The trophic dynamics of the broadnose sevengill shark *(Notorynchus cepedianus)* in False Bay, South Africa, using multiple tissue stable isotope analysis.

Leigh de Necker

DNCLEI001

Thesis presented for the degree of Master of Science

Department of Biological Sciences

University of Cape Town

March 2017

MSc candidate:

Leigh de Necker
1. Department of Biological Sciences, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa

2. Shark Spotters, P. O. Box 22581, Fish Hoek, 7974, South Africa
leighdenecker@gmail.com

Primary supervisor:

Prof. M. Justin O’Riain
Department of Biological Sciences, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa
Justin.ORiain@uct.ac.za

Co- supervisor:

Dr Alison Kock
1. South African Institute for Aquatic Biodiversity, Private Bag 1015, Grahamstown 6140, South Africa.

2. Shark Spotters, P. O. Box 22581, Fish Hoek, 7974, South Africa
Alison.Kock@gmail.com

Co- supervisor:

Dr Matthew Lewis
Department of Archaeology, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa
matt.lewis.uct@gmail.com

Co-supervisor:

Dr Adam Barnett
College of Marine & Environmental Sciences, James Cook University, Cairns, Queensland, 4878, Australia
adam.barnett@jcu.edu.au

DECLARATION

I, Leigh de Necker, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed by candidate

Signature Removed

Leigh de Necker

ETHICS STATEMENT

Animal ethics for capture, sampling and release was obtained from the University of Cape Town (2010/V13/AH) and South African Institute for Aquatic Biodiversity (2015-06).

Signed by candidate

Signature Removed

Leigh de Necker

ABSTRACT

Despite their important ecological role, there is limited quantitative information on the trophic ecology of large, apex predator sharks. This is largely a consequence of their occupying naturally low population densities, being highly mobile and elusive and ranging over large distances. Stable isotopes provide a low cost, non-lethal method for investigating the short and long-term diet of a predator, which when combined with prey data can be used to understand the trophic interactions and the potential regulatory effects they may have on the structure and function of marine ecosystems. In this study, I used non-lethal stable isotope analysis to investigate the trophic dynamics of sevengill sharks, *Notorynchus cepedianus*, within False Bay, South Africa. A total of 39 muscle biopsies (33 female, six male), and 28 blood plasma samples (25 female, three male) were collected from sevengill sharks. These were analysed together with 161 prey samples from 32 different species, including cephalopods, crustaceans, teleosts, chondrichthyans and marine mammals. In addition, seven white shark, *Carcharodon carcharias,* muscle samples were collected from False Bay and analysed for comparison with those of sevengills. Sevengills in False Bay had the highest $\delta^{15}N$ values of all species sampled in this study (including white sharks) and appear to feed predominantly on a variety of coastal prey species from various functional groups. A stable isotope mixing model revealed that inshore chondrichthyans were their most important prey, with Cape fur seals and inshore teleost species also being important prey groups. There was no apparent seasonal shift in the diet of sevengills, despite clear seasonal aggregation in coastal kelp forests during the summer months. $\delta^{15}N$ decreased significantly with sevengill size, with immature females having higher $\delta^{15}N$ values than mature females, while $\delta^{13}C$ increased with sevengill size. These trends are hypothesised to be linked to dietary shifts associated with the relative use of different habitat types when individuals become sexually mature. There is a need to combine stable isotope data with movement patterns and habitat use to better understand the relationship between isotope ratios and habitat use. Ongoing research on sevengill and white shark movement patterns in False Bay, together with the results presented in this study, will provide important information on the trophic and ecological role that two top predatory sharks play in False Bay.

Keywords: *Notorynchus cepedianus*, sevengill shark, stable isotopes, trophic ecology

ACKNOWLEDGEMENTS

Firstly, I would like to thank my primary supervisor, Justin O'Riain, for believing in me and believing in this project. I would have been completely lost without your constant encouragement and guidance. Whenever I lost my way, you were always there to give me direction and put me back on track with my work. Thank you for the invaluable knowledge you contributed to this process and further, for trusting me and my ability to not entirely make a mess of this opportunity. To my co-supervisor, Alison Kock, I do not really know where to begin. I am very selective of my role models, but you are without a doubt, one of the most influential people in my life. Your passion and love for sharks is truly inspiring and having the opportunity to share this with you and learn from you, has been the most incredible experience. Thank you for your endless support and for always being available for my silly questions- for not only being a great teacher, but a great friend. I appreciate your sincerity and kindness and for always trusting in my passion, when my intellect (and sanity) was hard to find. I would also like to thank Adam Barnett, for all the time and effort put into reading through some of the terrible drafts I forwarded for review. Adam, your knowledge and thorough feedback has proven to be invaluable to this work and for that I am truly grateful. A further thank you to Katja Abrantes for all your input and expertise. Finally, to Matthew Lewis, thank you for all the time you put into helping me make sense of stable isotopes and providing me with all the contacts and resources I needed for both my lab work and the dreaded statistics and models that followed. You made what was could have been a terrifying and daunting task, turn out to be months of rather enjoyable and therapeutic lab work. Further, thank you for your genius in SIAR and for helping me run the models over and over and over again until I was satisfied with my prey groupings. Your patience is truly appreciated as well as your encouragement when I doubted my ability to take on stable isotopes.

Funding for this project was primarily made possible from the Two Oceans Aquarium in collaboration with Woolworths (through sales of the "shark/seventy-four" Ocean Promise reusable shopping bag), with additional financial or equipment support from the University of Cape Town and Save Our Seas Foundation. Further, I would like to thank the National Research Foundation (NRF) for funding my tuition and personal

living expenses. Without these contributions, the opportunity for me to do this dream project would not be possible.

A special mention and thanks to Nigel Hussey, for his prompt, thorough and enthusiastic responses to my panicked emails in trying to make sense of lipid extraction in shark plasma. Further, for sharing isotope data on Cape fur seals and white sharks in South Africa, without hesitation. I am honoured to have the expert in the field of shark stable isotopes make such a willing and enthusiastic contribution to my work. Your humility and charisma are truly admirable.

I would like to thank Lab 3.20, for their interest in my work, constant enthusiasm and endless amounts of humour during my brief visits to campus. I would also like to thank the University of Cape Town for all the resources and facilities used as part of this project. Thanks to the Biological Sciences Systematics lab for facilitating part of my sample preparations. A massive thank you to Ian Newton, John Lanham and the UCT Archaeology stable isotope lab. Ian and John, thank you for all your help and guidance in answering my questions relating to the preparation and processing of my samples, as well as running the samples and returning the results even when I had unreasonable deadlines. I am truly grateful to you and your willingness to assist me on demand throughout the days- weeks- months I camped out in your lab. You made the most daunting, yet one of the most important parts of my research a lot less stressful than it could have been.

A big thank you to Shark Spotters for taking me in as part of their team. I am privileged and so proud to be associated with such an awesome organization. A special mention and thanks must go to our research technician, Dave van Beuningen. As a team of three females and one Dave, you had to put up with a lot. Thank you for being eager to lend a hand where ever and whenever we needed you, for fixing all the things we broke and/or don’t know how to fix, and for making really cool gadgets to make our research easier. Fieldwork would have been a lot more challenging without your skills and odd but sharp sense of humour.

Another important thank you to the team from the Two Oceans Aquarium- Georgina Cole, Kevin Spiby, Deen Hill, Simon Brill, Nicholas Nicolle, Paul van Nimwegen and Tinus Beukes. Without you, I would not have my all-important sevengill shark muscle and plasma samples so a massive thank you for your time, effort, patience, expertise and muscle power in safely catching, sampling and releasing the stars of this research. I would like to thank Charlene Da Silva from DAFF, for samples of some of the more challenging to collet chondrichthyan prey species. Tamzyn Zweig and Meaghan McChord from the South African Shark Conservancy for their contribution of prey samples. Thanks to Nicola Okes, Gary Carstens and the friendly local Kalk Bay fishermen for their contributions of prey samples. Thank you to Alison Kock and Adrian Hewitt, for their invaluable contribution of white shark samples. A special thank you to Bennie De Bruin, from Mandurah Seafoods, for his keen interest in this research and willingness to contribute samples from key prey species.

A very special and sentimental thank you to my family. Firstly, my mom, Sue- my passion and love for the ocean comes straight from you. To my dad, without your sacrifices and financial assistance I would not be close to where I am today, not only in my career, but in all areas of life. To my little sister, Taryn, for always making me feel like a superhero, and like my achievements and hard work matter and are noticed and that I have the ability to inspire the most important people in my life. Thank you my beautiful family, that no matter how tough things get and through all the hard times, you have always encouraged me to follow my heart and follow my dreams. You have believed in me and been proud of me through my successes and failures. I love you and am grateful beyond words for your unconditional love. To my uncle, Robbie Miller and cousin, Calven Miller, for getting me off my feet at the start of this adventure and my life in Cape Town. Furthermore, through this journey I have had a number of people who I adopted as parents since I moved away from home, Leon and Carol Jordaan, Petronel and Bennie Potgieter, Gavin Goldblatt, Tessa and Dennis Valdes and Charmaine Carstens. I cannot express how grateful I am to you for the occasional meal, accommodation and transport. Your kindness and generosity, without obligation, as well as overall good life advice and support is something money cannot buy.

Another very important and white shark sized thank you to Morne Hardenberg and Stephen Swanson of Shark Explorers. Thank you for taking the chance on an overly enthusiastic scuba diver girl a few years ago, who wanted nothing more than to live her dream of working with sharks. Thank you for recognizing my raging passion and allowing me to step into a reality that turned out to be more amazing than my wildest dreams. You opened up the door that led to the opportunity for me to do this project and set me on the path to an amazing future of working with these animals I love so much. Thank you for being my shark family and for believing in me. To the rest of the Shark Explorers team, thank you for sharing this passion and for all the love and enthusiasm, Monique Olivier, Nina Daniels, Marty Schouten, and Brother E. Thank you to Rob and Karen Lawrence for thinking that I am kind of smart and that my passion, love and respect for sharks would be useful to African Shark Eco- Charters. I am truly grateful for the opportunity to share my skills and knowledge through working with you and white sharks.

To my friends who have become like family over the past few years, there is a long list. Each one has made a special, unique contribution in my life and through this indescribable journey. Thank you for the fantastic people you are, for your endless support, and for maintaining my sanity while also allowing me the space to express my insanity. I cannot thank you enough, Gary Carstens, Cailyn Sonderup, Tia Jordan, Roxi Walmsley, Gareth Jordaan, Monique Olivier, Gideon Strydom, Charles Kinzer, Chi Ingledew, Jess Joyner, Jessica Comley, and my underwater hockey team mates. Thank you for your constant interest in my work and concern over my temperamental state of mind and for allowing me to share the highs and lows of this process with you! I am truly blessed and endlessly grateful.

Lastly, to my best friend, the Orc, Tammy Engelbrecht. We started this journey into sevengill shark research together and nobody understands the rollercoaster ride its been better than you. You know the excitement that comes with the highs of the fieldwork and successful sample collections, and you understand the lows of failed attempts in the lab and the red ink spilt on your thesis after your supervisor has reviewed it. You have been the person to get me through the toughest parts, when I've felt stupid, self-esteem gone, wanting to give up, cry, scream, run away, you were there to motivate me, encourage me and support me like only you know how sometimes with tough love, other times with compassion, good food and wine. Not only have you been the greatest support for this thesis, but in every area of my life over the past few years. Thank you for putting up with me. I could write a whole new thesis on how grateful I am to you, but without getting overly sentimental, I just want to say thank you, for being the best friend and non-biological shark sister ever!

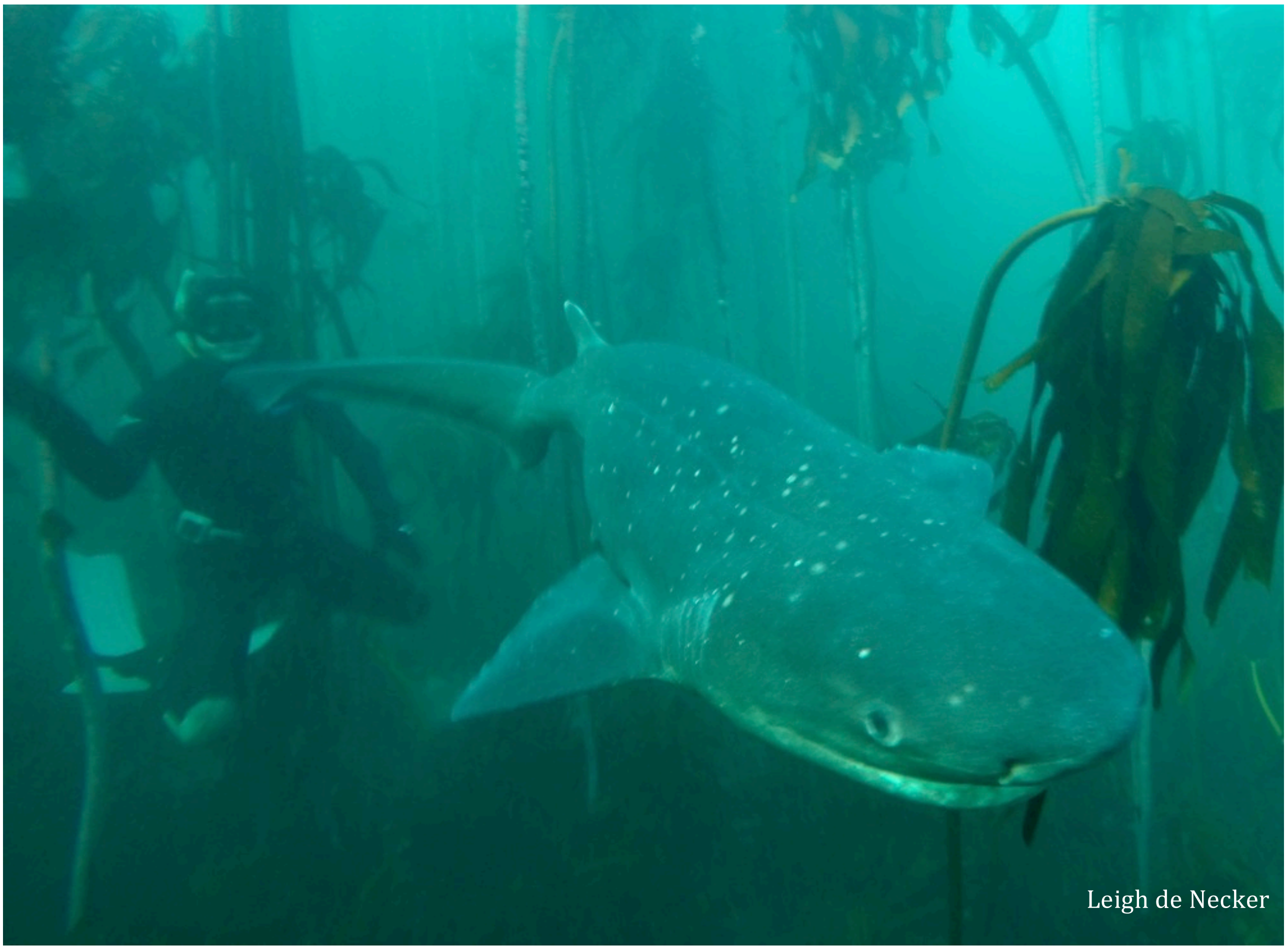
TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION



Leigh de Necker

1.1. The role of apex predators in marine ecosystems

Apex predators play an important part in preserving the health, diversity and stability of ecosystems (Estes *et al*. 1998; Heithaus *et al*. 2008; Baum & Worm 2009, Barnett *et al*. 2012). The removal of top predators from marine food webs can lead to trophic cascades that could ultimately alter ecosystem structure and function (Williams *et al.* 2004, Dulvy *et al.* 2008, Heithaus *et al.* 2008, Baum & Worm 2009, Quevedo *et al.* 2009). For example, Estes *et al.* (1998) found that the deforestation of kelp beds by sea urchins in the nearshore ecosystems of western Alaska, was the ultimate consequence of cascading effects of the rapid decline of sea otters, the primary regulators of the sea urchin population.

Large predatory sharks consume marine mammals, large teleosts and other chondrichthyan species (Ferretti *et al.* 2010), and therefore occupy trophic positions at, or near, the top of marine food webs (Stevens *et al.* 2000, Heithaus *et al.* 2008). Despite their important ecological role, there is limited quantitative information available on the trophic ecology of large, apex predator shark species (Wetherbee & Cortés 2004, Braccini 2008). This is largely a consequence of the difficulties associated with studying species that occur at naturally low population densities, are generally highly mobile and elusive and range over large distances (Heithaus *et al.* 2002, Matich & Heithaus 2014). Quantifying the spatial and temporal feeding ecology of these species is essential for understanding trophic interactions and the potential regulatory effects they may have on the structure and function of marine ecosystems (Braccini 2008, Barnett *et al.* 2010a, Hussey *et al.* 2012a). Furthermore, such information is vital for setting up integrated and effective conservation and management strategies for predators and ecosystems.

1.2. Analysing stomach contents and stable isotopes to understand trophic dynamics in sharks

Biologists have resorted to indirect methods to understand trophic relationships amongst large marine species, including gut content analysis and more recently, stable isotope analysis from a variety of animal tissues. Stomach content analysis has been the most popular method employed in studies investigating the diet and trophic dynamics of sharks (Wetherbee & Cortés 2004). There are however a number of limitations associated with this method, foremost of which is the difficulty associated with sampling large, living predators (Madigan *et al.* 2012). Consequently, most stomach content studies have relied on lethal sampling methods (Ebert 2002, Crespi-Abril *et al.* 2003, Lucifora *et al.* 2005), which provides only a snapshot of an individual's diet. In addition, many sharks are captured with empty stomachs and unidentifiable prey items, therefore, large sample sizes are invariably required to provide a representative diet for a given species (Wetherbee & Cortés 2004). For threatened and protected species in particular, lethal sampling is not recommended.

