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Individual and Sexual Variation in the Trophic
Ecology of the White Shark (*Carcharodon*
carcharias)

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University of Sussex
Faculty of Life Science
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Declaration

I confirm that the work submitted is my own, except where work which has been formed as part of jointly-authored publications is included. The contributions of other authors are indicated for published chapters listed below. This thesis has not been and will not be submitted to another institution for the award of any other degree.

List of published chapters and contributions:

Chapter 2. French, G.C.A., Stürup, M., Rizzuto, S., van Wyk, J.H., Edwards, D., Dolan, R.W., Wintner, S.P., Towner, A.V., Hughes, W.O.H. (2017) **The tooth, the whole tooth, and nothing but the tooth; tooth shape and ontogenetic shift dynamics in the white shark.** *Journal of Fish Biology*. **91:** 1032-1047.

GCAF, JHvW, AVT and WOHH conceived the study and designed the methodology; GCAF, MS, SR, DE, RWD, SW and WOHH collected the data; GCAF analysed the data and led the writing of the manuscript. All authors contributed critically to drafting the manuscript.

Chapter 3. French, G.C.A., Rizzuto, S., Stürup, M., Inger, R., Barker, S., van Wyk, J.H., Towner, A.V., Hughes, W.O.H. (in review) **Sex, size and isotopes; cryptic trophic ecology of an apex predator, the white shark** *Carcharodon carcharias*. *Marine Biology* (under review).

GCAF, WOHH, JHvW and AT conceived and designed the study. GCAF, SR and MS conducted the fieldwork. SB and GCAF performed experiments. GCAF analysed the data. GCAF wrote the manuscript. WOHH aided in manuscript writing. RI provided statistical advice. All authors provided editorial advice.

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GCAF, WOHH, JvW, and AVT conceived and designed the study. GCAF, SR and MS collected the biopsy samples. JD and M Sprague performed the fatty acid lab work. GCAF analyzed the data. GCAF wrote the manuscript. WOHH aided in manuscript writing, all other authors provided editorial advice.

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Abstract

Individual and sexual variation are widespread across the animal kingdom, and can have significant implications for species and population ecology and conservation.

Ontogenetic shifts in diet and habitat use are prevalent in species that exhibit large changes in body size from birth/hatching to maturity, and can alter an individual's role in communities and ecosystems. The role of these phenomenon in the ecology of mobile top predators is especially important to understand, as these species are often vital for maintaining food web stability and ecosystem linkage. White sharks (*Carcharodon carcharias*) are highly migratory top predators, listed as Vulnerable on the International Union for the Conservation of Nature's Red List, and are reported to undergo an ontogenetic dietary shift. Despite being protected across parts of their range, they are still subject to multiple anthropogenic threats. This work incorporates tooth shape, stable isotope, and fatty acid analyses to investigate individual and sexual variation in white shark ontogenetic shift dynamics and trophic ecology. Evidence for individual and sexual variation across populations is reviewed, and the associated conservation implications discussed, highlighting important current issues and areas for future research that will benefit white shark conservation management.

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Chapter 1 General Introduction

Intraspecific variation within populations, whether morphological, physiological, or behavioural is an inherent facet of species biology and evolution. It is well documented that demographic differences, such as sex and life stage/size can significantly influence individual, population and community ecology (Clutton-Brock et al. 1982; Polis 1984; Forero et al. 2002; Morris 2003; Ruckstuhl and Neuhaus 2005). The effects of sex and life stage can also have complex interactions which lead to disparate ecological outcomes (Lejeune et al. 2017). Additionally, the significance of intraspecific variation in studies of ecology has only more recently been recognised and is still not well understood in some cases, while often being ignored in others; an oversight which can obfuscate our understanding of a species' role within an ecosystem (Bolnick et al. 2003, 2011; Réale et al. 2007; Dall et al. 2012). These sources of variation can have significant implications for species and population conservation, and so it is of great importance that they are accounted for and understood (Ruckstuhl and Neuhaus 2005; Biro and Post 2008; Réale et al. 2010; Matich et al. 2011; Wolf and Weissing 2012). It is especially important to understand these mechanisms and outcomes in highly mobile top predators, as these species are integral for food web stability and ecosystem linkage (Sweitzer et al. 1997; Estes et al. 1998, 2011; Schreiber et al. 2011; Nifong et al. 2015).

19 **1.1. Ontogeny/size**

20 **1.1.1. Ontogenetic shifts**

21 In general, animals increase in size throughout their lifetime. Increase in body size can
 22 require a switch towards food sources with higher energetic rewards, at the same time as
 23 altering metabolic requirements (Werner and Hall 1974; Werner and Mittelbach 1981;
 24 Olson 1996; Scharf et al. 2000; Sherwood et al. 2002; Jackson et al. 2004; Glazier et al.
 25 2015). Distinct ontogenetic shifts in trophic ecology are common in species which have
 26 very large differences in body size from birth/hatching to maturity, and so are
 27 particularly prevalent in fish and reptiles (Wilson 1975; Werner and Gilliam 1984). For
 28 example, young komodo dragons (*Varanus komodoensis*) < 1 kg in weight forage
 29 mostly on insects, small rodents, reptiles, and birds, while adult dragons which weigh >
 30 20 kg include large ungulates in their prey base (Purwandana et al. 2016). Size-based
 31 ontogenetic shifts such as this can have significant effects on predator-prey dynamics
 32 and population stability (McCauley et al. 1996; Olson 1996; Scharf et al. 2000) and the
 33 differences in trophic ecology between size/age classes can be so great that in some
 34 cases they can be considered different ecological species (Polis 1984), performing
 35 different functional roles in communities and ecosystems (Hutchinson 1957; Werner
 36 and Gilliam 1984; McCauley et al. 1996; Olson 1996; Scharf et al. 2000; Grubbs 2010).

37 *1.1.1.2. Allometric scaling of trophic structures*

38 Large increases in body size are not the only morphological features that facilitate
 39 ontogenetic shifts in trophic ecology. Allometric scaling of body structures used in
 40 feeding such as gape size, dentition, and jaw musculature can also be key in dietary
 41 shifts (Wilson 1975; Polis 1984; Peters 1986; Scharf et al. 2000; Huber et al. 2008).

42 Allometric scaling in this context means a disproportionate change in a physical feature
43 in comparison to change in overall body size. For example, in the turtle *Sternotherus*
44 *minor* the size of the jaw muscle exhibits a disproportionate increase in size with
45 increased turtle length, which facilitates a shift towards hard-bodied prey (Pfaller et al.
46 2011).

47 1.1.1.3. *Habitat use*

48 Ontogenetic changes in diet are typically concurrent with changes in habitat use and
49 movement patterns, where larger individuals select habitats which support their new
50 prey choices/nutritional requirements, have greater movement capability, and are able to
51 use habitats that may pose more risk to smaller individuals (Werner and Gilliam 1984;
52 Gilliam and Fraser 1987; Werner and Hall 1988; Lima and Dill 1990; Law 1991;
53 McCauley et al. 1996; Morris 2003; Keren-Rotem et al. 2006; Purwandana et al. 2016).
54 Referring to the komodo dragon example given earlier, the demonstrated ontogenetic
55 shift in diet is accompanied by a significant increase in home range size, and a switch
56 from arboreal to terrestrial habitat use (Purwandana et al. 2016). Ontogenetic shift
57 dynamics need to be integrated into the identification of critical habitats such as nursery
58 areas (Nagelkerken et al. 2015) and ontogenetic differences in habitat and resource
59 requirements should be important considerations in conservation management plans.
60 Increased movement between habitats can also result in larger size classes playing an
61 important role in nutrient distribution and ecosystem linkage (Nifong et al. 2015), which
62 may provide key information for effective landscape scale ecosystem management.

63 **1.2. Sex**

64 Ecological differences between the sexes of a species is prevalent across taxa (Clutton-
65 Brock et al. 1982; Le Boeuf et al. 2000; Phillips et al. 2004; Mowat and Heard 2006;
66 Beck et al. 2007; Thiemann et al. 2008; Patrick and Weimerskirch 2014; Lejeune et al.
67 2017). Mechanisms underlying sex-mediated differences in ecology include sexual
68 dimorphism and sexual segregation, which are explained below.

69 **1.2.1. Sexual dimorphism**

70 Sexual dimorphism is evident throughout the animal kingdom, and has a key role in
71 understanding ecology (Selander 1972; Belovsky and Jordan 1978; Clutton-Brock et al.
72 1982; Shine 1989; Magurran and Garcia 2000). Sexual dimorphism in size and
73 morphology can be influenced by one or a combination of, fecundity selection (e.g.
74 increased fecundity in larger females), sexual selection (mating displays, mate
75 acquisition, choice, searching, cooperation) and ecological divergence (Shine 1989). In
76 this context, sexual dimorphism in body size or foraging apparatus can present similar
77 ecological effects to ontogenetic shifts, where in this case the larger sex, or the sex that
78 has developed more exaggerated trophic structures may exploit different food resources
79 and/or habitats than the other sex, comprising intersexual niche divergence (Selander
80 1972; Shine 1989). Sexual dimorphism features heavily in theories of sexual
81 segregation, described below.

82 **1.2.2. Sexual segregation**

83 Sexual segregation is typically split into two categories; social segregation and habitat
84 segregation (Conradt 2005; Ruckstuhl 2007), both of which can be influenced by sexual

size dimorphism (Ruckstuhl and Neuhaus 2005). Social segregation occurs when the two sexes form different groups outside of the mating season but use the same areas and habitats, while habitat segregation occurs when the sexes use different habitats, which may or may not be within the same area. Both types of segregation can lead to spatial separation of the sexes (Conradt 2005; Ruckstuhl and Clutton-Brock 2005; Wearmouth and Sims 2008) and it is important to distinguish between them in order to understand their ecological implications (Bowyer 2004). The majority of research into this subject has been on sexually dimorphic, social species where sexual segregation is particularly prevalent, and studies have especially focussed on ungulates (Ruckstuhl 2007) though application to the marine environment has been recognised as important (Wearmouth and Sims 2008). Sexual segregation is an important consideration in wildlife conservation (Rubin and Bleich 2005) and ecological differences between the sexes are likely to become important in species conservation under future climate change and human exploitation scenarios (Paiva et al. 2017).

There are five main hypotheses of the proximate causes of sexual segregation; 1) predation risk 2) forage selection 3) activity budget 4) thermal niche – fecundity 5) social factors (Ruckstuhl and Neuhaus 2005).

1.2.2.1. Predation risk

Formerly known as the reproductive strategy hypothesis (Main et al. 1996), the predation risk hypothesis predicts that the sex most vulnerable to predation will select safer habitats. Often this means that the larger sex will be able to exploit more risky habitats and food resources, though females may also choose safer habitats for parturition and rearing of progeny (Ruckstuhl and Neuhaus 2005; Croft et al. 2006).

108 *1.2.2.2. Forage selection*

109 The forage selection hypothesis is based on differences in the nutritional needs of the
110 sexes, which typically results in habitat segregation (Main et al. 1996; Ruckstuhl and
111 Neuhaus 2000). The theory refers specifically to the fact that larger animals have a
112 proportionately larger gut that inherently improves digestion efficiency and allows for a
113 diet that is comparatively lower quality than that needed by a smaller animal that has
114 less efficient digestion (Gross 1998; Ruckstuhl and Neuhaus 2000). Furthermore,
115 different forage needs due to gestation, lactation and other reproductive factors also fall
116 under this hypothesis (Robbins 1983; Ruckstuhl and Clutton-Brock 2005), as does
117 competitive exclusion as a result of one sex being better morphologically adapted to
118 exploit certain food patches (Ruckstuhl and Neuhaus 2005; Wearmouth and Sims
119 2008).

120 *1.2.2.3. Activity budget*

121 Under this hypothesis, sexual segregation occurs when the sexes (in species that are
122 either size dimorphic or have different reproduction-related needs), have different
123 nutritional needs, which causes them to exhibit differences in activity rhythm, also
124 described as having incompatible activity budgets (Ruckstuhl 1998, 1999; Conradt
125 1998). These differences in time allocation to tasks such as foraging, movement rates,
126 and predator vigilance cause mixed-sex groups to split apart and form same-sex groups,
127 potentially causing both social and habitat segregation (Conradt 1998; Ruckstuhl 1999,
128 2007; Ruckstuhl and Neuhaus 2005).

129 *1.2.2.4. Social factors*

130 Here, an affinity for the same sex, or avoidance of/conflict with the opposite sex causes
131 habitat segregation (Bon 1991; Main et al. 1996; Ruckstuhl and Neuhaus 2000; Conradt
132 2005; Parker 2006). Same sex affinities can provide benefits in mate acquisition and
133 location of breeding sites for naïve individuals, and assist males by providing
134 opportunities to practice fighting skills, develop dominance hierarchies and assess the
135 competitive value of their rivals (Main et al. 1996; Ruckstuhl and Neuhaus 2000;
136 Ruckstuhl 2007). Conflict avoidance can be both physical, for example female
137 avoidance of sparring males, and evolutionary, where the optimal reproductive
138 outcomes for both sexes cannot be achieved simultaneously due to differential
139 investment in procreation (Parker 2006; Ruckstuhl 2007). In solitary species, the
140 affinities and splits caused by these social factors would take the form of size, or
141 reproductive state, dependent habitat use (Wearmouth and Sims 2008). A relatively new
142 social factor that seems particularly prevalent in marine systems is female avoidance of
143 males due to sexual harassment/mating coercion, which can cause females both physical
144 harm and energetic costs (Wearmouth et al. 2012; Galezo et al. 2017).

145 *1.2.2.5. Thermal niche – fecundity*

146 This hypothesis is based on an assumption that the sexes select habitats that have
147 different temperatures, reflecting those at which their fecundity is maximised (Sims
148 2005). This has mostly been proposed in ectotherms (Robichaud and Rose 2003; Sims
149 2005; Wearmouth and Sims 2008) but has also been found in mammals (Altringham
150 and Senior 2005; Angell et al. 2013).

151 **1.3. Individual variation**

152 **1.3.1. Trophic/resource polymorphism and individual specialists**

153 Individual trophic specialisation has been identified in a wide range of organisms
 154 (Bolnick et al. 2003; Araújo et al. 2011). There are subtle differences between the most
 155 commonly used nomenclature for this phenomenon, namely resource polymorphism,
 156 which centres around discrete intraspecific morphs (Wimberger 1994; Skúlason and
 157 Smith 1995; Smith and Skúlason 1996) and individual specialisation, where an
 158 individual's comparatively narrow niche width is not attributable to sex, age, or discrete
 159 morphological group (Bolnick et al. 2003). Morphs are sometimes referred to as
 160 ‘ecotypes’, especially when genetic differences exist between them (Snorrason et al.
 161 1994; Mowat and Heard 2006; Kobler et al. 2009; Shafer et al. 2014; Jeglinski et al.
 162 2015).

163 Specialisation within trophic ecology is influenced by competition, habitat and
 164 prey availability, morphology, genetics, and behaviour (Meyer 1990b; Ehlinger 1990;
 165 Wainwright et al. 1991; Bolnick et al. 2003; Araújo et al. 2011; Shafer et al. 2014;
 166 Newsome et al. 2015; Marklund et al. 2018). Consistent selection for specific prey can
 167 increase individual foraging efficiency (Reilly et al. 1992; Araújo and Gonzaga 2007)
 168 and decrease intraspecific competition (Roughgarden 1972; Bolnick et al. 2003;
 169 Swanson et al. 2003). However, this specialisation can also increase some specialist's
 170 exposure to parasites and disease (Curtis et al. 1995; Lloyd-Smith et al. 2005; Johnson
 171 et al. 2009) and can limit food web connectivity in addition to leaving species or
 172 populations more vulnerable to ecosystem fragmentation and the risk of extinction
 173 compared to generalists (Purvis et al. 2000; Layman et al. 2007b; Quevedo et al. 2009).
 174 Dietary specialisation in top predators can have especially strong effects on food webs,

175 through differential prey choice and hunting tactics (Schmitz and Suttle 2001; Schreiber
 176 et al. 2011) and recent studies are revealing that specialisation in top predators is more
 177 prevalent than previously thought (e.g. (Matich et al. 2011; Nifong et al. 2015;
 178 Rosenblatt et al. 2015)).

179 **1.3.2. Behaviour – personality and behavioural syndromes**

180 Personality can be defined as intra-individual behavioural differences that are consistent
 181 through time and across contexts (Gosling 2001; Wolf and Weissing 2012). The term
 182 personality is often used interchangeably with ‘behavioural syndrome’, though
 183 behavioural syndromes are suites of behavioural traits correlated across individuals,
 184 which result in behavioural types (Sih et al. 2004b; Réale et al. 2007; Dingemanse et al.
 185 2012). The ecological implications of personality and behavioural syndrome differences
 186 are significant, including life-history trade-offs, food-web stability and at an
 187 evolutionary scale, speciation (Sih et al. 2004a, 2012; Réale et al. 2007; Wolf and
 188 Weissing 2012; Dall et al. 2012; Dingemanse et al. 2012). One of the most simple and
 189 often described behavioural syndromes is the bold-shy axis, where some individuals can
 190 be categorised as ‘bold’, while others are ‘shy’ (Réale et al. 2007). Correlations between
 191 shyness and boldness and individual ecology have been found in many species (e.g.
 192 Conrad et al. 2011; Sih et al. 2012; Patrick and Weimerskirch 2014; Pruitt and Keiser
 193 2014) and present a more nuanced phenotypic polymorphism than the morphological
 194 type described above, where individuals with no apparent morphometric differences can
 195 present very different ecologies. For example, ‘bold’ black-browed albatross
 196 (*Thalassarche melanophrys*), categorised as such by their response to a novel object,
 197 forage in a different habitat to ‘shy’ individuals (Patrick and Weimerskirch 2014).
 198 Interestingly, these foraging locations have different fitness implications for males and

199 females (Patrick and Weimerskirch 2014), which highlights the importance of assessing
200 intraspecific variation in combination with sex differences.

201 **1.3.3. Pace-of-Life-Syndrome Hypothesis**

202 The POLS hypothesis comprises intrinsic links between individual behaviour,
203 physiology, and life history parameters where for example, individuals that are
204 considered ‘bold’ in their behaviour have faster growth rates, earlier onset of maturity,
205 and other physiological differences, such as ability to cope with stress, in comparison to
206 ‘shy’ individuals (Ricklefs and Wikelski 2002; Biro and Stamps 2008; Réale et al.
207 2010). This has implications for ontogenetic shift dynamics, where some individuals
208 may undergo ontogenetic shifts earlier or later than others.

209 Individual differences in life history parameters and behaviour have significant
210 effects on individual exposure to anthropogenic threats such as risk of fishing mortality,
211 in addition to effects on population stability and growth rates (Biro and Post 2008;
212 Wilson et al. 2011; Wolf and Weissing 2012; Härkönen et al. 2014). It is therefore
213 critical that these sources of variation are well understood, especially in already
214 threatened species.

215 **1.4. Model system: The white shark**

216 **1.4.1. Description**

217 The white shark (*Carcharodon carcharias*) is a large predatory fish, characterised by its
218 conical snout, torpedo shaped body, large serrated teeth, and lunate caudal fin shape
219 (Compagno 2001). Colouration is variable, but typically grey (ranging from light to

220 dark) dorsally, countershaded ventrally with white (Compagno 2001). Controversy
221 reigns over the maximum size and age attained by white sharks, though the most
222 recently accepted reliable estimates are 600 cm (Castro 2012) and 70 years+ (Hamady
223 et al. 2014).

224 As a Lamniform, white sharks possess the ability to maintain the temperature of
225 their eyes, brain, stomach, and muscles several degrees above that of the water in which
226 they are swimming (Carey et al. 1982; Block and Carey 1985; McCosker 1987; Wolf et
227 al. 1988). The physiological structures behind this ability are termed the retia mirabilia,
228 and comprise a system of vascular tissue that acts as a counter-current heat exchanger,
229 utilising metabolic heat to warm the various body parts to which they are adjacent
230 (Carey and Teal 1969; Carey et al. 1982). Elevated muscular temperatures allow white
231 sharks to increase their muscle power output (Hartree and Hill 1921), while increased
232 ocular temperature is believed to improve visual capabilities (Block and Carey 1985);
233 both of these factors facilitating the ability to predate highly mobile and sometimes
234 large prey. Such prey items are subsequently rapidly digested, as a consequence of
235 heightened stomach temperatures (McCosker 1987). The ability to maintain a relatively
236 warm brain is hypothesised to aid in mitigating against rapid and substantial changes in
237 temperature by providing a thermal buffer for the nervous system (Block and Carey
238 1985).

239 **1.4.2. Distribution**

240 White sharks use both coastal and pelagic habitats around the globe (Compagno 2001),
241 where they undertake both return coastal migrations, and movement between the coast
242 and open ocean (Bonfil et al. 2005, 2010; Weng et al. 2007a; Jorgensen et al. 2010;
243 Block et al. 2011; Duffy et al. 2012; Domeier and Nasby-Lucas 2013; Bonfil and

OBrien 2015), which are transoceanic in some cases (Bonfil et al. 2005; Duffy et al. 2012; Del Raye et al. 2013). Genetically distinct populations are located in South Africa, Australia/New Zealand, Northeast Pacific, Northwest Pacific, Northwest Atlantic and the Mediterranean (Pardini et al. 2001; Jorgensen et al. 2010; Tanaka et al. 2011; Gubili et al. 2011, 2012; Andreotti et al. 2015).

1.4.3. Conservation

1.4.3.1. Intrinsic vulnerability to anthropogenic threats

White sharks have long lifespans, take up to three decades to reach sexual maturity, and have relatively low fecundity (Myers and Worm 2003; Hamady et al. 2014; Natanson and Skomal 2015); like many elasmobranchs, this renders them sensitive to over-fishing and exploitation, leading to significant population declines (Myers and Worm 2003; Baum et al. 2003; Worm et al. 2013; Dulvy et al. 2014). Removal of mobile top predators such as the white shark can have significant impacts on marine food webs and ecosystems, causing consumptive and behaviour-mediated trophic cascades (Heithaus et al. 2008; Ferretti et al. 2010; Ruppert et al. 2013; Rasher et al. 2017), and disrupting ecosystem connectivity (Lundberg and Moberg 2003; McCauley et al. 2012; Rosenblatt et al. 2015).

1.4.3.2. Conservation status

White sharks are listed as Vulnerable on the International Union for the Conservation of Nature's (IUCN) Red List (Fergusson et al. 2009), in addition to Appendix II of the Convention on International Trade in Endangered Species of flora and fauna (CITES), and both Appendices of the Convention on Conservation of Migratory Species. National

266 protection has been granted in South Africa, Australia, USA, Namibia, Israel and Malta
 267 (Fergusson et al. 2009).

268 *1.4.3.3. Existing threats*

269 Although white sharks are listed under several pieces of protective legislation, they are
 270 still subject to threats across their range, partly because their wide ranging movement
 271 patterns mean that the sharks travel into areas where they are not protected (Bonfil et al.
 272 2005, 2010; Weng et al. 2007a; Fergusson et al. 2009; Blower et al. 2012; Domeier and
 273 Nasby-Lucas 2013). White shark fins are also illegally traded for both food and trophies
 274 (Shivji et al. 2005), and species-specific identification of fins within markets remains an
 275 issue (Cardeñosa et al. 2017). Targeted and non-targeted sport and trophy fishing is still
 276 prevalent across their range, in addition to bycatch in commercial fisheries (Baum et al.
 277 2003; Fergusson et al. 2009; Lyons et al. 2013b) and swimmer protection programmes
 278 in South Africa and Australia continue to catch white sharks despite both countries
 279 listing them as protected species (Dudley and Simpfendorfer 2006; Fergusson et al.
 280 2009). Shark nets and especially targeted culls in response to shark bites in Australia are
 281 still utilised and regularly proposed, despite lack of public support (Pepin-Neff and
 282 Wynter 2017). The media play a pivotal role in the public perception of sharks, often
 283 irresponsibly portraying them in an overly negative manner that has been shown to
 284 influence policy and fisher attitudes (Neff and Hueter 2013; Neff 2015; McCagh et al.
 285 2015; Nosal et al. 2016; Drymon and Scyphers 2017; Pepin-Neff and Wynter 2018).

286 Several emerging global threats are likely to affect white sharks.

287 Bioaccumulation of anthropogenic toxins has been recorded in white sharks across
 288 populations (Schlenk et al. 2005; Mull et al. 2013; Lyons et al. 2013a; Marsili et al.
 289 2016), with deleterious effects recognised in South Africa (Marsili et al. 2016). Ocean

290 warming and acidification reduce shark hunting efficacy while increasing their
291 energetic demands (Dixon et al. 2015; Pistevos et al. 2015, 2017; Rosa et al. 2017) and
292 anthropogenic noise pollution has been identified as posing extreme and negative fitness
293 consequences for the world's fishes (Cox et al. 2018). Cage diving ecotourism is
294 popular at major white shark aggregations in South Africa, Australia and Mexico, and
295 there is evidence that these activities alter the shark's movement patterns, though it has
296 not yet been ascertained whether these changes may be detrimental to the sharks
297 (Laroche et al. 2007; Bruce and Bradford 2013; Huvaneers et al. 2013; Towner et al.
298 2016).

299 As the white shark is a globally threatened, highly mobile top predator,
300 understanding the dynamics of ontogenetic shifts and sexual and individual variation in
301 the species is of significant importance to our understanding of its ecology and
302 conservation management.

303 **1.4.4. Diet and ontogenetic shift**

304 White sharks are documented to experience a distinct ontogenetic shift in prey
305 preference when they reach approximately three metres in length, characterised by a
306 change in primary prey from piscine species in smaller sharks, to incorporating hunting
307 of marine mammals in larger individuals (Tricas and McCosker 1984; Klimley 1985;
308 Cliff et al. 1989; Estrada et al. 2006; Hussey et al. 2012b). The reported shark length at
309 which the shift occurs varies between 2.0 m and 3.4 m total body length (Table 1.1). It
310 was previously believed that only large white sharks scavenged from whale carcasses,
311 but young individuals have also been documented feeding at carcasses (Dicken 2008).
312 The Mediterranean population is the only one known to potentially partially specialise

on turtles (Fergusson et al. 2000) and relatively little is known about the ecology of the population off the coast of Japan in the northwest Pacific (Tanaka et al. 2011).

Table 1.1: Shark total length (TL) at which the ontogenetic shift is reported to occur in white shark populations.

TL at which ontogenetic shift is reported to occur (m)	Population	Reference	Notes
2.5	Australia, South Africa	(Cliff et al. 1989; Bruce 1992)	
2.7	Australia	(Malcolm et al. 2001)	
2 - 3	All	(Compagno 2001)	
3.0	Australia	(Bruce et al. 2006)	Smallest size visiting seal colonies
3.41	North Atlantic	(Estrada et al. 2006)	Stable isotope study
2.66	South Africa	(Hussey et al. 2012b)	Smallest individual found to have seal remains in stomach

This shift in diet is believed to be facilitated by a change in tooth shape, from relatively cuspidate to broad; hypothesised to increase handling efficiency of marine mammal blubber (Tricas and McCosker 1984; Frazzetta 1988). Tooth shape change through ontogeny has not been studied in the context of sexual or individual variation in white sharks, despite evidence of both sexual and individual variation in tooth shape of

324 elasmobranchs (e.g. Taniuchi 1970; Litvinov 1983, 2003; Hubbell 1996; Kajiura and
325 Tricas 1996) and the evidence presented below.

326 With the exclusion of the smallest size classes, which are not present, size
327 segregation at aggregations associated with pinniped colonies has little support
328 (Domeier and Nasby-Lucas 2007, 2008; Duffy et al. 2012; Kock et al. 2013; Bruce and
329 Bradford 2015), though some stratification of aggregation attendance by size is evident
330 in South Africa (see section 1.5.2.) and fine-scale habitat use has been shown to differ
331 temporally between size classes at Guadalupe Island (Hoyos-Padilla et al. 2016). A
332 size-based feeding hierarchy was noted among bait-attracted sharks by Strong et al.
333 (1992).

334 **1.4.5. Sexual dimorphism**

335 Significant sexual dimorphism is evident in *C. carcharias* with females achieving
336 greater length and mass in comparison to their male cohorts (Compagno 2001).
337 Differences in age and length at maturity are evident, where males have been shown to
338 mature at 350 - 410 cm, while females only reach maturity at 400 - 500 cm (Table 1.2.),
339 and the most recent estimate places male maturity at 26 years and female at 33 years
340 (Natanson and Skomal 2015). There is evidence that these sizes and ages differ between
341 populations (Tanaka et al. 2011) and that growth rates differ between the sexes, where
342 females grow faster than males (Tanaka et al. 2011; Hamady et al. 2014), though this is
343 yet to be confirmed.

Table 1.2: Size (total length (m)) and maturity classes of male and female white sharks.

Maturity Class	Male	References	Female	References
Young of the Year	≤ 1.75	(Francis 1996)	≤ 1.75	(Francis, 1996)
Juvenile	2 - 3	(Bruce & Bradford, 2012)	2 - 3	(Bruce & Bradford, 2012)
Sub-Adult	3 - 3.5	(Pratt, 1996; Bruce & Bradford, 2012)	3 - 4.5	(Francis, 1996; Compagno, 2001; Bruce & Bradford, 2012)
Adult	≥ 3.6	(Pratt, 1996)	≥ 4.5	(Francis, 1996; Compagno, 2001)

1.4.6. Sexual segregation

Habitat segregation and differences in movement patterns have been recorded in the northeast Pacific (Anderson & Pyle, 2003; Jorgensen et al., 2010; Domeier & Nasby-Lucas, 2012, 2013; Weng & Honebrink, 2013), Australia/New Zealand (Bruce et al. 2006; Robbins 2007; Robbins and Booth 2012; Francis et al. 2015; Bruce and Bradford 2015) and South Africa (Cliff et al. 1989, 2000; Pardini et al. 2001; Zuffa et al. 2002; Bonfil et al. 2005; Kock et al. 2013; Towner et al. 2013a, 2016; Hewitt et al. 2018). Some of these differences have been attributed to female gestation and pupping (Anderson & Pyle, 2003; Domeier & Nasby-Lucas, 2013) and different nutritional requirements (Robbins 2007; Weng et al. 2007a; Jorgensen et al. 2010; Kock et al.

