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Elucidating Complex Animal Interactions in the Modern World: Determining the occurrence and effects of intraguild predation within food webs

Krystle-Blue D. Pahl
University of Windsor

Elucidating Complex Animal Interactions in the Modern World: Determining the occurrence and effects of intraguild predation within food webs

By

K. Blue Pahl

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Elucidating Complex Animal Interactions in the Modern World: Determining the occurrence and effects of intraguild predation within food webs

by

K. Blue Pahl

APPROVED BY:

_____

O. Love
Great Lakes Institute for Environmental Research

_____

D. Higgs
Department of Integrative Biology

_____

D. Yurkowski
Special Committee Member

_____

N. Hussey, Advisor
Department of Integrative Biology

December 16, 2020

DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

I. Co-Authorship

I hereby declare that this thesis incorporates material that is result of joint research, as follows: Introduction of the thesis (i.e. Chapter 1) was co-authored with David Yurkowski, Kirsty Lees and Nigel Hussey under the supervision of Nigel Hussey. Chapter 2 was co-authored with David Yurkowski, Sabine Wintner, Matthew Dicken, Geremy Cliff and Nigel Hussey under the supervision of Nigel Hussey. Chapter 3 was co-authored with David Yurkowski and Nigel Hussey under the supervision of Nigel Hussey. In all cases, the key ideas, primary contributions, experimental designs, data analysis, interpretation, and writing were performed by K. Blue Pahl. N. Hussey helped conceive the ideas, designed methodology, collect data, provided detailed feedback on refinement of ideas and editing of the manuscript in all cases; Sabine Wintner, Geremy Cliff and Matthew Dicken collected data for Chapter 2 and 3; David Yurkowski helped design methodology, contributed to the refinement of statistical analysis, provided feedback on refinement of ideas and editing of the manuscript in all cases; Kirsty Lees provided feedback on refinement of ideas and editing of the manuscript for Chapter 1.

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II. Previous Publication

This thesis includes 2 original papers that have been previously published/submitted for publication in peer reviewed journals, as follows:

Chapter [1]	Pahl, K.B., Yurkowski, D.J, Lees, K.J., Hussey, N.E. (2020) Measuring the occurrence and strength of intraguild predation in modern food webs. *Food Webs* doi.org/10.1016/j.fooweb.2020.e00165	Publication status* Published
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ABSTRACT

Animal interactions structure food webs with stability being contingent on the presence and strength of multi-species interactions. Intraguild predation (IGP) is a complex interaction that can impact species at the individual, population and community levels, ultimately determining the strength, direction and linearity of trophic cascades and species abundance across trophic levels. IGP occurs among a minimum of three species; a predator (IGpredator) that kills and consumes a prey (IGprey) with which it competes for a common resource. Through a systematic literature search, I determined traditional to modern approaches to measure the occurrence and effect of IGP and then identified the research effort afforded to the different implication levels and IGP effects characterized by Polis et al. (1989). I highlighted IGP effects that require focused attention and provided recommendations on methods that could be used to address knowledge gaps.

To understand the role of IGP in higher order predators, I focused on the large shark assemblage given their largely unknown role in top down control and limited IGP studies to date. The large shark assemblage exhibits high phenotypic plasticity that results in varied functional roles (e.g. secondary vs. tertiary piscivores) suggesting complex IGP interactions occur. Stable isotope analysis (SIA) provides an approach to reconstruct consumer diet to examine IGP, however, a detailed understanding of tissue preparation techniques is first required to ensure accurate interpretation of results. Elasmobranch liver is a useful high turnover tissue for IGP studies, but it contains high lipid levels and is expected to retain urea and TMAO for osmotic balance which can bias isotopic values. I found that deionized water washing for urea and TMAO removal was not required as $\delta^{15}N$ values were not modified following treatment. Residual lipid within lipid extracted liver samples, however, required the development of C:N thresholds to derive ecologically relevant liver isotopic values. A preliminary comparison between muscle and liver tissue highlighted the value of liver for understanding short vs. long term movements and its application for IGP studies.

The occurrence, class and consistency of IGP among large sharks was examined using published stomach content data and prey contributions from stable isotope mixing models. IGP was present among all sharks with the strength and class varying by species, ontogeny and over time (i.e. daily vs. annually). Understanding shark functional roles within marine food webs can improve management practices through the lens of multi-species interactions; targeted conservation on shark species involved in moderate levels of IGP with high connectance among species may enhance food web stability.

DEDICATION

To my raison d’être; Coral Lynn. Everything I do, I do so that you might have a brighter future than the one that is currently projected. I will work to ensure that you and your children will be able to enjoy marine food webs as much as I have throughout my life.

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white (GRE; *Carcharodon carcharias*), c) sand tiger (RAG; *Carcharias taurus*) and d) scalloped hammerhead (SCA; *Sphyrna lewini*), as well as all species combined (e). The grey area indicates the 95% confidence intervals for the linear regression. The black dotted line is the 1:1 line, the point at which no difference exists between treatment groups (LE vs. LEWW).

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CHAPTER 1

Measuring the Occurrence and Strength of Intraguild Predation in Modern Food Webs

1.1 Introduction

Hierarchical animal interactions drive the structure of food webs and ultimately determine ecosystem stability and function. An understanding of species interactions within food webs allows one to predict the consequences of species depletion or loss from perturbations such as overexploitation, habitat loss and climate change (Pimm, 1980; Polis and Strong, 1996). Animal interactions have traditionally been studied by examining the inter-relationship between two species. This, however, ignores the fact that multi-species interactions can have complex, indirect effects within food webs. Intraguild predation (IGP) is a more holistic approach to study animal interactions. A form of omnivory, IGP is a multi-trophic interaction that forms a 'trophic loop'—a closed pathway of trophic links (Neutel et al. 2002). Intraguild predation simultaneously combines predation and competition among a minimum of three species; an intraguild predator (IGpredator) that kills and consumes an intraguild prey (IGprey), with which it competes for a common resource (Polis et al. 1989).

The seminal theoretical IGP framework by Polis et al. (1989) identified two main descriptors involved in this multi-species interaction: symmetry and age structure. Symmetry is classified as either asymmetrical, with one clearly defined IGpredator and IGprey, or, symmetrical whereby role reversal between IGPconsumers (i.e. IGpredator and IGprey) occurs. Age-structure may be unimportant or important, and IGP may occur solely between individuals in a particular age class. Four main IGP classes are currently defined: i) asymmetrical age-structure unimportant, ii) asymmetrical age-structure important, iv) symmetrical age-structure unimportant and iv) symmetrical age-structure important (Figure 1.1).

Intraguild predation is highly relevant in the modern era of ecosystem-based studies as the occurrence of trophic loops can shape individual-, population- and community-level processes (McCann et al. 1998). Intraguild predation interactions, for example, can determine the strength, direction and linearity of trophic cascades, developmental

bottlenecks, and biomass availability across trophic levels (Holt and Polis, 1997). This review provides i) an overview of the methods available to measure the occurrence and strength of IGP within food webs from qualitative observation, to complex quantitative simulation models and ii) determines the overall research effort focused on IGP since Polis et al. (1989) from the individual to the community level to identify IGP knowledge gaps that require focused study.

1.2 Methods Used to Study the Occurrence and Strength of IGP

Confirmation of the presence of IGP within a food web requires verification of predation and consumption of an IGprey by an IGpredator, that the IGPconsumers are sympatric (i.e. have overlapping niches and occupy the same environment at the same time) and compete for a common resource (Guzmán et al. 2016; Fonseca et al. 2017). Without sufficient evidence of consumption after a kill, a predator may merely exhibit an extreme form of interference competition whereby one predator reduces competition through killing, commonly termed 'interspecific killing' (Palomares and Caro, 1999). In a systematic review of literature that examined lethal interactions among apex vertebrae predators, 48% of the studies failed to mention consumption of a prey item by the predator (Lourenço et al. 2014). Verification of predation, consumption and competitive interactions is thus fundamental to accurately describe IGP within the context of a food web. This section explores existing methods to measure the occurrence and strength of IGP in the literature to date, as well as provides novel approaches that may be used in future IGP studies. For a complete list of approaches discussed refer to Table 1.1.

1.2.1 Direct Observation

Historically, the existence of IGP among species was determined through direct observation of predation and consumption of IGprey by an IGpredator (Polis and McCormick, 1986). Opportunistic, direct observation of IGP is still reported in the modern literature, for example, Fallows et al. (2015) confirmed IGP by a Cape fur seal (*Arctocephalus pusillus;* IGpredator), on a juvenile blue shark (*Prionace glauca*; IGprey), with videography and photography (Figure 1.2A). Recording technologies, including photography (Gilman, 2016; Figure 1.2B), videography (Oppenheim and Wahle, 2013) and

audio recordings (Bright, 2008), can be used to document IGP interactions in real-times, and near real-time. Tracking technology, through radio (Figure 1.2C) and satellite telemetry (Figure 1.2D), provide an alternative approach to document IGP through attachment of a tag to an animal that transmits location data in real-time (active radio telemetry) or near real-time (acoustic/ratio and satellite telemetry; Cooke et al. 2004; Hussey et al. 2015). Radio-telemetry has documented IGP interactions among large terrestrial species such as cheetahs, wild dogs and lions (Swanson et al. 2014), medium-sized species such as weasels and voles (Brandt and Lambin, 2007), foxes and coyotes (Kozlowski et al. 2012), as well as smaller species including insects (e.g. Mormon crickets and digger wasps; Srygley and Lorch, 2016). It has also facilitated the study of IGP of hawks (e.g. sharp-shinned and Cooper's hawks; Roth and Lima, 2007) and owls (e.g. tawny and little owls; Michel et al. 2016), fish (e.g. pike; Cucherousset et al. 2009), crocodiles (Hutton, 1989) and revealed insights into IGP among invasive species (e.g. Jackson chameleon; Van Kleeck et al. 2018).

Passive satellite telemetry has enabled the study of IGP in logistically challenging environments, for example, to examine changes in the sheltering behaviour of female polar bears (*Ursus maritimus*) in response to cannibalism by males (Ferguson et al. 1997). A recent technological advancement, stomach temperature pills (STPs; Figure 1.2E), provides evidence for predation events by homeothermic predators, such as narwhal (Heide-Jørgensen et al. 2014), in near real-time. Placed within the stomach, STPs monitor fluctuations in the gut temperature and relay data to a satellite transmitter attached to the predator, indicating the time and location of a suspected predation event (Heide-Jørgensen et al. 2014).

1.2.2 Retrospective Observation

Recording and Tracking Technology

Retrospective observation of IGP can be documented through passive photography and videography, whereby the device is set to document interactions at programmed recording intervals with data stored and evaluated after the event (Fedriani et al. 2000; Rich et al. 2017). For example, remote cameras were used to retrospectively assess the interactions between three sympatric species: the dingo (*Canis dingo*), European red fox (*Vulpes*

vulpes) and feral cat (*Felis catus;* Greenville et al. 2014). Spatiotemporal interactions among the three species provided evidence of IGP through confirming high dietary overlap and consumption of the feral cat by the red fox (Greenville et al. 2014). Similarly, videography documented the efficiency of an ectoparasite, *Tamarixia radiata*, as a biological control for a population of Asian citrus psyllid, *Diaphrina citri*, a citrus pest that transmits *Candidatus Liberibacter spp.*, the causative agent of Citrus Greening disease (Halbert and Manjunath, 2004; Grafton-Cardwell et al., 2013). Video surveillance of species' activity patterns among citrus habitats examined the frequency and nature of *D. citri* interactions, including IGP, to determine the effectiveness of biological control for future integrated pest management approaches (Kistner et al. 2017; Figure 1.3A).

Acoustic telemetry, a passive archival approach to monitor movements of fully aquatic species, remotely collects data on tagged fish when in a range of fixed receivers or hydrophones. Data are subsequently downloaded periodically from recovered receivers (Hammerschlag et al., 2011; Tickler et al. 2019). Acoustic telemetry revealed that juvenile cutthroat trout, *Oncorhynchus clarki utah*, are most at risk of predation by adult conspecifics in early August when they are habitat-restricted due to hypoxia and higher lake temperatures (Baldwin et al. 2002). More recently, acoustic tags with a digestible fuse called predation detection acoustic tag (PDAT; Schultz et al. 2017; Figure 1.3B) and predation tag (PT; Halfyard et al. 2017; Figure 1.3C), have been developed to identify predation events post-consumption. Similar technology may also be effectively integrated into archival satellite tags widening the applicability of this approach (Meyer and Holland, 2012).

Integrative approaches that combine telemetry and archival biologgers with sensors (e.g. impedance tags, PDATs, accelerometers) and cameras can provide novel methods that reduce uncertainty in the identification and quantification of IGP events. For example, confirmation of predation has been validated through combined temperature and gastric pH data in an acoustic pH transmitter (Papastamatiou et al. 2007). Inserted into the stomachs of captive adult blacktip reef sharks, *Carcharhinus melanopterus*, the pH transmitters identified rapid increases in gastric pH associated with prey consumption (Papastamatiou et al. 2007). In Adélie penguins, *Pygoscelis adeliae*, the application of two accelerometers (placed on the head and body) coupled with a camera determined the type

and number of foraging events (Watanabe and Takahashi, 2013), while animal-borne miniaturized mobile transceivers equipped with an accelerometer provided a framework for examining conspecific interactions among Greenland sharks (Barkley et al. 2020). These integrative approaches provide novel techniques with applications for studying IGP.

Dietary Analysis of IGP

Traditionally diet composition of predatory species is retrospectively studied through fecal (Lockie, 1959) and stomach content analysis (SCA; Hyslop, 1980) as both methods are relatively cost-effective and can be non-invasive.

Fecal Analysis

Fecal analysis is performed through the collection and examination of prey items in fecal matter (see Figure 1.3D). Once collected, prey is identified via hard boney parts that remain undigested, such as otoliths and dentaries from fish, insect exoskeletons, mammalian bones, fur and cranial structures (Trites and Joy, 2005). The use of fecal analysis in terrestrial studies has provided much insight into inter- and intra-specific behaviours of predators. The presence of undigested guard hairs in the feces of the eastern chimpanzee (*Pan troglodytes schweinfurthii*), for example, revealed rare cannibalistic behaviour (Walker et al. 2018). Similarly, Gormezan and Rockwell (2013) examined the scat of polar bears, *U. maritimus*, and determined >6% of diet composition consisted of conspecifics. The authors also compared the current diet of polar bears with historical data collected 40 years ago and found an increase in prey such as snow geese, eggs and caribou. The study suggested that polar bears are opportunistic omnivores by incorporating novel resources into their diet and using IGP and cannibalism as adaptive foraging strategies in response to climate-induced shifts in available resources (Gormezano and Rockwell, 2013). Typically restricted to terrestrial studies, this method also has been used in the marine environment to study sperm whales (*Physeter macrocephalus*; Smith and Whitehead, 2000).

Stomach Content Analysis

Stomach content analysis (SCA) requires discerning gut contents through various stomach-emptying procedures including lethal dissection and non-lethal alternatives such as gastric

lavage (Light et al. 1983), physical eversion of the stomach (Bush, 2003) and injection of an emetic to induce stomach eversion (Sims et al. 2000). Typically, SCA involves quantitative metrics such as count, volume and weight, similar to fecal analysis (Hynes 1950; Hyslop 1980; Figure 1.3E). For example, SCA examined the diet composition and possibility of IGP among three coexisting pelagic fish species of the North Sea (Raab et al. 2012). The three fish, European anchovy (*Engraulis encrasicolus*), juvenile herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) forage primarily on zooplankton, however, can also incorporate fish into their diets (Huse and Toresen 1996). Symmetrical IGP (see Figure 1.1) is likely between the anchovy and sprat, as both species were found to consume fish (Raab et al. 2012). Alternatively, asymmetrical IGP (see Figure 1.1) is expected between herring and other fish species as the herring diet contained no fish, suggesting they can act as IGprey.

Dietary composition studies have also studied IGP through SCA in terrestrial environments. Examination of gut contents provided a method for evaluating niche partitioning between the native canid, red fox (*V. Vulpes*), and invasive golden jackal (*C. aureus*). Previous studies of newly invaded environments by the jackal (e.g. Hungary) demonstrated high dietary overlap between the golden jackal and red fox; the fox was also found in the diet of the jackal (Lanszki et al. 2006). For the coexistence of IGPconsumers, however, traditional IGP theory requires that IGprey (red fox) be superior at common resource acquisition (Polis et al. 1989; Holt and Polis, 1997). Stomach content analysis of carnivores in environments where the golden jackal had previously become established supported IGP theory, as the red fox continued to consume small rodents as a primary prey source while jackals (IGpredator) experienced substantial niche partitioning, shifting to a scavenger diet consisting of ungulate and domestic animal carcasses (Tsunoda et al. 2017).

1.2.3 Markers and/or Tracers

Complementary techniques to SCA/fecal analysis include i) biological markers and ii) chemical tracers. Biological markers include identification of IGP species via enzymes, amino acids, DNA sequences, fatty acids and sterols, while chemical tracers include detection of elemental ratios such as carbon (^{12}C:^{13}C) and nitrogen (^{14}N:^{15}N), as well as additional elements such as mercury and sulphur. Each detection technique involves the

identification of specific tracers from IGP species, however, there are fundamental differences between methods. Integrated stomach/fecal and tracer approaches improve the resolution of IGP quantification through species-level identification of prey, providing greater confidence in results (Jarman et al. 2004).

Biological Markers

Early predation studies (the late 1970s to early 1990s) on invertebrates used gel electrophoresis (Wool et al. 1978; Castañera et al. 1983), enzyme-linked immunosorbent assays (ELISA; Ragsdale, 1980) and gas chromatography-mass spectrometry (GC-MS; Knutsen and Vogt, 1985) for quantitative estimation of prey item consumption. While gel electrophoresis is a simple and affordable method used to sort prey enzymes and proteins by length, the test has limited sensitivity and is therefore incapable of identifying specific prey items from closely related species. Alternatively, ELISAs are highly sensitive as they immobilize antigens for identification of prey items through binding of specific prey monoclonal antibodies; however, generating prey-specific antibodies is time-consuming and therefore ELISAs are often used for identification of a single prey item rather than entire predatory diets (Traugott et al. 2013). Studies using GC-MS identify prey through prey chemicals found in predators, with prey detection rates and the accuracy of this method variable as target chemicals of prey (e.g. defensive chemicals) can be broken down or remain unmetabolized in the predator and therefore undetectable (Aebi et al. 2011). Despite the limitations, these methods are occasionally still used (Symondson, 2002; Aebi et al. 2011; Hagler et al. 2020). Technological advancements, however, have improved the accuracy and resolution of dietary analyses and more advanced approaches, including DNA analysis, are now commonly used in IGP studies.

Diagnostic PCR Used to Detect DNA Sequences

Application of DNA analysis to examine multi-species interactions of suspected IGpredators using gut contents or fecal matter is a promising tool for future IGP studies. DNA analyses can be grouped into two different techniques; barcoding approaches and diagnostic polymerase chain reaction (PCR; Traugott et al. 2013). The first approach compares DNA sequences from prey to a barcode database of previously identified species

(e.g. GenBank; Barnett et al. 2010). DNA analysis can be more targeted, however, if prior knowledge of consumer diet exists through group-specific PCR primers (Valentini et al. 2009). Diagnostic PCR involves the search for a specific species ('singleplex PCR') or several prey taxa simultaneously ('multiplex PCR') using PCR amplification of group-specific primers or species-specific targets (Tollit et al. 2009; Traugott et al. 2013). A study by Gagnon et al. (2011) successfully detected IGP among four closely related coccinellid species using PCR gut content analysis. Moreover, the study raised concern over variability in the detection of prey DNA post-feeding due to species-specific differences in rates of decay and variability among the size of prey items. This led to the development of an index of exponential decay approach (DS_{50}). Initial IGP among two coccinellid pairs (*Harmonia axyridis-Coleomegilla maculata* and *Coccinella septempunctata-H. axyridis*) identified significant differences in IGP occurrence (17.9% and 8.6%, respectively), yet DS_{50} correction determined similar IGP occurrence between the pairs (scores of 0.07 and 0.08, respectively; Gagnon et al. 2011).

Other sources of DNA, such as saliva, blood, and hair, can also be used to identify IGP events. For example, amplified DNA from the saliva and hair of IGpredators were collected from carcasses of fishers (*Martes pennant;* see Figure 1.3F & G), a small endangered mammal native to North America, and primers specific to the DNA sequences of four suspected IGpredators were used to confirm predation by the domestic dog, mountain lion (*Puma concolor*), bobcat (*Lynx rufus*) and coyote (*C. latrans*). Also, fisher skulls were examined for injuries sustained before death to identify which IGpredator was involved. This integrated approach allowed the preferential selection of IGprey by IGpredators to be examined; large IGpredators (e.g. Mountain lion) foraged primarily on large male fishers, while smaller IGpredators (e.g. bobcat) preferred small female fishers (Wengert et al. 2013).

Fatty Acid Analysis for IGP Identification via Fatty Acid Signatures

Fatty acids (FA) can also be used to investigate IGP (Iverson et al. 1997). Fatty acid profiles work on the premise that the average FA composition of prey eaten by a consumer experience minimal change upon assimilation and are reflected in predator tissues (Iverson et al. 1995; Kirsch et al. 1998; 2000). The distinction of prey items within a consumer's

diet is possible as some primary producers (e.g. plants, bacteria, fungi, algae) can synthesize unique FAs (e.g. γ-linolenic acid by protozoa; Lechevalier and Lechevalier, 1988). Consequently, FAs can differentiate prey items by taxa (e.g. mammals, birds and insects; Colombo et al. 2016), species (e.g. 18:3ω3 is specific to macroalgae species; Meyer et al. 2019), biome (e.g. terrestrial vs. aquatic), geographic range (e.g. latitude and temperature; Colombo et al. 2016) and phylogeny (Colombo et al. 2016; Meyer et al. 2019). Pethybridge et al. (2014) observed differences in the FA profiles of the liver and muscle tissue in white sharks, *Carcharodon carcharias*, suggesting turnover rates or consumer ontogenetic diet shifts were responsible for the discrepancy between tissues. Although it was not the focus of the study, similarities in FA profiles between white sharks and suspected IG_{prey} (e.g. marine mammals) could be used to indicate the presence of IGP in future studies.

Chemical Markers and/or Tracers

Bulk Stable Isotope Analysis

In the context of IGP, isotopic ratios of animal tissues act as the building blocks to determine the association between IGpredator and IGprey (Peterson and Fry, 1987) through quantification of proportional IGprey isotopic contributions to the overall isotopic diet composition of an IGpredator ("you are what you eat"; DeNiro and Epstein, 1981). Stable isotopes of carbon (^{12}C:^{13}C; $\delta^{13}C$; Inger and Bearhop, 2008), nitrogen (^{14}N:^{15}N; $\delta^{15}N$; Martínez del Rio et al. 2009), sulphur (^{32}S:^{34}S; $\delta^{34}S$; Goodenough, 2014) and mercury (Lourenço et al. 2011), among others, can be used to investigate the occurrence and strength of IGP. Establishing predator-prey interactions using bulk-stable isotope analysis (SIA) is contingent on knowledge of tissue turnover rates and fractionation (the expected enrichment between predator and prey; Inger and Bearhop 2008). Identification of specific prey species within a consumer diet may be difficult, however, (e.g. Hobson 1993) as each prey item must be isotopically distinct (Harrigan et al. 1989; Doucett et al. 1996; Phillips et al. 2005). Modern mixing models, for example, SIAR and MixSIAR (Parnell et al., 2010; Stock et al., 2018), apply a Bayesian approach to SIA, incorporating species-specific isotopic values (i.e. distinct prey groups, IGpredator and IGprey) while accounting for variation in model parameters such as fractionation (Bond and Diamond, 2011), ultimately

determining the relative contribution of IGprey items to total predator diet and inferring the strength of IGP (Rickers et al. 2006; Yurkowski et al. 2017).

An effective method to indirectly identify IGP, SIA monitored shifts in species interactions among two wolf spider species *Alopecosa cuneata* (IGpredator) and *Pardosa palustris* (IGprey), a common resource (*Heteromurus nitidus*) and an alternative resource (*Drosophila melanogaster*; Rickers et al. 2006). Niche overlap between *A. cuneata* and the smaller *P. palustris* provides an ideal environment for asymmetrical age-structure important IGP (see Figure 1.1). To ensure accurate interpretation of IGpredator dietary switches, a marked difference in the $\delta^{13}C$ values between the alternative resource and IGprey tissues were established via ^{13}C enrichment of *D. melanogaster* before the study. Consumption of enriched *D. melanogaster* would, therefore, result in inflated $\delta^{13}C$ values of IGpredator when consuming more of the alternate resource (Rickers et al. 2006). The occurrence of IGP was confirmed through a marked decrease in $\delta^{13}C$ values observed in IGpredator tissue (Rickers et al. 2006).

Compound-Specific Stable Isotope Analysis (CSIA)

Compound-specific stable isotope analysis (CSIA) of select molecules, often amino acids and fatty acids, is assumed to address disadvantages in bulk-SIA, for example, variability in baselines and among trophic discrimination factors (Blanke et al. 2017) through analyzing 'source' and 'trophic' molecules within a single tissue (McClelland and Montoya 2002; Chikaraishi and Naraoka, 2003). Source amino acids (e.g. phenylalanine) have $\delta^{15}N$ signatures that are conserved across trophic levels and therefore act as a baseline, while trophic amino acids (e.g. glutamic acid) experience enrichment with each trophic level (Chikaraishi et al. 2009). Through improved precision of trophic position estimates and enhanced resolution of trophic interactions using CSIA of amino acids, Chikaraishi et al. (2014) were able to resolve the trophic structure of complex marine and terrestrial food webs, ultimately providing additional support for the prevalence of omnivory, including IGP, among food webs.

1.2.4 Modeling Approaches

A hierarchy exists in a food web with individual-, population- and community-level processes interacting to form a complex network. An understanding of these multi-species, multi-level interactions is required before a more complete understanding of the structure and function of a food web is possible (Beckner, 1974). Since the acknowledgement of IGP in food webs by Polis et al. (1989), the study of multi-species interactions has followed a step-wise progression; early IGP calculations were first incorporated into existing, commonly used equations for individual species competition and predation (e.g. Schoener's exploitative competition model; Schoener, 1976). Subsequent community models, or simple one-dimensional IGP models, accounted for additional food web complexity by incorporating factors such as foraging strategy and trophic level (Rosenheim and Corbett, 2003). Further complexity was incorporated through IGP model comparisons and by including the relative strength of species interactions, resulting in two-dimensional food web matrices (Arim and Marquet, 2004). Following the incorporation of additional variables and spatiotemporal parameters, three-dimensional ecosystem-based models were formed (e.g. Ecopath with Ecosim; Pauly and Christensen, 1995; Pauly et al. 1998). A holistic approach is necessary for the development of ecosystem-based management as system-wide conservation strategies have become a growing concern. This section is not a systematic review of all species interaction models that account for IGP, but rather provides a broad overview of the development and application of IGP modelling approaches to date.

Community Models

Common Mechanistic Models to Estimate the Effects of IGP

To quantify the impacts of IGP at a population- and community-level, Holt and Polis (1997) incorporated IGP interactions into three commonly used mechanistic models: i) a general resource-consumer model, ii) the Lotka-Volterra model for a food chain and iii) the exploitative competition model of Schoener (1976). A mechanistic model of IGP examines individual growth rate functions for the IGpredator, IGprey and the common resource (P, N and R, respectively). Growth rate equations for species in a community consisting of variables for species responses to competing organisms [e.g. $\alpha(R, N, P)N$ is the IGpredator response to IGprey], a term for reproduction (e.g. b) and a term for the rate

of mortality (e.g. *m;* Holt and Polis, 1997). Holt and Polis (1997) incorporated IGP into mechanistic models through the addition of terms such as, β, which represents the energetic benefit received by the IGpredator from IGprey consumption, and a recruitment term for the common resource [e.g. $R\phi(R)$; Holt and Polis, 1997]. Traditional IGP models are assumed to be asymmetrical, (Figure 1.1) and exist as IGP 'community modules', a closed system whereby only the IGP species are interacting (Holt, 1997; Holt and Polis, 1997). One-dimensional models, therefore, provide the theoretical framework necessary to monitor changes in population dynamics in response to IGP and allow the possibility for non-linear functional responses (i.e. the feeding rate of a predator as a function of prey abundance; Holling, 1959; Skalski and Gilliam, 2001).

