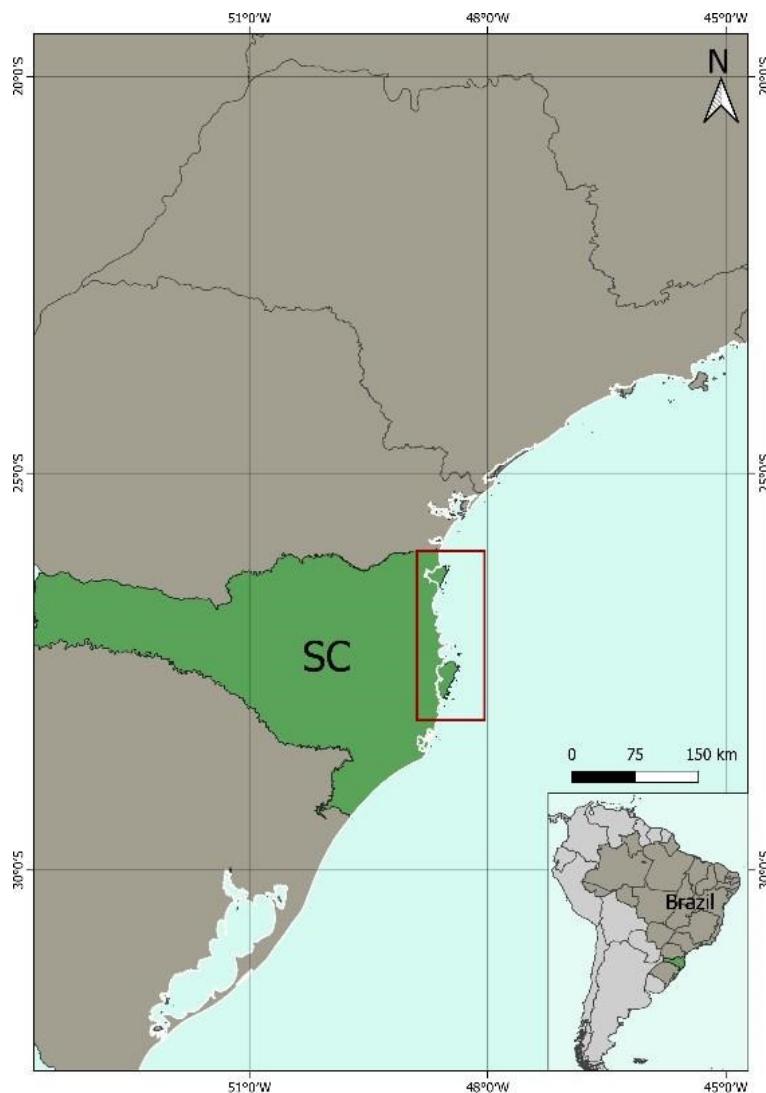
overlapping with the feeding areas used by many Procellariiformes outside the breeding period (Neves et al. 2006).

Here, we used stable isotopes analysis of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  to describe the isotopic niche partitioning among Procellariiformes species and between males and females during the non-breeding period in the southwest Atlantic Ocean. We expected to observe higher isotopic niche overlap among the species of Procellariiformes recognized to strongly interact with fishing vessels in Brazilian waters (Neves et al 2006a,b; Neves et al. 2007; Bugoni et al. 2009)

## METHODS

### Study Area

The feathers used for stable isotopes analysis were sampled from stranded Procellariiformes recorded in the coast of Santa Catarina, from Itapoá ( $26^{\circ}03'47''\text{S}$ ;  $48^{\circ}37'05''\text{W}$ ) to Florianópolis ( $27^{\circ}35'49''\text{S}$ ;  $48^{\circ}32'56''\text{W}$ ) (Fig 1). The coast of Santa Catarina, in southern Brazil, is composed of a mosaic of bays, sandy coastline, rocky coastal islands, estuaries, lagoons, and mangrove forests. The area is under the influence of the Subtropical Convergence Zone, where the waters from the north, brought by Brazil Current meet the sub-Antarctic waters rich in nutrients brought by the Malvinas Current (Möller et al. 2008). In addition to marine currents, discharge of freshwater from the La Plata River and the Patos Lagoon Estuary also influences nutrient dynamics in the coastal zone, resulting in high nutrient concentration and biological productivity (Muelbert et al. 2008).



**Fig 1** Location of the study area in the coast of Santa Catarina (SC), Southern Brazil. Red square represents the area in which stranded Procellariiformes were recorded and feathers were collected for stable isotope analyses

### Samples

Feathers of Procellariiformes were obtained from beach surveys conducted by the Santos Basin Beach Monitoring Project (PMP-BS), which was developed as a requirement set by Brazilian Institute of the Environment (IBAMA) to evaluate possible impacts on marine tetrapods. The surveys resulted from oil and gas production and transport by Petrobras at the pre-salt province. We also obtained feathers of stranded individuals from the sample bank of Albatross Project (BAAP). All the carcasses of Procellariiformes detected from waterline to foredunes were recorded, counted, identified and also removed from the beach or marked to avoid recounting. In the field, the survey team identified the

