TROPHIC ECOLOGY OF LARGE MARINE PREDATORS IN THE SOUTHWEST ATLANTIC

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Contents

Statement of Originality

I hereby certify that this thesis entitled “*Trophic ecology of large marine predators in the Southwest Atlantic*” is an original piece of research and has not previously been submitted for a degree or diploma in any university. This work was prepared and written by me. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person except where due reference is made in the thesis itself. All assistance in the fieldwork and preparation of this thesis has been explicitly acknowledged in the corresponding section. All references and sources of information used in this thesis are listed within.

This work was undertaken in the Marine Predator Research Group in the Department of Biological Sciences at Macquarie University under the supervision of Professor Robert Harcourt (Macquarie University), Dr Vincent Raoult (University of Newcastle and Macquarie University) and Dr David Slip (Taronga Institute of Science and Learning). Financial support was provided by a co-funded scholarship from Macquarie University and *Agencia Nacional de Investigación e Innovación* (Uruguay) and by a grant awarded by PADI Foundation (United States). Fieldwork and sample collection were conducted under permit 252/2018 issued by the National Directorate of Aquatic Resources (DINARA).

Sabrina Riverón Mato
Date: June 29, 2022

Acknowledgements

My PhD journey was not smooth, but I trust it was worth the effort to make it through to the end. Who could have imagined that a global pandemic would arrive mid PhD! My second season of fieldwork in Uruguay was in the early months of 2020, and as a result, I was no longer allowed to return to Australia to analyse my samples and work on the thesis in the lab with my lab mates due to severe travel restrictions. My entire thesis progression was done remotely, for the first 6 months of the pandemic my partner and I were forced to sleep on a mattress on the floor and live and work in a tiny room, as well as deal with health problems and loss of loved ones. The pandemic had wider ramifications, with several critical issues with shipping samples to stable isotope laboratories, which had major staffing problems and inefficiencies that caused terrible delays compounded by communication problems. Looking back now, I can see the enormous difficulties I needed to overcome. For this reason, I do not have enough words to thank those who made this thesis a reality.

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Dedication

I want to dedicate this thesis to my late mother, Maria Luisa, who always encouraged me to pursue my dreams even though they were literally on the other side of the world.

Abstract

Globally marine ecosystems are changing dramatically under pressures from human activities, with potentially major disruption of the ecological roles of higher marine predators. As key players in the structure of marine ecosystems (either by direct or indirect effects on the food chain), loss of, or significant reductions in predators may result in top-down trophic cascades, which may then jeopardise the health of entire ecosystems. Understanding the trophic ecology of higher predators is a fundamental goal if we are to understand their responses to a changing environment. In this thesis, I assessed how different factors affect the trophic niches of higher marine predators. In Chapter Two, I used stable isotope analysis of tissues from two sympatric species with contrasting foraging strategies from the Falkland Islands (South Atlantic) the South American sea lion (*Otaria byronia*) and the South American fur seal (*Arctocephalus australis*), to understand how foraging strategies and competition affect individual specialisation. I found that foraging behaviour influenced the degree of individual specialisation. In the Falklands, the pelagically foraging fur seals feed in a dynamic environment with abundant but similar prey and are specialised populations of generalist individuals. By contrast, the benthically foraging sea lions fed in habitats with diverse but less abundant prey and had more generalist populations composed of highly specialised individuals. In Chapter Three, I tested the plasticity of population isotopic trophic niches in two widely distributed sympatric species (*O. byronia* and *A. australis*) from two contrasting environments, the Falkland Islands and the Río del Plata, Uruguay. There was an unexpected, complete inversion of the population trophic niche size for both species between the two sites. This striking result demonstrates considerable plasticity in the foraging niche and illustrates that predators may show divergent foraging strategies in different environments. Here it appears that when conditions dictate, populations of generalist individuals can result in broad population niches and that, if necessary, populations of specialist

individuals can be confined to very narrow population niches. Finally, in Chapter Four, I explored how morphology (teeth shape and body length) influence trophic niche using a comparative analysis of two sympatric sharks of similar size but contrasting feeding modes. One, the grey nurse shark (*Carcharias taurus*), captures and swallow their prey whole with spear-shaped teeth, and the other, the sevengill shark (*Notorynchus cepedianus*), has multi-cuspid cutting teeth used to serrate larger prey. Using stable isotopes and stomach contents, I show that as body length increases, both species consumed species from higher trophic levels, but non-gape limited sevengill sharks consistently preyed over higher trophic levels and were less restricted by mouth size.

Overall, in this thesis, I hypothesised that differences in individual specialisation were related to prey availability and habitat differences. However, regardless of the degree of individual specialisation displayed by the individuals within a population, the population may be either specialist or generalist depending on the regional characteristics of the environment and associated prey available. Morphological characters play an important role in the predator-prey selection and may be the primary mechanism facilitating resource partitioning in large sympatric predators. Differences in trophic morphological traits, individual specialisation, and regional environmental characteristics on population niche plasticity are all key factors that should be considered to better understand how predators' prey choices shape trophodynamics in marine ecosystems.

Chapter One - General Introduction

1.1. Food webs in a changing environment

A food web describes the trophic interactions between consumers and resources within an ecosystem (de Ruiter et al., 2005). All organisms need energy and nutrients to grow, survive and reproduce; therefore, understanding how the resources are acquired and how each component of a food web interacts is one of the main focuses of trophic ecology (Garvey & Whiles, 2017; Smith & Smith, 2017). As habitat characteristics affect the dynamic and structure of the ecosystems impacting the food web components, how ecosystemic attributes (*e.g.,* nutrient cycle, habitat structure) interplay with feeding interactions is of particular interest in trophic ecology (Timpane-Padgham et al., 2017). Moreover, changes in the degree of interactions between the components of a food web could impact community dynamics and the persistence of populations in an ecosystem. One clear example is how the decrease in abundance of a species, *e.g.* sea otters, can drive cascading effects and change the whole structure of an entire ecosystem, *e.g.* loss of kelp forest (*e.g.*, Duffy, 2003; Estes et al., 2011). Improving our understanding of food web functioning provides information on the dynamics and structure of ecosystems, and how these properties are affected by environmental variability and anthropogenic disturbances (de Ruiter et al., 2005).

Trophic niche is an essential concept in ecology. It refers to the trophic resources used by an organism or a group of individuals (*e.g.*, populations, species), determining their role in the ecosystem (Lunghi et al., 2018). The trophic niche is shaped by a combination of factors, which can be divided into biotic and abiotic factors (Figure 1.1). Biotic factors are those associated with the living organisms in a food web and can be divided into intrinsic and extrinsic factors.

Intrinsic factors are inherent characteristics of individuals, including physiological, morphological, and behavioural features, that enhance or constrain individuals' feeding abilities (Bolnick et al., 2003; Horn & Ferry-Graham, 2006). Extrinsic factors refer to the biotic interactions with other organisms of the food web (*e.g.*, intra and interspecific competition, predation). For example, a decrease in available resources may lead to an increase in competition, which may change a population's trophic niche (Svanbäck & Bolnick, 2007). Finally, abiotic factors are associated with physical characteristics of the environment, such as salinity and temperature, which determine, in part, the presence, distribution and abundance of prey and their consumers in the ecosystem (*e.g.*, Castillo et al., 1996; Bailleul et al., 2007; Domenici et al., 2007). Abiotic factors vary both temporally and regionally, which can result in variations in the trophic niche of species across either scale (*e.g.*, Drago et al., 2010; Ciancio et al., 2021). These variations are more pronounced in marine ecosystems than in terrestrial ones, making them highly dynamic environments and more susceptible to rapid environmental changes such as climate change (Gattuso et al., 2015; Antão et al., 2020). Therefore, a better understanding of how organisms and food webs behave in response to variations of factors that may affect their trophic niches has become increasingly important to predict the future response of populations to environmental changes.

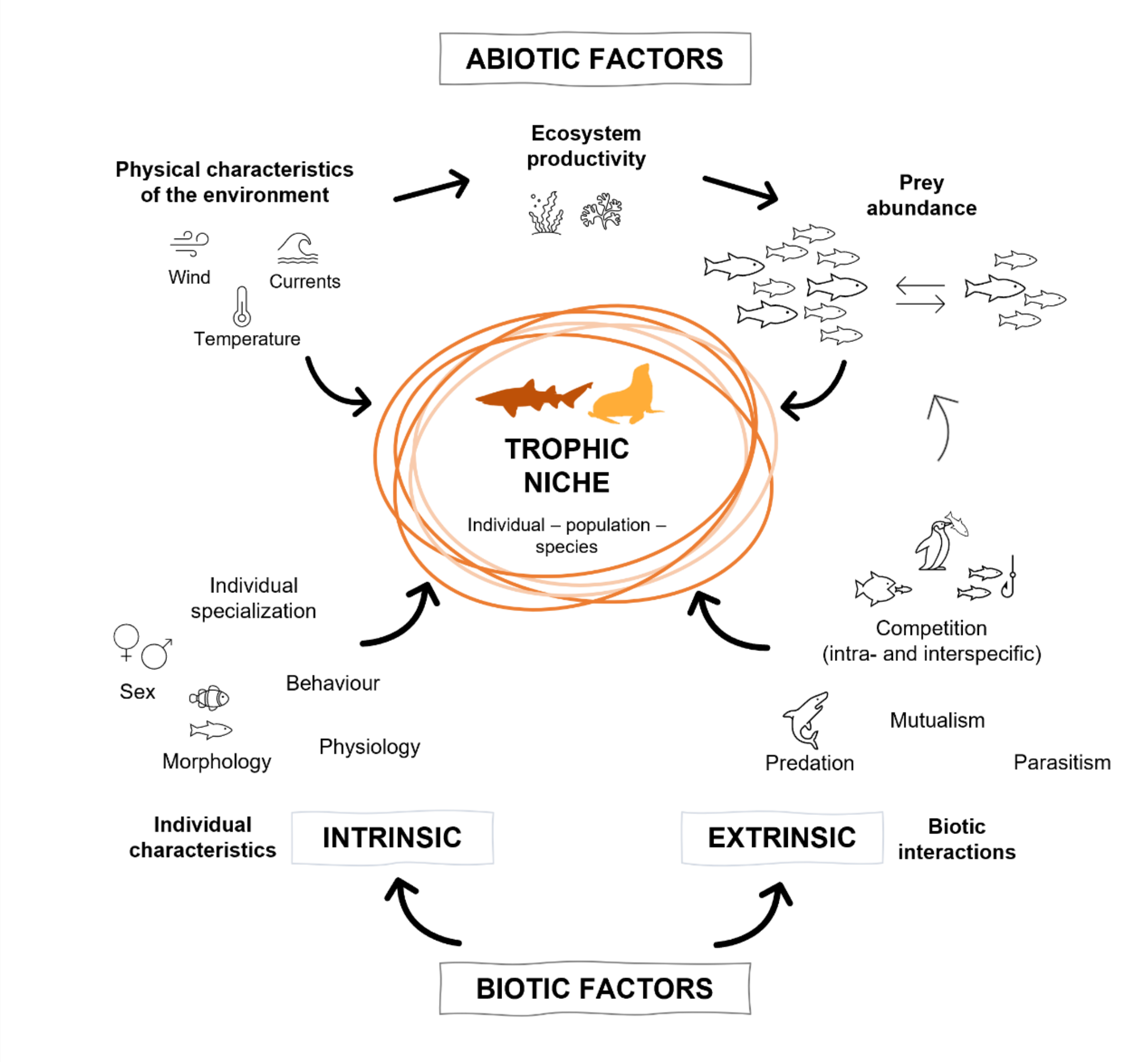

Figure 1.1. **Biotic and abiotic factors are shapers of trophic niches**. Abiotic factors refer to the physical characteristics of the environment (*e.g.*, temperature, salinity, winds). These parameters, at least partly, determine the presence, abundance, and distribution of prey in the environment, affecting consumers' trophic niches. Abiotic factors are differentiated into intrinsic and extrinsic; the former refers to individual characteristics that may enhance or constrain individuals' feeding abilities. Biotic extrinsic factors are the interactions between organisms of the ecosystem, such as resource competition (intra and interspecific), predation, and mutualisms, among others. The combination of these factors delineates and shapes the trophic niches of organisms.

1.2. Role of high-order marine predators in marine ecosystems

High-order marine predators play a crucial role in ecosystem engineering, affecting food web dynamics in different ways (reviewed by Estes et al., 2016). Predators can affect prey abundance and distribution through its direct predation and/or the costs of antipredator behavioural responses, also known as the risk effect (Lima & Dill, 1990; Creel & Christianson, 2008). In addition, many marine predators are highly mobile organisms that use multiple habitats, playing an essential role in connecting ecosystems and redistributing nutrients between different habitats (*e.g.*, McCauley et al., 2012; Williams et al., 2018; Kiszka et al., 2022). As marine ecosystems are changing dramatically under pressures from human activities, a potentially major disruption of the ecological roles of higher-order marine predators is evident (*e.g.,* Hughes, 2000; Learmonth et al., 2006; Schumann et al., 2013; Hobday et al., 2015). As key players in the structure of marine ecosystems (either by direct or indirect effects on the food chain), loss of, or significant reductions in predators may result in top-down trophic cascades, which may then jeopardise the health of entire ecosystems (Heithaus et al., 2008; Steneck, 2012). Understanding the trophic ecology of marine predators is a fundamental goal if we are to understand their responses to a changing environment.

1.3. The Patagonian Continental Shelf and its marine predators

The Patagonian continental shelf is located on the Atlantic coast of South America (23-55°S) and is characterised by different topographic, climatic, and oceanographic characteristics along its range (Piola et al., 2018) (Figure 1.2). It is one of the widest and flattest shelves in the world, with substantial variation in width across its latitudinal range (10–800 km) (Longhurst, 2007; Gil et al., 2019). The area encompasses one of the largest estuaries (Río de la Plata estuary) and one of the most energetic regions of the world ocean (Brazil-Falkland Confluence), making it a highly dynamic ecosystem affected by climate phenomena like El Niño-La Niña (Mianzan et al., 2001; Acha et al., 2008; Piola & Matano, 2017). Moreover, it is a highly productive marine region that sustains multiple fisheries and a diverse community of marine predators (Croxall & Wood, 2002; Brazeiro et al., 2003; Acha et al., 2008; Mandiola et al., 2016; Baylis et al., 2019a; García-Alonso et al., 2019). These include large sharks as well as significant colonies of pinnipeds (> 100,000 individuals).

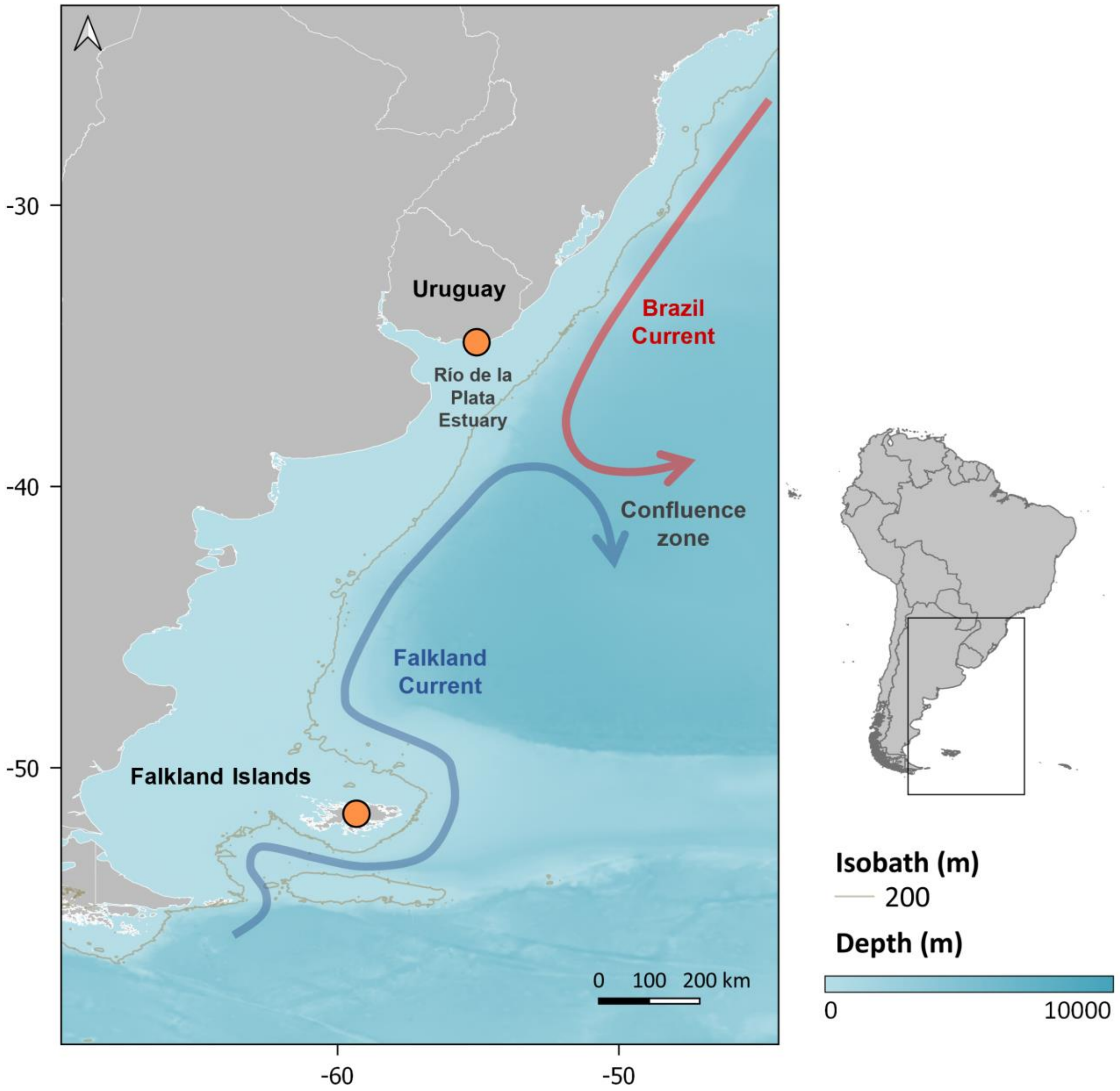

Figure 1.2. Map showing the Patagonian continental shelf, including: the two studied localities (Uruguay and Falkland Islands, orange dots), Río de la Plata estuary, the two main currents in the area (Falkland and Brazil Currents) with their respective confluence zone (Brazil-Falkland Confluence), and the 200 meters isobath.

In many of coastal areas around the world, pinniped (fur seals and sea lions) and shark species play an important role in marine ecosystems. Four key species of large-bodied marine predators inhabit the coastal region of the Patagonian Shelf, most of them in sympatry (co-occurring in the same geographical area, and therefore, frequently encounter one another;

Futuyma & Kirkpatrick, 2017). The sevengill shark (*Notorynchus cepedianus*) and grey nurse shark (*Carcharias taurus*), are widely distributed coastal species in almost every ocean basin (Ebert et al., 2021). Both species interact with local fisheries through commercial exploitation, bycatch and/or competition for resources. For the Patagonian shelf region there are neither local nor regional estimates of their abundance.

South American sea lions (*Otaria byronia*) and South American fur seals (*Arctocephalus australis*) are sympatric marine predators distributed on both Atlantic and Pacific coast of South America. On the Atlantic coast, they can be found from southern Brazil to Tierra de Fuego (including the Falkland Islands), while in the Pacific coast, from Tierra de Fuego up to Perú (Crespo & de Oliveira, 2021; Crespo et al., 2021). *Otaria byronia* is a species also known as *Otaria flavescens*; in this thesis, I will be using the scientific name recommended by the Marine Mammal Society, which is *Otaria byronia* (Committee on Taxonomy, 2022). Both species have breeding colonies close to the latitudinal extremes of the Patagonian shelf, in Uruguay (35°S) and the Falklands Islands (52°S) (Páez, 2006; Baylis et al., 2019a; Crespo & de Oliveira, 2021; Crespo et al., 2021). These colonies, approximately 1,850 km apart, are in two contrasting environments: an oceanic archipelago closer to the southern extreme of their Atlantic range (Falkland Islands) and an estuarine environment near the northern one (Uruguay). Both species were commercially exploited during the early 20th century until the 90s (Crespo et al., 2021; Crespo & de Oliveira, 2021). However, despite their recovery in the last decades, the current abundance of South American sea lions is far less than their pre-harvesting period (Romero et al., 2017). The latest population estimates of South American fur seals based on pup abundance data is ~120,000 individuals in the Falkland Islands (Baylis et al., 2019b; Crespo & de Oliveira, 2021) and ~350,000 individuals in Uruguay (Enrique Páez, personal communication, 2022, National Directorate of Aquatic Resources-DINARA). The later estimate made in Uruguay disagrees with a previous estimate of ~150,000 individuals made through aerial surveys (Franco-Trecu et al., 2019). The high discrepancy is probably the result of using different estimation methodologies and performing the surveys at different periods of the breeding season. In this thesis, I will consider the estimations based on pup abundance, due to it comparability with the Falkland Islands data and the methodology is more accurate for the type of geographical topology of the Uruguayan colonies. Abundance estimates of South American sea lion population in the Falkland Islands is ~7,500 individuals (Crespo et al., 2012), and between 13,000-14,000 individuals in Uruguay (Páez, 2006; Crespo et al., 2012). Both species move throughout the entire southwestern Atlantic and individuals from Uruguayan populations are repopulating areas in northern and central Patagonia (Crespo et al., 2015**;** Giardino et al., 2016). In addition, these species have different foraging strategies; South American fur seals are known to be generalist, pelagic foragers (Thompson et al., 2003), while South American sea lions are specialist, benthic foragers (Thompson et al., 1998; Riet-Sapriza et al., 2013; Baylis et al., 2015). This scenario facilitates the study of the trophic ecology of marine predators, both at a local and regional scale, and facilitates the assessment of how geographical differences may shape the feeding ecology of species with different foraging strategies. Moreover, both species are central place foragers, and their reproductive success is strongly associated with local food availability (Jönsson, 1997; Costa, 2008), making them good indicators of change.

1.4. Thesis aims

The overall objective of the thesis is to improve our understanding of how different abiotic and biotic factors of marine ecosystems may affect the trophic niches of marine predators. In particular, I focus on how intrinsic factors of individuals, such as morphology and individual specialisation, and extrinsic factors like regional differences may shape the trophic niche of marine predators. This new information will improve our understanding of how trophic niches vary along different biotic and abiotic gradients and help predict trophic niche responses in a changing environment. To achieve this goal, the main aims of this thesis are:

1. Explore the trophic ecology of sympatric species with different foraging modes by quantifying individual specialisation (Chapter Two).
2. Assess niche plasticity of two widely distributed sympatric species in two contrasting environments under different biotic and abiotic factors (Chapter Three).
3. Assesses how different trophic morphologies (teeth shape and body size) affect prey choice selectivity in large marine predators with contrasting foraging strategies (Chapter Four).

1.5. Thesis structure

This thesis is composed of five chapters. The first chapter is a General Introduction to the thesis. The General Introduction is followed by three data chapters, each of which addresses one of the main objectives of the thesis (Chapters Two, Three and Four). Chapter Five provides a synthesis of the findings and provides future recommendations. Finally, as an Appendix, a fourth manuscript is included. This final manuscript originated from an knowledge gap identified during the thesis project design, which could be answered using my data set. This work is associated with the processing of samples for stable isotope analysis.

In **Chapter Two**, I explore how foraging strategies may affect individual specialisation, and ultimately how individual specialization influences the population trophic niche in two sympatric marine predators (Riverón et al., 2021). I compare two pinniped species, the South American sea lion (*Otaria byronia*) and South American fur seal (*Arctocephalus australis*) which differ in foraging strategies (benthic and pelagic forager, respectively). I use stable isotope analysis of carbon and nitrogen ($\delta^{13}C$, $\delta^{15}N$) of consecutive, longitudinal segments of whiskers to determine the degree of individual specialisation associated with the foraging strategy of each predator. I discuss how pelagic and benthic foraging strategies could be influenced by environmental factors and explain the patterns found.

Trophic ecology studies commonly assume some degree of stability in the trophic niches of different populations of the same species, mainly due to the relative stability of species-specific characteristics. However, some variation in trophic niche metrics between population niches has been observed and primarily attributed to regional differences in resource availability. In **Chapter Three**, I assess the degree of niche plasticity between two contrasting environments of South American sea lion and South American fur seal, two widely distributed pinniped species. I use stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ to characterise their isotopic niche in both regions, comparing their relative isotopic niche size. The results prove unexpected and have implications for the way we perceive trophic niche plasticity in the marine environment. I discuss the ecological implication of these findings and propose some scenarios to explain the degree of niche plasticity found in the two species.

In **Chapter Four**, I further explore the effect of intrinsic individual factors that could constrain the trophic niche in marine predators, using two shark species as case-study. I focus on morphological characteristics, specifically, tooth shape and body length, and how they shape the trophic niche of two sympatric species of sharks of similar body size (and by implication gape) but contrasting feeding modes. The grey nurse shark (*Carcharias taurus*) captures prey with grasping teeth, while the sevengill shark (*Notorynchus cepedianus*) has multi-cuspid cutting teeth used to serrate larger prey. I combine stable isotope analysis ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) and stomach content analyses to elucidate how trophic morphologic shapes food web structure. I discuss how morphology might facilitate resource partitioning in large sympatric predators and its importance to ecological studies involving prey choice.

Finally, the **Appendix (Chapter 6)** fills a knowledge gap to do with the effect of lipid extraction on stable isotope values of sulfur, carbon, and nitrogen ($\delta^{34}S$, $\delta^{13}C$, $\delta^{15}N$) (Riverón et al., 2022). This is the first study to assess how a commonly used procedure in stable isotopes analysis may alter sulfur isotopic values in skin samples of pinnipeds and muscle and liver samples of shark species. I demonstrate that lipid extraction can lead to significant variation in sulfur values in tissues with high lipid content. I propose a plausible explanation for this significant variation in sulfur isotopic values arising from lipid extraction and provide correction factors.

This thesis follows Macquarie University's guidelines for "Thesis by Publications". Chapter Two and the Appendix Chapter have been already peer-reviewed and published. Published papers have been reformatted to minimise differences in style throughout the thesis manuscript, and the main text remains unchanged.

1.6. References

Acha, M. E., Mianzan, H., Guerrero, R., Carreto, J., Giberto, D., Montoya, N., & Carignan, M. (2008). An overview of physical and ecological processes in the Rio de la Plata Estuary. *Continental Shelf Research*, *28*, 1579–1588. https://doi.org/10.1016/j.csr.2007.01.031

Antão, L. H., Bates, A. E., Blowes, S. A., Waldock, C., Supp, S. R., Magurran, A. E., Dornelas, M., & Schipper, A. M. (2020). Temperature-related biodiversity change across temperate marine and terrestrial systems. *Nature Ecology and Evolution*, *4*, 927–933. https://doi.org/10.1038/s41559-020-1185-7

Bailleul, F., Charrassin, J. B., Monestiez, P., Roquet, F., Biuw, M., & Guinet, C. (2007). Successful foraging zones of southern elephant seals from the Kerguelen Islands in relation to oceanographic conditions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *362*, 2169–2181. https://doi.org/10.1098/rstb.2007.2109

Baum, J. K., & Worm, B. (2009). Cascading top-down effects of changing oceanic predator abundances. *Journal of Animal Ecology*, *78*(4), 699–714. https://doi.org/10.1111/j.1365-2656.2009.01531.x

Baylis, A. M. M., Orben, R. A., Arnould, J. P. Y., Peters, K., Knox, T., Costa, D. P., & Staniland, I. J. (2015). Diving deeper into individual foraging specializations of a large marine predator, the southern sea lion. *Oecologia*, *179*(4), 1053–1065. https://doi.org/10.1007/s00442-015-3421-4

Baylis, A. M. M., Tierney, M., Orben, R. A., Warwick-Evans, V., Wakefield, E., Grecian, W. J., Trathan, P., Reisinger, R., Ratcliffe, N., Croxall, J., Campioni, L., Catry, P., Crofts, S., Boersma, P. D., Galimberti, F., Granadeiro, J., Handley, J., Hayes, S., Hedd, A., Masello, J. F., Montevecchi, W. A., Pütz, K., Quillfeldt, P., Rebstock, G. A., Sanvito, S., Staniland, I. J., & Brickle, P. (2019a). Important at-sea areas of colonial breeding marine predators on the southern Patagonian shelf. *Scientific Reports*, *9*(8517), 1–13. https://doi.org/10.1038/s41598-019-44695-1

Baylis, A. M. M. , Orben, R. A., Arkhipkin, A. A. , Barton, J., Brownell Jr, R. L., Staniland, I. J., & Brickle, P. (2019b). Re-evaluating the population size of South American fur seals and conservation implications. *Aquatic Conservation: Marine and Freshwater Ecosystems, 29,* 1988–1995. https://doi.org/10.1002/aqc.3194

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., Forister, M. L., & Forister. (2003). The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist*, *161*(1), 1–28. https://doi.org/10.2307/3078879

Brazeiro, A., Acha, M., Mianzan, H., Gómez-Erache, M., & Fernández, V. (2003). Aquatic priority areas for the conservation and management of the ecological integrity of the Rio de la Plata and its maritime front. In *Technical Report PNUD Project: GEF RLA/99/G31* (Issue March 2014).

https://doi.org/10.1093/jnci/djr261

Castillo, J., Barbieri, M. A., Gonzalez Castillo, A., Barbieri, J., & Gonzalez, A. (1996). Relationships between sea surface temperature, salinity, and pelagic fish distribution off northern Chile. In *ICES Journal of Marine Science* (Vol. 53). https://academic.oup.com/icesjms/article/53/2/139/791578

Ciancio, J. E., Yorio, P., Buratti, C., Colombo, G. Á., & Frere, E. (2021). Isotopic niche plasticity in a marine top predator as indicator of a large marine ecosystem food web status. *Ecological Indicators*, *126*, 107687. https://doi.org/10.1016/j.ecolind.2021.107687

Committee on Taxonomy. (2022). List of marine mammal species and subspecies. *Society for Marine Mammalogy*, www.marinemammalscience.org, consulted on November 29th, 2022.

Costa, D. P. (2008). A conceptual model of the variation in parental attendance in response to environmental fluctuation: foraging energetics of lactating sea lions and fur seals. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *17*, S44–S52. https://doi.org/10.1002/aqc

Creel, S., & Christianson, D. (2008). Relationships between direct predation and risk effects. *Trends in Ecology and Evolution*, *23*(4), 194–201. https://doi.org/10.1016/j.tree.2007.12.004

Crespo, E. A., Oliva, D., Dans, S. L. & Sepúlveda, M. (2012). Estado de situación del lobo marino común en su área de distribución. Editorial Universidad de Valparaíso, Valparaíso, Chile.

Crespo, E. A., Schiavini, A., García, N. A., Franco-Trecu, V., Goodall, R. N. P., Rodríguez, D., Stenghel Morgante, J., & de Oliveira, L. R. (2015). Status, population trend and genetic structure of South American fur seals *Arctocephalus australis* in southwestern Atlantic waters. *Marine Mammal Science*, *31* (3), 866–890. https://doi.org/10.1111/mms.12199

Crespo, E. A., & de Oliveira, L. R. (2021). South American fur seal (*Arctocephalus australis*, Zimmerman, 1783). In G. Heckel & Y. Schramm (Eds.), *Ecology and conservation of pinnipeds in Latin America* (First edition, pp. 13–29). Springer International Publishing. https://doi.org/10.1007/978-3-030-63177-2_2

Crespo, E. A., de Oliveira, L. R., & Sepúlveda, M. (2021). South American sea lion (*Otaria flavescens*, Shaw 1800). In G. Heckel & Y. Schramm (Eds.), *Ecology and conservation of pinnipeds in Latin America* (First edition, pp. 93–118). Springer International Publishing. https://doi.org/10.1007/978-3-030-63177-2_6

Croxall, J. P., & Wood, A. G. (2002). The importance of the Patagonian Shelf for top predator species breeding at South Georgia. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *12*, 101–118. https://doi.org/10.1002/aqc.480

de Ruiter, P. C., Wolters, V., & Moore, J. C. (2005). Dynamic food webs: Multispecies assemblages, ecosystem development, and environmental change. In P. C. de Ruiter, V. Wolters, & J. C. Moore

(Eds.), *Dynamic Food Webs* (First edition). Academic Press.

Domenici, P., Claireaux, G., & McKenzie, D. J. (2007). Environmental constraints upon locomotion and predator-prey interactions in aquatic organisms: An introduction. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *362*, 1929–1936. https://doi.org/10.1098/rstb.2007.2078

Drago, M., Cardona, L. L., Crespo, E. A., García, N. A., Ameghino, S., & Aguilar, A. (2010). Change in the foraging strategy of female South American sea lions (Carnivora: Pinnipedia) after parturition. *Scientia Marina*, *74*(3), 589–598. https://doi.org/10.3989/scimar.2010.74n3589

Duffy, J. E. (2003). Biodiversity loss, trophic skew and ecosystem functioning. *Ecology Letters*, *6*, 680–687. https://doi.org/10.1046/j.1461-0248.2003.00494.x

Ebert, D. A., Dando, M., & Fowler, S. (2021). *Sharks of the world: A complete guide*. Princeton University Press.

Estes, J. A., Heithaus, M., McCauley, D. J., Rasher, D. B., & Worm, B. (2016). Megafaunal impacts on structure and function of ocean ecosystems. *Annual Review Of Environment and Resources*, *41*, 83–116. https://doi.org/10.1146/annurev-environ-110615-085622

Estes, J. A., Terborgh, J., Brashares, J. S., Power, M. E., Berger, J., Bond, W. J., Carpenter, S. R., Essington, T. E., Holt, R. D., Jackson, J. B. C., Marquis, R. J., Oksanen, L., Oksanen, T., Paine, R. T., Pikitch, E. K., Ripple, W. J., Sandin, S. A., Scheffer, M., Schoener, T. W., Shurin, J. B., Sinclair, A. R. E., Soulé, M. E., Virtanen, R., & Wardle, D. A. (2011). Trophic downgrading of planet Earth. *Science*, *333*(6040), 301–306. https://doi.org/10.1126/science.1205106

Franco-Trecu, V., Drago, M., Grandi, M. F., Soutullo, A., Crespo, E. A. & Inchausti, P. (2019). Abundance and population trends of the South American fur seal (*Arctocephalus australis*) in Uruguay. Aquatic Mammals, 45 (1), 48-55. https://doi.org/10.1578/AM.45.1.2019.48

Futuyma, D.J., & Kirkpatrick, M. (2017). *Evolution*. Fourth Edition. Sinauer Associates Inc.

García-Alonso, J., Lercari, D., & Defeo, O. (2019). Río de la Plata: A neotropical estuarine system. In E. Wolanski, J. W. Day, M. Elliott, & R. Ramachandran (Eds.), *Coasts and estuaries: The future* (First edition, pp. 45–56). Elsevier Inc. https://doi.org/10.1016/B978-0-12-814003-1.00003-4

Garvey, J. E., & Whiles, M. R. (2017). *Trophic ecology* (First edition). CRC Press, Taylor & Francis Group.

Gattuso, J. P., Magnan, A., Billé, R., Cheung, W. W. L., Howes, E. L., Joos, F., Allemand, D., Bopp, L., Cooley, S. R., Eakin, C. M., Hoegh-Guldberg, O., Kelly, R. P., Pörtner, H. O., Rogers, A. D., Baxter, J. M., Laffoley, D., Osborn, D., Rankovic, A., Rochette, J., Sumaila, U. R., Treyer, S., & Turley, C. (2015). Contrasting futures for ocean and society from different anthropogenic CO_2 emissions scenarios. *Science*, *349*(6243). https://doi.org/10.1126/science.aac4722

Giardino, G. V., Mandiola, M. A., Bastida, J., Denuncio, P. E., Bastida, R. O., & Rodríguez, D. H. (2016).

Travel for sex: long-range breeding dispersal and winter haulout fidelity in southern sea lion males. *Mammalian Biology*, *81* (1), 89–95. https://doi.org/10.1016/j.mambio.2014.12.003

Gil, M. N., Giarratano, E., Barros, V., Bortolus, A., Codignotto, J. O., Schenke, R. D., Góngora, G. M. E., Lovrich, G., Monti, A. J., Pascual, M., Rivas, A. L., & Tagliorette, A. (2019). Southern Argentina: The Patagonian Continental Shelf. In C. Sheppard (Ed.), *World Seas: an Environmental Evaluation* (Second edition, Vol. 1, pp. 783–811). Elsevier. https://doi.org/10.1016/B978-0-12-805068-2.00040-1

Heithaus, M. R., Frid, A., Wirsing, A. J., & Worm, B. (2008). Predicting ecological consequences of marine top predator declines. *Trends in Ecology and Evolution*, *23*(4), 202–210. https://doi.org/10.1016/j.tree.2008.01.003

Hobday, A. J., Arrizabalaga, H., Evans, K., Nicol, S., Young, J. W., & Weng, K. C. (2015). Impacts of climate change on marine top predators: Advances and future challenges. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 113, 1-13. https://doi.org/10.1016/j.dsr2.2015.01.013

Horn, M. H., & Ferry-Graham, L. A. (2006). Feeding mechanisms and trophic interactions. In L.G. Allen, D. J. Pondella, & M. H. Horn (Eds.), *The Ecology of Marine Fishes: California and Adjacent Waters* (First edition, pp. 387–410). University of California Press. https://doi.org/10.1525/california/9780520246539.003.0014

Hughes, L. (2000). Biological consequences of global warming: is the signal already apparent? *Trends Ecology and Evolution*, 15(2), 56-61. https://doi.org/10.1016/S0169-5347(99)01764-4

Jönsson, K. I. (1997). Capital and income breeding as alternative tactics of resource use in reproduction. *Oikos*, *78*, 57–66. https://doi.org/10.2307/3545800

Kiszka, J. J., Woodstock, M. S., & Heithaus, M. R. (2022). Functional roles and ecological importance of small cetaceans in aquatic ecosystems. *Frontiers in Marine Science*, *9*, 803173. https://doi.org/10.3389/fmars.2022.803173

Learmonth, J. A., Macleod, C. D., Santos, M. B., Pierce, G. J. & Robinson, R. A. (2006). Potential effects of climate change on marine mammals. *Oceanography and Marine Biology: An Annual Review*, 44, 431-464.

Lima, S. L. & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, 68, 619–640. https://doi.org/10.1139/z90-092

Longhurst, A. R. (2007). The Atlantic Ocean. In A. R. Longhurst (Ed.), *Ecological geography of the sea* (Second edition, pp. 131–273). Academic Press. https://doi.org/https://doi.org/10.1016/B978-012455521-1/50010-3

Lunghi, E., Cianferoni, F., Ceccolini, F., Mulargia, M., Cogoni, R., Barzaghi, B., Cornago, L., Avitabile, D., Veith, M., Manenti, R., Ficetola, G. F., & Corti, C. (2018). What shapes the trophic niche of European plethodontid salamanders? *PLoS ONE*, *13*(10), e0205672. https://doi.org/10.1038/sdata.2018.83

Mandiola, A., Alemany, D., Dassis, M., Pon, J. P. S., Prosdocimi, L., Mianzan, H., Acha, E. M., Rodrı, D., Favero, M., Copello, S., & Leo, A. P. De. (2016). Key ecological areas and opportunities for marine conservation. *Marine Science*, *73*(6), 1579–1588.

McCauley, D. J., Young, H. S., Dunbar, R. B., Estes, J. A., Semmens, B. X., & Micheli, F. (2012). Assessing the effects of large mobile predators on ecosystem connectivity. *Ecological Applications*, *22*(6), 1711–1717. https://doi.org/10.1890/11-1653.1

Mianzan, H., Acha, E., Guerrero, R., Macchi, G., & Bremec, C. (2001). The Río de la Plata estuary, Argentina-Uruguay. In U. Seeliger & B. Kjerfve (Eds.), *Coastal marine ecosystems of Latin America. Ecological studies* (Vol. 144, pp. 185–204). Springer.

Páez, E. (2006). Situación de la administración del recurso lobos y leones marinos en Uruguay. In R. Menafra, L. Rodríguez-Gallego, F. Scarabino, & D. Conde (Eds.), *Bases para la conservación y el manejo de la costa uruguaya* (First edition, pp. 577–583). Vida Silvestre Uruguay.

Piola, A. R., & Matano, R. P. (2017). Ocean Currents: Atlantic Western Boundary—Brazil Current/Falkland (Malvinas) Current. *Reference Module in Earth Systems and Environmental Sciences*, 0–7. https://doi.org/10.1016/b978-0-12-409548-9.10541-x

Piola, A. R., Palma, E. D., Bianchi, A. A., Castro, B. M., Dottori, M., Guerrero, R. A., Marrari, M., Matano, R. P., Möller, O. O., & Saraceno, M. (2018). Physical oceanography of the SW Atlantic shelf: A review. In M. S. Hoffmeyer, M. E. Sabatini, F. P. Brandini, D. L. Calliari, & N. H. Santinelli (Eds.), *Plankton ecology of the southwestern Atlantic: From the subtropical to the subantarctic realm* (First edition, pp. 37–56). Springer. https://doi.org/10.1007/978-3-319-77869-3_2

Riet-Sapriza, F. G., Costa, D. P., Franco-Trecu, V., Marín, Y., Chocca, J., González, B., Beathyate, G., Louise Chilvers, B., & Hückstädt, L. A. (2013). Foraging behavior of lactating South American sea lions (*Otaria flavescens*) and spatial–temporal resource overlap with the Uruguayan fisheries. *Deep Sea Research Part II: Topical Studies in Oceanography*, *88–89*, 106–119. https://doi.org/10.1016/j.dsr2.2012.09.005

Riverón, S., Raoult, V., Baylis, A. M. M., Jones, K. A., Slip, D. J., & Harcourt, R. G. (2021). Pelagic and benthic ecosystems drive differences in population and individual specializations in marine predators. *Oecologia*, *196*(3), 891–904. https://doi.org/10.1007/s00442-021-04974-z

Riverón, S., Raoult, V., Slip, D. J., & Harcourt, R. G. (2022). Lipid extraction has tissue-dependent effects on isotopic values (δ^{34}S, δ^{13}C, δ^{15}N) from different marine predators. *Rapid Communications in Mass Spectrometry*. https://doi.org/10.1002/rcm.9346

Romero, M. A., Grandi, M. F., Koen-Alonso, M., Svendsen, G., Ocampo-Reinaldo, M., García, N. A., Dans, S. L., González R., & Crespo E. A. (2017). Analysing the natural population growth of a large marine mammal after a depletive harvest. *Scientific Reports*, *7*, 5271. https://doi.org/10.1038/s41598-017-05577-6

Schumann, N., Gales, N. J., Harcourt, R. G., & Arnould, J. P. Y. (2013). Impacts of climate change on Australian marine mammals. *Australian Journal of Zoology*, 61, 146-159. https://doi.org/10.1071/ZO12131

Smith, T. S., & Smith, R. L. (2017). *Elements of ecology* (Eighth edition). Pearson Benjamin Cummings.

Steneck, R. S. (2012). Apex predators and trophic cascades in large marine ecosystems: Learning from serendipity. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(21), 7953–7954. https://doi.org/10.1073/pnas.1205591109

Svanbäck, R., & Bolnick, D. I. (2007). Intraspecific competition drives increased resource use diversity within a natural population. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1611), 839–844. https://doi.org/10.1098/rspb.2006.0198

Thompson, D., Duck, C. D., McConnell, B. J., & Garrett, J. (1998). Foraging behaviour and diet of lactating female southern sea lions (*Otaria flavescens*) in the Falkland Islands. *Journal of Zoology*, *246*, 135–146. https://doi.org/10.1017/S0952836998010024

Thompson, D., Moss, S., & Lovell, P. (2003). Foraging behaviour of South American fur seals *Arctocephalus australis*: extracting fine scale foraging behaviour from satellite tracks. *Marine Ecology Progress Series*, *260*, 285–296. https://doi.org/10.3354/meps260285

Timpane-Padgham, B. L., Beechie, T., & Klinger, T. (2017). A systematic review of ecological attributes that confer resilience to climate change in environmental restoration. *PLoS ONE*, *12*(3), 1–23. https://doi.org/10.1371/journal.pone.0173812

Williams, J. J., Papastamatiou, Y. P., Caselle, J. E., Bradley, D., & Jacoby, D. M. P. (2018). Mobile marine predators: An understudied source of nutrients to coral reefs in an unfished atoll. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1875). https://doi.org/10.1098/rspb.2017.2456

Chapter Two

Pelagic and benthic ecosystems drive differences in population and individual specializations in marine predators

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2.1. ABSTRACT

Individual specialization, which describes whether populations are comprise of dietary generalists or specialists, has profound ecological and evolutionary implications. However, few studies have quantified individual specialization within and between sympatric species that are functionally similar but have different foraging modes. We assessed the relationship between individual specialization, isotopic niche metrics and foraging behaviour of two marine predators with contrasting foraging modes: pelagic foraging female South American fur seals (*Arctocephalus australis*) and benthic foraging female South American sea lions (*Otaria byronia)*. Stable isotope analysis of carbon and nitrogen was conducted along the length of adult female vibrissae to determine isotopic niche metrics and the degree of individual specialization. Vibrissae integrated time ranged between 1.1 and 5.5 years, depending on vibrissae length. We found limited overlap in dietary niche-space. Broader population niche sizes were associated with higher degrees of individual specialization, while narrower population niches with lower degrees of individual specialization. The degree of individual specialization was influenced by pelagic and benthic foraging modes. Specifically, South American fur seals, foraging in dynamic pelagic environments with abundant but similar prey, comprised specialist populations composed of generalist individuals. In contrast, benthic South American sea lions foraging in habitats with diverse but less abundant prey had more generalist populations composed of highly specialized individuals. We hypothesize that differences in specialization within and between populations were related to prey availability and habitat differences. Our study supports growing literature highlighting that individual specialization is a critical factor in shaping ecological niche of higher marine predators.

2.2. INTRODUCTION

Understanding marine higher predators' trophic ecology is a fundamental goal in ecology because marine higher predators shape the structure and function of marine ecosystems, either by direct or indirect effects on the food chain (Estes et al., 2016). Marine higher predators, such as pinnipeds, occupy a diverse range of habitats but are broadly characterised by either pelagic or benthic foragers. Pelagic and benthic marine systems have different resource dynamics and productivity pathways that influence the life-history traits of the consumers they support (Duffill Telsnig et al., 2019). Specifically, the structure and function of food webs is one of the factors that influences consumer populations (Frederiksen et al., 2006; Thompson et al., 2012). For marine predators, pelagic food webs tend to have low species diversity but highly abundant prey, and the primary source of carbon in these food webs is phytoplankton (Agnew, 2002; Takai et al., 2007). In contrast, benthic food webs have multiple different carbon sources (*e.g.,* terrestrial, benthic), with higher species richness but lower abundance of individuals within each species (Cury et al., 2000; Snelgrove, 2001). The higher number of benthic and demersal species in benthic habitat is attributed to its increased habitat heterogeneity compared to pelagic environments, especially in inshore areas (*e.g.*, Cherel et al., 2011). Given this dichotomy, we hypothesized that pelagic predators will prey on fewer prey species but of higher abundance, while benthic foragers will feed on a more diverse prey base, with specialization potentially arising due to competition for the limited resources. Therefore, at the individual level, benthic foragers should have higher levels of individual specialization, while pelagic predators should be generalists. Consumer preferences for resources are determined by the availability of resources in the environment, and the predators foraging modes are based on factors such as habitat and individuals' natural history (*i.e.* sex, ontogenetic stage) (Duffill Telsnig et al., 2019). Although individual preferences within a population are likely to drive population-scale niche variations, comparing dietary preferences and individual specializations between benthic and pelagic foragers has rarely been tested in ecological studies (*e.g.,* Rossman et al., 2015; Baylis et al., 2015a). Understanding how marine predators adapt to differences in prey availability of dissimilar marine habitats will allow us to predict future responses as these environments continue to change.

At a population level, differences in the trophic ecology of pelagic and benthic foragers may be quantified by characterizing the ecological niche in a multidimensional space (Hutchinson, 1957). Niche size is determined by the variability within a set of ecological metrics for a population, which describes its population resource use (Bearhop et al., 2004; Polechová & Storch, 2019). By using niche size to characterize trophic ecology, populations can be separated into specialists when they have narrower niches or generalists when they have broader niches (Peers et al., 2012). Generalist populations with broader niches are likely favoured in heterogeneous and stable environments (*e.g.*, benthic ecosystems), whereas specialist populations with narrower niches are favoured in highly dynamic ecosystems and homogenous environments (*e.g.*, pelagic ecosystems) (Futuyma & Moreno, 1988; Kassen, 2002). Ultimately, populations may be comprised of specialist individuals, or generalist individuals, or a combination of both. Individual specialization has important ecological and evolutionary implications because it generates variability within populations, ultimately affecting the population niche metrics. Intra-population niche variation provides populations with the capacity to adapt to changing environments (Bolnick et al., 2003; Svanbäck & Bolnick, 2007).