The addition of IGP into the resource-consumer model identified criteria necessary for coexistence among IGP species: i) superior exploitation of the common resource by the IG_{prey} following the R* rule: $R^*_N < R^* < R^*_{P,}$ where R is the growth rate of the IGprey, resource and IGpredator, respectively (Holt et al. 1994; Grover, 1995), ii) an immediate energetic gain by the IGpredator from IGprey consumption, and iii) an intermediate level of common resource productivity in environments with a productivity gradient (Holt and Polis, 1997). The addition of IGP into the resource-consumer model demonstrated that while the opportunity for stable coexistence of IGP species exists, under the criteria listed, the possibility for all criteria to be met at once is rare (Polis and Holt, 1992). The incorporation of IGP into the Lotka-Volterra food chain model required several assumptions: the common resource had a logistic growth rate in the absence of IGpredator and IGprey, the growth rate of the IGPconsumers were proportional to the rate of prey consumption, and the IGPconsumers exhibited linear functional responses, therefore IGpredator and IGprey foraging rates were proportional to prey density (Holt and Polis, 1997). The Lotka-Volterra IGP model resulted in five possible equilibria: i) all species with a density of zero, ii) the dominance of the common resource while the IGPconsumers have a density of zero, and iii) coexistence of the IGP species and two possible alternative stable states. Alternative stable states exist whereby the system can experience different configurations dependent on initial parameters found within the model community (e.g. species densities; Beisner et al. 2003). The two alternative stable equilibria were iv) the IGpredator and common resource coexist while the IGprey is absent, or v) the IGprey and

common resource coexist while the IGpredator density is zero. The model ultimately demonstrated that the long-term coexistence of IGP species may not be possible, despite mutual invasibility, due to unstable community dynamics driving species to low abundances (Holt and Huxel, 2007). The possibility for stable coexistence of species within an IGP module is therefore predicted by the Lotka-Volterra IGP model to be minimal (Holt and Polis, 1997). Altering Schoener's exploitative competition model (Schoener, 1976) to include IGP resulted in hyperbolic isoclines for the IGPconsumer growth rates that expanded the range for alternative stable states, IGP species coexistence and reversal of competitive dominance between IGPconsumers. Furthermore, Ruggieri and Schreiber (2005), determined an additional alternative stable state within the IGP community, that is, the contingent coexistence of the IGP species or displacement of the IGprey depending on initial species densities. The alternative stable state, therefore, allowed for IGPconsumer coexistence such that IGprey density was not sufficiently reduced. A perturbation resulting in the loss of IGprey abundance, however, resulted in the permanent exclusion of the IGprey by the IGpredator from the system (Ruggieri and Schreiber, 2005).

Results from empirical studies in support of theoretical IGP model predictions vary (Rosenheim et al. 1995; Mylius et al. 2001; Janssen et al. 2007) with most experimental evidence found in laboratory microcosm and parasitoid communities (Morin and Lawler, 1996; Amarasekare 2000; Arim and Marquet, 2004). Morin (1999), for example, examined the influence of bacterial concentrations (common resource) on the density of ciliates in a freshwater microbial food web. The relationship between *Blepharisma americanum* (IGpredator), and *Colpidium striatum* (IGprey) in a laboratory microcosm supported traditional IGP theory predictions; the IGpredator was excluded at low bacterial concentrations and coexistence of IGP species occurred at higher common resource concentrations (Morin, 1999). Other laboratory experiments have confounding results at low common resource concentrations whereby IG_{prey} is excluded or there is no change in population density (Lawler and Morin, 1993; Janssen et al. 2006).

Although theoretical IGP models predict competitive exclusion or instability among IGP species, coexistence is commonly found (Brodeur and Rosenheim, 2000; Mylius et al. 2001; Arim and Marquet, 2004). The discrepancy between theory and observation may be a result of external factors in the food web that stabilize IGP species

interactions and allow for coexistence; an interaction that IGP theoretical models would otherwise predict to result in IGP species extinctions (Wootton, 2017). Traditional theoretical models may, therefore, be limiting the possibility of species coexistence through assumptions such as the requirement for equilibrium dynamics, limited species numbers to focused community modules (i.e. three or four species; Holt, 1997) and ignoring external factors such as environmental habitat structure (Janssen et al. 2007). A meta-analysis of IGP studies investigating the effect of habitat structure on IGP species indicated that complex habitats facilitate coexistence of IGP species (Janssen et al. 2007). The incorporation of additional species interactions, such as commensalism, into the Lotka-Volterra IGP model of a microzooplankton community further highlighted scenarios for IGP species coexistence (Löder et al., 2014). Similarly, integration of a fourth species, thus an additional trophic link, into the Lotka-Volterra IGP model by Hall (2011), demonstrated that a specialized natural enemy can stabilize an IGP community and increase the opportunity for species coexistence regardless of the efficiency of the IGprey at common resource acquisition. Through the preferential attack of a predator on the IGpredator, the stable presence of all IGP species was possible, even when the specialist predator was superior at competition for the common resource (Hall, 2011). Moreover, the presence of a fourth species as an alternative prey source within a community may also allow for the coexistence of IGP species (Holt and Huxel, 2007).

Game Theoretical Model

The IGP game-theoretical model of habitat use predicts species distributions in the presence of asymmetrical IGP (Figure 1.1) based on 5 factors: efficiency of resource exploitation, habitat complexity, dietary overlap, resource productivity and the availability of an additional resource (Heithuas, 2001). Through the incorporation of flexibility in species distributions, Heithuas (2001) determined IGP species coexistence occurred when dietary overlap was low and the shared resource had intermediate productivity. Additionally, coexistence of IGPconsumers was possible in habitats with high resource productivity through the addition of an alternative resource, a fourth species, for consumption by the IGpredator. The game-theoretical IGP model provides novel insights into the indirect influence of alternative prey resources for the IGpredator on community

structure, and the spatial distribution of IGprey in response to i) habitat safety, ii) dietary overlap and iii) the balance between resource availability and predation risk (Heithuas, 2001).

Identifying Conditions Necessary for Alternative Stable States

The progression of IGP modelling continued through the comparison of model results from communities with different species compositions (Verdy and Amarasekare, 2010). For example, changes to common resource availability and productivity can alter community outcomes depending on the growth rate trajectories (i.e. logistic vs. exponential growth rates) and functional responses (i.e. Type I or II; Holling, 1959) of species within a community (Takimoto et al. 2007). Through comparing a tritrophic IGP model with one IGP species at each of the three trophic levels, to a model with four trophic levels through the addition of prey for the common resource, Takimoto et al. (2007) demonstrated that the possibility for alternative stable states was similar for each model, with results depending on the growth rates of the species within the system. Species growth rates were controlled by two main drivers: i) the identity of the IGPconsumer with the competitive advantage for common resource exploitation, and ii) the efficiency of energy transfer from the common resource to the IGpredator measured via body size (Takimoto et al. 2007).

Empirical studies have often failed to identify alternative stable states and therefore the frequency of this phenomenon was unknown in natural environments. Verdy and Amarasekare (2010) developed a model to predict the biological conditions necessary for the presence of alternative stable states in communities with IGP by examining community functional responses that were linear vs. non-linear (i.e. Type I or Type II, respectively; Holling, 1959) under two common resource growth rate trajectories, logistic vs. chemostatic (i.e. a constant environment with a growth rate of zero, $r = 0$). Model results highlighted three alternative stable state scenarios. Scenario i) the stable presence of an IGPconsumer; this scenario required the common resource exhibit logistic or chemostatic growth and the IGPconsumers have linear or non-linear functional responses. Scenario ii) the coexistence of IGPconsumers or dominance by the IGpredator; scenario ii required the common resource have a chemostatic growth rate and the IGPconsumers have linear or non-linear functional responses. Scenario iii) the coexistence of IGP species or competitive

dominance by the IGprey; the last alternative stable state required the common resource exhibit logistic growth and the IGPconsumers have non-linear functional responses (Verdy and Amarasekare, 2010). In a system with IGP interactions producing alternative stable states, the community can shift between scenarios in response to perturbation, thus impacting community diversity and ecosystem stability that result in changes to the structure and dynamics of entire ecosystems (Verdy and Amarasekare, 2010). Models with the ability to predict community composition based on IGP species growth rate dynamics and functional responses may, therefore, become an integral tool necessary for conservation efforts and ecosystem-based management strategies in the future.

Food Web Models

Early mathematical approaches failed to provide a mechanistic explanation for the full complexity of food webs. The 'niche model', for example, examined the strength of species interactions and estimated the factors (e.g. looping, omnivory, IGP) that contributed most to the complexity of food web structure (Williams and Martinez, 2000). This approach, based on an earlier 'cascade model' (Cohen et al. 1990), accounted for trophic similarity, length and number of food-chains in food webs by employing connectance (i.e. the proportion of links or species interactions observed) and species number as empirical parameters.

The incidence of IGP in food webs was first quantified using data from previously published food web studies (Arim and Marquet, 2004). Unlike earlier studies that assumed omnivory and IGP were destabilizing and rare in food webs (Pimm and Lawton, 1978; Pimm, 1982), this two-dimensional model quantitatively established that IGP is common in food webs. Species were categorized into 'trophic groups', a biologically meaningful way of classifying species using both functional role (Cohen et al. 1990) and foraging type (Arim and Marquet, 2004). The relative contribution of trophic groups to the overall prevalence of IGP in food webs was developed from food web matrices and analyzed using a null model approach. IGP existed in more than half (58–87%) of all food webs analyzed, with each trophic group participating in IGP at a different frequency (Arim and Marquet, 2004).

Simulation Models

Simulation models expand on previous one- and two-dimensional IGP models, providing a method to predict how an ecosystem may change in response to proposed management strategies or perturbations (e.g. global climate change; Fulton, 2010). An outline for the existing multi-species model categories was originally presented by Hollowed et al. (2000) and updated by Plagányi (2007). The categories include species number (single- vs. multi-species; Hollowed et al. 2000), trophic level (lower vs. higher trophic level; Daewel et al. 2014), model complexity (whole ecosystem vs. single-species; Plagányi, 2007) and unit of measurement (biomass- vs. size-based; De Roos et al. 2003). While this review does not provide a comprehensive list of the existing simulation models, a broad overview of the main models for which IGP can be incorporated is addressed along with examples.

Multi-Species Individual-Based Models

Individual-Based Models (IBM) consider the entire life cycle of an individual species and how its interactions impact ecosystem dynamics (Plagányi, 2007). To account for multi-species interactions such as IGP, IBM is expanded to form a multi-species individual-based model. OSMOSE, for example, simulates interspecific species interactions among fish of higher trophic levels via predation, under the assumption that predation is non-selective, dependent on the predator-prey size ratios and their spatiotemporal occurrence (Shin and Cury, 2001). OSMOSE has a hierarchical structure, with fish grouped by species, age-structure, size and weight, allowing for the study of species- and size-specific trophic interactions. The level of IGP can be simulated through the assessment of fish group movements across a closed-boundary, two-dimensional grid (Shin et al. 2004; Irigoien and De Roos 2011).

A study by Andonegi et al. (2013) compared single-stock assessments of two economically important fish species from the Bay of Biscay, the European anchovy, *Engraulis encrasicolus* and sardine, *Sardina pilchardus*, with predictions from several models, including OSMOSE. A size-based link was revealed between the anchovy population and eight other important species within the bay system including Atlantic mackerel, *Scomber scomber* and Atlantic bluefin tuna, *Thunnus thynnus*. Although the level of IGP between the sardine and anchovy was unknown, OSMOSE allowed

investigation of possible direct and indirect effects of IGP through simulating different fish population sizes within the system. Results indicated annual variability in anchovy and sardine populations, whereby changes to the anchovy population dynamics were directly linked to population changes in other fish species within the system, for example, mackerel and sardine (Andonegi et al. 2013).

Minimal Realistic Models

Minimal realistic models (MRM), or dynamic multi-species models, limit the number of species included by restricting the model to a subset of the ecosystem (Punt and Butterworth, 1995). Most simulation models are categorized as MRM including GADGET (Begley, 2005) and MULTSPEC (Bogstad et al. 1997) and capture IGP by including age-structured interactions (Plagányi, 2007). An age-structured MRM was used, for example, to improve the stock assessment of the South African hake fishery comprised of the shallow-water Cape hake, *Merluccius capensis* and the deep-water Cape hake, *M. paradoxus*. The hake species were known to engage in IGP and cannibalism, however, total allowable catch (TAC) was traditionally estimated using single-stock models that failed to include species interactions and food web dynamics (Ross-Gillespie, 2016). Intraguild predation was incorporated into the existing stock assessment model through the inclusion of an additional hake mortality parameter, predation by conspecifics. The MRM output reflected population oscillations, similar to those reported in the early 20th century when the development of the *M. capensis* fishery caused *M. paradoxus* populations to increase in response to predatory release. Modern populations of *M. paradoxus* have decreased and MRM predictions reflected a greater depletion than previous models had suggested. By including IGP and multi-species interactions into fish population assessments, more reliable data is available that can improve the sustainability, management and economic viability of fisheries (Ross-Gillespie, 2016).

Dynamic System Models

Dynamic system models account for the driving forces within an ecosystem, for example through top-down or bottom-up approaches and provide user control over external factors such as temperature and pH (Fulton and Smith, 2004*a*; Condie et al. 2014). Typically

restricted to a subset of species, these models provide detail about target species that more complex modelling, such as whole ecosystem models, cannot provide (Plagányi, 2007). For example, ATLANTIS examines the response of 'network motifs', interconnected patterns within food webs that cannot be explained by chance, such as IGP that form trophic loops, to perturbations such as climate change and overharvesting (Fulton et al. 2004*b*&*c*). Small changes to closed loops have been shown to drive diverse responses that alter the direction and strength of predicted population trends, suggesting motifs heavily influence system functioning (Condie et al. 2014). When applied in a fisheries context, population recovery of the eastern gemfish, *Rexea solandri*, slowed despite fishery closures and historically low TAC (Little and Rowling, 2010). The unexpected ecological response was considered to be in response to strong IGP interactions between IGpredator the arrow squid, *Nototodarus gouldi*, an omnivore that forages on IG_{prey} juvenile gemfish thus impeding its recovery (Condie et al. 2014).

Whole Ecosystem Models

Whole ecosystem models incorporate all trophic levels to form a three-dimensional model that captures the full structural complexity of food webs (Plagányi, 2007). Examples of whole ecosystem models include Ecopath with Ecosim (EwE v6.6.1; Ecopath International Initiative, 2020), bioenergetic, allometric and trophodynamic models (e.g. Koen-Alonso and Yodzis, 2005). Formed from the combination of Ecopath (Polovina, 1984; Christensen and Pauly, 1992), Ecosim (Walters et al. 1997) and Ecospace (Walters et al. 1999), EwE describes temporal changes in biomass between groups of species in response to complex interactions (Christensen and Walters, 2004) and can be used to study IGP (Walters and Martell, 2004). Ecopath estimates how changes in production and loss of species biomass, resulting from fishing activity, affect food web structure (Pauly et al. 1998). Ecosim (v2004; Entsminger, 2019) incorporates temporal changes in initial system variables, such as species' life-histories or increases in IGP, and Ecospace is a spatially explicit model for fishing effort and biomass distribution (Walters et al. 1999).

Changes to food web structure in response to increased IGP among shark populations were measured by Kitchell et al. (2002) through an EwE of the Central North Pacific. The study performed two simulations: a baseline scenario and an apex shark

scenario. The baseline scenario simulated the response of the food web to fishery management practices, such as longline fisheries, by including the trophic position of focal sharks as estimated from stomach content data reported in the literature. The apex shark scenario was simulated under the assumption that sharks play a greater top-down role than assumed. This was achieved by assigning large sharks with a higher trophic position value to reflect increased IGP through the consumption of elasmobranchs. Overall, the apex shark scenario found by including a low level of IGP (i.e. 5% shark consumption) strong non-linear responses were observed throughout the entire food web relative to limited effects from the baseline scenario (Kitchell et al. 2002).

There is a multitude of simulation models available that can be used in future IGP studies, with tradeoffs between model complexity and confidence in model results. Existing IGP models can act as a starting point for future IGP studies by providing a tool for improved realism when studying multi-species interactions. Model requirements may be difficult to meet, specifically when studying data-deficient target species. The selection of an IGP model will involve an examination of the available information and the model criteria. When possible, several different models may be used to avoid bias and misleading conclusions (Koen-Alonso and Yodzis; 2005).

1.3 The Status of IGP Studies After 30 years

The seminal IGP review by Polis, Myers and Holt (1989) described the food web implications and broad suite of ecological effects of species involved in IGP. Here I used Polis et al. (1989) as a framework for a systematic assessment of the research effort conducted to date at three distinct IGP 'implication levels': i) individual, ii) population and iii) community. Though the implication levels were not defined by Polis et al. (1989), it is assumed that the individual level is the study of one entity, the population level examines characteristics among a group of individuals from the same species (Mendelian population; Dobzhansky, 1950), and community-level studies investigate characteristics of a network of multiple populations. Within each level, IGP can influence or be influenced by different characteristics, known as 'IGP effects' (Polis et al. 1989, Holt and Polis 1997).

The status of current IGP knowledge was determined using a 'two-tiered approach' to ensure all existing studies were captured for each topic: i) a broad systematic search of

existing literature on IGP using the terms ‘intraguild predation’ and ‘cannibalism’ (an intra-specific interaction), the implication-level, and synonyms for the IGP effects within the level, and ii) a narrower systematic search, including only the key terms specified in Polis et al. (1989; Figure 1.4). The search results from i) and ii) were compared and results were found to be comparable providing confidence that the research effort was thorough (Figure 1.4). IGP effects with more or less research effort are identified and recommendations are made for novel methods that can be used in future IGP studies to bridge knowledge gaps.

Individual-Level Implications

Interactions at the individual level operate as the biological building blocks for food web structure. Individuals may experience shifts in fitness, behaviour, morphology, chemical and life-history characteristics in response to IGP interactions that alter the stability and function of an entire ecosystem (Johnson, 2000, Finke and Denno, 2005). Additionally, several individual-level characteristics can facilitate an ideal environment for the occurrence of IGP (Nilsson-Örtman et al. 2014).

Many of the pioneering studies of IGP focused on observational changes to individual species traits to measure individual fitness levels and energetic gains acquired by the IGpredator from the consumption of IGprey (see 1.1 Direct Observation; Walter, 1987; Wissinger, 1988). Effort is given to individual characteristics influenced by IGP, which vary considerably across studies, with behavioural changes in response to IGP (e.g. adaptive foraging; Wootton, 2017) the most frequently studied both at the individual level (n = 76, 62%; Figure 1.4) and across all IGP effects combined (n = 16, 13%). Some examples of behavioural changes include changes in mobility of IGprey (Lucas et al. 1998), spatial avoidance of IGpredators by IGprey (Tannerfeldt et al. 2002) and increased growth rate of IGpredators (Takatsu and Kishida, 2015). By contrast, individual changes in chemical, morphological and fitness characteristics in response to IGP have received less study (5.5–6.5%, Figure 1.4). Modern technological advancements and analytical methods, such as molecular tools, provide opportunities for increased understanding of IGP influences on species chemical traits (Thomas et al. 2013). Hautier et al. (2008) used gas chromatography-mass spectrometry analysis (GC-MS) to monitor IGP in coccinellid species via alkaloids, a defensive chemical produced to deter predation by ants (Marples,

1993), birds (Marples et al. 1989) and conspecifics (Glisan King and Meinwalk, 1996). GC-MS can detect exogenous alkaloids, for example Adaline, from IGprey within the gut of an IGpredator post-consumption, confirming the occurrence of IGP (Hautier et al. 2008; 2011).

Population-Level Implications

The outcome of interspecific interactions can have broad implications on species populations. When studying IGP at the population level, the size, stability and resilience of IGP species are examined (Figure 1.4). Changes to population size in response to IGP (n=49, 42%; Figure 1.4) has been heavily studied in the literature, whereas minimal focus has been given to the resilience of IGP species (predator, prey and resource) in response to IGP (3-13%), both at the population level (n = 3, <3%) and across all implication levels (n ~5, 1%; Figure 1.4). Early IGP population studies focused on population size likely because it can be easily monitored via counts of individuals (Connell, 1983; Polis and McCormick 1986). Studying changes in the resilience of a resource, for example, is not as straight forward as there is no simple metric for 'resilience'.

One area of study that has examined the effects of IGP on a common resource is through the biological control of agricultural pests (Finke and Denno 2005; Frank, 2010). Pest species are typically herbivores, therefore crop yield and profit are dependent on the successful management of these species. Exotic predators introduced to consume a pest are often assumed to be safe provided they consume only the target pests (Sheppard, 2003), however, exotic predators may experience additional interactions, such as IGP with native predatory species, that can inhibit pest control and thus fail to reduce pest population density (Pearson and Callaway, 2005). For example, IGP interactions were found among predatory species used for pest control of the green peach, cabbage (Snyder et al. 2006), potatoes, (Lucas et al. 1998), grain (Sheppard et al. 2005) and milkweed (Lucas, 2005). Intraguild predation has also been observed among biological control species and plant pathogens (Martin and Hancock, 1987; Tixier et al. 2013). Although concentrated study effort has focused on pest control, few studies measure changes in the resilience of the pest in response to IGP, focusing instead on the success or failure of the biological control program, often through examining crop yield (Finke and Denno 2005; Frank, 2010).

Community-Level Implications

Understanding IGP at the community level provides insight into changes to guild structure, community diversity, community stability and overall food web structure (Polis et al. 1989). The response of food web structure to IGP has received the greatest research effort of community-level IGP effects (n = 117; 36%; Figure 1.4), while community stability and diversity has received a moderate level of study (27-29%; Figure 1.4). The intense effort afforded to understand structural changes of food webs may coincide with growing concern over the impacts of biodiversity loss on ecosystems (Dirzo and Raven 2003; Ceballos et al. 2015), and recent IGP studies have revealed stabilizing properties of moderate levels of IGP within communities (Rudolf, 2007; Miller and Rudolf, 2011). Consequently, IGP community-based models provide a promising approach for predicting food web responses to perturbations and to mediate additional loss of species diversity (Urbani and Ramos-Jiliberto, 2010).

Changes to the 'guild' structure, i.e. structural changes to the group of species in a community that use similar resources (Polis et al. 1989), is one IGP effect that has received minimal research effort (n = 28, 9%; Figure 1.4). This knowledge gap may exist as several generations may be necessary before the observation of guild structure changes are detected (Briggs and Borer, 2005). The financial and logistical challenges of long-term monitoring often result in the use of short-term experiments to extrapolate long-term predictions (Brown et al. 2001; Hastings, 2004). Modern technological advancements provide increased opportunity to study long-term changes in IGP that are necessary to fully understand how species respond to climate change. For example, Yurkowski et al. (2017) used SIA to investigate changes in foraging patterns of an IGpredator beluga (*Delphinapterus leucas*) on IGprey Greenland halibut (*Reinhardtius hippoglossoides*) in response to increases in abundance of a common resource, capelin (*Mallotus villosus*). The study identified an overall decrease in asymmetrical IGP across two decades resulting from a northward distribution shift of capelin with climate change (Yurkowski et al. 2017).

1.4 Conclusions

In the modern age of ecosystem-based studies, it is increasingly important to account for the total complexity of a food web when attempting to understand species interactions and

their consequences. Intraguild predation does not account for all species interactions but, it incorporates several species across different trophic levels. For improved comparison among IGP studies, verification of essential interactions (i.e. competition and predation) and classification of IGP type (i.e. symmetry and age-structure) is encouraged. Diverse methods from qualitative direct observation to integrated telemetry and sensor approaches afford exciting opportunities to investigate the occurrence and strength of IGP in modern food webs.

Traditional IGP models demonstrated several population dynamics that occur when IGP is present in a food web, including coexistence, alternative stable states, competitive exclusion or instability of species populations. The possibility for the coexistence of IGP species, however, was assumed rare and unstable in traditional IGP models. Added realism, through the inclusion of model parameters, reconciled the discrepancy between empirical and theoretical studies, demonstrating the increased opportunity for IGP species coexistence. Improved resolution of IGP models, through growth from one dimensional to ecosystem-level frameworks in conjunction with more robust computer processing, is now providing methods for more accurate stock assessments in fisheries and improved management practices to ensure resource sustainability.

Future IGP research effort focused on chemical and morphological changes in individual-level IGP studies and the resilience of common resources to IGP at population and community levels is required. Rapid advancements in methodological approaches (such as compound-specific isotope analysis of individual amino/fatty acids), the continuing development of novel sensors (such as predation tags) and simulation modelling provide avenues for exploring IGP with opportunities to address knowledge gaps through sophisticated experimental designs. Interdisciplinary approaches will improve confidence in IGP study results, while modern multi-species modelling will progress the quantification of IGP in an ecosystem context. Through a multifactorial approach that accounts for system complexity, the study of IGP can better predict how food webs are and will respond to perturbations in turn improving our understanding of the underlying mechanisms responsible for ecosystem function.

1.5 Overall Thesis Objectives

This thesis examined intraguild predation in modern food webs. Several knowledge gaps were addressed with this study including a review and synthesis on the available methods to study intraguild predation (Chapter 1). Given it has been over three decades since the seminal work of Polis, Myers and Holt (1989), a review of the literature examining available study techniques, technological advancements and the research effort that has been afforded to the implication levels (i.e. individual, population and community) and IGP effects (e.g. behavioural changes, resilience of resource and community stability) was required and provides focused attention on the IGP effects that require additional research effort.

When present at intermediate levels and among several species, complex interactions can mediate the impact of species loss within a food web (Holt and Huxel, 2007; Hall, 2011). To understand the role of IGP in marine food webs, a large shark assemblage was used as a model group of species. Large predatory sharks can function as marine apex predators, controlling marine food webs through a strong top-down effect. High phenotypic plasticity, however, among this group may drive varying functional roles, thus examination into the level of species connectance and shark involvement in complex interactions such as IGP may help further elucidate the functional roles of sharks in marine food webs. Stable isotope analysis (SIA) can allow one to reconstruct consumer diet, and thus provide IGP estimates among large sharks. In Chapter 2, shark liver tissue was examined as a possible short-term indicator for shark diet composition studies with a view to also understand IGP. Elasmobranch liver tissue is complex as it is the site of energy storage and is also expected to contain urea and TMAO for osmotic balance. Lipid, urea and TMAO, however, can confound stable isotope results and thus must be removed prior to stable isotope analysis. Due to the complex nature of elasmobranch liver tissue, few studies have previously explored the use of liver tissue in shark stable isotope studies. Given the high lipid content and presence of urea and TMAO in liver tissue, it was hypothesized that: i) lipid would remain in liver tissue samples despite lipid extraction with chloroform-methanol (3 rounds), ii) urea and TMAO would be removed from liver tissue following water washing thus resulting in increases in $\delta^{15}N$ values, iii) C:N thresholds would provide ecologically relevant liver isotopic values and iv) $\delta^{13}C$ mean isotopic

differences between tissue pairs (muscle and liver tissue) would correlate with known movement behaviour of focal shark species.

The prevalence, classes and consistency of IGP across short and long-term time scales were then examined in chapter three by considering shark diet using two methods: i) stomach content analysis and ii) stable isotope analysis of shark tissues (i.e. muscle and liver). It was hypothesized that the prevalence and strength of IGP would vary across shark species. The large sharks examined in the study ranged from secondary piscivores to tertiary piscivores, with tertiary piscivores feeding at trophic positions of ≥ 4, thus assuming the role of apex predators in marine food webs and are expected to participate in IGP interactions as the IGpredator. Furthermore, it was hypothesized that IGP would vary with body size given known ontogenetic diet shifts reported across large shark species, for example higher rates of elasmobranch consumption over ontogeny, driving stronger IGP interactions (Fu et al. 2016). Finally, it was hypothesized that IGP would vary across different time scales for individual species (i.e. short vs. long term) given known seasonal migration patterns through distinct ecosystem components with varying prey availability (Bonfil, 2005, Nalesso et al. 2019). Loss of prey density and biodiversity can result in higher incidence of IGP through increased competition for limited resources, while species rich environments are expected to have lower incidence of IGP due to alternative resource availability (Holt & Huxel, 2007).

