UNIVERSITY OF SOUTHAMPTON

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

School of Ocean and Earth Science

THE TROPHO-SPATIAL ECOLOGY OF DEEP-SEA SHARKS AND CHIMAERAS FROM A STABLE ISOTOPE PERSPECTIVE

by

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ABSTRACT

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Chondrichthyans (sharks, rays and chimaera) are one of the most speciose groups of higher order predators on the planet and are often cited as playing an important functional role in many ecosystems. However, most studies to date have focused on oceanic and shelf habitats, and there is limited information on the ecological role that chondrichthyans play in the deep-sea. This research aims to examine the trophic and spatial ecology of deep-sea chondrichthyans using stable isotope analysis. Stable isotopes of carbon and nitrogen vary among different trophic levels and between spatially separated areas, and therefore provide a potential tool for uncovering some ecological characteristics of deep-water chondrichthyans.

In this study, I found that on a global scale, oceanic sharks appear to transfer nutrients over large spatial scales, whereas sharks found in shelf habitats couple ecologically varied food webs close to their capture location. Although global data is limited for deep-sea sharks, in the northeast Atlantic it appears that sharks found on seamounts are more tightly coupled to pelagic production than their counterparts on the continental slopes. Continental slope habitats may provide access to more isotopic niches, where sharks integrate nutrients from benthic and pelagic nutrient pathways. On the other hand, chimaeras appear to fill a unique role feeding on benthic prey items that are inaccessible to other fishes (e.g hard shelled benthic animals). Depth gradients in nutrient availability are reflected in the bathymetric distribution patterns of chondrichthyan families, with depth segregations likely reducing interspecific competition for resources. For some of the largest shark species in this ecosystem, such as Portuguese dogfish (*Centroscymnus coelolepis*) and leafscale gulper shark (*Centrophorus squamosus*), whole life-history ecology was recovered from sequential analysis of eye lens proteins. Both these species appear to undertake relatively consistent latitudinal migrations linked with ontogeny and reproductive development.

This study reveals the ecological characteristic of diverse deep-sea chondrichthyan assemblages, and how trophic and spatial behaviours facilitate the transfer of nutrients in these ecosystems. Subsequently, chondrichthyans likely play an important role in deep-sea ecosystems and should be managed appropriately within fisheries.

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Declaration of Authorship

I, Christopher S. Bird, declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

THE TROPHO-SPATIAL ECOLOGY OF DEEP-SEA SHARKS AND CHIMAERAS FROM A STABLE ISOTOPE PERSPECTIVE

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. None of this work has been published before submission, although chapter 3 is currently in review.

Signed: ....

Date: 03-11-2017 ....

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Chapter 1: Thesis Introduction

Chondrichthyan fishes (sharks, rays and chimaeras) are one of the most speciose groups of higher trophic predators on the planet and can be found in most marine ecosystems (Ebert *et al.*, 2013). It has recently been reported however that one-quarter of all chondrichthyan species are threatened by extinction, largely due to overexploitation and habitat destruction (Dulvy *et al.*, 2014, Fernandes *et al.*, 2017). Chondrichthyans, especially sharks, are targeted in many global fisheries and also retained as valuable by-catch in other mixed fisheries, where they are harvested for fins, meat, liver oil, and leather (Worm *et al.*, 2013, Dulvy *et al.*, 2014, Dent and Clarke, 2015). Although a highly diverse group, the life history characteristics of many species are typified by slow growth rates, long gestation periods, and low fecundity that culminate in them having limited recovery potential from overexploitation (Stevens *et al.*, 2000, Dulvy and Reynolds, 2002, Garcia *et al.*, 2008, Simpfendorfer and Kyne, 2009). Despite improvements to management and conservation strategies for some species (White *et al.*, 2017), many chondrichthyan fishes are still threatened (Dulvy *et al.*, 2014, Davidson and Dulvy, 2017), with many populations showing limited signs of recovery (Neat *et al.*, 2015, Fernandes *et al.*, 2017). Developing a thorough understanding of the ecological role of this group will be paramount for determining the consequences of their removal, and aid in the development of robust management and conservation strategies (Stevens *et al.*, 2000, Ellis *et al.*, 2008, Heithaus *et al.*, 2008, Clarke, 2009, Simpfendorfer *et al.*, 2011, Heupel *et al.*, 2014, Grubbs *et al.*, 2016, Hays *et al.*, 2016, Davidson and Dulvy, 2017).

Although commonly regarded as being important components of ecosystem function and structure, and often cited as ‘apex predators’ in marine ecosystems, our understanding of their ecological role is continually developing (Stevens *et al.*, 2000, Kitchell *et al.*, 2002, Heithaus *et al.*, 2008, Ferretti *et al.*, 2010, Heupel *et al.*, 2014). There is now growing evidence that many chondrichthyans are mesopredators in most marine ecosystems, with only some species occupying apex positions in some habitats (Heupel *et al.*, 2014, Frisch *et al.*, 2016, Roff *et al.*, 2016). As predators, chondrichthyans have the capacity to interact with various ecosystem components through predation on other animals (Matich *et al.*, 2011), exchanging nutrients between adjacent habitats (McCauley *et al.*, 2012), imposing risk/fear effects on other animals (Heithaus *et al.*, 2008), and scavenging carrion (Jamieson *et al.*, 2011). They themselves are also prey, being consumed by larger sharks, marine mammals and cephalopods (Pyle *et al.*, 1999, Braid and Bolstad, 2014, Frisch *et al.*, 2016). Determining the ecological role of a species is further complicated however, as many species exhibit strong ontogenetic shifts in diet (Moura *et al.*, 2008, Kim *et al.*, 2012b), habitat use (Guttridge *et al.*, 2012, Carlisle *et al.*, 2015) and migratory movements (Chin *et al.*, 2013, Lea *et al.*, 2015a), as well as spatial segregations by size, sex and maturity (Sims, 2005, Heupel *et al.*, 2007, Mucientes *et al.*, 2009, Holt *et al.*, 2013, Moura *et al.*, 2014), which may influence the role that these fish play across a broad range of temporal and spatial scales. For example, daily or seasonal segregations and/or aggregations have the capacity to impose localised ecological interactions, such as predation or fear effects, at those specific areas during those times, further adding complexity to ecological roles (Heupel and Simpfendorfer, 2005).

Most of what we infer about the ecological role of chondrichthyans has been derived from studies from coastal and coral reef habitats (Dulvy *et al.*, 2014, Heupel *et al.*, 2014, Cotton and Grubbs, 2015, Frisch *et al.*, 2016, Roff *et al.*, 2016), with studies on oceanic and in particular deep-sea habitats, still relatively rare. Deep-sea habitats are typically classified as waters deeper than 200 m, and generally mark the edge between continental and insular shelf habitats and that of the upper slopes, with bathymetric reliefs continuing down to the abyssal plains (Kyne and Simpfendorfer, 2010). The majority of deep-sea chondrichthyans are associated with demersal habitats between 200 m – 3500 m (herein referred to as deep-sea) and are typically associated with continental slopes, seamounts and oceanic ridges (Priede *et al.*, 2006, Cotton and Grubbs, 2015, Musick and Cotton, 2015). Deep-sea chondrichthyan species account for 46% of global chondrichthyan diversity (Dulvy *et al.*, 2014), yet over half (57.6%) are currently listed as data deficient on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Figure 1.1). Most deep-sea species are still lacking basic information on aspects such as taxonomy, abundance, stock structure, productivity, spatial distributions, and migration patterns (Kyne and Simpfendorfer, 2010, Ramos *et al.*, 2013, Veríssimo *et al.*, 2014, Cotton and Grubbs, 2015). This of concern considering deep-sea species are some of the most biological vulnerable chondrichthyans, having especially conservative life history stages and some of the lowest potential recovery rates of any fish (Clarke *et al.*, 2003a, Garcia *et al.*, 2008, Simpfendorfer and Kyne, 2009, Kyne and Simpfendorfer, 2010, Rigby and Simpfendorfer, 2013). Although there has been considerable literature stemming from early fisheries surveys (Compagno *et al.*, 1991, Gordon and Swan, 1997, Wetherbee, 2000, Graham *et al.*, 2001), there are still large gaps in our understanding about the trophic and spatial ecology of deep-sea chondrichthyans (Cotton and Grubbs, 2015). Addressing some of these knowledge gaps will be pertinent for improving our understanding of any ecosystem services and functions provided by chondrichthyans, and subsequently aid in the development of sustainable fishing operations of deep-sea fish stocks.

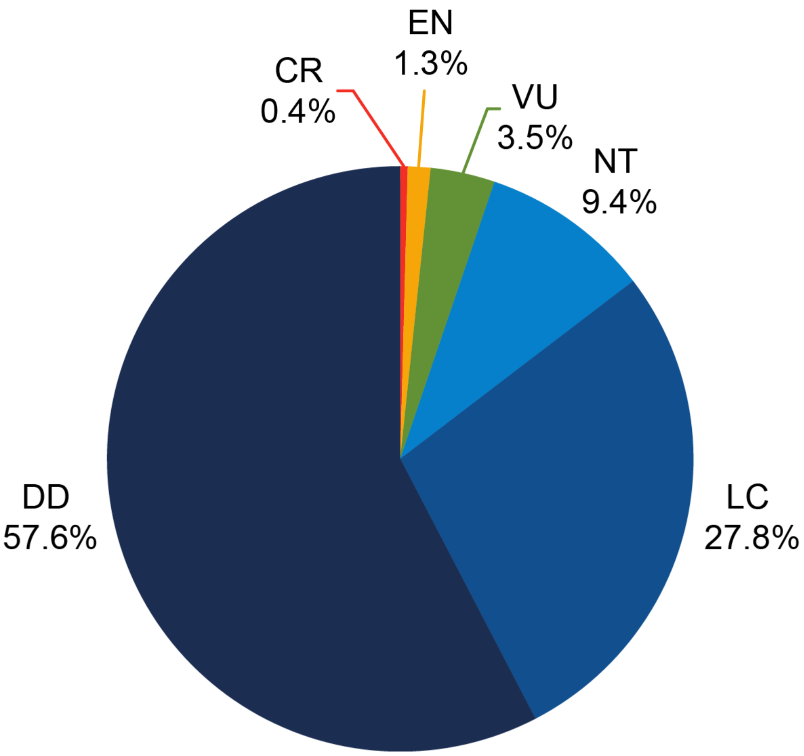

Figure 1.1: Proportional classification of deep-sea chondrichthyans listed on the IUCN Red List of Threatened Species. Modified from results from Dulvy *et al.* (2014). DD – data deficient, LC – least concerned, NT – near threatened, VU – vulnerable, EN – endangered, CR – critically endangered.

1.1 Deep-sea Chondrichthyans

1.1.1 Evolutionary Ecology

In order to fully understand the nature of modern day shark assemblages, it is important to consider the evolutionary processes that established modern day taxa. The dominant shark order found in most deep-sea ecosystems is the dogfish (Squaliformes), with all six families being found almost exclusively in deep-sea habitats (Figure 2.1). Catsharks (order Carcharhiniformes, family Scyliorhinidae/ Pentanchidae), six-gill sharks (order Hexanchiformes, families Hexanchidae and Chlamydoselachidae) and bramble sharks (Echinorhinoformes) can also be found in many deep-water assemblages but they are typically less well represented in terms of abundance (Gordon and Swan, 1997, Neat *et al.*, 2008).

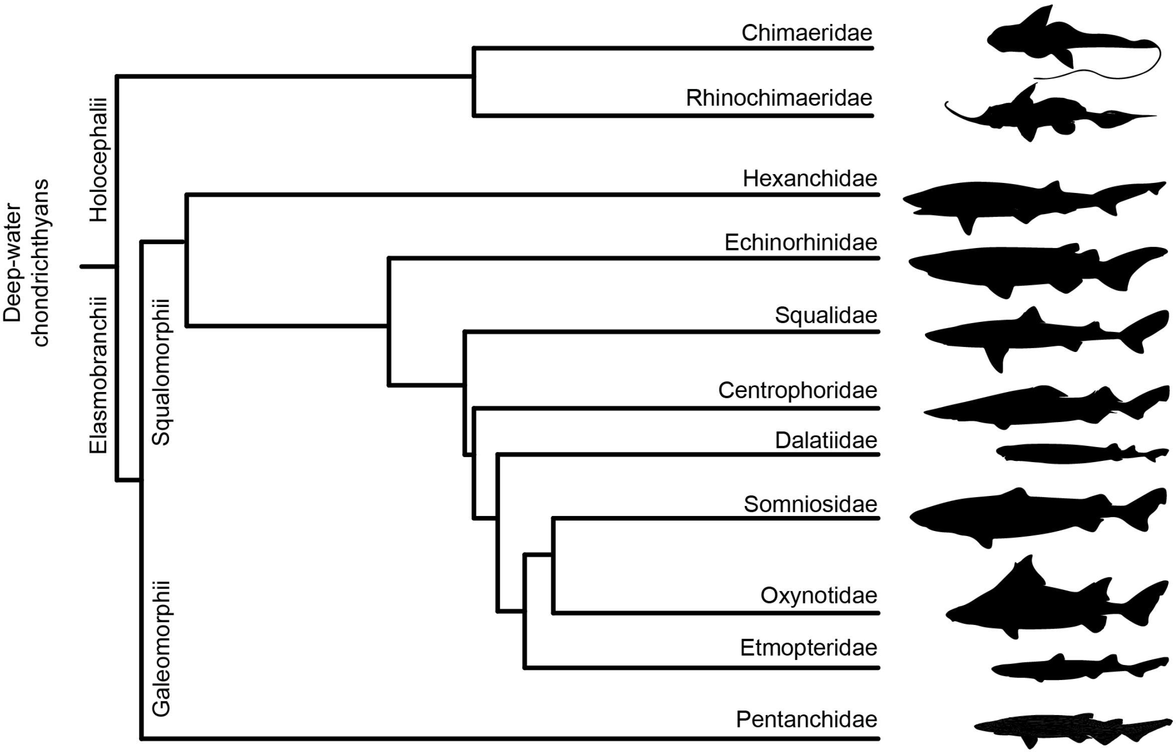

Figure 1.2: Graphical representation of major deep-sea chondrichthyan groups adapted from Straube *et al.* (2015) and Inoue *et al.* (2010). Node lines are not to scale, nor do they reflect accurate estimations of divergence times.

Early chondrichthyans would have probably occupied shallow water habitats before radiating into deep-sea environments (Priede and Froese, 2013). Although modern sharks (neoselachians) have been present since the Palaeozoic (~290mya), the first unequivocal Squaliformes appeared in the fossil record during the Barremian (Late Cretaceous; ~130mya) possibly due to the competitive release from larger hybodont 'sharks' that became extinct during a similar period (Maisey *et al.*, 2004). During the following Turonian (89.8 – 93.9mya) and Santonian (83.6 – 86.3mya), most extant Squaliform families emerge in the fossil record, including Squalidae, followed by Centrophoridae, Somniosidae, Dalatiidae, and then later Oxynotidae and Etmopteridae (Guinot *et al.*, 2012). The emergence and radiation of these Squaliform families, coincides with the global oceanic anoxic event (OAE) at the Cenomanian-Turonian (C/T) boundary (Adnet *et al.*, 2008). Although the causes of OAE are still ambiguous, anoxic environmental conditions were associated with elevated oceanic temperatures and increased sea level rises, and have been linked with the significant mass extinction of many marine taxa across a range of habitats (Raup and Sepkoski, 1986, Schlanger *et al.*, 1987, Adnet *et al.*, 2008). It is believed that the following disappearance of large marine predators and the reorganisation of benthic marine food webs, combined with the deepening of thermal stratification layers, may have opened up deep-water habitats to opportunistic early Squaliformes (Adnet and Cappetta, 2001, Klug and Kriwet, 2010, Priede and Froese, 2013).

Although the mechanisms for the initial emergence and radiation of early deep-water Squaliformes families remains somewhat ambiguous, there is agreement that it was succeeded by a secondary radiation event following the Cretaceous-Tertiary (K/T) mass extinction event (Adnet and Cappetta, 2001). Global mass extinctions in both marine and terrestrial faunas are believed to have been a consequence of the Chixulub meteor impact at the end of the Maastrichtian (~65mya) (Schulte *et al.*, 2010). The subsequent ‘impact-winter’ reduced primary production, oxygen availability and ambient temperatures, as well as increased ocean acidity, which culminated in catastrophic effects for life on earth, causing many ecosystems and food-webs to collapse (Alegret *et al.*, 2012, Robertson *et al.*, 2013). Squaliform diversity was largely unaffected across the K/T boundary in comparison to shallow water taxa suggesting that the deep-sea may have provided a refuge during stressful environmental crises and/or provided substantial scavenging opportunities (Adnet and Cappetta, 2001, Kriwet and Benton, 2004). The smaller body sizes and the versatile cutting-blade dentition characteristic of Squaliformes species may have allowed opportunistic adaptive trophic diversification within a recovering post-crisis ocean, particularly for scavenging carrion (Adnet *et al.*, 2008, Underwood *et al.*, 2016). Smaller body sizes are believed to be favoured following post-crises (Lilliput Effect), with the associated biological, behavioural and ecological properties allowing greater survivorship, and facilitating increased levels of speciation, in stressed environments (Urbanek, 1993, Twitchett, 2007, Berv and Field, 2017). Although rarely considered in studies of chondrichthyan evolution (Mutter and Neuman, 2009), the Lilliput Effect may help explain the prevalence of smaller bodied, highly-speciose groups of Squaliform shark in the deep-sea but more studies of relative shark tooth size across evolutionary time would be needed to confirm this.

Today, chondrichthyans are found in relatively high numbers in many deep-sea environments and compose a significant proportion of ichthyofaunal assemblages (Gordon and Duncan, 1985). Due to the stark environmental differences between deep-sea and shallow water habitats (e.g. oceanic, shelf, coastal, reef), it is currently unclear if the same ecological principles that have been inferred for shallow water chondrichthyans (e.g. (Heithaus *et al.*, 2008, Heupel *et al.*, 2014)) can be applied to these habitats. Modern day deep-sea environments are topographically diverse and compose one of the largest biomes on the planet (Ramirez-Llodra *et al.*, 2010). The abiotic and biotic characteristics of these environments will have a strong influence on the structure and function of ichthyofaunal assemblages, and ultimately, the ecological role of sharks in these environments.

1.2 Deep-sea Environments

The deep-sea is typically characterised by diminished light, low temperatures, limited nutrients but relatively stable environmental conditions (Thurber *et al.*, 2014). Historically considered to be an area of the ocean devoid of life, it is now known that continental slopes, islands, seamounts, and oceanic ridges support some of the highest species diversities on the planet (Grassle and Maciolek, 1992, Levin *et al.*, 2001).

Continental slopes connect shallow shelf seas to abyssal plains along steep seabed reliefs, accounting for ~11% of the total ocean floor (Ramirez-Llodra *et al.*, 2010). Spanning both large latitudinal and bathymetric ranges (~ 200 – 2000 m), these dynamic habitats express strong regional differences in environmental (e.g. temperature, salinity, oxygen) and geological factors (e.g. topography, substrate type) (Koslow, 1993, Gordon *et al.*, 1995, Neat *et al.*, 2008, Bergstad, 2013, Pajuelo *et al.*, 2016). In contrast, seamounts (and the slopes of oceanic islands) are relatively isolated topographic structures, and are generally located near mid-ocean spreading ridges that create distinct regional oceanographic/hydrological conditions (Hulley, 1992, Pitcher *et al.*, 2008, Rowden *et al.*, 2010b). Although topographically distinct, bathyal habitats support rich and abundant deep-sea fish stocks (Gordon and Bergstad, 1992, Gordon *et al.*, 1996, Gordon and Swan, 1997, Kelly *et al.*, 1998, Menezes *et al.*, 2006, Neat *et al.*, 2008).

In the northeast Atlantic, although deep-sea fish assemblages have high species diversities, the most dominant fish are alepocephalids (slick heads), macrourids (grenadiers), and squaliformess (dogfish) (Gordon and Duncan, 1985, Kelly *et al.*, 1998). This is generally true for most habitats with there being a high degree of species overlap between geographic regions (Koslow, 1993, Bergstad *et al.*, 2012, Bergstad, 2013). At the local regional level, ichthyofaunal assemblages are often strongly structured by depth, with bathymetric zonation patterns (depth ranges that share similar species compositions) coinciding with different water masses (Merrett, 1987, Koslow, 1993, Williams *et al.*, 2001, Cartes *et al.*, 2004, King *et al.*, 2006, Menezes *et al.*, 2006, Bergstad *et al.*, 2012, Menezes *et al.*, 2015). In addition to environmental factors, bathymetric trends in nutrient availability also have a strong influence on the structure and function of deep-sea ecosystems (Crabtree *et al.*, 1991, Trueman *et al.*, 2014, Mindel *et al.*, 2016a, Woolley *et al.*, 2016). Determining the extent to which chondrichthyans utilise different nutrient pathways will be fundamental in determining their ecological role and understanding the trophic interactions that exist in these ecosystems.

1.2.1 Nutrient Dynamics

At depths below 200m, diminishing light availability prohibits autotrophic photosynthetic production. Instead, deep-sea ecosystems are predominantly reliant on the vertical transport of nutrients from surface waters (Shanks and Trent, 1980, Miquel *et al.*, 1994, Romero-Romero *et al.*, 2016). Autotrophic nutrients in the form of particulate organic matter (POM) are transported to deep-sea ecosystems both passively through the precipitation of POM and actively via vertically migrating mesopelagic fauna (Billett *et al.*, 1983, Merrett, 1987, Angel and Boxshall, 1990, Mauchline and Gordon, 1991, Trueman *et al.*, 2014). Precipitation of POM, often termed marine snow (Shanks and Trent, 1980), includes substances such as planktonic aggregates, faecal pellets and animal moults that form a significant source of nutrients for bacteria and benthic food webs (Billett *et al.*, 1983, Lampitt *et al.*, 2001, Drazen *et al.*, 2008, Wei *et al.*, 2010). Organic matter is typically integrated into benthic food webs through detritivores and suspension feeders, which in turn are predated by benthic feeding fishes (Iken *et al.*, 2001). Although nutrient availability decreases logarithmically with depth and increasing distance from land (Lampitt *et al.*, 2001, Rex *et al.*, 2006, Buesseler *et al.*, 2007), large animal biomasses and diversities are supported at intermediate depths, in part due to the alternative nutrient subsidies provided by diel vertically migrating animals (Mauchline and Gordon, 1991, Priede *et al.*, 2010, Trueman *et al.*, 2014). The sinking of animal carcasses (carrion) also provides an important source of nutrients, particularly for deep-sea fish that are able to scavenge (Mahaut *et al.*, 1990, Drazen *et al.*, 2008, Jamieson *et al.*, 2011).

Mesopelagic (200 – 1000m) fauna (teleosts, crustaceans, cephalopods) that migrate between surface water layers at night and deep-waters during the day (collectively diel vertical migrators), establish important biological linkages between photic production and demersal fish assemblages (Mauchline and Gordon, 1991, Davison *et al.*, 2013, Sutton, 2013, Trueman *et al.*, 2014). This pelagic production becomes accessible to demersal fishes when diel vertical migrations are truncated by the seafloor, or when horizontal currents cause lateral impingements on the seabed (Isaacs and Schwartzlose, 1965, Angel and Boxshall, 1990, Mauchline and Gordon, 1991, Porteiro and Sutton, 2007). Mauchline and Gordon (1991) found that the distributions and diets of benthopelagic species at Rockall Trough, northeast Atlantic, coincided with the daytime depths of pelagic plankton and micronekton. Furthermore, they ascribed the high benthopelagic fish biomasses at intermediate depths (800 – 1500 m) to the bathymetric range where diel vertical migrators are maximally impinged onto the benthos. This was later supported by Trueman *et al.* (2014) who demonstrated that over 50% of the demersal fish biomass between 500 and 2000m was supported by pelagic nutrient subsides provided by diel vertical migrators. Mesopelagic nutrient subsidies have been demonstrated in multiple locations and appear to be of ubiquitous importance in sustaining bathyal fish assemblages (Fock *et al.*, 2002, Porteiro and Sutton, 2007, Sutton *et al.*, 2008, Romero-Romero *et al.*, 2016, Stasko *et al.*, 2016).

The strong bathymetric trends in nutrient dynamics consequentially have a strong impact on the prevalence of different trophic guilds. Within slope fish communities, benthic feeding fishes dominate in upper slopes (<500m) and lower slopes (>1500m), whereas middle slope depths are dominated by benthopelagic and pelagic-feeding fishes (Trueman *et al.*, 2014). Mindel *et al.* (2016a) found that the functional trait morphologies of various deep-sea teleosts varied as a function of depth and was closely linked with the availability of benthic and pelagic nutrient sources at those depths. Similarly, the general trend of increasing fish body size with depth is believed to be a mechanism that enables scavengers to endure longer periods of starvation between increasingly patchy distributions of carrion (Collins *et al.*, 2005). Although sharks have typically been classified as benthopelagic predators, and chimaera as benthic feeders (Mauchline and Gordon, 1983, Dunn *et al.*, 2010b), their regular appearance at baited underwater cameras suggests that scavenging may be an important component of their diet (Mahaut *et al.*, 1990, King *et al.*, 2006, Jamieson *et al.*, 2011). Additionally, chondrichthyans are distributed over wide bathymetric ranges (Gordon and Swan, 1997, Campbell *et al.*, 2010, Dunn *et al.*, 2013) and will therefore be exposed to bathymetric gradients in nutrient availability. It may therefore be expected that their trophic characteristics will vary as a function of depth, but to what extent resources are partitioned in this group is still unclear.

Although fishery survey assessments (Collins *et al.*, 2005, Priede *et al.*, 2010, Mindel *et al.*, 2016a, Mindel *et al.*, 2016b) and stomach content studies (Mauchline and Gordon, 1983, Ebert *et al.*, 1992, Dunn *et al.*, 2010b) have provided invaluable insight into some of the ecological aspects of deep-sea chondrichthyans (later discussed), stable isotope analyses may provide additional information. By tracing the isotopes of essential carbon and nitrogen nutrients through food webs, it is possible to determine the flow of energy through ecosystems and therefore, the associated trophic interactions (Peterson and Fry, 1987, Hobson, 1999). The stable isotope characteristics of food webs also vary spatially, so can provide additional information on where an animal may have been feeding. Stable isotope analysis may therefore provide a powerful tool to develop a deeper understanding of the ecological characteristics of chondrichthyan fishes.

1.3 Stable Isotope Ecology

1.3.1 Trophic Ecology

In ecology, the isotopic ratios of carbon (expressed as $\delta^{13}C$) and nitrogen (expressed as $\delta^{15}N$) are now routinely used to elucidate the trophic structure and nutrient pathways supporting food webs (Peterson and Fry, 1987, Wada *et al.*, 1991). Established during photosynthetic fractionation, the stable isotope composition of carbon and nitrogen in structural nutrients of primary production are transferred through food webs, via consumption, in a predictable manner so that it is possible to trace the trophic interactions among animals retrospectively. $\delta^{15}N$ values are typically used to infer trophic level, whereas $\delta^{13}C$ values may distinguish between sources of primary productivity supporting food webs (Fry and Sherr, 1989, Vander Zanden *et al.*, 1997, Davenport and Bax, 2002, Post, 2002, Jennings and van der Molen, 2015). During trophic interactions, consumer tissues are enriched in the heavier isotope of nitrogen (^{15}N) resulting in more positive $\delta^{15}N$ values. This ‘trophic enrichment’ occurs due to the preferential excretion of light ^{14}N during metabolism, and the retention of heavier ^{15}N within consumer tissues (Post, 2002). The trophic discrimination factor (TDF), or the difference between prey and consumer tissue values, is approximately 3.4‰ between trophic levels (Post, 2002), although a scaled approach may be more realistic, whereby TDFs decrease with increases in predator size (Hussey *et al.*, 2013).

Conversely, carbon isotopes undergo minimal enrichment during trophic interactions (~0-1‰) and large variations in $\delta^{13}C$ values within a food web are commonly taken to indicate different primary production types (i.e. phytoplankton, macroalgae, seagrasses) (Fry and Sherr, 1989, Davenport and Bax, 2002). $\delta^{13}C$ values vary among different primary production types due to variations in the enzymatic pathways adopted by different plants during photosynthesis (Peterson and Fry, 1987, O'Leary, 1988, Fry and Sherr, 1989), with $\delta^{13}C$ values being typically lower in marine phytoplankton (~ -22‰) than terrestrial plants (~ -13‰). In deep-sea demersal ecosystems, stable isotopes can also be used to distinguish between benthic and pelagic nutrient pathways (Figure 1.3), with benthic food webs being more enriched than pelagic food webs due to the increasing utilisation of organic matter that has been subjected to microbial degradation or trophic recycling (Bergmann *et al.*, 2009, Boyle *et al.*, 2012, Trueman *et al.*, 2014, Stasko *et al.*, 2016). In addition to trophic linked differences, stable isotope values can also vary between geographic locations, providing information on, not only what an animal fed on, but also where those food webs were located.

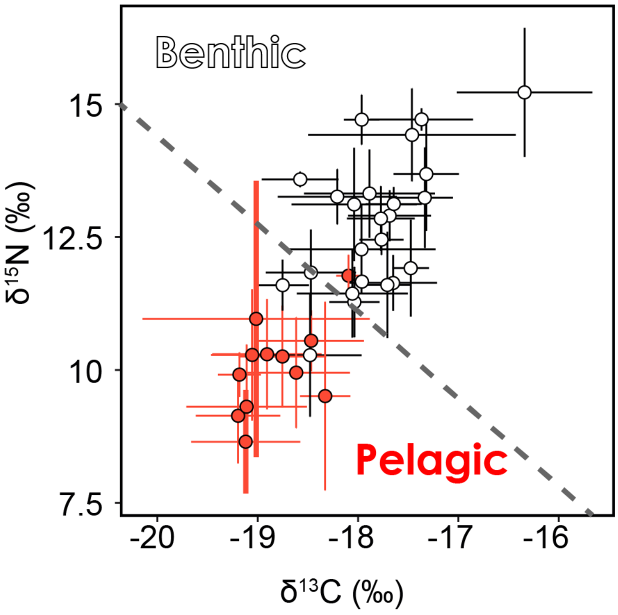

Figure 1.3: Modified figure from Trueman *et al.* (2014) demonstrating the stable isotopic distinction between benthic and pelagic feeding demersal fish from Rockall Trough. Additional data has been added from Vieira *et al in prep*. Individual points are mean species values with standard error bars for pelagic (red) and benthic (clear) feeding fish.

1.3.2 Spatial Ecology

Baseline stable isotope values can vary between different geographic regions, and as such can be used to reveal migratory movement behaviours (Hobson *et al.*, 2010, Trueman *et al.*, 2012, McMahon *et al.*, 2013). In marine environments, $\delta^{15}N$ values vary depending on the nitrogen source utilised by primary production and its isotopic composition (NO_3^-, N^2, NH_3), together with additional biological transformation (N^2 fixation, denitrification, nitrification) among different water masses (Montoya, 2007, Graham *et al.*, 2010, Somes *et al.*, 2010, McMahon *et al.*, 2013, Lorrain *et al.*, 2015). $\delta^{13}C$ values in phytoplankton vary as a function of dissolved CO_2 concentration, cell geometry, taxonomy, and community dynamics (Magozzi *et al.* (2017) and references therein), which are all influenced to some extent by temperature (Rau *et al.*, 1997, MacKenzie *et al.*, 2011, Magozzi *et al.*, 2017). Geographic variations in temperature therefore establish strong $\delta^{13}C$ gradients across ocean basins (Magozzi *et al.*, 2017), which change abruptly at ocean frontal regions (Cherel and Hobson, 2007, Louzao *et al.*, 2017). Models estimating the geographic distribution of baseline stable isotope values (termed 'isoscapes') have advanced in recent years, providing useful tools in identifying spatial movement behaviours of marine animals (Graham *et al.*, 2010, McMahon *et al.*, 2013, MacKenzie *et al.*, 2014, Magozzi *et al.*, 2017).

Animals feeding in one area but then caught in another isotopically distinct location will express stable isotope values that reflect their dietary origin and thus movements (Hobson *et al.*, 2010, MacKenzie *et al.*, 2011). Stable isotope-based geolocation to foraging grounds has been applied to seabirds (Cherel and Hobson, 2007, Meier *et al.*, 2017), tuna (Lorrain *et al.*, 2015), salmon (MacKenzie *et al.*, 2012), turtles (Vander Zanden *et al.*, 2015), whales (Mendes *et al.*, 2007), and seals (Beltran *et al.*, 2015). There have been limited studies however looking at the migratory movements of sharks using this method, and none applied to deep-sea habitats. Carlisle *et al.* (2012) analysed the stable isotope composition of collagen in sequential samples along the diameter of the vertebrae of salmon sharks (*Lamnus ditropis)* revealing off-shore oceanic nursery grounds and ontogenetic movements into more neritic and coastal waters at the onset of maturity. Just as informative as migratory movements, locational residency within isotopically distinct regions has also been demonstrated in several species of sharks. Sevengill sharks (*Notorynchus cepedianus*; (Abrantes and Barnett, 2011) and sharpnose sharks (*Rhizoprionodon taylori*) (Munroe *et al.*, 2015) expressed stable isotope values that were reflective of their prospective capture locations. Both authors concluded that within the timeframe of tissue turnover, spatially separated populations had assimilated local food web signatures through relatively little geographic movement (Abrantes and Barnett, 2011, Munroe *et al.*, 2015).

Because stable isotopes are reflective of previous feeding behaviours (months to years), in theory every animal is 'tagged', thus allowing for the potential retrospective recovery of potential movement behaviours (Trueman *et al.*, 2012). The results are obviously much coarser than GPS satellite telemetry studies (m's) and instead of providing a physical location of an animal, can provide locations of the spatial origin of assimilated dietary nutrients in the range of 100-200 km (Trueman *et al.*, 2017). Satellite telemetry can provide information on where an animal is moving, while stable isotopes can tell you where an animal has fed.

1.3.3 Depth Gradients

One other consideration for deep-sea ecosystems, is the systematic transformation of stable isotope values with depth. In deep-sea environments, surface-derived nutrients are typically partitioned between a detrital benthic pathway, and a biological pelagic pathway mediated by vertically migrating organisms (Drazen *et al.*, 2008, Boyle *et al.*, 2012, Trueman *et al.*, 2014). Benthic food webs utilise POM that may have been subjected to microbial degradation during precipitation through the water column or undergone substantial reworking through previous trophic interactions (resuspended organic matter, faecal pellets, animal tissue etc.; (Drazen *et al.*, 2008, Bergmann *et al.*, 2009)). Furthermore, this enrichment increases with depth, as nutrients progressively undergo additional degradation and recycling (Mintenbeck *et al.*, 2007, Trueman *et al.*, 2014). Consequently benthic feeding consumers express stable isotope values that are enriched in comparison to pelagic food webs, and also show bathymetric enrichment (Trueman *et al.*, 2014).

Pelagic food webs on the other hand are tightly coupled to pelagic production by diel vertically migrators, so that the nutrients assimilated are relatively 'fresh' (Sutton *et al.*, 2008, Choy *et al.*, 2012). Micronektonic communities can also express bathymetric enrichments in stable isotope values due to the integration of degraded precipitating POM, but they are typically not detected in pelagic and benthopelagic consumers due to foraging over large vertical and horizontal ranges (Trueman *et al.*, 2014, Choy *et al.*, 2015). Consumers feeding over either benthic or pelagic trophic pathways will consequently express stable isotope values relating to the bathymetric origin of their diets. This isotopic distinction provides a useful means of elucidating broad-scale trophic interactions of chondrichthyans in these environments, in addition to providing information relating to spatial ecology.

1.3.4 Stable Isotopic Applications in the Study of Shark Ecology

When combined, stable isotope analysis (SIA) of $\delta^{13}C$ and $\delta^{15}N$ provides a powerful tool to examine the tropho-spatial ecology of sharks. The stable isotope values of a consumer's tissues provide an integrated overview of actually assimilated prey items, so overcome some of the limitations of 'snap-shot' stomach content assessments (Cortés, 1997, Hussey *et al.*, 2012a). SIA has been used extensively in shark ecology (reviewed by Shipley *et al.* (2017a) and Shiffman *et al.* (2012)), and have provided information on resource use (Matich *et al.*, 2011, Heithaus *et al.*, 2013, Matich *et al.*, 2017), trophic levels (Estrada *et al.*, 2003, Hussey *et al.*, 2013), trophic structure

(Layman *et al.*, 2007, Churchill *et al.*, 2015b, Hussey *et al.*, 2015), ontogenetic behavioural shifts (Estrada *et al.*, 2006, Carlisle *et al.*, 2015), and spatial movement patterns (Carlisle *et al.*, 2012, Munroe *et al.*, 2015). Furthermore, differences in the metabolic turnover rates of different tissues types, can provide ecological information pertaining to a range of timeframes (MacNeil *et al.*, 2005). In sharks, plasma, muscle and cartilage are typically reflective of feeding behaviours from the previous months, years and decades respectively (MacNeil *et al.*, 2005, Caut *et al.*, 2009, Kim *et al.*, 2012a). Sequential analysis of incrementally grown tissues such as vertebrae has also allowed the construction of stable isotopic profiles relating to the whole life ecology of an individual, providing valuable insight into ontogenetic behavioural shifts and the ecology of cryptic life stages (Estrada *et al.*, 2006, Carlisle *et al.*, 2015, Christiansen *et al.*, 2015).

The application of stable isotope analysis may therefore provide a powerful tool to retrospectively uncover the cryptic ecology of chondrichthyan assemblages (an overview of the stable isotopic gradients found in the marine environment can be found in Figure 1.4). However, due to the number of spatial and trophic influences on stable isotope ecology, and their confounding interactions (Christiansen *et al.*, 2015), subsequent inferences of stable isotope patterns are best used in complement to other methodological approaches. The descriptive resolution of stable isotope analysis may not be as accurate as satellite telemetry studies or long-term dietary descriptions, but when applied to data-poor deep-sea environments, have the potential to provide new insight. Stable isotope studies on chondrichthyan fishes must also consider confounding influences of biochemical contaminants such as urea, trimethylamine oxide (TMAO) and lipids on recorded stable isotope values (reviewed by (Shipley *et al.*, 2017a)), and must adopt methodological practices that account for these effects before making ecological interpretations.

1.3.5 Lipid and Urea Contaminations

Urea, in conjunction with TMAO, are used by chondrichthyan fishes to osmoregulate (Hammerschlag, 2006). As waste products, urea and TMAO (herein after collectively referred to as urea) have relatively high concentrations of the lighter isotope of nitrogen, ^{14}N, which is preferentially excreted (Gannes *et al.*, 1998). Accordingly, tissue samples with high dissolved urea contents can express lower δ^{15}N values than that of true protein (Churchill *et al.*, 2015a, Carlisle *et al.*, 2016, Li *et al.*, 2016b). Lipids on the other hand are depleted in ^{13}C (~6-8‰) in relation to pure protein and can accordingly lower δ^{13}C values in tissues with high lipid contents (DeNiro and Epstein, 1977, Post *et al.*, 2007, Hussey *et al.*, 2012b). The extent of contamination of these compounds is typically quantified through examination of the elemental %C to %N ratio (C:N) (Carlisle *et al.*, 2016). Urea contamination causes lower C:N values and lipid contamination results in higher C:N values (Sweeting *et al.*, 2006, Carlisle *et al.*, 2016). Samples with C:N values between ~3-3.5 are considered to reflect pure protein (Post *et al.*), although the interaction between urea and lipid contamination has recently be demonstrated to confound interpretations in chondrichthyans (Carlisle *et al.*, 2016).

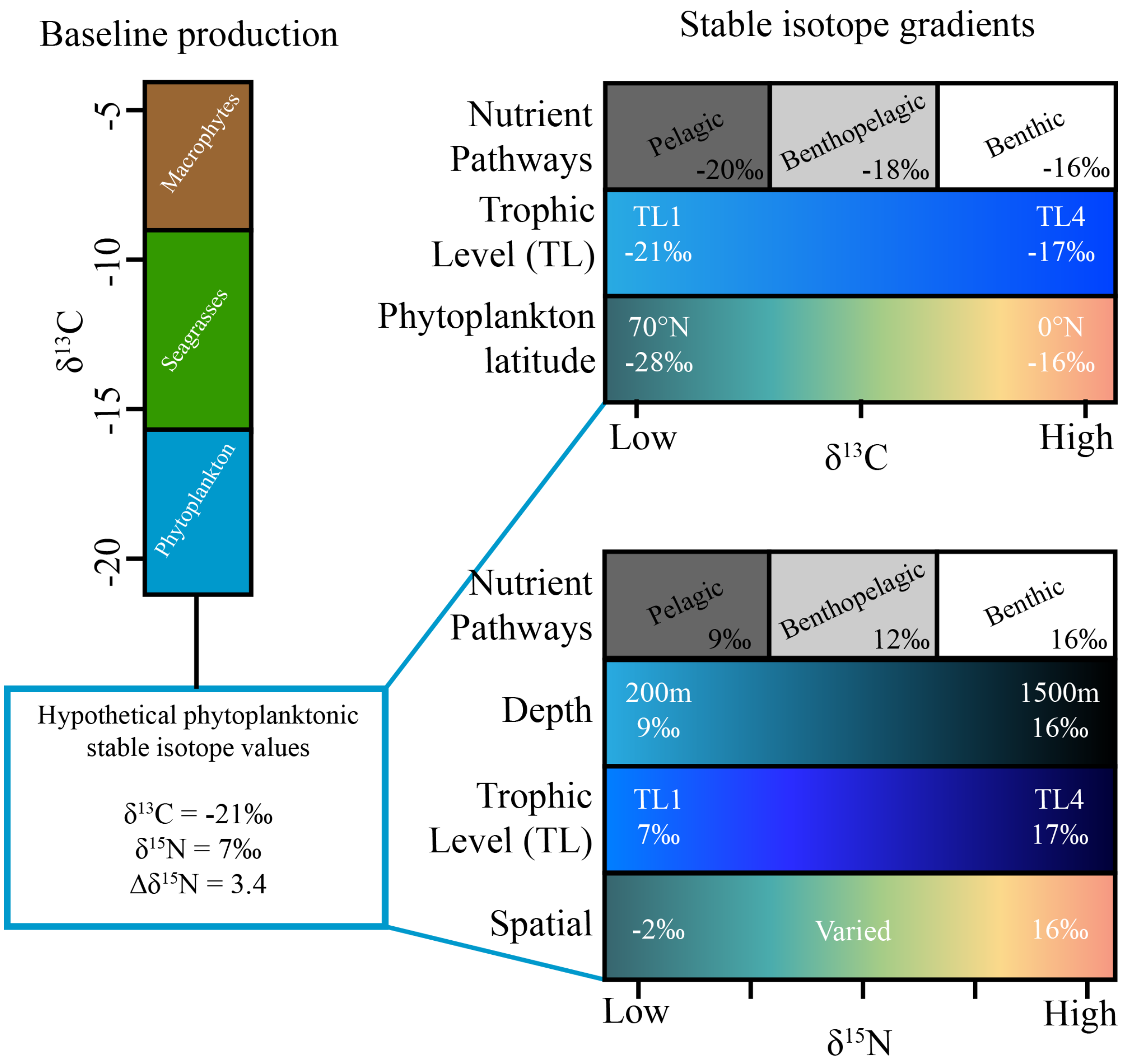

Figure 1.4: Schematic representation of stable isotopic gradients in the marine environment. Panels give approximate examples of the scale of phytoplanktonic pelagic enrichment under several ecological scenarios.