Stable isotope ratios are widely used to investigate both the ecology of a species and to infer population and individual specialization (*e.g.*, Newsome et al., 2010; Hussey et al., 2012; Kernaléguen et al., 2015). The chemical composition of an organism is directly influenced by what it consumes and where its lives (Newsome et al., 2007), which makes tissue isotopic ratios an effective tool in trophic ecology. Nitrogen isotope ratios ($\delta^{15}N$) are used as a proxy of the trophic level of organisms (Post, 2002) and carbon isotope ratios ($\delta^{13}C$) are used to determine the source of primary production of carbon, thereby providing information on foraging modes strategies and locations (*e.g.,* offshore vs inshore, benthic vs pelagic) (Newsome et al., 2007). Pelagic sources are known to typically have lower $\delta^{13}C$ values than benthic sources, allowing to assess differences in the foraging habitats of consumers (*e.g.*, Cherel & Hobson, 2007; Ceia et al., 2018). Stable isotope analysis is a more robust tool to infer individual temporal consistencies in diet-resource use than traditional dietary proxies (*e.g*., stomach content analysis, scats analysis). The latter only provides a snapshot of the individual degree of specialization (Araújo et al., 2007), while different animal tissues integrate dietary information over different temporal ranges and stable isotopes allow us to assess degrees of individual specialization. Within metabolically active tissues (Bearhop et al., 2004), there are short-term tissues, those that integrate dietary information from weeks to a few months (1-2) (*e.g.*, plasma and liver) and long-term tissues, which integrate dietary data from several months to years (*e.g.*, muscle and bone). Metabolically inert tissues, such as whiskers, remain unchanged once they have been synthesized, providing trophic information about the specific time period they were formed (Newsome et al., 2010). If this inert tissue grows continually and is retained, longitudinal sampling of the tissue can provide long-term information and reveal the trophic history of an individual and by deduction, their degree of specialization (Newsome et al., 2010; Shiffman et al., 2012; Raoult et al., 2019).

South American fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria byronia*), are sympatric breeding otariids that have different foraging modes. Specifically, adult female fur seals are typically characterized as pelagic foragers (Harcourt et al., 2002; Thompson et al., 2003), while adult female sea lions are benthic foragers (Thompson et al., 1998; Riet-Sapriza et al., 2013; Baylis et al., 2015a). Accordingly, these species provide a useful model to explore how individual specialization might vary by foraging mode in functionally equivalent species, which could provide insights into the factors that drive dietary specialization. Given adult females of both species are income breeding central place foragers, foraging trip duration is limited by the fasting ability of nutritionally dependent offspring (Jönsson, 1997; Costa, 2008). The average lactation length for both species is about 10 months, and during this time, foraging trips of lactating females become longer as the pup grows older (Baylis et al., 2015b; Jones et al., 2020). Therefore, during lactation adult females must compete for resources around a central place, which presumably leads to increased intraspecific competition (Costa, 2008). In pinnipeds, stable isotope analysis of vibrissae (also known as whiskers) contain dietary information assimilated over several years, providing a simple and reliable method by which to investigate their trophic ecology (*e.g.*, Newsome et al., 2009; Lowther et al., 2013; McHuron et al., 2016).

Here, we explore the trophic ecology of sympatric breeding species with different foraging modes by quantifying individual specialization. Specifically, our aims were to (1) estimate niche sizes and the degree of resource partitioning at a population level, and (2) the degree of individual specialization at the intra-population level. From there we will assess the relationship between individual specialization and population niche size in order to discuss the role of foraging strategy and competition on individual specialization. In so doing, we will better understand the role that individual variability plays in the trophic ecology of marine predators.

2.3. MATERIALS AND METHODS

Study Site

The study was conducted in the Falkland Islands, South Atlantic (Figure 2.1), where both species forage over the Patagonian shelf and shelf slope (Thompson et al., 2003; Baylis et al., 2015a, 2018). The majority of adult female sea lions were sampled from Big Shag Island (*n* = 27), the largest South American sea lion breeding colony in the Falkland Islands (Baylis et al., 2015b), from February to April over four separate years between 2011 and 2017. Female sea lions were also sampled at Kelp Island (*n* = 2), Turn Island (*n* = 1) and Cape Dolphin (*n* = 1). Female South American fur seals were sampled at two breeding colonies during austral winter in 2015 (North Fur Island (*n* = 5) and Volunteer Rocks (*n* = 4)), and one breeding colony in austral winter in 2018 (Bird Island, *n* = 13) (Figure 2.1). Adult female sea lions from which whiskers were collected foraged in either inshore (coastal) or offshore waters (outer Patagonian shelf) based on tracking data (Baylis et al., 2015a, 2016). As a result, data for South American sea lions were grouped based on habitat use (coastal-offshore and coastal-inshore).

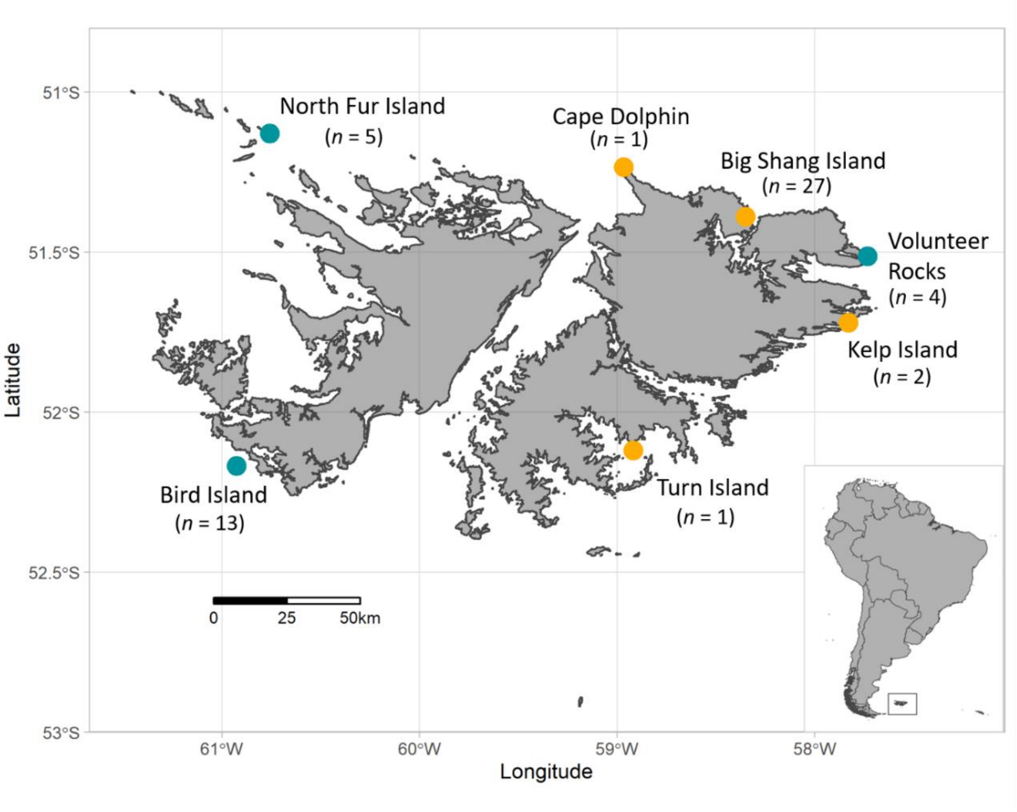


Figure 2.1. Sample locations of South American fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria byronia*) at the Falkland Islands in the South Atlantic. South American sea lion study colonies (orange filled circles): Big Shag Island (*n* = 27), Kelp Island (*n* = 2), Turn Island (*n* = 1) and Cape Dolphin (*n* = 1). South American fur seal study colonies (cyan filled circles): Volunteer Rocks (*n* = 4), North Fur Island (*n* = 5) and Bird Island (*n* = 13).

Sample collection

Whiskers were collected as part of biologging studies (Baylis et al., 2015a, 2016, 2018). Nursing adult females were selected at random and chemically restrained (Baylis et al., 2015c). The longest whisker from the right side of the snout was removed by snipping it as close to the skin as possible using cutting pliers. The samples were labelled and stored dry in plastic bags until laboratory analysis. Standard Length (TL) of adult females was measured whenever possible according to the Committee on Marine Mammals (1967).

Stable isotope analysis

Vibrissae growth rate estimates for South American fur seal adult females are 0.08 mm per day (de Lima et al., 2019). However, there are no specific studies of vibrissae growth rate of South American sea lion females. Consequently, a mean growth rate of 0.11 mm/day^{-1} from Steller sea lions (Hirons et al., 2001) was used for this species. Because whiskers from adults usually reach an average of 10 centimetres in length, they can integrate trophic information over multiple years.

Whiskers were individually cleaned, placed in an ultrasonic bath of distilled water for 5 minutes and dried using 95% ethanol (Kernaléguen et al., 2012). Each whisker was then inspected under a microscope for any remaining contaminants and, if necessary, the cleaning process repeated. Vibrissae were cut into 5 mm long consecutive segments starting from the proximal (facial) end. Each fragment corresponded to a time period of 62 days for South American fur seals and 45 days for South American sea lions according to the growth rates used. Based on the requirements of the isotope ratio mass spectrometer, the target mass for each segment was 0.5 mg (minimum mass to reliably secure an isotopic measurement). To achieve the target mass, it was necessary to sub-sample each 5 mm section from the proximal end of each segment. Finally, samples were packed in tin containers and sent for analysis.

Stable isotope values of $\delta^{13}C$ and $\delta^{15}N$ of samples from 2011, 2013, 2014 and 2015 were determined by a Carlo-Erba elemental analyser interfaced with a Finnigan Delta Plus XP mass spectrometer (Light Stable Isotope Lab, University of California Santa Cruz, Santa Cruz, USA). Samples from 2017 and 2018 were analysed by a Thermo-Fisher-Scientific Delta XP Plus Isotope Ratio Mass Spectrometer (Scottish Universities Environmental Research Centre, Scotland, United Kingdom). Stable isotope ratios were measured in part per mil (‰) deviation from international standards (Vienna-Pee Dee Belemnite for carbon and atmospheric air N_2 for nitrogen). Measurement precision (standard deviation) was based on within-run replicate measures of the laboratory standards (Light Stable Isotope Lab: pugel, and Scottish Universities Environmental Research Centre: GEL, ALAGEL and GLYGEL). Samples published in Baylis et al. (2015a) had a precision of 0.06 ‰ for $\delta^{13}C$ and 0.08 ‰ for $\delta^{15}N$, samples analysed in Baylis et al. (2016, 2018), had a precision of 0.03‰ for $\delta^{13}C$ and 0.06‰ for $\delta^{15}N$. New samples analysed specifically for this paper, had a measurement precision of < 0.17‰ and < 0.22‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Data analysis

Adult female South American sea lion and South American fur seal $\delta^{13}C$ and $\delta^{15}N$ isotope values were compared using linear mixed effect models (LME), with individual included as a random effect and species as a fixed effect. We used a low order correlation structure (corARMA, $p = 2$) to account for temporal autocorrelation between successive vibrissae samples. Independent slopes and intercepts were considered for each individual and each species. As South American sea lion females show distinctive intra-population foraging modes (coastal-offshore and coastal-inshore) identified by telemetry and isotopic data (Baylis et al., 2015a, 2016), the same procedure was conducted to compare between these modes (using foraging modes as fixed effect). Potential differences among colonies of South American fur seals (Baylis et al., 2018) were not tested as the sample size was too low to generate reliable statistical results. To fit the linear mixed effect models, we used the function '*lme*' included in the R package '*nlme*'. The best model fit was selected using the Akaike's Information Criterion (AIC) and, visual exploration of the residual plots and the raw data. The model was adjusted separately for $\delta^{13}C$ and $\delta^{15}N$ isotope values as response variables. In addition, to test for differences among species and South American sea lion foraging modes we used a Wilcoxon Rank Sum Test on mean $\delta^{13}C$ and $\delta^{15}N$ values for each individual. Normality and homogeneity of variances of the isotopic data were previously tested using the Shapiro-Wilks and the Levene test, respectively.

To assess resource partitioning between and within species we quantified isotopic niche size and overlap using the R package '*nicheROVER*'. Isotopic niche size for each population was defined as a probabilistic region of 95%, consisting of the most likely values of the two-dimensional stable isotope measurements. This was developed using a Bayesian framework with a Normal-Inverse-Wishart (NIW) with a default 'non-informative' prior (Swanson et al., 2015). Markov Chain Monte Carlo (MCMC, with default parameters) estimation of the overlap between species was used to estimate the probability that a randomly drawn individual from one species would be found in the same niche as another, and vice versa (Swanson et al., 2015). The overlap probability was reported as the mean of the posterior distribution and 95% credible intervals for each pairwise group comparison. In addition, as South American sea lion females show distinctive intra-population foraging strategies (coastal-offshore and coastal-inshore, Baylis et al. 2015a, 2016), we also conducted this analysis grouping the data in three clusters (offshore-fur seals, coastal-offshore sea lions and coastal-inshore sea lions). Bayesian estimation of the isotopic niche space of each species (Standard ellipse area, SEA) was calculated from carbon and nitrogen isotopic values using 100,000 posterior draws in the R package SIBER (Jackson et al., 2011). We used the mean isotopic values for each individual to conduct these analyses, as sequential segments of each whisker are not independent and so do not comply with the assumption of sample independence.

Specialist individuals were defined by Bolnick et al. (2003) as when their trophic niche is substantially narrower than the mean population's niche for reasons not attributable to difference in sex, age, or measurable morphological characters associate to their trophic habits (*e.g.*, body size, mouth width, etc.). We used stable isotope values to calculate the specialization index (S) to estimate the degree of individual specialization (Roughgarden, 1972; Bolnick et al., 2002), based on isotopic variation within and between individuals of a population. The Within Individual Component (WIC) is the variance of the isotopic signal along each whisker, while the Between Individual Component (BIC) is calculated as the total variance between individuals of the sampled populations (Roughgarden, 1972). The specialization index (S) is defined as:

$$S = \frac{WIC}{TNW}$$

where Total Niche Width (TNW) is the sum of the WIC and BIC. Accordingly, S values vary from 0 to 1, with 1 representing a complete overlap between the individual and population isotopic niche and so representing an extreme generalist individual. Lower values of S represent lower inter-individual overlap and higher degrees of specialization. We considered a specialist individual to be an individual that occupied < 50% of the total niche of the sampled population, or a specialization index < 0.5 (Hückstädt et al., 2012). S was calculated separately for each species and for each isotope ($\delta^{13}C$ and $\delta^{15}N$). In addition, S was estimated for the two foraging strategies (coastal-inshore and coastal-offshore) of southern female sea lions. Since S was estimated from isotopic values, it is not possible to determine whether the degree of individual specialization is due to diet, foraging area or both. Morphological differences in body size may provide access to greater depths for larger animals allowing them to access more diverse prey items. To account for this variable and ensure that we were measuring individual specialization as defined by Bolnick et al. (2003), we assessed the relationship between the individual isotopic niche specialization (*S*) and the total animal length (as a proxy of their body size) using a linear regression model for each species, and carbon and nitrogen separately.

2.4. RESULTS

We analysed 22 whiskers from South American fur seals, which ranged from 60–160 mm (mean ± SD; 105 ± 22.5 mm) in length, represented between 2.1–5.5 years per individual, and provided an average of 21 ± 4.5 segments per individual. Thirty-one (31) whiskers from South American sea lions were analysed, which ranged from 45–210 mm (122.5 ± 40 mm) in length, represented 1.1–5.2 years per individual, and produced an average of 24 ± 7.8 segments per individual. Total length of females ranged from 121 to 147 cm in South American fur seals and from 158 to 202 cm in South American sea lions.

There were significant differences in mean isotope values between adult female South American fur seals and South American sea lions, with South American sea lions having significantly higher $\delta^{13}C$ values (−13.7 ± 0.7 ‰ vs. −15.4 ± 0.2 ‰, respectively) and $\delta^{15}N$ values (16.4 ± 1.0 vs. 14.2 ± 0.4 ‰, respectively) (Wilcoxon's rank sum test, *p*-value < 0.001 for both $\delta^{13}C$ and $\delta^{15}N$). Previously identified intra-specific foraging groups of female South American sea lions (coastal-offshore vs coastal-inshore) (Baylis et al., 2015b, 2016), were confirmed with the isotopic data, consolidating the use of two intra-specific groups with different foraging behaviour for subsequent analyses (Figure 2.2). Adult females that foraged inshore were significantly more enriched in ^{13}C (−12.6 ± 0.2 ‰) and ^{15}N (17.7 ± 1.0 ‰) than those that foraged offshore (^{13}C: −14.0 ± 0.4 ‰, ^{15}N: 16.0 ± 0.5 ‰) (Wilcoxon's Rank-Sum test, *p*-value < 0.001 for both ^{13}C and ^{15}N).

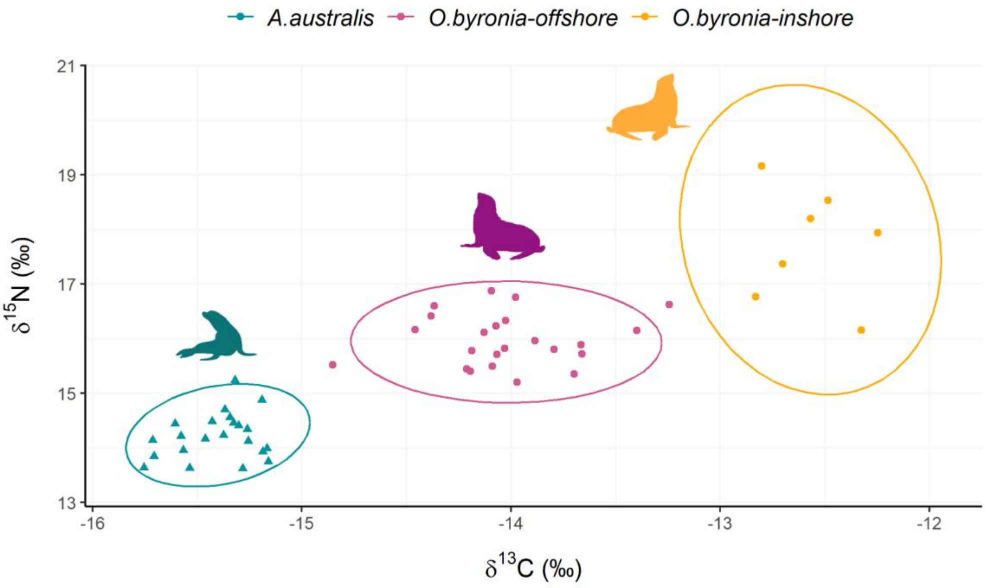


Figure 2.2. Isotope bi-plots of $\delta^{15}N$ and $\delta^{13}C$ values of South American fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria byronia*). Including the two intra-specific groups of *O. byronia* with different foraging behaviour, inshore and offshore ecotype. Each point represents the mean value for an individual's whisker. Solid lines represent the 95% normal confidence ellipses for each group considered.

Isotopic niche size and overlap

The isotopic niche of South American fur seals (1.4 ± 0.3 ‰^{2}) was six times smaller than South American sea lions (9.2 ± 1.6 ‰^{2}). South American sea lion that foraged offshore had narrower niche sizes (3.0 ± 0.63 ‰^{2}) than those that foraged inshore (3.7 ± 1.6 ‰^{2}) (Figure 2.2). Moreover, the inshore group showed higher variability in isotopic values (Online Resource, Figure 2.S1). The probability of overlap between the isotopic niches approached zero for each of the groups (Table 2.1; Online Resource, Figure 2.S2).

Bayesian estimation of the isotopic niche space of the two species shows that South American fur seals females are specialized at the population level, with a narrower iso-space compared to the female South American sea lion population (Figure 2.3). The latter was characterized by a wider isotopic niche space *i.e.* they form a generalist population.

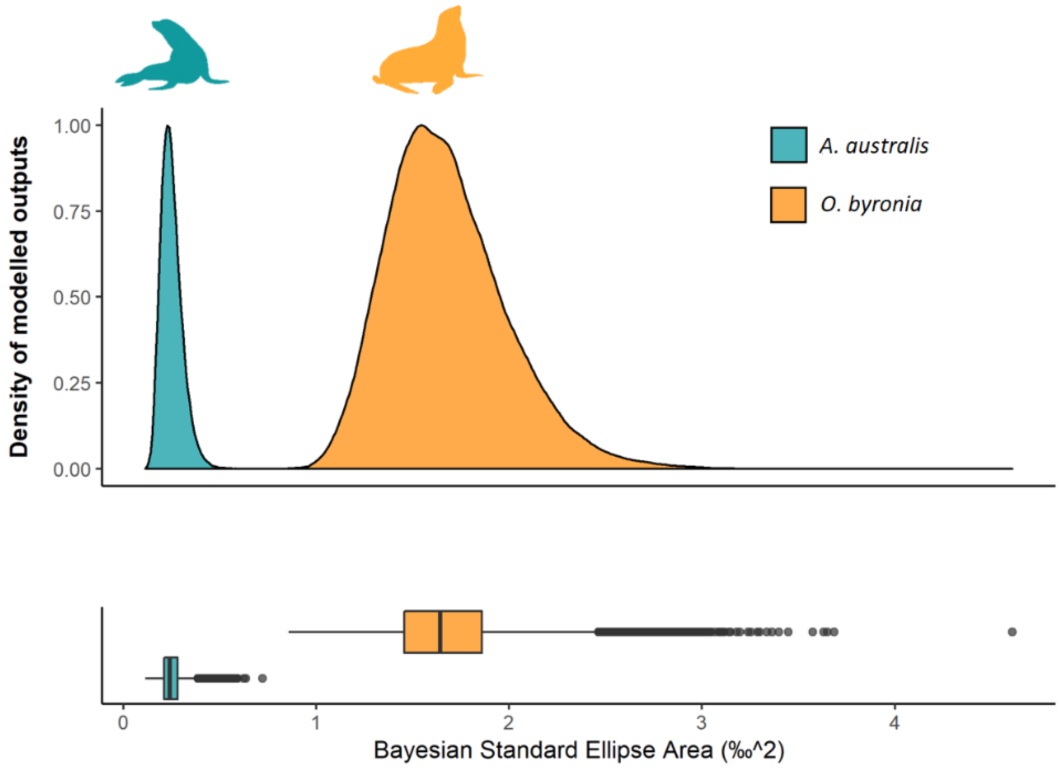


Figure 2.3. Density plot obtained from a Bayesian estimation of the isotopic niche space (Bayesian Standard Ellipse Area) calculated from the carbon and nitrogen isotopic values of South American fur seals (*Arctocephalus australis*) and South American sea lion (*Otaria byronia*). Peaks indicate the most frequently isotopic niche space modelled by the Bayesian estimation. Boxplot representing the median, inter-quartile range (rectangle), 95% range (continuous line) and outliers (filled points).

Table 2.1. Isotopic niche overlap (%) between South American fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria byronia*) females. Values are the probability (posterior means and 95% credible intervals) that an individual from one group (rows) is found within the niche area of the other group (columns), generated by "*nicheROVER*" (Swanson et al., 2015).

	A. australis	***O. byronia* – offshore**	***O. byronia* – inshore**
A. australis	NA	0.07 (0.00 – 0.00)	0.00 (0.00 – 0.00)
***O. byronia* – offshore**	0.05 (0.00 – 1.00)	NA	0.30 (0.00 – 1.00)
***O. byronia* – inshore**	0.00 (0.00 – 0.00)	0.16 (0.00 – 2.00)	NA

Individual isotopic niche specialization

At the individual level, the specialization index value of female South American fur seals (Figure 2.4) for both carbon (0.81 ± 0.08) and nitrogen (0.74 ± 0.07), indicated that South American fur seal females were generalists, with low variability among individuals. By contrast, South American sea lion females were characterized by a high degree of specialization, with specialization indexes for both carbon (0.31 ± 0.16) and nitrogen (0.22 ± 0.11) lower than 0.5, with higher variability among individuals (Figure 2.4). Time series of $\delta^{13}C$ and $\delta^{15}N$ isotope values of each individual in both species show relative long-term stability (over many years) in their foraging habits (Online Resource, Fig. 2.S3, 2.S4). There were exceptions for two South American sea lion females, which had more generalist (> 0.5) specialization indexes. Specifically, one individual (GPS1_2013) that foraged offshore based on tracking data (Baylis et al., 2016), showed a decrease in carbon isotopic signal along the whisker suggesting that this individual had switched from foraging in inshore to offshore habitats (Fig. 2.S4). The other individual, South American sea lion (6074_2011), reflected in their carbon isotopic signal along the whisker that it uses both offshore and inshore habitats, switching between them (Fig. 2.S4). No significant relationship was found between the total animal length of each individual and its individual specialization index ($p > 0.05$) for either species.

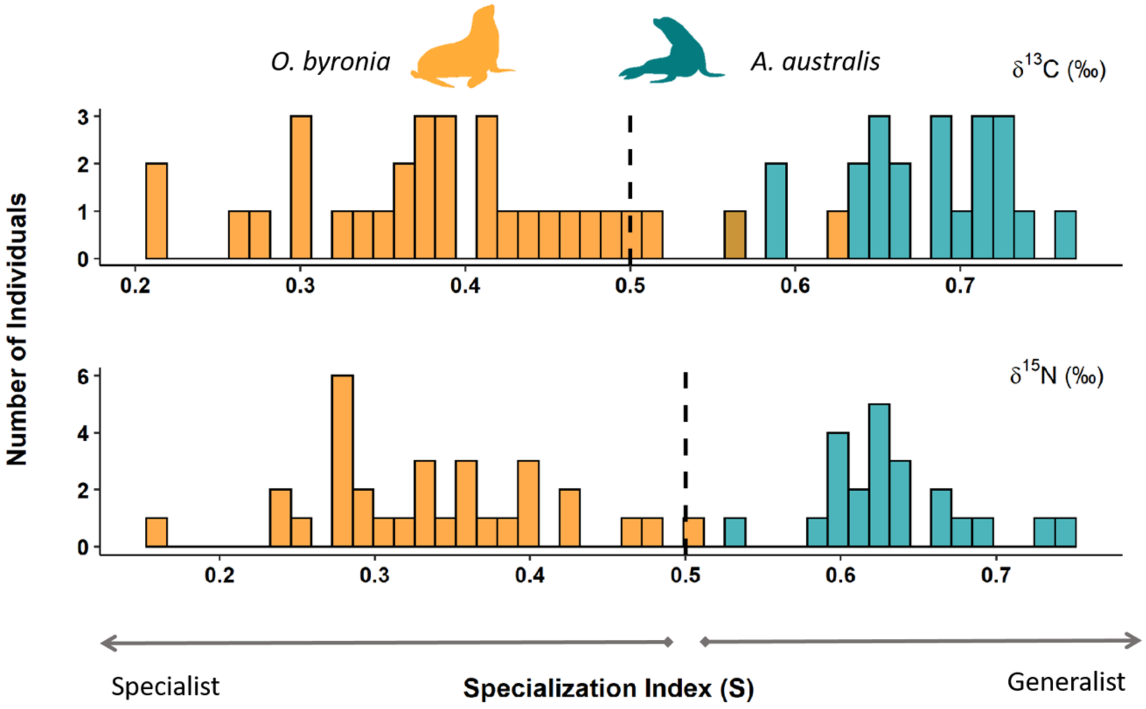


Figure 2.4. Specialisation index (*S*) for individual adult female South American fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria byronia*) from $\delta^{13}C$ (a) and $\delta^{15}N$ (b) isotopic values. The frequency distribution reveals that all South American fur seals females were generalist individuals, while South American sea lions had high degrees of specialisation, with two exceptions (GPS1_2013 and 6074_2011) classified as generalist individuals. A specialisation index of 1 classifies an individual as an isotopic niche generalist while an index closer to 0 is a highly specialised individual.

When we focus on South American sea lion female population and considered its foraging groups separately (Figure 2.5), coastal inshore individuals are generalist based on its $\delta^{13}C$ values (S = 0.9 ± 0.1) but specialists if we based on $\delta^{15}N$ values (S = 0.3 ± 0.1). However, offshore coastal animals appear to be comprised of both specialist and generalist individuals from the $\delta^{13}C$ and $\delta^{15}N$-based specialization indices (S = 0.5 ± 0.2 and 0.5 ± 0.1, respectively).

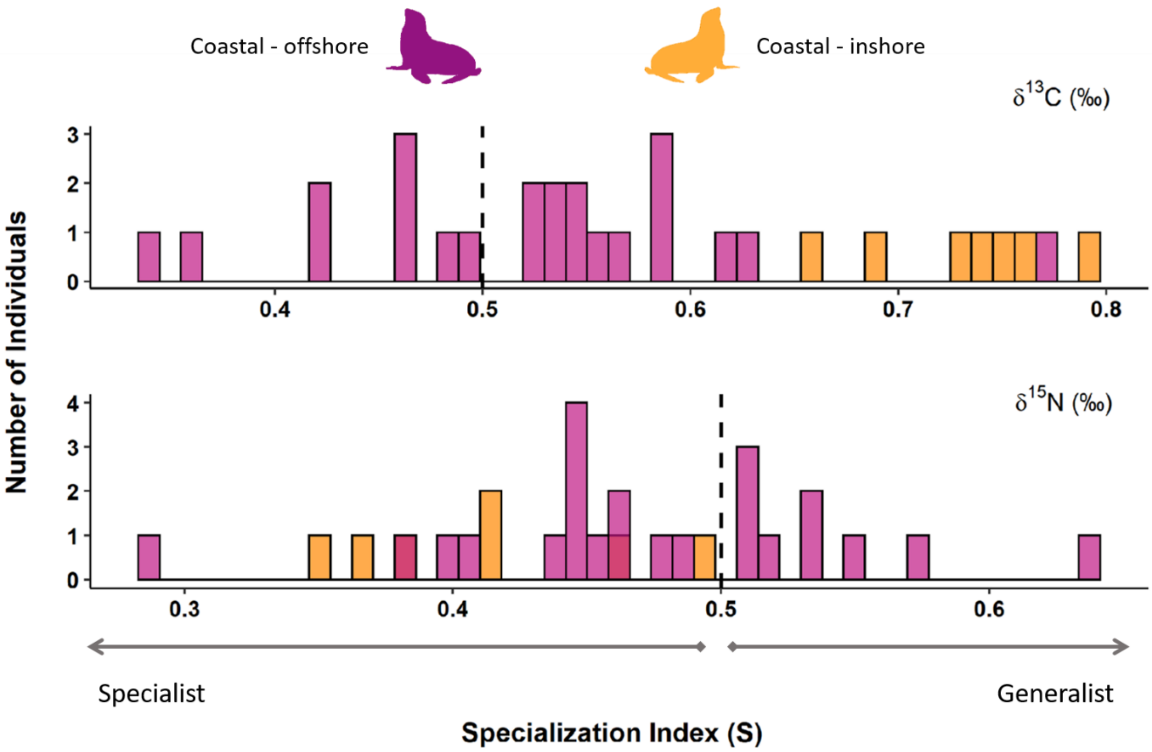


Figure 2.5. Specialization index (*S*) of adult female South American sea lions (*Otaria byronia*) for each foraging strategy. Values were computed using δ^{13}C (a) and δ^{15}N (b) isotopic values separately. The frequency distribution reveals that inshore foragers are more generalist in isotopic niche based on δ^{13}C isotopic values and more specialized based on δ^{15}N isotopic values. Offshore individuals show diverse degrees of specialization in isotopic niche based on δ^{15}N values, but were never as generalist as the inshore ecotype based on for δ^{13}C. A specialization index of 1 classifies an individual as an isotopic niche generalist, while an index closer to 0 is a highly specialized individual.

2.5. DISCUSSION

We found profound differences in the degree of individual specialization and intra-population competition between species characterized by a pelagic foraging mode (South American fur seals) and a benthic foraging mode (South American sea lions). Pelagic consumer populations that typically foraged in offshore habitats had narrower isotopic niches and were composed of generalist individuals with lower degrees of niche specialization (Figure 2.6). This could imply lower intra-population competition. By contrast, benthic predators that foraged within inshore habitats had a broader population isotopic niche, comprised of highly specialized individuals, presumably due to higher levels of intra-population competition (Figure 2.6). These traits associated with pelagic and benthic modes were also observed at an intra-population level, *i.e.* among the inshore and offshore foraging modes within South American sea lion females. Closer to the coast, the isotopic niches of the populations were broader with individuals more specialized and feeding at higher trophic levels. The isotopic specialization of individuals could be determined by long-term fidelity for a preferred prey type, foraging strategy or feeding area, or even a combination of these factors.

Individuals from the same population are predicted to compete for limited resources more than coexisting individuals from sympatric species due to the higher similarity in their resources used (Alley, 1982; Chesson, 2000). Our findings were broadly consistent with these predictions. As expected, the isotopic niche of South American sea lions and South American fur seals did not overlap, which likely reflects differences in preferred prey types, foraging habitat and individual-based characters (*e.g.*, body size, physiology capacity). Although, the populations of both species are growing in the Falklands, with fur seals increasing more rapidly than sea lions (Baylis et al., 2015b, 2019a), interspecific competition is unlikely. This is due to differences in body size and therefore diving physiology, which define, at least in part, foraging mode (Costa et al., 2004). Limited dietary studies on both species confirm differences in preferred prey at the Falkland Islands. While pelagic schooling Falkland herring (*Spattus fuegensis*) and Patagonian longfin squid (*Loligo gahi*) are important prey for South American fur seals, benthic species, such notothenid fishes and octopuses, predominate in the diets of South American sea lions (Thompson et al., 1998; Baylis et al., 2014). Dietary differences between fur seals and sea lions at the Falkland Islands are consistent with those seen in other breeding locations, for example Uruguay and Argentina, where dietary differences are attributed to pelagic and coastal foraging strategies (Franco-Trecu et al., 2012, 2014, 2017; Drago et al., 2017; Szteren et al., 2018). Similar patterns have also been found in sympatric otariid species with different foraging modes elsewhere. For example, adult females Galapagos fur seals (pelagic foragers) have a smaller population isotopic niche area than Galapagos sea lions (benthic foragers), and there is little overlap between the species (Páez-Rosas et al., 2014b).

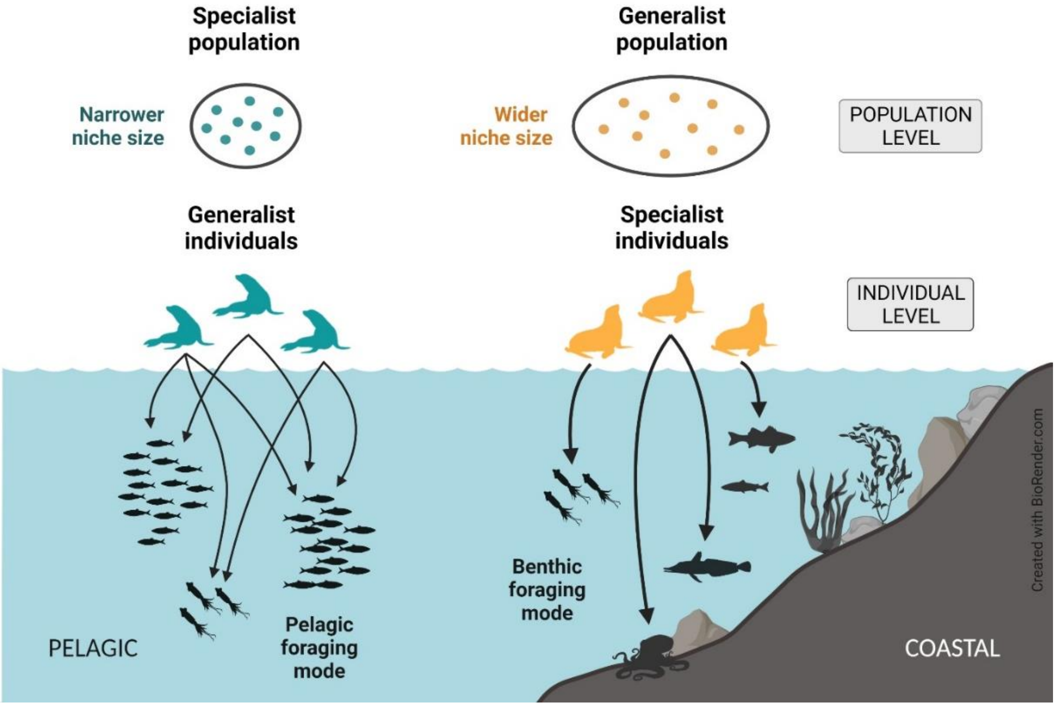


Figure 2.6. Isotopic population niche differentiation and degree of individual specialization in marine predators inhabiting distinct ecosystems and consuming different prey types. Pelagic predators (South American fur seals) foraging in dynamic environments with abundant but similar prey, form specialist populations (narrower isotopic niche size) of generalist individuals. By contrast, benthic predators (South American sea lion) foraging in coastal habitats with diverse but less abundant prey, form more generalist populations (wider isotopic niche size) of highly specialized individuals. The higher diversity of benthic and demersal prey species from benthic habitats is attributed to their increased habitat heterogeneity compared to pelagic environments.

Although isotopic niche overlap, and therefore competition, was low between species, competition within species was likely to be high owing to central place foraging constraints. To mitigate intra-population competition, conspecific competitors could switch prey resources, leading to ecological diversification and higher degrees of individual specialization (Araújo et al., 2007; Svanbäck & Bolnick, 2007). This is a particularly appealing hypothesis in the context of benthic foraging South American sea lions that displayed broader isotopic niches with high individual specialization, which presumably reduced intra-population competition. Coastal and benthic waters support higher prey diversity than open ocean environments (Ray, 1991; Angel, 1993; Cury et al., 2000) due to the spatial heterogeneity of these environments (Figure 2.6) (Ray, 1991; Snelgrove, 2001). However, benthic fish biomass tends to be lower than pelagic biomass (May & Blaber, 1989; Duffill Telsnig et al., 2019). When marine predators feed on coastal and demersal species with higher diversity but low abundance, they must cope with limited resources, and this may drive individual specialization to offset intra-population competition. Behaviourally-driven specialization of populations can also occur for similar reasons in sympatric coastal species (*e.g.*, Aurioles-Gamboa et al., 2013; Raoult et al., 2015). Ecological diversification of individual foraging strategies increases the isotopic population niche size (Bearhop et al., 2004; Araújo et al., 2011), as in South American sea lion females. Ecological diversification is found in other species that predominantly forage in inshore areas, such as Galapagos sea lion females (*Zalophus wollebaeki*), New Zealand sea lions (*Phocarctos hookeri*) and bottlenose dolphins (*Tursiops truncatus*) (Wolf et al., 2008; Chilvers & Wilkinson, 2009; Aurioles-Gamboa et al., 2013). The isotopic breadth of a population could be also broader in coastal environments due to a greater diversity of isotopic pathways including benthic, pelagic and terrestrial influences (Ray, 1991) that span a broad range of δ^{13}C values (-39 to -10‰) (Hemminga & Mateo, 1996; Sikes et al., 2009). The closer to the coast, the greater the availability of different prey types for a population to exploit to avoid competition, which has been identified here by broader ranges of carbon and nitrogen isotopic composition relative to pelagic predators (Figure 2.2).

The intra-population diversification of foraging strategies that we report in benthic South American sea lion females (coastal-inshore and coastal-offshore foraging groups identified through our stable isotope analysis) is supported by biologging data (Baylis et al., 2015a, 2016). In those studies, there was evidence of further specialization within the coastal-offshore group, as two different diving modes were found (benthic and pelagic dives). Given stable isotopes indicated long-term fidelity to inshore and offshore foraging patterns, it is plausible that some individual specialization between these foraging patterns might reflect specialization in predation techniques. Capturing and consuming a wide diversity of prey within different habitats requires different predatory skills (Hocking et al., 2017). Specialization is likely to be beneficial when prey items are in low abundance and occur in dissimilar habitats (*e.g.*, benthic, pelagic, rocky reef, bare sediment) because it may be more efficient for a benthic predator to specialize and increase the likelihood that they can successfully capture and handle difficult prey, such as skates or octopuses, leading to a decrease in intra-population competition. Moreover, there are costs to fitness associated with learning, which limits the number of new strategies that a predator can learn to efficiently handle new prey items (Baird et al., 1992; Araújo et al., 2008; Hocking et al., 2017). Hence, specialization in sea lions will lead to enhanced efficiency in handling prey types, reducing intra-population competition and ultimately expanding the population niche.

In contrast to sea lions, pelagic foraging South American fur seals exhibited smaller niche size and were generalist individuals. This implies that intra-population competition might be higher in fur seals due to overlapping individual niches. However, the stable isotopic signature of pelagic ecosystems depends on a single planktonic carbon source (Yoshii et al., 1999; Takai et al., 2007), which could reduce the isotopic variability among pelagic prey species, masking potential use of different resources by pelagic consumers. For example, variance in $\delta^{13}C$ and $\delta^{15}N$ of secondary consumers in pelagic food webs is 4-5 times lower than in benthic food webs from south-eastern Australia (0.8 vs 3.5 for $\delta^{13}C$, 1.1 vs 5.5 for $\delta^{15}N$) (Davenport & Bax, 2002). Nevertheless, the abundance of preferred prey is a key factor controlling intra-population competition (Svanbäck et al., 2011). In the context of fur seals, the high degree of trophic overlap between the individual niches from the same population could be explained by a relaxation of competition due to their highly abundant preferred prey, reducing the need for individuals to specialize and allowing them to share abundant resources (Forero et al., 2004; Svanbäck et al., 2011; Mancini & Bugoni, 2014). This finding is consistent with South American fur seal dietary studies, given diet is dominated by a few abundant prey items (Naya et al., 2002; Baylis et al., 2014), whereby distribution and abundance of prey depend on the highly dynamic marine systems and seasonality of their habitat (Agnew, 2002; Arkhipkin et al., 2012, 2013). The lower degree of individual specialization in South American fur seals is also consistent with studies at other breeding locations (Franco-Trecu et al., 2014; de Lima et al., 2019). The narrower isotopic niche of fur seals could reflect lower diversity of prey, the use of similar foraging areas, or both. Pelagic marine ecosystems are highly variable with lower species richness than coastal ecosystems (Cury et al., 2000; Suca et al., 2018). Pelagic food resources are distributed in patches associated with high productivity areas, however, are highly dynamic (Boyd, 1996; Agnew, 2002; Harcourt et al., 2002). Despite their variability, when they are found, the high abundance and density of pelagic prey makes it worthwhile for predators to spend time and energy in their search and handling (Chilvers & Wilkinson, 2009). Pelagic marine predators, like the South American fur seal, mainly rely on these patchy resources (*e.g.*, Benoit-Bird et al., 2013; Baylis et al., 2014) associated with high prey densities (Georges et al., 2000; Kuhn et al., 2015). Prey species of pelagic predators from the neritic region of the Patagonian shelf and slope near the Falkland Islands have an order of magnitude higher biomass than the surrounding oceanic waters (Agnew, 2002; Baylis et al., 2019b). This high level of prey abundance supports the coexistence of multiple marine predator species (Agnew, 2002; Baylis et al., 2019b).

Ecological implications

Different degrees of individual specialization imply different responses of populations to biological and environmental changes in the ecosystem. Specialist individuals with different foraging strategies facilitate resource partitioning (Swanson et al., 2003; Svanbäck & Bolnick, 2007; Cloyed & Eason, 2017), and are responsible for sustaining ecological variability within populations (Svanbäck & Bolnick, 2007). This diversification allows populations to adapt to new environmental and biological scenarios. Ecological processes at an intra-population level could have more substantial effects on community compositions than those occurring at an inter-specific level (Des Roches et al., 2018), highlighting the importance of considering individual variation in ecological studies.

Globally, marine ecosystems are changing dramatically because of anthropogenic activities (Halpern et al., 2008; Hoegh-Guldberg & Bruno, 2010), negatively affecting local populations that cannot adapt quickly enough to reduced resources and habitat degradation. In species with highly specialized individuals and populations, constrained by for example long-term individual fidelity to diet, foraging strategy, or area, populations may be more susceptible to environmental changes as they will be less likely to be able to rapidly change their trophic habits (Gallagher et al., 2015). However, if a generalist population is composed of individuals that specialize on different resources, as found in these South American sea lion females and Galapagos sea lions (Páez-Rosas et al., 2014a), this may provide the population with capacity to adapt to a changing environment, even if some components of the population cannot. Furthermore, species such as South American fur seals that are composed of generalist individuals, are more likely to be able to adapt foraging strategies to overcome rapid environmental changes. Recently, female Guadalupe fur seals (*Arctocephalus townsendi*), previously considered offshore predators, showed adaptability by exploiting novel prey species when subject to a warming event in 2015, thereby broadening their isotopic niche (Amador-Capitanachi et al., 2020). Understanding individual variability in niche size and intra-population competition is a significant factor to consider when predicting the resilience of populations facing environmental changes. Without this knowledge we will fail to outline effective management programs in wild populations.

Conclusions

Marine predators inhabiting distinct ecosystems and feeding on different prey types showed isotopic niche differentiation and different degrees of individual specialization (Figure 2.6). Pelagic marine predators that inhabit dynamic environments with abundant but a low diversity of prey, comprise specialized populations of generalist individuals. By contrast benthic demersal foragers in environments with diverse but less abundant prey comprise more generalist populations of highly specialized individuals. These results highlight the importance of including individual variability in foraging modes when investigating trophic ecology in marine ecosystems. Ultimately, understanding the degree of individual specialization will help to understand how populations will respond to an increasingly stochastic marine environment.

2.6. REFERENCES

Agnew D. J. (2002). Critical aspects of the Falkland Islands pelagic ecosystem: distribution, spawning and migration of pelagic animals in relation to oil exploration. *Aquatic Conservation: Marine Freshwater Ecosystems*, *12*, 39–50. https://doi.org/10.1002/aqc.474

Alley, T. R. (1982). Competition theory, evolution, and the concept of an ecological niche. *Acta Biotheoretica*, *31*(3), 165–179. https://doi.org/10.1007/BF01857239

Amador-Capitanachi, M. J., Moreno-Sánchez, X. G., Ventura-Domínguez, P. D., Juárez-Ruiz, A., González-Rodríguez, E., Gálvez, C., Norris, T., & Elorriaga-Verplancken, F. R. (2020). Ecological implications of unprecedented warm water anomalies on interannual prey preferences and foraging areas of Guadalupe fur seals. *Marine Mammal Science*, *36*, 1254–1270. https://doi.org/10.1111/mms.12718

Angel, M. V. (1993). Biodiversity of the pelagic ocean. *Conservation Biology*, *7*(4), 760–772. https://doi.org/10.1046/j.1523-1739.1993.740760.x

Araújo, M. S., Bolnick, D. I., Machado, G., Giaretta, A. A., & Dos Reis, S. F. (2007). Using $\delta^{13}C$ stable isotopes to quantify individual-level diet variation. *Oecologia*, *152*(4), 643–654. https://doi.org/10.1007/s00442-007-0687-1

Araújo, M. S., Guimarães, P. R., Svanbäck, R., Pinheiro, A., Guuimarães, P., Dos Reis, S. F., & Bolnick, D. I. (2008). Network analysis reveals contrasting effects of intraspecific competition on individual vs. population diets. *Ecology*, *89*(7), 1981–1993. https://doi.org/10.1890/07-0630.1

Araújo, M. S., Bolnick, D. I., & Layman, C. A. (2011). The ecological causes of individual specialisation. *Ecology Letters*, *14*(9), 948–958. https://doi.org/10.1111/j.1461-0248.2011.01662.x

Arkhipkin, A., Brickle, P., Laptikhovsky, V., & Winter, A. (2012). Dining hall at sea: feeding migrations of nektonic. *Journal of Fish Biology*, *81*, 882–902. https://doi.org/10.1111/j.1095-8649.2012.03359.x

Arkhipkin, A., Brickle, P., & Laptikhovsky, V. (2013). Links between marine fauna and oceanic fronts on the Patagonian shelf and slope. *Arquipelago: Life and Marine Sciences*, *30*, 19–37.