Given complex multi-species interactions have been shown to influence food webs at each level within an ecosystem, identifying the prevalence of IGP interactions among large marine predators can improve our understanding of the functional roles of these species. Moreover, many shark species are considered essential for maintaining stability within food webs through top-down control; a loss of marine apex predators, for example was shown to promote mesopredator release and trophic cascades (Myers et al. 2007). Loss of shark populations, however, are occurring globally (MacNeil et al. 2020) with 30% of shark and ray species having been identified as threatened with extinction this year (IUCN, 2020). Loss of biodiversity is occurring at an unprecedented rate, with continued loss expected given the Anthropocene (Ceballos et al. 2015). It is therefore of critical conservation importance to understand the mechanisms that drive different functional roles

within the large shark assemblage which may improve management strategies through enhanced forecasting of community structure and species interaction effects in the future.

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Table 1.1 The diverse suite of methodological approaches available used to estimate the occurrence and strength of intraguild predation (IGP)

Estimating Intraguild Predation				
Categories	**Methods**		**Example Studies**	
			Author	***Study Species***
Direct Observation	Opportunistic Observation		Fallows et al. (2015)	Cape fur seal (*Arctocephalus pusillus*) and blue shark (*Prionace glauca*)
	Recording	Photography	Gilman, R.T. (2016)	Salticid (*Hyllus brevitarsus*) and large orb weaver (*Nephila senegalensis*)
		Videography	Oppenheim & Wahle (2013)	American Lobster (*Homarus americanus*)
		Audio Recordings	Bright, A (2008)	Feather pecking and non-feather pecking laying flocks
	Tracking	Radio-telemetry	Swanson et al. (2014)	African lions (*Panthera leo*), cheetahs (*Acinonyx jubatus*) and African wild dogs (*Lycaon pictus*)
			Brandt and Lambin (2007)	Weasel (*Mustela nivalis*) and field vole (*Microtus agrestis*)
			Kozlowski et al. (2012)	Kit foxes (*Vulpes macrotis*) and coyotes (*Canis lactrans*)
		Satellite-telemetry	Srygley and Lorch (2016)	Mormon crickets (*Anabrus simplex*), digger wasps (*Palmodes laeviventris* and *P. Hesperus*)
			Ferguson et al. (1997)	Polar bears (*Ursus maritimus*)
		Stomach Temperature Pills	Heide-Jørgense et al (2014)	Narwhal (*Monodon monoceros*)
Retrospective Observation	Recording	Photography	Greenville et al. (2014)	Dingo (*C. dingo*), European red fox (*V. vulpes*) and feral cat (*Felis catus*)
		Videography	Kistner et al. (2017)	Asian citrus psyllid (*Diaphrina citri*), predators (e.g. lacewigs and hover flies) and Argentine ants (*Linepithema humile*)
	Tracking	Acoustic-telemetry	Baldwin et al. (2002)	Cuthroat Trout (*Oncorhynchus clarki utah*)
		Predation Detection Acoustic Tags (PDAT)	Schultz et al. (2017)	Chinook Salmon (*Oncorhynchus tshawytscha*) and Striped Bass (*Morone saxatilis*)
		Integrative biologging e.g. Accelerometry & video e.g. Acoustic pH transmitter	Watanabe & Takahashi (2013)	Adélie penguins (*Pygoscelis adeliae*)
			Papastamatiou et al. (2007)	Blacktip reef sharks (*Carcharhinus melanopterus*)
	Faecal analysis		Walker et al. (2018)	Eastern chimpanzees (*Pan troglodytes schweinfurthii*)
	Stomach content analysis		Raab et al. (2012)	Anchovy (*Engraulis encrasicolus*), herring (*Clupea harengus*) and sprat (*Sprattus sprattus*)
			Tsunoda et al. (2017	Golden jackal (C. *aureus*) and red fox (*V. Vulpes*)
Markers and/or Tracers	Biological Markers	Gel eletrophoresis	Wool et al. (1978)	Internal parasite (*Aphidius matricariae*) in *Myzus persicae*
		Enzyme-Linked Immunosorbent Assay	Ragsdale (1980)	Detection of *Nezara viridula* in predators
		Gas Chromatography Mass Spectrometry (GC-MS)	Knutsen and Vogt (1985)	Lobsters (*Homarus Gammarus* (L.)) and shrim (*Artemia sauna* (L.))
		DNA analysis	Wengert et al. (2013)	Fisher (*Martes pennanti*), domestic dog, mountain lion (*Puma concolor*), bobcat (*Lynx rufus*) and coyote (*Canis latrans*)
		Fatty acid analysis	Pethybridge et al. (2014)	White shark (*Carcharodon carcharias*)
	Chemical Tracers	Stable Isotope Analysis (SIA)	Rickers et al. (2006)	Wolf spider species (*Alopecosa cuneata* and *Pardosa palustris*), springtail (*Heteromurus nitidus*) and fruit fly (*Drosophila melanogaster*)
		Compound-Specific Stable Isotope Analysis (CSIA)	Chikaraishi et al. (2014)	200 free-roaming organisms, representing 39 species in coastal marine (a stony shore) and 38 species in terrestrial (a fruit farm) environments

Estimating Intraguild Predation			
Categories	**Models**	**Example Studies**	
		Author	***Model Topic(s)***
Community Models	Traditional IGP	Grover (1995)	General Resource-Consumer Model identified criteria necessary for coexistence among IGP species.
		Löder et al. (2014)	Lotka – Volterra IGP Model of a microzooplankton community further highlighted scenarios for IGP species coexistence.
		Ruggieri and Schreiber (2005)	Incorporation of IGP into Schoener's Exploitative Competition Model resulted in the expanded range for alternative stable states. This study found an alternative stable state called 'contingent coexistence of the IGP species or displacement of the IGP' depending on initial species densities.
	Game Theoretical	Heithuas (2001)	IGP species coexistence occurred when dietary overlap was low, the shared resource had intermediate productivity and when an alternative resource (i.e. fourth species) was added for the IGpredator when resource productivity was high.
	Model comparisons	Takimoto et al. (2007)	3 vs. 4 trophic level model – alternative stable states were dependent on growth rates.
		Verdy and Amarasekare (2010)	Predicted biological conditions necessary for alternative stable states in communities with IGP.
Food Web Models	Niche	Williams and Martinez (2000)	Estimated the factors that contribute most to the complexity of a food web structure.
	Food web matrix	Arim and Marquet (2004)	Examined food web studies and determined IGP is ubiquitous in food webs.
Simulation Models	Multi-species individual-based	Andonegi et al. (2013)	OSMOSE used to assess stock populations of European anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*)
	Minimum Realistic (MRM)	Ross-Gillespie (2016)	GADGET used to improve stock assessment of South African hake fishery comprised of shallow-water Cape hake (*Merluccius capensis*) and deep-water Cape hake (*M. paradoxus*)
	Dynamic System	Condie et al. (2014)	ATLANTIS determined lack of population recovery by eastern gemfish (*Rexea solandri*) was likely due to IGP interactions with arrow squid (*Nototodarus gouldi*)
	Whole Ecosystem	Kitchell et al. (2002)	EwE used to determine that predation by apex sharks on sharks (i.e. IGP) results in strong non-linear responses in food webs.

Figure 1.1 Schematic of intraguild predation (IGP) and the two descriptors responsible for the different classes of IGP a) symmetry and b) age-structure. Symmetry can either be asymmetrical with one clearly defined IGpredator and one clearly defined IGprey, or, symmetrical whereby role reversal between the IGpredator and IGprey is possible. Similarly, age structure can be unimportant for the interaction, or important whereby only certain age classes of species are involved in IGP interactions. The four resulting IGP classes are i) asymmetrical age-structure unimportant, ii) asymmetrical age-structure important, iii) symmetrical age-structure unimportant and iv) symmetrical age-structure important IGP.

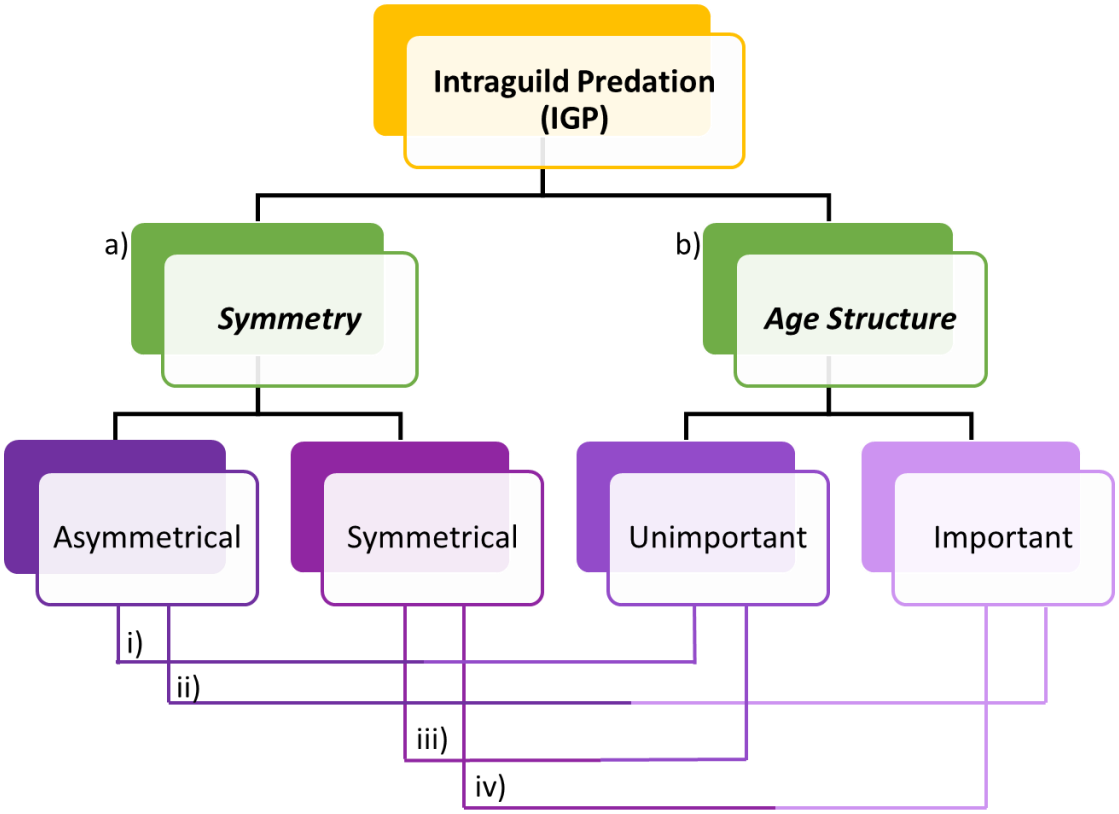


Figure 1.2 Examples of direct observation of intraguild predation. A) Opportunistic observation of a cape fur seal (*Arctocephalus pusillus*) consuming a blue shark (*Prionace glauca*) [from Fallows et al. (2015)]. B) Photographic recording of intraguild predation between a salticid (*Hyllus brevitarsus*) and an orb weaver (*Nephila senegalensis*) [from Gilman, R.T. (2016)]. C) Example of the use of radio telemetry on kissing bugs (*Triatoma gerstaeckeri*) [From Hamer et al. (2018)]. D & E) Tracking equipment MK10-AL satellite and STP3 stomach temperature pill, respectively, deployed on leatherback turtles (*Dermochelys coriacea*) [From Casey et al. (2010)].

Figure 1.3 Examples of methods used for the retrospective observation of intraguild predation. A) Recording technology, videography, used to study intraguild predation among Asian citrus psyllid (*Diaphrina citri*), predators (e.g. lacewigs and hover flies) and Argentine ants (*Linepithema humile*) [From Kistner et al. (2017)]. B & C) Predator detection tags [from Schultz et al. (2017) and Halfyard et al. (2017), respectively]. D) Fecal pellets collected from the European hare (*Lepus europaeus*) for fecal analysis [From Rodrigues et al. (2019)]. E) Stomach contents collected from a tiger shark (*Galeocerdo cuvier*) for stomach content analysis [From Dicken et al. (2017)]. F & G) Collection of predator saliva and hair, respectively, from the carcass of a fisher (*Martes pennanti*) for DNA analysis [from Wengert et al. (2013) study].

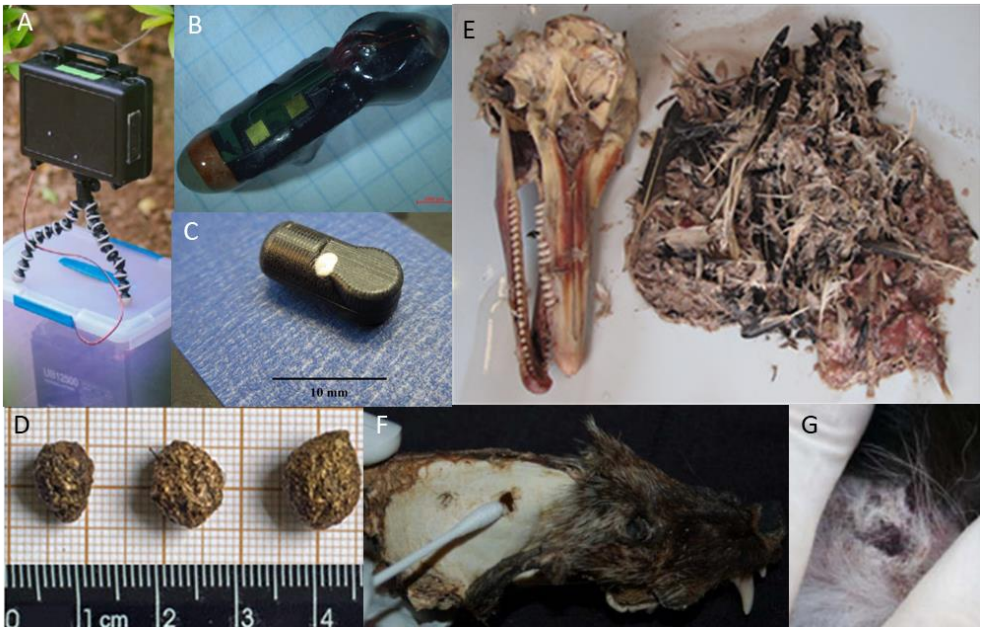
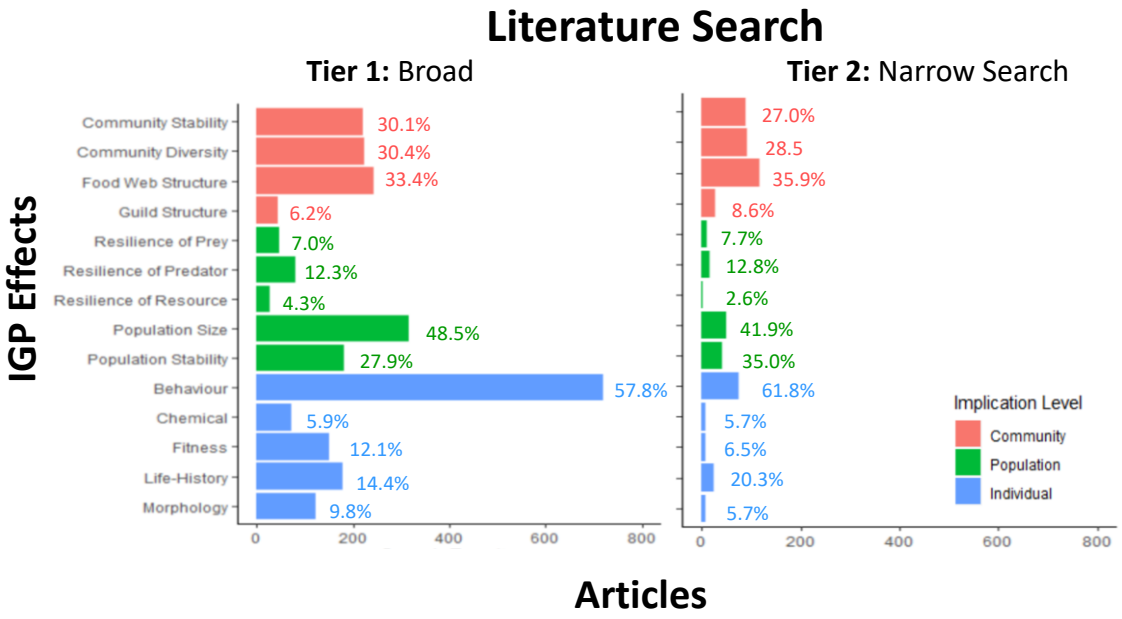


Figure 1.4 Literature search results grouped by tier whereby Tier 1 is a broad overview search (N Tier 1 = 2628) that includes key terms 'intraguild predation' and 'cannibalism' as well as the implication-level and synonyms for the intraguild predation (IGP) effect. Tier 2 is a narrow search (N Tier 2 = 566), removing 'cannibalism' and synonyms, using only the terms listed in the seminal work of Polis et al. (1989). The IGP effects from the Polis et al. (1989) paper are listed on the y-axis and each colour delineates the implication-level at which IGP effects can occur; the individual (blue; Tier 1 *n*= 1244, Tier 2 *n* = 123), population (green; Tier 1 *p* = 653, Tier 2 *p* = 173) and community-levels (red; Tier 1 *c* = 731, Tier 2 *c* = 326). The percent of each IGP effect relative to the total number of search results for each implication level are provided at the end of each bar.



CHAPTER 2

Determining the appropriate pre-treatment procedures and the utility of liver tissue for bulk stable isotope ($\delta^{13}C$ and $\delta^{15}N$) studies in sharks

2.1 Introduction

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes provide a valuable tool to address complex questions pertaining to elasmobranch ecology (Hussey et al., 2012a; Shipley et al., 2017*a*), based on the premise that the consumption of prey by a predator results in systematic prey isotopic fractionation that is reflected in consumers' tissues (Post, 2002). Fractionation of carbon (^{13}C:^{12}C; denoted as $\delta^{13}C$) and nitrogen (^{15}N:^{14}N; denoted as $\delta^{15}N$) are used to identify foraging location via basal carbon sources (e.g. coastal vs. pelagic; McConnaughey & McRoy, 1979; Hussey et al., 2011) and estimate consumer trophic position (Zanden & Rasmussen, 1999; Hussey et al., 2014), respectively. Isotopic incorporation rates are tissue-specific given each tissue has a distinct metabolic pathway (Tieszen et al. 1983; Logan & Lutcavage 2010). Comparative stable isotope analysis (SIA) between tissue types consequently provides a method to assess variability in individual movement/foraging behaviours over time (Bearhop et al., 2004) and has been applied across several different taxa including mammals, birds, and fish (Dalerum & Angerbjörn, 2005; Trakimas et al., 2011; Yurkowski et al., 2016). Specifically for elasmobranchs, the comparison of isotopic ratios from metabolically active tissues that have a fast turnover rate (i.e. plasma) with less metabolically active tissues with slow turnover rates (i.e. muscle), has; i) improved our understanding of temporal variation in consumer foraging patterns (MacNeil et al., 2005) ii) highlighted the influence of body size on isotopic variability among tissues (Matich et al., 2019), iii) provided insight into species-specific trophic ecology within a population (Ferreira et al., 2017), and iv) determined the level of individual specialization exhibited by large sharks (Matich et al. 2011). While muscle tissue, blood plasma and red blood cells have been investigated for use in SIA of elasmobranchs (Logan & Lutcavage, 2010; Kim & Koch, 2012), the potential use of liver tissue as a short-term indicator of diet and habitat use has remained relatively unexplored (Hussey et al., 2012a; MacNeil et al., 2006).

Elasmobranch liver is a highly complex tissue due to its role in osmotic regulation and energy storage (Hamlett, 1999; Hoffmayer et al., 2006). The synthesis of urea ($CO(NH_2)_2$) and presence of trimethylamine n-oxide (TMAO; C_3H_9NO; to counteract the fact that urea inhibits protein binding and folding; Yancey, 2005) is key to maintain osmotic balance (Hazon et al. 2003; Hammerschlag, 2006). Acclimatization to reduced salinity environments by lemon sharks (*Negaprion brevirostris*), for example, results in increased urea and extracellular solute (e.g. TMAO) excretion (Goldstein et al., 1968). The urea:TMAO relationship is depth dependent; at shallow depths (i.e. 50-90 m) the ratio is 2.96 and decreases to 0.67 at greater depths (i.e. 1911-2165 m). This suggests that TMAO may counteract hydrostatic pressure as TMAO concentrations increase in elasmobranch species at greater depths (Laxson et al., 2011). In addition, urea and TMAO aid with buoyancy control as the compounds have a combined 'lift contribution' of approximately $5.7 g1^{-1}$; for a *C. obscurus* with an overall mass of 93.5g in water, for example, the total lift of urea and TMAO is estimated to be 26.6g (Withers et al., 1994). The chemical composition of urea and TMAO (herein referred to as urea) present a unique challenge for SIA as both compounds contain carbon and are depleted in ^{15}N (Goldstein et al., 1968), while concentrations vary within and among individuals and species and are dependent on the environment they inhabit. Higher concentrations of urea typically lower $\delta^{15}N$ values resulting in decreased C:N values. Given non-extracted elasmobranch tissues generally have very low C:N (<3.0), removal of urea can result in C:N values that indicate low lipid content (i.e. C:N of 3.0) despite lipids still present in the tissue (Carlisle et al., 2016; Li et al., 2016).

Lipids can also bias carbon isotope values given they are depleted in ^{13}C relative to proteins and carbohydrates (6-8‰; DeNiro & Epstein, 1977; Yurkowski *et al*., 2015). Aside from unique species such as the Greenland shark (*Somniosus microcephalus;* Shipley et al., 2017*b*), most elasmobranch tissues are known to be low in lipids (e.g. muscle tissue; Hussey et al., 2012*b*), with the exception of liver (Bone and Roberts, 1969; Speers-Roesch and Treberg, 2010). The total lipid content of elasmobranch livers varies by species and geographic location, ranging from 51-81% (wet mass; Pethybridge et al., 2014) in white sharks (*Carcharodon carcharias*) to 26-45% in bigeye thresher sharks (*Alopias superciliosus*; Jayasinghe et al., 2003). Similarly, Remme et al. (2006) found that liver

tissue of deep-sea elasmobranchs; Leafscale gulper shark (*Centrophorus squamosus)*, Portuguese (*Centroscymnus coelolepis)* and black dogfish (*Centrocyllium fabricii*), had lipid concentrations ranging from 35-50% wet mass. High lipid content exists in the liver because it is the site of lipid synthesis and energy storage in elasmobranchs (Hoffmayer et al. 2006; Remme et al. 2006). Lipid content of tissues is often inferred through C:N values, whereby tissue samples with a C:N <3.5 are considered not to require lipid extraction as the sample is representative of pure protein (Post et al., 2007; but see also Carlisle et al., 2016 for C:N > 3.2). Tissues containing both urea and lipid, such as elasmobranch liver, however, may confound interpretation of C:N values due to the presence of increased nitrogen (%N; Carlisle et al., 2016) and require caution in interpretation. As a result, the ecological application of SIA in elasmobranch liver tissue requires consideration of tissue preparation techniques to remove urea/lipid compounds that may have confounding effects.

Urea is typically removed from elasmobranch tissue samples using standard water washing methods (Li et al., 2016). For example, Burgess and Bennett (2017) reported that C:N and $\delta^{15}N$ muscle tissue values of bluespotted maskray (*Neotrygon kuhlii*) increased significantly following urea removal as would be expected (Kim & Koch, 2012; Carlisle et al., 2016). Similarly, Li et al. (2016) examined the use of different tissue preparation methods including water washing (WW), lipid extraction (LE) and combined lipid extraction and water washing (LEWW) on muscle tissue of seven pelagic sharks. Results found that LEWW was most effective at urea removal given the %N was reduced, $\delta^{15}N$ significantly increased and the C:N increased from 2.6 to 3.1, indicating the removal of ^{15}N-depleted urea. For lipids, several extraction techniques exist, but the modified chloroform-methanol approach of Bligh and Dyer (1959) is commonly used across elasmobranch species and tissue types (MacNeil et al., 2005; Kinney et al., 2011).

While urea and lipid concentrations vary by elasmobranch tissue type, species- and habitat-specific variation in these compounds is also expected (Logan & Lutcavage, 2010; Shipley et al., 2017*c*). Consequently, it is broadly recognized that urea and lipid extraction are required, or at least preliminary tests should be conducted on a study- or species-specific basis (Hussey et al., 2012*b*). Given liver tissue contains high lipid content and is the site of urea synthesis, I investigated the need and effectiveness of: i) deionized water

washing to remove urea, and ii) chloroform-methanol for extraction of lipids. I then; i) established C:N thresholds for deriving ecologically relevant liver isotopic values given complications of removing all lipid from liver tissue and ii) undertook a preliminary comparison of $\delta^{13}C$ values between tissue pairs sampled from individual animals (muscle and liver) to test if observed isotopic differences were correlated to known movement behaviour. Tests were conducted on liver and muscle tissue of four large shark species sampled from KwaZulu-Natal (KZN), South Africa: the dusky (*Carcharhinus obscurus)*, sand tiger (*Carcharias taurus*), scalloped hammerhead (*Sphyrna lewini)* and white shark (*Carcharodon carcharias*).

2.2 Materials and Methods

Ethical Statement

All research in this investigation was conducted under annually renewed operating (OC/OCS/020) and research permits issued by the Department of Environmental Affairs, South Africa. Samples were collected from dead specimens, caught in the KZN bather protection programme, and hence ethical approval was not required.

Sampling Collection and Analyses

All sampled sharks were caught in nets and/or drumlines in association with the KZN bather protection programme. In 2014, a total of 22.4 km of netting remained across 37 beaches, with 79 drumlines found adjacent to the nets at 18 of the beaches. The nets are approximately 213.5 m long, placed 300-500 m from shore, at a depth of 10-14 m. For further information regarding net locations and net installations please refer to Dudley *et al.* (2005). Anchored drumlines consist of a Mustad 4480DT 14/0 J hook (Gjøvik, Norway) baited with *jacopever* spp. (Scorpaenidae) or southern rover (*Emmelichthys nitidus*) suspended 4 m beneath a large float (Cliff & Dudley, 2011). The equipment is serviced approximately 18-20 times per month with deceased sharks found in good condition (i.e. not decomposed) transported to the main KZN Sharks Board laboratory in Durban for further processing. Prior to dissection, sex and morphometric measurements were recorded [including PCL (cm), and total mass (kg)]. Sample collection included the excision of ~5

g of white muscle tissue from anterior to the first dorsal fin. During shark dissection, the complete liver was extracted from each individual and liver tissue samples (~5 g) were obtained from the bottom left lobe. All tissue samples were frozen at -20˚C.

Shark tissue samples were freeze dried for 48 h and finely ground using a mortar and pestle. Tissue samples were then sub-sampled with ~5 mg removed for each treatment; i) lipid extraction (LE) and ii) lipid extraction and water washing (LEWW). Liver tissues grouped within the LEWW treatment group underwent urea removal through the addition of 4 mL of deionized water. The solution was mixed with the sample for 1 min and then left at room temperature for 24 hr. Liver samples were then centrifuged, excess water removed from the sample using a pipette and the above process was repeated three times before a second round of freeze drying. All sub-samples underwent lipid extraction using chloroform-methanol (adapted from Hussey et al., 2012*a*). In brief, dried, ground samples were placed in 2 mL cryovials and vortexed with 2:1 chloroform-methanol (~1.9 mL) for 10 seconds and left in a 30˚C water bath for 24 h to promote solvent extraction. The remaining sample was then centrifuged for 5 minutes before the residual solvent was filtered out. The addition of chloroform-methanol, agitation, and filtration of solvent was repeated a further two times (n = 3 extractions) before the remaining solvent was left to evaporate from the sample for 48 hrs. Only one round of lipid extraction was necessary for muscle given its low lipid content (Hussey et al., 2012*b*). Following the processing steps of each treatment, sub-samples were weighed (~400-600 μg) into tin capsules and analyzed using a Thermo-Delta 5 Plus continuous flow isotope mass-spectrometer (Thermo Finnigan, Bremen, Germany) equipped with a zero blank auto-sampler and a 4010 Elemental Analyzer (Costech International S.P.A., Milan Italy).