2013; Bruce and Bradford 2015), while others hypothesise influence of thermal niche where females use warmer water conditions to increase growth rate and/or development of embryos (Robbins, 2007; Towner et al., 2013a). As yet, there has not been a cohesive review of sexual segregation in white sharks, or its potential conservation implications. Furthermore, many studies of the trophic ecology of white sharks do not separate samples into the sexes, or consider the interaction of both sex and size. Given the evidence for both ontogenetic and sexual variation in white shark ecology, this represents a major gap in the scientific literature for this species.

1.4.7. Individual variation

The study of individual variation in shark behaviour and ecology is a burgeoning field, and few studies have tackled it explicitly in white sharks. Individual variation in response to ecotourism activities has been recorded (Laroche et al. 2007; Huveneers et al. 2013), in addition to predatory behaviour (Huveneers et al. 2015; Towner et al. 2016), and individual dietary specialisation has been revealed in the northeast Pacific and Australia (Kim et al. 2012; Pethybridge et al. 2014).

1.5. Study population - South African white sharks

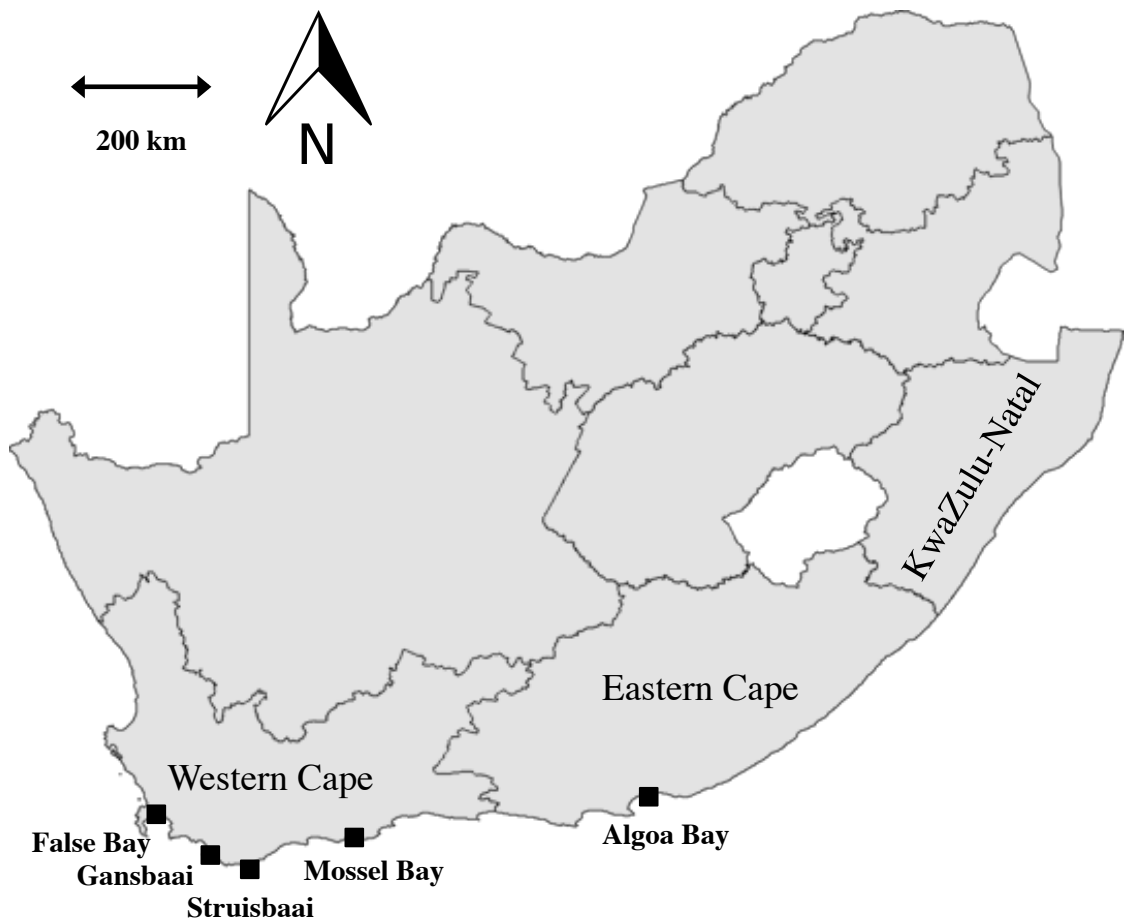
1.5.1. Population status and size

South Africa's white shark population is genetically distinct, though there is a degree of movement and gene flow between South Africa and the Australia/New Zealand population (Pardini et al. 2001; Bonfil et al. 2005; Andreotti et al. 2015). Recently, controversy has emerged over population estimates for South Africa, with some

378 claiming a size of 808 - 1008 and 972 - 1586 (Cliff et al. 1996; Towner et al. 2013b)
 379 while others claim the very low number of 353 - 522 (Andreotti et al. 2016); a figure
 380 that has been contested (Irion et al. 2017).

381 1.5.2. Population distribution

382 Ferreira and Ferreira (1996) provided the first description of South Africa's discrete
 383 white shark coastal aggregations (False Bay, Gansbaai, Struisbaai, Mossel Bay and
 384 Algoa Bay), and Cliff et al. (1989) documented that white sharks are common off the
 385 coast of KwaZulu-Natal (KZN) (Figure 1.1).



386

387 **Figure 1.1 Locations of the five major coastal aggregations of white sharks in South**
 388 **Africa.**

389 The sharks are known to undertake return coastal migrations between the coastal
 390 aggregation sites, KwaZulu-Natal, and Mozambique, as well as oceanic return
 391 migrations within the western Indian Ocean (Cliff et al. 1996; Ferreira and Ferreira
 392 1996; Bonfil et al. 2005; Jewell et al. 2011). There is some stratification of age class
 393 between the aggregations; Algoa Bay is believed to serve as a white shark nursery (Cliff
 394 et al. 1996; Dicken 2008), KZN sharks are mostly juvenile and young of the year (Cliff
 395 et al. 1989), the Mossel Bay aggregation is largely juvenile with some sub-adults
 396 (Ryklief et al. 2014), and Gansbaai and False Bay sharks are dominated by sub-adults of
 397 both sexes as well as some juveniles and adult males (Kock et al. 2013; Towner et al.
 398 2013a; Hewitt et al. 2018). All of the coastal aggregations are in proximity to large
 399 pinniped colonies, except for Struisbaai (Dudley 2012). Mature females are notable by
 400 their scarcity from all of these aggregations, and have instead been documented in the
 401 tropical waters of the Western Indian Ocean, where sightings of large sharks have been
 402 made in Mozambique, Madagascar, Kenya, Seychelles, Mauritius and Zanzibar (Cliff et
 403 al. 2000; Bonfil et al. 2005). No pregnant females have yet been recorded in South
 404 Africa (Francis 1996).

405 **1.5.3. National conservation plan**

406 The United Nation's Food and Agriculture Organisation (FAO) International Plan of
 407 Action for the conservation and management of sharks (IPOA-Sharks, 1999), requires
 408 member states to develop their own, self funded, National shark POA's (NPOA-
 409 Sharks). As an FAO member state, and following its guiding marine biodiversity
 410 legislation (Objectives and Principles of the Marine Living Resource Act 1998), South
 411 Africa has recently finalised the NPOA-Sharks South Africa (NPOA-Sharks South

412 Africa), within which is highlighted the need for better understanding of all aspects of
413 shark behaviour for the benefit of optimal conservation efforts.

414 **Aims of this Thesis**

415 This thesis aims to assess and review the role of sexual and individual variation in the
416 trophic ecology of the white shark, particularly in ontogenetic shift dynamics. The work
417 uses data collected from the Gansbaai aggregation of white sharks in South Africa, for
418 which trophic studies have not yet been conducted, in addition to previously published
419 data. Questions asked in this thesis challenge long-held paradigms in white shark
420 biology, especially concerning tooth shape change through ontogeny. Trophic ecology
421 is investigated through tooth shape metrics, stable isotope and fatty acid analyses. A
422 timely review of sexual and individual variation in the species provides a cohesive
423 overview of research to date, and highlights conservation management implications.
424 Direct incorporation of size, sex and individual differences in trophic modelling will
425 provide important insight into the ecology of a highly mobile, threatened top predator.
426 These insights could be transferred across species and ecosystems, and provide a basis
427 for better-informed management of ecologically important wildlife.

428 Chapter 2 The tooth, the whole tooth and nothing
429 but the tooth: tooth shape and ontogenetic shift
430 dynamics in the white shark *Carcharodon*
431 *carcharias*.

2.1. Abstract

Ontogenetic dietary shifts are widespread across the animal kingdom, and often involve associated morphological changes in foraging phenotype. These changes may differ between sexes or vary between individuals, and are important factors in the ecology of species. While such factors have received much attention in terrestrial systems, they are much less well understood in marine taxa. The white shark *Carcharodon carcharias* is a marine apex predator that is accepted to provide a classic example of an ontogenetic dietary shift, with an associated change in tooth morphology from cuspidate to broad. Our results however, which include measurements obtained using a novel photographic method, reveal significant differences between the sexes in the relationship between tooth cuspidity and shark total length (TL), and a novel ontogenetic change in male tooth shape. Males exhibit broader upper first teeth and increased distal inclination of upper third teeth with increasing length, while females do not present a consistent morphological change. Substantial individual variation, with implications for pace of life syndrome, was present in males, and tooth polymorphism was suggested in females. Sexual differences and individual variation may play major roles in ontogenetic changes in tooth morphology in white sharks, with potential implications for their foraging biology. Such individual and sexual differences should be included in studies of ontogenetic shift dynamics in other species and systems.

2.2. Introduction

Ontogenetic shifts in ecological niche are widespread across the animal kingdom, and represent changes in resource use with size, from birth/hatching to maximum size (Werner and Gilliam 1984). In some species, ontogenetic shifts in diet are generally characterized by a change from smaller size classes consuming a limited range of relatively small prey species, to larger size classes consuming a wider range of prey items with a larger mean body size (Wilson 1975). Such shifts in diet can be accompanied, or even made possible, by allometric scaling of morphological features, in which one morphological feature changes disproportionately to general body growth. In some species, there may be phenotypic polymorphism in the ontogenetic change in morphology and diet, resulting in trophic polymorphism (Hutchinson 1957; van Valen 1965; Meyer 1989, 1990a).

The ecological importance of ontogenetic dietary shifts and associated morphological changes, and of sexual or individual variation in them, may be particularly significant in marine apex predators such as sharks because of their often keystone ecology and vulnerable conservation status (Matich and Heithaus 2015). It is becoming increasingly clear that sharks exhibit sexual and individual differences in diet and habitat use, and allometric scaling of morphological features through ontogeny. For example, bull sharks (*Carcharhinus leucus*), tiger sharks (*Galeocerdo cuvier*), and other large pelagic sharks show individual variation in diet (Heithaus et al. 2002; Matich et al. 2011; Kiszka et al. 2015), and female scalloped hammerheads (*Sphyrna lewini*) shift to offshore habitats at a smaller size than males, where access to pelagic prey and improved foraging success allow them to grow faster than their male counterparts (Klimley 1987). Bull, tiger, blacktip (*Carcharhinus limbatus*), and horn sharks (*Heterodontus francisci*) show allometric changes in head shape and musculature

(Huber et al. 2006; Kolmann and Huber 2009; Habegger et al. 2012; Fu et al. 2016), and bull, tiger and white (*Carcharodon carcharias*) sharks show this with caudal-fin shape (Lingham-Soliar 2005; Irschick and Hammerschlag 2015). Allometric scaling of mouth length and width is also evident in the viper dogfish (*Trigonognathus kabeyai*) (Yano et al. 2003).

Individual variation in tooth morphology, a mechanistic facilitator of shark diet (Frazzetta 1988; Compagno 1990) has been reported for sand tiger (*Carcharias taurus*), blue (*Prionace glauca*), and porbeagle (*Lamna nasus*) sharks (Litvinov 1983; Shimada 2002a; Lucifora et al. 2003; Litvinov and Laptikhovsky 2005). Sexual dimorphism in tooth shape has been linked to different diets (Litvinov and Laptikhovsky 2005), but can also be an adaptation that gives males greater purchase when holding on to females during copulation (Kajiura and Tricas 1996). Quantifying ontogenetic change is logistically challenging in large pelagic elasmobranchs due to their intolerance of captivity, cryptic habitat use, wide-ranging movements, relatively low abundance and handling difficulty. As such, many ontogeny studies have been limited to dead specimens.

The white shark is a classic example of a morphological, diet-related change through ontogeny. White sharks are a member of the Lamniformes, an order for which tooth morphology is an informative defining character (Compagno 1990). It is widely accepted that white sharks undergo an ontogenetic shift in prey preference (Tricas and McCosker 1984; Cliff et al. 1989; Bruce 1992; Compagno 2001; Estrada et al. 2006; Hussey et al. 2012b). Stomach content and stable isotope analyses indicate that this shift constitutes a change in trophic level, from a predominantly piscivorous diet when young, to marine mammals making up the major component of diet when older (Tricas and McCosker 1984; Klimley 1985; Cliff et al. 1989; Estrada et al. 2006; Hussey et al.

2012b). The estimated length at which they undergo this dietary shift varies between 2 m and 3.4 m body length (Cliff et al. 1989; Bruce 1992; Compagno 2001; Estrada et al. 2006; Bruce et al. 2006; Hussey et al. 2012b), and is generally considered to occur in both sexes at the same size, despite the fact that white sharks are sexually dimorphic, with males reaching maturity at approximately 3.5 m and females at 4.5 m in length (Francis 1996; Pratt 1996; Compagno 2001; Bruce and Bradford 2012). This dietary shift is widely accepted to be facilitated by a change in tooth morphology, from relatively pointed (cuspidate) teeth with serrational cusplets adapted to puncturing piscivorous prey, to broader teeth lacking serrational cusplets that are better suited to handling mammalian prey (Tricas and McCosker 1984; Frazzetta 1988; Hubbell 1996; Whitenack and Motta 2010; Bemis et al. 2015) (Figure 2.1).

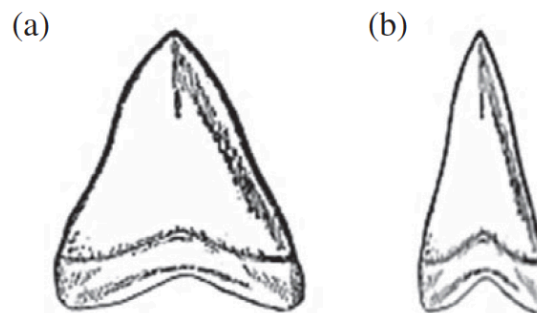


Figure 2.1: Illustrations of variation in *Carcharodon carcharias* tooth breadth and cuspidity (a) a broad and (b) a cuspidate tooth.

However, the primary reliance of adult white sharks on marine mammal prey is arguably overstated (Fergusson et al. 2009), and there is mounting evidence of individual dietary variation that does not appear to be related to sex or age (Estrada et al. 2006; Hussey et al. 2012b; Carlisle et al. 2012; Kim et al. 2012; Hamady et al. 2014; Pethybridge et al. 2014; Christiansen et al. 2015; Towner et al. 2016). Individual and

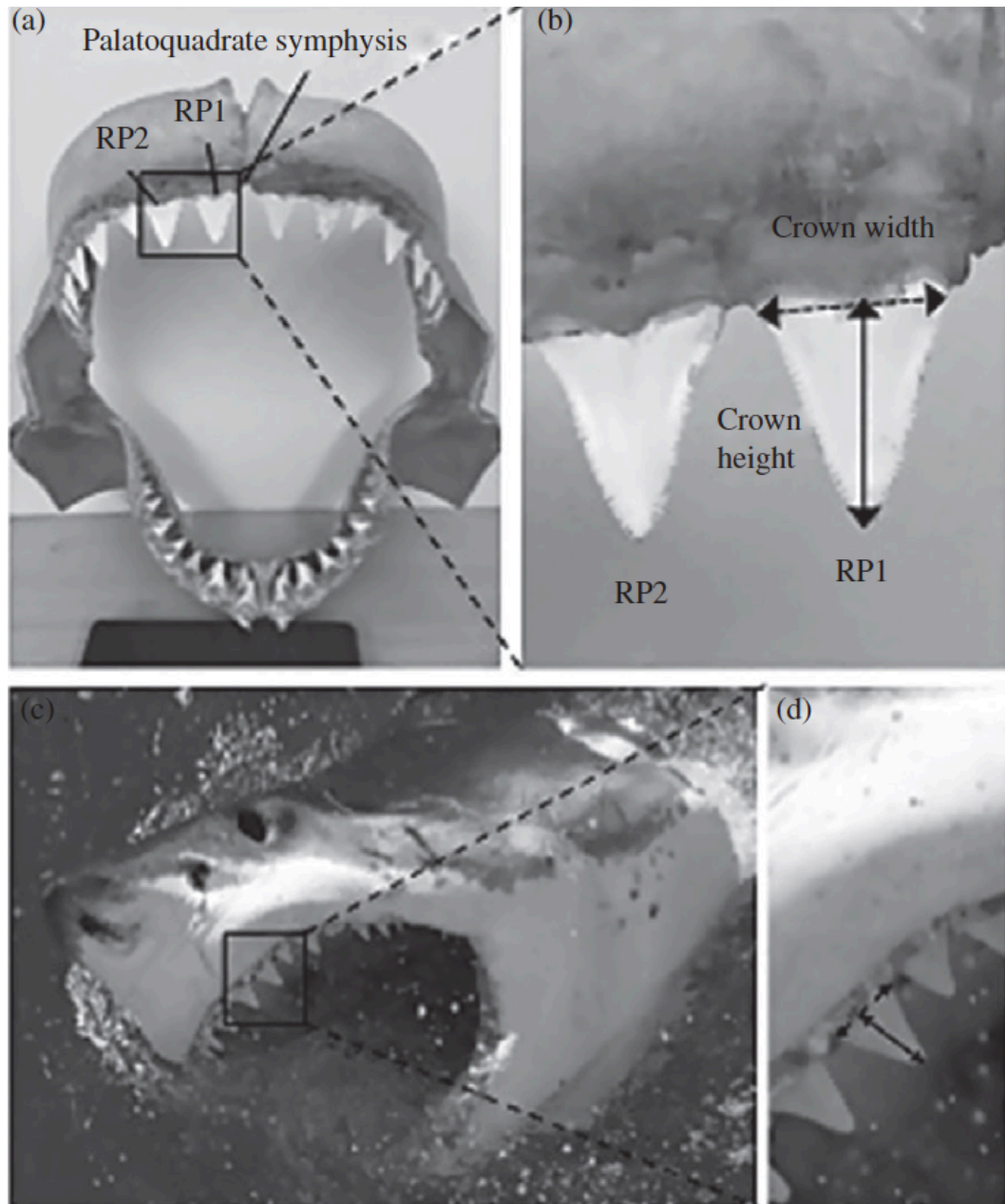
521 sexual differences in foraging strategy have been found (Huveneers et al. 2015; Towner
522 et al. 2016), and there are questions over whether the dietary shift occurs at all for some
523 individuals (Estrada et al. 2006; Hussey et al. 2012b). Tooth shape in adult white sharks
524 has also been reported as highly variable, with some large sharks retaining the more
525 cuspidate tooth shape of juveniles (Hubbell 1996; Castro 2012). However, the only
526 previous explicit investigations of tooth morphometrics in relation to sex and body
527 length included only tooth height (Randall 1973, 1987; Mollet et al. 1996; Shimada
528 2002b), a metric which does not capture tooth cuspidity. As tooth cuspidity is
529 considered to play an important role in the ontogenetic dietary shift, this leaves a
530 substantial gap in our understanding of the dynamics of this shift, including within and
531 between the sexes.

532 Morphological changes through ontogeny are difficult to measure in wild
533 animals, especially those inhabiting marine environments, and even more so in wide-
534 ranging apex predators. White sharks provide an excellent opportunity to study these
535 changes because their predictable aggregation at certain pinniped colonies, and the ease
536 with which they can be lured to boats and photographed makes photographic analysis of
537 live sharks a potentially valuable source of information on tooth morphology. Here we
538 examine the ontogenetic change in tooth cuspidity by integrating published data and
539 tooth measurements from jaws of dead sharks with a new non-invasive method of
540 quantifying tooth morphology for live sharks from photographs, and examine how the
541 ontogenetic change in tooth morphology differs between sexes and individuals.

542 **2.3. Methods**

543 **2.3.1. Tooth cuspidity**

544 Teeth are described as per the system detailed by Moyer et al. (2015) and Bemis et al.
545 (2015), in which teeth are given a code based on their location in the left or right side
546 of the jaw (L and R, respectively), in Meckel's or palatoquadrate cartilage (M and P,
547 respectively), and then numbered distally to medially, relative to the appropriate
548 symphysis (Figure 2.2a, 2.3a). We used measurements of tooth crown height and width,
549 as described in Hubbell, (1996), to calculate tooth cuspidity, dividing the crown height
550 by the crown width to produce what we have termed the tooth index value (Figure
551 2.2b). The presence of serrational cusplets are not mentioned in the published datasets,
552 and were not observed in any of the specimens that we measured. For analyses of the
553 relationship between tooth cuspidity and shark length, all tooth measurements were
554 taken from RP1 or LP1 teeth (Figure 2.2).



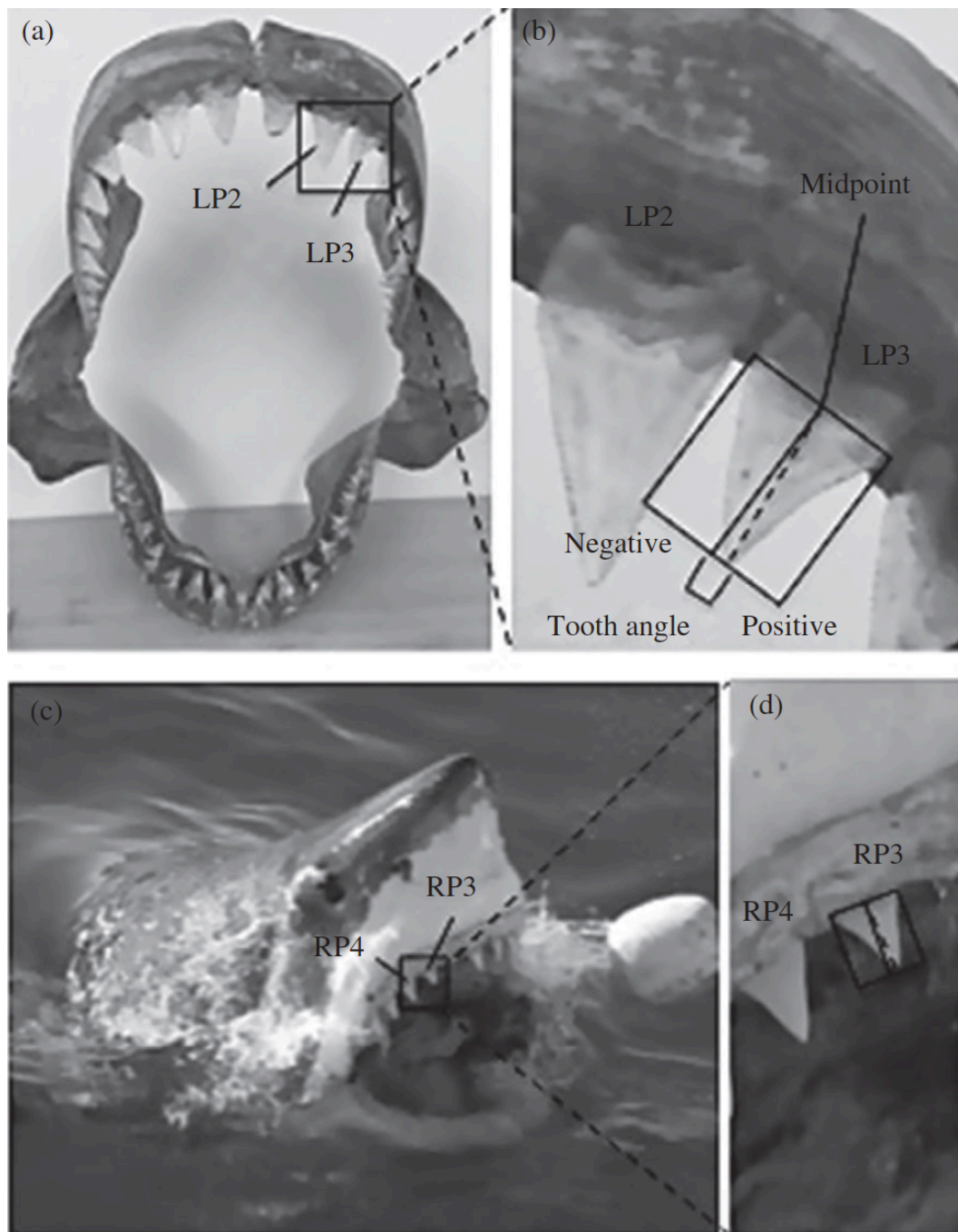
555 **Figure 2.2** Calculating tooth index value for RP1 and RP2 teeth (a) Position of first (1) and
 556 second (2) right (R) palatoquadrate (P) teeth in *Carcharodon carcharias* from a jaw held in the
 557 KwaZulu-Natal Sharks Board jaw collection, with (b) an enlarged view of RP1 and RP2
 558 showing crown height and base length measurements. (c) Photograph of a live *C. carcharias*
 559 showing left (L) P1 and LP2 teeth with (d) an enlarged view of the teeth showing height and
 560 base length measurements
 561 of the LP2 tooth.

562
 563 We included P1 data from 23 live sharks in Gansbaai, South Africa (34.5805° S,

19.3518° E), using a novel photographic method and ImageJ software (Abramoff et al. 2004) described below. We included measurements taken manually from teeth of 50 jaws in the jaw collection held by the KwaZulu-Natal Sharks Board (KZNSB) South Africa, and P1 crown height and width data from 55 sharks, published by Hubbell (1996), and Mollet et al. (1996), where in the latter, crown height was termed “UA1E2” and crown width “UA1W”). KZNSB sharks were caught as part of a bather safety program, and jaws either dried or frozen at time of measurement. The Gansbaai and KZNSB sharks both came from the same South Africa population. The sharks in Hubbell (1996) and Mollet et al. (1996) came from multiple populations (Australia-New Zealand, South Africa, northeast Pacific, northwest Atlantic).

2.3.2. Tooth angle

The intermediate upper tooth (R/LP3, Figure 2.3a, b, c, d) is markedly different in shape from the P1 and P2 teeth, in that it typically displays asymmetry, and an approximately straight medial edge (Applegate and Espinosa-Arrubarrena 1996; Hubbell 1996). The angle of the tip of the crown in comparison to the tooth midpoint shows greater variation in this tooth than the equivalent angles of the P1 and P2 teeth (Hubbell 1996), and was thus selected as another potential metric for analysing relationships between tooth morphology and shark length (Figure 2.3b, d). One P3 tooth per shark was selected, and ImageJ software was used to measure the angle (lateral or medial) of the tip of the tooth crown in relation to the midpoint of the tooth base (Hubbell 1996); Figure 2.3b, d). Medial inclinations were denoted by positive angles, and distal inclinations as negative (Figure 2.3b). We combined P3 angle measurements derived from photographs of live sharks (see below), and photographs of jaws held by the KZNSB, with data published by Hubbell (1996).



588 **Figure 2.3 Measuring P3 tooth angle** (a) Derivation of *Carcharodon carcharias* tooth
 589 angle from the third (3) left (L) palatoquadrate (P) (LP3) tooth from a jaw held in the KwaZulu-
 590 Natal Sharks Board jaw collection, with (b) an enlarged view of LP2 and LP3 teeth showing the
 591 tooth midpoint and tooth angle on the LP3 tooth. (c) Photograph of a live *C. carcharias*
 592 showing RP3 and RP4 teeth of a live shark with (d) an enlarged view of the teeth showing tooth
 593 angle measurement of the LP3 tooth.

594 **2.3.3. Shark length**

595 Shark lengths (total length) were directly measured for sharks in the KZNSB and
596 published datasets. For live sharks in Gansbaai, lengths were estimated in the field by
597 visually comparing the free-swimming sharks to an object of known length (a 4.7 m
598 length cage diving cage), fixed to the side of the boat, as has been done in many
599 previous studies (Kock et al. 2013; Towner et al. 2013a, 2016).

600 **2.3.4. Photographic method**

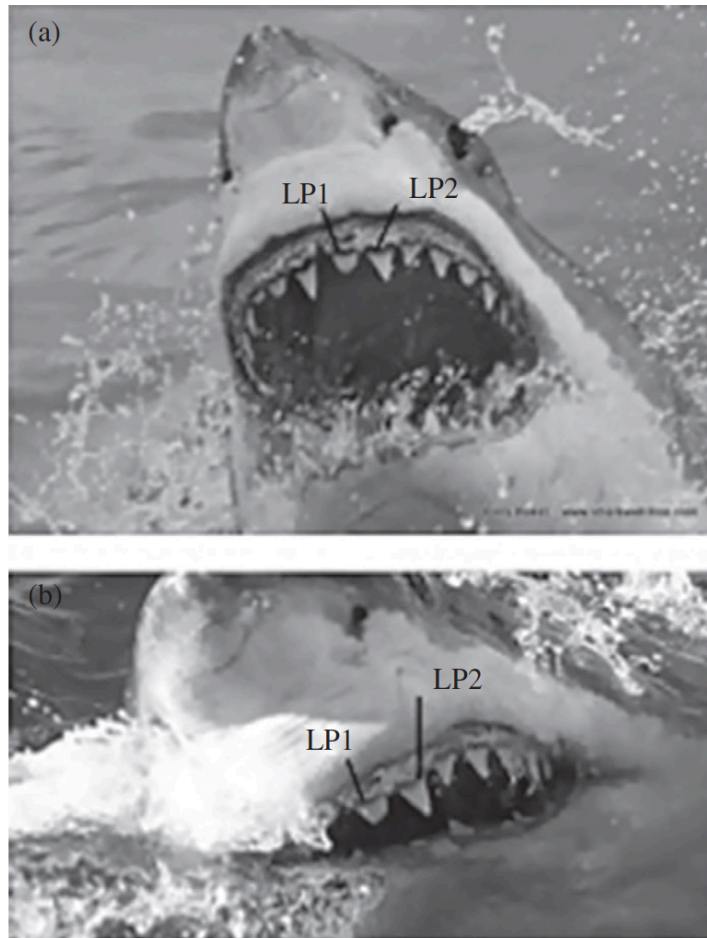
601 We took measurements of crown height, width, and angle from photographs of both live
602 sharks and KZNSB jaws (Figures 2.2c, d, 2.3, 2.4). Live sharks were photographed
603 from a cage diving vessel operated by Marine Dynamics, based in Gansbaai, South
604 Africa. The photographs were taken when sharks were interacting with stimuli (salmon
605 head bait and a wooden seal decoy), during three field trips: August-October 2014,
606 February-April 2015, and June 2015. Sharks were individually identified using
607 photographs of the first dorsal fin and DARWIN ID software, with digital traces of the
608 outline of the fin being matched by the software and confirmed by eye (Stanley 1995;
609 Towner et al. 2013b). We gave tooth images a quality score rating of 1–6, based on their
610 resolution, clarity and angle relative to the camera, and only images with a score of four
611 or above were included in analyses, based on the results of the repeatability of the
612 method, described below. These images were imported into ImageJ software where
613 measurements of crown height, crown width and tooth angle were taken in pixels.
614 Height and width measurements were taken three times, and averages used in the
615 calculation of tooth index values.

