Carl Robert Priester

Trophic ecology and coastal habitat use of two sympatric shark species in the Azores using CNS stable isotope analysis.

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

2020

Carl Robert Priester

Trophic ecology and coastal habitat use of two sympatric shark species in the Azores using CNS stable isotope analysis.

Mestrado em Biologia Marinha

Supervisors:

Pedro Afonso

Co-supervisor

David Abecasis

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

2020

Declaração de autoria de trabalho

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

I declare that I am the author of this work, which is original and unpublished. Authors and works consulted are duly cited in the text and are included in the list of references.

____

Carl Robert Priester, 06.10.2020

Acknowledgements

First and foremost, I would like to thank my parents for the unconditional mental and financial support in pursuing a career that is publicly sometimes perceived as holidays by the sea and hugging dolphins. Their education and examples have led me to see things in the naturally curious and scientifically critical way I do. Tausend Dank!

Of course, I thank my friends near and far for all the good comments, ideas, distractions, and great meals in the last days of intense writing.

Nature, for keeping always keeping me sane, grounded and inspired.

And last but not least, I am grateful for Pedro and Jan and their Hawaiian friendship making this master thesis not only possible but also highly interesting. Mahalo!

“Progress is man's ability to complicate simplicity.”

— Thor Heyerdahl

Table of Contents

I English abstract

The anthropogenic exploitation of marine resources has severely altered ecosystems and caused drastic declines of large predatory fishes. Amongst these, sharks are the species of major conservation concern due to their critical role as top predators and high vulnerability to overfishing. The Azores are an oceanic archipelago in the mid-North Atlantic thought to serve as essential fish habitat (EFH) for some oceanic or semi-oceanic sharks such as coastal pupping and nursery grounds for tope (*Galeorhinus galeus*, Linneaus 1758) and smooth hammerhead (*Sphyrna zygaena*, Linneaus 1758) sharks. Yet, to date, the dependency of those juvenile sharks on coastal resources has not been investigated and crucial information on their trophic ecology is missing. This knowledge gap is relevant as it would allow to ascertain the importance of these areas for conservation and help developing management plans.

Here, simultaneous $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ (CNS) stable isotope analysis is used to investigate the trophic ecology, ontogenetic shifts and habitat use of the coastal life stages of *G. galeus* and *S. zygeana* around the Azores. A Bayesian ellipse approach (nicheROVER) and generalized additive mixed models (GAMM) are applied and interpreted in reference to isotope values of coastal and pelagic food web samples. The results show high diet overlap between tope and juvenile smooth hammerhead sharks with coastal-associated values. Tope shark showed a significant ontogenetic shift to higher trophic level coastal-benthic prey with growing size. Smooth hammerhead sharks exhibited significant decreases in $\delta^{34}S$, also suggesting a shift towards more coastal-benthic prey with increasing size. The diet of both species support their co-occurrence in shared nurseries with no evidence of sexual segregation or interspecific niche partitioning, but instead highly trophic competition, emphasizing the importance of healthy coastal habitats for conservation of these highly mobile sharks in the wider Atlantic.

Keywords:

Stable isotope analysis, trophic ecology, essential fish habitats, shark nursery, *Sphyrna zygaena*, *Galeorhinus galeus*

II Resumo

A exploração antropogénica dos recursos marinhos alterou gravemente o funcionamento dos ecossistemas e causou declínios drásticos da maioria dos grandes peixes predadores. Destes, os tubarões oceânicos e semi-oceanicos são a maior preocupação para a conservação marinha devido ao seu papel ecológico essencial como predadores de topo e à sua excepcional vulnerabilidade à sobrepesca. Estes tubarões utilizam frequentemente habitats costeiros como zonas de refúgio e alimentação nas fases iniciais da vida. Aqui, os juvenis são expostos a pressões antropogénicas crescentes, tais como a degradação do habitat e a pressão da pesca, que podem ter repercussões nas populações através da redução do recrutamento. Os Açores são um arquipélago oceânico do Atlântico norte central, que contém habitats essenciais (Essential Fish Habitats - EFH) para várias espécies de elasmobrânquios. Observações de agregações abundantes e regulares de tubarão martelo (*Sphyrna zygaena*, Linneaus 1758) e cação (*Galeorhinus galeus*, Linneaus 1758) em redor das ilhas sugerem a presença de habitats essenciais para alimentação e reprodução. No entanto, faltam informações cruciais sobre as suas estratégias de alimentação, competição e história de vida para identificar áreas de conservação prioritárias e desenvolver planos de gestão sustentável. Com os avanços tecnológicos e a diminuição dos custos de análise, o uso de isótopos estáveis tem-se estabelecido como uma ferramenta versátil e altamente informativa para investigar a ecologia aquática. Seguindo o conceito *"você é o que come"*, a composição dos isótopos estáveis em proteínas pode elucidar as fontes alimentares, os habitats associados e mudanças ontogenéticas, mesmo com tamanhos de amostra reduzidos. A razão isotópica do nitrogénio (^{15}N:^{14}N) entre presas e consumidores tem sido utilizado para examinar a ecologia da alimentação, a posição trófica e a estrutura da teia alimentar, enquanto que a razão isotópica de carbono (^{13}C:^{12}C) entre consumidores primários e predadores permite examinar a utilização do habitat animal e as fontes de presas. Embora o enxofre (^{34}S:^{32}S) seja menos utilizado, os baixos factores de fraccionamento trófico em combinação com diferenças pronunciadas entre diferentes fontes de enxofre, tornam-no uma ferramenta altamente discriminatória entre fontes pelágicas e costeiras ou bentónicas. A análise simultânea de isótopos estáveis de carbono, nitrogénio e enxofre (CNS) constitui assim um poderoso instrumento para reduzir as lacunas de conhecimento existentes sobre a ecologia trófica da fase costeira do tubarão martelo e cação nos Açores.

Nesta tese foi utilizada a análise de isótopos estáveis de CNS para investigar a ecologia trófica, o uso do habitat costeiro e as mudanças ontogenéticas do cação (*G. galeus)* e tubarão-martelo (*S. zygeana)*. As amostras de teias alimentares costeiras e pelágicas revelaram diferenças significativas nos três isótopos, confirmando os gradientes isotópicos da literatura. Foi aplicada uma abordagem de elipse Bayesiana para calcular regiões de nicho de 95% (nicheROVER) entre as duas espécies e as teias alimentares costeira e pelágica, e para estimar contribuições relativas e sobreposição de nichos nas três dimensões isotópicas. Para determinar a variação trófica inter e intra-específica ao longo da ontogenia das espécies, foram estimados modelos mistos aditivos generalizados (Generalized Additive Mixed Model – GAMM) dos três isótopos estáveis como variáveis de resposta. As primeiras iterações do modelo incluíram o comprimento e o sexo (isolado e em interacção com espécies) como efeitos fixos. Para ter em conta potenciais alterações da linha de base ao longo do período de amostragem, a data da amostragem foi implementada como efeito aleatório. Devido aos efeitos não-significativos do sexo (em interacção com espécie e isoladamente) em todos os isótopos, estes efeitos foram excluídos dos modelos subsequentes. Finalmente, para avaliar a viabilidade de tecidos alternativos para estudos futuros foi testado o uso de muco e do centro de vértebras.

As regiões de nicho mostraram uma elevada sobreposição entre as duas espécies, sobretudo a sobreposição do tubarão-martelo com cação, e sobrepuseram-se sobretudo às regiões costeiras. O comprimento total mostrou efeitos altamente significativos por espécie em todos os isótopos: como previsto, $\delta^{13}C$ e $\delta^{15}N$ aumentaram significativamente com o tamanho no cação enquanto $\delta^{34}S$ diminuiu significativamente no martelo com o tamanho. Aplicando as diferenças pronunciadas entre as teias alimentares costeiras e pelágicas, os resultados sugerem uma elevada contribuição da dieta costeira/bentónica tanto nos cações como nos tubarões-martelos. Os resultados sugerem um aumento ontogenético da alimentação costeira/bentónica juntamente com a posição trófica no cação, e uma ligeira diminuição do nível trófico e elevada dependência da dieta costeira/bentónica do tubarão martelo durante a sua fase costeira juvenil, em concordância com estudos no oceano Pacífico. A grande sobreposição dos nichos isotópicos sugere um elevado potencial de concorrência inter- e intra-específica. No entanto, os dados indicam vários mecanismos de possível partição de nicho. As fêmeas adultas de cação alimentam-se de níveis tróficos mais elevados, indicando menor competição com os tubarões mais pequenos, mas potencial pressão de predação. Os juvenis das duas espécies apresentam a maior sobreposição, mas a grande variação observada em

todos os isótopos aponta para a influência de outras causas de variação individual para além da espécie, sexo ou tamanho. A análise futura de presas bentónicas e de diferentes tecidos poderá revelar informações em falta sobre a alimentação das presas bentónicas, e melhorar a resolução temporal desta dinâmica ao longo da vida.

A exploração do uso de muco (não-invasivo) e dos incrementos vertebrais *centra* para aumentar a resolução temporal na SIA revelou elevada viabilidade em ambos os casos para estudos futuros. A primeira aplicação de SIA em muco CNS mostrou diferenças significativas entre músculo e muco, mas os factores de discriminação dos tecidos da dieta e os tempos de rotação precisam de ser quantificados para permitir uma interpretação precisa. A amostragem não invasiva e a análise bem sucedida do muco epidérmico torna-o um tecido altamente informativo com impacto reduzido nos tubarões estudados. As vertebras indicam variação entre diferentes camadas de crescimento e diferem dos valores musculares. Para avaliar as mudanças ontogenéticas da dieta dos adultos e determinar a transição de habitats costeiros para habitats pelágicos, sugere-se uma análise isotópica estável de secções incrementais de vértebras *centra* tanto de tubarão-martelo como de cação.

No conjunto, a potencial competição pelos recursos costeiros em áreas putativas de maternidade partilhadas mostrada neste trabalho reitera a importância de ecossistemas costeiros saudáveis para as populações destas espécies vulneráveis ao nível do Atlântico. A diminuição da abundância de peixes costeiros pode provocar uma sobreposição e competição crescentes das fases juvenis com efeitos negativos sobre a integridade das suas populações. Esta conclusão enfatiza a prioridade de mais investigação e subsequente protecção das maternidades comuns de tubarões de ilhas oceânicas e ecossistemas associados.

III Glossary and Abbreviations

C	Stable carbon isotopes
C:N	Elemental mass ratio of carbon to nitrogen
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
DTDF	Diet–Tissue Discrimination Factor – is the diet and tissue specific factor of change of the ratio of the heavy to the light stable isotope.
DOP	Departamento de Oceanografia e Pescas, Horta, Faial
EFH	Essential Fish Habitat
EMH	Essential Megafauna Habitat
GAMM	Generalized Additive Mixed Model
GG	Tope shark *(Galeorhinus galeus)*
ICCAT	International Commission for the Conservation of Atlantic Tunas
Isotope fractionation	The alteration of the ratio of the heavy rare isotope, to the light one during some reactions (e.g. lipid synthesis)
IUCN	International Union for the Conservation of Nature
N	Stable nitrogen isotopes
S	Stable sulphur isotopes
SIA	Stable Isotope Analysis
SZ	Smooth hammerhead shark *(Sphyrna zygaena)*
TEF	Trophic Enrichment Factor - in other studies sometimes referred to as trophic shift or diet-consumer difference, is the average increase of the isotope ratio per trophic transfer in a food web.
TL	Total Length, in this study measured in cm
Turnover time	The time it takes for a tissue to reach isotopic equilibrium after a diet switch. Depends on the replacement of proteins in the tissue (thus resulting in short-, medium- and long turnover tissues).

IV Table of Figures

V Table of Tables

VI Introduction

VI.1 Global status of shark conservation

Overfishing is one of the most impactful marine anthropogenic disturbances and in combination with climate change has led to the collapse of coastal ecosystems (Jackson et al. 2001) and a rapid decline of large predatory elasmobranchs in recent years (Baum et al. 2003, Worm et al. 2013, Dulvy et al. 2014). Due to their low fecundity, late maturity and wide-ranging migratory patterns (Frisk et al. 2005, Dulvy et al. 2014, Gallagher et al. 2014) sharks are especially vulnerable to fishing pressure and other anthropogenic threats. Large predators are key functional groups of all ecosystems around the world, balancing and controlling the populations of lower trophic levels (Heithaus et al. 2012). Top predator declines can evoke cascading top-down effects due to their high trophic position in most food webs, impacting the coastal food webs in their entirety (Myers et al. 2007, Ferretti et al. 2010, Heithaus et al. 2012). Due to their distribution in vast geographical ecosystems, often extending across Exclusive Economic Zones, many shark population metrics remain unquantified due to deficient data (Castro et al. 1999, Red-List 2018). In combination with their conservative life-history traits and complex migratory habits, this is hampering elasmobranch research and conservation efforts (Castro et al. 1999). The integration of essential fish habitats (EFH) into ecosystem-based management has been emphasised as a key factor to attain sustainable and sound fisheries management and legislations (Rosenberg et al. 2000, Vaz and Le Pape 2019). EFH was defined as “waters and substrate necessary to fish for spawning, breeding, feeding or growth to maturity.” by the Magnus-Stevens Act (16 U.S.C. § 1801 et seq.). Sharks frequently use coastal habitats as nursery areas associated with a reduced risk of predation (Springer 1967). High abundances and regular aggregations of sharks in the Azores suggest the use of local resources and EFHs (Afonso et al. 2014b, Afonso et al. 2020). Nevertheless, with only fragmented information about the life history, spatial habitat uses and feeding grounds, the knowledge about the extent and location of such EFH and nurseries remains scarce for most shark species.

VI.2 Sharks around the Azores

The Azores are an oceanic archipelago of nine major volcanic islands located on the junction of three tectonic plates in the mid-Atlantic (36°-40°N; 25°-31°W see Figure VI.1). Through the tectonically intricate geology and the interaction with the southern branch of the North Atlantic Current and the Azores current, its marine domain is characterised by strong currents, eddies, upwelling regions and productive seamounts (Amorim et al. 2017, Caldeira and Reis 2017).

> *“The wider Azores emerge as a singular multispecies oceanic EMH [essential megafauna habitat] hotspot on a migratory crossroads, linking the eastern to western basin margins as well as the cold productive boreal waters to the tropical and equatorial seas.”*
>
> Afonso et al. (2020)

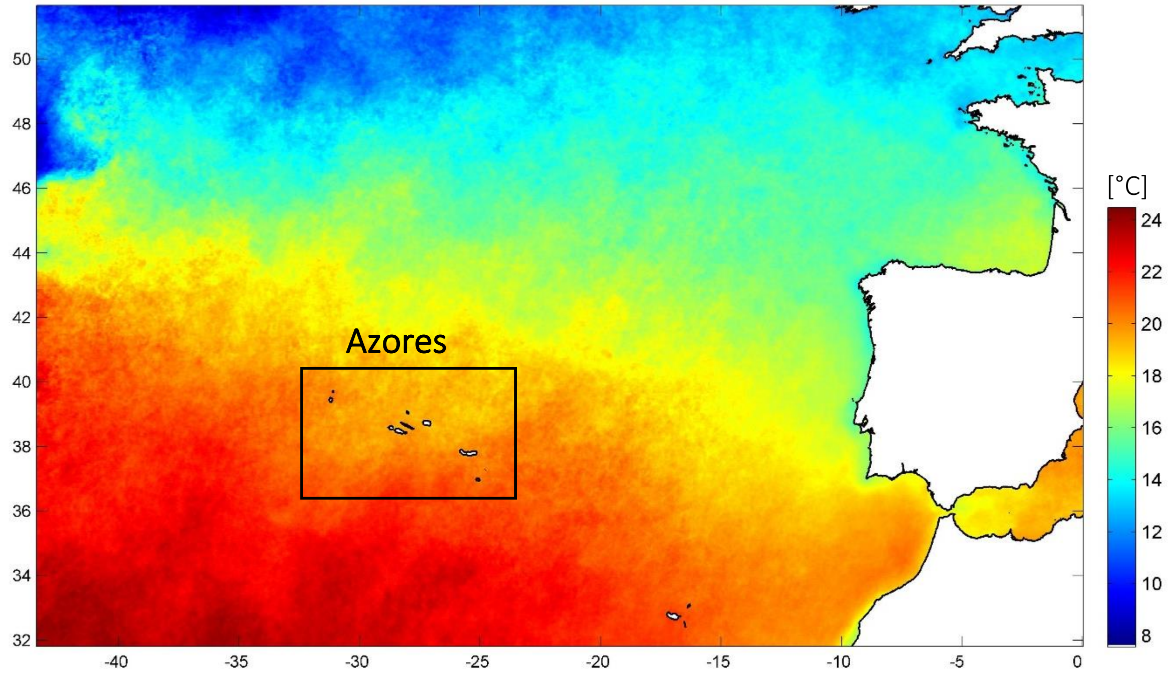

Figure VI.1 - Map of the Azores and the 2019 annual MODIS Terra L3 sea surface temperature of the North-East Atlantic.

Along with other megafauna, 39 different shark species have been identified to use the archipelagos habitats temporarily or as residents (Das and Afonso 2017). The Azores thus provide important habitats to these species - both oceanic and coastal - and are of potentially crucial relevance for their larger Atlantic populations (Afonso et al. 2014a, Das and Afonso 2017). A network of over 110,000 km^2 (ca. 10% of the exclusive economic zone) of marine protected areas (Abecasis et al. 2015) limits the fishing pressure locally, but direct and indirect effects of the present fisheries still impact the fish populations. In their proposed action plan, Afonso et al. (2020) highlight the priority of identifying the location of multi-specific local megafauna hotspots and the relevant processes to establish “sites of priority for full protection”. Sharks often use inshore coastal habitats for nursing, with large numbers of juvenile individuals aggregating in the shallow waters (Heupel et al. 2007). This has been attributed to evasion of predators, shelter and the productivity classically hypothesised to be so high, that that food is not a limiting factor (Springer 1967). Yet, more recent studies have questioned this resource abundance (see Heupel et al. 2007 and references therein) providing evidence for competition and niche partitioning on various levels (Bethea et al. 2004, Kinney et al. 2011). (Springer 1967). The spatial overlap with anthropogenic pressures such as habitat destruction and recreational and commercial fisheries, renders coastal shark species or life stages especially vulnerable and could reduce prey resources (Knip et al. 2010). In the Azores, the two most abundant shark species present in coastal waters are the smooth hammerhead shark, *Sphyrna zygaena* (Linnaeus, 1785) and tope shark, *Galeorhinus galeus* (Linnaeus, 1758) (Das and Afonso 2017, GAMPA 2020, Santos et al. 2020).

VI.3 Study Species

VI.3.1 Tope shark - *Galeorhinus galeus*

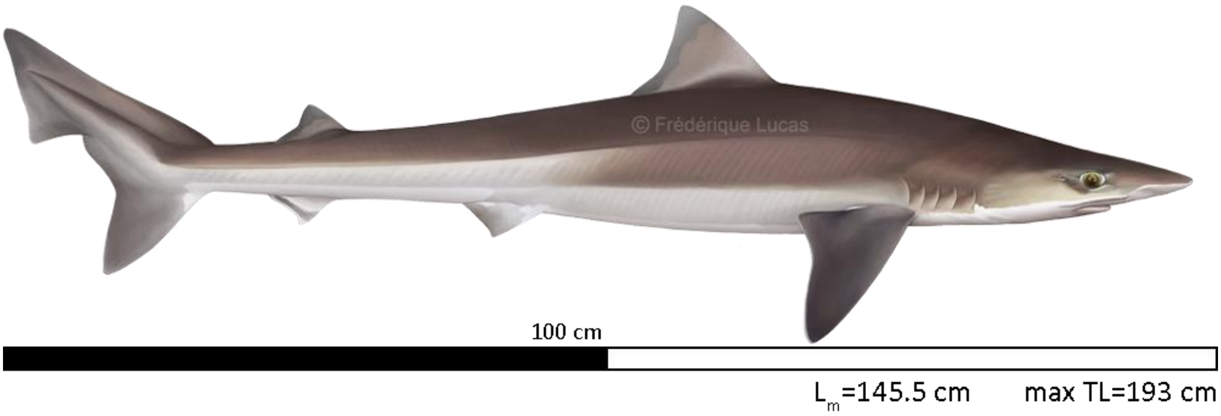

Figure VI.2 - Tope Shark (*Galeorhinus galeus*) with L_m - total length at maturity and the maximum total length from FishBase (Froese and Pauly 2010) – copyright Frédérique Lucas

The tope shark, *Galeorhinus galeus* (Linnaeus, 1758) (see Figure VI.2), is a medium-sized cosmopolitan predator that can be found in temperate shelf- and coastal waters down to depths of 1100 m (Cox and Francis 1997, Ebert and Stehmann 2013). Their life history is characterized by slow growth, late maturity (50% maturity females 155 cm, 17 years; males 121 cm, 12 years) and high longevity (max. 59 years, L_∞ = 201 cm) (Dureuil and Worm 2015). A global genetic study of tope sharks revealed significant structure with several distinct populations, the European Atlantic constituting one (Chabot 2015). Adults in the North Atlantic have been tracked performing long-distance oceanic migrations over 2500 km (Holden and Horrod 1979, Colloca et al. 2019, Thorburn et al. 2019) before most likely returning to their natal area in coastal waters (Fitzmaurice et al. 2003). Here the pregnant females aggregate in shallow areas and bays for pupping, while adult males have been found to stay in deeper waters (Compagno 1984). Reproduction occurs as aplacental viviparity with an average litter size of 20 to 35 pups (Ebert 2003). Stomach content analysis studies around the world have found their diet to consist mainly of teleosts, cephalopods and invertebrates, with varying proportions at different locations and by size (Ellis et al. 1996, Cortés 1999, Walker 1999, Lucifora et al. 2006). In the Azores, Morato et al. (2003) found an 81% contribution of teleosts to tope shark diet, with few highly important species.

Around the local islands, tope sharks have been observed to aggregate in nurseries and adult grounds, migrating between the different islands of the Archipelago and Macaronesia (Afonso unpublished data). Here they are often caught by commercial and recreational fisheries as both by-catch and target species (GAMPA 2020). Even though, the latest European assessment was listed as “data deficient” (Ferretti et al. 2015), the most recent global assessment classified them as “critically endangered” on the IUCN Red List (Walker 2020). Due to its conservative life-history characteristics and aggregating nature, fisheries can have severe impacts making tope sharks prone to overexploitation. As a consequence, local landings (commercial and fisheries-independent surveys) have been declining up to 68% in the last 4 years (GAMPA 2020). The coastal waters of the Azores could constitute important EFHs, relevant for the larger North Atlantic tope shark populations (Das and Afonso 2017, unpublished data) and the concern over the data-poor situation and increased vulnerability has been reiterated (GAMPA 2020, Santos et al. 2020).

VI.3.2 Smooth hammerhead shark - *Sphyrna zygaena*

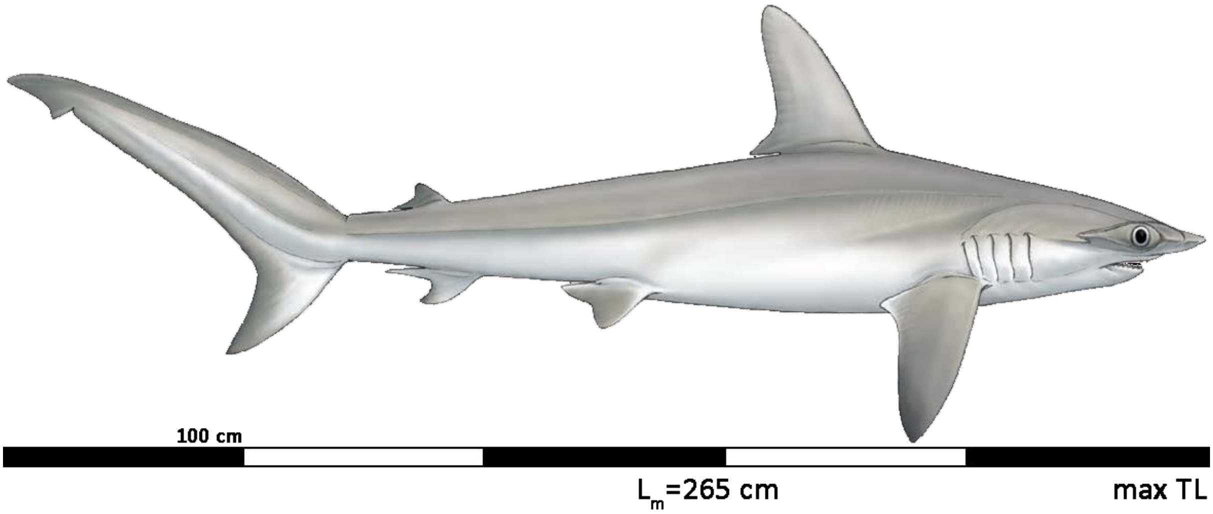

Figure VI.3 - Smooth hammerhead shark (*Sphyrna zygaena*) with L_m - total length at maturity and maximum total length from FishBase (Froese and Pauly 2010) – modified from (Ebert 2014)

The smooth hammerhead shark, *Sphyrna zygaena*, (Linnaeus, 1758) (see Figure VI.3) is a coastal-pelagic and semi-oceanic species (Compagno 1984) that can be found circumglobally in warm temperate to tropical waters and depths to 260 m (Santos and Coelho 2018). The North-East Atlantic smooth hammerhead population is listed as “vulnerable” on the IUCN Red List (Casper 2009). Of eight shark species and groups, Baum et al. (2003) found hammerhead sharks to be the most affected, exhibiting population declines of 89% in the North-west Atlantic Ocean since 1986. Although landings of smooth hammerheads are prohibited in International Commission for the Conservation of Atlantic Tunas (ICCAT) fisheries (ICCAT 2008), and their trade is protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the effectiveness of these measures has been questioned (Santos and Coelho 2019). Illegal trade of the highly valuable fins persists (Cardeñosa et al. 2018) and the high long-line catch rates are aggravated by a high capture and post-release mortality (Coelho et al. 2012, Ellis et al. 2017).