Aurioles-Gamboa D, Rodríguez-Pérez MY, Sánchez-Velasco L, Lavín MF (2013) Habitat, trophic level, and residence of marine mammals in the Gulf of California assessed by stable isotope analysis. Mar Ecol Prog Ser 488:275–290. https://doi.org/10.3354/meps10369

Baird, R. W., Abrams, P. A., & Dill, L. M. (1992). Possible indirect interactions between transient and resident killer whales: implications for the evolution of foraging specializations in the genus *Orcinus*. *Oecologia*, *89*(1), 125–132. https://doi.org/10.1007/BF00319024

Baylis, A. M. M., Arnould, J. P. Y., & Staniland, I. J. (2014). Diet of South American fur seals at the Falkland Islands. *Marine Mammal Science*, *30*(3), 1210–1219. https://doi.org/10.1111/mms.12090

Baylis, A. M. M., Orben, R. A., Arnould, J. P. Y., Peters, K., Knox, T., Costa, D. P., & Staniland, I. J. (2015a). Diving deeper into individual foraging specializations of a large marine predator, the southern sea lion. *Oecologia*, *179*(4), 1053–1065. https://doi.org/10.1007/s00442-015-3421-4

Baylis, A. M. M., Orben, R. A., Arnould, J. P. Y., Christiansen, F., Hays, G. C., & Staniland, I. J. (2015b). Disentangling the cause of a catastrophic population decline in a large marine mammal. *Ecology*, *96*(10), 2834–2847. https://doi.org/10.1890/14-1948.1

Baylis, A. M. M., Page, B., Staniland, I., Arnould, J. P. Y., & Mckenzie, J. (2015c). Taking the sting out of darting: Risks, restraint drugs and procedures for the chemical restraint of Southern Hemisphere otariids. *Marine Mammal Science*, *31*(1), 322–344. https://doi.org/10.1111/mms.12148

Baylis, A. M. M., Orben, R. A., Costa, D. P., Arnould, J. P. Y., & Staniland, I. J. (2016). Sexual segregation in habitat use is smaller than expected in a highly dimorphic marine predator, the southern sea lion. *Marine Ecology Progress Series*, *554*, 201–211. https://doi.org/10.3354/meps11759

Baylis, A. M. M., Tierney, M., Orben, R. A., Staniland, I. J., & Brickle, P. (2018). Geographic variation in the foraging behaviour of South American fur seals. *Marine Ecology Progress Series*, *596*, 233–245. https://doi.org/10.3354/meps12557

Baylis, A. M. M., Orben, R. A., Arkhipkin, A. A., Barton, J., Brownell, R. L., Staniland, I. J., & Brickle, P. (2019a). Re-evaluating the population size of South American fur seals and conservation implications. Aquatic Conservation: Marine and Freshwater Ecosystems, 29(11), 1988–1995. https://doi.org/10.1002/aqc.3194

Baylis, A. M. M., Tierney, M., Orben, R. A., Warwick-Evans, V., Wakefield, E., Grecian, W. J., Trathan, P., Reisinger, R., Ratcliffe, N., Croxall, J., Campioni, L., Catry, P., Crofts, S., Boersma, P. D., Galimberti, F., Granadeiro, J., Handley, J., Hayes, S., Hedd, A., Masello, J. F., Montevecchi, W. A., Pütz, K., Quillfeldt, P., Rebstock, G. A., Sanvito, S., Staniland, I. J., & Brickle, P. (2019b). Important at-sea areas of colonial breeding marine predators on the southern Patagonian shelf. *Scientific Reports*, *9*(8517), 1–13. https://doi.org/10.1038/s41598-019-44695-1

Bearhop, S., Adams, C. E., Waldron, S., Fuller, R. A., & Macleod, H. (2004). Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology*, *73*(5), 1007–1012. https://doi.org/10.1111/j.0021-8790.2004.00861.x

Benoit-Bird, K. J., Battaile, B. C., Nordstrom, C. A., & Trites, A. W. (2013). Foraging behavior of northern fur seals closely matches the hierarchical patch scales of prey. *Marine Ecology Progress Series*, *479*, 283–302. https://doi.org/10.3354/meps10209

Bolnick, D. I., Yang, L. H., Fordyce, J. A., Davis, J. M., & Svanback, R. (2002). Measuring individual-level resource specialization. *Ecology*, *83*(10), 2936–2941. https://doi.org/10.1890/0012-9658(2002)083[2936:MILRS]2.0.CO;2

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., Forister, M. L., & Forister. (2003). The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist*, *161*(1), 1–28. https://doi.org/10.2307/3078879

Boyd, I. L. (1996). Temporal scales of foraging in a marine predator. *Ecology*, *77*(2), 426–434. https://doi.org/10.2307/2265619

Ceia, F.R., Cherel, Y., Paiva, V. H. & Ramos, J. A. (2018). Stable isotope dynamics (δ^{13}C and δ^{15}N) in neritic and oceanic waters of the North Atlantic inferred from GPS-tracked Cory's shearwaters. *Frontiers in Marine Science*, *5*, 377. https://doi.org/10.3389/fmars.2018.00377

Cherel, Y. & Hobson, K. A. (2007). Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. *Marine Ecology Progress Series*, *329*, 281-287. https://doi.org/10.3354/meps329281

Cherel, Y., Koubbi, P., Giraldo, C., Penot, F., Tavernier, E., Moteki, M., Ozouf-Costaz, C., Causse, R., Chartier, A., & Hosie, G. (2011). Isotopic niches of fishes in coastal, neritic and oceanic waters off Adélie land, Antarctica. *Polar Science*, *5*, 286–297. https://doi.org/10.1016/j.polar.2010.12.004

Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, *31*, 343–366. https://doi.org/10.1146/annurev.ecolsys.31.1.343

Chilvers, B. L., & Wilkinson, I. S. (2009). Diverse foraging strategies in lactating New Zealand sea lions. *Marine Ecology Progress Series*, *378*, 299–308. https://doi.org/10.3354/meps07846

Cloyed, C. S., & Eason, P. K. (2017). Niche partitioning and the role of intraspecific niche variation in structuring a guild of generalist anurans. *Royal Society Open Science*, *4*(3). https://doi.org/10.1098/rsos.170060

Committee on Marine Mammals (1966-67). (1967). Standard measurements of seals. *Journal of Mammalogy*, *48*(3), 459. https://doi.org/10.2307/1377778

Costa, D. P., Kuhn, C. E., Weise, M. J., Shaffer, S. A. & Arnould, J. P. Y. (2004). When does physiology limit the foraging behaviour of freely diving mammals? *International Congress Series*, *1275*, 359-366. https://doi.org/10.1016/j.ics.2004.08.058

Costa, D. P. (2008). A conceptual model of the variation in parental attendance in response to environmental fluctuation: foraging energetics of lactating sea lions and fur seals. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *17*, S44–S52. https://doi.org/10.1002/aqc

Cury, P., Bakun, A., Crawford, R. J. M., Jarre, A., Quiñones, R. A., Shannon, L. J., & Verheye, H. M. (2000). Small pelagics in upwelling systems: patterns of interaction and structural changes in 'wasp-waist' ecosystems. *ICES Journal of Marine Science*, *57*, 603–618. https://doi.org/10.1006/jmsc.2000.0712

Davenport, S. R., & Bax, N. J. (2002). A trophic study of a marine ecosystem off southeastern Australia using stable isotopes of carbon and nitrogen. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*, 514–530. https://doi.org/10.1139/f02-031

de Lima, R. C., Franco-Trecu, V., Vales, D. G., Inchausti, P., Secchi, E. R., & Botta, S. (2019). Individual foraging specialization and sexual niche segregation in South American fur seals. *Marine Biology*, *166*(3), 1–12. https://doi.org/10.1007/s00227-019-3480-x

Des Roches, S., Post, D. M., Turley, N. E., Bailey, J. K., Hendry, A. P., Kinnison, M. T., Schweitzer, J. A., & Palkovacs, E. P. (2018). The ecological importance of intraspecific variation. *Nature Ecology and Evolution*, *2*(1), 57–64. https://doi.org/10.1038/s41559-017-0402-5

Drago, M., Cardona, L., Franco-Trecu, V., Crespo, A., Vales, D., Borella, F., Zenteno, L., Gonzáles, E. M., & Inchausti, P. (2017). Isotopic niche partitioning between two apex predators over time. *ARPN Journal of Engineering and Applied Sciences*, *86*(4), 766–780. https://doi.org/10.1111/1365-2656.12666

Duffill Telsnig, J. I., Jennings, S., Mill, A. C., Walker, N. D., Parnell, A. C., & Polunin, N. V. C. (2019). Estimating contributions of pelagic and benthic pathways to consumer production in coupled marine food webs. *Journal of Animal Ecology*, *88*, 405–415. https://doi.org/10.1111/1365-2656.12929

Estes, J. A., Heithaus, M., McCauley, D. J., Rasher, D. B., & Worm, B. (2016). Megafaunal impacts on structure and function of ocean ecosystems. *Annual Review OfEnvironment and Resources*, *41*, 83–116. https://doi.org/10.1146/annurev-environ-110615-085622

Forero, M. G., Bortolotti, G. R., Hobson, K. A., Donazar, J. A., Bertelloti, M., & Blanco, G. (2004). High trophic overlap within the seabird community of Argentinean Patagonia: A multiscale approach. *Journal of Animal Ecology*, *73*(4), 789–801. https://doi.org/10.1111/j.0021-8790.2004.00852.x

Franco-Trecu, V., Aurioles-Gamboa, D., Arim, M., & Lima, M. (2012). Prepartum and postpartum trophic segregation between sympatrically breeding female *Arctocephalus australis* and *Otaria flavescens*. *Journal of Mammalogy*, *93*(2), 514–521. https://doi.org/10.1644/11-MAMM-A-174.1

Franco-Trecu, V., Aurioles-Gamboa, D., & Inchausti, P. (2014). Individual trophic specialisation and niche segregation explain the contrasting population trends of two sympatric otariids. *Marine Biology*, *161*, 609–618. https://doi.org/10.1007/s00227-013-2363-9

Franco-Trecu, V., Drago, M., Costa, P., Dimitriadis, C., & Passadore, C. (2017). Trophic relationships in apex predators in an estuary system: A multiple-method approximation. *Journal of Experimental Marine Biology and Ecology*, *486*, 230–236. https://doi.org/10.1016/j.jembe.2016.10.017

Frederiksen, M., Edwards, M., Richardson, A. J., Halliday, N. C., & Wanless, S. (2006). From plankton to top predators: Bottom-up control of a marine food web across four trophic levels. *Journal of Animal Ecology*, *75*(6), 1259–1268. https://doi.org/10.1111/j.1365-2656.2006.01148.x

Futuyma, D. J., & Moreno, G. (1988). The evolution of ecological specialization. *Annual Review of Ecology and Systematics*, *19*, 207–233. https://www.jstor.org/stable/2097153

Gallagher, A. J., Hammerschlag, N., Cooke, S. J., Costa, D. P., & Irschick, D. J. (2015). Evolutionary theory as a tool for predicting extinction risk. *Trends in Ecology and Evolution*, *30*(2), 61–65. https://doi.org/10.1016/j.tree.2014.12.001

Georges, J., Bonadonna, F., & Guinet, C. (2000). Foraging habitat and diving activity of lactating Subantarctic fur seals in relation to sea-surface temperatures at Amsterdam Island. *Marine Ecology Progress Series*, *196*, 291–304. https://doi.org/10.3354/meps196291

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., Bruno, J. F., Casey, K. S., Ebert, C., Fox, H. E., Fujita, R., Heinemann, D., Lenihan, H. S., Madin, E. M. P., Perry, M. T., Selig, E. R., Spalding, M., Steneck, R., & Watson, R. (2008). A global map of human impact on marine ecosystems. *Science*, *319*, 948–952. https://doi.org/10.1111/2041-210X.12109

Harcourt, R. G., Bradshaw, C. J. A., Dickson, K., & Davis, L. S. (2002). Foraging ecology of a generalist predator, the female New Zealand fur seal. *Marine Ecology Progress Series*, *227*, 11–24. https://doi.org/10.3354/meps227011

Hemminga, M. A., & Mateo, M. A. (1996). Stable carbon isotopes in seagrasses: Variability in ratios and use in ecological studies. *Marine Ecology Progress Series*, *140*, 285–298. https://doi.org/10.3354/meps140285

Hirons, A. C., Schell, D. M., & St. Aubin, D. J. (2001). Growth rates of vibrissae of harbor seals (Phoca vitulina) and Steller sea lions (Eumetopias jubatus). *Canadian Journal of Zoology*, *79*, 1053–1061.

https://doi.org/10.1139/z01-055

Hocking, D. P., Marx, F. G., Park, T., Fitzgerald, E. M. G., & Evans, A. R. (2017). A behavioural framework for the evolution of feeding in predatory aquatic mammals. *Proceedings of the Royal Society B: Biological Sciences*, *284*, 20162750. https://doi.org/10.1098/rspb.2016.2750

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. *Science*, *328*, 1523–1528. https://doi.org/10.1126/science.1189930

Hückstädt, L. A., Koch, P. L., McDonald, B. I., Goebel, M. E., Crocker, D. E., & Costa, D. P. (2012). Stable isotope analyses reveal individual variability in the trophic ecology of a top marine predator, the southern elephant seal. *Oecologia*, *169*, 395–406. https://doi.org/10.1007/s00442-011-2202-y

Hussey, N. E., MacNeil, M. A., Olin, J. A., McMeans, B. C., Kinney, M. J., Chapman, D. D., & Fisk, A. T. (2012). Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. *Journal of Fish Biology*, *80*(5), 1449–1484. https://doi.org/10.1111/j.1095-8649.2012.03251.x

Hutchinson, G. E. (1957). Concluding remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, *22*, 415–427. https://doi.org/10.1201/9781315366746

Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, *80*(3), 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

Jones, K. A., Ratclffe, N., Voltier, S. C., Newton, J., Forcada, J., Dickens, J., Stowasser, G., Staniland, I. J., Ratcliffe, N., Votier, S. C., Newton, J., Forcada, J., Dickens, J., Stowasser, G., & Staniland, I. J. (2020). Intra-specific niche partitioning in Antarctic fur seals, Arctocephalus gazella. *Nature Scientific Reports*, *10*, 3238. https://doi.org/10.1038/s41598-020-59992-3

Jönsson, K. I. (1997). Capital and income breeding as alternative tactics of resource use in reproduction. *Oikos*, *78*, 57–66. https://doi.org/10.2307/3545800

Kassen, R. (2002). The experimental evolution of specialists, generalists, and the maintenance of diversity. *Journal of Evolutionary Biology*, *15*, 173–190. https://doi.org/10.1046/j.1420-9101.2002.00377.x

Kernaléguen, L., Cazelles, B., Arnould, J. P. Y., Richard, P., Guinet, C., & Cherel, Y. (2012). Long-term species, sexual and individual variations in foraging strategies of fur seals revealed by stable isotopes in whiskers. *PLoS ONE*, *7*(3), e32916. https://doi.org/10.1371/journal.pone.0032916

Kernaléguen, L., Arnould, J. P. Y., Guinet, C., & Cherel, Y. (2015). Determinants of individual foraging specialization in large marine vertebrates, the Antarctic and subantarctic fur seals. *Journal of Animal Ecology*, *84*, 1081–1091. https://doi.org/10.1111/1365-2656.12347

Kuhn, C. E., Sterling, J. T., & Zeppelin, T. K. (2015). Linking northern fur seal behavior with prey distributions: The impact of temporal mismatch between predator studies and prey surveys. *Animal Biotelemetry*, *3*(26), 1–12. https://doi.org/10.1186/s40317-015-0064-5

Lowther, A. D., Harcourt, R. G., & Goldsworthy, S. D. (2013). Regional variation in trophic ecology of adult female Australian sea lions inferred from stable isotopes in whiskers. *Wildlife Research*, *40*, 303–311. https://doi.org/10.1071/wr12181

Mancini, P. L., & Bugoni, L. (2014). Resources partitioning by seabirds and their relationship with other consumers at and around a small tropical archipelago. *ICES Journal of Marine Science*, *71*(9), 2599–2607. https://doi.org/10.1038/278097a0

May, J. L., & Blaber, S. J. M. (1989). Benthic and pelagic fish biomass of the upper continental slope off eastern Tasmania. *Marine Biology*, *101*, 11–25.

McHuron, E. A., Walcott, S. M., Zeligs, J., Skrovan, S., Costa, D. P., & Reichmuth, C. (2016). Whisker growth dynamics in two North Pacific pinnipeds: Implications for determining foraging ecology from stable isotope analysis. *Marine Ecology Progress Series*, *554*, 213–224. https://doi.org/10.3354/meps11793

Naya, D. E., Arim, M., & Vargas, R. (2002). Diet of South american fur seals (*Arctocephalus australis*) in Isla de Lobos, Uruguay. *Marine Mammal Science*, *18*(3), 734–745. https://doi.org/https://doi.org/10.1111/j.1748-7692.2002.tb01070.x

Newsome, S. D., Rio, Martinez del, C., Bearhop, S., & Phillips, D. L. (2007). A niche for isotope ecology. *Frontiers in Ecology and the Environment*, *5*(8), 429–436. https://doi.org/10.1890/060150.01

Newsome, S. D., Tinker, M. T., Monson, D. H., Oftedal, O. T., Ralls, K., Staedler, M. M., Fogel, M. L., & Estes, J. A. (2009). Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). *Ecology*, *90*(4), 961–974. https://doi.org/10.1890/07-1812.1

Newsome, S. D., Clementz, M. T., & Koch, P. L. (2010). Using stable isotope biogeochemistry to study marine mammal ecology. *Marine Mammal Science*, *26*(3), 509–572. https://doi.org/10.1111/j.1748-7692.2009.00354.x

Páez-Rosas, D., Riofrío-Lazo, M., & Aurioles-Gamboa, D. (2014a). Flexibility in the foraging strategies of the Galapagos sea lion inferred from a multiple approach analysis. In J. Denkinger & L. Vinueza (Eds.), *The Galapagos Marine Reserve. Social and Ecological Interactions in the Galapagos Islands* (First edition, pp. 71–80). Springer International Publishing. https://doi.org/10.1007/978-3-319-02769-2_4

Páez-Rosas, D., Rodríguez-Pérez, M., & Riofrío-Lazo, M. (2014b). Competition influence in the segregation of the trophic niche of otariids: A case study using isotopic bayesian mixing models in Galapagos pinnipeds. *Rapid Communications in Mass Spectrometry*, *28*, 2550–2558. https://doi.org/10.1002/rcm.7047

Peers, M. J. L., Thornton, D. H., & Murray, D. L. (2012). Reconsidering the specialist-generalist paradigm in niche breadth dynamics: resource gradient selection by Canada lynx and bobcat. *PLoS ONE*, *7*(12), e51488. https://doi.org/10.1371/journal.pone.0051488

Polechová, J., & Storch, D. (2019). Ecological niche. In B. Fath (Ed.), *Encyclopedia of Ecology* (Second edition, pp. 72–80). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-409548-9.11113-3

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, *83*(3), 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Raoult, V., Gaston, T. F., & Williamson, J. E. (2015). Not all sawsharks are equal: Species of co-existing sawsharks show plasticity in trophic consumption both within and between species. *Canadian Journal of Fisheries and Aquatic Sciences*, *72*, 1769–1775. https://doi.org/10.1139/cjfas-2015-0307

Raoult, V., Broadhurst, M. K., Peddemors, V. M., Williamson, J. E., & Gaston, T. F. (2019). Resource use of great hammerhead sharks (*Sphyrna mokarran*) off eastern Australia. *Journal of Fish Biology*, *95*, 1430–1440. https://doi.org/10.1111/jfb.14160

Ray, G. C. (1991). Coastal-zone biodiversity patterns: Principles of landscape ecology may help explain the processes underlying coastal diversity. *BioScience*, *41*(7), 490–498. https://doi.org/10.2307/1311807

Riet-Sapriza, F. G., Costa, D. P., Franco-Trecu, V., Marín, Y., Chocca, J., González, B., Beathyate, G., Louise Chilvers, B., & Hückstädt, L. A. (2013). Foraging behavior of lactating South American sea lions (*Otaria flavescens*) and spatial–temporal resource overlap with the Uruguayan fisheries. *Deep Sea Research Part II: Topical Studies in Oceanography*, *88–89*, 106–119. https://doi.org/10.1016/j.dsr2.2012.09.005

Rossman, S., Ostrom, P. H., Stolen, M., Barros, N. B., Gandhi, H., Stricker, C. A., Wells, R. S. (2015). Individual specialization in the foraging habits of female bottlenose dolphins living in a trophically diverse and habitat rich estuary. *Oecologia*, *178*(2), 415-425. . https://doi.org /10.1007/s00442-015-3241-6

Roughgarden, J. (1972). Evolution of niche width. *The American Naturalist*, *106*(952), 683–718. https://doi.org/10.1086/282892

Shiffman, D. S., Gallagher, A. J., Boyle, M. D., Hammerschlag-Peyer, C. M., & Hammerschlag, N. (2012).

Stable isotope analysis as a tool for elasmobranch conservation research: A primer for non-specialists. *Marine and Freshwater Research*, *63*, 635–643. https://doi.org/10.1071/MF11235

Sikes, E. L., Uhle, M. E., Nodder, S. D., & Howard, M. E. (2009). Sources of organic matter in a coastal marine environment: Evidence from n-alkanes and their $\delta^{13}C$ distributions in the Hauraki Gulf, New Zealand. *Marine Chemistry*, *113*, 149–163. https://doi.org/10.1016/j.marchem.2008.12.003

Snelgrove, P. V. R. (2001). Diversity of marine species. In J. K. Cochran, H. J. Bokuniewicz, & P. L. Yager (Eds.), *Encyclopedia of Ocean Sciences* (Third editition, pp. 694–701). Academic Press. https://doi.org/10.1016/B978-0-12-813081-0.00289-5

Suca, J. J., Pringle, J. W., Knorek, Z. R., Hamilton, S. L., Richardson, D. E., & Llopiz, J. K. (2018). Feeding dynamics of Northwest Atlantic small pelagic fishes. *Progress in Oceanography*, *165*, 52–62. https://doi.org/10.1016/j.pocean.2018.04.014

Svanbäck, R., & Bolnick, D. I. (2007). Intraspecific competition drives increased resource use diversity within a natural population. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1611), 839–844. https://doi.org/10.1098/rspb.2006.0198

Svanbäck, R., Rydberg, C., Leonardsson, K., & Englund, G. (2011). Diet specialization in a fluctuating population of *Saduria entomon*: A consequence of resource or forager densities? *Oikos*, *120*(6), 848–854. https://doi.org/10.1111/j.1600-0706.2010.18945.x

Swanson, B. O., Gibb, A. C., Marks, J. C., & Hendrickson, D. A. (2003). Trophic polymorphism and behavioral differences decrease intraspecific competition in a cichlid, *Herichthys minckleyi*. *Ecology*, *84*(6), 1441–1446. https://doi.org/10.1890/02-0353

Swanson, H. K., Lysy, M., Power, M., Stasko, A. D., Johnson, J. D., & Reist, J. D. (2015). A new probabilistic method for quantifying *n*-dimensional ecological niches and niche overlap. *Ecology*, *96*(2), 318–324. https://doi.org/https://doi.org/10.1890/14-0235.1

Szteren, D., Aurioles-Gamboa, D., Labrada-Martagón, V., Hernández-Camacho, C. J., & De María, M. (2018). Historical age-class diet changes in South American fur seals and sea lions in Uruguay. *Marine Biology*, *165*(59), 17. https://doi.org/10.1007/s00227-018-3315-1

Takai, N., Hirose, N., Osawa, T., Hagiwara, K., Kojima, T., Okazaki, Y., Kuwae, T., Taniuchi, T., & Yoshihara, K. (2007). Carbon source and trophic position of pelagic fish in coastal waters of south-eastern Izu Peninsula, Japan, identified by stable isotope analysis. *Fisheries Science*, *73*, 593–608. https://doi.org/10.1111/j.1444-2906.2007.01372.x

Thompson, D., Duck, C. D., McConnell, B. J., & Garrett, J. (1998). Foraging behaviour and diet of lactating female southern sea lions (*Otaria flavescens*) in the Falkland Islands. *Journal of Zoology*, *246*, 135–

146. https://doi.org/10.1017/S0952836998010024

Thompson, D., Moss, S., & Lovell, P. (2003). Foraging behaviour of South American fur seals *Arctocephalus australis*: extracting fine scale foraging behaviour from satellite tracks. *Marine Ecology Progress Series*, *260*, 285–296. https://doi.org/10.3354/meps260285

Thompson, R. M., Brose, U., Dunne, J. A., Hall, R. O., Hladyz, S., Kitching, R. L., Martinez, N. D., Rantala, H., Romanuk, T. N., Stouffer, D. B., & Tylianakis, J. M. (2012). Food webs: Reconciling the structure and function of biodiversity. *Trends in Ecology and Evolution*, *27*(12), 689–697. https://doi.org/10.1016/j.tree.2012.08.005

Wolf, J. B. W., Harrod, C., Brunner, S., Salazar, S., Trillmich, F., & Tautz, D. (2008). Tracing early stages of species differentiation: Ecological, morphological and genetic divergence of Galápagos sea lion populations. *BMC Evolutionary Biology*, *8*, 150. https://doi.org/10.1186/1471-2148-8-150

Yoshii, K., Melnik, N. G., Timoshkin, O. A., Bondarenko, N. A., Anoshko, P. N., Yoshioka, T., & Wada, E. (1999). Stable isotope analyses of the pelagic food web in Lake Baikal. *Limnology and Oceanography*, *44*(3), 502–511. https://doi.org/10.4319/lo.1999.44.3.0502

SUPPLEMENTARY MATERIAL

Chapter 2. Pelagic and benthic ecosystems drive differences in population and individual specializations in marine predators.

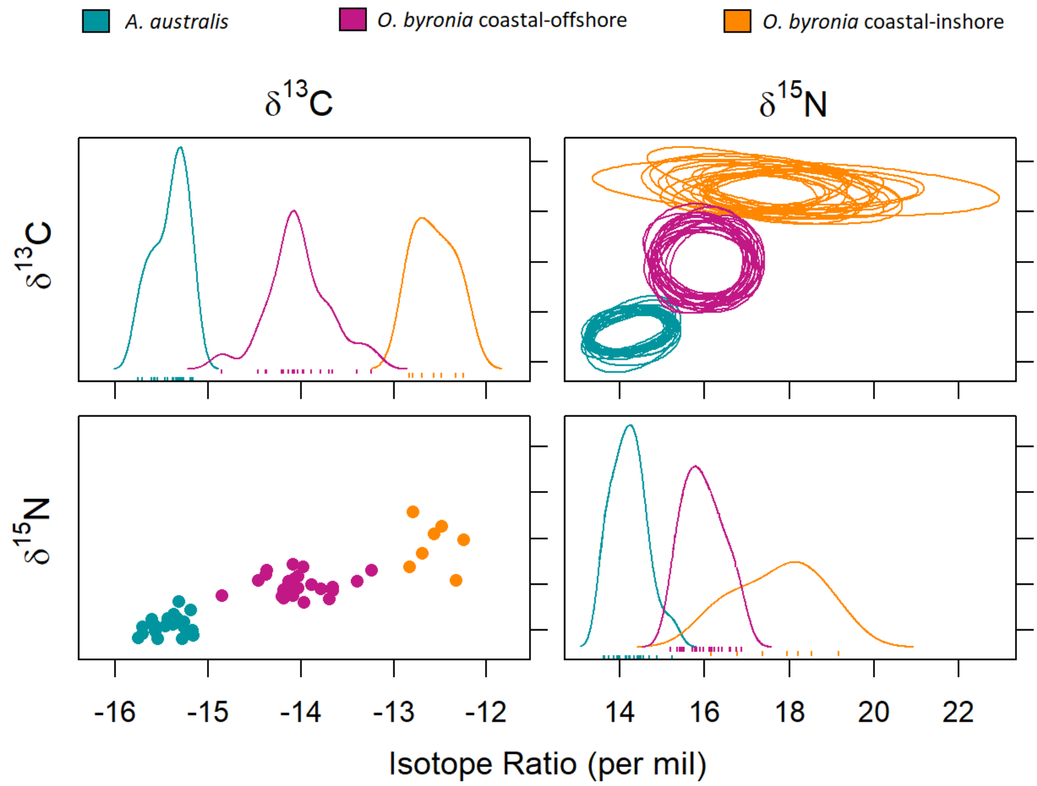


Figure 2.S1. Twenty random elliptical projections of trophic niche region for each group (top-right), density plots distributions (diagonal) and scatterplot of the raw data (bottom-left) for each pairwise combination of stable isotope data in South American sea lion (*Otaria byronia)* females (coastal-inshore and coastal-offshore) and South American fur seal (*Arctocephalus australis*) from Falkland Islands.

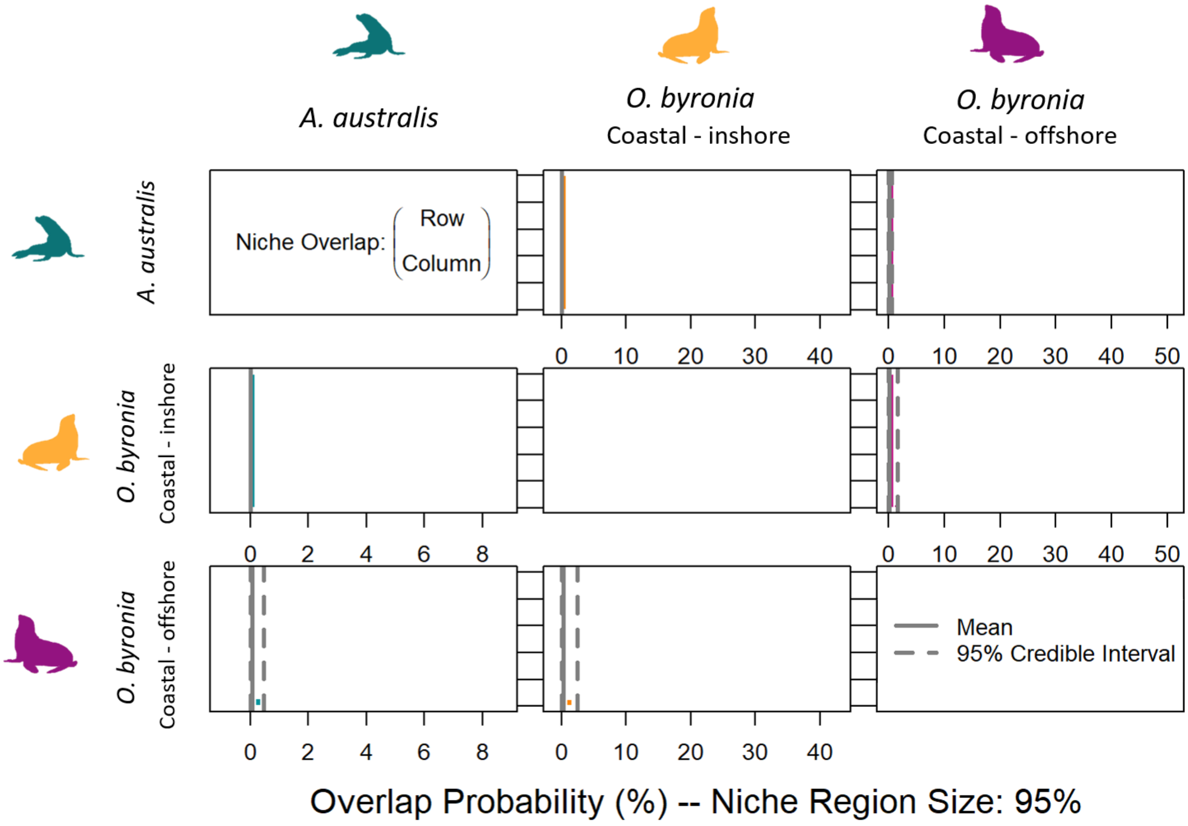
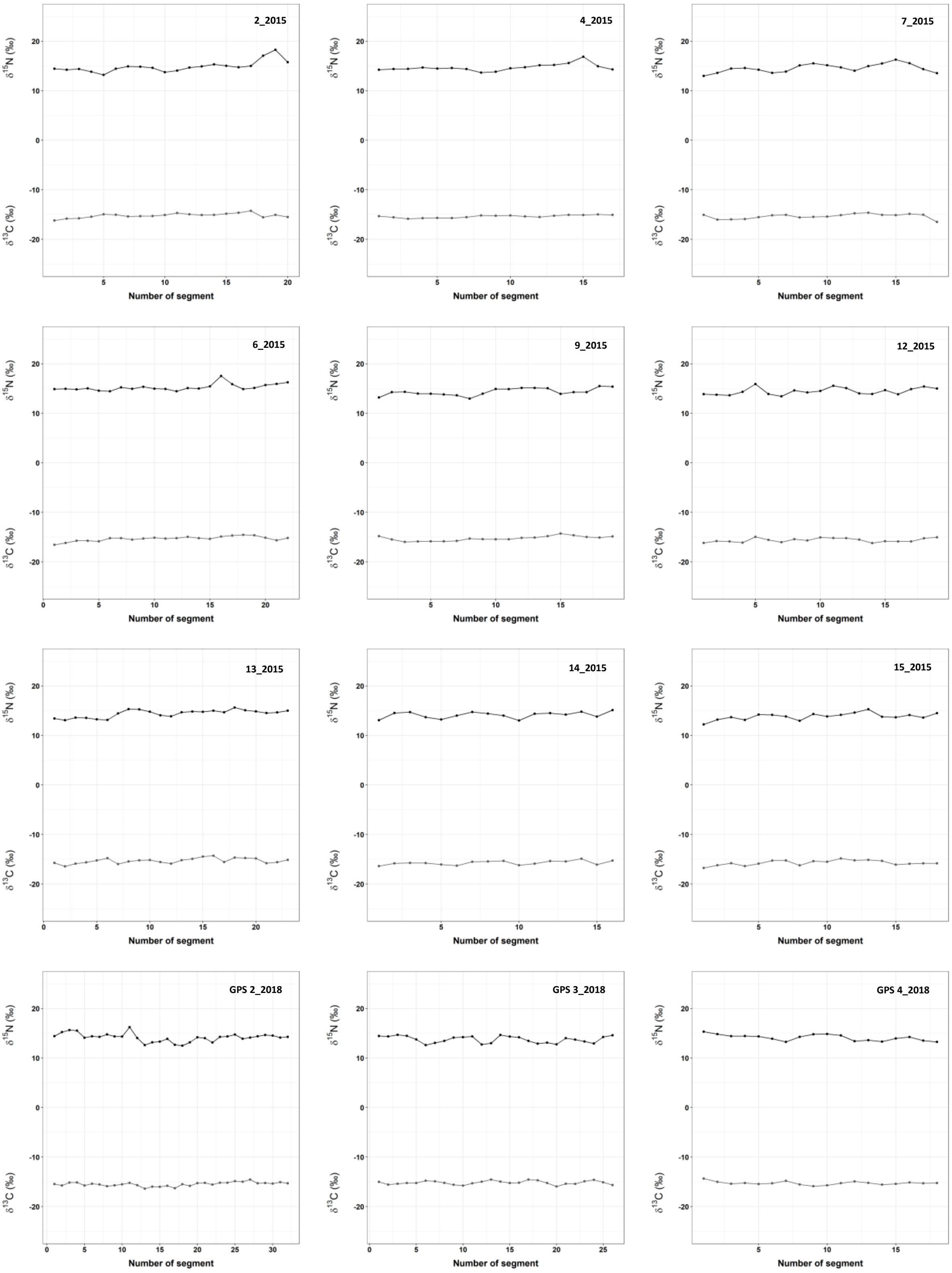


Figure 2.S2. Overlap probability of South American fur seal (*Arctocephalus australis*) females and South American sea lion (*Otaria byronia*) females (coastal-offshore and coastal-inshore foraging modes) from the Falkland Islands. Posterior distributions of the probabilistic niche overlap (%) for a specified niche region of 95%, representing the probability that an individual from one group (rows) is found within the niche region of the other group (columns). Continuous grey line represents mean overlap metric of the posterior distributions and discontinuous grey line represents the 95% Bayesian credible intervals calculated by doing 1,000 elliptical projections using a Bayesian framework.

Figure 2.S3. Temporal variation of δ^{13}C and δ^{15}N values of the sequential vibrissae segments from each adult female South American fur seals (*Arctocephalus australis*) from the Falkland Islands. The first sample is closest to the root of the vibrissae.



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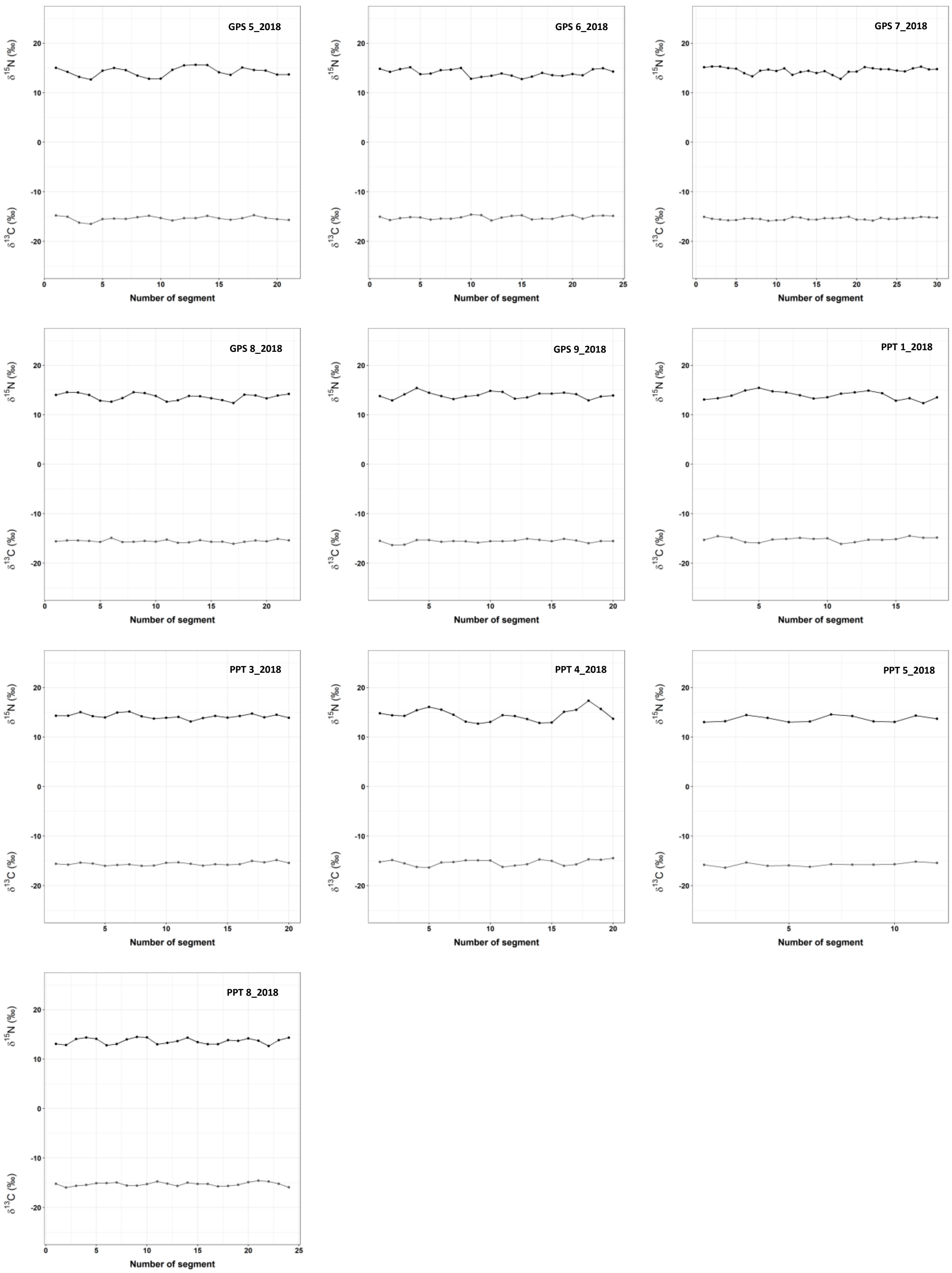
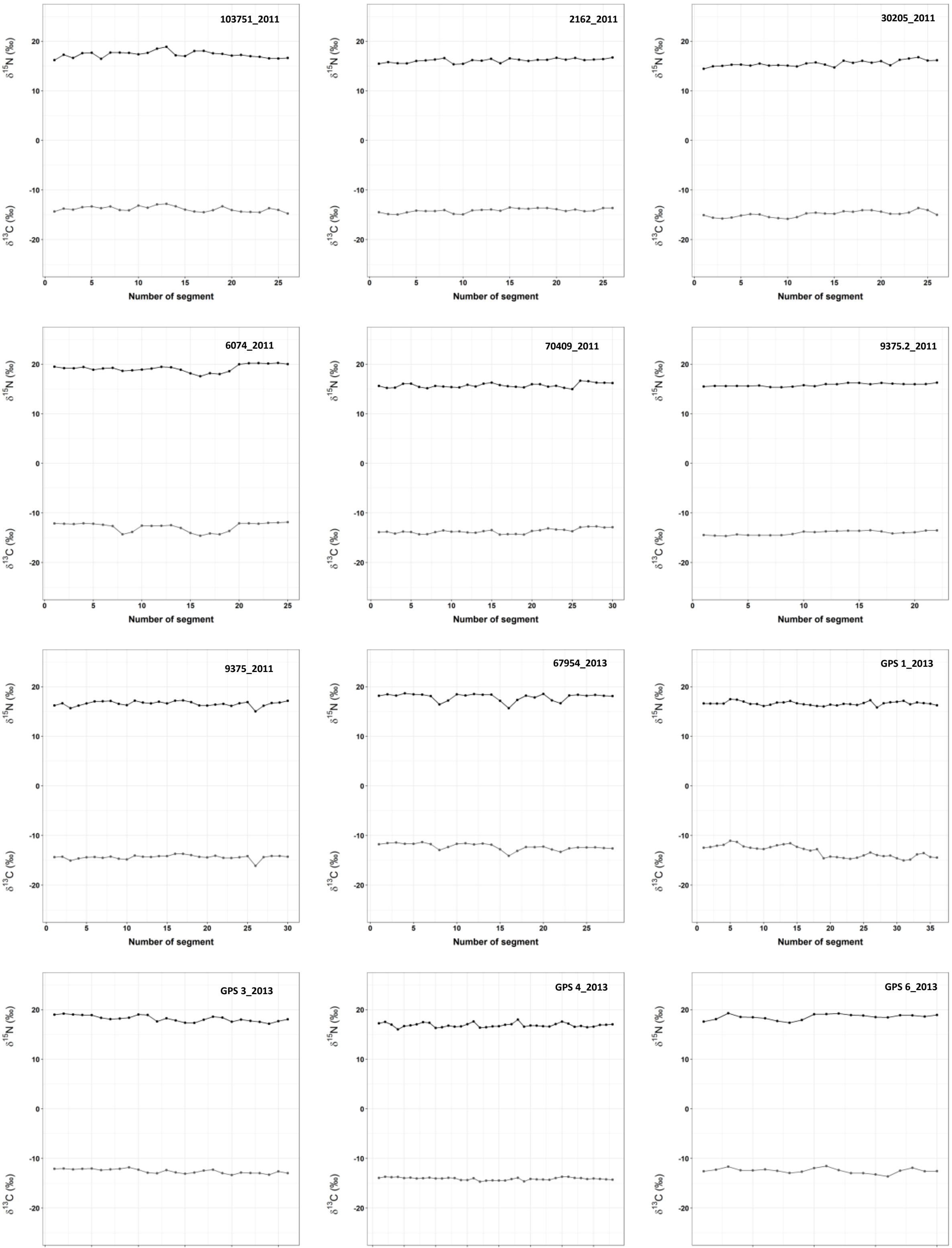


Figure 2.S4. Temporal variation of $\delta^{13}C$ and $\delta^{15}N$ values of sequential whiskers segments from each adult female South American sea lions (*Otaria byronia*) from the Falkland Islands. The first sample is closest to the root of the vibrissae.



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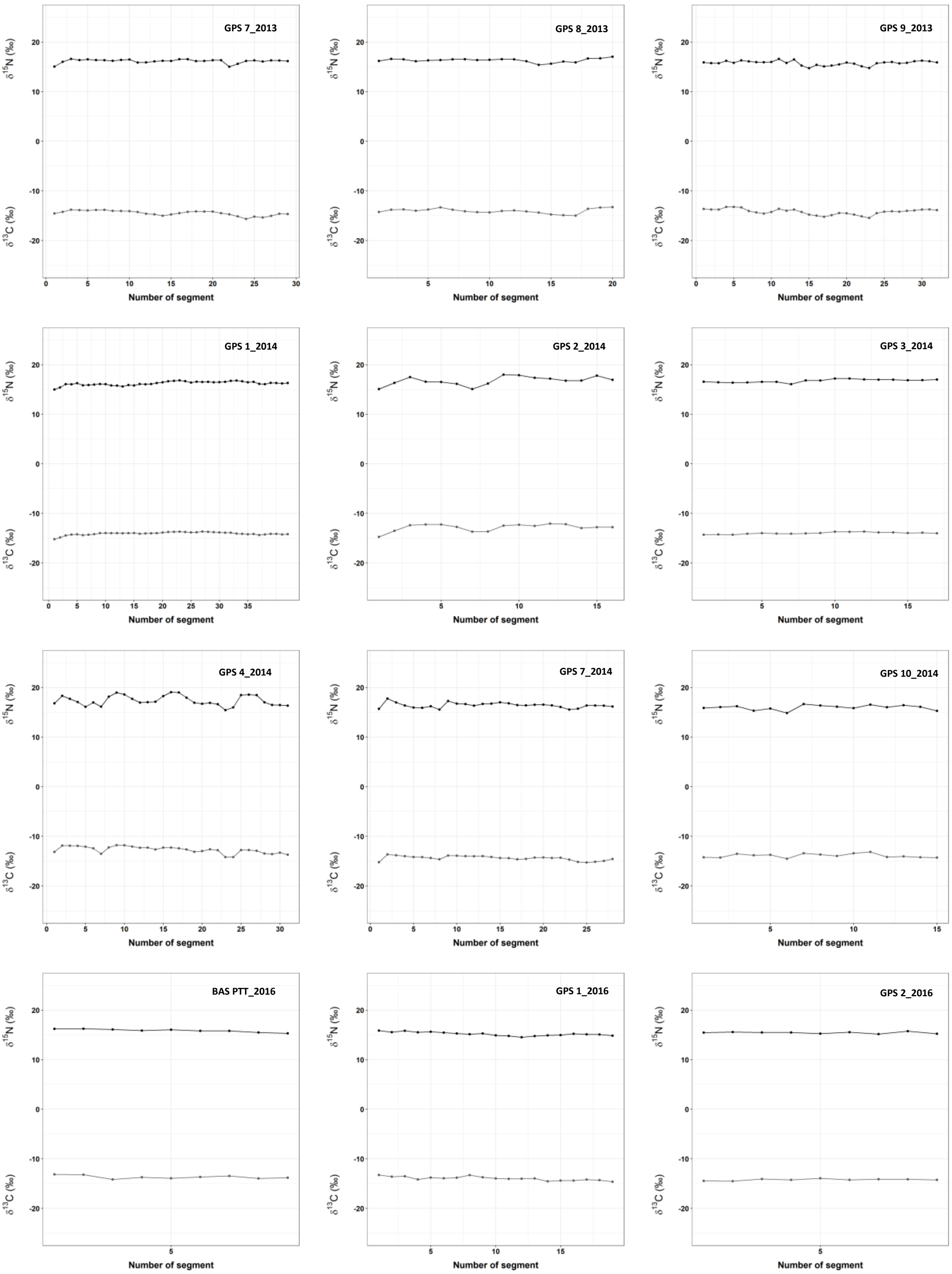

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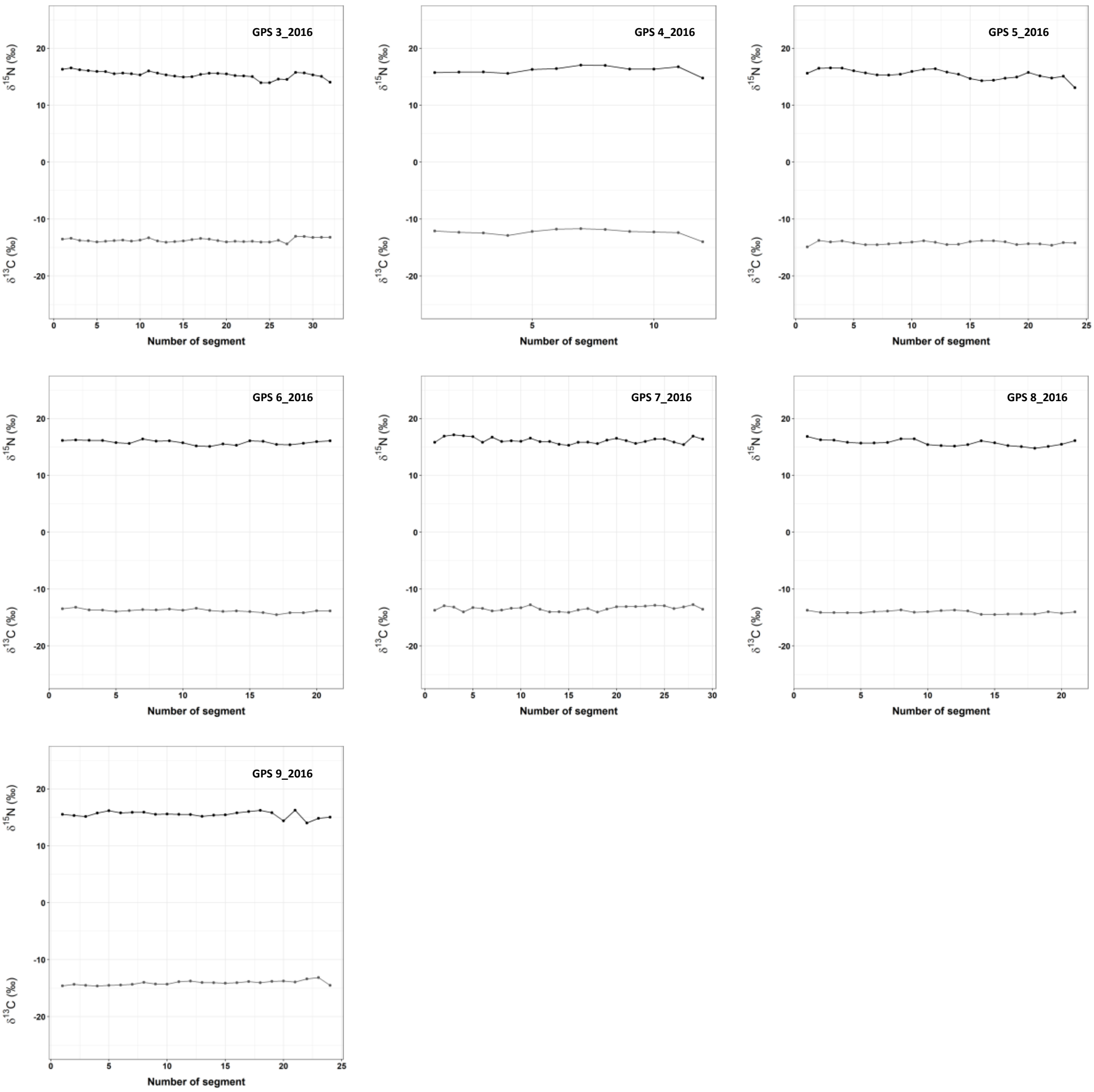

Chapter Three

Plasticity is the key: two sympatric species in contrasting environments switch roles, between niche generalist and niche specialist

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Robert G. Harcourt

3.1. ABSTRACT

Niche plasticity, the degree to which species populations can change their resource and habitat use, is generally believed to be constrained due to the life histories of species. This leads to the assumption that the niche and trophic role of a given species in one area will be similar elsewhere, but this assertion is rarely tested. We compared niche metrics in two sympatric pinniped species that share an extensive range, the South American sea lion (SASL, *Otaria byronia*) and the South American fur seal (SAFS, *Arctocephalus australis*) from two different environments. We collected samples from the northern and southern extremes of their Atlantic distribution resulting in contrasting environments, one under the influence of the Río de la Plata estuary and the Brazil-Falkland confluence (Uruguay; SAFS *n* = 35, SASL *n* = 44), and the other, from an oceanic archipelago (Falkland Islands; SAFS *n* = 22, SASL *n* = 31). We used stable isotopes of δ^{15}N and δ^{13}C to characterise their isotopic niche in both regions. High levels of niche plasticity were observed between regions for both species. In Uruguay, SAFS females had an isotopic niche size ~4.3 times larger than SASL females (10.3 ± 1.8 ‰^{2} and 2.4 ± 0.4‰^{2}, respectively). To our surprise, the complete opposite pattern was observed in the Falklands, where SASL females had an isotopic niche size ~6.8 times larger than SAFS females (9.2 ± 1.7 ‰^{2}, 1.4 ± 0.3 ‰^{2}, respectively). This remarkable inversion in niche metrics and therefore in the roles of these species (generalist or specialist) demonstrates that niche plasticity can be greater than commonly assumed. These findings highlight the risk of extrapolating results on trophic role from single studies.