Resulting isotope ratios were expressed in delta (δ) notation defined as the deviation from a standard reference material in per mil (‰) following the equation: $\delta X(‰) = ((R_{sample}/R_{standard})-1) \times 1000$, where X is ^{15}N or ^{13}C, while R_{sample} and $R_{standard}$ are the isotopic ratios (heavy:light) of the sample with respect to the sample and reference material (Peterson & Fry, 1987). . The standards for N_2 and CO_2 were atmospheric nitrogen and Vienna Pee Dee Belemnite (V-PDB) carbonate, respectively. The precision assessed by the standard deviation of replicate analyses of four standards; bovine liver (i.e. NIST1577c), an internal lab standard (i.e. tilapia muscle), L-glutamic acid (i.e. USGS 40) and urea (n=33

for all), was determined to be ≤0.19‰ for $\delta^{15}N$ and ≤0.11‰ for $\delta^{13}C$ for all standards. Accuracy, based on repeat sampling of certified values of USGS 40 (n=33 for $\delta^{13}C$ and $\delta^{15}N$), was analyzed throughout runs and showed a difference of 0.09‰ for $\delta^{15}N$ and -0.07‰ for $\delta^{13}C$ from the certified value. Instrumentation accuracy was validated every tenth run using NIST standards 8573, 8547 and 8574 for $\delta^{15}N$ and 8542, 8573 and 9574 for $\delta^{13}C$. The mean difference from the certified values for each standard were -0.13, -0.13 and -0.04‰ for $\delta^{15}N$ and -0.06, 0.02 and 0.16‰ for $\delta^{13}C$, respectively.

2.2.1 Urea Effects on Shark Liver Isotope Values

To examine if a significant change in shark liver isotopic values ($\delta^{13}C$ and $\delta^{15}N$) occurred following WW (i.e. testing between LE vs. LEWW liver tissue), Student paired t-tests (for parametric data) and Wilcoxon signed rank tests (for non-parametric data) were conducted for each shark species, as well as for all shark species combined. The direction and magnitude of change in isotopic values was then explored by conducting a linear regression on isotope values between treatment groups (e.g. $\delta^{15}N_{LE}$ vs. $\delta^{15}N_{LEWW}$) compared to a null hypothesis of no change/difference (i.e. a 1:1 relationship for $\delta^{15}N_{LE}$ and $\delta^{15}N_{LEWW}$).

2.2.2 Lipid Extraction & C:N Thresholds

To determine the effectiveness of lipid extraction at removing lipids from shark liver tissue, the C:N ratio (i.e. calculated as weight %) was first assessed based on values <3.5 indicating lipid-free tissue (Post et al., 2007). Linear regression analysis of $\delta^{13}C$ vs. C:N was then examined for all shark species combined and for each shark species individually based on the expectation that if all lipid was successfully removed, a non-significant relationship between $\delta^{13}C$ and C:N would occur. Given the high lipid content in elasmobranch liver and high C:N ratios reported for lipid extracted liver of teleost fish (Stowasser et al., 2009), it was predicted that standard lipid extraction procedures (even repeated 3 times) may not be effective at removing lipid from all samples. Consequently ^{13}C-depleted liver values for samples with remaining lipid would drive a high C:N ratio, resulting in a significant negative relationship between $\delta^{13}C$ and C:N. Under this scenario, I undertook stepwise linear regression analysis at 0.1 increments, from the highest recorded lipid extracted C:N value per species until a non-significant trend was identified ($p \geq 0.05$).

The C:N value at this point was defined as the 'C:N threshold' as it indicates successful lipid extraction and provides ecologically relevant data on a species-by-species basis.

2.3 Preliminary Muscle-Liver Tissue Comparison

To undertake a preliminary assessment of the value of liver for understanding movement behaviour of the focal sharks, the difference between muscle and liver $\delta^{13}C$ values was examined for each species relative to known movement patterns. First tissue-specific fractionation was accounted for by correcting liver and muscle $\delta^{13}C$ isotopic values using Caut *et al.* (2009) consumer estimates for diet tissue discrimination factors (DTDF) for each tissue type ($\Delta^{13}C_{liver}$ = 0.77 ± 0.30; $\Delta^{13}C_{muscle}$ = -0.248$\delta^{13}C$-3.4770). Then the difference in mean $\delta^{13}C$ values was examined between i) $liver_{LEWW}$ and $muscle_{LE}$ samples before the C:N threshold was applied (i.e. $\delta^{13}C_{Diff} = \delta^{13}C\ Mus_{LE} - \delta^{13}C\ Liver_{LEWW}$) and ii) the $\delta^{13}C_{Diff}$ that was deemed acceptable following the C:N threshold. After the removal of data ≥ C:N threshold for each shark species, an overall decrease in the difference in $\delta^{13}C$ between tissue types was expected as a result of removing lipid biased samples. The $\delta^{13}C_{Diff}$ between C:N threshold corrected $liver_{LEWW}$ and $muscle_{LE}$ was then examined in the context of the known movement ecology of each shark species.

Monthly catch rates of sharks in beach protection nets were used as a proxy for residency and seasonal movements of each species in KwaZulu-Natal (KZN) in conjunction with available literature. Sub-adult/adult sand tigers undergo seasonal movements between the temperate Eastern Cape and subtropical/tropical waters of KZN/Mozambique (Dicken et al., 2007); while dusky sharks are caught throughout the year in KZN, with seasonal peaks in adult captures occurring in July associated with the sardine run (Dudley et al., 2005). Tag-recapture data further indicate that juvenile dusky sharks undertake small scale movements between KZN and the temperate Eastern Cape (Bass *et al*. 1973; Hussey et al., 2009) while larger individuals connect shelf and pelagic food webs (Hussey et al., 2012*c*). The dusky and sand tiger sharks are therefore considered to undertake restricted movements confined mostly to the continental shelf and shelf edge. In contrast, the scalloped hammerhead and white sharks show distinct seasonal catch rates in KZN beach protection nets (Cliff et al., 1989; Dudley & Cliff, 2010). The white shark undertakes extensive coastal movements between the Southern Cape, KZN and

Mozambique, but also pelagic movements into tropical waters of the broader Indian Ocean (Cliff et al. 2000) while the scalloped hammerhead shark moves southward to the Eastern Cape (Diemer et al., 2011), connects shelf and pelagic food webs and likely moves northward into tropical areas during winter months (Hussey et al., 2012*a*). Given the catch rates/known variability in movement routes among marine habitats and reported isotopic turnover rates of ~166 days for liver (95% turnover; *Potamotrygon motoro;* MacNeil et al., 2006) and 341 ± 39 days for muscle tissue (95% turnover; *C. plumbeus*; Logan & Lutcavage, 2010), I expected: i) minimal differences in $\delta^{13}C$ values between muscle (Mus_{LE}) and liver ($Liver_{LEWW}$) for sand tiger (RAG) and dusky sharks (DUS) and ii) greater differences between tissue types for white (GRE) and scalloped hammerhead (SCA) sharks. It was noted that isotopic turnover rates do not likely match seasonal movements, consequently isotopic differences between tissues may be marginal unless strong isotopic gradients exist along movement routes. Normality and homogeneity of variance was tested for all data prior to conducting statistical tests. All statistical analyses were performed in RStudio (version 1.2.1578, R Development Core Team) with statistical significance (α) set to 0.05.

2.4 Results

2.4.1 Urea Effects on Shark Liver Isotope Values

When testing for an effect of water washing on urea removal from elasmobranch liver tissue, no significant difference in $\delta^{15}N$ was found across treatment groups (i.e. $\delta^{15}N_{LiverLE}$ vs. $\delta^{15}N_{liverLEWW}$; n=56, p=0.54; Table 2.1). At the species level, the mean difference in $\delta^{15}N$ liver values (i.e. $\delta^{15}N_{LiverLE}$ - $\delta^{15}N_{liverLEWW}$) was -0.06 ± 0.41‰, -0.01 ± 0.17‰, -0.07 ± 0.46‰ and -0.11 ± 0.26‰ for the DUS, RAG, SCA and GRE, respectively (mean difference ± SD; Table 2.1). A significant increase in %N of liver tissue of 1.46 ± 0.83% was observed following WW (t=13.20, p<0.001) for all sharks combined (Table 2.1). At the species level, the scalloped hammerhead showed the greatest %N increase of 1.77 ± 0.90%, while the white shark had the smallest increase (0.75 ± 0.67%; Table 2.1). Pre-treatment of shark liver tissue through water washing also resulted in a significant increase in $\delta^{13}C$ (V=1332, p<0.001; Table 2.1) and %C values (t=18.47, df=55, p<0.001; Table 2.1) between treatments. The mean $\delta^{13}C_{Diff}$ between LE and LEWW liver samples was 0.54 ±

0.63‰, 0.33 ± 0.20‰, 0.42 ± 0.53‰ for the DUS, RAG and SCA, respectively (Table 2.1). No significant difference in $\delta^{13}C$ values was found for GRE treatment groups (-0.08 ± 0.80‰; p=0.74; Table 2.1). Following WW, the C:N ratio decreased for DUS, RAG and SCA sharks by 0.23 ± 0.46, 0.16 ± 0.11 and 0.22 ± 0.33, respectively, while the C:N value for GRE remained constant (t=-.77, df=11, *p*=0.46; Table 2.1). As expected, there was a positive linear relationship between treatment groups for $\delta^{15}N$ (F=720.4, $r^2 = 0.93$, $p<0.001$; Figure 2.1E) and $\delta^{13}C$ (F=185.8, $r^2 = 0.77$, $p<0.001$; Figure 2.2E) for all sharks combined, with some variation at the species level (Figure 2.1A-D & Figure 2.2A-D).

2.4.2 Lipid Extraction & C:N Thresholds

At the species level, C:N ratios for both $liver_{LE}$ and $liver_{LEWW}$ treatments exceeded the accepted 3.5 ratio for pure protein, with mean values ranging from 3.9 ± 0.3 (3.25 – 4.88) for the GRE to 4.2 ± 0.3 (3.49 – 4.99) for SCA sharks (Table 2.1). In addition, a negative linear relationship was observed between $\delta^{13}C$ (both $liver_{LE}$ and $liver_{LEWW}$) and C:N values of liver tissue for each individual species and all species combined (F=119.8, $r^2 = 0.28$, df = 309, $p < 0.01$). Lipid effects were therefore assumed present in the $liver_{LE}$ and $liver_{LEWW}$ samples (Post et al., 2007) and 'C:N thresholds' were estimated for each shark species for each treatment type. The C:N thresholds determined for $liver_{LE}$ were 5.0, 4.6, 4.5 and 4.0 and for $liver_{LEWW}$ were 4.0, 3.6, 4.7 and 3.9 for DUS, RAG, SCA and GRE, respectively (Figure 2.3). Variation in C:N threshold values between $liver_{LE}$ and $liver_{LEWW}$ treatment groups is likely a result of smaller sample sizes for $liver_{LE}$; DUS, RAG, SCA and GRE sample sizes were 85, 63, 60 and 46, respectively, for $liver_{LEWW}$ tissue and 15, 15, 15 and 12 respectively, for $liver_{LE}$ samples (Figure 2.3).

2.4.3 Preliminary Muscle-Liver Tissue Comparisons

As expected, the application of $liver_{LEWW}$ C:N thresholds and thus removal of lipid-biased tissue values from the data, increased the mean $\delta^{13}C$ values of DTDF-corrected liver tissue by 0.30‰, 0.82‰ and 0.29‰ for DUS, RAG and GRE, respectively, while the $\delta^{13}C$ for SCA liver did not change. No arithmetic correction was applied to the data given a lack of species-specific lipid normalization models for the focal species in this study and previous complications over attempting this for elasmobranchs (Carlisle et al., 2016; Shipley et al.,

2017). The sample sizes, however, were reduced from 79, 45, 57 and 43 to 54, 11, 55 and 26 for DUS, RAG, SCA and GRE, respectively following the application of the C:N thresholds. Comparison of C:N threshold and DTDF-corrected $\delta^{13}C$ $liver_{LEWW}$ and $muscle_{LE}$ values for the four shark species followed expected trends, with the magnitude of the observed isotopic differences between tissue pairs corresponding with catch rates/known movements of each species. For the regionally-resident dusky and sand tiger sharks, tissue $\delta^{13}C$ values were similar with $\delta^{13}C_{Diffs}$ of 0.24 ± 0.99‰ (n=54) and 0.57 ± 0.38‰ (n = 11) for DUS and RAG, respectively (Figure 2.4). The scalloped hammerhead and white sharks, that are considered to undertake larger scale movements across distinct environments/latitudes, had larger $\delta^{13}C_{Diffs}$ of 1.24 ± 0.63‰ (n = 55) and 1.08 ± 0.71‰ (n=26), respectively (Figure 2.4).

2.5 Discussion

Understanding the requirement for and effectiveness of urea and lipid extraction from shark liver tissue is important for accurate ecological interpretation of $\delta^{13}C$ and $\delta^{15}N$ values. To date, no consensus has been reached regarding pre-treatment of elasmobranch liver tissues for SIA. The suspected presence of ^{15}N-depleted urea and TMAO in liver indicated the need to remove this compound and thus an examination of the overall effectiveness of water washing was warranted (Hussey et al., 2011; Carlisle et al., 2016). Successful removal of both urea and TMAO through WW was expected to result in an overall increase in $\delta^{15}N$ values and a decrease in %N. In contrast, no difference in $\delta^{15}N$ values across treatment groups was found and there was a marginal increase in %N. Furthermore, marked increases in $\delta^{13}C$ and an overall decrease in C:N were observed for the dusky, sand tiger and scalloped hammerhead sharks. The use of deionized water washing for the removal of urea and TMAO as pre-treatment for SIA is therefore not necessary for liver tissue for these four elasmobranch species. Logan and Lutcavage (2010) found no significant difference in $\delta^{15}N$ values among treatment groups (bulk tissue, lipid-extracted tissue, urea-extracted tissue, and urea extract) of skate blood (*Leucoraja* spp.) and spiny dogfish (*Squalus acanthias*) muscle tissue. Similarly, Shipley et al. (2017*c*) reported no significant differences between bulk and WW $\delta^{15}N$ values of nurse shark muscle (*Ginglymostoma cirratum*), southern stingray fins (*Hypanus americanus*) and Atlantic chupare stingray

(*Styracura schmardae*) fins. Shipley et al. (2017*c*) suggested the lack of change in $\delta^{15}N$ values of water washed muscle and fin may be due to small concentrations of urea in those tissues. Although urea synthesis takes place in elasmobranch liver, reabsorption occurs in the kidneys, suggesting urea and TMAO are released from the liver following synthesis and stored in elasmobranch blood plasma (Yancey, 1994; Ballantyne, 1997), lowering concentrations of these compounds in the liver. Urea concentrations in blood plasma of the lesser spotted dogfish (*Scyliorhinus canicular*), for example, are 1000x greater than that of liver and red blood cells (Walsh et al., 1994). Furthermore, the use of chloroform-methanol as a solvent for lipid extraction of elasmobranch tissues has been shown to remove urea (Hussey et al., 2010, 2012; Li et al., 2016) and therefore three rounds of washing with this solvent may have resulted in the removal of urea from liver tissue prior to WW.

But why was there a marginal increase in $\delta^{13}C$ values following WW? Given the high concentrations of lipid in shark tissue, it is possible that additional ^{13}C-depleted lipids may have been removed with deionized water. Li et al. (2016) observed similar increases in $\delta^{13}C$ values following WW of muscle tissue from silky (*C. falciformis*), blue (*Prionace glauca*), smooth hammerhead (*S. zygaena*), scalloped hammerhead (*S. lewini*), shortfin mako (*Isurus oxyrinchus*), pelagic thresher (*Alopias pelagicus*) and oceanic whitetip (*C. longimanus*) sharks. The C:N ratios of the seven shark species, however, increased following WW as would be expected, indicating removal of urea and/or TMAO (Li et al. 2016). In contrast, an unexpected decrease in C:N ratio and an increase in %N and %C were observed in shark liver tissue following LEWW, a trend that requires further investigation. The polarity and chemical composition of TMAO [C_3H_9NO] and urea [$CO(NH_2)_2$], as well as the polarity of the solvent used for lipid extraction (i.e. chloroform-methanol), may explain unexpected isotopic trends following WW. For example, Connan et al. (2019) observed identical trends to those reported here for lipid-extracted/water washed samples compared to lipid-extracted samples of cape jaw (*Oplegnathus conwayi*) and *C. taurus* muscle and *C. taurus* red blood cell samples treated with chloroform-methanol (i.e. 1 and 2 rinses). The removal of structural lipids with a polar solvent may result in the co-extraction of lipids and lipophilic amino acids (Sweeting et al., 2006; Connan et al., 2019). The strong polarity of chloroform-methanol may therefore effectively remove structural lipids in lipid-concentrated tissues such as liver, freeing low-weight

amino acids (Mathew & Shamasundar, 2002; Murthy & Rajanna, 2011). Loss of both lipids and amino acids could counteract the removal of urea following WW, resulting in an overall increase in $\delta^{13}C$ and a decrease in C:N. The C:N of liver samples following LEWW was ~4.0 for the dusky, sand tiger and scalloped hammerhead sharks. Although a C:N ratio of <3.5 is the universally accepted standard used to indicate a pure muscle sample (i.e. unbiased; Post *et al*. 2007), C:N ratios are tissue-specific, related to amino acid composition (McMahon et al., 2015, 2010) and therefore elasmobranch liver tissue may be delipidated at C:N ratios of ~4.0. Examination of tiger shark (*G. cuvier*) liver tissue amino acid composition (Scott et al. 1976) resulted in an overall C:N of ~4.1, confirming that the C:N ratio of fully delipidated elasmobranch liver is above the universally accepted delipidated muscle C:N ratio of 3.5. Through examining the relationship between $\delta^{13}C$ and C:N for each shark species and accepting some lipid bias still present in liver samples, our 'C:N threshold' approach provides a conservative method to derive ecologically viable species-specific C:N values for each treatment type (i.e. LE vs. LEWW). It is important to test on a species-by-species basis, however, that all urea has been removed from the liver sample otherwise the C:N threshold approach could be compromised by higher levels of percent nitrogen if urea were present (Hussey et al., 2010; Carlisle et al., 2016). Moreover, given liver lipid content varies by species, life-stage and potentially related to condition/movement phase (Hussey et al., 2009), investigators should ensure there is no life-stage bias in their data following the application of C:N thresholds (i.e. all juveniles are removed following the application of the C:N thresholds if variation in isotope values by life-stage is a key factor under investigation).

Given a larger sample size of $liver_{LEWW}$ samples, DTDF-corrected $\delta^{13}C$ values between liver (LEWW) and muscle (LE) tissue pairs were compared before and after the application of the C:N thresholds. Application of the $liver_{LEWW}$ C:N thresholds ultimately lowered $\delta^{13}C$ mean differences between muscle and liver tissues for each species as expected. Furthermore, the tissue comparison demonstrated the value of liver tissue isotope values for examining the ecology of these species. Variability existed between $\delta^{13}C$ values of $muscle_{LE}$ and $liver_{LEWW}$ pairs at the species level, with the magnitude in the $\delta^{13}C$ difference broadly matching predictions based on monthly catch-rates and known latitude/habitat movement patterns. The isotopic similarity between $\delta^{13}C$ values of dusky

and sand tiger tissue pairs was likely a combination of high site fidelity, small-scale migratory patterns, generalist foraging strategies and consumption of prey with similar isotopic signatures over time (Hussey et al., 2009; Dudley & Cliff, 2010; Smale et al. 2012).

The larger differences in $\delta^{13}C$ values between tissue pairs of the white and scalloped hammerhead sharks, likely reflect large scale seasonal movement patterns across isotopically distinct locations that may also drive diet switches over set time scales. For example, white sharks undertake primarily large coastal movements between temperate and tropical waters, but transoceanic migration is also documented (Bonfil, 2005). White shark diet may therefore, based on geographic location, be a major driver of the observed difference in $\delta^{13}C$ values between tissues. Similarly, while scalloped hammerhead sharks can be highly resident, large scale movements across pelagic waters are recorded (Nalesso et al., 2019). These movements are likely to occur in KZN given this region (including the Eastern Cape; Diemer et al., 2011) represents their most southern distribution and seasonal variation in water temperatures will drive northward migrations.

2.6 Conclusion

Standardized tissue-specific sample preparation is required for the accurate interpretation of stable isotope data especially when making comparative analyses across tissues, individuals and species. While evaluations have been conducted to determine the appropriate SIA preparation protocols for some elasmobranch tissues (e.g. white muscle; Li et al., 2016), the use of elasmobranch liver tissue as a short-term diet indicator is relatively unexplored and therefore no standardized protocol currently exist. It is widely accepted that the presence of lipid, urea and TMAO within elasmobranch tissues can bias isotopic data and ecological interpretation (Hussey et al. 2011; Kim et al. 2012; Shipley et al., 2017a), therefore, chemical extraction procedures and water washing have become standard practices to remove these compounds. The current study demonstrated that treatment of $liver_{LE}$ tissue with deionized water for the removal of urea and TMAO among dusky, sand tiger, scalloped hammerhead and white sharks is not required, given $\delta^{15}N$ values did not change following treatment.

In terms of lipid removal, three consecutive chloroform-methanol extractions resulted in some liver samples that were still lipid biased. It is proposed that multiple extractions are required and that deriving species-specific C:N thresholds provides a tool to determine reliable $liver_{LE}$ values in sharks. It is also noted that expected C:N ratios of delipidated shark liver tissue may be higher than muscle based on different amino acid composition. Elasmobranch liver tissue provides a valuable short-term indicator of diet/movement and the approaches presented here will assist the application of this tissue to understand elasmobranch trophic ecology across different temporal and spatial scales.

2.7 References

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Table 2.1 Summary of liver stable isotope values following treatment (LE vs. LEWW) for large sharks caught in beach protection nets off the coast of KwaZulu-Natal, South Africa. The mean (± SD) $\delta^{15}N$, %N, $\delta^{13}C$, %C and C:N for each shark species is provided for the two defined treatment types; lipid extracted (LE) and lipid extracted water washed (LEWW). The mean difference and level of significance between treatments are detailed. Acronyms for shark species include DUS: *Carcharhinus obscurus*, RAG: *Carcharias taurus*, SCA: *Sphyrna lewini* and GRE: *Carcharodon carcharias*. (**) Indicates $p<0.001$, (*) indicates $p<0.01$ between $liver_{LE}$ and $liver_{LEWW}$ treatment groups.

Table 2.1

Species	n	Parameter	*Liver Pairs* Liver (LE)	Liver (LEWW)	Mean Difference	Significance
Combined (ALL)	56	**δ^{15}N** (‰)	14.6 ± 1.3	14.6 ± 1.2	0.03 ±0.34	V = 723 *p* = 0.54
		%N	11.4 ± 1.0	12.9 ± 0.9	1.46 ± 0.83	t = 13.20 **
		δ^{13}C (‰)	-16.7 ± 1.2	-16.4 ± 1.0	0.32 ± 0.59	V = 1332 **
		%C	46.5 ± 1.6	50.7 ± 1.5	4.18 ± 1.70	t = 18.47 **
		C:N	4.1 ± 0.4	4.0 ± 0.3	-0.15 ± 0.32	V = 333 **
DUS	14	**δ^{15}N** (‰)	13.7 ± 0.8	13.8 ± 0.8	-0.06 ±0.41	V=51 *p* = 0.95
		%N	11.0 ± 1.3	12.6 ± 1.0	1.56 ± 0.93	t = 6.28 **
		δ^{13}C (‰)	-17.0 ± 1.0	-16.5 ± 0.6	0.54 ± 0.63	t = 3.22 *
		%C	46.1 ± 1.8	50.2 ± 0.9	4.07 ± 1.89	t = 8.03 **
		C:N	4.2 ± 0.6	4.0 ± 0.3	-0.23 ± 0.46	V = 88 ***p < 0.05***
RAG	15	**δ^{15}N** (‰)	15.4 ± 0.7	15.4 ± 0.6	-0.01±0.17	t = -0.28 *p* = 0.78
		%N	11.5 ± 0.8	13.2 ± 0.8	1.62 ± 0.41	t = 15.30 **
		δ^{13}C (‰)	-16.1 ± 0.7	-15.8 ± 0.6	0.33 ± 0.20	t = 6.60 **
		%C	47.1 ± 1.7	51.5 ± 1.6	4.48 ± 1.70	t = 13.47 **
		C:N	4.1 ± 0.4	4.0 ± 0.4	-0.16 ± 0.11	t = -5.93 **
SCA	15	**δ^{15}N** (‰)	13.5 ± 0.9	13.4 ± 0.6	-0.07 ±0.46	t = -0.61 *p* = 0.54
		%N	11.1 ± 0.7	12.9 ± 0.7	1.77 ± 0.90	t = 7.64 **
		δ^{13}C (‰)	-17.7 ± 1.1	-17.2 ± 1.1	0.42 ± 0.53	t = 3.07 *
		%C	46.2 ± 1.3	50.9 ± 1.0	4.62 ± 1.70	t = 10.52 **
		C:N	4.2 ± 0.3	4.0 ± 0.3	-0.22 ± 0.33	t = -2.61 ***p* < 0.05**
GRE	12	**δ^{15}N** (‰)	16.0 ± 0.6	15.9 ± 0.6	-0.11 ±0.26	t = -1.38 *p* = 0.19
		%N	12.2 ± 0.8	12.9 ± 0.8	0.75 ± 0.67	t = 3.92 *
		δ^{13}C (‰)	-15.7 ± 1.3	-15.8 ± 0.9	-0.08 ± 0.80	t = -0.33 *p* = 0.74
		%C	46.8 ± 1.8	50.2 ± 1.9	3.41 ± 1.81	t = 6.51 **
		C:N	3.9 ± 0.4	3.9 ± 0.3	0.05 ± 0.24	t = 0.77 *p* = 0.46

Figure 2.1 The relationship between lipid extracted (LE) and lipid extracted water washed (LEWW) liver tissue $\delta^{15}N$ values (i.e. $\delta^{15}N_{LiverLE}$ vs. $\delta^{15}N_{LiverLEWW}$) for four shark species; A) dusky (DUS; *Carcharhinus obscurus*), B) white (GRE; *Carcharodon carcharias*), C) sand tiger (RAG; *Carcharias taurus*) and D) scalloped hammerhead (SCA; *Sphyrna lewini*), as well as all species combined E). The grey area indicates the 95% confidence intervals for the linear regression. The black dotted line is the 1:1 line, the point at which no difference exists between treatment groups (LE vs. LEWW).