Stable isotope analysis provides a practical and cost effective alternative, or complementary method, addressing many of the limitations of stomach contents for evaluating dietary and trophic relationships in marine systems (Domi *et al.* 2005, Martinez del Rio *et al.* 2009, Shiffman *et al.* 2012, Hussey *et al.* 2012a,b). Predator stable isotope values reflect those of assimilated nutrients from ingested prey integrated over longer time periods, thus providing insights into long-term diet (Domi *et al.* 2005, MacNeil *et al.* 2006). Furthermore, analysis of isotope values of a predator may reveal whether an animal's stable isotope values are near equilibrium with their prey and the dietary breadth or niche calculations of a population can be made to highlight intra- and inter-individual variation in resource use (MacNeil *et al.* 2005, Hussey *et al.* 2012a, Matich & Heithaus 2014, Matich *et al.* 2015). Stable isotope research can therefore provide a better understanding of a species' role within the ecosystem, and may thus prove more informative to conservation and management strategies (Hussey *et al.* 2012a).

1.2.1. Carbon and nitrogen stable isotopes

Stable isotope analysis is based on the premise that, through normal metabolic functions, the heavier, rare isotope of an organism is retained, whilst the lighter, more common isotope is excreted (Post 2002). Specific ratios of heavy to light isotopes indicate particular resource use which may be used to infer both diet and/or habitat. By analysing a particular tissue/s, the isotopic value of an individual can be determined, and once compared with other organisms within the relevant trophic system, the trophic position (in both level and habitat) in the food web can be estimated (Post 2002, Boyle *et al.* 2012).

Carbon and nitrogen are the most commonly used elements for examining an organism's diet and trophic structure, as they provide clear indications of the dietary niche of a consumer (Martinez del Rio *et al.* 2009, Hussey *et al.* 2012a). Ratios of heavy-to-light carbon and nitrogen isotopes increase with trophic transfers, providing a chemical tracer for links in diet (Logan & Lutcavage 2010). Although carbon isotope ratios (^{13}C:^{12}C) vary among different primary producers, they stay reasonably constant as they move from prey to predator providing researchers with an indication of a consumer's original source of dietary carbon (Estrada *et al.* 2006). Ratios of ^{13}C:^{12}C may therefore be used to infer a consumer's primary prey, the food chain in which it is foraging, habitat use and migration patterns (Estrada *et al.* 2006, Newsome *et al.* 2010, Hussey *et al*. 2012a).

The marked increase in nitrogen isotope ratios (^{14}N:^{15}N) from predator to prey serves as a good indicator of an organism's relative trophic position within a food web. $\delta^{15}N$ has been used as a direct indicator of the trophic level of the predator (Post 2002). However, recently it has been suggested that caution be used in using $\delta^{15}N$ as a direct indicator of trophic level due to variability that exists in nitrogen discrimination factors as well as biases linked with isotopic ratios of the trophic baseline (Cresson *et al*. 2014, Hussey *et al*. 2014). However, within a constrained system, inferences can be made on relative trophic position using $\delta^{15}N$ values.

Together, carbon and nitrogen stable isotopes allow for the inference of diet, foraging ecology and food web dynamics and structure (Estrada *et al.* 2006, Hussey *et al.* 2012a, Shiffman *et al.* 2012). For example, Estrada *et al.* (2003) used this approach and found the stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in the tissues of mako sharks, *Isurus oxyrinchus,* were closely related to those of their prey. Variation in isotopic ratio values amongst individual members of these pelagic predators was attributed to differences in the habitat that they were foraging in i.e. inshore versus offshore waters (Estrada *et al.* 2003).

1.2.2. Tissue types

Limited research has been done to understand how stable isotopes pass through the digestive system and how they are incorporated into the tissues of sharks, but it has been shown that stable isotope values are correlated with metabolic turnover rates of particular tissues (MacNeil *et al.* 2005). This implies that the stable isotopes of each metabolically distinct tissue can be used as a representation of a different feeding period (MacNeil *et al.* 2005). Metabolically active tissues with different turnover rates can therefore be compared in order to examine temporal dietary shifts (Logan & Lutcavage 2010, Matich & Heithaus 2014). By comparing $\delta^{15}N$ and $\delta^{13}C$ values from different tissues (liver, muscle and cartilage), MacNeil *et al.* (2005) found that multiple-tissue sampling is able to capture detailed feeding relationships and seasonal feeding dynamics of sharks and their particular prey. Sampling multiple tissues with different isotopic incorporation rates from an individual provide insights not only into foraging and diet over varying temporal scales, but also highlight seasonal dietary switching or shifts, habitat use and potential resource partitioning within a population.

Comparisons between different tissues, should be conducted with caution, since stable isotope values of consumer tissues are different to those of their prey due to isotopic discrimination and the same organism can have different discrimination factors in different tissues (Hussey *et al.* 2010a, Hussey *et al.* 2012a). It is therefore important to understand the isotopic discrimination values of the different tissues being used when examining temporal variability in trophic interactions (Hussey *et al.* 2012a, Matich & Heithaus 2014).

The most commonly sampled tissue for stable isotope analyses in elasmobranchs is white muscle tissue (Hussey *et al.* 2012a). The growth rate of most elasmobranch species is slow compared to teleosts and therefore white muscle from sharks can provide a long-term integrated measure of their feeding habits (Hussey *et al.* 2012a). Stable isotope values ($\delta^{15}N$ and $\delta^{13}C$) of muscle tissue have been used to infer trophic position (Estrada *et al.* 2003, Hussey *et al.* 2012a), variation in diet and resource use of different species (Domi *et al.* 2005, Kim *et al.* 2011), the role of a species in food webs and whole ecosystems (Abrantes & Barnett 2011), and to elucidate movement patterns (Hobson 1999, Abrantes & Barnett 2011, Hussey *et al.* 2011). Furthermore, muscle tissue can be sampled from multiple individuals of varying size, either sex and different life stages of maturity for an integrated assessment of ontogenetic dietary shifts (Papastamatiou *et al*. 2010, Abrantes & Barnett 2011, Hussey *et al*. 2011).

Work on elasmobranchs to date indicates that muscle tissue has an isotopic turnover rate of 390 - 540 days (Matich *et al.* 2011), whole blood turns over between 240 - 300 days (MacNeil *et al.* 2006, Matich *et al.* 2011) and plasma has a faster isotopic turnover rate of 72 - 102 days (Kim *et al.* 2012a, Matich *et al*. 2011). Therefore, stable isotope analysis of blood plasma can identify dietary shifts made by sharks over smaller time intervals than muscle tissue (Hussey *et al.* 2012a). Liver and red blood cells are also used for stable isotope analysis and have turnover rates slower than plasma, but faster than muscle and cartilage (Malpica-Cruz *et al.* 2012). It is important to note that tissue-specific incorporation of stable isotopes can vary with body size (Martinez del Rio *et al.* 2009, Matich *et al.* 2011), and between species and can further be affected by variability in environmental conditions (Newsome *et al.* 2010).

1.2.3. Stable isotope mixing models

The development of stable isotope mixing models has allowed for improved quantification and understanding of complex diets (Hussey *et al.* 2010b). Stable isotope values reflect an integrated value of prey consumed over time. By using isotopically distinct prey groups, relative proportions of particular prey and the degree to which predators rely on certain prey groups can be estimated using stable isotope mixing models. Certain of these models account for uncertainty in prey values and trophic discrimination factors when estimating a predator's proportional dietary inputs. With adequate sampling of predators and potential prey and accurate trophic discrimination factor values of predators, stable isotope analysis and the use of mixing models is a powerful tool for studying trophic interactions (Madigan *et al.* 2012).

1.3. Study species: the broadnose sevengill shark, *Notorynchus cepedianus*

1.3.1. Distribution

The broadnose sevengill shark, *Notorynchus cepedianus* (sevengill) (Chondrichthyes: Hexanchidae), is widely distributed in temperate waters globally. They occur in shallow coastal areas to deeper offshore waters (Last & Stevens 2009, Braccini 2008, Abrantes & Barnett 2011) up to 360 m (Stehfest *et al.* 2014). The species has also been recorded aggregating seasonally in shallow coastal habitats (Ebert 1989, Lucifora *et al.* 2005, Barnett *et al.* 2010a, Williams *et al.* 2012). In Tasmania (Australia), these sharks move into coastal areas to exploit seasonally abundant prey resources (Barnett *et al.* 2010b, Barnett & Semmens 2012).

The sevengill is vulnerable to fisheries outside of protected areas (Cedrola *et al.* 2009) and since the coastal zone is generally more heavily fished, they may be exposed to higher fishing exploitation rates than their more pelagic counterparts (Barnett *et al*. 2012, Best *et al*. 2013). Limited research and fisheries data is available on the species to determine whether documented patterns of decline are occurring throughout its distributional range (Cedrola *et al.* 2009). Sevengills are consequently listed as Data Deficient by the IUCN Red List Assessments (Compagno *et al.* 2005).

1.3.2. Diet and feeding ecology

A study by Cortés (1999) determined the trophic positions of 149 shark species. The study found sharks in the order Hexanchidae to be positioned at the highest trophic level, with the sevengill, exhibiting the highest trophic position of all shark species. Surprisingly, sevengills were found to even occupy a trophic position higher than the largest of the predatory sharks, white sharks, *Carcharodon carcharias* (Cortés 1999).

There have been a number of dietary studies across the distribution range of sevengills, including California, USA (Ebert 1989), southern Africa (Ebert 1991a), north Patagonia, Argentina (Lucifora *et al.* 2005) and south-east Tasmania (Barnett *et al.* 2010b, Abrantes & Barnett 2011). Together these studies reveal that sevengills have a diverse diet, which includes chondrichthyans, teleosts, marine mammals, molluscs and crustaceans. Sharks of the genus *Mustelus* are one of the most common prey items (Ebert 1989, 1991a, Lucifora *et al.* 2005, Barnett *et al.* 2010b, Abrantes & Barnett 2011) across the different studies.

To date there has only been one other study exploring aspects of the trophic ecology of sevengill sharks in southern Africa. Ebert (1991a) analysed stomach contents of sevengills around the Western Cape and found chondrichthyans comprised the majority of their diet with the striped catshark (*Poroderma africanum),* the smoothhound shark (*Mustelus mustelus)* and the leopard catshark *(Poroderma pantherinum)* being the top three prey species consumed. Marine mammals were the second most abundant prey group, with Cape fur seals (*Arctocephalus pusillus pusillus*) being by far the most common prey species in this category and only the occasional dolphin (e.g. dusky dolphin, *Lagenorhynchus obscurus*), recorded. Teleost fish were the third most important prey group with no particular species being consumed in abundance. Invertebrates and algae were also found in stomach contents of sevengills, but these did not constitute a significant contribution to their overall diet off the Western Cape.

Ebert (2002) found that sevengill sharks in both California and southern Africa showed an ontogenetic shift in their diet. Smaller sharks consumed a higher proportion of chondrichthyans, while larger individuals had more mammalian prey, such as, cetaceans and Cape fur seals (Ebert 2002, Lucifora *et al.* 2005). Such shifts in diet with age may be attributed to the increased body and gape size which contributes to improved hunting and prey handling skills, expanded range and exploitation of new habitats (Lowe *et al.* 1996, Braccini 2008). To date, no stable isotope studies have been conducted to examine the feeding ecology of sevengills in South Africa.

In this study, I explore stable isotopes in a large shark species, the broadnose sevengill shark, *Notorynchus cepedianus,* within False Bay, South Africa. I hypothesise that the stable isotope values of sevengills will reflect a diverse diet, typical of generalist shark species. The spatial ecology of this species is currently being studied in conjunction with the white shark, *Carcharodon carcharias*, by colleagues, as part of a broader study to understand the trophic role and ecology of apex predatory sharks in False Bay. I predict that there would be a seasonal dietary shift in the diet of the sevengills and further expect the diet of the sevengill to include similar prey species to that of the white shark, where larger individuals would be expected to consume more high δ^{15}N prey than smaller conspecifics.

1.4. Objectives

The primary objective of this study was to analyse stable isotopes from blood plasma and muscle tissue from sevengills and to use these samples to investigate:

1. effects of lipid and urea extraction on δ^{13}C and δ^{15}N of muscle tissue
2. inter-tissue variation between muscle and plasma of individual sevengills
3. the relative contribution of prey groups to the diet of sevengills using a mixing model
4. inter-annual variation in the diet of sevengills
5. seasonal differences in the diet of sevengills
6. differences between sexes of sevengills
7. relationships between individual size and δ^{13}C and δ^{15}N of sevengills
8. a comparison of the isotope values of the two top predatory sharks (sevengills and white sharks) and their potential prey with the ultimate goal of understanding the relative trophic dynamics within False Bay, South Africa.

CHAPTER 2

MATERIALS AND METHODS

© Morne Hardenberg

2.1. Study Site

The study site, Castle Rock (S34°14.356' E018°28.826') is a Marine Protected Area (MPA) in False Bay, South Africa. The Castle Rock region stretches from Rumbly Bay, just south of Miller's Point, to Baboon Rock, south of Partridge Point (Figure 1). The region has been a MPA, (declared under the Marine Living Resources Act of 1998), since 2004. Miller's Point is fairly shallow reaching a maximum depth of approximately 12 meters, with an average depth of about eight meters. The area is characterised by granite reefs and kelp forests, with sandy patches in between. Many of the reefs are relatively large areas consisting of ridges, gullies and boulders.

Figure 1. Satellite image showing False Bay and highlighting the Castle Rock Marine Protected Area and Miller's Point study site (Adapted from Google Maps, 2015).

2.2. Sampling protocol

2.2.1. Capture of sevengills

Sevengills were captured (as part of an ongoing study led by Shark Spotters, in association with the Two Oceans Aquarium and the South African Institute for Aquatic Biodiversity), using a 50 - 100 cm length of wire trace, with size 12 circle hooks (crimped on to the one end to flatten the barb), and a long-line clip attached to the other end to allow for the quick release of the gear from the hook and main-line. A braided rope was tied off to the boat with a sinker on the other end used as a hand line. All shark handling and sampling procedures followed a strict protocol to ensure both people and shark safety. A qualified veterinarian was present to oversee the work. Once a shark had been caught and had tired itself out on the end of the line for <5 min, it was maneuvered into a specially designed cradle attached to the side of the boat. For smaller sharks, a stretcher was placed underneath the shark for the animal to be lifted onto the boat, making it easier to perform sampling procedures. A rope was tied around the tail of all captured sharks to restrict their movement. A designated person was responsible for maintaining control of the head and providing oxygenated water flow over the gills while the shark was out of the water. Once all sampling had taken place, the hook was removed and the sharks were returned to the cradle or stretcher and allowed to recover while under supervision. Sharks were considered to have recovered once they showed signs of being able to swim off comfortably on their own, after which they were released. Animal ethics for capture, sampling and release was obtained from the University of Cape Town (2010/V13/AH) and South African Institute for Aquatic Biodiversity (2015-06).

2.2.2. On-board sampling and storage protocol

All individuals were sexed and then pre-caudal length (PCL) and total length (TL) were measured. Approximately 1 g of white muscle tissue was excised anterior to the first dorsal fin using an 8 mm biopsy punch. An 18-gauge needle was used to collect 15 - 20 ml of blood from the caudal vein. Whole blood was immediately transferred into lithium (Li) heparin microtainer (BD) tubes. Both blood and muscle samples were immediately placed on ice. Muscle tissue was subsequently frozen at –20°C until processing commenced.

Since it was not practical to centrifuge blood samples on the boat, centrifugation (30 000 rpm for five minutes) took place immediately upon return to land using a centrifuge (Gemmy Industrial Corp, model PLC-03). Plasma fractions were separated from whole blood and pipetted into tubes before being frozen at –20°C. Kim & Koch (2012) examined the effects of Lithuim (Li) heparin on stable isotope analyses by comparing plasma and red blood cell (RBC) samples collected in no-additive tubes with those in Li heparin tubes. Their results revealed that plasma and RBC isotope values were practically identical in no- additive versus Li heparin collection tubes. It was therefore concluded that Li heparin tubes do not introduce error to isotope values and results could be compared to no-additive samples with confidence.

2.2.3. Prey sampling

A list of potential prey species was compiled using the results from stomach content analyses of sevengills in False Bay (Ebert 1991a), East London, and Swakopmund, in Central Namibia (Table 1). Few teleost species were identifiable (Ebert 1991a) and I thus sampled the most abundant teleost species found within False Bay (Lamberth *et al.* 1994, Table 1). Samples were collected opportunistically using a variety of methods including: 1) the regurgitated stomach contents of sevengills that were captured as part of this study; 2) dead fish captured by recreational and trek net fishermen in False Bay; 3) seal carcasses found in the waters of False Bay; and 4) frozen samples obtained by colleagues for other research projects at the University of Cape Town. Additional chondrichthyan samples were collected from a shark processing factory located in Strand, Western Cape, South Africa. Muscle tissue samples were excised from the dorsal section anterior to the dorsal fin in teleosts and sharks (following Hussey *et al.* 2010a), and from the mid-dorsal regions in seals (Olin *et al.* 2013). All tissue samples were stored on ice and then frozen at –20°C before laboratory preparations (Kim & Koch 2012).

Table 1. A list of the potential prey species of sevengill sharks *(Notorynchus cepedianus)* in southern Africa. "X" denotes the presence of a particular species recorded in the stomach contents of sevengills caught in the Western Cape of South Africa and those recorded in stomachs of sevengills caught elsewhere around the coast of southern Africa (Eastern Cape, south and central Namibia). The remaining prey listed were collected opportunistically in False Bay and analysed as potential prey of sevengills, despite not being recorded previously in stomach content studies.