To correct for biases introduced from urea and lipid contaminations, ecological studies often apply chemical extractions (Folch *et al.*, 1957, Bligh and Dyer, 1959) or mathematical corrections (McConnaughey and McRoy, 1979, Kiljunen *et al.*, 2006, Post *et al.*, 2007, Hoffman and Sutton, 2010). Chemical extractions benefit from directly removing urea and lipids but can result in the alteration of δ^{15}N values through the separation of polar and non-polar amino acids with differing δ^{15}N values or possibly (depending on the extraction method used) the introduction of additional nitrogen-containing chemical compounds (Sweeting *et al.*, 2006). Additionally, chemical extractions are costly, laborious, and have varying efficiencies between species (Post *et al.*, 2007, Churchill *et al.*, 2015a). Because urea is water soluble, simply washing tissue samples in distilled water prior to analysis is sufficient to remove urea (Carlisle *et al.*, 2016, Li *et al.*, 2016b). For lipids however, it has been advised that chemical extractions are necessary for correct interpretation of results, especially when C:N ratios are greater than ~3.5 (Post *et al.*, 2007, Hussey *et al.*, 2012b, Carlisle *et al.*, 2016, Li *et al.*, 2016b).

An alternative approach for accounting for lipids is the application of mathematical corrective models. Once corrected for urea (through water washing), the C:N ratio can be used as a proxy for lipid contamination and accordingly used to adjust the observed δ^{13}C values (Post *et al.*, 2007, Reum, 2011). Most carbon correction models are developed for large C:N ranges (4-10) and can be effective for estimating δ^{13}C values associated with pure protein (Kiljunen *et al.*, 2006, Post *et al.*, 2007, Hoffman and Sutton, 2010). Carbon corrections have been developed for a wide range of animals (Kiljunen *et al.*, 2006) including specific models for deep water fishes (Hoffman and Sutton, 2010) and chondrichthyans (Logan and Lutcavage, 2010, Reum, 2011). Carbon corrective models benefit from being able to be applied retrospectively to samples with known C:N ratios, providing a cost effective and time efficient manner to correct for lipid contamination (Sweeting *et al.*, 2006, Post *et al.*, 2007). Mathematical corrections have recently come under scrutiny however, as most studies are applying the mathematical corrections to studies that have not accounted for urea (Carlisle *et al.*, 2016, Shipley *et al.*, 2017c). If urea is accounted for however (e.g. with water washing), mathematical corrections provide a robust, cost effective measure for correcting for contamination effects, especially if C:N ratios are between 3.5 and 4, where corrective effects will be minimal.

1.4 Deep-water Chondrichthyan Ecology

Most of what we know about the trophic ecology of deep-water chondrichthyans has been derived from stomach content assessments (Mauchline and Gordon, 1983, Ebert *et al.*, 1992, Ebert *et al.*, 1996). Chimaeras are consistently described as epibenthic predators (Mauchline and Gordon, 1983, Moura *et al.*, 2005, Dunn *et al.*, 2010a), whereas the trophic guild classifications are much less clear for deep-water sharks. Studies regularly report spatially varied diets containing crustaceans, cephalopods, teleosts and marine mammals from pelagic, mesopelagic and benthic habitats (Macpherson, 1983, Kazunari, 1991, Ebert *et al.*, 1992, Cortés, 1999, Dunn *et al.*, 2010b, Pethybridge *et al.*, 2011, Albo-Puigserver *et al.*, 2015). Trophic inferences are further confounded by the high incidents of empty stomachs and recent feeding observations being poorly representative of long-term diets (Cortés, 1997).

Ecosystem modelling approaches, using *Ecopath with Ecosim* (EwE) have also been widely used to determine food web structure of marine ecosystems (Kitchell *et al.*, 2002, Christensen and Walters, 2004), with some applications to deep-sea environments (Heymans *et al.*, 2011, Tecchio *et al.*, 2013). In a study of the Catalan Slope in the Mediterranean, Tecchio *et al.* (2013) found that demersal sharks may act as a keystone top predator group, feeding across a wide range of prey, and having a high structuring impact on the other food web components. It is often hard to validate these models however, and they require a relatively good understanding of the ecosystems in the first place in order to build them (Heymans *et al.*, 2011).

Increasingly, considerable work has been conducted using complementary biochemical approaches such as stable isotope analysis (reviewed by Shipley *et al.* (2017a)). Using a combination of stomach content analysis, fatty acid profiling, and stable isotope analysis, demersal sharks in New Zealand were demonstrated to adopt a range of trophic behaviours closely related to taxonomic classifications with chimaeras (Chimaeriformes) being epibenthic, catsharks (Carcharhinifromes) feeding on cephalopods, small dogfish (Squaliformes) feeding on mid-trophic teleosts and cephalopods, and large dogfish feeding on bathypelagic teleosts, squid and marine mammals (Pethybridge *et al.*, 2010, Pethybridge *et al.*, 2011, Pethybridge *et al.*, 2012). Churchill *et al.* (2015b) and Shipley *et al.* (2017d) report the trophic interactions between upper-slope deep-water shark communities and suggest a high amount of trophic overlap between species. Lastly, Barría *et al.* (2015) comparing shark and ray taxa across the northeast Mediterranean, report similar feeding strategies among shark groups and suggest most species fill an apex predatory role in these ecosystems. These studies have typically been constrained to a small number of individuals and/or species however, and none have accounted for potential bathymetric differences in the observed patterns. Additionally, these data are often considered in isolation due to the limited access of both data and distributional data from the wider ichthyofaunal community. There is therefore great scope to conduct a large-scale stable isotopic assessment of deep-sea chondrichthyan assemblages over a range of spatial scales and using a variety of supplementary data sources, to reveal novel insight into the ecology of this group.

Furthermore the mechanisms constraining chondrichthyan fishes to waters less than 3500 m has been widely debated, with implications that may influence the ecology of sharks in deep-sea environments. It has been speculated that the high energetic requirements needed to maintain an oil-rich liver for buoyancy (Priede *et al.*, 2006) or obtain sufficient protein-stabilising trimethylamine N-oxide (TMAO) from dietary items (Laxson *et al.*, 2011) may physiologically constrain sharks to more productive deep-sea depths. More recently, Musick and Cotton (2015) suggested the high trophic levels and relatively limited modes of feeding mechanisms (mainly active piscivores and macronekton foragers) restrict sharks to depths where these prey are sufficiently abundant (Musick and Cotton, 2015). All three studies have alluded to some trophic interaction being important in the observed bathymetric distributions of sharks, but definitive evidence is still lacking. Stable isotope analysis of deep-sea chondrichthyan fishes may therefore be able to improve our understanding of these ecosystems, and the ecological roles of sharks in these habitats.

1.4.1 Fisheries Vulnerability

Although ecological data may be limited for most deep-water chondrichthyans, there is a reasonable amount of biological data on some species. Deep-water chondrichthyans are typified by slow growth rates, low fecundity, late ages of sexual maturity and long gestation periods, which culminate in most deep-water sharks having poor rates of recovery following exploitation (Garcia *et al.*, 2008, Simpfendorfer and Kyne, 2009, Kyne and Simpfendorfer, 2010, Rigby and Simpfendorfer, 2013). For example, female leafscale gulper sharks (*Centrophorus squamosus*) may not reach sexual maturity until they are 35 years old (Clarke *et al.*, 2002a), produce 1-10 offspring (Figueiredo *et al.*, 2008), have reproductive recovery times greater than a year and live until they are about 70 years (Clarke *et al.*, 2002a, Bañón *et al.*, 2006). While age estimations for deep-water chondrichthyans have often used vertebral or fin spine ring counts (Clarke *et al.*, 2002b, Irvine *et al.*, 2006, Coelho and Erzini, 2008b) these techniques are not applicable to all species (Moore *et al.*, 2013). Recently, radio bomb carbon in the eye lenses of the Greenland shark (*Somniosus microcephalus*) provided estimates of 392 ± 120 years of age in the largest individuals (Nielsen *et al.*, 2016). While these estimates of longevity are associated with high levels of uncertainty and likely overestimated, the general consensus is that deep-sea sharks are slower growing and longer lived than their shallower water counterparts. For example, some Centrophoridae and Somniosidae species are believed to live to between 35-70 years (Simpfendorfer and Kyne, 2009, Cotton *et al.*, 2013). Accordingly, deep-sea chondrichthyans may be one of the most biologically vulnerable groups to overexploitation (Clarke *et al.*, 2003b, Garcia *et al.*, 2008, Simpfendorfer and Kyne, 2009). While the realistic extinction risks to deep-sea sharks may be lower than other shark species due to the relatively low levels of current exploitation and generally broad geographic distributions, being constrained to specific bathymetric depths increases their susceptibility to local extirpation (Priede *et al.*, 2006, Musick and Cotton, 2015). With limited knowledge on the stock structure and migratory movements of most deep-sea shark species, precautionary management measures are favoured until biological assessments can be improved (Clarke *et al.*, 2015).

Following the progression of fisheries into deep-sea habitats (Morato *et al.*, 2006b, Watson and Morato, 2013), there has been a concern with regard to the sustainability of fishery operations (Koslow *et al.*, 2000, Gordon, 2003, Norse *et al.*, 2012), especially for vulnerable deep-sea shark species (Garcia *et al.*, 2008, Simpfendorfer and Kyne, 2009, Kyne and Simpfendorfer, 2010, Rigby and Simpfendorfer, 2013). Deep-sea sharks are caught both in targeted long-line fisheries, for their meat and liver (Piñeiro *et al.*, 2001, Pajuelo *et al.*, 2010), and as incidental by-catch in mixed trawl fisheries and long-line fisheries targeting black scabbard fish (*Aphanopus carbo*) (Clarke *et al.*, 2003b, Coelho and Erzini, 2008a, Oliver *et al.*, 2015). In light of reported population declines (Kelly *et al.*, 1997, Kelly *et al.*, 1998, Graham *et al.*, 2001), catch limits were imposed on deep-sea shark populations in Europe in 2005, before zero total allowable catch (TAC) quotas were imposed in 2010. Despite management efforts, Neat *et al.* (2015) found that there has been limited sign of recovery in some deep-sea shark species, especially in larger dogfish (*C. squamosus* and *C. coelolepis*). While these management strategies prohibited the landing of deep-water sharks, deep-sea trawl and long-line fisheries still operate at these depths, exposing deep-water shark to high levels of capture rates. Due to the stresses imposed by fishing, high levels of mortality are likely still prevalent, even though sharks are released (Shipley *et al.*, 2017b).

As of December 2016, zero TAC management quotas were lifted for deep-sea sharks in the long-line black scabbard fishery, allowing the landing of 10 tonnes of sharks in 2017 and 2018 as 'unavoidable bycatch' and 'in order to collect scientific information' (Council Regulation (EU) 2016/2285; (The European Commission, 2016). Additionally, the repealing of Council Regulation (EC) 2347/2002 in 2016 (The European Union, 2016), restricted bottom-trawling activities to depths at, or above, 800m, which has the potential to offer further protection to the large abundance and diversity of vulnerable fishes below this depth. It is therefore an appropriate time to try to address some of the gaps in our understanding of this group. Furthermore, establishing baseline ecological data on deep-sea fish assemblages may provide greater monitoring potential moving forward, increasing the potential to assess any effects of newly imposed bathymetric limitations to trawl bans. It is hoped this thesis may provide support to those scientific studies, and that the inferences made on the spatial-temporal ecology of deep-water chondrichthyans may be useful for supporting the on-going efforts to improve the sustainability of deep-sea exploitation.

1.5 Thesis Aims and Objectives

There is growing concern of the effects that fishing operations are having on marine environments, with extinction risks being high for large ocean predators such as chondrichthyans (Ferretti *et al.*, 2010, Dulvy *et al.*, 2014, Fernandes *et al.*, 2017). In order to improve the sustainability of current fishing practises, there is an increased need to understand the ecological role that sharks and their relatives may be playing in marine ecosystems (Heupel *et al.*, 2014). We are currently lacking basic ecological information on many shark species, but details on deep-sea populations are notably scant (Ramos *et al.*, 2013, Dulvy *et al.*, 2014, Cotton and Grubbs, 2015). Furthermore, the inaccessibility and obscure nature of deep-sea ecosystems make direct observations challenging therefore requiring alternative approaches to better understand ecological dynamics.

This thesis therefore aims to examine the tropho-spatial ecology of chondrichthyan fishes with a primary focus on deep-sea shark populations in the northeast Atlantic. The objectives are:

- To compile published stable isotope data on global shark populations in order to identify fundamental ecological differences between sharks from different habitats (i.e. oceanic, shelf and bathyal). Stable isotope analysis has now been extensively used to infer the ecological characteristics of sharks, and may provide strong comparative insight to deep-sea habitats. I take this opportunity to compile the large majority of published stable isotope data on sharks in order to better understand global stable isotopic ecology of this group.

- Explore how the nutrient dynamics of bathyal ecosystems vary between continental slope and seamount ecosystems, using sharks as ecological samplers. Bathyal ecosystems are reliant on the vertical transport of surface derived nutrients but it is currently unclear how the stark topographic differences between continental slopes and seamounts affect the underlying trophic structure of ichthyofaunal food webs.

- Determine the ecological role that demersal chondrichthyans play in the continental slope ecosystem of Rockall Trough, UK. I build on the earlier work of Trueman *et al.* (2014), looking specifically at the ecological role that sharks and chimaeras play across a bathymetric range (500 – 2000m), and how they may be ecologically different from benthic and benthopelagic teleosts.

- Recover whole-life stable isotopic profiles from two threatened deep-water shark species; Portuguese dogfish (*Centroscymnus coelolepis*) and leafscale gulper sharks (*Centrophorus squamosus*). Neonatal and juveniles sharks of both these species are seldom seen and have led many to speculate about the occurrence of spatially distant nursery habitats and reproductively linked ocean-scale migrations. Using eye lenses as chronological recorders of whole life history ecology, I hope to uncover key trophic and spatial ecological characteristics relating to cryptic life history stages.

1.6 Thesis Structure

In order to achieve these goals, I adopted stable isotopic approaches using newly collected data and globally compiled published data. Stable isotopic data is considered in relation to fisheries independent survey methods, global geochemical isoscape models and previously published stomach content data. The thesis progresses with narrowing ecological scales, starting with global ecological processes (chapter 2), through to bathyal nutrient dynamics in the northeast Atlantic (chapter 3), trophic interactions of a chondrichthyan assemblage at Rockall Trough (chapter 4) and ending with the recovery of life-history ecology of two vulnerable species of deep-sea shark (chapter 5).

Chapter 2 compares globally compiled $\delta^{13}C$ data from sharks caught in oceanic, shelf and bathyal habitats, to $\delta^{13}C$ estimates of global particulate organic carbon from a newly developed isoscape model. I test hypotheses of differences in resource use and spatial migrations between sharks ascribed to each habitat.

Chapter 3 examines the trophic dynamics of bathyal ecosystems at continental slope and seamount locations. Whole shark assemblages are used to test for differences in underlying nutrient dynamics between different bathyal systems. Using three common sympatric species caught at each capture location, I use $\delta^{15}N$ values as a proxy of benthic-pelagic coupling to determine how reliant each respective ecosystem is on pelagic production.

Chapter 4 examines the ecological structure of a deep-sea chondrichthyan assemblage across a bathymetric gradient. Newly collected stable isotope samples from sharks and chimaeras caught during a 2015 Marine Science Scotland survey at Rockall Trough are compared to the broader ichthyofaunal community. These data are considered in conjunction with a medium term fisheries independent trawl survey from the same region to better understand the ecological processes determining the observed assemblage structure.

Chapter 5 recovers $\delta^{13}C$ and $\delta^{15}N$ values from sequential layers of eye lenses of two threatened deep-water shark species; Portuguese dogfish (*Centroscymnus coelolepis*) and leafscale gulper sharks (*Centrophorus squamosus*), from the northeast Atlantic. I report stable isotope values related to maternal ecology, neonatal first feeding and adult ecology. In conjunction with published demographic distributions I infer ocean-basin migratory movement patterns of these species.

Chapter 6 will briefly summarise my findings, discuss some of the limitations of stable isotope analysis, suggest future work, and highlight how these results could support future fisheries management decisions.

Chapter 2 - 5 have been prepared for publication so there will be some overlap between this introductory chapter and the respective introductions and methodologies.

Chapter 2: A global perspective on the trophic geography of sharks

This chapter is a reproduction of text currently in review with *Nature Ecology and Evolution,* and as such, is written in the style of the journal.

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2.1 Abstract

Sharks are a diverse group of mobile predators that forage across varied spatial scales and have the potential to shape or stabilise food webs. Recent declines in shark biomass may have broader ecological consequences if sharks display common behavioural traits across taxa and regions. By tracking the original site of photosynthetic fixation of carbon atoms ultimately assimilated into muscle tissues of 5394 sharks from 114 species, we identify globally consistent biogeographic traits in trophic interactions between sharks found in different habitats. We show that populations of shelf-dwelling sharks appear to derive a substantial proportion of their carbon from regional pelagic sources, but contain individuals that forage within additional isotopically diverse local food webs, such as those supported by C4 plant sources, benthic production and macrophytes. In contrast, oceanic sharks appear to utilise energy and nutrients derived from a relatively narrow latitudinal range. Global-scale compilations of stable isotope data combined with biogeochemical modelling generate novel hypotheses regarding animal behaviours that can be tested with additional methodological approaches.

2.2 Introduction

Sharks are one of the most speciose groups of predators on the planet, and can be found over a broad range of habitats in every ocean (Ebert *et al.*, 2013). Globally, population declines have been reported in many species of sharks, largely due to fishing pressures and habitat degradation over the last century (Ferretti *et al.*, 2010, Worm *et al.*, 2013, Dulvy *et al.*, 2014). However, the impacts of these declines on broader ecosystem structure and function remain uncertain (Kitchell *et al.*, 2002, Myers *et al.*, 2007, Heithaus *et al.*, 2008, Heupel *et al.*, 2014, Grubbs *et al.*, 2016, Roff *et al.*, 2016, Ruppert *et al.*, 2016). Global-scale ecological consequences from declining shark numbers are likely, and may be apparent if shark taxa perform broadly similar functions across different regions and habitat types, such that local effects scale across wide geographic regions. In marine systems, the impact of an individual on the wider ecosystem is strongly influenced by trophic interactions (McCann *et al.*, 1998). Thus, the composition and spatial origin of diet plays an important part in shaping the ecological roles of individuals, species, and functional groups. Here, we use the term ‘trophic geography’ to refer to the spatial relationship between an animal and its assimilated food. Broadly quantifying the trophic geography of marine consumers is particularly challenging, because the spatial and temporal scales over which individuals forage can extend for thousands of kilometres and over months to years. Nevertheless, trophic geography may provide critical information on how food webs are structured, and the biological connectivity of these ecosystems.

Stable isotope analysis provides a potentially useful approach to address this challenge. Extensive use of stable isotope analysis in localised studies of marine food webs has provided a wealth of published information on trophic ecology across broad geographic regions, and numerous ecosystems within those regions. Of particular utility, the stable isotopic composition of carbon ($\delta^{13}C$) in marine food webs provides spatial and trophic information on nutrient assimilation, because of the predictable variation in $\delta^{13}C$ values with latitude and among different primary production types, such as phytoplankton (-24‰ to -18‰), macrophytes (-27‰ to -8‰), and seagrasses (-15‰ to -3‰) (Fry and Sherr, 1989, Laws *et al.*, 1995, McMahon *et al.*, 2013). The carbon stable isotope composition of primary producers is directly assimilated by consumers through feeding, and provides a biochemical tracer linking a consumer to the basal source of carbon and/or latitudinal origin of the food webs that support tissue growth (Hobson, 1999). The extent of fractionation of carbon stable isotopes during photosynthesis by algal phytoplankton varies strongly with latitude, and to a lesser extent with nutrient contents, due to latitude-dependent variation in variables such as cell size, growth rates and the concentration and isotopic composition of dissolved CO_2 (Laws *et al.*, 1995, Magozzi *et al.*, 2017). The stable isotopic composition of algal phytoplankton has been simulated using isotope-enabled biogeochemical models (Magozzi *et al.*, 2017), providing global-scale predictions of latitude-dependent variation in $\delta^{13}C$ values. Carbon stable isotopes can thus be used as an indicator of the latitudinal origin of nutrients assimilated by mobile marine consumers, providing insight into cross-ecosystem foraging without the need to directly track movements of individual animals (Fry and Sherr, 1989, Hobson, 1999). Sharks assimilating food fuelled by primary production source(s) in one region but captured in an isotopically distinct second region should exhibit anomalous isotopic compositions compared to primary producers in the capture location. Here, we compare latitudinal trends in $\delta^{13}C$ values observed in muscle tissues of sharks found on continental shelves, open oceans, and deep-sea habitats, with those predicted for phytoplankton at capture locations to establish global patterns of trophic geography in sharks.

We compile a global-scale database of $\delta^{13}C$ values of white muscle tissue from 5394 individual sharks from 114 species associated with continental shelves (neritic waters <200m in depth), oceanic (open-ocean waters but mainly occurring <200m) and deep-sea (continental slopes and seamounts ≥200m) habitats (Appendix A.1, Figure 2.1). Habitats were classified based on where species are most regularly observed and reported across the literature. We compare observed shark $\delta^{13}C$ values ($\delta^{13}C_S$) to biomass-weighted annual average $\delta^{13}C$ values predicted for phytoplankton ($\delta^{13}C_P$) within biogeographically distinct ecological regions (Longhurst Biogeographical Provinces) corresponding to shark capture locations (Figure 2.2). We test the null hypothesis that sharks feed exclusively within the phytoplankton-derived food webs of their capture locations by comparing the observed and predicted latitudinal trends in $\delta^{13}C$ values. Capture location $\delta^{13}C_P$ values are calculated from a carbon-isotope-enabled global ocean-ecosystem model (Figure 2.1). Please refer to Magozzi *et al.* (2017) for full details of the model used to predict baseline $\delta^{13}C_P$. Global-scale isoscapes are not available for sources of other marine production, thus we cannot discount the possibility that all sources of production show consistent latitudinal gradients in $\delta^{13}C$ values. However, the isotopic offset between phytoplankton, seagrass, macrophytes and benthic production varies substantially between sites (Hobson, 1999). Furthermore, variables such as cell size, growth rates and dissolved CO_2 concentrations, have less influence on the $\delta^{13}C$ values of alternative marine production sources (Laws *et al.*, 1995). We therefore expect that $\delta^{13}C$ values of alternative primary production sources will vary more at the local level, and varying contributions of different production sources within shark food webs will predominantly influence the variance seen in shark $\delta^{13}C$ values. A detailed description of the considerations and rationale behind the isotopic comparisons are given in the supplementary material.

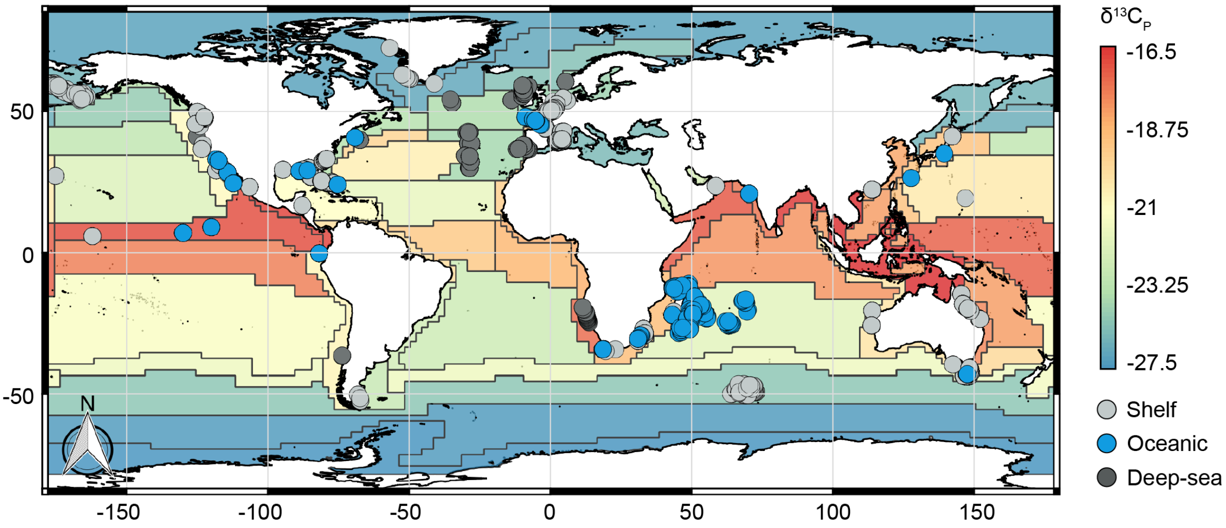


Figure 2.1: Distribution of compiled shark data overlaid on a $\delta^{13}C$ annually averaged particulate organic carbon isoscape ($\delta^{13}C_P$) within Longhurst Biogeographical Provinces from the median sampling year, 2009. Coloured points signify habitat classification of those samples. Most studies were only able to provide one location for multiple samples and in some cases, just a landing dock.

2.3 Results

Carbon isotope values of shark muscle ($\delta^{13}C_S$) co-vary negatively with latitude for oceanic and shelf sharks, but the relationship between latitude and $\delta^{13}C_S$ values differs among habitats (Figure 2.2). In continental shelf waters, latitudinal trends observed in shark muscle were similar to those estimated from biochemical models. The observed rate of change in $\delta^{13}C$ values per degree of latitude was -0.11 for sharks and -0.13 for plankton, although these rates were statistically distinguishable (ANCOVA $F_{11.864}$, p=0.0006).

The average isotopic offset between plankton and shelf sharks (the difference in intercept values between the best fit linear regressions) is 4.6‰, close to the expected trophic offset of 4.5‰, given that the median trophic level for sharks is estimated at 4.1 (Cortés, 1999), and the mean isotopic difference between sharks and their prey, i.e. trophic discrimination factor for $\delta^{13}C$, is 1.1‰ (Table 2.1). Best-fit generalised additive models (GAMs) indicate that the largest amount of deviance in $\delta^{13}C_S$ in shelf sharks is explained by latitude (42.0%), with very little effect of shark size (3.1%), and a combined explanatory deviance of 46.7% (Table 2.3). Regional biogeography among continental shelf sharks are not ubiquitous though, and across all latitudes, the range of $\delta^{13}C_S$ values within a given single-species population of shelf sharks is higher than that of oceanic or deep-sea sharks (Figure 2.2).

Table 2.1: List of trophic discrimination factors used to calculate trophic offset values for shark samples

Species	$\Delta^{13}C$ (SD), ‰	Reference
Scyliorhinus canicula	0.80 (0.10)	Caut et al., 2009
Carcharias taurus & Negaprion brevirostris	0.84 (0.23)	Hussey et al., 2010
Triakis semifasciata	1.00	Malpica-cruz et al., 2012
Triakis semifasciata	1.70 (0.50)	Kim et al., 2012a
Mean	1.10	

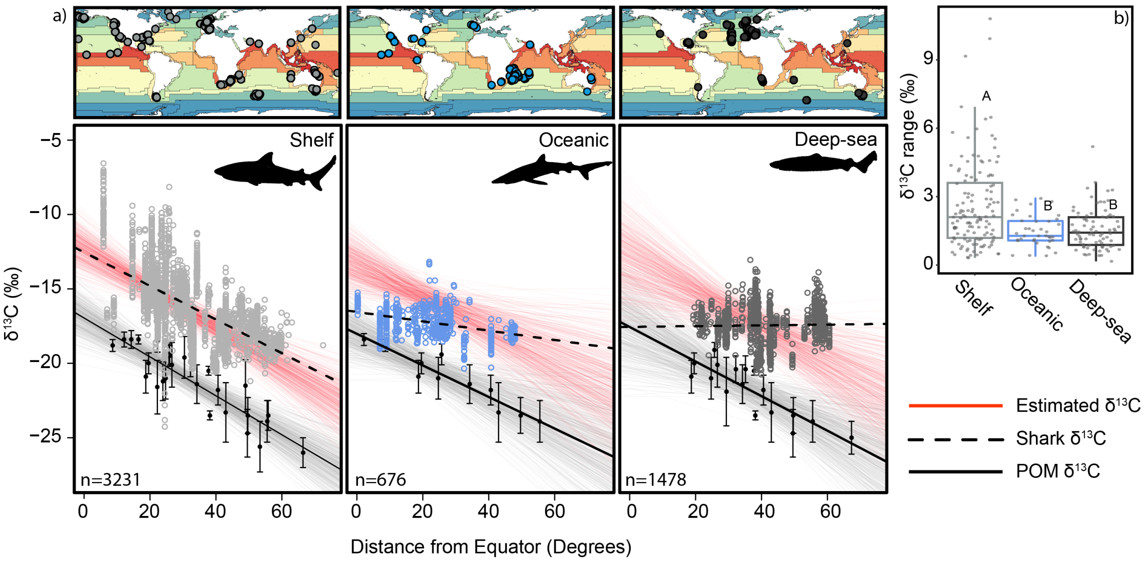


Figure 2.2**:** a) The relationship between carbon isotope compositions of modelled particulate organic carbon $\delta^{13}C$ ($\delta^{13}C_P$) from Longhurst Biogeographic Provinces associated with shark capture locations (solid black line), and shark muscle stable carbon isotope ($\delta^{13}C_S$) values (dashed black line & open circles) and latitude. Confidence envelopes around linear regression lines (grey solid lines) reflect 500 Monte Carlo iterations considering variance in $\delta^{13}C_P$ values within each Longhurst Biogeographic Provinces, and in red the same latitudinal trends predicted for $\delta^{13}C_S$ with an offset of 4.6‰ added corresponding to the mean offset between $\delta^{13}C_P$ and $\delta^{13}C_{S,}$ and corresponding to likely trophic effects on $\delta^{13}C$ values. Maps provide individual shark sample locations overlaid with the $\delta^{13}C_P$ isoscape from Figure 2.1. b) Boxplots of the observed $\delta^{13}C_S$ ranges of shark populations in each habitat. Horizontal line is the mean $\delta^{13}C_S$ range across shark populations within that habitat. Boxes contain 50% of the data and lines correspond to 95% confidence interval. Letters signify analysis of variance, Tukey HSD results for significant difference, with the same letters representing mean values that are not significantly different from each other.

Conversely, while oceanic and shelf sharks were sampled from a similar latitudinal range (albeit that we were lacking stable isotope samples from oceanic sharks from higher latitudes), the observed latitudinal trends in $\delta^{13}C_S$ values from oceanic sharks are less steep than those predicted for phytoplankton from the corresponding Longhurst Biogeographic Province (ANCOVA: $F_{205.63}$, p<0.001; Figure 2). Irrespective of capture latitude, the observed range of $\delta^{13}C_S$ values in oceanic sharks was small (-17.22 ± 0.99‰) across the sampling range. The lack of covariance of $\delta^{13}C_S$ with latitude suggests oceanic sharks assimilate the majority of their carbon from a relatively restricted latitudinal range, although temporal differences in habitat use and $\delta^{13}C$ values of prey coupled with relatively slow isotopic turnover rates of muscle in elasmobranchs could potentially mask variability driven by latitude (discussed further in supplementary methods). Best-fit GAM models indicate that only 20.2% and 4.8% of the deviance in oceanic shark muscle isotope values is explained by latitude and shark size, respectively (Table 2.3).

Despite the concentration of deep-sea samples from the North Atlantic, latitudinal trends in $\delta^{13}C_S$ for deep-sea sharks do not co-vary with latitude (R^2 = <0.001, p = 0.314) or with $\delta^{13}C_P$ (ANCOVA: $F_{1581.9}$, p<0.001; Figure 2), displaying patterns similar to those seen in oceanic sharks. Body size explained 25.3% and depth of capture 17.6% of the deviance in carbon isotope compositions of deep-sea sharks (Table 2.3), which implies that their trophic ecology is strongly depth and size-structured (i.e. marine animals feeding on things smaller than themselves), consistent with other fishes from continental slopes (Trueman *et al.*, 2014).

Habitat	$\delta^{13}C_P$ - soild				$\delta^{13}C_S$ - dashed			
	intercept	slope	R^2	p	intercept	slope	R^2	p
Shelf	-16.87	-0.13	0.61	<0.001	-12.54	-0.11	0.37	<0.001
Oceanic	-17.75	-0.11	0.80	<0.001	-16.55	-0.03	0.17	<0.001
Deep-sea	-16.74	-0.12	0.67	<0.001	-17.55	<-0.01	<0.001	0.314

Table 2.2**:** Regression coefficients for modelled particulate organic matter (POM) $\delta^{13}C$ ($\delta^{13}C_P$) and observed $\delta^{13}C$ shark muscle ($\delta^{13}C_S$) values from Figure 2.2.

2.4 Discussion

Carbon stable isotope compositions estimated from phytoplankton and those measured in shelf sharks ($\delta^{13}C_S$) express similar latitudinal trends. The observed similarity in latitudinal isotopic trends between phytoplankton and sharks is consistent with our null hypothesis that shelf shark populations are supported primarily by phytoplanktonic production close to their capture location.

The relatively high intraspecific variability observed in carbon stable isotope composition of shelf sharks compared to oceanic and deep-sea populations (Figure 2.2) suggests that while population median isotopic compositions imply that the bulk of assimilated food is supported by phytoplankton production, individuals within populations of shelf sharks assimilate nutrients from additional isotopically-distinct sources. Shelf, and particularly coastal, ecosystems provide access to a wider diversity of ecological and isotopic niches than oceanic ecosystems, including seagrasses, benthic production, macroalgae and coral (Fry and Sherr, 1989, Briand *et al.*, 2016). In most shelf habitats, pelagic phytoplankton yields more negative δ^{13}C values than alternative carbon sources (Fry and Sherr, 1989). Thus, foraging across isotopically distinct coastal food webs will tend to produce less negative δ^{13}C values than predicted based on local phytoplankton, such as those observed here.

		Parametric Coefficients				Significance of smooth terms						
Habitat	Smooth Term	Estimate	S.E	t	Pr(>|t|)	edf	Ref.df	F	p value	n	DE%	AIC
Shelf	POC ~ DistEq	-20.89	0.02	-1008	<0.001	2.00	2.00	2969.00	<0.001	3029	**66.3**	9397
	DistEq	-16.17	0.03	-523	<0.001	2.00	2.00	1098.00	<0.001	3029	42.0	11824
	logTL	-16.17	0.04	-404	<0.001	1.91	1.99	45.66	<0.001	3029	3.1	13381
	DistEq x logTL	-16.17	0.03	-545	<0.001				<0.001	3029	**46.7**	11570
	-DistEq					2.00	2.00	1248.30	<0.001			
	-logTL					1.00	1.00	267.50	<0.001	DIFF		
											19.6	
Oceanic	POC ~ DistEq	-20.24	0.02	-811	<0.001	2.00	2.00	1918.00	<0.001	651	**85.6**	1264
	DistEq	-17.22	0.03	-495	<0.001	1.83	1.97	79.37	<0.001	651	20.2	1697
	logTL	-17.22	0.04	-453	<0.001	1.97	2.00	15.51	<0.001	651	4.8	1813
	DistEq x logTL	-17.22	0.03	-499	<0.001					651	**21.7**	1689
	-DistEq					1.87	1.98	68.24	<0.001			
	-logTL					1.83	1.97	5.18	0.004			
										DIFF	**63.9**	
Bathyal	POC ~ DistEq	-21.75	0.03	-838	<0.001	1.98	2.00	1604.00	<0.001	1452	**68.8**	4091
	DistEq	-17.42	0.03	-598	<0.001	1.98	2.00	22.89	<0.001	1452	3.1	4431
	logTL	-17.42	0.03	-681	<0.001	1.97	2.00	248.00	<0.001	1452	25.3	4052
	Depth	17.42	0.03	-598	<0.001	1.97	2.00	138.00	<0.001	1452	17.6	3764
	DistEq x logTL	-17.42	0.03	-695	<0.001					1452	**28.5**	3994
	-DistEq					1.99	2.00	31.61	<0.001			
	-logTL					1.99	2.00	255.40	<0.001			
										DIFF	**40.3**	
	DistEq x logTL x Depth	-17.42	0.03	-667	<0.001					1452	**34.0**	3994
	-DistEq					1.97	2.00	10.23	<0.001			
	-logTl					1.96	2.00	121.23	<2E-16			
	-Depth					1.81	1.96	38.82	<2E-16			
										DIFF	**34.8**	

Table 2.3**:** Results from the most parsimonious generalised additive models (GAMS). Particulate organic carbon (POC) models describe POC δ^{13}C as a function of distance from equator (DistEq). δ^{13}C shark models describe ä^{13}C trends in shark muscle as a function of DistEq, log total length (logTL) and depth (only for deep-sea sharks) where all measurements were available. SE is standard error, edf is estimated degrees of freedom and DE% is the deviance explained by the explanatory variables. Difference is the difference between POC and shark models for the independent variables.

We infer that at the population level, shelf sharks act as generalist predators, but populations of at least some of those species are likely to be composed of individuals that specialise across a broad range of food webs during the timescale of isotopic turnover (likely 1-2 years, (Kim *et al.*, 2012a)). The range of $\delta^{13}C_S$ values observed within populations of shelf sharks is greater in latitudes lower than around 40 degrees (Figure 2.2), potentially indicating greater reliance on food webs supported by a range of non-phytoplankton based resources such as seagrasses and coral reefs in tropical settings. These inferences regarding the range of primary production sources fuelling shark populations could be further tested using essential amino acid carbon isotope fingerprinting (McMahon *et al.*, 2016).

Pairing stable isotope analysis with more traditional habitat use methodologies could improve the resolution of shark behaviour on continental shelves. Tracking studies demonstrate that while spatial residency and/or repeated return-migrations (philopatry) are common traits among sharks that use continental shelves, some species are capable of undertaking large oceanic migrations (e.g. white and tiger sharks), and philopatry is still under investigation (Chapman *et al.*, 2015). Some species, identified *a priori* here as shelf sharks (i.e. tiger, white, bull sharks etc.), use multiple habitats, and undertake offshore migrations in excess of 1000 km (Lea *et al.*, 2015b). The isotopic compositions of sharks classified as mixed habitat species diverge in latitudes lower than 35° (Figure 2.3). Among studies of species capable of utilising multiple habitats, the majority of populations surveyed displayed $\delta^{13}C$ values more consistent with obligate shelf sharks than oceanic sharks (Figure 2.3). This suggests that while some shelf shark species may be highly migratory, the carbon supporting tissue growth is largely assimilated from foraging within shelf areas. The mixed habitat assigned sharks from lower latitudes have stable isotope values that suggest that they may have previously fed on prey that was derived from higher latitudes. Indeed, these corresponding studies collected tissue samples on board oceanic longliners and suggest that they may have migrated from elsewhere (Li *et al.,* 2016a). The Greenland shark is also higher than would be expected for a shelf shark from this latitude, and these patterns may be due to feeding on marine and terrestrial mammals (Hansen *et al.*, 2012). Further studies looking at species-specific ecology within the context of global patterns would be advantageous but are beyond the scope of this study.

In contrast to shelf sharks, stable carbon isotope compositions of oceanic sharks and local phytoplankton do not co-vary, and oceanic shark populations sampled within these studies show similar carbon isotope values across all reported capture latitudes (Figure 2.2). The limited isotopic variability seen in oceanic sharks, could, reflect either derivation of the majority of nutrients from a restricted latitudinal range, or extensive foraging across large latitudinal gradients to produce a consistent weighted average value. In both cases derivation of nutrients with relatively low $\delta^{13}C$ values (i.e. from higher latitudes) is needed to explain the relatively ^{13}C depleted values seen in sharks caught at low latitudes. Oceanic sharks are not commonly found in latitudes greater than *c.*50° N or S, limiting the potential to balance any diet sources with more positive δ^{13}C values, and we therefore infer that the majority of nutrients assimilated had a similar relatively ^{13}C depleted stable isotopic composition, consistent with derivation of carbon from phytoplankton-based food webs (including mesopelagic food webs) from intermediate latitudes between *c.*30-50 degrees from the equator. The uncertainty surrounding the predictions of baseline $\delta^{13}C_P$, capture locations, and isotopic turnover rates limit our ability to definitively identify preferential foraging latitudes however. Oceanic sharks could also potentially be intercepting migratory prey that has originated from a restricted latitudinal range, such as squid (Ichii *et al.*, 2009). Regardless of the mechanism(s), our data imply that intermediate latitude areas may provide globally important sources of energy and nutrients for the oceanic shark populations sampled in these studies.