Figure 2.1

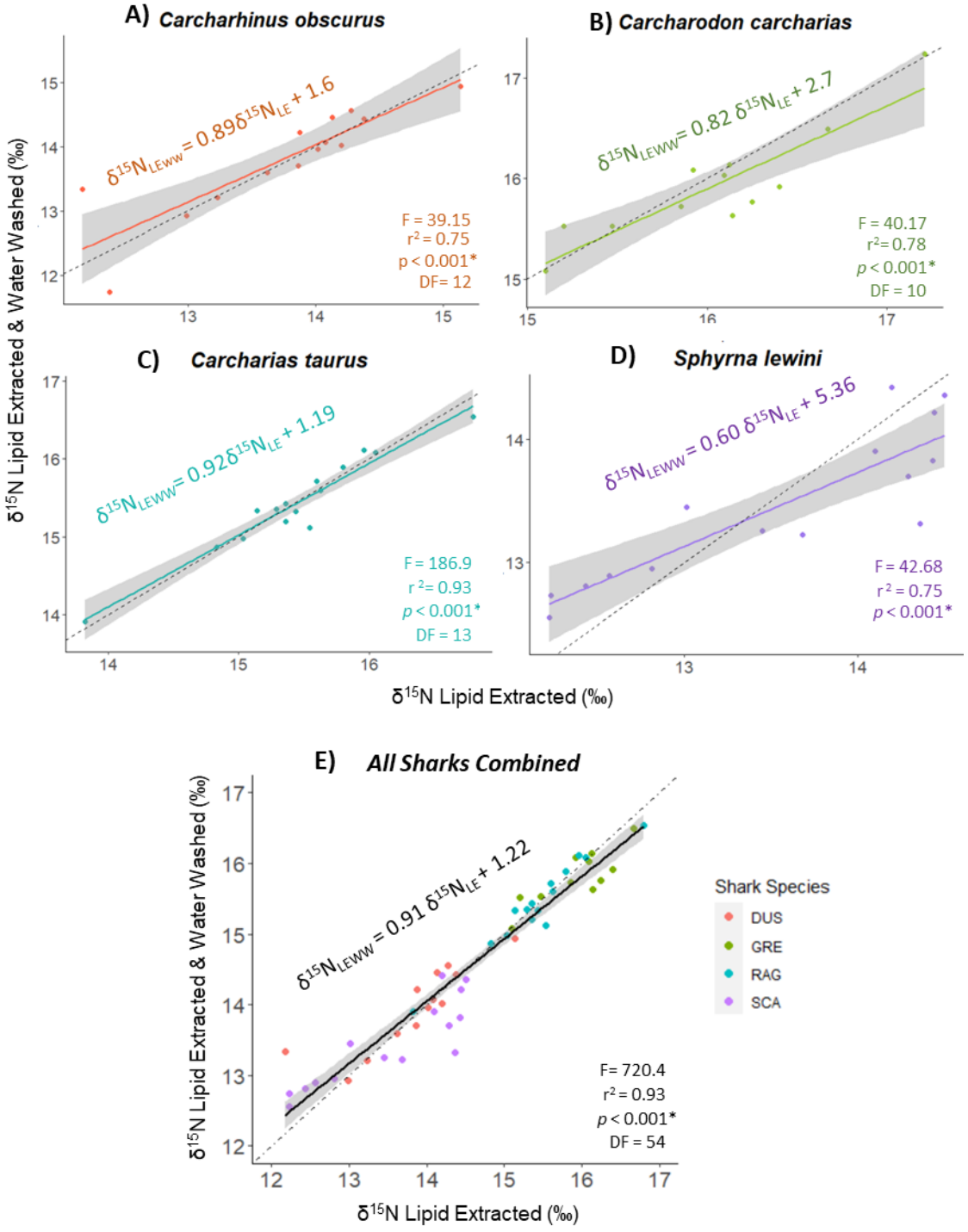

A) Carcharhinus obscurus
δ15NLEWW = 0.89δ15NLE + 1.6
F = 39.15
r2 = 0.75
p < 0.001*
DF= 12
B) Carcharodon carcharias
δ15NLEWW = 0.82 δ15NLE + 2.7
F = 40.17
r2 = 0.78
p < 0.001*
DF = 10
C) Carcharias taurus
δ15NLEWW = 0.92δ15NLE + 1.19
F = 186.9
r2 = 0.93
p < 0.001*
DF = 13
D) Sphyrna lewini
δ15NLEWW = 0.60 δ15NLE + 5.36
F = 42.68
r2 = 0.75
p < 0.001*
δ15N Lipid Extracted & Water Washed (‰)
δ15N Lipid Extracted (‰)
E) All Sharks Combined
δ15NLEWW = 0.91 δ15NLE + 1.22
F = 720.4
r2 = 0.93
p < 0.001*
DF = 54
Shark Species
DUS
GRE
RAG
SCA

Figure 2.2 The relationship between lipid extracted (LE) and lipid extracted water washed (LEWW) liver tissue $\delta^{13}C$ values plotted by treatment type (i.e. $\delta^{13}C_{LiverLE}$ vs. $\delta^{13}C_{LiverLEWW}$) for four shark species; A) dusky (DUS; *Carcharhinus obscurus*), B) white (GRE; *Carcharodon carcharias*), C) sand tiger (RAG; *Carcharias taurus*) and D) scalloped hammerhead (SCA; *Sphyrna lewini*), as well as all species combined E). The grey area indicates the 95% confidence intervals for the linear regression. The black dotted line is the 1:1 line, the point at which no difference exists between treatment groups (LE vs. LEWW).

Figure 2.2

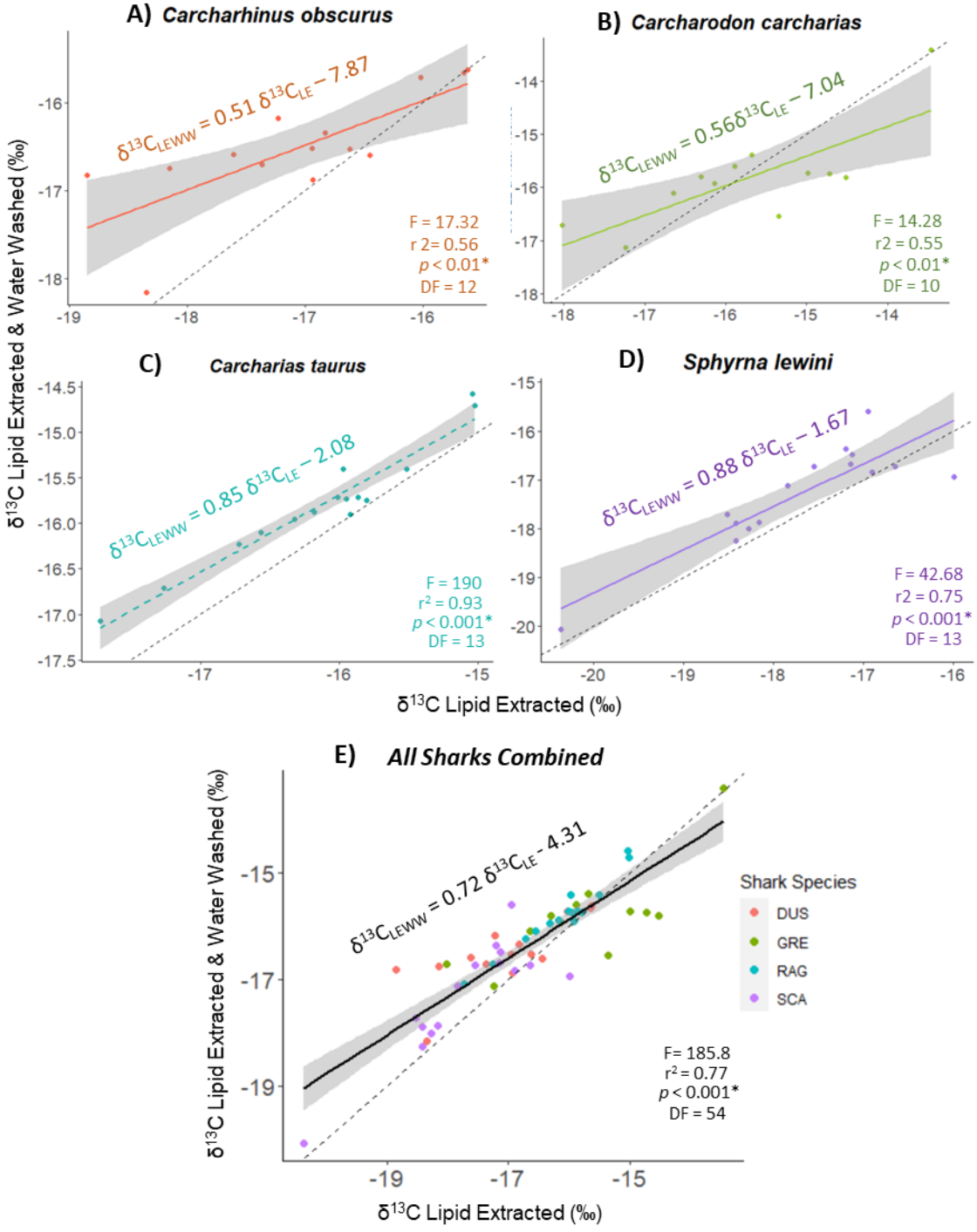

A) Carcharhinus obscurus
δ13CLEWW = 0.51 δ13CLE − 7.87
F = 17.32
r 2= 0.56
p < 0.01*
DF = 12
B) Carcharodon carcharias
δ13CLEWW = 0.56δ13CLE − 7.04
F = 14.28
r2 = 0.55
p < 0.01*
DF = 10
C) Carcharias taurus
δ13CLEWW = 0.85 δ13CLE − 2.08
F = 190
r² = 0.93
p < 0.001*
DF = 13
D) Sphyrna lewini
δ13CLEWW = 0.88 δ13CLE − 1.67
F = 42.68
r2 = 0.75
p < 0.001*
DF = 13
δ13C Lipid Extracted & Water Washed (‰)
δ13C Lipid Extracted (‰)
E) All Sharks Combined
δ13CLEWW = 0.72 δ13CLE - 4.31
F= 185.8
r² = 0.77
p < 0.001*
DF = 54
Shark Species
DUS
GRE
RAG
SCA
δ13C Lipid Extracted & Water Washed (‰)
δ13C Lipid Extracted (‰)

Figure 2.3 The relationship between lipid extracted (LE) and lipid extracted water washed (LEWW) liver tissue $\delta^{13}C$ values and C:N ratio for four shark species; dusky (DUS), sand tiger (RAG), scalloped hammerhead (SCA) and white (GRE), as well as all species combined. The grey area indicates the 95% confidence intervals for linear regressions. Tissue samples in red were lipid extracted only, while teal points were lipid extracted and water washed. C:N thresholds to derive reliable $\delta^{13}C$ data following LE are 5.0, 4.6, 4.5 and 4.0 for DUS_{LE}, RAG_{LE}, SCA_{LE} and GRE_{LE}, respectively. The C:N thresholds for liver following lipid extraction and water washing (LEWW) are 4.0, 3.6, 4.7 and 3.9 for DUS_{LEWW}, RAG_{LEWW}, SCA_{LEWW} and GRE_{LEWW}, respectively.

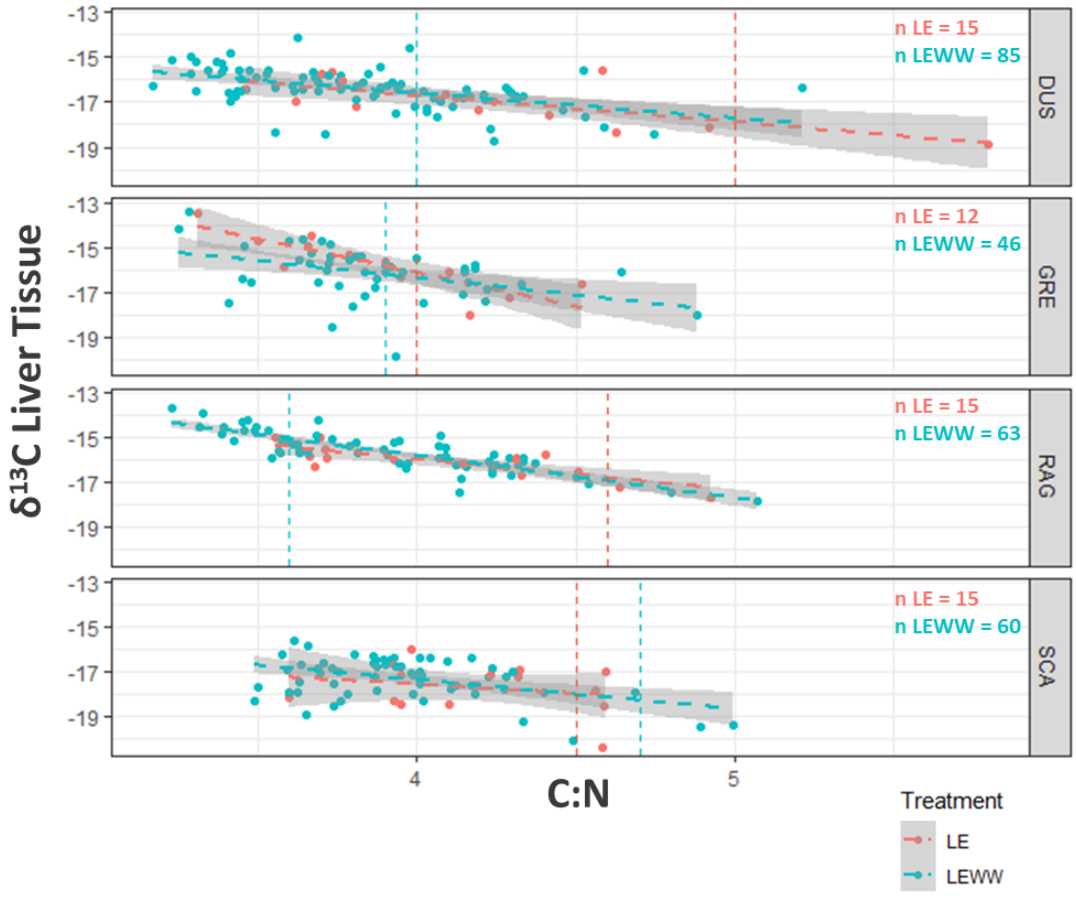
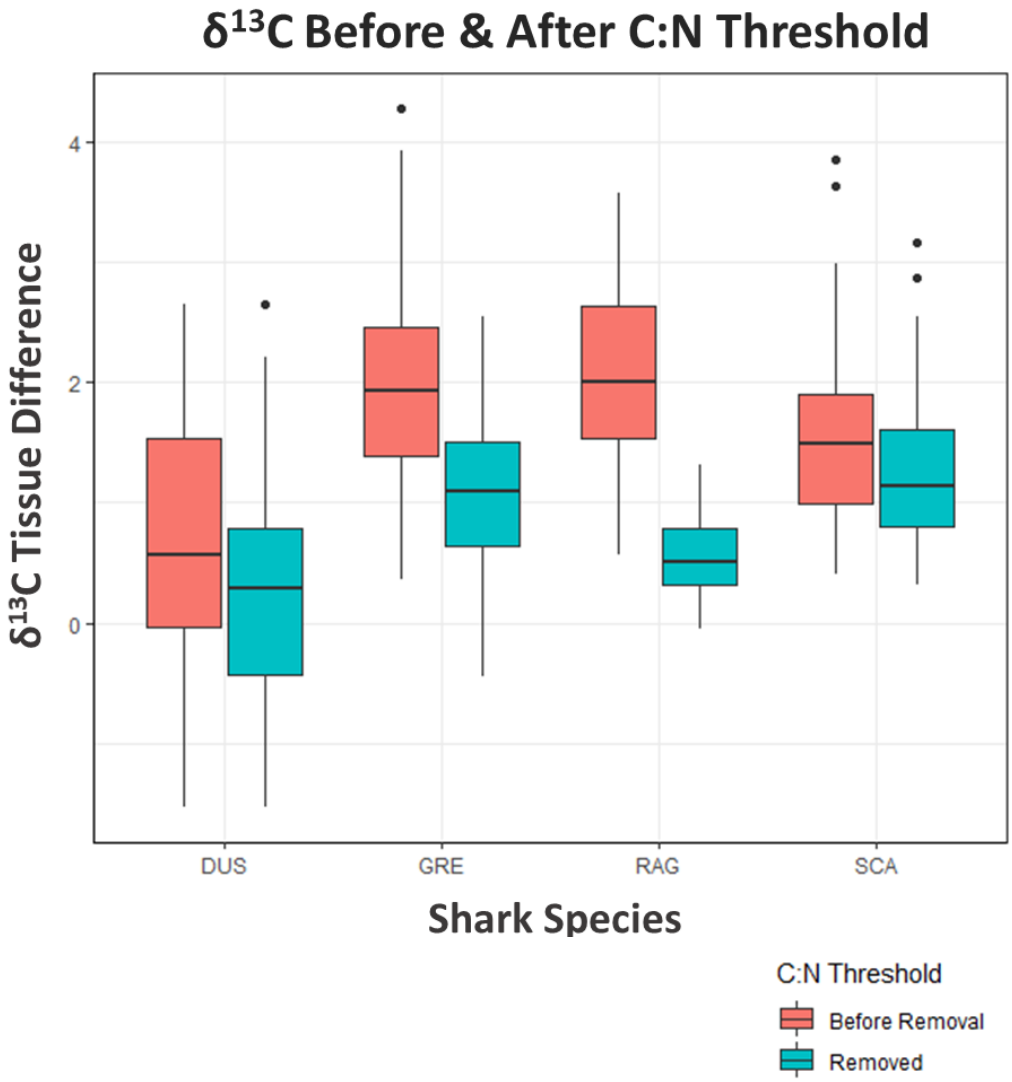


Figure 2.4 The difference in $\delta^{13}C$ values between lipid extracted (LE) muscle and lipid extracted water washed (LEWW) liver tissue before and after C:N thresholds are applied. The difference in $\delta^{13}C$ (i.e. $\delta^{13}C_{Diff} = \delta^{13}C_{MusLE} - \delta^{13}C_{liverLE}$) is calculated for each shark species; dusky (DUS), sand tiger (RAG), scalloped hammerhead (SCA) and white (GRE). The species-specific C:N threshold points applied are those determined for LEWW liver tissue (4.0, 3.6, 4.7, 3.9 for DUS, RAG, SCA and GRE, respectively). All tissue values have been corrected with tissue-specific diet tissue discrimination factors (DTDF) to allow ecological interpretation.



CHAPTER 3

It's a Shark Eat Shark World: Identifying the Occurrence and Class of Intraguild Predation Among Large Predatory Sharks

3.1 Introduction

The sixth mass global extinction, the Anthropocene, is currently underway, causing loss of biodiversity at unprecedented rates (Ceballos et al. 2015). Conservation and scientific investigation into mechanisms that drive losses in biodiversity are required to mitigate continued species loss and to protect the existing levels of biodiversity (Duffy et al. 2017). With 30% of shark and ray species currently identified as threatened with extinction (IUCN, 2020), it is of critical conservation importance to understand the functional role of these species within aquatic food webs. Many shark populations have already experienced widespread global decline across tropical oceans in response to anthropogenic stressors such as overexploitation through industrialized fishing, gillnets, longlines and the shark fin trade (MacNeil et al. 2020). Given large predatory sharks can exert strong top-down control in marine food webs, loss of shark populations has been shown to alter food web structure. For example, theoretical studies using ecosystem models have predicted that reduced large shark populations can result in trophic cascades (Ferretti et al. 2010). A high degree of phenotypic plasticity, however, exists among the elasmobranch assemblage with large sharks occupying different functional roles. For example, examination of the large shark assemblage off South Africa revealed that large sharks are secondary and tertiary piscivores occupying trophic levels (TL) ranging from 3.2 to 6.1 (Hussey et al. 2014). Similarly, life history stage (i.e. juvenile vs. adult) and size classes (i.e. small, medium and large; defined by total length) have also been used to discern ecological roles given variation over ontogeny (Heupel et al. 2014). Although the magnitude of ecosystem level impacts is commonly determined by the identity of species at risk of extinction, it is species interactions that define functional roles within an ecosystem (Cardinale et al. 2006).

Intraguild predation – a multi-trophic interaction that involves omnivory – is prevalent across food webs (Arim & Marquet 2004). Intraguild predation (IGP) occurs among a minimum of three participants: a top predator, known as the IGpredator, that kills and consumes an IGprey with which it competes for a common resource (Polis et al. 1989).

This complex interaction simultaneously combines competition and predation to form a trophic loop. The presence of IGP can lead to individual-, population- and community-level implications through direct effects on biomass availability across trophic groups and indirect effects such as trophic cascades and bottlenecks (Holt and Polis, 1997). There are several different categories of IGP that can occur within a community that are classified using two descriptors: i) symmetry: which can be asymmetrical, whereby the IGpredator remains the IGpredator throughout the interaction, or symmetrical; whereby both predatory IGP species interact as IGpredator and IGprey and ii) age structure: whereby age-class of the IGpredator and IGprey play a role in IGP interactions. Four main classes of IGP can occur: 1) symmetrical where age-structure is important, 2) symmetrical with age-structure being unimportant, 3) asymmetrical where age-structure is important and 4) asymmetrical with age-structure being unimportant (Polis et al. 1989; Pahl et al. 2020).

Loss of species and alterations to species interactions within food webs can drive changes to biodiversity (Hooper et al. 2012; Duffy et al. 2017) that shift the overall function of a food web (Loreau 2010; Cardinale et al. 2012). The stability of a food web in response to species losses may, however, be contingent on the presence and strength of complex species interactions that have been shown to mediate the impact of loss of apex predators (see Ferrettii et al. 2010). While the coarse-level functional roles of large shark species have been investigated (Heupel, 2014; Hussey et al. 2014), limited knowledge exists on the broad suite of IGP interactions that occur. To address this knowledge gap and provide improved understanding of the connectance of large sharks in marine food webs, the first examination of the occurrence, strength and class of IGP was examined among sharks. Eleven large shark species, referred to hereafter as the 'large shark assemblage', were examined: blacktip (BLA; *Carcharhinus limbatus*), bull (ZAM; *Carcharhinus leucas*), copper (COP; *Carcharhinus brachyurus*), dusky (DUS; *Carcharhinus obscurus*), java (JAV; *Carcharhinus amboinensis*), sand tiger (RAG; *Carcharias taurus*), scalloped hammerhead (SCA; *Sphyrna lewini*), spinner shark (SPN; *Carcharhinus brevipinna*), smooth hammerhead (SMO; *Sphyrna zygaena*), tiger (TIG; *Galeocerdo cuvier*) and white shark (GRE; *Carcharodon carcharias*). By reconstructing shark diet composition using two approaches; stomach content analysis and stable isotope analysis (SIA) this study examined i) the class and strength of IGP present among each shark species and ii) the

variability in IGP strength and occurrence over different time scales. It was hypothesized that IGP strength and class would vary i) across shark species, ii) shark ontogeny and iii) across different time scales (short-term vs. long-term). Given large sharks occupy secondary piscivore to tertiary piscivore roles (Hussey et al. 2014) variation in functional roles among large shark species is expected to result in variability in the strength and class of IGP experienced across different species. Additionally, increased body size and thus gape size, allows shark species to consume increased prey species and sizes, including IGprey, over ontogeny (Fu et al. 2016). Finally, many shark species undertake seasonal migration that would be expected to drive variability in IGP across different time scales dependent on prey types available (Bonfil, 2005, Nalesso et al. 2019).

Through improved understanding of shark involvement in IGP interactions, finer resolution into shark functional roles within marine food webs is possible which can help with the development of holistic management strategies that take into account species involved in complex interactions and how these multi-species interactions contribute to food web stability and structure.

3.2 Methods

3.2.1 Shark Sample Collection

Shark samples were collected from KwaZulu-Natal bather protection nets installed off the coast of Durban, South Africa, in 1952. The nets were approximately 213.5 m long and were placed 300-500 m from the shore at a depth of 10-14 m. In 2014, the beach protection programme remained active across 37 beaches with netting totaling 22.4 km (Petta et al. 2020). Additional information regarding the nets, their installations and locations can be found at Dudley et al. (2005). Drumlines have also been established adjacent to the nets at 18 beaches. Southern rover (*Emmelichthys nitidus*) and/or jacopever species (*Scorpaenidae*) were baited on a Mustad 4480DT 14/0 J hook (Gjøvik, Norway) and suspended 3- 4 m below a large float. All bather protection equipment was serviced 18-20 times per month and inspected daily for newly deceased sharks that were transported to the KZN Sharks Board (KZNSB) laboratory in Durban for additional processing. Sharks were then stored at -20ºC until dissection. During dissection, species, sex, morphological measurements, such as precaudal length (PCL, cm; Dudley et al. 2005), and maturity were

recorded. Maturity was determined following Bass et al. (1973), whereby males were considered sexually mature when the claspers were fully grown and rigid as a result of calcification. Adolescent males had signs of clasper growth, but they were not yet rigid, while immature males had short, soft claspers. Sexually mature females had distinct ova within the ovary, expanded uteri that formed loose sacs and a ruptured hymen. Adolescent females had similar ova and uteri, however, the hymen remained intact, while immature females had thin, tight-walled uteri tubes with an intact hymen (Bass et al. 1973). Dissection involved the removal of stomach and gut contents, that were sorted and identified to the lowest possible taxon (see 'Stomach Content Analysis' below; sampling ranged between 1985 and 2018) and tissue samples were taken for analysis (see "Stable Isotope Analysis" below; sampling since 2005).

3.2.2 Stomach Content Analysis

Identified prey items (at the species level) were grouped into ecologically relevant functional groups; classified by taxonomic class, then further subdivided into functional groups (i.e. shark, ray, cetacea, pinniped) following Cortés (1999) and Hussey et al. (2015). The main prey groups examined in this study were therefore: Elasmobranchii (i.e. sharks, rays, unknown elasmobranchs), Mammalia (i.e. pinniped, cetacea, unknown and terrestrial), Actinopterygii (i.e. teleosts), Malacostraca (i.e. crustacea) and Cephalopoda.

Stomach content data from previously published studies was used to determine the class and prevalence of IGP within the diet of each focal shark species (see Table 3.1). The IGP class was determined by examining the percent mass (%M) of each prey group within the shark diet. Although several metrics exist for stomach content analysis, % mass is standardized such that all prey items total 100%, while metrics such as percent frequency (%F) and the index of relative importance (%IRI) can have totals that exceed 100%. Using % mass, the percent contribution of each prey group to the total predator diet was determined. To then estimate IGP for each species, Mammalia and Elasmobranchii prey were summed (i.e. *%IGP in Shark diet = %M Mammalia + %M Elasmobranchii*). Several stomach content studies for the focal species were divided by size categories (i.e. approximating juvenile, adolescent and adult), while others combined all data (see Table 3.2 for predator size categories). Assumptions made during the evaluation and calculation

of IGP for each shark species were as follows; guitarfish and dogfish were classified as 'sharks' and batoids were classified as 'rays'. Mammalian aquatic prey species were assumed IGprey, as pinnipeds and cetaceans are known to consume many of the same resources as sharks (e.g. *Teleostei* species such as *Pomadasys commersoni* and Cephalopoda such as *Loligo* spp. ; Young and Cockcroft 1994). Terrestrial mammalian species were not included in IGP calculations. Using the above approach, four IGP calculations were conducted to provide a range of IGP estimates: 1) a conservative IGP estimate whereby only identified shark and Mammalia species were included, 2) the unknown shark categories were included (i.e. 'small sharks', 'large sharks' and 'unknown shark remains'), 3) unknown sharks and rays were included in the IGP estimate and 4) the most liberal IGP estimate whereby unknown sharks, rays and Elasmobranchii were included.

Identification of IGP class by symmetry (i.e. asymmetrical or symmetrical) required examination of both focal shark prey items and prey of the IGprey. Sharks identified as prey of a focal shark, for example, were examined. The presence of the focal shark within the diet of the shark prey identified symmetrical IGP was present. Alternatively, the absence of the focal shark from the prey shark diet resulted in classification of the IGP interaction as asymmetrical. Although shark stomach content analysis often contains unidentified large sharks, small shark and shark remains, the classification of 'symmetrical IGP' required the taxonomic identification of a prey item down to the species-level. The occurrence of symmetrical IGP among the large shark assemblage may therefore be more prevalent among large sharks than the conservative estimates provided in this study.