Keywords: niche, plasticity, *Otaria byronia*, *Arctocephalus australis*, stable isotopes, foraging, generalist, specialist

3.2. INTRODUCTION

Niche plasticity refers to the degree that populations of species can change their resource and habitat use, and therefore determine the niche width and ecological role of a species. Niche plasticity is generally considered to be constrained by the life history of the species, and this leads to the potentially misleading assumption that conspecific individuals are ecologically equivalent (see Bolnick et al., 2003; Riverón et al., 2021). This assumption is often scaled to a species level, leading to an inherent assumption that a given species will remain generalist or specialist, even if there may well be variability with niche breadth as ecosystems vary (*e.g.*, by productivity Drago et al., 2016; Chiu-Werner et al., 2019; Lesser et al., 2020; Troina, et al., 2020a; Ciancio et al., 2021).

A main driver of niche plasticity is the variation in prey availability, whether seasonal (*e.g.*, Chiu-Werner et al., 2019) or regional (*e.g.*, Ciancio et al., 2021). Variability in marine prey distribution and abundance is driven by ecosystemic primary productivity, which in turn, is determined by regional oceanographic characteristics, such as temperature, upwelling regions and bathymetric topography (*e.g.*, Rutherford et al., 1999; Rykaczewski & Checkley, 2008; Kwasniewski et al., 2010). Differences in prey availability may induce changes in community composition, which may change the competitive forces for resources among species of similar requirements (Valladares et al., 2015; Mittelbach & McGill, 2019). How malleable a population is in response to ecosystemic change is likely to reflect long term persistence in a dynamic environment (Van Buskirk, 2012; Evans & Moustakas, 2018). Therefore, in the context of global acceleration in changing marine environments (Hoegh-Guldberg & Bruno, 2010; Antão et al., 2020), we need an understanding of the degree of the niche plasticity in predator populations.

Pinnipeds are in many ways an ideal taxa within which to assess niche plasticity since they are well studied, have broad geographic distributions and individuals can travel great distances to forage (Pagel et al., 1991; Jefferson et al., 2015). Moreover, adult females are central-place foragers, with foraging spatially and temporally constrained during pup rearing to areas near the breeding colonies (Costa, 2008; Ladds et al., 2020). South American sea lions (SASL, *Otaria byronia*) and South American fur seals (SAFS, *Arctocephalus australis*) are sympatric species with a broad latitudinal distribution and contrasting foraging strategies (Jefferson et al., 2015; Crespo & de Oliveira, 2021; Crespo et al., 2021; Riverón et al., 2021). On the Atlantic coast, there are breeding colonies of both species in Uruguay, Argentina (Provinces of Chubut, Santa Cruz, and Tierra de Fuego), and in the Falkland Islands (Würsig et al., 2018). This allowed us to compare the trophic niches of both species at the extremes of their range in contrasting environments: to the north, a wide estuarine environment in Uruguay and to the south, a small oceanic archipelago, the Falkland Islands. SAFS and SASL have different foraging strategies, SAFS are known to be generalist pelagic foragers (Thompson et al., 2003), while the SASL is a specialist benthic forager (Thompson et al., 1998; Riet-Sapriza et al., 2013; Baylis et al., 2015). This presented the opportunity to examine niche plasticity and the role of species in food webs, and where we expected to observe moderate changes in niche size between the two species.

3.3. METHODS

Study site

The study was conducted at the northern and southern extremes of the Atlantic distribution of SAFS and SASL. In the far north, sampling occurred at two breeding colonies of SASL and SAFS in Uruguay (Figure 3.1), at Isla de Lobos in Punta del Este (35°1’36’’ S; 54°53’2’’ W) and at Isla Rasa in Cabo Polonio-Rocha (35°24’11’’ S; 53°46’10’’ W). The two colonies are approximately 130 km apart and are the northernmost breeding colonies of the Atlantic distribution for both species (Bastida et al., 2007). Isla de Lobos is located at the mouth of the estuary of the Río de la Plata (south-eastern outer limit) and is 8 km away from the coast. Isla Rasa is located further to the northeast of the Atlantic coast with greater oceanic influence and closer to the coast (~600 m), of Cabo Polonio. Near the southern limit of the Atlantic distribution of both species, we included data collected from breeding colonies in the Falkland Islands (51°47’47’’ S; 59°31’25’’ W; Riverón et al., 2021).

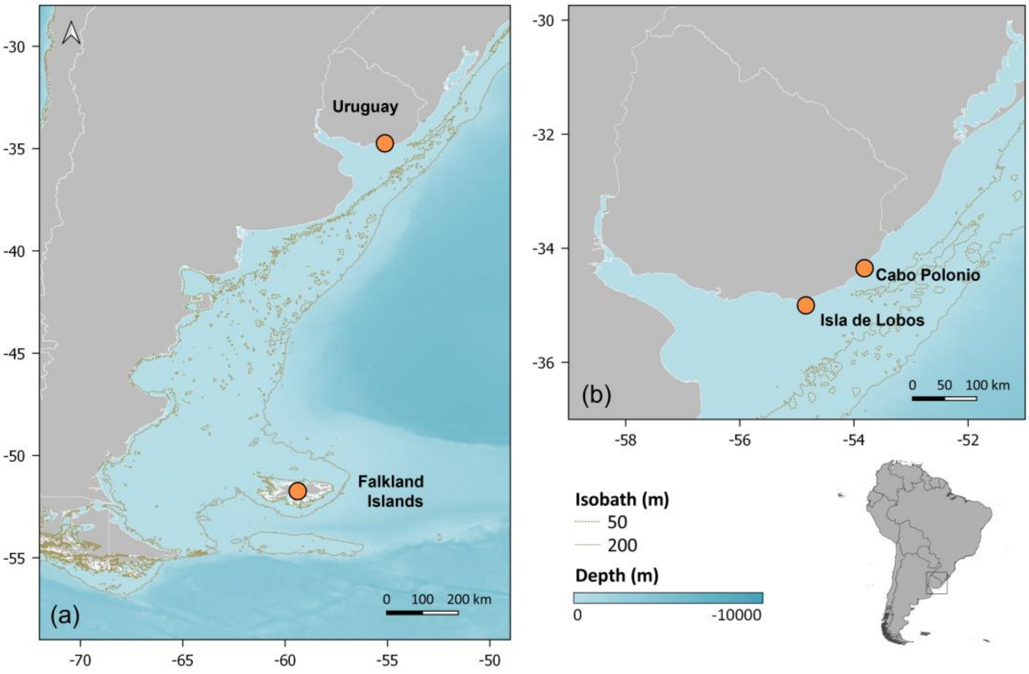

Figure 3.1. (a) Map of South America showing the two study localities (orange circles): Uruguay and the Falkland Islands. (b) Río de la Plata region and the two colonies sampled in Uruguay: Cabo Polonio and Isla de Lobos. For details of the Falkland Islands sampling, refer to Riverón et al. (2021) (Chapter 2).

The study sites are located close to the latitudinal edges of the Patagonian Shelf ~1850 km apart (35°S Uruguay, 52°S Falkland Islands) and offer contrasting marine ecosystems with distinctive biotic and abiotic characteristics (Figure 3.1). Both regions are characterized by high productivity that sustains fisheries and populations of marine predators (Croxall & Wood, 2002; Brazeiro et al., 2003; Acha et al., 2008; Mandiola et al., 2016; Baylis et al., 2019; García-Alonso et al., 2019). The Falkland Islands are an oceanic archipelago located to the southern end of the Patagonian Shelf, where productivity is associated with the subantarctic nutrient rich waters and upwelling oceanic regimes (Matano & Palma, 2008; Arkhipkin et al., 2013). Further north, the shelf narrows considerably (from ~830 km to ~200 km), and the cold nutrient-rich waters of the Falkland Current meets the mouth of Río de la Plata estuary (~38°S) with the warm and nutrient-poor waters of the Brazil Current, creating the Brazil-Falkland Confluence (Piola & Matano, 2017; Piola et al., 2018). The high productivity in this area relies on plant detritus from the large freshwater discharges of the Río de la Plata, the fifth largest estuary in the world, and from marine phytoplankton from upwelling events (Acha et al., 2008; Piola & Matano, 2017). The discharge of the Río de la Plata waters and the seasonal thermal regimen of the two main currents make it a highly variable ecosystem (Acha et al., 2008; Heileman, 2009).

Sample collection

In Uruguay, skin samples from pups were collected during two consecutive reproductive seasons (2018-2019 and 2019-2020) at the two reproductive colonies (Isla de Lobos and Cabo Polonio, Figure 3.1). Samples were collected in collaboration with the National Directorate of Aquatic Resources (DINARA) during their routine annual population monitoring. A small piece of the flipper tip was cut off with surgical scissors and stored at -20°C until further processing in the laboratory. Flipper clips were collected from fresh carcasses stranded at the colonies and from live animals during the tagging and measuring process conducted by DINARA. Fieldwork and sample collection were conducted under permit number 252/2018 issued by DINARA.

Samples from the Falkland Islands were whiskers collected from nursing females as part of biologging studies (Baylis et al., 2015, 2016a, 2018).

Stable isotope analysis

Stable isotope analysis is a commonly used tool to quantify ecological niche dimensions in marine mammal populations (reviewed by Newsome et al., 2007). Stable isotopes values of nitrogen ($\delta^{15}N$) provide a proxy of the trophic level of an organism, while stable isotopes of carbon ($\delta^{13}C$) and sulfur ($\delta^{34}S$) help us identify the sources of primary energy of an ecosystem (*i.e.*, coastal vs oceanic, pelagic vs benthic) (Peterson et al., 1979; Post, 2002; Shipley & Matich, 2020; Riverón et al., 2021). Moreover, the integration of $\delta^{34}S$ increases the resolution of identification of primary producers, even in estuarine and coastal environments with several potential sources of organic matter (*e.g.*, Connolly et al., 2004; Niella et al., 2021) such as Río de la Plata. Therefore, the inclusion of $\delta^{34}S$ is a powerful tool to assess differences between the two breeding colonies in the Río de la Plata region.

Skin samples from flipper tips were rinsed with de-ionized water to eliminate any residue that could affect the isotopic signal. The flipper samples were dissected, preserving the epidermis and dermis layers for analysis, and cut into small pieces prior to oven-drying at 60°C for 72 hours. Dried samples were ground to a fine powder using an MM200 ball mill (Retsch GmbH, Haan, Germany). As tissue lipid content may vary between species and bias their isotopic values, it is recommended to perform a lipid extraction when comparing different species, and no species- and tissue-specific information on the lipid content is available (Newsome et al., 2010; Hussey et al., 2012). Moreover, no information on the possible effects of lipid extraction on sulfur isotopic values existed at the time of analysis, as Chapter 6 (Appendix data chapter;

Riverón et al., 2022) was published long afterwards. Lipid extraction was conducted using a 2:1 chloroform-methanol solution adapted from Folch et al. (1957) on approximately 1 gram of tissue. The samples were dried at 60°C for 48 hours or until the solvent completely evaporated to remove the remaining solvent. Approximately 9 mg of dried sample was weighed in tin containers and sent for analysis. For details on the processing of whiskers samples for stable isotope analysis refer to Riverón et al. (2021) (Chapter 2).

Stable isotope values of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ from Uruguayan colonies were determined at two facilities by a vario-PYRO-cube elemental analyser coupled to an Isoprime100 mass spectrometer at the Central Science Laboratory of the University of Tasmania (CSL-UTAS, Australia) and by a continuous flow-isotope ratio mass spectrometer Hydra 20-22 coupled with Europa EA-GSL elemental analyser at Griffith University Stable Isotope Laboratory (SIL-GU, Australia). The amount of skin sample, after laboratory processing was insufficient to provide reliable results on sulfur measurements for some of the samples, therefore only those with enough material were included in the analyses, all from the Griffith University lab. Stable isotope abundances are reported in delta (δ) values as deviations from international standards in part per mille (‰), using air for ^{15}N and Pee Dee Belemnite for ^{13}C at CLS-UTAS, and using IAEA-N1 and IAEA-N2 for ^{15}N, IAEA-CH-6 for ^{13}C and IAEA-S1, IAEA-S2 and IAEA-S3 for ^{34}S at SIL-GU. The measurement precision of isotopic values from SIL-GU was 0.0-0.1‰ for $\delta^{15}N$ and $\delta^{13}C$, and 0.1-0.3‰ for $\delta^{34}S$. Isotopic values from CSL-UTAS had a measurement precision of ~0.1‰ for $\delta^{15}N$ and $\delta^{13}C$. For samples from the Falkland Islands, only $\delta^{13}C$ and $\delta^{15}N$ isotopic values were determined, using the international standards Vienna-Pee Dee Belemnite for ^{13}C and atmospheric air N_2 for ^{15}N. Falkland samples had a measurement precision of < 0.17‰ and < 0.22‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Refer to Riverón et al. (2021) for details about laboratories and equipment used for whisker samples. Isotopic values are expressed as means and their associated standard deviation (SD).

Pup skin samples were used as a proxy of the isotopic signal of adult females for both species (Jenkins et al., 2001; Aurioles et al., 2006; Lowther & Goldsworthy, 2011). Pups were sampled during their first month after birth and at this age their nutrition is fully dependent of their mother’s milk. As mammalian skin has a slow turnover rate of months (Voigt et al., 2003; Alves-Stanley & Worthy, 2009; Clark et al., 2019), skin samples of newborn pups can be used to assess the trophic ecology of adult females. We recognise that there are likely to be differences in mother-pup enrichment (Drago et al., 2015), however these should be consistent between sites and so relative differences should still be robust.

Statistical analyses

All statistical analyses were performed in R software version 4.2.0 (R Core Team, 2022). Isotopic parameters of the Uruguayan colonies were determined based on $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values of pup skin samples. As differences in foraging behaviour may exist at small geographic scales (*e.g.*, Tollit et al., 1998; Deagle et al., 2009; Wege et al., 2019; Grandi et al., 2021), Uruguayan colonies were initially assessed to test for possible differences in their isotopic niches. These results helped us to interpret the isotopic niche sizes for each species in the region. Consequently, when differences in mean isotopic values between colonies were detected, their level of significance was tested using a Wilcoxon's Rank-Sum test, since not all the variables had a normal distribution. Normality was assessed using the Shapiro-Wilks test. We quantified the relative isotopic niche size of each colony, using the R package ‘*nicheROVER*’. Due we did not reach a minimum number of $\delta^{34}S$ values for both species in each colony, only $\delta^{13}C$ and $\delta^{15}N$

values were used for niche size estimates. The isotopic niche size was estimated through a Bayesian framework using a Normal-Inverse-Wishart (NIW) with a default 'non-informative' prior (Swanson et al., 2015). The isotopic niche size of each population was defined as a probabilistic region of 95% (alpha = 0.95), consisting of the most likely values of the two-dimensional stable isotope measurements.

To compare the isotopic niches of SAFS and SASL at both extremes of their Atlantic distribution (Uruguay, Falkland Islands), stable isotope ranges and estimation of the relative isotopic niche size for each species in the two regions were estimated. Isotopic niche metrics estimates for the Uruguayan populations were made from skin isotopic values, while for the Falklands populations, the mean isotopic values for each sequentially sampled whisker were used (see Riverón et al. 2021, Chapter 2, for details of whisker processing). The use of tissues with long-term temporal windows gives a better approximation of the population niche size, as it integrates possible variations in the population isotopic niche that may exist in association with seasonal changes. As mean isotopic values for each sequentially sampled whisker represent long temporal series (months-years) (*e.g.*, Lowther et al., 2011; Chilvers, 2019) and skin (including epidermis and dermis) is a tissue with a slow turn-over rate (several months) (Voigt et al., 2003; Clark et al., 2019), both are good estimates of an individual long-term trophic ecology. Given that our aim was not to make a direct comparison between isotopic values of each region, differences between tissues in their isotopic turnover (Newsome et al., 2010) and latitudinal differences in the isotopic baselines between regions (*e.g.*, Glew et al., 2021; Troina et al., 2020; Vales et al., 2013), should not affect broad estimates of specialisation. Here we focus on the overall patterns shown by species niche size and breadth, looking at the distributions and spread of each tracer for the population rather than absolute stable isotope values, which reflect the niche of the species.

Only $\delta^{13}C$ and $\delta^{15}N$ values were available for whisker samples, therefore, niche metric estimates for the regional comparison were made only with these two tracers. Range of stable isotope values for each species in both regions were calculated as a basic measurement of the dispersion of each isotope. We estimated the relative isotopic niche size with '*nicheROVER*'. The alpha values were set to 0.40 and 0.95, to obtain estimation of isotopic niche sizes corresponding to areas which encompass the 40% and the 95% of the most likely values of isotopic values for both species. In addition, we conducted a Bayesian estimation of the isotopic niche space (SEA_B, Bayesian Standard Ellipse Area) for each species based in carbon and nitrogen isotopic values using 100,000 posterior draws in the R package *"SIBER"* (Jackson et al. 2011). The SEA_B values were used to construct density plots for both species in each study region and correspond to *c.* 40% of the data.

3.4. RESULTS

The total number of samples analysed for $\delta^{13}C$ and $\delta^{15}N$ from Uruguayan colonies were 35 SAFS (Cabo Polonio *n* = 18, Isla de Lobos *n* = 17) and 44 SASL (Cabo Polonio *n* = 18, Isla de Lobos *n* = 26). From the Falklands Islands 22 samples of SAFS and 31 SASL were analysed for the same elements. Only 28 samples of SAFS (Cabo Polonio *n* = 18, Isla de Lobos *n* = 10) and 16 SASL (Cabo Polonio *n* = 9, Isla de Lobos *n* = 7) had sufficient material to obtain reliable measurements for $\delta^{34}S$.

Isotopic mean values of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) were significantly higher in adult female SASL ($\delta^{15}N$ = 20.4 ± 0.4‰, $\delta^{13}C$ = -13.5 ± 0.3‰) than SAFS ($\delta^{15}N$ = 17.7 ± 1.6‰, $\delta^{13}C$ = -15.0 ± 0.5‰) from Uruguay (Table 3.1, Figure 3.S1). Less pronounced but still significant differences in mean isotopic values of sulfur ($\delta^{34}S$) were also detected between the two species (SASL = 16.9 ± 0.6‰, SAFS = 17.3 ± 0.6‰) (Table 3.1, Figure 3.S1). Mean isotopic values for SAFS and SASL adult females from the Falkland Islands from Riverón et al. (2021) were also significantly different in $\delta^{15}N$ and $\delta^{13}C$ values between species (presented in Table 3.1).

Table 3.1. Mean isotopic values of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulfur ($\delta^{34}S$) in pup skin samples of South American fur seals (SAFS, *Arctocephalus australis*, *n*=35) and South American sea lions (SASL, *Otaria byronia*, *n*=44) from Uruguay and the Falkland Islands. Mean values of $\delta^{34}S$ were calculated with a smaller sample size (SASL: *n*=28 and SASL: *n*=16). The *p*-value obtained from the Wilcoxon's Rank Sum test for species comparisons in their isotopic values is shown. Data are expressed as means ± standard deviation. Significance levels: *<0.05, **<0.01, ***<0.001.

	Arctocephalus australis South American fur seal	***Otaria byronia*** South American sea lion	***p*-value**
Uruguay			
$\delta^{15}N$ (‰)	17.7 ± 1.6	20.4 ± 0.4	< 0.001***
$\delta^{13}C$ (‰)	-15.0 ± 0.5	-13.5 ± 0.3	< 0.001***
$\delta^{34}S$ (‰)	17.3 ± 0.6	16.9 ± 0.6	0.015*
Falkland Islands (*)			
$\delta^{15}N$ (‰)	14.2 ± 0.4	16.4 ± 1.0	< 0.001***
$\delta^{13}C$ (‰)	-15.4 ± 0.2	-13.7 ± 0.7	< 0.001***

(*) From Riverón et al. (2021)

Significant differences between colonies of SAFS were detected in $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values (Table 3.2, Figure 3.S2), with $\delta^{15}N$ values ~1.5‰ higher in individuals from the Isla de Lobos. Differences in $\delta^{13}C$ and $\delta^{34}S$ values were less than 0.5‰ approximating the analytical error of the measurement equipment. For South American sea lions only sulfur isotopic values (~0.7‰) were significantly different between colonies but again this difference approximate instrument measurement error (Table 3.2, Figure 3.S2). Isotopic niche sizes (alpha = 0.95) estimated from the δ^{13}C and δ^{15}N ratios of SAFS (CP: 8.9 ± 2.2 ‰^{2}, IL: 8.7 ± 2.2 ‰^{2}) and SASL (CP: 1.9 ± 0.5 ‰^{2}, IL: 2.4 ± 0.5 ‰^{2}) pups were roughly the same at the two colonies (Table 3.2, Figure 3.S3).

Table 3.2. Stable isotope values of nitrogen (δ^{15}N), carbon (δ^{13}C) and sulfur (δ^{34}S) in pup skin samples of South American fur seal (*Arctocephalus australis*) and South American sea lion (*Otaria byronia*) from the two Uruguayan colonies (Cabo Polonio and Isla de Lobos). Results of the Wilcoxon Rank Test (*p*-value) are shown. Data are expressed as means ± standard deviation. Isotopic values are in parts per mill (‰). Significance levels: *<0.05, **<0.01, ***<0.001.

	Arctocephalus australis South American fur seal			***Otaria byronia*** South American sea lion		
	Cabo Polonio	**Isla de Lobos**	***p*-value**	**Cabo Polonio**	**Isla de Lobos**	***p*-value**
δ^{15}N	17.0 ± 1.7	18.4 ± 1.2	0.002**	20.4 ± 0.4	20.3 ± 0.4	0.954
δ^{13}C	-15.2 ± 0.5	-14.8 ± 0.5	0.005**	-13.6 ± 0.3	-13.5 ± 0.4	0.199
δ^{34}S	17.4 ± 0.6	17.0 ± 0.5	0.027*	17.2 ± 0.5	16.5 ± 0.5	0.014*

Isotopic patterns of each species were inverted at both extremes of their Atlantic distribution (Figure 3.2 – 3.3). In Uruguay, SAFS adult females had an isotopic niche size of 10.3 ± 1.8 ‰^{2}, approximately 4.3 times larger than SASL (2.4 ± 0.4‰^{2}). This pattern was the opposite in the Falkland Islands, where SASL adult females had an isotopic niche size (9.2 ± 1.7 ‰^{2}), approximately 6.8 times larger than that of SAFS (1.4 ± 0.3 ‰^{2}). Adult females SAFS in Uruguay had a range of δ^{15}N values 4.5 times larger than SASL, and both species has similar ranges of δ^{13}C (~2‰, Table 3.3). In contrast, adult females SASL in the Falkland Islands, the range of δ^{15}N values was 2.5 times larger than SAFS, and that of δ^{13}C, 4.3 times larger (Table 3.3). Comparing the isotopic niche sizes of each species between the regions, SAFS females had an isotopic niche size ~7.5 times larger in Uruguay than in the Falkland Islands (Table 3.3). By contrast the isotopic niche size of SASL females was ~4 times larger in the Falkland Islands than in Uruguay (Table 3.3). For both species, variations in isotopic niche size were mainly driven by differences in the dispersion in δ^{15}N between regions.

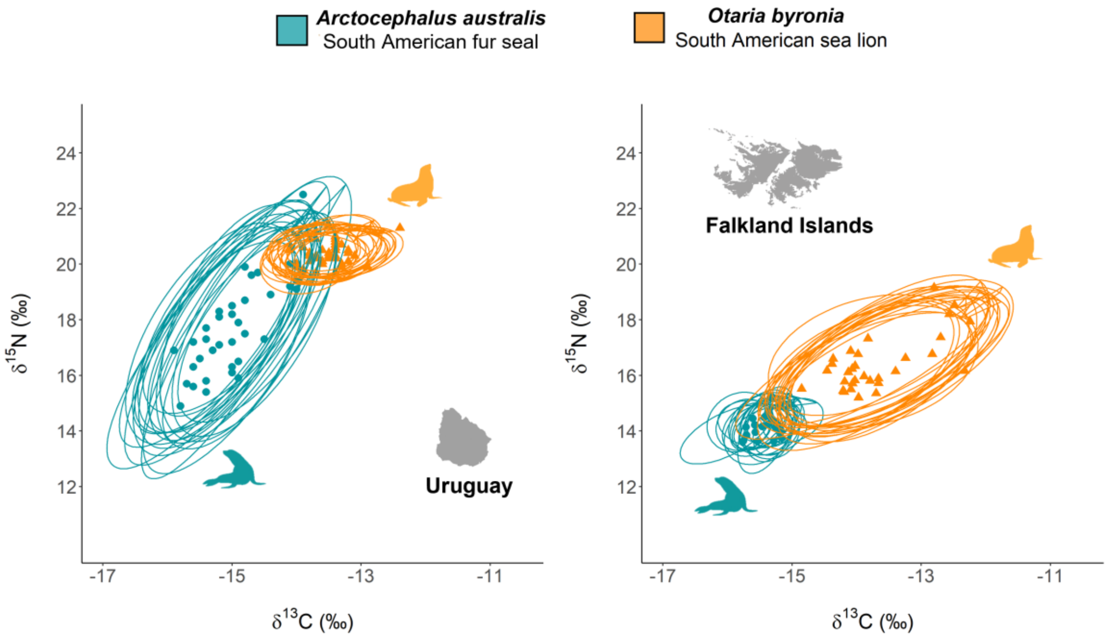

Figure 3.2. Isotopic niches from carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) for South American sea lion (*Otaria byronia*) and South American fur seal (*Arctocephalus australis*) from Uruguay and the Falkland Islands. Standard Ellipse Area (95%) showing first 20 simulation draws.

Table 3.3. Bayesian estimates of isotopic niche size (N_R) calculated from isotopic values of $\delta^{13}C$ and $\delta^{15}N$ of South American fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria byronia*) from Uruguay and the Falkland Islands. Data with standard deviation. Estimates of the niche size were conducted for alpha equal to 0.40 and 0.95.

	Arctocephalus australis South American fur seal		***Otaria byronia*** South American sea lion	
	Falklands	**Uruguay**	**Falklands**	**Uruguay**
N_R (‰^{2}) 95%	1.35 ± 0.30	10.33 ± 1.80	9.17 ± 1.71	2.36 ± 0.36
N_R (‰^{2}) 40%	0.25 ± 0.06	1.90 ± 0.33	1.68 ± 0.31	0.41 ± 0.06
$\delta^{15}N$ range (‰)	1.60	7.60	4.00	1.70
$\delta^{13}C$ range (‰)	0.60	2.00	2.60	1.70

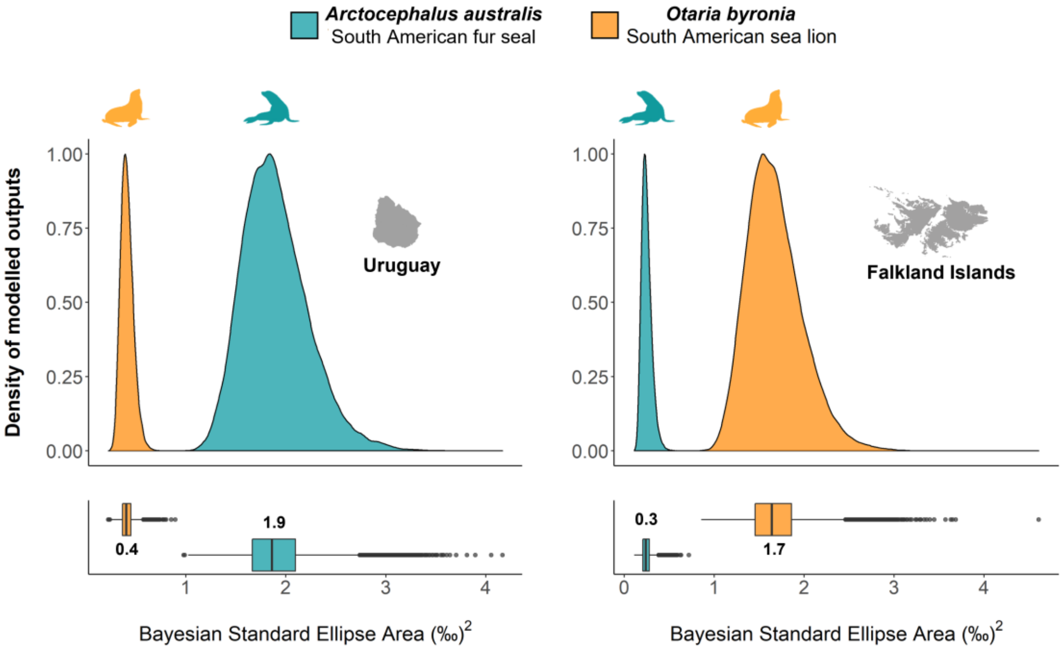

Figure 3.3. Posterior density plot obtained from a Bayesian estimation of the isotopic niche space (Bayesian Standard Ellipse Area) calculated from the carbon and nitrogen isotopic values of South American fur seals (*Arctocephalus australis*) and South American sea lions (*Otaria byronia*) from Uruguay and the Falkland Islands. Boxplot representing the median, inter-quartile range (rectangle), 95% range (continues line) and outliers (filled points) for ease of interpretation.

3.5. DISCUSSION

To our surprise, the degree of niche plasticity in SAFS and SASL within the Atlantic distribution was considerably higher than expected. Isotopic niches sizes of each species inverted at both extremes of their Atlantic distribution, and therefore their population ecological role switched from specialist to generalist, and vice versa. The detection of a complete inversion of the ecological role in two sympatric species from two different ecosystems is remarkable and demonstrates that the niche plasticity of a species can be greater than commonly assumed, and that assigning a fixed trophic role (generalist/specialist) to a species may be inappropriate.

We propose that differences in trophic role in populations of the same species may be driven by differences in local prey abundance and diversity, arising from the contrasting regional differences between ecosystems, and these drivers may be stronger at determining trophic role than intrinsic factors associated with life histories alone. Moderate variations in trophic niche size within the same species between regions and associated to differences in prey availability were previously registered in other marine predators, such as bony fishes: Lesser et al. (2020); cetaceans: Troina et al. (2020a) and penguins: Ciancio et al. (2021). Higher levels of productivity in the environment could lead to narrower population niche size, as the abundance of prey increase and allow predators to focus on preferred resources (Lesser et al., 2020). Moreover, the level of individual specialization (generalists and specialists) within a given population would produce differential responses to environmental changes in the diversity or abundance of prey (Bolnick et al., 2003; Araújo et al., 2011).

In this study we see how generalists may become specialists as a function of reduced spatial environment heterogeneity (Van Tienderen, 1991). Generalist populations (broader niche size) can become specialists (narrower niche size) when competition for resources is relaxed (see Araújo et al., 2011). This may happen because of an increase in prey availability, either by higher prey abundance, increased species diversity or greater prey accessibility. Some examples include different taxonomic groups and ecosystems: bony fishes from marine and lake ecosystems (*e.g.*, Araujo et al., 2008; Lesser et al., 2020), marine mammals (*e.g.,* Drago et al., 2016; Troina et al., 2020a), seabirds (*e.g.*, Chiu-Werner et al., 2019; Ciancio et al., 2021), invertebrates (*e.g.,* Svanbäck et al., 2011) and terrestrial mammals (*e.g.,* Darimont et al., 2009). The narrow niche size of SASL females in Uruguay is likely an indicator of high abundance of their preferred prey, which relaxes intraspecific competition and reduces the need for individuals to specialise on less preferred prey to reduce levels of competition. In line with this hypothesis, so far only one foraging strategy (benthic) has been identified in SASL females from Uruguay (Riet-Sapriza et al., 2013; Rodríguez et al., 2013). In contrast, the larger niche size of SASL females in the Falkland Islands reflects three different foraging strategies within their population (Baylis et al., 2016b; Riverón et al., 2021). Diversification in foraging behaviour in SASL implies higher levels of intraspecific competition with lower abundance of any particular prey type, resulting in individuals specialising on diverse resources. Regional plasticity in SASL diving behaviour and physiology occurs across its distribution, and regional differences in continental shelf extent have been proposed as a likely explanation (Hückstädt et al., 2016). Niche size variation in females between colonies occurs in northern Patagonia, and is attributed to differences foraging areas and prey choice (Grandi et al., 2021). Moreover, temporal variability in their niche sizes was registered in both species in the Río de la Plata region (Drago et al., 2017; Szteren et al, 2018) attributed to changes in the prey availability through history. Therefore, niche plasticity of a population depends not only upon the environmental characteristics of the area, but also the community composition and the ability of the species to coexist with sympatric species that have similar requirements (Carscadden et al., 2020).

Similarly, as spatial heterogeneity increases in the environment, specialist populations may become generalists (Van Tienderen, 1991). As proposed in Riverón et al. (2021), even though SAFS females from the Falkland Islands show differences in foraging grounds and diving behaviours (Baylis et al., 2018), their narrow pelagic isotopic signal suggests that SAFS consume abundant but similar pelagic prey. In contrast, the SAFS in the Uruguayan estuary ecosystem can choose between two highly productive areas: the shallow continental shelf where they may prey on pelagic and small demersal species, or at the shelf-front edge where they may prey on pelagic species. The most recent diet studies in Río de la Plata show that SAFS not only prey on pelagic species, but also on demersal species commonly found on the continental shelf (Frau, 2009; Denuncio et al., 2021). Biologging data of females from Isla de Lobos confirmed that SAFS are shallow divers whose foraging areas are S-SW of Isla de Lobos within the continental shelf (< 60 m depth) (Franco-Trecu, 2015). Our results confirm that SAFS females probably have at least two foraging strategies most likely associated with differences in distance to preferred foraging areas. Females from Isla de Lobos are closer to *Río de la Plata* and have higher values of $\delta^{13}C$ and $\delta^{15}N$ compared to females from *Cabo Polonio* (~130 km Northeast), suggesting that females from *Cabo Polonio* prefer to prey on a pelagic food web, and those from *Isla de Lobos* forage over the continental shelf. These two different strategies would explain the larger trophic niche shown by SAFS in Uruguay. Since the mid-twentieth century there has been a progressive convergence in the niche of SAFS and SASL, and it has been hypothesised that this may reflect an increased preference of SAFS for small demersal species in their diet (Drago et al., 2017; Szteren et al., 2018). Given that the abundance of SAFS populations in Uruguay has been increasing since 1950s (Lima & Paez, 1995; Franco-Trecu et al., 2019), an increase in intraspecific competition may be the driver for diversification in their diet.

This study has identified a very high degree of niche plasticity in a high trophic level marine predator with a range in niche size of ~7.3-times for SAFS. The previous highest range of change registered for a marine predator population within a latitudinal range in Patagonian coastal waters, was maximum of ~3.8-time differences in their niche sizes between regions (Ciancio et al., 2021). This points to the potential variability of species population niche metrics, especially among populations of widely distributed species.

Ecological implications

In this work, we confirmed that the trophic niche size of a species in different geographical areas may vary markedly, indicating that niches can be extremely plastic. We found a complete inversion of the species trophic role in different populations of the same species (one changing from a specialist population to generalist one, and vice versa). These findings question the assumption that niches size among populations of the same species are relatively fixed and in indeed that there are species-specific traits determining niche size. This is predicated by the switch from generalist to specialist for a taxa broadly assumed to be a generalist (SASL), and the switch from specialist to generalist for a taxa broadly assumed to be specialist throughout its range (SAFS). Higher degrees of niche plasticity are expected to be found in species with broad geographic distribution as their range encompasses different ecosystems that could drive differences trophic niche size and here we find role reversals for two species with broad, sympatric ranges. Generalist populations are known to be more resilient than specialist populations to rapid changes in the environment. The niche size of a population is commonly used to determine whether a species is a generalist or a specialist. The plasticity displayed in this study suggests that care must be taken in inferring intrinsic constraints on species.

3.6. REFERENCES

Acha, M. E., Mianzan, H., Guerrero, R., Carreto, J., Giberto, D., Montoya, N., & Carignan, M. (2008). An overview of physical and ecological processes in the Rio de la Plata Estuary. *Continental Shelf Research*, *28*, 1579–1588. https://doi.org/10.1016/j.csr.2007.01.031

Alves-Stanley, C. D., & Worthy, G. A. J. (2009). Carbon and nitrogen stable isotope turnover rates and diet-tissue discrimination in Florida manatees (*Trichechus manatus latirostris*). *Journal of Experimental Biology*, *212*, 2349–2355. https://doi.org/10.1242/jeb.027565

Antão, L. H., Bates, A. E., Blowes, S. A., Waldock, C., Supp, S. R., Magurran, A. E., Dornelas, M., & Schipper, A. M. (2020). Temperature-related biodiversity change across temperate marine and terrestrial systems. *Nature Ecology and Evolution*, *4*, 927–933. https://doi.org/10.1038/s41559-020-1185-7

Araújo, M. S., Guimarães, P. R., Svanbäck, R., Pinheiro, A., Guuimarães, P., Dos Reis, S. F. & Bolnick, D. I. (2008). Network analysis reveals contrasting effects of intraspecific competition on individual vs. population diets. *Ecology*, *89,* 1981–1993. https://doi.org/10.1890/07-0630.1

Araújo, M. S., Bolnick, D. I., & Layman, C. A. (2011). The ecological causes of individual specialisation. *Ecology Letters*, *14*(9), 948–958. https://doi.org/10.1111/j.1461-0248.2011.01662.x

Arkhipkin, A., Brickle, P., & Laptikhovsky, V. (2013). Links between marine fauna and oceanic fronts on the Patagonian shelf and slope. *Arquipelago: Life and Marine Sciences*, *30*, 19–37.

Aurioles, D., Koch, P. L., & Le Boeuf, B. J. (2006). Differences in foraging location of Mexican and California elephant seals: Evidence from stable isotopes in pups. *Marine Mammal Science*, *22*(2), 326–338. https://doi.org/10.1111/j.1748-7692.2006.00023.x

Bastida, R., Rodríguez, D., Secchi, E., & Silva, V. da. (2007). *Mamíferos acuáticos de Sudamérica y Antártida* (First edition). Vázquez Manzini Editores.

Baylis, A. M. M., Orben, R. A., Arnould, J. P. Y., Peters, K., Knox, T., Costa, D. P., & Staniland, I. J. (2015). Diving deeper into individual foraging specializations of a large marine predator, the southern sea lion. *Oecologia*, *179*(4), 1053–1065. https://doi.org/10.1007/s00442-015-3421-4

Baylis, A. M. M., Orben, R. A., Costa, D. P., Arnould, J. P. Y., & Staniland, I. J. (2016a). Sexual segregation in habitat use is smaller than expected in a highly dimorphic marine predator, the southern sea lion. *Marine Ecology Progress Series*, *554*, 201–211. https://doi.org/10.3354/meps11759

Baylis, A. M. M., Kowalski, G. J., Voigt, C. C., Orben, R. A., Trillmich, F., Staniland, I. J., & Hoffman, J. I. (2016b). Pup vibrissae stable isotopes reveal geographic differences in adult female southern sea lion habitat use during gestation. *PLoS ONE*, *11*(6), e0157394. https://doi.org/10.1371/journal.pone.0157394

Baylis, A. M. M., Tierney, M., Orben, R. A., Staniland, I. J., & Brickle, P. (2018). Geographic variation in the foraging behaviour of South American fur seals. *Marine Ecology Progress Series*, *596*, 233–245. https://doi.org/10.3354/meps12557

Baylis, A. M. M., Tierney, M., Orben, R. A., Warwick-Evans, V., Wakefield, E., Grecian, W. J., Trathan, P., Reisinger, R., Ratcliffe, N., Croxall, J., Campioni, L., Catry, P., Crofts, S., Boersma, P. D., Galimberti, F., Granadeiro, J., Handley, J., Hayes, S., Hedd, A., Masello, J. F., Montevecchi, W. A., Pütz, K., Quillfeldt, P., Rebstock, G. A., Sanvito, S., Staniland, I. J., & Brickle, P. (2019). Important at-sea areas of colonial breeding marine predators on the southern Patagonian shelf. *Scientific Reports*, *9*(8517), 1–13. https://doi.org/10.1038/s41598-019-44695-1

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., Forister, M. L., & Forister. (2003). The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist*, *161*(1), 1–28. https://doi.org/10.2307/3078879

Brazeiro, A., Acha, M., Mianzan, H., Gómez-Erache, M., & Fernández, V. (2003). Aquatic priority areas for the conservation and management of the ecological integrety of the Rio de la Plata and its maritime front. In *Technical Report PNUD Project: GEF RLA/99/G31* (Issue March 2014). https://doi.org/10.1093/jnci/djr261

Carscadden, K. A., Emery, N. C., Arnillas, C. A., Cadotte, M. W., Afkhami, M. E., Gravel, D., Livingstone, S. W., & Wiens, J. J. (2020). Niche breadth: Causes and consequences for ecology, evolution, and conservation. *The Quarterly Review of Biology*, *95*(3), 179–214.

Chilvers, B. L. (2019). Whisker stable isotope values indicate long-term foraging strategies for female New Zealand sea lions. *Endangered Species Research*, *38*, 55–66. https://doi.org/10.3354/ESR00934

Chiu-Werner, A., Ceia, F. R., Cárdenas-Alayza, S., Cardeña-Mormontoy, M., Adkesson, M., & Xavier, J. (2019). Inter-annual isotopic niche segregation of wild humboldt penguins through years of different El Niño intensities. *Marine Environmental Research*, *150*(July), 104755. https://doi.org/10.1016/j.marenvres.2019.104755

Ciancio, J. E., Yorio, P., Buratti, C., Colombo, G. Á., & Frere, E. (2021). Isotopic niche plasticity in a marine top predator as indicator of a large marine ecosystem food web status. *Ecological Indicators*, *126*, 107687. https://doi.org/10.1016/j.ecolind.2021.107687

Clark, C. T., Horstmann, L., & Misarti, N. (2019). Lipid normalization and stable isotope discrimination in Pacific walrus tissues. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-42095-z

Connolly, R. M., Guest, M. A., Melville, A. J. & Oakes, J. M. (2004). Sulfur stable isotopes separate producers in marine food-web analysis. *Oecologia, 138*(2), 161-167. https://doi.org/10.1007/s00442-003-1415-0

Costa, D. P. (2008). A conceptual model of the variation in parental attendance in response to environmental fluctuation: foraging energetics of lactating sea lions and fur seals. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *17*, S44–S52. https://doi.org/10.1002/aqc

Crespo, E. A., & de Oliveira, L. R. (2021). South American fur seal (*Arctocephalus australis*, Zimmerman, 1783). In G. Heckel & Y. Schramm (Eds.), *Ecology and conservation of pinnipeds in Latin America* (First edition, pp. 13–29). Springer International Publishing. https://doi.org/10.1007/978-3-030-

63177-2_2

Crespo, E. A., de Oliveira, L. R., & Sepúlveda, M. (2021). South American sea lion (*Otaria flavescens*, Shaw 1800). In G. Heckel & Y. Schramm (Eds.), *Ecology and conservation of pinnipeds in Latin America* (First edition, pp. 93–118). Springer International Publishing. https://doi.org/10.1007/978-3-030-63177-2_6

Croxall, J. P., & Wood, A. G. (2002). The importance of the Patagonian Shelf for top predator species breeding at South Georgia. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *12*, 101–118. https://doi.org/10.1002/aqc.480

Darimont, C. T., Paquet, P. C., & Reimchen, T. E. (2009). Landscape Heterogeneity and Marine Subsidy Generate Extensive Intrapopulation Niche Diversity in a Large Terrestrial Vertebrate. *Journal of Animal Ecology, 78*(1), 126–133. http://www.jstor.org/stable/27696348

Deagle, B. E., Kirkwood, R., & Jarman, S. N. (2009). Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Molecular Ecology*, *18*(9), 2022–2038. https://doi.org/10.1111/j.1365-294X.2009.04158.x

Denuncio, P., Gana, J. C. M., Giardino, G. V., Rodríguez, D. H., & Machovsky-Capuska, G. E. (2021). Prey composition and nutritional strategies in two sympatric pinnipeds. *Journal of Experimental Marine Biology and Ecology*, *545*, 151629. https://doi.org/10.1016/j.jembe.2021.151629

Drago, M., Cardona, L., Franco-Trecu, V., Crespo, A., Vales, D., Borella, F., Zenteno, L., Gonzáles, E. M., & Inchausti, P. (2017). Isotopic niche partitioning between two apex predators over time. *ARPN Journal of Engineering and Applied Sciences*, *86*(4), 766–780. https://doi.org/10.1111/1365-2656.12666

Drago, M., Franco-Trecu, V., Cardona, L., & Inchausti, P. (2015). Diet-to-female and female-to-pup isotopic discrimination in South American sea lions. *Rapid Communications in Mass Spectrometry*, *29*, 1513–1520. https://doi.org/10.1002/rcm.7249

Drago, M., Franco-Trecu, V., Cardona, L., Inchausti, P., Tapia, W., & Páez-Rosas, D. (2016). Stable isotopes reveal long-term fidelity to foraging grounds in the galapagos sea lion (*Zalophus wollebaeki*). *PLoS ONE*, *11*(1), e0147857. https://doi.org/10.1371/journal.pone.0147857

Evans, M. R., & Moustakas, A. (2018). Plasticity in foraging behaviour as a possible response to climate change. *Ecological Informatics*, *47*, 61–66. https://doi.org/10.1016/j.ecoinf.2017.08.001

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, *226*(1), 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5

Franco-Trecu, V. (2015). *Tácticas comportamentales de forrajeo y apareamiento y dinámica poblacional de dos especies de otáridos simpátricas con tendencias poblacionales contrastantes* [PhD thesis, Universidad de la República]. Colibri Repository. https://www.colibri.udelar.edu.uy/jspui/

Franco-Trecu, V., Drago, M., Grandi, M. F., Soutullo, A., Crespo, E. A., & Inchausti, P. (2019). Abundance and population trends of the South American fur seal (*Arctocephalus australis*) in Uruguay. *Aquatic Mammals*, *45*(1), 48–55. https://doi.org/10.1578/AM.45.1.2019.48

Frau, M. R. (2009). *Dieta del lobo fino (Arctocephalus australis) en Isla de Lobos (Maldonado-Uruguay) durante el período de reproducción* [Bachelor thesis, Universidad de la República]. Colibri Repository. https://www.colibri.udelar.edu.uy/jspui/.