Of the Sphyrnidae family, the smooth hammerhead is most tolerant of temperate waters with an Atlantic latitudinal range from Argentina to the British Isles (Froese and Pauly 2010) as well as the tropics (Santos and Coelho 2019). Tagging studies have tracked movements of over 6600 km across the equator and recorded a large amount of their time present in surface waters (50% < 10 m) with only sporadic deeper dives (Santos and Coelho 2018). Compared with the greater hammerhead (*Sphyrna mokorran*) and scalloped hammerhead (*Sphyrna lewini*), only recently studies have started to unveil detailed spatial movement (Francis 2016, Santos 2017, Santos and Coelho 2019, Logan et al. 2020) and relatively large knowledge gaps remain concerning the coastal resource use. Females are thought to come to shallow water to give birth as placental viviparity to young of 50-60 cm TL (Compagno 1998). Large aggregations of juveniles at coastal nursery grounds have been observed (Compagno 1998) and philopatric- and natal homing behaviour has been suggested (Félix-López et al. 2019). Smooth hammerheads are specialist predators and their preferred diet consists mainly of teleosts, cephalopods and other elasmobranchs (Compagno 1984, Smale 2010) with varying compositions in different studies around the world (Ochoa Díaz 2009, Smale 2010, Gonzalez-Pestana et al. 2017, Estupiñán-Montaño et al. 2019). Several studies suggest an ontogenetic shift from continental coastal nurseries to pelagic-oceanic habitats and diet at ~ 150 cm TL (Bornatowski et al. 2014, Gonzalez-Pestana et al. 2017, Estupiñán-Montaño et al. 2019). Around the Islands of the Azores, juvenile smooth hammerhead sharks (below ~ 150 cm TL) have been observed in high abundance in shallow coastal waters in the summer months, while adults (larger than 2 m) are only rarely seen (Afonso unpublished data, Das and Afonso 2017). Despite the existence of putative EFH, to date, no study has been conducted on the resource use and trophic ecology of this species in the Azores or other oceanic islands.

To complement the available data and contribute to filling the existing knowledge gaps about the trophic ecology and putative communal nursery use, a methodology is needed that can decrypt the trophic interactions of the study species in space and throughout their ontogeny.

VI.4 The power of stable isotope analysis

Stable isotope analysis (SIA) has become a highly influential tool in aquatic ecology (Post 2002) giving novel insights into the feeding ecology of marine species and food webs, energy fluxes and migrations. It is thus a highly suitable tool to close the existing knowledge gaps. After a brief explanation of the assumptions underlying SIA, I explore the current state of the art of SIA in the field of elasmobranch habitat ecology and resulting study questions.

Isotopes are atoms of the same element that differ only in the number of neutrons. In contrast to radioactive isotopes, stable isotopes exhibit no radioactive decay and thus exist globally in stable proportions (e.g.: $^{13}C/^{12}C$). Although in most chemical and physical reactions they function equally to their lighter analogue, some mass-dependent reactions can cause preferential incorporation of one or the other in the product. This alteration of the ratio of the heavy rare isotope, to the light one, is called *isotope fractionation*. When proteins are accreted in body tissues such as muscles, they contain a fractionated signature of the isotopic composition of the diet. Tissues can be analysed for heavy and light stable isotopes of specific elements (i.e.: ^{13}C and ^{12}C) using mass spectrometry, and the inherent ratio between the isotopes can be calculated. This ratio is standardised by comparing the ratio of isotopes of the unknown sample with a recognized standard. Controlled diet experiments in the laboratory can elucidate not only the degree of isotope fractionation from the diet into the tissue of interest (*diet-tissue determination factor – DTDF,* see Figure VI.4) but also the amount of time it takes for the tissue to reach a stable isotope composition after a diet switch (turnover time). Knowing these parameters and ratios of potential sources, the analysis of tissues can give insights about the incorporated elemental source and pathways. Following the principle "*You are what you eat*" (Peterson and Fry 1987), stable isotopes can thus be used as "ecological tracers" in food webs and ecosystems. Traditionally, in fish ecology, muscle is used for SIA to get a time-integrated signal of dietary pathways during the incorporation of the tissue proteins. Nevertheless, all tissues containing synthesised proteins can be as ecological tracers and yield varying fingerprints through different metabolism- and protein fractionation pathways. Through the analysis of different elements, the advance in spectrometry and the more thorough understanding of fractionation pathways, SIA have become a powerful technique to elucidate a broad array of study questions in ecology.

VI.4.1 A brief manual of the toolbox

Two of the most essential considerations for the design and interpretation of stable isotope analysis of different tissues are a) their molecular turnover time and b) fractionation pathways and resulting enrichment factors. The turnover time of the tissue proteins dictates the temporal resolution and integrative nature of the stable isotopes. The analysed stable isotope ratios thus reflect a time-integrated signal of the environmental conditions of the study organism throughout the turnover time. When interpreting for example diet-related stable isotope signatures, slow turnover tissues (i.e.: muscle) can mask abrupt diet shifts (through the integration of the two different diet signals), while on the other hand, revealing insights into the diet integrated over a longer period. The analysis of a combination of tissues with differing turnover times can hence yield more comprehensive and insightful results and is highly recommended (MacNeil et al. 2005, Hussey et al. 2012, Kim and Koch 2012).

Proteins of different tissues can be synthesized through different pathways, during which the fractionation of the inherent stable isotope ratios can be altered. To account for the differences in fractionation, discrimination factors were established. As these depend on the proteins of the diet (Florin et al. 2011) and the tissues' fractionation pathways these are often termed diet-tissue discrimination factors (DTDF) and are specific for each isotope combination (Tieszen et al. 1983, Hobson and Clark 1992). Many tissues are analysed for the stable isotope ratio of the organic compounds, most frequently proteins. Other tissue components, such as lipids or urea, with stable isotope ratios differing from those of pure protein, can lead to variations even within the same tissue and thus confound the interpretation (Post et al. 2007, Carlisle et al. 2017). To ensure a pure and accurate signal, samples with high lipid or urea content (C:N ratio of all samples < 3.5 (Post et al. 2007)) can be washed or processed with extraction methods (see Hussey et al. 2012, Kim and Koch 2012). As lipid-extraction processes have been shown to bias the protein stable isotope ratio of some species (Post et al. 2007), mathematical correction models have been developed and applied successfully (Kiljunen et al. 2006, Post et al. 2007, Logan et al. 2008, Carlisle et al. 2017). Several studies have suggested that mathematical lipid-correction only produces accurate results when using C:N ratios of washed urea-extracted samples (Li et al. 2016, Carlisle et al. 2017).

VI.4.2 Different isotopes – different tools

Carbon: ($^{13}C/^{12}C$)

Carbon atoms are an essential part of most organic molecules. Out of its 15 isotopes two are stable: ^{12}C and ^{13}C. Due to the fractionation pathways of the photosynthesis, organic molecules are usually depleted in the heavy isotope, resulting in a negative $\delta^{13}C$ value. The varying photosynthetic pathways of different plants and primary producers (i.e.: C3, C4, phytoplankton and macroalgae) can lead to pronounced differences (Figure VI.4). Due to very little alteration of the stable isotope ratios by consumers (DeNiro and Epstein 1978), the signals of the primary producers can be retraced through the entire food web (Fry and Sherr 1989). Several primary production gradients enable the tracking of the underlying sources: a) globally, benthic algae are significantly enriched in 13C when compared to pelagic ones (France 1995); b) shelf primary productivity is enriched in 13C compared to slope ecosystems further offshore (Perry et al. 1999) and terrestrial signatures are higher than marine ones due to the dependence on $[CO_2]_{aq}$ and the growth rate, which vary by latitude (Peterson and Fry 1987, Laws et al. 1995). In recent efforts, these habitat-specific differences have been compiled to produce georeferenced maps of carbon isotopes, called isoscapes (McMahon et al. 2013, Trueman and Glew 2019).

Nitrogen ($^{15}N/^{14}N$)

Nitrogen is the most common element in the earth's atmosphere and the central building block of amino acids. Nitrogen has two stable isotopes: ^{14}N and ^{15}N. In their ground-breaking study, DeNiro and Epstein (1981) found, that the isotopic composition ($\delta^{15}N$) of an animal closely reflects the one of its diet with a relatively consistent enrichment of ^{15}N. This regular TEF has since been used by ecologists to calculate the trophic position of organisms (Figure VI.4 and Hobson 1993, Torres et al. 2014, Seubert et al. 2019). Like carbon, nitrogen can be assimilated by the primary producers via different pathways impacting the degree of isotope fractionation (Peterson and Fry 1987). Hence, to obtain accurate trophic level results, the $\delta^{15}N$ value of the base of the food web needs to be subtracted from the study animals one (e.g. Estrada et al. 2003).

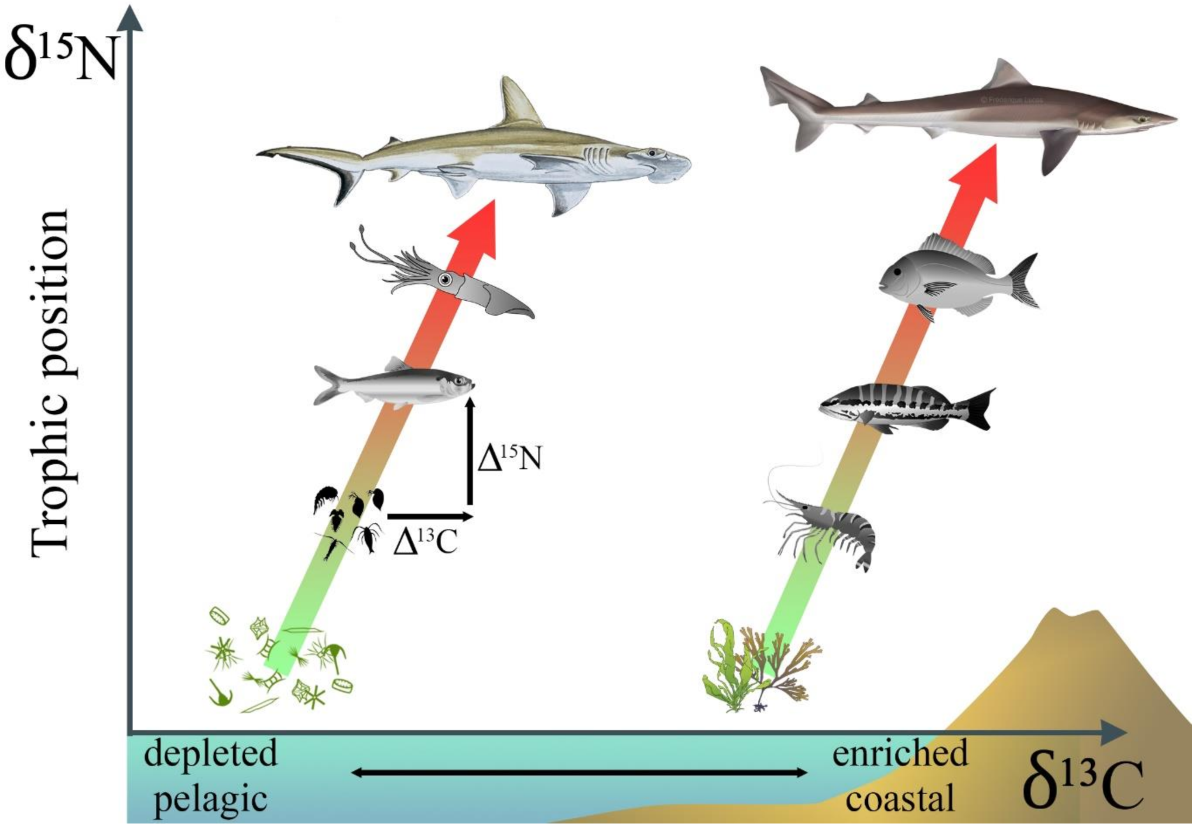

Figure VI.4 - Contextualization of the enrichment of $\delta^{13}C$ and $\delta^{15}N$ throughout a pelagic phytoplankton-based and a benthic algal-based food-chain where $\Delta^{13}C$ and $\Delta^{15}N$ are the trophic enrichment factors.

Sulphur ($^{34}S/^{32}S$)

Of the 20 proteinogenic amino acids, only two contain sulphur: methionine and cysteine. Nevertheless, both amino acids play essential roles in proteins and are incorporated in most proteins (Brosnan and Brosnan 2006). Sulphur has four stable isotopes of which ^{32}S and ^{34}S are the naturally most abundant (Hobson 1999). In marine environments, sulphur is most commonly present in two forms: as sulphates in the water column (enriched ~ +20‰) or as bacterially reduced sulphides in the sediment (depleted ~ -24‰) (Peterson and Fry 1987), while freshwater sulphates from precipitation range between +2 to +16‰ (Peterson and Fry 1987). In combination with a low TEF (-0.9 – 1.9‰ - McCutchan et al. (2003)), the broad ranges of the different sulphur sources make $\delta^{34}S$ a highly powerful ecological tracer of the primary production sources (Mekhtiyeva et al. 1976, Peterson and Fry 1987). In a review of 14 estuarine and marine food web CNS SIA studies, Connolly et al. (2004) found sulphur to provide the highest degree of differentiation between producers, separating over 40% of cases that were tied using carbon or nitrogen. Two main sulphur gradients are commonly utilized in ecological studies: differences between terrestrial and marine primary production sources in coastal ecology (e.g. Fry 1983, Peterson et al. 1986, Hesslein et al. 1991) and the difference between pelagic and benthic producers (Hobson 1999, Mittermayr et al. 2014, Curnick et al. 2019). The addition of sulphur as a dimension in stable isotope studies can add a significant amount of detail and information on the n-dimensional hypervolume of ecological niches (Hutchinson 1957) and habitat use (Shipley and Matich 2020).

VI.5 State of the SIA-art in shark ecology

Especially for the study of large and vulnerable migratory predators the use of SIA can be a very powerful tool (Hussey et al. 2012, Kim and Koch 2012). A Web of Science search using the terms: *"stable isotope analysis (shark* OR elasmobranch*)"* yielded a total of 337 publications with a maximum of 47 publications per year in 2019 (access date: 27.08.2020). In shark ecology, it has been used in the scope of various contexts: trophic position, diet and niche characterisation, niche partitioning, habitat use and ontogenetic shifts (e.g.: Estrada et al. 2006, Hussey et al. 2011, Kinney 2011). Most of these studies have focused on SIA of muscle tissue; but vertebrae, liver, blood, and other tissues have also been used.

Hussey et al. (2012), and Kim and Koch (2012) compiled comprehensive reviews about different tissue types, preservation, preparation and use of different isotopes for elasmobranch SIA.

Several controlled diet laboratory studies have elucidated the turnover time of tissues and DTDFs through controlled diet shifts providing the necessary parameters for interpretation (MacNeil et al. 2005, Hussey et al. 2009, Logan and Lutcavage 2010, Kim et al. 2011, Malpica-Cruz et al. 2012). Muscle turnover times from controlled diet experiments vary between around a year (MacNeil et al. 2006, Logan and Lutcavage 2010) to several years (Kim et al. 2012, Malpica-Cruz et al. 2012) and depend on the element, size growth rate of the study organisms (see Weidel et al. 2011). Mucus protein turnover quantifications are much more scarce but studies of trout and catfish indicate a more rapid turnover of several months depending on size (Church et al. 2009, Heady and Moore 2013, Maruyama et al. 2017). Vertebrae centra are accreted throughout time with the organic matrix being preserved in as metabolically inert compounds(Campana et al. 2002).

A Web of Science search returned eight peer-reviewed publications concerning the trophic or feeding ecology of the two species (Table VI.1). Most of these studies have investigated the feeding ecology and niche in other parts of the globe, especially in the Pacific Ocean. To my knowledge, no published stable isotope analysis studies have focused on the smooth hammerhead shark in the North Atlantic Ocean and only two studies of tope shark could be found here, with one conducted in the Azores.

Table VI.1 - Peer-reviewed stable isotope analysis studies of tope (Galeorhinus galeus - GG) and smooth hammerhead shark (Sphyrna zygaena - SZ), the analysed isotopes, tissue and sample size (n), mean results ± standard deviation or ranges trophic position (TP) and context of the studies.

AUTHORS	SPECIES	LOCATION	ISOTOPES	TISSUES	N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	TP	CONTEXT
ALFARO-CORDOVA ET AL. (2018)	GG, SZ	Peru	$\delta^{13}C$, $\delta^{15}N$	Muscle	11 45	GG: -14.74 ± 0.58 SZ: -15.67 ± 0.52	14.99±1.13 15.74 ± 1.88	GG: 4.46-4.31 SZ: 4.81 – 3.89	Interspecific trophic interactions, niche overlapping with 2 other species
BOTTO ET AL. (2011)	GG	Argentina and Uruguay	$\delta^{13}C$, $\delta^{15}N$	Muscle	1	-16.2	19.5		Trophic structure of an estuarine ecosystem
DAVENPORT AND BAX (2002)	GG, SZ	Australia	$\delta^{13}C$, $\delta^{15}N$	Muscle	13 1	GG: -17.0 ± 0.6 SZ: –16.8	GG: 13.3 ± 0.5 SZ: 14.2		Trophic structure of larger ecosystem
DOMI ET AL. (2005)	GG	Celtic Sea	$\delta^{13}C$, $\delta^{15}N$	Muscle and liver	6	-15.5 ±0.5	16.7±0.9		Feeding ecology of 5 commercial shark species
LOOR-ANDRADE ET AL. (2015)	SZ	Peru	$\delta^{13}C$, $\delta^{15}N$	Muscle, Vertebrae	64 20	-16.1 ± 0.4	14.8 ± 0.8		Trophic niche overlap with scalloped hammerhead
MILLER ET AL. (2010)	GG	California	$\delta^{13}C$, $\delta^{15}N$	Muscle	1	-16.5	15.4	3.8	Trophic structure of larger ecosystem
OCHOA DÍAZ (2009)	SZ	Baja California	$\delta^{13}C$, $\delta^{15}N$	Muscle	38	-14.9 - -15.81	17.64 – 19.86	3.7 – 4.1	Trophic spectrum of SZ
TORRES ET AL. (2014)	GG	Azores	$\delta^{13}C$, $\delta^{15}N$	Muscle	124	-18.6 - -17.2	12.3 - 14.8	4.01-4.36	Trophic and feeding ecology,

SIA studies of tope sharks found high trophic positions and indications of a piscivorous diet (see reference in Table VI.1). Smooth hammerhead publications reveal lower trophic positions with coastal and oceanic diet and indications of a narrow, specialized niche.

Torres et al. (2014) laid an important foundation for the tope shark ecology in the Azores by studying their trophic ecology with a combination of SCA data, trace metals and SIA. Using $\delta^{13}C$, $\delta^{15}N$ and SI data of 4 fish species, cephalopods, and invertebrates from other publications, the authors calculated the trophic position and a mixing model of the different sources. Overall, no significant differences were found for $\delta^{13}C$ and $\delta^{15}N$ between sex but between size for both. $\delta^{15}N$ values were depleted relative to other studies (Pinnegar et al. 2002, Domi et al. 2005) which are attributed to baseline differences. They identified a significant increase of TP by size and a total variation between 3.78 to 4.66 depending on the prey values used for the calculation. Their MixSIR model identified teleosts as main prey with *Trachurus picturatus* having the highest contribution out of the 4 fish. They proposed the existence of a Mid-Atlantic tope shark population with a piscivore diet. The authors recommend the use of multiple tissues in the future to shed detail on temporal changes. Alfaro-Cordova et al. (2018) compared the isotopic niches of tope and smooth hammerhead sharks at the Peruvian coast using CN SIA of muscle. Although no overlap of ellipses was found, the differences between the two groups were insignificant.

All the studies so far used $\delta^{13}C$ and $\delta^{15}N$ relying on the well-defined baseline differences between different carbon sources and trophic enrichment of nitrogen. Even though $\delta^{34}S$ has been found to be a highly discriminative ecological tracer, only 9 publications with the topic *"(sulfur OR sulphur) stable isotope analysis (elasmobranchs* OR shark*)"* were found, with merely four focusing on sharks as their main study species. Most of these studies reiterate the feasibility of sulphur stable isotope analysis. Sulphur traditionally needed to be analysed from a duplicate sample with more tissue because of its low concentration compared to carbon and nitrogen. This made the analysis more expensive, susceptible to errors and needing larger sample amounts (Hansen et al. 2009). Due to these complications, $\delta^{34}S$ is rarely analysed in ecological studies. Yet, the unique coupling of a high sensitivity elemental analyser to a conventional isotope ratio mass spectrometer (EA-IRMS/ GCC-IRMS) (Hansen et al. 2009) allows the analysis of C, N and S using very small tissue amounts. This not only makes the simultaneous study of C, N, S isotopes possible but also enables the successful analysis of minuscule tissue amounts sampled non-lethally during tagging studies.

While mucus stable isotope analysis of fish as a non-invasive tissue is just starting to gain a foothold the merits of this easily accessible epidermal layer are still lagging application in shark science. To my knowledge, only one study has been published on mucus SIA of elasmobranchs, namely giant manta rays (Burgess et al. 2018). The authors highlight the feasibility of the sampling and analysis which could prove extremely useful for closing existing ecological knowledge gaps of protected and sensitive shark species.

VI.6 Study questions

The regular aggregations of juveniles of both species around the islands of the Azores indicate the presence of EFHs, especially nursery areas (Afonso unpublished data, GAMPA 2020). As part of a larger project called “Island Shark”, telemetric, genetic, and video survey studies are being conducted and have started to unveil the fine-scale movement and population connectivity of these species around the archipelago (Afonso unpublished data). This data suggests a high use of coastal habitats with considerable spatial overlap of juveniles of both species indicating communal shark nurseries. With indications of different vertical habitat use and proximate availability of coastal, pelagic, and benthic prey, the respective dietary contributions could result in different niches. Thus, the importance of the local ecosystems for their diet, the trophic ecology and niche use of these sympatric sharks remain to be identified to coerce a comprehensive picture of EFHs and their putative communal nursery use around the archipelago. The existing ecological knowledge gaps can be summarised as:

1. **What roles do Azorean coastal systems play for the diet of tope and smooth hammerhead sharks and throughout their ontogeny?**
2. **Do the two species compete for resources and if so, in which life stages?**

Forming a collaboration between the sampling effort of the DOP of the Azores, and the advanced SIA facilities of the GEOMAR Helmholtz Centre for Ocean Research, Kiel, Germany, this study aims at elucidating the trophic ecology of tope and smooth hammerhead sharks and closing existing knowledge gaps. Using SIA of C, N and for the first time, S of muscle, mucus, and vertebrae centra has the potential to reveal novel and valuable insights about these shark species. Closing the knowledge gap of the trophic and ontogenetic role of the coastal Azores ecosystems for the Atlantic shark populations is of crucial importance for the definition of nurseries, essential fish habitats and resultingly the design of MPAs and efficient management of these charismatic predators.

As this study includes several novel experimental approaches, the study questions can be divided into biological- and methodological questions:

Biological questions

1) What characterises the trophic ecology of each species around the Azores?
    a) Do they rely more on coastal or pelagic resources?
    b) Do the species segregate based on sex?
    c) Does the diet change throughout the ontogeny, and if yes how?
2) How does the trophic ecology of the two species differ?
    a) Are niches partitioned or do the species compete?

➔ Do these conclusions differ from studies of continental/pelagic environments?

Hypotheses:

Based on established evolutionary models of trophic variation (see Figgener et al. 2019 and references therein) I expect the highest trophic variation between the two species, decreasing with declining competition and evolutionary differentiation (see Figure VI.5).

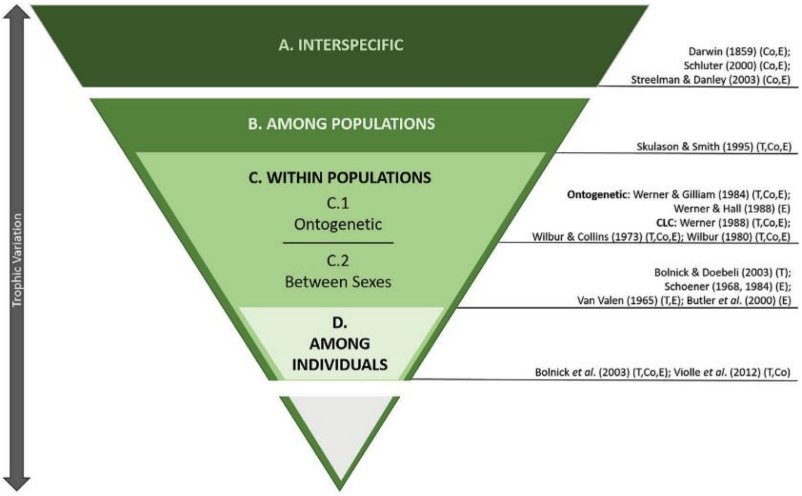

Figure VI.5 - Conceptual model of the nested hierarchy of trophic variation by Figgener et al. (2019) with conceptual (Co), theoretical (T) and empirical (E) respective studies, CLC are complex life cycles. In this study, I cover the levels C, D and A.