2.3.5. Statistical analyses

To investigate scaling relationships between shark length and P1 tooth index, both variables were \log_{10} transformed, sorted into male and female datasets, and analysed with linear regression. \log_{10} transformations are typically used to increase linearity of allometric relationships, which tend to form curves as they are a power function, e.g. (Huber et al. 2006; Kolmann and Huber 2009; Habegger et al. 2012). If the predicted isometric slope of 1 fell outside of the 95% confidence intervals of the regression slope, the relationship was considered allometric (Sokal and Rohlf 1995). To identify discrete tooth index groupings (e.g. pre- and post-ontogenetic shift and/or polymorphs) in P1 teeth, hierarchical cluster analyses were applied to P1 tooth index data. The NbClust function (Charrad et al. 2014) in R statistical software (version 3.2.4.) (R 2017) was used to identify the optimal number of clusters with which to perform the cluster analyses *a priori*. A Mann-Whitney U test and one-way ANOVA were applied to data from males and females, respectively, to test for differences in shark length between tooth clusters (male data were non-normal; female data had more than two clusters). Linear regression analyses were further applied separately to male and female P3 tooth angle and shark length data, and an isometric slope of 1 used to determine allometry. \log_{10} transformations were not used for these data, as they included negative and positive values.

We conducted tests of both accuracy and repeatability to determine the robustness of the photographic methodology (Jeffreys et al. 2013). We used the white shark jaw collection held by the KZNSB to assess the accuracy of our photographic method for measuring tooth cuspidity (Figure 2.2a, b). We measured LM1 and LM2 teeth of 35 jaws using a tape measure in situ, and used photographs of the same jaws to

640 measure the same teeth digitally, in pixels, using ImageJ software. We used linear
 641 regression to compare the tooth index values produced from manual and digital
 642 measurements. We further compared digital measurements, obtained from multiple
 643 photographs of the same teeth of live Gansbaai sharks, to assess the repeatability of our
 644 photographic method (Figure 2.4). This dataset included teeth from both the upper and
 645 lower jaw, in any position visible, provided the quality of the image met the
 646 requirements described above. The teeth of eleven individual sharks, totalling 12 unique
 647 teeth, each measured at least twice, were included in a repeatability calculation
 648 described by Lessells and Boag (1987). This calculation uses the mean square values
 649 produced by a one way analysis of variance (ANOVA) (IBM SPSS v22) ($MS_W =$
 650 within group variance, $MS_A =$ among group variance) as such; Repeatability (r) = $S^2_A /$
 651 $S^2 + S^2_A$, where $S^2 = MS_W$, $S^2_A = (MS_A - MS_W)/n_0$, $n_0 = [1/(a-1)] * [\sum n_i - \sum n_i^2 / \sum n_i]$, a
 652 = number of groups, and n_i = sample size of the i th group. Two repeatability scores were
 653 calculated: using teeth with a quality score of three and above ($n=46$), or four and above
 654 ($n=25$).



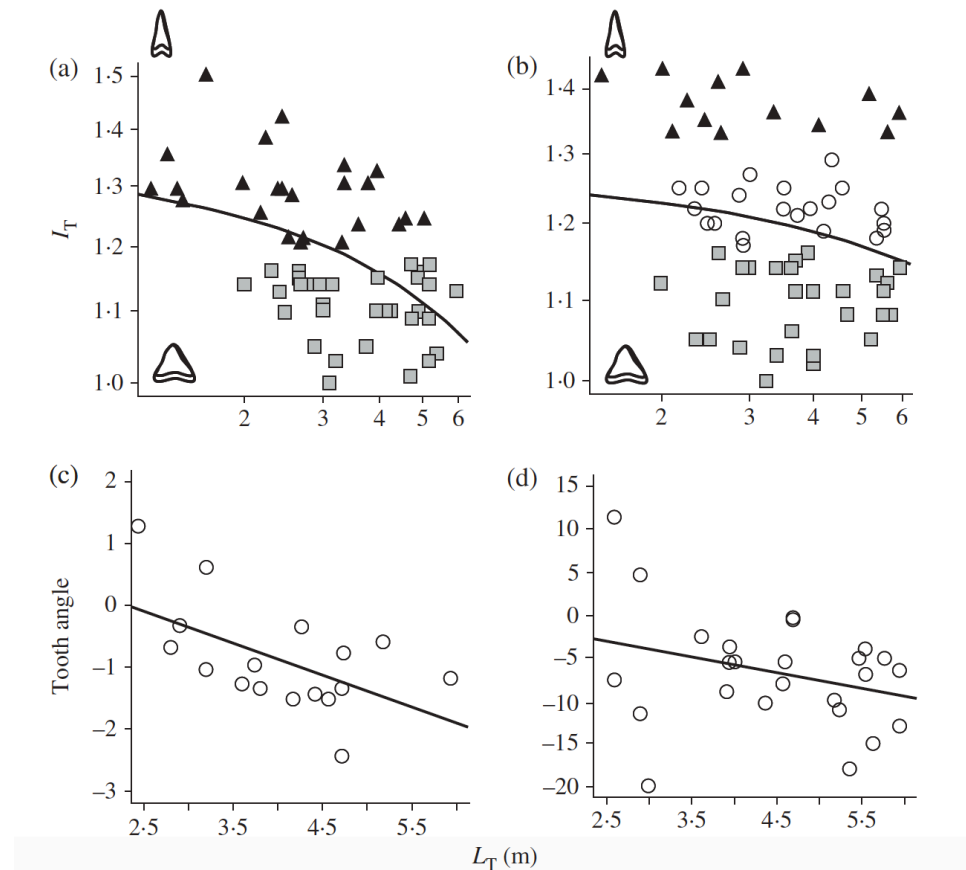
655 **Figure 2.4. Repeat photographs of the same tooth** (a) 15 March 2015 (K. Baker,
 656 www.sharkwatch.sa) and (b) 24 March 2015 showing position of first (1) and second (2) left (L)
 657 palatoquadrate (P) teeth in the individually identified *Carcharodon carcharias* 'Rosie II' used
 658 in the repeatability test of the photographic method.

659 2.4. Results

660 2.4.1. Tooth cuspidity and shark length

661 P1 tooth index in male white sharks was significantly related to body length (linear
 662 regression, $F_{1,55} = 20.6$, $P < 0.001$, 95% confidence interval on slope -0.17 and -0.07, R^2
 663 = 0.25), and was negatively allometric, with the predicted isometric slope of 1 being
 664 outside the 95% confidence intervals of the regression slope (Figure 2.5a). Tooth index

665 in female sharks also decreased significantly with body length (linear regression, $F_{1,61} =$
 666 4.0, $P = 0.05$, 95% confidence interval on slope -0.14 and -1.23, $R^2 = 0.05$), but showed
 667 isometry (predicted isometric slope of 1 was inside of the 95% confidence intervals)
 668 (Figure 5b). Additionally, there was much greater variability in the relationship for
 669 females than for males ($R^2 = 0.05$ and $R^2 = 0.25$, respectively) (Figure 2.5b).



670 **Figure 2.5 Relationships between \log_{10} P1 tooth index (I_T) and \log_{10} total body length (L_T)**

671 for (a) male and (b) female *Carcharodon carcharias*. Broad and cuspidate tooth types are
 672 illustrated on the y-axes. Males formed two clusters, with teeth that were relatively cuspidate
 673 (black triangles) or relatively broad (grey squares), whereas females formed three clusters, with
 674 teeth that were relatively cuspidate (black triangles), intermediate (open circles) or relatively
 675 broad (grey squares) (c) The relationships between the angle of the third palatoquadrate (P3)
 676 tooth and total body length (L_T) for male and (d) female *C. carcharias*.

677

678 **2.4.2. Tooth angle and shark length**

679 The angle of the P3 tooth was significantly related to shark length in male sharks (linear
 680 regression, $F = 6.85$, $P = 0.019$; 95% confidence interval on slope -0.94 and -0.1, $R^2 =$
 681 0.31) in an isometric relationship, as the predicted isometric slope was 1 (Figure 2.5c).
 682 In female sharks, the angle of the P3 tooth was not related to shark length (linear
 683 regression, $F = 2.62$, $P = 0.146$, 95% confidence interval on slope -4.35 and 0.69, $R^2 =$
 684 0.05) (Figure 2.5d). The P1 teeth of male sharks formed two clusters; one where teeth
 685 were relatively cuspidate, and another where teeth were broader (Figure 2.5a). The
 686 lengths of sharks in the two tooth clusters were significantly different (Mann-Whitney
 687 U test, $U = 191$, $P < 0.001$). Female P1 teeth separated into three clusters that
 688 represented cuspidate, intermediate and broad teeth (Figure 2.5b), and shark length did
 689 not significantly differ between these clusters (one way ANOVA, $F_{1,62} = 0.234$, $P =$
 690 0.63, 95% confidence interval on slope -0.14 and 0.22, $R^2 = 0.01$).

691 **2.4.3. Accuracy and repeatability of the photographic method**

692 There was a significant, positive relationship between the measurements taken directly
 693 from teeth and from photographs (P1 and P2: linear regression, $F_{1,34} = 43.02$, $P < 0.001$,
 694 95% confidence interval: 0.57 - 1.08, $R^2 = 0.57$; P1 only: linear regression, $F_{1,16} = 61.0$,
 695 $P < 0.001$, 95% confidence interval: 0.73 - 1.27, $R^2 = 0.8$) (Figure 2.6a and b,
 696 respectively). Digital images of only the P1 tooth were therefore substantially more
 697 accurate than those of the P2 tooth.

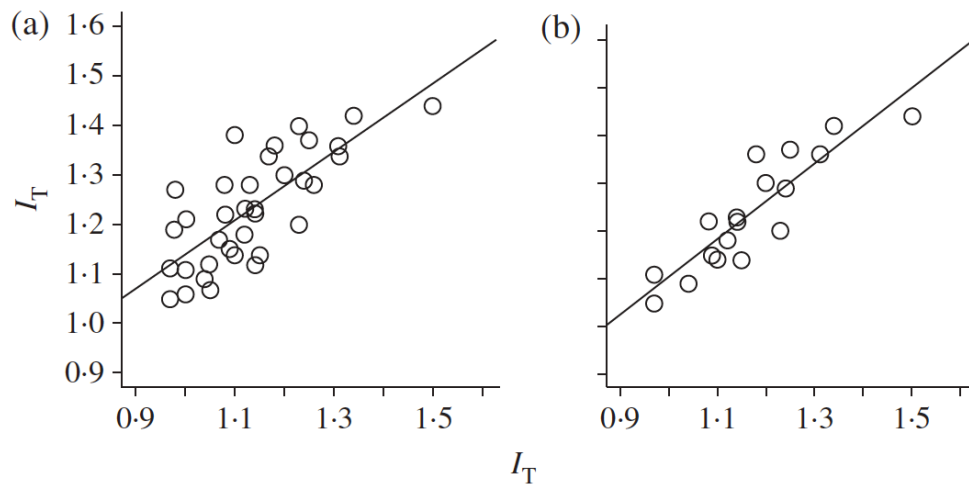


Figure 2.6 Accuracy regressions. Relationship between *Carcharodon carcharias* tooth index values (I_T) from (a) measurements from photographs of first (1) and second (2) palatoquadrate (P) teeth from photographs and (b) manual measurement of PI teeth of only *C. carcharias* caught by the KwaZulu-Natal Sharks Board.

Tooth measurements showed high repeatability, which was substantially greater when using images ranked four or more (Table 2.1), and therefore only those were considered in analyses of tooth index and shark length.

Table 2.1 Repeatability (R) of tooth index (I_T) values obtained from photographs of teeth with image quality scores (Q) ≥ 3 and ≥ 4 n , Number of images.

Q	n	Group mean I_T	d.f.	c.v. (%)	95% C.I.	R	P
≥ 3	46	1.09	45	0.092	1.17	0.57	<0.001
≥ 4	25	1.10	24	1.32	0.57	0.86	<0.001

709 **2.5. Discussion**

710 The results show that white sharks exhibit an ontogenetic shift in tooth shape, but that
711 this relationship differs between sexes, and shows substantial individual variation.
712 Males showed a distinct increase in P1 tooth breadth with length, and a change in angle
713 of the P3 tooth, both of which were far less pronounced in females. Measurements taken
714 from photos were accurate and repeatable, suggesting that use of photos of live sharks
715 could be a valuable source of data for future studies.

716 The results confirm that male white sharks undergo an ontogenetic shift in tooth
717 shape. Upper first teeth of male sharks become significantly more broad with increasing
718 shark length, showing negative allometry, and male sharks clustered into cuspidate and
719 broad-toothed groups that significantly differed in shark length, with the more cuspidate
720 group containing smaller sharks than the broad group. These two clusters likely
721 represent pre- and post-ontogenetic shift individuals. This ontogenetic change in white
722 sharks is commonly believed to facilitate the inclusion of marine mammals into their
723 diet (Tricas and McCosker 1984; Klimley 1985; Frazzetta 1988; Cliff et al. 1989;
724 Hubbell 1996; Estrada et al. 2006; Hussey et al. 2012b). The medial angle of the P3
725 tooth was also found to scale significantly with shark length in males, in an isometric
726 relationship. This tooth has been hypothesised to be a specialised tool for inflicting
727 large, disabling wounds on pinniped prey due to its shape and location on the strongest
728 part of the jaw (Martin et al. 2005). An increase in the distal inclination of the tooth tip,
729 as evidenced in males, could be a further adaptation for handling and despatching
730 marine mammals. Alternatively, this change in angle could assist in the handling of
731 females during copulation, during which male sharks bite females in the gill, head, and
732 pectoral regions (Kajiura and Tricas 1996; Pratt and Carrier 2001).

733 Although shark lengths in the cuspidate and broad clusters of males were
 734 significantly different, providing further evidence of a distinct change in tooth shape
 735 through ontogeny, there was significant variation and overlap in size. This indicates that
 736 there may be individual variation in the length at which male sharks undergo the
 737 ontogenetic shift. Males reach sexual maturity at a similar size to that at which they
 738 undergo the ontogenetic shift in tooth morphology (Cliff et al. 1989). This suggests that
 739 the ontogenetic shifts in diet and tooth shape are intrinsically linked to sexual maturity.
 740 In animals, individual variation in life history traits such as the onset of maturity,
 741 coupled with behavioural changes such as changes in habitat use and diet, can be
 742 components of a Pace-of-Life Syndrome, in which life-history trade-offs produce
 743 consistent behavioural differences in areas such as activity level, movement patterns,
 744 boldness and aggressiveness (Ricklefs and Wikelski 2002; Stamps 2007; Wolf et al.
 745 2007; Biro and Stamps 2008; Réale et al. 2010). For example, in the house mouse (*Mus*
 746 *musculus*), size and age at maturity is linked to activity level, growth rate, fecundity,
 747 adult body size, and longevity, with ‘fast paced’ mice being more active, faster growing,
 748 and reaching maturity at a smaller size and younger age than ‘slow paced’ mice (Wirth-
 749 Dzieciolowska et al. 1996; Wirth-Dzieciolowska and Czumińska 2000; Wirth-
 750 Dzieciolowska et al. 2005). The higher energetic needs of individuals which mature
 751 more quickly, require morphological and physiological adaptations that enable them to
 752 consume the necessary volume or type of sustenance (Biro and Stamps 2008). In the
 753 case of white sharks, this could pertain to broader teeth facilitating the incorporation of
 754 energy rich marine mammals into their diet. White sharks exhibit sexual and individual
 755 differences in migratory behaviour (Weng et al. 2007a; Block et al. 2011; Domeier and
 756 Nasby-Lucas 2012; Kock et al. 2013), that will affect the water temperatures individuals
 757 inhabit and, because white sharks are endothermic (Carey et al. 1982), therefore the

758 energetic demands of thermoregulation, producing individual variation in
759 energetic demands that may influence pace-of-life strategies. Elevated hunger and
760 activity levels increase risk of fishing mortality, and can lead to rapid depletion of fast
761 paced genotypes (Young et al. 2006; Biro and Post 2008; Mittelbach et al. 2014;
762 Härkönen et al. 2014).

763 Female white shark teeth were found to scale with isometry in relation to shark
764 length, and the observed level of variation made any overall relationship weak.
765 Additionally, the facts that the angle of the intermediate tooth did not scale with shark
766 length and the cluster analysis suggested three tooth groups as opposed to the two
767 groups in males, demonstrate that ontogenetic shifts in tooth shape likely differ between
768 males and females. That these tooth types were independent of shark length, suggests
769 that female white sharks may exhibit phenotypic polymorphism. Stable isotope analyses
770 suggest that some females do not undergo an ontogenetic dietary shift, and can show
771 consistent dietary specialisation instead (Estrada et al. 2006; Hussey et al. 2012b; Kim
772 et al. 2012; Pethybridge et al. 2014; Christiansen et al. 2015). However, the mechanism
773 behind such specialisation has not been elucidated. Tooth polymorphism facilitates
774 niche polymorphism in sympatric populations of some fish species (Meyer 1990b), and
775 has been linked to dietary specialisation in other shark species (Litvinov 1983; Litvinov
776 and Laptikhovsky 2005). As tooth shape is generally accepted to relate to the
777 exploitation of different prey types in white sharks (Tricas and McCosker 1984;
778 Frazzetta 1988; Hubbell 1996), it is reasonable to suggest that sharks with cuspidate,
779 intermediate or broad teeth feed preferentially on different prey, constituting trophic
780 polymorphism in females. Potential consequences of specialisation in white shark diets
781 include altered food web structure if changes in resource availability affect tooth
782 morphs differently (Christiansen et al. 2015), and differing levels of bioaccumulation of

783 toxins (Young et al. 2006; Biro and Post 2008; Mittelbach et al. 2014; Härkönen et al.
784 2014), an issue already known to pose a significant threat to white sharks generally
785 (Schlenk et al. 2005; Mull et al. 2013; Lyons et al. 2013a; Marsili et al. 2016). While we
786 cannot rule out geographic variation in female shark tooth shape, it seems less likely as
787 no such variation was evident in male teeth.

788 One of the major limitations in establishing the ontogenetic relationships
789 between morphology, diet and maturity, especially in threatened species, is sample size.
790 For sharks, the majority of tooth data currently available is from a limited number of
791 jaw collections, harvested from dead specimens. Our study shows that our novel
792 photographic method produces accurate and repeatable tooth shape data of live white
793 sharks in the field, providing that image quality is controlled, and these data can be used
794 to study the ontogenetic dietary shift. The increase in accuracy when comparing digital
795 and manual measurements of P1 teeth and pooled P1 and P2 teeth is likely due to
796 parallax error, induced by P2 teeth not being exactly front-on to the camera due to their
797 position in the jaw. This highlights the importance of ensuring that the position of the
798 tooth relative to the camera is directly parallel.

799 We have developed a non-lethal research method that can be used to provide
800 sample sizes that better elucidate the onset and occurrence of ontogenetic shifts within
801 and between populations, in addition to individual variation, sexual dimorphism and
802 polymorphism in white sharks, and potentially other sharks as well. Ontogenetic shift
803 dynamics are a major component of elasmobranch life history. Consideration of sexual
804 and individual variation in ontogenetic shift dynamics will therefore be important both
805 for understanding the ecology of a species, and for the development of effective
806 management strategies.

- 807 Chapter 3 Sex, size and isotopes; cryptic trophic
808 ecology of an apex predator, the white shark
809 *Carcharodon carcharias*

810 **3.1. Abstract**

811 Demographic differences in resource use are key components of population and species
812 ecology across the animal kingdom. White sharks (*Carcharodon carcharias*) are
813 migratory, apex predators, which have undergone significant population declines across
814 their range. Understanding their ecology is key to ensuring that management strategies
815 are effective. Here we carry out the first stable isotope analyses of free-swimming white
816 sharks in South Africa. Biopsies were collected in Gansbaai, (34.5805° S, 19.3518° E)
817 between February and July 2015. We used SIBER (Stable Isotope Bayesian Ellipses in
818 R) and traditional statistical analyses to quantify and compare isotopic niches of male
819 and female sharks of two size classes, and analyse relationships between isotopic values
820 and shark length. Our results reveal cryptic trophic differences between the sexes and
821 life stages. Males, but not females, were inferred to feed in more offshore or westerly
822 habitats as they grow larger, and only males exhibited evidence of an ontogenetic niche
823 shift. Lack of relationship between $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and female shark length may be caused
824 by females exhibiting multiple migration and foraging strategies, and a greater
825 propensity to travel further north. Sharks < 3 m had much wider, and more diverse
826 niches than sharks > 3 m, drivers of which may include individual dietary specialisation
827 and temporal factors. The differences in migratory and foraging behaviour between
828 sexes, life stages, and individuals will affect their exposure to anthropogenic threats,
829 and should be considered in management strategies.

830 **3.2. Introduction**

831 Patterns of resource use are a key component in the ecology of species, and such data
832 are vital for ensuring that wildlife management and conservation measures are
833 successful. Individual variation in resource use has been highlighted as a critical topic in
834 further understanding species, and community ecology (Bolnick et al. 2003, 2011;
835 Réale et al. 2010; Sih et al. 2012; Dall et al. 2012), particularly in the case of predators
836 (Schreiber et al. 2011), and is emerging as an important facet in the study of
837 elasmobranchs (Matich et al. 2011; Jacoby et al. 2014; Huvaneers et al. 2015; Matich
838 and Heithaus 2015; Towner et al. 2016). Ecological differences between males and
839 females in elasmobranchs are already recognised as prevalent (Sims 2005), and form
840 another important consideration in the understanding of their ecology, and consequently
841 their effective management. The niche concept (Hutchinson 1957), has been recognised
842 as a tool for quantifying resource specialisation and overlap between individuals, and
843 species (van Valen 1965; Kohn 1968; Cody 1974). This concept has recently been
844 reinvigorated by construction of the isotopic niche, in which stable isotope ratios of
845 Carbon and Nitrogen (in δ denomination) of study organism tissue are plotted in
846 bivariate space (Bearhop et al. 2004; Layman et al. 2007a; Newsome et al. 2007). The
847 isotopic constituents of an animal's tissues reflect the isotopic composition of the
848 organisms on which they feed, with nitrogen isotopes ($\delta^{15}\text{N}$) being considered to
849 provide reliable reflections of trophic position (Post 2002) and carbon isotopes ($\delta^{13}\text{C}$)
850 indicating habitat use (DeNiro and Epstein 1978).

851 The white shark (*Carcharodon carcharias*) is the world's largest non-filter
852 feeding fish (Compagno 2001), and is currently listed as Vulnerable on the IUCN Red
853 List (Fergusson et al. 2009), due to significant population declines, largely attributed to
854 targeted overfishing and bycatch, which has resulted in relatively small contemporary

855 populations across its range (Baum et al. 2003; Gubili et al. 2011; Blower et al. 2012;
 856 Nasby-Lucas and Domeier 2012; Towner et al. 2013b). Upon reaching approximately 3
 857 m in length, white sharks are thought to undergo an ontogenetic shift in diet, from being
 858 largely piscivorous to a greater emphasis on marine mammals (Tricas and McCosker
 859 1984; Casey and Pratt 1985; Cliff et al. 1989; Compagno 2001; Hussey et al. 2012b).
 860 There is suggestion of individual dietary specialisation in white sharks (Estrada et al.
 861 2006; Hussey et al. 2012b; Carlisle et al. 2012; Kim et al. 2012; Hamady et al. 2014;
 862 Pethybridge et al. 2014; Christiansen et al. 2015), evidence of individual variation in
 863 predatory behaviours (Huvaneers et al. 2015; Towner et al. 2016), and sexual
 864 differences in movement patterns (Pardini et al. 2001; Anderson and Pyle 2003;
 865 Domeier and Nasby-Lucas 2007; Weng et al. 2007a; Jorgensen et al. 2010; Domeier
 866 and Nasby-Lucas 2012; Robbins and Booth 2012; Bruce and Bradford 2012; Kock et al.
 867 2013).

868 The South African population of white sharks has five main coastal aggregation
 869 sites (from west to east: False Bay, Gansbaai, Struisbaai, Mossel Bay and Algoa Bay).
 870 These aggregations are not genetically distinct (Andreotti et al. 2015), with sharks
 871 migrating between them, and further along the South African coast to KwaZulu-Natal
 872 (KZN), Mozambique, and the western Indian Ocean (Cliff et al. 1989; Ferreira and
 873 Ferreira 1996; Bonfil et al. 2005; Jewell et al. 2011). Some segregation by shark size is
 874 apparent between the sites, with average size typically increasing from west to east
 875 (Cliff et al. 1989; Ferreira and Ferreira 1996; Dicken 2008; Kock et al. 2013; Towner et
 876 al. 2013a; Rykief et al. 2014; Hewitt et al. 2018). Apart from Struisbaai, these
 877 aggregation sites are typified by the presence of large pinniped colonies (Dudley 2012).
 878 Mature females are largely absent from all of these aggregations, instead being recorded

879 in the more tropical waters of the Western Indian Ocean (Cliff et al. 2000; Bonfil et al.
880 2005).

881 Previous studies of diet in South African white sharks, both through gut content
882 analysis and isotopic analyses, have been based on samples from relatively small sharks
883 caught in the nets of a bather safety programme managed by the KZN Sharks Board
884 (Cliff et al. 1989; Hussey et al. 2012b; Christiansen et al. 2015), and have not included
885 an analysis of niche space. Christiansen et al. (2015) have urged that isotopic results be
886 interpreted within a multidisciplinary framework, in order to obtain the most accurate
887 and useful data, from which management decisions can be deduced. Biopsy sampling
888 provides a non-lethal method of collecting shark tissue for stable isotope analysis,
889 which may be of particular benefit for elasmobranchs, many of which are undergoing
890 severe population declines at a global scale and require informed conservation
891 management (Myers and Worm 2003; Worm et al. 2013; Dulvy et al. 2014). Here, in
892 addition to traditional statistics, we use metrics derived from stable isotope bivariate
893 plots (Layman et al. 2007a; Jackson et al. 2011) to visualise and quantify the variation
894 in niche among potential pre and post ontogenetic shift male and female sharks, and
895 interpret our results in the context of published diet, telemetry, sighting and capture
896 data, in the first isotopic study of free-swimming white sharks in South Africa.

897 **3.3. Methods**

898 **3.3.1. Data collection**

899 Tissue biopsy samples were collected from white sharks between February and July
900 2015, within the designated white shark cage-diving area in Gansbaai, South Africa.
901 Collection took place from either a 9 m research catamaran or a 14 m custom-built

902 shark cage-diving catamaran, owned and operated by the Dyer Island Conservation
 903 Trust and Marine Dynamics Shark Tours. Sharks were brought close to the vessels
 904 using fish oil chum and a salmon head bait lure. Photographs were taken for individual
 905 identification based on distinguishing marks and DARWIN dorsal fin ID software
 906 (<http://darwin.eckerd.edu/>). Finn Larsen Ceta darts (4 x 0.9 cm) affixed to a biopsy pole
 907 were used to excise cores of tissue, comprising muscle and dermis, from the dorsal
 908 surface of free-swimming sharks.

909 Shark sex was classified by the presence or absence of claspers, and only
 910 samples from the 26 sharks of known sex were included in the study. Shark total length
 911 was estimated by comparison of free-swimming sharks with a 4.7 m object of known
 912 length (Kock et al. 2013; Towner et al. 2013a). For the SIBER analyses (see below)
 913 sharks were classified as either < 3 m (six females, five males), or > 3 m (ten females,
 914 five males) to reflect pre-and post-ontogenetic shift life stages (Tricas and McCosker
 915 1984; Casey and Pratt Jr 1985; Cliff et al. 1989; Compagno 2001; Hussey et al. 2012b).

916 **3.3.2. Stable isotope analysis**

917 Twenty-six samples were prepared for stable isotope analysis. Muscle and dermis have
 918 different isotopic turnover rates, and muscle isotopic turnover can take up to two years
 919 (Martinez del Rio et al. 2009; Logan and Lutcavage 2010; Hussey et al. 2012c). Only
 920 muscle was used for analysis. Ethanol was removed from the tissues by blowing with
 921 nitrogen for 20 min at 30°C using a Techne dri-block DB.2A, and samples were freeze-
 922 dried overnight. Storage of fish muscle in ethanol causes small but directionally uniform
 923 changes to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Arrington and Winemiller 2002), and so would not
 924 affect between-sample comparisons. Dried samples were homogenised using scissors,
 925 weighed and placed into tin capsules. Lipid and urea extraction are recommended prior

to isotope analysis of elasmobranch tissues as presence of lipids, trimethylamine and urea can affect isotopic values and ratios, which precludes accurate estimation of trophic position and diet reconstruction (Fisk et al. 2002; Hussey et al. 2012a). Lipid and urea extraction were not performed, because our main aim was to perform comparative analyses within our own samples, and no effect of increasing animal size has been detected (Hussey et al. 2012a).

Stable isotope ratios were measured using continuous flow isotope ratio mass spectrometry using a Sercon Integra integrated elemental analyser and mass spectrometer. Stable isotope ratios are reported as δ -values and expressed in ‰, according to the following: $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, and R_{standard} is the ratio of the international references PDB for carbon and AIR for nitrogen. Replicate analyses of internal lab standard alanine yielded standard deviations of 0.15‰ for $\delta^{15}\text{N}$ and 0.09‰ for $\delta^{13}\text{C}$. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data were averaged between the two analytical runs and tested for outliers using the package ‘Outliers’ (Komsta 2011) in R statistical software version 3.3.1., which was used for all analyses (R 2017). Data points that fell outside of 95% of the normal distribution were removed to create an ‘outlier-removed’ dataset.