species (or the lowest taxonomic level), assigned individual identification code, recorded geographical coordinates and carcass condition. Animals found alive were transferred to local rehabilitation centers for potential posterior reintroduction. Those found dead, whenever possible, were taken to laboratories to be necropsied. Sex was determined through macroscopic analyses of gonads during necropsy. The carcasses were individually frozen for preservation and feathers were posteriorly sampled. Primary and body feathers were selected according to their availability, since studies suggest no significant differences in their isotopic values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Jaeger et al. 2009). We selected eight Procellariiformes species (*Ardenna gravis*, *Calonectris borealis*, *Macronectes giganteus*, *Oceanites oceanicus*, *Procellaria aequinoctialis*, *Puffinus puffinus*, *Thalassarche chlororhynchos* and *T. melanophris*) belonging to three different families (Diomedeidae, Oceanitidae and Procellariidae). For all species, a minimum of six individuals was sampled. The sex of each individual was determined through macroscopic analyses of gonads during necropsy (Wibbels 2003) and genetic analyzes was performed to confirm the taxonomic classification of *C. borealis*. The feathers of each individual were cleaned separately with distilled water, with a pattern of five repetitions each, and dried in a laboratory stove at 60°C for 24 hours. After dried, feathers were individually cut with a steel scissor until the smallest fragment possible. One sample of each individual, weighing between 0.8 and 2mg, was separated and weighed in a tin capsule (3 mm × 5 mm). The isotopic measurements were determined by a Delta Plus and a mass spectrometer coupled with an elemental analyzer (Model Carlo Erba 1110, Milan, Italy). No lipid correction was applied since feather samples had weight percent C:N ratios <3.5, indicating insignificant lipid contents for marine animals (Post et al. 2007; Kojadinovic et al. 2012). Stable isotope ratios were expressed in  $\delta$  notation as parts per thousand (‰) deviation from the international standards V-Pee Dee belemnite (carbon) and atmospheric air (nitrogen), using the equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where  $X$  are  $^{13}\text{C}$  or  $^{15}\text{N}$  and  $R$  are the corresponding ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  (Peterson and Fry 1987). The accuracy of the isotopic ratio measurements was  $\pm 0,3\text{ ‰}$  e  $\pm 0,4\text{ ‰}$  for  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$ , respectively. The stable isotope analyses were performed at the Center for Nuclear Energy in Agriculture (CENA) at the University of São Paulo, Brazil.

## Data Analyses

We tested carbon and nitrogen data for normality using a Shapiro-Wilk test. Carbon distribution was not normal ( $W = 0.87$ ,  $p$ -value  $<0.05$ ), and thus we used a Kurskal-Wallis with a Dunn test as post hoc to compare  $\delta^{13}\text{C}$  mean values among species. The Mann-Whitney U test was used to compare  $\delta^{13}\text{C}$  values between males and females of each species. Nitrogen distribution was normal ( $W = 0.99$ ,  $p >0.05$ ), so we performed an one-way analysis of variance (ANOVA) with a Tukey's post hoc test to compare the  $\delta^{15}\text{N}$  values between species. The Student's t-test was used to compare possible differences in the  $\delta^{15}\text{N}$  values between males and females of each species. It was not possible to compare  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  mean values between males and females of *O. oceanicus* since only males were identified. We considered the confidence interval of 95% for all analyses ( $p<0.05$ ).

We compared the isotopic niche width of each species by generating bivariate ellipses in SIBER (Stable Isotope Bayesian Ellipses in R; Jackson et al. 2011), which employs Markov-Chain Monte Carlo (MCMC) simulations to construct parameters of ellipses based on sampling points. We estimated the standard ellipse area corrected for small sample sizes (SEAc, expressed as  $\%\text{o}^2$ ), which represents the mean core area of each species' isotopic niche (Jackson et al. 2011; Layman et al. 2007). We used the SEAc to estimate the proportional niche width overlap among species. Additionally, we calculated the Bayesian standard ellipse area (SEAb) to obtain unbiased estimates of the isotopic niche widths with credibility intervals (Jackson et al. 2011). To test for significant differences, we ran 20,000 MCMC iterations and constructed 95% credible intervals around the mean of each species. The isotopic niche width was compared between males and females only for *P. puffinus* and *T. chlororhynchos*, since the other species presented less than 5 individuals in each group and the ellipses estimative is sensible to small samples.