Existing information (telemetry, baited remote underwater videos and experimental fishing (Afonso unpublished data)) reveals different local depth distributions of both juvenile and adult stages of both species respectively. I hypothesize juvenile smooth hammerheads to occupy shallower water depths and feeding on more pelagic sources (higher $\delta^{13}C$, $\delta^{34}S$) and juvenile tope sharks mostly below 30 m with a predominantly benthic diet (lower $\delta^{34}S$). As the sharks leave the coastal nursery grounds, I hypothesise these differences to become more pronounced with age as adult topes are most frequently caught at depths down to 650 m around the Azores (Santos et al. 2020) (high $\delta^{13}C$, low $\delta^{34}S$), while the smooth hammerhead sharks stay in the upper water column (Santos and Coelho 2019) with the proportional importance of pelagic food sources increasing (low $\delta^{13}C$, high $\delta^{34}S$) (see Figure VI.4 and Figure VI.6). Hence, I expect a higher level of niche overlap of the two species during juvenile life stages with increasing niche partitioning throughout their life cycle.

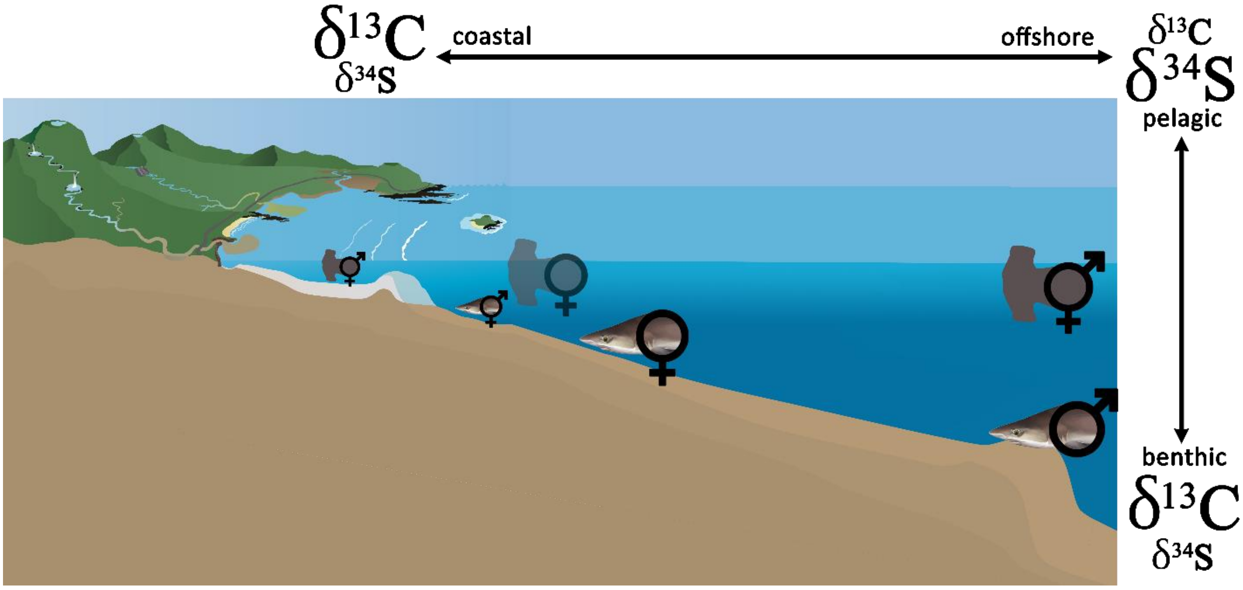

Figure VI.6 - Conceptual model of the habitat use of tope (G. galeus) and smooth hammerhead shark (S. zygaena) along common $\delta^{13}C$ and $\delta^{34}S$ gradients.

Intra-specific differences have been found in both species as ontogenetic shifts (Thorburn et al. 2019, Rosende-Pereiro et al. 2020) and sexual segregation (Compagno 1984, Lucifora et al. 2004). I expect a pronounced shift in trophic level (increasing $\delta^{15}N$) as both species grow and consume higher trophic level prey. Furthermore, as explained above, the shift to offshore waters (and deeper waters for tope) are expected to be reflected as an ontogenetic shift in $\delta^{13}C$ and $\delta^{34}S$.

While no study of smooth hammerheads has found significant differences between sex, male tope sharks have been found to stay further offshore in slope proximity (lower $\delta^{13}C$), while the females stay further inshore (higher $\delta^{13}C$) which could be reflected in the ^{13}C signatures.

The analysis of 12 framework samples of the coastal and pelagic food web is expected to reveal which isotopes reflect the most pronounced gradients in the local ecosystems and inform about the signature characteristics of the two endpoints, putting otherwise relative values into perspective.

Methodological study questions

To the best of our knowledge, this study constitutes the first $\delta^{34}S$ SIA of these two study species and the first CNS analysis of shark mucus. While this study was not designed to assess methodological questions the novelty of the approach leaves me to answer some ground-laying questions that ought to be addressed for future studies:

- How does $\delta^{34}S$ perform compared to $\delta^{13}C$ and $\delta^{15}N$ and what gradients does it distinguish in the North-Atlantic?
- Is the SIA of mucus and vertebrae centra feasible in this context and how do they compare to muscle tissue?
- How can the non-lethal sampling of muscle and mucus SIA samples be conducted?

Hypotheses:

I expect sulphur to have high discriminatory power, potentially differentiating between benthic and pelagic sources and coastal and offshore diet more robustly than carbon. As observed in other studies, I also expect a larger variation of sulphur within groups (McCutchan et al. 2003, Connolly et al. 2004).

Characterizing the trophic ecology and habitat use of the two study species using these novel approaches, could provide major contributions to the closure of existing knowledge gaps and inform management about the priorities of EFH in the Azores.

VII References

Abecasis, R. C., P. Afonso, A. Colaço, N. Longnecker, J. Clifton, L. Schmidt, and R. S. Santos. 2015. Marine conservation in the Azores: evaluating marine protected area development in a remote island context. Frontiers in Marine Science **2**:104.

Afonso, P. Unpublished data

Afonso, P., J. Fontes, E. Giacomello, M. C. Magalhães, H. R. Martins, T. Morato, V. Neves, R. Prieto, R. S. Santos, and M. A. Silva. 2020. The Azores: a mid-Atlantic hotspot for marine megafauna research and conservation. Frontiers in Marine Science.

Afonso, P., F. Vandeperre, J. Fontes, and F. Porteiro. 2014a. Conservation of pelagic elasmobranchs in the Azores. INTERNET RESOURCES:25.

Afonso, P., F. Vandeperre, J. Fontes, and F. Porteiro. 2014b. Conservation of pelagic elasmobranchs in the Azores. Page 25 *in* H. R. Martins, editor. ARQUIPELAGO Life and Marine Sciences. The sea of the Azores: scientific forum for decision support. University of the Azores.

Alfaro-Cordova, E., A. D. Solar, A. Gonzalez-Pestana, N. Acuña-Perales, J. Coasaca, F. Cordova-Zavaleta, J. Alfaro-Shigueto, and J. C. Mangel. 2018. Isotopic niches of four commercially important pelagic elasmobranch species captured by the small-scale driftnet fishery of northern Peru. Latin american journal of aquatic research **46**:482-488.

Amorim, P., A. D. Perán, C. K. Pham, M. Juliano, F. Cardigos, F. Tempera, and T. Morato. 2017. Overview of the ocean climatology and its variability in the Azores region of the North Atlantic including environmental characteristics at the seabed. Frontiers in Marine Science **4**:56.

Baum, J. K., R. A. Myers, D. G. Kehler, B. Worm, S. J. Harley, and P. A. J. S. Doherty. 2003. Collapse and conservation of shark populations in the Northwest Atlantic. **299**:389-392.

Bethea, D. M., J. A. Buckel, and J. K. Carlson. 2004. Foraging ecology of the early life stages of four sympatric shark species. Marine Ecology Progress Series **268**:245-264.

Bornatowski, H., A. F. Navia, R. R. Braga, V. Abilhoa, and M. F. M. Corrêa. 2014. Ecological importance of sharks and rays in a structural foodweb analysis in southern Brazil. ICES Journal of Marine Science **71**:1586-1592.

Botto, F., E. Gaitán, H. Mianzan, M. Acha, D. Giberto, A. Schiariti, and O. Iribarne. 2011. Origin of resources and trophic pathways in a large SW Atlantic estuary: an evaluation using stable isotopes. Estuarine, Coastal and Shelf Science **92**:70-77.

Brosnan, J. T., and M. E. Brosnan. 2006. The sulfur-containing amino acids: an overview. The Journal of nutrition **136**:1636S-1640S.

Burgess, K., M. Guerrero, A. Richardson, M. Bennett, and A. Marshall. 2018. Use of epidermal mucus in elasmobranch stable isotope studies: a pilot study using the giant manta ray (Manta birostris). Marine & Freshwater Research **69**:336-342.

Caldeira, R., and J. C. Reis. 2017. The Azores confluence zone. Frontiers in Marine Science **4**:37.

Campana, S. E., L. J. Natanson, and S. Myklevoll. 2002. Bomb dating and age determination of large pelagic sharks. Canadian Journal of Fisheries and Aquatic Sciences **59**:450-455.

Cardeñosa, D., A. T. Fields, E. A. Babcock, H. Zhang, K. Feldheim, S. K. Shea, G. A. Fischer, and D. D. Chapman. 2018. CITES-listed sharks remain among the top species in the contemporary fin trade. Conservation Letters **11**:e12457.

Carlisle, A. B., S. Y. Litvin, D. J. Madigan, K. Lyons, J. S. Bigman, M. Ibarra, and J. J. Bizzarro. 2017. Interactive effects of urea and lipid content confound stable isotope analysis in elasmobranch fishes. Canadian Journal of Fisheries Aquatic Sciences **74**:419-428.

Casper, B. M., Domingo, A., Gaibor, N., Heupel, M.R., Kotas, E., Lamónaca, A.F., Pérez-Jimenez, J.C., Simpfendorfer, C., Smith, W.D., Stevens, J.D., Soldo, A. & Vooren. 2009. Sphyrna zygaena. The IUCN Red List of Threatened Species. last accessed 12 November 2018

Castro, J. I., C. M. Woodley, and R. L. Brudek. 1999. A preliminary evaluation of the status of shark species. Food & Agriculture Org.

Chabot, C. 2015. Microsatellite loci confirm a lack of population connectivity among globally distributed populations of the tope shark Galeorhinus galeus (Triakidae). Journal of Fish Biology **87**:371-385.

Church, M. R., J. L. Ebersole, K. M. Rensmeyer, R. B. Couture, F. T. Barrows, and D. L. Noakes. 2009. Mucus: a new tissue fraction for rapid determination of fish diet switching using stable isotope analysis. Canadian Journal of Fisheries and Aquatic Sciences **66**:1-5.

Coelho, R., J. Fernandez-Carvalho, P. G. Lino, and M. N. Santos. 2012. An overview of the hooking mortality of elasmobranchs caught in a swordfish pelagic longline fishery in the Atlantic Ocean. Aquatic Living Resources **25**:311-319.

Colloca, F., D. Scannella, M. L. Geraci, F. Falsone, G. BATISTA, S. Vitale, and M. Di Lorenzo. 2019. British sharks in Sicily: records of long distance migration of tope shark (Galeorhinus galeus) from North-eastern Atlantic to Mediterranean Sea. Mediterranean Marine Science **20**.

Compagno, L. 1998. Sphyrnidae. Hammerhead and bonnethead sharks. FAO identification guide for fishery purposes. The Living Marine Resources of the Western Central Pacific. FAO, Rome. pp:1361-1366.

Compagno, L. J. 1984. Sharks of the world: an annotated and illustrated catalogue of shark species known to date.

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

Cortés, E. 1999. Standardized diet compositions and trophic levels of sharks. ICES Journal of Marine Science **56**:707-717.

Cox, G. J., and M. Francis. 1997. Sharks and rays of New Zealand. Canterbury University Press.

Curnick, D. J., A. B. Carlisle, M. J. Gollock, R. J. Schallert, and N. E. Hussey. 2019. Evidence for dynamic resource partitioning between two sympatric reef shark species within the British Indian Ocean Territory. Journal of Fish Biology **94**:680-685.

Das, D., and P. Afonso. 2017. Review of the diversity, ecology, and conservation of elasmobranchs in the Azores region, mid-north Atlantic. Frontiers in Marine Science **4**:354.

Davenport, S. R., and N. J. Bax. 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.

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

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

Domi, N., J.-M. Bouquegneau, and K. Das. 2005. Feeding ecology of five commercial shark species of the Celtic Sea through stable isotope and trace metal analysis. Marine Environmental Research **60**:551-569.

Dulvy, N. K., S. L. Fowler, J. A. Musick, R. D. Cavanagh, P. M. Kyne, L. R. Harrison, J. K. Carlson, L. N. Davidson, S. V. Fordham, M. P. Francis, C. M. Pollock, C. A. Simpfendorfer, G. H. Burgess, K. E. Carpenter, L. J. Compagno, D. A. Ebert, C. Gibson, M. R. Heupel, S. R. Livingstone, J. C. Sanciangco, J. D. Stevens, S. Valenti, and W. T. White. 2014. Extinction risk and conservation of the world's sharks and rays. Elife **3**:e00590.

Dureuil, M., and B. Worm. 2015. Estimating growth from tagging data: an application to north-east Atlantic tope shark Galeorhinus galeus. Journal of Fish Biology **87**:1389-1410.

Ebert, D. 2003. Sharks, rays, and chimaeras of California. Univ of California Press.

Ebert, D. A. 2014. On Board Guide for the Identification of Pelagic Sharks and Rays of the Western Indian Ocean. FAO.

Ebert, D. A., and M. F. Stehmann. 2013. Sharks, batoids and chimaeras of the North Atlantic. FAO, Roma (Italia).

Ellis, J., S. McCully Phillips, and F. Poisson. 2017. A review of capture and post-release mortality of elasmobranchs. Journal of Fish Biology **90**:653-722.

Ellis, J., M. Pawson, and S. Shackley. 1996. The comparative feeding ecology of six species of shark and four species of ray (Elasmobranchii) in the north-east Atlantic. Journal of the Marine Biological Association of the United Kingdom **76**:89-106.

Estrada, J. A., A. N. Rice, M. E. Lutcavage, and G. B. Skomal. 2003. Predicting trophic position in sharks of the north-west Atlantic Ocean using stable isotope analysis. Journal of the Marine Biological Association of the United Kingdom **83**:1347-1350.

Estrada, J. A., A. N. Rice, L. J. Natanson, and G. B. Skomal. 2006. Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. Ecology **87**:829-834.

Estupiñán-Montaño, C., L. Cedeño-Figueroa, J. F. Estupiñán-Ortiz, F. Galván-Magaña, A. Sandoval-Londoño, D. Castañeda-Suarez, and C. J. Polo-Silva. 2019. Feeding habits and trophic level of the smooth hammerhead shark, Sphyrna zygaena (Carcharhiniformes: Sphyrnidae), off Ecuador. Journal of the Marine Biological Association of the United Kingdom **99**:673-680.

Félix-López, D. G., N. Bolaño-Martinez, P. Díaz-Jaimes, E. C. Oñate-González, J. S. Ramírez-Pérez, E. García-Rodríguez, D. Corro-Espinosa, J. E. Osuna-Soto, and N. C. Saavedra-Sotelo. 2019. Possible female philopatry of the smooth hammerhead shark Sphyrna zygaena revealed by genetic structure patterns. Journal of Fish Biology **94**:671-679.

Ferretti, F., A. Soldo, R. Walls, B. Casper, A. Domingo, N. Gaibor, M. R. Heupel, J. Kotas, A. Lamónaca, W. D. Smith, J. Stevens, Vooren, , and J. C.M. & Pérez-Jiménez. 2015. Sphyrna zygaena. The IUCN Red List of Threatened Species 2015. last accessed 05 September 2020

Ferretti, F., B. Worm, G. L. Britten, M. R. Heithaus, and H. K. Lotze. 2010. Patterns and ecosystem consequences of shark declines in the ocean. Ecol Lett **13**:1055-1071.

Figgener, C., J. Bernardo, and P. T. Plotkin. 2019. Beyond trophic morphology: stable isotopes reveal ubiquitous versatility in marine turtle trophic ecology. Biological Reviews **94**:1947-1973.

Fitzmaurice, P., G. Keirse, P. Green, and M. Clarke. 2003. Tope Tagging in Irish Waters (1970-2002). Central Fisheries Board.

Florin, S. T., L. A. Felicetti, and C. T. Robbins. 2011. The biological basis for understanding and predicting dietary-induced variation in nitrogen and sulphur isotope ratio discrimination. Functional Ecology **25**:519-526.

France, R. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Marine Ecology Progress Series **124**:307-312.

Francis, M. 2016. Distribution, habitat and movement of juvenile smooth hammerhead sharks (Sphyrna zygaena) in northern New Zealand. New Zealand Journal of Marine and Freshwater Research **50**:506-525.

Frisk, M. G., T. J. Miller, and N. K. Dulvy. 2005. Life Histories and Vulnerability to Exploitation of Elasmobranchs: Inferences from Elasticity, Perturbation and Phylogenetic Analyses. Journal of Northwest Atlantic Fishery Science **35**:27-45.

Froese, R., and D. Pauly. 2010. FishBase. https://www.fishbase.de/ last accessed 04.10.2020

Fry, B. 1983. Fish and shrimp migrations in the northern Gulf of Mexico analyzed using stable C, N, and S isotope ratios. Fish Bull **81**:789-800.

Fry, B., and E. B. Sherr. 1989. δ 13 C measurements as indicators of carbon flow in marine and freshwater ecosystems. Pages 196-229 Stable isotopes in ecological research. Springer.

Gallagher, A. J., N. Hammerschlag, D. S. Shiffman, and S. T. Giery. 2014. Evolved for Extinction: The Cost and Conservation Implications of Specialization in Hammerhead Sharks. BioScience **64**:619-624.

GAMPA. 2020. Coastal Fisheries Resources of the Azores: an X-Ray. IMAR/Okeanos.

Gonzalez-Pestana, A., N. Acuna-Perales, J. Coasaca-Cespedes, F. Cordova-Zavaleta, J. Alfaro-Shigueto, J. C. Mangel, and P. Espinoza. 2017. Trophic ecology of the smooth hammerhead shark (Sphyrna zygaena) off the coast of northern Peru. Fishery Bulletin **115**.

Hansen, T., A. Burmeister, and U. Sommer. 2009. Simultaneous δ15N, δ13C and δ34S measurements of low-biomass samples using a technically advanced high sensitivity elemental analyzer connected to an isotope ratio mass spectrometer. Rapid Communications in Mass Spectrometry **23**:3387-3393.

Heady, W. N., and J. W. Moore. 2013. Tissue turnover and stable isotope clocks to quantify resource shifts in anadromous rainbow trout. Oecologia **172**:21-34.

Heithaus, M. R., A. Wirsing, and L. Dill. 2012. The ecological importance of intact top-predator populations: a synthesis of 15 years of research in a seagrass ecosystem. Marine and Freshwater Research **63**:1039-1050.

Hesslein, R., M. Capel, D. Fox, and K. Hallard. 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.

Heupel, M. R., J. K. Carlson, and C. A. Simpfendorfer. 2007. Shark nursery areas: concepts, definition, characterization and assumptions. Marine Ecology Progress Series **337**:287-297.

Hobson, K. A. 1993. Trophic relationships among high Arctic seabirds: insights from tissue-dependent stable-isotope models. Marine Ecology-Progress Series **95**:7-7.

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

Hobson, K. A., and R. G. Clark. 1992. Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. The Condor **94**:189-197.

Holden, M., and R. Horrod. 1979. The migrations of tope, Galeorhinus galeus (L), in the eastern North Atlantic as determined by tagging. ICES Journal of Marine Science **38**:314-317.

Hussey, N. E., J. Brush, I. D. McCarthy, and A. T. Fisk. 2009. δ15N and δ13C diet–tissue discrimination factors for large sharks under semi-controlled conditions. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology **155**:445-453.

Hussey, N. E., S. F. Dudley, I. D. McCarthy, G. Cliff, and A. T. Fisk. 2011. Stable isotope profiles of large marine predators: viable indicators of trophic position, diet, and movement in sharks? Canadian Journal of Fisheries and Aquatic Sciences **68**:2029-2045.

Hussey, N. E., M. A. MacNeil, J. A. Olin, B. C. McMeans, M. J. Kinney, D. D. Chapman, and A. T. Fisk. 2012. Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. J Fish Biol **80**:1449-1484.

Hutchinson, G. E. 1957. Cold spring harbor symposium on quantitative biology. Concluding remarks **22**:415-427.

ICCAT. 2008. Recommendation by ICCAT on hammerhead sharks (Family Sphyrnidae) caught in association with fisheries managed by ICCAT.

Jackson, J. B., M. X. Kirby, W. H. Berger, K. A. Bjorndal, L. W. Botsford, B. J. Bourque, R. H. Bradbury, R. Cooke, J. Erlandson, and J. A. Estes. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science **293**:629-637.

Kiljunen, M., J. Grey, T. Sinisalo, C. Harrod, H. Immonen, and R. I. Jones. 2006. A revised model for lipid-normalizing δ13C values from aquatic organisms, with implications for isotope mixing models. Journal of applied ecology **43**:1213-1222.

Kim, S. L., D. R. Casper, F. Galván-Magaña, R. Ochoa-Díaz, S. B. Hernández-Aguilar, and P. L. Koch. 2011. Carbon and nitrogen discrimination factors for elasmobranch soft tissues based on a long-term controlled feeding study. Environmental Biology of Fishes **95**:37-52.

Kim, S. L., C. M. del Rio, D. Casper, and P. L. Koch. 2012. Isotopic incorporation rates for shark tissues from a long-term captive feeding study. J Exp Biol **215**:2495-2500.

Kim, S. L., and P. L. Koch. 2012. Methods to collect, preserve, and prepare elasmobranch tissues for stable isotope analysis. Environmental Biology of Fishes **95**:53-63.

Kinney, M. J. 2011. The communal nursery area paradigm revisited: niche overlap versus niche separation among juvenile shark species of Cleveland Bay. James Cook University.

Kinney, M. J., N. E. Hussey, A. T. Fisk, A. J. Tobin, and C. A. Simpfendorfer. 2011. Communal or competitive? Stable isotope analysis provides evidence of resource partitioning within a communal shark nursery. Marine Ecology Progress Series **439**:263-276.

Knip, D. M., M. R. Heupel, and C. A. Simpfendorfer. 2010. Sharks in nearshore environments: models, importance, and consequences. Marine Ecology Progress Series **402**:1-11.

Laws, E. A., B. N. Popp, R. R. Bidigare, M. C. Kennicutt, and S. A. Macko. 1995. Dependence of phytoplankton carbon isotopic composition on growth rate and [CO2) aq: theoretical considerations and experimental results. Geochimica et cosmochimica acta **59**:1131-1138.

Li, Y., Y. Zhang, N. E. Hussey, and X. Dai. 2016. Urea and lipid extraction treatment effects on δ15N and δ13C values in pelagic sharks. Rapid Communications in Mass Spectrometry **30**:1-8.

Logan, J. M., T. D. Jardine, T. J. Miller, S. E. Bunn, R. A. Cunjak, and M. E. Lutcavage. 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. Journal of Animal Ecology **77**:838-846.

Logan, J. M., and M. E. Lutcavage. 2010. Stable isotope dynamics in elasmobranch fishes. Hydrobiologia **644**:231-244.

Logan, R. K., J. J. Vaudo, L. L. Sousa, M. Sampson, B. M. Wetherbee, and M. S. Shivji. 2020. Seasonal Movements and Habitat Use of Juvenile Smooth Hammerhead Sharks in the Western North Atlantic Ocean and Significance for Management. Frontiers in Marine Science **7**.

Loor-Andrade, P., F. Galván-Magaña, F. R. Elorriaga-Verplancken, C. Polo-Silva, and A. Delgado-Huertas. 2015. Population and individual foraging patterns of two hammerhead sharks using carbon and nitrogen stable isotopes. Rapid Communications in Mass Spectrometry **29**:821-829.

Lucifora, L. O., V. B. García, R. C. Menni, and A. H. Escalante. 2006. Food habits, selectivity, and foraging modes of the school shark Galeorhinus galeus. Marine Ecology Progress Series **315**:259-270.

Lucifora, L. O., R. C. Menni, and A. H. Escalante. 2004. Reproductive biology of the school shark, Galeorhinus galeus, off Argentina: support for a single south western Atlantic population with synchronized migratory movements. Environmental Biology of Fishes **71**.