3.3.3. Statistical analysis

General linear models (glms) were used to assess the relationship between outlier-removed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, and for relationships between and shark total length (m) and sex respectively. Models specified a Gaussian distribution and identity link function, and all two-way interactions were included in the full models. Backwards step-wise elimination of variables, using Akaike Information Criterion (AIC) (Akaike 1973), and variable significance, was used to pare models. F-values were produced by

950 comparing full and null models in an ANOVA. Differences in median $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
 951 between the sexes were analysed for both averaged and outlier-removed data sets, using
 952 independent samples Mann Whitney U tests, and differences in the variance of these
 953 data were tested using a Fligner-Killeen test. For the statistical analyses described
 954 above, P-values were considered significant if ≤ 0.05 . To investigate dietary
 955 specialisation, we used the pamk function in R package ‘fpc’ to determine the optimal
 956 number of clusters for a k-means cluster analysis of averaged $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and
 957 averaged $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with outliers removed. This method uses optimum average
 958 silhouette width to suggest the number of data clusters based on mediods (Hennig
 959 2015).

960 We used the SIBER package in R to compute the size and overlap of isotopic
 961 niches for < 3 m, and > 3 m male and female sharks, and compared results produced
 962 from analyses run with averaged, and outlier-removed data sets (Jackson et al. 2011).
 963 Isotopic niches based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were plotted in SIBER, and values of niche size
 964 produced from estimates of small sample size corrected standard ellipse areas (SEAc)
 965 and total area (TA) of convex hulls. Bayesian estimates of standard ellipse area were
 966 generated using 10000 repetitions, and the probabilities of each demographic group
 967 (“Group A”) being smaller than the other demographic groups in turn (“Group B”) were
 968 calculated and plotted with 50%, 75% and 95% credible intervals. Layman metrics were
 969 computed for each group, providing values of nitrogen range (NR), carbon range (CR),
 970 mean distance to centroid (CD), mean nearest neighbour distance (MNND), and the
 971 standard deviation of MNND (SDMNND) (Layman et al. 2007a). Wider nitrogen and
 972 carbon isotope ranges suggest wider trophic diversity and a greater number of basal
 973 food sources exploited respectively, while CD provides a metric of the average degree
 974 of trophic diversity. MNND gives a measure of trophic similarity within each group,

975 where smaller numbers would indicate that individuals within a group have more
 976 similar ecologies, and SDMNNND provides a similar measure, but less influenced by
 977 sample size. Isotopic niche overlap was calculated as the % of a group's SEAc that
 978 overlapped with the SEAc of another group.

979 **3.4. Results**

980 **3.4.1. General linear models of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, sex and length**

981 Two $\delta^{13}\text{C}$ and two $\delta^{15}\text{N}$ outliers (each from a separate individual, all juveniles) were
 982 identified, resulting in 24 samples being included in glm analyses, and 22 included in
 983 SIBER analyses. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were significantly related (Linear regression: R^2
 984 = 0.15, $F_{(1,20)} = 4.66$, $P = 0.043$, confidence interval on the slope 0.01 and 0.69; Figure
 985 3.1a), with larger males in particular exhibiting a conspicuous linear trend. There was
 986 no effect on $\delta^{15}\text{N}$ of shark sex or length (General linear model: $F_{(1,2)} = 0.89$, $P = 0.24$),
 987 but there was a significant interaction between the effects of shark sex and length on
 988 $\delta^{13}\text{C}$ (General linear model: $F_{(1,2)} = 3.57$, $P = 0.018$). There was no relationship between
 989 $\delta^{13}\text{C}$ and female length (Figure 3.2a), but it was negatively correlated with the length of
 990 male sharks (Figure 3.2b). There was also no relationship between $\delta^{15}\text{N}$ and female
 991 length (Figure 3.2c), but while there was no significant relationship between $\delta^{15}\text{N}$ and
 992 male length, there was a decreasing trend (Figure 3.2d). Overall, neither $\delta^{13}\text{C}$ nor $\delta^{15}\text{N}$
 993 differed between males and females (averaged data: Mann-Whitney $U = 70$, $N_1 = 10$,
 994 $N_2 = 16$, $P = 0.91$, and $U = 71$, $N_1 = 10$, $N_2 = 16$, $P = 0.86$, respectively; outlier-
 995 removed data: Mann-Whitney $U = 86$, $N_1 = 9$, $N_2 = 15$, $P = 0.78$, and $U = 87$, $N_1 = 9$,
 996 $N_2 = 15$, $P = 0.74$ respectively), and the variances of the data also did not differ
 997 between males and females for either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (averaged data, $df = 1$ in all cases:

998 Chi-Square test: $\chi^2 < 0.001$, $P = 0.95$; $\chi^2 = 0.52$, $P = 0.47$ respectively; outlier-removed
 999 data: Chi-square test: $\chi^2 = 0.005$, $P = 0.95$; $\chi^2 = 0.516$, $P = 0.47$ respectively).

1000 **3.4.2. Cluster analysis**

1001 The pamk function revealed that paired $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data split optimally into three
 1002 clusters for the averaged data, heavily influenced by the inclusion of outliers (Figure
 1003 3.1a). Cluster one comprised sharks with moderate $\delta^{15}\text{N}$, and low $\delta^{13}\text{C}$ values, while
 1004 cluster 2 was typified by sharks with relatively high $\delta^{15}\text{N}$ and moderate to high $\delta^{13}\text{C}$,
 1005 and cluster three contained juveniles with low $\delta^{15}\text{N}$ but relatively high $\delta^{13}\text{C}$ values
 1006 (Figure 3.1a). In the outlier-removed dataset, the data split into two clusters, where
 1007 sharks grouped into cluster two exhibited slightly higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than
 1008 cluster 1 (Figure 3.1b). The average lengths of female sharks within these clusters were
 1009 almost identical (3.6 and 3.68m respectively), but there was a distinctive difference in
 1010 the average male shark lengths of the two clusters (3.67 and 3.0m respectively). As this
 1011 dataset was less biased by outlying data points, it likely reflects a more accurate
 1012 clustering of the isotopic data within the Gansbaai aggregation.

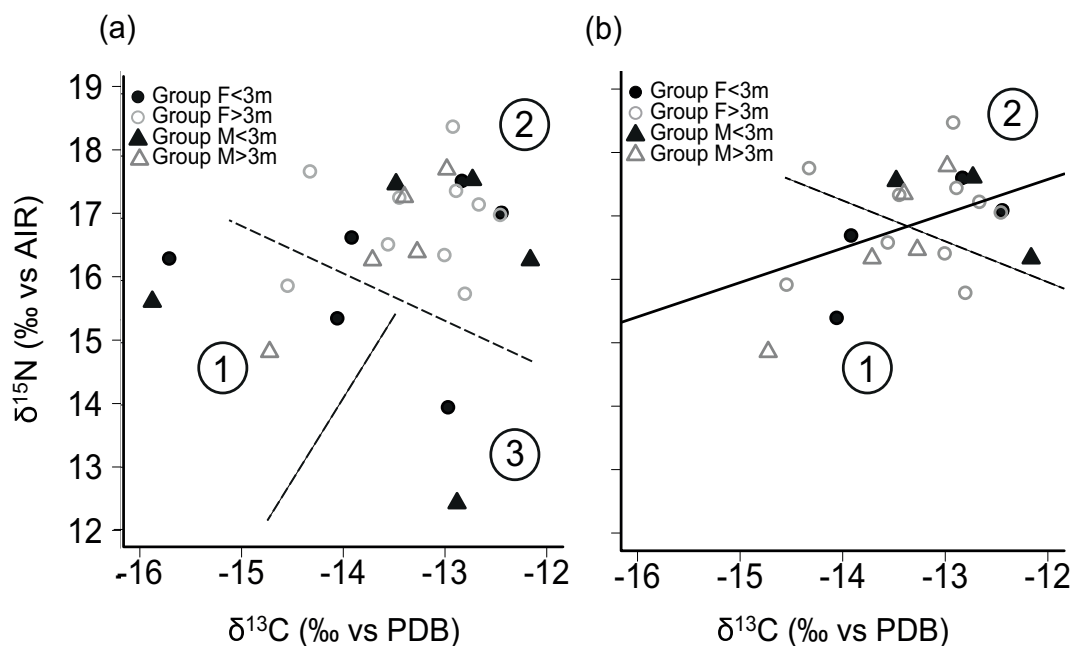


Figure 3.1 Cluster and regression analyses a) K-means cluster analysis of averaged

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data for white sharks (*Carcharodon carcharias*) from the Gansbaai aggregation

separated by sex and size category: female sharks < 3 m (closed black circles $n = 6$), female

sharks > 3 m (open grey circles $n = 10$), male sharks < 3 m (closed black triangles $n = 5$), and

male sharks > 3 m (open grey triangles $n = 5$). Three clusters were indicated in the analysis (1,

2, 3 demarcated by a dashed line) b) Linear regression ($y = 0.35x - 19.17$, $R^2 = 0.15$, $P = 0.043$)

and k-means cluster analysis results of averaged and outlier-removed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data; female

sharks < 3 m (closed black circles $n = 4$), female sharks > 3 m (open grey circles $n = 10$), male

sharks < 3 m (closed black triangles $n = 3$), and male sharks > 3 m (open grey squares $n = 5$);

two clusters were indicated by the analysis (1 and 2, demarcated by a dashed line).

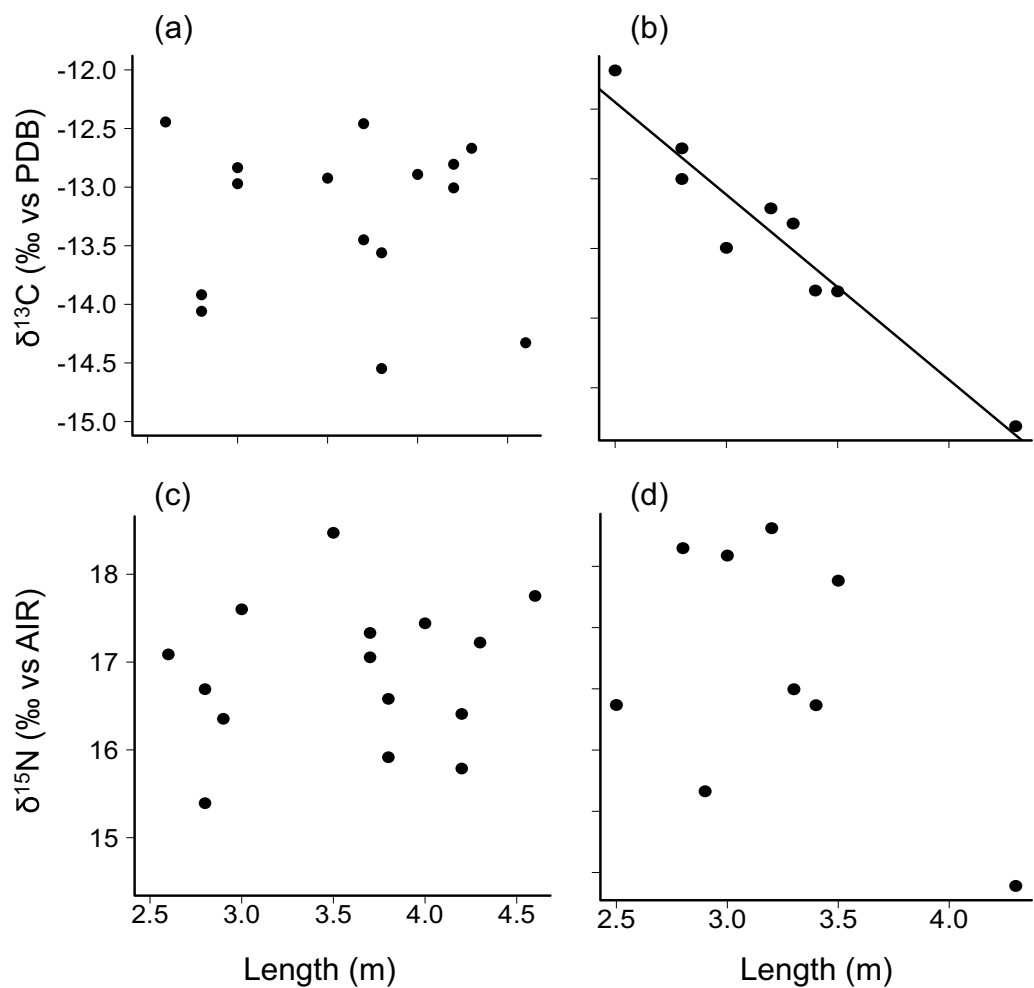


Figure 3.2 Relationships between male and female shark length and stable isotope values
a) female length and $\delta^{13}\text{C}$, b) male length and $\delta^{13}\text{C}$, c) female length and $\delta^{15}\text{N}$, and d) male length and $\delta^{15}\text{N}$, for white sharks sampled at the Gansbaai aggregation.

3.4.3. SIBER analysis

In the averaged data, both female and male sharks > 3 m had markedly smaller isotopic niche regions than sharks < 3 m, as indicated by estimates of SEAc, TA, and probabilities generated by SIBER analysis (Tables 3.1 and 3.2, Figure 3.3a). Large (> 3 m) males had the smallest isotopic niche, while small (< 3 m) males had the largest, and greatest trophic diversity (Tables 3.1 and 3.2). The greatest difference in isotopic niche size was for smaller males, with the niche of male sharks < 3 m being significantly

larger than that of males or females > 3 m at the 75% credible interval limit (Figure 3.3b), and overlapping all other groups by 100% (Table 3.3). The smallest overlap in SEAc was between larger and smaller males, with males > 3 m only overlapping with 9.02% of the niche for males < 3 m. Smaller females had 1.6 times greater overlap with larger females than they did with larger males, and overlap between larger and smaller females was three times greater than the overlap between larger and smaller males. Both nitrogen and carbon ranges were greater in smaller sharks, and values of CD, MMND and SDNND showed that for the most part, larger sharks had the least trophic diversity, most similar ecologies, and even distribution of trophic niches (Table 3.1).

The isotopic niches of < 3 m sharks were greatly reduced in the outlier-removed dataset (Table 3.1, Figure 3.3c), and Layman metrics became roughly similar across groups (Table 3.1, Figure 3.3d). The biggest change in isotopic niche overlap was between larger males and smaller females, which changed from 92.5% to 8.7% with the removal of outliers. However, females consistently exhibited greater niche overlap than males, and females < 3 m had much greater overlap with males < 3 m than was true for males > 3 m.

Table 3.1: Layman metrics and standard ellipse areas (SEAc) generated for female white sharks less than 3 m in length ($F < 3$), females over 3 m ($F > 3$), males less than 3 m ($M < 3$) and males over 3 m ($M > 3$). TA = convex hull total area; SEAc = small sample size corrected standard ellipse area; NR = range of $\delta^{15}\text{N}$ values; CR = range of $\delta^{13}\text{C}$ values; CD = mean distance to centroid; MNND = mean nearest neighbour distance; SDNND = standard deviation of nearest neighbour distance. White cells = averaged $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data, grey cells = averaged and outlier-removed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data.

Group	TA	SEAc	NR	CR	CD	MNND	SDNND
F < 3m	5.89	6.13	3.64	3.27	1.5	1.26	0.52
F > 3m	4.08	2.05	2.68	2.1	0.94	0.64	0.33
M < 3m	10.24	12.87	5.19	3.72	2.16	1.99	1.46
M > 3m	0.96	1.15	2.92	1.74	0.97	0.78	0.57
F < 3m	1.39	2.24	2.21	1.62	1.02	0.98	0.38
F > 3m	4.08	2.05	2.68	2.1	0.94	0.64	0.33
M < 3m	0.50	1.81	1.28	1.32	0.76	0.97	0.37
M > 3m	0.96	1.15	2.92	1.74	0.97	0.78	0.57

Table 3.2: SEAc size probabilities. Probability that the standard ellipse area (SEAc) of the isotopic niche of each sex-size demographic group of white sharks ("Group A") was smaller than the other groups ("Group B"). Probabilities are for female (F) or male (M) white sharks < 3 m or > 3 m in total body length. White cells = averaged $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data, grey cells = averaged and outlier-removed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data.

	Group B			
Group A	F < 3m	F > 3m	M < 3m	M > 3m
F < 3m		0.06	0.83	0.15
F > 3m	0.94		1.00	0.63
M < 3m	0.17	0.00		0.03
M > 3m	0.85	0.37	0.97	
F < 3m		0.32	0.45	0.45
F > 3m	0.68		0.61	0.63
M < 3m	0.55	0.39		0.50
M > 3m	0.55	0.37	0.50	

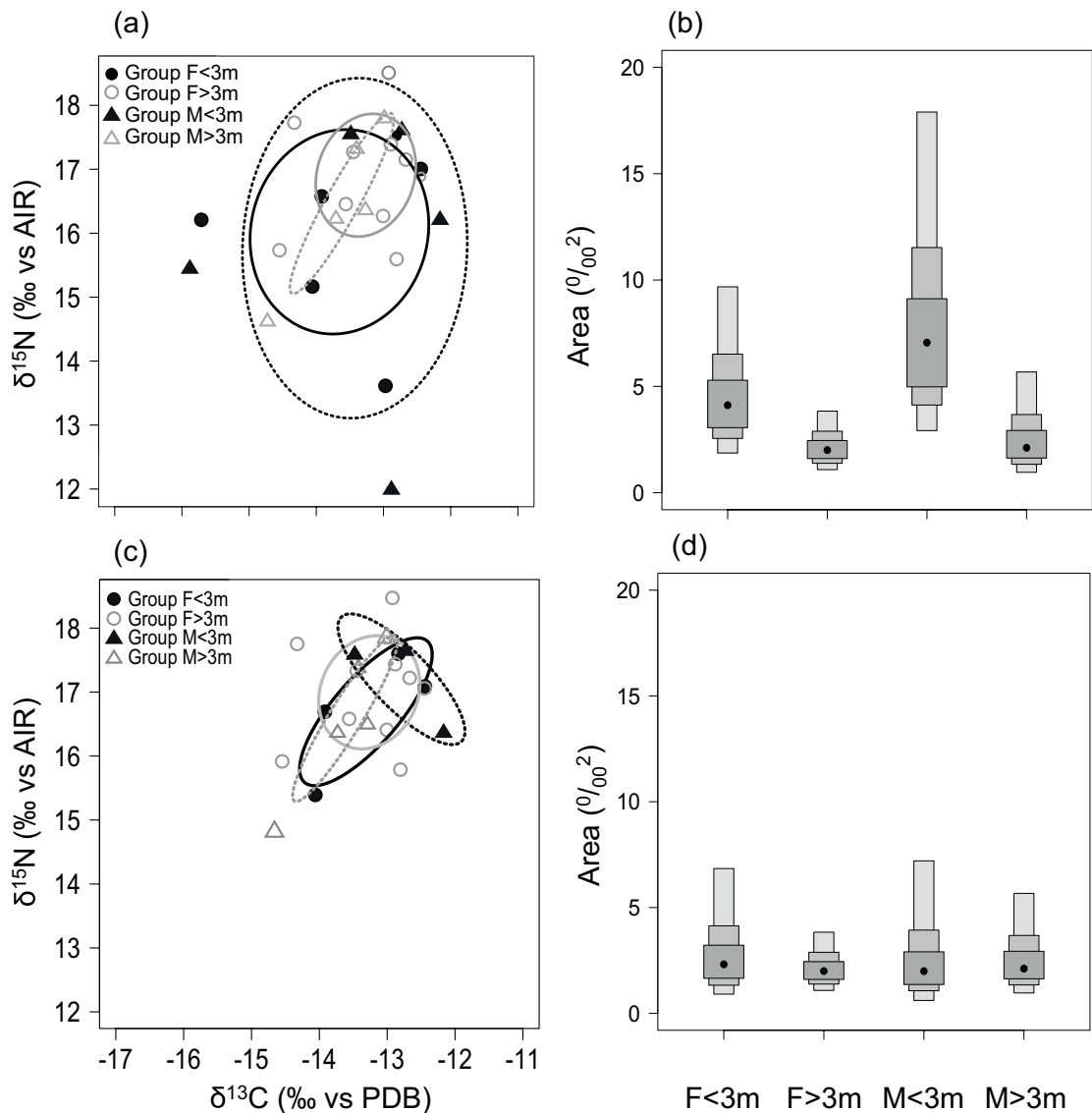


Figure 3.3: Isotopic niches of 22 white sharks sampled at the Gansbaai aggregation.

a) SIBER generated biplots of averaged $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with small sample size corrected standard ellipse areas (SEAc) for female sharks < 3 m (closed black circles, solid black line $n = 6$), female sharks > 3 m (open grey circles, solid grey line $n = 10$), male sharks < 3 m (closed black triangles, dashed black line $n = 5$), and male sharks > 3 m (open grey triangles, dashed grey line $n = 5$). b) Credible intervals (95%, 75%, 50%) of Bayesian estimates of SEAc for averaged $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for female sharks < 3 m, female sharks > 3 m, male sharks < 3 m, male sharks > 3 m. c) Averaged and outlier-removed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with small sample size corrected standard ellipse areas (SEAc), for female sharks < 3 m (closed black circles, solid black line $n = 4$), female sharks > 3 m (open grey circles, solid grey line $n = 10$), male sharks < 3 m (closed black triangles, dashed black line $n = 3$), and male sharks > 3 m (open grey

triangles, dashed grey line $n = 5$). d) Credible intervals (95%, 75%, 50%) of Bayesian estimates of SEAc for averaged and outlier-removed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for female sharks < 3 m, female sharks > 3 m, male sharks < 3 m, male sharks > 3 m.

Table 3.3: Percentage overlap of SEAc for a sex-size demographic group of white sharks (Group A) with the SEAc of the other groups (Group B). Percentages are for female (F) or male (M) white sharks less than 3 m or over 3 m in total body length. White cells = averaged $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data, grey cells = averaged and outlier-removed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data.

	Group B			
Group A	F < 3m	F > 3m	M < 3m	M > 3m
F < 3m		28.21	100.00	17.28
F > 3m	84.52		100.00	35.67
M < 3m	47.65	15.93		9.02
M > 3m	92.50	63.71	100.00	
F < 3m		66.07	32.14	44.64
F > 3m	72.20		43.90	35.61
M < 3m	39.78	49.72		14.92
M > 3m	8.70	63.48	23.48	

3.5. Discussion

Our results reveal isotopic differences between sexes of white sharks. Male sharks exhibited clear change in $\delta^{13}\text{C}$ with increasing shark length, while females retained a more homogenous isotopic niche through ontogeny. Male $\delta^{15}\text{N}$ values also showed a decreasing trend with increasing shark length, and $\delta^{15}\text{N}$ values were significantly related to $\delta^{13}\text{C}$ for outlier-removed shark data. Averaged data revealed differences in niche size between size classes of shark, which were greatly reduced when outliers were removed. Though SIBER sample sizes were comparatively small, we believe that the results can still provide useful insights, especially when interpreted within the context of available literature.

1095 The change in $\delta^{13}\text{C}$ values with increasing male length, the evident male length
 1096 differences between clusters based on both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data, and the significant
 1097 relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ overall, are indicative of an ontogenetic change in
 1098 food web, and potentially a concurrent change in diet, in male sharks. Our $\delta^{13}\text{C}$ results
 1099 suggest that males either feed further offshore, or in more westerly coastal habitats as
 1100 they age (Hill et al. 2006; Hill and McQuaid 2008), which could explain the observed
 1101 relative lack of males caught in KZN, and a paucity of males at the Western Cape in the
 1102 summer (Cliff et al. 2000; Kock et al. 2013; Towner et al. 2013a). Previous studies in
 1103 South Africa and globally have also shown that white sharks utilise offshore areas more
 1104 as they age (Boustany et al. 2002; Bonfil et al. 2005; Bruce et al. 2006; Weng et al.
 1105 2007a; Domeier and Nasby-Lucas 2008; Bonfil et al. 2010; Hussey et al. 2012b; Smale
 1106 and Cliff 2012; Carlisle et al. 2012; Hoyos-Padilla et al. 2016), but have not detected
 1107 the male bias evident in our results. While we did not find a significant relationship
 1108 between male length and $\delta^{15}\text{N}$, males, and particularly those > 3 m, did show an overall
 1109 trend for depletion of $\delta^{15}\text{N}$ with increasing length, which may have been weakened by a
 1110 relatively small sample size. Depletion in $\delta^{15}\text{N}$ has been found previously in the largest
 1111 white sharks of other studies, and suggests that pelagic prey items are an important part
 1112 of male diet as they age (Hussey et al. 2012b; Smale and Cliff 2012; Carlisle et al.
 1113 2012).

1114 Females did not exhibit the relationships between length and $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ found
 1115 in males, which could be due to multiple factors. Satellite tracking and sighting data of
 1116 South African white sharks indicates that only large individuals cross the Mozambique
 1117 Basin to Madagascar, with only mature females travelling up to the northern Mascarene
 1118 Plateau (Cliff et al. 2000; Zuffa et al. 2002; OCEARCH 2017). Our muscle samples
 1119 represent a relatively slow isotopic turnover rate, and therefore long-term diet and

1120 habitat use (MacNeil et al. 2006), comprising the average isotopic uptake over up to two
 1121 years (Martinez del Rio et al. 2009; Logan and Lutcavage 2010; Hussey et al. 2012a). If
 1122 females are roaming over a larger area than males, as appears the case in South Africa
 1123 and as has been found in the northeastern Pacific population (Jorgensen et al. 2010;
 1124 Domeier and Nasby-Lucas 2012), a greater degree of averaging of the $\delta^{13}\text{C}$ signatures
 1125 of several habitats is likely, resulting in less clear cut trends. Alternatively, the lack of
 1126 relationships for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and female shark length could be explained by
 1127 dietary specialisation, which has been identified in northeastern Pacific and Australian
 1128 white sharks (Kim et al. 2012; Pethybridge et al. 2014). Specialisation on piscine prey
 1129 and/or lack of ontogenetic dietary shift in females is further suggested by the fact that
 1130 females within the two clusters identified in the outlier-removed data were of the same
 1131 average length, and that large females consistently exhibited greater isotopic niche
 1132 overlap with smaller sharks than larger males did. Additionally, females lack a
 1133 significant ontogenetic change in tooth shape (French et al. 2017) which is reported to
 1134 facilitate a change in diet from largely fish based, to heavily reliant on marine mammals
 1135 (Tricas and McCosker 1984; Frazzetta 1988), and greater reliance on fish in the females
 1136 compared to males studied here is supported by fine-scale habitat use and seasonal
 1137 abundance of sharks acoustically tagged in False Bay, Gansbaai and Mossel Bay (Kock
 1138 et al. 2013; Jewell et al. 2013; Towner et al. 2013a; Jewell et al. 2014; Towner et al.
 1139 2016). Lastly, there is evidence of multiple coastal migration strategies in females that
 1140 may preclude clear isotopic trends. Easterly migrations to the coast of KZN peak either
 1141 in mid-winter or mid-summer, with a capture bias towards females (Cliff et al. 1989;
 1142 OCEARCH 2017). These peaks coincide respectively with either a mass migration
 1143 event of *Sardinops sagax* pilchard (the ‘sardine run’; (Whitehead et al. 1985) that
 1144 attracts high densities of the mesopredator prey of white sharks (Cliff et al. 1989;

Dudley et al. 2005; Dudley and Cliff 2010), or abundance of high densities of dusky shark (*Carcharhinus obscurus*) and reef manta ray (*Manta alfredi*) prey species (Smale 1991; Dudley et al. 2005; Marshall and Bennett 2010a, b). Females attending the Gansbaai aggregation could be following one of two strategies during summer, either staying at the Western Cape to feed on elasmobranchs and teleosts, or migrating east to take advantage of shark and ray prey availability in Algoa Bay, KZN and Mozambique. Sharks that migrate in midwinter seem likely to be exploiting prey availability associated with the sardine run, be it the sardines themselves (Dudley and Cliff 2010), or the mesopredators that the sardines attract.

While we found overlap between isotopic niches of all demographic groups, similar to other South African white shark diet studies, we also found evidence of expanded and diverse niches in juvenile sharks in comparison to those > 3 m (Cliff et al. 1989; Hussey et al. 2012b; Christiansen et al. 2015), where all our outliers were juveniles. This concurs with expanded habitat use found in smaller white sharks in South Africa (Jewell et al. 2013). Christiansen et al. (2015) suggested multiple reasons why South Africa's young white sharks show such diversity in isotopic signatures, including individual variation, spatial segregation, and maternal influences. In the case of smaller sharks at the Gansbaai aggregation, temporal variation could also play a strong role in their isotopic diversity, representing a function of the time since they undertook the westerly coastal migration for the first time (Cliff et al. 1989, 1996; Ferreira and Ferreira 1996; Dicken 2008; Kock et al. 2013; Towner et al. 2013a; Ryklier et al. 2014; Hewitt et al. 2018). Kelp detritus contributes significantly to the coastal food web of South Africa (Bustamante and Branch 1996; Miller and Page 2012), and recorded variation in $\delta^{13}\text{C}$ values of kelp could also partially explain the variation in SIBER niches between juveniles and larger sharks as juveniles make comparatively

more use of coastal habitat as opposed to the pelagic or tropical habitats utilised by larger individuals (Cliff et al. 2000; Zuffa et al. 2002; Bonfil et al. 2005; Hussey et al. 2012b; Smale and Cliff 2012; OCEARCH 2017). However, this would not account for the concurrent variation in $\delta^{15}\text{N}$ values found in Christiansen et al. (2015) and this study.

Our results, combined with multifaceted evidence of individual and sexual variation in diet, movement, and foraging strategies in South Africa and globally, suggest that sex and individual specialisation are key drivers in ecological variation in white sharks, which remain important through ontogeny (Estrada et al. 2006; Hussey et al. 2012b; Carlisle et al. 2012; Kim et al. 2012; Kock et al. 2013; Pethybridge et al. 2014; Huveneers et al. 2015; Christiansen et al. 2015; Towner et al. 2016). Intraspecific trait variation in a predator population has important implications for community ecology and species conservation (Bolnick et al. 2003, 2011; Schreiber et al. 2011; Mittelbach et al. 2014). In South Africa, the sexes exhibit ontogenetic differences in habitat use, migration patterns and diet, and juvenile sharks have expanded niches compared to larger sharks, which may be the result of multiple factors including specialisation and temporal effects. These sex, age, and individual driven differences should be considered in conjunction with exposure to spatially explicit threats, such as fisheries and pollution when developing management strategies, and explicitly included in ecological studies of the species.