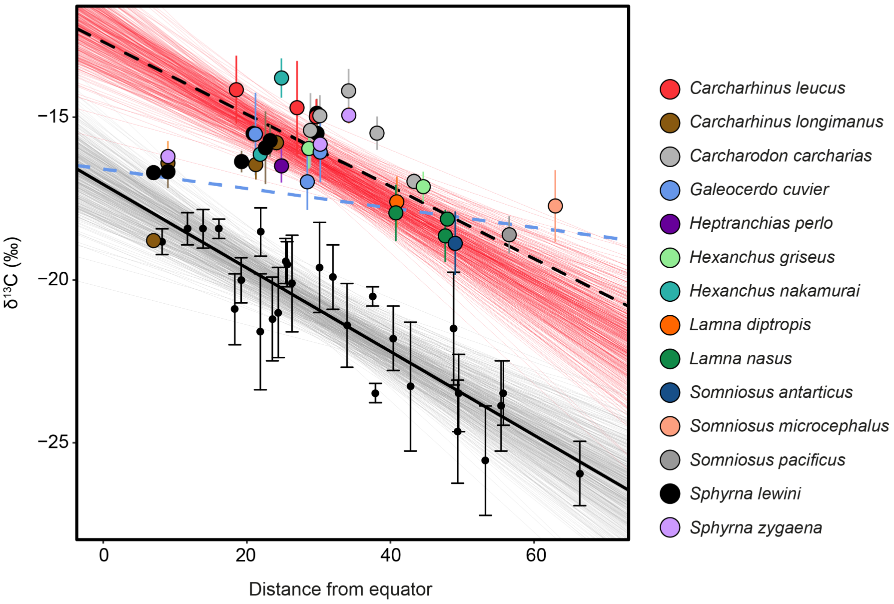


Figure 2.3: Latitudinal trends in mean δ^{13}C values (with standard deviation bars) for shelf shark species (and one oceanic species: *C. longimanus*) that have the potential to migrate over multiple habitats (i.e either shelf and deep-sea habitats or shelf and oceanic habitats). Each point represents mean values for a population within an individual study with each colour corresponding to a different species. The latitudinal δ^{13}C regression observed for oceanic shark muscle is also provided (dashed blue). 500 Monte Carlo iterations of the latitudinal trends in $\delta^{13}C_P$ observed in shelf sharks, considering the variance within each Longhurst Biogeographic Province (dark grey solid lines), and the latitudinal trends predicted for $\delta^{13}C_S$ with a trophic offset of 4.6‰ (red solid lines - see Methods section and Figure 2.2).

Our inferences of regionally-restricted foraging areas are consistent with latitudinal trends in oceanic productivity and satellite telemetry studies of several oceanic shark species (Scales *et al.*, 2014b, Queiroz *et al.*, 2016). Pelagic ecosystems at intermediate latitudes are typically characterized by strong thermal gradients that act to concentrate ocean productivity in frontal and eddy systems (Figure 2.4), which subsequently attract and support oceanic consumers including cetaceans, fishes, seabirds and marine turtles (Tittensor *et al.*, 2010, Block *et al.*, 2011, Scales *et al.*, 2014b). Tracking data from some oceanic shark species show high residency within intermediate latitudes (Block *et al.*, 2011, Hazen *et al.*, 2013, Queiroz *et al.*, 2016), and our interpretation of the stable isotope data supports these predictions of centralised foraging locations. Migrations away from productive foraging grounds may provide optimal habitat for behaviours such as breeding, pupping, and avoiding intraspecific competition and harassment (Campana *et al.*, 2011, Queiroz *et al.*, 2016). Oceanic sharks have distributional ranges spanning ocean basins (Compagno, 2001). Therefore, recognising that most of the carbon assimilated into their muscle tissues is derived from photosynthesis occurring in a relatively limited latitudinal region highlights the global importance of regional food webs. More observations of oceanic sharks and/or potentially migratory prey from tropical waters are required to test our hypotheses of centralised foraging. Undertaking similar global compilations of satellite telemetry and stomach content data could improve our interpretation of these large-scale ecological data.

Similar latitudinal isotopic gradients are observed between oceanic and deep-sea sharks, which may imply a shared nutrient resource supporting sharks in both habitats (Figure 2.5). Deep-sea sharks rely on the vertical flux of nutrients derived mainly from surface phytoplanktonic production (Trueman *et al.*, 2014), and may therefore be expected to closely track the stable isotopic composition of surface production. However, the concentration of deep-sea shark samples from the North Atlantic Ocean (74%) make it difficult to determine the tropho-spatial dynamics of this group, because the ameliorating effects of the Gulf Stream suppresses latitudinal variation in $\delta^{13}C_P$ (Figure 2.1). Latitudinal trends are further complicated by the strong effect of body size and depth (Table 2.3), whereby some species of deep-sea shark express bathymetric segregations by size (Moura *et al.*, 2014). While movement data for most deep-sea shark species is limited, some larger species undertake long-distance migrations possibly linked to ontogeny, but may also undertake diel vertical migrations linked with foraging (Veríssimo *et al.*, 2011, Rodríguez-Cabello *et al.*, 2016). More research is needed to fully understand the trophic geography of deep-sea sharks and their functional roles in deep-sea ecosystems.

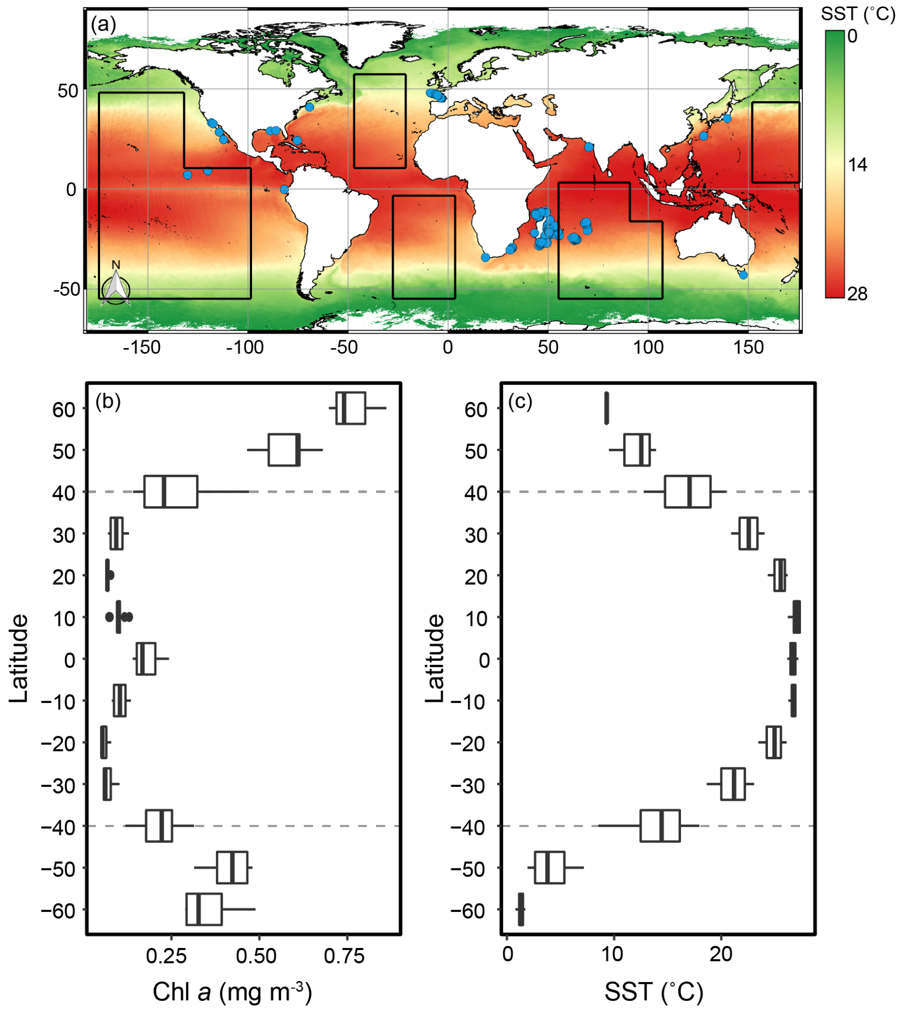


Figure 2.4: a): Boxes represent extent of environmental chlorophyll *a* (Chl *a*) and sea surface temperature (SST) data used to calculate latitudinal trends. Blue points represent locations of oceanic shark samples. Chl *a* (b) and SST (c) for median sampling year, 2009, within oceanic waters. Vertical line represents average value for that latitudinal bin, box represents 50% spread of the data and horizontal lines are 95% of the data. Latitude where abrupt shift in environmental data occurs shown with dashed grey line.

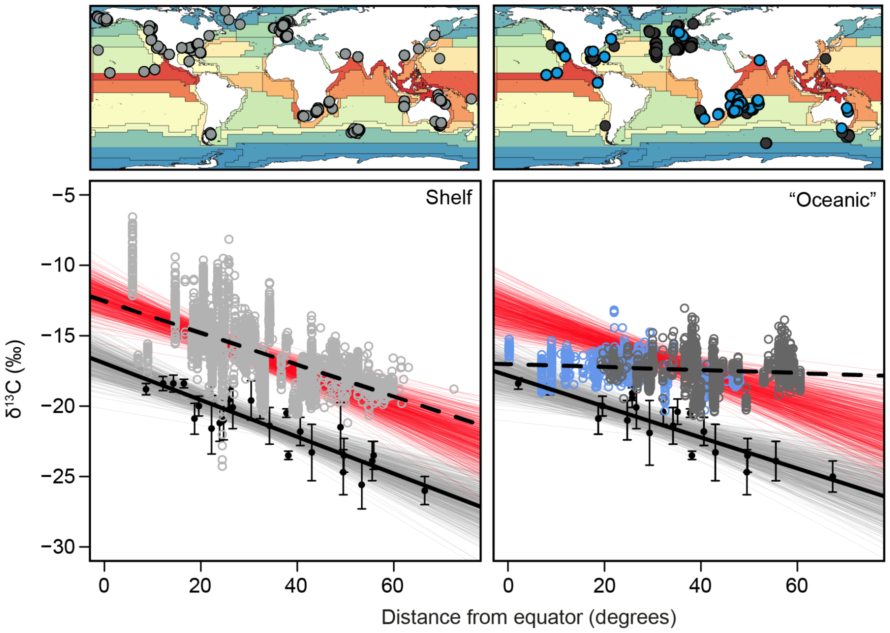


Figure 2.5: Linear regressions for shark muscle $\delta^{13}C$ ($\delta^{13}C_S$) (dashed line), corresponding predictions of planktonic $\delta^{13}C$ ($\delta^{13}C_P$) (solid line) with distance from the equator. Grey lines represent 500 Monte Carlo iterations of latitudinal trends in $\delta^{13}C_P$, taking into consideration the variance in the Longhurst Biogeographical Provinces. The red lines represent the predicted trophic offset of baseline $\delta^{13}C$. Oceanic and deep-sea sharks have been combined within the "oceanic" plot. Individual points correspond to $\delta^{13}C$ values from individual sharks from shelf (grey open circles), oceanic (blue open circles) and deep-sea (dark grey open circles) sharks. Maps represent sampling distributions overlaid on the $\delta^{13}C$ isoscape from Figure 2.1.

2.5 Concluding Remarks

Nearly a quarter of all chondrichthyan species are evaluated as threatened on the IUCN Red List of Threatened Species, raising concerns on the future of many populations and the resulting effects such declines may have on ecosystem function (Heithaus *et al.*, 2008, Ferretti *et al.*, 2010, Dulvy *et al.*, 2014, Heupel and Simpfendorfer, 2014). Concurrent declines in species with shared trophic geographies help identify common risks associated with fishing or climate change. While it is beyond the scope of this study, and these data, to predict the effects of further removal of sharks from the oceans, we generate novel hypotheses that warrant further investigation, specifically (1) that phytoplankton-based food webs provide the majority of carbon to shelf shark populations, (2) that within populations of shelf sharks, individuals specialise on food webs supported by carbon sources of production characterised by higher $\delta^{13}C$ and (3) that oceanic sharks derive the majority of their nutrients from a restricted latitudinal range in sub-tropical regions. It is also unclear whether the trophic geographies observed in sharks are also seen in other globally distributed, mobile, higher order predators, such as cetaceans, teleosts and seabirds. Future studies adopting similar protocols as those used here could identify global trophic geographic trends in other taxa.

We have provided evidence suggesting that on a global scale, sharks typically forage within spatially restricted, regional seascapes. Conservation of shelf marine environments is increasingly addressed through the creation of marine protected areas (MPAs) (Edgar *et al.*, 2014). MPAs may be effective measures for protecting locally-resident shelf shark species, providing they encompass the range of adjacent habitats and core areas utilized by these shark populations (Heupel *et al.*, 2015, White *et al.*, 2017). Although the distributional ranges for most oceanic sharks are expansive, core intermediate latitudes appear to be important for the provision of nutrients and energy. Productive intermediate latitudes are also targeted by pelagic fisheries, which increase the susceptibility of oceanic sharks to exploitation (Queiroz *et al.*, 2016). Establishing management and protective strategies that encompass all critical habitats utilised by a species is complex. However, our results suggest that oceanic sharks may benefit from global strategies that mitigate deleterious impacts on intermediate-latitude food webs and from fishing practices that minimise shark mortality in these areas (Scales *et al.*, 2014b, Queiroz *et al.*, 2016).

Electronic tagging has revolutionized shark spatial ecology, providing detailed records of the movement of individual animals (Block *et al.*, 2011, Chapman *et al.*, 2015). We add to this growing picture of individual behaviour by reconstructing the trophic geography of shark populations. Tracking nutrients rather than individual animals provides a link between the presence of an animal in an area and the importance of that area for provisioning, enhancing our knowledge of the extent and scale of connectivity between oceanic habitats. Locating ecologically-relevant provisioning areas may also assist effective design and placement of marine protected areas, particularly in open ocean and deep-water habitats.

2.6 Methods

Raw carbon stable isotope data (bulk tissue δ^{13}C values) were compiled from 54 publications and 7 unpublished datasets yielding measurements from 5602 individual sharks of 117 species. Where possible, information such as location, body size, sample size, lipid extraction method, and date were reported. The majority of studies were only able to provide a general area of capture and the mapped locational assignment was taken as the median of the latitudinal and longitudinal ranges of these areas. Likewise, some studies sampled landing docks so were only able to provide the area of that landing dock. The locations provided by these studies were of the landing docks and it was assumed that fishers were catching sharks in waters in the vicinity of the landing port. Species habitat preferences were categorised using published information from their prospective papers (Appendix A.1) and on the advice of the corresponding authors. Species that had multiple habitat descriptions were classified as shelf sharks. Examples of this are *Hexanchus* spp, which are classified here as shelf sharks (n=198). Although typically quoted as deep-sea sharks, all species in this study occur consistently over the shelf so were not considered as obligate deep-sea shark species.

Samples from two plankivorous species (*Rhinocodon typus*: *n* = 26; (Borrell *et al.*, 2011, Hussey *et al.*, 2015), *Megachasma pelagios:* n*=2; Wyatt unpublished*), from ecotourism provisioning sites (*Carcharhinus perezii*; n = 23; (Maljković and Côté, 2011)), and from a riverine study (*Carcharrhinus leucas*; n = 125; (Matich *et al.*, 2011)) were excluded as the study focuses on marine predators under natural conditions. Within the studies comprising the dataset, five chemical treatments were used (none: *n* = 2386; water washed: 1407; 2:1 chloromethanol: 748; cyclohexane: 696; and petroleum ether: 157). Tests for lipid extraction effects were not significant and it is assumed that any effect associated with chemical pre-treatment methods are spatially averaged across the data. Samples with a C:N ratio greater than 10 were removed as it is highly unlikely that the δ^{13}C value of these samples represents muscle protein. A further 314 samples with C:N ratios ranging between 4-10 were subjected to mathematical correction for lipid influences on δ^{13}C values (Kiljunen *et al.*, 2006). All other values were used under the assumption that published values were representations of true isotopic composition of muscle protein. The data compiled will form the "Chondrichthyan Stable Isotope Data Project" and we invite the utilisation of these data and addition of new data to help build on the global geographic trends observed here.

For each major ocean, annual mean sea surface temperature (SST) and chlorophyll *a* concentrations (Chl *a*) were derived from the moderate-resolution imaging spectroradiometer (MODIS) 9km AQUA night time sea surface temperatures and 9km MODIS AQUA CHL-a concentration data (NASA Oceancolor) for the median sampling year for the shark data, 2009 (Figure 2.4). Environmental data extraction was constrained to oceanic waters within areas highlighted on the map (Figure 2.4).

2.6.1 δ^{13}C Baseline Predictions

A mechanistic model predicting the spatio-temporal distribution of global δ^{13}C values of particulate organic matter ($\delta^{13}C_P$) was used to interpret shark isotope data (Magozzi *et al.*, 2017). Briefly, the model estimates δ^{13}C values in phytoplankton from ocean carbon chemistry, phytoplankton composition and phytoplankton growth rate variables output from the NEMO-MEDUSA biogeochemical model system at 1 degree and monthly resolution. Biomass weighted annual average phytoplankton δ^{13}C values together with associated spatial and temporal standard deviations were averaged across Longhurst Biogeochemical Provinces (Figure 2.1). Model-predicted baseline δ^{13}C values were then inferred for the capture location for each individual shark data point.

2.6.2 Mathematical Models

The relationship between latitude and carbon stable isotope composition for both phytoplankton ($\delta^{13}C_P$) and shark muscle ($\delta^{13}C_S$) was modelled using linear regression (Figure 2.2, Table 2.2). For phytoplankton, we recovered the median and standard deviation of annual average $\delta^{13}C_P$ values simulated within each Longhurst Province with a corresponding shark sample. We then ran 500 repeated (Monte Carlo) linear regressions to account for the spatial variation in predicted $\delta^{13}C_P$ values within each biogeographic province. We predicted null hypothesis shark isotope compositions by adding 4.6‰ (reflecting 4.1 as the median trophic level of sharks and using published experimental studies of trophic discrimination factors for $\delta^{13}C$ values in elasmobranch tissues of 1.1‰ (Table 2.1) to the intercept of each of the 500 simulated regression models. ANCOVA analyses were run to compare the slopes of regressions within a given habitat and between comparable variables between habitats ($\delta^{13}C_S$, $\delta^{13}C_P$). ANOVA with post-hoc Tukey HSD were used to test for significant differences between population carbon ranges among habitats.

Generalised additive models (GAMs) were developed to describe latitudinal trends in $\delta^{13}C_S$. Specific habitat models were used to determine the amount of deviance that could be explained by single and multiple explanatory variables including distance from the equator and predicted $\delta^{13}C_P$ (Table 2.3). A depth parameter was also added to the deep-sea shark models. $\delta^{13}C_P$ values were modelled separately from corresponding capture locations as a function of distance from the equator. By comparing the amount of deviance explained within both the $\delta^{13}C_S$ and $\delta^{13}C_P$ models, it was possible to determine how much of the predicted $\delta^{13}C_P$ patterns were captured within $\delta^{13}C_S$ values. All models were limited to two smoothing knots in order to make models comparable and interpretable. Model comparisons were drawn using Akaike's information criterion (AIC) to determine the most parsimonious model. Final models were visually inspected using standard residual q-q plots to assess model suitability.

2.7 Supplementary Information

2.7.1 Linking the $\delta^{13}C$ values of sharks to simulated $\delta^{13}C$ values of phytoplankton

The stable isotopic composition of a consumer's tissue reflects a time-integrated average of the composition of primary production fuelling the base of the food web, modified by isotopic fractionation during metabolism (i.e. preferential excretion of light isotopes and resulting enrichment of heavy isotopes in assimilated body proteins). We therefore consider four variables when predicting carbon stable isotopic compositions in shark tissues:

1. Variation in the isotopic composition of primary production between Longhurst Biogeographic Provinces.
2. Spatio-temporal variation of primary production within Longhurst Biogeographic Provinces.
3. Trophic fractionation between sharks and primary production.
4. Turnover rate of shark tissues, and implications for stable isotopes.

To determine the variation in the stable isotopic composition of carbon in primary production between Longhurst Biogeographic Provinces, we took biomass-weighted annual average predictions of $\delta^{13}C$ values at one-degree intervals, and calculated the median of the annually averaged $\delta^{13}C$ values within each biogeographic province. The biomass-weighted annual average simply weights the stable isotopic composition of diatoms and non-diatoms predicted at monthly intervals by the proportion of total annual production produced in that month. Thus in temperate latitudes with seasonal plankton blooms, the biomass weighted average is closer to the isotopic composition of plankton grown under bloom conditions.

The use of annual (biomass weighted) average $\delta^{13}C_P$ values could in theory complicate spatial interpretations, however seasonal short-term variations in primary production $\delta^{13}C$ values are attenuated through food chains, as the isotopic incorporation (turnover) rate increases with body size, and thus trophic level in size structured marine ecosystems. As an illustration, we simulate temporal fluctuations in the stable isotopic compositions of successive trophic levels sustained by phytoplankton in temperate latitudes characterised by extreme seasonal variations in $\delta^{13}C_P$ values. We simulate the $\delta^{13}C$ values in tissues of consumers at sequential trophic levels with isotopic equilibration rates of two, four, and six months respectively. By trophic level three (with an isotopic equilibration rate of six months, which is lower than predicted for most sharks), the amplitude of seasonal fluctuations in $\delta^{13}C$ values is reduced from >6‰ in phytoplankton to less than 2‰. Seasonal fluctuations in $\delta^{13}C_P$ values are therefore effectively smoothed through trophic attenuation, justifying the use of a constant biomass-weighted annual average reference value.

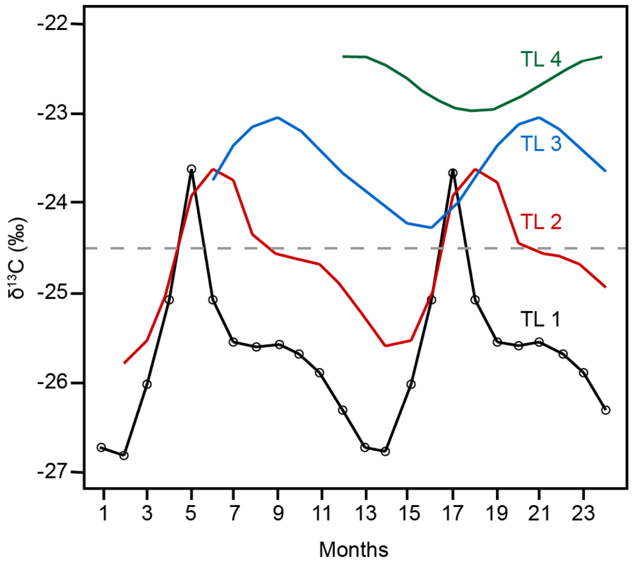


Figure 2.6**:** Effect of trophic attenuation: The black points show two 12-month cycles of $\delta^{13}C$ values of phytoplankton production simulated by the Magozzi *et al.* (2017) NEMO MEDUSA isotopic extension for the North East Atlantic around the coast of west Ireland, a region characterised by a strong seasonal plankton bloom. Note the pronounced enrichment in $\delta^{13}C$ values associated with bloom conditions in May-July. The dashed black line indicates the biomass-weighted annual average $\delta^{13}C_{POM}$ value, the relatively positive average $\delta^{13}C$ value indicating that the bulk of all production occurs during bloom conditions. Animals feeding at successive trophic levels are simulated assuming constant feeding levels through the year and a trophic fractionation factor of 1‰. For trophic level (TL) 2 (red), an isotopic incorporation rate of 2 months was assumed, for TL 3 (blue) the incorporation rate is 4 months and for TL 4 (green) it is 6 months. Note that by TL 4 even the pronounced seasonal fluctuations in $\delta^{13}C_{POM}$ values of >3‰ are essentially attenuated to less than 0.5‰ through successive temporal smoothing, and the biomass weighted annual average is a reasonable approximation. In reality attenuation is likely to be more pronounced as feeding is not constant through the year but weighted to times of year with greater production.

We considered the effect of spatial variability in $\delta^{13}C_P$ values within Longhurst Provinces by calculating the standard deviation and maximum range of $\delta^{13}C$ values within each province. We estimated uncertainty around latitudinal trends in $\delta^{13}C_P$ values characterising Longhurst Provinces with a Monte Carlo simulation where we sampled $\delta^{13}C_P$ values randomly from a Gaussian distribution taking the $\delta^{13}C$ value in the latitudinal mid-point of the province as the mean and using the measured standard deviation.

The rate of isotopic turnover (the rate at which new atoms of carbon are assimilated from diet into tissues through growth and tissue turnover) varies between taxa and under differing conditions. We use as a first approximation scaling relationships between body size and turnover rate (Thomas and Crowther, 2015) to estimate likely rates of turnover. Sharks with body masses between 10^4g and 10^5g are predicted to exhibit isotopic half-lives on the order of 100-200 days, with temperature influencing rates so that turnover will be greater at higher temperatures. Thus, a 10kg shark moving between feeding sites separated by 10 degrees of latitude (reflecting 1.2‰ difference in baseline δ^{13}C values on average) would need to forage for 100 days in the new environment to express an isotopic shift in excess of 0.5‰. Isotopic turnover rate does, of course, limit the sensitivity to movement across isotopic gradients: animals foraging across isotopic gradients at timescales significantly shorter than isotopic turnover rates will form tissues with isotopic compositions reflecting a weighted average of the ingested food sources over the timescale of equilibration. If populations are comprised of individuals switching feeding between two isotopically-distinct resources, the median composition of the population will trend towards a value between the two sources, and the isotopic variance within the population will reduce as the rate at which the individuals switch resources (e.g. move between habitats) increases. Thus if individuals forage across a latitudinal range, the slope of the isotopic gradient reflected in the population mean of tissue isotopic compositions will reduce, the extent of any reduction depending on the relative proportion of tissues assimilated outside of the catch location during the period of isotopic equilibration, and the mean geographic distance between assimilation site and capture location.

Shelf sharks display population mean latitudinal slopes that are similar to that of local phytoplankton (Figure 2.2), implying little attenuation of isotopic gradients due to latitudinal mixing. Oceanic sharks, however, show shallow isotopic gradients that could, in theory, reflect either derivation of the majority of nutrients from restricted latitude, or foraging across large latitudinal gradients. In both cases derivation of nutrients with lower δ^{13}C values (i.e. from higher latitudes) is needed to explain the shallow latitudinal gradients seen. Nutrients assimilated from regions characterised by primary production with higher δ^{13}C (i.e. low latitude areas) must therefore be balanced by nutrients assimilated from isotopically depleted (high latitude) regions. Oceanic sharks are not commonly found in latitudes greater than *c.*50°N or S, limiting the potential to balance isotopically positive dietary sources, and we therefore infer that the majority of nutrients assimilated had a similar and relatively ^{13}C-depleted isotopic composition, consistent with derivation from intermediate latitudes between *c.*30-50 degrees from the equator. The δ^{13}C values of oceanic shark tissues captured at 10 degrees average around 17.5‰. A simple two component mass balance considering diet δ^{13}C end member values estimated from phytoplankton models implies that between *c.*65-100% of all nutrients must be derived from latitudes between 30 and 50 degrees.

2.7.2 Interpretation of Shelf Variation

Shelf dwelling shark populations display greater ranges in δ^{13}C values than slope or pelagic dwelling sharks. This variation could be caused by increased spatial variation in regional phytoplankton baselines in coastal systems, or through increasing use of food webs supported by isotopically distinct sources.

Variations in $\delta^{13}C$ values within Longhurst Biogeographic Provinces increase towards high latitudes that are characterised by steep latitudinal temperature gradients (Figure 2.2). Isotopic variation in shelf shark populations, however, is greater in tropical latitudes indicating that isotopic variability is not primarily controlled by spatial variation within the local biogeographic province.

Sharks are typically viewed as generalist consumers, however generalism may occur through individuals foraging across a wide range of resources or through populations comprised of individuals specialising on locally abundant, discrete sources. Individual generalism would tend to produce relatively low between-individual isotopic variability; therefore the high variance seen within populations of shelf sharks implies individual-level food web specialisation within the timescale of isotopic incorporation. Median $\delta^{13}C$ values in shelf sharks populations are indistinguishable from those predicted for sharks feeding on local phytoplankton-dominated food webs. The null hypothesis that shelf sharks derive all carbon from local food webs supported by phytoplankton is therefore partially supported as latitudinal trends in $\delta^{13}C$ values are indistinguishable from those predicted for phytoplankton, but the range in $\delta^{13}C$ values observed in shelf sharks strongly implies additional utilisation of food webs characterised by high $\delta^{13}C$ values.

The $\delta^{13}C$ of shelf shark muscle muscles are relatively high compared to local phytoplankton at both low and high latitude ranges, likely as a consequence of a greater proportion of individual shelf sharks feeding on prey supported by primary production with higher $\delta^{13}C$, or at a higher trophic level than currently prescribed. The very high $\delta^{13}C$ values seen in some individual sharks (i.e. 5‰ heavier than predicted) cannot realistically be explained through increased trophic level, and thus strongly imply derivation of a substantial proportion of their nutrients from more coastal food webs characterised by higher $\delta^{13}C$, such as seagrasses, macrophytes, coral reefs, and/or terrestrially supported food webs (Fry and Sherr, 1989).

Chapter 3: Mesopredatory deep-sea sharks reveal differences in food web structure between seamounts and continental slopes across the northeast Atlantic.

3.1 Abstract

Using higher trophic predators, such as sharks, as integrators of ecological variability, may provide useful information on the underlying nutrient dynamics of local food webs. Here we use stable isotope analysis of whole shark assemblages to compare the nutrient dynamics and trophic structure between continental slope ecosystems (Rockall Trough and Portuguese slopes) and several seamounts across the northeast Atlantic. Furthermore, we directly compare the isotopic characteristics of three abundant squaliform species that are common at each sampling location in order to infer species-specific differences in trophic ecology, and therefore local nutrient availability. Bulk stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) showed lower species-specific variability on seamounts suggesting sharks may have less access to isotopically diverse prey items. The three commonly sampled sharks had lower $\delta^{15}N$ values at seamount locations, suggesting that they were feeding at comparatively lower trophic levels (i.e. had diets more tightly coupled to baseline production). These results are highly suggestive of sharks, and therefore ichthyofaunal assemblages, on seamounts being more reliant on pelagic production than those found on continental slopes, likely due to the stark topographic differences between the two habitats. The sampled seamounts may provide a lower diversity of trophic niches than continental slopes that may cause higher order predators to be more vulnerable to over exploitation than their counterparts found on continental slopes; something that should be considered in future sustainability assessments of these ecosystems.

3.2 Introduction

The deep-sea is the largest biome on the planet, encompassing waters deeper than 200 m and 73% of the world's oceans (Ramirez-Llodra *et al.*, 2010). Bathymetric heterogeneities across the deep-sea create a diversity of habitats and ecosystems that support a broad range of faunal assemblages (Grassle and Maciolek, 1992). Deep-sea continental slopes, seamounts and oceanic ridges support high biomasses of fishes that are of commercial interest (Kelly *et al.*, 1998, Large *et al.*, 2003, Morato *et al.*, 2006b, Ramirez-Llodra *et al.*, 2010). Due to the slow life history dynamics expressed by many deep-sea fish species (Koslow *et al.*, 2000, Gordon, 2003, Garcia *et al.*, 2008, Simpfendorfer and Kyne, 2009), many of these stocks are susceptible to overexploitation (Connolly and Kelly, 1996, Kelly *et al.*, 1998, Gordon, 2003, Large *et al.*, 2003). Stock collapses have been observed in several deep-water fisheries, most notably the orange roughy (Branch, 2001), with other groups exhibiting substantial population declines, such as deep-sea sharks (Baker *et al.*, 2009, Neat *et al.*, 2015). There is therefore an increased need to better understand the ecological processes governing these environments in order to improve management of deep-sea resources.

Despite the obvious differences in the topography of continental slopes, seamounts and oceanic ridges (Boehlert and Genin, 1987, Genin, 2004, Priede *et al.*, 2013), many demersal fish assemblages in the northeast-central Atlantic are dominated by similar benthopelagic fish species (Mauchline and Gordon, 1991, Bergstad *et al.*, 2012, Trueman *et al.*, 2014). On continental slopes, high benthopelagic biomasses are sustained through the vertical transport of nutrients from productive photic zones, primarily by vertically migrating mesopelagic prey, such as teleosts, crustaceans and cephalopods (Mauchline and Gordon, 1991, Davison *et al.*, 2013, Sutton, 2013, Trueman *et al.*, 2014). Demersal ichthyofaunal assemblages are provided with substantial pelagic nutrient subsidies where the vertical range of migrating prey impinge directly on, or within swimming distance to, the seafloor (Mauchline and Gordon, 1991, Porteiro and Sutton, 2007). Benthic food webs are primarily supported by the passive flux of organic matter directly onto the seabed, which is prone to significant reworking and recycling by microbial communities (Drazen *et al.*, 2008, Jeffreys *et al.*, 2011).

On seamounts, the nutrient dynamics supporting benthopelagic teleosts remain ambiguous, with several theories proposed for the local bio-physical processes that sustain local fish populations (Pitcher *et al.*, 2008, Rowden *et al.*, 2010a). For example, Taylor columns, which are anti-cyclonic circulations associated with seamounts, have been proposed to promote nutrient upwelling and nutrient retention around seamounts that subsequently support primary and secondary production (Pitcher *et al.*, 2008). On the other hand, topographic trapping hypotheses, similar to those proposed for continental slopes, suggest that pelagic prey items impinge on seamounts during diel vertical migrations or are advected by localised lateral currents (Isaacs and Schwartzlose, 1965, Boehlert, 1988, Genin, 2004, Rowden *et al.*, 2010a). To date, most studies have considered the processes at single site locations and there has been limited work to see how local processes may vary between different deep-sea habitats (Tracey *et al.*, 2004, Howell *et al.*, 2010).

Our ability to quantify and compare the ecological structure of deep demersal habitats is limited by a paucity of directly comparable survey data using the same fishing gear (Bergstad, 2013) and corresponding trophic information (Cortés, 1997). Geochemical tracking of nutrient sources supporting consumers in contrasting habitats provides an alternative tool to test for differences in food web structure. In marine food webs, the stable isotope compositions of carbon ($^{12}C/^{13}C$; expressed as '$\delta^{13}C$') and nitrogen ($^{14}N/^{15}N$; expressed as '$\delta^{15}N$') within consumer tissues vary primarily as a function of the isotopic composition of primary production and trophic distance from primary production (Layman *et al.*, 2007, Drazen *et al.*, 2008, Bergmann *et al.*, 2009, Trueman *et al.*, 2014, Stasko *et al.*, 2016). Consequently, variations in the stable isotope composition of the structural nutrients carbon and nitrogen have been routinely used to trace energy and nutrient flow through ecosystems (Peterson and Fry, 1987, Pinnegar and Polunin, 1999, West *et al.*, 2006).

As a functional group, sharks are common in many deep-sea food webs (Macpherson and Roel, 1987, Gordon and Swan, 1997). As typically opportunistic mesopredators or higher order predators with cosmopolitan diets (Cortés, 1999), sharks have the capacity to feed over multiple nutrient pathways (Mauchline and Gordon, 1983), providing an integrated view of nutrient supply and food web structure. As ‘ecological-samplers’, the stable isotope values expressed in the white muscle of sharks reflect the local resources available within those food webs, and the rate at which these resources are transferred from primary production to mesopredators and higher-order predators. The isotopic range exhibited by a population in two-dimensional stable isotope space (termed the ‘isotopic niche’; (Layman *et al.*, 2007, Newsome *et al.*, 2007, Jackson *et al.*, 2011) can be used as an indication of resource breadth utilised by a population, integrating information on both trophic interactions and habitat use (Newsome *et al.*, 2007, Rader *et al.*, 2017). Additionally, relative δ^{15}N values within an assemblage can be used to indicate the degree of benthic-pelagic coupling (Kopp *et al.*, 2015), once spatial variations in baseline δ^{15}N have been accounted for (Somes *et al.*, 2010).

The continental slopes and seamounts of the northeast Atlantic support a diversity of deep-sea fisheries, including both trawling and long-lining operations (Ramos *et al.*, 2013, ICES, 2017). To what extent the nutrient dynamics vary between these deep-sea habitats remains unclear however, but could potentially impact the level of exploitation that can be sustained by prospective stocks. Using deep-sea shark assemblages as indicators of underlying nutrient dynamics, we record the stable isotopic properties of deep-sea shark assemblages. The null hypothesis is the stable isotope values presented in the muscle tissue of sharks, will be similar between the sampled seamounts and continental slope areas, implying the underlying food web and nutrient dynamics are similar. For this purpose, we sample the most locally abundant squaliform shark species from continental slope regions and oceanic seamounts in the northeast Atlantic Ocean. To account for differences in species composition between locations, we compared the stable isotope values of three common sympatric species found at all locations: Portuguese dogfish (*Centroscymnus coelolepis*), birdbeak dogfish (*Deania calcea*) and lantern sharks (*Etmopterus spinax* and *E. pusillus*). The stable isotopic values expressed in the white muscle of sharks may provide a time-integrated assessment of the trophic structure of these continental slope and oceanic seamount sites, and provide an indication of the variety of resources available to different demersal fish assemblages.

3.3 Materials and Methods

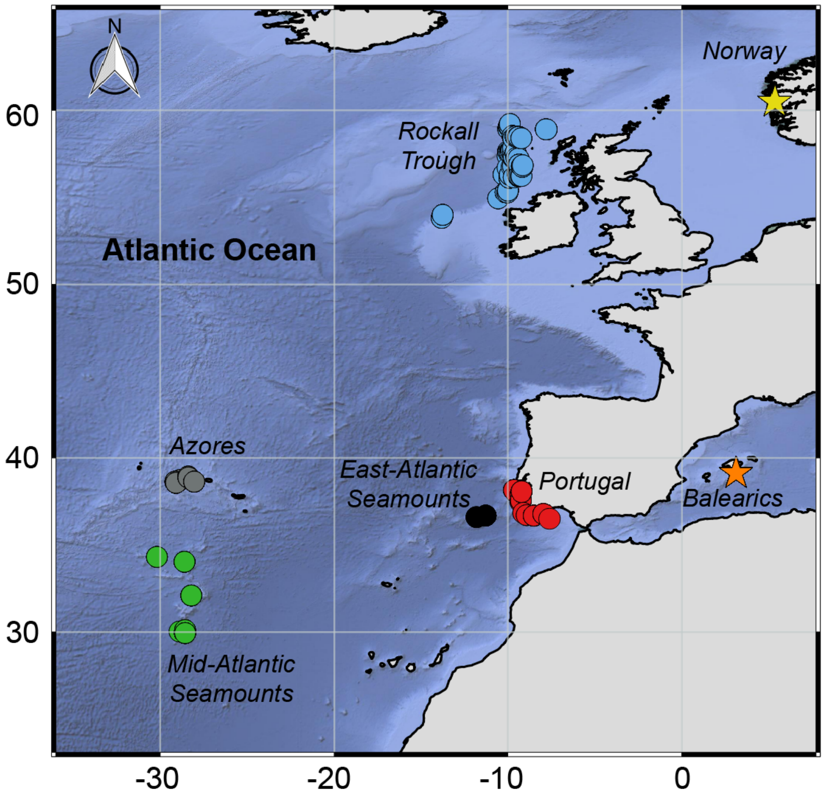


Figure 3.1: Distribution of shark samples from Norway (yellow), Rockall Trough (blue), Azores (grey), mid-Atlantic seamounts (green), east Atlantic seamounts (black), Portugal (red) and the Balearic Islands (orange). Stars represent locations where only *Etmopterus spinax* samples were acquired.

3.3.1 Sampling

White muscle samples were collected from 596 deep-water squaliform sharks (Squaliformes) from across the northeast Atlantic (Figure 3.1). Core sampling took advantage of annual-fisheries surveys run by Marine Science Scotland (MSS; Rockall Trough), Instituto Português do Mar e da Atmosfera (IPMA; Portugal) and Department of Oceanography and Fisheries of the University of the Azores (DOP; Azores and Seamounts) (Table 3.1). Additional samples were acquired from Sesimbra, Portugal from by-catches of the commercial black scabbard long-line fishery. As such, precise capture depths are lacking for this group of samples but the fishing fleet operates between 800m – 1450 m (Bordalo-Machado and Figueiredo, 2009). Additional stable isotope data was integrated from 45 sharks sampled from Rockall Trough in 2006 and 2012 from Trueman *et al.* (2014). Lastly, *Etmopterus spinax* samples were acquired opportunistically from research conducted by the Universite Catholique de Louvain in Norway, and from the Mediterranean International Trawl Survey (MEDITS) around the Balearic Islands, Mediterranean Sea (Table 3.1).

The Rockall Trough (ROC) and the Portuguese slope (POR) were classified as continental slope habitats and were considered separately throughout. Samples from the Mid-Atlantic seamounts (Atlantis, Great Meteor, Irving, Tyro seamounts (SMC)), Azores (Condor seamount (SMC)) and East Atlantic Seamounts (Gorringe (SME)) were grouped together as seamounts (SMO) due to limited samples from each individual location (Figure 3.1, Table 3.1). Whole shark assemblages were compared among the three core sampling sites, i.e. Rockall Trough, the continental slopes off Portugal and grouped seamounts, with further between-region comparisons concentrating on three species found at all locations: *Etmopterus* spp (lanternsharks), *D. calcea* and *C. coelolepis*. The results from *E. spinax* and *E. pusillus* were pooled due to low sample sizes of each species separately. Both species are of a similar size (<50 cm), occur at a similar depth range, and have a similar trophic ecology (Coelho and Erzini, 2007, Coelho and Erzini, 2008b). Due to their small size and similar diets of pelagic micronekton (Neiva *et al.*, 2006), lanternsharks are expected to accumulate site-specific isotopic signals relatively quickly and therefore reflect spatial differences in baseline stable isotope values.

Table 3.1: Overview of sampling methods used to collect shark samples from continental slopes and seamounts and the laboratory used to process the corresponding samples (Lab). Institutes conducting the research surveys are: Department of Oceanography and Fisheries of the University of the Azores (DOP), Instituto Português do Mar e da Atmosfera (IPMA), Marine Science Scotland (MSS), Mediterranean International Trawl Survey (MEDITS) and Universite catholique de Louvain (UCL). Only *Etmopterus spinax* samples were obtain from sites with ‘*’. n is the number of samples collected.