Species name	Common name	Western Cape	Southern Africa
Chondrichthyans			
Poroderma africanum	Pyjama shark	X	X
Poroderma pantherinum	Leopard catshark	X	X
Haploblepharus edwardsii	Puffadder shyshark	X	X
Haploblepharus pictus	Dark shyshark	X	X
Skate species	*Skate species*	X	X
Raja straeleni	Biscuit skate		X
Dasyatis chrysonota	Blue stingray	X	X
Raja miraletus	Brown ray		X
Torpedo marmorata	Marbled electric ray		X
Pteroplatytrygon violacea	Pelagic stingray		
Callorhinchus capensis	St. Joseph's shark	X	X
Squalus acutipinnis	Bluntnose spiny dogfish	X	X
Mustelus palumbes	Whitespotted smooth hound shark	X	
Mustelus mustelus	Smooth hound shark	X	X
Galeorhinus galeus	Soupfin shark	X	X
Syphyrna species	Hammerhead shark		
Carcharhinus brachyurus	Bronze whaler	X	
Prionace glauca	Blue shark		
Isurus oxyrinchus	Shortfin mako shark		
Teleosts			
Pterogymnus laniarius	Panga		
Pachymetopon blochii	Hottentot		
Chrysoblephus laticeps	Red Roman		
Boopsoidea inornata	Fransmadam		
Trachurus trachurus	Horse mackerel		X
Sardinops sagax	Pilchard/Sardine	X	
Thyrsites atun	Snoek		
Seriola lalandi	Giant yellowtail		
Mammals			
Arctocephalus pusillus pusillus	Cape fur seal	X	X

2.3. Sample preparation

All muscle tissue (sevengills and prey) was freeze dried for 24 hours and sevengill plasma samples were dried for 48 hours using a Scanvac Coolsafe 55-4 cooling trap (Labogene, Lynge, Denmark). All muscle samples were then homogenised using a Retsch MM 400 mixer mill (VERDER Group, Netherlands).

2.3.1. Lipid extraction

Lipids are depleted in ^{13}C relative to carbohydrates and proteins (DeNiro & Epstein 1977) with δ^{13}C values up to 5‰ lower than associated proteins (Newsome *et al.* 2010). Although the overall reported increases in δ^{13}C values are small, and lipid content is reportedly low in elasmobranch tissue, there is variation between species. Hussey *et al.* (2012b) examined the effects of lipid extraction on muscle tissue for 21 elasmobranch species and found a mean increase in δ^{13}C after extraction. Thus, it is recommended that lipid extraction be performed (Logan & Lutcavage 2010, Kinney *et al.* 2011, Hussey *et al.* 2012a, Olin *et al.* 2013) to standardise data among individuals, between different tissues in conspecifics and across species within a food web (Hussey *et al.* 2012a).

Lipid extraction was undertaken by agitating the dried powdered muscle tissue in a 2:1 chloroform–methanol solution for 24 hours. The tissue and solvent were centrifuged for three minutes using an Eppendorf 5415 D centrifuge (Eppendorf, USA) and then decanted before a second addition of 2:1 chloroform–methanol was added. This was followed by a further round of agitation and centrifuging before the final decant.

2.3.2. Urea extraction

Urea is a waste product of metabolism in elasmobranch species and consequently is expected to be ^{15}N depleted, and has been shown to reflect artificially lower δ^{15}N values and a lower trophic position estimates than expected (Fisk *et al.* 2002, Logan & Lutcavage 2010). In light of this, the removal of urea is recommended to elucidate accurate trophic position estimates and relative food web position of elasmobranchs and for diet reconstruction (Hussey *et al.* 2012a). All chondrichthyan muscle samples were therefore rinsed three times with distilled water to remove excess urea after lipid extraction.

2.3.3. Lipid and urea extraction tests

2.3.3.1. Muscle

Sample sizes for lipid extraction were based on the amount of each sample available to run multiple tests. Lipid extraction tests were performed on paired samples from nine sevengill, four soupfin and four smooth hound sharks, to confirm whether lipid and urea extraction was required: (i) to remove lipids given reported low lipid content and, (ii) to determine if $\delta^{15}N$ values were affected and whether this relates to the retention of isotopically light urea by elasmobranchs. This process was repeated for 15 teleost and six mammal (Cape clawless otter, *Aonyx capensis*) samples to determine effects of $\delta^{13}C$ and $\delta^{15}N$ values on other major groups.

2.3.3.2. Plasma

Since plasma is the vehicle for gut-tissue and inter-tissue transport of lipids in elasmobranchs, non-esterified fatty acid concentrations can vary in elasmobranch plasma depending on the timing of the last meal (Hussey *et al.* 2012a). Elasmobranch plasma is a promising tissue for diet reconstruction, but contains high concentrations of urea and Trimethylamine N-oxide (TMAO) that could bias $\delta^{13}C$ and $\delta^{15}N$ values (Hussey *et al.* 2012a). Due to uncertainty in the literature on the appropriate sample preparation protocol for elasmobranch plasma, 12 sevengill samples were analysed using the same method described above to derive non-lipid extracted (BULK), lipid extracted (LE) and lipid extracted and water rinsed (LEWR) isotope values. Due to further uncertainty associated with water rinsing of plasma samples, all plasma samples used for analysis were lipid extracted, but not water rinsed.

2.4. Stable isotope analysis

For all muscle and plasma samples, the mixture of tissue and solvent was filtered and the resulting residue was freeze dried for 24 hours to evaporate any remaining solvent (MacNeil *et al.* 2005, Hussey *et al.* 2010a, Hussey *et al.* 2012b). Between 0.35 mg and 0.45 mg of tissue samples from sharks and prey species were weighed into tin capsules on a Sartorius M2P microbalance. The cups were then folded tightly to enclose the sample. Samples were then combusted in a Flash 2000 organic elemental analyzer and the gases passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit (Thermo Scientific, Bremen, Germany) within the Archaeology Department, University of Cape Town, South Africa.

The in-house standards used were: Choc (a commercial chocolate/egg mixture, USA), Valine (DL Valine purchased from Sigma-Aldrich, South Africa), and seal bone crushed, demineralized and dissolved in acid, and then reconstituted in gel form (Stable isotope laboratory, Archaeology Department, University of Cape Town). All the in-house standards have been calibrated against IAEA (International Atomic Energy Agency) standards.

2.5. Stable isotope calculations

The isotope ratios were expressed in the conventional δ notation as parts per thousand (‰) deviations from the international standards: atmospheric nitrogen (N_2) for $\delta^{15}N$ and PeeDee Belemnite for $\delta^{13}C$:

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

where X is ^{15}N or ^{13}C, and R is the corresponding ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$. These values were then plotted on a dual isotope plot with nitrogen on the *y*-axis and carbon on the *x*-axis.

2.6. Statistical analyses

All statistical analyses (confidence level of 95%) of isotope data were carried out using Statistica 12 (2014).

2.6.1. Lipid and urea extraction effects

Student t-tests were used for paired samples to compare $\delta^{13}C$ and $\delta^{15}N$ between lipid extracted (LE) and untreated (BULK) muscle tissue for elasmobranch, mammal and teleost groups. One-way ANOVA with Post Hoc (Tukey HSD) tests were used to investigate differences in total percentage $\delta^{15}N$ and $\delta^{13}C$ between non-lipid extracted (BULK), lipid extracted (LE) and lipid extracted and water rinsed (LEWR) plasma of sevengill sharks.

2.6.2. Differences between tissues

Due to the small sample size of plasma, Wilcoxon matched-pairs tests were used to determine whether plasma and muscle isotope values differed significantly.

2.6.3. Inter-annual variation

The possibility of inter-annual variation in isotopic values of female sevengills was analysed using stable isotope values from 2013, 2014, and 2015. One-way ANOVA with Post Hoc (Tukey HSD) test was used to determine whether there was any variability between the sampling years. Separate Kruskal-Wallis tests were performed to determine whether there was a difference between the three years for immature and mature female sharks.

2.6.4. Seasonal variation

Given the low sample size for each season, a Mann-Whitney U test was used to determine whether there was a difference between summer (December - February) and winter (June - August) isotope values of sevengill plasma samples. Summer samples consisted of both immature and mature females and therefore, further Mann-Whitney U tests were performed to determine whether there was a difference between immature and mature females. Since winter samples consisted of mature and immature female sharks, as well as males, a Kruskal-Wallis test was performed to determine whether any sex/maturity differences were present within the winter sampled sharks.

2.6.5. Sex differences

Due to the low samples size of male sharks, Mann-Whitney U tests were used to determine whether significant differences exist between male and female sevengills.

2.6.6. Size effects

All male sevengills sampled were considered mature at a size of >160 cm (Ebert 1996). Female sharks were grouped as immature (160 - 219 cm) and mature (>220 cm) according to Ebert (1996). Linear regression analyses were performed to investigate the relationship between shark length and $\delta^{15}N$ and $\delta^{13}C$ values of sevengills. Student t-tests were performed to determine whether there was a significant difference in isotope values between immature and mature female sevengills.

2.7. Diet mixing models

A Bayesian mixing model run in the Stable Isotope Analysis in R package (SIAR v. 4.2) was used to generate estimates of proportional composition of sevengill diets. Such models (MixSIR and SIAR) have been developed to allow for incorporation of uncertainty associated with the multiple prey sources of a consumer (Inger *et al.* 2010). The uncertainty is incorporated into the model run in SIAR through addition of error around predator $\delta^{15}N$ and $\delta^{13}C$ values, $\delta^{15}N$ and $\delta^{13}C$ values of prey and trophic enrichment factors (TEFs) to the model input (Inger *et al.* 2010).

SIAR was used to generate a series of proportional prey contributions to the diet of sevengills belonging to different demographic groups (immature females (160 – 219 cm), mature females (>220 cm) and male sharks; Ebert 1996). Trophic enrichment factors (TEFs) used for the models were 2.29 for $\delta^{15}N$ and 0.9 for $\delta^{13}C$. These factors were derived by Hussey *et al*. (2010b) for large sand tiger (*Carcharias taurus*) and lemon shark (*Negaprion brevirostris*) under a controlled feeding regime.

2.7.1. Grouping of prey species

Since many of the prey species had similar isotopic values, their biology, preferred habitat and feeding habits (Boyle *et al.* 2012) as well as their known contributions to sevengill diet (Ebert 1991a) were considered for accurate grouping (Table 2). Mean and standard deviation $\delta^{15}N$ and $\delta^{13}C$ values were plotted for each prey group on a dual-isotope graph. One-way ANOVA with Post Hoc (Tukey HSD) tests were used to determine whether $\delta^{15}N$ and $\delta^{13}C$ values of prey groups were significantly different from one another (Table 5).

Table 2. Potential prey groups of sevengill sharks *(Notorynchus cepedianus).*

Group	Species name	Common name
Catsharks	*Poroderma africanum* *Poroderma pantherinum* *Haploblepharus edwardsii* *Haploblepharus pictus*	Pyjama shark Leopard catshark Puffadder shyshark Dark shyshark
Demersal chondrichthyans	*Skate species* *Raja straeleni* *Dasyatis chrysonota* *Raja miraletus* *Torpedo marmorata* *Pteroplatytrygon violacea* *Callorhinchus capensis* *Squalus acutipinnis*	*Skate species* Biscuit skate Blue stingray Brown ray Marbled electric ray Pelagic stingray St. Josephs shark Bluntnose spiny dogfish
Inshore sharks	*Mustelus palumbes* *Mustelus mustelus* *Galeorhinus galeus* *Syphyrna zygaena* *Carcharhinus brachyurus*	White-spotted smoothhound shark Smooth hound shark Soupfin shark Smooth hammerhead shark Bronze whaler
Offshore sharks	*Prionace glauca* *Isurus oxyrinchus*	Blue shark Shortfin mako shark
Inshore teleosts	*Pterogymnus laniarius* *Pachymetopon blochii* *Chrysoblephus laticeps* *Boopsoidea inornata* *Chelidonichthys capensis*	Panga Hottentot Roman Fransmadam Cape gurnard
Small offshore teleosts	*Trachurus trachurus* *Sardinops sagax* *Thyrsites atun*	Horse mackerel Pilchard/Sardine Snoek
Large offshore teleosts	*Seriola lalandi* *Thunnus albacares*	Giant yellowtail Yellow-fin Tuna
Seals	*Arctocephalus pusillus pusillus*	Cape fur seal
Squid	*Loligo reynaudii*	Cape Hope squid
Crayfish	*Jasus lalandii*	West coast rock lobster

2.8. Preliminary comparisons of sevengills with white sharks

Towards the end of the study, seven white shark, *Carcharodon carcharias*, samples were contributed for comparisons with sevengill samples. These samples were collected from white sharks in False Bay between 2010 and 2012 as part of a separate study. Due to the small sample size, Mann-Whitney U tests were used to determine whether there was a significant difference in isotope values between white sharks and sevengills in False Bay. Information on sex was not available for all the white sharks sampled, but details on size indicated that all sharks were likely immature (between 3 and 3.5 m TL), regardless of sex (Compagno 2001). Mann-Whitney U tests were used to compare isotope values of immature white sharks with immature sevengills. Estimates of proportional contributions of prey groups to the diet of white sharks were not modeled due to the small sample size.

CHAPTER 3

RESULTS

A total of 39 muscle samples (33 female, six male), and 28 blood samples (25 female, three male) from sevengills sampled between 2013 and 2015 were analysed. A total of 161 prey samples from 32 different species, including cephalopods, crustaceans, teleosts, chondrichthyans and marine mammals were analysed (Table 1 and 2). For accurate seasonal comparisons, only plasma samples collected in true summer (December - February: n = 6) and true winter (June - August: n = 6) were analysed.

3.1. Lipid and urea extraction tests

3.1.1. Muscle

The process of lipid extraction on the muscle tissue of sevengills and their elasmobranch prey led to a significant increase in $\delta^{15}N$ values (p = 0.0001 and p = 0.0004 respectively). Lipid extraction also resulted in an increase in $\delta^{13}C$ for all groups, however, this increase was only significant for sevengills (p = 0.016). There was no significant effect of lipid extraction on teleost and mammal $\delta^{15}N$ or $\delta^{13}C$ values, but since an increase was observed in overall means of both $\delta^{15}N$ and $\delta^{13}C$ for all groups tested, lipid extractions were performed on all samples and all analyses were done on lipid extracted muscle samples (Table 3).

Table 3. Mean ± SD (‰) $\delta^{15}N$ and $\delta^{13}C$ values of non-lipid extracted (BULK) and lipid extracted (LE) muscle tissue of sevengill sharks *(Notorynchus cepedianus)*, elasmobranchs, teleosts and mammals.

		$\delta^{15}N$		**$\delta^{13}C$**	
Treatment	**N**	**BULK**	**LE**	**BULK**	**LE**
Sevengills	9	16.51 ± 0.57	18.02 ± 0.70	–14.46 ± 0.56	–13.75 ± 0.55
Elasmobranch	8	14.82 ± 0.36	15.60 ± 0.31	–14.91 ± 0.40	–14.62 ± 0.35
Teleosts	15	14.56 ± 1.06	14.99 ± 1.00	–16.08 ± 0.91	–15.92 ± 1.02
Mammals	6	17.74 ± 3.61	18.16 ± 3.67	–19.24 ± 3.10	–18.92 ± 3.12

3.1.2. Plasma

Lipid extraction resulted in an increase in both $\delta^{13}C$ and $\delta^{15}N$ values of plasma, however, this increase was smaller for samples that were further treated with a water rinse (LEWR). The observed enrichment relative to BULK tissue was only significant for $\delta^{13}C$ of LE samples (n = 7, p = 0.007) (Figure 2).

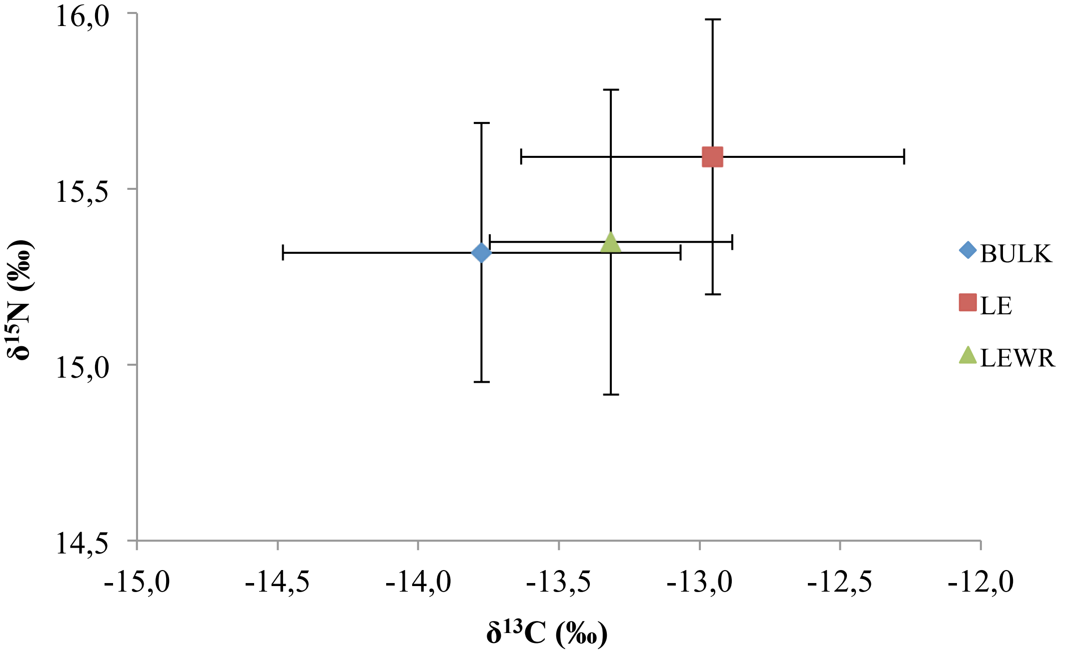


Figure 2. Mean ± SD (‰) δ^{13}C and δ^{15}N of sevengill shark *(Notorynchus cepedianus)* plasma after three different sample preparation treatments (BULK, LE: lipid extracted, LEWR: lipid extracted and water rinsed).

3.2. Inter-tissue comparisons

δ^{13}C values were variable for both plasma (mean ± SD: –13.20 ± 0.70) and muscle (mean ± SD: –13.07 ± 0.90) samples but there was no significant difference in δ^{13}C between the tissues (T = 14.00, p = 1.00). Muscle tissue revealed significantly higher δ^{15}N values (mean ± SD: 17.20 ± 0.25) compared to plasma (mean ± SD: 15.51 ± 0.43) (T = 0.00, p = 0.02) (Table 4, Figure 3).