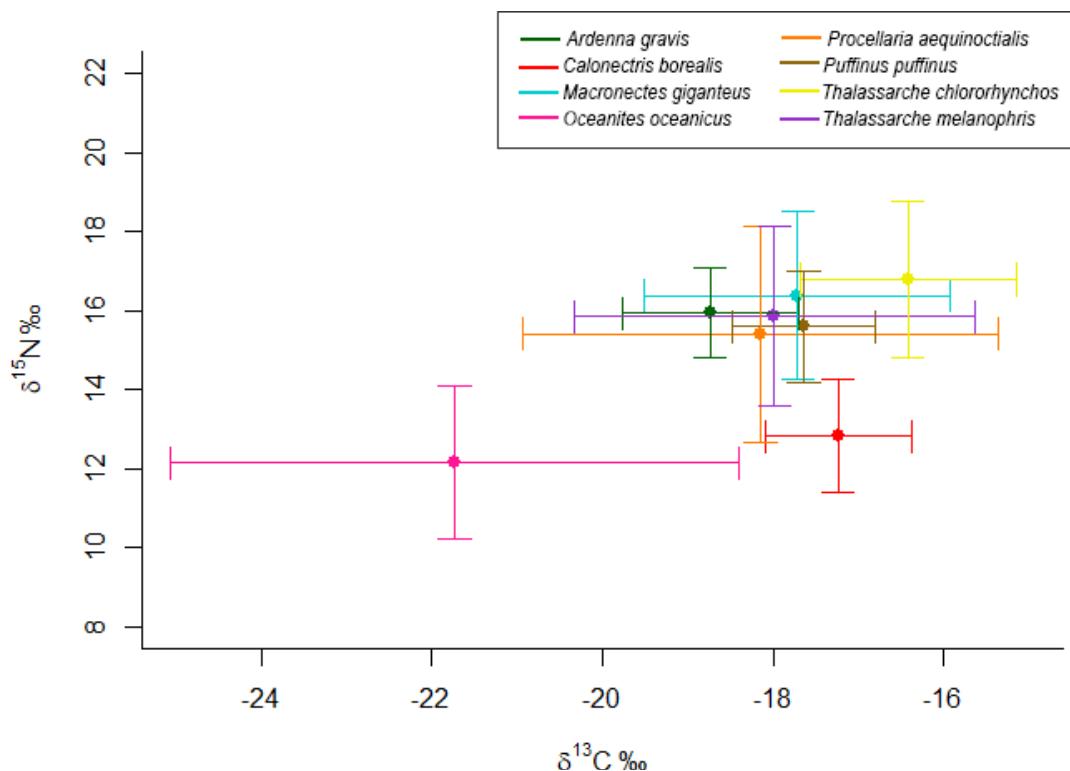
## RESULTS

We observed no significant difference in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for the majority of the species of Procellariiformes analyzed. However,  $\delta^{13}\text{C}$  differed significantly between some species, such as *C. borealis*, which presented enriched values of  $\delta^{13}\text{C}$  when comparing to *O. oceanicus* ( $p<0.05$ ), and *T. chlororhynchos* which differed from both *O. oceanicus* and *A. gravis*, also showing enriched values of  $\delta^{13}\text{C}$  compared to these species

(Table1). We found no significant difference of  $\delta^{15}\text{N}$  between *C. borealis* and *O. oceanicus*, although both species differed greatly from all other species in terms of the trophic level consumed (Fig 2). No significant differences of both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were observed between males and females of Procellariiformes species.

**Table 1** Number of Procellariiformes individuals of each species (N ind), males and females (M/F) and the values (‰) of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (mean and standard deviation) for each group analyzed.

Species	N ind (M/F)	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C(M)}$	$\delta^{13}\text{C(F)}$	$\delta^{15}\text{N(M)}$	$\delta^{15}\text{N(F)}$
<b>Diomedeidae</b>							
<i>Thalassarche chlororhynchos</i>	10(5/5)	-16.4‰±1.2	16.7‰±1.9	-16.7‰±1.2	-16.1‰±1.3	17.0‰±1.4	13.5‰±2.7
<i>Thalassarche melanophrys</i>	14(4/3)	-17.9‰±2.3	15.8‰±2.2	-16.8‰±1.2	-19.9‰±3.7	16.8‰±2.1	16.7‰±2.4
<b>Oceanitidae</b>							
<i>Oceanites oceanicus</i>	6(3/0)	-21.7‰±3.3	12.1‰±1.9	--	--	--	--
<b>Procellariidae</b>							
<i>Ardenna gravis</i>	7(2/2)	-18.7‰±1.0	15.9‰±1.1	-19.8‰±0.7	-18.5‰±0.1	15.4‰±0.3	15.7‰±1.5
<i>Calonectris borealis</i>	10(5/5)	-17.2‰±0.8	12.8‰±1.1	-17.2‰±1.0	-17.2‰±1.0	12.4‰±0.8	13.0‰±1.7
<i>Macronectes giganteus</i>	10(5/2)	-17.7‰±1.7	16.3‰±2.1	-17.7‰±1.0	-16.2‰±0.8	16.7‰±2.2	16.7‰±0.2
<i>Procellaria aequinoctialis</i>	9(3/5)	-18.1‰±2.8	15.4‰±2.7	-16.9‰±2.2	-18.5‰±3.3	16.5‰±2.9	15.1‰±2.9
<i>Puffinus puffinus</i>	22(11/11)	-17.6‰±0.8	15.9‰±1.4	-17.3‰±1.1	-17.9‰±0.3	15.9‰±1.6	15.2‰±1.1



**Fig 2** Mean values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for *Ardenna gravis*, *Calonectris borealis*, *Macronectes giganteus*, *Oceanites oceanicus*, *Procellaria aequinoctialis*, *Puffinus puffinus*, *Thalassarche chlororhynchos* and *T. melanophris*

**Table 2** Dunn's-test comparing the mean values of  $\delta^{13}\text{C}$  between Procellariiformes species. **C.b** = *Calonectris borealis*; **M.g** = *Macronectes giganteus*; **O.o** = *Oceanites oceanicus*; **P.a** = *Procellaria aequinoctialis*; **A.g** = *Ardenna gravis*; **P.p** = *Puffinus puffinus*; **T.c** = *Thalassarche chlororhynchos*; **T.m** = *Thalassarche melanophris*.

\* Represents significant values ( $p < 0.05$ ).