Row Labels	Code	Gear	Years	Institute	n	Depth (m)	Lab
Azores	AZO	Longline	2011, 2012, 2014	DOP	81	450-1325	Elemtex
Portugal	POR	Longline	2014	IPMA	168	525-1100	Elemtex
Rockall	ROC	Trawl	2004, 2012, 2015	MSS	203	500-1800	OEA, SUERC
Seamounts	SMC	Longline	2015	DOP	77	575-1975	SUERC
Seamounts East	SME	Longline	2015	DOP	16	575-975	SUERC
Mediterranean*	MED	Trawl	2014	MEDITS	33	230-754	Elemtex
Norway*	NOR	Longline	2014	UCL	18	230-230	Elemtex

Total length (cm), sex and species identification were recorded for each specimen before 1 cm^3 of white muscle was removed from the dorsal side proximal to the first dorsal fin and wrapped in aluminium foil. Samples were then frozen to at least -20°C before being transported back to the University of Southampton for analysis. Samples were kept frozen throughout transit by packaging in insulated bags and were never out of the freezer for more than 12 hours. Frozen muscle samples were washed by vigorously shaking with distilled water 3 times to remove urea, before freezing again at -20°C. Samples were then freeze-dried at -58°C for 48 hours prior to homogenisation, and 0.7mg per sample was weighed into tin capsules for stable isotope analysis.

3.3.2 Stable Isotope Analysis

Stable isotope values are expressed in parts per thousand (‰) with reference to a standard using; $\delta X = 1000[R_{sample} \times R_{standard}^{-1}]$, where X is either ^{13}C or ^{15}N, R_{sample} is the ratio of heavy to light isotope in the sample and $R_{standard}$ is the isotopic ratio for a given standard. $\delta^{13}C$ values are expressed with reference to the universal standards of PeeDee Belemnite and $\delta^{15}N$ values are expressed relative to atmospheric N_2. Depending on the timing of the sampling, bulk carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis was performed at one of 3 different laboratories (Table 3.1). Scottish University Environmental Research Council (SUERC) samples were run on a continuous flow Elementar vario PYRO cube elemental analyser coupled with a Thermo Scientific Delta V plus isotope ratio mass spectrometer (IRMS) at the NERC LSMSF, East Kilbride facility. OEA Labs and Elemtex samples were run on a Thermo EA 110 elemental analyser linked to a Europa Scientific 2020 isotope ratio mass spectrometer running in continuous flow mode. USGS 40 glutamic acid samples were used to assess accuracy and precision in all laboratories and a separate single batch of in-house glutamic acid standard was analysed multiple times throughout each analysis run at each facility to ensure comparability between laboratories. $\delta^{13}C$ values for the in-house glutamic acid were on average 0.24 ± 0.40‰ more positive from laboratory 1 than those obtained from other facilities (Figure 3.2), and subsequently this difference was subtracted from all shark $\delta^{13}C$ values from this facility. All muscle samples were subjected to mathematical lipid corrections as outlined in Kiljunen *et al.* (2006).

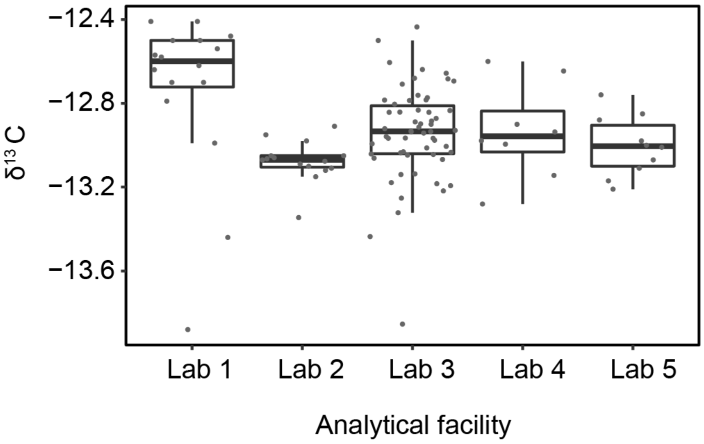


Figure 3.2: Boxplots displaying mean (thick horizontal), 25th and 75th percentiles (box) and 1.5 x the interquartile range (vertical lines) for $\delta^{13}C$ values of in-house glutamic acid standard run at each of the stable isotopic analytical facilities.

3.3.3 Data Analysis

$\delta^{13}C$ and $\delta^{15}N$ values were plotted in 2-dimensional space to calculate the size-corrected standard ellipse areas (SEAc) for each shark species at each of the three main study sites (ROC, POR, SMO) using the R-package SIBER (Jackson *et al.*, 2011). Linear mixed effects (LME) models were run using *nlme4* software packages (Pinheiro *et al.*, 2009) to test for differences in $\delta^{15}N$ values between continental slope and seamounts groups. Total length was used as a fixed effect with an interaction of habitat (seamount or slope). Species and sex were set as random terms, to account for any sex-based differences within species due to potential spatial segregations (Moura *et al.*, 2014). Finally, to test for species differences in stable isotope values for sharks at each location (*Etmopterus* spp,, *D. calcea* and *C. coelolepis*), ANOVA was used. ANOVA tests were also used to test for regional differences in stable isotope values of the same species from different locations. All analyses were carried out using R-cran (R Development Core Team, 2016)

3.4 Results

Species availability was different among sampling locations (Table 3.2, Figure 3.3) but was reflective of the most abundant species per location using each of the fishing methods applied. Eight species were collected from ROC (204 individuals) and from POR (168 individuals), and 7 species from SMO (174 individuals). The highest mean $\delta^{15}N$ values were recorded in *C. coelolepis* from Rockall Trough (14.41 ± 0.59‰) and Portugal (14.86 ± 0.59‰), and in *Centroscymnus owstonii* from the seamounts (13.58 ± 0.68‰). The highest mean $\delta^{13}C$ values were recorded in *Centrophorus squamosus* in Rockall Trough (-15.91 ± 0.89‰), in *C. coelolepis* Portugal (-16.29 ± 0.81‰), and in *C. owstonii* at the seamounts (-15.59 ± 0.0.83). The lowest $\delta^{15}N$ values were observed in *Deania profundorum* in Portugal (11.31 ± 0.65‰) and seamounts (11.15 ± 0.39 ‰), but *E. spinax* had the lowest average values at Rockall Trough (11.60 ± 0.84‰). The lowest mean $\delta^{13}C$ values were recorded in *E. spinax* at Rockall Trough (-17.85 ± 0.46‰) and *Deania profundorum* at the seamounts (-18.32 ± 0.09) and off Portugal (-17.66 ± 0.66‰). Considering all species at each location, the size-corrected standard ellipse areas (SEAc) were significantly smaller in seamount shark species compared to continental slope habitats (ΔSEAc = 0.415, ANOVA: F=6.651, p=0.01).

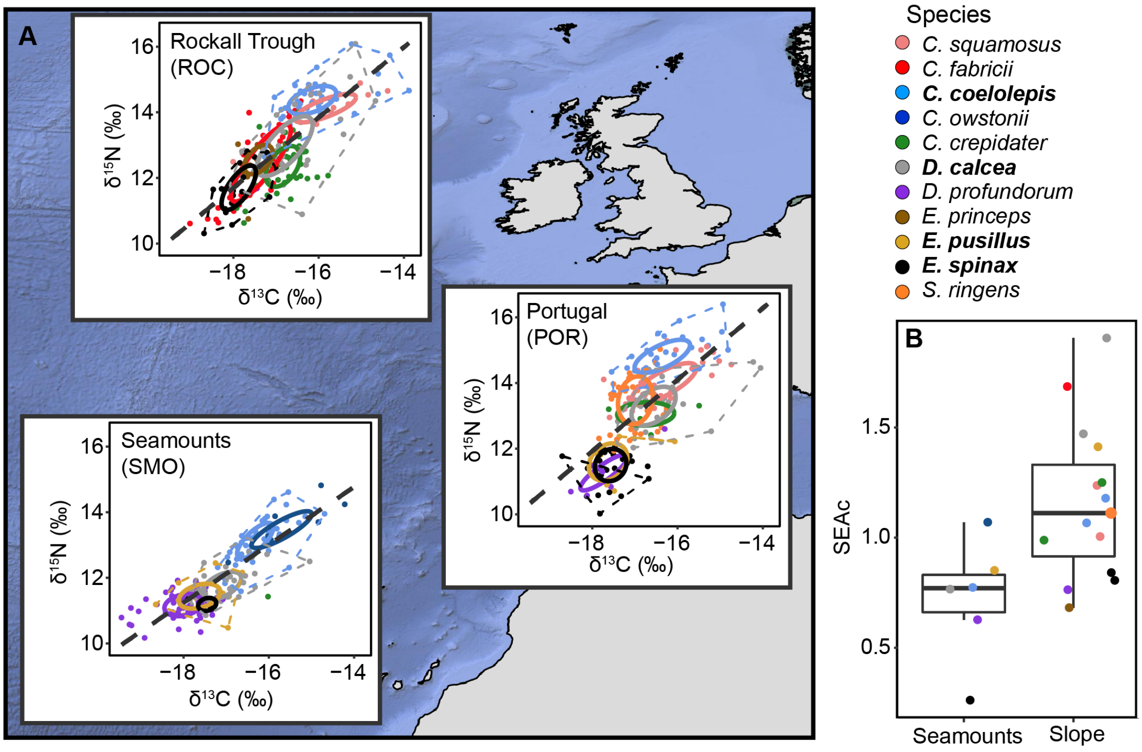


Figure 3.3: A) Bulk δ^{13}C and δ^{15}N values for squaliform shark species sampled across the northeast Atlantic. Individual colours represent different species with corrected standard ellipse areas (SEAc; solid line) and convex hulls (dashed lines). Species names in bold are those found at all study sites. B) Box and whisker plot for population SEAc for seamount and continental slopes (Rockall and Portugal).

Table 3.2: Mean stable isotope values ± standard deviations for Rockall Trough (ROC), Portugal (POR) and seamounts (SMO) with sample size (*n*) total length (TL), sex ratio of female (F) to male (M), and depth range of capture in metres (Depth).

Habitat	Species	*n*	δ^{15}N	δ^{13}C	C:N	TL (cm)	Sex Ratio (F:M)	Depth (m)
Rockall	*Centrophorus squamosus*	14	14.09 ± 0.59	-15.91 ± 0.89	3.42 ± 0.17	86 - 122	1.00	750 - 1200
(Slope)	*Centroscyllium fabricii*	32	12.43 ± 1.2	-17.38 ± 0.74	3.59 ± 0.25	20 - 90	1.13	1000 - 1500
	Centroscymnus coelolepis	38	14.42 ± 0.59	-16.04 ± 0.75	3.58 ± 0.23	70 - 116	3.75	1050 - 1800
	Centroselachus crepidater	42	12.32 ± 0.72	-16.86 ± 0.47	3.42 ± 0.18	28 - 88	1.80	730 - 1500
	Deania calcea	42	13.09 ± 1.08	-16.66 ± 0.78	3.29 ± 0.23	61 - 102	0.35	500 - 1500
	Deania profundorum	1	11.68	-17.45	3.16	69 - 69	0.00	500
	Etmopterus princeps	16	12.48 ± 0.61	-17.42 ± 0.37	3.44 ± 0.13	41 - 82	1.29	1200 - 1800
	Etmopterus spinax	19	11.6 ± 0.84	-17.85 ± 0.46	3.26 ± 0.34	20 - 55	0.73	500 - 850
Portugal	*Centrophorus squamosus*	29	14.06 ± 0.66	-16.29 ± 0.81	3.29 ± 0.14	85 - 136	0.81	1100
(Slope)	*Centroscymnus coelolepis*	28	14.86 ± 0.59	-16.28 ± 0.74	3.51 ± 0.13	69 - 115	1.00	1100
	Centroselachus crepidater	5	13.05 ± 0.39	-16.67 ± 0.76	3.45 ± 0.22	69 - 86	4.00	800
	Deania calcea	32	13.3 ± 0.7	-16.44 ± 0.76	3.43 ± 0.14	71 - 112	1.29	1100
	Deania profundorum	8	11.31 ± 0.65	-17.66 ± 0.66	3.44 ± 0.15	30 - 42	1.00	574 - 800
	Etmopterus pusillus	7	11.62 ± 0.63	-17.46 ± 0.65	3.74 ± 0.18	20 - 46	1.33	574 - 800
	Etmopterus spinax	25	11.42 ± 0.56	-17.58 ± 0.44	3.61 ± 0.18	19 - 49	1.50	525 - 670
	Scymnodon ringens	34	13.41 ± 0.78	-16.98 ± 0.45	3.35 ± 0.18	34 - 99	1.83	553 - 1100
Seamounts	*Centroscymnus coelolepis*	49	13.19 ± 0.77	-16.26 ± 0.52	3.5 ± 0.15	79 - 112	0.20	1125 - 1975
	Centroscymnus owsonii	9	13.58 ± 0.68	-15.59 ± 0.83	3.52 ± 0.1	75 - 114	2.00	1175 - 1325
	Centroselachus crepidater	1	11.43	-16.02	3.49	81 - 81	1.00	1100
	Deania calcea	39	11.82 ± 0.51	-17.05 ± 0.57	3.36 ± 0.12	70 - 112	0.77	825 - 1225
	Deania profundorum	55	11.15 ± 0.39	-18.06 ± 0.53	3.27 ± 0.18	42 - 98	1.20	575 - 1000
	Etmopterus pusillus	16	11.45 ± 0.48	-17.58 ± 0.55	3.74 ± 0.29	29 - 47	0.60	450 - 975
	Etmopterus spinax	5	11.25 ± 0.23	-17.47 ± 0.28	3.65 ± 0.13	29 - 44	4.00	550 - 750

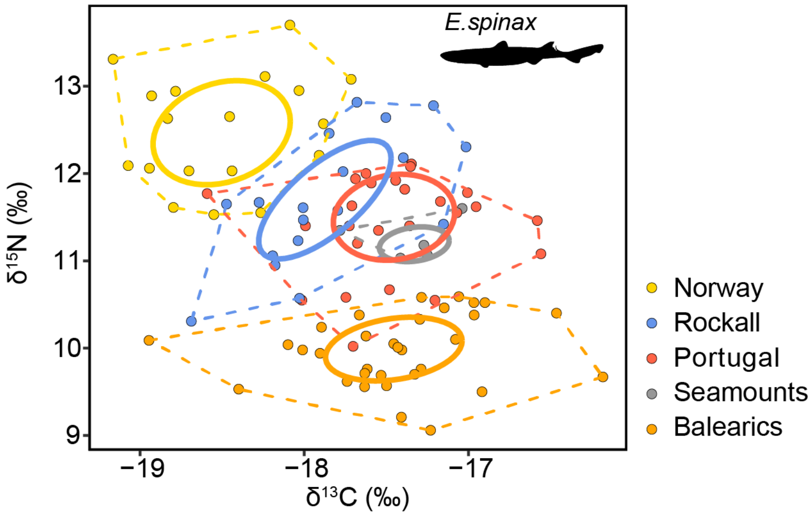


Figure 3.4: $\delta^{13}C$ and $\delta^{15}N$ values for *E. spinax* from Azores (grey), Balearic Islands (orange), Norway (yellow), Portugal (red), and Rockall Trough (blue) with 40% standard ellipse areas (SEA; solid lines) and convex hull areas (dashed lines).

Across the whole sampling region, the stable isotope values for *Etmopterus spinax* were distinct between areas (Figure 3.4), consistent with latitudinal gradients that would be expected in baseline $\delta^{13}C$. The *Etmopterus* species complex was therefore assumed to be a good representation of local baseline stable isotope values, and provide a suitable species with which to compare the stable isotopic ecology of some sympatric shark species.

The results from the linear mixed effect models highlighted that habitat type (seamount or continental slope) has a significant influence on $\delta^{15}N$ ($F=33.70$, $p<0.01$), with values being statistically lower in seamount habitats after accounting for total length and sex-species interactions. There was no detectable influence of habitat type on $\delta^{13}C$ values though ($F=1.21$, $p=0.27$). To further determine regional differences, common species at Rockall Trough, Portugal and seamount sites were considered separately. *Etmopterus* spp. $\delta^{13}C$ values were significantly different between areas ($F=4.82$, $p=0.01$), whereas $\delta^{15}N$ values were invariant among sampling locations ($F=1.51$, $p=0.23$). The absolute differences between regions (as symbolised by 'Δ' symbol) were significantly different for *D. calcea* for $\delta^{13}C$ ($F=9.48$, $p<0.001$) and $\delta^{15}N$ ($F=36.5$, $p<0.001$), with seamounts having lower $\delta^{15}N$ values than Rockall ($\Delta\delta^{15}N = 1.27‰$, $p<0.001$) and Portugal ($\Delta\delta^{15}N = 1.48‰$, $p<0.001$). For *C. coelolepis*, $\delta^{13}C$ values were similar between each sampling location ($F=0.541$, $p=0.58$) but seamount locations had lower $\delta^{15}N$ values compared to Rockall ($\Delta\delta^{15}N = -1.23$, $p<0.001$) and Portugal ($\Delta\delta^{15}N = -1.67$, $p<0.001$) (Figure 3.5).

Within each sampling location, the size distribution of sampled individuals was similar between locations (Figure 3.6). $\delta^{15}N$ values were different between species, apart from in the seamount locations, where $\delta^{15}N$ values for *D. calcea* and *Etmopterus* spp. were similar (F=92.97, p=0.16) (Figure 3.5). Likewise, $\delta^{13}C$ values were different between species at Rockall Trough but in Portugal, *C. coelolepis* and *D. calcea* values were similar (F=33.05, p=0.63). Additionally, the species-specific SEAc were more distinct in Rockall Trough and Portugal, than in the seamounts, where there was higher overlap among species (Figure 3.6).

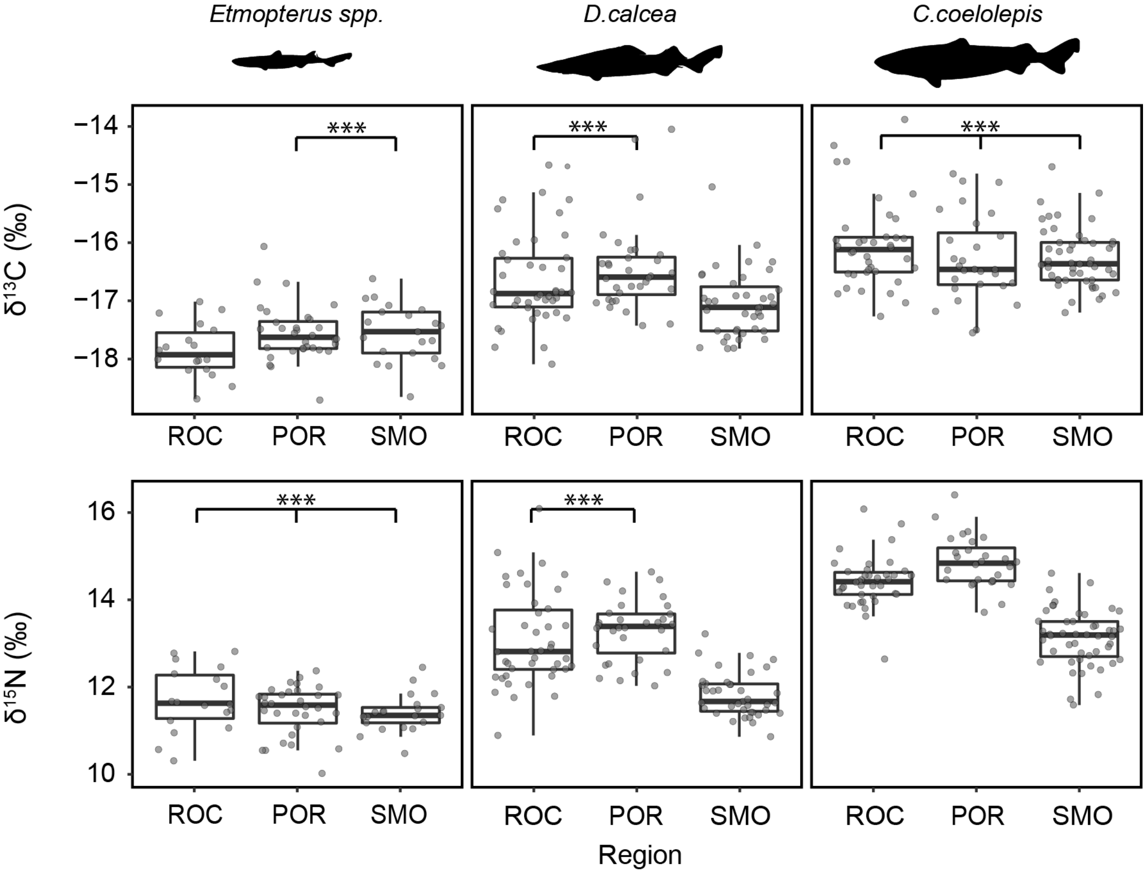


Figure 3.5: $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) values for *Etmopterus* spp. (left), *D. calcea* (middle), and *C. coelolepis* (right), with horizontal middle line being the mean, outer box representing 50% confidence levels and the vertical line being 95% confidence limits. Asterisks horizontal bars connect those sites that were statistically similar as determined from a one-way ANOVA post-hoc Tukey HSD test.

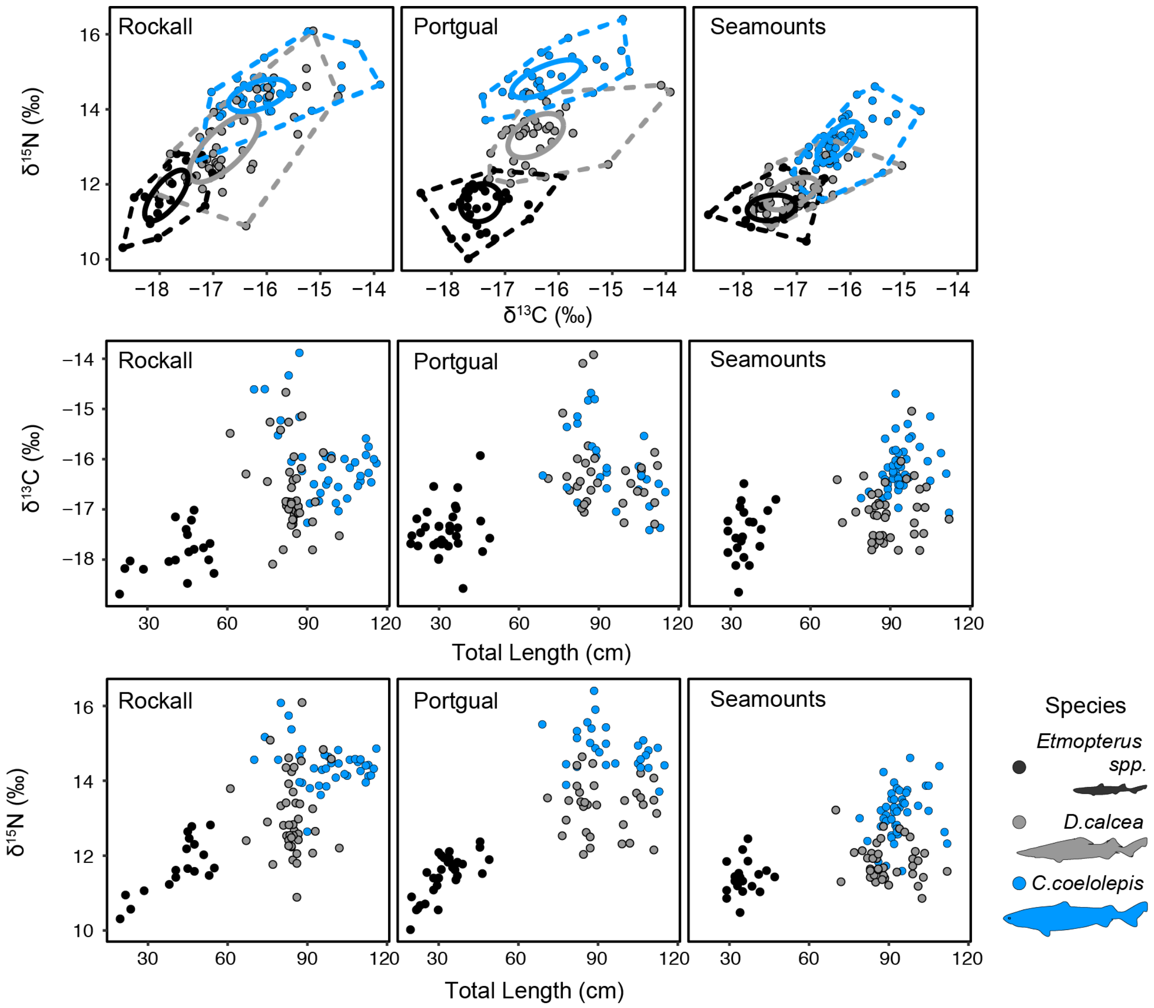


Figure 3.6: Bi-plot of $\delta^{13}C$ and $\delta^{15}N$ (top) with SEAc (solid line) and convex hulls (dashed lines), $\delta^{13}C$ and total length (middle), and $\delta^{15}N$ and total length (bottom) for *Etmopterus* spp. (black), *D. calcea* (grey) and *C. coelolepis* (blue) for Rockall Trough (left), Portugal (middle) and Seamounts (right).

3.5 Discussion

Using sharks as ecological samplers, we falsify the null hypothesis that sharks sampled at seamounts and on continental slopes have the same stable isotopic relationships. Shark populations from the continental slope sites have relatively high $\delta^{15}N$ values and greater stable isotopic niche areas than their counterparts on the seamounts. These trends were observed both within common sympatric species (Figure 3.5) as well as across whole shark assemblages (Figure 3.3). These results suggest that underlying nutrient dynamics supporting fish assemblages may be different between the seamounts and the continental slope sites. Demersal food webs at the sampled seamounts appear to be more reliant on pelagic resources, which are isotopically light and relatively homogenous. On the other hand, sharks from the two continental slope regions appear to have access to more varied, isotopically enriched resources such as those from benthic food webs. These results do not preclude the utilisation of benthic resources by sharks on seamounts, but instead imply that those resources found on seamounts have lower stable isotope values and are more closely coupled to pelagic production. We argue that these isotopic differences between slopes and seamounts are most likely a result of differences in nutrient availability and trophic structure between habitats.

Within the core sampling regions (Rockall Trough and Portuguese slopes, and seamounts), the latitudinal variability in $\delta^{13}C$ values observed in *E. spinax* are consistent with $\delta^{13}C$ baseline differences among our sampling regions (Somes *et al.*, 2010, Magozzi *et al.*, 2017) and suggests that small-bodied lanternsharks are a suitable reference species to compare trophic comparisons between regions. Accordingly, differences in the relative isotopic spacing between larger sharks and *Etmopterus* spp. among regions is most likely a product of differences in local nutrient dynamics between the sampled regions (Figure 3.6). As $\delta^{15}N$ values primarily vary with trophic level (i.e. distance to primary production), the lower $\delta^{15}N$ values measured in larger species *Deania calcea* and *Centroscymnus coelolepis* at seamount locations implies that demersal food webs at these seamounts are more closely coupled to pelagic production than those from the continental slope habitats. Furthermore, the lower isotopic niche areas observed for seamount sharks (Figure 3.3) is consistent with these inferences, suggesting that these seamounts may provide fewer isotopic/ecological niches than the adjacent continental slopes; with most production coming from pelagic pathways.

Nutrient availability at the sampled seamounts appears to be driven mostly by pelagic production. Indeed, seamounts are commonly observed to have higher epibenthic megafauna biomass (i.e. hard corals, sponges, asteroids) than adjacent continental slopes, with the majority of this biomass being dominated by filter feeding organisms (McClain *et al.*, 2010, Rowden *et al.*, 2010b). The seamounts sampled here are primarily of volcanic origin, characterised by hard substrate and steep reliefs. These substrates provide limited habitats for soft substrate benthic organisms, such as deposit feeders and infauna, but instead are usually dominated by epibenthic filter feeding organisms (McClain *et al.*, 2010). Filter feeding benthic epifauna express lower stable isotope values due to the assimilation of rapidly settling phytodetritus (Bergmann *et al.*, 2009, Christiansen *et al.*, 2009). This is in agreement with other trophic studies of seamount fish assemblages, which have commonly reported a dominance of pelagic nutrients to the diets of benthopelagic fishes (Koslow, 1997, Christiansen *et al.*, 2009, Colaço *et al.*, 2013, Preciado *et al.*, 2017).

The inference that there are less isotopic niches available on these sampled seamounts than the slopes is supported by the findings from other localised studies. Preciado *et al.* (2017) noted that benthic resources on seamounts were impoverished compared to other regions, which they suggest is linked with the low levels of organic matter in the sediments. In fact, it has been proposed that benthic food webs on seamounts may be relatively simple (i.e. reliant on direct pelagic production; Duineveld *et al.* (2004)). The decrease in isotopically varied nutrient sources and/or prey items on seamounts is further supported by trophic studies of benthopelagic fishes. Several seamount studies have reported high incidences of pelagic prey in demersal fish stomach contents (Fock *et al.*, 2002, Christiansen *et al.*, 2009), whereas fish on continental slopes have highly varied diets composed of benthic and pelagic prey items (Mauchline and Gordon, 1985, Macpherson and Roel, 1987, Mauchline and Gordon, 1991, Dunn *et al.*, 2010b). Using stable isotopes from a wide range of animals on the Condor seamount, Colaço *et al.* (2013) noted the importance of mesopelagic organisms in coupling pelagic and demersal habitats, a finding that has been echoed from the Galacia Bank seamount (Preciado *et al.*, 2017). Our results are consistent with these findings, and suggest that there is tight benthic-pelagic coupling of demersal fish food webs in seamount ecosystems. However, it has also been suggested that the increased complexity added by the structure of epibenthic fauna, promotes the retention of resuspended organic matter (Genin *et al.*, 1986) and of complex trophic architectures (Samadi *et al.*, 2008). Our data do not support high food web complexity at the sampled seamounts, but instead highlight a dominance of pelagic nutrient subsidies for these food webs.

In turn, the increased δ^{15}N values and larger isotopic niche areas for sharks sampled on continental slopes could be indicative of having access to both pelagic and benthic resources. Continental slopes typically support a higher proportion of deposit feeders compared to filter feeders (Iken *et al.*, 2001, Rowden *et al.*, 2010b). Benthic deposit feeders generally have elevated stable isotope values as they assimilate organic matter that has been subjected to increased recycling, reworking, and microbial degradation, (Iken *et al.*, 2001, Bergmann *et al.*, 2009). The diversity of substrates and reliefs (i.e. shallow banks, canyons, steep faces etc.) on continental slopes (Neat *et al.*, 2008) may provide increased ecological niches for lower trophic organisms (Tracey *et al.*, 2004, Rowden *et al.*, 2010b). However, Trueman *et al.* (2014) found that the role of benthic resources may be less important than pelagic resources in supporting local fish assemblages, especially for the dominant benthopelagic feeding teleosts. Likewise, Romero-Romero *et al.* (2016) found that phytodetrital organic matter was the most important source of nutrients supporting food webs in a submarine canyon. Considering all the above information, our results of increased δ^{15}N values and larger isotopic niche areas may be specific to the trophic ecology of deep-sea sharks on continental slopes, and highlight their ability to feed over multiple trophic pathways.

Indeed, stomach content studies of both *D. calcea* and *C. coelolepis* have reported both benthic and pelagic sources of nutrients (Macpherson, 1983, Mauchline and Gordon, 1983, Dunn *et al.*, 2010b, Pethybridge *et al.*, 2011, Dunn *et al.*, 2013). Continental slopes may also provide increased scavenging opportunities due to the larger spatial areas where carrion can settle (Priede *et al.*, 1994, Collins *et al.*, 2005). Increased access to scavenging opportunities, especially those provided by higher trophic cetaceans with high δ^{15}N values (Duineveld *et al.*, 2007), may also increase the range of isotopic niches with which deep-sea sharks are able to forage over.

While there is considerable evidence to suggest that there may be decreased diversities of nutrients sources on seamounts, there is also evidence to suggest that the diversity of fishes and other fauna do not vary between some spatially adjacent seamounts of continental slopes (Koslow, 1993, Howell *et al.*, 2010). The underlying trophic architecture of seamounts and continental slope habitats may therefore be specific to the sampled areas and further work will be needed to ascertain the local biophysical properties that underpin the trophic structure of deep-sea food webs.

One ecological aspect that may limit the use of deep-sea sharks as local ecological samplers is the potential for large-scale movements in some species. Moving and feeding across isotopically varied seascapes has the potential to expose higher order predators to a range of isotopically varied food webs (Graham *et al.*, 2010, McMahon *et al.*, 2013). The relatively lower δ^{15}N values for both *D. calcea* and *C. coelolepis* compared to *Etmopterus* spp. on seamounts could therefore be related to the migratory movements of both of these species from areas of inherently lower δ^{15}N values. Both species are believed to undertake large migratory movements across the northeast Atlantic, linked with ontogeny and the reproductive cycle (Moura *et al.*, 2014, Catarino *et al.*, 2015). Thus, the lower δ^{15}N values observed in *D. calcea* and *C. coelolepis* could be due to substantial feeding in areas of lower baseline δ^{15}N values prior to their capture on the seamounts. While possible, this seems unlikely considering the broad range of seamounts sampled across the region (Figure 3.1). On the contrary, it could be argued that the smaller isotopic niches areas observed at the seamounts could be linked with some degree of residency on seamount habitats, limiting the assimilation of nutrients from other sources (Rader *et al.*, 2017). Without finer scale reconstruction of the migratory movements of these species, it is not possible to disentangle any spatial effects within the observed patterns. Nevertheless, the increased benthic-pelagic coupling of these seamounts appears to be the most parsimonious explanation of the observed patterns.

The potentially lower diversity of foraging opportunities, in addition to the spatially constrained nature of the seamounts sampled here, may cause associated fish populations to be more vulnerable to overfishing and climate change than their continental slope counterparts. Seamount fishes have higher intrinsic biological vulnerabilities than other deep-sea fishes (i.e. slower growth, longer longevity, lower fecundity; (Koslow, 1997, Morato *et al.*, 2006a), which may be linked to the limited nutrient resources. The distributions of diel vertically migrating production are tightly coupled to overlaying oceanographic conditions (e.g. sea surface temperature, particulate organic matter flux, oxygen content; (Boehlert and Genin, 1987, Klevjer *et* *al.*, 2016) and may therefore be affected by climate-driven environmental changes (Sweetman *et al.*, 2017). Alterations to the spatial distributions of productivity or ocean stratification structure will also likely influence the transport of nutrients to seamount habitats more than continental slopes (Smith *et al.*, 2008, Sweetman *et al.*, 2017). Continental slope ecosystems may provide higher trophic consumers with alternative foraging opportunities during perturbations through benthic trophic pathways and connectivity to wider latitudinal areas. Seamount habitats may accordingly be able to sustain lower levels of fishing than continental slopes and management efforts should reflect these differential ecosystem dynamics in future assessments. The degree of connectivity between seamounts and continental slope habitats may affect local life history parameters of those species and may influence the degree to which seamounts can be recolonized after fisheries induced depletions. Stock assessments may benefit from setting specific quotas for seamounts, taking into consideration the standing stock biomass, connectivity to other regions, and the species composition of these seamounts.

In summary, using sharks as ecological samplers, we find evidence to suggest that the nutrient dynamics supporting deep-sea fish assemblages may be distinct between some the slope and seamount communities sampled here. The seamounts studied here, appear to provide fewer isotopically varied ecological niches than the continental slope sites, possibly due to their narrow and steep topographies constraining seamount food webs to pelagic nutrient pathways. The apparent increased benthic-pelagic coupling inferred from the seamount sites may not be a common trait observed between all seamounts and continental slopes, but rather specific to those sites sampled here. Further studies of more varied seamount communities will be needed to ascertain the ubiquity of these ecological processes. The biophysical interaction between pelagic production and topography appears to play an important, but currently overlooked, role in the nutrient dynamics of bathyal food webs, and should be further quantified to help assess sustainable areas for exploitation of deep-sea resources.

Chapter 4: Ecological characteristics of sharks and chimaeras in continental slope ecosystems

4.1 Abstract

About half of all global chondrichthyan diversity resides in deep-sea habitats (200 - 3500m), of which the dogfish (Squaliformes), catsharks (Carcharhiniformes) and chimaeras (Chimaeriformes) are the most abundant orders. In the northeast Atlantic, deep-water chondrichthyans have been depleted from over-fishing, and despite management efforts there have been limited signs of recovery. There is therefore an increasing need to better understand the ecological processes supporting deep-water environments and chondrichthyan assemblages. Here we use bulk stable isotope analysis from 1324 deep-sea fish (409 chondrichthyans and 915 teleosts) and a long-term (2000 – 2015) deep-water trawl fisheries survey to uncover the spatial and trophic dynamics of the deep-sea chondrichthyan community at Rockall Trough, UK. The trophic ecology of the chondrichthyan fishes matches their phylogenetic distinctness, with catsharks, dogfish, and chimaera having isotopic values different from each other, and intermediate to sympatric benthic and benthopelagic feeding teleosts. Sharks appear to have a complex ecology in Rockall Trough, displaying varying degrees of overlap with benthic and benthopelagic teleosts across their bathymetric range. Chimaeras on the other hand, are isotopically enriched compared to all other fishes, which is likely reflective of their unique diet on hard shelled benthic prey. We also found that chondrichthyan families were typically segregated by depth, with peak relative biomasses occurring at different depths. This can largely be attributed to differences in trophic ecology, and chondrichthyan families may vertically segregate to reduce interspecific competition for resources. Our results support the adoption of bathymetric limitations for trawl fisheries in order to reduce the by-catch of large ecologically distinct dogfish at intermediate depths (700 – 1200m).

4.2 Introduction

Chondrichthyan fishes (sharks, rays and chimaeras) are generally higher order predators that have the potential to impact a broad range of ecosystem functions and services however their ecological role, and the impact that fishing is having in many habitats, is still ambiguous (Stevens *et al.*, 2000, Heithaus *et al.*, 2008, Baum and Worm, 2009, Heupel *et al.*, 2014, Grubbs *et al.*, 2016, Roff *et al.*, 2016, Ruppert *et al.*, 2016). While neritic and oceanic habitats have been relatively well studied, 46% of global chondrichthyan diversity resides in deep-sea habitats (i.e. deeper than 200 m in depth) (Dulvy *et al.*, 2014, Cotton and Grubbs, 2015). As a group, 57.8% of deep-sea chondrichthyan species are currently listed as data deficient on the International Union for the

Conservation of Nature (IUCN) Red List (Dulvy *et al.*, 2014), meaning that basic information need on stock ecology is still lacking.

Deep-water chondrichthyans are characterised by life history attributes such as slow growth, low fecundity, long reproductive cycles, and high longevity, making them one of the most biologically vulnerable groups to fisheries overexploitation (Garcia *et al.*, 2008, Simpfendorfer and Kyne, 2009, Bergstad, 2013, Rigby and Simpfendorfer, 2013). As global commercial fisheries have increasingly deepened onto continental slopes (Norse *et al.*, 2012, Watson and Morato, 2013), the limited recovery potential of many chondrichthyan populations has caused substantial declines in many stocks (Koslow *et al.*, 2000, Graham *et al.*, 2001, Priede *et al.*, 2011, Neat *et al.*, 2015). With limited knowledge on the ecological role that sharks and their relatives play in these environments, it is still unclear what effect, if any, their removal will have on the ecology of continental slope ecosystems.

Continental slopes connect shallow shelf habitats to abyssal plains, spanning a broad bathymetric range (200 m – 2000 m) and accordingly experiencing a wide range of hydrographical, environmental and biological conditions (Gordon *et al.*, 1995) that influence species compositions, functional morphologies, and faunal abundances and distributions (Koslow, 1993, Cartes *et al.*, 2004, Sutton, 2013, Mindel *et al.*, 2016b). Pelagic subsidies provided via the diurnal vertical movement of large numbers of invertebrates and fishes (diel vertical migrators), have a strong influence on the observed faunal zonation patterns (Trueman *et al.*, 2014). In shallow slope habitats (<500m), the direct impingement of diel vertical migrators on the benthos supports high biomasses of benthic and pelagic fishes (Trueman *et al.*, 2014, Stasko *et al.*, 2016). With increases in depth, demersal habitats extend beyond the lower vertical range of the vertically migratory fauna, so that benthopelagic feeding fishes dominate in biomass at intermediate depths (700 m – 1500 m) due to their ability to vertically access productivity (Trueman *et al.*, 2014, Mindel *et al.*, 2016a). To what extent the chondrichthyan community is supported by, and therefore influences, different energetic pathways remains unclear however, but may determine their ecological role in comparison to the wider teleost community.

Current ecological knowledge on deep-water chondrichthyans has largely been derived from trawl survey data and stomach content assessments (Mauchline and Gordon, 1983, Ebert *et al.*, 1992, Bulman *et al.*, 2002, Dunn *et al.*, 2013). Such studies have provided great insight in these ecosystems but sampling techniques are prone to significant sampling biases and may not be truly representative of long-term ecological processes (Merrett *et al.*, 1991, Gordon and Bergstad, 1992, Cortés, 1997, Clarke *et al.*, 2005). An increasingly complementary technique to these studies is the application of stable isotope analysis (SIA), as it provides a time-integrated assessment of nutrient flow, food web structure, and spatial patterns of nutrient assimilation (Peterson and Fry, 1987, Hobson, 1999, Post, 2002).

Stable isotope analysis of bulk carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are typically used to trace the origin of nutrient pathways supporting consumers' diets (Fry, 2007), and as such may allow the estimation of the relative use of different energetic pathways within the chondrichthyan community. In demersal ecosystems, benthic food webs express stable isotope values that are more enriched in $\delta^{13}C$ (Δ~1-2‰) and $\delta^{15}N$ (Δ~2-4‰) than benthopelagic food webs, particularly at mid-slope depths (1000 m - 1500 m; (Drazen *et al.*, 2008, Bergmann *et al.*, 2009, Trueman *et al.*, 2014, Stasko *et al.*, 2016). This isotopic distinction is largely driven by the relative distance of each trophic pathway from baseline production, with benthic food webs utilising recycled/reworked particulate organic matter (POM) and benthopelagic food webs being more tightly coupled with surface production via direct access to the pelagic-coupled diel vertical migrators (Polunin *et al.*, 2001, Drazen *et al.*, 2008, Bergmann *et al.*, 2009, Trueman *et al.*, 2014). Accordingly, comparing stable isotope values of chondrichthyans with teleosts from each trophic pathway may allude to the energetic pathways supporting local populations and the ecological niches they occupy.