1190 Chapter 4 Complex size and sex effects on trophic
1191 ecology in a highly mobile top predator.

4.1. Abstract

Top predator trophic ecology can have strong effects on food webs, with the sex and size of predators having significant, but often overlooked, effects on this. Stable isotope and fatty acid analyses provide non-lethal methods of assessing diet and habitat use in wild animals, and in combination can provide both long and short-term information on predator foraging and movement patterns, though dietary interpretation of predator fatty acids are less well understood than for stable isotopes. Here we use fatty acids to analyze the effects of sex and size on the trophic ecology of a large marine top predator, the white shark (*Carcharodon carcharias*), which is classically thought of as exhibiting an ontogenetic dietary shift. We compare these results to the stable isotopes $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ to aid interpretation and explore assumptions of some fatty acid biomarkers. We found novel fatty acid differences between the sexes and sizes of South African white sharks indicating that both are important factors in their ecology. While we found evidence from fatty acid signatures of the generally recognized dietary shift from piscivory to marine mammal prey at ~ 3.0 m body length, there were lower levels of fatty acids associated with marine mammals in large sharks ($> \sim 4.0$ m in body length), which could indicate a second ontogenetic dietary shift. Dietary specialization, influenced by sex and less so by size, was also detected, while the fatty acid signatures of large female sharks suggested extended use of tropical habitats, which may expose them to greater risk of fishing mortality. Fatty acid signatures can be a useful tool to complement stable isotope analysis in elucidating the trophic ecology of marine predators, and that sex, size, and individual variation needs to be considered when designing management strategies.

4.2. Introduction

Top predators exert strong forces on food webs, and their removal can have cascading trophic effects with ecosystem-wide consequences in both terrestrial and aquatic environments (Estes et al. 2011). Food web alteration occurs through both direct consumption of prey and behaviour-mediated effects of fear of predation (Beckerman et al. 1997; Brown et al. 1999; Pace et al. 1999; Fortin et al. 2005; Preisser et al. 2005; Burkholder et al. 2013; Rasher et al. 2017), in addition to mesopredator competitive release (Ritchie and Johnson 2009). Dietary choice in even a small number of individual top predators can also result in changes at prey, community, and ecosystem scales (Sweitzer et al. 1997; Estes et al. 1998). The global human-induced predator population reduction is one of mankind's most prevalent, negative influences on the natural environment (Estes et al. 2011). Given the vital role of top predators in ecosystems and communities, and their history of population declines due to anthropogenic activities, it is important that their ecology is well understood if population, species, community, and ecosystem-scale conservation efforts are to be effective. This is especially important in the context of highly mobile predators, as these species can play significant roles in ecosystem connectivity and nutrient regulation (Lundberg and Moberg 2003; McCauley et al. 2012; Nifong et al. 2015).

Sex and size differences in diet have been found in top predators across taxa (e.g. bears (Mowat and Heard 2006; Thiemann et al. 2008), seals (Le Boeuf et al. 2000; Beck et al. 2007), birds (Phillips et al. 2004; Patrick and Weimerskirch 2014) and reptiles (Nifong et al. 2015)) but are not always considered in dietary analyses. Sexual dimorphism and ontogenetic changes in diet with body size can be particularly significant in species which have very large differences in body size from birth/hatching to maturity (Wilson 1975; Werner and Gilliam 1984), and failure to include sex and size

in ecological studies can result in underestimation of niche breadth (Clutton-Brock et al. 1982; Polis 1984; Forero et al. 2002), ultimately limiting our understanding of the ecology of populations or species. Dietary specialisation in top predators can have further effects on food webs, through differential prey choice and hunting tactics (Schmitz and Suttle 2001; Schreiber et al. 2011), and recent studies are revealing that some predators previously considered generalist are in fact made up of subsets of specialists (e.g. Matich et al., 2011; Rosenblatt et al., 2015).

Non-lethal methods of studying predator trophic ecology are especially important in the case of threatened species, and stable isotope and fatty acid analyses of biopsies can provide such methods for the study of diet and habitat use in wild animals (Budge et al. 2006; Layman et al. 2012). Ratios of isotopes $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ compared to international standard values can respectively be used to infer trophic level and habitat use (DeNiro and Epstein 1978; Post 2002). Prey fatty acids undergo very little modification during transference to predator tissues so can also provide relatively detailed dietary information (Iverson et al. 2004; Budge et al. 2006). Whereas stable isotopes provide information on relatively long-term dietary changes, fatty acid signatures and give insight into relatively short-term changes, including those with season (Iverson et al. 2002). Certain fatty acids and fatty acid ratios are considered biomarkers, which can indicate trophic position, nutritional condition, and predation on different food sources (Graeve et al. 1994; Sargent et al. 1999; Iverson et al. 2004; El-Sabaawi et al. 2009; Mohan et al. 2016), although these are often applied across taxa and species without consideration of potential differences in physiological processes. Comparison of these biomarkers, for example to stable isotopes, to better understand measures of trophic level and habitat use is important to develop our understanding of their suitability in different model systems. Combination of stable isotope and fatty acid

analyses can therefore be particularly useful in the study of food webs and the foraging ecology of predators (Smith et al. 1996; Hooker et al. 2001; Herman et al. 2005; Williams et al. 2008; Budge et al. 2008; El-Sabaawi et al. 2009; Belicka et al. 2012).

The white shark (*Carcharodon carcharias*) is a highly migratory top predator (Compagno 2001), listed as Vulnerable on the IUCN Red List of Threatened Species due to a variety of anthropogenic causes (Fergusson et al. 2009). White sharks display sexual dimorphism in size, and the size at which different maturity stages are reached, with females attaining longer total lengths and reaching maturity at greater size than males (Francis 1996; Pratt 1996; Compagno 2001). White sharks also undergo an ontogenetic dietary shift at approximately 3 m in length, when they begin to switch from piscivory to incorporating marine mammals into their diet (Tricas and McCosker 1984; Casey and Pratt 1985; Cliff et al. 1989; Compagno 2001; Hussey et al. 2012b), though sexual and individual variation in this shift have been detected (Kim et al. 2012; French et al. 2017). Fatty acids have only recently been used to infer diet and dietary specialism in white sharks in Australia (Pethybridge et al. 2014), and validation of fatty acid interpretation and clarification of drivers of dietary specialism remain important issues (Kim et al. 2012; Pethybridge et al. 2014; Christiansen et al. 2015).

Here we combine fatty acid and stable isotope analyses to explore the effects of sex and size on the trophic ecology of white sharks, as an example of a large, marine, migratory top predator, to gain insight into the interpretation of fatty acid data for the species and the complementarity of the methods in general. Our study focuses on individuals from the South African population, understanding the ecology of which is especially important in light of a recent, though perhaps overly negative, population estimate of only 438 individuals (Andreotti et al. 2016), evidence of deleterious effects

of bioaccumulated toxins (Marsili et al. 2016), and potential for trophic specialism to have food web effects (Christiansen et al. 2015).

4.3. Methods

4.3.1. Sample collection and analysis

Muscle biopsies and shark sex, length, and individual identification data were collected from free-swimming white sharks in Gansbaai, South Africa over two field trips in 2015 (Feb - April, June - July) following the methodology described in (French et al. 2017). In brief, biopsies were collected using Finn Larsen Ceta darts (4 x 0.9 cm) affixed to a biopsy pole and samples were stored immediately in ethanol. Shark length (total length, m) was estimated by comparison to a 4.7 m object of known length (Kock et al. 2013; Towner et al. 2013a), sex was determined by the presence or absence of claspers, and individuals were identified using distinguishing marks and DARWIN dorsal fin ID software (<http://darwin.eckerd.edu/>). For the Principal Component Analyses (see below) sharks were classified as either < 3 m total body length (five females, five males), or > 3 m (ten females, five males) to reflect pre- and post- the generally recognised ontogenetic dietary shift (Tricas and McCosker 1984; Casey and Pratt 1985; Cliff et al. 1989; Compagno 2001; Hussey et al. 2012b).

Total lipid was extracted from muscle biopsies by homogenising in 20 volumes of ice-cold chloroform:methanol (2:1, v/v) using an Ultra-Turrax tissue disrupter (Fisher Scientific, Loughborough, UK) according to Folch et al. (1957). Non-lipid impurities were isolated by washing with 0.88% (w/v) KCl and the upper aqueous layer removed by aspiration and the lower solvent layer containing the lipid extract dried under oxygen-free nitrogen and overnight desiccation *in vacuo* before making up to a 10

mg.ml⁻¹ concentration. Fatty acid methyl esters (FAME) from total lipid extracts were prepared by acid-catalysed transmethylation at 50°C for 16 h according to Christie (1993). FAME were extracted and purified according to Tocher and Harvie (1988), and separated by gas-liquid chromatography using a Fisons GC-8160 (Thermo Scientific, Milan, Italy) equipped with a 30 m x 0.32 mm i.d. x 0.25 µm ZB-wax column (Phenomenex, Cheshire, UK), 'on column' injection and flame ionisation detection. Hydrogen was used as carrier gas with an initial oven thermal gradient from 50°C to 150°C at 40°C.min⁻¹ to a final temperature of 230°C at 2°C.min⁻¹. Individual FAME were identified by comparison to known standards (Restek 20-FAME Marine Oil Standard; Thames Restek UK Ltd., Buckinghamshire, UK) and published data (Tocher and Harvie, 1988). Data were collected and processed using Chromcard for Windows (Version 1.19; Thermoquest Italia S.p.A., Milan, Italy).

Stable isotope analyses are from Chapter 3, and were quantified using a Sercon Integra integrated elemental analyser and mass spectrometer. Stable isotope ratios are reported as δ-values and expressed in ‰, according to the following: $\delta X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$, where X is ¹³C or ¹⁵N and R is the corresponding ratio ¹³C/¹²C or ¹⁵N/¹⁴N, and R_{standard} is the ratio of the international references PDB for carbon and AIR for nitrogen. Replicate analyses of internal lab standard alanine yielded standard deviations of 0.15 for δ¹⁵N and 0.09 for δ¹³C.

4.3.2. Statistical analyses

Only dietary fatty acids (Iverson et al. 2004), and only those representing at least 0.05% of the total lipids on average, were included in statistical analyses, comprising 22 individual fatty acids (Table 4.1). All statistical analyses were conducted in R 3.3.1. (R 2017). Fatty acids are described as A:BωD, where A represents the number of carbon

atoms, B the number of double bonds in the carbon chain and ω D is the position of the first double bond from the terminal methyl end of the molecule. Some fatty acids are abbreviated; docosahexaenoic acid, 22:6 ω 3 (DHA), eicosapentaenoic acid 20:5 ω 3 (EPA) and arachidonic acid 20:4 ω 6 (ARA). Lipid classes include saturated fatty acids SFA, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA).

Principal Component Analysis (PCA) was used to identify which fatty acids had the most influence on variation within the fatty acid dataset. As PCA is a parametric test, fatty acid % data were logit transformed before PCA analysis (Budge et al. 2006; Warton and Hui 2011), which was performed using the factoMineR package (Le et al. 2008). $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and demographic group (females less or greater than 3 m in length ($F < 3$ m, $F > 3$ m), males less or greater than 3m length ($M < 3$ m, $M > 3$ m)) were treated as supplementary variables for comparison to PCA results. The centre of gravity of each group, calculated as the barycentre of individuals within the group, was plotted and the *dimdesc* function was used to calculate the correlation coefficient and associated P value between the supplementary variables and the axes of the principle components. 95% confidence ellipses were plotted around each demographic group's centre of gravity.

We applied general linear models with Gamma link functions to test for associations between $\delta^{15}\text{N}$, PCA identified fatty acids (14:0, 16:0, ARA, 18:1 ω 9, and DHA), and shark sex and length, including second order interactions. Interactions between isotopes and length would indicate changes through ontogeny while isotope:sex interactions would signify differences in diet and/or habitat use between the sexes. Length:sex interactions would suggest differences between the life stages of the sexes, and interactions between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ could indicate either foraging on different prey in the same location or the same prey in different habitats. Data were

assessed for outliers using Cleveland dot plots (Cleveland 1993) and the Gamma family was chosen for its appropriateness in analysing proportional data and utility in reducing the effects of outlying data in response variables (Zuur et al. 2010). Third order interactions weren't used due to low sample size and to avoid false inference (Crawley 2013), and Trip wasn't included in any interactions due to low sample size (Zuur et al. 2010). Some fatty acids with extreme outlying data points were logit transformed (+20) prior to modelling. The link function that produced the best model fit, assessed on inspection of standard residuals, was used in each case. Using the MuMIn package (Bartoń 2017), models were run in every possible sequence and those with δ small sample size corrected Akaike Information Criterion (AICc) (Hurvich and Tsai 1989) < 6 were averaged to produce final model estimates (Burnham and Anderson 2003; Richards 2007). Several fatty acid biomarkers used to infer 1) trophic position, 18:1 ω 9/18:1 ω 7 (El-Sabaawi et al. 2009), 2) nutritional condition, physiological stress or benthic input, AA/EPA, ω 3/ ω 6 (Sargent et al. 1999; El-Sabaawi et al. 2009) and 3) diatom vs. dinoflagellate food webs DHA/EPA (Graeve et al. 1994), were also modeled as described above, to test for their validity for use as biomarkers in white sharks and to provide further insight into their trophic ecology.

To identify possible dietary groups, we performed hierarchical cluster analysis on the full fatty acid dataset. The analysis was applied to a Bray-Curtis similarity matrix, and the number of clusters to split the data into was determined a priori using the NbClust function (Charrad et al. 2014).

1382 **4.4. Results**

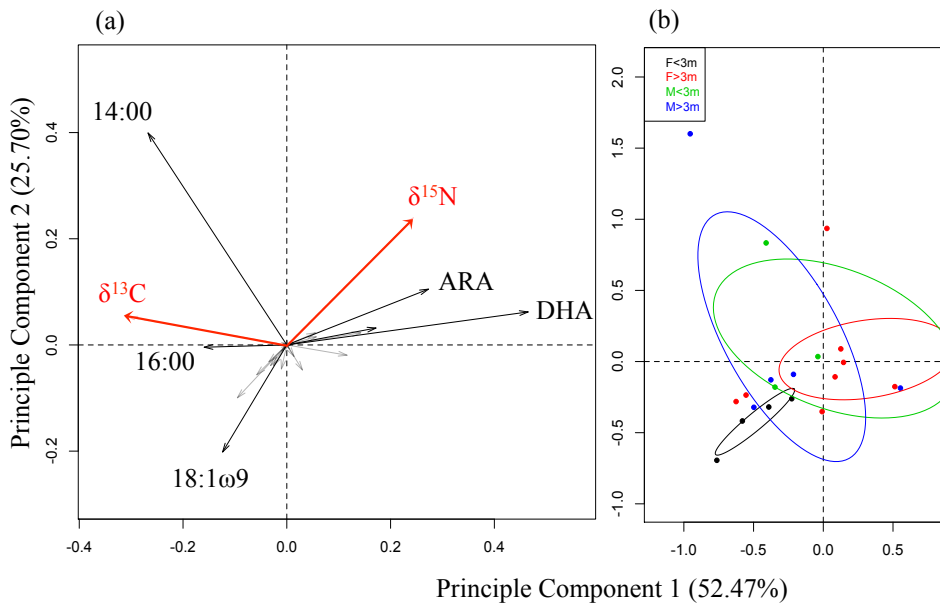
1383 **4.4.1. Fatty acid profiles**

1384 A total of 23 muscle samples were prepared for fatty acid analyses, comprising 14
1385 females and 9 males of varying length (Table 4.1). Fatty acids are expressed as
1386 percentages of total fatty acids \pm one standard error. The most abundant individual fatty
1387 acids were 16:0 (average $29.5 \pm 0.91\%$), 18:1 ω 9 (average $16.2 \pm 0.93\%$), 18:00
1388 (average $14.8 \pm 0.65\%$), and ARA (average $5.78 \pm 0.55\%$). ω 6 PUFA made up a larger
1389 component than ω 3 PUFA with a ω 3/ ω 6 ratio of 0.61. Further fatty acid profile data
1390 and discussion are presented in Supplementary Data.

1393 **4.4.2. Principal component analysis**

1394 Principal Components 1 and 2 together explained 78% of the variation
1395 data, with Principal Component 1 being driven primarily by the fatty ac
1396 and DHA, and Principal Component 2 being driven primarily by 14
1397 (Figure 4.1a). There was considerable overlap between the four demog
1398 fatty acid Principal Components 1 and 2, though smaller females r
1399 smallest ellipse, and only marginal overlap with larger females and
1400 (Figure 4.1b).

1401



1402

1403 **Figure 4.1: Principal component analysis results** of logit transformed dietary

1404 Top five most influential PC1 and PC2 fatty acids, overlaid with δ¹⁵N (‰) and
1405 b) PC1 and 2 individual sharks split into demographic groups (F < 3 m = females
1406 in total length (black), F > 3 m = females over 3 m in total length (red), M < 3
1407 than 3 m in total length (green), M > 3 = males over 3 m in total length (blue).
1408 confidence ellipses around each group.

1409 **4.4.3. Fatty acid GLMs of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, sex and length**

1410 Both the fits and the explanatory power of the general linear models varied between
 1411 fatty acids, but explanatory power (R^2) was generally high (Table 4.2; Supplementary
 1412 Data). Models for 14:0, and ARA/EPA had no significant relationships with any of the
 1413 explanatory factors (Table 4.2). There were significant interactions between the effects
 1414 of sex, size and isotopes for several fatty acids: $\delta^{15}\text{N}$:Sex and $\delta^{15}\text{N}$:Length were
 1415 significant interactions for four fatty acids, $\delta^{13}\text{C}$:Sex, $\delta^{13}\text{C}$:Length, and Length:Sex were
 1416 significant interactions for two fatty acids (Table 4.2). The interaction between $\delta^{15}\text{N}$ and
 1417 $\delta^{13}\text{C}$ was also significant for 16:0, and there was a significant effect of Trip for 18:1 ω 9
 1418 (Table 4.2).

1419 Model 16:0 had a significant interaction between $\delta^{15}\text{N}$ and sex, where males and
 1420 females had positive and negative relationships respectively (Figure 4.2a). Smaller
 1421 sharks exhibited a positive relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, but this relationship was
 1422 reversed in larger sharks (Figure 4.2b). Further, less clear results for model 16:0 are
 1423 presented in Supplementary Data. In the ARA model, sex interacted significantly with
 1424 $\delta^{15}\text{N}$, where females had a positive relationship between ARA and $\delta^{15}\text{N}$, while this
 1425 relationship was negative in males (Figure 4.2c).

1426 In model 18:1 ω 9, $\delta^{15}\text{N}$ had a significant interaction with shark length, where
 1427 smaller sharks displayed a positive slope, and larger sharks a negative slope (Figure
 1428 4.2d). Trip also had a significant effect on 18:1 ω 9, which was higher in Trip 1, though
 1429 boxplots of the data exhibited overlap (Figure 4.2e). The trophic marker 18:1 ω 9/18:1 ω 7
 1430 was found to be significantly influenced by sex mediated differences in shark length,
 1431 where 18:1 ω 9/18:1 ω 7 decreased with increasing female length, but increased with
 1432 increasing length in males (Figure 4.2f). Results and figures for models DHA,
 1433 DHA/EPA and ω 3/ ω 6 were heavily influenced by a small number of extreme values,

1434 despite the mitigation of using a Gamma link and are presented in Supplementary Data.

1435

1436 **Table 4.2: δ AICc < 6 averaged general linear models** of fatty acids (%), $\delta^{15}\text{N}$ (‰), $\delta^{13}\text{C}$ (‰),

1437 and total shark length (m), with 95% confidence intervals. Sig = significance of the P-value for

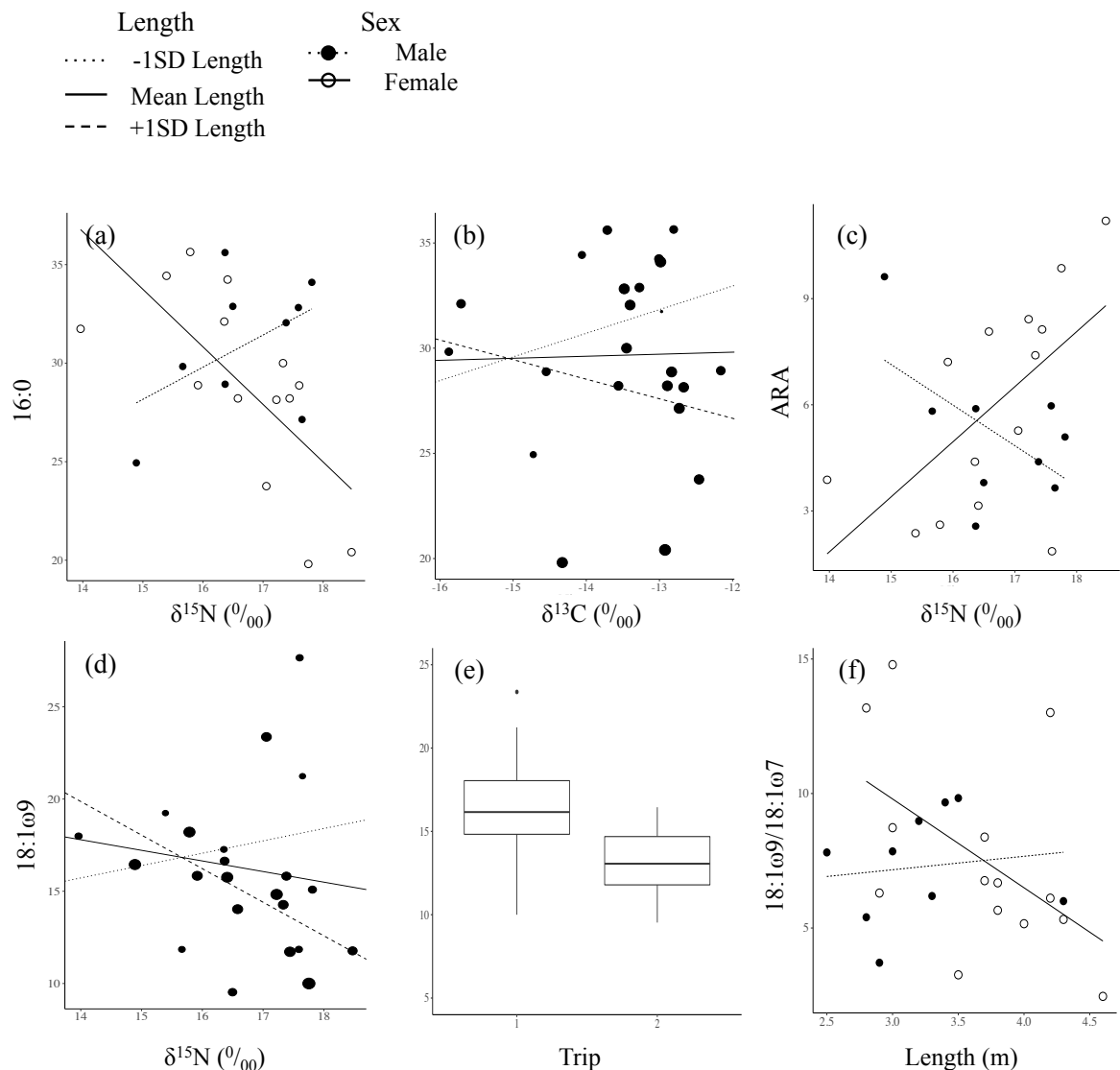
1438 a term in the model (. = < 0.1; * = < 0.05; ** = < 0.005; *** = < 0.0005).

	Variable	Estimate	P-Value	Sig	95% CI's	
					2.50%	97.50%
Model: 14:0	Intercept	0.10045	0.03	*	0.0433	0.0681
$F_{7,15} = 0.85$	Sex	-0.01905	0.50		-0.0109	0.0088
$\text{AICc} = 35.85$	$\delta^{13}\text{C}$	0.00322	0.28		-0.0015	0.0006
$R^2 = 0.29$	Length	-0.01392	0.24		-0.0037	0.0044
	$\delta^{15}\text{N}$	0.00016	0.73		-0.0008	0.0007
Family: Gamma	Sex: $\delta^{13}\text{C}$	-0.00115	0.30		-0.0020	0.0009
Link: Inverse	Sex: $\delta^{15}\text{N}$	0.00011	0.91		-0.0023	0.0006
	$\delta^{13}\text{C}$:Length	-0.00101	0.24		-0.0015	0.0011
Model: 16:0	Intercept	0.02577	0.85		-0.2400	0.2915
$F_{10,12} = 3.33$	$\delta^{15}\text{N}$	0.00422	0.62		-0.0123	0.0208
$\text{AICc} = 140.25$	Sex	0.05199	0.37		-0.0628	0.1668
$R^2 = 0.74$	$\delta^{15}\text{N}$:Sex	-0.00493	0.01	*	-0.0088	-0.0010
	$\delta^{13}\text{C}$	0.00353	0.87		-0.0402	0.0473
Family: Gamma	Length	-0.05018	0.37		-0.1604	0.0600
Link: Inverse	$\delta^{13}\text{C}$:Length	-0.00519	0.02	*	-0.0095	-0.0009
	$\delta^{13}\text{C}$:Sex	-0.00491	0.03	*	-0.0092	-0.0006
	Trip	0.00123	0.66		-0.0042	0.0066
	$\delta^{13}\text{C}$: $\delta^{15}\text{N}$	0.00330	0.03	*	0.0003	0.0063
	Length: $\delta^{15}\text{N}$	0.00397	0.04	*	0.0001	0.0078
Model: 18:1ω9	Intercept	0.68318	0.26832		-0.5265	1.8928
$F_{11,11} = 2.14$	$\delta^{13}\text{C}$	0.00671	0.88174		-0.0817	0.0951
$\text{AICc} = 132.51$	Length	-0.21188	0.08686	.	-0.4544	0.0307
$R^2 = 0.72$	$\delta^{15}\text{N}$	-0.05809	0.08214	.	-0.1236	0.0074
	Sex	0.04047	0.39502		-0.0528	0.1337
Family: Gamma	Trip	0.01601	0.03907	*	0.0008	0.0312
Link: Inverse	Length: $\delta^{15}\text{N}$	0.01596	0.00303	**	0.0054	0.0265
	Length:Sex	-0.02176	0.09554	.	-0.0474	0.0038
	$\delta^{13}\text{C}$: $\delta^{15}\text{N}$	-0.00635	0.22518		-0.0166	0.0039
	$\delta^{13}\text{C}$:Sex	0.00461	0.42755		-0.0068	0.0160
	$\delta^{15}\text{N}$:Sex	0.00007	0.99205		-0.0134	0.0135
	$\delta^{13}\text{C}$:Length	-0.00029	0.95608		-0.0106	0.0100

Model: ARA	Intercept	0.76590	0.6278		-72.424	74.064
$F_{9,13} = 3.59$	Length	-0.04734	0.7005		-28.505	22.357
AICc = 107.47	Trip	-0.06166	0.0592	.	0.030	4.809
$R^2 = 0.71$	$\delta^{15}\text{N}$	-0.05337	0.6864		-6.064	7.547
	$\delta^{13}\text{C}$	0.06638	0.7387		-10.849	10.959
Family: Gamma	Sex	-0.49677	0.4995		-29.028	52.247
Link: Inverse	$\delta^{15}\text{N}:\text{Sex}$	0.08068	0.0146	*	-3.034	0.299
	$\delta^{13}\text{C}:\delta^{15}\text{N}$	-0.02864	0.1846		-4.532	-0.211
	Length: $\delta^{15}\text{N}$	-0.00547	0.8443		-0.719	3.084
	$\delta^{13}\text{C}:\text{Length}$	0.01852	0.4826		0.005	2.784
Model: DHA	Intercept	0.03433	0.69736		-0.138685	0.207344
$F_{11,11} = 4.98$	Length	0.02890	0.01911	*	0.004730	0.053078
AICc = 33.82	$\delta^{15}\text{N}$	0.00126	0.8212		-0.009644	0.012159
$R^2 = 0.83$	Sex	-0.02023	0.32769		-0.060734	0.020278
	Length: $\delta^{15}\text{N}$	-0.00159	0.00397	**	-0.002667	0.000507
Family: Gamma	$\delta^{15}\text{N}:\text{Sex}$	0.00168	0.00789	**	0.000440	0.002915
Link: Inverse	$\delta^{13}\text{C}$	0.00432	0.59337		-0.011543	0.020188
	$\delta^{13}\text{C}:\text{Length}$	0.00095	0.07239	.	-0.000086	0.001982
	$\delta^{13}\text{C}:\delta^{15}\text{N}$	-0.00093	0.05772	.	-0.001898	0.000031
	$\delta^{13}\text{C}:\text{Sex}$	0.00146	0.05943	.	-0.000058	0.002973
	Length:Sex	0.00152	0.21944		-0.000909	0.003958
	Trip	0.00015	0.82726		-0.001177	0.001472
Model: (18:109/18:107)	Intercept	0.18502	0.865		-1.949	2.319
$F_{11,11} = 1.59$	Trip	0.04211	0.1911		-0.021	0.105
AICc = 118.43	Length	-0.15229	0.7157		-0.972	0.667
$R^2 = 0.61$	$\delta^{13}\text{C}$	-0.00520	0.9406		-0.142	0.132
	Sex	0.20137	0.427		-0.295	0.698
Family: Gamma	Length:Sex	-0.10960	0.0468	*	-0.218	-0.002
Link: Inverse	$\delta^{15}\text{N}$	-0.03235	0.7201		-0.209	0.145
	$\delta^{13}\text{C}:\text{Length}$	-0.03109	0.2393		-0.083	0.021
	Length: $\delta^{15}\text{N}$	0.04086	0.0808	.	-0.005	0.087
	$\delta^{13}\text{C}:\delta^{15}\text{N}$	-0.00631	0.7519		-0.045	0.033
	$\delta^{15}\text{N}:\text{Sex}$	-0.03776	0.1541		-0.090	0.014
	$\delta^{13}\text{C}:\text{Sex}$	-0.01464	0.6946		-0.088	0.058
Model: (ARA/EPA)	Intercept	0.08368	0.936		-1.96	2.12
$F_{9,13} = 2.4$	Length	0.10192	0.75		-0.52	0.73
AICc = 90.46	Sex	-0.01968	0.893		-0.31	0.27
$R^2 = 0.62$	$\delta^{13}\text{C}$	-0.04809	0.709		-0.30	0.20
	Trip	-0.01819	0.639		-0.09	0.06
Family: Gamma	$\delta^{13}\text{C}:\text{Length}$	0.04630	0.077	.	-0.01	0.10