	<b>C.b</b>	<b>M.g</b>	<b>O.o</b>	<b>P.a</b>	<b>A.g</b>	<b>P.p</b>	<b>T.c</b>
<b>M.g</b>	1.00	-	-	-	-	-	-
<b>O.o</b>	0.03*	0.14	-	-	-	-	-
<b>P.a</b>	1.00	1.00	0.54	-	-	-	-
<b>A.g</b>	0.47	1.00	1.00	1.00	-	-	-
<b>P.p</b>	1.00	1.00	0.16	1.00	1.00	-	-
<b>T.c</b>	1.00	1.00	0.00*	0.99	0.04*	0.61	-
<b>T.m</b>	1.00	1.00	0.09	1.00	0.99	1.00	1.00

**Table 3** One-way ANOVA (p-adjusted values) comparing the mean values of  $\delta^{15}\text{N}$  between Procellariiformes species. **C.b** = *Calonectris borealis*; **M.g** = *Macronectes giganteus*; **O.o** = *Oceanites oceanicus*; **P.a** = *Procellaria aequinoctialis*; **A.g** = *Ardenna gravis*; **P.p** = *Puffinus puffinus*; **T.c** = *Thalassarche chlororhynchos*; **T.m** = *Thalassarche melanophrys*. \* Represents significant values ( $p<0.05$ ).

	<b>C.b</b>	<b>M.g</b>	<b>O.o</b>	<b>P.a</b>	<b>A.g</b>	<b>P.p</b>	<b>T.c</b>
<b>M.g</b>	0.00*	-	-	-	-	-	-
<b>O.o</b>	0.99	0.00*	-	-	-	-	-
<b>P.a</b>	0.07	0.95	0.03*	-	-	-	-
<b>A.g</b>	0.02*	0.99	0.01*	0.99	-	-	-
<b>P.p</b>	0.00*	0.95	0.00*	0.99	0.99	-	-
<b>T.c</b>	0.00*	0.99	0.00*	0.76	0.98	0.72	-
<b>T.m</b>	0.00*	0.99	0.00*	0.99	1.00	0.99	0.93

**Table 4** Results of Mann-Whitney ( $\delta^{13}\text{C}$ ) and Students' T-test ( $\delta^{15}\text{N}$ ) comparing the mean values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ( $p<0.05$ ) between males and females of each Procellariiformes species.

<b>Species</b>	<b>Mann-Whitney (<math>\delta^{13}\text{C}</math>)</b>	<b>Students' t-test (<math>\delta^{15}\text{N}</math>)</b>
<i>Thalassarche chlororhynchos</i>	0.30	0.97
<i>Thalassarche melanophrys</i>	0.41	0.07
<i>Oceanites oceanicus</i>	-	-
<i>Ardenna gravis</i>	0.33	0.62
<i>Calonectris borealis</i>	0.61	0.44
<i>Macronectes giganteus</i>	0.60	0.97
<i>Procellaria aequinoctialis</i>	0.57	0.55
<i>Puffinus puffinus</i>	0.15	0.29

*Oceanites oceanicus* had the largest standard ellipse area (SEAc: 15.1‰<sup>2</sup> and SEAB: 14.3‰<sup>2</sup>, 95% CI: 4.4‰<sup>2</sup> – 34.8‰<sup>2</sup>) followed by *P. aequinoctialis* (SEAc: 10.1‰<sup>2</sup> and SEAB: 11.9‰<sup>2</sup>, 95% CI: 6.1‰<sup>2</sup> – 26.3‰<sup>2</sup>) and *T. chlororhynchos* (SEAc: 9.5‰<sup>2</sup> and SEAB: 3.2‰<sup>2</sup>, 95% CI: 5.7‰<sup>2</sup>–16.9‰<sup>2</sup>) (table 2). On the other hand, *A. gravis* presented