This study builds upon considerable work already conducted on the demersal fish assemblages of Rockall Trough, UK (Mauchline and Gordon, 1985, Trueman *et al.*, 2014, Neat *et al.*, 2015), thus providing a strong background on which to explore ecological interactions at the geochemical level (Gordon and Duncan, 1985, Mauchline and Gordon, 1985, Gordon *et al.*, 1996, Kelly *et al.*, 1998, Neat and Campbell, 2011). Rockall Trough is located 100 km off the west coast of Scotland and Ireland, and is of commercial importance to many fisheries (Kelly *et al.*, 1998). Our aim is to uncover the ecological properties of sharks and chimaeras in a temperate, continental slope ecosystem, in relation to teleost fishes. We combine the largest stable isotope dataset of any deep-water chondrichthyan assemblage and accompanying sympatric teleost dataset, with fisheries independent trawl survey data to identify key tropho-spatial dynamics along a bathymetric gradient. Furthermore, we examine resource-partitioning mechanisms among the chondrichthyan community to better understand how continental slope ecosystems are able to support such abundance and diversities of species with overlapping distributions.

4.3 Material and Methods

4.3.1 Trawl Survey

The structure of the fish community at Rockall Trough was determined using survey data from the F.R.V. *Scotia* (Marine Scotland Science; (Neat *et al.*, 2008, Neat *et al.*, 2010)) between the years 2000 - 2015 (Figure 4.1). Data were restricted to surveys conducted during the regular biennial Deep-water Time Series trawls in August-September. Trawl surveys were limited to daylight hours and, accordingly the ecological inferences of the ichthyofaunal community reflect daytime distributions. The fishing gear used was dual-warp bottom trawl with rock-hopper ground gear (Jackson BT-184 bottom trawls; (Neat *et al.*, 2010)). Surveys operate between depths of 300 m and 2100 m along the continental slopes of Rockall Trough and the adjacent seamounts, with concentrated effort at 500 m, 1000 m, 1500 m and 1800 m (Figure 4.1). In order to account for differences in fishing effort between depths and minimise the potential for skewed data from aggregating species, catch per unit effort (CPUE) measurements were calculated for each haul using total $\log_n$ biomass divided by the total time spent trawling at each depth across the survey period. Although survey methods can have a large influence on the catch composition of demersal fish assemblages (Gordon and Duncan, 1985, Merrett *et al.*, 1991, Clarke *et al.*, 2005), the survey methods used produce catch composition similar to that of the commercial fishing fleets (Clarke *et al.*, 2015), thus providing relevant information on ecosystem components exposed to fishing pressures.

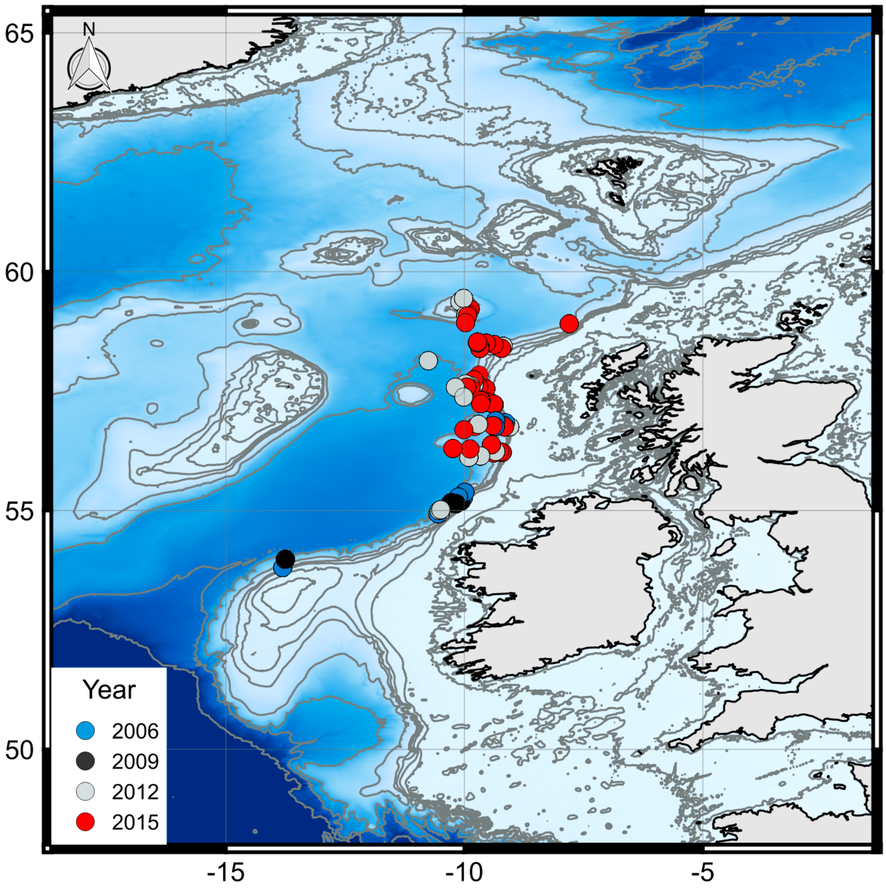


Figure 4.1: Map of trawl locations where stable isotope samples were collected during 2006, 2009, 2012 and 2015.

4.3.2 Functional Guilds

Teleosts were assigned *a priori* to either benthic or benthopelagic feeding strategies as determined from published stomach content and morphological observations (Froese and Pauly, 2015). Benthic feeding fishes were those species with a dominance of epibenthic or hyperbenthic prey items in their stomach, whereas benthopelagic fishes were those that were reported to feed upon pelagic and benthopelagic fauna (Mauchline and Gordon, 1985, Trueman *et al.*, 2014). No *a priori* trophic classifications were made for chondrichthyans due to the ambiguous data. Instead, chondrichthyans were separated into the three main taxonomic Orders: Squaliformes (dogfish), Carcharhiniformes (catsharks) and Chimaeriformes (chimaeras).

4.3.3 Tissue Sampling

Newly collected white muscle (1 cm^3) from 805 fish were collected in 2012 (n=563) and 2015 (n=242) on board the R.V. *Scotia,* and added to the 519 fish samples included in Trueman *et al.* (2014) (Table 4.1). All samples were collected from core sampling depths of 500 m, 1000 m, 1500 m, 1800 m and 2000 m, representing a total of 409 chondrichthyans (Table 4.2), 436 benthic teleosts, and 479 benthopelagic teleosts (Table 4.5). Sampling was designed to represent the range of the dominant species and sizes classes at each depth (Figure 4.5). For each fish, length and/or weight were measured. For species with missing size parameters, weight or length were calculated using length-weight relationships produced by Marine Science Scotland from deep-water trawl surveys (Neat *et al.*, 2010). Due to the range of different length measurements for different species (i.e. total length, precaudal length etc.), fish sizes are represented as $\log_n$ mass.

White muscle was removed from the dorsal region, individually wrapped in aluminium foil and immediately frozen at -20°C. Once in the laboratory, chondrichthyan muscle tissue was thoroughly washed in distilled water (repeated three times) to eliminate urea, refrozen at -20°C for 24 hours and freeze dried at -52°C for 48 hours. Dried muscle samples were homogenised before approximately 0.7 mg of powdered muscle was transferred into tin capsules for stable isotope analysis.

4.3.4 Stable Isotope Analysis

Tissues collected in 2012 were analysed at OEA, Cornwall, UK, on a Thermo EA 110 elemental analyser linked to a Europa Scientific 2020 isotope ratio mass spectrometer running in continuous flow mode. Samples collected in 2015 were analysed using a continuous flow Elementar vario PYRO cube elemental analyser coupled with a Thermo Scientific Delta V plus isotope ratio mass spectrometer (IRMS) at the NERC LSMSF, East Kilbride. All other samples were processed as described in Trueman et al. (2014). To ensure comparability between laboratories, samples from a single batch of USGS 40 glutamic acid were analysed throughout all analyses. No significant differences in $\delta^{13}C$ (ANOVA: $F_{2.50,}$ p=0.089) or $\delta^{15}N$ (ANOVA: $F_{1.50}$, p=0.23) were found between laboratories.

Stable isotope values are reported using the 'δ' notation and expressed in parts per thousand (‰) relative to a reference standard using the following equation: $\delta X = 1000[R_{sample} \text{ x } R_{standard}^{-1}]$, where X is the heavy isotope, either ^{13}C or ^{15}N, R_{sample} is the ratio of heavy to light isotope in the muscle sample, and $R_{standard}$ is the isotopic ratio for a given standard. ^{13}C standards are with reference to the PeeDee Belemnite and ^{15}N are with reference to atmospheric N_2. C:N ratios for chondrichthyans were 3.33 ± 0.23 and therefore samples were considered to represent true protein values (Post *et al.*, 2007) and did not warrant further lipid extraction. Furthermore, there was no relationship between $\delta^{13}C$ and C:N for any species, further supporting that lipid extractions were not necessary.

Although lipid content was likely low, some chondrichthyan (n=65) and teleost (n=163) samples had C:N ratios greater than 3.5, suggesting potential lipid contamination. We subsequently subjected all samples to mathematical lipid corrections as per Kiljunen *et al.* (2006) that was developed for a wide range of taxa that we felt was more appropriate for our dataset than the teleost specific lipid correction method developed by Hoffman and Sutton (2010). This non-linear mathematical correction has little effect on samples with a C:N between 3-4 but will correct samples that fall significantly out of this range.

4.3.5 Statistical Analysis

Best-fit polynomial regressions were fitted to total haul CPUE for each functional guild (i.e. benthic teleosts, benthopelagic teleosts, catsharks, dogfish and chimaeras) as a function of depth (Table 4.1). The average proportional biomasses within hauls were calculated for individual chondrichthyan families along the sampling depth range.

To ensure comparability between sampling years, generalised linear models (GLMs) were ran separately for $\delta^{13}C$ and $\delta^{15}N$ with interactions between sampling year and $\log_5$ mass. There was no observable effect of year of sampling on $\delta^{13}C$ (p=0.296) or $\delta^{15}N$ (p=0.565) values recorded in fish species sampled throughout the study, thus validating the comparability of our data.

In order to determine the likely nutrient pathways supporting the chondrichthyan groups, we first tested the isotopic discrimination of teleost groups, into benthic and benthopelagic guilds, using linear discriminant analyses (LDA) of $\delta^{13}C$, $\delta^{15}N$ and $\log_5$ mass at each depth interval. Where classification of nutrient pathways differed between isotopic (direct, individual-based data) and morphological or stomach content (indirect species-level inferences from literature) data, feeding guild was assigned based on isotopic classifications.

Linear models of $\delta^{13}C$ and $\delta^{15}N$ against size ($\log_5$ mass) were independently applied to each teleost feeding guild at each depth, and then applied to the chondrichthyan fishes (Figure 4.2). If chondrichthyan fish size-related stable isotope values were within 1 standard deviation (1 s.d.) of either of the corresponding $\delta^{13}C$ or $\delta^{15}N$ teleost feeding models, that individual was considered to have similar ecological characteristics as that teleost feeding guild at that depth (Figure 4.2). The proportion of distinct chondrichthyan fishes within both 1 s.d. and 2 s.d. of both benthic and benthopelagic feeding models was calculated, as a proxy for ecological uniqueness across their bathymetric range (Table 4.4).

Lastly, sample size corrected standard ellipse areas SEAc (ellipse containing 40% of the data) were calculated for $\delta^{13}C$ and $\delta^{15}N$ bi-plots for each functional group at each depth, as graphical representations of teleost stable isotope distributions.

All analyses were performed using R (R Development Core Team, 2016), and the freely available base statistical packages, plyr (Wickham, 2011), ggplot2 (Wickham, 2009) and SIBER (Jackson *et al.*, 2011).

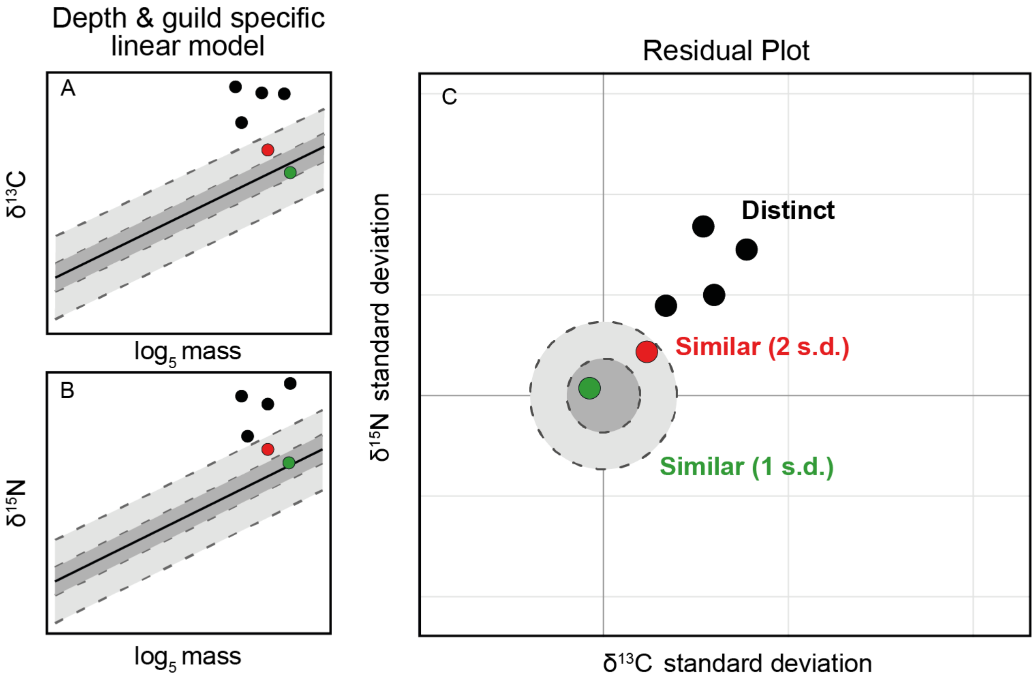


Figure 4.2: Schematic representation of dual $\delta^{13}C$ (A) and $\delta^{15}N$ (B) linear model residual plot (C) used to distinguish distinct size-related isotopic characteristics of chondrichthyan community. Individuals within 1 standard deviation (s.d.) (red) or 2 s.d. of either stable isotope model were classified as similar, whereas those individuals that expressed values greater than 1 s.d. or 2.s.d. were classified as distinct.

4.4 Results

4.4.1 Rockall Trough Demersal Fish Community and Biomass Trends

In total, teleost fishes made up 82.4% of the total trawled fish biomass at Rockall Trough with sharks and chimaeras making up 8.6% and 8.8% respectively. CPUE of the different fish groups (Figure 4.3) display strong variations with depth and were best described by cubic or quadratic polynomial regression (Figure 4.3, Table 4.1). In shallower depths (<500m), hauls were dominated by benthic feeding teleosts, with chimaeras being the dominant chondrichthyan (Figure 4.3). Between 700 and 1500 m, benthopelagic teleosts dominated in addition to increased dogfish (Figure 4.3). Beyond 1500 m, benthopelagic teleost biomass decreased and chimaeras and catsharks increased in relative and total biomass (Figure 4.3).

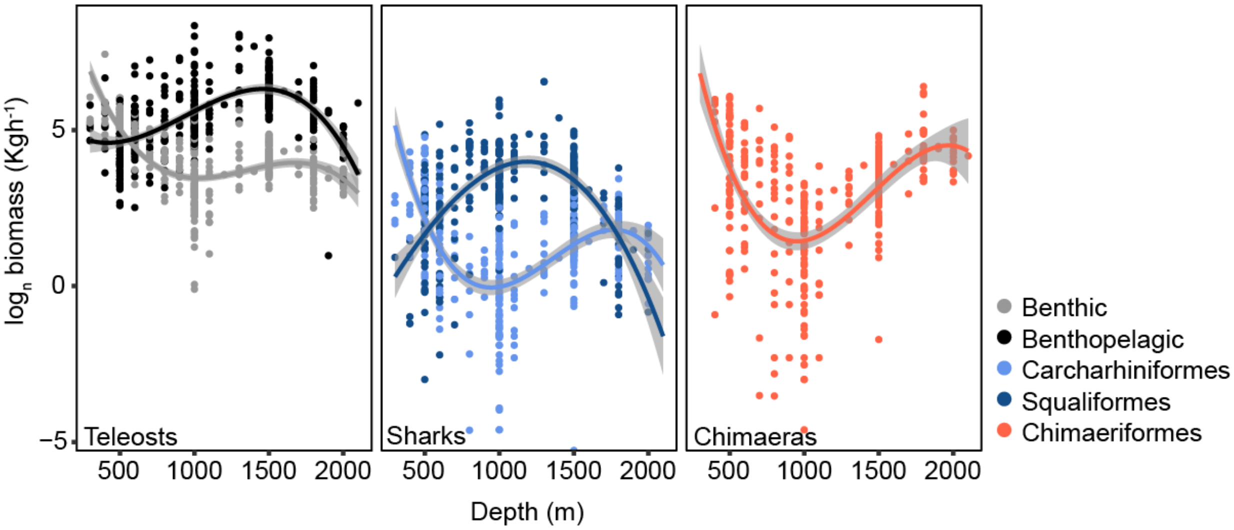


Figure 4.3: CPUE of total haul biomasses ($\log_5$ kg h^{-1}) of fish feeding groups across the bathymetric sampling range. Individual points represent the total $\log_n$ biomass per hour for a haul. Polynomial regressions are overlaid in corresponding colours and standard errors shown in light grey (coefficients in
Table 4.1).

Table 4.1: Linear regression coefficients for regressions in Figure 4.3, where; $\log_n$ biomass (y) = z + $ax + bx^2 + cx^3$, where x is depth (m). B – benthic teleosts, BP – benthopelagic teleosts.

Guild	z	*a*	*b*	*c*
B	5.80	-11.40	11.86	-9.82
BP	7.84	10.75	-12.10	-8.06
Catshark	1.74	-4.49	19.60	-15.78
Chimaera	4.05	8.05	24.77	-13.65
Dogfish	4.22	5.33	-24.72	-2.10

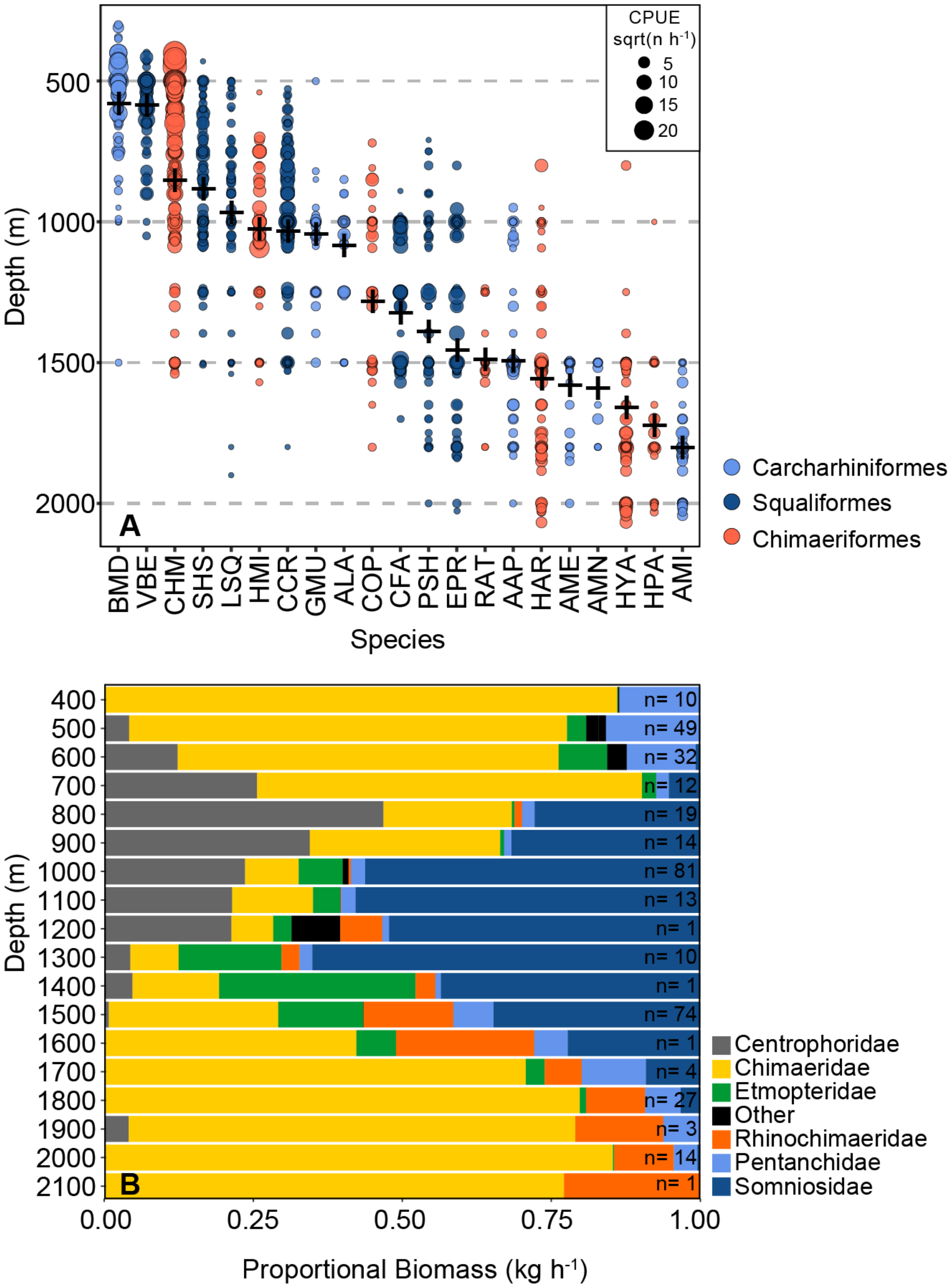


Figure 4.4: (A) Distributional ranges for chondrichthyans at Rockall Trough. Filled circles size is proportional to the square root abundance of that species within that haul with colours corresponding to Carcharhiniformes (light blue), Squaliformes (dark blue) and Chimaeriformes (red). Black crosses indicate the median capture depth for that species. Codes are given in Table 4.2. (B) Average proportional biomass for each chondrichthyan family at each depth. Each colour represents a different family as displayed in the legend. The number of hauls at that depth over the survey period is given along the right side.

The dominant chondrichthyan orders, in terms of biomass, at Rockall Trough were Carcharhiniformes (catsharks; 8 species), Squaliformes (dogfish; 9 species), and Chimaeriformes (chimaeras; 7 species). There was evidence of strong vertical structure between taxonomic orders with peak relative abundances occurring at different depths (Figure 4.4). Peak relative abundances were 400 m and 2000 m for Pentanchidae, 700 m for Centrophoridae, 1300 m for Somniosidae and 1400 m for Etmopteridae (Figure 4.4).

4.4.2 Stable Isotope Analysis

Chondrichthyan stable isotope values displayed large amounts of overlap between species at all depths (Figure 4.5, Table 4.2). Catsharks had the lowest stable isotope values (δ^{13}C: -17.46 ± 0.55‰, δ^{15}N: 11.90 ± 0.87‰) of the sampled chondrichthyans and generally displayed similar stable isotope values among species (Table 4.2). For dogfish, stable isotope values were more variable, displaying stable isotope values intermediate (δ^{13}C: -16.97 ± 0.83‰, δ^{15}N: 12.96 ± 1.23‰) to the chimaeras and catsharks (Table 4.2). The highest stable isotope values for a dogfish was the single sample from *Oxynotus paradoxus* (SBS). Chimaeras were enriched in δ^{13}C (mean: -16.24 ± 0.45‰) and δ^{15}N (mean: 14.03 ± 1.37‰) and values were consistently higher than those of benthic feeding fishes (Figure 4.5). Benthic feeding fishes typically had higher δ^{13}C and δ^{15}N values than benthopelagic feeding fishes (Figure 4.5). Stable isotope ratios became more enriched for benthic feeding fishes and less enriched for benthopelagic feeding fishes with increasing depth, until 1500m, before returning to values similar to shallower depths at 2000m (Figure 4.5). *A priori* defined benthic and benthopelagic feeding teleosts displayed isotopic separation across all sampling depths, with δ^{13}C, δ^{15}N and $\log_n$ mass correctly discriminating 89.8% of benthic individuals and 91.2% of benthopelagic individuals in an LDA analysis (Table 4.3).

Table 4.2: Stable isotope summary data for the chondrichthyan community. N is the number of samples analysed for stable isotopes. TL represents total length (cm) for the catsharks and the dogfish, and represents pre-caudal length in the chimaera (given in centimetres). Values within parenthesis are standard deviations. Codes represent species names used in Figure 4.4 & 4.5.

Order	Family	Species	Code	*n*	Total Length (cm)	Depth (m)	$\delta^{13}C$	$\delta^{15}N$	C:N
Carcharhiniformes	Pentanchidae	*Apristurus aphyodes*	*AAP*	25	37-56	1000-1800	-17.10 (0.55)	11.8 (0.78)	3.4 (0.15)
Carcharhiniformes	Pentanchidae	*Apristurus laurussonii*	*ALA*	28	20-77	700-1500	-17.61 (0.54)	12.29 (1.03)	3.56 (0.27)
Carcharhiniformes	Pentanchidae	*Apristurus manis*	*AMN*	3	65-92	1500-1800	-18.57 (0.10)	11.47 (0.42)	3.24 (0.04)
Carcharhiniformes	Pentanchidae	*Apristurus melanoasper*	*AME*	11	39-74	1500-1800	-17.79 (0.48)	11.55 (0.77)	3.59 (0.18)
Carcharhiniformes	Pentanchidae	*Apristurus microps*	*AMI*	31	32-70	1500-2000	-17.34 (0.43)	11.96 (0.88)	3.47 (0.22)
Carcharhiniformes	Pentanchidae	*Apristurus sp*	*APU*	7	19-37	1000-2000	-17.75 (0.14)	11.06 (0.24)	3.47 (0.05)
Carcharhiniformes	Pentanchidae	*Galeus melastomus*	*BMD*	9	57-73	500-700	-17.69 (0.27)	12.08 (0.4)	3.27 (0.1)
Carcharhiniformes	Pentanchidae	*Galeus murinus*	*GMU*	6	22-44	1000-1200	-17.00 (0.45)	11.72 (0.73)	3.54 (0.17)
Squaliformes	Centrophoridae	*Centrophorus squamosus*	*LSQ*	14	86-122	700-1200	-15.91 (0.89)	14.09 (0.58)	3.41 (0.16)
Squaliformes	Centrophoridae	*Deania calceus*	*SHS*	41	64-102	700-1500	-16.84 (0.65)	13.05 (1.02)	3.28 (0.22)
Squaliformes	Centrophoridae	*Deania profundorum*	*DPR*	1	69	500	-17.45	11.68	3.16
Squaliformes	Etmopteridae	*Centroscyllium fabricii*	*CFA*	26	20-90	1000-1500	-17.86 (0.46)	12.21 (1.15)	3.52 (0.23)
Squaliformes	Etmopteridae	*Etmopterus princeps*	*EPR*	16	41-82	1200-1800	-17.42 (0.37)	12.48 (0.6)	3.43 (0.13)
Squaliformes	Etmopteridae	*Etmopterus spinax*	*VBE*	18	20-55	500-700	-17.85 (0.46)	11.7 (0.73)	3.33 (0.19)
Squaliformes	Oxynotidae	*Oxynotus paradoxicus*	*SBS*	1	76	500	-15.26	15.08	3.61
Squaliformes	Somniosidae	*Centroscymnus coelolepis*	*PSH*	38	74-116	1000-1800	-16.28 (0.58)	14.41 (0.58)	3.57 (0.22)
Squaliformes	Somniosidae	*Centroscymnus crepidater*	*CCR*	42	28-89	700-1500	-17.00 (0.43)	12.32 (0.72)	3.41 (0.18)
Chimaeriformes	Chimaeridae	*Chimaera monstrosa*	*CHM*	12	30-73	500-700	-16.75 (0.38)	13.36 (0.66)	3.23 (0.14)
Chimaeriformes	Chimaeridae	*Chimaera opalescense*	*COP*	11	54-84	1000-1500	-16.69 (0.3)	14.91 (0.91)	3.34 (0.2)
Chimaeriformes	Chimaeridae	*Hydrolagus affinis*	*HYA*	14	57-119	1500-2000	-16.37 (0.78)	15.71 (1.19)	3.72 (0.27)
Chimaeriformes	Chimaeridae	*Hydrolagus mirabilis*	*HMI*	24	27-41	700-1000	-16.84 (0.29)	12.8 (0.83)	3.34 (0.08)
Chimaeriformes	Chimaeridae	*Hydrolagus pallidus*	*HPA*	9	41-98	1500-2000	-16.67 (0.22)	14.65 (1.27)	3.39 (0.14)
Chimaeriformes	hinochimaerida	*Hariotta raleighana*	*HAR*	11	28-70	1500-2000	-16.36 (0.39)	13.47 (0.97)	3.63 (0.19)
Chimaeriformes	hinochimaerida	*Rhinochimaera atlantica*	*RAT*	11	52-81	1500	-15.99 (0.12)	14.42 (0.52)	3.21 (0.07)

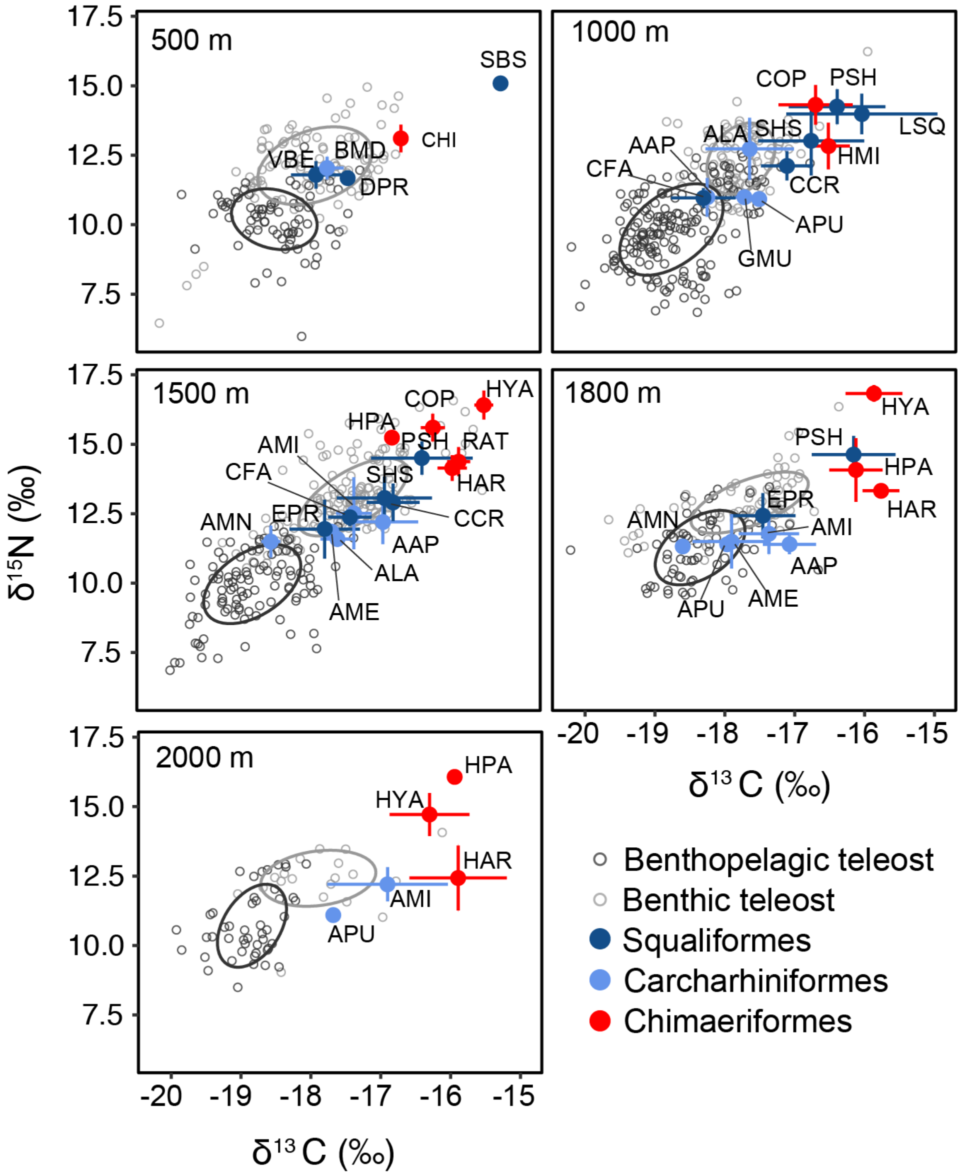


Figure 4.5: Stable isotope data of individual teleosts (open circles) and mean values for chondrichthyans (filled circles) at different depths (500 - 2000 m). Lines represent one standard deviation of the mean. Text codes represent species names as referred to in Table 4.2 and full teleost species lists can be found in Table 4.5.

4.4.3 Residual Analysis

Chondrichthyan fishes typically displayed size-related stable isotope values that were distinct from either benthic or benthopelagic feeding fish guilds across their bathymetric range (Figure 4.6, 4.7, Table 4.4), particular with regard to benthopelagic fishes. For a given size, dogfish and catsharks stable isotope values were intermediate to that of teleost fishes, particularly with regard to δ^{15}N (Figure 4.6). While size-specific isotope values of catsharks became more distinct with depth, they were generally more similar to benthic teleosts in shallower depths and benthopelagic teleosts in deeper depths (Figure 4.7, Table 4.4). Dogfish stable isotope values were more similar to benthic feeding fishes across their depth range, with the exception being at 1000m (Table 4.4). At 1000m, 93.4% of all dogfish had size-related stable isotope values that were distinct from either benthic and benthopelagic linear models (60.7% at 2 s.d.). In shallower depths, chimaeras were dissimilar to benthic and benthopelagic fish stable isotope linear models, with only 7.1% and 8.3% of individuals being similar to benthic feeding fishes at 1800 m and 2000 m within 1 s.d., respectively (Figure 4.7, Table 4.4).

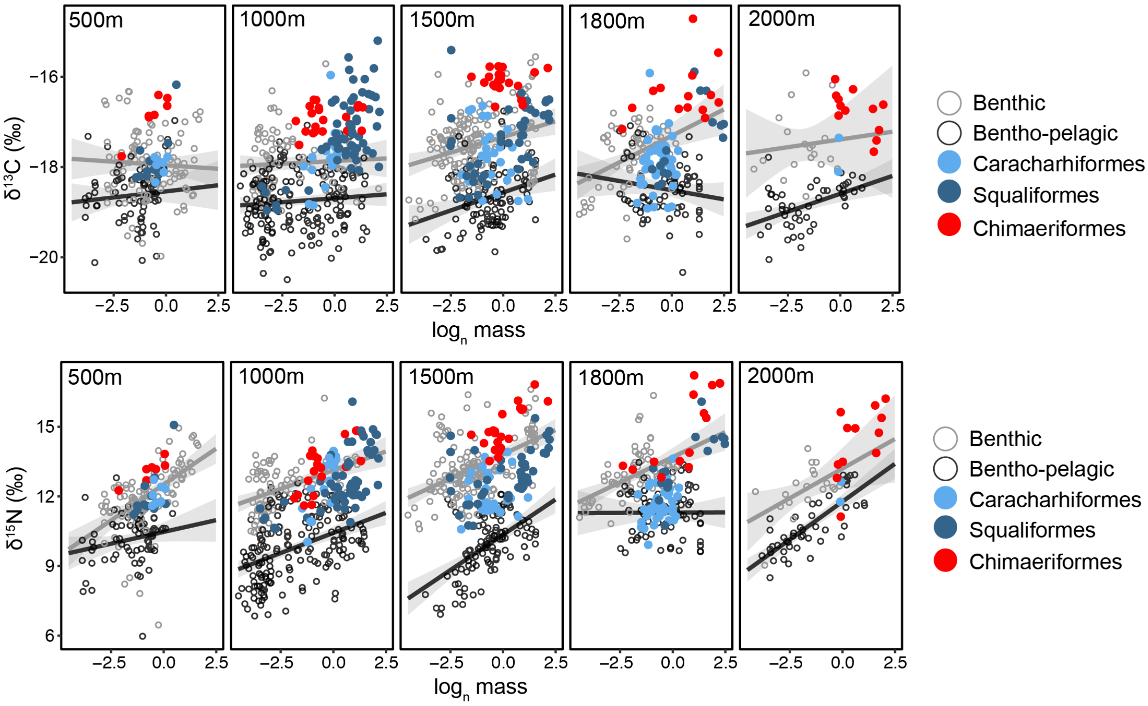


Figure 4.6: Isotope-weight relationships for the fish community along a depth gradient. Open circles are individual values from either benthic (light grey) or benthopelagic (dark grey) feeding teleosts, with linear regressions in corresponding colours. Filled circles are individual values for Carcharhiniformes (light blue), Squaliformes (dark blue) and Chimaeriformes (red).

Table 4.3: Proportion of *a priori* assigned benthic and benthopelagic teleosts correctly clustered using parameters $\delta^{13}C$, $\delta^{15}N$ and $\log_5$ mass in a Linear Discriminant Analysis (LDA)

Depth	Benthic	Benthopelagic
500	0.87	0.84
1000	0.87	0.88
1500	0.98	0.96
1800	0.93	0.9
2000	0.84	0.98
Total	0.9	0.91

Table 4.4: Proportional similarity of chondrichthyan species to benthic (%B) and benthopelagic (%BP) linear models for both $\delta^{13}C$ and $\delta^{15}N$ with $\log_5$ mass, and dissimilarity to both feeding guilds (%U) within one standard deviation (^{1}) and two standard deviations (^{2}) – Figure 4.7.

Guild	**Depth**	***n***	**B^1 (%)**	**BP^1 (%)**	**U^1 (%)**	**B^2 (%)**	**BP^2 (%)**	**U^2 (%)**
Catsharks	500	8	1.00	0.00	0.00	1.00	0.75	0.00
	1000	23	0.17	0.09	0.74	0.78	0.57	0.09
	1500	26	0.19	0.04	0.77	0.73	0.19	0.15
	1800	41	0.05	0.15	0.80	0.34	0.54	0.20
	2000	3	0.00	0.00	1.00	0.67	0.00	0.33
Total		101	0.19	0.09	0.72	0.60	0.46	0.15
Dogfish	500	12	0.83	0.08	0.17	0.92	0.67	0.08
	1000	61	0.03	0.03	0.93	0.36	0.26	0.61
	1500	43	0.42	0.02	0.56	0.93	0.12	0.02
	1800	15	0.40	0.07	0.53	0.73	0.33	0.20
Total		131	0.27	0.04	0.69	0.64	0.26	0.32
Chimaera	500	7	0.00	0.00	1.00	0.86	0.00	0.14
	1000	20	0.00	0.00	1.00	0.10	0.00	0.90
	1500	23	0.00	0.00	1.00	0.57	0.00	0.43
	1800	14	0.07	0.00	0.93	0.36	0.00	0.64
	2000	12	0.08	0.00	0.92	0.25	0.00	0.75
Total		76	0.03	0.00	0.97	0.38	0.00	0.62

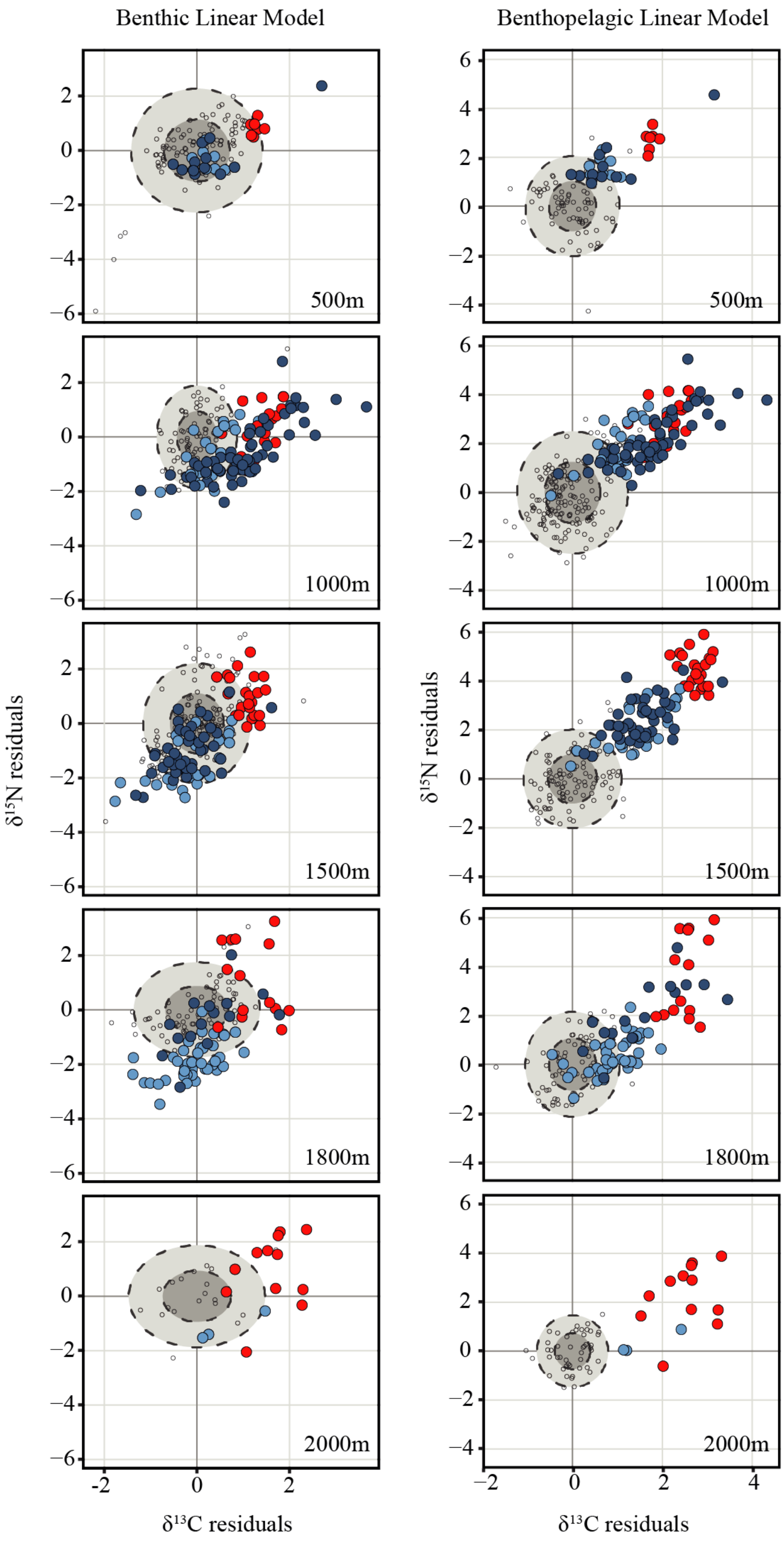


Figure 4.7: Chondrichthyan residual plot for benthic (left) and benthopelagic (right) teleost size-related stable isotope linear models. Filled circles are residual values for $\delta^{13}C$ and $\delta^{15}N$ vs size ($\log_5$ mass) for individual Carcharhiniformes (light blue), Squaliformes (dark blue) and Chimaeriformes (red). Open light grey circles are residual values for individual teleost fishes for corresponding teleost fishes. Filled dark grey and light grey circles with dashed lines represent one and two standard deviations on each axis of the linear models at that depth, respectively.