Table 4. Difference in isotope values between muscle and plasma of individual female sevengill sharks *(Notorynchus cepedianus)*.

Shark ID	Sample date	Shark Size (cm)	Muscle δ^{15}N	Plasma δ^{15}N	Muscle-Plasma δ^{15}N	Muscle δ^{13}C	Plasma δ^{13}C	Muscle-Plasma δ^{13}C
NC 014	17/04/14	225	16.95	14.88	2.07	–11.90	–14.05	2.15
NC 015	17/04/14	220	17.24	15.88	1.36	–12.09	–12.60	0.51
NC 017	30/05/13	232	17.60	16.05	1.55	–12.48	–13.12	0.64
NC 021	18/02/15	232	16.99	15.47	1.52	–14.10	–12.58	–1.52
NC 031	06/03/13	212	17.31	15.04	2.27	–13.92	–14.31	0.39
NC 039	05/03/13	231	16.95	15.55	1.40	–13.45	–12.92	–0.53
NC 019	18/02/15	233	17.34	15.73	1.61	–13.58	–12.79	–0.79

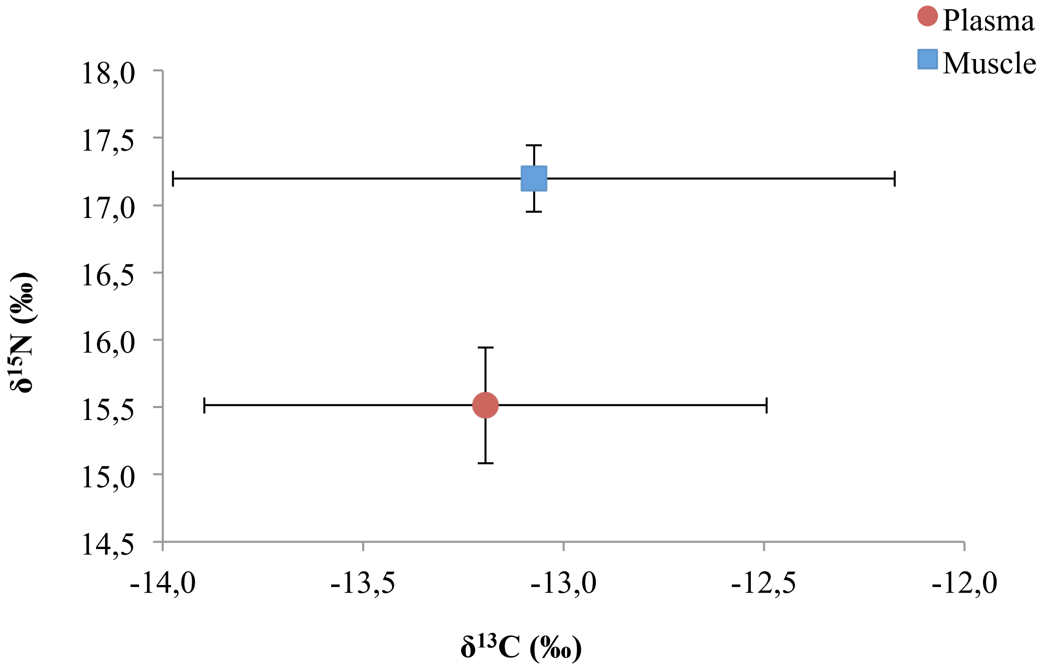


Figure 3. Mean ± SD $\delta^{13}C$ and $\delta^{15}N$ of sevengill shark *(Notorynchus cepedianus)* lipid extracted plasma and muscle.

3.3. Predators and prey in False Bay

Sevengills had the highest $\delta^{15}N$ values (mean ± SD: 17.78 ± 0.49) followed by white sharks (mean ± SD: 17.10 ± 0.56) (Figure 4). Seals were ranked the highest amongst all prey species tested ($\delta^{15}N$ mean ± SD: 16.93 ± 0.65), while crayfish were positioned with the lowest $\delta^{15}N$ values (mean ± SD: 11.79 ± 0.07) (Table 5, Figure 4). Inshore and offshore prey groups were isotopically distinct, with offshore sharks, teleosts and squid having lower $\delta^{13}C$ (mean ± SD: –15.89 ± 0.31) than inshore prey groups ($\delta^{13}C$ mean ± SD: –14.22 ± 0.90) (Table 5; 6, Figure 4). The species grouped as “Catsharks” were high in ^{13}C relative to all other prey groups (Table 5; 6, Figure 4).

Table 5. Mean ± SD (‰) $\delta^{15}N$ and $\delta^{13}C$ values of sevengill shark *(Notorynchus cepedianus)* potential prey groups.

Prey group	**$\delta^{13}C$**	**$\delta^{15}N$**
Catsharks	–12.86 ± 0.91	15.22 ± 0.56
Seals	–14.95 ± 0.33	16.93 ± 0.65
Demersal chondrichthyans	–14.69 ± 0.25	14.64 ± 0.48
Inshore sharks	–14.85 ± 0.38	15.69 ± 0.41
Inshore teleosts	–14.96 ± 0.37	14.90 ± 0.32
Offshore sharks	–15.82 ± 0.01	15.65 ± 0.15
Large offshore teleosts	–15.98 ± 0.21	14.73 ± 0.39
Small offshore teleosts	–15.50 ± 0.26	12.58 ± 0.44
Crayfish	–13.73 ± 0.29	11.79 ± 0.07
Squid	–16.25 ± 0.33	14.44 ± 0.53

Table 6. Post Hoc (Tukey HSD) test, highlighting significant differences: $p < 0.05$ (*), $p < 0.005$ (**), $p < 0.0005$ (***) in $\delta^{15}N$ and $\delta^{13}C$ between potential prey groups of sevengill sharks *(Notorynchus cepedianus)*.

Prey group	Seals		Catsharks		Demersal chondrichthyans		Inshore teleosts		Small offshore teleosts		Large offshore teleosts		Offshore sharks		Inshore sharks		Crayfish		Squid	
	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Seals			***	***	***		***		***	***	***	***			***		***	*	***	***
Catsharks	***	***				***		***	***	***		***		***		***	***			***
Demersal chondrichthyans	***			***					***	***		***			***		***			***
Inshore teleosts	***			***					***	***		***		***	**		***			***
Small offshore teleosts	***	***	***	***	***	***	***	***			***		***		***	***		***	***	
Large offshore teleosts	***	***		***		***		***	***						***	***	***	***		
Offshore sharks				***				***	***						***	***	***	***		
Inshore sharks	***			***	***		**		***	***	***	***		***			***		*	***
Crayfish	***	*	***		***		***			***	***	***	***	***	***				***	***
Squid	***	***		***		***		***	***						***	***	***	***		

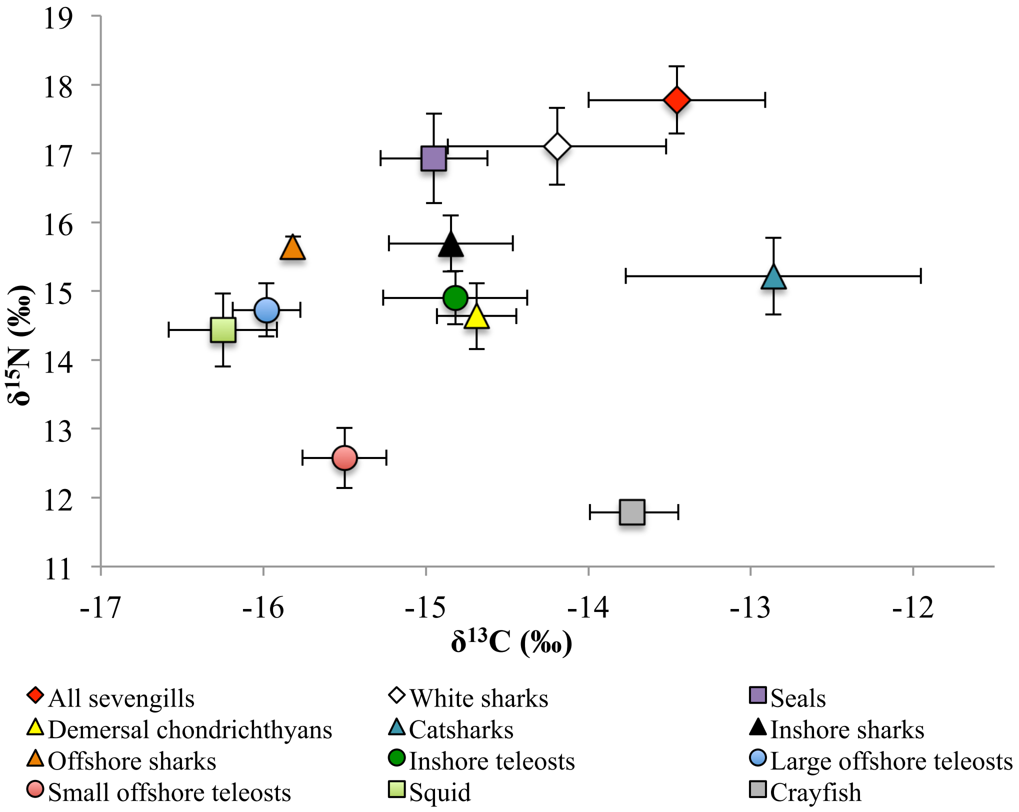


Figure 4. Dual- isotope plot showing mean ± SD $\delta^{13}C$ and $\delta^{15}N$ values of key predator species and potential prey groups found within False Bay.

3.4. Sevengill diet

3.4.1. Isotope mixing models

The relative contributions of the various prey groups, estimated using SIAR, indicated that demersal chondrichthyans, inshore sharks and teleosts appear to be of similar importance to the diets of both immature and mature female sharks (Table 7, Figure 5). Immature females had a greater proportion of high $\delta^{15}N$ prey in their diet (e.g. seals) relative to mature females whose diet consisted of more high $\delta^{13}C$ prey that was lower in $\delta^{15}N$ (e.g. catsharks) in their diet (Table 7, Figure 5). Catsharks may have comprised more than 50% of mature female sevengill diet, but no more than 25% of immature females or males. Seals on the other hand, comprised no more than 21% of mature female diet, but comprised a relatively large proportion of immature female diet (16% - 47%).

Table 7. The median, minimum and maximum percentages of different prey in the diet of female immature (163 – 219 cm TL) and mature (> 220 cm TL) and male sevengill sharks *(Notorynchus cepedianus)*. Values are those of 95% credibility intervals (CI) from a Bayesian isotope mixing model (SIAR).

	Immature females			**Mature females**			**Males**		
Prey groups	Min	Max	Median	Min	Max	Median	Min	Max	Median
Catsharks	7.8	25	16.4	18	52	35	0	22	11
Seals	16	47	31.5	0	21	10.5	2.6	37	19.8
Demersal chondrichthyans	0	30	15	0.5	40	20.3	1.2	42	21.6
Inshore sharks	0	44	22	0	32	16	0.2	42	21.1
Inshore teleosts	0	34	17	0	40	20	0.6	42	21.3

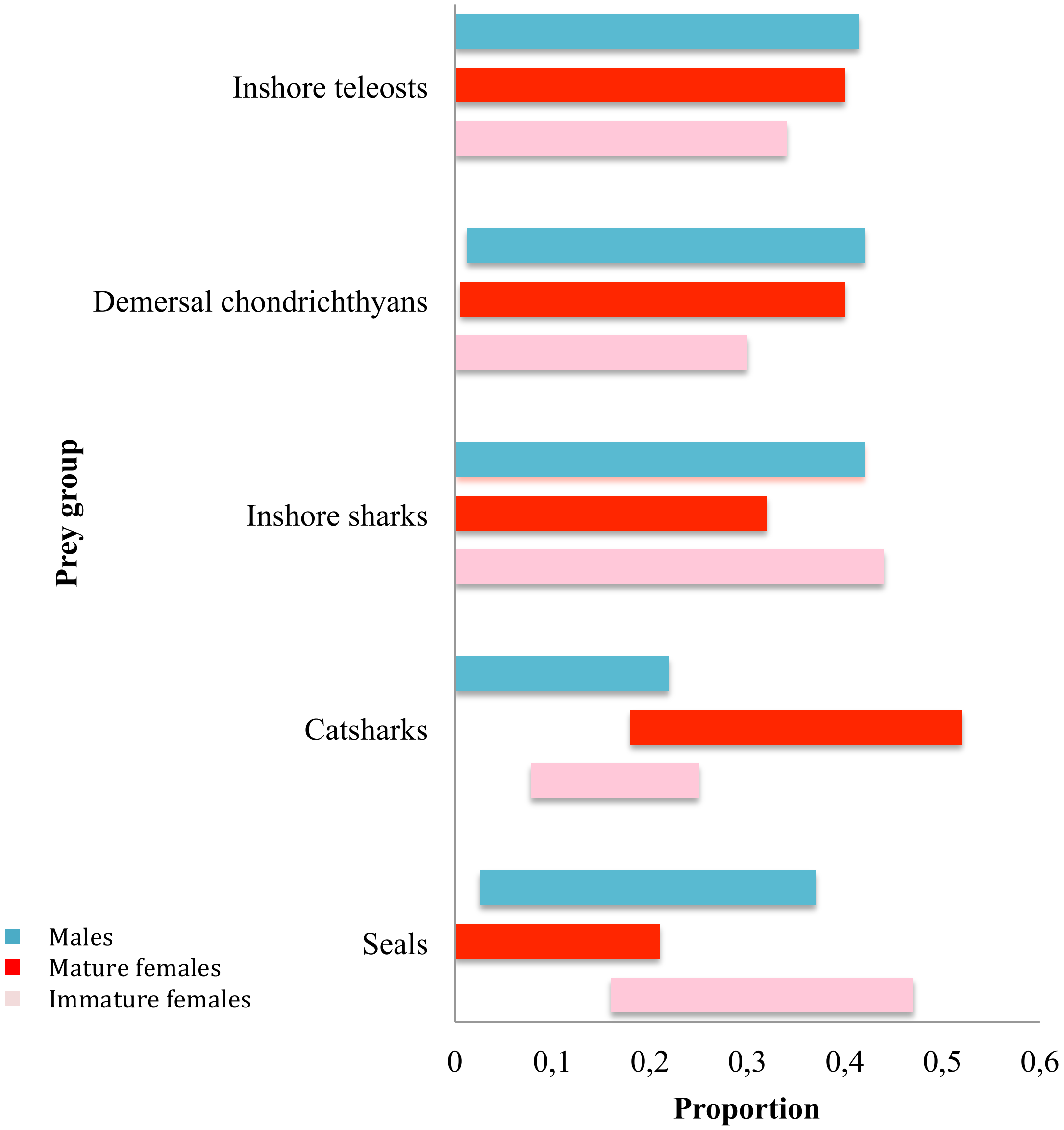


Figure 5. Range of proportional contributions of five prey groups to the diet of male and both immature (163 - 219 cm) and mature (> 220 cm) female sevengill sharks *(Notorynchus cepedianus)*. Values represent 95% credibility intervals generated by a Bayesian isotope mixing model (Stable-Isotope Analysis in R, SIAR).

Stable isotope values for individual sevengills fell within a polygon defined by the observed values for five prey groups (seals, catsharks, demersal chondrichthyans, inshore sharks, inshore teleosts) after TEF correction. The position of sevengill isotope values in isotopic space were scattered between these predominantly inshore prey values. Some values overlapped more closely with particular prey groups, while others fell between two or more of the groups. No clear overlap was observed between sevengill values and offshore prey groups or the crayfish prey group (Figure 6) and therefore these prey groups were not included in the isotope mixing model.

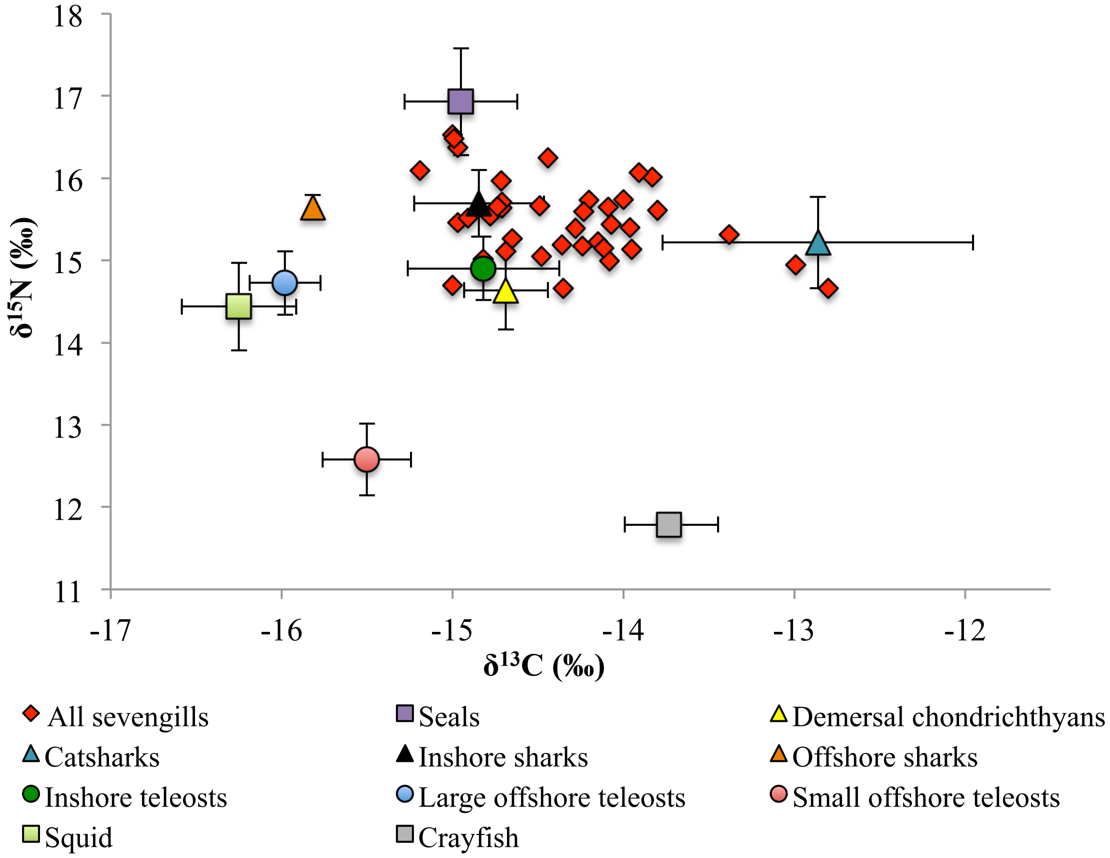


Figure 6. Dual stable isotope plot showing δ^{13}C and δ^{15}N values of potential prey and of individual sevengill sharks *(Notorynchus cepedianus)* corrected for trophic fractionation (δ^{13}C TEF = –0.9 and δ^{15}N TEF = –2.29) and mean ± SD δ^{13}C and δ^{15}N.