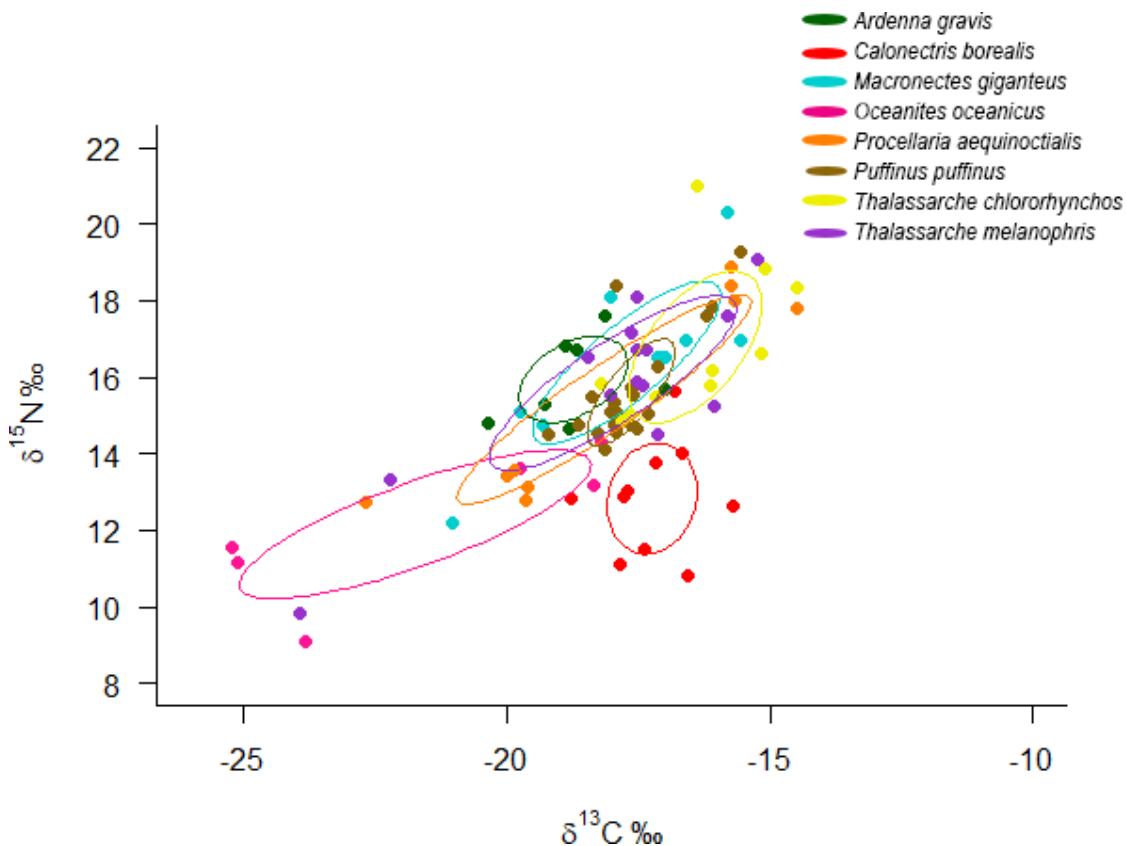
the smaller standard ellipse area (SEAc: 3.9‰<sup>2</sup> and SEAB: 2.9‰<sup>2</sup>, 95% CI: 1.5 – 7.3‰<sup>2</sup>). Based on SEAc estimates, *T. melanophris* has the highest ellipses overlap with *P. aequinoctialis* (8.1‰<sup>2</sup>) and *M. giganteus* (6.6‰<sup>2</sup>), which also overlapped with *P. aequinoctialis* (5.3‰<sup>2</sup>) (Fig 3). The ellipses of both albatross species (*T. chlororhynchos* and *T. melanophris*) overlapped in 3.8‰<sup>2</sup>. The ellipse area estimated for *A. gravis* and *P. puffinus* differed from *P. aequinoctialis* and *M. giganteus* ( $p < 0.05$ ) but overlapped with both albatross species and between each other (0.3‰<sup>2</sup>). Although the mean values of  $\delta^{13}\text{C}$  differed between *A. gravis* and *T. chlororhynchos*, the species ellipses area overlapped in 0.02‰<sup>2</sup>.

When comparing males and females, males of both *T. chlororhynchos* (SEAc: 11.9‰<sup>2</sup> and SEAB: 8.9‰<sup>2</sup>, 95% CI: 0.4 - 22.5‰<sup>2</sup>) and *P. puffinus* (SEAc: 2.6‰<sup>2</sup> and SEAB: 2.9‰<sup>2</sup>, 95% CI: 1.5-5.7‰<sup>2</sup>) presented larger niche width than females (Fig 4). *Puffinus puffinus* males and females had a short overlap of 0.8‰<sup>2</sup> with males showing higher individual variation in  $\delta^{13}\text{C}$ , whereas females varied mostly in  $\delta^{15}\text{N}$ . On the other hand, males and females of *T. chlororhynchos* overlapped 2.5‰<sup>2</sup> of ellipses area, with males presenting potential individual variation in  $\delta^{15}\text{N}$  and females in  $\delta^{13}\text{C}$  values (Fig. 4).