4.5 Discussion

The trophic ecology of the sharks and chimaeras at Rockall Trough can largely be distinguished by their taxonomic phylogeny, with chimaeras (Chimaeriformes), catsharks (Carcharhiniformes), and dogfish (Squaliformes) expressing distinct stable isotope values between each other, and from the sympatric teleost community. For a given size, the chondrichthyan fishes appear to be feeding on different proportional components of the local food web. The ecological properties for the catsharks and dogfish appear complex however. While from a stable isotope perspective, both catsharks and dogfish were intermediate to the teleost feeding guilds, the bathymetric biomass trends of dogfish were more similar to benthopelagic-feeding teleosts and the catsharks displayed similar trends to the benthic-feeding teleosts. The chimaeras on the other hand were consistently distinct from any other fish across the bathymetric range, suggesting that they may have a distinct ‘hyper-benthic’ diet. Our inferences from these data is that while chimaeras potentially occupy a unique ecological role at Rockall Trough, the sharks have a more complex trophic ecology, potentially feeding across both benthic and benthopelagic nutrient pathways.

4.5.1 Benthopelagic Predators

The strong opposing biomass patterns observed between benthic teleosts, catsharks and chimaeras, and those of the benthopelagic teleosts and dogfish (Figure 4.3), may give insight into fundamental features of the nutrient availability within continental slope environments. The highest biomasses of dogfish coincide with the peak biomasses of the maximum daytime depth of the mesopelagic diel vertical migrators (~700 m – 1500 m) and, consequently, with the highest biomass of benthopelagic teleosts (Gordon and Duncan, 1985, Mauchline and Gordon, 1991, Merrett *et al.*, 1991). Both horizontal and vertical impingement of the diel vertical migrators at these depths, concentrate mesopelagic fauna near the seabed, providing substantial nutrient subsidies to demersal fishes (Mauchline and Gordon, 1991, Trueman *et al.*, 2014). Although dogfish and benthopelagic teleosts display similar bathymetric distributions in terms of biomass, dogfish size-related stable isotope values are distinct from benthopelagic fishes, displaying intermediate values and in some cases, stronger overlap with benthic-feeding teleosts (Table 4.4). These conflicting results could be established by dogfish feeding on high-trophic benthopelagic fauna and/or obtaining substantial contributions from benthic prey.

Stomach content studies suggest dogfish have a varied trophic ecology, reporting diverse diets of benthic, benthopelagic and mesopelagic teleosts, crustaceans and cephalopods, in addition to scavenging of cetacean carrion in the case of larger dogfish (Macpherson, 1983, Mauchline and Gordon, 1983, Ebert *et al.*, 1992, Pethybridge *et al.*, 2011, Iitembu and Richoux, 2015). Being highly mobile predators with dentition capable of feeding on a broad size-spectrum of fauna (Underwood *et al.*, 2016), dogfish distributional patterns coincide with the highest faunal biomasses associated with diel vertically migrating production and other benthopelagic teleosts. Tracking studies from *C. squamosus*, recorded distinct diurnal vertical migrations between 600 m and 1200 m that was linked with foraging on diel vertically migrating fauna (Rodríguez-Cabello *et al.*, 2016) and is supported by the current study. Although it is likely that large dogfish are associated with the high biomasses of benthopelagic fishes, they are capable of exploiting addition nutrient pathways. For example, dietary studies from *C. coelolepis*, highlight a prevalence of scavenged cetacean carrion (Mauchline and Gordon, 1983, Carrasson *et al.*, 1992, Pethybridge *et al.*, 2011) and other elasmobranch species, including cannibalism (Cartes *et al.*, 2016). The consistently high stable isotope values of *C. coelolepis* across the bathymetric range support these findings, and suggest that they may be some of the highest trophic predators in these ecosystems. The most parsimonious explanation for the intermediate, sometimes more 'benthic', stable isotope values observed in large dogfish is that feeding behaviours are likely opportunistic and across multiple trophic pathways.

Dogfish stable isotope values were most distinct at 1000 m, especially for $\delta^{13}C$ (Figure 4.6, Table 4.4). At 1000m, large dogfish species (*C. squamosus*, *D. calcea* and *C. coelolepis*), dominate in terms of biomass (Figure 4.4). While it could be concluded that these dogfish have a distinct trophic ecology from the other fishes at this depth, an alternative explanation could be that migratory movements have resulted in the mixing of individuals from more isotopically enriched areas, such as southerly latitudes (McMahon *et al.*, 2013, Magozzi *et al.*, 2017). Indeed, *C. squamosus*, *D. calcea* and *C. coelolepis* are all hypothesised to undertake large oceanic scale migrations linked with reproductive development (Veríssimo *et al.*, 2011, Verissimo *et al.*, 2012, Moura *et al.*, 2014, Catarino *et al.*, 2015). Stable isotope values may therefore reveal some degree of cryptic movement behaviours in these species (McMahon *et al.*, 2013, Moura *et al.*, 2014), but more research will be needed across the northeast Atlantic to disentangle trophic and spatial components of the observed patterns.

Of notable interest in these results is the high stable isotope value recorded in *Oxynotus paradoxus* (Figure 4.5). This elusive species is rarely observed in trawl surveys and this was the first record in this survey. Although very little is known about rough sharks (*Oxynotus* spp*,*) studies from New Zealand suggest that they may have a specialist diet on chimera egg cases (Finucci *et al.*, 2016). Likewise, observations from a captive specimen in an aquarium, reported a diet exclusively of elasmobranch egg cases (Guallart *et al.*, 2015). Considering the high stable isotope values observed in the chimaeras at Rockall Trough, this result adds to the growing evidence that rough sharks may have a specialist diet on chondrichthyan egg cases.

4.5.2 Micronekton Specialists

At depths on either side of peak benthopelagic biomasses (i.e. <700 m and >1200 m), smaller dogfish species increase in biomass (i.e. mainly lanternsharks; Etmopteridae), followed by increases in catsharks (*Apristurus* spp.) moving progressively deeper (Figure 4.4). Similar to large dogfish, the high proportion of distinct/intermediate size-related stable isotope values, especially below 1000m, could indicate a distinct stable isotopic ecology in relation to other teleost fishes (Figure 4.6, Table 4.4). It is inferred that these species are likely benthopelagic predators, feeding on proportionally higher trophic level, pelagically-coupled prey than the teleost community. This is in agreement with stomach content studies and fatty acid profiling that regularly reports lanternshark and catshark diets being derived of micronektonic invertebrates, and mesopelagic teleosts and cephalopods (Mauchline and Gordon, 1983, Carrasson *et al.*, 1992, Ebert *et al.*, 1992, Ebert *et al.*, 1996, Hallett and Daley, 2011, Pethybridge *et al.*, 2011). Furthermore, the cranial morphology and associated distribution of ampullae of Lorenzini in lanternshark and catshark species, support the predation of fast moving micronekton prey such as decapod and mysid crustaceans, and cephalopods (Atkinson and Bottaro, 2006, Moore and McCarthy, 2014).

At intermediate depths (700-1200 m), rapid consumption of nutrients by the high biomasses of meso-benthopelagic fishes may reduce the rate of carbon sequestering into deeper environments (Buesseler *et al.*, 2007). This may potentially limit the capacity for high biomass benthic food webs or micronekton communities in the benthic boundary layer (BBL), at depths where benthopelagic fauna dominate (Bergmann *et al.*, 2009, Trueman *et al.*, 2014). At shallower depths, both benthic and benthopelagic feeding fishes have direct access to the diel vertically migrating fauna (Trueman *et al.*, 2014). At depths beyond the dominance of benthopelagic consumers, phytodetrital deposits predominantly reach the seafloor and support micronekton biomass in the BBL (including Mysidacea and Decapoda; (Hargreaves, 1984, Hargreaves, 1985, Mauchline and Gordon, 1991)). At Rockall Trough, day time peak abundances of near-bottom micronekton occurred at ~500 - 600 m and ~1300 – 1500 m (Hargreaves, 1984, Hargreaves, 1985), which coincide with peak biomasses of small dogfish and catshark species (Figure 4.3, 4.4, 4.5), which we suggest reflects their preference for these prey.

Although stable isotope values of micronekton are not available at this area, values from other locations report low stable isotope values that reflect a diet of planktonic fauna and detritus (Hargreaves, 1985, Romero - Romero *et al.*, 2016, Preciado *et al.*, 2017), and would be aligned with the stable isotope data here. It is likely that catsharks and smaller dogfish are in competition for pelagically-coupled micronektonic resources, but are both dissimilar from other sympatric teleosts. It could be proposed that the presence of large higher trophic dogfish in middle slope depths could have competitively excluded smaller dogfish and catsharks to less prey rich bathymetric depths, where species diversified trophic behaviours to exploit smaller micronektonic prey.

4.5.3 Chimaeras

The strong similarities between the biomass trends of the benthic-feeding teleosts and chimaeras suggest that their distributional patterns are limited to areas that support sufficient benthic biomass (Figure 4.3). Generally, benthic megafaunal biomass decreases with depth, although it appears relatively high within our sampling range (i.e <2000 m) (Lampitt *et al.*, 1986, Paterson and Lambshead, 1995, Howell *et al.*, 2002). While clearly benthic consumers, the consistently distinct stable isotope values across their bathymetric distribution (Table 4.4, Figure 4.7) suggest that chimaeras have a different trophic ecology from other benthic teleosts. Stomach content studies demonstrate a varied diet of epibenthic and infaunal invertebrates (Mauchline and Gordon, 1983, Macpherson and Roel, 1987, Dunn *et al.*, 2010b, Pethybridge *et al.*, 2011). Possessing unique crushing dentition, chimaeras are possibly able to exploit components of the benthic food webs that are not available to other teleosts (i.e. crushing shells of bivalves and gastropods), and these data provide evidence to suggest that chimaera occupy a keystone role within this ecosystem.

Although we lack stable isotope values of potential benthic prey items from this region, studies from other deep-sea food webs suggest that suspension feeders and subsurface deposit feeders have some of the highest stable isotope values (Iken *et al.*, 2001), which may be reflected in the high stable isotope values of chimaeras. The high relative biomasses of chimaera species in both shallow (<500 m) and deeper (>1500 m) slope habitats is likely due to their ability to occupy distinct ecological niches in benthic food webs (Gordon *et al.*, 1996, Neat *et al.*, 2008), with little competition from other fishes. It could also be suggested that the presence of larger dogfish at intermediate depths could also have competitively excluded chimaera from these depths. Indeed, chimaera remains have been reported from the stomachs of *C. coelolepis* (Mauchline and Gordon, 1983) and the presence of a large dorsal fin spines suggests they may be subjected to pressures from large predators. Although chimaera are of low economic interest with regards to fisheries management, these data suggest that they may be playing an important, yet underappreciated, role in continental slope ecosystems. By feeding on prey inaccessible to other fishes, it could be speculated that chimaera connect otherwise disjunct trophic pathways, facilitating the transfer and recycling of nutrients to other components of the food web. More work will need to be conducted to support these inferences however.

4.5.4 Bathymetric Constraints

The absence of chondrichthyans from the abyssal plains has been widely debated with suggestion that the high energetic demands of maintaining lipid-rich livers require them to remain close to abundant prey in shallower environments (Priede *et al.*, 2006, Laxson *et al.*, 2011, Musick and Cotton, 2015). Our data strongly support these ideas, suggesting the bathymetric ranges of chondrichthyans closely match the distributional ranges of their predominantly pelagic-derived prey preferences. At about 2000 m, Rockall Trough flattens out, and in addition to hydrographical changes that may affect nutrient availability (New and Smythe-Wright, 2001), the increased distance from slope-affiliated production provides less access to benthopelagic subsidies (Trueman *et al.*, 2014). Large chondrichthyan species commonly found at depths greater than 1800 m, (e.g. *C. coelolepis*, *E. princeps, H. affinis*), have been reported to be scavengers and have well developed olfactory senses in order to facilitate foraging of carrion (Clarke and Merrett, 1972, Priede *et al.*, 2010, Neat and Campbell, 2013, Yopak *et al.*, 2014). Scavenging allows these species to access large pelagic carrion beyond the bathymetric ranges of diel vertically migrating prey, but will still be limited to depths proximal to slope associated productivity. Additionally, the dominance of large *Hydrolagus* species at depth suggests that benthic prey quality may not be high enough to support substantial shark biomasses, but may be sufficient for chimaeras.

Being bathymetrically constrained to depths where slope associated production is sufficient (i.e. <2500 m), chondrichthyans are particularly vulnerable to fishing pressures as they have limited capacity to seek refuge in deeper waters beyond fishing activities (Musick and Cotton, 2015). Furthermore, differences in species bathymetric distributions will make certain species more vulnerable to fishing than others (Clarke *et al.*, 2015, Neat *et al.*, 2015). For some species of dogfish and chimaera, bathymetric segregations have been observed relating to sex and reproductive stage (Jakobsdóttir, 2001, Holt *et al.*, 2013) that presumably reduce intraspecific competition and provide more favourable conditions for progeny where predation risks may be lower (Sims, 2005, Heupel *et al.*, 2007). Future studies should focus on the vertical structure of chondrichthyan stocks in more detail to assess the impact fishing may have on vertically segregated species, and how their removal may impact wider slope ecology.

4.6 Conclusion

By combining large trawl survey data with stable isotope information it has been possible to allude to some ecological properties of the sharks and chimaeras in the Rockall Trough. When considering each dataset independently, the ecological characteristics of this assemblage are ambiguous, highlighting the necessity for combining methodological approaches to ecological studies. Sharks appear to occupy a range of stable isotopic niches at Rockall Trough, and while as a group may not provide a unique functional role in relation to the whole teleost assemblage, at the species level, it could be argued that sharks may be deriving nutrients from a wider range of sources than any single species of teleost. This may suggest that the functional role of one shark species may be similar to that of multiple teleost fishes. For chimaeras, there is strong evidence to suggest that they may have a unique ecological role in this assemblage through accessing benthic trophic pathways not utilised by many other fish. The apparent broad ecological complexities of the deep-sea chondrichthyans have the capacity to link otherwise separate habitats and may stabilise broader ecosystem functions (McCann *et al.*, 2005, Blanchard *et al.*, 2011, Plank and Law, 2012, Rooney and McCann, 2012). The removal of coupling agents, such as sharks, through fishing may therefore act to destabilise ecosystem states and reduce their resilience to future perturbations from fisheries pressures and climate change (Tecchio *et al.*, 2013, Britten *et al.*, 2014).

These results support the adoption of bathymetric limits for trawl fisheries operations proposed by Clarke *et al.* (2015), to control the bycatch of sharks and their relatives. We strengthen their propositions by highlighting the important ecological role that sharks play on broader ecosystem functions, especially in intermediate depths (700 m– 1500 m). These ecological characteristics are likely consistent across slope communities that display similar chondrichthyan assemblages (Compagno *et al.*, 1991, Dunn *et al.*, 2013, Barría *et al.*, 2015). European deep-sea longline fisheries typically operate in intermediate depth ranges, where large dogfish species occur, and may have a disproportionally high impact on the wider chondrichthyan community (Pajuelo *et al.*, 2010, Ramos *et al.*, 2013). Removal of these large, ecologically distinct species, which have complex spatial distributions (Moura *et al.*, 2014), has the capacity to impact ecosystems at scales that transcend local fishing activities.

In light of the ecological complexities of this group, fisheries managers should explore by-catch mitigation strategies (i.e. bathymetric limits, fishing gear modification) that will reduce the mortality of potentially ecologically important chondrichthyan fishes. One such modification could be the adoption of monofilament tracers in the deep-sea black scabbard longline fisheries. The cusp-like dentition of the Squaliformes lower jaw could sever monofilament, reducing their capture on hooks while retaining the target black scabbard fish. Although these methods have been trialled in the Portuguese black scabbard fishery with promising results (Ramos *et al.*, 2013), there is little economic incentive to adopt these strategies, and deep-sea sharks remain a substantial by-catch in these fisheries. It will also be useful to resolve the complex migratory movement patterns of deep-sea sharks and chimaeras (possibly using stable isotope approaches) to further strengthen management strategies.

Lastly, these data provide a baseline assessment of the trophic structure of the deep-sea fish assemblage at Rockall Trough. Through regular collection of stable isotopic data, it would be possible to monitor any potential recoveries within the fishery, following the implementation of bathymetric trawl limitations (Clarke *et al.*, 2015). Time series analysis of stable isotopes could also provide insight into changes in trophic levels of these species, food web length, as well as the diversity of prey sources available to different components of the food web (Jennings *et al.*, 2002). Utilisation of stable isotope analysis in fisheries monitoring programs could therefore be a useful tool to inform fisheries managers on the state of Rockall Trough deep-sea fish stocks.

4.7 Supplementary Information

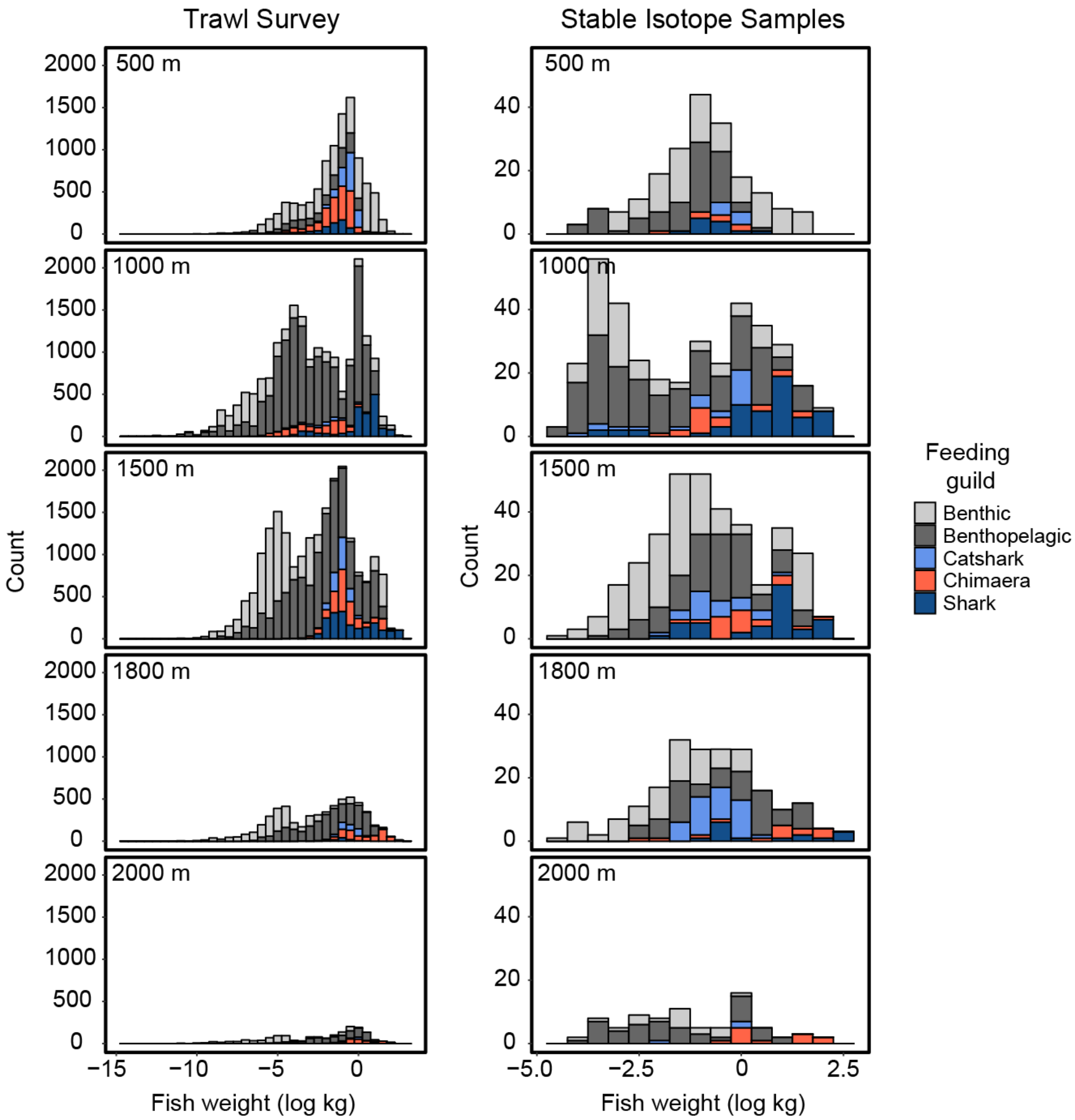


Figure 4.8: Size distribution of each feeding guild at each of the main sampling depths for the Marine Science Scotland trawl surveys from 2000 - 2015 (left) and our stable isotope sampling of the fish community (right). Note different scale to x-axis.

Table 4.5: Stable isotope values with standard deviations (in brackets) and feeding guild assignment for deep-sea fish sampled at Rockall Trough. N is the number of samples.

Feeding Guild	Order	Species	N	$\delta^{13}C$ (s.d)	$\delta^{15}N$ (s.d)	C:N (s.d)	Length (cm)
Benthic	Aulopiformes	*Bathypterois dubius*	7	-17.82 (0.18)	14.71 (0.47)	3.11 (0.06)	22.64 (2.98)
	Beryciformes	*Hoplostethus atlanticus*	62	-17.41 (0.51)	12.55 (1.49)	3.72 (0.42)	28.17 (19.91)
	Gadiformes	*Brosme brosme*	3	-17.21 (0.51)	14.71 (0.21)	3.2 (0.01)	66.15 (8.04)
		Caelorinchus caelorinchus	15	-17.48 (0.46)	11.64 (0.52)	3.32 (0.19)	35.17 (6.9)
		Caelorinchus labiatus	7	-17.63 (0.22)	12.45 (0.29)	3.08 (0.01)	19.46 (1.58)
		Chalinura mediterranea	85	-17.99 (0.73)	12.23 (0.86)	3.16 (0.16)	50.49 (22.62)
		Coryphaenoides guentheri	36	-17.44 (0.73)	13.29 (1.15)	3.16 (0.05)	43.37 (11.26)
		Merluccius merluccius	15	-18.05 (0.6)	13.26 (0.52)	3.24 (0.12)	65.75 (10.62)
		Molva dypterygia	6	-18.23 (0.44)	12.94 (1)	3.13 (0.05)	91.25 (20.76)
		Molva molva	7	-17.99 (1.12)	12.55 (2.73)	3.12 (0.03)	78.38 (15)
		Mora moro	24	-18.13 (0.63)	12.61 (1.16)	3.12 (0.03)	47.06 (10.07)
		Nezumia aequalis	35	-17.65 (0.44)	12.59 (0.67)	3.15 (0.12)	20.97 (3.35)
		Phycis blennoides	27	-18.47 (0.62)	11.46 (1.43)	3.09 (0.05)	37.65 (11.61)
		Trachyrhynchus murrayi	44	-17.47 (0.32)	12.95 (0.45)	3.16 (0.02)	45.92 (9.42)
	Notacanthiformes	*Halosauropsis macrochir*	16	-17.18 (0.15)	13.57 (0.64)	3.38 (0.3)	61.06 (5.37)
		Polyacanthonotus rissoanus	9	-17.11 (0.85)	14.6 (1.25)	3.83 (0.63)	48.27 (11.66)
	Ophidiiformes	*Cataetyx laticeps*	9	-16.18 (0.67)	15.22 (1.21)	3.23 (0.17)	78.62 (3.85)
		Spectrunculus grandis	10	-17.49 (0.25)	12.78 (0.4)	3.14 (0.04)	43.85 (4.25)
	Pleuronectiformes	*Glyptocephalus cynoglossus*	9	-17.55 (0.23)	11.6 (1)	3.21 (0.04)	38.09 (6.17)
	Scorpaeniformes	*Helicolenus dactylopterus*	10	-17.31 (0.17)	11.91 (0.91)	3.28 (0.13)	24.3 (3.88)
Benthopelagic	Anguilliformes	*Serrivomer beani*	10	-18.84 (0.33)	10.06 (0.58)	3.22 (0.03)	45.68 (8.37)
		Synaphobranchus kaupi	17	-17.68 (0.48)	11.53 (1.04)	6.64 (1.79)	56.99 (7.52)
	Gadiformes	*Antimora rostrata*	20	-18.46 (0.34)	12.56 (0.59)	3.12 (0.06)	50.69 (10.97)
		Coryphaenoides rupestris	105	-18.77 (0.52)	10.32 (1.06)	3.17 (0.14)	60.56 (17.42)
		Halargyreus johnsonii	10	-19.04 (0.21)	9.91 (0.42)	3.11 (0.02)	26.43 (2.78)
		Lepidion eques	36	-18.02 (0.57)	11.11 (1.05)	3.2 (0.06)	24.17 (6.19)
		Micromesistius poutassou	25	-18.61 (0.44)	10.25 (0.96)	3.15 (0.05)	28.3 (6.83)
	Osmeriformes	*Alepocephalus agassizi*	34	-18.84 (0.56)	11.03 (0.92)	3.19 (0.26)	45.66 (17.74)
		Alepocephalus bairdii	127	-18.89 (0.51)	9.48 (1.13)	3.37 (0.45)	48.81 (23.25)
		Argentina silus	35	-18.37 (0.58)	9.8 (1.12)	3.39 (0.26)	37.99 (3.35)
		Bathylagus euryops	10	-18.82 (0.32)	9.95 (0.76)	3.28 (0.17)	20.97 (3.43)
		Xenodermichthys copei	33	-18.79 (0.59)	8.86 (1.19)	4 (0.42)	19.42 (2.79)
	Perciformes	*Aphanopus carbo*	13	-17.85 (0.26)	11.98 (0.6)	3.54 (0.56)	88.68 (14.31)
		Epigonus telescopus	4	-18.16 (0.25)	9.51 (1.77)	3.31 (0.13)	28.53 (19.4)

Chapter 5: Isotopic-life histories of deep-water sharks recovered from eye lens proteins: a first look

Some of the laboratory work conducted in this chapter was undertaken with the support of a Masters student, Sarah Alewijnse, as part of her thesis.

5.1 Abstract

Our current knowledge of the ecology and biology of deep-water sharks is generally limited and one major gap is a lack of information on life history dynamics. In several threatened deep-water shark species such as the Portuguese dogfish (*Centroscymnus coelolepis*) and leafscale gulper shark (*Centrophorus squamosus*), neonatal and juvenile individuals are seldom seen in the same area as adults, leading to speculation on the existence of large-scale migrations and utilisation of specific pupping areas. Here we apply stable isotope analysis to incrementally sampled eye lenses to provide a 'first-step' approach in recovering the life-long stable isotope profiles for these two species. Isotopic life histories differ between species, but three distinct isotopic life history stages were detected, which were infer pertain to: maternal ecology during oocyte development, early juvenile life stages, and adult ecology. While stable isotope patterns alone are insufficient to provide detailed insight into ontogenetic shifts in ecology, we argue that the observed patterns are most consistent with speculated migratory movement patterns of these species. These results are consistent with pupping and first-feeding consistently occurring in lower latitudes, potentially in nursery regions, and that neonatal *C. squamosus* feed at lower trophic levels than neonatal *C. coelolepis*. If the observed patterns are linked with latitudinal movements, these results suggest that adult *C. coelolepis* may migrate back to similar isotopic environments as their mothers and use consistent areas for oocyte development between generations. These data support the use of eye lens tissues as a promising tool for uncovering life-long ecological trends of individual sharks but more work is need to understand eye lens development and the associated assimilation of stable isotope information within eye lens proteins.

5.2 Introduction

Understanding the trophic, spatial and behavioural ecology of an animal is essential to identify critical life stages and corresponding space use throughout an individual's life cycle. Such knowledge can be used to better inform management and conservation strategies (Ellis *et al.*, 2008, Hays *et al.*, 2016), especially for those animals that are vulnerable to overexploitation, such as those found in the deep-sea (Simpfendorfer and Kyne, 2009, Dulvy *et al.*, 2014). Deep-sea sharks, those found deeper than 200 m, are one of the most biological vulnerable groups of fish (Kyne and

Simpfendorfer, 2010, Bergstad, 2013, Rigby and Simpfendorfer, 2013) due to conservative life history traits, yet our inability to observe their behavioural ecology directly has limited our understanding of this group. Traditional deep-sea survey methods have provided invaluable insight into species distribution patterns and biological parameters (Clarke, 2000, Bulman *et al.*, 2002), but inferring population-scale behavioural ecology across ocean basins from catch survey data is challenging (Cotton and Grubbs, 2015). Furthermore, individual-based movement patterns are almost completely unknown for many deep-water shark species, with only a few studies being successful in recovering movement data (Rodríguez-Cabello and Sánchez, 2014, Daley *et al.*, 2015, Shipley *et al.*, 2017b). It is therefore necessary to develop or apply new methodologies to deep-water shark populations to address the large gaps in our understanding.

5.2.1 Stable Isotope Ecology

Stable isotope analysis (SIA) is now commonly used to examine trophic and spatial ecology from individual to ecosystem scales, and has been increasingly applied to deep-sea ecosystems where direct observations of animal behaviour are challenging (Trueman *et al.*, 2014, Romero-Romero *et al.*, 2016, Preciado *et al.*, 2017, Shipley *et al.*, 2017a). The relative proportion of stable isotopes of carbon (expressed as $\delta^{13}C$ values) vary considerably between different primary production sources and trophic pathways (i.e. phytoplankton, seagrasses or macrophytes) and also between different locations depending on the isotopic composition of inorganic carbon and the physiology of primary producers (DeNiro and Epstein, 1978, Trueman *et al.*, 2012). Stable nitrogen isotope ratios (expressed as $\delta^{15}N$ values) are modified during nutrient assimilation and excretion (termed trophic enrichment) and are typically used to estimate trophic levels of consumers (Post, 2002). The isotopic composition of nitrogen in marine primary production also varies spatially depending on biogeochemical properties of the water mass and thus can also be used to estimate vertical and horizontal location in the ocean (Lin *et al.*, 2012, McMahon *et al.*, 2013, Trueman *et al.*, 2017).

In fish ecology, SIA is typically performed on muscle tissues and can provide insight into tropho-spatial ecology over the timescale of muscle growth or turnover. Application of SIA to metabolically inert, incrementally grown tissues, such as vertebrae, has also proved useful in uncovering whole life-history ecological profiles for some shark species (Estrada *et al.*, 2006, Carlisle *et al.*, 2015). Unfortunately, many deep-sea sharks lack significantly large vertebrae to provide detailed isotopic life histories. One relatively novel approach to examining whole-life history ecology is the use of eye lenses (Wallace *et al.*, 2014). Eye lenses are metabolically inert, incrementally grown, proteinaceous tissues that grow isometrically throughout ontogeny (Grainger, 1992). Fish eye lenses are typically spherical, and the core of the eye lens represents the oldest tissue, with concentric lens layers deposited as the fish grows (Bloemendal *et al.*, 2004). Layering of eye lens proteins does not appear to be regular, thus prohibiting age estimation, but lens diameter can be used to estimate total body length since lenses grow isometrically (Zigman and Yulo, 1979, Siezen, 1989, Litherland *et al.*, 2009). Eye lens proteins can therefore provide an ontogenetic isotopic life history profile recording aspects of the tropho-spatial ecology of an individual. Due to isometric growth between lens diameter and body length, and the slowing of growth rates after maturity (Clarke *et al.*, 2002b), eye lens records in deep-sea sharks will mainly reflect juvenile portions of life history, which we know very little about, with relatively low temporal resolution available for adult time periods.

5.2.2 Vulnerable Species

Within the Northeast Atlantic, the Portuguese dogfish (*Centroscymnus coelolepis*) and the leafscale gulper shark (*Centrophorus squamosus*), are currently listed on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, largely due to high capture rates in both target and non-target fisheries over the past few decades (Piñeiro *et al.*, 2001, Clarke *et al.*, 2003b, Ramos *et al.*, 2013). Despite European wide implementation of total allowable catch quotas (TACs) since 2005, neither species has shown significant signs of recovery (Neat *et al.*, 2015). Both species are believed to undertake large-scale migrations linked with reproductive development (Figueiredo *et al.*, 2008, Veríssimo *et al.*, 2011, Verissimo *et al.*, 2012), however neonatal and juvenile specimens are seldom reported from existing deep water fisheries (Moura *et al.*, 2014). It has been hypothesised that both species may utilise nursery areas rarely sampled by fishing surveys (Veríssimo *et al.*, 2011, Moura *et al.*, 2014) but empirical evidence of common nursery areas is missing.

Here we sample the eye lens proteins of the Portuguese dogfish (*C. coelolepis*) and the leafscale gulper (*C. squamosus*) sharks, from the Northeast Atlantic to provide a preliminary attempt at uncovering both feeding and migratory aspects of this species throughout ontogeny. We attempt to address several questions: Do *C. coelolepis* and *C. squamosus* share common stable isotope histories and are there sex-based differences within each species? Is there evidence to support the use of common pupping areas (nursery habitats)? Can we detect migrations linked with ontogenetic development? Resolving currently missing ontogenetic tropho-spatial information will be pertinent for improving our understanding of the stock dynamics of populations threatened by fisheries overexploitation (Ellis *et al.*, 2008, Neat *et al.*, 2015).

5.3 Materials and Methods

5.3.1 Sample Collection

The left eye lens and ~1 cm^3 of white muscle were removed from 13 *C. coelolepis* and 6 *C. squamosus* collected opportunistically during Marine Science Scotland deep-water trawl surveys at Rockall Trough, in 2015 and 2016 (Table 5.1). An additional 5 *C. coelolepis* eye lenses were collected from fisheries discards in Sesimbra, Portugal, in 2015. Eye lenses and muscle tissue were wrapped in aluminium foil and immediately frozen at -20°C before being transported frozen to laboratories at the University of Southampton. Additionally, eye lenses were also collected to develop isometric diameter-length regressions but were not subjected to isotopic analyses.

A Masters student that was under the supervision of the author conducted laboratory tissue preparations. The outer lens capsule was removed before sequential layers of tissue were peeled from the lens using a scalpel, while frozen, according to Parry (2003). Lens diameter was measured after each peel using electronic callipers with a precision of 0.001 mm. Tissue samples were placed into 10 ml plastic vials, frozen at -20°C for 24 hours and then freeze-dried at -53°C for 24 hours. White muscle samples were thoroughly washed with distilled water 3 times before being frozen at -20°C for 24 hours and then freeze-dried at -53°C for 48 hours. All samples were then individually homogenised before ~0.7 mg of tissue was weighed into tin capsules for stable isotope analysis.

5.3.2 Critical Size Estimations

Linear regression models were used to estimate the relationship between shark total length and eye lens diameter. Measurements of analysed (n=26) and non-analysed eye lenses measurements (n=19) were combined with measurements from individuals in museum collections (n=14; Table 5.3). Eye lens diameter at birth was then calculated from published body lengths at birth (Table 5.2) from the corresponding species-specific linear regressions (Figure 5.1). These were combined with measured lens diameters from neonatal museum specimens to calculate a range of possible eye lens diameters equivalent to size at birth. Similarly, sex-specific size at maturity (L_{50}) estimates were acquired for each species from published literature and converted to eye lens diameter using the isometric linear regressions (Figure 5.1). Estimates of size at birth and size at 50% maturity (L_{50}) were then used to classify the eye lens samples into pre-birth, immature and mature sections, using the median of the size range estimates as a threshold.

Table 5.1: Biological information of individuals sampled, where T.L is the total length in centimetres and *n* is the number of layers recovered and analysed for bulk δ^{13}C and δ^{15}N. Mature stages (M) are defined as those individuals with gametes presents and immature (IM) are the converse.

Species	Area	*n*	I.D	T.L (cm)
C. coelolepis	Portugal	11	PP1	105.0
C. coelolepis	Portugal	10	PP2	112.5
C. coelolepis	Portugal	12	PP3	106.0
C. coelolepis	Portugal	6	PP4	86.0
C. coelolepis	Portugal	10	PP5	88.5
C. coelolepis	Rockall	7	PR1	116.0
C. coelolepis	Rockall	10	PR2	108.0
C. coelolepis	Rockall	13	PR3	114.0
C. coelolepis	Rockall	9	PR4	110.0
C. coelolepis	Rockall	8	PR5	104.0
C. coelolepis	Rockall	11	PR6	88.0
C. coelolepis	Rockall	9	PR7	95.0
C. coelolepis	Rockall	9	PR8	97.0
C. coelolepis	Rockall	13	PR9	115.0
C. coelolepis	Rockall	11	PR10	112.0
C. coelolepis	Rockall	8	PR11	105.0
C. coelolepis	Rockall	10	PR12	92.5
C. coelolepis	Rockall	12	PR13	95.5
C. squamosus	Rockall	13	LR1	121.0
C. squamosus	Rockall	13	LR2	107.0
C. squamosus	Rockall	11	LR3	101.0
C. squamosus	Rockall	12	LR4	96.0
C. squamosus	Rockall	13	LR5	110.0
C. squamosus	Rockall	12	LR6	104.5
C. squamosus	Rockall	8	LR7	96.0
C. squamosus	Rockall	16	LR8	101.0

5.3.3 Stable Isotope Analysis

Stable isotope values are reported relative to universal standards of Vienna PeeDee Belemnite for δ^{13}C and atmospheric N_2 for δ^{15}N. All eye lens tissue was analysed using a vario ISOTOPE select elemental analyser coupled with Isoprime100 isotope ratio mass spectrometer at the University of Southampton. Muscle samples were analysed separately using a continuous flow Elementar vario PYRO cube elemental analyser coupled with a Thermo Scientific Delta V plus isotope ratio mass spectrometer (IRMS) at the NERC LSMSF, East Kilbride facility. USGS 40 glutamic acid standards were analysed at both facilities to ensure comparability between laboratories (t-test for differences between laboratories: δ^{13}C (t=-1.47, p=0.154) and δ^{15}N (t=-0.77, p=0.448)). All C:N ratios were <3.5‰ indicating low lipid contents as expected for crystalline lens proteins and no chemical extraction or mathematical corrections were applied. All muscle samples were subjected to mathematical carbon lipid corrections as defined by Kiljunen *et al.* (2006).

5.3.4 Statistical Analysis

Generalised additive mixed models (GAMM) were developed to compare whole eye lens profiles for $\delta^{13}C$ and $\delta^{15}N$ for both species using the *mgcv* package (Wood and Wood, 2016). GAMMs included eye lens diameter as an unconstrained smoothing function with species, area and an interaction between species and sex as fixed effect factors. Individual ID was included as a random factor to account for between individual variations in profiles. ANOVA models were used to distinguish differences between maternal and first-feeding stable isotope values. Life history regions were defined using estimated sizes at birth from isometric regressions. Lastly, 40% standard ellipse areas were created for shark muscle values from different locations using newly collected samples and data from Newman *et al.* (2011), Trueman *et al.* (2014) and Iitembu and Richoux (2015) using the freely available SIBER package (Jackson *et al.*, 2011). All analyses were conducted using the statistical programming package R (R Development Core Team, 2016).

5.4 Results

5.4.1 Embryonic Lens Development

Eye lens diameter at birth was estimated to be between 5.00 and 6.27 mm for *C. coelolepis* based on museum specimens and back calculations from published total length measurements of 280 - 330mm (Table 5.2). Using the same methods, *C. squamosus* eye lens diameter at birth was estimated to be 8.8 - 10 mm (Table 5.2). Mean stable isotope values for lens core material was -15.15 ± 0.45‰ for $\delta^{13}C$ and 12.05 ± 0.56‰ for $\delta^{15}N$ in *C. coelolepis*, and -15.24 ± 0.45‰ for $\delta^{13}C$ and 12.36 ± 0.62‰ for $\delta^{15}N$ in *C. squamosus*. Although quite variable between individuals, $\delta^{13}C$ values were generally relatively stable before the predicted size at birth (Figure 5.2), with *C. coelolepis* showing more individual variability and a lower range of mean $\delta^{13}C$ values than *C. squamosus*. On the contrary, $\delta^{15}N$ values were similar between species, generally increasing until size at birth for both species (Figure 5.2).

5.4.2 Whole Lens Profiles

Using GAMMs the two species expressed significantly different $\delta^{13}C$ (t=-3.21, p=0.001) and $\delta^{15}N$ profiles (t=-5.20, p<0.001) across the diameter of the eye lens. In *C. coelolepis*, male $\delta^{13}C$ profiles were generally lower than females (t=-2.18, p=0.030) but similar for $\delta^{15}N$ (t=0.361, p=0.718). In *C. squamosus*, male $\delta^{13}C$ values were higher than females (t=3.01, p=0.003) but indistinguishable with regard to $\delta^{15}N$ (t=0.727, p=0.468).