3.4.2. Inter-annual variation

Plasma samples were collected in 2013 (n = 16), 2014 (n = 10) and 2015 (n = 13). One-way ANOVA Post Hoc Tukey test found no significant difference in isotope values across the three years (δ^{15}N: MS = 0.26, df = 36, $p > 0.05$ and δ^{13}C: MS = 0.28, df = 36, $p > 0.05$).

3.4.3. Seasonal variation

Sevengill plasma isotope values for summer (mean ± SD δ^{15}N: 15.59 ± 0.38 and δ^{13}C: -12. 83 ± 0.31, n = 6) and winter (δ^{15}N: 15.56 ± 0.55 and δ^{13}C: -13.18 ± 0.67, n = 6) were not significantly different (δ^{15}N: U = 16.00, Z = 0.24, p = 0.81 and δ^{13}C: U = 14.00, Z = 0.56, p = 0.58). There were also no significant differences in plasma isotope values between mature (n = 2) and immature (n = 4) sevengills sampled in summer (δ^{15}N: U = 4.00, Z = 0.00, p = 1.00 and δ^{13}C: U = 3.00, Z = 0.23, p = 0.82), or between mature (n = 2) and immature (n = 2) female sharks sampled during winter (Kruskal-Wallis two-tailed test: H (2, N = 6) p = 0.65).

3.4.4. Sex and size- based differences in sevengill shark diet

No significant differences were observed between the combined samples for all males and females in terms of either δ^{13}C (U = 61.50, Z = 1.44, p = 0.15) or δ^{15}N (U = 89.50, Z = 0.35, p = 0.73). Mature males however had higher δ^{13}C values than mature females (U = 15.00, Z = 2.06, p = 0.04) while δ^{15}N values were similar (U = 26.00, Z = –1.10, p = 0.27).

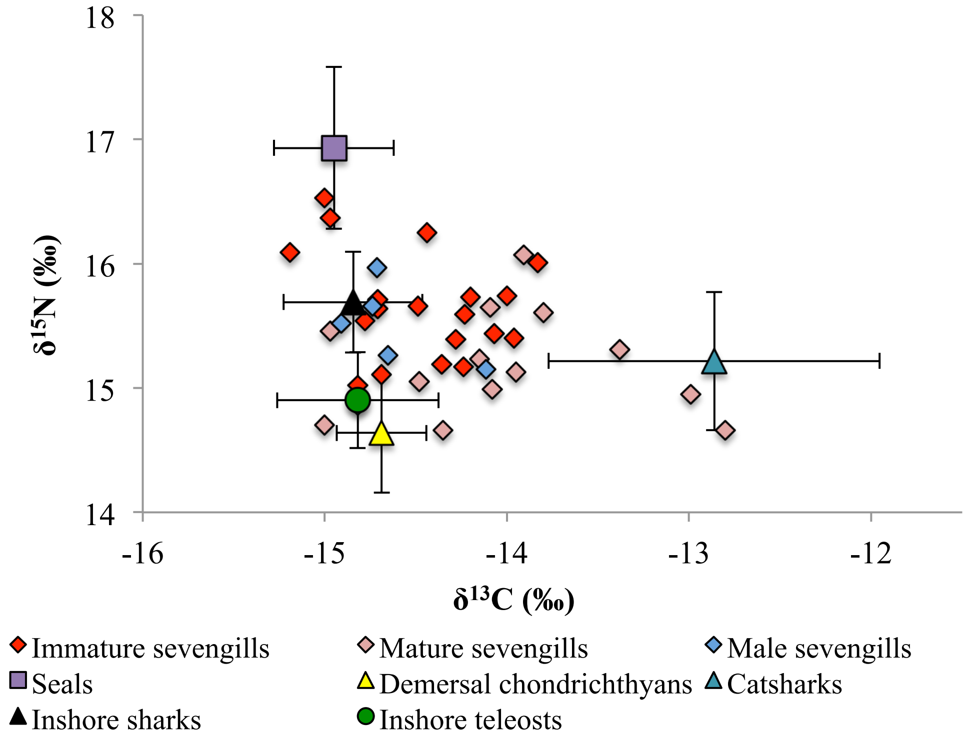


Figure 7. Dual- isotope plot of δ^{13}C and δ^{15}N of individual immature female, mature female and male sevengill sharks *(Notorynchus cepedianus)* from False Bay (TEF correction values: δ^{13}C –0.9 and δ^{15}N –2.29) and mean ± SD δ^{13}C and δ^{15}N of their prey groups.

3.4.5. The effects of shark size on sevengill $\delta^{15}N$ and $\delta^{13}C$ values

δ^{15}N values decreased with increasing size of female sevengills ($R^2 = 0.347$, $F_{1,31} = 16.467$, $p = 0.0003$) and immature sharks had significantly higher δ^{15}N than mature sharks ($t = 3.240$, $p = 0.003$) (Table 8, Figure 8.1). δ^{13}C values increased with increasing size of female sevengills ($R^2 = 0.236$, $F_{1,30} = 9.577$, $p = 0.004$) and immature sharks had significantly lower δ^{13}C than mature sharks ($t = -2.740$, $p = 0.010$) (Table 8, Figure 8.2).

Table 8. Mean ± SD total length (TL), $\delta^{15}N$ and $\delta^{13}C$ values of immature (160- 219 cm) and mature (> 220 cm) female sevengill sharks *(Notorynchus cepedianus)*.

	N	TL (cm)		$\delta^{15}N$		$\delta^{13}C$	
		Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
All sevengills	39	160 to 236	202.40 ± 22.17	16.95 to 18.82	17.78 ± 0.49	−14.29 to −11.90	−13.46 ± 0.54
All females	33	160 to 236	205.12 ± 23.01	16.95 to 18.82	17.79 ± 0.51	−14.29 to −11.90	−13.40 ± 0.56
Immature females	20	160 to 216	189.90 ± 15.92	17.31 to 18.82	17.99 ± 0.46	−14.29 to −12.93	−13.60 ± 0.40
Mature females	13	220 to 236	228.54 ± 5.55	16.95 to 18.36	17.48 ± 0.43	−14.10 to −11.90	−13.10 ± 0.66
Males	6	181 to 195	187.42 ± 5.20	17.15 to 18.26	17.69 ± 0.39	−14.01 to −13.22	−13.76 ± 0.28

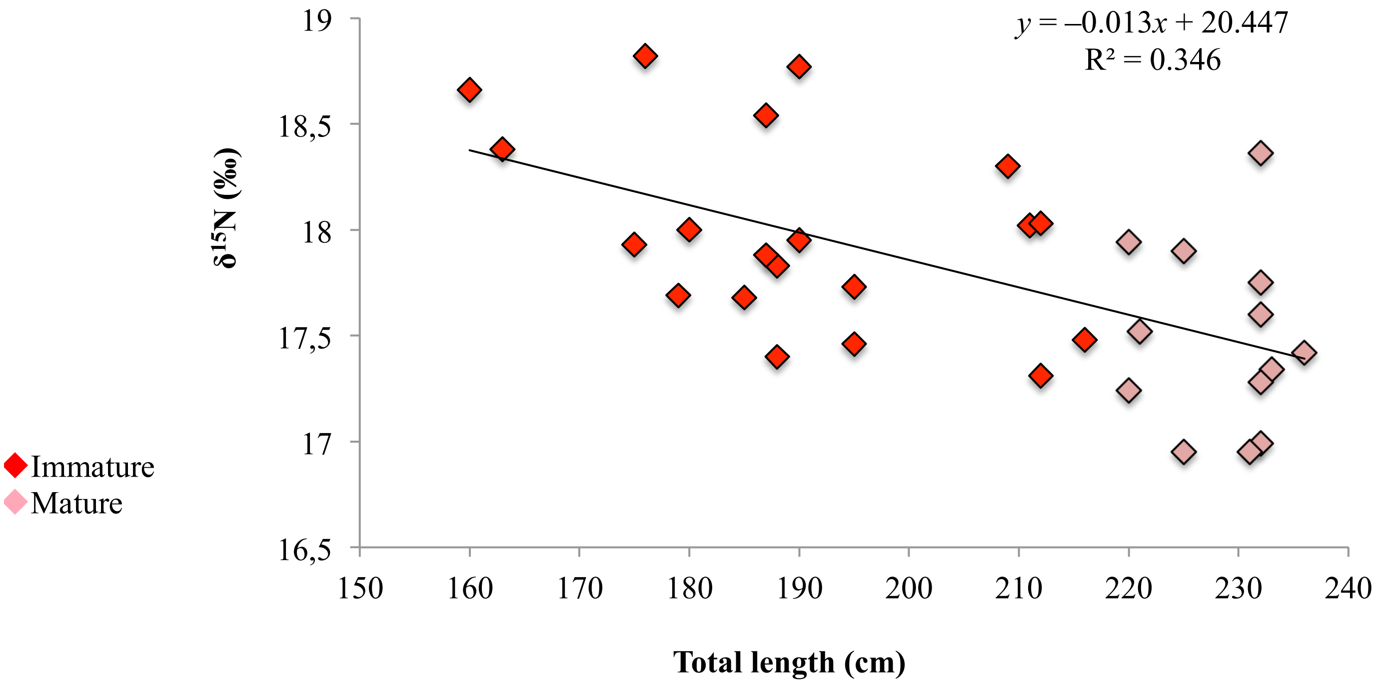


Figure 8.1. The relationship between δ^{15}N and total length of female sevengill sharks *(Notorynchus cepedianus)*. Immature: 163 - 219 cm and mature sharks: >220 cm.

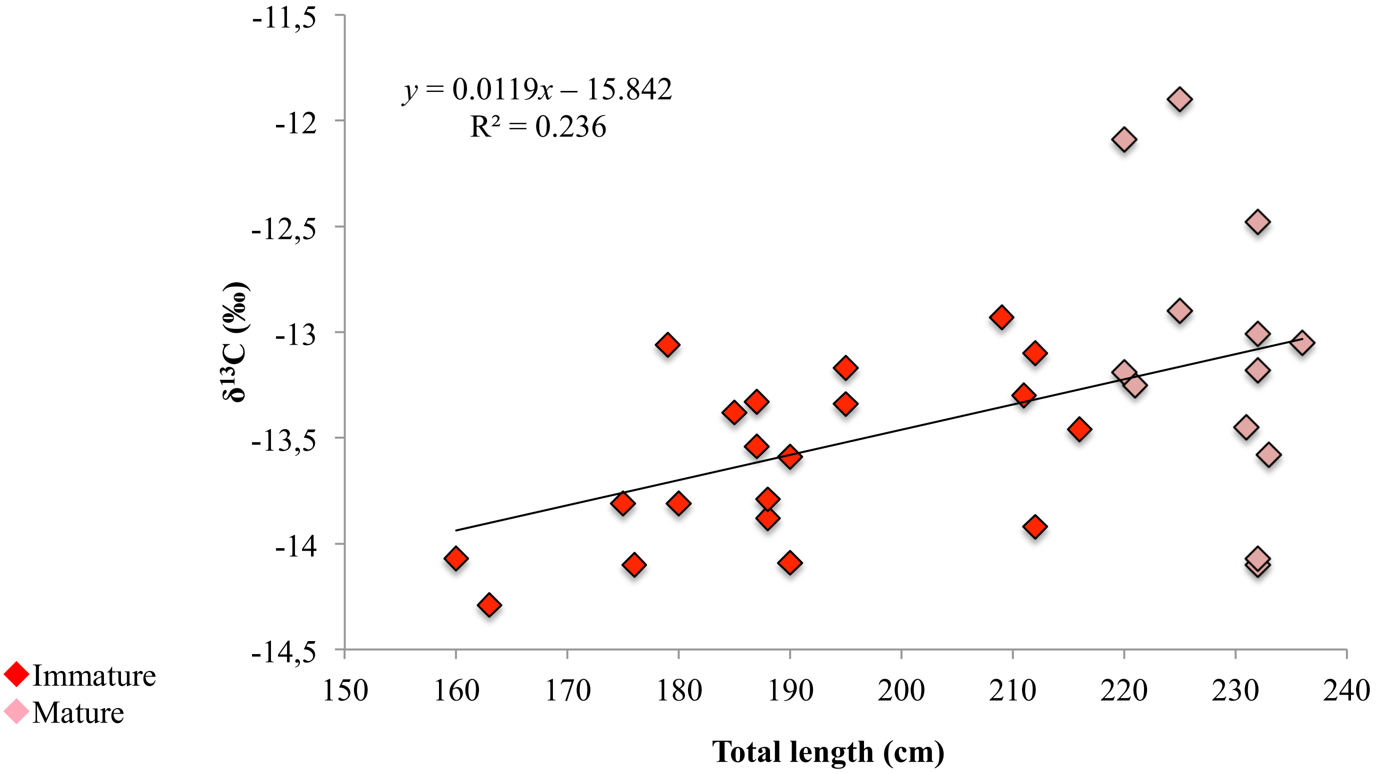


Figure 8.2. The relationship between δ^{13}C and total length of female sevengill sharks *(Notorynchus cepedianus)*. Immature: 163 - 219 cm and mature sharks: >220 cm.

3.5. Comparing isotope values of sevengills and white sharks

Both white shark and sevengills exhibited a broad range of carbon and nitrogen values (δ^{15}N: 16.37 to 18.04; δ^{13}C: –15.25 to –13.20 and δ^{15}N: 16.95 to 18.82; δ^{13}C: –14.29 to –11.90, respectively) (Figure 9). The mean δ^{15}N values of sevengills (mean ± SD: 17.78 ± 0.49, n = 39) were significantly (U = 49.50, p = 0.01) higher than those of white sharks (mean ± SD: 17.1 ± 0.56). Similarly mean ± SD δ^{13}C (13.46 ± 0.54) were significantly higher (U = 51.00, p = 0.01) than in white sharks (mean ± SD: –14.19 ± 0.67) (Figure 4). Since all white sharks sampled were classified as immature, I removed all mature sevengill samples and repeated the comparison. Sevengill isotope values were still significantly higher than white sharks (U = 31.00, Z = 2.13, p = 0.03 for δ^{13}C and U = 16.50, Z = 2.93, p = 0.00 for δ^{15}N).

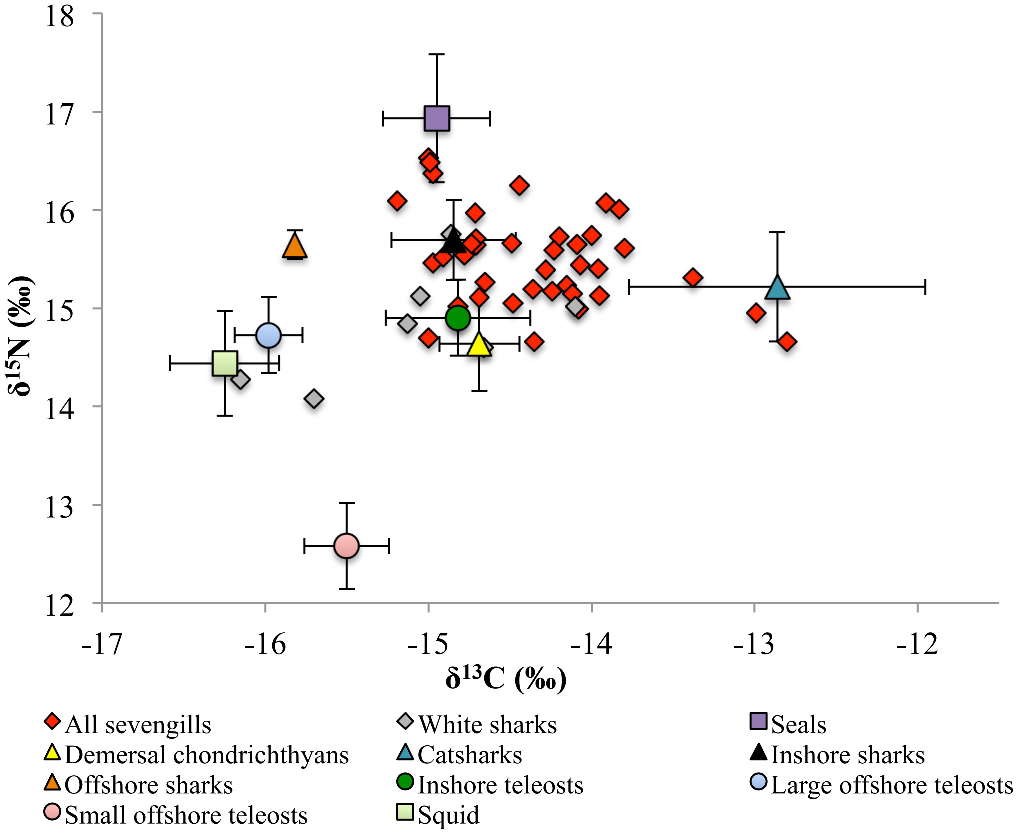


Figure 9. Dual isotope plot showing δ^{13}C and δ^{15}N for six individual white sharks *(Carcharodon carcharias)* and 39 sevengill sharks *(Notorynchus cepedianus)* (TEF corrected δ^{13}C –0.9 and δ^{15}N –2.29) and the mean ± SD δ^{13}C and δ^{15}N of their potential prey groups.