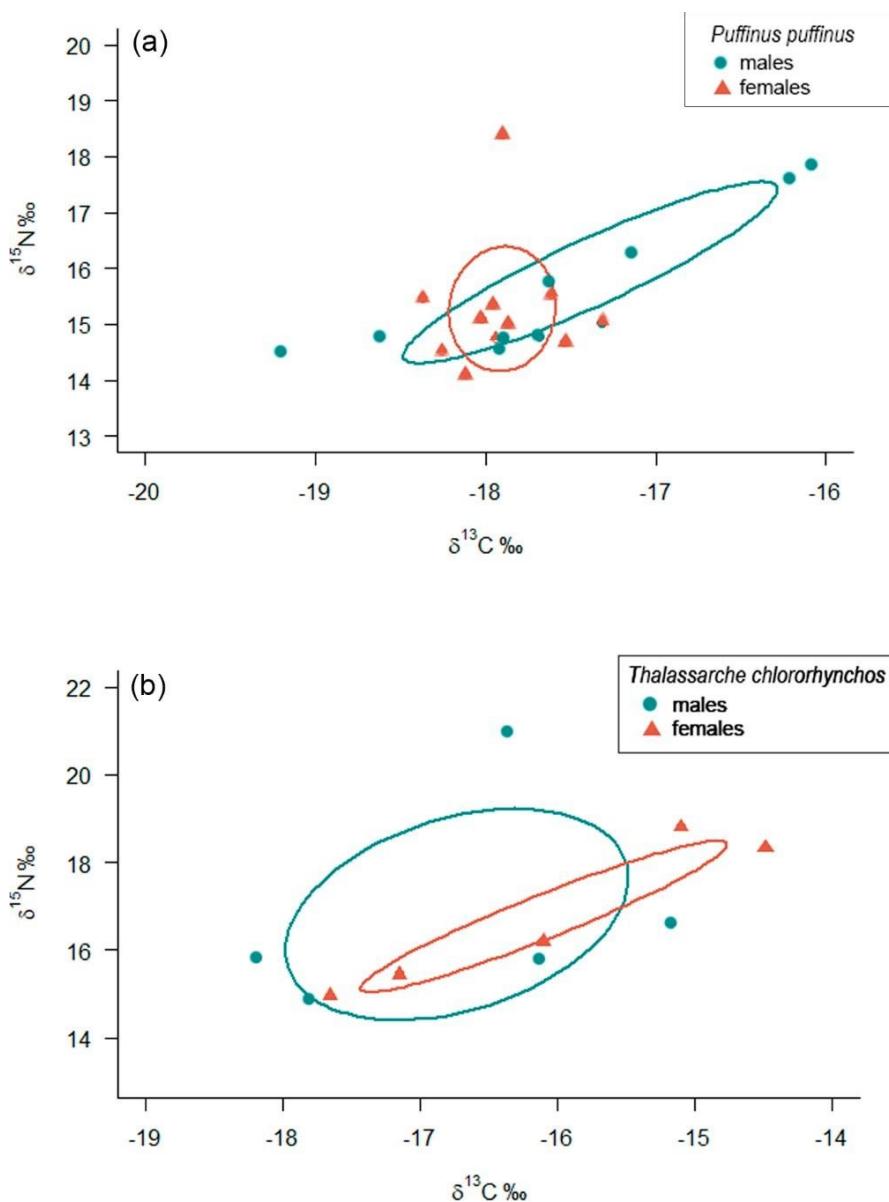
**Table 5:** Ellipse areas (TA; SEA. SEAc; SEAB) and confidence interval (CI) estimated for Procellariiformes species: *Ardenna gravis*, *Calonectris borealis*, *Macronectes giganteus*, *Oceanites oceanicus*, *Procellaria aequinoctialis*, *Puffinus puffinus* (males and females), *Thalassarche chlororhynchos* (males and females) and *Thalassarche melanophris*. Values are presented in ‰<sup>2</sup>. **TA** = total area; **SEA** = standard ellipse area; **SEAC** = standard ellipse area corrected for small samples sizes; **SEAB** = Bayesian standard ellipse area; **CI (95%)** = confidence interval.

Species	TA	SEA	SEAc	SEAB	CI (95%)
<i>Ardenna gravis</i>	4.8	3.3	3.9	2.9	1.5 – 7.3
<i>Calonectris borealis</i>	8.2	3.8	4.3	3.6	1.9 – 7.2
<i>Macronectes giganteus</i>	13.5	6.7	7.6	7.4	3.8 – 15.2
<i>Oceanites oceanicus</i>	13.4	12.1	15.1	14.3	4.4 – 34.8
<i>Procellaria aequinoctialis</i>	15.1	8.8	10.1	11.9	6.1 – 26.3
<i>Puffinus puffinus</i> (both)	8.3	2.4	2.5	2.5	1.7 – 3.9
males	3.9	2.3	2.6	2.6	1.5 – 5.7
females	2.4	1.1	1.3	1.3	0.5 – 1.9
<i>Thalassarche chlororhynchos</i> (both)	11.4	6.4	9.5	6.2	3.2 – 12.7

males	8.9	8.8	11.9	8.9	0.4 – 22.5
females	2.5	2.3	3.1	4.1	1.5 – 11.8
<i>Thalassarche melanophrys</i>	23.5	8.8	7.2	9.7	5.7 – 16.9



**Fig 3**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  biplot illustrating the isotopic niche of *Ardenna gravis*, *Calonectris borealis*, *Macronectes giganteus*, *Oceanites oceanicus*, *Procellaria aequinoctialis*, *Puffinus puffinus*, *Thalassarche chlororhynchos* and *Thalassarche melanophrys* in Santa Catarina coast, southern Brazil. Each point represents an individual and ellipses represent the standard ellipse area corrected for small sample sizes (SEAc).



**Fig 4** δ<sup>13</sup>C and δ<sup>15</sup>N biplot illustrating the isotopic niche of males and females of (a) *Puffinus puffinus* and (b) *Thalassarche chlororhynchos* in Santa Catarina coast, southern Brazil. Each point represents an individual (circle: males; triangle: females) and ellipses represent the standard ellipse area corrected for small sample sizes (SEAc).

## DISCUSSION

To our knowledge, this is the first study to access the isotopic niche partitioning among Procellariiformes species through feathers of stranded individuals at Southwest Atlantic Ocean. The isotope signatures of Procellariiformes species analyzed corresponded to the values expected for Subantarctic to Subtropical waters (Phillip et al. 2009). With exception to *O. oceanicus*, this result suggests that all species analyzed moult in the Subtropical regime of the South Atlantic Ocean, highlighting the importance of this area for the Procellariiformes community. In general, our results corroborated our initial hypothesis, showing a broad isotopic niche overlap for the species of Procellariiformes recognized to interact with fishing vessels. This result is of particular concern, since Procellariiformes represent the most threatened order among birds, accounting for 70% of worldwide seabird longline fishery bycatch (Brothers et al. 1999).

Many seabirds, in addition to being active predators, also capture prey found dead or debilitated on the sea surface. This behavior makes them susceptible to supplement the diet with fishing discards and attempt to steal baits on hooks, which may result in accidental capture by fishing gears (Neves et al. 2006b). *Thalassarche chlororhynchos*, *T. melanophris* and *P. aequinoctialis* depart from their colonies in the Southern Atlantic to the feeding areas around April, inhabiting waters of Southern Brazil during the non-breeding period (Neves et al. 2006a). These three species are also recognized as the main captured by longline fleet in Brazil (Neves et al. 2007, Bugoni et al. 2008) since the periods of higher fishery activities matches these species non-breeding period (Neves et al. 2006a).