3.2.3 Stable Isotope Analysis

For a subset of sharks (n = 691 individuals) caught in the beach protection nets since 2005, approximately 5 g of white muscle was sampled from the base of the first dorsal fin for stable isotope analysis. Stable isotope analysis (SIA) was used to compliment SCA as it provides insight into individual diet composition over time (Bearhop et al. 2004). Prey items, assimilated into consumer tissues following digestion and fractionation, are detected through elemental ratios of carbon and nitrogen isotopes ('you are what you eat'; Peterson

and Fry 1987). The systematic fractionation of nitrogen isotopes (^{15}N:^{14}N) from prey to predatory tissues can be used to estimate food chain length and trophic position, while the conservative fractionation of carbon isotopes (^{13}C:^{12}C) can identify predator habitat use and migratory patterns (Hussey et al. 2012a). In elasmobranch predators, muscle tissue has a 95% turnover of ~341 days (Logan & Lutcavage, 2010), providing an indication of consumer annual foraging patterns. In terms of IGP, comparison of muscle tissue with prey proportions determined through stomach content analysis was used to assess variability in IGP interactions over two time scales; long term (>1 year) and short term (days; Tieszen et al. 1983; Hobson & Welch, 1992). Elasmobranch tissue samples, however, contain several molecules that have been shown to cause biased SIA results (Hussey et al. 2012a, b; Shipley et al. 2017; Pahl et al. 2020 in review). Archived muscle tissue samples collected from focal sharks were therefore subsampled (0.5-1.0g), ground into a powder and underwent pre-treatment for the extraction of these compounds prior to SIA (Post et al. 2007).

a) Lipid & Urea Extraction

The presence of lipid within elasmobranch tissue has been shown to bias carbon isotopic values as lipids are depleted in ^{13}C relative to proteins and carbohydrates (6-8‰; DeNiro and Epstein 1977; Yurkowski et al. 2015). To allow for comparable $\delta^{13}C$ values between individuals, it is standard procedure to remove lipids from tissue samples via lipid extraction (Bligh and Dyer 1959). Different elasmobranch tissues, however, contain different lipid concentrations. Although most elasmobranch tissues have low lipid concentrations, there are exceptions such as Greenland shark tissues (*Somniosus microcephalus*; Shipley et al. 2017); therefore to avoid confounding interpretations of ecological relationships between shark species, as well as within individuals, muscle tissue underwent standard chemical lipid extraction with chloroform-methanol as per Bligh and Dyer (1959).

Urea [$CO(NH2)_2$,] and trimethylamine n-oxide [TMAO; C_3H_9NO] stored in elasmobranch tissues for osmotic regulation have also been shown to bias SIA results as both compounds are depleted in ^{15}N (Gannes et al. 1997; del Rio et al. 2009; Wolf et al. 2009). High concentrations of these molecules in tissue samples can thus result in

artificially low $\delta^{15}N$ values (Laxson et al. 2011). To avoid unintentional bias in SIA results, urea and TMAO are often removed with lipids through a combined lipid extraction (LE) and deionized water washing (Kim & Koch, 2012; Li et al. 2006). Several studies, however, have shown that lipid extraction, following the method proposed by Folch et al. (1957), with 2:1 chloroform methanol can remove urea and TMAO, shown through reductions in %N, and increases in both C:N and $\delta^{15}N$ (Hussey et al. 2010, 2012; Li et al. 2016). Furthermore, deionized water washing of elasmobranch tissue samples may not be necessary for the removal of urea and TMAO as Shipley et al. (2017) found no significant difference in $\delta^{15}N$ values of nurse shark (*Ginglymostoma cirratum*) muscle tissue following water washing. Therefore, given the low concentration of urea expected in muscle tissue, this pre-treatment step was not conducted.

The lipid extraction procedure was adapted from Hussey et al. 2012. In brief, 0.9 mL of 2:1 chloroform-methanol was added to ground, dried muscle sample in 2 mL cryovials and vortexed for 10 seconds. To promote solvent extraction, the vortexed cryovials were left in a 30˚C water bath for 24 h. After 5 minutes of centrifugation, the solvent was filtered out and the sample was left for 48hrs to allow any residual solvent to evaporate from the sample. Following lipid extraction, approximately 400-600 µg of the sample was weighed into tin capsules that were analyzed for ^{13}C:^{12}C and ^{15}N:^{14}N ratios using a Thermo-Delta 5 Plus continuous flow isotope mass-spectrometer (Thermo Finnigan, Bremen, Germany) equipped with a zero blank auto-sampler and a 4010 Elemental Analyzer (Costech International S.P.A., Milan Italy).

Isotopic ratios were defined by the deviation from a standard reference material in per mil (‰) and expressed in delta (δ) notation following the equation $\delta X(‰) = ((R_{sample}/R_{standard})-1) \times 1000$. The R_{sample} and $R_{standard}$ are the isotopic ratios (heavy:light) of the sample with respect to the sample and reference material, and X is ^{15}N or ^{13}C (Peterson and Fry 1987). The standards used for ^{15}N:^{14}N and ^{13}C:^{12}C were atmospheric nitrogen and Vienna Pee Dee Belemnite (V-PDB) carbonate, respectively. Precision was accessed through the standard deviation of replicate analysis of bovine liver (i.e. NIST1577c), tilapia muscle, L-glutamic acid (i.e. USGS 40) and urea (n = 33 for all). Precision was determined to be ≤0.09‰ for $\delta^{15}N$ and ≤0.11‰ for $\delta^{13}C$ for all standards. Accuracy was calculated by repeat sampling of L-glutamic acid throughout runs resulting in a difference of 0.09‰ for

$\delta^{15}N$ and -0.07‰ for $\delta^{13}C$ from the certified value. Instrument accuracy was validated through the use of NIST standards 8573, 8547 and 8574 for $\delta^{15}N$ and 8542, 8573 and 9574 for $\delta^{13}C$ every tenth run. The resulting mean difference from the certified values of each standard were -0.13, -0.13 and -0.04‰ for $\delta^{15}N$ and -0.06, 0.02 and 0.16‰ for $\delta^{13}C$, respectively.

b) Prey Groups

Prey groups within shark diet were standardized by categorizing derived prey isotope data by taxonomic class. Each shark predator species had the same five prey groups: Elasmobranchii, Mammalia, Actinopterygii, Malacostraca and Cephalopoda. Small sample sizes and minimal overall contribution to shark diet resulted in the removal of gastropods and bivalves from the study. The mean isotopic values estimated per functional prey group, however, differed by shark species as only prey species identified in the stomach contents of a shark predator were included in the calculation of mean $\delta^{13}C$ and $\delta^{15}N$ prey values. Some shark species therefore had four prey groups, as Mammalia were not identified in the shark diet via stomach content analysis. The mean isotopic values for each prey group were determined through a weighted mean calculation to avoid sample size bias. To account for tissue-specific fractionation, $\delta^{13}C$ and $\delta^{15}N$ prey isotopic values were corrected using Caut et al. (2009) consumer estimates for diet tissue discrimination factors (DTDF). Each prey $\delta^{13}C$ value was corrected through the addition of the DTDF estimate (i.e. $\Delta^{13}C = -0.248\delta^{13}C-3.477$) to the $\delta^{13}C$ prey value. Similarly, prey $\delta^{15}N$ values were corrected using the equation $\Delta^{15}N = -0.281\delta^{15}N-5.879$ (as per Caut et al. 2009).

c) Bayesian Mixing Model

The contribution of the prey items to the total diet of shark predators was estimated using the Bayesian mixing model R package 'SIMMR' (Stable Isotope Mixing Models in R; Parnell, 2016). The probability distribution of the contribution of prey items; called 'sources', to the 'mixture' (i.e. predator diet) was evaluated using mean and standard deviations of the isotopic prey values (i.e. both $\delta^{13}C$ and $\delta^{15}N$), mean and standard deviation estimates for DTDFs of each prey group and the raw $\delta^{13}C$ and $\delta^{15}N$ values for the consumer of interest. Shark species categorized into size classes in the stomach content

studies were also grouped into equivalent size categories for the Bayesian mixing models (see size classes in Table 3.2). SIMMR output underwent a three-step evaluation process to determine if the model output was appropriate and ecologically viable for consideration and comparison with stomach content analysis. Three criteria had to be met for inclusion: i) the distribution of the five prey sources had to cover the geometric mixing space of the consumer data within the mixing model, ii) the prey groups had to be isotopically distinct and thus have minimal overlap, and iii) the SIMMR estimates of diet contribution had to be biologically credible given the known diet from stomach content analysis. Refer to Table 3.2 in the Appendix for a list of the three categories and the decision process.

3.2.4 Correlation Between Stomach Content and Stable Isotope Analysis

To compare the prevalence of intraguild predation predicted by the two dietary composition methods (i.e. stomach content analysis and stable isotope analysis), Shapiro-Wilk normality tests were performed, followed by Spearman Rho correlation analyses. All statistical analyses were performed using R version 4.0.2 (R Core Team 2020), in RStudio (version 1.3.1093, R Development Core Team) with statistical significance (α) set to 0.05.

3.3 Results

3.3.1 Stomach Content Analysis

Each of the focal sharks identified in the large shark assemblage participated in at least one form of intraguild predation, with asymmetrical IGP being the most prevalent IGP interaction among the predators (Figure 3.1; Table 3.3-3.5). Five shark species were involved in asymmetrical age-structure important (Table 3.3) and unimportant IGP (Table 3.4), while symmetrical IGP was less common within the large shark assemblage with only 2 (symmetrical age-structure important) and 3 (symmetrical age-unknown) species involved (Figure 3.1; Table 3.6). The dusky shark participated in each class of IGP and was the only species that engaged in cannibalism, with this interaction present throughout dusky ontogeny (i.e. within each size class; Table 3.6). White sharks had the largest occurrence of IGP in their diet, with a liberal estimate of 94.4% (i.e. Table A.1-A.3 estimates summed with conservative estimates from Table 3.3 – 3.6) and a conservative estimate of 79.8% (Table 3.3-3.6); white sharks occupied the role of IGpredator most

frequently among the large shark assemblage (Figure 3.2). Inversely, the spinner shark experienced the lowest rate of IGP occurrence, with stomach contents indicating between 0.5 – 1.0% of spinner diet as IGprey (Figure 3.2). Unexpectedly, the spinner shark was found to engage in symmetrical IGP with the dusky shark and therefore occasionally acted as IGpredator to the dusky shark (Table 3.6). When conservative IGP estimates were examined, the dusky and bull sharks had IGP estimates that exceeded those of the tiger shark (i.e. conservative estimate of 32.9% IGP), 57% and 44.7% IGP, respectively (Figure 3.2). The conservative IGP estimate in java shark diet, 32.7%, was half the value predicted by the liberal IGP estimates (65.2%; Figure 3.2). Similarly, copper shark IGP were highly variable between liberal vs. conservative estimates, ranging from 35.4% to 14.1% (see Figure 3.2).

3.3.2 Stable Isotope Analysis

Following the three step evaluation approach (see Table A.4), the white shark, scalloped hammerhead and smooth hammerhead sharks were deemed acceptable to include in the comparison (Figure 3.3). For the remainder of species, estimation of prey contributions was confounded by either consumer values falling outside of the geometric mixing space of the mixing model, overlapping prey sources that limited the model's ability to accurately predict the prey contributions to predator diet and/or spurious dietary estimates (see Figure A.1 in Appendix). For the white shark, SIMMR estimated the highest occurrence of IGP in its diet, 89.6 ± 7% (mean ± SD) for all white sharks combined (Figure 3.4). Variability in IGP, however, existed over white shark ontogeny with mean values ranging from 61.6 ± 14.1%, to 89 ± 9% to 83 ± 14.6% for small, intermediate and medium sized sharks, respectively (Figure 3.4). Large white sharks were removed from the analysis given a small sample size (n = 2). Scalloped hammerhead sharks exhibited similar variability in IGP over ontogeny ranging from 47.2 ± 7.1%, to 25.3 ± 13.5% to 74.6 ± 10.9% for small, medium and large life stages (Figure 3.5). IGP for scalloped hammerheads was primarily driven by the consumption of Elasmobranchii prey as Mammalia were not a prey source in their diet. Smooth hammerhead sharks exhibited the lowest occurrence of IGP among the three predatory shark species, with a mean estimate of 28.1 ± 5.9% (Figure 3.5).

3.3.3 Correlation Between Stomach Content and Stable Isotope Analysis

Although the mean contribution of prey items determined through stomach content analysis was found to have a normal distribution (p = 0.33), stable isotope prey estimates did not, and therefore a Spearman's rank Correlation Rho test was used to evaluate the relationship between IGP estimated using the two diet composition methods. There was no significant correlation between IGP occurrence by stomach content and stable isotope analyses (p = 0.48, roh = 0.06; Figure 3.6). Instead, stable isotope mixing model estimates of prey contributions for %IGP in diet were typically higher than the liberal estimates based on stomach content analysis (Figure 3.7).

3.4 Discussion

To our knowledge, this is the first study to examine intraguild predation among a large shark assemblage. Using an integrated approach through incorporating both published stomach content data and established prey contributions from stable isotope mixing models, the prevalence, classes and consistency of IGP across species were examined over short-term (i.e. stomach contents) and long-term (i.e. muscle tissue) temporal scales. Stomach content analysis identified that each member within the South African large shark assemblage participated in some form of IGP, with most shark species involved in asymmetrical IGP (10 out of 11 species), assuming the role of IGpredator. Blacktip, spinner, java, dusky and sand tiger sharks were also involved in symmetrical IGP, thus acting as both IGpredator and IGprey within the marine food web. Variability in IGP was shown over shark ontogeny with the prevalence of IGP in shark diet often increasing with predator size class. Among scalloped hammerhead sharks, for example, the intensity of IGP observed through stomach content analysis increased by 1.6 to 2 fold over each size class, as would be expected given larger shark size classes can consume additional prey sources through increased gape size and are known to undergo ontogenetic diet shifts (Fu et al. 2016).

Identifying and understanding the level of occurrence of IGP among the large shark assemblage is important as several of these shark species have been identified as tertiary piscivores: bull, java, sand tiger and white sharks. Dusky and scalloped hammerhead sharks are also considered tertiary piscivores given the high proportion of elasmobranchs in their

diet (Hussey et al. 2014). These apex predators structure marine food webs via a top-down approach through direct consumption of mesopredators, and indirect effects such as behavioural modification of prey sources (Paine 1980; Baum and Worm 2009). A loss in apex predator control can therefore result in a mesopredator release, whereby mesopredator population densities increase within a food web due to the absence of predation (Ward and Myers 2005). A mesopredator release that propagates down the food web, termed a trophic cascade, alters prey density, biomass or productivity across multiple trophic levels (Pace et al. 1999). For example, reduced sea otter (*Enhydra lutris*) populations due to increased predation by killer whales (*Orcinus orca*) in Alaska released sea urchin prey that led to overgrazing on kelp forests (Estes 1998). In agreement, theoretical models of systems with IGP have shown that the loss of an apex predator (IGpredator) involved in strong IGP with a mesopredator (IGprey) can drive mesopredator release that depletes the population of the shared resource (Terborgh et al., 2010). Speculation on the rise in global cephalopod populations between 1974 and 1994 was attributed to overfishing of predatory fish involved in top-down control of cephalopods through predation (Caddy & Rodhouse, 1998). This study was later supported by a central North Pacific Ecosim model that found loss of apex predators; sperm whales (*Physeter catodon*; trophic level 4.7), resulted in a mesopredator release of large squid and epipelagic fishes (Essington, 2007). In the context of the South African marine food web, if a closed IGP loop were present, and thus IGP occurred among a shark predator, an IGprey and a common resource, without access to alternative prey, the loss of the IGpredator (the shark) would result in a mesopredator release of the IGprey and a decrease in population density of the common resource. Many species within the large shark assemblage, however, are considered generalists and therefore have access to alternative prey. The presence of alternative prey allows the predator to switch between prey items based on prey availability/abundance which can vary over time (i.e. seasonally). Loss in prey abundance can therefore result in increased strength of IGP interactions, while the presence of alternative prey for IGprey can increase connectance within a food web and moderate trophic cascades by reducing fluctuations in species population densities (Polis and Strong, 1996). The dusky shark may therefore occupy an important functional role within the marine food web as it is involved in all forms of IGP, thus maintaining high connectance among species. Furthermore, the dusky

shark is also involved in cannibalism; an interaction shown to promote IGP species coexistence through improved IGpredator exploitation of the common resource at low resource population densities (Rudolf, 2007). Cannibalism among the IGpredator can also prevent IGprey exclusion at high common resource productivities. Maintaining biodiversity within ecosystems through species engaged in low to moderate levels of IGP may therefore act as a stabilizing mechanism within the food web (Holt and Huxel 2007). Given eleven species within the South African large shark assemblage engaged in IGP, the biodiversity and redundancy in functional roles among these shark species may thus provide stability. Reduced biodiversity, such as the loss of coastal shark populations from overfishing and habitat modification (Roff et al. 2018), may alter population and community dynamics within a food web, especially in the presence of strong IGP interactions. Strong IGP interactions, such as those among white sharks (94.4% IGP liberal, 79.8% conservative IGP estimate), can destabilize food webs through strong predation, which drives oscillations in prey population densities that can ultimately result in the loss of IGP species coexistence (McCann et al. 1998).

The prevalence of IGP across shark species estimated through stomach content analysis was often less than the predicted IGP occurrence in shark diet via stable isotope analysis. Variability in IGP may therefore occur across different time intervals, with the contribution of elasmobranchs and mammals in shark diet changing on an annual vs. daily basis. Differences in IGP between methods may also be an artifact of the prey sources consumed by shark species that undergo large scale migratory patterns. White and scalloped hammerhead sharks are known to travel between temperate and tropical waters (Bonfil, 2005), and across pelagic waters (Nalesso et al. 2019), respectively, thus these shark species may feed on different prey types defined by habitat and availability. Location of shark capture may also drive differing IGP estimates between stomach contents and stable isotopes as coastal environments are often utilized as shark nursery grounds. Caught in coastal beach nets, shark predators in this study may have experienced biased IGP estimates over short term time scales due to the location of shark capture and increased availability of juvenile sharks as prey. Alternatively, given the inherent challenge of identifying isotopically distinct prey groups, mixing model limitations may have resulted in higher IGP estimates than those of stomach contents as a consequence of overlapping

prey sources. For example, Elasmobranchii occupy several trophic levels, with the feeding behaviour within the prey group ranging from rays that consume benthic species (e.g. *Raja miraletus*), to those that prefer crustaceans (e.g. *Rhinobatos annulatus*) to primary and secondary piscivores (e.g. *Rhynchobatus djiddensis*, *Carcharhinus brachyurus*, respectively). This range of feeding behaviours drives high standard deviations within the Elasmobranchii isotopic prey group. With a standard deviation that can span 4‰ for $\delta^{15}N$ and 2‰ $\delta^{13}C$ (e.g. Java; see A.1), overlap with other prey source values, such as Actinopterygii, occurred which could confound mixing model results. Moreover, capturing the full scope of shark diet given the highly mobile nature of these consumers is a challenge as isotopic mixing models are limited in the number of prey sources (i.e. 5) that can be included. Future work on IGP among apex predators may benefit from removing low contribution prey sources such as cephalopods and malacostraca from shark diet estimates to provide additional flexibility among prey sources of isotopic mixing models. Although Gastropoda and Bivalvia were removed as prey sources in this study due to low contribution, Cephalopoda and Malacostraca do not occupy large components of large shark diets, therefore with their removal finer resolution of more dominant prey groups would be possible through the division of Elasmobranchii, for example, into rays and sharks, or large sharks and small sharks.

By addressing the knowledge gap over the role of sharks in complex multispecies interactions, improved understanding of shark connectance within food webs is possible. Several shark species were involved in IGP only as IGpredators including: bull, copper, scalloped, smooth, tiger and white sharks, while other shark species were identified to have more flexible roles, acting as both IGpredator and IGprey. This variation highlights the diverse roles members of the large shark assemblage play in modulating food webs. When considering conservation and management actions, species within marine food webs cannot be managed in isolation, instead predator-prey interactions must be accounted for and understood for effective management practices (Baum and Worm, 2009). Identifying shark species involved in intermediate IGP interactions with high species connectance, as well as species involved in the strongest IGP interactions (e.g. white, tiger, dusky and bull sharks) will help focus conservation efforts on species that provide food web stability, as

well as avoidance of predicted destabilization of food webs resulting from the loss of strong IGpredators that can lead to trophic cascades.

3.5 References

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Table 3.1 List of previously published studies used in Chapter 3 of this thesis for stomach content analysis and IGP estimates.

Shark	Species	Stomach with Prey	Author	Year	Book/Journal
BLA	*Carcharhinus limbatus*	442	Dudley & Cliff	1993	South African Journal of Marine Science
COP	*Carcharhinus brachyurus*	119	Smale	1991	South African Journal of Marine Science
		413	Cliff & Dudley	1992	South African Journal of Marine Science
		15	Sauer & Smale	1991	South African Journal of Marine Science
DUS	*Carcharhinus obscurus*	725	Dudley et al.	2005	African Journal of Marine Science
		67	Smale	1991	South African Journal of Marine Science
		900	Hussey et al.	2011	NRC Research Press
JAV	*Carcharhinus amboinensis*	103	Cliff & Dudley	1991	South African Journal of Marine Science
RAG	*Carcharias taurus*	100	Smale et al.	2005	African Journal of Marine Science
SCA	*Sphyrna lewini*	1018	Hussey et al.	2011	NRC Research Press
SMO	*Sphyrna zygaena*	144	Smale	1991	South African Journal of Marine Science
		933	Dicken et al	2018	African Journal of Marine Science
SPN	*Carcharhinus brevipinna*	379	Allen & Cliff	2000	South African Journal of Marine Science
TIG	*Galeocerdo cuvier*	39	Bass et al.	1975	Oceanographic research institute
		628	Dicken	2017	PLOS One
ZAM	*Carcharhinus leucas*	247	Cliff & Dudley	1991	South African Journal of Marine Science
GRE	*Carcharodon carcharias*	225	Hussey et al.	2012	*Global perspectives on the biology and life history of the white shark*

Table 3.2 The size classes for each shark predators within the large shark assemblage. Sizes measurements are in cm.

Shark	Species	Small	Intermediate	Medium	Large
BLA	*Carcharhinus limbatus*	Grouped together			
COP	*Carcharhinus brachyurus*	≤ 200			> 200
DUS	*Carcharhinus obscurus*	<100	100 - 139	140-209	≥ 210
JAV	*Carcharhinus amboinensis*	Grouped together			
RAG	*Carcharias taurus*	≤ 200			> 200
SCA	*Sphyrna lewini*	<110		110-140	> 140
SMO	*Sphyrna zygaena*	≤ 200			> 200
SPN	*Carcharhinus brevipinna*	Grouped together			
TIG	*Galeocerdo cuvier*	<150		150-220	> 220
ZAM	*Carcharhinus leucas*	Grouped together			
GRE	*Carcharodon carcharias*	<185	185-234.9	235 -284.9	≥ 285

Table 3.3 Shark species involved in **asymmetrical** age-structure **important** intraguild predation (IGP) determined from stomach contents. IGP in shark diet was calculated through the sum of Mammalia and Elasmobranchii prey groups (i.e. *%IGP in Shark diet = %M Mammalia + %M Elasmobranchii*).

IGPredator	Size Class	IGPrey Class	IGPrey Species	%Mass by Species	%IGP
Copper	Small	Elasmobranchii	Lesser sandshark (*Acroteriobatus annulatus*)	6.8	6.8
	Large	Elasmobranchii	Dogfish (*Squalus megalops*)	14.1	14.1
Dusky	Medium	Elasmobranchii	SCA, Banded catshark (*Halaelurus lineatus*), African Angelshark (*S. africana*), SMO	6.8, 0.2, 0.5, 9.4	16.9
	Large	Elasmobranchii	COP, SAN, SMO, Sphyrnidae	0.8, 1.4, 0.8, 7.1	10.1
White	Intermediate	Elasmobranchii	SAN (*C. plumbeus*), Guitarshark, Milk, Thresher, Unknown guitarfish, *Sphyrnidae*	1.94, 0.78, 2.52, 1.63, 0.06, 1.14	13.62
		Mammalia	Pinniped, Unidentified whale	5.4, 0.15	
	Medium	Elasmobranchii	RAG (*C. taurus*), COP (*C. brachyurus*), Guitarfish (*Rhinobatidae*), *Sphyrnidae*	6.16, 13.92, 0.49, 6.28	44.54
		Mammalia	Pinniped, Whale	14.86, 2.83	
	Large	Elasmobranchii	Whale shark (*Rhinodon typus*), *Sphyrnidae*	0.1, 9.48, 34.83	65.54
		Mammalia	Pinniped, unidentified whale	21.13	
Scalloped Hammerhead	Medium	Elasmobranchii	Dogfish	1.8 (F)	1.8(F)
	Large	Elasmobranchii	Angelshark	4.8 (M)	4.8
Tiger	Small	Mammalia	Marine mammals x 2	0.11, 0.25	0.36
	Medium	Elasmobranchii	Dogfish (*Squalidae*), *Sphyrnidae* (Hammerhead), Angelshark (*Squatinidae*)	0.06, 0.06, 0.27	4.14
		Mammalia	*Physeteridae*, *Balaenopteridae*, *Mammalia* x 2	0.19, 2.49, 0.31, 0.76	
	Large	Elasmobranchii	*Sphyrnidae* x 2, whale shark, angel sharks, *Triakidae*	1.29, 2.41, 0.4, 1.14, 0.33	10.23
		Mammalia	*Physeteridae*, *Balaenopteridae* x2, *Mammalia* x2	1.22, 0.84, 0.46, 0.41, 1.73	

Table 3.4 Shark species involved in **asymmetrical** age-structure **unimportant** intraguild predation (IGP) determined from stomach contents. IGP in shark diet was calculated through the sum of Mammalia and Elasmobranchii prey groups (i.e. *%IGP in Shark diet = %M Mammalia + %M Elasmobranchii*).

IGPredator	Size Class	IGPrey Class	IGPrey Species	%Mass by Species	%IGP
Dusky	Small	Elasmobranchii	Milk shark, Guitarfish (*Rhinobatidae*)	0.8, 0.4	3.7
		Mammalia	Cetacea	2.5	
	Medium	Elasmobranchii	*Rhinobatidae*	1.9	6.1
		Mammalia	Cetacea, Unidentified *Mammalia*	1.7, 2.5	
	Large	Elasmobranchii	*Rhinobatidae*	0.3	6.6
		Mammalia	Dolphin, whale, bottlenose (*T. aduncus*)	0.6, 5.3, 0.4	
White	Small	Elasmobranchii	DUS (*C. obscurus*), Dogfish	13.16, 2.48	22.13
		Mammalia	Cetacea (*D. delphis*)	6.49	
	Intermediate	Elasmobranchii	DUS (*C. obscurus*)	18.14	37.12
		Mammalia	*D. delphis*, *T. aduncus*, Dolphin, Cetacea, Mammalia	5.45, 0.53, 11, 1.56, 0.44	
	Medium	Elasmobranchii	DUS (*C. obscurus*)	10.57	22.03
		Mammalia	*T. aduncus*, dolphin, *Grampus griseus*	0.1, 7.64, 3.72	
	Large	Mammalia	*D. delphis*, *T. aduncus*, whale, dolphin	1.39, 12.83, 0.01	14.23
Sand Tiger	Small	Elasmobranchii	COP (*C. brachyurus*), Lesser sandshark	2.62, 15.19	17.81
	Large	Elasmobranchii	COP (*C. brachyurus*), Lesser sandshark	1.98, 8.16	10.14
Scalloped Hammerhead	Small	Elasmobranchii	Catshark (*Scyliorhinidae*), *Rhinobatidae*	2.39 (M), 2.76 (M), 3.51 (F), 0.44 (F)	5.15(M), 3.95(F)
	Medium	Elasmobranchii	*Scyliorhinidae*, *Rhinobatidae*	6.64(M), 5.34(M), 1.11(F), 1.63(F)	11.98(M), 4.54(F)
	Large	Elasmobranchii	*Scyliorhinidae*, *Rhinobatidae*	8.01 (M), 11.54 (M)	19.55 (M)
Tiger	Small	Elasmobranchii	*Rhinobatidae* x2, *Scyliorhinidae*	0.25, 2.7, 0.05	22.8
		Mammalia	*Odontoceti*, *Delphinidae* x2, *Mysticeti*, *Pinnipedia*	7.43, 3.04, 1.56, 7.64, 0.13	
	Medium	Elasmobranchii	Other sharks: guitarfish x5, catshark	1.04, 0.72, 0.13, 0.02, 2.59, 0.02	28.71
		Mammalia	*Physeteridae*, *Balaenopteridae*, *Mammalia* x 2	0.03, 8.62, 2.17, 0.26, 1.02, 10.99, 1.1	
	Large	Elasmobranchii	*Rhinobatidae* x2	0.02, 0.45	22.5
		Mammalia	*0dontoceti*, *Delphinidae*, *Mysticeti*	4.63, 1.3, 16.1	

Table 3.5 Shark species involved in **asymmetrical** age-structure **unknown** intraguild predation (IGP) determined from stomach contents. IGP in shark diet was calculated through the sum of Mammalia and Elasmobranchii prey groups (i.e. *%IGP in Shark diet = %M Mammalia + %M Elasmobranchii*).