3.5.1. *$\delta^{13}C$ and $\delta^{15}N$ for white sharks and prey*

Stable isotope values for individual white sharks after TEF correction were scattered between all prey groups (Figure 10). White sharks were between 300 and 350 cm in length and linear regressions showed no significant relationship between size and $\delta^{13}C$ ($R^2 = 0.036$, $p > 0.05$) or $\delta^{15}N$ values ($R^2 = 0.016$, $p > 0.05$).

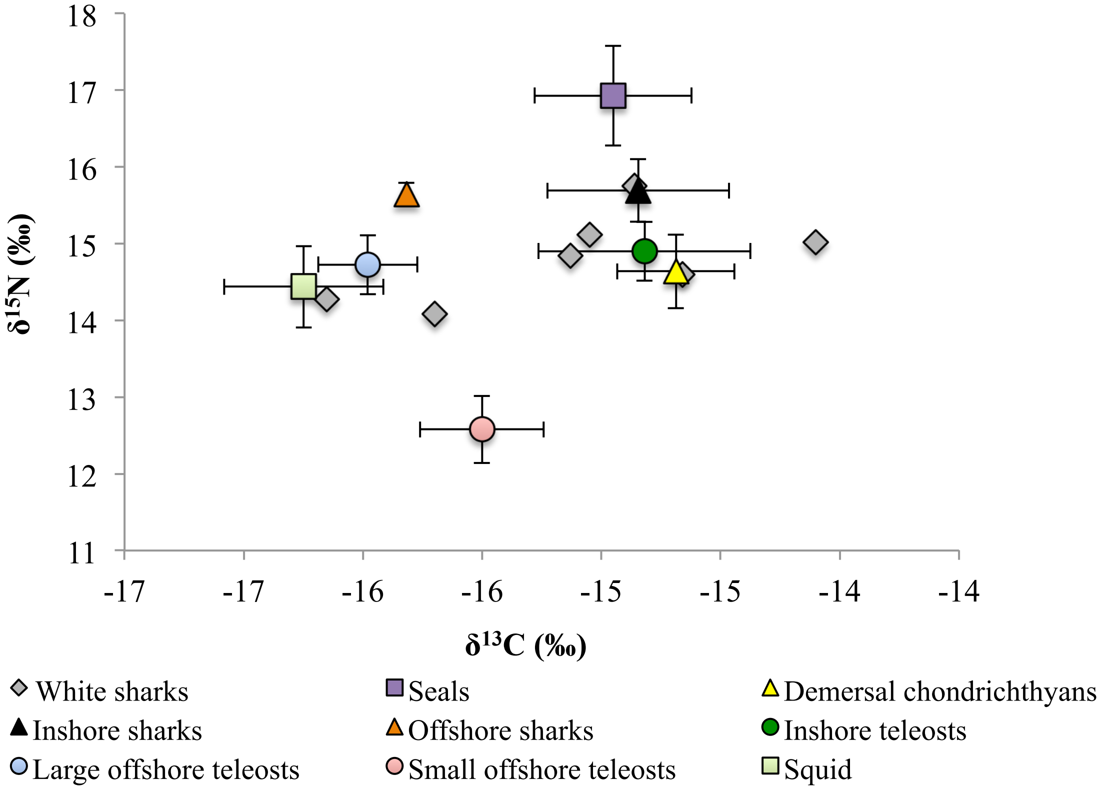


Figure 10. Dual isotope plot showing $\delta^{13}C$ and $\delta^{15}N$ of six individual white sharks *(Carcharodon carcharias)* from False Bay (TEF corrected $\delta^{13}C$ –0.9 and $\delta^{15}N$ –2.29) and mean ± SD $\delta^{13}C$ and $\delta^{15}N$ of their potential prey groups.

CHAPTER 4
DISCUSSION


Leigh de Necker

4.1. Extraction methods and inter-tissue comparisons for deriving isotope values in sevengills

The effect of lipid extraction on the isotope values of plasma and muscle tissue of sevengills and their prey was an increase in $\delta^{13}C$ (Table 3, Figure 2). Although the overall increase in $\delta^{13}C$ values observed in this study were small, and lipid content is reportedly low in elasmobranch tissue, variation has been found between groups and species (Hussey *et al.* 2012b). Lipid extraction was thus performed to standardise data among individuals, between different tissues in conspecifics and across species within a food web.

Detailed inter-tissue comparisons were limited in our study because of the low sample size of plasma samples obtained and the limited size range of sharks included in the analyses. Although no significant difference was observed between $\delta^{13}C$ values of plasma and muscle of sevengills, there was a significant variation in $\delta^{15}N$ with values of muscle tissue being consistently higher than those of plasma (Table 4, Figure 3). Matich *et al.* (2010) found a similar relationship between $\delta^{15}N$ values in the blood and muscle of bull and tiger sharks in addition to large variation in $\delta^{13}C$. Comparisons within or between species should thus control for both extraction methods and the type of tissue sampled when attempting to elucidate possible differences that may be attributed to habitat use, body condition and/or seasonality between individuals of the same or different species. To avoid confounding effects associated with comparing different tissues, I only compare samples from muscle tissue following lipid extraction. The only exception to this was the use of plasma (which has a faster rate of turnover) for the seasonal dietary comparisons of sevengills.

4.2. Predators and prey in False Bay

Similar to findings by Cortés (1999) and Abrantes and Barnett (2011), sevengills in this study had the highest $\delta^{15}N$ values of all predator and prey species sampled, including the larger white shark (Figure 4). This suggests that sevengills occupy a higher trophic position relative to all other species analysed in this study. Sevengill isotope values clustered predominantly around prey groups that are known to frequent inshore habitats (Figure 6). This supports previous studies in both the Western Cape (Ebert 1991a) and Tasmania (Abrantes & Barnett 2011) which used a combination of diet and habitat use to reach the conclusion that the sevengill spends a large amount of time in coastal areas. However, the diet of the sevengill also included prey species from a variety of habitats, including reef, demersal and, to a lesser extent, pelagic habitats (Figure 6). Foraging in diverse habitats in addition to consuming prey from different functional groups supports previous conclusions (Barnett *et al.* 2010b) and the main prediction of this study, that the sevengill is a generalist apex predator feeding opportunistically on seasonally abundant prey.

Chondrichthyans emerged as a major prey group for sevengills in False Bay which mirrors stomach content data for the species in southern Africa (Ebert 1991a), California (Ebert 1989), Argentina (Lucifora *et al.* 2005) and Australia (Braccini 2008; Barnett *et al.* 2010b) (Figure 11). Teleosts were also an important prey group, in California and Argentina in particular, with seals making up a smaller contribution to the overall diet. The exception to this trend is southern Africa, where marine mammals comprise a higher proportion of the diet than teleosts (Ebert 1991a) (Figure 11).

Ebert (1991a) analysed the stomach contents of sevengills from four different regions off southern Africa and revealed that while chondrichthyans were found to be the most important prey group for sharks in the Eastern Cape, Western Cape and central Namibian regions, mammals (Cape fur seals in particular) were the most important prey group for sharks in southern Namibia. Delphinids were the main group of mammals preyed upon by sharks in the Eastern Cape, which only has two small seal rookeries. By contrast, the other regions contain various larger rookeries, with the southern Namibian region having the highest concentration of seal colonies in southern Africa (Oosthuizen 1991). Similarly, stomach contents of sevengills from Patagonian waters contained larger proportions of pinnipeds than cetaceans, which was likely related to the higher abundance of pinnipeds in the area (Crespi-Abril *et al.* 2003). Together these data suggest that sevengills are opportunistic and consume prey according to their relative abundance in each region.

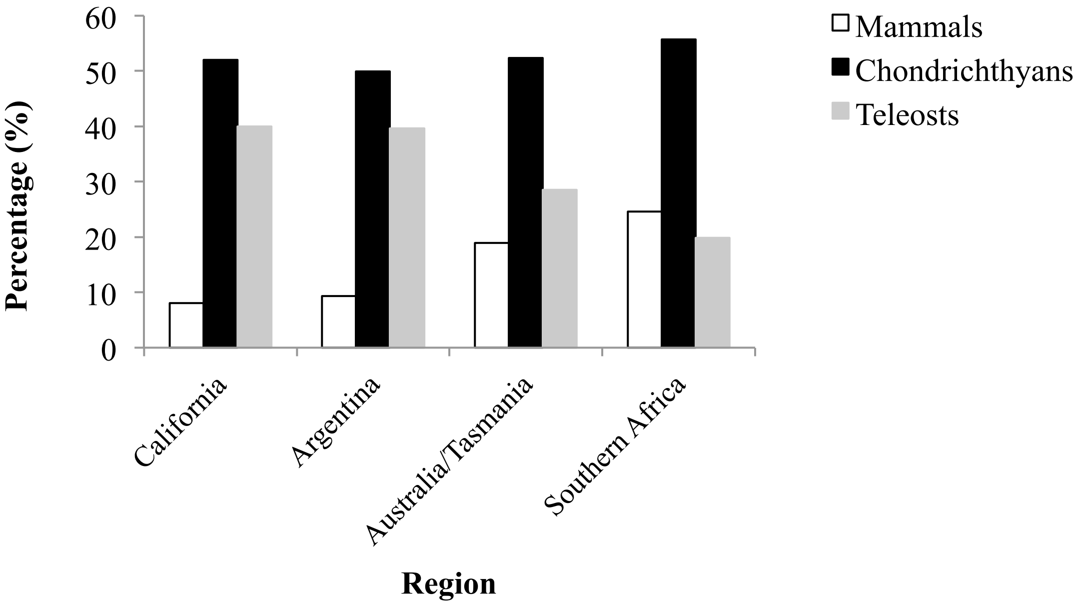


Figure 11. The percentage of mammal, chondrichthyan and teleost prey found in stomach contents of sevengill sharks *(Notorynchus cepedianus)* in four regions around the world. Adapted from Ebert 1989 (California); Lucifora *et al.* 2005 (Argentina); Braccini 2008, Barnett *et al.* 2010b (Australia).

The relative proportions of the contribution of various prey groups to immature and mature female and male sevengill diet, as estimated by SIAR, indicated that demersal chondrichthyans, inshore sharks and teleosts appear to be of similar importance (Table 7, Figure 5). Larger sharks included a greater proportion of prey with lower $\delta^{15}N$ values in their diet, i.e. catsharks, whereas smaller sevengills, had a greater contribution of prey with relatively higher $\delta^{15}N$, but low $\delta^{13}C$ values e.g. seals, in their diet (Table 7, Figure 5). The presence of seals in the diet of sevengills in False Bay was confirmed when individuals that were being sampled on board the boat regurgitated seal remains. Together these findings support those of Ebert (1991a, 2002), who showed that chondrichthyans were the most important prey group for sevengills caught in the Western Cape, and further that immature female sharks aggregated in areas where marine mammals are readily available. What is not clear from this or previous studies is whether sevengills are showing a preference for specific prey or are foraging opportunistically on the most available prey in particular areas, while also segregating for purposes such as reproduction or competition.

The high δ^{13}C values reported in this study for sevengills (Figure 4) suggests they are consuming prey that live and feed within high δ^{13}C habitats. In the marine environment these are typically benthic (Boyle *et al.* 2012), inshore environments, with organisms living in close proximately to kelp forests having the highest δ^{13}C values of all consumers (Kaehler *et al.* 2000). Miller's Point is a well-established sevengill aggregation site within False Bay and many of the individuals in this study were captured near this site. Miller's Point is characterised by extensive kelp forests with an abundance of catshark prey (De Vos *et al.* 2015a). Thus the high δ^{13}C values for sevengills in this study may reflect their preference for an inshore demersal habitat that is dominated by kelp and where the most abundant prey is high δ^{13}C catshark species (Table 5, Figure 4).

Similar results were obtained for other predator and prey species in this study, with inshore prey groups having higher δ^{13}C values than offshore groups, and larger predatory groups having higher δ^{15}N values (Figure 4). There was no significant difference in δ^{13}C values of seals, large inshore sharks, inshore teleosts and demersal chondrichthyans, with a considerable amount of overlap between the latter two groups (Table 5 & 6, Figure 4). These similarities in δ^{13}C suggest that the species in these groups feed in similar habitats (Kim *et al.* 2012a), ie. inshore areas and kelp beds. Differences in δ^{15}N however, suggest that they occupy different trophic levels, with seals having the highest δ^{15}N values of all potential prey species (Table 5, Figure 4).

Larger animals generally have the ability to consume larger, higher trophic level prey (Arim *et al.* 2010), therefore, it is not surprising that large offshore teleosts were significantly higher δ^{15}N values, relative to small offshore teleost species (Table 5, Figure 4). There was no significant difference in δ^{15}N between offshore and inshore sharks, or between large offshore and inshore teleost species (Table 6). This is supported by average trophic level calculations from Cortés (1999), who found species in offshore and inshore shark groups to occupy similar trophic levels of 4.2 and 4.1, respectively. However, differences between inshore and offshore teleost and shark groups are most notable in their δ^{13}C values, where significantly higher stable isotope values were observed in inshore groups (Table 5 & 6, Figure 4). Although the species in each of these groups appear to occupy similar trophic positions, the results here suggest they spend different amounts of time feeding in select habitats (e.g.

inshore versus offshore and benthic versus pelagic) that have marked differences in $\delta^{13}C$ values. In addition, the variability within and overlap between some groups is expected and largely attributed to overlapping diets (Daly *et al.* 2013).

4.3. Variation in sevengill shark diet

4.3.1. Seasonal variation

No inter-annual variation in isotope values was apparent over the three year sampling period and thus "year" was excluded as a variable in this analysis. No significant difference was found between summer and winter plasma samples of sevengills sampled in False Bay. Although sample sizes are small and the results should thus be considered as preliminary, the lack of seasonal variation was unexpected. Sevengills in other parts of the world have been shown to engage in seasonal shifts in habitat use, which is attributed to seasonal changes in the abundance of prey (Barnett *et al.* 2010b, Barnett *et al.* 2011, Barnett & Semmens 2012, Williams *et al.* 2012, Barnett *et al.* 2012). In False Bay, sevengills are known to exhibit marked seasonal shifts in presence along the inshore region (Engelbrecht, unpublished data). Although the reason(s) for these shifts in habitat use are unknown and may be attributed to predator avoidance, reproduction and/or foraging. I had nevertheless predicted that they would be reflected by seasonal variation in their isotopic values. The lack of seasonal variation suggests that changes in habitat use do not correspond to a shift in diet which is supported by the fact that many of the important prey species e.g. puffadder shyshark, dark shyshark, smoothhound shark, pyjama shark, leopard catshark (De Vos *et al.* 2015a), and Cape fur seals (Laroche *et al.* 2008, De Vos *et al.* 2015b), are abundant all-year round within False Bay.

4.3.2. Sex differences

Similar to previous studies for sevengills (Abrantes & Barnett 2011) and other elasmobranchs (Espinoza *et al.* 2015) there were no differences in isotope values of all size/life stage categories of male and female sevengills (Figure 7, Table 8). There was however, a significant difference in $\delta^{13}C$ values between mature female and mature male sevengill sharks (Figure 7, Table 8). Significant interactions between sex and size have been observed between male and female brown smoothhound sharks, *Mustelus henlei*, which were shown to feed from different $\delta^{13}C$ sources as they grew larger (Espinoza *et al.* 2015). Larger female sevengills may show a shift in diet for reasons relating to reproduction, or move to different areas in order to avoid males and their persistent and violent harassment in attempting to mate (Ebert 2002). Sexual segregation is common among elasmobranch species (Wearmouth & Sims 2008) and has been reported amongst a variety of species, including scalloped hammerhead sharks, *Sphyrna lewini* (Klimley 1987), shortfin makos, *Isurus oxyrhinchus* (Mucientes *et al.* 2009) and white sharks (Kock *et al.* 2013). δ^{13}C values of sevengills in south-east Tasmania differed between sexes and this difference was suggested to be associated with differences in movement patterns between males and females (Abrantes & Barnett 2011).

4.3.3. Size differences

It is hypothesised that a positive relationship exists between body size and trophic position in most trophic structures, whereby larger animals are better equipped to satisfy increased energetic demand and sustain viable population sizes at higher trophic positions, where fewer resources are available (Arim *et al.* 2010). This has been shown through stable isotope studies on a variety of apex predatory shark species, including white sharks, (Hussey *et al.* 2012b) and bull sharks, *Carcharhinus leucas* (Daly *et al.* 2013); which revealed that larger sharks showed higher δ^{15}N values, indicative of them occupying a higher trophic position than smaller conspecifics. It is thus surprising that in this study δ^{15}N decreased with an increase in body size for female sevengills ranging from 160 cm - 236 cm TL (Figure 8.1). This finding further contradicts previous studies on ontogenetic dietary shifts using stomach contents of sevengill sharks which showed an increase in consumption of higher trophic level prey, such as marine mammals and other sharks, with an increase in size (Ebert 2002, Lucifora *et al.* 2005, Braccini 2008).

However, full ontogenetic isotope profiles and diet shifts could not be determined from my data due to the absence of neonate and juvenile sharks in the sample. Therefore, the relationship between size and trophic position remains unclear (Arim *et al.* 2010). Abrantes and Barnett (2011) found that the largest animals (>250 cm TL) in their study in Tasmania, also had lower δ^{15}N than the smaller size class (189 - 250 cm TL). Similarly, Kim *et al.* (2012b) found substantial ontogenetic and individual variation in diet within a white shark population in the northeast Pacific and suggested that high δ^{15}N values of young sharks could be a result of consistent scavenging on high trophic level prey. Further studies on white sharks in the Pacific (Carlisle *et al.* 2012) and dusky sharks *(Carcharhinus obscurus)* in Kwa-Zulu Natal, South Africa (Hussey *et al.* 2012b), also found a decrease in $\delta^{15}N$ with an increase in size of large female sharks. Both studies concluded that the observed pattern is likely indicative of changes in habitat use and hence prey consumed, possibly related to the onset of maturity. Sevengill females mature at 220 cm TL (Ebert 1996) and adults have a reproductive cycle of two to three years (Awruch *et al.* 2014), during which their movement patterns are different to immature female sevengills, which are seldom present in nursery areas (Ebert 1991a, 1996, 2002). These differences in habitat use may explain the observed differences in stable isotope composition with maturity (Abrantes & Barnett 2011).