Fishery discards can also supplement the diet of seabirds with reliable resources that would not be naturally accessible, and therefore, can result in a spatiotemporal overlap of species that could otherwise segregate at the feeding areas (Jiménez et al. 2017). Therefore, the abundance and predictability of this food resource could be a determinant of the trophic structure for seabirds. The hierarchy in access to discards in seabirds communities is related to the body size (Ballance et al. 1997; Barnes et al. 1997), where the preferential access to feeding discards is toward larger species, such as those of the genus *Thalassarche* and *Macronectes*. As we expected, the main species recognized to interact with fishing vessels (*T. chlororhynchos*, *T. melanophris*, *M. giganteus* and *P.*

*aequinoctialis*) were those presenting the highest isotopic niche overlap, suggesting that similar values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  may be a consequence of consuming fishing discards and baits (e.g Bugoni et al. 2010). However, it is important to consider the possibility that two or more species consuming different resources in the same area can have identical stable isotopes signatures, since different combination of prey species may reflect the same isotopic values for the consumer (Bond and Jones 2009).

Although seabirds can follow fishing vessels in order to obtain food, some species remain around for a short period of time, while others are too small to swallow the baits on hooks, such as *O. oceanicus*, *C. borealis* and *P. puffinus* (Neves et al. 2006b). In this study, *C. borealis* and *O. oceanicus* differed from other species in terms of  $\delta^{15}\text{N}$ , indicating the consumption of prey in the lowest trophic level within the community. This result also suggests that, unlikely other species analyzed, fishery discards are not the main component of their diets. The isotopic signature of blood samples collected from *C. borealis* following fishing vessels in the Southwestern Atlantic Ocean also showed that fishery discards does not play a major role in the species diet (Bugoni et al. 2010).

*Calonectris borealis* and *P. puffinus* perform transequatorial migrations from different breeding colonies in the North Hemisphere to the feeding areas in South Atlantic. The majority of *P. puffinus* world population breed in Britain and Ireland islands, spending the boreal winter off the coast of South America, with the majority of ringing recoveries coming from Brazil (Perrins and Brooke 1976; Thompson 2002; Hamer 2003). However, studies approaching the isotopic niche of these species during the non-breeding period are scarce (De Felipe et al. 2019; Roscales et al. 2011). We firstly expected that *P. puffinus* would segregate the isotopic niche from the species that interact with fishing vessels, but a great isotopic niche overlap was observed between the *P. puffinus* and both species of albatross, suggesting the consumption of preys on the same trophic level and in similar foraging areas.

*Ardenna gravis* also performs transequatorial migration, but toward the feeding areas in the North Hemisphere after breeding in the South Atlantic Ocean (Warham, 1996). *Ardenna gravis* and *P. puffinus* presented similar values of both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , indicating their coexistence in the same area and the consumption of prey in the same trophic level. Moreover, both *A. gravis* and *P. puffinus* differed their isotopic niche from other species

with larger body size and that are strongly recognized to interact with fishing vessels to obtain food (*M. giganteus* and *P. aequinoctialis*), respecting the hierarchy in access to discards, in which individuals with larger body sizes tend to be favored (Ballance et al. 1997; Barnes et al. 1997).

Foraging strategies can vary among species regarding individual preferences, sex, age and breeding status, which can expand the population niche and shape the trophic structure of communities and ecosystems (Annett and Pierotti 1999; Bolnick et al. 2003). Here, we found no significant difference in the mean values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between males and females of Procellariiformes species, indicating the use of similar foraging areas and the consumption of preys on the same trophic level during the non-breeding period. Therefore, males and females of different species may be potentially vulnerable to the same impacts (e.g climatic events, environmental conditions and bycatch). Considering the bycatch of Procellariiformes males and females attending longline fishing vessels in the Southwestern Atlantic Ocean, Bugoni et al. (2011) found that both sexes have similar access to discards and baits, even in species with more pronounced sexual dimorphism in size. Thus, differential at sea distribution of males and females is more likely to explain the skewed adult sex ratio in seabirds bycatch, especially when distant from breeding grounds (Bugoni et al. 2011).

Although no differences between sex were observed for Procellariiformes species, males of *P. puffinus* appeared to have higher individual variation in  $\delta^{13}\text{C}$ , suggesting broader foraging areas than females, which varied mostly in terms of  $\delta^{15}\text{N}$ . On the other hand, males of *T. chlororhynchos* showed higher individual variation in  $\delta^{15}\text{N}$  and females in  $\delta^{13}\text{C}$ . In many cases, the type of prey consumed (Hunter 1987) and sexual segregation is related to the degree of sexual size dimorphism (Catry et al. 2005; Wearmouth and Sims 2008), which can result in the exclusion of the smaller ones from areas and resources preferred by larger individuals, or lead to segregation by specialization (Catry et al. 2005; Phillips et al. 2011). In addition, competition can increase if the availability and abundance of resources during winter is limited, leading to sex differences in order to allow the coexistence of males and females during the non-breeding period (Phillips et al. 2008).