IGPredator	Size Class	IGPrey Class	IGPrey Species	%Mass by Species	%IGP
Blacktip	Together	Mammalia	Cetacea	1.7	5.1
		Elasmobranchii	Milk Shark, Guitarfish, Catshark, *Sphyrnidae*	1.3, 1, 0.1, 1	
Copper	Together	Mammalia	Unidentified Mammalia, Dolphin	1.8, 0.1	3.6
		Elasmobranchii	Dogfish (*Squalidae*), African Angelshark (*Squatina Africana*)	0.9, 0.8	
Dusky	Intermediate	Elasmobranchii	Milk shark, Sphyrnidae	1.1, 4.9	6.5
		Mammalia	Unidentified Mammalia	0.5	
White	Together	Elasmobranchii	COP, Dogfish, Lesser guitarfish, Milk shark, Guitarfish, DUS, SCA, RAG	2.6, 2.4, 0.1, 3.4, 1.3, 16.9, 2.8, 1.7	72
		Mammalia	Cetacea (*D. delphis*), dolphin, pinniped	13.6, 17.6, 9.6	
Java	Together	Elasmobranchii	Sphyrnidae, Guitarfish, giant guitarfish, Blackspot, Milk, Catshark	7.2, 4.1, 1.6, 3.8, 0.8, 0.6	18.1
Sand Tiger	Together Smale (2005)	Elasmobranchii	Spurdog, COP, Smooth-hound, Milk, Houndshark, Catshark x 2, Shyshark, Lesser sandshark	0.49, 2.05, 3.03, 5.09, 2.42, 3.64, 0.47, 0.85, 0.12, 8.95	27.11
	Together	Elasmobranchii	Brown shyshark	6.44	6.44
	Large	Elasmobranchii	Shortnose spurdog (*S. megalops*), Smooth-hound x 2 (*M. mustelus*, *M. palumbes*), Milk (*R. acutus*), Sharptooth Houndshark (*Triakis megalopterus*), Catshark x 2 (*Halaelurus natalensis*, *Poroderma pantherinum*),Shyshark (*Haploblepharus fuscus*)	0.56, 3.41, 5.73, 2.73, 4.1, 0.53, 0.96, 0.14	18.16
Scalloped Hammerhead	Together	Elasmobranchii	Other Sharks: Banded catshark (*H. lineatus*), African angelshark (*S. africana*), lesser guitarfish (*R. annulatus*), Greyspot guitarfish (*R. leucospilus*), Puffadder shyshark (*Haploblepharus edwardsii*)	1.1, 1.6, 0.6, 1.6, 0.2	5.1
Smooth Hammerhead	Small	Elasmobranchii	Dogfish (*S. megalops*)	1.3	1.3
	Together	Elasmobranchii	Sphyrnidae	0.81	0.81
Spinner	Together	Elasmobranchii	Sphyrnidae, lesser guitarshark	0.3, 0.1	0.4
Bull	Together	Elasmobranchii	DUS, SCA, RAG, GRE, BLA, Milk, SMO, *S. africana*, *R. dijddensis*, Guitarfish, catshark, blackspot, zebra, bignose, smooth-hound, shortfin mako	6.9, 1.2, 0.3, 3.4, 0.6, 1.1, 2.1, 0.2, 14.1, 3.7, 0.1, 0.1, 1, 4, 0.2, 0.5	44.7
		Mammalia	Cetacea, Mammalia	5.1, 0.1	

Table 3.6 Shark species involved in **symmetrical** age-structure **important**, age-structure **unknown** intraguild predation (IGP) and **cannibalism** calculated from stomach contents. IGP in shark diet was calculated through the sum of Mammalia and Elasmobranchii prey groups (i.e. *%IGP in Shark diet = %M Mammalia + %M Elasmobranchii*).

Symmetry	Age-Structure	IGPredator	Size Class	IGPrey Class	IGPrey Species	%Mass by Species	%IGP
Symmetrical	**Important**	**Dusky**	Medium	Elasmobranchii	BLA (*C. limbatus*), SPN (*C. brevipinna*)	0.1, 1.8	1.9
			Large	Elasmobranchii	RAG (*C. taurus*)	6	6
		Sand Tiger	Together	Elasmobranchii	DUS (*C. obscurus*)	4	4
			Large	Elasmobranchii	DUS	4.5	4.5
	Unknown	**Blacktip**	Together	Elasmobranchii	Dusky	9.4	9.4
		Java	Together	Elasmobranchii	Dusky	14.6	14.6
		Spinner	Together	Elasmobranchii	Dusky	0.1	0.1
Cannibalism	**Unknown**	**Dusky**	Small	Elasmobranchii	Dusky	2.6	2.6
			Intermediate	Elasmobranchii	Dusky	2.9	2.9
			Medium	Elasmobranchii	Dusky	3.3	3.3
			Large	Elasmobranchii	Dusky	5.5	5.5

Figure 3.1 Total number of sharks involved in each class of intraguild predation including (from the bottom to the top): asymmetrical age-structure important, asymmetrical age-structure unimportant, asymmetrical age-structure unknown, cannibalism, symmetrical age-structure important, symmetrical age-structure unknown and intraguild predation of unknown symmetry or age-class, determined from stomach content analysis. The bar colour identifies the shark species that participates in the intraguild predation interaction.

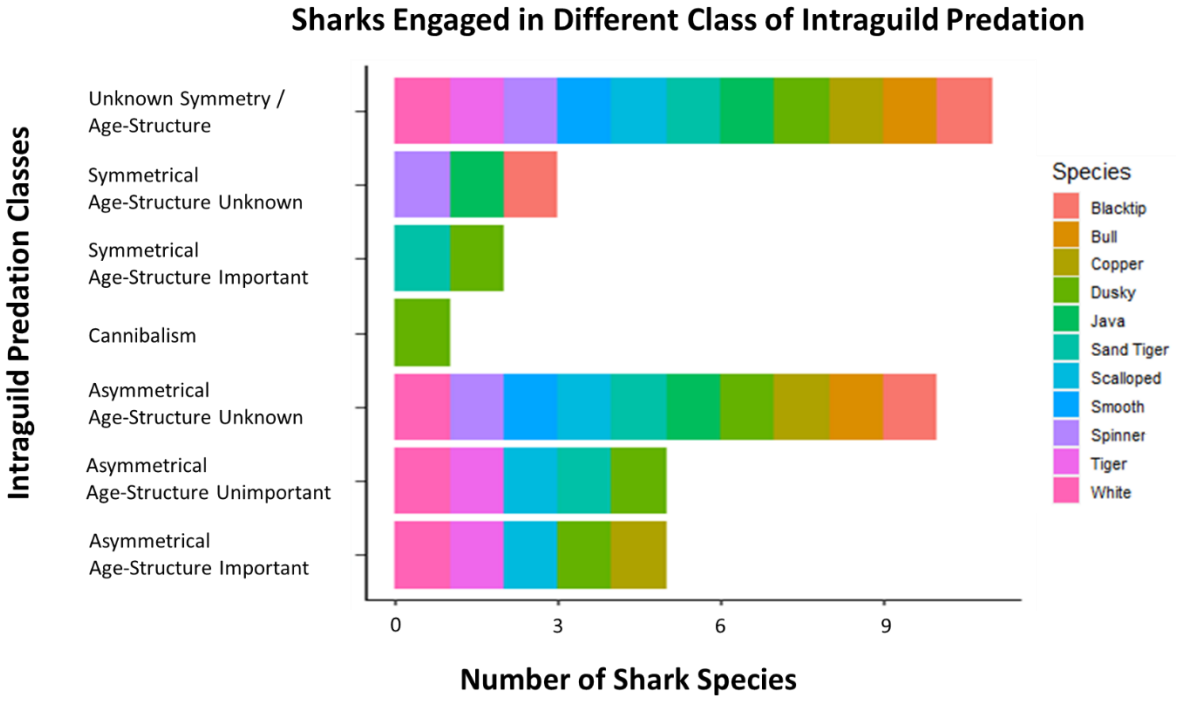


Figure 3.2 The strength of intraguild predation (IGP) within shark diet was estimated from stomach content analysis and represented as percent (%) of diet. The position of the shark along the y-axis depicts the percent IGP within the shark diet from 0% (at the bottom) to 100% (at the top of the y-axis). The liberal estimate of intraguild predation within shark diet is given in bold and the conservative estimate (see methods) is given in brackets.

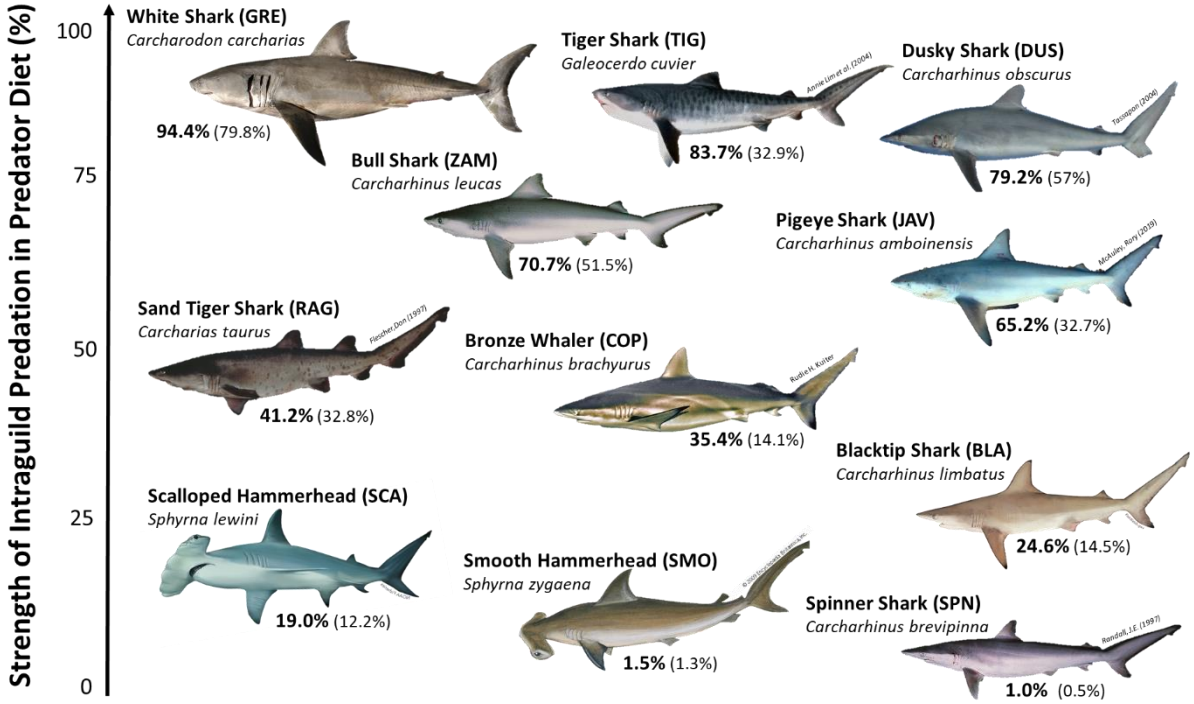


Figure 3.3 SIMMR isospace plots for the smooth hammerhead, scalloped hammerhead and white sharks. Consumer $\delta^{13}C$ and $\delta^{15}N$ (expressed in ‰) values are represented as points on the plot, while crosses are weighted mean isotopic prey source values (center) and error bars represent standard deviations (outer edges). In the bottom right corner are the size classes examined for each shark predator, and in the top left corner are the prey sources used in the mixing model each denoted by a separate colour.

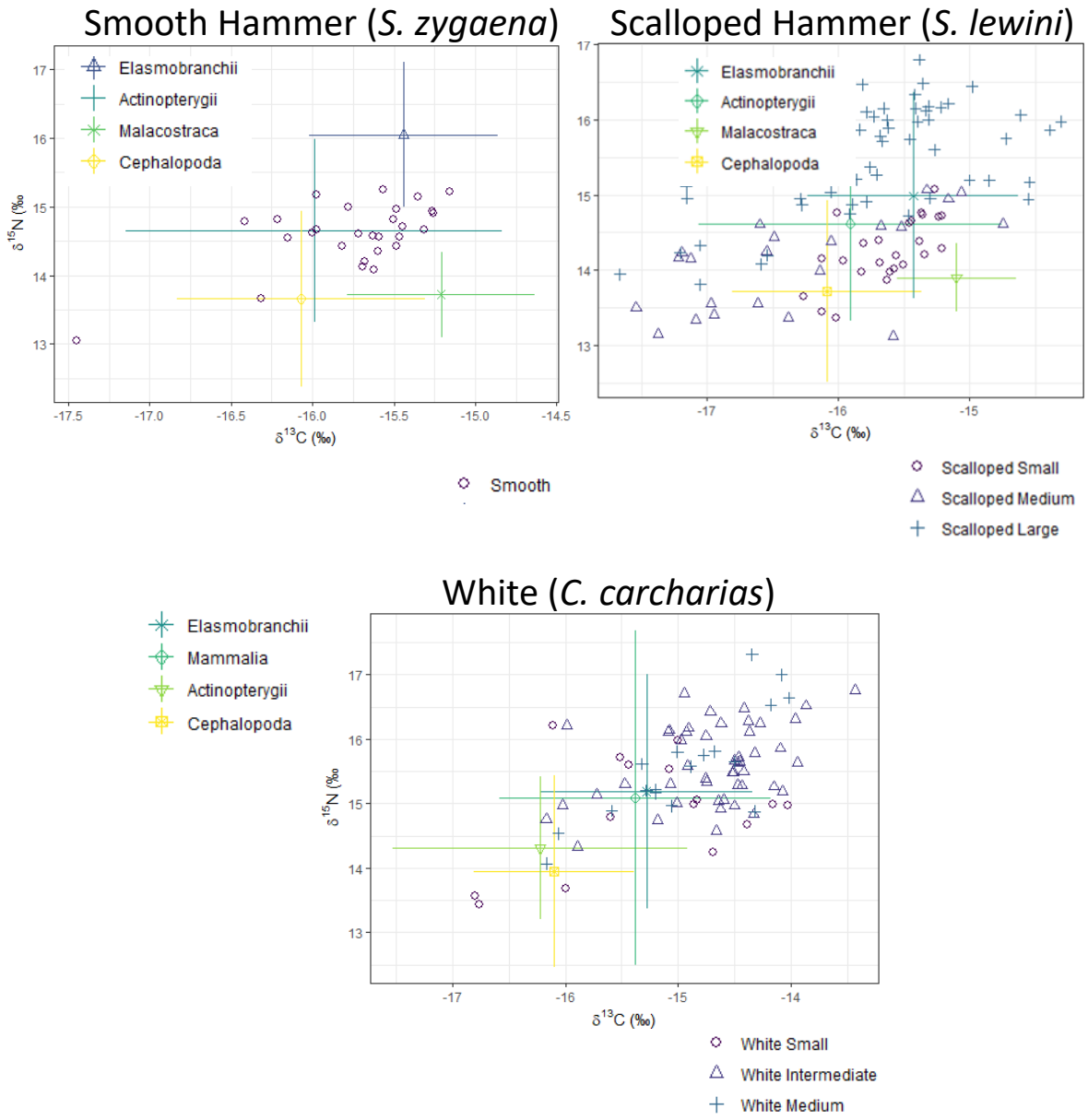


Figure 3.4 Prey source contributions to the white shark diet grouped together and by size classes: small, intermediate and medium sizes. Each box and whisker plot display the range between the 25% and 75% confidence intervals, with error bars extending to the minimum and maximum values (2.5% and 97.5%, respectively). The median is represented by the center vertical line within the box. Predator size classes are found in Table 3.2. Sample sizes of white shark by size class were: n small = 15, n intermediate = 48, n medium = 18. Together the white shark sample size was 81.

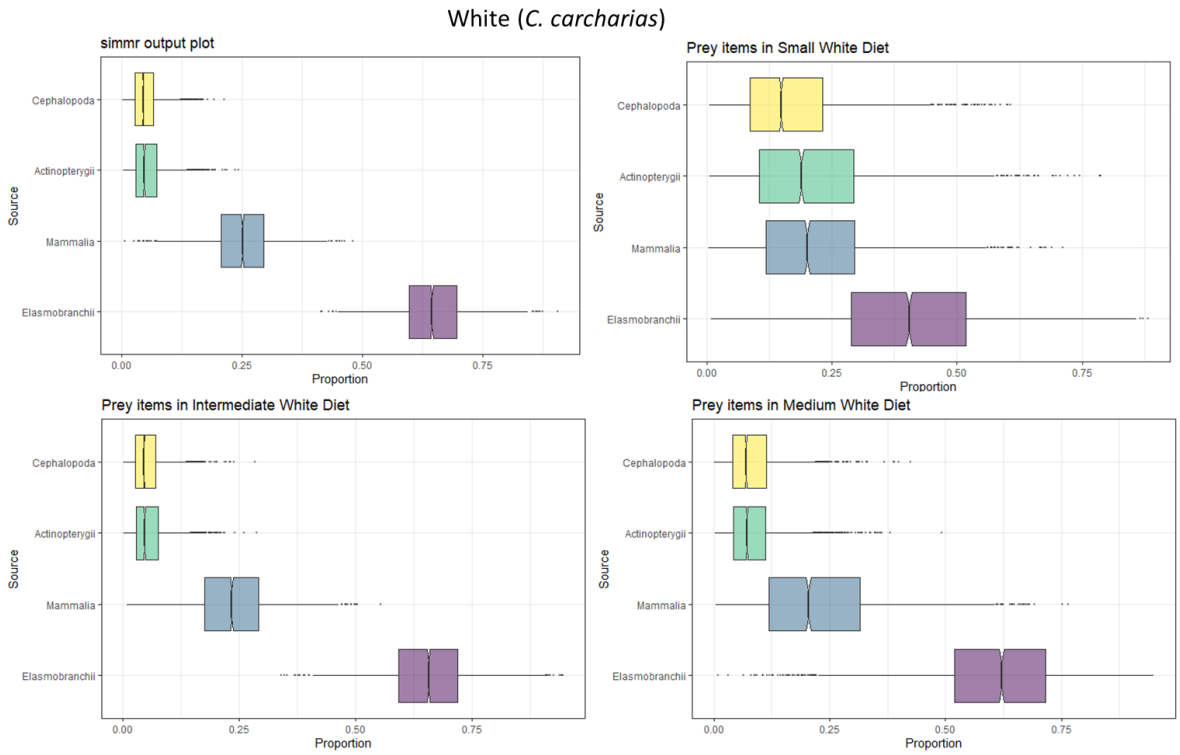


Figure 3.5 Prey source contributions to the scalloped hammerhead shark diet grouped together and by size classes: small, medium and large sizes. Prey source contributions to the smooth hammerhead shark diet grouped together. Each box and whisker plot display the range between the 25% and 75% confidence intervals, with error bars extending to the minimum and maximum values (2.5% and 97.5%, respectively). The median is represented by the center horizontal line within the box. Predator size classes are found in Table 3.2. Sample sizes per scalloped hammerhead size class include: n small = 25, n medium = 22 and n large = 53. Smooth hammerheads have a total sample size of n = 28.

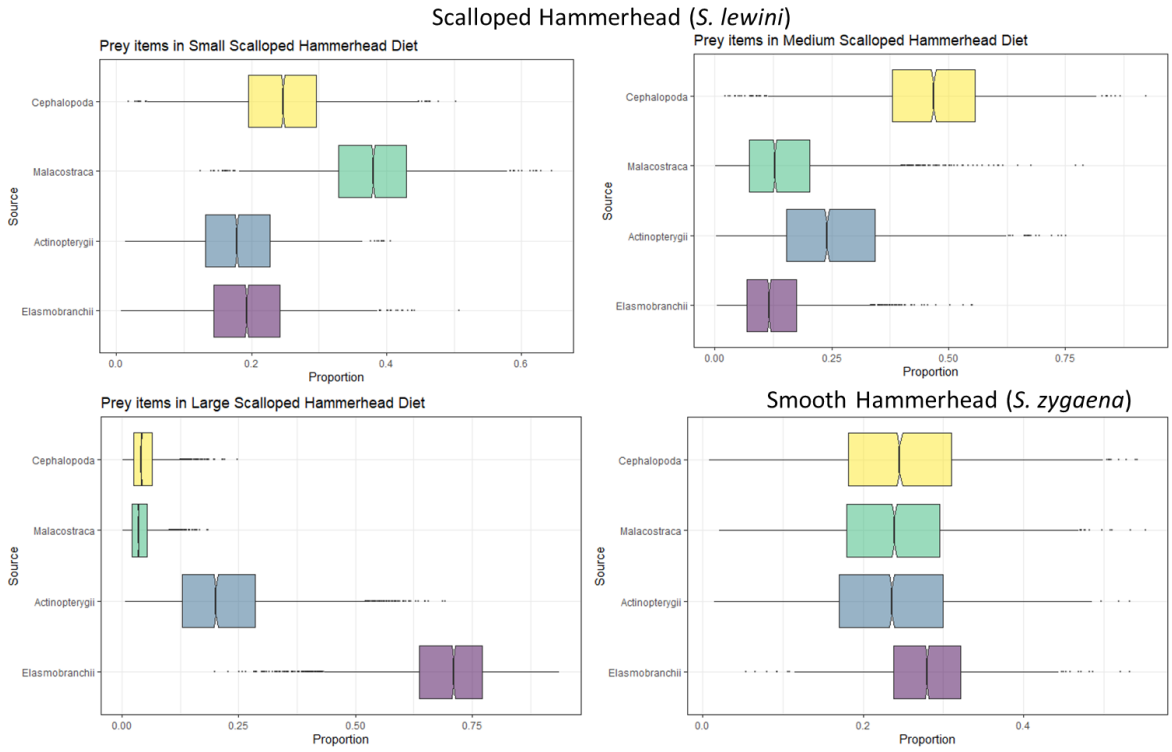


Figure 3.6 Scatterplot of white shark, scalloped hammerhead and smooth hammerhead IGP estimates by size class determined via stomach content analysis compared with stable isotope analysis. The gray dotted line represents the 1:1 line. No correlation was found between the IGP estimates of the two methods (p = 0.48, rho = 0.06).

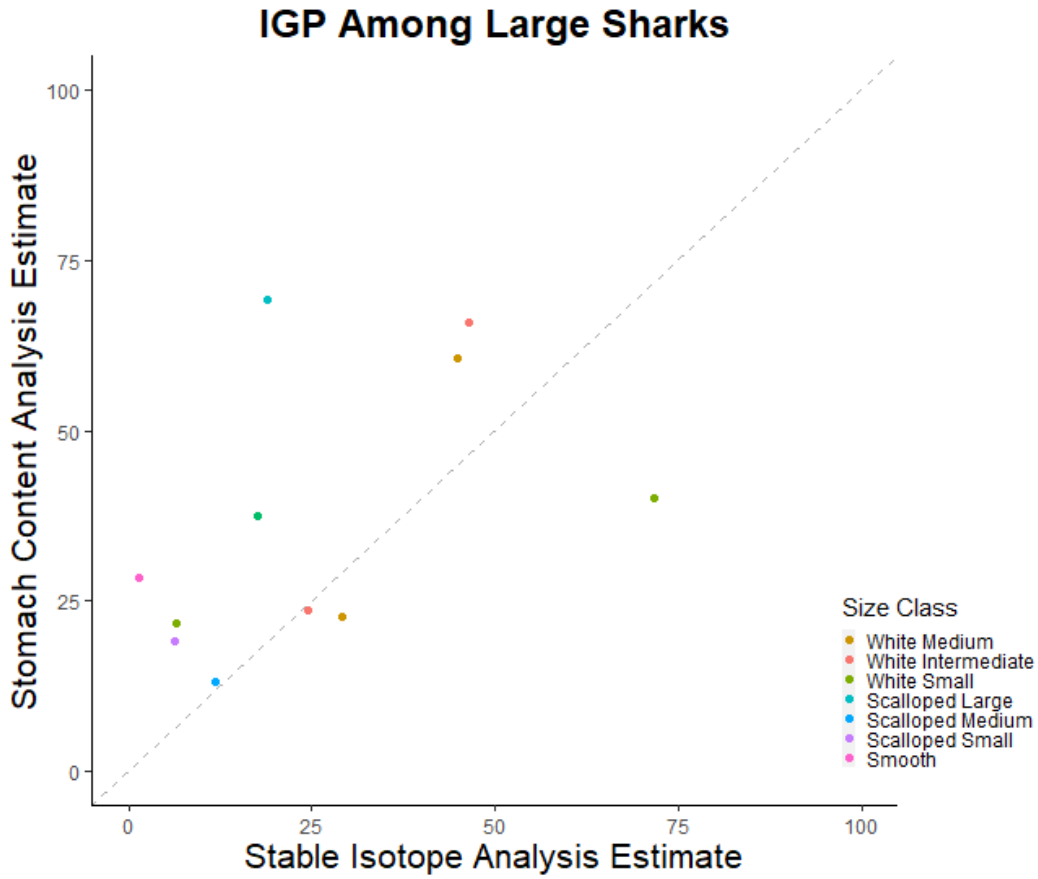
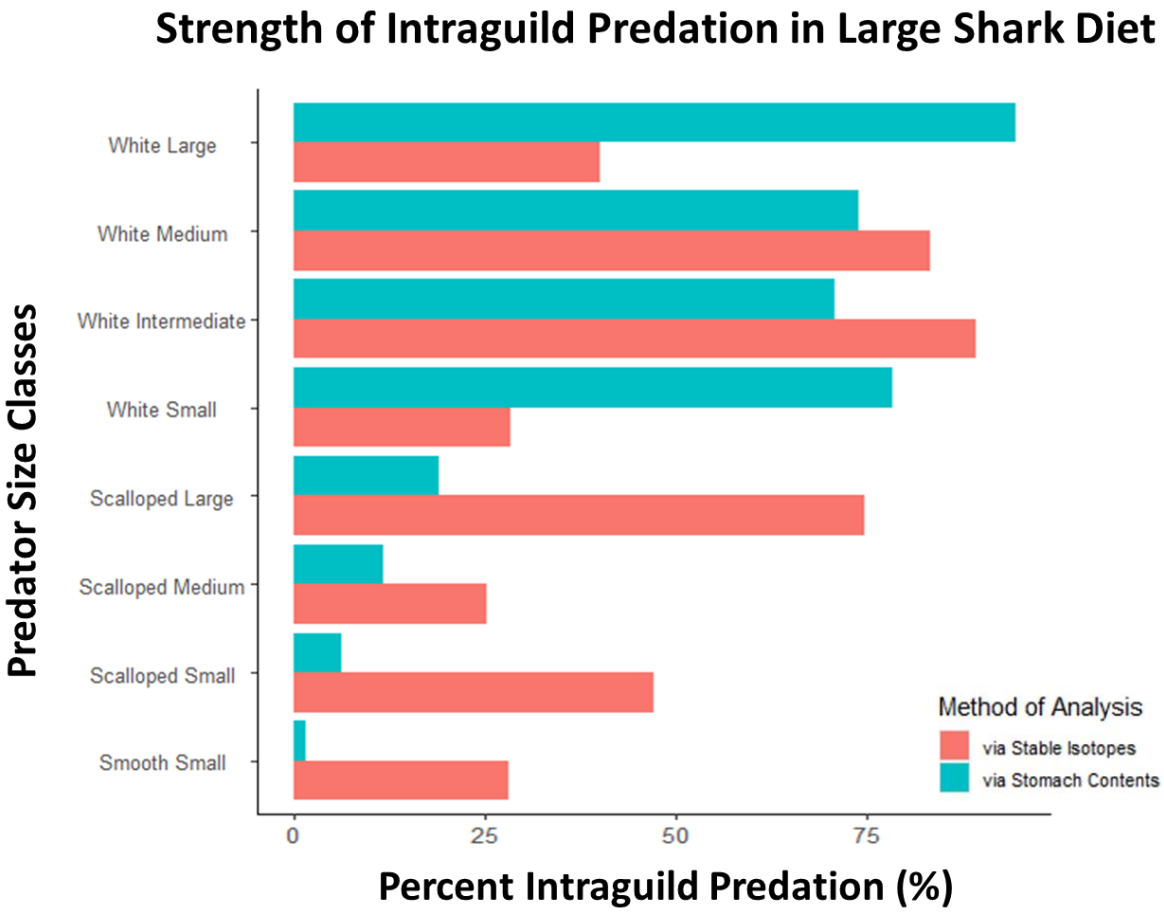


Figure 3.7 Intraguild predation estimates in shark diet (%) calculated through stable isotope analysis (shown in red) and stomach content analysis (shown in blue).