García-Alonso, J., Lercari, D., & Defeo, O. (2019). Río de la Plata: A neotropical estuarine system. In E. Wolanski, J. W. Day, M. Elliott, & R. Ramachandran (Eds.), *Coasts and estuaries: The future* (First edition, pp. 45–56). Elsevier Inc. https://doi.org/10.1016/B978-0-12-814003-1.00003-4

Glew, K. S. J., Espinasse, B., Hunt, B. P. V., Pakhomov, E. A., Bury, S. J., Pinkerton, M., Nodder, S. D., Gutiérrez-Rodríguez, A., Safi, K., Brown, J. C. S., Graham, L., Dunbar, R. B., Mucciarone, D. A., Magozzi, S., Somes, C., & Trueman, C. N. (2021). Isoscape models of the southern Ocean: Predicting spatial and temporal variability in carbon and nitrogen isotope compositions of particulate organic matter. *Global Biogeochemical Cycles*, *35*, e2020GB006901. https://doi.org/10.1029/2020GB006901

Grandi, M. F., Vales, D. G., Crespo, E. A., & Loizaga, R. (2021). Variation in trophic resources in female South American sea lions at a small geographic scale. *Marine Mammal Science*, *37*(1), 314–327. https://doi.org/10.1111/mms.12746

Heileman, S. (2009). XVI South West Atlantic. In K. Sherman & G. Hempel (Eds.), *The UNEP large marine ecosystem report: a perspective on changing conditions in LMEs of the world's regional seas, Vol. 182* (pp. 699–746). United Nations EP.

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. *Science*, *328*, 1523–1529.

Hückstädt, L. A., Tift, M. S., Riet-Sapriza, F., Franco-Trecu, V., Baylis, A. M. M., Orben, R. A., Arnould, J. P. Y., Sepulveda, M., Santos-Carvallo, M., Burns, J. M., & Costa, D. P. (2016). Regional variability in diving physiology and behavior in a widely distributed air-breathing marine predator, the South American sea lion (*Otaria byronia*). *The Journal of Experimental Biology*, *219*(15), 2320–2330. https://doi.org/10.1242/jeb.138677

Hussey, N. E., MacNeil, M. A., Olin, J. A., McMeans, B. C., Kinney, M. J., Chapman, D. D. & Fisk, A. T. (2012). Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions.

Journal of Fish Biology, 80(5), 1449–1484. https://doi.org/10.1111/j.1095-8649.2012.03251.x

Jefferson, T. A., Webber, M. A., & Pitman, R. L. (2015). Pinnipeds. In T. A. Jefferson, M. A. Webber, & R. L. Pitman (Eds.), *Marine Mammals of the World* (Second edition, pp. 358–522). Elsevier. https://doi.org/10.1016/B978-0-12-409542-7.50005-6

Jenkins, S. G., Partridge, S. T., Stephenson, T. R., Farley, S. D., & Robbins, C. T. (2001). Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. *Oecologia*, *129*(3), 336–341. https://doi.org/10.1007/s004420100755

Kroeker, K. J., Bell, L. E., Donham, E. M., Hoshijima, U., Lummis, S., Toy, J. A., & Willis-Norton, E. (2020). Ecological change in dynamic environments: Accounting for temporal environmental variability in studies of ocean change biology. *Global Change Biology*, *26*(1), 54–67. https://doi.org/10.1111/gcb.14868

Kwasniewski, S., Gluchowska, M., Jakubas, D., Wojczulanis-Jakubas, K., Walkusz, W., Karnovsky, N., Blachowiak-Samolyk, K., Cisek, M., & Stempniewicz, L. (2010). The impact of different hydrographic conditions and zooplankton communities on provisioning Little Auks along the West coast of Spitsbergen. *Progress in Oceanography*, *87*(1–4), 72–82. https://doi.org/10.1016/j.pocean.2010.06.004

Ladds, M., Rosen, D., Gerlinsky, C., Slip, D., & Harcourt, R. (2020). Diving deep into trouble: The role of foraging strategy and morphology in adapting to a changing environment. *Conservation Physiology*, *8*(1), 1–21. https://doi.org/10.1093/conphys/coaa111

Lesser, J. S., James, W. R., Stallings, C. D., Wilson, R. M., & Nelson, J. A. (2020). Trophic niche size and overlap decreases with increasing ecosystem productivity. *Oikos*, *129*(9), 1303–1313. https://doi.org/10.1111/oik.07026

Lima, M., & Paez, E. (1995). Growth and reproductive patterns in the South American fur seal. *Journal of Mammalogy*, *76*(4), 1249–1255. https://doi.org/10.2307/1382619

Lowther, A. D., & Goldsworthy, S. D. (2011). Detecting alternate foraging ecotypes in Australian sea lion (*Neophoca cinerea*) colonies using stable isotope analysis. *Marine Mammal Science*, *27*(3), 567–586. https://doi.org/10.1111/j.1748-7692.2010.00425.x

Lowther, A. D., Harcourt, R. G., Hamer, D. J., & Goldsworthy, S. D. (2011). Creatures of habit: Foraging habitat fidelity of adult female Australian sea lions. *Marine Ecology Progress Series*, *443*, 249–263. https://doi.org/10.3354/meps09392

Mandiola, A., Alemany, D., Dassis, M., Pon, J. P. S., Prosdocimi, L., Mianzan, H., Acha, E. M., Rodrı, D., Favero, M., Copello, S., & Leo, A. P. De. (2016). Key ecological areas and opportunities for marine conservation. *Marine Science*, *73*(6), 1579–1588.

Matano, R. P., & Palma, E. D. (2008). On the upwelling of downwelling currents. *Journal of Physical Oceanography*, *38*(11), 2482–2500. https://doi.org/10.1175/2008JPO3783.1

Mittelbach, G. G., & McGill, B. J. (2019). Species coexistence and niche theory. In *Community Ecology* (Second edition, pp. 141–157). Oxford University Press. https://doi.org/10.1093/oso/9780198835851.003.0008

Newsome, S. D., Clementz, M. T., & Koch, P. L. (2010). Using stable isotope biogeochemistry to study marine mammal ecology. *Marine Mammal Science*, *26*(3), 509–572. https://doi.org/10.1111/j.1748-7692.2009.00354.x

Niella, Y., Raoult, V., Gaston, T., Peddemors, V. M., Harcourt, R., Smoothey, A. F. (2021). Overcoming multi-year impacts of maternal isotope signatures using multi-tracers and fast turnover tissues in juvenile sharks. *Chemosphere*, *269*, 129393. https://doi.org/10.1016/j.chemosphere.2020.129393

Pagel, M. D., May, R. M., & Collie, A. R. (1991). Ecological aspects of the geographical distribution and diversity of mammalian species. *The American Naturalist*, *137*(6), 791–815.

Peterson, B. J., Howarth, R. W., Garritt, R. H. (1979). Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science*, *227*(4692), 1361-1363. https://doi.org/10.1126/science.227.4692.1361

Piola, A. R., & Matano, R. P. (2017). Ocean Currents: Atlantic Western Boundary—Brazil Current/Falkland (Malvinas) Current. *Reference Module in Earth Systems and Environmental Sciences*, 0–7. https://doi.org/10.1016/b978-0-12-409548-9.10541-x

Piola, A. R., Palma, E. D., Bianchi, A. A., Castro, B. M., Dottori, M., Guerrero, R. A., Marrari, M., Matano, R. P., Möller, O. O., & Saraceno, M. (2018). Physical oceanography of the SW Atlantic shelf: A review. In M. S. Hoffmeyer, M. E. Sabatini, F. P. Brandini, D. L. Calliari, & N. H. Santinelli (Eds.), *Plankton ecology of the southwestern Atlantic: From the subtropical to the subantarctic realm* (First edition, pp. 37–56). Springer. https://doi.org/10.1007/978-3-319-77869-3_2

Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, *83*(3), 703-718. doi:10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Riet-Sapriza, F. G., Costa, D. P., Franco-Trecu, V., Marín, Y., Chocca, J., González, B., Beathyate, G., Louise Chilvers, B., & Hückstädt, L. A. (2013). Foraging behavior of lactating South American sea lions (*Otaria flavescens*) and spatial–temporal resource overlap with the Uruguayan fisheries. *Deep Sea Research Part II: Topical Studies in Oceanography*, *88–89*, 106–119. https://doi.org/10.1016/j.dsr2.2012.09.005

Riverón, S., Raoult, V., Baylis, A. M. M., Jones, K. A., Slip, D. J., & Harcourt, R. G. (2021). Pelagic and benthic ecosystems drive differences in population and individual specializations in marine predators. *Oecologia*, *196*(3), 891–904. https://doi.org/10.1007/s00442-021-04974-z

Riverón, S., Raoult, V., Slip, D. J., & Harcourt, R. G. (2022). Lipid extraction has tissue-dependent effects on isotopic values (δ^{34}S, δ^{13}C, δ^{15}N) from different marine predators. *Rapid Communications in Mass Spectrometry*, *36*(18), e9346. https://doi.org/10.1002/rcm.9346

Rodríguez, D. H., Dassis, M., Ponce de León, A., Barreiro, C., Farenga, M., Bastida, R. O., & Davis, R. W. (2013). Foraging strategies of Southern sea lion females in the La Plata River Estuary (Argentina–Uruguay). *Deep Sea Research Part II: Topical Studies in Oceanography*, *88–89*, 120–130. https://doi.org/10.1016/j.dsr2.2012.07.012

Rutherford, S., D'Hondt, S., & Prell, W. (1999). Environmental controls on the geographic distribution of zooplankton diversity. *Nature*, *400*(6746), 749–753. https://doi.org/10.1038/23449

Rykaczewski, R. R., & Checkley, D. M. (2008). Influence of ocean winds on the pelagic ecosystem in upwelling regions. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(6), 1965–1970. https://doi.org/10.1073/pnas.0711777105

Svanbäck, R., Rydberg, C., Leonardsson, K., & Englund, G. (2011). Diet specialization in a fluctuating population of *Saduria entomon*: A consequence of resource or forager densities? *Oikos*, *120*, 848–854. https://doi.org/10.1111/j.1600-0706.2010.18945.x

Shipley, O. N., & Matich, P. (2020). Studying animal niches using bulk stable isotope ratios: An updated synthesis. *Oecologia*, *193*, 27-51. doi: 10.1007/s00442-020-04654-4

Swanson, H. K., Lysy, M., Power, M., Stasko, A. D., Johnson, J. D., & Reist, J. D. (2015). A new probabilistic method for quantifying *n*-dimensional ecological niches and niche overlap. *Ecology*, *96*(2), 318–324. https://doi.org/https://doi.org/10.1890/14-0235.1

Szteren, D., Aurioles-Gamboa, D., Labrada-Martagón, V., Hernández-Camacho, C. J., & De María, M. (2018). Historical age-class diet changes in South American fur seals and sea lions in Uruguay. *Marine Biology*, *165*(59), 17. https://doi.org/10.1007/s00227-018-3315-1

Thompson, D., Duck, C. D., McConnell, B. J., & Garrett, J. (1998). Foraging behaviour and diet of lactating female southern sea lions (*Otaria flavescens*) in the Falkland Islands. *Journal of Zoology*, *246*, 135–146. https://doi.org/10.1017/S0952836998010024

Thompson, D., Moss, S., & Lovell, P. (2003). Foraging behaviour of South American fur seals *Arctocephalus australis*: extracting fine scale foraging behaviour from satellite tracks. *Marine Ecology Progress Series*, *260*, 285–296. https://doi.org/10.3354/meps260285

Tollit, D. J., Black, A. D., Thompson, P. M., Mackay, A., Corpe, H. M., Wilson, B., Van Parijs, S. M., Grellier, K., & Parlane, S. (1998). Variations in harbour seal Phoca vitulina diet and dive-depths in relation to foraging habitat. *Journal of Zoology*, *244*, 209–222. https://doi.org/10.1017/S0952836998002064

Troina, G. C., Botta, S., Dehairs, F., Di Tullio, J. C., Elskens, M., & Secchi, E. R. (2020a). Skin $\delta^{13}C$ and $\delta^{15}N$ reveal spatial and temporal patterns of habitat and resource use by free-ranging odontocetes from the southwestern Atlantic Ocean. *Marine Biology*, *167*(12), 186. https://doi.org/10.1007/s00227-020-03805-8

Troina, G. C., Dehairs, F., Botta, S., Tullio, J. C. Di, Elskens, M., & Secchi, E. R. (2020b). Zooplankton-based $\delta^{13}C$ and $\delta^{15}N$ isoscapes from the outer continental shelf and slope in the subtropical western South Atlantic. *Deep-Sea Research Part I: Oceanographic Research Papers*, *159*, 103235. https://doi.org/10.1016/j.dsr.2020.103235

Vales, D. G., Saporiti, F., Cardona, L., De Oliveira, L. R., Dos Santos, R. A., Secchhi, E. R., Aguilar, A., & Crespo, E. A. (2013). Intensive fishing has not forced dietary change in the South American fur seal *Arctophoca* (= *Arctocephalus*) australis off Río de la Plata and adjoining areas. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *24*(6), 745–759. https://doi.org/10.1002/aqc.2397

Valladares, F., Bastias, C. C., Godoy, O., Granda, E., & Escudero, A. (2015). Species coexistence in a changing world. *Frontiers in Plant Science*, *6*, 866. https://doi.org/10.3389/fpls.2015.00866

Van Buskirk, J. (2012). Behavioural plasticity and environmental change. In U. Candolin & B. B. M. Wong (Eds.), *Behavioral responses to a changing world: mechanisms and consequences* (First edition, pp. 145–158). Oxford University Press.

Van Tienderen, P. H. (1991). Evolution of generalists and specialists in spatially heterogeneous environments. Evolution, 45(6), 1317-1331.

Voigt, C. C., Matt, F., Michener, R., & Kunz, T. H. (2003). Low turnover rates of carbon isotopes in tissues of two nectar-feeding bat species. *Journal of Experimental Biology*, *206*(8), 1419–1427. https://doi.org/10.1242/jeb.00274

Wege, M., De Bruyn, P. J. N., Hindell, M. A., Lea, M. A., & Bester, M. N. (2019). Preferred, small-scale foraging areas of two Southern Ocean fur seal species are not determined by habitat characteristics. *BMC Ecology*, *19*, 36. https://doi.org/10.1186/s12898-019-0252-x

Würsig, B., Thewissen, J. G. M., & Kovacs, K. M. (Eds.). (2018). *Encyclopedia of marine mammals* (Third edition). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-804327-1.00034-0

Supplementary Material

Chapter 3. Plasticity is the key: two sympatric species in contrasting environments switch roles, between niche generalist and niche specialist

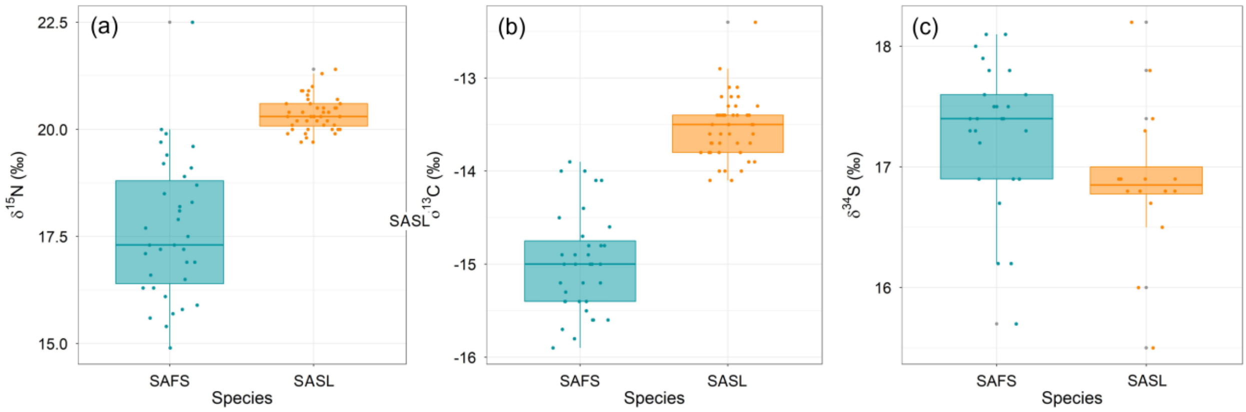

Figure 3.S1. Boxplot of stable isotopic values of δ^{13}C, δ^{15}N, δ^{34}S for South American fur seals (SAFS, *Arctocephalus australis*) and South American sea lions (SASL, *Otaria byronia*) pups from Uruguay. Horizontal lines indicate median; shaded boxes include the interquartile range showing upper (75%) and lower (25%) quartiles. Grey points indicate extreme values, outside 1.5 times the interquartile range above the upper and below the lower quartiles.

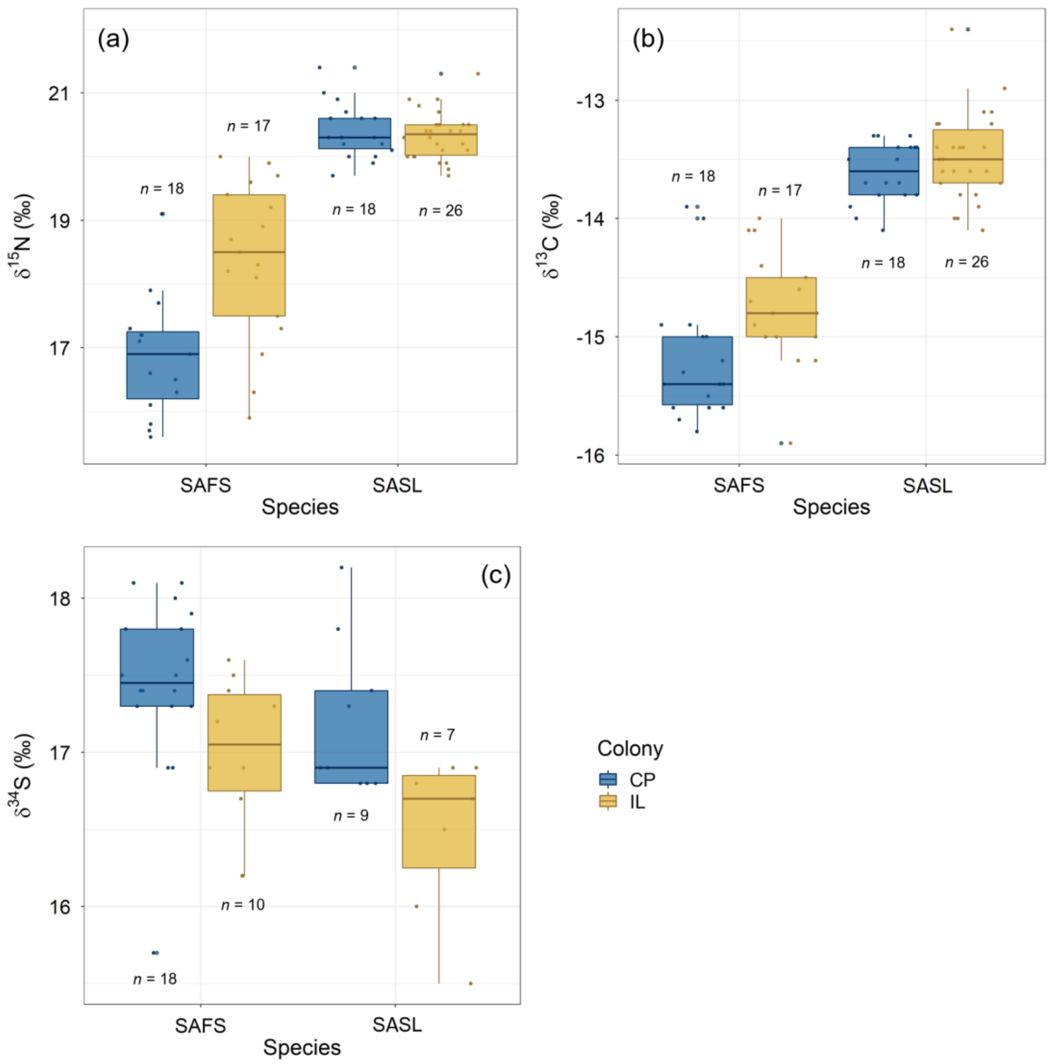

Figure 3.S2. Boxplot of stable isotopic values of δ^{13}C, δ^{15}N, δ^{34}S for South American fur seals (SAFS, *Arctocephalus australis*) and South American sea lions (SASL, *Otaria byronia*) from the two reproductive colonies in Uruguay: Cabo Polonio (CP) and Isla de Lobos (IL). Horizontal lines indicate median; shaded boxes include the interquartile range showing upper (75%) and lower (25%) quartiles. Grey points indicate extreme values, outside 1.5 times the interquartile range above the upper and below the lower quartiles.

Chapter Four

Morphology influences niche dynamics in two large, sympatric sharks

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Santiago Silveira, Robert G. Harcourt

4.1. ABSTRACT

The availability of prey in an environment does not ensure that it will be consumed by a predator:prey must also be detected, captured and successfully handled. Marine predator morphology imposes limitations on prey selection due to biomechanical constraints, making some prey functionally inaccessible. Morphological factors including tooth shape, body size and mouth gape therefore impose constraints on predator trophic niches. We assessed how two important components of trophic morphology (tooth shape and body length) influence prey selectivity and trophic niche in two large-bodied sympatric sharks with contrasting feeding modes. The first species captures prey using spear-shaped, grasping teeth (grey nurse shark, *Carcharias taurus*) while the second has multi-cuspid cutting teeth used to serrate larger prey (sevengill shark, *Notorynchus cepedianus*). Stomach content analysis and isotopic values of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ from muscle and liver were used to characterize trophic niche and prey selection. As gape-limited grey nurse sharks grew, their consumption of teleosts decreased inversely to chondrichthyans. By contrast, non-gape limited sevengill sharks consumed teleosts and chondrichthyans in similar proportions, along with marine mammals, but with no clear relationship to body size. As body length increased, both species consumed prey from higher trophic levels (higher $\delta^{15}N$), but sevengill sharks accessed prey at relatively higher trophic levels. Values of $\delta^{13}C$ and $\delta^{34}S$ remained substantially unchanged with body length since mouth gape and dentition are less limiting for these tracers. Morphological characters play an important role in predator prey selection and may be the primary mechanism facilitating resource partitioning in large sympatric predators. Their inclusion in ecological studies can help understand prey choice and how this shapes trophodynamics in marine ecosystems.

Keywords: stomach contents, stable isotopes, prey choice, sharks, *Carcharias taurus*, *Notorynchus cepedianus*, Southwestern Atlantic Ocean, trophic ecology

4.2. INTRODUCTION

The feeding ecology of marine predator populations, and therefore their trophic niche, is defined by a combination of biotic and abiotic factors. Biotic factors refer to all living organisms in an ecosystem, which together constitute the food web. Abiotic factors, such as temperature and salinity (Castillo et al., 1996; Harrison & Whitfield, 2006; Bailleul et al., 2007; Smyth & Elliott, 2016), determine in part the presence, distribution and abundance of prey and their consumers within an ecosystem (Domenici et al., 2007). Together, biotic and abiotic components of an ecosystem are drivers of inter and intraspecific competition for resources (Connell, 1983; Chase et al., 2002; Ward et al., 2006; Svanbäck & Bolnick, 2007; Bolnick et al., 2011). A decrease in prey abundance may lead to increased competition among its consumers, or if the resource is more abundant, competition may relax. However, the presence of potential prey in the environment does not imply that a predator will invariably feed upon this prey. Predators have intrinsic factors that enhance or constrain their feeding abilities, including morphological, physiological, and behavioural features (Horn & Ferry-Graham, 2006). Morphological characteristics form one of the final modes of prey selection which determines whether a predator is capable of consuming prey once all other requirements, such as prey availability, have been met. Therefore, these morphological characteristics, under strong selective pressure, exert differential constraints on large marine predators with different morphological features (Ferry, 2015; Bazzi et al., 2021).

Feeding phenotypes have different evolutionary pathways, originating from adaptative radiations that begin with habitat differentiation and continue with the evolution of divergent feeding morphological structures (Streelman & Danley, 2003; Grossnickle, 2020; Bazzi et al., 2021). Optimal foraging theory proposes that changes in the trophic morphology (morphological characters associated with the feeding ecology of individuals) will maximise the energy gain from feeding on a preferred available prey (MacArthur & Pianka, 1966; Pyke, 1984; Winkler et al., 2017). However, despite its fundamental role in feeding ecology, other than size, morphology is often an underappreciated parameter in trophic models (Ferry-Graham et al., 2002). This is despite the likelihood that this mechanism may help explain the ecological patterns obtained through other analytical approaches (*e.g.*, stomach content and stable isotope analysis) (Verde Arregoitia et al., 2017; Keppeler et al., 2020).

Morphological structures involved in feeding are key to determining which available prey items will ultimately become part of an animal’s diet (Horn & Ferry-Graham, 2006). Therefore, a strong relationship between diet and morphology has been reported for many taxonomic groups, including terrestrial mammals (Grossnickle, 2020), sharks (Bazzi et al., 2021), bony fishes (Ferry-Graham et al., 2001; Olivier et al., 2019), marine turtles (Figgener et al., 2019) and invertebrates (DeVries, 2017). The high degree of accuracy with which feeding habits can be identified using morphological structures makes it possible even to infer the dietary habits of extinct species based on characteristics of living equivalents, *e.g.*, shark (Bazzi et al., 2021) and mammalian (Croft et al., 2011; Verde Arregoitia et al., 2017) teeth used as references for the fossil record.

Individuals seek to maximise their net energy gain from predation, and this involves a combination of time and energy loss due to the predation event, which includes searching, handling, processing, and prey digestion (Willson & Hopkins, 2011; Hocking et al., 2021). Increased body size usually improves a predator’s ability to handle larger prey and therefore access a new range of prey items (Wainwright & Richard, 1995; MacNulty et al., 2009; Cuthbert et al., 2020). Variation in diet associated with changes in predator body size occurs throughout ontogeny, either as a direct consequence of increased body size or due to changes in their feeding structures (Stoner & Livingston, 1984; Ward-Campbell & Beamish, 2005; Powter et al., 2010; Ferrara et al., 2011; Davis et al., 2012; Wilga et al., 2016; Türtscher et al., 2022). For most marine predators, optimisation of the feeding process is explicitly related to the mouth and associated structures. Mouth gape is closely associated with the maximum prey size that a predator can consume, especially those that swallow their prey whole (Karpouzi & Stergiou, 2003; Mihalitsis & Bellwood, 2017). Species that can shear their prey into smaller pieces can target individuals larger than their gape size, freeing them from these constraints and providing them with a greater range of prey sources (Lucifora et al., 2005; Ferrara et al., 2011). Among dietary structures, teeth morphology has been the most correlated with the feeding habits of predators (Wilga & Ferry, 2015; Van Valkenburgh, 2019). Consequently, it is expected that differences in predator body size, mouth gape and tooth shape will determine the trophic niche; as will limit predators to specific trophic levels by constraining their access to different types of prey.

Along with mammals, sharks have the highest diversity in tooth morphology shape, so distinctive that they can be used to identify individual species (Corn et al., 2016). Sevengill sharks (*Notorynchus cepedianus*) and grey nurse sharks (*Carcharias taurus*) are two sympatric species of coastal sharks with similar body sizes (85-325 cm grey nurse sharks; 35-295 cm sevengill sharks) (Ebert et al., 2021). As body size is positively correlated with gape size (Karpouzi & Stergiou, 2003; Ladds et al., 2020), we can also expect that similar body sizes in both species will have similar gape sizes. However, as they have distinct tooth morphologies (Figure 4.1), these two species can be used to assess how morphological factors help to determine trophic niche. Grey nurse sharks have spear-shaped teeth adapted to capture and swallow their prey whole with minimal handling (Frazzetta, 1988; Lucifora et al., 2001; Ferrara et al., 2011; Lucifora et al., 2009a). This type of dentition limits their ability to consume prey larger than their mouth gape, restricting the trophic levels over which they can feed. Previous work has shown that nearly 94% of grey nurse shark prey is consumed whole (Lucifora et al., 2009a). In contrast, sevengill sharks have multi-cusped cutting teeth and cut their prey to pieces prior to consumption (Ebert, 1991). Therefore, they can access large prey from higher trophic levels (*e.g.*, marine mammals) that are much larger than their mouth gape (Crespi-Abril et al., 2003; Lucifora et al., 2005). As sevengill sharks can theoretically access a greater variety of prey sizes regardless of their body size, ontogenetic changes in prey choice should be more apparent in grey nurse sharks than in sevengill sharks. We hypothesise that, despite having otherwise similar body characteristics (size, mouth gape), sevengill sharks will have access to a much broader range of prey sizes than grey nurse sharks, and this will be reflected in a broader trophic niche and access to prey over higher trophic levels.

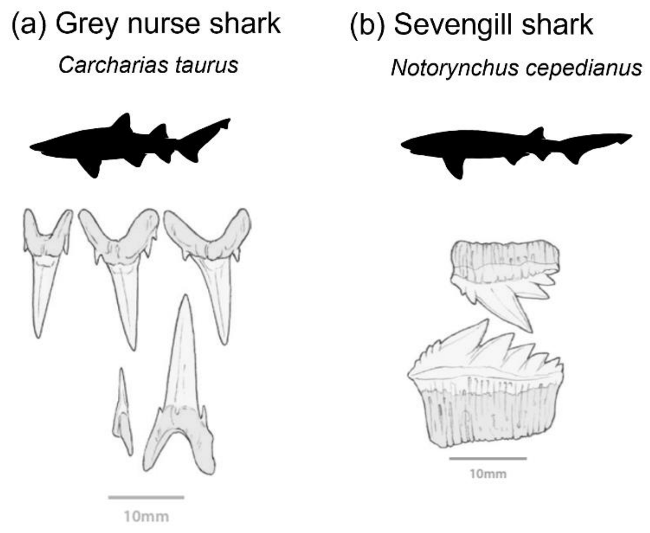

Figure 4.1. Tooth morphology of grey nurse sharks (a, *Carcharias taurus*) and sevengill sharks (b, *Notorynchus cepedianus*). Modify from Ebert et al. (2021).

Stomach content and stable isotope analysis are widely used to study diet and estimate the trophic niche breadth of species. Stable isotopes of carbon ($\delta^{13}C$) and sulfur ($\delta^{34}S$) indicate resource use by consumers (*e.g.*, coastal vs oceanic, benthic vs pelagic), while relative trophic position is indicated by nitrogen isotopic values ($\delta^{15}N$) (Post, 2002). This study assesses how two different facets of trophic morphology (teeth shape and body size) affect prey choice selectivity in large predators with contrasting foraging strategies. To test our hypothesis about differences in diet breadth, we quantified the diet of each species using stomach content analysis. We characterized consumers' isotopic niches and estimated their trophic role using isotopic values of $\delta^{13}C$, $\delta^{34}S$ and $\delta^{15}N$. Stability over time in prey choice was assessed by analysing isotopic values in the liver and muscle, which represent tissues with short- and long-term dietary incorporation rates, respectively (Hussey et al., 2012). Finally, we looked at how different estimates of foraging niche varied with body size. This information allowed us to test the hypothesis that predators with cutting teeth access prey from a broader range of trophic levels than sympatric predators of similar body size but with grasping teeth.

4.3. METHODS

Study Site and Sampling

Muscle and liver samples of dead grey nurse and sevengill sharks were obtained from artisanal and recreational fisheries located along the Atlantic coast of Uruguay (Figure 4.2). Both species co-occur in the same areas during spring and summer when they approach the coast and have access to the same food resources (Silveira et al., 2018; Praderi, 1985). It is even common for individuals of the two species to be caught from the same fishing haul. Outside of the fishing season, there is no information on the habitat use or movement patterns of these two species. Fieldwork was conducted over two consecutive shark fishing seasons between spring

(September-December) and summer (December-February) of 2018-2019 and 2019-2020. Individuals were identified, sexed, and measured in centimetres for total length (TL, *sensu* Ebert et al., 2021). Liver samples were obtained from the posterior portion of either lobe, while white muscle samples were excised from the ventral region close to the base of the pelvic fins. Whenever possible, the stomach was removed and sealed with a zip tie at the oesophagus and another at the anterior end of the spiral valve intestine to avoid losing contents. Samples were stored at -20°C until they were processed in the laboratory. Samples were collected in collaboration with local fishers in the frame of the participatory fishing monitoring program carried out by the Atlantic Fisheries Management Unit of the National Directorate of Aquatic Resources (UGEPA-DINARA), under the permit 252/2018 issued by DINARA.

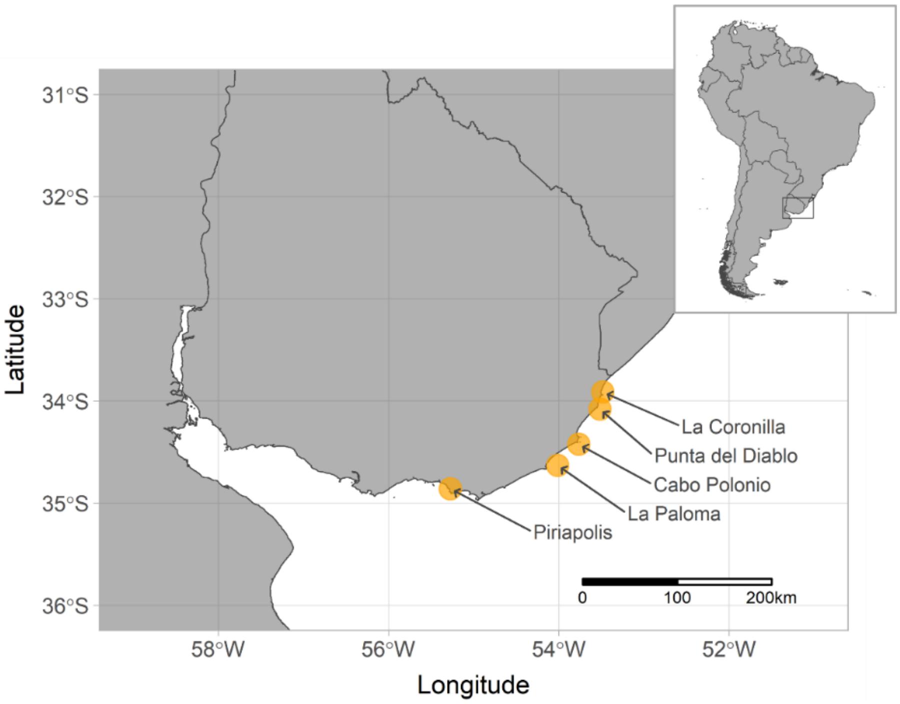

Figure 4.2. Localities along the Atlantic coast of Uruguay (yellow circles) where samples were obtained from artisanal and recreational fisheries.

Stable isotope analysis

Muscle and liver samples of grey nurse (*n* = 104) and sevengill sharks (muscle *n* = 34, liver *n* = 31) were cleaned with de-ionized water to avoid any residue that could affect the isotopic signals. Samples from the 2018-2019 fishing season were freeze-dried, while samples from 2019-2020 were oven-dried at 60°C for 72 hours. Previous work has shown that freeze-dried and oven-dried samples do not show significant differences in their isotopic values (Akamatsu et al., 2016; Bashir et al., 2020). Dried samples were ground into a fine powder and homogenised using an IKA® A11 Basic Analytical Mill (IKA-Werke GmbH & Co. KG, Staufen, Germany).

Lipid extraction was conducted on approximately 1 gram of tissue using a 2:1 chloroform-methanol solution adapted from (Folch et al., 1957). In liver samples with high lipid content, such as grey nurse (Davidson & Cliff, 2011) and sevengill sharks (Pethybridge et al., 2010), the process had to be repeated until the supernatant was clear to ensure a successful lipid removal (Medeiros et al., 2015). To eliminate the remaining solvent, samples were dried at room temperature for 48 hours or until the solvent completely evaporated.

Shark tissues are rich in urea and Trimethylamine N-Oxide (TMAO), which enables them to maintain an osmotic balance with their environment (Yancey, 2015) but also affects the $\delta^{15}N$ and $\delta^{13}C$ isotopic values leading to biases in ecological interpretations (Kim & Koch, 2012; Li et al., 2016; Bennett-Williams et al., 2022). Therefore, urea extraction was performed after lipid extraction following an adaptation of the Kim and Koch (2012) protocol. Samples were rinsed with 5ml of deionised water, with a reaction time of 10min and vortexed for 1min, after which were centrifuged, and the supernatant was discarded. This procedure was repeated three consecutive times. Samples were dried for 24 hours in a drying oven at 60°C or until the sample was dried entirely (usually no more than 48 hours). Finally, dried samples were weighed in tin capsules and sent for analysis.

Stable isotope values of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ were analysed at the Stable Isotope Laboratory at the University of Hong Kong, Hong Kong, using a continuous flow-isotope ratio mass spectrometer Thermo Scientific™ EA IsoLink™ IRMS System (Thermo Fisher Scientific Inc., Massachusetts, US). The following international standards were used for data normalisation: USGS-40 and USGS-41a for ^{15}N and ^{13}C; IAEA-S-1 and IAEA-S-2 for ^{34}S. Analytical accuracy was evaluated using the reference material USGS-42 ($\delta^{15}N = 0.1 – 0.3$, $\delta^{13}C = 0.0 – 0.1$ and $\delta^{34}S = 0.1 – 1.0$).

Stomach Content Analysis

Stomach contents were rinsed with fresh water, and items were sorted using a series of 4 mm, 2 mm and 1 mm metal mesh sieves. Prey items were identified to the lowest possible taxonomic group. Hard parts (*e.g.*, otoliths, pharyngeal teeth plates and other bones) were dried for identification. Otoliths were photographed with a stereomicroscope Nikon SMZ-745T (Nikon Instruments Inc., Melville, United States of America) for identification using regional otolith guides as reference (Volpedo & Echeverría, 2000; Rossi-Wongtschowski et al., 2014; Giaretta et al., 2017; Volpedo et al., 2017). To identify fish remains in advanced stages of digestion, the taxonomic determination was based on its extracted otoliths (whenever possible) or skeletal morphology (axial, cranial and appendicular). Bone identification was conducted using reference literature of the prey species (Dyer, 2006; Deli Antoni et al., 2008; Tombari et al., 2010; Rodrigues & Bemvenuti, 2011; Marceniuk et al., 2012; Perez Comesaña et al., 2014;

Bemis et al., 2019; Colautti et al., 2020). Items identified as parasites (*e.g.*, isopods, nematodes), secondary prey items (*e.g.,* small bivalves) and other non-dietary items (*e.g.*, fishing gear fragments, sand) were excluded from the analysis.

To describe the dietary composition of each species, we used a combination of a presence-absence method (Frequency of occurrence, %F) and a numerical method (%N) (Hynes, 1950; Hyslop, 1980; Amundsen & Sánchez-Hernández, 2019). These approaches are recommended in species with a high digestibility rate, such as sharks, since they are less affected by the decomposition of prey items and give an unambiguous interpretation for diet than volumetric or gravimetric methods (Baker et al., 2014; Amundsen & Sánchez-Hernández, 2019). Frequency of occurrence is defined as the number of stomachs in which a specific prey type is expressed as a percentage of the total number of stomachs with any prey type (Hyslop, 1980). We calculated %N as the total number of individuals of a particular prey type as a proportion of the total number of prey items in all stomachs (Hynes, 1950). Only stomachs with prey content were considered for calculations of %F and %N.

Statistical analyses

For each shark species, a cumulative prey curve was used to determine if the number of stomachs analysed was enough to accurately describe the diet of a particular predator (Ferry & Cailliet, 1996; Cortés, 1997). Cumulative prey curves were constructed using the software *EstimateS* version 9.1.0 (Colwell et al., 2004). The order in which the stomachs were analysed was randomised 100 times and the mean cumulative number of new prey items was plotted against the number of stomachs examined. Individuals with empty stomachs and unidentified prey items were excluded from the calculations. The sample size was considered sufficient to describe diet if the curve reached an asymptote (Magurran, 2004).

To assess whether the consumption of different prey types varies with body size, we analysed the variation in the proportion of prey consumed and the isotopic values of $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ with TL. We defined the proportion of prey consumed as the frequency of each prey type found in one stomach, with three main categories considered: actinopterygii (bony fishes), chondrichthyes and marine mammals. A generalised linear model was fitted for each species of shark. As the values for the proportion of prey are constrained between 0 and 1, we adjusted a function with a binomial conditional distribution, which used a default logit link function. The proportion of prey consumption was used as a response variable, and as explanatory variable TL, the type of prey categorization was included in the model as an interaction. Marine mammal category was not included in the grey nurse shark model, as no marine mammals were recorded as prey items.

Since maternal influence can bias the isotopic values of neonate individuals in some shark species (Matich et al., 2010; Olin et al., 2011; Niella et al., 2021), individuals smaller or equal to the maximum reported birth size were removed from the analyses. The maximum TL at birth registered for grey nurse sharks were 110 cm (Gilmore et al., 1983) and 53 cm for sevengill sharks (Compagno, 1984). Linear models were fitted independently for each stable isotope and tissue type, using shark TL as a predictor variable. When a linear regression was fitted for the same tissue type and stable isotope for both species, the slopes were assessed and compared using a *t*-test. To conduct this analysis, the slopes were obtained and compared adjusting a linear regression between $\delta^{15}N$ and TL by tissue type, with species included as interactions.

Different tissue types could have different tissue-specific isotopic enrichment factors (Hussey et al., 2010; Kim et al., 2012). Consequently, to make liver isotopic values of $\delta^{13}C$ and $\delta^{15}N$ directly comparable to muscle and assess stability over time, we corrected liver values using diet-tissue discrimination factors for lipid extracted samples published by Hussey et al. (2010). For grey nurse sharks, we used species specific values, and for sevengill, we used the generalized estimate values for '*all sharks*' (Hussey et al., 2010). The values of $\delta^{15}N$ and $\delta^{13}C$ of grey nurse shark liver were increased by 0.69 and 0.17‰, respectively. For sevengill sharks, liver $\delta^{15}N$ and $\delta^{13}C$ values were increased by 0.79 and 0.68‰, respectively. These values correspond to the differences between mean values of lipid-extracted muscle samples and mean values for lipid-extracted liver published by Hussey et al. (2010). No correction was performed to $\delta^{34}S$ values, as there is no tissue discrimination factor information on this isotope.

Linear models were fitted in R software version 4.1.0 (R Core Team, 2021) using the functions '*lm*' and '*glm*' from the package *'stats'.* Assumptions of the final models were verified by visual inspection of the residuals. The significance level was set at 0.05.

4.4. RESULTS

Stomach content analysis and prey consumption relative to body length

One hundred stomachs of grey nurse sharks and 33 from sevengill sharks were collected. A total of 92 (92%) stomachs from grey nurse sharks and 21 (62%) from sevengill sharks had at least one prey item. Cumulative diversity curves (Figure 4.S1) did not reach an asymptote for either species, indicating that a larger number of stomachs were needed for a more precise description of their diet in this region. Grey nurse sharks preyed mainly on teleosts (92.2 %N), followed by chondrichthyes (6.7 %N) (Table 4.S1). Among the most important items were two species of Scianidae (striped weakfish, *Cynoscion guatucupa* and the whitemouth croaker, *Micropogonias furnieri*) and specimens of the genera *Odontesthes* (silversides). Remains of marine mammals were not found in grey nurse shark stomachs. Based on prey items with low degrees of decomposition we observed that grey nurse sharks almost always swallowed their prey whole. In comparison, sevengill sharks had a more balanced diet between teleosts (44.7 %N) and chondrichthyans (34.2 %N), with some predation on marine mammals (7.9 %N) (Table 4.S1). No preferred prey item was identified within the defined prey categories, suggesting a more generalized diet. Consumption of marine mammals was recorded for three individuals, TL between 146-191 cm. Almost all the prey items recorded were highly digested, therefore it was not possible to observe whether the prey was eaten whole or in pieces.

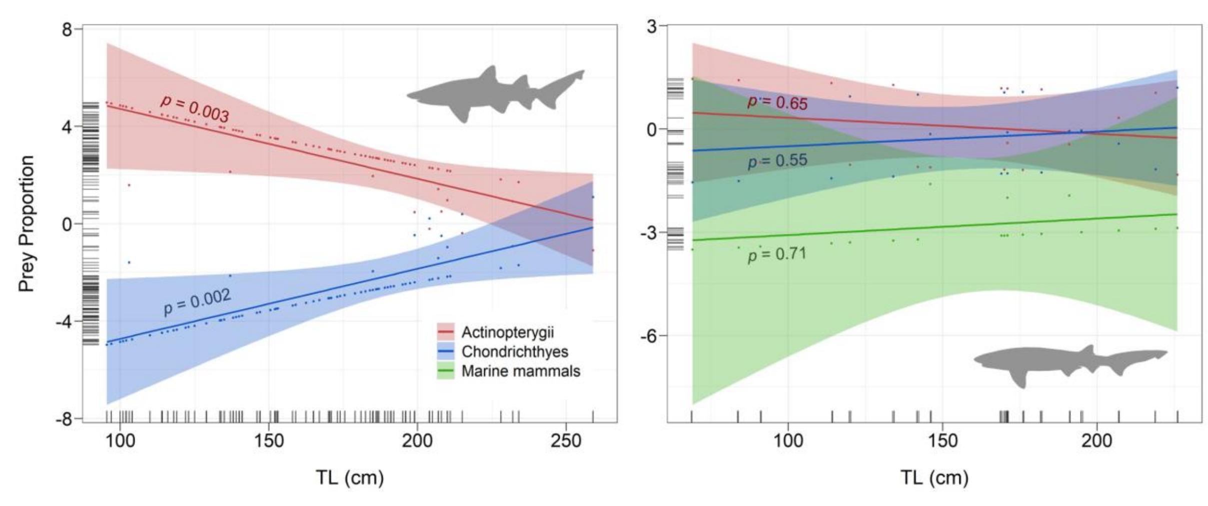

Figure 4.3. Variation in prey proportion (Logit transformed) as a function of total length (TL). For grey nurse sharks (*Carcharias taurus*, left panel), the fitted generalized linear models were significant for all categories of prey types consumed, but none were significant for sevengill sharks (*Notorynchus cepedianus*, right panel). Shaded areas represent 95% confidence intervals. Partial residuals are shown and rugs at the x and y-axis represent raw observed data. Categories of prey types: Actinopterygii (red), Chondrichthyes (blue) and Marine mammals (green).

Fitted generalized linear models for grey nurse sharks showed significant relationships in the proportion of consumed prey as function of TL, both for actinopterygii (*p*-value = 0.003) and chondrichthyes (*p*-value = 0.002). Decreased consumption of actinopterygii in larger individuals was associated with an increase in consumption of chondrichthyes (Figure 4.3). No significant relationships were observed between prey proportion as function of TL for sevengill sharks (actinopterygii *p*-value = 0.65, chondrichthyes *p*-value = 0.55, marine mammals *p*-value = 0.71; Figure 4.3). Consumption of cetaceans was recorded in two individuals with a TL of 146 and 171 cm, while only one individual of 191 cm fed on a pinniped.