Link: Log	$\delta^{15}\text{N}$	0.02242	0.846		-0.20	0.25
	Length:Sex	0.09128	0.177		-0.04	0.22
	Length: $\delta^{15}\text{N}$	-0.02653	0.323		-0.08	0.03
	$\delta^{13}\text{C}$: $\delta^{15}\text{N}$	0.02924	0.182		-0.01	0.07
Model: (w3/w6)	Intercept	3.41140	0.8875		-43.83	50.66
$F_{11,11} = 4.31$	Length	-6.84090	0.0149	*	-12.35	-1.34
$\text{AICc} = 1.94$	$\delta^{15}\text{N}$	-0.30510	0.8353		-3.18	2.57
$R^2 = 0.81$	Sex	6.27220	0.1791		-2.88	15.42
	Length: $\delta^{15}\text{N}$	0.41510	0.0078	**	0.11	0.72
	$\delta^{15}\text{N}$:Sex	-0.45620	0.0102	*	-0.80	-0.11
Family: Gamma	$\delta^{13}\text{C}$	-1.53430	0.5064		-6.06	2.99
Link: Log	$\delta^{13}\text{C}$: $\delta^{15}\text{N}$	0.24280	0.0949	.	-0.04	0.53
	Length:Sex	-0.37020	0.2851		-1.05	0.31
	Trip	-0.12900	0.5169		-0.52	0.26
	$\delta^{13}\text{C}$:Sex	-0.27490	0.1312		-0.63	0.08
	$\delta^{13}\text{C}$:Length	-0.15420	0.2694		-0.43	0.12
Model: (DHA/EPA)	Intercept	35.21630	0.031683	*	3.09	67.34
$F_{9,13} = 8.61$	$\delta^{13}\text{C}$	2.24710	0.025944	*	0.27	4.22
$\text{AICc} = 76.47$	Length	-11.47270	0.004867	**	-19.46	-3.49
$R^2 = 0.86$	$\delta^{15}\text{N}$	-0.42280	0.631201		-2.15	1.30
	Sex	-6.90870	0.048094	*	-13.76	-0.06
Family: Gamma	$\delta^{13}\text{C}$:Length	-0.68050	0.000558	***	-1.07	-0.29
Link: Log	$\delta^{13}\text{C}$:Sex	-0.86000	0.000387	***	-1.34	-0.39
	Length: $\delta^{15}\text{N}$	0.33510	0.056855	.	-0.01	0.68
	Length:Sex	-1.55080	0.001345	**	-2.50	-0.60
	$\delta^{13}\text{C}$: $\delta^{15}\text{N}$	-0.15660	0.353059		-0.49	0.17

1439



1440

1441 **Figure 4.2: Significant averaged general linear model results** (see Table 4.2 for full models)

1442 and two-way interactions of a) fatty acid 16:0 and $\delta^{15}\text{N}$, influenced by sex; b) fatty acid 16:0

1443 and $\delta^{13}\text{C}$, influenced by shark length (m); c) fatty acid ARA and $\delta^{15}\text{N}$ influenced by shark sex;

1444 d) 18:1 ω 9 and $\delta^{15}\text{N}$, influenced by shark length; e) the effect of Trip on 18:1 ω 9; f)

1445 18:1 ω 9/18:1 ω 7 and shark length, influenced by sex. Symbol size reflects shark length, with

1446 larger symbols denoting longer sharks and slopes are fitted to -1 standard deviation of length,

1447 mean length, and + 1 standard deviation length, to illustrate the interaction effect. N.B. For

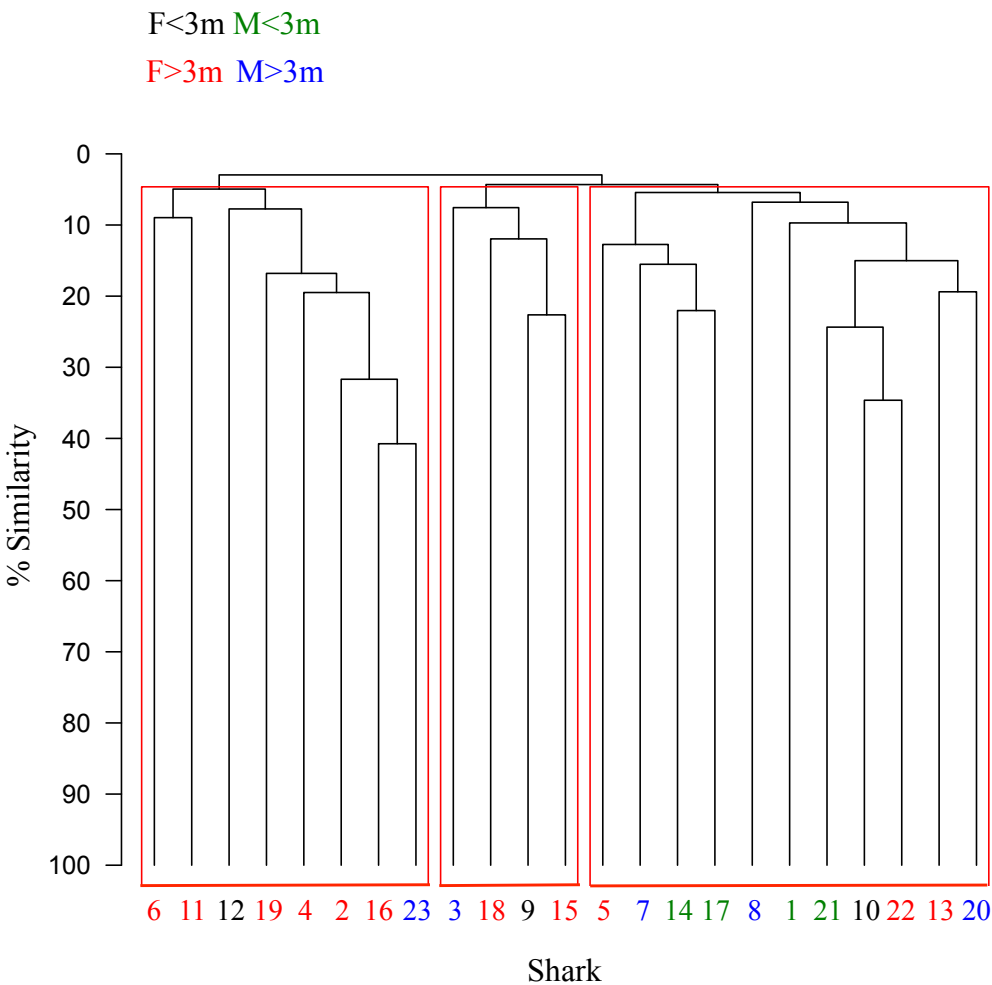
1448 illustrative purposes, the plots are based on models with a Gaussian distribution, while the

1449 statistical models all utilised a Gamma distribution (Table 4.2).

1450 **4.4.4. Hierarchical cluster analysis**

1451 The NbClust analysis revealed that the optimal number of clusters in the fatty acid
1452 principal components was three (Figure 4.3). Cluster 1 was dominated by large females
1453 with high 14:0, ARA/EPA and DHA/EPA (7 females, 1 male, average length = 3.7 m),
1454 Cluster 2 comprised only 4 sharks all of average length with the lowest 18:1 ω 9 and
1455 DHA and highest 16:00 (3 females, 1 male, average length = 3.5 m), and Cluster 3
1456 contained 11 sharks that were relatively small in size and had the highest 18:1 ω 9 (7
1457 males, 4 females, average length = 3.3m).

1458



1459 **Figure 4.3: Dendrogram of hierarchical cluster analysis**, based on a Bray-Curtis similarity
1460 matrix, of dietary fatty acids. Red boxes denote clusters identified by the NbClust function in R.

1461 **4.5. Discussion**

1462 The combined analysis of fatty acids and stable isotopes reveals a complex picture of
1463 trophic ecology in a top predator, which is significantly influenced by both sex and size.
1464 Hierarchical cluster analysis further indicated dietary specialization, which was also
1465 influenced by sex and size. Fatty acid biomarkers that are commonly used to infer
1466 trophic position, nutritional condition, and habitat use in diverse animals also had
1467 complex relationships with shark demographics and stable isotopes, calling their
1468 applicability across taxa into question.

1469 As a consequence of our comparatively low $\omega 3$ levels, influenced largely by lack of
 1470 DHA, ours is the first study to report a $\omega 3/\omega 6$ ratio of < 1 in white sharks, and the third
 1471 to find this low ratio in a shark species, the other being whale sharks in Mozambique
 1472 and three tropical euryhaline sharks (Rohner et al. 2013; Every et al. 2016). Fatty acid
 1473 signatures of muscle and sub-dermal tissue differ significantly in white sharks (Meyer et
 1474 al. 2017), and while the low levels of DHA in our sample more closely resemble sub-
 1475 dermal levels, other fatty acids responsible for separation of the tissue types, such as
 1476 ARA and EPA, more closely match muscle. While every care was taken to ensure that
 1477 samples comprised pure muscle, it is possible that some contamination with sub-dermal
 1478 tissue occurred, though this would have likely produced results more consistent with
 1479 findings in Meyer et al. (2017). Our results may instead reflect use of tropical habitat,
 1480 especially by females (see Supplementary Data for detailed discussion).

1481 Fatty acids that contributed the most to Principal Component axes were 16:0, ARA,
 1482 18:1 ω 9 and DHA. These are all thought to relate to the contribution of fish/cephalopod
 1483 vs. marine mammal prey in the diet of marine predators (Pethybridge et al. 2014).
 1484 Detailed discussion of PCA results in relation to female size are presented in
 1485 Supplementary Data.

1486 18:1 ω 9 is very high in the blubber of cape fur seals (*Arctocephalus pusillus*
 1487 *pusillus*) and dusky dolphin (*Lagenorhynchus obscurus*) in South Africa (Grahl-Nielsen
 1488 et al. 2010). 18:1 ω 9 as a reflection of marine mammal input is supported by our results,
 1489 where it increased in smaller sharks of < 3 m, and decreased in sharks > 4 m. This likely
 1490 reflects the accepted ontogenetic shift towards marine mammals in sharks approaching
 1491 3 m (Tricas and McCosker 1984; Cliff et al. 1989; Bruce 1992; Compagno 2001;
 1492 Estrada et al. 2006; Hussey et al. 2012b), and suggests a decreasing dependency on
 1493 them in larger sharks, which could constitute a secondary ontogenetic shift, perhaps

1494 involving increased consumption of lower trophic value cephalopods (Smale and Cliff
 1495 2012). The effect of Trip on 18:1 ω 9 is discussed in Supplementary Data. 16:0 had
 1496 complex relationships with shark sex, length, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ (Figure 4.2 and
 1497 Supplementary Data), which may indicate multiple sources and locations of high 16:0
 1498 prey exploited disproportionately by different demographic groups (Post 2002; Hill et
 1499 al. 2006; Hill and McQuaid 2008; Allan et al. 2010). Our DHA model indicated several
 1500 interactions between model variables, but extreme data points makes drawing
 1501 conclusions from them risky. High ARA can be associated with tropical habitat use in
 1502 elasmobranchs (Dunstan et al. 1988; Couturier et al. 2013; Rohner et al. 2013). We
 1503 found that females had a clear positive relationship between $\delta^{15}\text{N}$ and ARA, while
 1504 males did not. This could reflect the overall, long-term higher trophic level feeding of
 1505 larger females, which are more likely to make excursions to the tropics (Cliff et al.
 1506 2000; Zuffa et al. 2002; OCEARCH 2017) and consequently may provide an important
 1507 link between temperate and tropical ecosystems in the South Atlantic and Western
 1508 Indian Oceans. Extended travel outside of South Africa where they are not protected
 1509 may also expose large females to greater fishing mortality.

1510 It would be expected that the trophic biomarker 18:1 ω 9/18:1 ω 7 would have a
 1511 relationship with $\delta^{15}\text{N}$ as both are used to reflect trophic position (Post 2002; El-
 1512 Sabaawi et al. 2009). The lack of relationship in our findings suggests that either: 1)
 1513 18:1 ω 9/18:1 ω 7 is not a valid trophic marker for white sharks, 2) $\delta^{15}\text{N}$ isn't a reliable
 1514 indicator of trophic level in white sharks, 3) the timescales reflected by stable isotope
 1515 and fatty acid are too different to compare 18:1 ω 9/18:1 ω 7 and $\delta^{15}\text{N}$, or 4) our sample
 1516 size wasn't large enough to detect a relationship. Compound-Specific Isotope Analysis
 1517 of Individual Amino Acids (CSIA-AA) would help to resolve whether $\delta^{15}\text{N}$ is reflecting
 1518 trophic position (McClelland and Montoya 2002). Nutritional condition index ω 3/ ω 6

1519 decreased with increasing $\delta^{15}\text{N}$ in smaller sharks, but increased in larger sharks. Similar
 1520 to bull sharks (*Carcharhinus leucus*) this pattern could reflect increased foraging
 1521 success in more experienced larger sharks in comparison to smaller individuals that
 1522 have only more recently joined the Gansbaai aggregation (Hobson et al. 1993; Martin et
 1523 al. 2005; Belicka et al. 2012). While our ARA/EPA biomarker wasn't significantly
 1524 related to any demographic or isotopic variable, it was higher than for white sharks
 1525 sampled three years earlier in KwaZulu-Natal (4.44 compared to 3.82) (Davidson et al.
 1526 2011). Differences in this ratio between Gansbaai and KwaZulu-Natal may be dietary in
 1527 nature, though elevated ARA/EPA can be a symptom of physiological stress in fish, for
 1528 example inflammatory response (Sargent et al. 1999). White sharks sampled in
 1529 Gansbaai in 2012 were found to have dangerously high levels of ecotoxins, derived
 1530 from human-sourced pollutants and ascribed to recent use of the insecticide
 1531 Dichlorodiphenyltrichloroethane (DDT) and oil transport (Marsili et al. 2016). Sexually
 1532 immature sharks exhibited signs of estrogenic effects, which can cause feminization and
 1533 subsequently impaired reproductive success (Jobling et al. 1998; Harris et al. 2011;
 1534 Marsili et al. 2016). It is possible that the effect of these toxins has also resulted in an
 1535 elevated ARA/EPA ratio in the shark fatty acids.

1536 Dietary clusters have previously been recorded in white sharks through both stable
 1537 isotope and fatty acid analyses, but drivers behind the clustering have not been
 1538 identified (Kim et al. 2012; Pethybridge et al. 2014). Our fatty acid cluster analysis
 1539 results suggest dietary specialization that is at least in part influenced by sex and size.
 1540 These results are in-keeping with evidence of individual variation in hunting tactics,
 1541 also influenced by sex, from behavioural data and tooth morphometrics (Towner et al.
 1542 2016; French et al. 2017) and seasonal movement patterns in nearby False Bay (Kock et
 1543 al. 2013). Isotope values were fairly uniform across these clusters, which may highlight

1544 the usefulness of the more short-term, comparatively detailed analyses of multiple fatty
1545 acids in highly mobile, top predator ecology. Cluster 1 contained all of the largest
1546 females and the high DHA/EPA and ARA/EPA of the group could reflect wide ranging
1547 movement and resultant physiological stress (Graeve et al. 1994; Sargent et al. 1999).
1548 Cluster 2 was the most different to other clusters and had the lowest DHA and 18:1 ω 9
1549 and highest 16:0 which may indicate feeding on coastal fish (Dunstan et al. 1988;
1550 Schmidt-Nielsen 1997; Pethybridge et al. 2011a). The relatively small sharks of Cluster
1551 3 which contained the highest proportion of males (including all smaller males) had the
1552 highest 18:1 ω 9, which likely indicates preference for marine mammals.

1553 Our results suggest that sex, size, and individual dietary specialization are
1554 important facets in the trophic ecology of a top predator, with the result that a generalist
1555 predator in fact consists of specialized subsets of individuals which may exhibit multiple
1556 ontogenetic dietary shifts. At least some females may be opting to forage more heavily
1557 on fish than on pinnipeds, which males, especially smaller individuals, appear to favor
1558 until they reach approximately 4 m in length. The combination of stable isotope and
1559 fatty acid analyses provided complementary insights into long-term and short-term
1560 aspects of shark diet and highlighted shortcomings in our understanding of data
1561 interpretation. This temporal contrast could be especially useful in the study of
1562 ontogenetic dietary shift dynamics within predator populations. Future predator studies
1563 would benefit from the combination of stable isotope or ideally CSIA-AA, and fatty
1564 acid analyses with telemetry to provide a better understanding of how to produce valid
1565 diet and habitat use interpretations across taxa. Mixing models such as QFASA (Happel
1566 et al. 2016) would also assist in identification of different sources of fatty acids, which
1567 may be exploited differently by demographic groups and/or specialists. Clear
1568 differences in the ecology of the sexes and sizes of white sharks should be considered in

1569 their management, particularly in relation to tropical habitat use in large females and
1570 exposure to toxins.

1571 Chapter 5 A review of sexual and individual
1572 variation in the white shark (*Carcharodon*
1573 *carcharias*)

1574 **5.1. Summary**

1575 Sexual and individual phenotypic variations are widespread drivers of population
1576 ecology across taxa, and have significant implications for conservation management. As
1577 a top predator, white sharks (*Carcharodon carcharias*), are important for the healthy
1578 functioning of their environments, but are currently listed as Vulnerable on the IUCN
1579 Red List. To date, the occurrence and implications of sexual and individual variation
1580 have not been synthesised for this species, representing a significant gap in the
1581 understanding of their ecology, and potentially, effective conservation management.
1582 This review describes sexual and individual variation in white sharks in the context of
1583 1) sexual dimorphism and life history; 2) diet; 3) migration patterns and habitat use; 4)
1584 behaviour, and discusses the consequent ecological and management implications for
1585 the species. Females and some individuals may be disproportionately exposed to
1586 fisheries interactions, swimmer safety programmes, and bioaccumulation of toxins.
1587 Furthermore, the potential deleterious effects of cage-diving ecotourism may affect
1588 some individuals more than others, and males may be more affected by climate-
1589 mediated changes in ocean conditions. The aggregated evidence presented here strongly
1590 suggests that sex and individual variation should be explicitly considered in the analysis
1591 and interpretation of data in studies of white shark ecology and factored into
1592 conservation management strategies.

1593 **5.2. Introduction**

1594 White sharks are the largest of the warm-bodied, fast-swimming Lamnidae (Compagno
 1595 2001), reaching maximum lengths of approximately six meters (Castro 2012). They
 1596 have an International Union for the Conservation of Nature (IUCN) Red List global
 1597 categorisation of Vulnerable and are protected in several countries, in addition to being
 1598 listed under Appendix II of the Convention on International Trade in Endangered
 1599 Species of flora and fauna (CITES), and both Appendices of the Convention on
 1600 Conservation of Migratory Species (Fergusson et al. 2009). The species is found in six
 1601 more-or-less discrete populations in South Africa, Australia/New Zealand, northeast
 1602 Pacific, northwest Pacific, northwest Atlantic and the Mediterranean (Pardini et al.
 1603 2001; Jorgensen et al. 2010; Gubili et al. 2011, 2012). White sharks utilise both coastal
 1604 and pelagic habitat (Compagno 2001), and are highly migratory within and between the
 1605 coastal and offshore areas of their population range (Weng et al. 2007a; Bonfil et al.
 1606 2010; Jorgensen et al. 2010; Block et al. 2011; Domeier and Nasby-Lucas 2013; Bonfil
 1607 and O'Brien 2015), and in some cases transoceanic (Pardini et al. 2001; Bonfil et al.
 1608 2005; Duffy et al. 2012; Del Raye et al. 2013). While white sharks are relatively well
 1609 protected by various legislation, they remain victims of fisheries bycatch, bather safety
 1610 nets, deliberate culling, negative consequences of ocean warming and acidification, and
 1611 the deleterious effects of bioaccumulation of environmental toxins (Schlenk et al. 2005;
 1612 Fergusson et al. 2009; Mull et al. 2013; Lyons et al. 2013a, b; Dixon et al. 2015;
 1613 Pistevidos et al. 2015, 2017; Marsili et al. 2016; Rosa et al. 2017).

1614 Large sharks are typified by long life spans, delayed maturity and low fecundity
 1615 in comparison to bony fish (Myers & Worm 2005); traits that make them vulnerable to
 1616 the threats of fishing pressure, pollution, and habitat changes that have led to significant
 1617 population declines in many species (Myers and Worm 2003; Baum et al. 2003;

1618 Cavanagh et al. 2007; Worm et al. 2013; Dulvy et al. 2014). These large sharks have a
 1619 disproportionate influence on marine ecosystems, exerting strong top-down forces on
 1620 communities (Heithaus et al. 2008; Ferretti et al. 2010; Estes et al. 2011; Ruppert et al.
 1621 2013; Burkholder et al. 2013; Rasher et al. 2017), and are considered vital to
 1622 maintaining ecosystem health.

1623 Sexual and individual phenotypic variation can significantly influence many key
 1624 aspects of the ecology and biology of species, from diet to life history parameters,
 1625 behaviour, and movement patterns (e.g. Bolnick et al. 2003; Sims 2005; Sih et al. 2012;
 1626 Wolf and Weissing 2012). This variation is important in the context of species and
 1627 population conservation, and should be explicitly addressed in management plans. Sex
 1628 driven differences, particularly sexual dimorphism and spatial segregation, are prevalent
 1629 in elasmobranchs, and have direct influence on disparate exposure to anthropogenic
 1630 threats (Klimley 1987; Sims 2005; Mucientes et al. 2009; Domeier and Nasby-Lucas
 1631 2013). Individual variation in resource use is an important facet in the study of wildlife
 1632 ecology (Bolnick et al. 2003, 2011; Réale et al. 2010; Schreiber et al. 2011; Sih et al.
 1633 2012; Dall et al. 2012) and especially so in highly mobile predatory species due to
 1634 potential community and ecosystem level effects (Lundberg and Moberg 2003;
 1635 Quevedo et al. 2009; Schreiber et al. 2011; McCauley et al. 2012; Nifong et al. 2015).
 1636 While individual variation in behaviour and movement patterns have been well studied
 1637 in fish in general (Conrad et al. 2011; Mittelbach et al. 2014; Härkönen et al. 2014), it is
 1638 only recently that this field of research has been recognised as important in the study
 1639 and management of elasmobranchs (Matich et al. 2011; Jacoby et al. 2014; Huveneers
 1640 et al. 2015; Matich and Heithaus 2015).

1641 Although white sharks are relatively well studied in some respects, there remain
 1642 important gaps in our understanding of some of the more basic aspects of their life-

1643 history and behaviour, which hinders the development of effective species management
 1644 strategies. This review synthesises the evidence for sexual and individual variation in
 1645 white sharks in the context of 1) sexual dimorphism and life history; 2) diet; 3)
 1646 migration patterns and habitat use; 4) behaviour, and discusses the implications of these
 1647 for the ecology and management of the species.

1648 **5.3. Sexual Dimorphism and Life History**

1649 White sharks exhibit sexual size dimorphism, with females growing to and maturing at
 1650 a larger size than males (Francis 1996; Pratt 1996; Compagno 2001). Faster growth
 1651 rates have also been reported for females (Tanaka et al. 2011; Hamady et al. 2014),
 1652 though sample sizes were small. There is, however, evidence of individual variation in
 1653 the size at which individuals reach maturity stages. Significant variation in the size at
 1654 which male sharks in South Africa undergo the substantial increase in testes mass at the
 1655 onset of maturity was noted by Cliff et al. (1989), and variation in the size at which they
 1656 experience an ontogenetic shift in tooth shape has also been identified (French et al.
 1657 2017). Questions remain over individual differences in body length at maturity for
 1658 females (Francis 1996). French et al. (2017) recently presented evidence for Pace-of-
 1659 Life-Syndrome (POLS) in male sharks. The POLS hypothesis comprises intrinsic links
 1660 between individual behaviour, physiology and life history parameters where for
 1661 example, individuals with ‘bolder’ behaviour may have faster growth rates, earlier onset
 1662 of maturity and other physiological differences, such as ability to cope with stress, in
 1663 comparison to ‘shyer’ individuals (Ricklefs and Wikelski 2002; Réale et al. 2010). In
 1664 male white sharks, links between ontogenetic tooth shape change, onset of sexual
 1665 maturity, and foraging biology have been put forward as a basis for POLS (French et al.
 1666 2017).

1667 In addition to sexual dimorphism, regional and latitudinal variation in life
 1668 history parameters are common in elasmobranchs (Lombardi-Carlson et al. 2003;
 1669 Driggers et al. 2004; Neer and Thompson 2005; Walker 2007; Smart et al. 2015).
 1670 Tanaka et al. (2011) provided evidence for differential maturity and growth rates of
 1671 white sharks in the NWP population compared to other populations, suggesting that
 1672 NWP white sharks grow comparatively faster and mature at smaller sizes. This
 1673 population remains relatively understudied, and it is unclear what role regional variation
 1674 plays in white shark ecology.

1675 **Implications:**

1676 Differences in maturity and growth rates between the sexes and individuals will likely
 1677 result in differences in resource requirements, as the onset of sexual maturity can
 1678 require increased energy intake and specific nutrients (Robbins 1983). This can result in
 1679 spatial segregation, such as that exhibited by the scalloped hammerhead (*Sphyrna*
 1680 *lewini*), where females move offshore at a younger age than males, allowing them to
 1681 grow faster due to access to plentiful pelagic prey (Klimley 1987). Such spatial
 1682 segregation can lead to differences in exposure to anthropogenic threats (see section on
 1683 Migration Patterns and Habitat Use). Larger sharks, in addition to being
 1684 disproportionately important for population viability, are also disproportionately
 1685 removed by fishing (Ward and Myers 2005; Lucifora et al. 2009). In the case of white
 1686 sharks, this would pertain to females and potentially faster growing males, being fished
 1687 more heavily. Explicitly in the case of POLS, bolder fish genotypes that are also faster
 1688 growing, are also at greater risk of fishing mortality, due to elevated hunger levels to
 1689 sustain faster growth and greater levels of exploration; directly illustrating how POLS
 1690 can have ramifications for population survival (Young et al. 2006; Biro and Post 2008;

1691 Mittelbach et al. 2014; Härkönen et al. 2014). For white sharks, this is particularly
 1692 pertinent in the context of culling programmes and swimmer protection programmes,
 1693 which may disparately remove genotypes associated with greater movement activity
 1694 and bolder behaviour. Some evidence of disproportionate removal of faster growing
 1695 individuals is reported in South Africa (Wintner and Cliff 1999), but has not been found
 1696 in the northeast or northwest Pacific (Cailliet et al. 1985; Tanaka et al. 2011).
 1697 Population differences in size at maturity could have similar effects to intra-population
 1698 variation in maximum size and growth rates, and should be taken into account in the
 1699 development of regional management plans.

1700 **5.3. Migration Patterns and Habitat Use**

1701 Migration patterns and habitat use are the most intensively studied areas of white shark
 1702 ecology, thanks mostly to the development of acoustic and satellite tags, some of which
 1703 are able to provide data for up to multiple years (Dewar et al. 2004; Bonfil et al. 2005,
 1704 2010; Bruce et al. 2006; Weng et al. 2007a; Domeier and Nasby-Lucas 2008, 2013,
 1705 Jorgensen et al. 2010, 2012; Block et al. 2011; Duffy et al. 2012; Bradford et al. 2012;
 1706 Bruce and Bradford 2012, 2013, 2015; Nasby-Lucas and Domeier 2012; Kock et al.
 1707 2013; Jewell et al. 2013, 2014; Towner et al. 2016; Hoyos-Padilla et al. 2016). Some
 1708 researchers have also made use of photographic identification methods, while others
 1709 have simply recorded the sex and length of sharks, to monitor shark attendance patterns
 1710 at aggregation sites (Domeier and Nasby-Lucas 2007; Robbins 2007; Robbins and
 1711 Booth 2012; Nasby-Lucas and Domeier 2012; Towner et al. 2013a; Ryklief et al. 2014).
 1712 While some size segregation amongst white sharks is apparent across populations
 1713 (Klimley 1985; Robbins and Booth 2012; Jewell et al. 2013; Hoyos-Padilla et al. 2016),
 1714 several studies have identified strong sex-driven differences in movement and habitat

1715 use at both broad and fine scales (Anderson and Pyle 2003; Weng et al. 2007a; Domeier
 1716 and Nasby-Lucas 2008, 2012; Robbins and Booth 2012; Jorgensen et al. 2012; Kock et
 1717 al. 2013; Bruce and Bradford 2015; Towner et al. 2016), and more recently have also
 1718 described individual variation in these (Francis et al. 2015; Towner et al. 2016).

1719 White sharks in the northeastern Pacific have received the most attention in
 1720 terms of satellite tagging studies. This population is split into two discrete coastal
 1721 aggregations, Guadalupe Island, and central California, both of which utilise a pelagic
 1722 area (referred to as either the “Shared Offshore Forging Area” (SOFA) or the “White
 1723 Shark Café”), and in some cases also visit Hawaii (Weng et al. 2007a; Domeier and
 1724 Nasby-Lucas 2008; Nasby-Lucas et al. 2009; Jorgensen et al. 2010; Weng and
 1725 Honebrink 2013). At the central California aggregation, sex-specific visitation patterns
 1726 were first identified by Anderson and Pyle (2003), who found seasonal differences in
 1727 the arrival times of sharks at the Farallon Islands. It was later shown that the sharks
 1728 from this aggregation exhibit sex-specific use of the SOFA/café, where males
 1729 concentrate in a relatively small area, while females roam much more widely (Jorgensen
 1730 et al. 2010). Domeier and Nasby-Lucas (2012), addressed sexual differences in
 1731 migratory patterns for the Guadalupe Island aggregation explicitly, and revealed similar
 1732 usage patterns of the SOFA/café as the Californian sharks, showing that females move
 1733 more widely, and stay offshore for longer periods than males, where they experience
 1734 warmer temperatures generally, and a greater temperature range. More recently, these
 1735 authors further revealed that large females exhibit a biennial visitation pattern to
 1736 Guadalupe Island in contrast to the annual visits of males (Domeier and Nasby-Lucas
 1737 2013). During this extended migration period, females are believed to pup in the sea of
 1738 Cortez and the central Pacific coast of Baja California, Mexico (Domeier and Nasby-

1739 Lucas 2013). Seasonal differences in the visitation of males and females to Hawaii have
 1740 also been recorded (Weng and Honebrink 2013).