MacNeil, M. A., K. G. Drouillard, and A. T. Fisk. 2006. Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. Canadian Journal of Fisheries and Aquatic Sciences **63**:345-353.

MacNeil, M. A., G. B. Skomal, and A. T. Fisk. 2005. Stable isotopes from multiple tissues reveal diet switching in sharks. Marine Ecology Progress Series **302**:199-206.

Malpica-Cruz, L., S. Z. Herzka, O. Sosa-Nishizaki, and J. P. Lazo. 2012. Tissue-specific isotope trophic discrimination factors and turnover rates in a marine elasmobranch: empirical and modeling results. Canadian Journal of Fisheries and Aquatic Sciences **69**:551-564.

Maruyama, A., E. Tanahashi, T. Hirayama, and R. Yonekura. 2017. A comparison of changes in stable isotope ratios in the epidermal mucus and muscle tissue of slow-growing adult catfish. Ecology of Freshwater Fish **26**:636-642.

McCutchan, J. H., W. M. Lewis, C. Kendall, and C. C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos **102**:378-390.

McMahon, K. W., L. L. Hamady, and S. R. Thorrold. 2013. A review of ecogeochemistry approaches to estimating movements of marine animals. Limnology and Oceanography **58**:697-714.

Mekhtiyeva, V., M. VL, M. VL, P. RG, and G. Y. YA. 1976. Distributions and isotopic compositions of forms of sulfur in water animals and plants.

Miller, T. W., R. D. Brodeur, G. Rau, and K. Omori. 2010. Prey dominance shapes trophic structure of the northern California Current pelagic food web: evidence from stable isotopes and diet analysis. Marine Ecology Progress Series **420**:15-26.

Mittermayr, A., S. E. Fox, and U. Sommer. 2014. Temporal variation in stable isotope composition (δ13C, δ15N and δ34S) of a temperate Zostera marina food web. Marine Ecology Progress Series **505**:95-105.

Morato, T., E. Solà, M. P. Grós, and G. Menezes. 2003. Diets of thornback ray (Raja clavata) and tope shark (Galeorhinus galeus) in the bottom longline fishery of the Azores, northeastern Atlantic. Fishery Bulletin **101**:590-602.

Myers, R. A., J. K. Baum, T. D. Shepherd, S. P. Powers, and C. H. Peterson. 2007. Cascading effects of the loss of apex predatory sharks from a coastal ocean. Science **315**:1846-1850.

Ochoa Díaz, M. R. 2009. Espectro trófico del tiburón martillo Sphyrna zygaena (Linnaeus, 1758) en Baja California Sur: Aplicación de δ13C y δ 15N. Instituto Politécnico Nacional. Centro Interdisciplinario de Ciencias Marinas.

Perry, R. I., P. A. Thompson, D. L. Mackas, P. J. Harrison, and D. R. Yelland. 1999. Stable carbon isotopes as pelagic food web tracers in adjacent shelf and slope regions off British Columbia, Canada. Canadian Journal of Fisheries and Aquatic Sciences **56**:2477-2486.

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

Peterson, B. J., R. W. Howarth, and R. H. Garritt. 1986. Sulfur and carbon isotopes as tracers of salt-marsh organic matter flow. Ecology **67**:865-874.

Pinnegar, J. K., S. Jennings, C. O'brien, and N. Polunin. 2002. Long-term changes in the trophic level of the Celtic Sea fish community and fish market price distribution. Journal of applied ecology **39**:377-390.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**:703-718.

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

Red-List, I. 2018. The IUCN Red-list of Threatened Species. http://www.iucnredlist.org last accessed 04.10,2020

Rosenberg, A., T. E. Bigford, S. Leathery, R. L. Hill, and K. Bickers. 2000. Ecosystem approaches to fishery management through essential fish habitat. Bulletin of marine science **66**:535-542.

Rosende-Pereiro, A., J. R. Flores-Ortega, G. González-Sansón, and A. Corgos. 2020. Stomach content and stable isotopes reveal an ontogenetic dietary shift of young-of-the-year scalloped hammerhead sharks (Sphyrna lewini) inhabiting coastal nursery areas. Environmental Biology of Fishes **103**:49-65.

Santos, C. C., and R. Coelho. 2018. Migrations and habitat use of the smooth hammerhead shark (Sphyrna zygaena) in the Atlantic Ocean. PloS one **13**.

Santos, C. C., and R. Coelho. 2019. Distribution patterns and indicators of the smooth hammerhead shark (Sphyrna zygaena) in the Atlantic Ocean. Fisheries Research **212**:107-113.

Santos, C. C. N. d. 2017. Distribution patterns and habitat use of the smooth hammerhead shark (Sphyrna zygaena) in the Atlantic Ocean. Universidade de Lisboa.

Santos, R., A. Novoa-Pabon, H. Silva, and M. Pinho. 2020. Elasmobranch species richness, fisheries, abundance and size composition in the Azores archipelago (NE Atlantic). Marine Biology Research **16**:103-116.

Seubert, E. A., N. Hussey, S. P. Powers, J. F. Valentine, and J. M. Drymon. 2019. Assessing trophic flexibility of a predator assemblage across a large estuarine seascape using blood plasma stable isotope analysis. Food Webs **21**:e00132.

Shipley, O. N., and P. Matich. 2020. Studying animal niches using bulk stable isotope ratios: an updated synthesis. Oecologia:1-25.

Smale, M. J. 2010. Occurrence and feeding of three shark species,Carcharhinus brachyurus, C. obscurus and Sphyrna zygaena, on the Eastern Cape coast of South Africa. South African Journal of Marine Science **11**:31-42.

Springer, S. 1967. Social organization of shark population. Sharks, skate and rays:149-174.

Thorburn, J., F. Neat, I. Burrett, L.-A. Henry, D. M. Bailey, C. S. Jones, and L. R. Noble. 2019. Ontogenetic Variation in Movements and Depth Use, and Evidence of Partial Migration in a Benthopelagic Elasmobranch. Frontiers in Ecology and Evolution **7**.

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

Torres, P., R. T. da Cunha, R. Maia, and A. dos Santos Rodrigues. 2014. Trophic ecology and bioindicator potential of the North Atlantic tope shark. Science of the Total Environment **481**:574-581.

Trueman, C. N., and K. S. J. Glew. 2019. Isotopic tracking of marine animal movement. Pages 137-172 Tracking animal migration with stable isotopes. Elsevier.

Vaz, S., and O. Le Pape. 2019. Quantitative Mapping of Fish Habitat: From Knowledge to Spatialised Fishery Management. Pages 313-323 Oceanography Challenges to Future Earth. Springer.

Walker, T. 1999. Galeorhinus galeus fisheries of the world. FAO Fisheries Technical Paper.

Walker, T. I., Rigby, C.L., Pacoureau, N., Ellis, J., Kulka, D.W., Chiaramonte, G.E. & Herman, K. . 2020. *Galeorhinus galeus*. The IUCN Red List of Threatened Species **2020: e.T39352A2907336**.

Weidel, B. C., S. R. Carpenter, J. F. Kitchell, and M. J. Vander Zanden. 2011. Rates and components of carbon turnover in fish muscle: insights from bioenergetics models and a whole-lake 13C addition. Canadian Journal of Fisheries and Aquatic Sciences **68**:387-399.

Worm, B., B. Davis, L. Kettemer, C. A. Ward-Paige, D. Chapman, M. R. Heithaus, S. T. Kessel, and S. H. Gruber. 2013. Global catches, exploitation rates, and rebuilding options for sharks. Marine Policy **40**:194-204.

VIII Manuscript

VIII.1 Title page

Title: Trophic ecology and coastal habitat use of two sympatric shark species in the Azores using CNS stable isotope analysis.

Author: Carl Robert Priester [1]

1 - Faculdade de Ciências e Tecnologia, Universidade do Algarve, 8005-139 Faro, Portugal

Keywords: Stable isotope analysis, trophic ecology, essential fish habitats, shark nursery, *Sphyrna zygaena*, *Galeorhinus galeus*

VIII.2 Abstract

The anthropogenic exploitation of marine resources has severely altered ecosystems and caused drastic declines of large predatory fishes. Amongst these, sharks are the species of major conservation concern due to their critical role as top predators and high vulnerability to overfishing. The Azores are an oceanic archipelago in the mid-North Atlantic thought to serve as essential fish habitat (EFH) for some oceanic or semi-oceanic sharks such as coastal pupping and nursery grounds for tope (*Galeorhinus galeus*, Linneaus 1758) and smooth hammerhead (*Sphyrna zygaena*, Linneaus 1758) sharks. Yet, to date, the dependency of those juvenile sharks on coastal resources has not been investigated and crucial information on their trophic ecology is missing. This knowledge gap is relevant as it would allow to ascertain the importance of these areas for conservation and help developing management plans.

Here, simultaneous $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ (CNS) stable isotope analysis is used to investigate the trophic ecology, ontogenetic shifts and habitat use of the coastal life stages of *G. galeus* and *S. zygeana* around the Azores. A Bayesian ellipse approach (nicheROVER) and generalized additive mixed models (GAMM) are applied and interpreted in reference to isotope values of coastal and pelagic food web samples. The results show high diet overlap between tope and juvenile smooth hammerhead sharks with coastal-associated values. Tope shark showed a significant ontogenetic shift to higher trophic level coastal-benthic prey with growing size. Smooth hammerhead sharks exhibited significant decreases in $\delta^{34}S$, also suggesting a shift towards more coastal-benthic prey with increasing size. The diet of both species support their co-occurrence in shared nurseries with no evidence of sexual segregation or interspecific niche partitioning, but instead highly trophic competition, emphasizing the importance of healthy coastal habitats for conservation of these highly mobile sharks in the wider Atlantic.

VIII.3 Introduction

In recent decades, overfishing and climate change have caused collapses of coastal ecosystems (Jackson et al. 2001) often resulting in a rapid decline of large predatory elasmobranchs (Baum et al. 2003, Worm et al. 2013, Dulvy et al. 2014). Due to their conservative life-history characteristics such as low fecundity, late maturity and wide-ranging migratory patterns (Frisk et al. 2005, Dulvy et al. 2014, Gallagher et al. 2014), sharks are particularly vulnerable to anthropogenic threats. Large predators take up essential roles in ecosystems around the world, balancing and controlling the populations of lower trophic levels (Heithaus et al. 2012) and their declines can evoke cascading top-down effects often reverberating through entire food webs (Myers et al. 2007, Ferretti et al. 2010, Heithaus et al. 2012). Consequently, their loss has been termed the most pervasive anthropogenic impact on nature (Estes et al. 2011). Due to the highly mobile nature and vast distribution extending across Exclusive Economic Zones, many shark populations remain data deficient or poorly assessed (Castro et al. 1999, Red-List 2018). which is hampering elasmobranch research and conservation efforts (Castro et al. 1999). Essential fish habitats (EFH) are habitats with key roles for reproduction, diet, or growth of fish populations. Nursery areas are one form of EFH and commonly characterised by regular and high abundances of juveniles and mature female sharks (Heupel et al. 2007) and are sometimes used communally by several species of sharks (Simpfendorfer and Milward 1993). If implemented correctly, the integration of communal shark nurseries into ecosystem-based management has been emphasised as a highly effective measure (Heupel et al. 2007, Kinney 2011). The Azores islands provide putative essential fish habitats to a variety of megafauna species (Afonso et al. 2020). Nevertheless, information about the coastal habitat use and diet throughout the ontogeny of many shark species is absent or scarce leaving acute knowledge gaps about the location of EFH and nursery areas.

The Azores are an oceanic archipelago situated in the Mid-North Atlantic within the confluence of productive temperate waters and the subtropical Azores current (Amorim et al. 2017, Caldeira and Reis 2017). Owing to its large abundance of elasmobranchs and cetaceans, the islands have been identified as a marine megafauna hotspot in the north Atlantic with increased conservation priority, offering essential habitats to a variety of resident and migratory species (Afonso et al. 2020). As part of their proposed action plan, Afonso et al. (2020) call for a multidisciplinary investigation on the location and driving processes of essential megafauna habitats to establish no-take MPAs and reduce the impact of fisheries. The tope shark *Galeorhinus galeus* (Linneaus 1758) and the smooth hammerhead *Sphyrna zyganea* (Linneaus 1758), can be found year-round in coastal waters. Regular aggregations of young-of-the-year and juveniles in shallow waters (Afonso unpublished data) suggest the presence of nurseries and pupping grounds (Afonso et al. 2014). The exploitation or habitat degradation of these essential fish habitats could have severe reverberations for the greater Atlantic populations of the two species and recent studies reiterate the urgent need for further research and management (Das and Afonso 2017, Afonso et al. 2020, GAMPA 2020, Santos et al. 2020).

The tope shark is a cosmopolitan predator that can be found in temperate coastal and shelf-waters (Cox and Francis 1997, Ebert and Stehmann 2013). It is characterised by slow growth, late maturity (Dureuil and Worm 2015) and aplacental viviparous reproduction (Compagno 1984). Large aggregations of juveniles have been found in shallow waters, while adults remain closer to the shelf down to 1100 m (Compagno 1984, Santos et al. 2020) and are capable of long-distance oceanic migrations (Holden and Horrod 1979, Colloca et al. 2019, Thorburn et al. 2019). While the North-East Atlantic population is listed as "data deficient" on the IUCN Red List (Walker 2006), the Azorean coastal fisheries monitoring report (GAMPA 2020) identified tope sharks as the most vulnerable coastal species with a severe decline in landings in recent years.

The smooth hammerhead (*Sphyrna zygaena*) is a coastal-pelagic predator and can be found circumglobally in tropic to temperate waters down to depths of 250 m (Compagno 1984, Santos and Coelho 2018). Large aggregations of juveniles have been observed (Compagno 1998), while the semi-oceanic adults live further offshore being capable of long migrations. Due to common misidentification with scalloped hammerhead (*Sphyrna lewini*) and less dedicated research, comparatively little is known about the smooth hammerhead's ecology (Camhi et al. 2009). Several studies have found "precipitous declines" in hammerhead sharks, often being the most pronounced of all studied sharks (see Gallagher et al. 2014 and references therein). While the latest European assessment was listed as "data deficient" (Ferretti et al. 2015), the most recent IUCN assessment classified tope shark as "critically endangered" (Walker 2020). Smooth hammerhead is globally listed as "vulnerable" on the IUCN red list (Casper 2009). Since 2014, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) prohibits the trade of smooth hammerhead sharks and all associated products (CITES 2020). In the Atlantic Ocean, the landing and retention of smooth hammerheads are prohibited in fisheries associated with ICCAT (ICCAT 2008). Evidence suggests, however, that these measures are not effective at curbing the decline of hammerheads as a) the illegal trade of valuable fins persists (Cardeñosa et al. 2018) and b) the hooking- and post release-mortality of *Sphyrna spp.* have been found to be high (Coelho et al. 2012, Ellis et al. 2017).

Telemetric and genetic data of both these species have been collected around the Azores to elucidate their habitat use and population structure. Nevertheless, essential information about the trophic ecology is lacking to evaluate the dietary importance of Azorean ecosystems for these vulnerable sharks, and vice versa.

Stable isotope analysis is a powerful and multifaceted tool with ever-increasing application in marine ecology (Post 2002, McMahon et al. 2013). It has been used successfully for more than three decades revealing novel insights into movement, trophic ecology, ontogenetic changes, and habitat use (Peterson and Fry 1987). Analysing the ratio of heavy to light stable isotopes within the protein matrix of tissues is commonly used to infer diet-related proxies based on the concept "*You are what you eat"* (Peterson and Fry 1987). Compared with traditional methods (such as stomach content analysis), stable isotope analysis merits the edification of assimilated sources, not merely ingested ones, and higher robustness at low sample sizes. Latter of which is especially desirable when working with protected and elusive species such as sharks. Knowledge about the distinct fractionation pathways of the different stable isotopes permits an ecological interpretation: $\delta^{13}C$ ($^{13}C/^{12}C$) has a very low fractionation when being assimilated and thus reflects the underlying differences in primary production (DeNiro and Epstein 1978, Fry and Sherr 1989). Pronounced and thus commonly used $\delta^{13}C$ gradients lie between coastal and offshore producers (Peterson and Fry 1987, Perry et al. 1999), benthic and pelagic producers (France 1995) and latitudinal differences (Laws et al. 1995, Trueman and Glew 2019). $\delta^{15}N$ ($^{15}N/^{14}N$) is consistently enriched upon each trophic transfer and thus often used to infer trophic positions (DeNiro and Epstein 1981, Hobson 1993). Similarly to carbon, $\delta^{34}S$ ($^{34}S/^{32}S$) has been found to undergo little fractionation in the food web, reflecting primary production sources (Mekhtiyeva et al. 1976, Peterson and Fry 1987). Studies have shown $\delta^{34}S$ to be a highly discriminative tracer distinguishing between pelagic vs. benthic and offshore vs. coastal primary production in cases and ecosystems that are tied using $\delta^{13}C$ (Connolly et al. 2004, Mittermayr et al. 2014). Nevertheless, due to high analytical costs, it is rarely used and to the best of our knowledge, no $\delta^{34}S$ stable isotope studies have been conducted on the two study species.

Stable isotopes can thus be used to infer bionomic and scenopoetic aspects of the post-competitive Eltonian niches (Elton 2001, Devictor et al. 2010) of organisms. Hutchinson (1957) defined ecological niches as the n-dimensional hypervolume where a stable population of a species can be sustained. Following this concept, the distribution and dispersion of stable isotopes can be analysed in a multidimensional space (see Swanson et al. 2015, Rossman et al. 2016). This allows the estimation and subsequent comparison of isotopic niche characteristics such as niche breadth, overlap and relative distance (Shipley and Matich 2020). The use of three, instead of the conventional two isotopes, adds a significant amount of detail, allowing the more accurate capture of ecological complexities (Swanson et al. 2015). Stable isotope values of potential food sources can add perspective to the results and be used to calculate the respective diet contributions using mixing models (e.g. R package SIAR (Parnell et al. 2010)).

The development of trophic ecology and habitat use throughout the ontogeny can be elucidated analysing samples of different age groups (see Li et al. 2016, Rosende-Pereiro et al. 2020). Here the turnover time of the sampled tissue dictates the temporal resolution through the integration of signals throughout this period (Tieszen et al. 1983). One approach to attain more temporal resolution is to analyse tissues that are resolved in a time-resolved manner (Hussey et al. 2012). Vertebrae centra are metabolically inert and grow as calcified layers, accreted over time, containing a high proportion of organic matrix (Ridewood and MacBride 1921, Campana et al. 2002). These attributes have been used successfully in elasmobranch ecology to reveal ontogenetic dynamics, making vertebrae SIA a lethal yet highly effective and powerful method (Estrada et al. 2006, Raoult et al. 2019). On the other end of the time-resolution spectrum lies the use of tissues with fast turnover times. Drawing on contrasting signatures following a diet shift the use of tissues with different turnover times is often recommended (MacNeil et al. 2005, Hussey et al. 2012). Commonly used elasmobranch tissues with relatively fast turnover are blood plasma and liver (Hussey et al. 2012) which need to be sampled invasively or lethally. Teleost mucus has been used as a fast to medium turnover tissue in stable isotope analysis for over a decade (Church et al. 2009) and only recently been applied to elasmobranchs for the first time (Burgess et al. 2018). Epidermal mucus is thought to act as an immune barrier, osmoregulation and friction reduction (Reif 1978, Shephard 1981) and can be sampled without surgery by simply scraping the skin (Lieber et al. 2013). To the best of our knowledge, no peer-reviewed SIA of shark mucus has yet been published.

To narrow the knowledge gaps about the role of coastal Azorean habitats of smooth hammerheads and tope sharks around the Azores I apply various tools of CNS SIA. I use 3D Bayesian ellipses niche analysis and generalized additive mixed models to characterise the trophic niche, ontogenetic shifts, and potential inter- and intraspecific trophic variation. This study constitutes the first $\delta^{34}S$ and mucus analysis of the two study species and has implications for the management of putative communal shark nurseries and its associated feeding grounds.

VIII.4 Material and Methods

In this study, carbon nitrogen and sulphur stable isotopes of muscle, mucus, and vertebrae centra samples were analysed. These were sampled between 2014 and 2019 during shark tagging campaigns around the island of Faial, Azores, Portugal (Figure VIII.1) and obtained from tope sharks sold at the local fish auction.

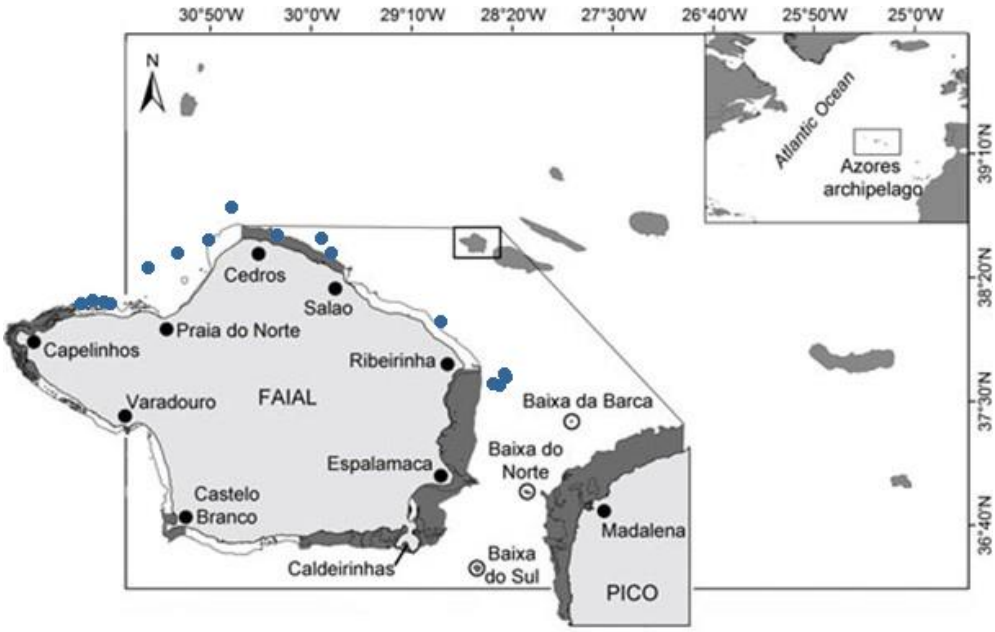

Figure VIII.1 - Map of the Azores and Faial, sampling locations in blue and present MPAs in the study area around the islands of Pico and Faial in dark grey – amended after Schmiing et al. (2015).

VIII.4.1 Muscle and Mucus

Individuals of tope sharks and smooth hammerhead sharks were caught with benthic longlines set within 1.5 nm of the coast (see Figure VIII.1) with approximately 100 circle hooks baited with squid or octopus. Using an alternating setup of 5 weights followed by a buoy after every 10 hooks, the longline was comprised of sections closer to the substrate and rising sections. The lines were deployed with a soak time of max. 2 hours between 40 m and 100 m to target the overlapping habitats of the coastal phase of both species, as well as one pelagic deployment targeted at smooth hammerhead sharks. All larger elasmobranchs were landed on the research vessel using a scoop net and retained in a large rehabilitation tank filled with oxygen saturated saltwater (see appendix figure i). If their vitality was stable (showing unimpeded reflexes - see (Fry and Sherr 1989), Davis (2010)), sharks were put into tonic immobility by turning them gently on their back and keeping them there until movement subsided. The immobilised individuals were carried to the nearby wet surgery table (see appendix figure ii) and after biometric data (total length, pre-caudal length, sex and hook position) were denoted, and if applicable tags deployed, mucus was scraped off the skin using the backside of a clean scalpel. The mucus was transferred to an Eppendorf vial and washed with 36 psu artificial saltwater. Secondly, a muscle sample was excised from the dorsal side of the main body close to the dorsal fin using a 6mm biopsy punch or a scalpel. This incision of the tough skin was then used to apply the standard spaghetti tags more easily. If there was any bleeding during the process, blood was sampled into vials.

After all necessary samples were obtained, the individual was released back into the rehabilitation tank. Once the sharks recovered from the surgery (commonly around 15 min), they were released from the side of the boat and their vitality was denoted. As studies have found changes in the isotopic composition of tissue samples stored in saltwater or ethanol (Lesage et al. 2010, Kim and Koch 2012), all SIA samples were stored in 2 ml Eppendorf vials in a freezer (-20°C) back at the research facility until freeze-dried for SIA.

VIII.4.2 Vertebrae Centra

The lethal vertebrae sampling requires special permits and only a few individuals were available. Tope shark samples were obtained from individuals bought on local fishing markets or individuals that died during the scientific fishing. Due to the protection status of smooth hammerhead sharks and international recommendations (ICCAT 2008, CITES 2020), fishermen are obligated to release all individuals caught and scientific fishing is restricted. The few individuals that died during the tagging campaigns, were dissected and vertebrae centra were sampled.

To obtain consistent and comparable results, the 12-16th vertebrae of the vertebral column were excised. The remaining tissue was removed manually, and vertebrae were cleaned with water before being air-dried. The dried vertebrae were sampled with a precision drill (1.5 mm drill bit) at the centre and 1-2 more layers toward the outside. To limit the dissolution of the inorganic matrix and maximize the one of collagen, Ethylenediaminetetraacetic acid (EDTA) was used for the demineralisation following Kim and Koch (2012).