CHAPTER 4

General Conclusion

4.1 Summary

I synthesized and provided new insight into complex multi-species interaction – intraguild predation (IGP) – and how it applies to the large shark assemblage. In the first chapter, the first review and synthesis of the methods that exist to study the occurrence and strength of IGP in food webs was provided since the original IGP review paper by Polis, Myers and Holt in 1989, more than three decades ago. Intraguild predation can be examined through direct observation, retrospective observation, chemical and/or biological markers and modeling approaches. A literature search of IGP effects at three implication levels: individual, population and community-level, determined high variability in research effort across different responses to IGP. For example, behavioural changes in response to IGP was the most studied IGP effect, while resilience of the resource was the least studied IGP effect. Given modern technological advancements, examples were provided of approaches that can be used in future IGP studies to fill knowledge gaps and enhance understanding of food web responses to complex multi-species interactions.

Chapter 2 determined that water washing was not an appropriate pre-treatment for the removal of urea and TMAO from liver tissue of four large shark species: dusky (*Carcharhinus obscurus*), sand tiger (*Carcharhinus taurus*), scalloped hammerhead (*Sphyrna lewini*) and white sharks (*Carcharodon carcharias*). The $\delta^{15}N$ values of lipid extracted (repeated three times with chloroform-methanol) and lipid extracted water washed (with deionized water) liver samples were not significantly different, suggesting that water washing did not remove urea and TMAO from liver tissue as previously assumed. Unexpectedly, %N, %C and $\delta^{13}C$ values were significantly different between treatment groups. It was suggested that liver tissue may not contain high concentrations of urea and TMAO and/or chloroform-methanol (i.e. a strong polar solvent) may have removed urea and TMAO prior to water washing and/or removed structural lipids that may have freed low weight amino acids that were subsequently removed with water washing. Given the high concentration of lipid in liver tissue, some lipid remained in some samples despite three rounds of lipid extraction. This was confirmed through the occurrence of a negative linear relationship between $\delta^{13}C$ and C:N values. To ensure only delipidated

samples were included in the analysis, C:N thresholds for each shark species per treatment group were determined, which improve ecological interpretation of isotopic results. A preliminary comparison between muscle and liver tissue showed $\delta^{13}C$ mean differences between tissues were larger for shark species that are known to undergo seasonal movements and thus have varying seasonal catch rates (i.e. scalloped hammerhead and white sharks), while regionally resident shark species with consistent catch rates (sand tiger and dusky) were shown to have smaller $\delta^{13}C$ mean differences between tissues. Given the turnover rate of the tissues, the magnitude of the $\delta^{13}C$ mean differences between tissues is likely an artifact of feeding on isotopically distinct prey groups from different habitats.

Chapter 3 determined a high occurrence of IGP among eleven shark species within the 'large shark assemblage' off the South African coast. Although most sharks (10 species) participated in IGP as the IGpredator (via asymmetrical IGP), five shark species had more flexible functional roles and thus participated in IGP as both IGpredator and IGprey (via symmetrical IGP). Variability in IGP class and strength was observed among the sharks with the dusky being the sole shark species engaged in all forms of IGP, as well as cannibalism. The dusky shark may therefore serve an important role within the marine food web as this shark provides increased connectance among species and engages in cannibalism which have both been shown to facilitate food web stability. Several shark species experienced increased prevalence of IGP in their diet with size (e.g. scalloped, copper and sand tiger sharks) as would be expected given known shark diet shifts with ontogeny and increased access to alternative prey sources. Comparison between stomach contents and isotopic mixing model results found no correlation between the methods suggesting that IGP may vary in shark species across different temporal scales (i.e. annually vs. daily). Differences in IGP between methods, however, may be an artifact of consumption of prey items from isotopically distinct environments during seasonal migrations. Artificially high stomach content IGP estimates may have also occurred given the capture of sharks from coastal waters with increased availability of juvenile shark prey due to nursery grounds. Finally, IGP estimates may show differences between methods as a consequence of confounded mixing model results.

4.2 Implications and Future Directions

In chapter one a schematic for a standardized method of IGP class identification was proposed, as the terminology associated with IGP within the literature often lacks consistency, which can be confusing and ultimately result in issues of replicability and comparisons between studies. For example, a review by Lourenço et al. (2014) examined 200 published papers on lethal interactions among vertebrate top predators and found that more than half (56%) of the studies had no definitive evidence of species competition. Given the level of competition within a predatory guild directly impacts the strength of IGP interactions, and the strength of complex multi-species interactions structure food webs, identification of IGP classes will provide improved understanding of the mechanisms driving species functional roles within food webs.

Opportunities exist to expand on the overall experimental design utilized in this thesis through the incorporation of additional tissues representing different temporal scales for comparative analysis with muscle and/or liver tissue. One tissue that has received minimal research effort in the literature is elasmobranch dermis. The opinion among the scientific community on the isotopic turnover rate of dermis is divided with some studies indicating dermis has a fast turnover rate (i.e. faster than liver tissue; Li et al. 2016; Marcus et al. 2019), while others assume a slow turnover rate (i.e. slower than muscle tissue; Ferreira et al. 2017, Preeble et al. 2018). Future studies would benefit from determining the metabolic turnover rate of dermis and thus the time scale that elasmobranch dermis represents as this tissue can provide a non-lethal, accessible tissue for sampling when using stable isotope analysis in sharks. By including tissues with faster turnover rates, such as plasma (72-102 days; Kim et al. 2012), or metabolically inert tissue that provide a complete timeline of prey consumption (i.e. vertebrae; Estrada et al. 2006), researchers may be able to categorize shark feeding strategies (i.e. delineate specialists from generalists; Shiffman et al. 2014), identify ontogenetic diet shifts (Estrada et al. 2006) and advance our understanding of IGP among marine predators through greater resolution into the consistency of the interaction over time. Comparative analysis with tissues indicating short-term diet may also enhance our understanding of shark movement behaviours and trophic ecology by highlighting seasonal movement patterns which may not be revealed in shark muscle tissue given the slow turnover rate (341 ± 39 days; 95% turnover; Logan &

Lutcavage, 2010). For example, Carlisle et al. (2012) examined stable isotope analysis of white shark dermis and muscle tissue to understand shark migratory patterns given they occupy offshore habitats for seven to eight months, as shown via pop-up archival transmitting (PAT tags; Weng et al. 2007, Jorgensen et al. 2010). Although it had been hypothesized that white sharks can fast for extended periods of time, the authors considered the likelihood that a shark undertaking long-distance migrations (approx. 4000 km; Del Raye et al. 2013) would fast for 7+ months to be low, and thus they assumed that the migrations to offshore habitats were foraging related. Mixing model results from muscle tissue found equal dietary contributions from coastal and offshore regions, however, model results that incorporated movement data indicated that sharks had higher rates of feeding in coastal environments, with limited foraging in offshore environments. Carlisle et al. (2012) thus suggested that foraging may not the main purpose of white shark movement behaviours, an idea supported by later work using biotelemetry to estimate white shark condition along these migratory routes (Del Ray et al. 2013).

Future studies can also benefit from determining the nitrogen isotopic ratio of trimethylamine oxide (TMAO). Urea and TMAO have been described as waste products of elasmobranch metabolism in previous studies (Kim & Koch, 2012; Churchill et al 2015; Carlisle et al. 2016) resulting in the assumption that both molecules are depleted in ^{15}N; given ^{14}N is preferential excreted as nitrogenous waste in elasmobranchs (Steel & Daniel, 1978; Logan & Lutcavage, 2010). Although the origin and synthesis pathways of TMAO are still debated, TMAO is synthesized and retained in elasmobranch tissues to counteract the effects of urea on protein destabilization (Seibel & Walsh, 2002) and therefore does not appear to be a nitrogenous waste product. By determining the isotopic ratio of TMAO, improved pre-treatment of elasmobranch tissues prior to stable isotope analysis is possible. This can provide more relevant measures of isotopic composition, improved ecological interpretation of isotopic results, and greater insight into unexpected isotopic changes that have been observed following pre-treatment procedures (e.g. water washing).

Examining IGP strength and class among large sharks may also be possible using the global stomach content dataset to determine if IGP correlates with geographic regions, ecosystems within those regions (e.g. pelagic vs. coastal vs. deep-sea habitats), as well as across ocean basins. While the spatio-temporal distribution of sharks has been examined

(Kai et al. 2017), to our knowledge no study has examined patterns in shark interactions relative to latitude and/or geographic region. Marine apex predators found in tropical locations with warmer water temperatures may experience reduced IGP prevalence and strength due to increased prey abundance and biodiversity, while colder regions or deep-sea habitats may experience increased IGP among predators due to limited resources in those environments. Given the complexity of IGP, however, the strength of the interactions within the environments would need to be assessed as warmer environments could alternatively increase predator population densities, thus increasing competition for resources and result in overall stronger IGP interactions.

A complex study design, such as a whole ecosystem modeling approach (e.g. Ecopath with Ecosim), could be used to model marine food webs, such as the Western Indian Ocean, to improve understanding on the overall food web response to shark interactions (Kitchell et al. 2002). Whole ecosystem models can incorporate changes in the external environment including; water temperatures, salinity and/or acidification, and stomach content data can be used in the model as predictive priors thus enhancing the resolution of the strengths of interactions taking place in the food web. Additional sources of variability in stable isotope signature, such as physiological attributes like age and sex can also be specified (Christensen & Pauly, 1992). A model like this would highlight the relationship between IGP strength and class with food web responses following species losses. Although estimates of IGP strength and class were provided in this study, I can only speculate as to which shark species might be most important for food web stability. With enhanced understanding of IGP among sharks comes improved understanding of shark functional roles within ecosystems. This, in turn, can improve management strategies by identifying species with a disproportionate effect on food web structure and ultimately enhance conservation efforts via targeted species.

4.3 References

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APPENDICES

Appendix A

Table A.1 Shark species involved in **possible** intraguild predation. Possible intraguild predation was calculated through the sum of unknown sharks, rays and Elasmobranchii remains (i.e. *$\%IGP_{possible}$= %M Unknown Sharks + %M Rays + %M Unknown Elasmobranchii*).

IGPredator	Size Class	IGPrey Class	IGPrey Species	%Mass by Species	%IGP
Blacktip	Together	Elasmobranchii	Unknown Sharks: small shark, large shark, unknown shark	1.1, 4.4, 1.7	10.1
			Rays: Backwater butterflyray (*Gymnura natalensis*), Batoid, Ray	1, 0.3, 1.5	
			Elasmobranchii	0.1	
Copper	Together	Elasmobranchii (Cliff & Dudley)	Unknown Sharks: small shark	0.3	0.5
			Rays: Bullray (*Aetomylaeus bovinus*)	0.2	
		Elasmobranchii (Sauer & Smale)	Elasmobranchii	34.54	34.54
	Small (<2m)	Elasmobranchii	Elasmobranchii	0.1	0.1
	Large (>2m)	Elasmobranchii	Unknown Sharks: Shark remains	15	16.4
			Rays: *Myliobatidae, Callorhinchus capensis*	0.5, 0.7	
			Elasmobranchii: Unidentified chondrichthyans	0.2	
Dusky	Small	Elasmobranchii	Unknown Sharks: Large shark, shark, small shark	0.5, 1.8, 14.1	26.1
			Rays: Flapnose (*Rhinoptera javanica*), Myliobatid, *Mobula*, Dasyatis, Batoid, Unidentified ray	1.4, 1.2, 0.1, 0.7, 0.3, 5.5	
			Elasmobranchii	0.5	
	Intermediate	Elasmobranchii	Unknown Sharks: Large shark, small shark	4.5, 10.6, 10.1	53.6
			Rays: *Myliobatis*, bullray (*A. bovinus*), *Mobula*, Dasyatid, Backwater Butterfly (*G. natalensis*), Batoid, Ray	0.9, 9, 1.5, 2.9, 3, 0.4, 10.7	
	Medium	Elasmobranchii	Unknown sharks: Carcharhinid, Small Shark, Large Shark	1.9, 3, 6.3	18.8
			Rays: Batoidea	6.2	
			Elasmobranchii	1.4	
	Large	Elasmobranchii	Unknown sharks: Carcharhinid, small sharks, large shark	9.3, 0.1, 17.1	30.3
			Rays: Flapnose (*R. javanica*), Batoidea	2.8, 1	

Table A.2 Shark species involved in **possible** intraguild predation. Possible intraguild predation was calculated through the sum of unknown sharks, rays and Elasmobranchii remains (i.e. $\%IGP_{possible}$= *%M Unknown Sharks + %M Rays + %M Unknown Elasmobranchii*).

IGPredator	Size Class	IGPrey Class	IGPrey Species	%Mass by Species	%IGP
White	Small	Elasmobranchii	Unknown sharks: Large shark, shark, small shark	14.83, 3.56, 34.47	56.17
			Rays: unidentified ray, giant manta (*Mobula birostris*)	0.16, 2.5	
			Elasmobranchii	0.65	
	Intermediate	Elasmobranchii	Unknown Sharks: Carcharhinidae, Small & Large shark	0.94, 11.22, 2.33, 2.14	20.1
			Rays: *A. narinari*, *A. bovinus*, batoidea	3.23, 0.01, 0.16	
			Elasmobranchii	0.07	
	Medium	Elasmobranchii	Unknown sharks: Small & Large shark, Carcharhinidae	3.14, 3.26, 0.02, 1.01	7.45
			Rays: unidentified stingray	0.01	
			Elasmobranchii	0.01	
	Large	Elasmobranchii	Unknown sharks: Small & Large shark, shark	2.24, 10.38, 1.96	14.6
			Elasmobranchii	0.02	
	Together	Elasmobranchii	Small & Large Shark	6.6, 8.5	15.1
Java	Together	Elasmobranchii	Unidentified shark, small shark	0.6, 8.5	32.5
			Rays: Eaglerays (*Myliobatidae*), *M. birostris*, *Mobula*, *G. natalensis*, Batoid	0.4, 9.3, 8.1, 3.6, 1.8	
			Elasmobranchii	0.2	
Sand Tiger	Together	Elasmobranchii	Rays: *Torpedo* sp., *Raja* remains, *Raja miraletis*, *M. aquila*, *Dasyatidae*	0.23, 0.25, 0.21, 1.55, 0.93	5.23
			Elasmobranchii	2.06	
	Together	Elasmobranchii	Rays: *Rajidae*, *A. annulatus*, *R. miraletis*	0.21, 27.4, 7.1	34.71
	Small	Elasmobranchii	Rays: *Torpedo*, *Raja* remains, *R. miraletis*	2.62, 0.05, 1.85	4.52
	Large	Elasmobranchii	Rays: *Raja* remains, *M. aquila*, *Dasyatidae*	0.28, 1.75, 1.05	5.4
			Elasmobranchii	2.32	
Smooth Hammerhead	Small	Elasmobranchii	Elasmobranchii: Unidentified Chondrichthyes	0.2	0.2
	Together	Elasmobranchii	Elasmobranchii	0.01	0.01
Spinner	Together	Elasmobranchii	Elasmobranchii	0.5	0.5

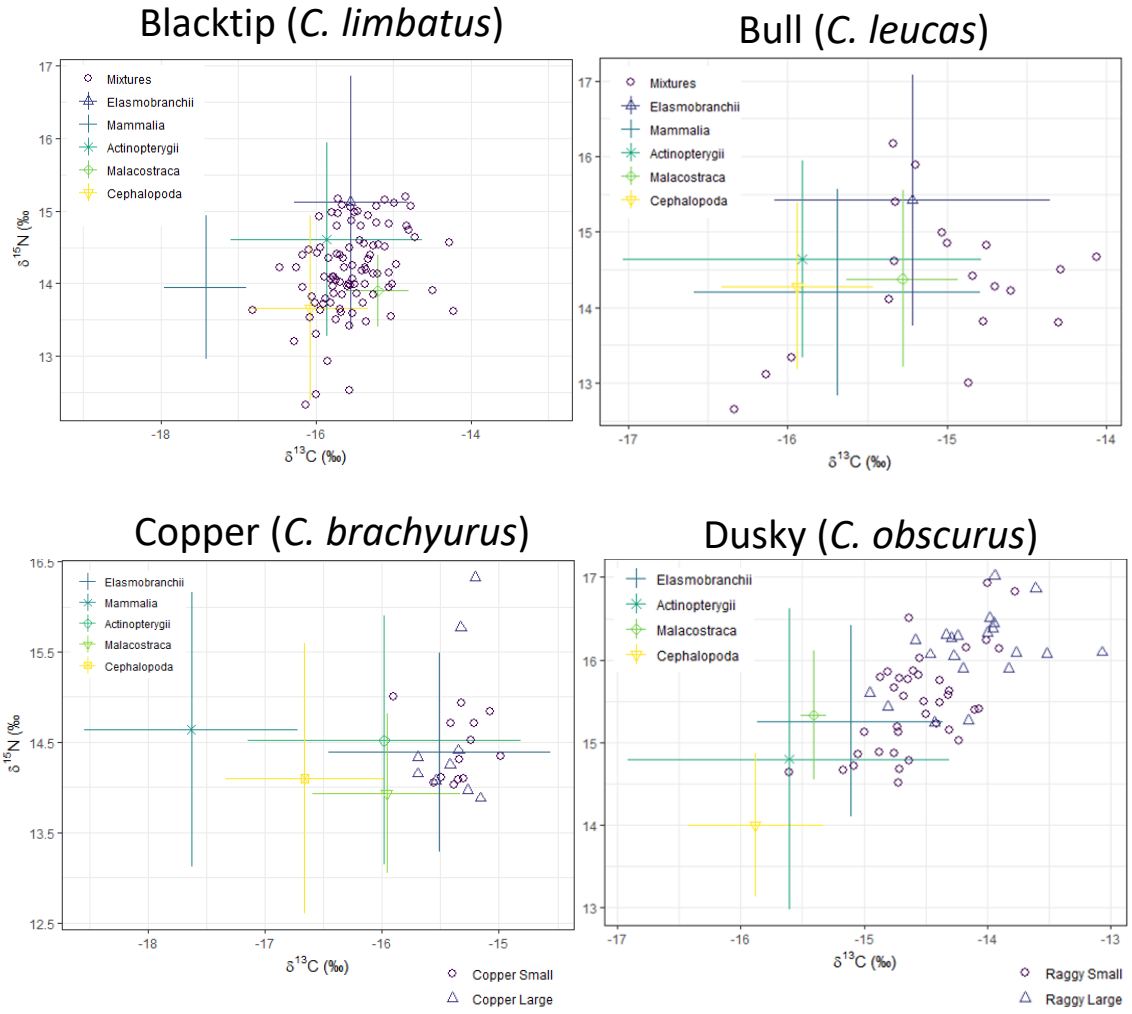
Table A.3 Shark species involved in **possible** intraguild predation. Possible intraguild predation was calculated through the sum of unknown sharks, rays and Elasmobranchii remains (i.e. *$\%IGP_{possible}$= %M Unknown Sharks + %M Rays + %M Unknown Elasmobranchii*).

IGPredator	Size Class	IGPrey Class	IGPrey Species	%Mass by Species	%IGP
Scalloped Hammerhead	Together	Elasmobranchii	Unknown Sharks: small sharks, dogfish (*Squalidae*), catshark (*Scyliorhinidae*), guitarfish (*Rhinobatidae*),	2.6, 0.1, 4.2, 3	12.4
			Rays: *Dasyatidae*, Backwater butterflyray (*G. natalensis*), Batoid, ray, skates	0.5, 1.3, 0.2, 0.1, 0.3	
			Elasmobranchii	0.1	
	Small	Elasmobranchii	Rays: *Rajidae*	1.88 (F), 0.29 (M)	2.09(F), 1.29(M)
			Elasmobranchii	0.21 (F), 1.07 (M)	
	Medium	Elasmobranchii	Rays: *Rajidae*, Butterflyray (*Gymnuridae*), *Dasyatidae*	3.02(F), 1.27(M), 2.7 (M)	3.02(F), 4.03 (M)
	Large	Elasmobranchii	Sharks: sharks, *Carcharhinidae*	6.96 (M), 1.25 (M)	13.6
			Rays: *Dasyatidae*, Butterflyray (*Brymnuridae*)	2.56 (M), 2.25 (M)	
			Elasmobranchii	0.58 (M)	
Tiger	Small	Elasmobranchii	Sharks: *Carcharhinidae* x3, *Odontaspididae*	0.25, 12.28, 0.25, 0.72	63.3
			Rays: *Torpedinidae*, *Myliobatidae* x4, *Dasyatidae* x2, *Gymnuridae*, *Batoidea*	0.4, 0.03, 6.07, 1.09, 7.29, 4.61, 0.33, 1.79, 0.79	
			Elasmobranchii x 4	0.79, 0.37, 12.39, 0.35	
	Medium	Elasmobranchii	*Carcharhinidae* x 5, *Lamnidae*, *Odontaspididae*	1.02, 0.15, 2.48, 0.23, 0.43, 0.78, 2.35	50.85
			Rays: *Torpedinidae* x 2, *Rajidae*, *Myliobatidae* x 7, *Dasyatidae* x3, *Gymnuridae*, *Batoidea*	0.27, 0.47, 0.06, 0.02, 0.5, 0.14, 1.62, 1.32, 15.8, 1.89, 5.78, 0.32, 0.29, 1.04, 1.91	
			Elasmobranchii x4	2.44, 0.93, 7.82, 0.79	
	Large	Elasmobranchii	Sharks: *Odontaspididae*, *Carcharhinidae* x5, *Lamnidae*	7.89, 0.37, 0.82, 1.84, 0.15, 4.12, 0.34	46.23
			Rays: eagle rays (*Myliobatidae*) x3, *Dasyatidae* x2, *Batoidea*	2.85, 6.83, 1.88, 2.65, 0.24, 0.05	
			Elasmobranchii x4	2.49, 0.37, 13.18, 0.16	
Bull	Together	Elasmobranchii	Sharks: small shark, L shark	0.4, 4.7	19.2
			Rays: *Mobula*, Batoidea, *M. birostris*, *Dasyatis marmorata*, *H. uarnak*, *A. bovinus*	1, 10.4, 0.2, 1.6, 0.1, 0.5	
			Elasmobranchii	0.3	

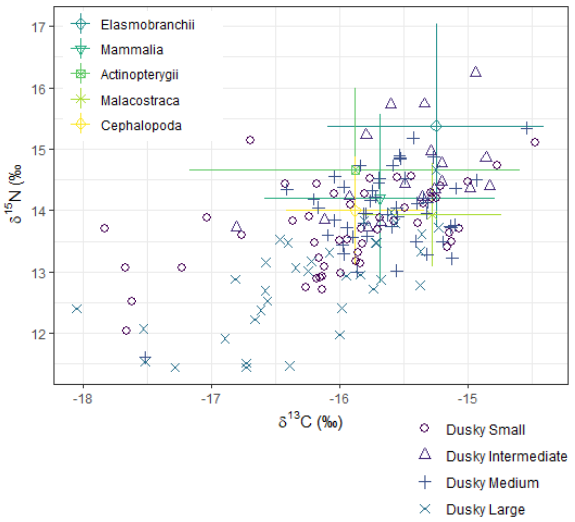
Table A.4 The decision tree used to determine viable Bayesian Mixing Model results and thus the shark species included in a comparison with intraguild predation estimates from stomach content analysis.

Shark Predators	Mixing Polygon Covered	Isotopically Distinct Prey Items	Biologically Credible Output	Decision
Blacktip (BLA)	Yes	Partially	No	Excluded
Bull (ZAM)	Partially	Yes	No	Excluded
Copper (COP)	Partially	No	No	Excluded
Dusky (DUS)	No	Partially	No	Excluded
Java (JAV)	No	Yes	Partially	Excluded
Sand tiger (RAG)	No	Partially	Partially	Excluded
Scalloped (SCA)	Partially	Partially	Partially	Included
Smooth (SMO)	Partially	Yes	Partially	Included
Spinner (SPN)	Partially	Partially	No	Excluded
Tiger (TIG)	No	Partially	No	Excluded
White (GRE)	Partially	No	Yes	Included

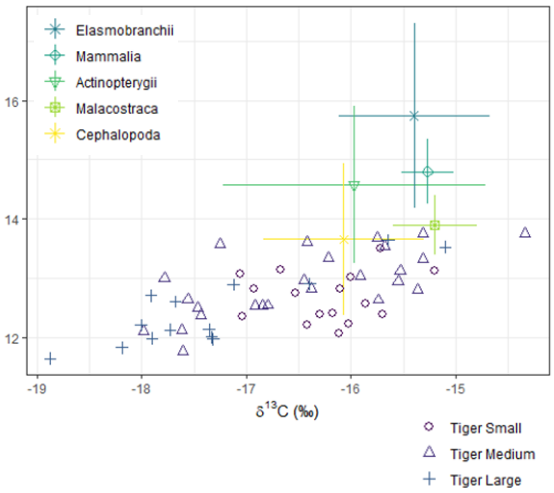
Figure A.1 SIMMR isospace plots for the blacktip, bull, copper, dusky, sand tiger, tiger, java and spinner sharks. Consumer $\delta^{13}C$ and $\delta^{15}N$ (expressed in ‰) values are represented as points on the plot, while crosses are weighted mean isotopic prey source values (center) and error bars representing standard deviations (outer edges). In the bottom right corner are the size classes examined for each shark predator, and in the top left corner are the prey sources used in the mixing model each denoted by a separate colour.



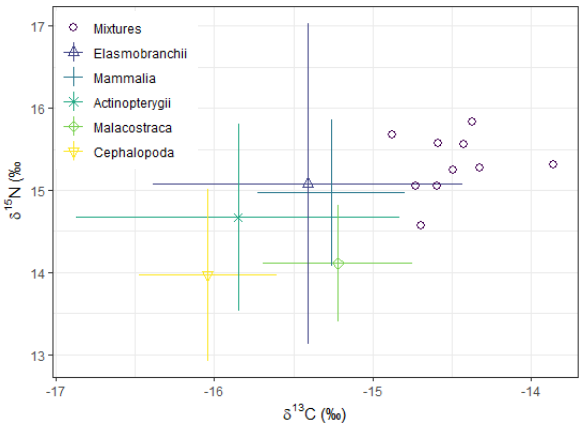
Sand Tiger (*C. taurus*)



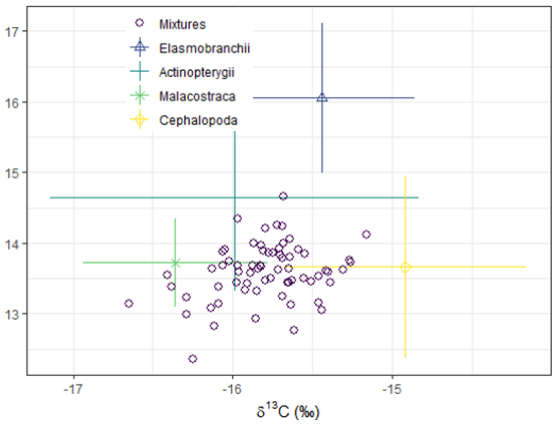
Tiger (*G. cuvier*)



Java (*C. amboinensis*)



Spinner (*C. brevipinna*)



VITA AUCTORIS

NAME:	Krystle-Blue Pahl
PLACE OF BIRTH:	Windsor, ON
YEAR OF BIRTH:	1988
EDUCATION:	St. Joseph's High School, Windsor, ON, 2005
	University of Windsor, B.Sc., Windsor, ON, 2011