1741 Seasonal sex-driven differences in coastal aggregation attendance have also been
 1742 identified in Australia and South Africa, with inferences for differentiation in prey
 1743 choice between the sexes (Robbins 2007; Robbins and Booth 2012; Kock et al. 2013;
 1744 Towner et al. 2013a; Bruce and Bradford 2015). In Australia there are marked
 1745 differences between the sexes in the seasonality of their attendance at the seal rookeries
 1746 of the Neptune Islands (Bruce et al. 2006; Robbins 2007; Robbins and Booth 2012;
 1747 Bruce and Bradford 2015). Males are in attendance year round, while female attendance
 1748 peaks specifically during the weaning period of Australian fur seals (*Arctocephalus*
 1749 *forsteri*), when the greatest number of female seals and pups will be frequenting the
 1750 water (Bruce and Bradford 2015). Sea surface temperature has also been linked to this
 1751 sexual segregation, with cooler conditions being associated with increased numbers of
 1752 male sharks, and female attendance tending to coincide with warmer conditions
 1753 (Robbins and Booth 2012). A similar pattern of visitation takes place in Gansbaai,
 1754 South Africa, with male abundance being associated with colder water temperatures and
 1755 female abundance coinciding with warmer temperatures (Towner et al. 2013a). The
 1756 authors hypothesised that this would result in warming female core temperatures, which
 1757 would increase their growth rate, enabling them to reach sexual maturity at the same age
 1758 as their male cohorts, and accelerate gestation in pregnant individuals. However, while
 1759 mature females may use tropical habitats for gestation and parturition, (e.g. in South
 1760 Africa: Cliff et al. 2000; Zuffa et al. 2002; OCEARCH 2017) females attending these
 1761 aggregations are generally not sexually mature so cannot be gravid (Towner et al.
 1762 2013a; Bruce and Bradford 2015). In addition, female visitation did not match warmer
 1763 temperatures in a long-term study at the Neptune Islands by Bruce and Bradford,

1764 (2015), and water temperatures are very unstable in Gansbaai during the female-specific
 1765 visitation season (Towner et al. 2013a). It therefore remains to be fully ascertained
 1766 whether sexual segregation is influenced by temperature itself, or by prey associated
 1767 with different temperatures.

1768 Analysis of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotopes in animal tissue provides information
 1769 on their diet and foraging habitat respectively (DeNiro and Epstein 1978; Post 2002). A
 1770 significant negative relationship between $\delta^{13}\text{C}$ and male shark length has been found in
 1771 Gansbaai, South Africa, indicating that they use either more pelagic or more westerly
 1772 habitats as they grow, while the lack of a similar isotopic trend in suggests they have
 1773 more varied, broad scale movement (Chapter 3). Two studies in the northeastern Pacific
 1774 have also detected a depletion in $\delta^{13}\text{C}$ with increased shark length, both of which were
 1775 dominated by male shark samples (Kerr et al. 2006; Carlisle et al. 2012), in addition to
 1776 evidence of depleted $\delta^{13}\text{C}$ in males from the northwest Atlantic (Hamady et al. 2014)
 1777 and KwaZulu-Natal, South Africa (Hussey et al. 2012b). This is suggestive that, the
 1778 lack of relationships between shark length and $\delta^{13}\text{C}$ in some other studies, may be
 1779 because differences between the sexes were not accounted for (Estrada et al. 2006; Kim
 1780 et al. 2012).

1781 In False Bay, South Africa, Kock et al. (2013) found that during the summer
 1782 months, females of all size classes move close inshore, while male sharks tend to move
 1783 away from the area. Disproportionate female use of inshore habitats has also recently
 1784 been identified in nearby Gansbaai, in addition to fine-scale sex-specific movement
 1785 (Towner et al. 2016). Of five sharks acoustically tracked in Gansbaai by Jewell et al.,
 1786 (2014), the single female of the sample was the only shark to utilise an area of reef as its
 1787 core habitat, as opposed to areas adjacent to a pinniped colony used by the males of
 1788 comparable size. Individual habitat partitioning and female preference for non-pinniped

1789 adjacent habitat is also suggested in a core habitat analysis of thirteen females
 1790 acoustically tracked in Mossel Bay, South Africa (Jewell et al. 2013). Here, some of the
 1791 sharks, including small (< 3 m) and a large (> 4 m) individual did not include the local
 1792 pinniped colony within their core habitat, instead using reef and rivermouth areas.

1793 **Implications**

1794 The collective data suggest that female movement patterns may put them at greater risk
 1795 of anthropogenic threats than males. When females pup in coastal areas, such as the Sea
 1796 of Cortez for example, the time spent in proximity to the coast exposes them to
 1797 increased risk of fishing mortality compared to males (Domeier and Nasby-Lucas
 1798 2013). Females in South Africa are caught more often in swimmer protection
 1799 programmes than males (Cliff et al. 1989), which may be caused by their apparent
 1800 propensity to use habitats closer to the shore (Kock et al. 2013; Towner et al. 2016).
 1801 Heavier utilisation of coastal habitats would also result in greater exposure to pollutants,
 1802 as detailed below. The fact that females rove more widely may further put them at
 1803 increased risk of encountering pelagic fisheries, especially if they move into areas
 1804 where they are not protected by law e.g. from South Africa to Mozambique and the
 1805 High Seas.

1806 If the sexes have disparate responses to thermal cues (Robbins and Booth 2012;
 1807 Towner et al. 2013a), warming caused by climate change could affect them differently.
 1808 The links between sea temperature, white shark physiology, and prey availability need
 1809 to be more clearly understood to ascertain the potential effects of climate change on the
 1810 sexes.

1811 **5.4. Diet**

1812 The diet of white sharks is varied, comprising largely teleost fish, elasmobranchs,
 1813 cephalopods, cetaceans and pinnipeds (Tricas and McCosker 1984; Cliff et al. 1989;
 1814 Bruce 1992; Compagno 2001; Hussey et al. 2012b).

1815 Stomach content analysis has not yielded dietary differences between the sexes
 1816 (Hussey et al. 2012b). However, this may due to the relatively coarse resolution of
 1817 stomach content data, which also only provides a very narrow snapshot of prey
 1818 selection. While several studies have investigated long-term diet using stable isotopes,
 1819 (Estrada et al. 2006; Kerr et al. 2006; Hussey et al. 2012b; Carlisle et al. 2012; Kim et
 1820 al. 2012; Malpica-Cruz et al. 2013; Jaime-Rivera et al. 2014; Christiansen et al. 2015),
 1821 only one has analysed data for males and females separately (Chapter 3). This study,
 1822 based on sharks sampled in Gansbaai, South Africa, revealed evidence for the sexes
 1823 foraging in different food webs, and a trend for reduced $\delta^{15}\text{N}$, inferring feeding at a
 1824 lower trophic level, in large males while no obvious trends in $\delta^{15}\text{N}$ were apparent in
 1825 females. Towner et al. (2016) reported that female white sharks tracked in Gansbaai
 1826 were more likely than males to adopt what the authors termed “patrolling”, as opposed
 1827 to “area restricted searching” foraging modes, and females there and in nearby False
 1828 Bay make exclusive use of near-shore habitats that coincides with peak abundance of
 1829 teleosts and elasmobranchs (Kock et al. 2013; Towner et al. 2016). Fatty acid data from
 1830 sharks in this aggregation, which likely represents shark diet from approximately 3 – 18
 1831 weeks (Beckmann et al. 2013, 2014) inferred greater recent consumption of marine
 1832 mammals in males, while females exhibited more individual variation, and higher input
 1833 of coastal fish (Chapter 4), matching inshore and reef-associated habitat use patterns
 1834 described above. Sex-specific foraging strategies have also been suggested for white
 1835 sharks in the northeastern Pacific population (Weng et al. 2007a; Jorgensen et al. 2010,

2012; Domeier and Nasby-Lucas 2013) in which males display more rapid oscillatory diving behaviour within a restricted offshore area, which could reflect foraging for squid (Weng et al. 2007a; Domeier and Nasby-Lucas 2012; Carlisle et al. 2012; Jorgensen et al. 2012; Domeier and Nasby-Lucas 2013).

Individual differences in prey choice have been identified and suggested in several studies across white shark populations (Estrada et al. 2006; Hussey et al. 2012b; Kim et al. 2012; Hamady et al. 2014; Pethybridge et al. 2014; Christiansen et al. 2015). Some sharks (notably females) appear not to undergo the generally recognised dietary shift from fish to mammal prey (Estrada et al. 2006; Hussey et al. 2012b), while others have been found to form groups of apparent specialisation that have not yet been explained by any biological or environmental factors (Kim et al. 2012; Pethybridge et al. 2014). Dietary specialisation seems particularly prevalent in females (Chapter 3, Chapter 4), a pattern that has been noted in other marine predators (Young and Cockcroft 1994; Connan et al. 2014).

The teeth of white sharks are generally accepted to undergo an ontogenetic change in shape, becoming broader as sharks age (Tricas and McCosker 1984; Frazzetta 1988; Hubbell 1996), and facilitating a transition from a largely piscivorous diet to one more heavily reliant on marine mammals (Cliff et al. 1989; Bruce 1992; Compagno 2001; Hussey et al. 2012b). However, anecdotal descriptions of tooth shape within the published literature suggest that tooth cuspidity in the largest sharks, especially females, is highly variable and does not always correspond to shark length (Hubbell 1996; Castro 2012). A recent quantitative investigation of the relationship between white shark length, sex, and tooth cuspidity found clear differences in tooth shape change through ontogeny between the sexes (French et al. 2017). Males did undergo the accepted broadening of teeth with increasing shark length, but notable individual variation in the

1861 shark's length at which their teeth changed shape was apparent. Females did not exhibit
 1862 a distinct ontogenetic change in tooth shape, and rather there was evidence of tooth
 1863 polymorphism between females. As tooth morphology is considered to facilitate
 1864 handling of specific prey types, it is reasonable to suggest that these differences in the
 1865 change in tooth shape reflect consequent differences in foraging ecology between the
 1866 sexes and among individuals. Several studies have found evidence of dietary clusters
 1867 (Kim et al. 2012; Pethybridge et al. 2014) and it is possible that these may be at least
 1868 partially explained by variation in the size at which some sharks change from cuspidate
 1869 to broad tooth morphologies, and the tooth shape polymorphism found in females. This
 1870 has been found for blue sharks (*Prionace glauca*) (Litvinov 1983; Litvinov and
 1871 Laptikhovsky 2005), small spotted catsharks (*Scyliorhinus canicula*) (Litvinov 2003)
 1872 and a classic example in a cichlid fish (*Cichlasoma citrinellum*) (Meyer 1990a, b).

1873 **Implications**

1874 Christiansen et al. (2015) mapped out the management consequences of specialisation
 1875 in white shark diet, concluding that multiple trophic roles within the species could alter
 1876 food web structure, and that declining resources would disparately affect different
 1877 individuals; factors of high and medium significance for management respectively.
 1878 Specialisation for different marine food webs, related to sex or size differences, can
 1879 have effects on levels of bioaccumulation of toxic substances, such as mercury,
 1880 polycyclic aromatic hydrocarbons, pesticides, and organochlorines (Loseto et al. 2008b,
 1881 a; Cardona-Marek et al. 2009; Gelsleichter and Walker 2010; St. Louis et al. 2011;
 1882 Lyons et al. 2013a). As apex marine predators, large sharks are particularly at risk of
 1883 bioaccumulation of these damaging materials (Gelsleichter and Walker 2010), and
 1884 white sharks have already been shown to contain very high, and potentially injurious

1885 levels of ecotoxins (Schlenk et al. 2005; Mull et al. 2013; Lyons et al. 2013a; Marsili et
 1886 al. 2016). High concentrations of ecotoxins found in the tissues of young of the year and
 1887 juvenile white sharks are the results of maternal offloading during gestation, and these
 1888 levels are affected by the trophic position and foraging habitat of females (Borga et al.
 1889 2004; Lyons et al. 2013a).

1890 **5.5. Behaviour**

1891 Consistent inter-individual differences in behaviour ('personalities' Gosling 2001; Wolf
 1892 and Weissing 2012) have been found across a wide range of fish (Conrad et al. 2011;
 1893 Mittelbach et al. 2014; Härkönen et al. 2014), including recently in several shark
 1894 species (Jacoby et al. 2014; Wilson et al. 2015; Finger et al. 2016; Byrnes and Brown
 1895 2016; Finger et al. 2018). The links between individual differences in behaviour and
 1896 POLS, and the associated ramifications, were discussed above. Individual differences in
 1897 the behaviour of white sharks has been inferred in several studies of the behaviour of
 1898 sharks around cage-diving vessels (Johnson and Kock 2006; Laroche et al. 2007; Bruce
 1899 and Bradford 2013; Huvaneers et al. 2013). For example, Huvaneers et al., (2015)
 1900 reported consistent individual differences in the way in which Australian white sharks
 1901 exploit the sun during predatory attempts on bait at a cage diving vessel. This has been
 1902 followed by the results of a sophisticated movement model, based on acoustic telemetry
 1903 data in South Africa, which revealed both individual and sex specific differences in
 1904 hunting strategy (Towner et al. 2016). Clearly individual differences in behaviour is
 1905 something that requires more scientific attention in the study of white shark ecology and
 1906 conservation.

1907 Implications

1908 Consistent inter-individual behavioural differences in white sharks have the potential to
1909 have significant ramifications for their conservation. As discussed under the POLS,
1910 differing personality types can lead to disparate exposure to threats, especially fishing,
1911 and therefore individual survival as well as population stability and growth rates (Biro
1912 and Post 2008; Wilson et al. 2011; Wolf and Weissing 2012; Mittelbach et al. 2014;
1913 Härkönen et al. 2014). The effects of variation in fish behavioural types, at the
1914 individual to the ecosystem level, are summarised in Figure 5.1 (Mittelbach et al. 2014);
1915 see also discussion of POLS under Sexual Dimorphism and Life History. In a species
1916 that is already considered Vulnerable to extinction due to overfishing, this issue is of
1917 urgent importance.
1918

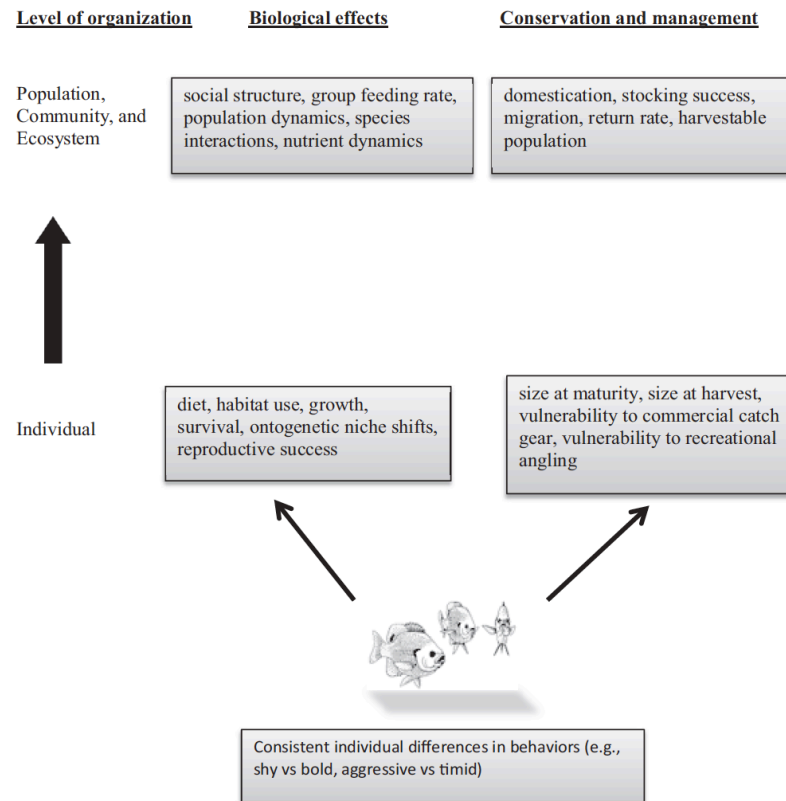


Figure 5.1 Consequences of variation in fish behavioural types, from Mittelbach et al., (2014).

Shark cage diving companies operate at white shark hotspots around the world and their effects on white sharks recorded so far include significant increase in residency time at the islands, changes in diel patterns, decrease in swimming depth, decrease in rate and area of movement, change in behavioural state and decreased times of arrival at cage-diving vessels (Laroche et al. 2007; Bruce and Bradford 2013; Huveneers et al. 2013; Towner et al. 2016). Evidence that some individuals are disproportionately effected by cage diving operations has been found in both South Africa and Australia (Johnson and Kock 2006; Laroche et al. 2007; Bruce and Bradford 2013; Huveneers et al. 2013), and changes in both short and long-term behaviour have been recorded for white sharks in general, with the ecological implications currently unknown (Bruce and Bradford 2013; Huveneers et al. 2013; Towner et al. 2016). In

1933 South Africa, some individuals were more consistently present at cage-diving vessels,
 1934 and may have learned to arrive at them more quickly over time (Johnson and Kock
 1935 2006; Laroche et al. 2007). Individual differences in response to cage-diving operators
 1936 found at the Neptune Islands in Australia has raised concerns over the energetic costs
 1937 due to distraction from feeding (Bruce and Bradford 2013; Huvaneers et al. 2013).
 1938 Shark ecotourism generates millions of US dollars every year, and has the potential to
 1939 benefit shark conservation through education, increased shark protection and provision
 1940 of alternative livelihoods to fishers (Gallagher and Hammerschlag 2011; Vianna et al.
 1941 2012; Cisneros-Montemayor et al. 2013; Gallagher et al. 2015; Haas et al. 2017). While
 1942 some studies have found negligible effects of shark ecotourism (Laroche et al. 2007;
 1943 Maljković and Côté 2011), and white shark cage-diving provides an opportunity to
 1944 improve their conservation status through participant education (Apps et al. 2016), the
 1945 overriding scientific stance is one of precaution, and more research into its potentially
 1946 negative effects, which can include among others - injury, impaired mobility, reduced
 1947 foraging success, energetic costs, change in habitat use and increased risk of disease
 1948 (Orams 2002; Gallagher et al. 2015). Research is urgently required to quantify more
 1949 fully the impacts of ecotourism on white sharks, and investigate whether some
 1950 individuals or one of the sexes is disproportionately exposed to its potentially
 1951 deleterious effects.

1952 **5.6. Discussion**

1953 It is clear from the available literature that sexual and individual differences exert strong
 1954 influences on white shark ecology across populations and contexts, and that these
 1955 differences have significant ramifications for their effective conservation management.
 1956 Such differences disproportionately predispose the sexes, and some individuals, to

1957 greater interaction with fishing apparatus, more prolonged exposure to environmental
 1958 pollutants, the effects of climate change, and the potential negative effects of
 1959 ecotourism activities.

1960 Our current understanding of individual and sexual variation in white shark
 1961 ecology is obfuscated by a lack of direct incorporation of sex in published analyses, in
 1962 addition to relatively small sample sizes. Samples are frequently simply split into shark
 1963 size classes, without consideration of sex or how sex and size may interact. As this
 1964 review shows that white sharks exhibit sexual dimorphism in size at maturation, tooth
 1965 morphology, movement patterns, habitat use, and diet, aggregation of data into size
 1966 classes without consideration of sex, often forced by small sample size, could have
 1967 serious implications for the usefulness and accuracy of results derived from such
 1968 studies.

1969 Several studies have shown that females range more widely than males, yet
 1970 others reveal that they spend a greater proportion of their time in coastal habitats (Zuffa
 1971 et al. 2002; Bonfil et al. 2005; Weng et al. 2007a; Domeier and Nasby-Lucas 2012,
 1972 2013; Weng and Honebrink 2013; Kock et al. 2013; Towner et al. 2016). Both of these
 1973 traits, and associated inferences for differences in diet, suggest that females are at
 1974 greater risk of encountering swimmer protection programmes and inshore fisheries, and
 1975 suffering greater exposure to, and contamination by, marine pollutants. Accumulation of
 1976 anthropogenic toxins, and interaction with fishing gear, both targeted and non-targeted,
 1977 are already recognised to pose serious threats to white sharks, despite their protected
 1978 status (Baum et al. 2003; Schlenk et al. 2005; Shivji et al. 2005; Fergusson et al. 2009;
 1979 Domeier and Nasby-Lucas 2012; Mull et al. 2013; Lyons et al. 2013a, b; Marsili et al.
 1980 2016), and if females are especially vulnerable, management strategies should reflect
 1981 this. Dietary specialisation has been found and/or inferred across populations (Estrada et

1982 al. 2006; Hussey et al. 2012b; Kim et al. 2012; Pethybridge et al. 2014; Christiansen et
 1983 al. 2015; French et al. 2017) and carries implications for food web effects, individual
 1984 survival, and exposure to pollutants. This phenomenon seems especially prevalent in
 1985 female white sharks, a trend also found in other marine predators (Young and Cockcroft
 1986 1994; Connan et al. 2014).

1987 Pace-of-Life-Syndrome hypothesis has recently been suggested in male white
 1988 sharks (French et al. 2017), suggesting that some males grow faster, mature more
 1989 quickly, and exhibit bolder behaviour than others (Ricklefs and Wikelski 2002; Réale et
 1990 al. 2010). These traits predispose fish to increased fishing mortality, and can result in
 1991 rapid depletion of genotypes (Biro and Post 2008; Conrad et al. 2011; Mittelbach et al.
 1992 2014). As culling efforts essentially comprise fishing for white sharks in the wake of a
 1993 shark-human interaction, they may be disproportionately attracting and removing faster
 1994 growing individuals, adding to the already negative effects of removing individuals
 1995 from an already depleted population. Pace-of Life Syndrome and its genetic component
 1996 in white sharks should be investigated promptly to assess potential impacts on
 1997 conservation of the species.

1998 Climate change, which is projected to result in steadily increasing seas surface
 1999 temperatures (Solomon et al. 2007), may also affect the sexes differently. Climate
 2000 change induced increases in ocean temperature and associated acidification negatively
 2001 affect shark growth and ability to hunt (Dixon et al. 2015; Pistevos et al. 2015, 2017;
 2002 Rosa et al. 2017). As males may be less varied in their habitat use and diet (Zuffa et al.
 2003 2002; Weng et al. 2007a; Domeier and Nasby-Lucas 2012; French et al. 2017;
 2004 OCEARCH 2017), they could also be less able to adapt to climate-mediated changes in
 2005 habitat and prey availability.

2006 Individual and sexual differences in behaviour have been demonstrated in
 2007 several sharks species, and suggested in white sharks (Laroche et al. 2007; Huvaneers et
 2008 al. 2013; Jacoby et al. 2014; Wilson et al. 2015; Huvaneers et al. 2015; Towner et al.
 2009 2016; Byrnes et al. 2016; Finger et al. 2016; Byrnes and Brown 2016; Finger et al.
 2010 2017, 2018). In addition to fishing mortality risks, individual differences in behaviour
 2011 could also result in some sharks being more affected by cage-diving ecotourism than
 2012 others (Laroche et al. 2007; Huvaneers et al. 2013). Suggested deleterious effects of
 2013 cage-diving include distraction from feeding and associated energy expenditure, decline
 2014 in predatory success, changes to predator/prey interactions, impairment of growth and
 2015 reproductive success, and reduced individual and population fitness (Laroche et al.
 2016 2007; Bruce and Bradford 2013; Huvaneers et al. 2013). Studies specifically assessing
 2017 the effects, in particular energetic costs, of cage-diving operations on individuals is
 2018 urgently required.

2019 Scientific studies of white shark ecology, especially diet and movement patterns,
 2020 often consider the population under investigation as a single unit. The evidence
 2021 reviewed here strongly suggests that sex and individual differences should be
 2022 considered explicitly in analyses of these data. Future research priorities should include
 2023 dietary specialisation and its drivers, differences in fisheries mortality and toxin
 2024 accumulation between the sexes, Pace-of-Life-Syndrome hypothesis, in particular in
 2025 males and combined with genetic testing, and the effects of cage diving ecotourism on
 2026 individuals.

Chapter 6 General Discussion

In this thesis I explore the roles of sexual and individual variation in white shark trophic ecology, and their effects on ontogenetic shift dynamics. These relationships are examined through tooth shape metrics, and stable isotopes and fatty acid analyses, in addition to a review of the existing literature.

Ontogenetic shifts in diet and habitat use, often facilitated by morphological changes in foraging apparatus, can have profound effects on an individual or age/size class's resource requirements, functional role, and conservation needs (Werner and Gilliam 1984; Polis 1984; Werner and Hall 1988; McCauley et al. 1996; Law and Dickman 1998; Scharf et al. 2000; Grubbs 2010). For the first time, I have shown that both sex and individual variation have strong effects on ontogenetic shift dynamics in white sharks, evidenced by tooth morphology (Chapter 2), stable isotope analysis (Chapter 3), fatty acid analysis (Chapter 4) and a review of the available evidence in the published literature (Chapter 5).

It was previously accepted that when white sharks reach approximately three meters length, they undergo an ontogenetic shift in diet that involves the inclusion of marine mammals as prey, and that this shift is facilitated by a change in tooth shape from cuspidate to broad (Tricas and McCosker 1984; Frazzetta 1988; Compagno 2001). This tooth shape change is cited ubiquitously in the white shark literature, despite the fact it was originally based on only 16 sharks (Tricas and McCosker 1984) and that the effect of sex on tooth cuspidity change through ontogeny has never been explored, only tooth height (Randall 1973, 1987; Mollet et al. 1996; Shimada 2002b). In Chapter 2, I aimed to explore whether this ontogenetic shift in tooth shape did indeed occur, and if it

2051 was influenced by sex and individual variation. I further aimed to extend our current
 2052 knowledge of ontogenetic tooth shape change in white sharks by including a novel
 2053 metric; the angle of the upper intermediate, or P3 tooth, as this measurement was found
 2054 to vary considerably between individuals (Hubbell 1996). Incorporating results from a
 2055 novel photographic method, for the first time we found evidence for both sexual and
 2056 individual variation in white shark ontogenetic tooth shape change, including a
 2057 previously unreported change in P3 tooth shape in male sharks which could be an
 2058 adaptation for handling marine mammal prey, or allow males to more effectively grasp
 2059 females during copulation (Chapter 2). Future research in this area should directly
 2060 compare tooth shape with dietary data, to determine whether tooth shape is related to
 2061 foraging or reproduction.

2062 Significantly, Chapter 2 provided the first argument for Pace-of-Life-Syndrome
 2063 (POLS) (Ricklefs and Wikelski 2002; Réale et al. 2010) in white sharks, at least in
 2064 males. Here, we linked individual variation in the size at which males underwent an
 2065 ontogenetic shift in tooth shape, to previously published data on variation in increase in
 2066 testes mass associated with sexual maturity (Cliff et al. 1989). Further evidence for
 2067 POLS was discussed in Chapter 5, and interestingly individual variation in growth rate
 2068 has recently been identified in juvenile lemon sharks (*Negaprion brevirostris*), with
 2069 links to personality suggested (Hussey et al. 2017). POLS, linked to physiology,
 2070 personality, and life history, could have serious implications for white shark
 2071 conservation management, as reviewed in Chapter 5. Given this information, and the
 2072 significant potential conservation management ramifications, studies directly testing for
 2073 POLS in white sharks, and other shark species, appear warranted.

2074 Chapter 3 represents the first stable isotope study on white sharks that examined
 2075 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ relationships with shark length separately for the sexes. The results from

Chapter 3 and examination of the literature in Chapter 5 suggest that failure to do so can confound interpretation of long-term patterns in trophic and ontogenetic differences between males and females. In concordance with the results of the tooth shape data analyses in Chapter 2, Chapter 3 revealed clearer evidence of a predictable ontogenetic shift in male sharks in contrast to females, especially in terms of habitat use. When considered alongside evidence from the northeast Pacific (Kerr et al. 2006; Carlisle et al. 2012), the northwest Atlantic (Hamady et al. 2014) and South Africa (Chapter 5), it seems that males across genetically distinct populations exploit pelagic food webs as they grow.

Chapters 3 and 4 further represent the first stable isotope and fatty acid analysis results from free-swimming white sharks in South Africa, and Chapter 4 is the first study to compare results from these analyses in the species. Interestingly, fatty acid results in Chapter 4 revealed a dietary separation between smaller and larger females that was not evident in stable isotope results (Chapter 3). It would be worthwhile to explore this further using mixing models, which would allow identification of the different prey species contributing to the diets of the female size classes. This finding highlights the usefulness of combining trophic biomarker methods to study ecology, as advocated by Christiansen et al., (2015). Fatty acids further indicated a potential second ontogenetic shift in sharks over four meters in length, involving a reduced reliance on marine mammals. This relationship was not significantly influenced by sex, which contrasts with the stable isotope results in Chapter 3, and patterns suggested in other stable isotope studies (Kerr et al. 2006; Hussey et al. 2012b; Carlisle et al. 2012; Hamady et al. 2014). These studies point towards a reduction in trophic level in large males and less clear patterns in females. It could be that the comparatively short-term nature of fatty acids fails to detect differences in trophic ecology picked up by more

2101 long-term representative stable isotope analysis (Iverson et al. 2002; MacNeil et al.
 2102 2005; Martinez del Rio et al. 2009; Logan and Lutcavage 2010; Hussey et al. 2012c).
 2103 However, stable isotopes, fatty acids, and a review of the current literature (Chapters 3,
 2104 4 and 5 respectively) all point towards wide ranging and varied movement patterns,
 2105 habitat utilization, and food web exploitation in female white sharks, with especial
 2106 importance of tropical ecosystems and coastal habitats.

2107 Because stable isotopes and fatty acids represent relatively long and short time
 2108 frames respectively, and because white sharks are highly mobile, future studies should
 2109 combine these analyses with telemetry to allow for more precise interpretation foraging
 2110 and food web utilization within an understood temporal context. Pooling of data
 2111 between research projects to boost sample size would also allow for more robust
 2112 statistical analyses. As ontogenetic changes can alter a species' functional role within an
 2113 ecosystem (Werner and Gilliam 1984; Polis 1984; Scharf et al. 2000; Grubbs 2010), it is
 2114 important that the ontogenetic shift dynamics identified here are understood.