A significant positive relationship between $\delta^{13}C$ and body size was evident (Figure 8.2) and mature female sevengills exhibited a wider range of $\delta^{13}C$ than immature females (Table 8). Daly *et al.* (2013) found that mature bull sharks, *Carcharhinus leucas*, exhibited a significantly wider range of $\delta^{13}C$ values than immature sharks and suggested that mature animals are likely to source a wider variety of prey over a wider range of habitats, a hypothesis supported by others (Papastamatiou *et al.* 2006, Kim *et al.* 2012b, Shiffman *et al.* 2014, Espinoza *et al.* 2015). It thus seems reasonable to suggest that mature sevengills may have a wider dietary range and potentially exhibit a niche shift consistent with an expanded foraging area. Differences in habitat use may result in differences in feeding behavior (Hussey *et al.* 2012b), but it is unlikely that foraging location is the only factor contributing to variability in isotope signatures. This variation may be attributed to habitat-specific variation in prey availability, or differences in cost-benefit ratios of potential prey among sexes, age and/or size classes of consumers (Quevedo *et al.* 2009, Kim *et al.* 2012b). Dietary differences associated with size and/or life history stage of sevengills may further be a strategy for reducing intraspecific competition (Ebert 2002, Cedrola *et al.* 2009). At this stage, we lack sufficient knowledge on sevengill movement patterns, habitat use, competition and prey availability in False Bay to determine the drivers of $\delta^{13}C$ variation in sevengills. My results are also similar to those of a study by Madigan *et al.* (2012), who found that the Pacific bluefin tuna *(Thunnus orientalis),* an apex predator in the California Current Large Marine Ecosystem, exhibited increasing $\delta^{13}C$ and decreasing $\delta^{15}N$ values with increasing size (Figure 8.1 & 8.2). They attributed these patterns to prey switching or differences in habitat use across the size ranges sampled. Mature female sevengill sharks exhibited a wider range of $\delta^{13}C$ relative to immature females (Table 8). As with my results, Daly *et al.* (2013) found that mature bull sharks, *Carcharhinus leucas*, in their study, exhibited a significantly wider range of $\delta^{13}C$ values relative to immature sharks and suggested that mature animals are likely to source a wider variety of prey over a wider range of habitats. Increased mobility in mature animals is not uncommon in marine communities (Gaines *et al.* 2007), and size based habitat partitioning and associated diet separation has been widely reported in sharks (Simpfendorfer *et al.* 2005, Papastamatiou *et al.* 2006, Kim *et al*. 2012b).

4.4. Individual dietary specialisation in sevengills

While sevengills are considered a generalist species as they consume a variety of prey (Braccini 2008), the variability in stable isotope results from sharks in False Bay suggests that some level of individual specialisation may be apparent in our sample (Figure 6, Figure 7). Diet is often regarded as a species-level trait, but it is important to consider the potential of individual variation that exists within a species (Araújo *et al.* 2011, Matich *et al.* 2011, Matich *et al.* 2015). To determine the functional role of an apex predator, such as the sevengill, intra-population variations in their diet and habitat use should be considered, as it may ultimately affect the food web dynamics of associated communities (Abrantes & Barnett 2011). It should not be discounted that high variability in $\delta^{13}C$ may be as a result of staggered arrival of different individuals into the study area, and so, although they may be feeding on the same prey, their isotope signatures are reflecting different times spent in these areas, rather than variations in overall diet composition (Abrantes & Barnett 2011). Patterns and variations in stable isotope values within populations and among individuals can further be driven by differences in foraging success (Hussey *et al.* 2012a), prey preferences (Kim *et al.* 2012b), seasonal food availability (Daly *et al*. 2013), quality of food consumed, metabolic processes, behavioural changes and fluctuations in environmental conditions (Matich *et al.* 2010). This, once again, highlights the importance of interpreting isotope results in conjunction with movement data. If sevengills permanently resided in these areas, identifying intrapopulation differences in diet may be possible (Abrantes & Barnett 2011), but this study lacks the necessary detail to confirm dietary specialisation of sevengills in False Bay. Additional research revealed distinct patterns in intraspecific habitat partitioning and recurring site fidelity in the use of the coastal habitats, suggested to be related to increased foraging success as a result of spatial familiarity and increased knowledge of local prey (Barnett *et al.* 2011). Variations in isotope data have also been found for white sharks (Kim *et al.* 2012b) and bull sharks (Daly *et al.* 2013) and suggest that individual specialisation is likely to take place within generalist populations. The variation reported in δ^{15}N values of white sharks is thought to be related to variable foraging strategies, whereby certain individuals may feed on particular prey types in particular habitats (Hussey *et al.* 2012b).

4.5. Inferring foraging behaviour from the isotope results of sevengills

Although various hunting strategies have been proposed for sevengills (Ebert 1991b, Lucifora *et al.* 2005, Braccini 2008), there is uncertainty regarding their ability to actively capture live seals, especially when compared to the effort and speed displayed by species like the white shark that actively hunt live mammal prey. It is thus widely assumed that sevengills consume most of their mammal prey by scavenging on carcasses (Crespi-Abril *et al.* 2003). However, according to Ebert (1991a), the frequency of occurrence of marine mammal remains in the stomach contents of sevengills is too high to be solely attributed to scavenging. Indeed the high amount of mammals consumed in all regions where diet studies are available for sevengills suggests they capture live mammals (Barnett *et al.* 2012). McMeans *et al.* (2013) found seals to be an important part of the diet of Greenland sharks, but also questioned their ability to successfully capture live seals, since they are slower swimmers than other sharks that have been observed to actively prey on pinnipeds. It was suggested that the Greenland shark may employ an alternate hunting strategy, capturing fast moving prey while they sleep in the water column (Leclerc *et al.* 2012), or that they consume seals through opportunistic scavenging (McMeans *et al.* 2013). Similar to the Greenland shark, the Pacific sleeper shark, *Somniosus pacificus*, is a large, seemingly sluggish looking shark that has been suggested to catch their fast moving prey at night, by vertically oscillating through the water column using olfactory cues (Hulbert *et al.* 2006). Barnett *et al.* (2010d) found sevengills in south-east Tasmania showed distinct diel differences in activity patterns likely related to foraging. The continuous oscillating movements through the water column recorded at night suggest that sevengills are predominantly nocturnal foragers, while during the day sharks were less active staying close to the sea floor, with burst speed events likely associated with opportunistic attacks. These behaviours, coupled with the frequent occurrence of benthic and marine mammal prey in the diet of sevengills, suggest that they may use similar strategies to species such as the Greenland shark and Pacific sleeper shark. Given the difficulty in directly observing predation events of sevengills, it is suggested that future work using accelerometers, cameras and telemetry be used to provide insight into the foraging behaviour of the species.

4.6. White sharks and sevengills

Interestingly, assuming similar TEFs for both species, higher $\delta^{15}N$ values for sevengills, placed them at a higher trophic level relative to the white sharks in this sample (Figure 10). This result is consistent with a study by Cortés (1999), who found sevengills (4.7) to occupy a higher trophic level than white sharks (4.5). Chondrichthyan prey appeared to be the most important prey group for both predators comprising 35.7% of the diet of white sharks and 40.7% of the diet of sevengills (Cortés 1999). The main proportional difference in diet was that sevengills consumed more mammal prey (35.5% vs 21.1%) and less teleosts (21.8% vs 35.5%), compared to white sharks (Cortés 1999). The higher proportion of mammal prey for sevengills in my study may be attributed to their feeding on seals all year round, whereas white sharks appear to switch from feeding predominantly on young-of-the-year seals in winter (De Vos *et al.* 2015b, Kock *et al.* 2013), to migratory elasmobranchs and fish during the summer months (Kock *et al.* 2013). In addition, the white sharks sampled in this study were all classified as immature (between 3 - 3.5 m TL) (Compagno 2001) and the consumption of marine mammals is known to increase with body size, particularly for sharks greater than 3 m TL (Hussey *et al.* 2012b).

Another possible reason for lower $\delta^{13}C$ and $\delta^{15}N$ values in white sharks may pertain to differences in broad scale habitat use by the two species. Offshore regions are known to have lower $\delta^{13}C$ and $\delta^{15}N$ relative to inshore areas (Kaehler *et al.* 2000, Hill *et al.* 2006), and while white sharks move between inshore and offshore environments (Carlisle *et al.* 2012), sevengills appear to be a predominantly coastal species (Ebert 1991a) frequenting inshore kelp forests (Engelbrecht, unpublished data).

Although both $\delta^{13}C$ and $\delta^{15}N$ values differed between white sharks and sevengills, differences in $\delta^{13}C$ were more marked. The variability in $\delta^{13}C$ values of species at similar trophic positions that occur in the same area may be a result of resource partitioning between the species, employed as a strategy to reduce competition (Papastamatiou *et al.* 2006, Kinney *et al.* 2011, Espinoza *et al.* 2015). For example, the significant increase in $\delta^{13}C$ values reported for silky sharks, *Carcharhinus falciformis,* relative to blue sharks, *Prionace glauca,* in the southwestern Indian Ocean was considered evidence for niche partitioning between the species, whereby silky sharks foraged in more inshore habitats compared to blue sharks (Rabehagasoa *et al.* 2012). Similarly, Kinney *et al.* (2011) found wide ranging $\delta^{13}C$ values among various species of sharks and predatory teleosts occupying similar trophic positions in their study in Cleveland bay, Australia. It is, however, important to interpret variation in isotope data with caution (Kinney *et al.* 2011), as variability may be a reflection of differences in spatial and seasonal distributions of a species (Espinoza *et al.* 2015) that may further vary with sex, size and/or life history stage. It is, therefore, essential to gain detailed knowledge on the movement patterns of a species in order to understand their feeding ecology (Espinoza *et al.* 2015). Further research into the spatial and temporal variability of the primary producers in False Bay will also provide insights into the variability associated with top predatory shark isotope values and how they may be linked to potential shifts in isotopic baselines. The assistance of other biomarkers, such as fatty acids and trace metal analyses, may also provide useful tools for gaining further ecological insights into the trophic dynamics of top predatory shark species.

Conclusions

This is the first study to use stable isotopes from muscle tissue and blood plasma of sevengills to gain insights into their diet and relative trophic position within False Bay, South Africa. Muscle tissue had higher $\delta^{13}C$ and $\delta^{15}N$ values than plasma and sample treatment and preparation affected both $\delta^{13}C$ and $\delta^{15}N$ values. This variation highlights the importance of consistency in sample preparation when comparing isotope values intra- and interspecifically. Estimates of isotopic fractionation, or at least of differences in fractionation between tissues, are necessary for comparisons between different tissues (Dalerum & Angerbjörn 2005). Due to the complexity associated with fractionation among species and between tissues, it is strongly suggested that efforts be made to perform laboratory experiments to improve our understanding of differential assimilation of dietary components, isotopic fractionation and metabolic routing (Dalerum & Angerbjörn 2005). It is further important to use species-specific fractionation values as far as possible when comparing values from different tissues (Dalerum & Angerbjörn 2005, Hussey *et al.* 2012b). To avoid confounding effects associated with comparing different tissues, all analyses were done on samples of the same tissue.

Sevengill sharks have the highest $\delta^{15}N$ values of all species sampled in this study, feeding on a variety of prey species from various functional groups and predominantly in coastal waters. These findings are in agreement with previous studies, both abroad and in southern Africa. Inshore chondrichthyans were found to be their most important prey, with Cape fur seals and inshore teleost species also being important prey groups. No seasonal shift in diet was observed which was unexpected due to the marked seasonal aggregation of the species in kelp forests in False Bay. It is possible that sevengills feed on similar prey in different areas, thus masking any temporal or spatial segregation. It is important to note that the sample size for plasma, that was used to investigate seasonal variation in isotope values was small ($n = 7$) and thus the results for seasonality should be considered preliminary.

The most surprising result in this study was the significant decrease in $\delta^{15}N$ with the increase in size of sevengills, where immature female sharks had higher $\delta^{15}N$ values than mature individuals. I had predicted that larger sharks would consume more mammalian prey species (e.g. seals), which had the highest $\delta^{15}N$ values of all prey.

The increase in $\delta^{15}N$ with size has however also been reported in another study on sevengills in Tasmania (Abrantes & Barnett 2011) and for white sharks both in False Bay and in the northeastern Pacific (Carlisle *et al.* 2012) suggesting that this relationship is not exceptional. It is possible that the abundant scavenging opportunities on seal pups around Seal Island in False Bay may provide immature sharks with access to large amounts of high $\delta^{15}N$ food while mature sharks might be restricted to coastal waters away from seal colonies for reasons related to reproduction.

The results of this study confirm that the sevengill is an apex predator and generalist species feeding on a variety of prey across diverse habitat types. It is possible that the marked variation in isotope values, particularly $\delta^{13}C$, reveals some level of individual dietary specialisation. However, elucidating such finer scale differences will require further research on the life history, movement patterns and feeding behaviour of sevengills in False Bay. When this work is mirrored by efforts on the sympatric white shark, the opportunity to explore the trophic and ecological role of top predatory sharks in a coastal bay will become an important milestone in shark ecology.


Leigh de Necker © African Shark Eco- Charters

5. REFERENCES

ABRANTES, K.G., BARNETT, A. 2011. Intrapopulation variations in diet and habitat use in a marine apex predator, the broadnose sevengill shark, *Notorynchus cepedianus*. *Marine Ecology Progress Series* 442: 133-148.

ARAÚJO, M.S., BOLNICK, D.I., LAYMAN, C.A. 2011. The ecological causes of individual specialisation. *Ecology Letters* 14(9): 948-958.

ARIM, M., ABADES, S., LAUFER, G., LOUREIRO, M., MARQUET, P.A. 2010. Food web structure and body size: trophic position and resource acquisition. *Oikos* 119(1): 147-153.

AWRUCH, C.A., JONES, S.M., GARCÍA ASOREY, M., BARNETT, A. 2014. Non-lethal assessment of the reproductive status of broadnose sevengill sharks (*Notorynchus cepedianus*) to determine the significance of habitat use in coastal areas. *Conservation Physiology* 2: doi: 10.1093/conphys/cou013.

BARNETT, A., STEVENS, J.D., FRUSHER, S.D., SEMMENS, J.M. 2010a. Seasonal occurrence and population structure of the broadnose sevengill shark *(Notorynchus cepedianus)* in coastal habitats of south-east Tasmania. *Journal of Fish Biology* 77: 1688-1701.

BARNETT, A., ABRANTES, K., STEVENS J.D., YICK, J, FRUSHER, S.D., SEMMENS, J.M. 2010b. Predator-prey relationships and foraging ecology of a marine apex predator with a wide temperate distribution. *Marine Ecological Progress* Series 416: 189-200.

BARNETT, A., ABRANTES, K., STEVENS J.D., BRUCE, B.D., SEMMENS, J.M. 2010c. Fine-Scale Movements of the Broadnose Sevengill Shark and its Main Prey, the Gummy Shark. *PLOS ONE* 5(12): e15464.

BARNETT, A., ABRANTES, K.G., STEVENS, J.D., SEMMENS, J.M. 2011. Site fidelity and sex-specific migration in a mobile apex predator: implications for conservation and ecosystem dynamics. *Animal Behaviour* 81: 1039-1048.

BARNETT, A., SEMMENS, J.M. 2012. Sequential movement into coastal habitats and high spatial overlap of predator and prey suggest high predation pressure in protected areas. *Oikos* 121: 882-890.

BARNETT, A., BRACCINI, J. M., AWRUCH, C.A., EBERT, D.A. 2012. An overview on the role of Hexanchiformes in marine ecosystems: biology, ecology and conservation status of a primitive order of modern sharks. *Journal of Fish Biology* 80: 966-990.

BAUM, J. K., WORM, B. 2009. Cascading top-down effects of changing oceanic predator abundances. *Journal of Animal Ecology* 78: 699-714.

BEST, L.N., ATTWOOD, C.G., DA SILVA, C., LAMBERTH, S.J. 2013. Chondrichthyan occurrence and abundance trends in False Bay, South Africa, spanning a century of catch and survey records. 2013. *African Zoology* 48(2): 201-227.

BOYLE, M.D., EBERT, D.A., CAILLIET, G.M. 2012. Stable-isotope analysis of a deep-sea benthic-fish assemblage: evidence of an enriched benthic food web. *Journal of Fish Biology* doi:10.1111/j.1095-8649.2012.03243.x.

BRACCINI, J.M. 2008. Feeding ecology of two high-order predators from south-eastern Australia: the coastal broadnose and the deepwater sharpnose sevengill sharks. *Marine Ecology Progress Series* 371: 273-284.

CARLISLE, A.B., KIM, S.L., SEMMENS, B.X., MADIGAN, D.J., JORGENSEN, S.J., PERLE, C.R., ANDERSON S.D., CHAPPLE, T.K., KANIVE, P.E., BLOCK, B.A. 2012. Using Stable Isotope Analysis to Understand the Migration and Trophic Ecology of Northeastern Pacific White Sharks (*Carcharodon carcharias*). *PLOS ONE* 7(2): e30492.

CEDROLA, P.V., CAILLE, G.M., CHIARAMONTE, G.E., PETTOVELLO, A.D. 2009. Demographic structure of broadnose sevengill shark, *Notorynchus cepedianus*, caught by anglers in southern Patagonia, Argentina. *Marine Biodiversity Records* 2: e138.

COMPAGNO, L.J.V. 2001. *Sharks of the World. An annotated and illustrated catalogue of the shark species known to date. Volume 2. Bullhead, mackerel and carpet sharks (Heterodontiformes, Lamniformes and Orectolobiformes). FAO Species Catalogue for Fisheries Purposes* No. 1, Vol. 2. Rome, FAO. 269 pp.