For *C. coelolepis*, there were generally two major isotopic trend shifts observed; one corresponding to approximately the predicted size at birth, and another before the onset of sexual maturity at eye lens diameter ~9 mm (estimated as ~60 cm total length) (Figure 5.3). Following birth, $\delta^{13}C$ values typically increased to a maximum of -13.84 ± 0.46‰ at 9.27 ± 0.90 mm eye lens diameter followed by a general decrease to -14.72 ± 0.74‰ in the outer cortex layers. $\delta^{15}N$ values also displayed a general isotopic enrichment after birth, with a maximum value of 15.11 ± 0.64‰ at 8.83 ± 1.60 mm of eye lens diameter, followed by a decrease to 14.13 ±0.77‰ in the outer layers (Figure 5.3). Male $\delta^{13}C$ values were generally lower than female $\delta^{13}C$ values (Figure 5.4). $\delta^{13}C$ trends are most likely related to migrations, while $\delta^{15}N$ trends represent trophic relationships, as well as spatial movements.

Table 5.2: Published length measurements and museum measurements (Table 5.3) for size at birth (SAB) and size at maturity for both species. Estimated lens diameters in millimetres (ELD) are calculated using the linear regression coefficients from independent regression models for *C. coelolepis* (a) and *C. squamosus* (b) (Figure 5.1).

Species	Reference	Size at Birth (mm)	ELD (mm)	Size at Maturity (mm)			
				Female	ELD (mm)	Male	ELD (mm)
C. coelolepis (a)	Girard and Du Buit, 1999	300	6.18	1020	12.60	860	11.17
	Veríssimo et al., 2003	268	5.90	-	-	-	-
	Figueiredo et al., 2008	310	6.27	1004	12.46	851	11.09
	Bañón et al., 2006	280	6.00	-	-	-	-
	Catarino et al., 2015	-	-	1090	13.22	830	10.90
	Museum	263	5.00	-	-	-	-
C. squamosus (b)	Girard and Du Buit, 1999	-		1240	16.35	980	14.26
	Figueiredo et al., 2008	450	10.00	1238	16.33	991	14.35
	Bañón et al., 2006	400	9.60	1250	16.43	1010	14.50
	White et al., 2010	400	9.60	-	-	-	-
	Museum	294	8.80	-	-	-	-

a. C. coelolepis diameter=0.00891* SAB + 3.22. R^2=0.86

b. C. squamosus diameter=0.00803 * SAB + 6.37. R^2=0.81

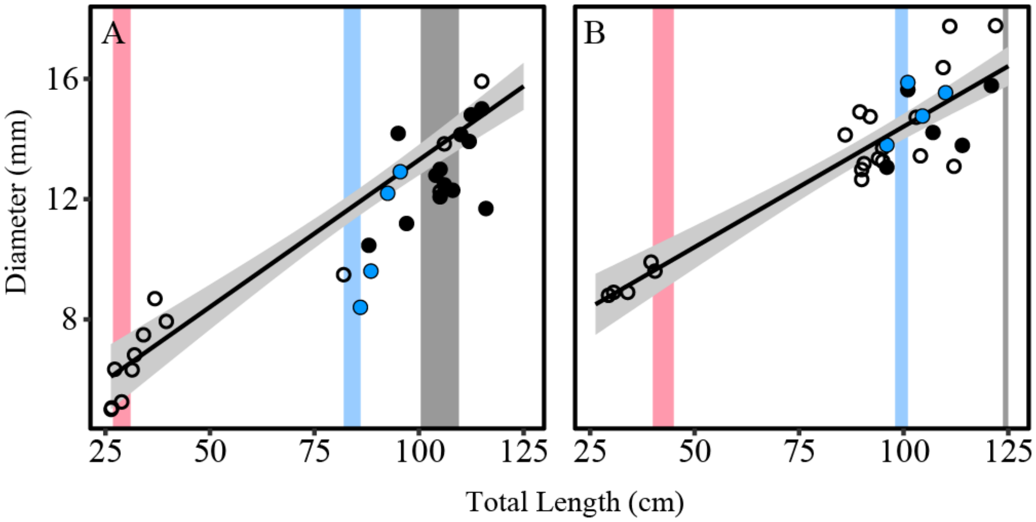


Figure 5.1: Linear regression with standard error (s.e) for lens diameter and total length of (A) *C. coelolepis* and (B) *C. squamosus* with published ranges of size at birth (red shading) and ranges of size at maturity (L_{50}) for males (blue shading) and females (grey shading). Open circles are lenses measured but not sampled and filled circles are males (blue) and females (black) sampled in this study.

Similar to *C. coelolepis,* there were two apparent shifts in the isotopic patterns in δ^{13}C for *C. squamosus*. The first major stable isotopic shift occurred before the predicted lens size at birth and then the second shift occurred at about an eye lens diameter of ~9mm (Figure 5.3). Unlike *C. coelolepis* however, δ^{15}N values remained generally constant across the eye lens layers (Figure 5.3). Mean maximum δ^{13}C values were -13.88 ± 0.27‰, which corresponded to an average eye lens length of 10.89 ± 1.43 mm. δ^{13}C values then typically decreased until -14.55 ± 0.25‰ in the outer most layer. Male δ^{13}C values were higher than females for similar eye lens diameters.

In both species, first feeding eye lens layers for both δ^{13}C (ANOVA: t=17.87, p<0.001) and δ^{15}N (ANOVA: t=9.27, p<0.001) were significantly more enriched than those corresponding to maternal (core) eye lens layer (Figure 5.5). Mature individuals were only available for *C. coelolepis*, with all *C. squamosus* individuals estimated to be immature. Those eye lens layers corresponding to mature life stages were less enriched in δ^{13}C and δ^{15}N for *C. coelolepis*, generally returning to values similar to that of pre-birth (maternal) layers (Figure 5.3). *C. squamosus* outer layers displayed a general trend of decreasing stable isotope values, but did not return to those values observed in their core lens layers (Figure 5.4).

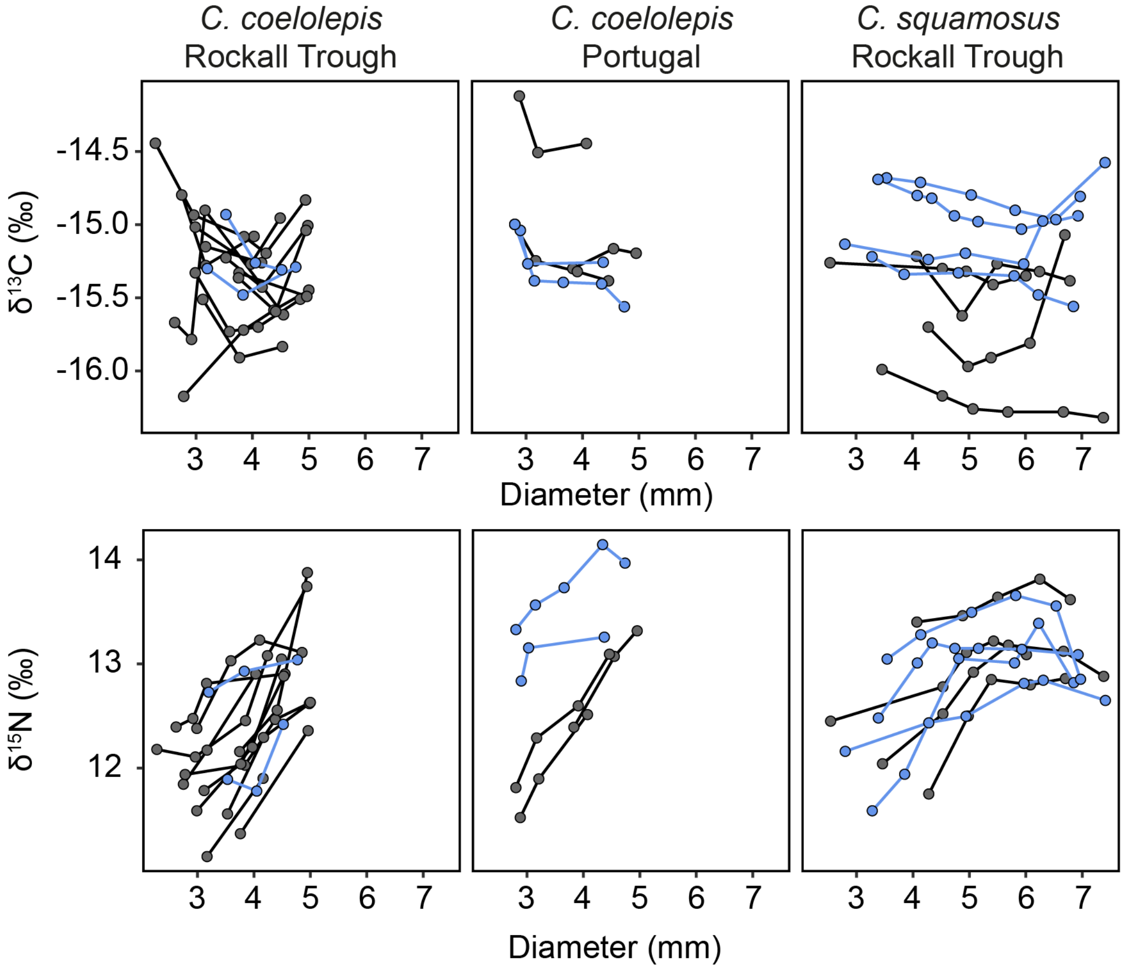


Figure 5.2: Stable isotope profiles (δ^{13}C: top; δ^{15}N: bottom) for just the core eye lens layers corresponding to embryonic life stages for females (black) and males (blue).

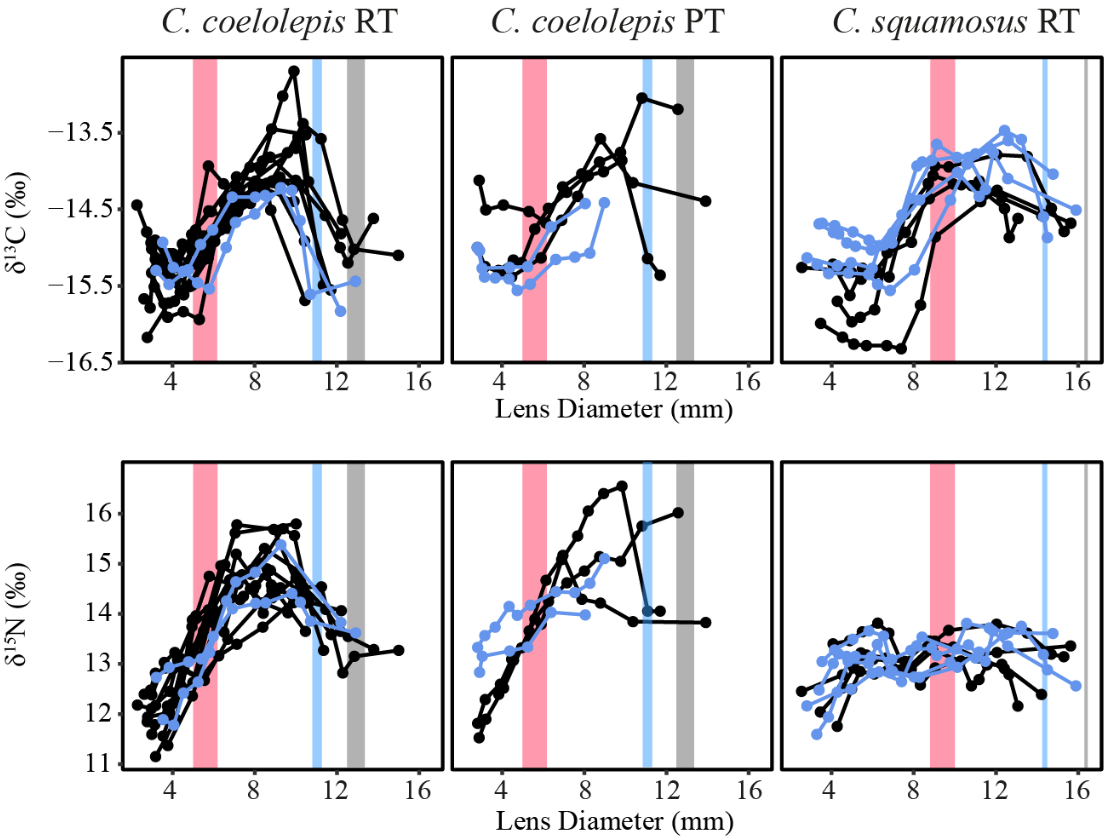


Figure 5.3: Individual δ^{13}C (top) and δ^{15}N (bottom) profiles recorded across the eye lens of female (black) and male (blue) individuals with estimated range of size at birth (red shaded box) and L$_{50}$ maturity size ranges for male (blue shaded box) and female (grey shaded box).

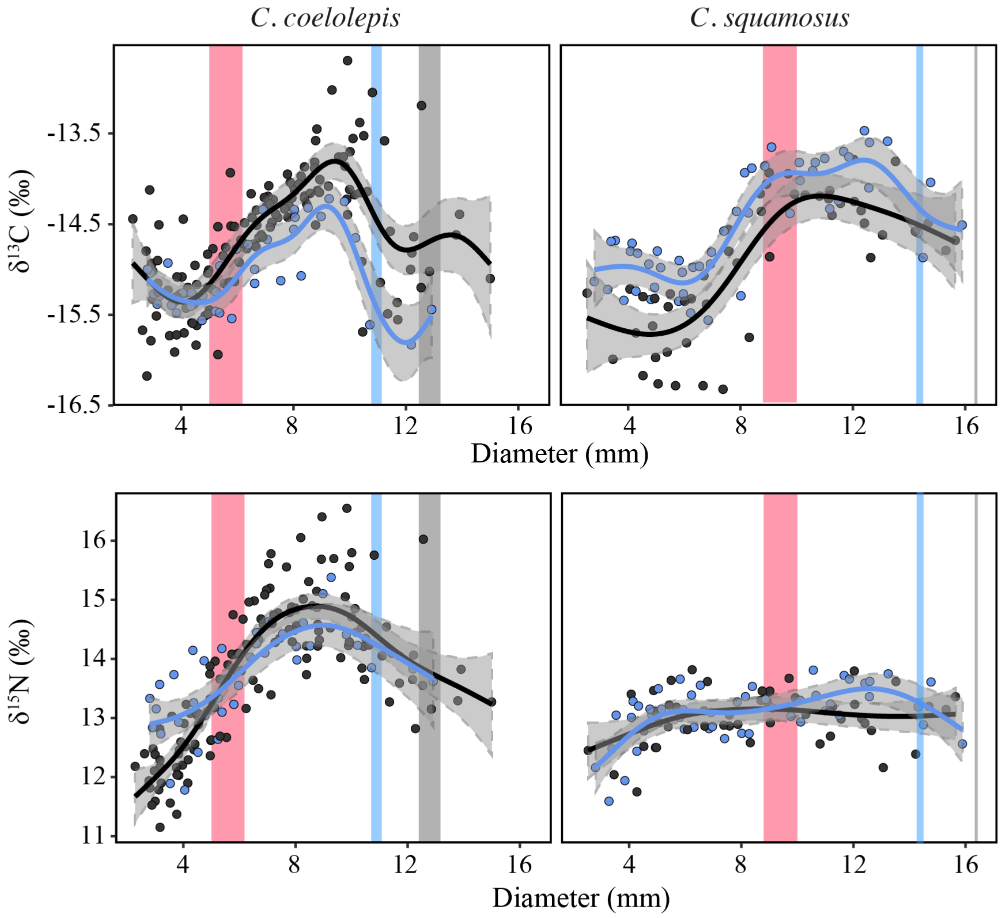


Figure 5.4: Grouped stable isotope eye lens profiles for all *C. coelolepis* (left) and *C. squamosus* (right) with separate GAM smoothed regressions fitted for females (black line) and males (blue line) and associated standard errors (grey shading). Estimated eye lens diameter at birth (red vertical bar) and L_{50} maturity estimations for female (grey vertical bar) and male (blue vertical bar).

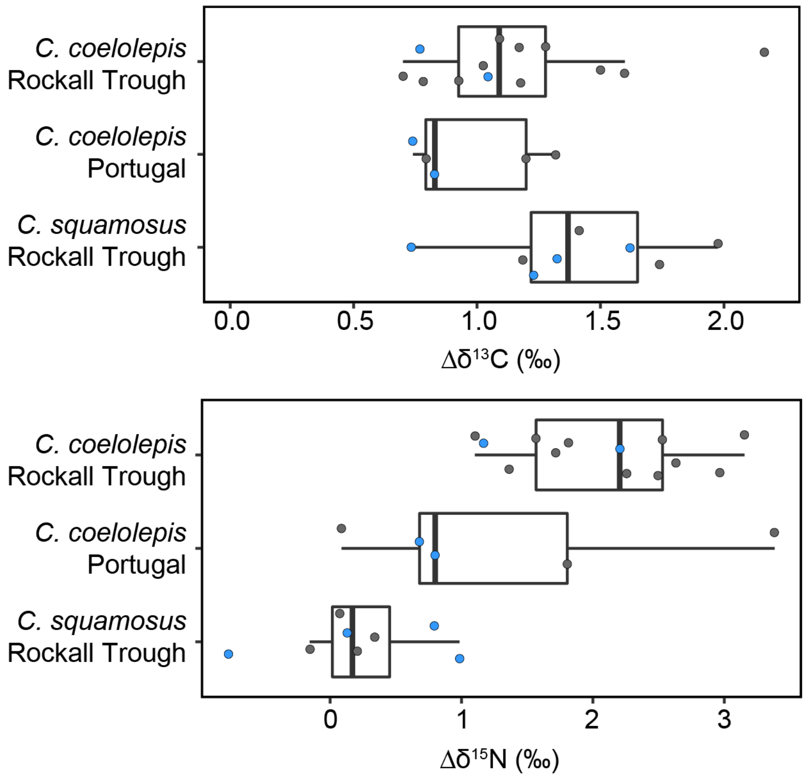


Figure 5.5: Isotopic difference between maternal and proposed first feeding stable isotope values for each shark population for $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom). Points represent individual differences with males in blue and females in grey. Bold vertical line is the average mean difference, the box represents 25% and 75% quartile ranges and the whisker is 1.5 x the inter-quartile range.

5.4.3 Muscle Data

Muscle stable isotope values were statistically similar for $\delta^{15}N$ (Δ-0.43 ± 0.77, t = 1.84, p =0.07) but significantly more enriched in $\delta^{13}C$ (Δ1.58±0.95‰, t=-10.23, p = <0.001) compared to outer eye lens layers for *C. coelolepis*. While muscle values were not available for the two specimens caught in 2016, the outermost layers were significantly lower in $\delta^{15}N$ (Δ1.97 ±0.21‰, t = 5.32, p=<0.001) and more enriched in $\delta^{13}C$ (Δ1.30 ± 0.34‰, t = -10.846, p<0.001) when compared to muscle values in the remaining samples.

For spatially compiled muscle samples, male *C. coelolepis* display clear stable isotopic differences between areas, with female populations expressing a high amount of isotopic similarity as demonstrated by overlapping SEAc's (Figure 5.6). Although white muscle data was only available for three different areas in the Atlantic Ocean for *C. squamosus,* populations in the Rockall Trough and Portugal had similar stable isotope values, with overlapping SEAc, and little distinction between males and females. Individuals from Namibia were isotopically distinct from European samples, although some individual points did overlap between the regions (Figure 5.6).

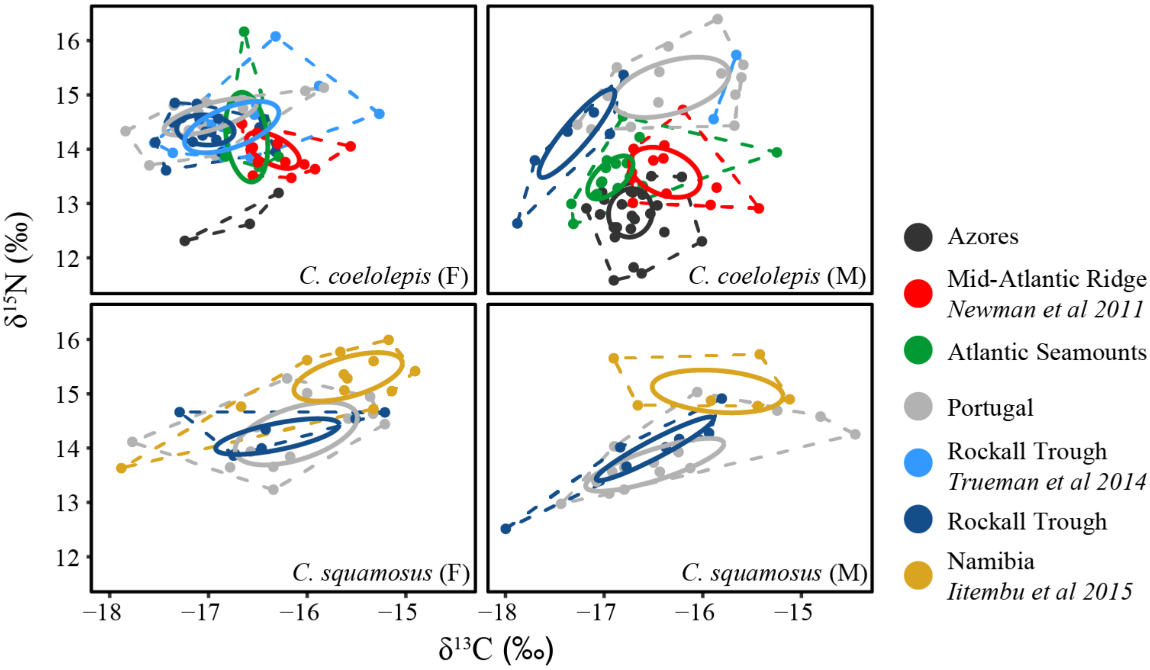


Figure 5.6: Muscle stable isotope values for female (left) and male (right) *C. coelolepis* (top) and *C. squamosus* (bottom). Newly sampled data from Azores (black), Portugal (grey), Rockall Trough (navy) and Atlantic Seamounts south of the Azores (green) were combined with published data from other Atlantic Ocean locations.

5.5 Discussion

We have recovered eye lens stable isotope values for two deep-water shark species, providing ecological details pertaining to whole life history ecology. *C. coelolepis* and *C. squamosus* have different stable isotope profiles as determined by GAMMs but within species, stable isotope profiles were similar, suggesting similar ontogenetic tropho-spatial shifts. We suggest that the observed abrupt shifts in stable isotope values are related to maternal ecology, first feeding, and migrations. The resolution of stable isotope data is coarse however, and incremental (temporal) trends can be interpreted in a variety of plausible ways dependent on trophic and spatial interactions (Christiansen *et al.*, 2015). Combining these data with published information on movement and feeding behaviours, we suggest that δ^{13}C lens profiles support hypotheses that both species are highly migratory. The patterns observed in δ^{15}N suggest that throughout ontogeny, these species have different trophic ecologies, with *C. coelolepis* appearing to undergo ontogenetic shifts or migratory movements, whereas *C. squamosus* has a similar trophic ecology throughout life.

5.5.1 Methodological Assessment

It should be noted that the sampling of shark eye lens is challenging. Protocol assessments for sampling fish eye lenses were assessed by Quaeck-Phillips 2017 (unpublished Ph.D thesis) who found that the methods adopted by Parry (2003) for eye lens peeling were most successful for obtaining sequential eye lens layers from sharks. Although the inner core lens layers were easier to sample (i.e. formed clearer banded layers that were easy to peel), the outer most eye lens layers were typically less defined, tacky in composition, and difficult to consistently peel. Accordingly, the thickness of each eye lens layer was not uniform across the diameter of the eye and it is unclear how much ecological time each layer relates to. Additionally, the low number of individuals sampled in this study limit abilities to infer population wide ecological processes, but do provide adequate samples for a preliminary assessment of this technique to deep-water shark species. Current baseline data on spatial gradients in baseline stable isotope values are confined to pelagic phytoplanktonic production (McMahon *et al.*, 2013, Magozzi *et al.*, 2017). While spatial gradients in deep-sea food webs are poorly documented, we assume that spatial isoscapes developed for surface production are likely reflected in deep-sea food webs due to their reliance on vertically fluxed pelagic nutrients (Mauchline and Gordon, 1991, Bergmann *et al.*, 2009, Trueman *et al.*, 2014). This is supported by Choy *et al.* (2012), that found the conservation of spatial gradients of stable isotope values in mesopelagic production in the Southern Ocean. Regardless of these challenges, and the uncertainties surrounding stable isotopic incorporation into lens tissues, the samples extracted in this study are believed to provide a good overview of the progression of stable isotope ecology throughout the development of these individual, relative to body size, and provide preliminary results that warrant further investigation.

5.5.2 Embryonic Development

Most Squaliformes (dogfish) are widely believed to reproduce via lecithotrophic viviparous development, meaning that embryonic development is supported by yolk sac nutrients followed by live-born neonates (Guallart and Vicent, 2001, Moura *et al.*, 2011). It has been estimated that due to the high energetic demands of producing large oocytes (Remme *et al.*, 2005), it may take females up to 2 years to become fully gravid (i.e. with ripe oocytes) (Girard and Du Buit, 1999, Veríssimo *et al.*, 2003). Although it was initially believed that mothers could exchange nutrients with developing embryos (Girard and Du Buit, 1999, Veríssimo *et al.*, 2003), Moura *et al.* (2011) suggested only water and minerals were exchanged with the embryos and all nourishment was provided by nutrients in the yolk sac.

The isotopic composition of oocytes, and therefore the developing embryos and their eye lenses, should in theory reflect the average maternal ecology when oocytes were developed. It was therefore surprising that there was considerable variation, especially in $\delta^{15}N$, in the stable isotope values of the inner eye lens layers. These results could be interpreted as some form of matrotrophic exchange of organic compounds during gestation, with embryo's receiving nutrients of varying stable isotopic ratios related to varying maternal diet (Girard and Du Buit, 1999, Veríssimo *et al.*, 2003, Paiva *et al.*, 2011, Cotton *et al.*, 2015). Alternatively, the observed variations could be linked with other physiological processes, for which we currently lack information, such as embryonic urea retention or catabolism of maternal nutrients. More studies are needed to understand Squaliformes embryonic nourishment and how this relates to pre-birth tissue compositions.

The high similarity of core stable isotope variations among *C. coelolepis* could however suggest that most mature individuals develop oocytes within isotopically similar environments (Figure 5.2) and are subjected to the same stable isotopic developments. On the contrary, *C. squamosus*, display quite large variability in δ^{13}C profiles suggesting that maturing females may occur over broader geographic regions (Figure 5.2). These results are consistent with trawl surveys, whereby mature female *C. coelolepis* are typically concentrated in Rockall Trough and the European continental slopes (Moura *et al.*, 2014) of the northeast Atlantic. Mature females for *C. squamosus* on the other hand are rarely seen in the northeast Atlantic (Clarke *et al.*, 2001, Figueiredo *et al.*, 2008) but there are some records suggesting that they may occur in northerly Icelandic waters and southerly waters located off Madeira and the Canary Islands (Moura *et al.*, 2014), something which may be reflected in these data.

5.5.3 First-Feeding

The abrupt enrichment of δ^{13}C values for inner layers (Figure 5.5) for both species, is consistent with the onset of post-partum feeding on prey that have dramatically different stable isotope values from maternal diets during oocyte development. Assuming that this is true, these data suggest that while estimates for *C. coelolepis* size at birth may be accurate, neonatal *C. squamosus* may be smaller than currently realised (Figure 5.3). While we cannot eliminate the possibility that neonatal *C. squamosus* diets are isotopically similar to maternal diets, it is more likely that the small sample sizes used to constrain isometric regressions (Figure 5.1) may have been inadequate to estimate eye lens diameters at birth. Future studies would benefit from measuring a wide size range of lens diameters and these results could be corroborated with the collection of more neonatal specimens.

In other stable isotope assessments of incrementally grown shark tissues (vertebrae), the first major shift in the stable isotopic trends is observed following pupping and the undertaking of first feeding (Estrada *et al.*, 2006, Carlisle *et al.*, 2015). Stable isotope values (particularly δ^{15}N) recorded in both neonatal white and salmon sharks, were lower than the pre-birth layers, which both authors conclude is consistent with lower trophic first-feeding (Estrada *et al.*, 2006, Carlisle *et al.*, 2015), which is expected in size-structured marine environments (Jennings and van der Molen, 2015). The results for *C. coelolepis* do not follow this same trend, but instead, δ^{15}N value prescribed to smaller sizes classes are higher than their mothers (i.e. inner eye lens layers derived from maternal yolk sac). It is unlikely that juvenile *C. coelolepis* are feeding at higher trophic levels than their mothers however. Both dietary and stable isotope assessments of *C. coelolepis* suggest that adults are some of the highest trophic level fish in continental slope ecosystems (Mauchline and Gordon, 1983, Carrassón and Cartes, 2002) and it seems unlikely that juveniles are of higher trophic levels. Alternatively, the enriched δ^{15}N may reflect a more benthic diet for neonatal and immature individuals, as suggest by Moura *et al.* (2014). Benthic food webs express enriched δ^{15}N values compared to pelagic production due to their increased reliance on reworked/recycled nutrients (Iken *et al.*, 2001, Bergmann *et al.*, 2009). However, the high δ^{15}N values (mean: 15.11 ± 0.64 ‰) are greater than other northeast Atlantic benthic feeders (i.e chimaeras) of a comparable size found at the same regions (Trueman *et al.*, 2014), which would not support a benthic diet in this region. Instead of reflecting trophic relationships, the increases in both δ^{13}C and δ^{15}N (Figure 5.4) are most likely explained by mothers provisioning oocytes and pupping in isotopically (spatially) distinct areas.

Baseline δ^{13}C and δ^{15}N of particulate organic matter generally increase towards the equator in the northeast Atlantic (Somes *et al.*, 2010, Magozzi *et al.*, 2017). The higher stable isotope ratios observed during inferred first feeding for *C. coelolepis* (Figure 5.5) could therefore be indicative of nursery areas situated in relatively lower (isotopically enriched) latitudes compared to where oocytes were provisioned. The similarity of post-birth stable isotope values across individuals further support shared ecological experiences of neonatal sharks (i.e. feeding on similar prey from a similar location), and is consistent with the existence of common nursery areas for pupping, which have been widely speculated (Heupel *et al.*, 2007, Moura *et al.*, 2014). Our inferences are in agreement with known distribution patterns of this species with mature individuals dominating catch records in the northeast Atlantic and neonatal individuals, although seldom reported, occurring in central eastern Atlantic locations (Girard and Du Buit, 1999, Clarke *et al.*, 2001, Veríssimo *et al.*, 2003, Figueiredo *et al.*, 2008, Moura *et al.*, 2014).

The utilisation of more southerly pupping regions could also be inferred for *C. squamosus* individuals. Although the estimated lens diameter at birth for *C. squamosus* was likely an overestimation, there are consistent sharp increases in δ^{13}C values at around 7 mm eye lens diameter (Figure 5.3) similar to that observed in *C. coelolepis*, which are consistent with the onset of first feeding. The most likely explanation for these observed patterns is that *C. squamosus* may also be pupping in spatially (and isotopically) distinct southerly locations compared to regions used for foraging during maternal egg production. Absolute δ^{13}C values in juvenile *C. squamosus* (-13.88 ± 0.27‰) are similar to those observed in *C. coelolepis* (-13.84 ± 0.46‰), which may indicate first feeding occurring in similar latitudinal areas. Pregnant females have been observed off the Madeira Archipelago (Severino *et al.*, 2009), and it is believed that there may be nursery areas located off the western coast of Africa (Moura *et al.*, 2014). It has also been proposed that there may also be a nursery area off Iceland (Moura *et al.*, 2014), but these data do not support such a location for these individuals.

Unlike *C. coelolepis*, δ^{15}N values in *C. squamosus* lens tissues are relatively invariant with increasing lens diameter, implying a constant trophic level throughout life. This however confounds our interpretation of the higher δ^{15}N values in *C. coelolepis* being reflective of spatial differences opposed to trophic differences. If our spatial inferences from δ^{13}C are true, these differences in δ^{15}N profiles could be linked with neonatal trophic differences. Several studies similarly suggest that *C. squamosus* feeds at the same trophic level throughout ontogeny (Iitembu and Richoux, 2015) and generally has a varied diet of benthic and benthopelagic teleosts and cephalopods (Mauchline and Gordon, 1983, Ebert *et al.*, 1992, Dunn *et al.*, 2010b). Assuming both species have pupping areas in lower latitudes, these data imply inter-specific resource partitioning in neonatal individuals, with *C. squamosus* feeding at lower/more pelagic trophic levels than *C. coelolepis,* or occurring in shallower waters. Increased surveys of deep-sea waters off of western Africa would be need to confirm these inferences however.

5.5.4 Inferred Migrations: *C. coelolepis*

The most parsimonious explanation for the observed stable isotope trends is that both species undergo large latitudinal migrations linked with using different geographic regions for different ontogenetic and reproductive stages, at least for the individuals sampled here. For *C. coelolepis* individuals, our interpretation of these results is that female oocyte/yolk development consistently takes place in relatively northerly latitudes, followed by migrations during gestation and pupping in lower latitudes, where neonatal first feeding occurs. Neonatal individuals then develop in lower latitudes before migrating north to undertake developmental and reproductive life history stages in more northerly areas. The convergence of outer lens layer stable isotope values to those similar to pre-birth (core lens layers) values (Figure 5.4) additionally suggest that egg maturation may occur in similar regions between generations.

The second notable stable isotope profile shift occurs at ~9 mm eye lens diameter (~60 cm), where δ^{13}C and δ^{15}N values become more negative. This shift coincides with reported sizes for transitions of juvenile individuals into immature subadult states (Moura *et al.*, 2014). Likewise, the smallest *C. coelolepis* observed in the northeast Atlantic are typically between ~65-75cm, further supporting the migration of subadults into the northeast Atlantic after leaving nursery areas (Girard and Du Buit, 1999, Clarke *et al.*, 2001, Veríssimo *et al.*, 2003, Figueiredo *et al.*, 2008, Moura *et al.*, 2014). Baseline particulate organic matter is more isotopically depleted in northerly latitudes (Somes *et al.*, 2010, Magozzi *et al.*, 2017), and it is assumed that these isotopic depletions are related to movement into higher latitudes. Migrations out of potential nursery regions may reduce intraspecific competition between neonates and juveniles (Heupel *et al.*, 2007). Furthermore, the enhanced productivity associated with higher latitudes (Gordon *et al.*, 1996, Wei *et al.*, 2010) may provide consistently used grounds for development of oocytes that supply alternative prey resources for energetically demanding oocyte development. The isotopic variability observed within Portuguese female *C. coelolepis* outer lens layers (Figure 5.3) could be linked with mixing of individuals from both southerly and northerly locations The orientation of the continental slope facilitates north-south migrations and the high incidence of pregnant and post-natal females in Portugal (Veríssimo *et al.*, 2003) mean that those individuals could have been undertaking migrations in different directions. These results should be considered with caution however, as it was noted that the outer eye lens layers were less defined than those of the inner eye lens, and the abrupt change in stable isotope values could be linked with biochemical properties associated with structural composition changes. While these results are aligned with current hypotheses of *C. coelolepis* migrations, further work is needed to confirm our inferences.

These data do provide support to the hypothesis from genetic studies that suggest *C. coelolepis* is highly migratory and sexes uses different geographic areas during ontogeny (Veríssimo *et al.*, 2011, Catarino *et al.*, 2015). δ^{13}C depletions occurred at smaller sizes in males than females (Figure 5.4), which is consistent with females maturing at larger sizes than males (Girard and Du Buit, 1999, Veríssimo *et al.*, 2003, Figueiredo *et al.*, 2008). The differences in stable isotope values in outer lens layers are also consistent with the documented sexual segregations of this species, with different maturity stages occurring at different geographic locations and depths (Yano and Tanaka, 1988, Moura *et al.*, 2014). Compiled white muscle stable isotope data display strong isotopic differences between sexes (Figure 5.6), with male populations being more spatially distinct than females. Stable isotope values of white muscle can provide ecological information pertaining to more recent ecology (1-2 years; (Kim *et al.*, 2012a)), and can therefore supplement the eye lens data. Sexual difference in white muscle data might reflect females moving and feeding across different regions during tissue turnover time, while males are relatively resident. These inferences are aligned with genetic and distributional data that suggest *C. coelolepis* undertake large-scale movements associated with reproductive development (Veríssimo *et al.*, 2011, Moura *et al.*, 2014). Sexual segregation is a well-documented mechanism used by sharks to reduce intraspecific competition for resources and limit sexual harassment (Sims, 2005, Wearmouth and Sims, 2008), something these data suggest may be occurring for *C. coelolepis*.

5.5.5 Inferred Migrations: *C. squamosus*

The lack of mature female *C. squamosus* in Rockall Trough limited sampling to immature individuals, although some mature males were sampled. The stable isotope profiles reported here may therefore not recorded ecological shifts related to reproductive development, and subsequently the inferences of the ecological life histories of this species are incomplete. The consistent depletion of $\delta^{13}C$ at about 9-10 mm eye lens diameter however, could suggest migrations away from pupping areas into more isotopically depleted environments (more north), as hypothesised in *C. coelolepis*. For $\delta^{13}C$, the outer lens layers do not return to values as seen in the core lens (Figure 5.4), which is in agreement with these immature individuals not reaching the same life history stage as their mothers. It could be argued that the gradual decrease in $\delta^{13}C$ values in the outer lens layers could be indicative of trophic switching (i.e. feeding on more pelagic animals) or migrating into more negative $\delta^{13}C$ POM areas (i.e. northerly areas). Iitembu and Richoux (2015) found no evidence of ontogenetic trophic switches for *C. squamous* off Namibia, also reporting consistent $\delta^{15}N$ values throughout ontogeny. The most parsimonious explanation of the observed depletion of $\delta^{13}C$ values is that these individuals began feeding in more northerly latitudes.

These results support the migration of individuals from southerly, possible nursery, areas (Severino *et al.*, 2009), into more northerly latitudes as they increase in size. Results from genetic studies suggest that *C. squamosus* is capable of undertaking long-distance, long-term migrations throughout life history (Verissimo *et al.*, 2012). Telemetry studies have demonstrated that over short time frames (months), *C. squamosus* can also exhibit relatively small-scale movements, instead undertaking diel vertical migrations likely linked with foraging (Daley *et al.*, 2015, Rodríguez-Cabello *et al.*, 2016). Although isotopic data from muscle was limited, the large amount of isotopic overlap between *C. squamosus* populations between Rockall Trough and Portugal, support some degree of mixing of these individuals and/or their prey over the time frame of white muscle turnover (1-2 years).

Similar to *C. coelolepis*, there were differences in $\delta^{13}C$ profiles between the sexes (Figure 5.4). The patterns of the overall isotopic profiles were the same, but $\delta^{13}C$ values were higher in males, implying potential contrasting movement patterns between the sexes or bathymetric segregations. Mature males and immature females dominate the northeast Atlantic Ocean, with females typically occurring deeper than the males (Clarke *et al.*, 2001). Additionally, genetic evidence suggests that males have larger dispersion distances than females (Verissimo *et al.*, 2012). Considering all information above, the observed eye lens differences are likely a reflection of differential habitat use between immature males and females, with isotopic values becoming more similar in later life stages coinciding with their similar capture locations in Rockall Trough. Again, the low sample sizes limit our ability to relate these spatial inferences to the population level, but provide a good first step on which to further test these hypotheses.

5.6 Conclusions

This preliminary study provides proof of concept of the use of sequential stable isotope analysis of eye lens tissues to provide ecological information related to individual whole-life histories. With limited application of this technique across other fish species (Wallace *et al.*, 2014), our understanding of the stable isotopic processes associated with eye lens development are still advancing. Competing stable isotopic gradients (spatial, depth, trophic) confound our abilities to definitively assign precise geographic assignment to inferred migrations but instead present the most parsimonious interpretation of these data in light of other published information. These results show great promise in uncovering cryptic life history ecologies of rarely observed life history stages and warrants more detailed investigations on a larger number of individuals to further refine ecological inferences.

Although this study was limited to only a few individuals, these results were consistent with both *C. coelolepis* and *C. squamosus* undertaking first feeding in environments characterised by relatively positive $\delta^{13}C$ and $\delta^{15}N$ values, which we suggest are in lower latitudinal waters, such as off western Africa. Neonatal and immature life stages are suggested to reside in these isotopically distinct areas before migrating into and feeding in northerly latitudes during maturation, where sexes spatially segregate to some degree. For these *C. coelolepis* it appears that individuals return to similar environments as their mothers alluding to the presence of important, consistently used oocyte maturation grounds for this species in the northeast Atlantic (Girard and Du Buit, 1999, Clarke *et al.*, 2001, Moura *et al.*, 2014)..

Putative migrations across the northeast Atlantic leave large squaliform sharks vulnerable to commercial fishing pressures throughout their distributional range. Large squaliform sharks are abundant between 700 m and 1200 m and likely provide an important ecological role in these habitats (*Bird et al in prep;* chapter 4). Additionally mature and/or pregnant individuals are typically found in shallower waters (Girard and Du Buit, 1999, Clarke, 2000, Veríssimo *et al.*, 2003, Moura *et al.*, 2014), increasing their risk of fisheries interaction and consequently their vulnerability to fisheries overexploitation (Kelly *et al.*, 1997, Simpfendorfer and Kyne, 2009, Ramos *et al.*, 2013, Neat *et al.*, 2015).

Furthermore, if pupping or nursery areas are located beyond European waters, pregnant females and juveniles could be exposed to fishing pressures within areas with limited fisheries management. This may explain the lack of recovery of some deep-water sharks in the northeast Atlantic (Neat *et al.*, 2015). These results support the establishment of bathymetric limits to bottom trawl fisheries to <700 m in order to protect highly vulnerable squaliformes sharks (Clarke *et al.*, 2015) but also highlight the need for international collaborative efforts to protect highly migratory populations that transcend political boundaries. With growing evidence to support the ecological importance of large sharks in marine ecosystems (Bird *et al in prep*: Chapter 2), it will be important to develop techniques that unveil necessary information needed to implement effective management strategies.