Stable isotope variation with body length

Isotopic values of nitrogen ($\delta^{15}N$) were consistently higher in sevengill sharks than in grey nurse sharks, for both tissue types (Table 4.1). Values were 1.5‰ and 1‰ higher in liver and muscle, respectively. The range of $\delta^{15}N$ values varied between 16.5 and 20.8‰ in grey nurse sharks, and between 17.8 and 21.7‰ in sevengill sharks. The range of values for $\delta^{15}N$ varied slightly when considering the liver-corrected values (see Methods): 17.2–20.8‰ in grey nurse sharks and 18.4–21.7‰ in sevengill sharks (Figure 4.5). Sevengill shark showed significant relationships in $\delta^{15}N$ values as a function of TL, explaining a large proportion of the variation for muscle (*p*-value < 0.001, R^2 = 0.67) and liver (*p*-value < 0.001, R^2 = 0.42) (Figure 4.5). Although the fitted linear models were significant for muscle and liver in grey nurse sharks, their explained variance was low (muscle: *p*-value = 0.02, R^2 = 0.06; liver: *p*-value = 0.03, R^2 = 0.05) (Figure 4.5). Both species showed a positive slope in their relationships between $\delta^{15}N$ and TL in muscle (grey nurse shark 0.004, sevengill shark 0.011) and liver (grey nurse shark 0.005, sevengill shark 0.006), being steeper for both tissue types in sevengill sharks. Comparison of the regression slopes was only significantly different for muscle (muscle $p < 0.05$, liver $p = 0.07$) when comparing both species by tissue type.

Table 4.1. Stable isotope values of nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$) and sulfur ($\delta^{34}S$) in muscle and liver of grey nurse sharks (*Carcharias taurus*) and sevengill sharks (*Notorynchus cepedianus*). Data are expressed as means ± standard deviation and correspond to isotopic values without any adjustment applied to liver samples, see Methods. Isotopic values are in parts per mill (‰).

	Carcharias taurus		***Notorynchus cepedianus***	
	Muscle (n = 104)	Liver (n = 104)	Muscle (n = 34)	Liver (n = 31)
$\delta^{15}N$	19.2 ± 0.5	17.8 ± 0.6	20.6 ± 0.9	19.5 ± 0.8
$\delta^{13}C$	-14.8 ± 0.3	-15.0 ± 0.4	-15.1 ± 0.4	-15.0 ± 0.4
$\delta^{34}S$	18.6 ± 0.9	18.1 ± 0.5	17.9 ± 0.9	17.6 ± 0.5

Values of stable isotope of carbon ($\delta^{13}C$) from liver samples increased significantly with TL in both grey nurse ($p < 0.001$, $R^2 = 0.12$) and sevengill sharks ($p = 0.01$, $R^2 = 0.20$) (Figure 4.5). However, no significant relationship was found in muscle of grey nurse ($p = 0.42$, $R^2 = 0.01$) and sevengill shark ($p = 0.89$, $R^2 = 0.00$) (Figure 4.5). Carbon isotopic values showed roughly the same values for both species and tissue type, and the lowest variance compared with sulfur and nitrogen values (Table 4.1). Both species varied between -15.8 to -13.9‰ in their $\delta^{13}C$ values. The range of $\delta^{13}C$ values varied slightly when the liver-corrected values were considered (see Methods): between -15.6 to -13.7‰ in grey nurse sharks and between -15.6 to -13.5‰ in sevengill sharks (Figure 4.5).

Sulfur stable isotope values ($\delta^{34}S$) did not exhibit any significant variation in relation to TL in muscle in either species (Figure 4.5), grey nurse shark (*p*-value = 0.23, $R^2 = 0.02$), sevengill shark (*p*-value = 0.13, $R^2 = 0.07$). In liver samples, grey nurse sharks showed a significant positive relationship between $\delta^{34}S$ and TL (*p*-value < 0.001, $R^2 = 0.15$), but not for sevengill sharks (*p*-value = 0.10, $R^2 = 0.09$). Values for $\delta^{34}S$ varied between 15.9 to 21.6‰ in grey nurse sharks, and between 15.7 to 20.2‰ in sevengill sharks (Figure 4.5). The level of variance was the same for both species, reaching higher values in muscle samples (Table 4.1). Differences between species in the mean values were less than 1‰ for both tissues (Table 4.1).

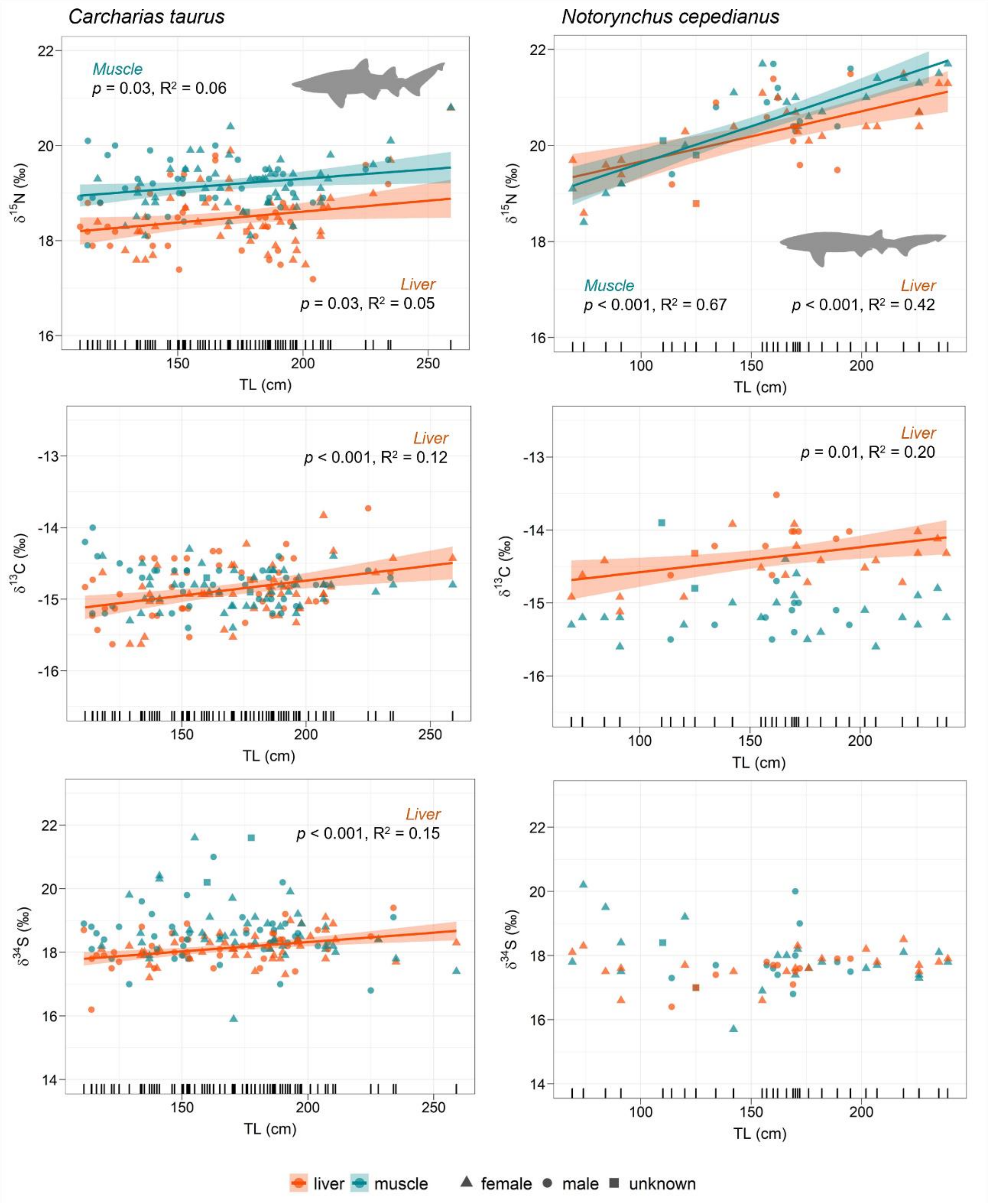

Figure 4.5. Linear models of δ^{15}N, δ^{13}C and δ^{34}S values as a function of total length (TL) for each tissue sample, muscle (cyan) and liver (orange) of the grey nurse shark (*Carcharias taurus*) and sevengill sharks (*Notorynchus cepedianus*). Liver isotopic values for δ^{15}N and δ^{13}C were adjusted to make them directly comparable to muscle values, see Methods. Shaded areas represent the 95% confidence intervals.

4.5. DISCUSSION

Morphological traits associated with feeding activities directly impact the type of prey a marine predator will be able to consume. Species with teeth morphology specialized in gripping and then swallowing their prey whole (*e.g.*, grey nurse sharks) appear to be constrained to consuming prey smaller than their gape size. By contrast, species adapted to cutting their prey into smaller pieces using serrating teeth (*e.g.*, sevengill sharks) can consume prey larger than their mouth gape. We found a significant positive relationship between body length and $\delta^{15}N$ values, which was steeper in the species capable of sectioning their prey. Larger body lengths would imply access to larger prey items from higher trophic levels. Values of $\delta^{13}C$ and $\delta^{34}S$ for both species and tissue types did not show differences of meaningful ecological significance (~1‰) that would imply differential resource use between the two species. These results suggest that both species feed on resources from similar trophic webs, supporting our contention that the main difference between the two species is the trophic level of the prey they feed on. Despite grey nurse sharks and sevengill sharks having similar body sizes and sharing the same feeding areas for at least part of the year (Silveira et al., 2018), their differences in tooth morphology lead them to select different prey, contributing, at least in part, to resource partitioning. Sevengill sharks showed a broader range of prey sizes and had access to higher trophic levels than grey nurse sharks. Our findings support the hypothesis that the broader prey size selection available to species with cutting-teeth allows them to occupy higher trophic levels (higher $\delta^{15}N$ values), preying on a wider range of prey sizes than gripping-teeth species of similar body size within the same ecosystem.

Morphology and feeding ecology

Based on our results, morphological traits directly involved in the feeding process (*e.g.,* teeth, mouth gape) are drivers of prey choice. As food intake is essential for organisms to grow, survive and reproduce, structures such as teeth are under strong selective pressures to maximise consumer efficiency to capture and process their prey (Grossnickle, 2020; Bazzi et al., 2021). The origin of the great diversity of tooth morphologies in sharks occurred as adaptive responses to survive changes in habitat and prey availability caused by severe climatic disruptions throughout shark evolution (Bazzi et al., 2021). Because trophic morphological traits were adapted to optimise predation of available resources, they become a primary factor controlling consumer diet choices. Some adaptive morphologies constrain or enhance the ability of predators to capture and consume available prey in the environment, helping to shape their trophic niche. Shark species with grasping teeth such as grey nurse sharks can predate slippery or fast-moving prey but are unable to tear their prey into pieces. Consequently, they must consume individuals smaller than their mouth gape, which limits the size of their target prey and restricts their trophic niche. In contrast, predators with teeth that allow them to cut their prey into smaller pieces such as sevengill sharks and white sharks (Ferrara et al., 2011), are able to access a wider variety of prey types, even larger than their mouth gape. As we initially hypothesised, sevengill sharks, consumed prey from different trophic levels, and this was reflected in a wider range and higher $\delta^{15}N$ values than grey nurse sharks. Grey nurse sharks rely on a piscivorous diet with restrictions on prey size imposed by gape, and so feed at lower trophic levels and had less variable $\delta^{15}N$ values.

Differences in body size may also lead to changes in prey choice (Graeb et al., 2006; Lucifora et al., 2009b; Davis et al., 2012). Predator and prey body size are positively correlated in marine environments (Cohen et al., 1993; Costa, 2009). Larger body sizes commonly lead to faster and stronger predators which facilitate the capture and consumption of larger prey (Wainwright & Richard, 1995; Ferrara et al., 2011; Cuthbert et al., 2020). An increase in body size is evident throughout the ontogeny of individuals, and corresponding changes in diet composition are seen in some shark species, either by changes in body size or due to changes in trophic morphology (McElroy et al., 2006; Baremore et al., 2009; Lucifora et al., 2009b; Dicken et al., 2017). These changes will allow older predators to access new foraging areas (*e.g.*, leaving their nursery area, increasing their home range due to lower predation risk by body size) or to access different types of prey (*e.g.*, a consequence of new teeth morphologies, improved prey handling by larger body size), unavailable for younger specimens. In general, body size has a positive relationship with mouth size (Karpouzi & Stergiou, 2003; Ladds et al., 2020), and a larger gape enables an animal to prey on larger prey items from higher trophic levels (Baremore et al., 2009; Powter et al., 2010; Grainger et al., 2020). However, body size may not always accurately reflect diet choice (Grossnickle, 2020; Keppeler et al., 2020), as it is under a number of selective pressures, not only associated with diet but also with other biological needs, such as reproduction, thermoregulation and predation risk (Kleiber, 1947; Barneche et al., 2018; Tan et al., 2021). For example, from our results, a sevengill shark of 207 cm total length can reach $\delta^{15}N$ values of 21.4‰, while a grey nurse shark of the same length reaches a $\delta^{15}N$ value of 19.1‰, approximately a difference of one trophic level. These differences cannot be explained by body size as they are individuals of the same length, and so their dental morphology may better explain their dietary differences. Although the proposed scenario is rather simplistic in that it does not consider additional individual factors involved in prey selection, such as differences in body mass or behaviour, it illustrates the key role of tooth morphology in prey selection. Consequently, for trophic ecology studies it appears that body size alone is not a sufficient estimator of a predator's diet, and trophic morphology traits (*e.g.*, dental or mandibular characters) must also be considered.

As expected, despite differences in tooth morphology, consumers showed an ontogenetic change in prey choice and trophic level. Ontogenetic changes in prey choice were more selective in grey nurse sharks than in sevengill sharks, probably due to this inability to consume prey larger than their gape. Larger grey nurse sharks consumed a higher proportion of chondrichthyes than smaller sharks, and previous work has found that this increase is mainly a consequence of an increase in consumption of benthic elasmobranchs (*e.g.*, eagle rays, angel sharks) (Lucifora et al., 2009a). Lucifora et al. proposed that bigger body sizes give the predator the strength to handle, kill and swallow this wide-bodied prey, which could be difficult and less efficient for a small shark with a smaller gape. For smaller individuals, feeding on unsuitable prey may lead to substantial costs in terms of handling and processing time, making the prey less attractive (Willson & Hopkins, 2011). In sevengill sharks, dietary change throughout ontogeny was less pronounced, probably due to their accessibility to prey on higher trophic levels at smaller body sizes than grey nurse sharks. In both species, there was a positive relationship between TL and $\delta^{15}N$ values, confirming that the overall increase in body size as an organism grows does provide accessibility to prey at higher trophic levels. However, grey nurse sharks showed a smaller positive slope than sevengill sharks. This was reflected in a smaller variation in the increase of $\delta^{15}N$ muscle values along with TL, 19.0‰ – 19.5‰ in grey nurse sharks and 19‰ – 22‰ in sevengill sharks (Figure 3.3). An ontogenetic increase in $\delta^{15}N$ values with body length has also been previously detected in Australian sevengill shark populations (Abrantes & Barnett, 2011). Isotopic values of nitrogen and their variation with body size were consistent in both tissue types for both species, suggesting long-term stability in their feeding habits. The ontogenetic change in body size increases predator accessibility to larger prey from higher trophic levels, however, the rate at which it increases will depend on other morphological traits directly related to feeding activity. Organisms with gripping teeth, without the possibility of severing their prey, show a more moderate increase than those with cutting teeth as they can access prey of higher trophic levels.

We were only able to include in the analyses a single species as representative of each type of tooth morphology in the studied ecosystem, as these are the only two sympatric species of large-bodied coastal sharks within the study area. This meant that we had no true 'control' species to validate tooth morphology as a trophic determinant, and we must be cautious when generalising such conclusions to other ecosystems and species. However, and consciously taking this into account, we are confident that the unique characteristics of our ecosystem allow us to consider this a natural experiment. This ecosystem encompasses two sympatric species of coastal sharks (at least for part of the year) (Silveira et al., 2018) that have access to the same food resource (Praderi, 1985), have approximately the same body size, display two starkly contrasting tooth morphologies, and can be sampled in sufficient numbers to test our main hypothesis. Indeed, this suite of characteristics make these species the best control available to compare trophic morphological characteristics and prey choices. However, in addition to trophic morphological traits, individual and species-specific behavioural or physiological traits may be bias our isotopic niche estimates. Some of these traits may include behavioural differences in predation tactics and specialisation (*e.g.,* Sims, 2003; Woo et al., 2008; Baylis et al., 2015; Watanabe et al., 2019), differential predation risk between species (*e.g.,* Lima & Dill, 1190; Hussey et al., 2017).

4.6. CONCLUSIONS

Trophic morphological traits play an important role in prey selection for predators and may be the primary mechanism in facilitating resource partitioning in sympatric species (DeVries, 2017; Figgener et al., 2019; Delariva & Neves, 2020). These morphological characters result from different evolutionary pathways that result in differential use of resources and strategies to coexist with other species and individuals. When a morphological trait is subjected to selective forces focused on optimizing feeding (*e.g.*, shape and structure of the teeth or jaw), it provides an excellent option for inferring the selection of prey by a predator. However, morphological traits that are under the effect of several selective forces (*e.g.*, reproduction, thermoregulation) besides feeding, such as body size, may not adequately reflect (or infer) predator prey selection.

In comparative trophic ecology studies with commonly used analyses, such as stable isotopes, fatty acids or stomach contents, including an assessment of trophic morphological traits may assist in interpreting how extrinsic and intrinsic factors affect the trophic ecology of a predator. We recommend not only focusing on the commonly studied extrinsic factors (inter- and intraspecific interactions, and abiotic factors) that might be affecting the trophic ecology of the groups under study, but also on intrinsic factors (morphological and behavioural traits) of the organisms to improve our interpretations.

In future studies, we recommend an evaluation of the effects of other types of tooth morphology on prey selection and trophic niches of predators. This will help to delineate general patterns in marine predators explaining how these inherent traits, constrain or enhance prey selectivity and shape their trophic niches.

4.7. REFERENCES

Abrantes, K. G., & Barnett, A. (2011). Intrapopulation variations in diet and habitat use in a marine apex predator, the broadnose sevengill shark *Notorynchus cepedianus*. *Marine Ecology Progress Series*, *442*, 133-148. https://doi.org/10.3354/meps09395

Amundsen, P. A., & Sánchez-Hernández, J. (2019). Feeding studies take guts – critical review and recommendations of methods for stomach contents analysis in fish. *Journal of Fish Biology*, *95*, 1364–1373. https://doi.org/10.1111/jfb.14151

Akamatsu, F., Suzuki, Y., Kato, Y., Yoshimizu, C., & Tayasu, I. (2016). A comparison of freeze-drying and oven-drying preparation methods for bulk and compound-specific carbon stable isotope analyses: examples using the benthic macroinvertebrates *Stenopsyche marmorata* and *Epeorus latifolium*. *Rapid Communications in Mass Spectrometry*, *30*, 137–142. http://doi.org/10.1002/rcm.7421

Araújo, M. S., Bolnick, D. I., & Layman, C. A. (2011). The ecological causes of individual specialisation. *Ecology Letters*, *14*(9), 948–958. https://doi.org/10.1111/j.1461-0248.2011.01662.x

Bailleul, F., Charrassin, J. B., Monestiez, P., Roquet, F., Biuw, M., & Guinet, C. (2007). Successful foraging zones of southern elephant seals from the Kerguelen Islands in relation to oceanographic conditions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *362*, 2169–2181. https://doi.org/10.1098/rstb.2007.2109

Baker, R., Buckland, A., & Sheaves, M. (2014). Fish gut content analysis: Robust measures of diet composition. *Fish and Fisheries*, *15*(1), 170–177. https://doi.org/10.1111/faf.12026

Baremore, I. E., Murie, D. J., & Carlson, J. K. (2009). Seasonal and size-related differences in diet of the Atlantic angel shark *Squatina dumeril* in the northeastern Gulf of Mexico. *Aquatic Biology*, *8*, 125–136. https://doi.org/10.3354/ab00214

Barneche, D. R., Ross Robertson, D., White, C. R., & Marshall, D. J. (2018). Fish reproductive-energy output increases disproportionately with body size. *Science*, *360*, 642–645. https://doi.org/10.1126/science.aao6868

Bashir, Z., Abdullah, M. M. & Rusli, M. U. (2020). Comparisons between tissues, preservation, and desiccation methods on stable isotopes $\delta^{13}C$ and $\delta^{15}N$ of spot-tail sharks (*Carcharhinus sorrah*) from the South China Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, *20*(9), 711-716. http://doi.org/10.4194/1303-2712-v20_9_01

Baylis, A. M. M., Orben, R. A., Arnould, J. P. Y., Peters, K., Knox, T., Costa, D. P., & Staniland, I. J. (2015). Diving deeper into individual foraging specializations of a large marine predator, the southern sea lion. *Oecologia*, *179*(4), 1053–1065. https://doi.org/10.1007/s00442-015-3421-4

Bazzi, M., Campione, N. E., Kear, B. P., Pimiento, C., & Ahlberg, P. E. (2021). Feeding ecology has shaped the evolution of modern sharks. *Current Biology*, *31*, 5138–5148. https://doi.org/10.1016/j.cub.2021.09.028

Bemis, K. E., Burke, S. M., St. John, C. A., Hilton, E. J., & Bemis, W. E. (2019). Tooth development and replacement in the Atlantic Cutlassfish, *Trichiurus lepturus*, with comparisons to other Scombroidei. *Journal of Morphology*, *280*, 78–94. https://doi.org/10.1002/jmor.20919

Bennett-Williams, J., Skinner, C., Wyatt, A. S. J., Mcgill, R. A. R., & Willis, T. J. (2022). A multi-tissue, multi-species assessment of lipid and urea stable isotope biases in mesopredator Elasmobranchs. *Frontiers in Marine Science*, *9*(821478). https://doi.org/10.3389/fmars.2022.821478

Bolnick, D. I., Amarasekare, P., Araújo, M. S., Bürger, R., Levine, J. M., Novak, M., Rudolf, V. H. W., Schreiber, S. J., Urban, M. C., & Vasseur, D. A. (2011). Why intraspecific trait variation matters in community ecology. *Trends in Ecology and Evolution*, *26*(4), 183–192. https://doi.org/10.1016/j.tree.2011.01.009

Castillo, J., Barbieri, M. A., Gonzalez Castillo, A., Barbieri, J., & Gonzalez, A. (1996). Relationships between sea surface temperature, salinity, and pelagic fish distribution off northern Chile. In *ICES Journal of Marine Science* (Vol. 53). https://academic.oup.com/icesjms/article/53/2/139/791578

Chase, J. M., Abrams, P. A., Grover, J. P., Diehl, S., Chesson, P., Holt, R. D., Richards, S. A., Nisbet, R. M., & Case, T. J. (2002). The interaction between predation and competition: a review and synthesis. *Ecology Letters*, *5*(2), 302–315. https://doi.org/10.1046/j.1461-0248.2002.00315.x

Cohen, J. E., Pimm, S. L., Yodzis, P., & Saldana, J. (1993). Body sizes of animal predators and animal prey in food webs. *The Journal of Animal Ecology*, *62*(1), 67. https://doi.org/10.2307/5483

Colautti, D. C., Miranda, L., Gonzalez-Castro, M., Villanova, V., Strüssmann, C. A., Mancini, M., Maiztegui, T., Berasain, G., Hattori, R., Grosman, F., Sanzano, P., Lozano, I., Vegh, S. L., Salinas, V., Del Ponti, O., del Fresno, P., Minotti, P., Yamamoto, Y., & Baigún, C. R. M. (2020). Evidence of a landlocked reproducing population of the marine pejerrey *Odontesthes argentinensis* (Actinopterygii; Atherinopsidae). *Journal of Fish Biology*, *96*(1), 202–216. https://doi.org/10.1111/jfb.14207

Colwell, R. K., Xuan Mao, C., & Chang, J. (2004). Interpolating, extrapolating, and comparing incidence-based species accumulation curves. *Ecology*, *85*(10), 2717–2727. www.google.com

Compagno, L. J. V. (1984). FAO species catalogue Vol . 4 , Part 1 Sharks of the world. *FAO Fisheries Synopsis*, *4*(125), 249.

Connell, J. H. (1983). On the prevalence and relative importance of interspecific competition: evidence from field experiments. *American Naturalist*, *122*(5), 661–696. https://doi.org/10.1086/284165

Corn, K. A., Farina, S. C., Brash, J., & Summers, A. P. (2016). Modelling tooth–prey interactions in sharks: The importance of dynamic testing. *Royal Society Open Science*, *3*(160141). https://doi.org/10.1098/rsos.160141

Cortés, E. (1997). A critical review of methods of studying fish feeding based on analysis of stomach contents: Application to elasmobranch fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*, 726–738. https://doi.org/10.1139/f96-316

Costa, G. C. (2009). Predator size, prey size, and dietary niche breadth relationships in marine predators. *Ecology*, *90*(7), 2014–2019. https://doi.org/10.1890/08-1150.1

Crespi-Abril, A. C., García, N. A., Crespo, E. A., & Coscarella, M. A. (2003). Consumption of marine mammals by broadnose sevengill shark *Notorynchus cepedianus* in the northern and central Patagonian shelf. *Latin American Journal of Aquatic Mammals*, *2*(2). https://doi.org/10.5597/lajam00038

Croft, D. A., Niemi, K., & Franco, A. (2011). Incisor morphology reflects diet in caviomorph rodents. *Journal of Mammalogy*, *92*(4), 871–879. https://doi.org/10.1644/10-MAMM-A-226.1

Cuthbert, R. N., Wasserman, R. J., Dalu, T., Kaiser, H., Weyl, O. L. F., Dick, J. T. A., Sentis, A., McCoy, M. W., & Alexander, M. E. (2020). Influence of intra- and interspecific variation in predator–prey body size ratios on trophic interaction strengths. *Ecology and Evolution*, *10*(12), 5946–5962. https://doi.org/10.1002/ece3.6332

Davidson, B., & Cliff, G. (2011). Liver lipids of female *Carcharias taurus* (spotted raggedtooth) sharks: A comparison between seasons. *Fish Physiology and Biochemistry*, *37*, 613–618. https://doi.org/10.1007/s10695-010-9463-y

Davis, A. M., Blanchette, M. L., Pusey, B. J., Jardine, T. D., & Pearson, R. G. (2012). Gut content and stable isotope analyses provide complementary understanding of ontogenetic dietary shifts and trophic relationships among fishes in a tropical river. *Freshwater Biology*, *57*(10), 2156–2172. https://doi.org/10.1111/j.1365-2427.2012.02858.x

Delariva, R. L., & Neves, M. P. (2020). Morphological traits correlated with resource partitioning among small characin fish species coexisting in a Neotropical river. *Ecology of Freshwater Fish*, *29*(4), 640–653. https://doi.org/10.1111/eff.12540

Deli Antoni, M. Y., Castro, M. G., & Díaz de Astarloa, J. M. (2008). Análisis de la morfología ósea de peces de la laguna costera Mar Chiquita, Argentina. *Revista de Biologia Marina y Oceanografia*, *43*(2), 355–380. https://doi.org/10.4067/s0718-19572008000200013

DeVries, M. S. (2017). The role of feeding morphology and competition in governing the diet breadth of sympatric stomatopod crustaceans. *Biology Letters*, *13*, 20170055. https://doi.org/10.1098/rsbl.2017.0055

Dicken, M. L., Hussey, N. E., Christiansen, H. M., Smale, M. J., Nkabi, N., Cliff, G., & Wintner, S. P. (2017). Diet and trophic ecology of the tiger shark (*Galeocerdo cuvier*) from South African waters. *PLoS ONE*, *12*(6), e0177897. https://doi.org/10.1371/journal.pone.0177897

Domenici, P., Claireaux, G., & McKenzie, D. J. (2007). Environmental constraints upon locomotion and predator-prey interactions in aquatic organisms: An introduction. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *362*, 1929–1936. https://doi.org/10.1098/rstb.2007.2078

Dyer, B. S. (2006). Systematic revision of the South American silversides (Teleostei, Atheriniformes). *Biocell*, *30*(1), 69–88.

Ebert, D. A. (1991). Observations on the predatory behaviour of the sevengill shark *Notorynchus cepedianus*. *South African Journal of Marine Science*, *11*(1), 455–465. https://doi.org/10.2989/025776191784287637

Ebert, D. A., Dando, M., & Fowler, S. (2021). *Sharks of the world: A complete guide*. Princeton University Press.

Ferrara, T. L., Clausen, P., Huber, D. R., McHenry, C. R., Peddemors, V., & Wroe, S. (2011). Mechanics of biting in great white and sandtiger sharks. *Journal of Biomechanics*, *44*, 430–435. https://doi.org/10.1016/j.jbiomech.2010.09.028

Ferry, L. A. (2015). Functional morphology: “Point and shoot” prey capture in fishes. *Current Biology*, *25*, R982–R984. https://doi.org/10.1016/j.cub.2015.09.023

Ferry, L. A., & Cailliet, G. M. (1996). Sample size and data analysis: are we characterizing and comparing diet properly? In D. MacKinlay & K. Shearer (Eds.), *Feeding Ecology and Nutrition in Fish. Symposium Proceedings* (pp. 71–80). American Fisheries Society.

Ferry-Graham, L. A., Bolnick, D. I., & Wainwright, P. C. (2002). Using functional morphology to examine the ecology and evolution of specialization. *Integrative and Comparative Biology*, *42*, 265–277. https://doi.org/10.1093/icb/42.2.265

Ferry-Graham, L. A., Wainwright, P. C., & Bellwood, D. R. (2001). Prey capture in long-jawed butterflyfishes (Chaetodontidae): The functional basis of novel feeding habits. *Journal of Experimental Marine Biology and Ecology*, *256*, 167–184. https://doi.org/10.1016/S0022-0981(00)00312-9

Figgener, C., Bernardo, J., & Plotkin, P. T. (2019). Beyond trophic morphology: stable isotopes reveal ubiquitous versatility in marine turtle trophic ecology. *Biological Reviews*, *94*, 1947–1973. https://doi.org/10.1111/brv.12543

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, *226*(1), 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5

Frazzetta, T. H. (1988). The mechanics of cutting and the form of shark teeth (Chondrichthyes, Elasmobranchii). *Zoomorphology*, *108*(2), 93–107. https://doi.org/10.1007/BF00539785

Giaretta, M. B., Siliprandi, C. C., Santificeturv, C., Brenha-Nunes, M. R., Conversani, V. R. M., & Rossi-Wongtschowski, C. L. D. B. (2017). Atlas of marine bony fish otoliths (sagittae) of southeastern-southern Brazil Part VI: Albuliformes, Anguiliformes, Osmeriformes, Stomiiformes, Aulopiformes, Myctophiformes, Ophidiiformes, Polimixiiformes, Batrachoidiformes and Lophiformes. *Brazilian Journal of Oceanography*, *65*(2), 258–308. https://doi.org/10.1590/S1679-87592017133806502

Gilmore, R. G., Dodrill, J. W., & Linley, P. A. (1983). Reproduction and embryonic development of the Sand tiger shark, *Odontaspis taurus* (Rafinesque). *Fishery Bulletin*, *81*(2), 201–225.

Graeb, B. D. S., Mangan, M. T., Jolley, J. C., Wahl, D. H., & Dettmers, J. M. (2006). Ontogenetic changes in prey preference and foraging ability of yellow perch: Insights based on relative energetic return of prey. *Transactions of the American Fisheries Society*, *135*, 1493–1498. https://doi.org/10.1577/t05-063.1

Grainger, R., Peddemors, V. M., Raubenheimer, D., & Machovsky-Capuska, G. E. (2020). Diet composition and nutritional niche breadth variability in juvenile white sharks (*Carcharodon carcharias*). *Frontiers in Marine Science*, *7*(422). https://doi.org/10.3389/fmars.2020.00422

Grossnickle, D. M. (2020). Feeding ecology has a stronger evolutionary influence on functional morphology than on body mass in mammals. *Evolution*, *74*(3), 610–628. https://doi.org/10.1111/evo.13929

Harrison, T. D., & Whitfield, A. K. (2006). Temperature and salinity as primary determinants influencing the biogeography of fishes in South African estuaries. *Estuarine, Coastal and Shelf Science*, *66*(1–2), 335–345. https://doi.org/10.1016/j.ecss.2005.09.010

Hocking, D. P., Park, T., Rule, J. P., & Marx, F. G. (2021). Prey capture and processing in fur seals, sea lions and the walrus. In C. Campagna & R. Harcourt (Eds.), *Ethology and behavioral ecology of marine mammals* (First edition, pp. 101–121). Springer Nature Switzerland AG.

Horn, M. H., & Ferry-Graham, L. A. (2006). Feeding mechanisms and trophic interactions. In L.G. Allen, D. J. Pondella, & M. H. Horn (Eds.), *The Ecology of Marine Fishes: California and Adjacent Waters* (First edition, pp. 387–410). University of California Press. https://doi.org/10.1525/california/9780520246539.003.0014

Hussey, N. E., Brush, J., McCarthy, I. D., & Fisk, A. T. (2010). $\delta^{15}N$ and $\delta^{13}C$ diet-tissue discrimination factors for large sharks under semi-controlled conditions. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *155*(4), 445–453. https://doi.org/10.1016/j.cbpa.2009.09.023

Hussey, N. E., MacNeil, M. A., Olin, J. A., McMeans, B. C., Kinney, M. J., Chapman, D. D., & Fisk, A. T. (2012). Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. *Journal of Fish Biology*, *80*(5), 1449–1484. https://doi.org/10.1111/j.1095-8649.2012.03251.x

Hussey, N. E., Dibattista, J. D., Moore, J. W., Ward, E. J., Fisk, A. T., Kessel, S., Guttridge, T. L., Feldheim, K. A., Franks, B. R., Gruber, S. H., & Weideli, O. C. (2017). Risky business for a juvenile marine predator? Testing the influence of foraging strategies on size and growth rate under natural conditions. *Proceedings of the Royal Society B: Biological Sciences*, *284*, 20170166. https://doi.org/https://doi.org/10.1098/rspb.2017.0166

Hynes, H. B. N. (1950). The food of fresh-water sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*), with a review of methods used in studies of the food of fishes. *Journal of Animal Ecology*, *19*(1), 36–58. https://doi.org/10.2307/1570

Hyslop, E. J. (1980). Stomach contents analysis—a review of methods and their application. *Journal of Fish Biology*, *17*, 411–429. https://doi.org/10.1111/j.1095-8649.1980.tb02775.x

Karpouzi, V. S., & Stergiou, K. I. (2003). The relationships between mouth size and shape and body length for 18 species of marine fishes and their trophic implications. *Journal of Fish Biology*, *62*(6), 1353–1365. https://doi.org/10.1046/j.1095-8649.2003.00118.x

Keppeler, F. W., Montaña, C. G., & Winemiller, K. O. (2020). The relationship between trophic level and body size in fishes depends on functional traits. *Ecological Monographs*, *90*(4), 1–19. https://doi.org/10.1002/ecm.1415

Kim, S. L., Casper, D. R., Galván-Magaña, F., Ochoa-Díaz, R., Hernández-Aguilar, S. B., & Koch, P. L. (2012). Carbon and nitrogen discrimination factors for elasmobranch soft tissues based on a long-term controlled feeding study. *Environmental Biology of Fishes*, *95*(1), 37–52. https://doi.org/10.1007/s10641-011-9919-7

Kim, S. L., & Koch, P. L. (2012). Methods to collect, preserve, and prepare elasmobranch tissues for stable isotope analysis. *Environmental Biology of Fishes*, *95*, 53–63. https://doi.org/10.1007/s10641-011-9860-9

Kleiber, M. (1947). Body size and metabolic rate. *Physiological Reviews*, *27*(4), 511–541.

Ladds, M. A., Pinkerton, M. H., Jones, E., Durante, L. M., & Dunn, M. R. (2020). Relationship between morphometrics and trophic levels in deep-sea fishes. *Marine Ecology Progress Series*, *637*(1978), 225–235. https://doi.org/10.3354/meps13243

Li, Y., Zhang, Y., Hussey, N. E., & Dai, X. (2016). Urea and lipid extraction treatment effects on δ^{15}N and δ^{13}C values in pelagic sharks. *Rapid Communications in Mass Spectrometry*, *30*, 1–8. https://doi.org/10.1002/rcm.7396

Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, *68*(4), 619–640. https://doi.org/10.1139/z90-092

Lucifora, L. O., García, V. B., & Escalante, A. H. (2009a). How can the feeding habits of the sand tiger shark influence the success of conservation programs? *Animal Conservation*, *12*(4), 291–301. https://doi.org/10.1111/j.1469-1795.2009.00247.x

Lucifora, L. O., García, V. B., Menni, R. C., Escalante, A. H., & Hozbor, N. M. (2009b). Effects of body size, age and maturity stage on diet in a large shark: Ecological and applied implications. *Ecological Research*, *24*(1), 109–118. https://doi.org/10.1007/s11284-008-0487-z

Lucifora, L. O., Menni, R. C., & Escalante, A. H. (2001). Analysis of dental insertion angles in the sand tiger shark, *Carcharias taurus* (Chondrichthyes: Lamniformes). *Cybium*, *25*(1), 23–31.

Lucifora, L. O., Menni, R. C., & Escalante, A. H. (2005). Reproduction, abundance and feeding habits of the broadnose sevengill shark *Notorynchus cepedianus* in north Patagonia, Argentina. *Marine Ecology Progress Series*, *289*, 237–244. https://doi.org/10.3354/meps289237

MacArthur, R. H., & Pianka, E. R. (1966). On optimal use of a patchy environment. *The American Naturalist*, *100*(916), 603–609. https://doi.org/10.1086/282454

MacNulty, D. R., Smith, D. W., Mech, L. D., & Eberly, L. E. (2009). Body size and predatory performance in wolves: Is bigger better? *Journal of Animal Ecology*, *78*, 532–539. https://doi.org/10.1111/j.1365-2656.2008.01517.x

Magurran, A. E. (2004). *Measuring biological diversity*. Blackwell Publishing.

Marceniuk, A. P., Menezes, N. A., & Britto, M. R. (2012). Phylogenetic analysis of the family Ariidae (Ostariophysi: Siluriformes), with a hypothesis on the monophyly and relationships of the genera.

Zoological Journal of the Linnean Society, *165*, 534–669. https://doi.org/10.1111/j.1096-3642.2012.00822.x

Matich, P., Heithaus, M. R., & Layman, C. A. (2010). Size-based variation in intertissue comparisons of stable carbon and nitrogen isotopic signatures of bull sharks (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*). *Canadian Journal of Fisheries and Aquatic Sciences*, *67*(5), 877–885. https://doi.org/10.1139/F10-037

McElroy, W. D., Wetherbee, B. M., Mostello, C. S., Lowe, C. G., Crow, G. L., & Wass, R. C. (2006). Food habits and ontogenetic changes in the diet of the sandbar shark, *Carcharhinus plumbeus*, in Hawaii. *Environmental Biology of Fishes*, *76*, 81–92. https://doi.org/10.1007/s10641-006-9010-y

Medeiros, L., da Silveira Monteiro, D., Petitet, R., & Bugoni, L. (2015). Effects of lipid extraction on the isotopic values of sea turtle bone collagen. *Aquatic Biology*, *23*(3), 191–199. https://doi.org/10.3354/ab00628

Mihalitsis, M., & Bellwood, D. R. (2017). A morphological and functional basis for maximum prey size in piscivorous fishes. *PLoS ONE*, *12*(9), 1–19. https://doi.org/10.1371/journal.pone.0184679

Niella, Y., Raoult, V., Gaston, T., Peddemors, V. M., Harcourt, R., & Smoothey, A. F. (2021). Overcoming multi-year impacts of maternal isotope signatures using multi-tracers and fast turnover tissues in juvenile sharks. *Chemosphere*, *269*, 129393. https://doi.org/10.1016/j.chemosphere.2020.129393

Olin, J. A., Hussey, N. E., Fritts, M., Heupel, M. R., Simpfendorfer, C. A., Poulakis, G. R., & Fisk, A. T. (2011). Maternal meddling in neonatal sharks: Implications for interpreting stable isotopes in young animals. *Rapid Communications in Mass Spectrometry*, *25*(8), 1008–1016. https://doi.org/10.1002/rcm.4946

Olivier, D., Lepoint, G., Aguilar-Medrano, R., Díaz, A. H. R., Sánchez-González, A., & Sturaro, N. (2019). Ecomorphology, trophic niche, and distribution divergences of two common damselfishes in the Gulf of California. *Comptes Rendus - Biologies*, *342*, 309–321. https://doi.org/10.1016/j.crvi.2019.11.001

Perez Comesaña, J. E., Bechthold, C., Arias, K., Carrizo, G. R., & Riestra, C. (2014). Estimación de la longitud de la pescadilla de red (*Cynoscion guatucupa*) (Actinopterygii: Sciaenidae) utilizando relaciones morfométricas de huesos del cráneo y de la cintura escapular, otolitos y medidas específicas del cuerpo. *Revista Del Museo Argentino de Ciencias Naturales, Nueva Serie*, *16*(1), 89–93. https://doi.org/10.22179/REVMACN.16.164

Pethybridge, H., Daley, R., Virtue, P., & Nichols, P. (2010). Lipid composition and partitioning of deepwater chondrichthyans: Inferences of feeding ecology and distribution. *Marine Biology*, *157*(6), 1367–1384. https://doi.org/10.1007/s00227-010-1416-6

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecological Society of America*, *83*(3), 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Powter, D. M., Gladstone, W., & Platell, M. (2010). The influence of sex and maturity on the diet, mouth morphology and dentition of the Port Jackson shark, *Heterodontus portusjacksoni*. *Marine and Freshwater Research*, *61*(1), 74–85. https://doi.org/10.1071/MF09021

Pyke, G. H. (1984). Optimal foraging theory: a critical review. *Annual Review of Ecology and Systematics*, *15*, 523–575. https://doi.org/10.1146/annurev.ecolsys.15.1.523

Rodrigues, F. L., & Bemvenuti, M. de A. (2011). Diet and feeding strategy of the silverside *Odontesthes bonariensis* (Valenciennes 1835) Atheriniformes: Atherinopsidae) from the Mirim Lagoon, Rio Grande do Sul, Brazil. *Pan-American Journal of Aquatic Sciences*, *6*(2), 161–169.

Rossi-Wongtschowski, C. L. D. B., Siliprandi, C. C., Brenha, M. R., Gonsales, S. de A., Santificetur, C., & Vaz-dos-Santos, A. M. (2014). Atlas of marine bony fish otoliths (sagittae) of southeastern- southern Brazil. Part I: Gadiformes (macrouridae, moridae, bregmacerotidae, phycidae and merlucciidae); Part II: Perciformes (carangidae, sciaenidae, scombridae and serranidae). *Brazilian Journal of Oceanography*, *62*(special issue), 1–103. https://doi.org/10.1590/S1679-875920140637062sp1

Silveira, S., Laporta, M., Pereyra, I., Mas, F., Doño, F., & Santana, O. (2018). Análisis de la captura de condrictios en la pesca artesanal oceánica de Uruguay, Atlántico Sudoccidental. *Frente Marítimo*, *Setiembre*, 301–324.

Sims, D. W. (2003). Tractable models for testing theories about natural strategies: foraging behaviour and habitat selection of free-ranging sharks. *JournalofFish Biology*, *63*(A), 53–73. https://doi.org/10.1046/j.1095-8649.2003.00207.x

Smyth, K., & Elliott, M. (2016). Effects of changing salinity on the ecology of the marine environment. In *Stressors in the Marine Environment* (First edition, pp. 161–174). Oxford University Press. https://doi.org/10.1093/acprof:oso/9780198718826.003.0009

Stoner, A. W., & Livingston, R. J. (1984). Ontogenetic patterns in diet and feeding morphology in sympatric sparid fishes from seagrass meadows. *Copeia*, *1984*(1), 174–187. https://doi.org/10.2307/1445050

Streelman, J. T., & Danley, P. D. (2003). The stages of vertebrate evolutionary radiation. *Trends in Ecology and Evolution*, *18*(3), 126–131. https://doi.org/10.1016/S0169-5347(02)00036-8

Svanbäck, R., & Bolnick, D. I. (2007). Intraspecific competition drives increased resource use diversity within a natural population. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1611), 839–844. https://doi.org/10.1098/rspb.2006.0198

Tan, H., Hirst, A. G., Atkinson, D., & Kratina, P. (2021). Body size and shape responses to warming and resource competition. *Functional Ecology*, *35*, 1460–1469. https://doi.org/10.1111/1365-2435.13789

Tombari, A. D., Gosztonyi, A., Echeverría, D. D., & Volpedo, A. V. (2010). Otolith and vertebral morphology of marine atherinid species (Atheriniformes, Atherinopsidae) coexisting in the southwestern Atlantic Ocean. *Ciencias Marinas*, *36*(3), 213–223. https://doi.org/10.7773/cm.v36i3.1692

Türtscher, J., Jambura, P. L., López-Romero, F. A., Kindlimann, R., Sato, K., Tomita, T., & Kriwet, J. (2022). Heterodonty and ontogenetic shift dynamics in the dentition of the tiger shark *Galeocerdo cuvier* (Chondrichthyes, Galeocerdidae) . *Journal of Anatomy*, *October 2021*, 1–21. https://doi.org/10.1111/joa.13668

Van Valkenburgh, B. (2019). Carnivore dental adaptations and diet: A study of trophic diversity within guilds. In J. L. Gittleman (Ed.), *Carnivore Behavior, Ecology, and Evolution* (First edit, pp. 410–436). Springer. https://doi.org/https://doi.org/10.1007/978-1-4757-4716-4_16

Verde Arregoitia, L. D., Fisher, D. O., & Schweizer, M. (2017). Morphology captures diet and locomotor types in rodents. *Royal Society Open Science*, *4*(1). https://doi.org/10.1098/rsos.160957

Volpedo, A. V., & Echeverría, D. D. (2000). *Catálogo y claves de otolitos para la identificación de peces del mar argentino*. Editorial Dunken.

Volpedo, A. V., Thompson, G. A., & Avigliano, E. (2017). *Atlas de otolitos de peces de Argentina*.

Wainwright, P. C., & Richard, B. A. (1995). Predicting patterns of prey use from morphology of fishes. *Environmental Biology of Fishes*, *44*, 97–113. https://doi.org/10.1007/BF00005909

Ward, A. J. W., Webster, M. M., & Hart, P. J. B. (2006). Intraspecific food competition in fishes. *Fish and Fisheries*, *7*, 231–261. https://doi.org/10.1111/j.1467-2979.2006.00224.x

Ward-Campbell, B. M. S., & Beamish, F. W. H. (2005). Ontogenetic changes in morphology and diet in the snakehead, *Channa limbata*, a predatory fish in western Thailand. *Environmental Biology of Fishes*, *72*(3), 251–257. https://doi.org/10.1007/s10641-004-1744-9

Watanabe, Y. Y., Payne, N. L., Semmens, J. M., Fox, A., & Huveneers, C. (2019). Swimming strategies and energetics of endothermic white sharks during foraging. *Journal of Experimental Biology*, *222*, jeb185603. https://doi.org/10.1242/jeb.185603

Wilga, C. A. D., Diniz, S. E., Steele, P. R., Sudario-Cook, J., Dumont, E. R., & Ferry, L. A. (2016). Ontogeny of feeding mechanics in smoothhound sharks: Morphology and cartilage stiffness. *Integrative and Comparative Biology*, *56*(3), 442–448. https://doi.org/10.1093/icb/icw078

Wilga, C. A. D., & Ferry, L. A. (2015). Functional anatomy and biomechanics of feeding in elasmobranchs. In R. E. Shadwick, A. P. Farrell, & C. J. Brauner (Eds.), *Fish Physiology, Volume 34, Part A* (pp. 153–187). Academic Press. https://doi.org/10.1016/B978-0-12-801289-5.00004-3

Willson, J. D., & Hopkins, W. A. (2011). Prey morphology constrains the feeding ecology of an aquatic generalist predator. *Ecology*, *92*(3), 744–754.