Samples were then freeze-dried for storage (see Table VIII.1).

Table VIII.1 - Overview of the number, total length (TL) range and stable isotope analysis (SIA) samples analysed in this study grouped by sex of tope (G. galeus) and smooth hammerhead shark (S. zygaena).

		TL range			**Analysed SIA samples**		
	n	Min	Mean	Max	Muscle	Mucus	Vertebrae centra
Galeorhinus galeus	**54**	**35**	**107.4**	**190**	**54**	**5**	**9**
F	28	35	120.8	190	28	2	6
M	26	69	94.5	133	26	3	3
Sphyrna zygaena	**36**	**54**	**96.0**	**159**	**36**	**12**	**8**
F	22	62	98.7	159	22	6	8
M	14	54	90.3	136	14	6	-
Grand Total	**90**				**90**	**17**	**17**

VIII.4.3 Framework samples

Framework samples (see Table VIII.2) were collected to test the difference between the coastal and pelagic isotopic differences. Here the sampled fish represent similar expected niches as sharks in these food webs, while krill were sampled to investigate underlying baseline differences. Samples were obtained from by-catch during the tagging campaign and from the catch of recreational fishermen in 2019 and 2020. The *Euphausiacea* samples were collected from washed-up krill on a beach on Faial. All framework samples were kept frozen before being freeze-dried. Based on local literature (GAMPA 2020 and references therein) and Fishbase (Froese and Pauly 2010) species were classified as coastal or pelagic.

Table VIII.2 - Framework samples from the Azorean coastal and pelagic food webs, sample number, trophic position, respective literature source and function in this study.

Species	n	Trophic position	Source	Function
Coastal food web				
Raja clavata	2	3.5-4.2	(Froese and Pauly 2010)	Representative
Pagrus pagrus	3	3.5-3.9	(Froese and Pauly 2010)	Representative
Diplodus sargus	1	3 – 3.4	(Froese and Pauly 2010)	Representative
Pelagic food web				
Acanthocybium solandri	1	>4	(Froese and Pauly 2010)	Representative
Euphausiidae	3	2-3	(Colaço et al. 2013)	Baseline
Coryphaena hippurus	3	>4	(Froese and Pauly 2010)	Representative

VIII.4.4 Bulk stable isotope analysis

Samples were processed following Kim and Koch (2012): All shark muscle samples were urea extracted by washing and sonicating with 10 ml of de-ionized water for 3 consecutive cycles. A subset of 10 sample replicates was urea and lipid extracted using petroleum ether. Vertebrae centra samples were demineralised using Ethylenediaminetetraacetic acid (EDTA) to limit the dissolution of the inorganic matrix and maximize the one of collagen.

All stable isotope preparation and analysis was performed in the laboratories of the Marine Evolutionary Ecology and the Ocean EcoSystems Biology group (GEOMAR, Helmholtz-Zentrum für Ozeanforschung Kiel, Düsternbrooker Weg 20, 24105 Kiel) with the help of Dr Thomas Hansen. Using a micro-scale (MC 5 Micro Balance; Sartorius; Göttingen, Germany), 50 ± 10 µg of tissue was weighed and placed into tin cups (3.2 x 4.0mm, Hekatech, Wegberg, Germany). To assure the complete exhaustive oxidation of Sulphur, 400 ±100 µg of Vanadium(*V*)oxide (V_2O_5) was added into the tin cups as a catalyst. These were then folded and compressed to small cubes making sure that no material adheres to the outside to eliminate possible cross-contamination. With the corresponding weight denoted, the tin cubes were placed in well plates to be analysed by the continuous flow isotope ratio mass spectrometer.

The mass spectrometer was calibrated against various primary standards and all isotope ratios were calculated according to a calibration curve developed using primary standards. The accuracy of the analysis was validated and calculated for a range of elemental mass using hay powder (ISCNS) standards (Hansen et al. 2009). Outside of this calibration range, the accuracy of measurements has not been tested and elevated elemental masses can contaminate the instruments.

The resulting values of the samples were corrected by hay powder (IS_{CNS}) standards as defined by:

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

where X stands for the isotopes ^{13}C, ^{15}N or ^{34}S and R represents the isotope ratio of the sample (R_{smpl}) and the standard (R_{stnd}).

A lipid extraction test was performed with 9 paired urea extracted and lipid extracted replicates (extracted using petroleum ether following Kim and Koch (2012)). Due to a low correlation of the $\Delta\delta^{13}C$ ($\delta^{13}C_U$ - $\delta^{13}C_{UL}$) and the C:N ratio (see appendix figure iii), which is commonly used to fit correction models (Post 2002), urea-extracted results were mathematically lipid corrected following the approach of Carlisle et al. (2017). Due to relatively low C:N ratios < 5, the linear model of (Logan et al. 2008) was applied with class-specific parameters:

$$\Delta\delta^{13}C = \beta_0 + \beta_1 Ln(C{:}N_U)$$

Where $\Delta\delta^{13}C$ is the difference between the carbon isotope ratio of urea extracted tissue, and urea and lipid extracted tissue ($\delta^{13}C_U$ - $\delta^{13}C_{UL}$), β_0 and β_1 are the parameters used for the different samples (given in Table VIII.3) and $C{:}N_U$ is the uncorrected elemental ratio between carbon and nitrogen of urea extracted tissue. As only lipid corrected $\delta^{13}C$ values were used for the main analysis, I refer to them as $\delta^{13}C$ and specifically mention if denoting uncorrected results.

Table VIII.3 - Model parameters used for the lipid-correction of $\delta^{13}C$ values of muscle samples.

Species	**β_0 parameter**	**β_1 parameter**	**Source**
Shark samples	-7.69 ± 0.82	6.74 ± 0.66	Carlisle et al. (2017)
Fish samples	-4.763 ± 0.1418	4.401 ± 0.0992	Logan et al. (2008)
Euphausiidae	-7.018 ± 1.8280	5.877 ± 1.3360	Logan et al. (2008)

VIII.4.5 Data analysis

Two outliers (one tope shark and one pelagic food web sample) were removed due to biologically unrealistic $\delta^{13}C$ values (> 58 ‰) and very high lipid content (> 17.8) respectively. Data were tested for normal distribution using the Shapiro-Wilk test, and homogeneous variances using the Levene's test prior to further analysis. Normally distributed samples were analysed for differences between the mean using the Student's t-test and non-normally distributed ones with the Wilcoxon test. Means between more than two groups were assessed using the analysis of variance (ANOVA) or the Kruskal-Wallis test.

The niche analysis was implemented using the probabilistic Bayesian n-dimensional approaches within the R package nicheROVER (Swanson et al. 2015). I used the recommended α=0.95 for the calculations of niche regions, which is defined as the parameter space where 95% of randomly sampled isotopic measurement can be found. Niche region ellipses of 15 draws of the Bayesian analysis were generated for each isotope pair in the plots. For the overlap metrics, a Monte Carlo estimate was obtained from 10 000 draws of the niche regions. Here the mean overlap metric can be defined as the 95% probability of species A to be found within the niche region of species B. This procedure was repeated 10 000 times to provide a posterior distribution of the probabilistic niche overlap and the 95% confidence intervals (for more detailed documentation see Swanson et al. (2015) and the R code in supplement).

To investigate inter- and intraspecific differences in relative coastal vs. pelagic diet contribution and trophic level, a generalized additive mixed model (GAMM) of each isotope was implemented using the gamm4 package (Wood et al. 2017). Initially, isotope ratio was implemented as the response variable of smoothed total length per species, species in interaction with sex, and species as fixed determinant effects with sampling date as a random effect to account for potential baseline changes throughout time. Insignificant determinant effects were eliminated from the model in subsequent iterations resulting in the final fixed effects smoothed total length per species and species. Assumptions of the final models were controlled using normal QQ plots, residual histograms, response versus fitted values and auto-correlation functions.

All data analysis was performed in R (R Core Team 2013) using significance levels of α=0.05.

VIII.5 Results

VIII.5.1 Coastal and pelagic food webs

All three isotopes show significant differences between the three food web groups. Coastal fish have more enriched $\delta^{13}C$ and $\delta^{15}N$ values than pelagic ones. Inversely, coastal $\delta^{34}S$ values are more depleted when compared with pelagic ones (see Figure VIII.2). In all cases, the most pronounced differences exist between coastal fish and pelagic krill.

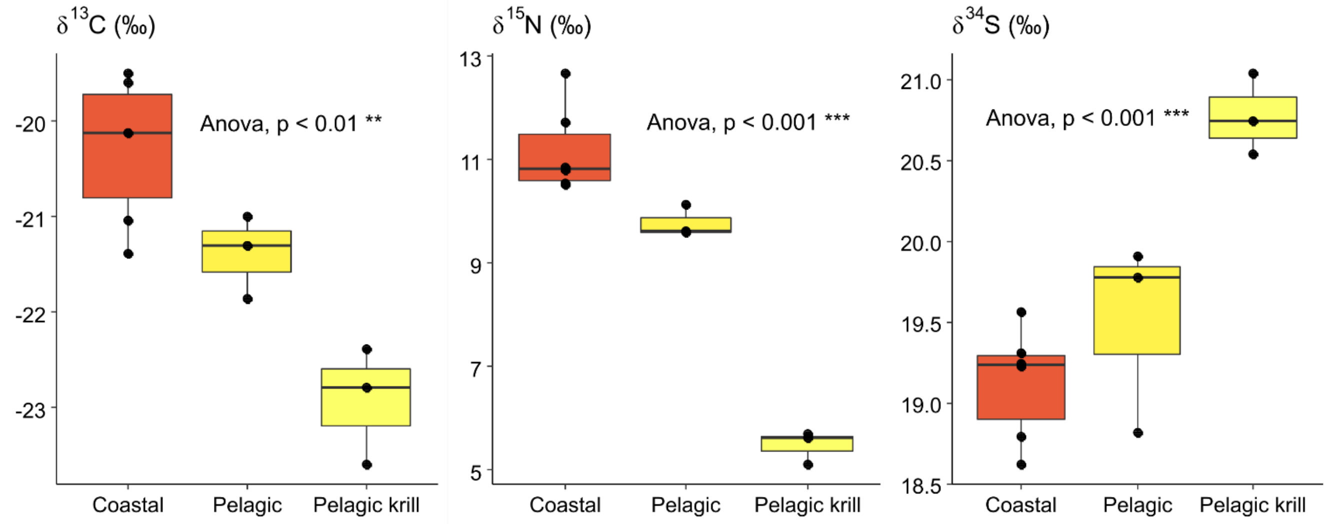

Figure VIII.2 - Boxplots of the lipid corrected $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values and statistical differences of the coastal fish, pelagic fish and pelagic prey from the Azores

VIII.5.2 Muscle

Overall the SIA results of tope and smooth hammerhead sharks exhibit similar mean values with differences between the variation (see Table VIII.4). Tope shark lipid corrected $\delta^{13}C$ have a broad range with the lowest values of all the groups (-15.84 ‰). Compared with smooth hammerhead they also show a broader range of $\delta^{15}N$ with the most enriched values overall (16.16‰). Sulphur ranges are wider for smooth hammerhead than tope sharks with the extreme minimum from the data ($\delta^{34}S$ 13.62 ‰). Shark muscle samples have elemental carbon to nitrogen ratios between 3.42 and 4.69, while food web samples were more enriched (up to 5.03).

Table VIII.4 - Summary of the lipid corrected $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ and C:N ratio stable isotope analysis results of G. galeus, S. zygaena and the coastal- and pelagic food webs.

	Mean ± standard deviation (min-max)							
Group	**δ^{13}C (‰)**		**δ^{15}N (‰)**		**δ^{34}S (‰)**		**Elemental C:N**	
G. galeus	-20.30 ± 1.15	(-22.47 - -15.84)	12.07 ± 1.42	(10.40 - 16.16)	18.12 ± 0.81	(16.17 - 19.87)	4.09 ± 0.24	(3.42 - 4.69)
S. zygaena	-19.98 ± 1.00	(-22.16 - -17.85)	12.51 ± 0.67	(10.37 - 13.42)	18.29 ± 1.17	(13.84 - 21.14)	4.08 ± 0.21	(3.65 - 4.65)
Coastal Food web	-20.29 ± 0.77	(-21.38 - -19.49)	11.19 ± 0.85	(10.53 - 12.68)	19.13 ± 0.35	(18.62 - 19.56)	4.28 ± 0.37	(3.94 - 4.88)
Pelagic Food web	-22.16 ± 0.97	(-23.60 - -21.00)	7.63 ± 2.38	(5.11 - 10.14)	20.14 ± 0.81	(18.82 - 21.04)	4.52 ± 0.33	(4.16 - 5.03)

The probabilistic Bayesian ellipses of the 3-dimensional 95% niche regions show an overlap between all groups with no isolated niche regions (see Figure VIII.3). Tope sharks overlap to a large degree with smooth hammerheads (65.97%) and to a low degree (< 20%) with the coastal and pelagic food web samples (see Table VIII.5). Here the overlap with coastal fish is slightly higher than with the pelagic samples. Smooth hammerhead sharks overlap to 86.41% with the tope shark niche region. Like tope sharks, their overlap with the food web ellipses is relatively low (< 20%) with a slightly higher overlap with the coastal than the pelagic niche region.

Table VIII.5 - Mean overlap of 95% niche region Bayesian ellipses of tope shark (G. galeus), smooth hammerhead shark (S. zygaena), and the coastal and pelagic food web samples. For the probability distribution and confidence intervals refer to appendix figure v.

Overlap of row with column	***G. galeus***	***S. zygaena***	**Coastal food web**	**Pelagic food web**
G. galeus	-	**65.97 %**	11.58 **%**	9.84 **%**
S. zygaena	**86.41 %**	-	11.33 **%**	9.33 **%**
Coastal food web	95.96 **%**	60.27 **%**	-	28.8 **%**
Pelagic food web	26.69 **%**	9.84 **%**	7.7 **%**	-

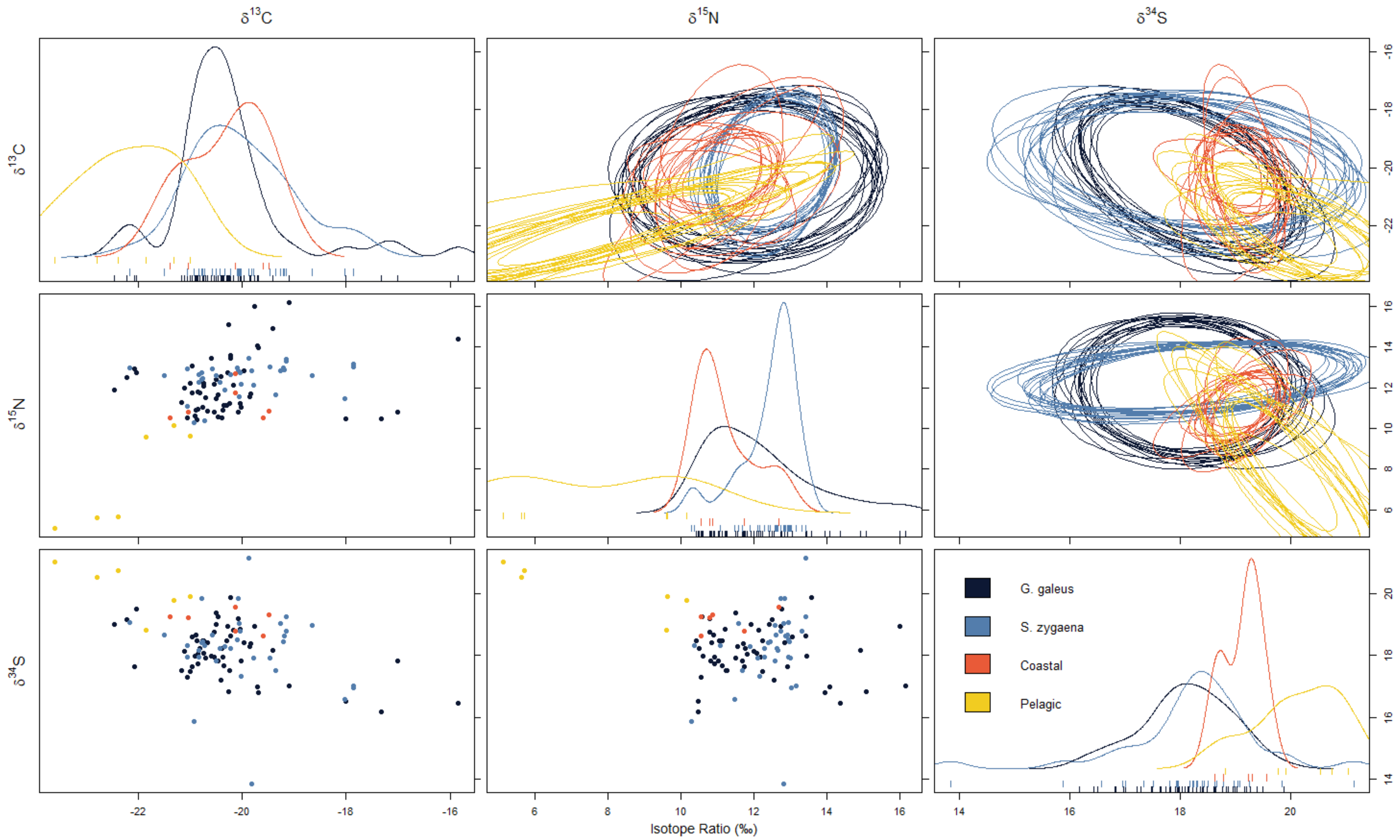

Figure VIII.3 - Paired plots of 15 95% niche regions, the distribution and scatter of $\delta^{13}C$*,* $\delta^{15}N$ *and* $\delta^{34}S$ *of tope shark (G. galeus), smooth hammerhead shark (S. zygaena) and the coastal and pelagic food web.*

Initial GAMMs returned insignificant effects of the interaction of species and sex, and independent sex as determinant factors. The final models show a high significance of species dependant size effects for all isotopes.

Table VIII.6 - Results of generalized additive mixed models (GAMM) of all three isotopes: eDf – effective degrees of freedom, the test statistic for parametric effects t statistic for non-parametric effects F statistic and p-values

Effect	eDf	Test statistic	p-value
$\delta^{13}C$ adjusted r²=0.17			
Intercept		-113.22	< 0.001 ***
Species		1.45	0.15
TL : G. galeus	1.86	7.14	< 0.01 **
TL : S. zygaena	2.16	1.73	0.15
$\delta^{15}N$ adjusted r²=0.357			
Intercept		60.61	< 0.001 ***
Species		1.22	0.23
TL : G. galeus	2.45	8.38	< 0.001 ***
TL : S. zygaena	1	0.82	0.37
$\delta^{34}S$ adjusted r²=0.061			
Intercept		138.76	< 0.001 ***
Species		-0.36	0.72
TL : G. galeus	1	0.80	0.38
TL : S. zygaena	1	7.14	< 0.01 **

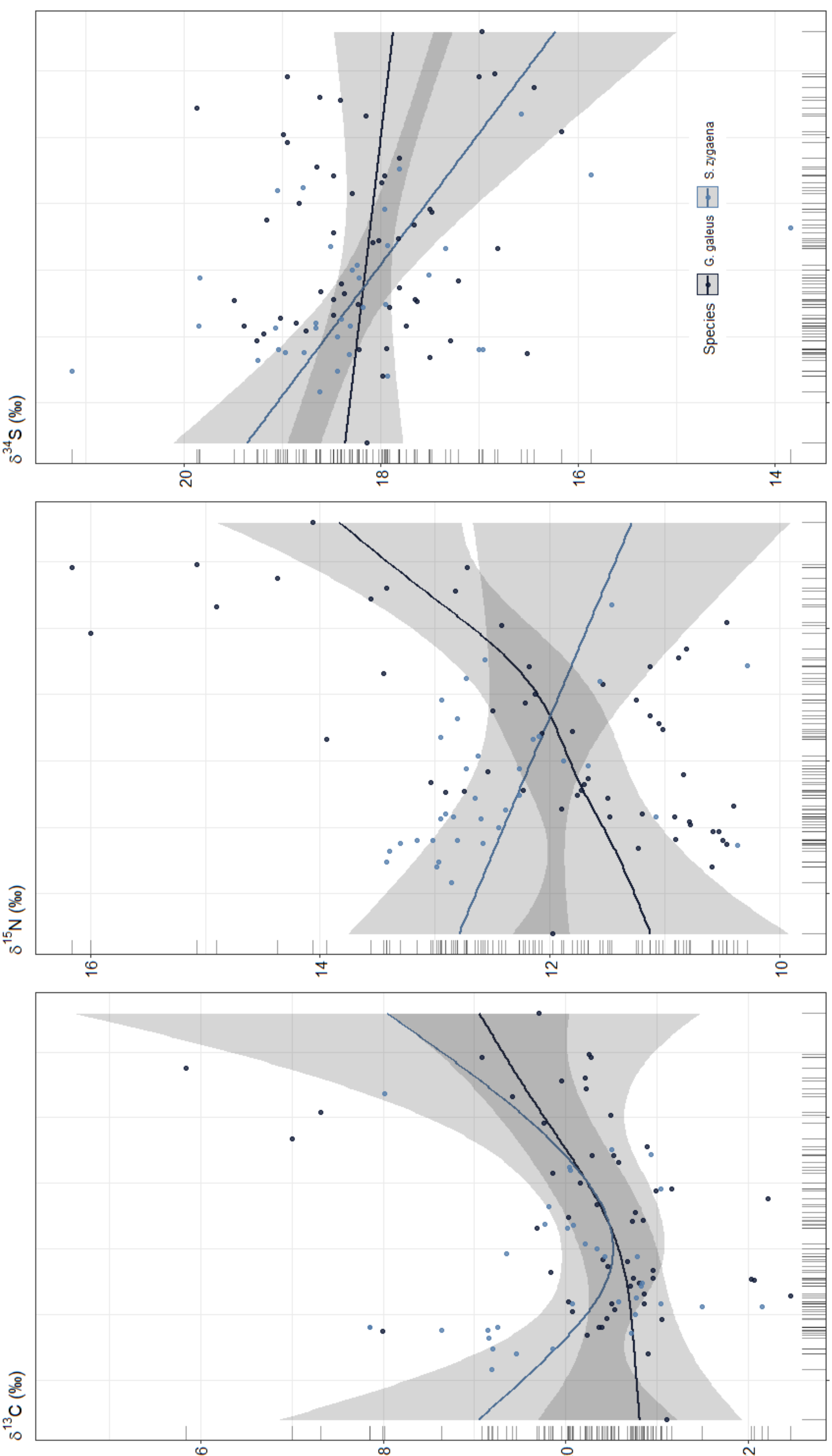

Figure VIII.4 - Generalized additive mixed model (GAMM) predictions for smoothed total length effects of tope (G. galeus) and smooth hammerhead shark (S. zygaena)

For the $\delta^{13}C$ model the smoothed effect of tope shark TL is highly significant (p-value < 0.01) increasing at a larger size (see Figure VIII.4). Smooth hammerheads show an insignificant u-shaped trend with size and the regression lines of both species converge around 120 cm TL. In $\delta^{15}N$, smoothed size of tope sharks is highly significant (p-value < 0.001) with enriched values at a larger size and smooth hammerheads revealing an insignificant negative trend (Figure VIII.4). The regression curves cross at 120 cm TL of both species. Patterns in $\delta^{34}S$ contrast carbon results: smooth hammerhead sharks smoothed size is highly significant (p-value < 0.01) with a linear decrease with total length and tope sharks exhibit insignificant declines (Figure VIII.4). Regression lines cross at ca. 100 cm TL. All models had a low r^2 (< 0.4) and considerable variation between fitted and predicted variables.

VIII.5.3 Other tissues

The pairwise comparison of muscle and mucus samples of the same individuals showed significant differences between the means for $\delta^{15}N$ and $\delta^{34}S$ (see Figure VIII.5). No isotope produced strictly systematic pairwise differences.