5.6.1 Future Work

It would be beneficial to sample these species more thoroughly and from other geographic locations to see how stable isotopic profiles vary between regions, thus confirming that the observed trends are primarily linked with spatial migrations opposed to trophic interactions. Sampling of central Atlantic continental slope regions would also be useful in confirming the presence of proposed first-feeding (nursery) habitats. We have also demonstrated the usefulness of using incremental analysis of eye lens layers to uncover the life-long ecology of difficult to observe cryptic species, especially when used in conjunction with additional data sources.

5.7 Supplementary Data

Table 5.3: Eye lens and total length measurements of embryonic/neonatal museum specimens.

Individual	Species	Total Length (cm)	Eye lens (mm)	Museum
NHM1	*C.coelolepis*	26.32	5.00	Natural History Museum
NHM2	*C.coelolepis*	26.47	5.05	Natural History Museum
NHM3	*C.coelolepis*	28.89	5.25	Natural History Museum
38452	*C.coelolepis*	27.20	6.33	Harvard Museum of Natural History
37420	*C.coelolepis*	32.00	6.81	Harvard Museum of Natural History
37424	*C.coelolepis*	31.40	6.31	Harvard Museum of Natural History
125402	*C.coelolepis*	39.50	7.93	Harvard Museum of Natural History
132510 A	*C.coelolepis*	36.80	8.69	Harvard Museum of Natural History
132510 B	*C.coelolepis*	34.10	7.48	Harvard Museum of Natural History
UF 159699	*C.squamosus*	29.40	8.80	Florida Museum of Natural History
UF 159283	*C.squamosus*	40.50	9.60	Florida Museum of Natural History
UF 159317	*C.squamosus*	39.60	9.90	Florida Museum of Natural History
USNM 161517	*C.squamosus*	30.60	8.90	Smithsonian Institution National Museum of Natural History
USNM 161518	*C.squamosus*	34.00	8.90	Smithsonian Institution National Museum of Natural History

Chapter 6: General Discussion

6.1 Summary of Findings

Developing a broadened understanding of the ecological complexities of shark populations will be vital for predicting the consequences of overfishing, developing sustainable fishing practices, and implementing effective conservation strategies. The ecological role of sharks in marine environments is still widely debated however, and there is an increasing need to attempt to address these questions using alternative, complementary methodological approaches (Kitchell *et al.*, 2002, Myers *et al.*, 2007, Heithaus *et al.*, 2008, Ferretti *et al.*, 2010, Heupel *et al.*, 2014, Frisch *et al.*, 2016, Grubbs *et al.*, 2016, Roff *et al.*, 2016, Ruppert *et al.*, 2016). The overall objectives of this research were to use stable isotope analysis to uncover aspects of the spatial and trophic ecology of sharks, taking a specific focus on data-limited deep-sea environments. Using stable isotopes as tracers of nutrient flow (carbon and nitrogen) through food webs it was possible to elucidate trophic and spatial interactions over a range of spatial and temporal scales.

As typically mobile, higher trophic level predators, chondrichthyans have the capacity to exert predatory pressures over a range of spatial scales by feeding and moving across a variety of habitats and food webs (Bauer and Hoye, 2014). Several isolated studies have demonstrated that sharks may play an important role in facilitating the transfer of nutrients between ecosystems (McCauley *et al.*, 2012, Papastamatiou *et al.*, 2015) but to what extent this process is ubiquitous across other shark populations was unclear. In order to identify how sharks link different ecosystems and habitats, chapter 2 compiled global $\delta^{13}C$ data from 5394 sharks from 114 species. This research revealed potential differences in the extent and nature of nutrient transfer and cross-ecosystem foraging between sharks found in oceanic and shelf habitats. Oceanic sharks appear to assimilate the majority of their nutrients from pelagic resources derived from a limited latitudinal range, which support the use of highly productive frontal zones in intermediate latitudes (Queiroz *et al.*, 2012, Scales *et al.*, 2014a). Conversely, sharks from shelf and coastal habitats appear to obtain most of their nutrients from local pelagic resources but also feed across other ecologically distinct food webs. It is argued that these trophic interactions could facilitated the coupling of food webs, something which has the capacity to promote ecosystem stability and resilience (McCauley *et al.*, 2012, Rooney and McCann, 2012, Britten *et al.*, 2014). This work also highlighted the paucity of data currently available on deep-sea sharks, with almost half of the data (48.2%) coming from samples collected for this thesis and the earlier work of Trueman *et al.* (2014). Of the available data, it was apparent that spatial trends in deep-sea sharks were more similar to those of oceanic sharks, suggesting that there may be similarities in the nutrient dynamics supporting these populations. Indeed, deep-sea ecosystems are primarily supported by vertically fluxed nutrients derived from oceanic phytoplanktonic production (Polunin *et al.*, 2001, Trueman *et al.*, 2014).

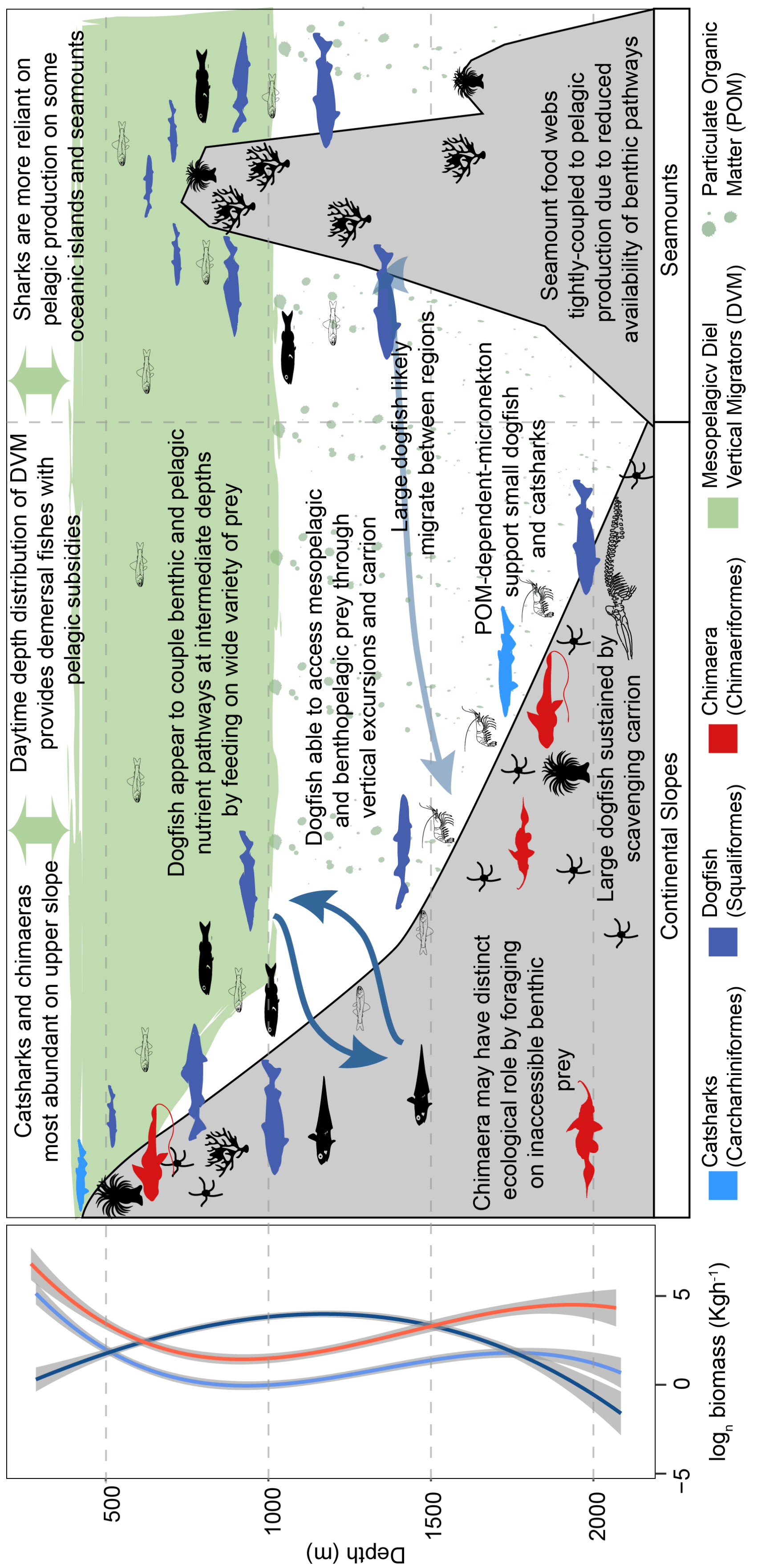
These data could be a reflection of both oceanic and deep-sea sharks being supported by mesopelagic nutrient subsidies provided by globally abundant diel vertically migrating fauna (Sutton, 2013, Trueman *et al.*, 2014, Howey *et al.*, 2016). This research generated several hypotheses that would warrant further investigation using different methodological approaches over global scales, and it is hoped these results support the development of this field. We further explored the nutrient dynamics supporting deep-sea sharks in chapter 3, by specifically examining the trophic ecology of assemblages found across the northeast Atlantic.

Deep-sea ichthyofaunal fish assemblages are reliant on the vertical transport of nutrients from productive surface waters to demersal habitats (Polunin *et al.*, 2001, Preciado *et al.*, 2017). Nutrients are typically partitioned between pelagic food webs, primarily facilitated by diel vertically migrating mesopelagic animals, and benthic food webs, which are reliant on the sedimentation of particulate organic matter (POM) and its resuspension (Iken *et al.*, 2001, Mintenbeck *et al.*, 2007, Trueman *et al.*, 2014). The findings in chapter 3 highlighted that the nutrient dynamics between several seamounts and two continental slope habitats may exhibit differences nutrient availability (Figure 6.1), and that sharks diets appear to be more tightly-coupled to pelagic production at seamounts than on continental slopes. While sharks on continental slopes still feed on prey from pelagic food webs, they may also have greater access to prey resources from isotopically enriched benthic food webs (i.e. provided by deposit feeding prey; Figure 6.1). These findings from the northeast Atlantic are supported by research that suggest that due to differences in topographic relief and local oceanography, seamounts support higher biomasses of filter feeding epibenthic fauna and less deposit feeders than continental slopes (Tracey *et al.*, 2004, Porteiro and Sutton, 2007, McClain *et al.*, 2010, Rowden *et al.*, 2010a, Rowden *et al.*, 2010b, Priede *et al.*, 2013). Stomach content studies also report dominant contributions from mesopelagic prey to predators on seamounts (Fock *et al.*, 2002, Preciado *et al.*, 2017), whereas fish on continental slopes often have more varied diets (Mauchline and Gordon, 1985, Macpherson and Roel, 1987). It appears that across the sampling region, continental slopes are able to support greater diversity of trophic pathways due to their shallower reliefs and closer proximity to continental shelf nutrient inputs (Cartes *et al.*, 2016, Romero-Romero *et al.*, 2016). Although it is unlikely that these findings are a ubiquitous feature across all seamount and slope habitats due to differences in geography, structure, and oceanography, these results do suggest trophic pathways can differ between deep-sea habitats. Establishing to what extent deep-sea chondrichthyans utilise benthic and/or pelagic food webs at continental slopes was the basis of the chapter 4.

Chondrichthyan assemblages associated with continental slopes can be highly diverse, have high biomass, and span large bathymetric ranges (Gordon and Swan, 1997, Neat *et al.*, 2015). In nutrient-limited environments such as bathyal ecosystems, this raises questions with regards to how sympatric species with overlapping distributions may partition resources. In chapter 4, the stable isotope characteristics of the dominant chondrichthyans at Rockall Trough, i.e. dogfish (Squaliformes), catsharks (Carcharhiniformes), and chimaeras (Chimaeriformes), were compared with those of benthic and benthopelagic teleosts. This research supported the partitioning of resources (prey and space) among sharks and chimaera fishes, with some degree of overlap between closely related species (Mauchline and Gordon, 1983, Dunn *et al.*, 2010a, Pethybridge *et al.*, 2011, Pethybridge *et al.*, 2012, Dunn *et al.*, 2013, Churchill *et al.*, 2015b, Shipley *et al.*, 2017d). As a group, dogfish and catsharks show a complex trophic ecology, demonstrating both ecological distinctiveness from the teleosts (especially at 1000m), and also potentially having broad intermediate diets assimilating nutrients from both pelagic and benthic trophic pathways that vary across their bathymetric range (Figure 4.6 & 4.7). The results from the chimaeras however were less ambiguous, demonstrating that they may be occupying a distinct isotopic niche in Rockall Trough, by feeding on isotopically enriched benthic prey that are inaccessible to other fishes (Figure 6.1). These stable isotope patterns were aligned with the limited stomach content studies from this region (Mauchline and Gordon, 1983) and elsewhere (Ebert *et al.*, 1992, Ebert *et al.*, 1996, Dunn *et al.*, 2010b, Pethybridge *et al.*, 2012), and suggest that chondrichthyans occupy trophic guilds that differ among, and may be similar within, taxonomic orders. By feeding on multiple components of these bathyal ecosystems, sharks have the capacity to couple discrete food webs and subsequently play an important role in maintaining ecosystem stability, and subsequently resilience (McCann *et al.*, 2005, Rooney *et al.*, 2006). These results provide strong evidence to suggest that chimaera have a distinct ecology in deep-sea ecosystems and may provide ecological functions and services not yet fully recognised.

To date, most studies of deep-sea chondrichthyan assemblages have not accounted for depth in their observed stable isotope results (Pethybridge *et al.*, 2012, Churchill *et al.*, 2015b, Shipley *et al.*, 2017d). Mindel *et al.* (2016b) found strong bathymetric trends in the morphological traits expressed by deep-sea fish, which were also linked with the availability of different nutrient pathways. The strong influence of bathymetric gradients on nutrient dynamics should therefore be considered in future assessments of deep-sea ecology. Ecological (isotopic) distinctness was most pronounced at 1000m where shark biomass is dominated by the large, higher trophic level gulper (Centrophoridae) and sleeper (Somniosidae) sharks (Figure 6.1). In addition to depth, time (i.e. ontogeny) and space should also be considered when analysing stable isotope profiles in deep-sea sharks, particularly those with high dispersal abilities. Indeed, both centrophorid and somniosid sharks include species that are proposed as highly migratory, utilising different parts of the northeast Atlantic during ontogeny and reproductive development (Veríssimo *et al.*, 2011, Verissimo *et al.*, 2012, Moura *et al.*, 2014) but direct evidence to support these claims is limited.

Figure 6.1: Summary illustration of some ecological characteristics of deep-sea chondrichthyans inferred from this thesis research. Biomass data (left) is modified Figure 4.2, showing average haul biomass for catsharks (light blue), dogfish (dark blue), and chimaera (red) from fisheries trawl survey data from Rockall Trough (2000-2015).



Sequential sampling of incrementally grown, inert, biological tissues for stable isotope analysis can provide information on life-history ecology (Estrada *et al.*, 2006, Carlisle *et al.*, 2015, Christiansen *et al.*, 2015). Deep-sea sharks often lack suitable tissues in which to conduct such studies, but one potential, currently underutilised tissue, are the crystalline proteins of eye lenses (Hunsicker *et al.*, 2010, Wallace *et al.*, 2014). A preliminary study of stable isotope profiles along the eye lens diameter of the Portuguese dogfish *C. coelolepis* and the leafscale gulper *C. squamosus* demonstrate that these species may undertake similar ontogenetic migrations but have different trophic ecologies. There was evidence to suggest that each species had a specific but consistent ecological profile that was most parsimoniously explained by migrations between different geographic areas associated with different life history stages (Moura *et al.*, 2014). Adult females appear to utilise higher latitudes for the development of oocytes, before pupping offspring in lower latitudes, possibly in nursery areas. Juvenile sharks appear to migrate back into higher latitudes to become sexually mature. Our inferences were supported by documented geographic gradients in baseline stable isotope values (McMahon *et al.*, 2013, Magozzi *et al.*, 2017), demographic distributional data for these species (Moura *et al.*, 2014), and genetic population structure assessments (Veríssimo *et al.*, 2011, Verissimo *et al.*, 2012). This was the first application of stable isotope analysis of eye lenses to deep-sea sharks and the results support the use of eye lens tissues for future ecological studies interested in examining the whole life history ecology of hard to study animals.

6.2 Methodological Assessment and Future Work

The use of stable isotope analysis has been routinely used to examine the ecological characteristics of marine ecosystems (Davenport and Bax, 2002, McMahon *et al.*, 2013), and more specifically, the ecology of chondrichthyan fishes (Hussey *et al.*, 2012a, Shiffman *et al.*, 2012, Shipley *et al.*, 2017a). While providing a relatively easy to implement methodological approach, there are some limitations that must be considered. Christiansen *et al.* (2015) demonstrated that several plausible, but distinct, ecological scenarios could result in the same stable isotope values. Interactions between trophic and spatial stable isotope factors can confound ecological interpretations, which can in turn be further affected by depth effects (Trueman *et al.*, 2014, Christiansen *et al.*, 2015). Accordingly, where possible throughout this thesis, stable isotope data were considered in conjunction with additional information including data from the wider ichthyofaunal community (Trueman *et al.*, 2014), long-term fisheries survey data (Neat *et al.*, 2010), geochemical modelling (Magozzi *et al.*, 2017), and considerable historic publications on stomach content analysis and distributional data (Mauchline and Gordon, 1983, Moura *et al.*, 2014).

The ecological inferences made about chondrichthyans in deep-sea ecosystems are somewhat hindered by the sampling of ecosystems that have already been impacted by fishing

(Gordon, 2003, Pajuelo *et al.*, 2010), and the observed mechanisms may not be the same as pristine environments. Using ecosystem modelling approaches at Rockall Trough, Heymans *et al.* (2011) suggested that deep-sea sharks would transition to incorporate more cephalopods in their diets due to fishing-induced reductions in teleost prey availability. It would be interesting to test the hypothesis of Heymans *et al.* (2011) by comparing the trophic ecology inferred from stomach content in Mauchline and Gordon (1983), and how this would compare to diet compositions now, after substantial fisheries exploitation. Furthermore, obtaining stable isotope data from other ecosystem components, such as mesopelagic micronekton, crustaceans and cephalopods, would have been potentially useful to identify specific prey utilised from each trophic pathway. Likewise, it would also be advantageous, especially for inferring migratory movements, to have isoscape maps for deep-sea food webs, and develop a better understanding of how spatial gradients in pelagic POM are transferred into deep-sea food webs. Accounting for these important trophic and spatial components would greatly reduce some of the ambiguity of confounding but plausible stable isotopic scenarios. There is great scope moving forward to use this thesis as a baseline to examine how the trophic ecology of chondrichthyans changes with time, climate change, and fishing pressure. For example, increases in $\delta^{15}N$ values, or increased stable isotopic niche areas, over time may suggest increasing food chain lengths or diversity of prey resources to fishes (Jennings *et al.*, 2008, Rader *et al.*, 2017). Additionally, it was demonstrated in chapter 3 that sharks are good ecological samplers of local nutrient dynamics and future studies could benefit in using higher predators as ecological indicators.

The use of eye lenses provides a promising new approach to uncovering the life long ecological characteristics of cryptic life history stages in marine animals. However, the sampling of eye lenses can be difficult, and the ease of obtaining sequential eye lens layers may differ among species (Quaeck-Davies *et al in prep*). The developmental physiology of the eye is not fully understood, especially with regard to the timing and mode of deposition of eye lens layers. Developmental studies looking at the ontogenetic development of the eye, and the integration of dietary stable isotope values, would greatly improve the robustness of this methodology. Furthermore, interpreting these data is confounded by spatial, temporal, and dietary sources of variance that are inherent to stable isotope ecology. Isotopic data should always be considered in conjunction with a wide as range of alternative data sources where possible (Christiansen *et al.*, 2015). It would be beneficial to obtain isotopic eye lens profiles from *C. squamosus* and *C. coelolepis* captured elsewhere in the world, in order to validate our inferences of trophic and spatial signals. Sampling deep-sea environments at lower latitudes could also validate the results from chapter 5. Recent trawl surveys from these regions documented large numbers of small *Centrophorus* species (Menezes *et al.*, 2015, Pajuelo *et al.*, 2016) that were of the predicted size of neonatal *C. squamosus* (Severino *et al.*, 2009). *Centrophorus* species are taxonomically difficult to identify (Veríssimo *et al.*, 2014) but considering the known distribution patterns of these species, it would be hugely beneficial to obtain additional biological and genetic information from areas, to support my inferences. Increased sampling of deep-water shark assemblages associated with the continental slopes of western Africa could greatly improve our understanding of the migratory movement patterns and stock structure of large migratory dogfish species in the northeast Atlantic.

Lastly, since this thesis research began, there has been considerable debate about the best methodological techniques to deal with the contamination of polar-compounds (lipids, urea, TMAO) in the tissues of chondrichthyans. The biggest area of contention is the interacting effect of urea and lipid on the C:N ratio of stable isotope data, which is then subsequently used to apply mathematical lipid corrections (Churchill *et al.*, 2015a, Lecea and Charmoy, 2015, Carlisle *et al.*, 2016, Li *et al.*, 2016b, Shipley *et al.*, 2017c). The general consensus is that urea and lipids should be chemically extracted in order to obtain the most accurate stable isotope data. What all of these studies fail to do however, is test the validity of applying urea extractions (e.g. by water washing) to obtain accurate C:N values before applying widely used mathematical corrections (Kiljunen *et al.*, 2006, Sweeting *et al.*, 2006, Post *et al.*, 2007). Here, all samples were thoroughly washed in water and mathematical corrections were then performed, and it is argued that the results presented here are accurate with regard to the questions that were addressed. All shark species sampled have expected low lipid content and mathematical corrections had little effect on the observed stable isotope values. Thus, it is hypothesised that developing species-specific lipid extraction techniques will have little to no effect on the inferences made herein. Moreover, the C:N ratios obtained from newly collected shark muscle samples subjected to water washing (3.44 ± 0.23) were all within the range of values for pure protein (3-3.5: (Post *et al.*, 2007)). It would be beneficial to validate these methods in a future study as the protocol followed here provides the most cost-effective and efficient methodological approach to studying shark ecology.

6.3 Management and Conservation Implications

This research has alluded to the potentially important role that sharks (in general; chapter 2), and deep-sea sharks play in demersal ecosystems (chapter 4), and the complex migratory movements some species undertake throughout life (chapter 5). It is therefore imperative to develop fisheries management strategies that limit the impact fishing may have on deep-water chondrichthyans. Clarke et al., (2015) found that the ratio of elasmobranch (sharks and rays) to commercially valuable fish ratio increased with depth, becoming significant between 600 - 800 m. The authors subsequently suggest imposing a bathymetric limit on trawl fisheries at 600 m. The results from this research support such a limit, as it was found that the sharks found at intermediate depths (700 – 1200 m), had a distinct isotopic ecology that could be linked with having a distinct ecological role at this depth (i.e. being the highest trophic level fish). These shallower depths are also where the largest dogfish species are most abundant (chapter 4), and contain species that are capable of undertaking large-scale migrations to complete their life cycles (chapter 5). The local removal of sharks from the upper slope could therefore have widespread deleterious impacts.

A European Commission Report on the impact of the Portuguese black scabbard fishery on deep-water sharks (Ramos *et al.*, 2013) suggested that it was important to improve information on species distribution patterns, as well as to identify biological sensitive areas, improve ecological understanding, and develop fisheries gears that minimise by-catch of vulnerable sharks. Our research addresses some of these knowledge gaps and provides further support in developing fishery management strategies that ensure that shark populations are sustainably exploited in deep-sea fisheries. This thesis presents findings that suggest that shark populations at seamounts may be more vulnerable to fisheries overexploitation than at continental slope habitats (chapter 3), and may subsequently be able to sustain lower fishing pressures. It also inferred that sharks and chimaera have a complex ecological role on continental slope ecosystems (chapter 4), and supported the existence of pupping grounds for two vulnerable deep-water shark species in lower latitude environments and the possibility of large scale, ontogenetically linked, latitudinal migrations (chapter 5). Due to the limitations of stable isotope data, it is essential that inferences on migratory movement behaviours and geographic habitat use (chapter 5) be validated by further deployment of telemetry technologies in other regions (Rodríguez-Cabello and Sánchez, 2014, Rodríguez-Cabello *et al.*, 2016), and improved deep-sea surveys at lower latitudes (Menezes *et al.*, 2015, Pajuelo *et al.*, 2016).

Satellite telemetry studies of deep-sea sharks are currently challenging due to high mortality rates sustained during fishing, and the inability to obtain satellite transmissions of locational information from underwater (in deep-water habitats). One potentially useful tag would be similar to an archival pop-off tag, like those used in Fisk *et al.* (2012) and Rodríguez-Cabello *et al.* (2016), but whereby the tag could release multiple geolocatory units over a prolonged period of time. While coarse in resolution, this type of tag may provide more detailed information on movement directions and the timings. Another useful tagging experiment could be the adoption of acoustic telemetry tags and the installation of acoustic receiver ‘curtains’ or ‘gates’ along the continental slope, similar to Daley *et al.* (2015). In an internationally co-ordinated effort, acoustic receiver curtains could be deployed at multiple locations along the continental slope (from Rockall Trough to Western Africa) to test the hypothesis of long-distance along slope migrations. While both studies are ambitious, with advances in telemetry technologies, they are areas to consider if our understanding of movement behaviours of deep-water sharks is to be improved.

If migratory movements do cross international boundaries, vulnerable deep-water shark species may be exposed to currently higher levels of fishing pressure than realised, especially for important life history stages (i.e. pregnant females and juveniles). In preliminary assessments of Moroccan fish landing ports by a UK-based charity Fin Fighters (http://www.finfighters.org), high numbers of deep-water sharks have been observed in multiple fish markets (Figure 6.2). This is of concern, as it provides clear evidence that despite not being landed in European waters, protected deep-sea species are being landed in considerable numbers in adjacent waters. These sharks were fished using long-lines in mixed fisheries, but the high numbers of processed carcasses suggest they may be targeting these fish (Figure 6.2). The species identified are mainly large squaliform sharks that we predict undertake large-scale migrations. This is of concern that these captured individuals may represent ecological important components of both local, and European, deep-sea ecosystems. It also highlights the complex nature of managing shark populations along continental slope habitats that transcend political boundaries, and the impact population depletions could have on the livelihood of local artisanal fishers.

As of December of 2016, the TAC management quotas were amended for deep-sea sharks, allowing European fisheries to land 10 tonnes of sharks in 2017 and 2018 for scientific research (Council Regulation (EU) 2016/2285; (The European Commission, 2016)). The enforcement of this new landing limit will have to be strictly monitored but the data obtained from these data could help improve the assessment of these stocks that may enable the development of sustainable fishing quotas.

6.4 Chondrichthyan Stable Isotope Data Project

Moving forward, the global shark stable isotope dataset assembled in chapter 2 will form the foundation for the ‘Chondrichthyan Stable Isotope Data Project’. This internationally collaborative group will look to maximise the use of the global compilation analysed here, and use these data to address other macro-ecological questions such as latitudinal niche breadth hypotheses (Vázquez and Stevens, 2004) or spatial variations in trophic levels (Duffy *et al.*, 2017). The project will provide a central depository of shark stable isotope data, which hopes to provide researchers access to global stable isotope data, and contact information of research scientists with data holdings. There has already been interest in developing research papers looking at stable isotopic niches area, ontogenetic diet shifts, and global trends in δ^{15}N values. It is hoped that these data can support local and global ecological studies long into the future, providing an invaluable data resource that will continue beyond this thesis research.

Figure 6.2: Collection of dogfish (Squaliformes) carcasses observed in fish markets in Morocco in 2015 by the charity Fin Fighters. Some species identified as *Deania sp*, *Centrophorus sp* and *Centroscymnus sp.* Used with permission from Fin Fighters.

Appendices

Appendix A

Appendix A.1: Primary habitat assignment for each shark species sampled in chapter 2 with corresponding paper provided. Those data points excluded from study (see Methods 2.3) indicated in parenthesis. Asterisks denote species that are likely to migrate over multiple habitats and appear in Figure 2.3.

Habitat	Species	*n*	Reference
Shelf	*Asymbolus rubiginosus*	3	(Abrantes and Barnett, 2011)
3231 (3412)	*Carcharhinus amblyrhynchos*	32	(Frisch *et al.*, 2016)
		10	(Speed *et al.*, 2012)
	Carcharhinus amboinensis	9	(Hussey *et al.*, 2015)
		10	(Kinney *et al.*, 2011)
	Carcharhinus brachyurus	4	(Hussey *et al.*, 2015)
		6	de Necker unpub
	Carcharhinus brevipinna	24	(Hussey *et al.*, 2015)
	Carcharhinus cautus	1	(Kinney *et al.*, 2011)
		5	(Vaudo and Heithaus, 2011)
	Carcharhinus fitzroyensis	8	(Kinney *et al.*, 2011)
	Carcharhinus galapagensis	12	(Papastamatiou *et al.*, 2015)
	*Carcharhinus leucas**	58	Brunnschweiller unpub
		18	(Daly *et al.*, 2013)
		11	(Hussey *et al.*, 2015)
		0 (125)	(Matich *et al.*, 2011)
	Carcharhinus limbatus	32	(Hussey *et al.*, 2015)
		36	(Plumlee and Wells, 2016)
	Carcharhinus macloti	10	(Kinney *et al.*, 2011)
	Carcharhinus melanopterus	45	(Frisch *et al.*, 2016)
		1	(Kinney *et al.*, 2011)
		63	(Papastamatiou *et al.*, 2010)
		53	(Speed *et al.*, 2012)
	Carcharhinus obscurus	1	(Frisch *et al.*, 2016)
		64	(Hussey *et al.*, 2015)
	Carcharhinus perezii	111 (132)	(Maljković and Côté, 2011)
		2	(Tilley *et al.*, 2013)
	Carcharhinus plumbeus	257	(Shiffman *et al.*, 2014)
	Carcharhinus signatus	4	(Churchill *et al.*, 2015b)
		3	(Tilley *et al.*, 2013)
	Carcharhinus sorrah	9	(Borrell *et al.*, 2010)
		10	(Kinney *et al.*, 2011)
	Carcharhinus tilstoni/limbatus	11	(Kinney *et al.*, 2011)

Carcharias plumbeus	6	(Hussey *et al.*, 2015)
Carcharias taurus	30	(Hussey *et al.*, 2015)
*Carcharodon carcharias**	4	(Abrantes and Barnett, 2011)
	21	(Carlisle *et al.*, 2012)
	36	(Hussey *et al.*, 2015)
	7	de Necker unpub
	26	(Malpica-Cruz *et al.*, 2013)
Cephaloscyllium laticeps	3	(Abrantes and Barnett, 2011)
Chiloscyllium plagiosum	80	(Wai *et al.*, 2011)
Chiloscyllium punctatum	21	(Vaudo and Heithaus, 2011)
Figaro boardmani	3	(Abrantes and Barnett, 2011)
	2	(Pethybridge *et al.*, 2012)
*Galeocerdo cuvier**	5	(Churchill *et al.*, 2015b)
	4	(Frisch *et al.*, 2016)
	18	(Hussey *et al.*, 2015)
Galeorhinus galeus	21	(Abrantes and Barnett, 2011)
	1	(Chouvelon *et al.*, 2012)
	3	(Kopp *et al.*, 2015)
	8	de Necker unpub
Haploblepharus edwardsii	5	de Necker unpub
Haploblepharus pictus	3	de Necker unpub
Heterodontus portusjacksoni	3	(Abrantes and Barnett, 2011)
Heptranchias perlo	2	(Shipley *et al.* 2017)
*Hexanchus griseus**	2	(Chouvelon *et al.*, 2012)
	9	(Churchill *et al.*, 2015b)
*Hexanchus nakamurai**	1	Kiszka unpub
	4	(Shipley *et al.* 2017)
*Lamna ditropis**	36	(Carlisle *et al.*, 2015)
*Lamna nasus**	7	(Cherel *et al.*, 2017)
	1	Chouvelon & Bustamente unpub
	3	(Teffer *et al.*, 2014)
Loxodon macrorhinus	83	(Kiszka *et al.*, 2014)
Mustelus antarcticus	48	(Abrantes and Barnett, 2011)
Mustelus asterias	11	(Chouvelon *et al.*, 2012)
	4	(Kopp *et al.*, 2015)
Mustelus canis-insularis	6	(Shipley *et al.* 2017)
Mustelus cf canis	6	(Churchill *et al.*, 2015b)

Mustelus manazo	10	(Borrell *et al.*, 2010)
Mustelus mustelus	4	(Chouvelon *et al.*, 2012)
	10	(Kopp *et al.*, 2015)
	9	de Necker unpub
Mustelus palumbes	2	de Necker unpub
Nebrius ferrugineus	7	(Frisch *et al.*, 2016)
Negaprion acutidens	6	(Frisch *et al.*, 2016)
	4	(Speed *et al.*, 2012)
Notorynchus cepedianus	140	(Abrantes and Barnett, 2011)
	39	de Necker unpub
	1	(Pethybridge *et al.*, 2012)
Poroderma africanum	11	de Necker unpub
Poroderma pantherinum	3	de Necker unpub
Pristiophorus cirratus	21	(Raoult *et al.*, 2015)
Pristiophorus nudipinnis	4	(Abrantes and Barnett, 2011)
	25	(Raoult *et al.*, 2015)
Pristis pectinata	2	(Borrell *et al.*, 2010)
Rhizoprionodon acutus	7	(Al-Reasi *et al.*, 2007)
	11	(Borrell *et al.*, 2010)
	3	(Hussey *et al.*, 2015)
	9	(Kinney *et al.*, 2011)
Rhizoprionodon taylori	10	(Kinney *et al.*, 2011)
	149	(Munroe *et al.*, 2015)
Rhizoprionodon terraenovae	50	(Plumlee and Wells, 2016)
Schroederichthys bivius	3	(Ciancio *et al.*, 2008)
Scoliodon laticaudus	220	(Wai *et al.*, 2011)
Scyliorhinus canicula	29	(Caut *et al.*, 2013)
	10	(Chouvelon *et al.*, 2012)
	25	(Cresson *et al.*, 2014)
	36	(Kopp *et al.*, 2015)
	72	(Valls *et al.*, 2014)
Scyliorhinus meadi	1	(Shipley *et al.* 2017)
Scyliorhinus stellaris	10	(Kopp *et al.*, 2015)
*Somniosus antarcticus**	20	(Cherel *et al.*, 2017)
*Somniosus microcephalus**	8	(Hansen *et al.*, 2012)
*Somniosus pacificus**	146 (159)	(Courtney and Foy, 2012)
Sphyrna lewini	24	(Bethea *et al.*, 2011)
	8	(Borrell *et al.*, 2010)
	38	(Hussey *et al.*, 2015)
	4	(Kinney *et al.*, 2011)

		44	(Kiszka *et al.*, 2014, Kiszka *et al.*, 2015)
		61	(Rojas *et al.*, 2014)
		8	(Li *et al.*, 2014)
		6	(Li *et al.*, 2016a)
	Sphyrna tiburo	24	(Bethea *et al.*, 2011)
		50	(Plumlee and Wells, 2016)
	Sphyrna zygaena	15	(Hussey *et al.*, 2015)
		2	de Necker unpub
		20	(Li *et al.*, 2016a)
	Squalus acanthias	20	(Abrantes and Barnett, 2011)
		7	(Chouvelon *et al.*, 2012)
		3	(Ciancio *et al.*, 2008)
		75	(Endo *et al.*, 2009)
		5	de Necker unpub
		2	(Pethybridge *et al.*, 2012)
		99	Quaeck unpub
	Squalus cubensis	22	(Shipley *et al.* 2017)
	Squalus suckleyi	76 (98)	(Reum and Essington, 2013)
	Stegostoma fasciatum	1	(Borrell *et al.*, 2010)
	Triaenodon obesus	9	Brunnschweiller unpub
		31	(Frisch *et al.*, 2016)
		4	(Speed *et al.*, 2012)
Oceanic	*Alopias pelagicus*	5	(Kiszka *et al.*, 2014)
676 (703)		40	(Polo-Silva *et al.*, 2013)
		13	(Li *et al.*, 2016a)
	Alopias superciliosus	7	(Li *et al.*, 2014)
	*Alopias vulpinus**	1	(Abrantes and Barnett, 2011)
		1	(Hussey *et al.*, 2015)
		28	(Teffer *et al.*, 2014)
	Carcharhinus falciformis	2	(Churchill *et al.*, 2015b)
		10	(Kiszka *et al.*, 2014, Kiszka *et al.*, 2015)
		82	(Radabaugh *et al.*, 2013)
		19	(Li *et al.*, 2014)
		46	(Li *et al.*, 2016a)
	*Carcharhinus longimanus**	13	(Kiszka *et al.*, 2015)
		13	(Madigan *et al.*, 2012)
		5	(Li *et al.*, 2014)

		7	(Li *et al.*, 2016a)
	Isurus oxyrinchus	4	(Hussey *et al.*, 2015)
		29	(Kiszka *et al.*, 2015)
		1	de Necker unpub
		10	(Madigan *et al.*, 2012)
		24	(Malpica-Cruz *et al.*, 2013)
		14	(Teffer *et al.*, 2014)
		5	(Li *et al.*, 2016a)
	Megachasma pelagios	0 (1)	Wyatt unpub
	Prionace glauca	38	Bustamente unpub
		24	Chouvelon & Bustamente unpub
		31	(Kiszka *et al.*, 2015)
		10	(Carlisle *et al.*, 2012)
		2	de Necker unpub
		9	(Madigan *et al.*, 2012)
		11	(Malpica-Cruz *et al.*, 2013)
		121	(Radabaugh *et al.*, 2013)
		18	(Li *et al.*, 2014)
		28	(Li *et al.*, 2016a)
	Pseudocarcharias kamoharai	5	(Kiszka *et al.*, 2015)
	Rhincodon typus	0 (3)	(Hussey *et al.*, 2015)
		0 (19)	(Borrell *et al.*, 2010)
		0 (4)	Wyatt unpub
Deep-sea 1487	*Apristurus aphyodes*	20	Bird unpub
		5	(Trueman *et al.*, 2014)
	Apristurus brunneus	2	(Boyle *et al.*, 2012)
	Apristurus laurussonii	15	Bird unpub
		13	(Trueman *et al.*, 2014)
	Apristurus manis	3	Bird unpub
		4	(Trueman *et al.*, 2014)
	Apristurus melanoasper	8	Bird unpub
		9	(Trueman *et al.*, 2014)
	Apristurus microps	21	Bird unpub
	Apristurus sinensis	1	(Pethybridge *et al.*, 2012)
	Apristurus sp.	7	Bird unpub
	Bythaelurus canescens	3	(Lopez *et al.*, 2013)
	Centrophorus cf. granulosus	51	(Churchill *et al.*, 2015b)
		1	(Iitembu and Richoux, 2015)
	Centrophorus moluccensis	16	(Le Bourg *et al.*, 2014)
	Centrophorus niaukang	40	(Churchill *et al.*, 2015b)
	Centrophorus sp.	9	(Shipley *et al.* 2017)
	Centrophorus squamosus	43	Bird unpub

	18	(Iitembu and Richoux, 2015)
Centrophorus zeehaani	2	(Pethybridge *et al.*, 2012)
Centroscyllium fabricii	17	Bird unpub
	15	(Trueman *et al.*, 2014)
Centroscymnus coelolepis	100	Bird unpub
	25	(Newman *et al.*, 2011)
	2	(Pethybridge *et al.*, 2012)
	15	(Trueman *et al.*, 2014)
Centroscymnus crepidater	22	Bird unpub
	5	(Chouvelon *et al.*, 2012)
	3	(Colaço *et al.*, 2013)
	21	(Pethybridge *et al.*, 2012)
	26	(Trueman *et al.*, 2014)
Centroscymnus owstonii	9	Bird unpub
	1	(Churchill *et al.*, 2015b)
	2	(Pethybridge *et al.*, 2012)
Centroscymnus plunketi	2	(Pethybridge *et al.*, 2012)
Dalatias licha	2	(Chouvelon *et al.*, 2012)
	2	(Pethybridge *et al.*, 2012)
Deania calcea	88	Bird unpub
	10	(Chouvelon *et al.*, 2012)
	2	(Churchill *et al.*, 2015b)
	9	(Iitembu and Richoux, 2015)
	2	(Pethybridge *et al.*, 2012)
	25	(Trueman *et al.*, 2014)
Deania profundorum	64	Bird unpub
	4	(Chouvelon *et al.*, 2012)
	5	(Colaço *et al.*, 2013)
	14	(Iitembu and Richoux, 2015)
Etmopterus baxteri	20	(Pethybridge *et al.*, 2012)

Etmopterus bigelowi	11	(Churchill *et al.*, 2015b)
Etmopterus granulosus	17	(Cherel *et al.*, 2017)
Etmopterus princeps	16	Bird unpub
	52	(Newman *et al.*, 2011)
Etmopterus pusillus	23	Bird unpub
	4	(Colaço *et al.*, 2013)
Etmopterus spinax	99	Bird unpub
	10	(Chouvelon *et al.*, 2012)
	6	(Colaço *et al.*, 2013)
Galeus arae	1	(Churchill *et al.*, 2015b)
Galeus melastomus	9	Bird unpub
	12	(Chouvelon *et al.*, 2012)
	73	(Cresson *et al.*, 2014)
	64	(Valls *et al.*, 2014)
Galeus murinus	6	Bird unpub
Scymnodon ringens	34	Bird unpub
Squalus cubensis	30	(Churchill *et al.*, 2015b)
Squalus megalops	2	(Abrantes and Barnett, 2011)
	57	(Le Bourg *et al.*, 2014)
	2	(Pethybridge *et al.*, 2012)
Squalus mitsukurii	140	(Churchill *et al.*, 2015b)
	12	Kiszka unoub
	2	(Pethybridge *et al.*, 2012)
Zameus squamulosus	2	Kiszka unpub
Grand Total:	*5394 (5561)*	

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