Winkler, N. S., Paz-Goicoechea, M., Lamb, R. W., & Pérez-Matus, A. (2017). Diet reveals links between morphology and foraging in a cryptic temperate reef fish. *Ecology and Evolution*, *7*, 11124–11134. https://doi.org/10.1002/ece3.3604

Woo, K. J., Elliott, K. H., Davidson, M., Gaston, A. J., & Davoren, G. K. (2008). Individual specialization in diet by a generalist marine predator reflects specialization in foraging behaviour. *Journal of Animal Ecology*, *77*, 1082–1091. https://doi.org/10.1111/j.1365-2656.2008.01429.x

Yancey, P. H. (2015). Organic osmolytes in elasmobranchs. In R. E. Shadwick, A. P. Farrell, & C. J. Brauner (Eds.), *Fish Physiology, Volume 34, Part B* (pp. 221–277). Academic Press. https://doi.org/10.1016/B978-0-12-801286-4.00004-6

Supplementary Material

Chapter 4. Morphology influences niche dynamics in two large, sympatric sharks

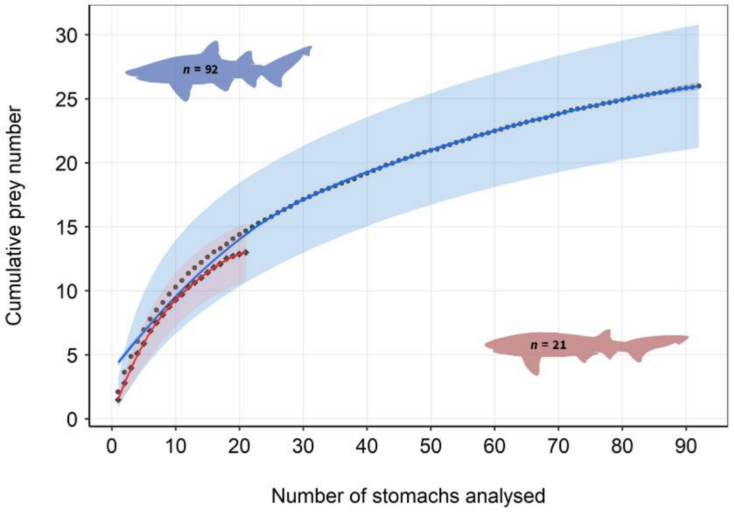

Figure 4.S1. Randomized cumulative prey curves estimated from the stomach content analysis of *Carcharias taurus* (grey nurse shark) and *Notorynchus cepedianus* (sevengill sharks) caught along the Uruguayan coast. The total number of stomachs analysed with at least one prey item was 92 for grey nurse sharks and 21 for sevengill sharks. The order in which the stomachs were analysed was randomised 100 times and the means (solid lines) and 95% confidence intervals (shaded area) are plotted.

Table 4.S1. Diet composition of *Carcharias taurus* (*n*=92, grey nurse shark) and *Notorynchus cepedianus* (*n*=21, sevengill sharks) caught along the Uruguayan coast. Results are presented in percentage by frequency of occurrence (%F) and percentage by number (% N).

		Carcharias taurus (*n*=92)		***Notorynchus cepedianus*** (*n*=21)	
Group/Taxa	**Prey**	**%N**	**%F**	**%N**	**%F**
Actinopterygii	**-**	**92.2**	**94.6**	**44.7**	**61.9**
Scianidae	*Micropogonias furnieri*	18.7	38.0	5.3	9.5
	Cynoscion guatucupa	17.0	35.9	-	-
	Macrodon atricauda	4.2	10.9	-	-
	Paralonchurus brasiliensis	1.4	4.3	-	-
	Menticirrhus americanus	0.7	2.2	-	-
	Umbrina canosai	0.4	1.1	-	-
Batrachoididae	*Porichthys porosissimus*	9.2	23.9	2.6	4.8
Atherinopsidae	*Odontesthes* sp	17.3	20.7	-	-
Stromateidae	*Peprilus* sp	7.8	13.0	2.6	4.8
Physidae	*Urophycis brasiliensis*	4.6	10.9	-	-
Clupeidae	*Brevoortia aurea*	1.4	3.3	-	-
Trichiuridae	*Trichiurus lepturus*	1.4	3.3	-	-
Mugilidae	*Mugil liza*	1.1	3.3	5.3	9.5
Congridae	*Conger orbignianus*	1.1	3.3	-	-
Sparidae	*Diplodus argenteus*	0.7	2.2	-	-
Ariidae	*Genidens barbus*	-	-	5.3	9.5
Unidentified Teleosts	-	4.6	14.1	23.7	28.6
Chondrichthyes	**-**	**6.7**	**12.0**	**34.2**	**52.4**
Subclass - Holocephali	-	0.4	1.1	-	-
Callorhinchidae	*Callorhinchus callorhynchus*	0.4	1.1	-	-
Subclass - Elasmobranchii	-	6.4	10.9	34.2	52.4
Rajidae	*Sympterygia acuta*	0.4	1.1	-	-
	Sympterygia acuta (egg capsules)	0.7	1.1	-	-
	Sympterygia bonapartii	0.4	1.1	-	-
Myliobatidae	*Myliobatis ridens*	0.7	1.1	5.3	9.5
	Myliobatis sp	-	-	2.6	4.8
Squatinidae	*Squatina guggenheim*	0.4	1.1	-	-
Triakidae	*Mustelus schmitti*	1.4	2.2	-	-
Unidentified Batoidea	-	0.4	1.1	7.9	14.3
Unidentified Selachimorpha	-	0.7	2.2	10.5	19.0
Unidentified Elasmobranchs	-	0.4	1.1	7.9	14.3
Unidentified fish species	-	0.7	2.2	2.6	4.8
Marine Mammals	**-**	**-**	**-**	**7.9**	**14.3**
Cetacea	Odontocetii	-	-	5.3	9.5
Carnivora	Otariidae	-	-	2.6	4.8

Chapter Five - General Conclusions

5.1. Main conclusions

Intrinsic biotic factors play a fundamental role as shapers of the trophic niche of marine predators, determining their role in the ecosystem (generalist and specialist species). Trophic niche size varied with the degree of individual specialisation of the populations. Populations composed of specialist individuals will have broader trophic niches (generalist population), whereas populations composed of generalist individuals will have narrower trophic niches (specialist populations). However, since trophic plasticity within species is higher than expected, we need to be careful in generalising this assumption, as these patterns could even be reversed with large regional differences in prey availability. In this thesis, the degree of individual specialisation was associated with the foraging habitat of the predator: temporal stable environments with a high diversity of prey but in low abundance (*e.g.*, benthic-coastal habitats) led to higher degrees of individual specialisation; whilst temporal dynamic environments with low variety of prey but in high abundance, promoted generalist individuals (*e.g.*, pelagic environments). Finally, trophic morphological characteristics are important determinants of trophic niches and may be the primary factor facilitating resource partitioning in species with different trophic morphologies. This thesis provides new insights into how abiotic and biotic factors define the trophic niche of marine predators, which will help predict responses of marine predators in a changing environment.

5.2. High-order marine predators in marine food webs

Currently, marine ecosystems are facing an accelerated change in both physical and biotic characteristics due to anthropogenic stressors, including chemical pollution, ocean warming and unsustainable exploitation of many resources (Halpern et al., 2008; Hoegh-Guldberg & Bruno, 2010; Bronselaer & Zanna, 2020). The acceleration in the rate of change in the last decade has led to an intensified need to improve our understanding of how food webs behave and respond to different external and internal factors. High-order marine predators are particularly susceptible to those changes which negatively affect their populations by jeopardising the health of marine ecosystems (Heithaus et al., 2008; Maxwell et al., 2013; Hazen et al., 2019; Nelms et al., 2021). As critical components of marine food webs, improving our understanding of how predators' trophic niches vary with different biotic and abiotic factors is fundamental to assessing if these species can overcome future environmental changes. In this thesis, I study how different biotic and abiotic factors may affect the trophic niche metrics of marine predators in the southwest Atlantic, with the aim of improving our understanding to help predict how marine predators may respond to change.

The trophic niche of organisms is determined, at least in part, by biotic factors that could be extrinsic or intrinsic to every organism (Figure 1.1). In Chapter Two, I explored how individual specialisation, an intrinsic factor, shapes the trophic population niche of two pinniped species and how this related to the foraging strategy of each predator. I showed that broader population trophic niches were associated with higher degrees of individual specialisation, while narrower population trophic niches were associated with lower degrees of individual specialisation. I proposed that the degree of individual specialisation is influenced by the foraging strategy of the predator, whether pelagic or benthic. Species that consumed pelagic species, such as South American fur seals (*A. australis*), typically foraged in a dynamic environment with abundant but similar prey and were comprised of specialist populations of generalist individuals. In contrast, coastal-benthic foragers such as South American sea lions (*O. byronia*), foraged on habitats with diverse but less abundant prey, and were composed of generalist populations of specialist individuals. I hypothesised that differences in the degree of individual specialization within and between populations were related to differences in habitat and prey availability. This hypothesis led me to the next chapter (Chapter Three), where I tested the former hypothesis by comparing population trophic niche metrics of the same species but in two contrasting environments. As in trophic ecology studies, the trophic niche of a species is commonly assumed to have some degree of stability due to species-specific life history constraints. Accordingly, I predicted that I would observe moderate variability in niche sizes between the different regions for both South American sea lions and South American fur seals, reflecting variability in prey availability but within the paradigm that the benthic South American sea lions would still be a generalist population and the epipelagic South American fur seals would still be a specialist population. This would be consistent with many other studies of these species which have found large variability in their diet breadth, particularly in South American sea lions (*e.g.*, Grandi et al., 2021), and long-term variability throughout years in face of a changing environment (Drago et al., 2017; Szteren et al., 2018). However, signs of niche stability over time have also been detected in adult males South American fur seals (Vales et al., 2020). To my surprise, I found that the degree of niche plasticity was far, far higher than expected. I found a switch in niche breadth between species at each end of their range. These results have important implications for trophic ecology studies, as they demonstrate there can be a very high level of trophic plasticity. This suggests that the trophic niche size of a species in different areas has the potential to change markedly, enough to provoke a complete inversion of the ecological role of the species. Regional differences in prey availability clearly have important consequences on the trophic niche metrics of a species.

Prey abundance and their presence in an environment do not imply that a predator will always be able to consume it (Horn & Ferry-Graham, 2006). Morphological characteristics are crucial to prey selection as these determine whether a predator can successfully detect, capture, handle and consume their prey once all other requirements, such as prey availability, have been met. In Chapter Four, I continued to explore the effects of intrinsic individual factors as shapers of the trophic niche of large-bodied marine predators. I focussed on the morphological characters of consumers that could constrain the prey choices of predators and therefore their trophic niche. I showed that morphological traits play an important role in prey choice and may be the primary mechanism facilitating resource partitioning within sympatric marine predators. Species that were able to dissect their prey before consuming it, such as sevengill sharks, have access to higher trophic levels of the food web regardless of ontogenetic stage. In contrast, species with morphological constraints that only allow them to eat their prey whole, such as grey nurse sharks, are limited in the trophic niche that they can access. As body size increased both types of predators consumed prey from higher trophic levels. However, those capable of cutting their prey into pieces consumed prey from relatively higher trophic levels. I found a strong relationship between prey selection and morphological traits subjected to strong selective forces that optimise feeding events, such as tooth shape and jaw morphology. This contrasts with morphological characters under multiple selective forces other than feeding which results in a weaker relationship between prey choice and the morphological character (*e.g.,* body size).

Including an assessment of trophic morphological traits may assist in interpreting how extrinsic and intrinsic factors affect the trophic ecology of a predator. I recommend not only focusing on the commonly studied extrinsic factors (inter- and intraspecific interactions, and abiotic factors) that might be affecting the trophic ecology of the groups under study, but also on intrinsic factors (morphological and behavioural traits) of the organisms to improve our ecological interpretations.

5.3. Caveats

Even though the project was designed to minimise its constraints while maximising the budget and timeframe available to address its objectives, there are some limitations that must be considered. The main caveats identified are listed below and will be discussed further in the following section of Further Directions.

- Due to my limited budget and time available for this project, we decided to use the more versatile, robust, and less expensive tool, stable isotope analysis. However, when using only stable isotopes to study the trophic ecology of populations, it is difficult to differentiate whether the results obtained are due to the diet of a predator, its habitat use or both. For this reason, it is recommended that stable isotope analysis be used in conjunction with other techniques such as eDNA or fatty acid analysis, which could help to better define the trophic niche. To minimise this problem, for Chapters 2 and 3, I used pinniped species for which there was previous information on their diet (scats and stomach content analysis) and habitat use (telemetry data) locally. Samples collected during the deployment of the telemetry devices in the Falklands Islands were included in the analysis. In the Uruguayan populations, there are previous studies using stable isotope analysis of δ^{13}C and δ^{15}N. However, samples from both breeding colonies have not been included so far. Moreover, I decided to include a third stable isotope (δ^{34}S) in the analysis as a novel approach to help to better differentiate the source of organic matter in complex ecosystems like the Rio de la Plata estuary. In contrast to the pinniped species, shark species in the region have been poorly studied, especially in Uruguay. For this reason, a dietary study based on stomach content analysis was included in the project, informing the stable isotope results. Due to the limited knowledge of these shark species in the region, although I used only two types of analysis, these results still provide baseline data on the trophic ecology of these two species, providing a platform upon to base future studies. During sample collection for this thesis, tissue samples were also collected to enable future studies using fatty acids analysis, metagenomic analysis of stomach contents and stable isotope analysis of items recovered from stomach contents.

- When pinnipeds were used as focal study species (Chapters 2 and 3), only adult females could be assessed. Consequently, we must be careful when extrapolating these results to the entire population of the species, as this only gives a partial picture of the trophic niche of these species in the studied regions. This is especially true for these two pinniped species which have a pronounced sexual dimorphism, with significant differences in body size and behaviour (females are central place foragers; while males are highly mobile), leading to different use of resources (*e.g.*, Koen-Alonso et al., 2000; Kernaléguen et al., 2016). Furthermore, these species are likely to show ontogenetic differences in resource use which should be considered.

- Despite the sample size of sevengill sharks exceeding the predefined minimum size to achieve ecologically robust results in stable isotope analysis, it was not possible to produce a complete description of their diet through the stomach content analysis as so many stomachs were empty (Chapter 4). Therefore, there is still some uncertainty identifying their diet preferences. Differences in samples size between species can be attributed to the fact that the sevengill shark fishing season in the region is short (~1-2 months) and these sharks are mainly targeted by recreational fisheries. Consequently, access to samples of sevengill sharks was more difficult than access to grey nurse sharks which are targeted by artisanal fisheries (with larger catches) and a longer fishing season (~3-4 months).
- In Chapter 4, I had few adult individuals for both species of sharks, especially females. Grey nurse sharks are known to show ontogenetic changes in their diet, increasing their consumption of elasmobranch as they become larger, mainly in individuals larger than 200 centimetres long (Lucifora et al., 2009). The under-representation of this cohort could lead to underestimates of the total population trophic niche, which needs to be considered when interpreting the results for the entire population.
- The lack of other large-bodied shark species inhabiting the coastal environments of Uruguay did not make it possible to evaluate other tooth morphologies or to duplicate those already represented. Species that share the same ecosystem, at least for some period, and with similar body sizes were chosen to reduce the possible biases produces by environmental differences (*e.g.*, prey availability) and differential prey accessibility due to differences in body sizes. As consequence, due to the low number of the species and types of teeth morphologies analysed, we urge caution when generalising these results to different shark species (or even different predators' taxa) and ecosystems.

However, the results obtained in Chapter 4, give us the first steps to propose and test an ecological hypothesis that can be extended to different ecosystems and predator species.

5.4. Future directions

The impact of intra- and interspecific competition on the trophic niche metrics of the populations studied could not be assessed for this thesis. These biotic extrinsic factors were initially one of the main objectives of the thesis. However, due to complications and delays caused by the COVID-19 pandemic, it could not be included. The available evidence confirms that different degrees of competition for resources can drive major effects on the allocation of resources among organisms in a community (*e.g.*, Svanbäck & Bolnick, 2005, 2007; Costa-Pereira et al., 2019). The four species of large-bodied marine predators studied in this thesis are sympatric coastal species inhabiting the coastal waters of Uruguay, which could exert some degree of competitive forces on each other. Therefore, this is an excellent opportunity to evaluate the inter-species competition between two large taxonomic groups of marine predators (sharks and pinnipeds). If the hypothesis that the non-diversification of foraging strategies in adult female South American sea lions in Uruguay is evidence of a high abundance of resources in the environment is correct (Chapter Three-this thesis), we should expect some overlap between the four species. In addition, I would expect sevengill sharks to be the species with the highest $\delta^{15}N$ values (and higher ranges of $\delta^{15}N$) due to the incidence of marine mammals in the diet (Chapter Four-this thesis, Praderi, 1985; Lucifora et al., 2005). Finally, sevengill sharks and South American fur seals will be generalist populations, as sevengill sharks can consume a wide range of prey sizes, and adult female South American fur seals show differentiation in their foraging strategies. In contrast, South American sea lions and grey nurse sharks will be specialist populations since female sea lions do not show differentiation in their foraging strategy (Chapter Three-this thesis), and grey nurse sharks, due to their morphological constraints can only consume a narrow range of prey sizes (Chapter Four-this thesis). An outline of the hypothetical scenario presented here, showing the relative position of the four species is shown in Figure 5.1. As there may be differences in resource use between sexes and across ontogeny within a population (*e.g.,* Koen-Alonso et al. 2000; Valés et al., 2015; Kernaléguen et al., 2016), not only adult females of pinniped populations will be included in the analysis, but also all population cohorts. The inclusion of cohorts will give us a more realistic and broader view of how these species are interacting in the region and how this shapes their trophic niches. Such a study would provide a nexus for the trophic characteristics examined in this thesis, allowing us to better understand the determinants of trophic niche in large marine predators. In addition, it would be one of the first to include a broad sympatric multispecies approach from two different taxonomic groups of predators to assess trophic interactions in a marine ecosystem. Prior to this study, trophic interactions between pinnipeds and sharks have not been assessed in the region and so these results will provide a more integrative view of the whole ecosystem. These results will provide a better understanding of the role of these predators in their ecosystems and assess their degree of vulnerability. These findings may also be used to inform conservation plans and improve the effectiveness of resource management in these areas.

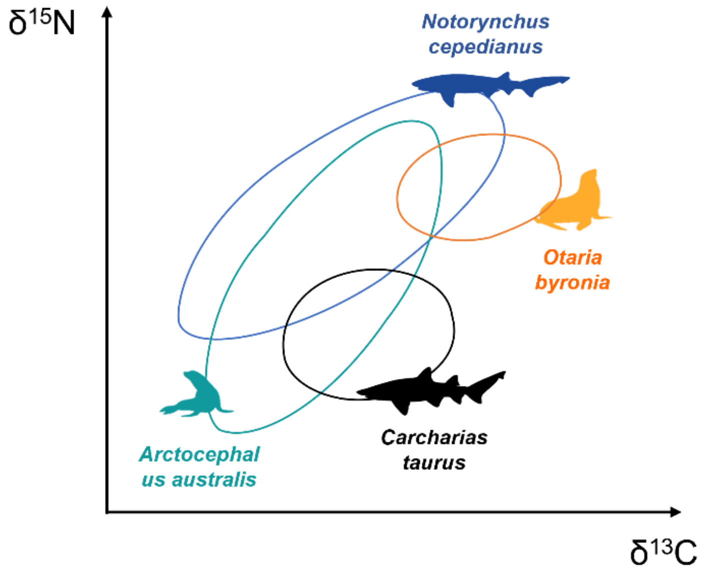

Figure 5.1. A hypothetical scenario showing the relative positions of the isotopic niches of four sympatric species of marine predators in the coastal ecosystem of Uruguay. Isotopic position of South American fur seal (*Arctocephalus australis*) and South American sea lions (*Otaria byronia*) was taken from Chapter 3. For sevengill sharks (*Notorynchus cepedianus*) and grey nurse sharks (*Carcharias taurus*), their relative position was inferred from the results obtained in this thesis.

Both studied species of coastal sharks have global conservation concern (Vulnerable: sevengill sharks and Critically Endangered: grey nurse shark) (Finucci et al., 2020; Rigby et al., 2021) as well as locally (Domingo et al., 2015). At present, the only two studies on the trophic ecology of grey nurse sharks and sevengill sharks in the *Río de la Plata* estuary region are from 40 years ago (Praderi, 1985) and the present study (Chapter Four-this thesis). Filling information gaps is vital to the development and implementation of management plans for the conservation and recovery of these species. Therefore, I strongly recommend complementing the thesis results through other study techniques that could not be included in this project. At the present, I have collected samples upon which to conduct fatty acid analysis, DNA metabarcoding for identification of prey items from stomach fluids, and stable isotope analysis of prey items found inside the stomachs analysed. Stable isotopes values of prey items will allow Mixing Models analysis and new estimates of their diet in two time periods (liver vs muscle samples). The DNA metabarcoding of stomach fluids will increase our samples size (especially from sevengill sharks) and the number of identified prey items from the stomach already analysed in this thesis, resulting in a new and complementary estimate of these shark species diets (Dunn et al., 2010; Amundsen & Sánchez-Hernández, 2019). Despite attempts to deploy five pop-up satellite archival tags (or PSATs) on grey nurse sharks and produce a preliminary description of their habitat use, this could not be achieved due to the COVID-19 pandemic and persistent unfavourable weather conditions. The use of transmitters to study the habitat use of grey nurse sharks is of great importance, as to date, nothing is known about the movement patterns and habitat use of this species outside of the fishing season. In the region, it has been proposed that individuals move to deeper waters until the next fishing season (Lucifora et al., 2002).

The results obtained in this thesis provide the basis for testing our hypotheses for a broader ecological context, assessing their appropriateness with other predator taxa and in different ecosystems.

In Chapter 2, I proposed that the degree of individual specialisation is influenced by the foraging strategy of the predator. Species that prey on temporal dynamic and structural homogenous environments with high abundance of prey (*e.g.*, pelagic) would comprise specialist populations of generalist individuals. In contrast, species preying on temporal stable and structural heterogeneous environments with diverse but less abundant prey (*e.g.*, coastal-benthic) would be composed of generalist populations of specialist individuals. Despite this hypothesis is also true for other marine predator species populations with different foraging strategies, such as pinnipeds (*e.g.*, Páez-Rosas et al., 2014, Baylis et al., 2015), cetaceans (*e.g.*, Aurioles-Gamboa et al., 2013) seabirds (*e.g.,* Polito et al. 2015) and fishes (*e.g.,* Quevedo et al., 2009). It would be interesting to test this hypothesis more rigorously in a global meta-analysis, including different marine predator taxa and ecosystems. I believe the most relevant discovery of this thesis is the demystification of the commonly held assumption that trophic niches have stability across populations of the same species. The results from Chapter 3 showed that different populations located in contrasting environments can have complete inversion in their niche metrics and therefore of populations' ecological role (generalist to specialist populations and vice versa). The extrapolation of data based on the study of a single population to another populations of the same species is commonly used. Consequently, it is necessary to test the niche plasticity of the study species before extrapolating data, especially when dealing with populations located in contrasting environments. I recommend, for future analyses, to evaluate the degree of niche plasticity in other species with a wide geographic range, especially in those that cover different environments. In turn, a more detailed study of the variation in the trophic niche metrics of these species would help us to identify the driver for niche plasticity. Previous isotopic studies on populations of both species throughout their distribution would facilitate this.

Finally, regarding the findings in Chapter 4, I recommend future evaluations to assess to what extent it is possible to generalise these results. An evaluation of the influences of different teeth morphologies on prey selection and trophic niches of predators will help to delineate general patterns in marine predators explaining how these inherent traits, constrain or enhance prey selectivity and shape their trophic niches.

5.5. References

Amundsen, P. A., & Sánchez-Hernández, J. (2019). Feeding studies take guts – critical review and recommendations of methods for stomach contents analysis in fish. *Journal of Fish Biology*, *95*(6), 1364–1373. https://doi.org/10.1111/jfb.14151

Aurioles-Gamboa, D., Rodríguez-Pérez, M. Y., Sánchez-Velasco, L., & Lavín, M. F. (2013). Habitat, trophic level, and residence of marine mammals in the Gulf of California assessed by stable isotope analysis. *Marine Ecology Progress Series*, *488*, 275–290. https://doi.org/10.3354/meps10369

Baylis, A. M. M., Orben, R. A., Arnould, J. P. Y., Peters, K., Knox, T., Costa, D. P., & Staniland, I. J. (2015). Diving deeper into individual foraging specializations of a large marine predator, the southern sea lion. *Oecologia*, *179*(4), 1053–1065. https://doi.org/10.1007/s00442-015-3421-4

Bronselaer, B., & Zanna, L. (2020). Heat and carbon coupling reveals ocean warming due to circulation changes. *Nature*, *584*(7820), 227–233. https://doi.org/10.1038/s41586-020-2573-5

Costa-Pereira, R., Araújo, M. S., Souza, F. L., & Ingram, T. (2019). Competition and resource breadth shape niche variation and overlap in multiple trophic dimensions. *Proceedings of the Royal Society B: Biological Sciences*, *286*, 20190369. https://doi.org/10.1098/rspb.2019.0369

Drago, M., Cardona, L., Franco-Trecu, V., Crespo, A., Vales, D., Borella, F., Zenteno, L., Gonzáles, E. M., & Inchausti, P. (2017). Isotopic niche partitioning between two apex predators over time. *ARPN Journal of Engineering and Applied Sciences*, *86*(4), 766–780. https://doi.org/10.1111/1365-2656.12666

Dunn, M. R., Szabo, A., McVeagh, M. S., & Smith, P. J. (2010). The diet of deepwater sharks and the benefits of using DNA identification of prey. *Deep-Sea Research Part I: Oceanographic Research Papers*, *57*(7), 923–930. https://doi.org/10.1016/j.dsr.2010.02.006

Finucci, B., Barnett, A., Cheok, J., Cotton, C. F., Kulka, D. W., Neat, F. C., Pacoureau, N., Rigby, C. L., Tanaka, S. & Walker, T. I. (2020). *Notorynchus cepedianus*. The IUCN Red List of Threatened Species 2020: e.T39324A2896914. https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T39324A2896914.en

Grandi, M. F., Vales, D. G., Crespo, E. A., & Loizaga, R. (2021). Variation in trophic resources in female South American sea lions at a small geographic scale. *Marine Mammal Science*, *37*(1), 314–327. https://doi.org/10.1111/mms.12746

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., Bruno, J. F., Casey, K. S., Ebert, C., Fox, H. E., Fujita, R., Heinemann, D., Lenihan, H. S., Madin, E. M. P., Perry, M. T., Selig, E. R., Spalding, M., Steneck, R., & Watson, R. (2008). A global map of human impact on marine ecosystems. *Science*, *319*, 948–952. https://doi.org/10.1111/2041-210X.12109

Hazen, E. L., Abrahms, B., Brodie, S., Carroll, G., Jacox, M. G., Savoca, M. S., Scales, K. L., Sydeman, W. J., & Bograd, S. J. (2019). Marine top predators as climate and ecosystem sentinels. *Frontiers in Ecology and the Environment*, *17*(10), 565–574. https://doi.org/10.1002/fee.2125

Heithaus, M. R., Frid, A., Wirsing, A. J., & Worm, B. (2008). Predicting ecological consequences of marine top predator declines. *Trends in Ecology and Evolution*, *23*(4), 202–210. https://doi.org/10.1016/j.tree.2008.01.003

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. *Science*, *328*, 1523–1529.

Horn, M. H., & Ferry-Graham, L. A. (2006). Feeding mechanisms and trophic interactions. In L.G. Allen, D. J. Pondella, & M. H. Horn (Eds.), *The Ecology of Marine Fishes: California and Adjacent Waters* (First edition, pp. 387–410). University of California Press. https://doi.org/10.1525/california/9780520246539.003.0014

Kernaléguen, L., Arnould, J. P. Y., Guinet, C., Cazelles, B., Richard, P., & Cherel, Y. (2016). Early-life sexual segregation: Ontogeny of isotopic niche differentiation in the Antarctic fur seal. *Scientific Reports*, *6*(August), 1–10. https://doi.org/10.1038/srep33211

Koen-Alonso, M., Crespo, E. A., Pedraza, S. N., García, N. A., & Coscarella, M. A. (2000). Food habits of the South American sea lion, Otaria flavescens, off Patagonia, Argentina. *Fishery Bulletin*, *98*(2), 250–263.

Lucifora, L. O., Menni, R. C., & Escalante, A. H. (2005). Reproduction, abundance and feeding habits of the broadnose sevengill shark *Notorynchus cepedianus* in north Patagonia, Argentina. *Marine Ecology Progress Series*, *289*, 237–244. https://doi.org/10.3354/meps289237

Lucifora, L. O., García, V. B., & Escalante, A. H. (2009). How can the feeding habits of the sand tiger shark influence the success of conservation programs? *Animal Conservation*, *12*(4), 291–301. https://doi.org/10.1111/j.1469-1795.2009.00247.x

Maxwell, S. M., Hazen, E. L., Bograd, S. J., Halpern, B. S., Breed, G. A., Nickel, B., Teutschel, N. M., Crowder, L. B., Benson, S., Dutton, P. H., Bailey, H., Kappes, M. A., Kuhn, C. E., Weise, M. J., Mate, B., Shaffer, S. A., Hassrick, J. L., Henry, R. W., Irvine, L., McDonald, B. I., Robinson, P. W., Block, B. A., & Costa, D. P. (2013). Cumulative human impacts on marine predators. *Nature Communications*, *4*, 1–9. https://doi.org/10.1038/ncomms3688

Nelms, S., Alfaro-Shigueto, J., Arnould, J., Avila, I., Bengtson Nash, S., Campbell, E., Carter, M., Collins, T., Currey, R., Domit, C., Franco-Trecu, V., Fuentes, M., Gilman, E., Harcourt, R., Hines, E., Hoelzel, A., Hooker, S., Johnston, D., Kelkar, N., Kiszka, J. J., Laidre, K. L., Mangel, J. C., Marsh, H., Maxwell, S. M., Onoufriou, A. B., Palacios, D. M., Pierce, G. J., Ponnampalam, L. S., Porter, L. J., Russell, D. J. F., Stockin, K. A., Sutaria, D., Wambiji, N., Weir, C. R., Wilson, B., & Godley, B. (2021). Marine mammal conservation: over the horizon. *Endangered Species Research*, *44*, 291–325. https://doi.org/10.3354/esr01115

Páez-Rosas, D., Rodríguez-Pérez, M., & Riofrío-Lazo, M. (2014). Competition influence in the segregation of the trophic niche of otariids: A case study using isotopic bayesian mixing models in Galapagos pinnipeds. *Rapid Communications in Mass Spectrometry*, *28*, 2550–2558. https://doi.org/10.1002/rcm.7047

Polito, M. J., Trivelpiece, W. Z., Patterson, W. P., Karnovsky, N. J., Reiss, C. S., & Emslie, S. D. (2015). Contrasting specialist and generalist patterns facilitate foraging niche partitioning in sympatric populations of Pygoscelis penguins. *Marine Ecology Progress Series*, *519*, 221–237. https://doi.org/10.3354/meps11095

Praderi, R. (1985). Relaciones entre *Pontoporia blainvillei* (Mammalia: Cetacea) y tiburones (Selachii) de aguas Uruguayas. *Comunicaciones Zoológicas Del Museo de Historia Natural de Montevideo*, *7*(151), 19.

Quevedo, M., Svanbäck, R., & Eklöav, P. (2009). Intrapopulation niche partitioning in a generalist predator limits food web connectivity. *Ecology*, *90*(8), 2263–2274. https://doi.org/10.1890/07-1580.1

Rigby, C. L., Carlson, J., Derrick, D., Dicken, M., Pacoureau, N., & Simpfendorfer, C. (2021). *Carcharias taurus*. The IUCN Red List of Threatened Species 2021: e.T3854A2876505. https://dx.doi.org/10.2305/IUCN.UK.2021-2.RLTS.T3854A2876505.en

Svanbäck, R., & Bolnick, D. I. (2005). Intraspecific competition affects the strength of individual specialization: An optimal diet theory method. *Evolutionary Ecology Research*, *7*, 993–1012.

Svanbäck, R., & Bolnick, D. I. (2007). Intraspecific competition drives increased resource use diversity within a natural population. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1611), 839–844. https://doi.org/10.1098/rspb.2006.0198

Szteren, D., Aurioles-Gamboa, D., Labrada-Martagón, V., Hernández-Camacho, C. J., & De María, M. (2018). Historical age-class diet changes in South American fur seals and sea lions in Uruguay. *Marine Biology*, *165*(59), 17. https://doi.org/10.1007/s00227-018-3315-1

Vales, D. G., Cardona, L., García, N. A., Zenteno, L., & Crespo, E. A. (2015). Ontogenetic dietary changes in male South American fur seals *Arctocephalus australis* in Patagonia. *Marine Ecology Progress Series*, *525*, 245–260. https://doi.org/10.3354/meps11214

Vales, D. G., Cardona, L., Loizaga, R., García, N. A., & Crespo, E. A. (2020). Long-Term Stability in the Trophic Ecology of a Pelagic Forager Living in a Changing Marine Ecosystem. *Frontiers in Marine Science*, *7*(February), 1–10. https://doi.org/10.3389/fmars.2020.00087

Chapter Six – Appendix

Lipid extraction has tissue-dependent effects on isotopic values ($\delta^{34}S$, $\delta^{13}C$, $\delta^{15}N$) from different marine predators

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Riverón, S., Raoult, V., Slip, D. J., & Harcourt, R. G. (2022). Lipid extraction has tissue-dependent effects on isotopic values ($\delta^{34}S$, $\delta^{13}C$, $\delta^{15}N$) from different marine predators. Rapid Communications in Mass Spectrometry. https://doi.org/10.1002/rcm.9346

6.1. ABSTRACT

RATIONALE: The use of sulfur isotopes to study trophic ecology in marine ecosystems has increased in the last decade. Unlike other commonly used isotopes (*e.g.*, carbon), sulfur can better discriminate benthic and pelagic productivity. However, how lipid extraction affects sulfur isotopic values has not been assessed, despite its frequent use to remove lipid effects on $\delta^{13}C$ values. **METHODS**: We used white muscle and liver samples from two species of sharks and skin samples from two species of pinnipeds (sea lion and fur seal) to assess the effects of lipid extraction on stable isotope values for $\delta^{34}S$, $\delta^{13}C$, $\delta^{15}N$. Isotopic values were determined by a continuous flow-isotope ratio mass spectrometer coupled to an elemental analyser (EA-IRMS). **RESULTS:** Lipid extraction significantly decreased $\delta^{34}S$ values in shark tissues, more so for liver than muscle (-4.6 ± 0.9 vs -0.8 ± 0.3‰, average change), with nearly no alteration to their standard deviations. Lipid extraction did not affect $\delta^{34}S$ values from pinniped skin samples (0.2 ± 0.8‰, average change). After lipid extraction, consistent increases in $\delta^{13}C$ values (0.2–7.3‰) were detected as expected, especially in tissue with high lipid content (C:N > 4). After lipid extraction, significant increases in $\delta^{15}N$ values (0.5–1.4‰) were found in shark muscle and liver tissues. For pinniped skin samples, $\delta^{15}N$ values were not significantly lower after lipid extraction (-0.4 – -0.1‰). **CONCLUSIONS:** Lipid extraction did not have a strong impact on $\delta^{34}S$ values of shark muscle and pinniped skin (≤1‰). However, our results suggest it is essential to consider the effects of lipid extractions when interpreting results from $\delta^{34}S$ values of shark liver tissue, as they significantly depleted values relative to bulk tissue (~5‰). This may reflect selective removal of sulpholipids and glutathione present in higher concentrations in the liver than in muscle and skin and requires further investigation.

Keywords: *Otaria byronia*, *Arctocephalus australis*, *Carcharias taurus*, *Notorynchus cepedianus*, stable isotope analysis

6.2. INTRODUCTION

Stable isotope analysis is one of the most widely used techniques for understanding spatial and trophic relationships in both ancient and contemporary ecosystems (Koch, 2008; Boecklen et al., 2011; Glibert et al., 2019). Trophic studies of aquatic ecosystems often use this approach due to the elusive nature of many aquatic organisms and the biases arising from traditional methods (*e.g.*, direct observation, stomach content analysis) (Kelly, 2000; Boecklen et al., 2011). Carbon and nitrogen are the most commonly used elements in stable isotope analysis since these are among the most abundant elements across all biological materials (Seyboth, Botta & Secchi, 2018), and their role in biological cycles and environmental gradients are well known (Peterson & Fry, 1987; Ohkouchi et al., 2015; Glibert et al., 2019). The use of different elements allows us to understand aspects of consumer ecology. Stable isotope ratio of nitrogen ($\delta^{15}N$) is a proxy of the trophic level of an organism, and $\delta^{13}C$ broadly indicates habitat use by identifying the source of primary energy (coastal vs oceanic, benthic vs pelagic) (Post, 2002; Shipley & Matich, 2020). With improvements and refinements in instrument sensitivity, other elements (*e.g.*, sulfur, oxygen, hydrogen) have become incorporated into stable isotope studies (Rossman et al., 2016), providing complementary or novel information on food webs and animal movements (*e.g.*, Hobson, 1999; Vander Zanden et al., 2016; Connan et al., 2019). The integration of a third element can improve ecological assessments by providing higher levels of resolution and greater discrimination power between different components of a trophic network (Connolly et al., 2004; Ramos & González-Solís, 2012; Rossman et al., 2016; Raoult et al., 2019). For example, the combined use of stable isotope ratios of sulfur ($\delta^{34}S$) and $\delta^{13}C$ has higher resolution capability for the identification of primary producers in an environment than carbon and nitrogen alone (Connolly et al., 2004), even in estuarine and coastal environments with several potential sources of organic matter (Peterson et al., 1985; Connolly et al., 2004; Niella et al., 2021).

Stable isotope ratios of sulfur have become increasingly important in trophic studies since the 2000s (Connolly & Schlacher, 2013; Nehlich, 2015). This expansion is a consequence of the recent advances in mass spectrometry that have simplified this complex analytical technique allowing it to be performed relatively routinely, reducing both cost and the total amount of sample required for measurement (Richards et al., 2003; Connolly & Schlacher, 2013). As sulfur has low trophic fractionation between consumers and their diet (~ 1.0‰) (Peterson et al., 1985; Nehlich, 2015; Pinzone et al., 2017), $\delta^{34}S$ allows a researcher to identify the contribution of assimilated prey with sulfur originating from different sources of primary production (benthic vs pelagic, terrestrial vs marine) (Hobson, 1999; Shipley et al., 2017a). Consequently, $\delta^{34}S$ has helped reveal complex trophic networks where carbon has failed to identify some of the trophic chain elements (Hesslein et al., 1991; Connolly et al., 2004) and provide higher resolution in ecological niche occupancy (Niella et al., 2021). In addition, $\delta^{34}S$ can improve diet quantification estimates from mixing models, with smaller confidence intervals around mean estimates of consumers' potential prey groups (Connolly et al., 2004). Although the use of sulfur in trophic ecology studies is increasing, there remain some methodological considerations *e.g.*, the effect of lipid extraction; that need to be undertaken to ensure repeatability and comparability between samples and studies.

Variation in lipid content between tissues and organisms can affect stable isotopic values and lead to misleading results and, therefore potentially erroneous ecological interpretations (Post et al., 2007; Logan et al., 2008; Boecklen et al., 2011). Lipids are approximately 6-8‰ depleted in ^{13}C relative to pure protein (DeNiro & Epstein, 1977); hence analyses of tissues with higher lipid content lead to lower $\delta^{13}C$ values. Chemical lipid extraction prior to undertaking $\delta^{13}C$ measurements avoids this problem and has been recommended for standardizing $\delta^{13}C$ for samples with high lipid content or when comparing across taxonomic groups that may have different lipid tissue content (Boecklen et al., 2011; Hussey et al., 2012). However, lipid extraction can also alter $\delta^{15}N$ by washing out nitrogenous compounds, causing an increase in the $\delta^{15}N$ values relative to non-extracted replicates (Pinnegar & Polunin, 1999; Sotiropoulos et al., 2004; Boecklen et al., 2011). Analysing $\delta^{13}C$ in samples with lipid extraction and $\delta^{15}N$ in non-lipid-extracted replicates overcomes this issue, but increases the costs, time of processing, amount of sample needed and use of hazardous chemicals such as chloroform and methanol (Radin, 1981; Sotiropoulos et al., 2004). A second option is to use mathematical corrections for isotopic ratios that take into account the effects of lipid extraction on the isotopic values of the different elements (*e.g.*, Sweeting et al., 2006; Li et al., 2016; Cloyed et al., 2020). However, knowledge of how lipid extraction affects the isotopic values for different tissue types and species is required to generate these mathematical corrections. Sulfur is an essential compound, primarily found in proteins with cysteine and methionine amino acids and sulpholipids (Farooqui & Horrocks, 1985; Brosnan & Brosnan, 2006; Koch, 2008). Therefore, the presence of sulfur in lipids could lead to biased sulfur isotopic values in samples with high lipid content, as outlined above with carbon. However, despite the increasing use of sulfur in ecological studies (Connolly & Schlacher, 2013; Nehlich, 2015), the potential influence of lipid extraction on sulfur isotopic values has only been tested on eggs of a few species of seabirds (Oppel et al., 2010; Elliott et al., 2014). Compared to the replicates without lipid extraction, the extracted egg yolks showed significant differences in $\delta^{34}S$ values with variable magnitude (-0.1 to 2.3‰) depending on the species analysed. Despite this evidence, the effect of lipid extraction on commonly used tissues (*e.g.*, muscle, liver) of non-avian marine predators has not been tested.

In this study, we compared the effects of two treatments, non-lipid extracted vs lipid extracted, on three different commonly used tissues of four marine predator species: muscle and liver of two species of sharks (*Carcharias taurus* and *Notorynchus cepedianus*), and skin samples from two species of pinniped (*Arctocephalus australis* and *Otaria byronia*). We assessed (1) the effect of lipid extraction on δ^{34}S, δ^{13}C and δ^{15}N values, and compared this with previously published information, and (2) provide correction factors for these species and tissues where required.

6.3. EXPERIMENTAL

Sample collection

Four species of coastal marine predators that occur off the Atlantic coast of Uruguay were included in this study. In January 2020, we collected muscle and liver samples from two shark species targeted by local artisanal fisheries: grey nurse shark (*Carcharias taurus*, *n* = 15) and sevengill shark (*Notorynchus cepedianus, n* = 15). Total length (TL) varied between 133.5–259 cm in grey nurse and 157–239 cm in sevengill sharks. Shark samples were collected in collaboration with the artisanal and recreational fisheries monitoring programmes of the National Directorate of Aquatic Resources (DINARA, by its acronym in Spanish). Between 2018 and 2020, we collected skin samples from fresh, stranded carcasses of two species of pinniped, the South American fur seal (*Arctocephalus australis, n* = 15, TL = 70–175 cm) and the South American sea lion (*Otaria byronia, n* = 16, TL = 105–255 cm). All samples were stored frozen at -

20°C until processed in the laboratory. Fieldwork and sample collection were conducted under the permit 252/2018 issued by DINARA.

Sample treatment

Skin samples from pinnipeds were dissected from hair and blubber using tweezers, keeping the epidermis and dermis layers for analysis. All samples (skin, muscle and liver) were rinsed with de-ionized water to eliminate any residue that could affect the isotopic signal and oven-dried at 60°C for 72 hours. Dried samples were ground to a fine powder using an IKA® A11 Basic Analytical Mill (IKA-Werke GmbH & Co. KG, Staufen, Germany) for liver and muscle samples, and a Retsch MM200 ball mill (Retsch GmbH, Haan, Germany) for skin samples. Each sample was split into two subsamples for different treatments, one for analysis without lipid extraction (referred to as bulk samples), the other for analysis after lipid extraction (referred to as Lipid Extracted samples, LE).

Lipid extraction was conducted using a 2:1 chloroform-methanol solution adapted from Folch et al. (1957) of approximately 1 gram of tissue. Shark liver typically has a high lipid content (~50% in grey nurse and sevengill sharks) (Pethybridge, 2010; Davidson & Cliff, 2011). Therefore, the process was repeated until the supernatant liquids were clear, indicating that lipids have been successfully removed (Medeiros et al., 2015). The samples were dried for 48 hours or until the solvent completely evaporated to remove the remaining solvent. The retention of urea and Trimethylamine N-Oxide (TMAO) in the tissues of elasmobranchs allows them to sustain osmotic balance and may influence the stable isotope values of δ^{15}N and δ^{13}C, leading to misleading interpretation of the data (Pethybridge, 2010; Li et al., 2016). We conducted urea extraction in shark tissues to study the effect of lipid extraction avoiding any biases produced by urea and TMAO, making our results comparable among species. All shark samples (including bulk and LE samples) were urea extracted following an adaptation of Kim and Koch (2012) protocol. Each sample was rinsed with 5ml of deionized water, allowing a reaction time of 10-min and vortexed for 1 min. Samples were centrifuged, and the supernatant was discarded. This procedure was repeated three consecutive times. Samples were oven-dried for 24 hours at 60°C or until the sample was dried (usually no more than 48 hours). Finally, dried samples were weighed into 2–2.5mg pellets and placed into tin containers and sent for analysis.

Stable isotope values of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ of pinniped samples were determined using a continuous flow-isotope ratio mass spectrometer Hydra 20-22 (Sercon Ltd., Cheshire, UK) coupled with Europa EA-GSL Elemental analyser (Europa Scientific Inc., Cincinnati OH), at Griffith University Stable Isotope Laboratory, Queensland, Australia. Stable isotope ratios were measured in part per mille (‰) deviation from international standards (for ^{15}N: IAEA-N1 and IAEA-N2, for ^{13}C: IAEA-CH-6, and for ^{34}S: IAEA-S1, IAEA-S2, IAEA-S3). The Standard deviation (SD) for measurements of known standards (bovine liver, Glycine NBS127, Glycine LSU 1 Delta, Hi Max, Low Mix) was $\delta^{15}N$ = 0.0–0.1‰, $\delta^{13}C$ = 0.0–0.1‰, and $\delta^{34}S$ = 0.1–0.3‰. Shark samples were analysed at the Stable Isotope Laboratory at the University of Hong Kong, Hong Kong, using a continuous flow-isotope ratio mass spectrometer Thermo Scientific™ EA IsoLink™ IRMS System (Thermo Fisher Scientific Inc., Massachusetts, US). The following international standards were used for data normalisation: USGS-40 and USGS-41a for ^{15}N and ^{13}C; IAEA-S-1 and IAEA-S-2 for ^{34}S. Analytical accuracy was evaluated using the reference material USGS-42 ($\delta^{15}N$ = 0.1–0.3‰, $\delta^{13}C$ = 0.0–0.1‰ and $\delta^{34}S$ = 0.1–0.7‰).

Statistical analyses

Paired *t*-tests were used to test for differences between bulk and lipid-extracted samples. The significance level was set at 0.05. The difference between paired observations was checked for normal distribution before analysis using the Shapiro-Wilks normality test. We used the standard deviation within non-lipid and lipid extracted samples to assess changes in the dispersion of the data sets. The carbon-to-nitrogen (C:N) ratio of bulk tissue was used as a proxy for the lipid content of the tissue (Post et al., 2007; Logan et al., 2008). The general trend is that higher C:N values indicate higher lipid content, with some possible exceptions (see Logan et al., 2008; Fagan et al., 2011). To assess whether the C:N ratio (as a proxy of lipid content) accounts for the differences between $\delta^{13}C$ and $\delta^{34}S$ before and after lipid extraction, we studied these relationships visually and with an adjusted model whenever possible. Final models were inspected for a normal residual distribution. All statistical analyses were performed in R software version 4.1.0 (R Core Team, 2021).

6.4. RESULTS AND DISCUSSION

Lipid extraction in shark tissues led to significant differences in $\delta^{34}S$, $\delta^{13}C$ and $\delta^{15}N$ values. In contrast, pinniped skin samples only showed significant differences in $\delta^{13}C$ values. Shark muscle and liver were the only tissues that showed significant decreases in $\delta^{34}S$ values due to lipid removal. As predicted, tissues with higher lipid content showed the greatest differences in $\delta^{34}S$ and $\delta^{13}C$ values due to lipid extraction.

The mean and standard deviation (SD) of isotopic ratios ($\delta^{34}S$, $\delta^{13}C$ and $\delta^{15}N$), C:N ratios and carbon (%C), nitrogen (%N) and sulfur content (%S) for each species, tissue, and treatment are shown in Table 1. Boxplots for each compound before and after lipid extraction are presented as supporting information (Figure 6.S1−6.S6).

Lipids were successfully removed after lipid extraction, even in shark liver samples with high lipid content (Pethybridge, 2010; Davidson & Cliff, 2011). The C:N ratios for liver from grey nurse sharks decreased from 13.6 ± 2.2 to 3.6 ± 0.2 after lipid extraction, and from 17.8 ± 3.7 to 3.8 ± 0.2 in sevengill sharks. In contrast, shark muscle samples with low lipid content (usually <1%) (*e.g*., Sargent et al., 1973; van Vleet et al., 1984; Pethybridge et al., 2010, 2014), had low variation in C:N ratios after lipid removal, from 2.8 ± 0.1 to 3.2 ± 0.0 in grey nurse sharks, and from 2.7 ± 0.1 to 3.1 ± 0.0 in sevengill sharks. Skin from pinnipeds also had low variation in C:N ratios after lipid extraction (from 3.2 ± 0.3 to 3.0 ± 0.2 in South American sea lions, and from 3.8 ± 0.8 to 3.2 ± 0.1 in south American fur seals), probably due to low lipid content in their skin.