In conclusion, our results indicated higher isotopic niche overlap between species that strongly interact with longline fishery, which suggest the consumption of similar items provided by fishery activities (i.e discards and baits). This perspective is of particular

concern since fishery activities and discards can influence seabirds distribution (Jiménez et al. 2017) and can lead to incidental capture, which is considered the main threat to seabirds populations during the non-breeding period (Neves et al. 2006a). In order to better understand the trophic relationships among Procellariiformes species and individuals, we suggest for future studies to include the SIA of primary producers and potential preys to accurately analyze the isotopic niche partitioning among species, individuals and the contribution of fishery activities on seabirds diet, distribution and isotopic niche partitioning.

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### *Conflicts of interest*

The authors declare no competing interests and that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## **4 CONCLUSÃO GERAL**

Os resultados obtidos aqui indicam a ocorrência de 19 espécies de Procellariiformes em áreas oceânicas adjacentes à costa de Santa Catarina. Dessas espécies, 5 estão ameaçadas de extinção. A ocorrência dessas espécies pode ser inferida através dos registros de encalhes e também pela análise de isótopos estáveis, a qual demonstrou que as espécies que habitam a região durante o período não reprodutivo possuem assinaturas isotópicas correspondentes ao regime Subtropical do Oceano Atlântico Sul. Nesse estudo, encontramos diversos indivíduos com sinais de interação com atividades pesqueiras, além de considerável sobreposição de nicho isotópico entre espécies, o que pode estar relacionado ao consumo de descartes de pesca. Assim, através das abordagens utilizadas aqui, chamamos atenção para a influência das atividades pesqueiras sobre a comunidade de Procellariiformes e para a importância de monitoramentos sistemáticos de encalhe como ferramenta para o estudo de espécies ameaçadas e de difícil acesso.

## **5 CONSIDERAÇÕES FINAIS**

Os albatrozes e petréis são aves pelágicas que habitam a terra, geralmente ilhas remotas, apenas para reprodução. Os Procellariiformes também representam a ordem com maior número de espécies ameaçadas dentre as aves e é durante o período não reprodutivo que se encontram mais suscetíveis a fatores climáticos e impactos antrópicos e são estimadas as maiores taxas anuais de mortalidade do grupo. Atualmente, a captura incidental pela pesca, especialmente a pesca de espinhel, representa a maior ameaça às populações de Procellariiformes. Devido à dificuldade de acessar essas populações de aves marinhas em seu habitat natural, especialmente quando se distribuem amplamente pelo oceano, existe uma carência muito grande de informações sobre a ecologia e distribuição dessas espécies fora do período reprodutivo.

Assim, através do estudo de animais encalhados e análise de isótopos estáveis, neste trabalho pudemos contribuir com informações sobre a ocorrência e ecologia trófica dos Procellariiformes durante o período não reprodutivo no Sul do Brasil. Até onde temos informação, esse trabalho também configura o primeiro estudo a analisar esses aspectos da comunidade de Procellariiformes que habita áreas oceânicas adjacentes à costa de Santa Catarina através da análise de indivíduos encalhados. Através do monitoramento de carcaças realizado pelo PMP-BS pudemos caracterizar a comunidade de

*Procellariiformes*, além de identificar potenciais ameaças antrópicas e investigar a partição de nicho isotópico entre diferentes espécies e também entre machos e fêmeas.

Ressaltamos a importância do monitoramento sistemático de animais encalhados como uma ferramenta para o estudo de espécies de difícil acesso e para obter informações sobre a ecologia e conservação dessas populações em curto prazo. Em geral, o monitoramento de encalhes pode ser mais viável em termos logísticos e financeiros em relação a outros métodos de monitoramento de aves marinhas, representando uma técnica relevante para países cujo financiamento para pesquisas é escasso, como é o caso do Brasil. Além disso, a análise de animais encalhados constitui um método não invasivo de acessar espécies de interesse conservacionista sem gerar distúrbios populacionais pela manipulação e captura de indivíduos.

A análise de isótopos estáveis também configura uma importante ferramenta para acessar a ecologia das populações de aves marinhas. A utilização das penas para essa análise é de extrema relevância, uma vez que elas refletem o habitat e o nível trófico consumido por essas espécies durante o período de muda, sobre o qual as informações são limitadas. A identificação das áreas e recursos utilizados pelos *Procellariiformes* é essencial para subsidiar medidas mitigatórias e estratégias de manejo e conservação, uma vez que essas espécies dependem de áreas chave para descanso, alimentação e descanso ao longo do seu ciclo anual. Assim, ressaltamos a importância do setor oeste do Oceano Atlântico Sul para diferentes espécies de *Procellariiformes* que habitam e forrageiam na região durante o período não reprodutivo.

Entretanto, para melhor compreender as causas dos encalhes as relações tróficas entre as espécies de *Procellariiformes* nós deixamos as seguintes sugestões: (i) incluir a análise de variáveis ambientais, climáticas e oceanográficas que podem influenciar a deriva de carcaça e dinâmica dos encalhes a fim de melhor investigar as potenciais causas de mortalidade e ocorrência de encalhes em áreas específicas; (ii) incluir as potenciais presas consumidas na análise de isótopos estáveis e utilizar modelos de mistura para investigar mais detalhadamente o nível de partição ou sobreposição de nicho isotópico entre espécies e entre machos e fêmeas, analisar a influência da pesca na dieta e distribuição das aves e poder inferir hábitos generalistas ou especialistas para as populações.