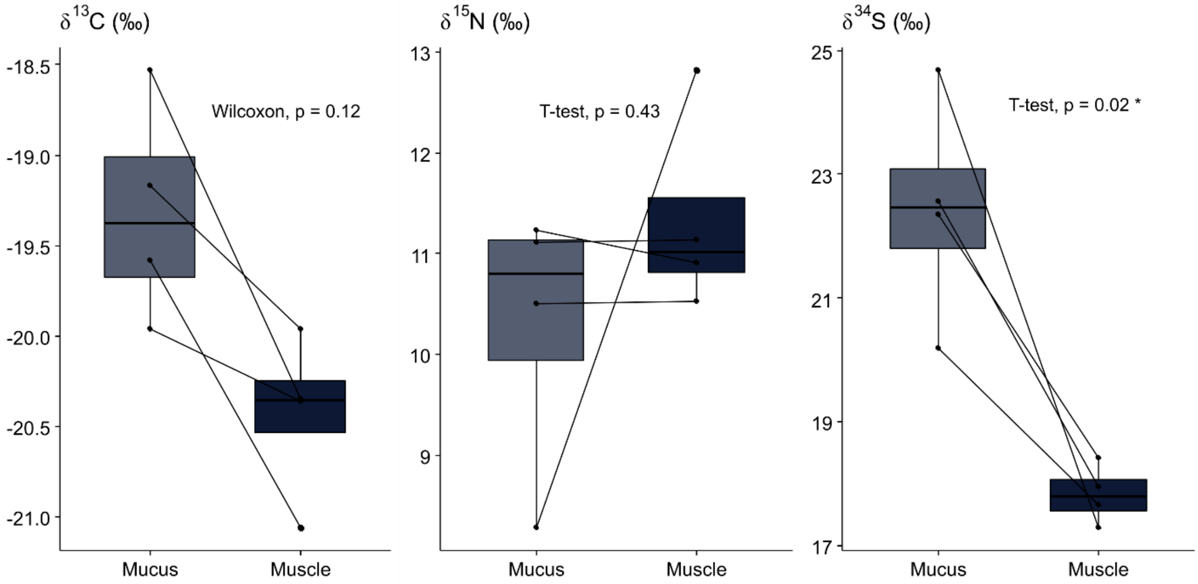

Figure VIII.5 - Boxplots of the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values and statistical differences of paired mucus and lipid corrected muscle samples of tope shark (G. galeus)

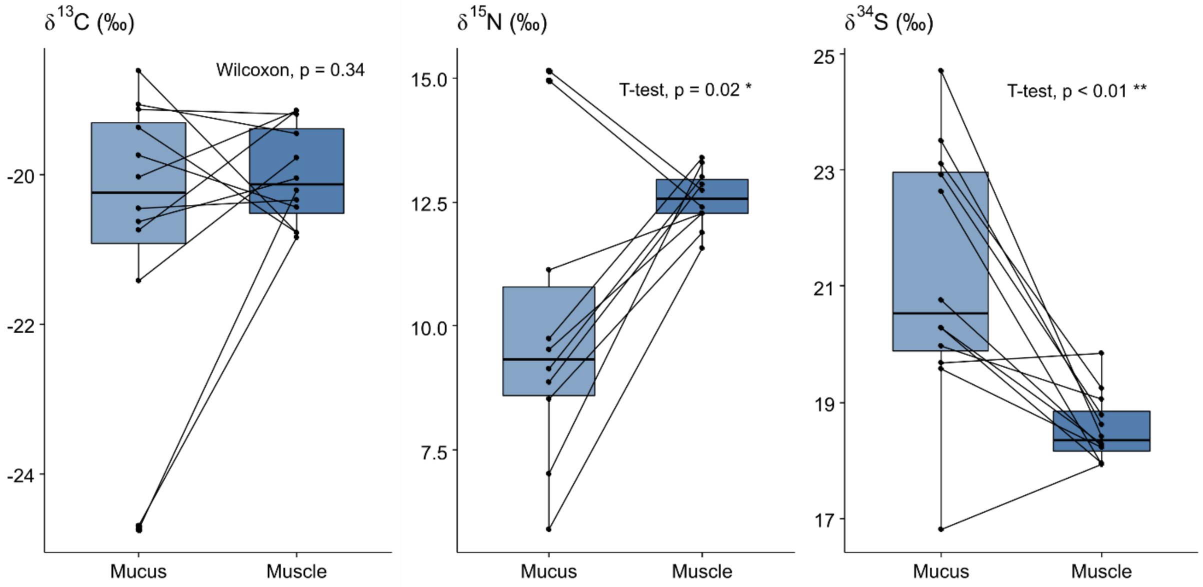

Figure VIII.6 - Boxplots of the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values and statistical differences of paired mucus and lipid corrected muscle samples of smooth hammerhead shark (S. zygaena)

Following the demineralization of Kim and Koch (2012), the vertebrae centra SIA was successful. Compared with muscle samples from the same individuals, vertebrae centra samples suggest enriched $\delta^{13}C$ values, while $\delta^{15}N$ and $\delta^{34}S$ appear more depleted (see Figure VIII.7), yet due to the low sample size, no statistical tests were applied.

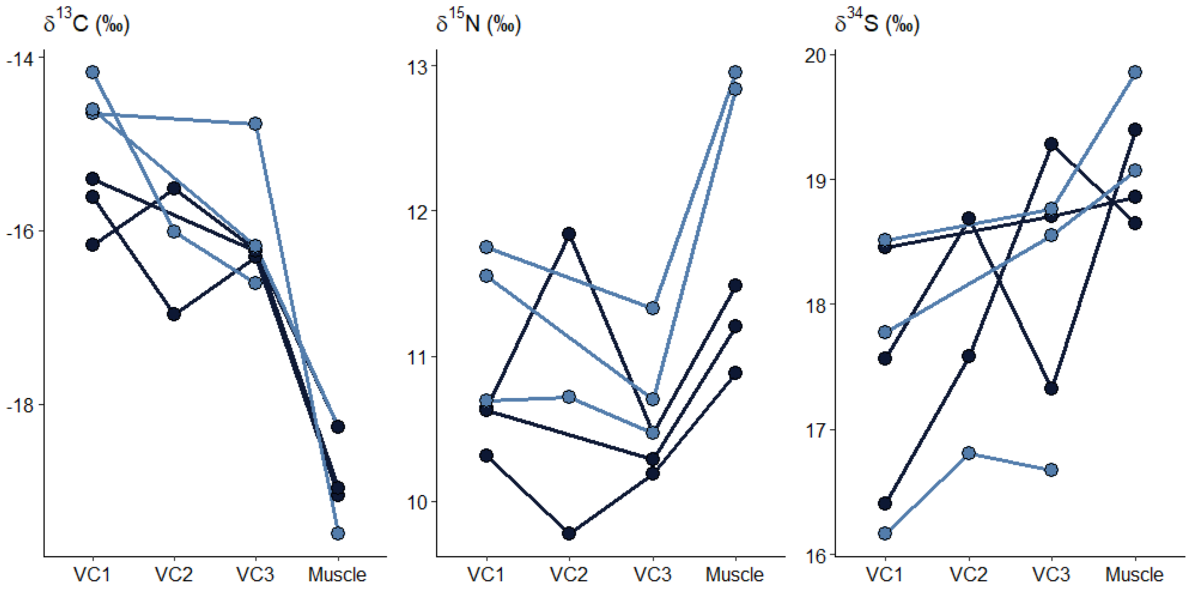

Figure VIII.7 - Points of the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values of the vertebrae centra sections (VC 1 = centre, VC 2=half radius, VC 3= outer edge) and corresponding muscle samples of tope (G. galeus - dark blue) and smooth hammerhead shark (S. zygaena - bright blue).

VIII.6 Discussion

The abundance of both juvenile smooth hammerhead and tope sharks around the Azores islands suggests the occurrence of shared shark nurseries in the coastal waters of this oceanic archipelago. Nevertheless, the scarce dietary information of both species produces a knowledge gap about the importance and potential competition for such coastal resources throughout their life history and particularly so in the first years of life. The results of this study indicate a large proportion of coastal/benthic diet with varying degree throughout the ontogeny of both species and suggest a high potential for competition between them within shared nurseries with several potential mechanisms of niche partitioning.

VIII.6.1 Coastal and pelagic food webs

The significant differences between the coastal and pelagic food web show the signatures of different organic matter sources at the base of the two food webs which form the interpretative framework of this study.

While direct the comparison of absolute stable isotope values is not advised without baseline references, the results of all three isotopes lie within biologically realistic ranges (see Table VI.1 and Peterson and Fry (1987)). The highly significant differences between coastal and pelagic $\delta^{13}C$ ratios with more enriched values in the coastal organisms fit gradients (Figure VIII.2) from the literature (DeNiro and Epstein 1978, Fry and Sherr 1989) and resulting expectations. Compared with zooplankton and krill values from Pajuelo et al. (2010) and Colaço et al. (2013), the krill $\delta^{13}C$ ratios of this study are around 2,5‰ more depleted. Fluctuations in $\delta^{13}C$ and $\delta^{15}N$ over time have been observed frequently in marine domains, and are commonly attributed to varying primary producer compositions (Post 2002) and thus termed baseline shifts. With its complex current patterns, periods of upwelling and unstable gulf stream meanders (Amorim et al. 2017, Caldeira and Reis 2017), shifts in baselines are probable. This emphasizes the importance of accounting for potential baseline shifts between the sampling years in mixing models (Woodland et al. 2012) and was the justification for including as random effects in the GAMM models within this study.

Compared with coastal samples, pelagic fish and krill were significantly depleted in ^{15}N (Figure VIII.2). Due to a high trophic enrichment factor of $\delta^{15}N$ lower values in the primary consumers were expected. The significantly lower $\delta^{15}N$ of pelagic fish, when compared with coastal fish of lower trophic positions furthermore suggest lower source values of the pelagic food web or a shorter food chain with less trophic transfers as suggested by Torres et al. (2014). The significant $\delta^{34}S$ differences show an opposite trend than C and N isotopes (Figure VIII.2): coastal samples exhibited the most depleted values while pelagic fish were more enriched, with krill forming the enriched extreme. Open-ocean sulphates are generally well mixed with a $\delta^{34}S$ of ca. 21‰ (Rees 1973). The krill values of this study hence reveal a clear and pelagic fishes a moderate ocean signature, while coastal fish exhibit more depleted values of increased sedimental or freshwater source influence (Peterson and Fry 1987).

As the coastal samples were obtained from relatively high trophic level predators (TP > 3), the underlying coastal baseline differences were assumed to be even more pronounced and relative isotopic trends rather than absolute values are thus used for interpretation. Depending on the source contribution, differences in carbon and sulphur isotopes could reveal the distinctive underlying sources (Connolly et al. 2004, Mittermayr et al. 2014). All interpretations within this study are restricted to distinguish between coastal/benthic versus pelagic food webs, and benthic food webs remain to be analysed further to determine benthic isotope characteristics.

VIII.6.2 The trophic ecology of coastal *Galeorhinus galeus*

The results from this CNS SIA study indicate tope shark to be a generalist coastal/benthic predator in the Azores, in agreement with past findings from the region and elsewhere. The mixed models reveal a significant ontogenetic change in carbon and nitrogen isotopes and insignificant trends in sulphur (Figure VIII.4). The smallest individual (35 cm TL) can be classified as a neonate (Lenanton et al. 1990), and the next largest individuals (≥ 60 cm) around 2 years or older (see growth curves within Francis and Mulligan 1998, Dureuil and Worm 2015). These young tope sharks reveal isotopic signatures characteristic of a lower trophic position with mixed but predominantly coastal diet. However, tope shark revealed more depleted $\delta^{13}C$ and enriched $\delta^{15}N$ and $\delta^{34}S$ values with increasing size (Figure VIII.4). This is indicative of a higher contribution of coastal/benthic prey and an increase in trophic position. Tope shark around the world have been found to inhibit high trophic positions (Davenport and Bax 2002, Domi et al. 2005, Botto et al. 2011) which can be attributed to their ability to cut their prey in pieces and thus overcoming gape restrictions even at an early age (Lucifora et al. 2006). While no trophic position calculation was performed in this study the elevated $\delta^{15}N$ of the large females alludes a high trophic level similar to (Torres et al. 2014). Using carbon and nitrogen SIA of tope sharks, Torres et al. (2014) found an increasing contribution of the benthopelagic blue jack mackerel (*Trachurus picturatus*) with size. According to carbon and sulphur gradients observed in the literature, the trends of tope sharks in this study could also imply the increasing importance of such benthopelagic prey. Confirming this hypothesis will require future analysis of benthic baseline and prey to determine differences between coastal and offshore benthic sources in the Azores.

Throughout the ontogeny, there was a large variation not explained by any of the included effects in all isotopes (low r^2 values in Table VIII.6 and insignificance of effects in early GAMMs). This result could suggest a generalist feeding or intra-specific niche partitioning on the individual non-gender-based level. Lucifora et al. (2006) found an ontogenetic shift from selective demersal-feeding juveniles to a more generalist strategy in adults and high plasticity in feeding strategy with season. Indeed, tope shark showed larger variation in both $\delta^{13}C$ and $\delta^{15}N$ in as larger sizes, potentially underscoring a more generalist feeding strategy of adult tope. Ongoing telemetric studies of the local population show differences in movement and habitat use between individuals (Afonso unpublished data), hinting towards a relevant degree of niche partitioning across life stages. Again, more diet studies need to be conducted to distinguish between generalist feeding habits and niche partitioning at an individual level. Sexual segregation of tope shark has been observed in many studies with males occupying deeper waters in shelf proximity (Compagno 1984, Lucifora et al. 2006, Ebert and Stehmann 2013, Thorburn et al. 2019), and the catch of large individuals of both sexes in more offshore benthic longlines around the islands (GAMPA 2020, Santos et al. 2020) suggests a similar pattern around the Azores. Yet, none of our coastal longlining campaigns captured any large male tope sharks (max. 133 cm TL), suggesting a more complex spatial heterogeneity (habitat-based) within the population. Adult male samples are needed to clarify this potential sexual segregation and potential diet shifts, a hypothesis certainly warranting future research.

VIII.6.3 The trophic ecology of juvenile *Sphyrna zygaena*

The results of juvenile smooth hammerhead muscle reveal isotopic signatures associated with predominantly coastal-benthic diet at an intermediate trophic position. Mixed models indicate no sexual segregation, but significant ontogenetic diet shifts throughout their coastal life stage (Figure VIII.4 and Table VIII.6). With a minimum size of 54 cm TL and observed umbilical scar and a maximum size of 159 cm TL with an approximate age of 6 (Rosa et al. 2017), this study covers neonates, young-of-the-year and juveniles of both males and females. Within the first years, carbon and nitrogen showed declining values. The interpretation of SIA results of new-born animals poses two main pitfalls that ought to be considered: a) the inclusion of maternal signals from gestation or placental nutrition (Jenkins et al. 2001, Olin et al. 2011) and b) potential differences in parameters such as DTDFs and turnover time (Weidel et al. 2011, Kim et al. 2012, Shipley and Matich 2020). Analysing muscle and liver C and N stable isotopes of new-born sharks, Olin et al. (2011) found significant decreases of both isotopic signatures with growth which they associated with the dilution of maternal signals. While not significant, the trends observed in the earliest life stage have been found in previous studies (Loor-Andrade et al. 2015, Gonzalez-Pestana et al. 2017) and could be caused by the dietary switch from maternal sources to prey. Thus, future analysis of this subject could finally shed light on the diet of the otherwise elusive pregnant female smooth hammerhead sharks and thus deserves attention.

Even though insignificant, the trends towards more depleted $\delta^{15}N$ values within the first years (Figure VI.1) parallels findings of both stomach content and stable isotope analysis in the Pacific, where authors found the highest specialization of smooth hammerhead sharks on coastal squid between 70 and 150 cm TL and the lowest trophic position throughout their life history (Gonzalez-Pestana et al. 2017, Estupiñán-Montaño et al. 2019). As our food web samples show differences in both trophic level and coastal versus pelagic feeding, the depleted nitrogen values could be interpreted as higher nutritional importance of pelagic sources. Nevertheless, both carbon and sulphur isotopes challenge this interpretation: Both $\delta^{13}C$ and $\delta^{34}S$ reveal trends towards increased coastal/benthic nutritional contribution (increasing values in $\delta^{13}C$ and decreasing values in $\delta^{34}S$ with size - Figure VIII.4). The high importance of coastal prey for juvenile smooth hammerhead sharks has been identified in various studies in continental coastal waters (Bolaño Martínez 2009, Smale 2010, Bornatowski et al. 2014, Loor-Andrade et al. 2015, Gonzalez-Pestana et al. 2017, Estupiñán-Montaño et al. 2019). The increased abundance of pelagic prey at the coast of oceanic islands in combination with the coastal diet of tope sharks as potential competitors warranted the hypothesis that, while in coastal habitats, smooth hammerhead they rely more and more on pelagic feeding as a mechanism of attaining oceanic-proficiency for later life stages. Our results do not support this hypothesis. Instead, they reveal an increasing reliance on coastal/benthic prey throughout their coastal phase. Four years of telemetry data of juvenile smooth hammerheads around Faial revealed repeated nocturnal movements to deeper waters over the shelf and break (Afonso unpublished data). Similarly, Santos and Coelho (2018) recorded deeper distributions at night in oceanic sub-adults. In other pelagic sharks, deep dives have been linked to foraging behaviour (Carey et al. 1990) and along with predator avoidance and orientation, the same has been suggested for smooth hammerhead (Francis 2016, Santos and Coelho 2018). Even though aggregating juveniles are often seen basking close to the surface, there are no reports of observations of them feeding on bait balls, a behaviour that even tope sharks occasionally display in the region. Thus, the hypothesis of sporadic deeper benthic foraging dives could help explain the isotopic signatures interpreted as "coastal" in this study. The future inclusion of benthic samples could help elucidate vertical gradients by distinguishing between coastal and offshore-benthic sources. Furthermore, stomach content data is urgently needed to attain insights into prey species, although such studies are nowadays challenging given the high vulnerability and full protection status of the species on the Azores. Samples of the locally abundant nektobenthic squid *Loligo forbesii* could provide valuable information about the contribution of cephalopods to their diet, which has proved to be important in other studies (Ochoa Díaz 2009, Bornatowski et al. 2014, Loor-Andrade et al. 2015, Gonzalez-Pestana et al. 2017).

VIII.6.4 Competition in shared nurseries

In recent years, the paradigm of shared shark nurseries has shifted from an assumed situation were abundant resources are unlimiting (Springer 1967) to another where communities are rich in competition and niche partitioning (Kinney et al. 2011). Our study also brings novel evidence to this growing body of literature, as the SIA results indicate significant overlap of trophic niches of tope and juvenile smooth hammerhead sharks in coastal habitats. This overlap points to a diet competition within the shared nurseries and to different ontogenetic strategies resulting in putative niche partitioning throughout their life histories.

The 95% niche regions and GAMMs of the three assessed isotopic dimensions show a large overlap between both species (> 60%). The relatively enriched ranges of $\delta^{13}C$ and depleted ranges of $\delta^{34}S$ of the sharks suggest high contribution of coastal feeding. Observations and preliminary telemetric and video survey data of both species (Afonso unpublished data) have indicated that both sharks extensively use the coastal habitats around on the islands' shelves. The results of this study also support the co-occurrence in shared coastal nurseries in the Azores through the species' assimilated diet. The frequent catch and persisting coastal signature of larger juveniles confirms that the young remain in insular coastal habitats for several years, agreeing with the definitions of nurseries proposed by Heupel et al. (2007). While this is the first study to explicitly show a shared use of nursery grounds by tope and smooth hammerhead sharks, other publications suggest their spatial overlap in coastal areas (Cartamil et al. 2011, Alfaro-Cordova et al. 2018).

Additionally, this study provides evidence that at least mature female tope shark also uses coastal habitats and its resources. Natal philopatry, whereby pregnant females return to their natal grounds to pup, has previously been suggested for both species (Fitzmaurice et al. 2003, Félix-López et al. 2019). In the case of tope shark, although some females appeared to be pregnant, the muscle samples (which integrate diet signals of over a year (Kim et al. 2012)) indicate that older individuals also relied on a coastal-benthic diet for a considerable amount of time prior to being caught. In contrast, female adult smooth hammerheads have only been spotted around the islands in the summer (Afonso – personal communication). Thus, the two species appear to differ drastically as to the dependency on coastal habitats in their adult phase. Further research is clearly needed to assess the temporal extent of the adult females' presence in the nurseries to elucidate whether they come to the islands exclusively to pup or for longer periods of time.

The shared habitat of the two shark species of differing size classes leads to the subsequent question whether the resources are shared or, instead, if trophic niches are partitioned. The results yield no indication of neither inter- nor intraspecific niche partitioning based on sex. This agrees with previous findings for both smooth hammerhead and tope sharks, where no significant differences between sexes within each species could be found (Torres et al. 2014, Gonzalez-Pestana et al. 2017, Alfaro-Cordova et al. 2018). While species as independent effect showed no significance, smoothed size by species was significant in all three isotope models providing evidence for different ontogenetic dynamics prior to major habitat shifts. The largest ontogenetic differences between isotopic values of the two species are present in $\delta^{15}N$. Here large mature female tope sharks show signatures associated with high trophic positions while smooth hammerheads of comparable sizes seem to feed at lower trophic levels (see Figure VIII.4). This finding suggests low competition of adult (female) tope sharks with smaller topes and smooth hammerheads.

The isotopic values of juveniles from both species were highly clustered for all isotopes and revealed the strongest dietary similarities at 100 to 125 cm TL. Similarly to the findings of Kinney et al. (2011), the overlap of isotopic niches with higher trophic level fishes suggests competition not only between the sharks but potentially also with other teleosts. With diverging strategies in late coastal stages, this could indicate a relevant degree of prior competition potentially leading to the subsequent resource partitioning as the energy demand of the sharks increases (Navia et al. 2007). The strong variation of similar sized specimens indicates inherent mechanisms of resource partitioning which remain unresolved by the models. Due to the integrative nature of the muscle SIA results, individuals seem to feed regularly on differing sources suggesting niche partitioning on an individual size and sex unrelated level. Acoustic telemetry around the islands similarly suggests pronounced differences between movement patterns which could be linked to such distinct feeding strategies (Afonso). As mentioned previously, the determination of isotopic characteristics of benthic diet remains to be analysed and could help elucidate possible niche partitioning through varying proportions of benthic feeding. Additionally, the comparison between tissues of contrasting turnover times could provide valuable information by improving the temporal resolution of change, which may remain masked by the slow turnover muscle data.

VIII.6.5 Feasibility of other tissues – Mucus and vertebrae centra

We analysed mucus and vertebrae samples, each paired with muscle samples of the same individual, to assess the feasibility of using these two contrasting tissues for future SIA.

To the best of our knowledge, this study constitutes the first CNS SIA of shark mucus. The significant differences in C and N suggest different DTDFs or turnover times as has been shown for teleosts (Church et al. 2009, Heady and Moore 2013). Controlled diet experiments are required to provide these fundamental tissue parameters to enable accurate ecological interpretation. The establishment of informative low-impact methodologies is highly desirable for the study of protected and elusive species such as large elasmobranchs. This study highlights the promising feasibility of using this minimally-invasive sampling for SIA of sharks.

As vertebrae centra are deposited concentrically, the outermost tissue reflects the most recent depositions (Ridewood and MacBride 1921). Compared with muscle samples from the same individuals, vertebrae centra samples exhibit significantly enriched $\delta^{13}C$ values (Figure VIII.7). Similar trends of ^{13}C enrichment have been found in scales (Perga and Gerdeaux 2003) and other vertebrae SIA studies (Estrada et al. 2006, Loor-Andrade et al. 2015) and can be attributed to different DTDFs and protein compositions (Winters 1972). N and S vertebrae isotopes are significantly depleted. Even though differences between the different sections were not significant, the results indicate a promising degree of variation. Analysing up to 7 different drill sections of mature smooth hammerhead sharks, Loor-Andrade et al. (2015) found significant ontogenetic trends in $\delta^{15}N$. Considering the relatively stable values found in the muscle from juveniles, it is not surprising that no significant trends could be found given the sample size and age of sampled individuals. Nevertheless, this methodological chapter reiterates the potential of SIA of time-resolved vertebrae centra to investigate ontogenetic shifts while limiting confounding inter-individual effects.

VIII.7 Conclusions

The results of this study provide evidence for the dietary importance of Azorean coastal ecosystems for North Atlantic populations of tope (*Galeorhinus galeus)* and smooth hammerhead sharks (*Sphyrna zygaena)*. CNS SIA reveals a high contribution of coastal/benthic prey for coastal life stages of both species. The data shows juveniles and adult female tope sharks to feed increasingly on coastal/benthic prey with significant increases of trophic position throughout their ontogeny. Smooth hammerhead juveniles below 160 cm TL exhibit lower trophic positions and the data suggests an increasing reliance on coastal/benthic prey. Both species show a high isotopic niche overlap implying relevant resource competition potentially alleviated by size structuring, benthic feeding, and individual-based niche partitioning. Overfishing has led to strong declines of coastal fish abundances. Such decreasing resources might provoke increasing overlap and competition of the juvenile life stages of these vulnerable shark species with potential reverberating effects on the integrity of their larger populations. This study thus provides evidence for the importance of intact coastal ecosystems for communal shark nurseries and stresses the relevance of their priority for protection.

VIII.8 Acknowledgements

I thank the entire Island Shark team for the collaboration and sample collection and Merlin Weichler for helping with the weighing of samples. Jorge Fontes and Mara Schmiing are acknowledged for technical and statistical advice. Lastly, I am extremely grateful for the valuable advice of Jan Dierking and the flawless and timely analysis by Thomas Hansen.

VIII.9 References

Afonso, P. Unpublished data

Afonso, P., J. Fontes, E. Giacomello, M. C. Magalhães, H. R. Martins, T. Morato, V. Neves, R. Prieto, R. S. Santos, and M. A. Silva. 2020. The Azores: a mid-Atlantic hotspot for marine megafauna research and conservation. Frontiers in Marine Science.

Afonso, P., F. Vandeperre, J. Fontes, and F. Porteiro. 2014. Conservation of pelagic elasmobranchs in the Azores. Page 25 *in* H. R. Martins, editor. ARQUIPELAGO Life and Marine Sciences. The sea of the Azores: scientific forum for decision support. University of the Azores.