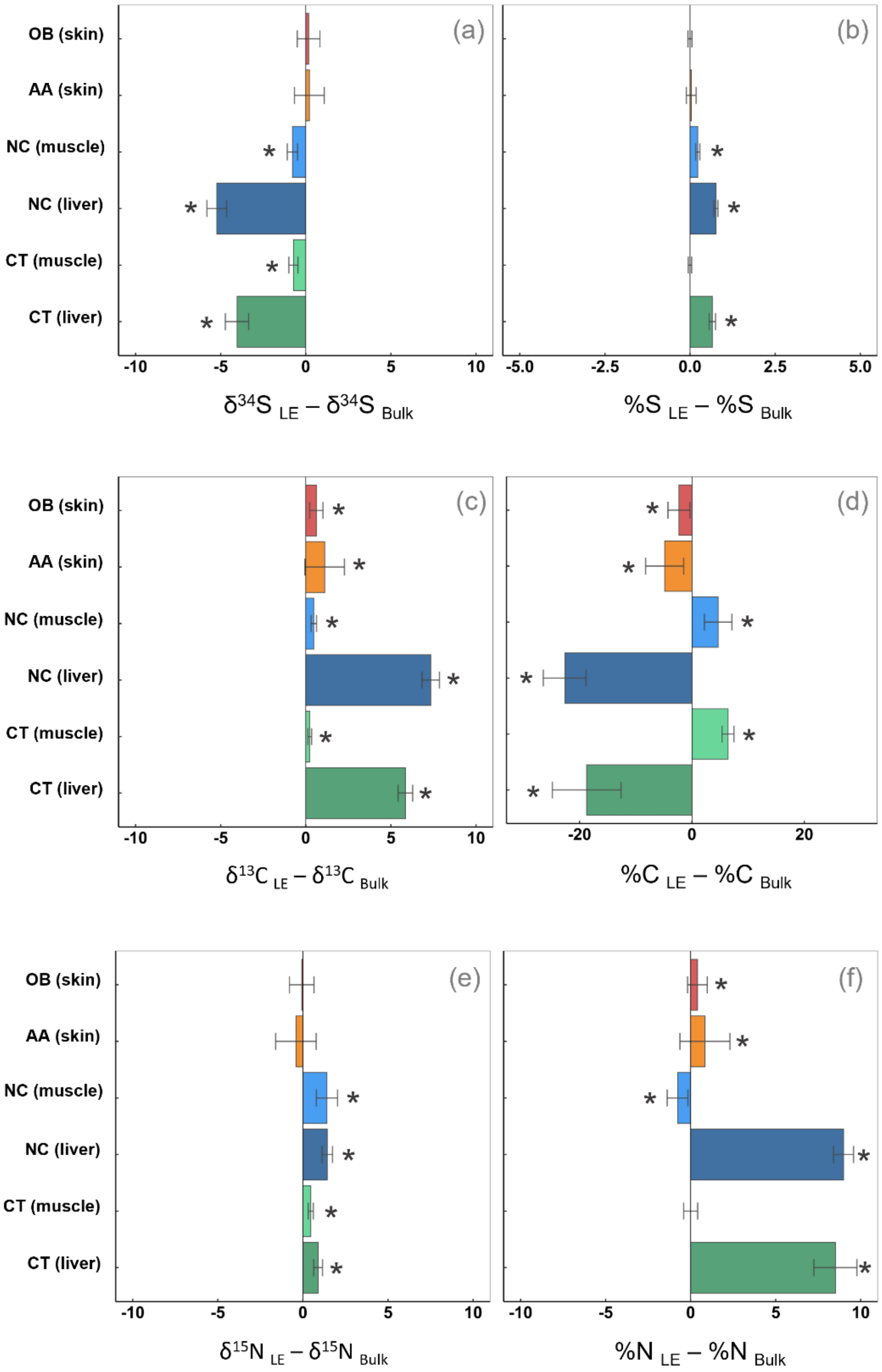

Figure 6.1. The effects of lipid extraction (LE) on isotopic ratios of sulfur ($\delta^{34}S$, a), carbon ($\delta^{13}C$, c) and nitrogen ($\delta^{15}N$, e) and their respective sulfur (%S, b), carbon (%C, d) and nitrogen content (%N, f) in skin samples from pinnipeds (*Otaria byronia* and *Arctocephalus australis*) and muscle and liver samples from sharks (*Carcharias taurus* and *Notorynchus cepedianus*). Positive values denote a higher concentration of the isotopic compound or higher values of isotopic ratios due to lipid extraction. Asterisks (*) indicate significant paired Student's *t*-test ($p < 0.05$). Whiskers represent the standard deviation (SD) of the mean for each parameter. OB: *Otaria byronia*, AA: *Arctocephalus australis*, NC: *Notorynchus cepedianus*, CT: *Carcharias taurus*.

Sulfur

Muscle and liver from shark species showed significant decreases in $\delta^{34}S$ values after lipid extraction (*p*<0.05, Table 6.1, Figure 6.1a). Liver samples of grey nurse shark and sevengill shark had the greatest decrease in $\delta^{34}S$ values (-4.0 ± 0.7‰ and -5.2 ± 0.6‰, respectively), differences that could have an impact on the ecological interpretation of these types of data. For example, $\delta^{34}S$ isotopic gradients in marine ecosystems typically range from ~20‰ (pelagic) to ~1‰ (benthic) (Krouse, 1989; Kharlamenko et al., 2001; Connolly et al., 2004; Koch, 2008). Distinct trophic groups in marine fishes can be differentiated by mean $\delta^{34}S$ values between 2-3% (Gajdzik et al., 2016), a difference smaller than the differences in $\delta^{34}S$ values resulting from lipid extraction. Therefore, care must be taken when comparing data from shark liver samples with different chemical treatments. Especially when the analysis of the liver, given its relatively fast turnover rate (MacNeil et al., 2005), has been critical to ecological studies of sharks revealing novel insights into their trophic ecology (*e.g.*, MacNeil et al., 2005; Matich et al., 2011; Raoult et al., 2019). Although shark muscle samples also showed a significant reduction in $\delta^{34}S$ values, these differences were less than 1‰ in both grey nurse sharks (-0.7 ± 0.3‰) and sevengill sharks (-0.8 ± 0.3‰), approximating the analytical accuracy of the instruments. Lipid extraction caused a significant but small increase in relative sulfur content in grey nurse shark liver (0.7 ± 0.1%) and muscle and liver samples of sevengill shark (0.2 ± 0.1% and 0.8 ± 0.1%, respectively). Previous works on tissue with high lipid content (egg yolk) of four species of seabirds (Oppel et al., 2010; Elliott et al., 2014) showed significant effects on $\delta^{34}S$ values after lipid removal, with variations in magnitude between species, ranging from -0.1 ± 0.9‰ to 2.3 ± 1.1‰. Oppel et al. (2010) suggested that the altered $\delta^{34}S$ values could result from an incidental loss of sulfur-bearing amino acids in proteins associated with polar structural lipids or sulpholipids. A decrease in $\delta^{34}S$ values after lipid extraction occurred in conjunction with an increase in sulfur content in both shark tissue types (Table 6.1, Figure 6.1a,b). The rise in sulfur percentage suggests that lipids found in liver tissue are sulfur-poor, and by extracting lipids 33−57% (Davidson & Cliff, 2011) and 48% (Pethybridge, 2010), in grey nurse sharks and sevengill sharks, respectively), the relative proportion of sulfur increases in the extracted replicate. In contrast, pinniped skin samples showed no significant differences in $\delta^{34}S$ and sulfur content values between bulk and LE samples (Table 6.1, Figure 6.1a,b). Lipid extraction did not cause significant changes in skin $\delta^{34}S$ values in either pinniped species (South American sea lion: $p = 0.34$, South American fur seal: $p = 0.37$). Variation in sulfur content after lipid extraction was negligible for both pinniped species (Table 6.1, Figure 6.1b).

The decrease in sulfur isotopic values in muscle and liver could be due to the removal of ^{34}S-enriched sulfur-containing lipids (sulpholipids) during lipid extraction. Sulpholipids have been reported in the liver and muscle of different terrestrial mammals (dogs, rabbits and humans) (Goldberg, 1961). Differences in the magnitude of the decrease between tissues could be explained by differences in the mean bulk C:N ratio (a proxy of lipid content). Sharks are characterised by high lipid content in the liver (Davidson & Cliff, 2011), which stores energy and provides buoyancy control, compared to muscle tissue in which lipid levels can be relatively low (Hussey et al., 2010; Hussey et al., 2012). Therefore, we expected that higher C:N values would be related to higher differences in $\delta^{34}S$ due to lipid extraction. However, we found the relationship between bulk C:N ratio and $\Delta\delta^{34}S = (\delta^{34}S_{LE} - \delta^{34}S_{Bulk})$ was unclear, suggesting that the lipid content alone does not explain the magnitude of decrease in $\delta^{34}S$ after lipid extraction (Figure 6.2b). This may be explained by the very small proportion of sulfolipids compared to total lipids (Kapoulas & Miniadis-Meimaroglou, 1985; Sikorski, 1990). Moreover, our results suggest that lipid extraction will affect $\delta^{34}S$ values differentially depending on the tissue and the species analysed. These results support previous studies conducted on seabird egg tissue (Elliott et al., 2014).

The decline of $\delta^{34}S$ values after lipid extraction may also be associated with the selective removal of another S-containing compound. Glutathione (GSH, $C_{10}H_{17}N_3O_6S$) is an antioxidant derived from the free amino acid cysteine, one of the few sulfur-bearing amino acids (Tcherkez & Tea, 2013). This molecule is synthesized mainly in the liver where it is found in higher concentrations than the rest of the body (Lu, 2013). Results from several terrestrial mammal studies showed that GSH is present at high levels in the liver, while muscle has lower reserves (Ingenbleek, 2006). GSH can be obtained directly by diet, although its origin is mainly endogenous and its main precursor, cysteine, is derived from the breakdown of dietary protein (Rakel, 2012; Lu, 2013). However, we found no information on its fractionation relative to dietary sulfur when it is synthesized in the body. Finally, GSH is extracted effectively with methanol (Muryanto et al., 2017), the same solvent discarded during our lipid extraction protocol. If the distribution of GSH is the same in shark muscle and liver as in terrestrial mammals, this may explain the differential decrease in sulfur between the two tissues. The removal of GSH by lipid extraction further explains the increase in %S as $\delta^{34}S$ decreases. Because of the elemental composition of GSH, the removal of GSH from the sample leads to an increase in %S relative to the total sample. This is because it loses 10 times more carbon and 3 times more nitrogen than sulfur. In turn, this means that the decrease in %C of lipid-extracted shark liver samples through chloroform-methanol protocols is caused not only by lipid removal but probably also by GSH removal. In addition, $\delta^{34}S$ values in the non-lipid extracted liver of grey nurse sharks (22.1 ± 0.6‰) and sevengill sharks (23.1 ± 0.7‰) exceeded the typical values of a fully pelagic consumer (~20‰, Koch (2008)). Given that these species do not feed exclusively on pelagic prey, their values should be in accordance with benthic and demersal diets (Lucifora et al., 2005; 2009). Therefore, this suggests that $\delta^{34}S$ values from lipid extracted liver samples may not exclusively reflect the diet of the consumer. Unfortunately, we could not find information to confirm that GSH is enriched in ^{34}S. Future work will be necessary to confirm our hypothesis. Studies that provide information on the biochemical composition of the extracts obtained by lipid extraction will allow us to begin to elucidate which compounds are being removed through lipid extraction.

Carbon and C:N ratios

Lipid extraction led to increases in δ^{13}C values and carbon content for all species and tissue types ($p < 0.05$) (Table 6.1, Figure 6.1c,d). Lipid extraction resulted in an increase in δ^{13}C values for all species. The smaller increases were in pinniped skin samples (0.6 − 1.1‰ mean values) and shark muscle samples (0.2 − 0.5‰) (Table 6.1, Figure 6.1c). Previously reported differences between lipid extracted and non-lipid extracted replicates in muscle δ^{13}C values in sevengill sharks (0.7 ± 0.6‰ (de Necker, 2017), align with our results (0.5 ± 0.2‰). The same is true for values previously reported for the grey nurse shark (~0.4‰ Hussey *et al.*, (2010) cf. 0.2 ± 0.1‰ – this study). Shark liver samples showed the highest increase in δ^{13}C values (5.9 − 7.3‰) (Table 6.1, Figure 6.1c). This is consistent with reports of extracts from the livers of marine mammals (*e.g.*, Ryan et al., 2013; Clark et al., 2019; Groß et al., 2021) and sharks (*e.g.*, Li et al., 2016). Lipid extraction resulted in a significant decrease (*t*-test, $p < 0.05$) in the amount of carbon detected in skin and liver samples, with the lowest reduction shown in pinniped skin samples (-2.3 − -4.9%) and the highest in shark liver samples (-18.7 – -22.7%) (Table 6.1, Figure 6.1d). However, shark muscle samples showed the opposite trend, and lipid extracted samples increased their amount of carbon (*t*-test, $p < 0.05$) between 4.7 and 6.4% compared to their non-lipid extracted replicates (Table 6.1, Figure 6.1d).

Table 6.1. Mean values and standard deviation (SD) of δ^{34}S, δ^{15}N and δ^{13}C (‰); sulfur, carbon and nitrogen content (%S, %C, %N) and C:N ratios for bulk (no-lipid extracted) skin samples from *Otaria byronia* and *Arctocephalus australis*, and for muscle and liver samples from *Notorynchus cepedianus* and *Carcharias taurus*. Differences between stable isotope values of Lipid Extracted (LE) and Bulk samples are presented as Δ (LE - Bulk) (‰). Results of paired *t*-test (*p*-value and *t*-value) comparing LE and Bulk samples of stable isotope ratio values (δ^{34}S, δ^{15}N and δ^{13}C) and isotopic content, are presented for all tissue types and species. Significance level based on α = 0.05. *n*: sample size.

Species	Tissue	*n*	Parameter	Mean ± SD	Δ (LE - Bulk)	Paired *t*-test	*t*-value
Otaria byronia	skin	16	C:N	3.2 ± 0.3	-0.3 ± 0.2	< 0.05	-4.0
			δ^{34}S	15.8 ± 0.8	0.2 ± 0.7	0.34	1.0
			%S	0.4 ± 0.1	0.0 ± 0.1	0.86	-0.2
			δ^{13}C	-14.6 ± 0.7	0.6 ± 0.4	< 0.05	6.4
			%C	46.6 ± 2.0	-2.3 ± 2.0	< 0.05	-4.7
			δ^{15}N	22.1 ± 0.8	-0.1 ± 0.7	0.70	-0.4
			%N	14.4 ± 0.6	0.4 ± 0.6	< 0.05	2.8
Arctocephalus australis	skin	15	C:N	3.8 ± 0.8	-0.6 ± 0.8	< 0.05	-3.3
			δ^{34}S	16.8 ± 0.6	0.2 ± 0.9	0.37	0.9
			%S	0.5 ± 0.1	0.0 ± 0.1	0.36	1.0
			δ^{13}C	-16.1 ± 1.2	1.1 ± 1.2	< 0.05	3.7
			%C	49.4 ± 3.1	-4.9 ± 3.4	< 0.05	-5.6
			δ^{15}N	20.3 ± 1.1	-0.4 ± 1.2	0.20	-1.3
			%N	13.3 ± 1.5	0.8 ± 1.5	< 0.05	2.2
Notorynchus cepedianus	muscle	15	C:N	2.7 ± 0.1	0.4 ± 0.1	< 0.05	22.6
			δ^{34}S	18.5 ± 0.3	-0.8 ± 0.3	< 0.05	-9.9
			%S	0.8 ± 0.0	0.2 ± 0.1	< 0.05	14.1
			δ^{13}C	-15.6 ± 0.3	0.5 ± 0.2	< 0.05	11.3
			%C	45.0 ± 1.8	4.7 ± 2.5	< 0.05	7.4
			δ^{15}N	19.7 ± 0.7	1.4 ± 0.6	< 0.05	8.7
			%N	16.6 ± 0.5	-0.8 ± 0.6	< 0.05	-4.9
Notorynchus cepedianus	liver	15	C:N	17.8 ± 3.7	-14.0 ± 3.6	< 0.05	-15.2
			δ^{34}S	23.1 ± 0.6	-5.2 ± 0.6	< 0.05	-35.2
			%S	0.3 ± 0.1	0.8 ± 0.1	< 0.05	50.2
			δ^{13}C	-22.3 ± 0.6	7.3 ± 0.5	< 0.05	55.8
			%C	71.9 ± 3.0	-22.7 ± 3.8	< 0.05	-23.1
			δ^{15}N	18.5 ± 0.5	1.4 ± 0.3	< 0.05	18.0
			%N	4.2 ± 0.7	9.0 ± 0.6	< 0.05	58.8
Carcharias taurus	muscle	15	C:N	2.8 ± 0.1	0.4 ± 0.1	< 0.05	24.8
			δ^{34}S	19.0 ± 0.3	-0.7 ± 0.3	< 0.05	-10.6
			%S	1.0 ± 0.1	0.0 ± 0.0	0.90	-0.1
			δ^{13}C	-15.0 ± 0.2	0.2 ± 0.1	< 0.05	7.9
			%C	45.5 ± 1.0	6.4 ± 1.1	< 0.05	23.4
			δ^{15}N	18.9 ± 0.6	0.5 ± 0.1	< 0.05	11.8
			%N	16.4 ± 0.4	0.0 ± 0.4	0.98	0.0
Carcharias taurus	liver	15	C:N	13.6 ± 2.2	-10.0 ± 2.2	< 0.05	-17.6
			δ^{34}S	22.1 ± 0.7	-4.0 ± 0.7	< 0.05	-22.9
			%S	0.4 ± 0.0	0.7 ± 0.1	< 0.05	27.2
			δ^{13}C	-21.0 ± 0.5	5.9 ± 0.4	< 0.05	52.9
			%C	68.0 ± 3.9	-18.7 ± 6.1	< 0.05	-11.9
			δ^{15}N	16.8 ± 0.9	0.9 ± 0.3	< 0.05	13.1
			%N	5.1 ± 0.6	8.5 ± 1.3	< 0.05	25.9

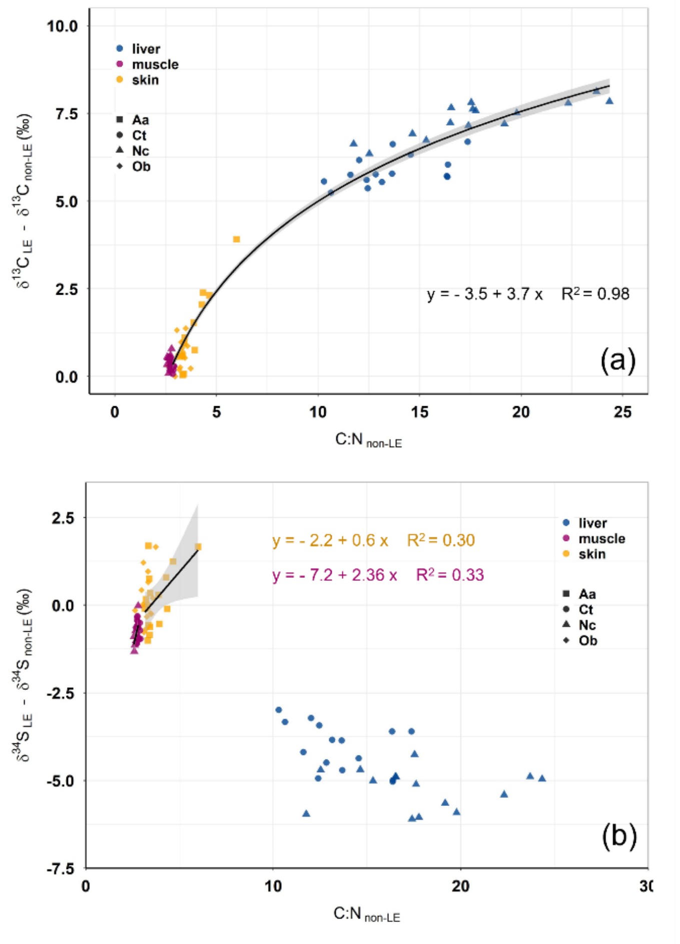

Figure 6.2. Relationship between C:N ratios from non-lipid extracted samples and the observed change in (a) δ^{34}S (δ^{34}S $_{\text{Lipid-Extracted}}$ - δ^{34}S $_{\text{non-Lipid Extracted}}$) and (b) δ^{13}C (δ^{13}C $_{\text{Lipid-Extracted}}$ - δ^{13}C $_{\text{non-Lipid Extracted}}$), due to lipid extraction in each tissue type. Liver and muscle samples from sharks and skin samples from pinnipeds are represented. Solid lines represent the corresponding adjusted linear and logarithmic models that were significant, with shaded areas representing the 95% confidence intervals. Ct (●): *Carcharias taurus*, Nc (▲): *Notorynchus cepedianus*, Aa (■): *Arctocephalus australis*, Ob (◆): *Otaria byronia*).

C:N ratios from bulk tissue showed a non-linear relationship with the observed change in δ^{13}C due to lipid extraction (Figure 6.2a), in accordance with previous work (*e.g.*, Logan et al., 2008; Ryan et al., 2012; Cloyed et al., 2020). After lipid extraction, all tissue types showed significant differences in C:N values (paired *t*-test, $p < 0.05$). Differences between C:N values of lipid extracted, and non-lipid extracted replicates of shark liver samples (tissue known for its high lipid content), decreased between -10.0 ± 2.2‰ (grey nurse sharks) and -14.0 ± 3.6‰ (sevengill sharks) (Table 6.1). For shark muscle samples (tissue known for its low lipid content), a 0.4 ± 0.1‰ difference in C:N values occurred, which is consistent with a mean difference of 0.5 ± 0.1‰ previously found in muscle samples of pelagic shark species (Li et al., 2016). C:N ratios of non-lipid extracted muscle samples for both species of shark had values ~2.7 ± 0.1‰, also consistent with previous studies of sharks, *i.e.* 3.1 ± 0.3‰ in bull sharks, *Carcharhinus leucas* (Matich et al., 2011), and < 3.0‰ in sandbar sharks, *Carcharhinus plumbeus* (Logan & Lutcavage, 2010).

Nitrogen

δ^{15}N values from muscle and liver samples of both species of shark significantly increased following lipid and urea extraction (paired *t*-test, p <0.05) (Table 6.1, Figure 6.1e). However, the magnitude of the increase was relatively small, from 0.5 ± 0.1‰ in muscle samples of grey nurse sharks to 1.4 ± 0.6‰ in muscle samples of sevengill sharks. Significant effects of lipid extraction in δ^{15}N values of muscle samples from sevengill sharks have been reported previously and provided a comparable difference between lipid extracted and non-lipid extracted replicates of 1.51 ± 0.61‰ (de Necker, 2017). Hussey et al. (2010) reported a smaller increase in δ^{15}N values after lipid extraction in muscle samples from grey nurse sharks of ~0.2‰. Significant increases in δ^{15}N values due to lipid extraction in muscle and liver have been found in other elasmobranch species (Logan & Lutcavage, 2010) and several marine and freshwater bony fishes (Sotiropoulos et al., 2004; Murry et al., 2006; Sweeting et al., 2006; Smyntek et al., 2007). However, it has been reported that lipid extraction does not change δ^{15}N values in several species of coastal elasmobranchs (Carlisle et al., 2017; Shipley et al., 2017b). In contrast to shark tissues, we found no significant effect of lipid extraction on δ^{15}N values on skin samples in either species of pinniped (South American sea lion: p = 0.70, South American fur seal: p = 0.20) (Table 6.1). This is consistent with reports of no changes produced on $\delta^{15}N$ by lipid extraction in skin samples from other groups of marine mammals (*i.e.*, cetaceans (Ryan et al., 2012; Cloyed et al., 2020), odobenids (Clark et al., 2019) and Sirenia (Cloyed et al., 2020). We found strong evidence that lipid extraction increased $\delta^{15}N$ values for our sharks but not our pinniped samples, supporting growing evidence that such effects can vary between species and tissue types (Pinnegar & Polunin, 1999; Logan et al., 2008; Yurkowski et al., 2015; Cloyed et al., 2020). Our data showed that we could perform stable isotope analysis of $\delta^{15}N$ and $\delta^{13}C$ on skin samples of South American fur seals and South American sea lions, without the need to separately analyse the elements to avoid biases in $\delta^{15}N$ due to lipid extraction.

Lipid extracted skin samples from pinnipeds were slightly higher in nitrogen, but by less than 1% for both species compared to non-lipid extracted samples (Table 6.1, Figure 6.1f). An increase in %N was found in the LE liver samples, which showed around 9% more nitrogen than the non-lipid extracted replicate. Urea and TMAO extraction led to an increase in %N, as reported in some shark species (Pahl et al., 2021). As urea and TMAO are synthesized in shark liver (Ballantyne, 1997), its presence could lead to a greater increase in %N in this tissue. Variation in nitrogen content between muscle replicates of grey nurse shark was negligible and not significantly different (p = 0.98). Muscle samples from sevengill sharks were the only tissue that showed a significant reduction in its nitrogen content (~ 1%, paired t-test: $p < 0.05$) along with an increase of $\delta^{15}N$ values after lipid extraction (Table 6.1, Figure 6.1f).

There are three possible explanations for the increase in $\delta^{15}N$ values after lipid and urea extraction, from liver samples. The effect of urea and TMAO extraction is the more obvious one, as both compounds are depleted in ^{15}N their removal can lead to an increase in $\delta^{15}N$ values (Carlisle et al., 2017; Pahl et al., 2021). In addition, chloroform and methanol are commonly used solvents in lipid extraction. Sotiropoulos et al. (2004) proposed that cellular proteins attached to polar structural lipids of the cell membrane could lead to the selective loss of amino acids when the structural lipids were removed together with the methanol fraction. The methanol phase retains 1% of total lipids content which could include a fraction with proteins attached (Folch, 1957; Bligh & Dyer, 1959). Since amino acids can differ in isotopic composition (*e.g.*, McClelland & Montoya, 2002; Schmidt et al., 2004), it may be that the amino acid-protein extracted in association with polar lipids are isotopically lighter than specific tissue proteins, thus increasing $\delta^{15}N$ values. Alternatively, the consistent increase in $\delta^{15}N$ values could be the result of removing nitrogenous wastes that resulted from cellular respiration (ammonia: NH_3, ammonium: NH_4^+) soluble in lipids and organic solvents being removed in the lipid extraction (Bearhop et al., 2000). We suggest that neither of these processes on their own explains the increase in $\delta^{15}N$ values, but rather either a combination of them, or an alternative hypothesis. The different patterns of $\delta^{15}N$ and %N values found after lipid and urea extractions support the hypothesis that structural and physiological differences between tissues produce these divergent patterns. The consistency of the effects of lipid extraction in $\delta^{15}N$ values in muscle of grey nurse shark and sevengill shark, between studies support this hypothesis. Our results highlight the urgency of conducting new experimental studies to understand these biochemical processes and how they differ between tissue types.

6.5. CONCLUSIONS AND RECOMMENDATIONS

Our work is the first study to assess the effect of lipid extraction on sulfur isotopic ratios of sharks and pinnipeds. Both species of pinniped showed a negligible increase in skin $\delta^{34}S$ after lipid extraction. Sharks similarly showed negligible changes in $\delta^{34}S$ values from muscle tissues. However, shark liver samples have high levels of enrichment of ^{34}S arising from the lipid and urea extraction treatments. We provide a general correction factor of -4.6 ± 0.9 to adjust for $\delta^{34}S$ values of shark liver samples that undergo lipid and urea extraction. Until the origin of variations in $\delta^{34}S$ values from shark liver samples can be clarified (*i.e.*, removal of sulpholipids and/or glutathione), we suggest caution when interpreting results. The effects of lipid extraction treatments on $\delta^{34}S$ values vary between tissue type and species of marine predators, in a similar fashion to $\delta^{15}N$. Consequently, in future research, if there is no information available on how $\delta^{34}S$ values react to lipid and urea extractions for the tissues of interest, we recommend *a priori* evaluation of the composition of the sample to detect and therefore account for possible effects of lipid and urea extractions. Our findings also demonstrated that stable isotope analysis of $\delta^{15}N$ and $\delta^{13}C$ on skin samples from South American sea lions and South American fur seals could be performed without the requirement to separately analyse elements to minimize lipid extraction-induced biases in $\delta^{15}N$.

6.6. REFERENCES

Ballantyne, J. S. (1997). Jaws: The Inside Story. The Metabolism of Elasmobranch Fishes. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *118*(4), 703–742. https://doi.org/10.1016/S0305-0491(97)00272-1

Bearhop, S., Waldron, S., & Furness, R. W. (2000). Influence of lipid and uric acid on $\delta^{13}C$ and $\delta^{15}N$ values of avian blood: Implications for trophic studies. *The Auk*, *117*(2), 504–507. https://doi.org/10.2307/4089734

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917. https://doi.org/10.1139/o59-099

Boecklen, W. J., Yarnes, C. T., Cook, B. A., & James, A. C. (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution, and Systematics*, *42*, 411–440. https://doi.org/10.1146/annurev-ecolsys-102209-144726

Brosnan, J. T., & Brosnan, M. E. (2006). The sulfur-containing amino acids: An overview. *The Journal of Nutrition*, *136*(6), 16365–16405. https://www.pkdiet.com/pdf/eaa/aminoacidwrksp.pdf

Carlisle, A. B., Litvin, S. Y., Madigan, D. J., Lyons, K., Bigman, J. S., Ibarra, M., & Bizzarro, J. J. (2017). Interactive effects of urea and lipid content confound stable isotope analysis in elasmobranch fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, *74*(3), 419–428. https://doi.org/10.1139/cjfas-2015-0584

Clark, C. T., Horstmann, L., & Misarti, N. (2019). Lipid normalization and stable isotope discrimination in Pacific walrus tissues. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-42095-z

Cloyed, C. S., DaCosta, K. P., Hodanbosi, M. R., & Carmichael, R. H. (2020). The effects of lipid extraction on δ^{13}C and δ^{15}N values and use of lipid-correction models across tissues, taxa and trophic groups. *Methods in Ecology and Evolution*, *11*(6), 751–762. https://doi.org/10.1111/2041-210X.13386

Connan, M., Dilley, B. J., Whitehead, T. O., Davies, D., McQuaid, C. D., & Ryan, P. G. (2019). Multidimensional stable isotope analysis illuminates resource partitioning in a sub-Antarctic island bird community. *Ecography*, *42*, 1948–1959. https://doi.org/10.1111/ecog.04560

Connolly, R. M., Guest, M. A., Melville, A. J., & Oakes, J. M. (2004). Sulfur stable isotopes separate producers in marine food-web analysis. *Oecologia*, *138*(2), 161–167. https://doi.org/10.1007/s00442-003-1415-0

Connolly, R. M., & Schlacher, T. A. (2013). Sample acidification significantly alters stable isotope ratios of sulfur in aquatic plants and animals. *Marine Ecology Progress Series*, *493*, 1–8. https://doi.org/10.3354/meps10560

Davidson, B., & Cliff, G. (2011). Liver lipids of female *Carcharias taurus* (spotted raggedtooth) sharks: A comparison between seasons. *Fish Physiology and Biochemistry*, *37*, 613–618. https://doi.org/10.1007/s10695-010-9463-y

de Necker, L. (2017). The trophic dynamics of the broadnose sevengill shark (*Notorynchus cepedianus*) in False Bay, South Africa, using multiple tissue stable isotope analysis [Master Thesis, University of Cape Town]. OpenUCT repository. https://open.uct.ac.za/handle/11427/25025

DeNiro, M. J., & Epstein, S. (1977). Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science*, *197*(4300), 261–263. https://doi.org/10.1126/science.327543

Elliott, K. H., Davis, M., & Elliott, J. E. (2014). Equations for lipid normalization of carbon stable isotope ratios in aquatic bird eggs. *PLoS ONE*, *9*(1), e83597. https://doi.org/10.1371/journal.pone.0083597

Fagan, K. A., Koops, M. A., Arts, M. T., & Power, M. (2011). Assessing the utility of C:N ratios for predicting lipid content in fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*(2), 374–385. https://doi.org/10.1139/F10-119

Farooqui, A. A., & Horrocks, L. A. (1985). On the role of sulfolipids in mammalian metabolism. *Molecular and Cellular Biochemistry*, *66*, 87–95. https://doi.org/10.1007/BF00231827

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, *226*(1), 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5

Gajdzik, L., Parmentier, E., Sturaro, N., & Frédérich, B. (2016). Trophic specializations of damselfishes are tightly associated with reef habitats and social behaviours. *Marine Biology*, *163*(12). https://doi.org/10.1007/s00227-016-3020-x

Glibert, P. M., Middelburg, J. J., McClelland, J. W., & Jake Vander Zanden, M. (2019). Stable isotope tracers: Enriching our perspectives and questions on sources, fates, rates, and pathways of major elements in aquatic systems. *Limnology and Oceanography*, *64*(3), 950–981. https://doi.org/10.1002/lno.11087

Goldberg, I. H. (1961). The sulfolipids. *Journal of Lipid Research*, *2*(2), 103–109. https://doi.org/10.1016/s0022-2275(20)39019-2

Groß, J., Fry, B., Burford, M. A., & Bengtson Nash, S. (2021). Assessing the effects of lipid extraction and lipid correction on stable isotope values (δ^{13}C and δ^{15}N) of blubber and skin from southern hemisphere humpback whales. *Rapid Communications in Mass Spectrometry*, *35*(16), 1–11. https://doi.org/10.1002/rcm.9140

Hesslein, R. H., Capel, M. J., Fox, D. E., & Hallard, K. a. (1991). Stable isotopes of sulfur, carbon, and nitrogen as indicators of trophic level and fish migration in the lower Mackenzie River Basin, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, *48*, 2258–2265. https://doi.org/10.1139/f91-265

Hobson, K. A. (1999). Tracing origins and migration of wildlife using stable isotopes: A review. *Oecologia*, *120*, 314–326. https://doi.org/10.1007/s004420050865

Hussey, N. E., Brush, J., McCarthy, I. D., & Fisk, A. T. (2010). δ^{15}N and δ^{13}C diet-tissue discrimination factors for large sharks under semi-controlled conditions. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *155*(4), 445–453. https://doi.org/10.1016/j.cbpa.2009.09.023

Hussey, N. E., MacNeil, M. A., Olin, J. A., McMeans, B. C., Kinney, M. J., Chapman, D. D., & Fisk, A. T. (2012). Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. *Journal of Fish Biology*, *80*(5), 1449–1484. https://doi.org/10.1111/j.1095-8649.2012.03251.x

Ingenbleek, Y. (2006). The nutritional relationship linking sulfur to nitrogen in living organisms. In *The Journal of nutrition Suplement* (pp. 1641–1651). https://doi.org/10.1093/jn/136.6.1641S

Kapoulas, V. M., & Miniadis-Meimaroglou, S. (1985). Composition and distribution of lipids in tissues of bogue (*Boops boops*). *Zeitschrift Für Naturforschung C*, *40*(7–8), 562–565. https://doi.org/10.1515/znc-1985-7-819

Kelly, J. F. (2000). Stable isotopes of carbon and in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology*, *78*, 1–27. https://doi.org/10.1139/z99-165

Kharlamenko, V. I., Kiyashko, S. I., Imbs, A. B., & Vyshkvartzev, D. I. (2001). Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. *Marine Ecology Progress Series*, *220*, 103–117. https://doi.org/10.3354/meps220103

Kim, S. L., & Koch, P. L. (2012). Methods to collect, preserve, and prepare elasmobranch tissues for stable isotope analysis. *Environmental Biology of Fishes*, *95*, 53–63. https://doi.org/10.1007/s10641-011-9860-9

Koch, P. L. (2008). Isotopic study of the biology of modern and fossil vertebrates. In R. Michener & K. Lajtha (Eds.), *Stable Isotopes in Ecology and Environmental Science* (Second edition, pp. 99–154). Wiley-Blackwell. https://doi.org/10.1002/9780470691854.ch5

Krouse, H. R. (1989). Sulfur isotope studies of the pedosphere and biosphere. In P. W. Rundel, J. R. Ehleringer, & K. A. Nagy (Eds.), *Stable isotopes in ecological research* (First editition, pp. 424–444). Springer-Verlag.

Li, Y., Zhang, Y., Hussey, N. E., & Dai, X. (2016). Urea and lipid extraction treatment effects on δ^{15}N and δ^{13}C values in pelagic sharks. *Rapid Communications in Mass Spectrometry*, *30*, 1–8. https://doi.org/10.1002/rcm.7396

Logan, J. M., Jardine, T. D., Miller, T. J., Bunn, S. E., Cunjak, R. A., & Lutcavage, M. E. (2008). Lipid corrections in carbon and nitrogen stable isotope analyses: Comparison of chemical extraction and modelling methods. *Journal of Animal Ecology*, *77*, 838–846. https://doi.org/10.1111/j.1365-2656.2008.01394.x

Logan, J. M., & Lutcavage, M. E. (2010). Stable isotope dynamics in elasmobranch fishes. *Hydrobiologia*, *644*, 231–244. https://doi.org/10.1007/s10750-010-0120-3

Lu, S. C. (2013). Glutathione synthesis. In *Biochimica et Biophysica Acta* (Vol. 1830, Issue 5, pp. 3143–3153). https://doi.org/10.1016/j.bbagen.2012.09.008

Lucifora, L. O., Menni, R. C., & Escalante, A. H. (2005). Reproduction, abundance and feeding habits of the broadnose sevengill shark *Notorynchus cepedianus* in north Patagonia, Argentina. *Marine Ecology Progress Series*, *289*, 237–244. https://doi.org/10.3354/meps289237

Lucifora, L. O., García, V. B., Menni, R. C., Escalante, A. H., & Hozbor, N. M. (2009). Effects of body size, age and maturity stage on diet in a large shark: Ecological and applied implications. *Ecological Research*, *24*(1), 109–118. https://doi.org/10.1007/s11284-008-0487-z

MacNeil, M. A., Skomal, G. B., & Fisk, A. T. (2005). Stable isotopes from multiple tissues reveal diet switching in sharks. *Marine Ecology Progress Series*, *302*, 199–206. https://doi.org/10.3354/meps302199

Matich, P., Heithaus, M. R., & Layman, C. A. (2011). Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. *Journal of Animal Ecology*, *80*(1), 294–305. https://doi.org/10.1111/j.1365-2656.2010.01753.x

McClelland, J. W., & Montoya, J. P. (2002). Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. *Ecology*, *83*(8), 2173–2180. https://doi.org/10.1890/0012-9658(2002)083[2173:TRATNI]2.0.CO;2

Medeiros, L., da Silveira Monteiro, D., Petitet, R., & Bugoni, L. (2015). Effects of lipid extraction on the isotopic values of sea turtle bone collagen. *Aquatic Biology*, *23*(3), 191–199. https://doi.org/10.3354/ab00628

Murry, B. A., Farrell, J. M., Teece, M. A., & Smyntek, P. M. (2006). Effect of lipid extraction on the interpretation of fish community trophic relationships determined by stable carbon and nitrogen isotopes. *Canadian Journal of Fisheries and Aquatic Sciences*, *63*, 2167–2172. https://doi.org/10.1139/F06-116

Muryanto, M. A., Nurdin, M., Hanifah, U., & Sudiyani, Y. (2017). Extraction of glutathione from EFB fermentation waste using methanol with sonication process. *AIP Conference Proceedings*, *1904*(020011). https://doi.org/10.1063/1.5011868

Nehlich, O. (2015). The application of sulphur isotope analyses in archaeological research: A review. *Earth-Science Reviews*, *142*, 1–17. https://doi.org/10.1016/j.earscirev.2014.12.002

Niella, Y., Raoult, V., Gaston, T., Peddemors, V. M., Harcourt, R., & Smoothey, A. F. (2021). Overcoming multi-year impacts of maternal isotope signatures using multi-tracers and fast turnover tissues in juvenile sharks. *Chemosphere*, *269*, 129393. https://doi.org/10.1016/j.chemosphere.2020.129393

Ohkouchi, N., Ogawa, N. O., Chikaraishi, Y., Tanaka, H., & Wada, E. (2015). Biochemical and physiological bases for the use of carbon and nitrogen isotopes in environmental and ecological studies. *Progress in Earth and Planetary Science*, *2*(1), 1–17. https://doi.org/10.1186/s40645-015-0032-y

Oppel, S., Federer, R. N., O'Brien, D. M., Powell, A. N., & Hollmén, T. E. (2010). Effects of lipid extraction on stable isotope ratios in avian egg yolk: Is arithmetic correction a reliable alternative? *Auk*, *127*(1), 72–78. https://doi.org/10.1525/auk.2009.09153

Pahl, K. B., Yurkowski, D. J., Wintner, S. P., Cliff, G., Dicken, M. L., & Hussey, N. E. (2021). Determining the appropriate pretreatment procedures and the utility of liver tissue for bulk stable isotope (δ13C and δ15N) studies in sharks. *Journal of Fish Biology*, *98*(3), 829–841. https://doi.org/10.1111/jfb.14635

Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, *18*, 293–320. https://doi.org/10.1146/annurev.es.18.110187.001453

Peterson, B. J., Howarth, R. W., & Garritt, R. H. (1985). Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science*, *227*(4692), 1361–1363. https://doi.org/10.1126/science.227.4692.1361

Pethybridge, H., Daley, R., Virtue, P., & Nichols, P. (2010). Lipid composition and partitioning of deepwater chondrichthyans: Inferences of feeding ecology and distribution. *Marine Biology*, *157*(6), 1367–1384. https://doi.org/10.1007/s00227-010-1416-6

Pethybridge, H. R. (2010). *Ecology and physiology of deepwater chondrichthyans off southeast Australia: mercury, stable isotope and lipid analysis* [PhD thesis, University of Tasmania and L'Université de Bordeaux]. Theses.fr repository. https://www.theses.fr/2010BOR14050

Pethybridge, H. R., Parrish, C. C., Bruce, B. D., Young, J. W., & Nichols, P. D. (2014). Lipid, fatty acid and energy density profiles of white sharks: Insights into the feeding ecology and ecophysiology of a complex top predator. *PLoS ONE*, *9*(5), e97877. https://doi.org/10.1371/journal.pone.0097877

Pinnegar, J. K., & Polunin, N. V. C. (1999). Differential fractionation of δ^{13}C and δ^{15}N among fish tissues: Implications for the study of trophic interactions. *Functional Ecology*, *13*(2), 225–231. https://doi.org/10.1046/j.1365-2435.1999.00301.x

Pinzone, M., Acquarone, M., Huyghebaert, L., Sturaro, N., Michel, L. N., Siebert, U., & Das, K. (2017). Carbon, nitrogen and sulphur isotopic fractionation in captive juvenile hooded seal (*Cystophora cristata*): Application for diet analysis. *Rapid Communications in Mass Spectrometry*, *31*, 1720–1728. https://doi.org/10.1002/rcm.7955

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, *83*(3), 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., & Montaña, C. G. (2007). Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, *152*, 179–189. https://doi.org/10.1007/s00442-006-0630-x

Radin, N. S. (1981). Extraction of tissue lipids with a solvent of low toxicity. *Methods in Enzymology*, *72*(C), 5–7. https://doi.org/10.1016/S0076-6879(81)72003-2

Rakel, D. (2012). *Integrative medicine* (D. Rakel (ed.); Third edititition). Saunders.

Ramos, R., & González-Solís, J. (2012). Trace me if you can: The use of intrinsic biogeochemical markers in marine top predators. *Frontiers in Ecology and the Environment*, *10*(5), 258–266. https://doi.org/10.1890/110140

Raoult, V., Broadhurst, M. K., Peddemors, V. M., Williamson, J. E., & Gaston, T. F. (2019). Resource use of great hammerhead sharks (*Sphyrna mokarran*) off eastern Australia. *Journal of Fish Biology*, *95*, 1430–1440. https://doi.org/10.1111/jfb.14160

Richards, M. P., Fuller, B. T., Sponheimer, M., Robinson, T., & Ayliffe, L. (2003). Sulphur isotopes in palaeodietary studies: A review and results from a controlled feeding experiment. *International Journal of Osteoarchaeology*, *13*, 37–45. https://doi.org/10.1002/oa.654

Rossman, S., Ostrom, P. H., Gordon, F., & Zipkin, E. F. (2016). Beyond carbon and nitrogen: Guidelines for estimating three-dimensional isotopic niche space. *Ecology and Evolution*, *6*(8), 2405–2413. https://doi.org/10.1002/ece3.2013

Ryan, C., McHugh, B., Trueman, C. N., Harrod, C., Berrow, S. D., & O'Connor, I. (2012). Accounting for the effects of lipids in stable isotope (δ^{13}C and δ^{15}N values) analysis of skin and blubber of balaenopterid whales. *Rapid Communications in Mass Spectrometry*, *26*(23), 2745–2754. https://doi.org/10.1002/rcm.6394

Ryan, C., McHugh, B., Trueman, C. N., Sabin, R., Deaville, R., Harrod, C., Berrow, S. D., & O'Connor, I. (2013). Stable isotope analysis of baleen reveals resource partitioning among sympatric rorquals and population structure in fin whales. *Marine Ecology Progress Series*, *479*, 251–261. https://doi.org/10.3354/meps10231

Sargent, J. R., Gatten, R. R., & McIntosh, R. (1973). The distribution of neutral lipids in shark tissues. *Journal of the Marine Biological Association of the United Kingdom*, *53*, 649–656. https://doi.org/https://doi.org/10.1017/S0025315400058847

Schmidt, K., McClelland, J. W., Mente, E., Montoya, J. P., Atkinson, A., & Voss, M. (2004). Trophic-level interpretation based on δ^{15}N values: Implications of tissue-specific fractionation and amino acid composition. *Marine Ecology Progress Series*, *266*, 43–58. https://doi.org/10.3354/meps266043

Seyboth, E., Botta, S., & Secchi, E. (2018). Using chemical elements to the study of trophic and spatial ecology in marine mammals of the southwestern Atlantic Ocean. In M. R. Rossi-Santos & C. W. Finkl (Eds.), *Advances in marine vertebrate research in Latin America* (First edition, pp. 221–248). Springer Cham. https://doi.org/10.1007/978-3-319-56985-7

Shipley, O. N., Brooks, E. J., Madigan, D. J., Sweeting, C. J., & Dean Grubbs, R. (2017a). Stable isotope analysis in deep-sea chondrichthyans: recent challenges, ecological insights, and future directions. *Reviews in Fish Biology and Fisheries*, *27*(3), 481–497. https://doi.org/10.1007/s11160-017-9466-1

Shipley, O. N., Murchie, K. J., Frisk, M. G., Brooks, E. J., O'Shea, O. R., & Power, M. (2017b). Low lipid and urea effects and inter-tissue comparisons of stable isotope signatures in three nearshore elasmobranchs. *Marine Ecology Progress Series*, *579*, 233–238. https://doi.org/10.3354/meps12264

Shipley, O. N., & Matich, P. (2020). Studying animal niches using bulk stable isotope ratios: An updated synthesis. *Oecologia*, *193*, 27–51. https://doi.org/10.1007/s00442-020-04654-4

Sikorski, Z. E. (1990). *Seafood: resources, nutritional composition, and preservation* (First edition). CRC Press.

Smyntek, P. M., Teece, M. A., Schulz, K. L., & Thackeray, S. J. (2007). A standard protocol for stable isotope analysis of zooplankton in aquatic food web research using mass balance correction models. *Limnology and Oceanography*, *52*(5), 2135–2146. https://doi.org/10.4319/lo.2007.52.5.2135

Sotiropoulos, M. A., Tonn, W. M., & Wassenaar, L. I. (2004). Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: Potential consequences for food web studies. *Ecology of Freshwater Fish*, *13*, 155–160. https://doi.org/10.1111/j.1600-0633.2004.00056.x

Sweeting, C. J., Polunin, N. V. C., & Jennings, S. (2006). Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Communications in Mass Spectrometry*, *20*, 595–601. https://doi.org/10.1002/rcm.2347

Tcherkez, G., & Tea, I. (2013). 32S/34S isotope fractionation in plant sulphur metabolism. *New Phytologist*, *200*, 44–53. https://doi.org/10.1111/nph.12314

Van Vleet, E. S., Candileri, S., McNeillie, J., Reinhardt, S. B., Conkright, M. E., & Zwissler, A. (1984). Neutral lipid components of eleven species of Caribbean sharks. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *79*(4), 549–554. https://doi.org/10.1016/0305-0491(84)90364-X

Vander Zanden, H. B., Soto, D. X., Bowen, G. J., & Hobson, K. A. (2016). Expanding the isotopic toolbox: Applications of hydrogen and oxygen stable isotope ratios to food web studies. *Frontiers in Ecology and Evolution*, *4*(20), 1–19. https://doi.org/10.3389/fevo.2016.00020

Yurkowski, D. J., Hussey, N. E., Semeniuk, C., Ferguson, S. H., & Fisk, A. T. (2015). Effects of lipid extraction and the utility of lipid normalization models on $\delta^{13}C$ and $\delta^{15}N$ values in Arctic marine mammal tissues. *Polar Biology*, *38*(2), 131–143. https://doi.org/10.1007/s00300-014-1571-1