2115

2116 In addition to the effects of sex on white shark trophic ecology and ontogenetic shift
 2117 dynamics, I aimed to improve our understanding of individual and sexual variation in
 2118 white shark ecology more generally, and if and how sex and individual variation may
 2119 interact. Individual variation can be strongly influenced by sex in marine predators
 2120 (Young and Cockcroft 1994; Kernaléguen et al. 2012; Connan et al. 2014; Smith et al.
 2121 2015), and this has actually been demonstrated in two South African white shark prey
 2122 species; cape fur seals (*Arctocephalus pusillus pusillus*) (Connan et al. 2014) and
 2123 common dolphin (*Delphinus delphis*) (Young and Cockcroft 1994). Similarly, size
 2124 and/or life stage can also have significant effects on individual variation (Scharf et al.
 2125 2000), which is already evident in white sharks (Hussey et al. 2012b; Christiansen et al.

2126 2015). Despite this, the only studies to have explicitly tested for individual variation in
 2127 white shark trophic ecology (Kim et al. 2012; Pethybridge et al. 2014) failed to
 2128 incorporate the combined influence of sex and size. I found evidence of individual
 2129 variation, influenced both by sex and size in Chapters 2, 3, 4 and 5 of this thesis.

2130 Stable isotope analysis highlighted prevalence of expanded trophic niche in
 2131 smaller sharks (Chapter 3), which concords with previously published white shark data
 2132 (Hussey et al. 2012b; Christiansen et al. 2015). Female white sharks pup in discrete
 2133 nursery areas and juveniles aggregate in specific habitats, usually close to the shore
 2134 (Klimley 1985; Dewar et al. 2004; Weng et al. 2007b; Bruce and Bradford 2012;
 2135 Domeier and Nasby-Lucas 2013; Lyons et al. 2013b; Harasti et al. 2017). Risk-benefit
 2136 tradeoffs related to foraging habitat and predation risk (Stamps 2007; Wolf et al. 2007)
 2137 have been suggested as mechanisms driving individual variation in diet and movement
 2138 patterns in juvenile bull (*Carcharhinus leucas*) and lemon sharks (Matich and Heithaus
 2139 2015; Finger et al. 2016; Hussey et al. 2017). Future research into whether young white
 2140 sharks also display individual variation in risk-benefit tradeoffs, or obtain their varied
 2141 isotopic signatures from different nursery grounds or maternal influence would benefit
 2142 our understanding of white shark ecology and management needs (Matich et al. 2015;
 2143 Christiansen et al. 2015).

2144 The results from Chapters 3 and 4 mean that Gansbaai is the first white shark
 2145 aggregation recorded to exhibit sexual and individual variation in both hunting behavior
 2146 (Towner et al. 2016) and diet, providing evidence for behavior-linked dietary
 2147 specialization. Trophic specialization, especially in females, was suggested by tooth
 2148 shape analysis (Chapter 2), stable isotope analysis (Chapter 3) and fatty acid analysis
 2149 (Chapter 4). It is possible that this trophic specialization, which was independent of

2150 size, is linked to personality differences in females that remain consistent through
2151 ontogeny and/or phenotypic polymorphism with regards to tooth shape type.

2152

2153 The underlying causes of sexual habitat segregation in elasmobranchs, including white
2154 sharks, is poorly understood (Wearmouth and Sims 2008). Competitive exclusion is
2155 thought to be unlikely (Bruce and Bradford 2015), as is female avoidance of male
2156 sexual coercion, given that segregation occurs between both mature and immature
2157 sharks (Kock et al. 2013; Towner et al. 2016). One theory behind sexual habitat
2158 segregation in white sharks is the thermal-niche hypothesis, where females are
2159 hypothesized to select warmer temperatures to increase their growth rate, enabling them
2160 to attain a larger size than their male conspecifics which may help them to cope better
2161 with bites endured copulation, and improve fecundity (Robbins 2007; Towner et al.
2162 2013a). However, some long-term studies have found no significant relationships
2163 between temperature and female attendance at aggregation sites (Bruce and Bradford
2164 2015). While there is some evidence, based on small sample sizes, that females may
2165 grow at a faster rate than males (Tanaka et al. 2011; Hamady et al. 2014), it is not yet
2166 certain if this is the case (Cailliet et al. 1985; Wintner and Cliff 1999; Kerr et al. 2006;
2167 Natanson and Skomal 2015; Andrews and Kerr 2015). It seems more likely that females
2168 achieve greater size in the same way as other members of the lamnidae family, where
2169 male growth rate reduces once they reach sexual maturity, which is at a smaller size
2170 than females, and females continue to grow (Campana et al. 2001; Natanson et al. 2002;
2171 Bishop et al. 2006). Also, the multiple migration strategies suggested in females in
2172 Chapter 3 don't lend support to the uniform habitat selection that would be expected if
2173 females were selecting for warm temperatures. However, until the growth rates and
2174 nutritional requirements of the sexes are better understood, it is not possible to discern

2175 whether thermal niche, forage selection, activity budget, or perhaps a combination of
2176 two or more factors are the reason behind the observed sexual segregation recorded in
2177 this thesis and other studies (Chapter 5).

2178 The patterns of individual and sexual variation in white shark trophic ecology
2179 identified in this thesis and elsewhere have clear implications for conservation
2180 management (Chapter 5). Through reviewing the available data in Chapter 5, I have
2181 highlighted that female white sharks are especially at risk from multiple threats that
2182 includes exposure to toxins, which will affect new generations of sharks through
2183 maternal offloading (Schlenk et al. 2005; Mull et al. 2013; Lyons et al. 2013a; Marsili et
2184 al. 2016), and fisheries interactions. In light of the evidence presented in this thesis, it
2185 seems likely that some individuals, and especially in males, grow faster than others.
2186 Given the potential for these individuals be disproportionately removed from the
2187 population via fishing mortality (Biro and Post 2008) and evidence that this may already
2188 be occurring (Wintner and Cliff 1999), swimmer safety programmes, culls, and any
2189 form of deliberate legal fishing for white sharks should be even more carefully
2190 considered.

2191

2192 Broadly speaking, this work has aimed to assess and review the roles of sexual and
2193 individual variation in the trophic ecology of the white shark. The evidence presented
2194 clearly shows that sexual and individual variation play major roles in white shark
2195 trophic ecology, particularly in ontogenetic shift dynamics, and future ecological studies
2196 should consider these factors in study design and analyses. There is a lot of work to be
2197 done in terms of understanding the proximate and ultimate causes of individual and
2198 variation in white sharks, and elasmobranchs generally. Determination of sex-specific
2199 growth rates and direct investigation of Pace-of-Life-Syndrome and personality in white

2200 sharks should be ranked amongst white shark research priorities. Finally, the
2201 implications of sexual and individual variation presented here should be directly
2202 incorporated into conservation management strategies.

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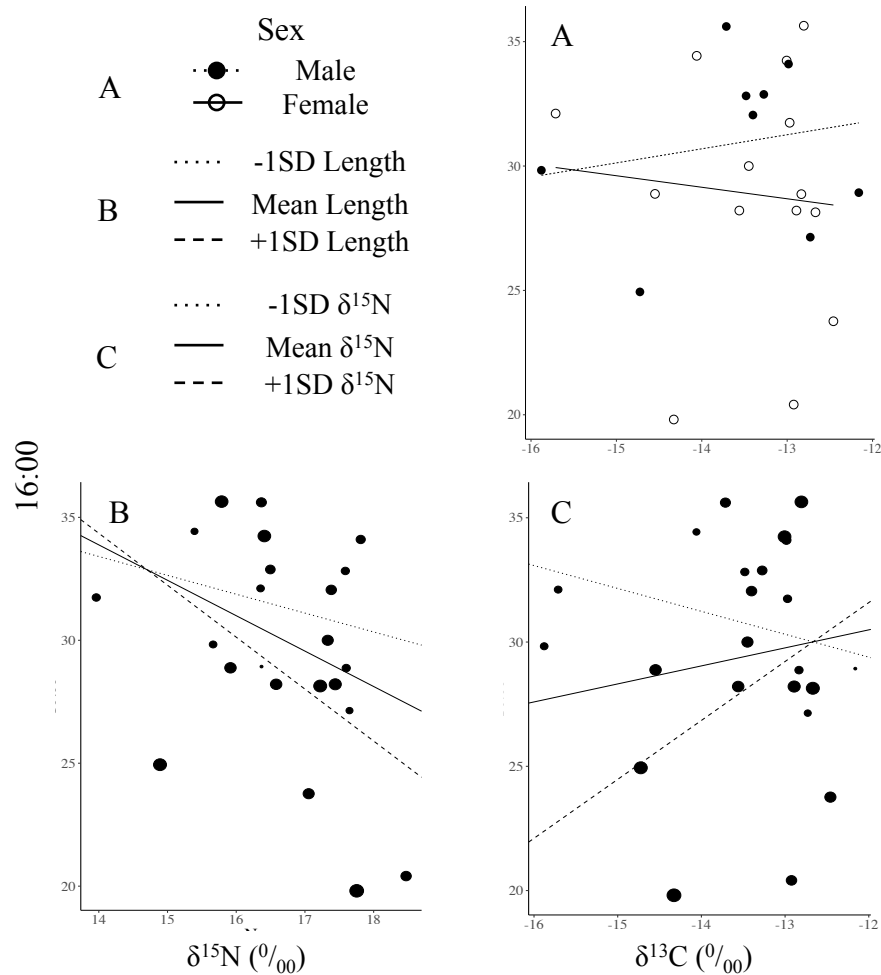
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3493

3494 **Supplementary Data**

3495 *Fatty Acid Profile*

3496 Saturated fatty acids were the most abundant (average $53.52\% \pm 1.5$) followed by
3497 monounsaturated (MUFA) (average $27.09\% \pm 1.1$) and polyunsaturated (PUFA)
3498 (average $16.30\% \pm 1.36$).



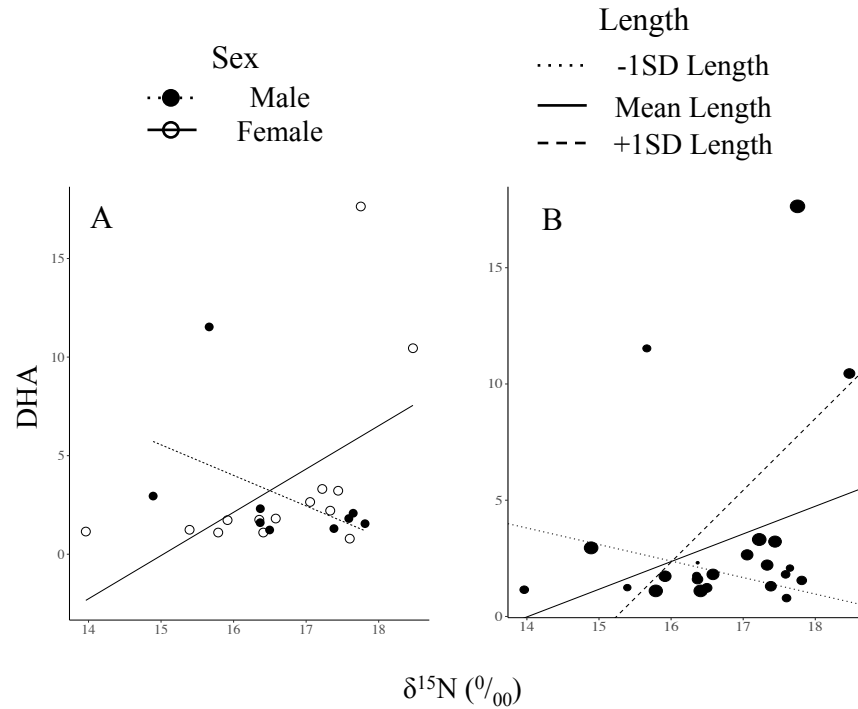
3499

3500 **Supplementary Figure 1:** Significant averaged general linear model (see Table 2 for full
3501 models) two-way interaction plots of fatty acid 16:0 (%) and A) $\delta^{13}\text{C}$ (‰), influenced by sex; B)
3502 $\delta^{15}\text{N}$ (‰) influenced by length, with lines of best fit of predicted values. Symbol size reflects

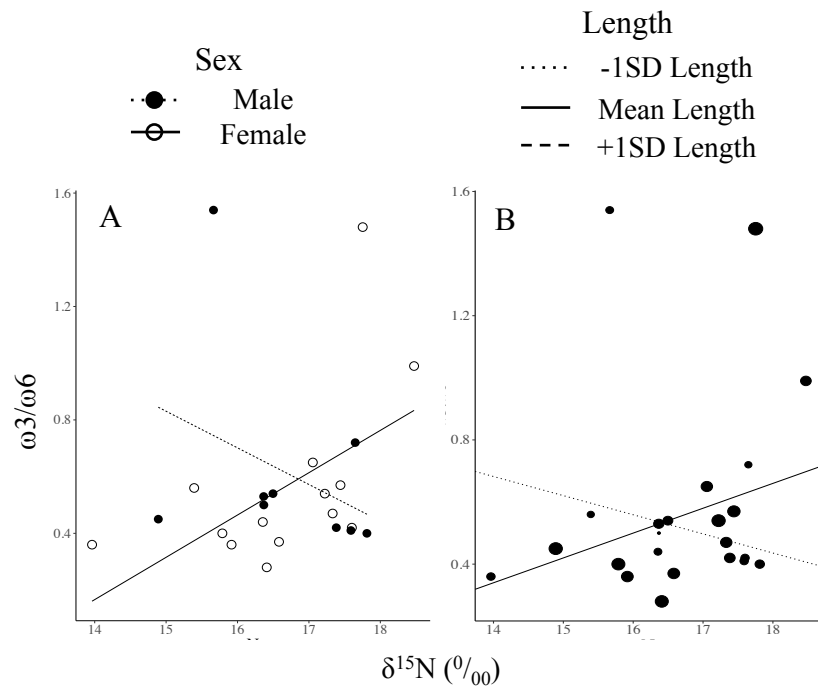
3503 shark length, with larger symbols denoting longer sharks and slopes are fitted to -1 standard
3504 deviation of length, mean length, and + 1 standard deviation length, to illustrate the interaction
3505 effect; C) $\delta^{13}\text{C}$ (‰) influenced by $\delta^{15}\text{N}$ (‰). Symbol size reflects $\delta^{15}\text{N}$, with larger symbols
3506 denoting higher values and slopes are fitted to -1 standard deviation of $\delta^{15}\text{N}$, mean $\delta^{15}\text{N}$, and + 1
3507 standard deviation $\delta^{15}\text{N}$, to illustrate the interaction effect. N.B. For illustrative purposes, the
3508 plots are based on models with a Gaussian distribution, while the statistical models all utilised a
3509 Gamma distribution (Table 4.2).

3510

3511 In the 16:00 model $\delta^{13}\text{C}$ had a significant interaction with sex, where males and females
3512 had positive and negative relationships respectively (Supplementary Figure 1A). There
3513 was also a significant interaction between $\delta^{15}\text{N}$ and shark length, where the slope of
3514 larger sharks was much more steeply negative than the slope for smaller sharks
3515 (Supplementary Figure 1B). Finally, there was a significant interaction between $\delta^{15}\text{N}$
3516 and $\delta^{13}\text{C}$, where 16:0 increased with mean and high levels of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, and was
3517 decreased when $\delta^{15}\text{N}$ $\delta^{13}\text{C}$ were low (Supplementary Figure 1C).



3518 **Supplementary Figure 2:** Significant averaged general linear model (see Table 2 for full
 3519 models) two-way interaction plots of fatty acid DHA (%) and A) $\delta^{15}\text{N}$ and sex; B) $\delta^{15}\text{N}$ and
 3520 shark length (m). Symbol size reflects shark length, with larger symbols denoting longer sharks
 3521 and slopes are fitted to -1 standard deviation of length, mean length, and + 1 standard deviation
 3522 length, to illustrate the interaction effect. N.B. For illustrative purposes, the plots are based on
 3523 models with a Gaussian distribution, while the statistical models all utilised a Gamma
 3524 distribution (Table 4.2).
 3525



3526

3527 **Supplementary Figure 3:** Significant averaged general linear model (see Table 2 for full3528 models) two-way interaction plots of fatty acid $\omega 3/\omega 6$ (%) and A) $\delta^{15}\text{N}$ and sex; B) $\delta^{15}\text{N}$ and

3529 shark length (m). Symbol size reflects shark length, with larger symbols denoting longer sharks

3530 and slopes are fitted to -1 standard deviation of length, mean length, and + 1 standard deviation

3531 length, to illustrate the interaction effect. N.B. For illustrative purposes, the plots are based on

3532 models with a Gaussian distribution, while the statistical models all utilised a Gamma

3533 distribution (Table 4.2).

3534

3535 DHA and $\omega 3/\omega 6$ had a significant interaction between $\delta^{15}\text{N}$ and sex, where females

3536 exhibited a positive relationship in contrast to the negative relationship in the male

3537 sample (Supplementary Figures 2A, 3A). $\delta^{15}\text{N}$ had further significant interactions with

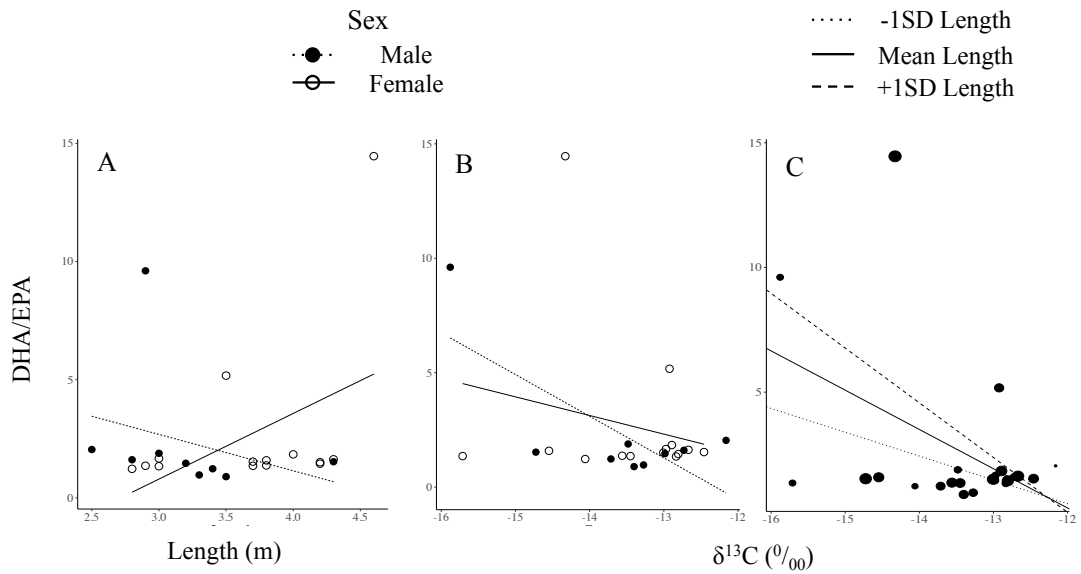
3538 shark length, revealing a negative relationship in smaller sharks, and a positive

3539 relationship in larger sharks (Supplementary Figures 2B, 3B). Both of these interactions

3540 were heavily influenced by a small number of large DHA $\omega 3/\omega 6$ values, despite the

3541 mitigation of using a Gamma link.

3542



3543

3544 **Supplementary Figure 4:** Significant averaged general linear model (see Table 2 for full
 3545 models) results of fatty acid DHA/EPA (22:6 ω 3/20:5 ω 3) (%) and two-way interactions between
 3546 A) shark length (m) influenced by shark sex, B) $\delta^{13}\text{C}$ influenced by shark sex, and C) $\delta^{13}\text{C}$
 3547 influenced by shark length (m). Symbol size reflects shark length, with larger symbols denoting
 3548 longer sharks and slopes are fitted to -1 standard deviation of length, mean length, and + 1
 3549 standard deviation length, to illustrate the interaction effect. N.B. For illustrative purposes, the
 3550 plots are based on models with a Gaussian distribution, while the statistical models all utilised a
 3551 Gamma distribution (Table 4.2.).

3552

3553 The model for diatom vs. dinoflagellate food webs, DHA/EPA, revealed a significant
 3554 effect of shark length in interaction with sex, where DHA/EPA increased with
 3555 increasing shark length in females, and decreased with increasing shark length in males
 3556 (Supplementary Figure 4A). In this model, sex also had a significant interaction with
 3557 $\delta^{13}\text{C}$, where both sexes exhibited a negative slope, but this was much steeper in females
 3558 (Supplementary Figure 4B). Finally, $\delta^{13}\text{C}$ had a further significant interaction with
 3559 shark length, where the negative slope of larger sharks was much steeper than the slopes
 3560 for mean sized and smaller sharks (Supplementary Figure 4C). Similarly to the results

for DHA, interactions were influenced by extreme data points, though the effects were dampened with the use of the Gamma link function.

Supplementary Discussion

Fatty Acid Profile of Muscle Tissue

Saturated fatty acid 16:00 was the major contributor to our high SFA results, levels of which were much greater in our samples compared to Australian sharks (29.50% vs. 18.55% respectively). 16:00 had complex relationships with shark sex, length, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Figure 1, Supplementary Figure 1), which may indicate multiple sources and locations of high 16:00 prey exploited disproportionately by different demographic groups (Post 2002; Hill et al. 2006; Hill and McQuaid 2008; Allan et al. 2010). Dominance of saturated fatty acids (SFA) has been recorded previously in white sharks from South Africa and Australia, but SFA levels in our samples were much higher – 53.52% compared to 34.82% and 34.7% for South Africa and Australia respectively (Davidson et al. 2011; Pethybridge et al. 2014). While our MUFA proportions were very similar to these studies (27.09% vs. 25.26% and 27.08%), our levels of PUFA were much lower (16.30% vs. 29.5% and 34.48%). Both of these studies demonstrated much higher levels of ω 3 PUFA (29.5% and 34.48% compared to our 6.07%), of which DHA was significantly higher (15.52% and 9.90% respectively) than our samples, which only averaged 3.36%. This low value is much closer to that found in sub-dermal tissue of whale sharks (*Rhincodon typus*), sampled further up the coast in Mozambique (Couturier et al., 2013). Low PUFA is more similar to dusky and spinner sharks sampled in South Africa (Davidson et al. 2011), which mostly consume elasmobranchs and teleosts, and teleosts respectively (Allen and Cliff 2000; Dudley et al. 2005), and

3586 are known prey of white sharks (Cliff et al., 1989; Hussey et al., 2012).

3587

3588 ***PCA and Female Size***

3589 In our samples, smaller and larger females were almost completely separated along the
 3590 PC1 axis, where larger females correlated more with greater amounts of ARA and
 3591 DHA. High levels of ARA and dominance of n6 pathways have been linked to tropical
 3592 marine ecosystems (Couturier et al., 2013; Sinclair et al., 1983). Previous research off
 3593 Mozambique, where large South African white sharks are known to visit during return
 3594 migrations (OCEARCH, 2017) found very high levels of ARA in the samples of whale
 3595 sharks (*Rhincodon typus*) and reef manta rays (*Manta alfredi*) (Couturier et al., 2013).
 3596 The largest female included in this study was a 4.6m individual, which has previously
 3597 been satellite tracked moving from Gansbaai, up the coast to Mozambique, and then
 3598 across to Madagascar, before returning to Gansbaai (OCEARCH 2017), and to date
 3599 only very large females are known to travel to the northern Mascarene plateau (Cliff et
 3600 al. 2000; Zuffa et al. 2002; OCEARCH 2017). Separation of female size classes on PC1
 3601 could therefore be caused by larger females making more extensive tropical migrations
 3602 than smaller females, which may be linked with a high DHA food source, though
 3603 reproductive state could also be a factor (Pethybridge et al. 2011b) . In this instance,
 3604 ARA and $\omega 6$ generally, could be a useful tool for detecting tropical habitat use. Smaller
 3605 ellipses in females generally, may point to a more restricted diet than in males, despite
 3606 overlap between all ellipses.

3607

3608 ***18:1 ω 9 and Trip***

3609 18:1 ω 9 was higher in sharks sampled during Trip 1 than Trip 2. This is surprising as
 3610 Trip 2 occurs within the peak season for seal predation by sharks in Gansbaai (Towner

3611 et al., 2013a). However, fatty acids have been shown to take up to 18 weeks to reflect
3612 dietary changes in shark muscle tissue (Beckmann et al., 2013, 2014), which would
3613 overlap with seasonal availability of aggregating whales in Gansbaai, which could be
3614 another source of high 18:1 ω 9 (Waugh et al., 2012). 18:1 ω 9 also increases with depth
3615 (Lewis 1967) and has been interpreted as evidence of deep diving behavior in whale
3616 sharks (Rohner et al., 2013), though this seems unlikely in white sharks.

1391 Table 4.1: Dietary fatty acids (%) with means > 0.05% included in analyses.

Group	14:0	16:0	18:0	16:1ω7	18:1ω9	18:1ω7	20:1ω11	20:1ω9	20:1ω7	22:1ω11	18:2ω6	18:3ω6	20:2ω6	20:3ω6	20:4ω6	22:4ω6	22:5ω6	18:3ω3	18:4ω3	20:5ω3	22:5ω3	22:6ω3
M<3m	3.99	28.93	15.94	1.5	16.48	2.11	0.18	0.8	0.14	0.48	2.29	0.36	0.1	0.00	5.89	0.99	0.19	0.52	0.00	1.13	0.92	2.31
F>3m	3.11	23.76	11.84	2.14	23.37	2.79	0.26	1.43	0.19	1.09	4.28	0.23	0.22	0.00	5.27	1.09	0.19	1.26	0.25	1.73	1.16	2.65
M>3m	3.5	32.05	14.64	0.93	15.82	1.61	0.29	0.96	0.00	1.04	2.86	0.41	0.00	0.26	4.39	0.65	0.00	0.27	0.00	1.45	0.56	1.3
F>3m	3.48	35.64	15.81	1.05	18.21	1.4	0.21	0.86	0.29	0.64	3.19	0.4	0.00	0.00	2.61	0.59	0.00	0.32	0.15	0.76	0.39	1.1
F>3m	3.41	28.21	15.99	1.59	14.03	2.48	0.2	1.05	0.23	0.65	1.74	0.35	0.25	0.00	8.07	1.43	0.22	0.28	0.00	1.32	1.05	1.81
F>3m	2.75	28.88	15.05	1.36	15.84	2.37	0.18	1.04	0.19	0.78	2.5	0.31	0.29	0.00	7.21	1.83	0.16	0.29	0.16	1.09	1.2	1.73
M>3m	3.02	35.61	15.91	1.08	16.64	1.72	0.24	1.14	0.16	1.01	3.25	0.45	0.16	0.00	2.57	0.46	0.00	0.43	0.00	1.3	0.3	1.6
M>3m	3.21	34.1	16.7	1.13	15.09	1.68	0.19	0.74	0.16	0.4	2.71	0.31	0.00	0.00	5.09	1.0	0.15	0.31	0.00	1.06	0.79	1.55
F<3m	2.96	34.43	16.96	1.27	19.24	1.46	0.22	1.11	0.00	1.28	2.9	0.48	0.00	0.00	2.37	0.41	0.00	0.47	0.00	1.01	0.57	1.24
F<3m	2.69	28.87	13.77	0.72	27.66	1.87	1.68	1.52	0.00	1.77	2.38	0.5	0.00	0.00	1.86	0.37	0.00	0.36	0.16	0.59	0.26	0.79
F<3m	3.0	31.74	16.75	1.58	17.99	2.06	0.27	1.42	0.00	1.2	2.58	0.58	0.00	0.00	3.88	0.8	0.00	0.31	0.00	0.69	0.68	1.15
F<3m	2.75	32.11	16.31	1.52	17.27	2.74	0.24	0.74	0.15	0.38	2.38	0.34	0.00	0.94	4.39	0.57	0.17	0.51	0.19	1.29	0.17	1.75
F>3m	3.7	34.24	14.65	2.0	15.76	2.58	0.23	0.5	0.00	0.33	5.6	0.00	0.00	1.32	3.15	0.65	0.00	0.55	0.17	0.73	0.41	1.1
M<3m	4.87	27.14	13.35	2.56	21.24	3.93	0.27	1.21	0.12	0.76	3.65	0.12	0.12	1.1	3.65	0.58	0.12	2.37	0.22	1.29	0.59	2.08
F>3m	10.47	28.21	13.07	1.45	11.72	2.27	0.17	0.88	0.19	0.61	1.69	0.00	0.00	0.18	8.13	1.27	0.27	0.27	0.2	1.75	1.15	3.22
F>3m	1.12	19.81	16.36	1.14	10.0	4.07	0.00	0.86	0.12	0.24	1.06	0.25	0.12	0.19	9.86	2.58	0.68	0.11	0.00	1.22	2.9	17.64
M<3m	1.48	29.83	15.57	1.26	11.85	3.19	0.21	0.78	0.00	0.41	2.04	0.00	0.00	0.22	5.82	1.45	0.47	0.3	0.25	1.2	2.07	11.53
F>3m	2.86	30.0	17.55	1.25	14.27	2.11	0.21	0.59	0.13	0.37	2.38	0.00	0.00	0.15	7.4	0.99	0.14	0.23	0.00	1.63	1.15	2.21
F>3m	1.66	28.14	16.94	1.73	14.83	2.79	0.21	0.79	0.17	0.38	2.31	0.00	0.00	0.00	8.42	1.54	0.33	0.22	0.00	2.03	1.19	3.31
M>3m	1.69	24.94	17.27	1.68	16.45	2.74	0.23	1.24	0.21	0.48	1.83	0.00	0.14	0.00	9.62	1.94	0.28	0.16	0.00	1.93	1.19	2.95
M<3m	10.76	32.82	16.12	0.97	11.85	1.51	0.24	0.68	0.14	0.37	1.98	0.00	0.3	0.15	5.97	0.79	0.14	0.19	0.00	0.96	0.89	1.81
F>3m	1.45	20.41	14.6	1.47	11.77	3.61	0.14	0.7	0.14	0.24	1.47	0.00	0.00	0.16	11.2	2.21	0.44	0.18	0.00	2.02	2.67	10.45
M>3m	24.46	32.88	11.28	0.97	9.53	1.54	0.2	0.5	0.00	0.38	1.38	0.00	0.12	0.00	3.8	0.62	0.12	0.13	0.19	1.27	0.47	1.23