Alfaro-Cordova, E., A. D. Solar, A. Gonzalez-Pestana, N. Acuña-Perales, J. Coasaca, F. Cordova-Zavaleta, J. Alfaro-Shigueto, and J. C. Mangel. 2018. Isotopic niches of four commercially important pelagic elasmobranch species captured by the small-scale driftnet fishery of northern Peru. Latin american journal of aquatic research **46**:482-488.

Amorim, P., A. D. Perán, C. K. Pham, M. Juliano, F. Cardigos, F. Tempera, and T. Morato. 2017. Overview of the ocean climatology and its variability in the Azores region of the North Atlantic including environmental characteristics at the seabed. Frontiers in Marine Science **4**:56.

Baum, J. K., R. A. Myers, D. G. Kehler, B. Worm, S. J. Harley, and P. A. J. S. Doherty. 2003. Collapse and conservation of shark populations in the Northwest Atlantic. **299**:389-392.

Bolaño Martínez, N. 2009. Ecología trófica de juveniles del tiburón martillo Sphyrna zygaena (Linnaeus, 1758) en aguas ecuatorianas. Instituto Politécnico Nacional. Centro Interdisciplinario de Ciencias Marinas.

Bornatowski, H., A. F. Navia, R. R. Braga, V. Abilhoa, and M. F. M. Corrêa. 2014. Ecological importance of sharks and rays in a structural foodweb analysis in southern Brazil. ICES Journal of Marine Science **71**:1586-1592.

Botto, F., E. Gaitán, H. Mianzan, M. Acha, D. Giberto, A. Schiariti, and O. Iribarne. 2011. Origin of resources and trophic pathways in a large SW Atlantic estuary: an evaluation using stable isotopes. Estuarine, Coastal and Shelf Science **92**:70-77.

Burgess, K., M. Guerrero, A. Richardson, M. Bennett, and A. Marshall. 2018. Use of epidermal mucus in elasmobranch stable isotope studies: a pilot study using the giant manta ray (Manta birostris). Marine & Freshwater Research **69**:336-342.

Caldeira, R., and J. C. Reis. 2017. The Azores confluence zone. Frontiers in Marine Science **4**:37.

Camhi, M. D., S. Valenti, S. Fordham, S. Fowler, and C. Gibson. 2009. The conservation status of pelagic sharks and rays: report of the IUCN shark specialist group pelagic shark red list workshop. IUCN Species Survival Commission Shark Specialist Group. Newbury, UK. x+ 78p.

Campana, S. E., L. J. Natanson, and S. Myklevoll. 2002. Bomb dating and age determination of large pelagic sharks. Canadian Journal of Fisheries and Aquatic Sciences **59**:450-455.

Cardeñosa, D., A. T. Fields, E. A. Babcock, H. Zhang, K. Feldheim, S. K. Shea, G. A. Fischer, and D. D. Chapman. 2018. CITES-listed sharks remain among the top species in the contemporary fin trade. Conservation Letters **11**:e12457.

Carey, F., J. Scharold, and A. J. Kalmijn. 1990. Movements of blue sharks (Prionace glauca) in depth and course. Marine Biology **106**:329-342.

Carlisle, A. B., S. Y. Litvin, D. J. Madigan, K. Lyons, J. S. Bigman, M. Ibarra, and J. J. Bizzarro. 2017. Interactive effects of urea and lipid content confound stable isotope analysis in elasmobranch fishes. Canadian Journal of Fisheries Aquatic Sciences **74**:419-428.

Cartamil, D., O. Santana-Morales, M. Escobedo-Olvera, D. Kacev, L. Castillo-Geniz, J. B. Graham, R. D. Rubin, and O. Sosa-Nishizaki. 2011. The artisanal elasmobranch fishery of the Pacific coast of Baja California, Mexico. Fisheries Research **108**:393-403.

Casper, B. M., Domingo, A., Gaibor, N., Heupel, M.R., Kotas, E., Lamónaca, A.F., Pérez-Jimenez, J.C., Simpfendorfer, C., Smith, W.D., Stevens, J.D., Soldo, A. & Vooren. 2009. Sphyrna zygaena. The IUCN Red List of Threatened Species. last accessed 12 November 2018

Castro, J. I., C. M. Woodley, and R. L. Brudek. 1999. A preliminary evaluation of the status of shark species. Food & Agriculture Org.

Church, M. R., J. L. Ebersole, K. M. Rensmeyer, R. B. Couture, F. T. Barrows, and D. L. Noakes. 2009. Mucus: a new tissue fraction for rapid determination of fish diet switching using stable isotope analysis. Canadian Journal of Fisheries and Aquatic Sciences **66**:1-5.

CITES. 2020. Checklist of CITES Species. http://checklist.cites.org last accessed 05. September 2020

Coelho, R., J. Fernandez-Carvalho, P. G. Lino, and M. N. Santos. 2012. An overview of the hooking mortality of elasmobranchs caught in a swordfish pelagic longline fishery in the Atlantic Ocean. Aquatic Living Resources **25**:311-319.

Colaço, A., E. Giacomello, F. Porteiro, and G. Menezes. 2013. Trophodynamic studies on the Condor seamount (Azores, Portugal, North Atlantic). Deep Sea Research Part II: Topical Studies in Oceanography **98**:178-189.

Colloca, F., D. Scannella, M. L. Geraci, F. Falsone, G. BATISTA, S. Vitale, and M. Di Lorenzo. 2019. British sharks in Sicily: records of long distance migration of tope shark (Galeorhinus galeus) from North-eastern Atlantic to Mediterranean Sea. Mediterranean Marine Science **20**.

Compagno, L. 1998. Sphyrnidae. Hammerhead and bonnethead sharks. FAO identification guide for fishery purposes. The Living Marine Resources of the Western Central Pacific. FAO, Rome. pp:1361-1366.

Compagno, L. J. 1984. Sharks of the world: an annotated and illustrated catalogue of shark species known to date.

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

Cox, G. J., and M. Francis. 1997. Sharks and rays of New Zealand. Canterbury University Press.

Das, D., and P. Afonso. 2017. Review of the diversity, ecology, and conservation of elasmobranchs in the Azores region, mid-north Atlantic. Frontiers in Marine Science **4**:354.

Davenport, S. R., and N. J. Bax. 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.

Davis, M. W. 2010. Fish stress and mortality can be predicted using reflex impairment. Fish and Fisheries **11**:1-11.

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

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

Devictor, V., J. Clavel, R. Julliard, S. Lavergne, D. Mouillot, W. Thuiller, P. Venail, S. Villeger, and N. Mouquet. 2010. Defining and measuring ecological specialization. Journal of applied ecology **47**:15-25.

Domi, N., J.-M. Bouquegneau, and K. Das. 2005. Feeding ecology of five commercial shark species of the Celtic Sea through stable isotope and trace metal analysis. Marine Environmental Research **60**:551-569.

Dulvy, N. K., S. L. Fowler, J. A. Musick, R. D. Cavanagh, P. M. Kyne, L. R. Harrison, J. K. Carlson, L. N. Davidson, S. V. Fordham, M. P. Francis, C. M. Pollock, C. A. Simpfendorfer, G. H. Burgess, K. E. Carpenter, L. J. Compagno, D. A. Ebert, C. Gibson, M. R. Heupel, S. R. Livingstone, J. C. Sanciangco, J. D. Stevens, S. Valenti, and W. T. White. 2014. Extinction risk and conservation of the world's sharks and rays. Elife **3**:e00590.

Dureuil, M., and B. Worm. 2015. Estimating growth from tagging data: an application to north-east Atlantic tope shark Galeorhinus galeus. Journal of Fish Biology **87**:1389-1410.

Ebert, D. A., and M. F. Stehmann. 2013. Sharks, batoids and chimaeras of the North Atlantic. FAO, Roma (Italia).

Ellis, J., S. McCully Phillips, and F. Poisson. 2017. A review of capture and post-release mortality of elasmobranchs. Journal of Fish Biology **90**:653-722.

Elton, C. S. 2001. Animal ecology. University of Chicago Press.

Estes, J. A., J. Terborgh, J. S. Brashares, M. E. Power, J. Berger, W. J. Bond, S. R. Carpenter, T. E. Essington, R. D. Holt, and J. B. Jackson. 2011. Trophic downgrading of planet Earth. Science **333**:301-306.

Estrada, J. A., A. N. Rice, L. J. Natanson, and G. B. Skomal. 2006. Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. Ecology **87**:829-834.

Estupiñán-Montaño, C., L. Cedeño-Figueroa, J. F. Estupiñán-Ortiz, F. Galván-Magaña, A. Sandoval-Londoño, D. Castañeda-Suarez, and C. J. Polo-Silva. 2019. Feeding habits and trophic level of the smooth hammerhead shark, Sphyrna zygaena (Carcharhiniformes: Sphyrnidae), off Ecuador. Journal of the Marine Biological Association of the United Kingdom **99**:673-680.

Félix-López, D. G., N. Bolaño-Martinez, P. Díaz-Jaimes, E. C. Oñate-González, J. S. Ramírez-Pérez, E. García-Rodríguez, D. Corro-Espinosa, J. E. Osuna-Soto, and N. C. Saavedra-Sotelo. 2019. Possible female philopatry of the smooth hammerhead shark Sphyrna zygaena revealed by genetic structure patterns. Journal of Fish Biology **94**:671-679.

Ferretti, F., A. Soldo, R. Walls, B. Casper, A. Domingo, N. Gaibor, M. R. Heupel, J. Kotas, A. Lamónaca, W. D. Smith, J. Stevens, Vooren, , and J. C.M. & Pérez-Jiménez. 2015. Sphyrna zygaena. The IUCN Red List of Threatened Species 2015. last accessed 05 September 2020

Ferretti, F., B. Worm, G. L. Britten, M. R. Heithaus, and H. K. Lotze. 2010. Patterns and ecosystem consequences of shark declines in the ocean. Ecol Lett **13**:1055-1071.

Fitzmaurice, P., G. Keirse, P. Green, and M. Clarke. 2003. Tope Tagging in Irish Waters (1970-2002). Central Fisheries Board.

France, R. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Marine Ecology Progress Series **124**:307-312.

Francis, M. 2016. Distribution, habitat and movement of juvenile smooth hammerhead sharks (Sphyrna zygaena) in northern New Zealand. New Zealand Journal of Marine and Freshwater Research **50**:506-525.

Francis, M. P., and K. P. Mulligan. 1998. Age and growth of New Zealand school shark, Galeorhinus galeus. New Zealand Journal of Marine and Freshwater Research **32**:427-440.

Frisk, M. G., T. J. Miller, and N. K. Dulvy. 2005. Life Histories and Vulnerability to Exploitation of Elasmobranchs: Inferences from Elasticity, Perturbation and Phylogenetic Analyses. Journal of Northwest Atlantic Fishery Science **35**:27-45.

Froese, R., and D. Pauly. 2010. FishBase. https://www.fishbase.de/ last accessed 04.10.2020

Fry, B., and E. B. Sherr. 1989. δ 13 C measurements as indicators of carbon flow in marine and freshwater ecosystems. Pages 196-229 Stable isotopes in ecological research. Springer.

Gallagher, A. J., N. Hammerschlag, D. S. Shiffman, and S. T. Giery. 2014. Evolved for Extinction: The Cost and Conservation Implications of Specialization in Hammerhead Sharks. BioScience **64**:619-624.

GAMPA. 2020. Coastal Fisheries Resources of the Azores: an X-Ray. IMAR/Okeanos.

Gonzalez-Pestana, A., N. Acuna-Perales, J. Coasaca-Cespedes, F. Cordova-Zavaleta, J. Alfaro-Shigueto, J. C. Mangel, and P. Espinoza. 2017. Trophic ecology of the smooth hammerhead shark (Sphyrna zygaena) off the coast of northern Peru. Fishery Bulletin **115**.

Hansen, T., A. Burmeister, and U. Sommer. 2009. Simultaneous δ15N, δ13C and δ34S measurements of low-biomass samples using a technically advanced high sensitivity elemental analyzer connected to an isotope ratio mass spectrometer. Rapid Communications in Mass Spectrometry **23**:3387-3393.

Heady, W. N., and J. W. Moore. 2013. Tissue turnover and stable isotope clocks to quantify resource shifts in anadromous rainbow trout. Oecologia **172**:21-34.

Heithaus, M. R., A. Wirsing, and L. Dill. 2012. The ecological importance of intact top-predator populations: a synthesis of 15 years of research in a seagrass ecosystem. Marine and Freshwater Research **63**:1039-1050.

Heupel, M. R., J. K. Carlson, and C. A. Simpfendorfer. 2007. Shark nursery areas: concepts, definition, characterization and assumptions. Marine Ecology Progress Series **337**:287-297.

Hobson, K. A. 1993. Trophic relationships among high Arctic seabirds: insights from tissue-dependent stable-isotope models. Marine Ecology-Progress Series **95**:7-7.

Holden, M., and R. Horrod. 1979. The migrations of tope, Galeorhinus galeus (L), in the eastern North Atlantic as determined by tagging. ICES Journal of Marine Science **38**:314-317.

Hussey, N. E., M. A. MacNeil, J. A. Olin, B. C. McMeans, M. J. Kinney, D. D. Chapman, and A. T. Fisk. 2012. Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. J Fish Biol **80**:1449-1484.

Hutchinson, G. E. 1957. Cold spring harbor symposium on quantitative biology. Concluding remarks **22**:415-427.

ICCAT. 2008. Recommendation by ICCAT on hammerhead sharks (Family Sphyrnidae) caught in association with fisheries managed by ICCAT.

Jackson, J. B., M. X. Kirby, W. H. Berger, K. A. Bjorndal, L. W. Botsford, B. J. Bourque, R. H. Bradbury, R. Cooke, J. Erlandson, and J. A. Estes. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science **293**:629-637.

Jenkins, S. G., S. T. Partridge, T. R. Stephenson, S. D. Farley, and C. T. Robbins. 2001. Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. Oecologia **129**:336-341.

Kim, S. L., C. M. del Rio, D. Casper, and P. L. Koch. 2012. Isotopic incorporation rates for shark tissues from a long-term captive feeding study. J Exp Biol **215**:2495-2500.

Kim, S. L., and P. L. Koch. 2012. Methods to collect, preserve, and prepare elasmobranch tissues for stable isotope analysis. Environmental Biology of Fishes **95**:53-63.

Kinney, M. J. 2011. The communal nursery area paradigm revisited: niche overlap versus niche separation among juvenile shark species of Cleveland Bay. James Cook University.

Kinney, M. J., N. E. Hussey, A. T. Fisk, A. J. Tobin, and C. A. Simpfendorfer. 2011. Communal or competitive? Stable isotope analysis provides evidence of resource partitioning within a communal shark nursery. Marine Ecology Progress Series **439**:263-276.

Laws, E. A., B. N. Popp, R. R. Bidigare, M. C. Kennicutt, and S. A. Macko. 1995. Dependence of phytoplankton carbon isotopic composition on growth rate and [CO2) aq: theoretical considerations and experimental results. Geochimica et cosmochimica acta **59**:1131-1138.

Lenanton, R., D. Heald, M. Platell, M. Cliff, and J. Shaw. 1990. Aspect of the reproductive biology of the Gummy Shark, Mustelus antarcticus Gunther, from waters off the South coast of Western Australia. Marine and Freshwater Research **41**:807-822.

Lesage, V., Y. Morin, È. Rioux, C. Pomerleau, S. H. Ferguson, and É. Pelletier. 2010. Stable isotopes and trace elements as indicators of diet and habitat use in cetaceans: predicting errors related to preservation, lipid extraction, and lipid normalization. Marine Ecology Progress Series **419**:249-265.

Li, Y., N. E. Hussey, and Y. Zhang. 2016. Quantifying ontogenetic stable isotope variation between dermis and muscle tissue of two pelagic sharks. Aquatic Biology **25**:53-60.

Lieber, L., S. Berrow, E. Johnston, G. Hall, J. Hall, C. Gubili, D. W. Sims, C. S. Jones, and L. R. Noble. 2013. Mucus: aiding elasmobranch conservation through non-invasive genetic sampling. Endangered Species Research **21**:215-222.

Logan, J. M., T. D. Jardine, T. J. Miller, S. E. Bunn, R. A. Cunjak, and M. E. Lutcavage. 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. Journal of Animal Ecology **77**:838-846.

Loor-Andrade, P., F. Galván-Magaña, F. R. Elorriaga-Verplancken, C. Polo-Silva, and A. Delgado-Huertas. 2015. Population and individual foraging patterns of two hammerhead sharks using carbon and nitrogen stable isotopes. Rapid Communications in Mass Spectrometry **29**:821-829.

Lucifora, L. O., V. B. García, R. C. Menni, and A. H. Escalante. 2006. Food habits, selectivity, and foraging modes of the school shark Galeorhinus galeus. Marine Ecology Progress Series **315**:259-270.

MacNeil, M. A., G. B. Skomal, and A. T. Fisk. 2005. Stable isotopes from multiple tissues reveal diet switching in sharks. Marine Ecology Progress Series **302**:199-206.

McMahon, K. W., L. L. Hamady, and S. R. Thorrold. 2013. Ocean ecogeochemistry: a review. Pages 327-374 Oceanography and Marine Biology: An Annual Review.

Mekhtiyeva, V., M. VL, M. VL, P. RG, and G. Y. YA. 1976. Distributions and isotopic compositions of forms of sulfur in water animals and plants.

Mittermayr, A., S. E. Fox, and U. Sommer. 2014. Temporal variation in stable isotope composition (δ13C, δ15N and δ34S) of a temperate Zostera marina food web. Marine Ecology Progress Series **505**:95-105.

Myers, R. A., J. K. Baum, T. D. Shepherd, S. P. Powers, and C. H. Peterson. 2007. Cascading effects of the loss of apex predatory sharks from a coastal ocean. Science **315**:1846-1850.

Navia, A. F., P. A. Mejía-Falla, and A. Giraldo. 2007. Feeding ecology of elasmobranch fishes in coastal waters of the Colombian Eastern Tropical Pacific. BMC ecology **7**:8.

Ochoa Díaz, M. R. 2009. Espectro trófico del tiburón martillo Sphyrna zygaena (Linnaeus, 1758) en Baja California Sur: Aplicación de δ13C y δ 15N. Instituto Politécnico Nacional. Centro Interdisciplinario de Ciencias Marinas.

Olin, J. A., N. E. Hussey, M. Fritts, M. R. Heupel, C. A. Simpfendorfer, G. R. Poulakis, and A. T. Fisk. 2011. Maternal meddling in neonatal sharks: implications for interpreting stable isotopes in young animals. Rapid Communications in Mass Spectrometry **25**:1008-1016.

Pajuelo, M., K. A. Bjorndal, J. Alfaro-Shigueto, J. A. Seminoff, J. C. Mangel, and A. B. Bolten. 2010. Stable isotope variation in loggerhead turtles reveals Pacific–Atlantic oceanographic differences. Marine Ecology Progress Series **417**:277-285.

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

Perga, M.-E., and D. Gerdeaux. 2003. Using the δ13C and δ15N of whitefish scales for retrospective ecological studies: changes in isotope signatures during the restoration of Lake Geneva, 1980–2001. Journal of Fish Biology **63**:1197-1207.

Perry, R. I., P. A. Thompson, D. L. Mackas, P. J. Harrison, and D. R. Yelland. 1999. Stable carbon isotopes as pelagic food web tracers in adjacent shelf and slope regions off British Columbia, Canada. Canadian Journal of Fisheries and Aquatic Sciences **56**:2477-2486.

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

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**:703-718.

R Core Team. 2013. R: A language and environment for statistical computing. Vienna, Austria.

Raoult, V., M. K. Broadhurst, V. M. Peddemors, J. E. Williamson, and T. F. Gaston. 2019. Resource use of great hammerhead sharks (Sphyrna mokarran) off eastern Australia. Journal of Fish Biology **95**:1430-1440.

Red-List, I. 2018. The IUCN Red-list of Threatened Species. http://www.iucnredlist.org last accessed 04.10,2020

Rees, C. 1973. A steady-state model for sulphur isotope fractionation in bacterial reduction processes. Geochimica et cosmochimica acta **37**:1141-1162.

Reif, W.-E. 1978. Wound healing in sharks. Zoomorphologie **90**:101-111.

Ridewood, W., and E. W. MacBride. 1921. VIII.—On the calcification of the vertebral centra in sharks and rays. Philosophical Transactions of the Royal Society of London. Series B, Containing Papers of a Biological Character **210**:311-407.

Rosa, D., R. Coelho, J. Fernandez-Carvalho, and M. N. Santos. 2017. Age and growth of the smooth hammerhead, Sphyrna zygaena, in the Atlantic Ocean: comparison with other hammerhead species. Marine Biology Research **13**:300-313.

Rosende-Pereiro, A., J. R. Flores-Ortega, G. González-Sansón, and A. Corgos. 2020. Stomach content and stable isotopes reveal an ontogenetic dietary shift of young-of-the-year scalloped hammerhead sharks (Sphyrna lewini) inhabiting coastal nursery areas. Environmental Biology of Fishes **103**:49-65.

Rossman, S., P. H. Ostrom, F. Gordon, and E. F. Zipkin. 2016. Beyond carbon and nitrogen: guidelines for estimating three-dimensional isotopic niche space. Ecology and evolution **6**:2405-2413.

Santos, C. C., and R. Coelho. 2018. Migrations and habitat use of the smooth hammerhead shark (Sphyrna zygaena) in the Atlantic Ocean. PloS one **13**.

Santos, R., A. Novoa-Pabon, H. Silva, and M. Pinho. 2020. Elasmobranch species richness, fisheries, abundance and size composition in the Azores archipelago (NE Atlantic). Marine Biology Research **16**:103-116.

Schmiing, M., H. Diogo, R. Serrão Santos, and P. Afonso. 2015. Marine conservation of multispecies and multi-use areas with various conservation objectives and targets. ICES Journal of Marine Science **72**:851-862.

Shephard, K. 1981. The influence of mucus on the diffusion of water across fish epidermis. Physiological Zoology **54**:224-229.

Shipley, O. N., and P. Matich. 2020. Studying animal niches using bulk stable isotope ratios: an updated synthesis. Oecologia:1-25.

Simpfendorfer, C. A., and N. E. Milward. 1993. Utilisation of a tropical bay as a nursery area by sharks of the families Carcharhinidae and Sphyrnidae. Environmental Biology of Fishes **37**:337-345.

Smale, M. J. 2010. Occurrence and feeding of three shark species,Carcharhinus brachyurus, C. obscurus and Sphyrna zygaena, on the Eastern Cape coast of South Africa. South African Journal of Marine Science **11**:31-42.

Springer, S. 1967. Social organization of shark population. Sharks, skate and rays:149-174.

Swanson, H. K., M. Lysy, M. Power, A. D. Stasko, J. D. Johnson, and J. D. Reist. 2015. A new probabilistic method for quantifying n-dimensional ecological niches and niche overlap. Ecology **96**:318-324.

Thorburn, J., F. Neat, I. Burrett, L.-A. Henry, D. M. Bailey, C. S. Jones, and L. R. Noble. 2019. Ontogenetic Variation in Movements and Depth Use, and Evidence of Partial Migration in a Benthopelagic Elasmobranch. Frontiers in Ecology and Evolution **7**.

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

Torres, P., R. T. da Cunha, R. Maia, and A. dos Santos Rodrigues. 2014. Trophic ecology and bioindicator potential of the North Atlantic tope shark. Science of the Total Environment **481**:574-581.

Trueman, C. N., and K. S. J. Glew. 2019. Isotopic tracking of marine animal movement. Pages 137-172 Tracking animal migration with stable isotopes. Elsevier.

Walker, T. I., Cavanagh, R.D., Stevens, J.D., Carlisle, A.B., Chiaramonte, G.E., Domingo, A., Ebert, D.A., Mancusi, C.M., Massa, A., McCord, M., Morey, G., Paul, L.J., Serena, F. & Vooren. 2006. Galeorhinus galeus. The IUCN Red List of Threatened Species. last accessed 12 November 2018

Walker, T. I., Rigby, C.L., Pacoureau, N., Ellis, J., Kulka, D.W., Chiaramonte, G.E. & Herman, K. . 2020. *Galeorhinus galeus*. The IUCN Red List of Threatened Species **2020: e.T39352A2907336**.

Weidel, B. C., S. R. Carpenter, J. F. Kitchell, and M. J. Vander Zanden. 2011. Rates and components of carbon turnover in fish muscle: insights from bioenergetics models and a whole-lake 13C addition. Canadian Journal of Fisheries and Aquatic Sciences **68**:387-399.

Winters, J. K. 1972. Variations in the natural abundance of carbon-13 in proteins and amino acids.

Wood, S., F. Scheipl, and M. S. Wood. 2017. Package 'gamm4'. American Statistician **45**:339.

Woodland, R. J., M. A. Rodríguez, P. Magnan, H. Glémet, and G. Cabana. 2012. Incorporating temporally dynamic baselines in isotopic mixing models. Ecology **93**:131-144.

Worm, B., B. Davis, L. Kettemer, C. A. Ward-Paige, D. Chapman, M. R. Heithaus, S. T. Kessel, and S. H. Gruber. 2013. Global catches, exploitation rates, and rebuilding options for sharks. Marine Policy **40**:194-204.