COMPAGNO, L.J.V., DANDO, M., FOWLER. S. 2005. *A field guide to the Sharks of the world*. Harper Collins, London, UK:London 368 pp.

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

CRESPI-ABRIL, A.C., GARCÍA, N.A., CRESPO, E.A. COSCARELLA, M.A. 2003. Consumption of marine mammals by broadnose sevengill shark Notorynchus cepedianus in the northern and central Patagonian shelf. *Latin American Journal of Aquatic Mammals 2*(2): 101-107.

CRESSON, P., RUITTON, S., OURGAUD, M., HARMELIN-VIVIEN, M. 2014. Contrasting perception of fish trophic level from stomach content and stable isotope analyses: A Mediterranean artificial reef experience. *Journal of Experimental Marine Biology and Ecology* 452: 54-62.

DALERUM, F., ANGERBJÖRN, A. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia 144*(4): 647-658.

DALY, R., FRONEMAN, P.W., SMALE, M.J. 2013. Comparative feeding ecology of bull sharks *(Carcharhinus leucas)* in the coastal waters of the southwest Indian Ocean inferred from stable isotope analysis. *PLOS ONE* 8(10): e78229.

DENIRO, M. J., EPSTEIN, S. 1977. Mechanisms of carbon isotope fractionation associated with lipid synthesis. *Science* 197: 261-263.

DE VOS, L., WATSON, R.G.A., GÖTZ, A., ATWOOD, C.G. 2015a. Baited remote underwater video system (BRUVs) survey of chondrichthyan diversity in False Bay, South Africa. *African Journal of Marine Science* 37(2): 209-218.

DE VOS, A., O'RIAIN, M.J., MEYER, M.A., KOTZE, P.G.H., KOCK, A.A., 2015b. Behavior of Cape fur seals *(Arctocephalus pusillus pusillus)* in relation to temporal variation in predation risk by white sharks *(Carcharodon carcharias)* around a seal rookery in False Bay, South Africa. *Marine Mammal Science 31*(3): 1118-1131.

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

DULVY, N.K., BAUM, J.K., CLARKE, S., COMPANGNO, L.J.V., CORTÉS, E., DOMINGO, A., FORDHAM, S., FOWLER, S., FRANCIS, M.P., GIBSON, C., MARTINEZ, J. 2008. You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. *Aquatic Conservation: Marine and Freshwater Ecosystems 18*(5): 459-482.

EBERT, D.A. 1989. Life History of the Sevengill Shark, *N. cepedianus* (Peron 1807), in two Northern California Bays. *California Fish and Game* 75: 102-112.

EBERT, D.A. 1991a. Diet of the sevengill shark *N. cepedianus* in the temperate coastal waters of Southern Africa. *South African Journal of Marine Science*: 11: 565-572.

EBERT, D.A. 1991b. Observations on the predatory behaviour of the sevengill shark *Notorynchus cepedianus. South African Journal of Marine Science* 11: 455-465.

EBERT, D.A. 1996. Biology of the sevengill shark *Notorynchus cepedianus* (Peron, 1807) in the temperate coastal waters of Southern Africa. *South African Journal of Marine Science* 17:93-103.

EBERT, D.A. 2002. Ontogenetic changes in the diet of the sevengill shark (*Notorynchus cepedianus*). *Marine and Freshwater Research* 53: 517-523.

ESPINOZA, M., MUNROE, S.E.M., CLARKE, T.M., FISK, A.T., WEHRTMANN, I.S. 2015. Feeding ecology of common demersal elasmobranch species in the Pacific coast of Costa Rica inferred from stable isotope and stomach content analyses. *Journal of experimental Marine Biology and Ecology* 470: 12-25.

ESTES, J.A., TINKER, M.T., WILLIAMS, T.M., DOAK, D.F. 1998. Killer whale predation on sea otters linking oceanic and nearshore ecosystems. *Science 282*(5388): 473-476.

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

ESTRADA, J.A., RICE, A.N., NATANSON, L.J., SKOMAL, G.B. 2006. Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. *Ecology* 87**:** 829-834.

FERRETTI, F., WORM, B., BRITTEN, G.L., HEITHAUS, M.R., LOTZE, H.K. 2010. Patterns and ecosystem consequences of shark declines in the ocean. *Ecology Letters* 13: 1055-1071.

FISK, A.T., TITTLEMIER, S.A., PRANSCHKE, J.L., NORSTROM, R.J. 2002. Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of Greenland sharks. *Ecology 83*(8): 2162-2172.

GAINES, S.D., GAYLORD, B., GERBER, L.R., HASTINGS, A., KINLAN., B.P. 2007. Connecting Places: The Ecological Consequences of Dispersal in the Sea. *Oceanography* 20(3): 90-99.

HEITHAUS, M.R., DILL, L.M., MARSHALL, G.J., BUHLEIER, B. 2002. Habitat use and foraging behaviour of tiger sharks (*Galeocerdo cuvier*) in a seagrass ecosystem. *Marine Biology* 140: 37-248.

HEITHAUS, M.R., FRID, A., WIRSING, A.J., WORM, B. 2008. Predicting ecological consequences of marine top predator declines. *Trends in Ecology and Evolution* 23(4): 202-210.

HILL, J.M., MCQUAID, C.D., KAEHLER, S. 2006. Biogeographic and nearshore-offshore trends in isotope ratios of intertidal mussels and their food sources around the coast of southern Africa. *Marine Ecology Progress Series* 318: 63-73.

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

HULBERT, L.B., SIGLER, M.F., LUNSFORD, C.R. 2006. Depth and movement behaviour of the Pacific sleeper shark in the north- east Pacific Ocean. *Journal of Fish Biology* 69: 406-425.

HUSSEY, N.E., MACNEIL, M.A., FISK, A.T. 2010a. The requirement for accurate diet-tissue discrimination factors for interpreting stable isotopes in sharks. *Hydrobiologia* 654: 1-5.

HUSSEY, N.E., BRUSH, J., MCCARTHY, I.D., FISK, A.D. 2010b. δ^{15}N and δ^{13}C diet discrimination factors for large sharks under semi-controlled conditions. *Comparative Biochemistry and Physiology Part A* 155: 445-453.

HUSSEY, N.E., MACNEIL, M.A., OLIN, J.A., MCMEANS, B.C., KINNEY, M.J., CHAPMAN, D.D., FISK, A.T. 2012a. Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. *Journal of Fish Biology* 80: 1449-1484.

HUSSEY, N.E., MCCANN, H., CLIFF, G., DUDLEY, S.F.J., WINTNER, S.P., FISK, A.T. 2012b. Size-based analysis of diet and trophic position of the white shark *Carcharodon carcharias* in South African waters. In: ML Domeier. *Global Perspectives on the Biology and Life History of the White Shark*. Boca Raton, FL: CRC Press. pp. 27-48.

HUSSEY, N.E., MACNEIL, M.A., MCMEANS, B.C., OLIN, J.A., DUDLEY, S.F.J., CLIFF, G., WINTNER, S.P., FENNESSY, S.T., FISK, A.T. 2014. Rescaling the trophic structure of marine food webs. *Ecology Letters* 17: 239-250.

INGER, R., JACKSON, A., PARNELL, A., BEARHOP, S. 2010. SIAR v4 (Stable Isotope Analysis in R): an ecologist's guide.

KAEHLER, S., PAKHOMOV, E.A., MCQUAID, C.D. 2000. Trophic structure of the marine food web at the Prince Edward Islands (Southern Ocean) determined by $\delta^{13}C$ and $\delta^{15}N$ analysis. 2000. *Marine Ecology Progress Series* 208: 13-20.

KIM, S.L., CASPER, D.R., GALVÁN-MAGAÑA, F., OCHOA-DIÁZ, R., HERNÁNDEZ-AGUILAR, S.B., KOCH, P.L. 2011. Carbon and nitrogen discrimination factors for elasmobranch soft tissues based on a long-term controlled feeding study. *Environmental Biology of Fishes* doi: 10.1007/s10641-011-9919-7.

KIM, S.L., DEL RIO, C.M., CASPER, D., KOCH, P.L. 2012a. Isotopic incorporation rates for shark tissues from a long-term captive feeding study. *The Journal of Experimental Biology* 215: 2495-2500.

KIM, S.L., TINKER, M.T., ESTES, J.A., KOCH, P.L. 2012b. Ontogenetic and among-individual variation in foraging strategies of northeast Pacific white sharks based on stable isotope analysis. *PLOS ONE* 7(9): e45068.

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

KINNEY, M.J., HUSSEY, N.E., FISK, A.T., TOBIN, A.J., SIMPENDORFER, C.A. 2011. Communal or competitive? Stable isotope analysis provides evidence of resource partitioning within a communal shark nursery. *Marine Ecology Progress Series* 439: 263-276.

KLIMLEY, A.P. 1987. The determinants of sexual segregation in the scalloped hammerhead shark, *Sphyrna lewini. Environmental Biology of Fishes* 18(1): 27-40.

KOCK, A., O'RIAIN, M.J., MAUFF, K., MEYER, M., KOTZE, D., GRIFFITHS, C. 2013. Residency, Habitat Use and Sexual Segregation of White Sharks, Carcharodon *carcharias* in False Bay, South Africa. *PLOS ONE* 8(1): e55048. doi:10.1371/journal.pone.0055048.

LAMBERTH, S.J., BENNETT, B.A. CLARK, B.M. 1994. Catch composition of the commercial beach-seine fishery in False Bay, South Africa. *South African Journal of Marine Science* 14(1): 69-78.

LAROCHE, R.K., KOCK, A.A., DILL, L.M., OOSTHUIZEN, W.H. 2008. Running the gauntlet: a predator- prey game between sharks and two age classes of seals. *Animal Behaviour* 76: 1901-1917.

LAST, P.R., STEVENS, J.D. 2009. Sharks and rays of Australia, 2nd edn. CSIRO Publishing, Collingwood.

LECLERC, L.E., LYDERDEN, C., HAUG, T., BACHMANN, L., FISK, A.T., KOVACS, K.M. 2012. A missing piece in the Arctic food web puzzle? Stomach contents of Greenland sharks sampled in Svalbard, Norway. *Polar Biology* 35: 1197-1208.

LOGAN, J.M., LUTCAVAGE, M.E. 2010. Stable isotope dynamics in elsmobranchs. *Hydrobiologia* 644: 231-244.

LOWE, C.G., WETHERBEE, B.M., CROW, G.L., TESTER, A.L. 1996. Ontogenetic dietary shifts and feeding behavior of the tiger shark, *Galeocerdo cuvier*, in Hawaiian waters. *Environmental Biology of Fishes* 47: 203-211.

LUCIFORA, L.O., MENNI, R.C., ESCALANTE, A.H. 2005. Reproduction, abundance and feeding habits of the broadnose sevengill shark *N. cepedianus* in north Patagonia, Argentina. *Marine Ecological Progress Series* 289: 237-244.

MACNEIL, M.S., SKOMAL, G.B., FISK, A.T. 2005. Stable isotopes from multiple tissues reveal diet switching in shark. *Marine Ecology Progress Series* 302: 199-206.

MACNEIL, M.A., DROUILLARD, K.G., FISK, A.T. 2006. Variable uptake and elimination of nitrogen stable isotopes between tissues in fish. *Canadian Journal of Fisheries and Aquatic Sciences* 63(2): 345-353.

MADIGAN, D.J., CARLISLE, A.B., DEWAR, H., SNODGRASS, O.E., LITVIN, S.Y., MICHELI, F., BLOCK., B.A. 2012. Stable Isotope Analysis Challenges Wasp-Waist Food Web Assumptions in an Upwelling Pelagic Ecosystem. *Nature: Scientific Reports* 2: 654. DOI: 10.1038/srep00654.

MALPICA-CRUZ, L., HERZKA, S.Z., SOSA-NISHiZAKI, O., LAZO, J.P. 2012. Tissue-specific isotope trophic discrimination factors and turnover rates in a marine elasmobranch: empirical and modeling results. *Canadian Journal of Fisheries and Aquatic Sciences* 69(3): 551-564.

MARTINEZ DEL RIO, C., WOLF, N., CARLETON, S.A., GANNES, L.Z. 2009. Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews* 84: 91-111.

MATICH, P., HEITHAUS, M.R., LAYMAN, C.A. 2010. Size-based variation in intertissue comparisons of stable carbon and nitrogen isotopic signatures of bull sharks (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*). *Canadian Journal of Fisheries and Aquatic Sciences* 67: 877-885.

MATICH, P., HEITHAUS, M.R., LAYMAN, C.A. 2011. Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. *Journal of Animal Ecology* 80: 294-305.

MATICH, P., HEITHAUS, M.R. 2014. Multi-tissue stable isotope analysis and acoustic telemetry reveal seasonal variability in the trophic interactions of juvenile bull sharks in a coastal estuary. *Journal of Animal Ecology* 83: 199-213.

MATICH, P., KISZKA, J.P., HEITHAUS, M.R., MOURIER, J., PLANES, S. 2015. Short-term shifts of stable isotope (δ^{13}C, δ^{15}N) values in juvenile sharks within nursery areas suggest rapid shifts in energy pathways. *Journal of Experimental Marine Biology and Ecology* 465: 83-91.

MCMEANS, B.C., ARTS, M.T., LYDERSEN, C., KOVACS, K.M., HOP, H., FALK-PETERSEN, S., FISK, A.T. 2013. The role of Greenland sharks (*Somniosus microcephalus*) in an Arctic ecosystem: assessed via stable isotopes and fatty acids. *Marine Biology* 160 (5): 1223-1238.

MUCIENTES, G.R., QUEIROZ, N., SOUSA, L.L., TARROSO, P., SIMS, D.W. 2009. Sexual segregation of pelagic sharks and the potential threat from fisheries. *Biology Letters* DOI: 10.1098/rsbl.2008.0761.

NEWSOME, S.D., CLEMENTZ, M.T., KOCH, P.L. 2010. Using stable isotope biogeochemistry to study marine mammal ecology. *Marine Mammal Science* 26: 509-572.

OLIN, J.A., HUSSEY, N.E., GRGICAK-MANNION, A., FRITTS, M.W, WINTNER, S.P., FISK, A.T. 2013. Variable δ^{15}N Diet-Tissue Discrimination Factors among Sharks: Implications for Trophic Position, Diet and Food Web Models. *PLOS ONE* 8(10): e77567.

OOSTEHUIZEN, W.H. 1991. General movements of South African (Cape) fur seals *Arctocephalus pusillus pusillus* from analysis of recoveries of tagged animals African *Journal of Marine Science* 11: 21-29.

PAPASTAMATIOU, Y.P., WETHERBEE, B.M., LOWE, C.G., CROW, G.L. 2006. Distribution and diet of four species of carcharhinid shark in the Hawaiian Islands: evidence for resource partitioning and competitive exclusion. *Marine Ecology Progress Series* 320: 239-251.

PAPASTAMATIOU, Y.P., FRIEDLANDER, A.M., CASELLE, J.E., LOWE, C.G. 2010. Long-term movement patterns and trophic ecology of blacktip reef sharks (*Carcharhinus melanopterus*) at Palmyra Atoll. *Journal of Experimental Marine Biology and Ecology* 386: 94-102.

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

QUEVEDO, M., SVANBÄCK, R., EKLÖV., P. 2009. Intrapopulation niche partitioning in a generalist predator limits food web connectivity. *Ecology* 90(8): 2263-2274.

RABEHAGASOA, N., LORRAIN, A., BACH, P., POTIER, M., JAQUEMET, S., RICHARD, P., MENARD, F. 2012. Isotopic niches of the blue shark *Prionace glauca* and the silky shark *Carcharhinus falciformis* in the southwestern Indian Ocean. *Endangered Species Research* 17: 83-92.

SHIFFMAN, D.S., GALLAGHER, A.J., BOYLE, M.D., HAMMERSCHLAG-PEYER, C.M., HAMMERSCHLAG, N. 2012. Stable isotope analysis as a tool for elasmobranch conservation research: a primer for non- specialists. *Marine and Freshwater Research* 63: 635-643.

SHIFFMAN, D.S., FRAZIER, B.S., KUCKLICK, J.R., ABEL, D., BRANDES, J., SANCHO, G. 2014. Feeding Ecology of the Sandbar Shark in South Carolina Estuaries Revealed through δ^{13}C and δ^{15}N Stable Isotope Analysis. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science* 6(1): 156-169.

SIMPFENDORFER, C.A., FREITAS, G.G., WILEY, T.R., HEUPEL, M.R. 2005. Distribution and Habitat Partitioning of Immature Bull Sharks (*Carcharhinus leucas*) in a Southwest Florida Estuary. *Estuaries* 28(1): 78-85.

STEHFEST, K.M., PATTERSON, T.A., BARNETT, A., SEMMENS, J.M. 2014. Intraspecific differences in movement, dive behavior and vertical habitat preferences of a key marine apex predator. *Marine Ecology Progress Series* 495: 249-262.

STEVENS, J.D., BONFIL, R., DULVY, N.K., WALKER, P.A. 2000. The effects of fishing on sharks, rays and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES Journal of Marine Science* 57: 476-494.

WEARMOUTH V.J., SIMS, D.W. 2008. Chapter 2 Sexual Segregation in Marine Fish, Reptiles, Birds and Mammals: Behaviour Patterns, Mechanisms and Conservation Implications. *Advances in Marine Biology* 54: 107-170.

WETHERBEE, B.M., CORTÉS, E. 2004. Food Consumption and Feeding Habits. In: *Biology of Sharks and Their Relatives,* Ch. 8. CRC Press LLC, Florida.

WILLIAMS, T.M., ESTES, J.A., DOAK, D.F., SPRINGER, A.M. 2004. Killer appetites: assessing the role of predators in ecological communities. *Ecology* 85(12): 3373-3384.

WILLIAMS, G.D., ANDREWS, K.S., KATZ, S.L., MOSER, M.L., TOLIMIERI, N., FARRER, D.A., LEVIN, P.S. 2012. Scale and pattern of broadnose sevengill shark Notorynchus cepedianus movement in estuarine embayments. *Journal of Fish Biology 80*(5): 1380-1400.