IX Appendix

Figure i (left). Adult Galeorhinus galeus in the rehabilitation tank after being dehooked from the longline.

Figure ii (right). Adult Galeorhinus galeus during the tagging and muscle sampling on the surgery table.

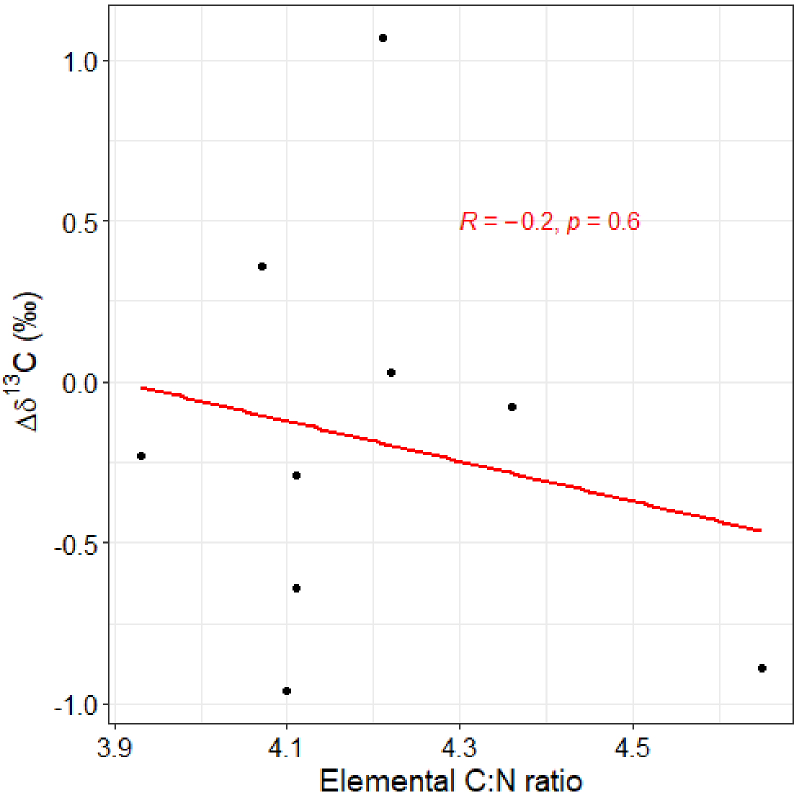

Figure iii. Elemental C:N ratio by $\Delta\delta^{13}C$ (urea extracted $\delta^{13}C$ – urea and lipid extracted $\delta^{13}C$) with the corresponding linear regression statistical values.

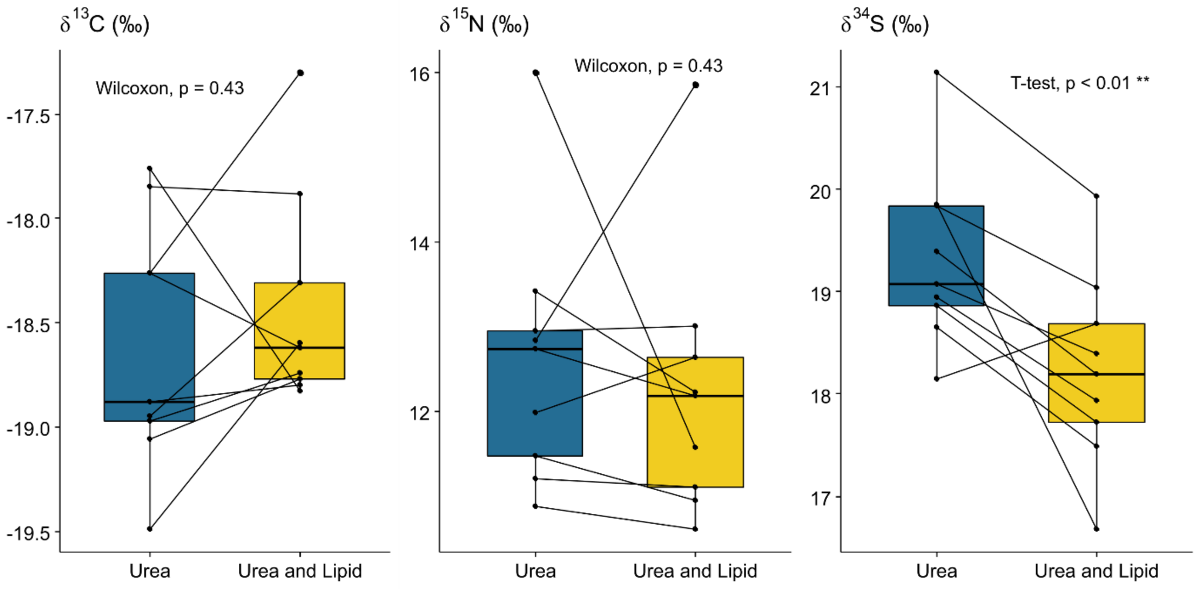

Figure iv. Boxplots of the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values and statistical differences of paired urea-, and urea and lipid extracted shark muscle samples.

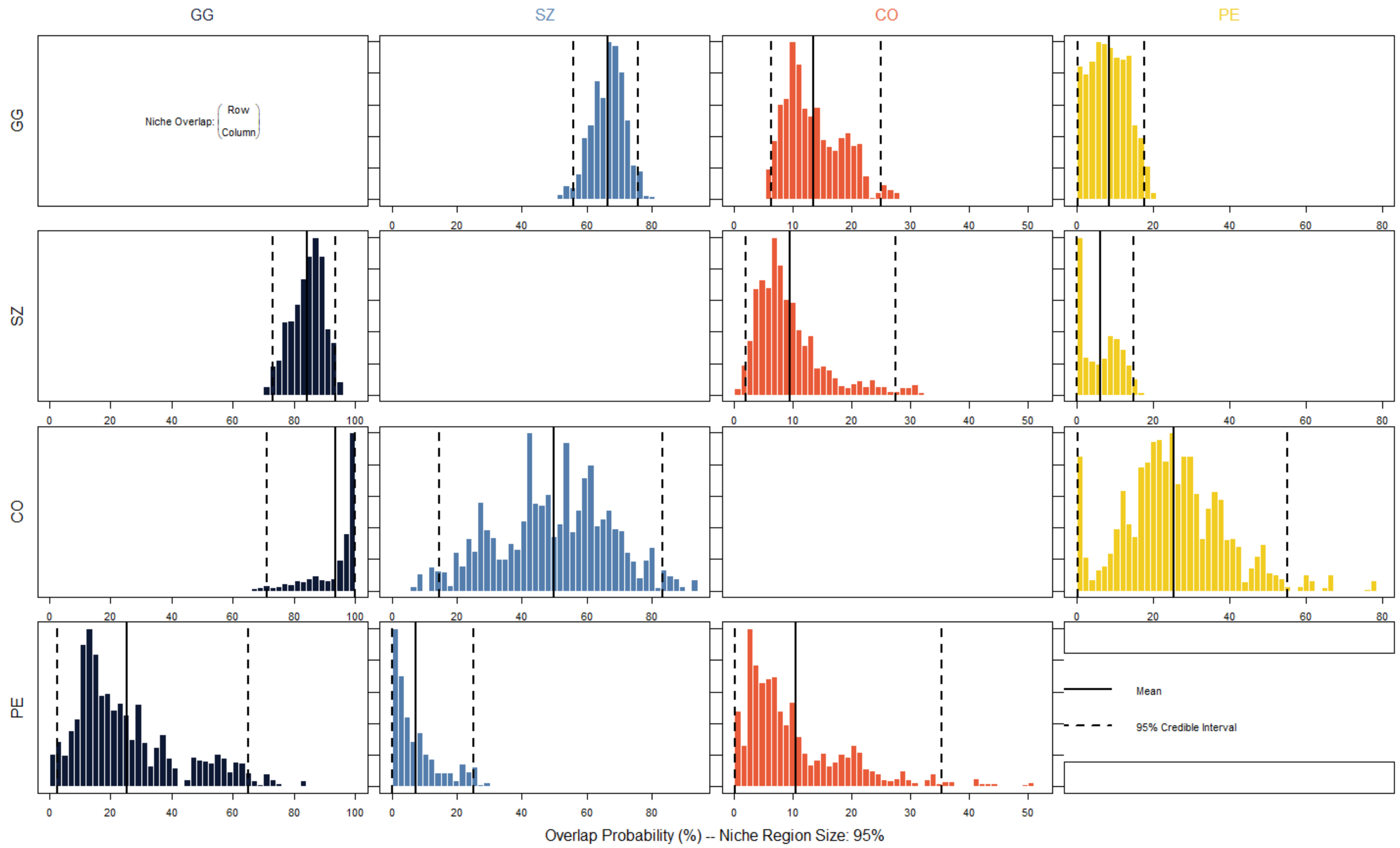

Figure v – Niche region overlap probability distribution for tope shark (G. galeus – GG), smooth hammerhead shark (S. zygaena – SZ), and coastal (CO) and pelagic (PE) food web samples based on 10 000 Monte Carlo draws and 95% confidence intervals.

Table i. Stable isotope results of all muscle and food web samples, where TL is the total length in cm, Corr. $\delta^{13}C$ is the lipid corrected $\delta^{13}C$ values, is the C:N are elemental carbon to nitrogen ratios.

Sample ID	**Species**	**Year**	**TL**	**Sex**	$\delta^{13}C$ (‰)	Corr. $\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	**C:N**
SZ001-MS1	*Sphyrna zygaena*	2013	147.5	F	NA	NA	NA	NA	NA
SZ002-MS1	*Sphyrna zygaena*	2013	62	F	-17.85	-19.86	13.42	21.14	4.22
SZ003-MS1	*Sphyrna zygaena*	2013	78	F	-19.49	-22.16	12.95	19.07	4.65
SZ016-MS1	*Galeorhinus galeus*	2013	35	F	-18.88	-21.11	11.98	18.14	4.36
GG007-MS1	*Galeorhinus galeus*	2014	123	M	-19.15	-21.16	11.25	17.5	4.22
SZ008-MS1	*Sphyrna zygaena*	2014	79	F	-19.16	-21.05	11.08	18.31	4.14
GG009-MS1	*Galeorhinus galeus*	2014	112	M	-18.81	-20.03	11.02	17.82	3.75
GG010-MS1	*Galeorhinus galeus*	2014	117	M	-19.09	-20.34	11.13	17.66	3.77
GG011-MS1	*Galeorhinus galeus*	2014	79	M	-19.04	-20.86	10.92	17.74	4.1
GG012-MS1	*Galeorhinus galeus*	2014	77	F	-19.06	-20.54	10.79	18.76	3.9
GG013-MS1	*Galeorhinus galeus*	2014	70.3	M	-19.02	-20.36	10.91	17.94	3.82
GG014-MS1	*Galeorhinus galeus*	2014	73.3	M	-19.19	-21.06	10.53	17.29	4.13
SZ015-MS1	*Sphyrna zygaena*	2014	81.5	F	-18.91	-20.78	12.39	18.4	4.13
SZ079-MS1	*Sphyrna zygaena*	2014	97	F	-18.95	-20.79	12.73	19.84	4.11
SZ017-MS1	*Sphyrna zygaena*	2014	87	M	-18.84	-20.84	12.27	17.95	4.21
GG018-MS1	*Galeorhinus galeus*	2014	108	M	-17.7	-19.68	13.94	16.81	4.2
GG019-MS1	*Galeorhinus galeus*	2014	95	F	-19.21	-20.68	10.84	18.4	3.89
GG020-MS1	*Galeorhinus galeus*	2014	110.5	M	-18.88	-20.73	12.07	18.08	4.12
GG021-MS1	*Galeorhinus galeus*	2018	190	F	-17.6	-19.71	14.06	16.97	4.28
GG031-MS1	*Galeorhinus galeus*	2018	173	F	-18.63	-20.28	12.72	18.95	4
GG034-MS1	*Galeorhinus galeus*	2018	173	F	-17.57	-19.09	16.16	17	3.92
GG037-MS1	*Galeorhinus galeus*	2018	174	F	-17.99	-20.26	15.07	16.84	4.38
GG049-MS1	*Galeorhinus galeus*	2018	125	M	-18.49	-20.16	12.13	18.83	4.01
GG050-MS1	*Galeorhinus galeus*	2018	133	M	-18.54	-20.59	13.45	17.99	4.24
GG004-MS1	*Galeorhinus galeus*	2018	148	F	-17.76	-19.76	16	18.95	4.21
SZ058-MS1	*Sphyrna zygaena*	2019	136	M	-17.46	-20.93	10.28	15.87	5.24
GG059-MS1	*Galeorhinus galeus*	2019	60	F	-18.87	-20.90	10.59	17.98	4.23
GG061-MS1	*Galeorhinus galeus*	2019	82	M	-19.74	-22.47	11.9	19.02	4.69
GG062-MS1	*Galeorhinus galeus*	2019	114	M	-18.88	-20.77	11.05	18.48	4.14
GG064-MS1	*Galeorhinus galeus*	2019	135.4	F	-18.42	-20.53	12.18	17.96	4.28
SZ068-MS1	*Sphyrna zygaena*	2019	131	F	-18.14	-20.04	12.73	18.79	4.15
SZ070-MS1	*Sphyrna zygaena*	2019	80	F	-18.41	-20.58	12.91	18.66	4.32
GG071-MS1	*Galeorhinus galeus*	2019	89	F	-18.56	-20.75	12.23	18.48	4.33
GG072-MS1	*Galeorhinus galeus*	2019	129	M	-17.78	-19.86	11.54	18.29	4.26
SZ073-MS1	*Sphyrna zygaena*	2019	75	F	-18.58	-20.77	12.45	18.44	4.33
SZ074-MS1	*Sphyrna zygaena*	2019	86	M	-18.64	-20.83	12.65	18.18	4.33
SZ076-MS1	*Sphyrna zygaena*	2019	78	F	-19.05	-21.50	12.6	18.66	4.5
SZ077-MS1	*Sphyrna zygaena*	2019	116	M	-18	-19.82	12.81	13.84	4.1

SZ078-MS1	*Sphyrna zygaena*	2019	109	M	-18.17	-20.09	12.95	18.51	4.16
GG055-MS1	*Sphyrna zygaena*	2019	79	F	-18.26	-20.08	12.84	19.85	4.1
GG080-MS1	*Galeorhinus galeus*	2019	96	M	-18.51	-20.41	12.54	17.21	4.15
GG081-MS1	*Galeorhinus galeus*	2019	111	F	-18.93	-20.85	11.807	18.02	4.16
GG085-MS1	*Galeorhinus galeus*	2019	119	F	-19.8	-22.22	12.5	19.16	4.48
GG087-MS1	*Galeorhinus galeus*	2019	87	M	-18.99	-20.81	11.76	18.23	4.1
GG095-MS1	*Galeorhinus galeus*	2019	92	M	-18.70	-20.96	13.037	18.61	4.37
GG094-MS1	*Galeorhinus galeus*	2019	151	F	-18.91	-20.50	12.42	18.99	3.96
GG098-MS1	*Galeorhinus galeus*	2019	152	F	-16.45	-17.32	10.46	16.17	3.56
GG097-MS1	*Galeorhinus galeus*	2019	139	F	-19.06	-20.90	10.88	18.65	4.11
GG101-MS1	*Galeorhinus galeus*	2019	122	F	-18.85	-20.99	12.22	17.48	4.3
GG106-MS1	*Galeorhinus galeus*	2019	89	M	-18.93	-20.96	11.73	17.65	4.23
GG103-MS1	*Galeorhinus galeus*	2019	91	M	-18.3	-19.83	11.7	18.37	3.93
GG104-MS1	*Galeorhinus galeus*	2019	142	F	-16.41	-17.01	10.81	17.81	3.42
GG105-MS1	*Galeorhinus galeus*	2019	88.5	M	-19.76	-22.04	12.75	19.49	4.39
GG108-MS1	*Galeorhinus galeus*	2019	86	M	-18.81	-20.71	11.5	17.91	4.15
SZ109-MS1	*Galeorhinus galeus*	2019	88	M	-19.62	-22.07	12.91	17.63	4.5
SZ111-MS1	*Sphyrna zygaena*	2019	138	F	-18.6	-20.50	12.57	17.81	4.15
GG088-MS1	*Sphyrna zygaena*	2019	108	M	-18.22	-20.02	12.15	17.34	4.09
GG112-MS1	*Galeorhinus galeus*	2019	79	M	-18.97	-20.50	11.48	19.39	3.93
SZ114-MS1	*Sphyrna zygaena*	2019	123	F	-18.94	-21.05	12.94	17.96	4.28
GG116-MS1	*Galeorhinus galeus*	2019	80	F	-18.26	-20.03	11.2	18.86	4.07
GG121-MS1	*Galeorhinus galeus*	2019	93.5	M	-18.53	-20.46	11.67	17.81	4.16
GG129-MS1	*Galeorhinus galeus*	2019	73.5	M	-18.92	-20.45	10.58	19.26	3.93
GG130-MS1	*Galeorhinus galeus*	2019	68.5	M	-16.87	-18.00	10.46	16.51	3.7
GG131-MS1	*Galeorhinus galeus*	2019	135.5	F	-18.64	-20.29	11.13	18.48	4
GG133-MS1	*Galeorhinus galeus*	2019	70	F	-18.81	-20.40	10.5	18.22	3.96
GG135-MS1	*Galeorhinus galeus*	2019	76	M	-18.56	-20.08	10.78	19.19	3.92
GG136-MS1	*Galeorhinus galeus*	2019	67	F	-18.62	-20.24	11.23	17.5	3.98
SZ140-MS1	*Sphyrna zygaena*	2019	68	F	-18.94	-20.73	10.37	18.32	4.08
SZ143-MS1	*Sphyrna zygaena*	2019	70	F	-16.44	-17.85	13.16	17	3.86
SZ144-MS1	*Sphyrna zygaena*	2019	60	M	-17.56	-19.46	12.99	17.93	4.15
SZ145-MS1	*Sphyrna zygaena*	2019	70	F	-16.48	-17.86	13.02	16.96	3.84
SZ146-MS1	*Sphyrna zygaena*	2019	62	F	-17.38	-19.20	12.97	18.44	4.1
SZ147-MS1	*Sphyrna zygaena*	2019	69	F	-17.6	-19.15	13.3	18.78	3.94
SZ148-MS1	*Sphyrna zygaena*	2019	66	M	-17.45	-19.15	13.4	19.25	4.03
SZ149-MS1	*Sphyrna zygaena*	2019	69	M	-17.61	-18.65	12.58	18.97	3.65
SZ150-MS1	*Sphyrna zygaena*	2019	54	M	-17.66	-19.19	12.86	18.62	3.93
SZ151-MS1	*Sphyrna zygaena*	2019	70	M	-17.74	-19.26	12.81	19.04	3.92
GG153-MS1	*Galeorhinus galeus*	2019	165	F	-18.22	-20.22	13.42	18.62	4.21
SZ155-MS1	*Sphyrna zygaena*	2019	97	M	-18.68	-20.43	12.27	18.22	4.06
SZ156-MS1	*Sphyrna zygaena*	2019	102	F	-18.55	-20.22	12.63	18.24	4.01
GG158-MS1	*Galeorhinus galeus*	2019	161	F	-18.24	-20.22	13.56	19.87	4.2
GG160-MS1	*Galeorhinus galeus*	2019	158	F	-17.53	-19.42	14.9	18.15	4.14

GG161-MS1	*Galeorhinus galeus*	2019	169	F	-14.86	-15.84	14.37	16.44	3.62
SZ163-MS1	*Sphyrna zygaena*	2019	130	F	-18.75	-20.06	11.57	19.05	3.8
SZ164-MS1	*Sphyrna zygaena*	2019	159	F	-16.89	-18.02	11.46	16.57	3.7
SZ165-MS1	*Sphyrna zygaena*	2019	109.3	M	-18.36	-19.77	12.1	17.93	3.86
SZ166-MS1	*Sphyrna zygaena*	2019	100	M	-18.84	-20.34	11.88	18.29	3.91
SZ167-MS1	*Sphyrna zygaena*	2019	98	F	-17.94	-19.35	11.67	17.51	3.86
GG168-MS1	*Galeorhinus galeus*	2019	164	F	-18.09	-19.96	12.82	18.41	4.13
GG174-MS1	*Galeorhinus galeus*	2019	83	F	-19.11	-20.86	10.4	18.48	4.06
Coastal Food Web									
CO001-MS0	*Diplodus sargus*	2020			-18.22	-19.49	10.85	19.31	3.94
CO002-MS0	*Raja clavata*	2019			-19.13	-21.04	10.79	19.23	4.55
CO003-MS0	*Raja clavata*	2019			-18.45	-20.13	12.68	19.56	4.32
CO004-MS0	*Pagrus pagrus*	2019			-18.8	-20.13	11.72	18.79	3.99
CO005-MS0	*Pagrus pagrus*	2019			-18.23	-19.59	10.53	18.62	4.02
CO006-MS0	*Pagrus pagrus*	2019			-19.17	-21.38	10.54	19.24	4.88
Pelagic Food Web									
PE001-MS0	*Euphausiacea*	2019			-21.21	-22.79	5.62	20.54	4.32
PE002-MS0	*Euphausiacea*	2019			-20.77	-22.39	5.69	20.74	4.35
PE003-MS0	*Euphausiacea*	2019			-21.36	-23.60	5.11	21.04	4.83
PE004-MS0	*Acanthocybium solandri*	2019			-19.51	-21.86	9.59	18.82	5.03
PE005-MS0	*Coryphaena hippurus*	2019			-19.51	-21.31	10.14	19.78	4.44
PE006-MS0	*Coryphaena hippurus*	2019			-19.49	-21.00	9.62	19.91	4.16

Table ii. Stable isotope results of all vertebrae centra samples, where GG is G. galeus, SZ is S. zygaena, Processing is the vertebrae increment: 1 – centre, 2 – half radius, 3 – outer edge, TL is the total length in cm, and C:N is the elemental carbon to nitrogen ratios.

Sample ID	**Species**	**Year**	**Processing**	**TL**	**Sex**	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	**C:N**
SZ001-VC1	SZ	2013	1	147.5	F	-14.18	10.69	16.17	3.89
SZ001-VC2	SZ	2013	2	147.5	F	-16.02	10.72	16.81	3.74
SZ001-VC3	SZ	2013	3	147.5	F	-16.61	10.47	16.67	3.76
SZ003-VC1	SZ	2013	1	78	F	-14.64	11.75	17.77	4.09
SZ003-VC2	SZ	2013	3	78	F	-14.77	11.33	18.55	3.94
SZ079-VC1	SZ	2019	1	79	F	-14.6	11.55	18.51	4.11
SZ079-VC2	SZ	2019	3	79	F	-16.18	10.7	18.76	4.48
GG097-VC1	GG	2019	1	139	F	-15.62	10.31	16.41	3.73
GG097-VC2	GG	2019	2	139	F	-16.97	9.78	17.58	3.86
GG097-VC3	GG	2019	3	139	F	-16.3	10.19	19.28	3.65
GG112-VC0	GG	2019	1	79	M	-16.17	10.64	17.56	3.84
GG112-VC1	GG	2019	2	79	M	-15.52	11.84	18.68	3.94
GG112-VC2	GG	2019	3	79	M	-16.22	10.47	17.32	3.6
GG116-VC1	GG	2019	1	80	F	-15.4	10.63	18.45	3.96
GG116-VC2	GG	2019	3	80	F	-16.23	10.29	18.7	4

Table iii. Stable isotope results of all mucus samples, where TL is the total length in cm, and C:N is the elemental carbon to nitrogen ratios.

Sample ID	**Specie**	**Year**	**TL**	**Sex**	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	**CN**
GG010-MC0	GG	2014	117	M	-18.53	11.11	20.18	3.56
GG013-MC0	GG	2014	70.3	M	-19.96	11.23	22.56	3.99
GG014-MC0	GG	2014	73.3	M	-19.58	10.5	24.68	3.49
GG168-MC0	GG	2019	164	F	-19.17	8.28	22.35	5.02
GG170-MC0	GG	2019	134	F	-21.39	6.49	22.48	4.59
SZ015-MC0	SZ	2014	81.5	F	-19.38	14.94	24.71	4.08
SZ079-MC0	SZ	2014	97	F	-18.62	15.14	19.68	4.38
SZ142-MC0	SZ	2019	125	F	-21.8	7.01	22.59	4.4
SZ144-MC0	SZ	2019	60	M	-19.07	9.13	22.63	4.01
SZ147-MC0	SZ	2019	69	F	-20.75	7.01	22.93	5.04
SZ148-MC0	SZ	2019	66	M	-20.04	9.75	23.1	3.95
SZ150-MC0	SZ	2019	54	M	-19.13	8.87	23.51	3.74
SZ155-MC0	SZ	2019	97	M	-19.75	9.51	19.57	4.03
SZ156-MC0	SZ	2019	102	F	-24.74	NA	20.76	5.33
SZ163-MC0	SZ	2019	130	F	-20.63	5.9	19.96	5.14
SZ165-MC0	SZ	2019	109.3	M	-21.43	NA	16.81	6.53
SZ166-MC0	SZ	2019	100	M	-20.45	8.52	20.27	4.36