Adaptations in allopatric populations of *Triakis megalopterus* isolated by the Benguela Current. Steps towards understanding evolutionary processes affecting regional biodiversity.

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Abstract

This study was initiated to gain a better understanding of evolution and adaptation of elasmobranchs by investigating how a putative biogeographic barrier, the Benguela Current, had influenced populations of a demersal shark species, *Triakis megalopterus*. It was hypothesized that the Benguela Current formed a biogeographic barrier in the distribution of *T. megalopterus* and was responsible for the divergence between South African (SA) and Angolan (AN) populations. Since elasmobranchs are generally characterized by a slow rate of evolutionary change and conservative morphology and life history traits, it was hypothesized that there would be limited genetic, morphological and life history divergence between the populations.

Both mtDNA Control Region (mtCR) and microsatellites (nDNA) were used to assess population connectivity and structure of *T. megalopterus*. The mtCR predominantly showed a northern (Angola, AN, and Namibia, NA) versus southern (Western Cape, WC, and Eastern Cape, EC) Benguela subsystem arrangement. This suggested that the formation of the Benguela Current had an influence on the genetic structure of *T. megalopterus* during the early Pleistocene. The nDNA, however, showed a distinct transoceanic, Atlantic (AN, NA, WC) versus Indian Ocean (EC) arrangement, and this was attributed to the more recent exposure of the Agulhas Bank and reduced rocky shore habitat during the glaciations of the late Pleistocene.

Traditional morphological analyses on full body and tooth morphology were used to assess phenotypic plasticity and/or adaptability of *T. megalopterus*. A novel method of geometric morphology, with potential for non-lethal application, was developed and tested to examine interpopulation divergence in shape. Traditional morphometrics showed significant divergence between populations and this variation was congruous with the mtCR haplotypes. However, the divergence in the truss variables was not concomitant to the haplotypes and

suggested that differences in shape may be attributed to phenotypic plasticity. There was limited divergence in the tooth morphology between populations. The divergence in several morphological characters associated with swimming speed and manoeuvrability may be attributed to both habitat structure and dominant prey in the different biogeographic zones.

The diet of *T. megalopterus* consisted primarily of crustaceans, teleosts and molluscs. The significant variation in the diet between populations suggested a generalist tooth configuration and broad trophic adaptability.

There was significant divergence in the interpopulation life history parameters. The AN population had the fastest growth, smallest size at maturity, and shortest longevity. Individuals in the EC population had the youngest age at maturity, while the WC population had the earliest parturition. This divergence may be attributed to the contrasting thermal regimes in the three biogeographic regions and the dissimilar exploitation rates of the three populations.

The results of this thesis demonstrated that a combination of the formation of the Benguela Current and sea level change most likely contributed to vicariance of three populations of *T. megalopterus*. The significant interpopulation morphological and life history divergence appeared to be both phenotypic and genetic, and suggested that contrasting environmental drivers can result in relatively rapid change in elasmobranchs.

"The sea, once it casts its spell, holds one in its net of wonder forever."
~Jacques Yves Cousteau~

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Granny, my biggest supporter, I did it! NOW you can pop the champagne.

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Chapter 1:
General introduction

The oceans cover 71% of the planet (Castro and Huber, 2003; Trujillo and Thurman, 2011), with a total volume of ± 1370 x 10^6 km^3 and around 300 times more habitable volume than land and fresh water systems combined (Lalli and Parsons, 1997). Five oceans surround the continents, namely the Pacific, Atlantic, Indian, Arctic, and Southern Oceans (Farndon, 2011). The coasts of southern Africa encounter two oceans, the southern Atlantic Ocean on the west and the Indian Ocean on the east, which merge at the southernmost tip of Africa, the meridian of Cape Agulhas (Stewart, 2008). The Atlantic and Indian Oceans are the second and third largest oceans respectively, correspondingly occupying surface areas of approximately 77 million and 69 million km^2 (McCutcheon and McCutcheon, 2003).

The oceans, each with their own set of distinct characteristics (Stewart, 2008), are connected via an intricate network of currents. These are primarily driven by wind, topographic features, the Earth's rotation (Coriolis Effect) and water temperature, and move warm and cold water great distances across the Earth's oceans (Levinton, 2001; Beesley *et al.*, 2008; Stewart, 2008). Due to the Coriolis Effect, currents rotate in circular paths (gyres) that revolve clockwise and counter-clockwise in the Northern and Southern Hemispheres, respectively (McCutcheon and McCutcheon, 2003; Farndon, 2011). Boundary currents, classified as either western or eastern boundary currents, flow parallel to either the eastern or western coastline, respectively (Stewart, 2008). Eastern boundary currents are characterized as relatively shallow, broad and slow/weak currents (Karleskint *et al.*, 2010) that transport cold water from the poles to the equator (Philander and Yoon, 1982). Western boundary currents are warm, deep, narrow and fast-flowing currents that form on the west side of ocean basins and transport water from the equator towards the Polar Regions (Cronin *et al.*, 2010; Karleskint *et al.*, 2010). Seas of the southern African coastal region are controlled by three ocean currents: Angola, Agulhas and Benguela (see **Chapter 2**, **Figure 2.1**).

The Benguela Current is arguably the most influential of these and is one of the five major continental margin upwelling systems of the world (Jahn *et al.*, 2003). Sedimentological, paleontological and geochemical data indicate the Benguela Current formation began off Northern Namibia in the late Miocene, ± 10 Ma. (Siesser, 1980; Diester-Haass *et al.*, 1990; Krammer *et al.*, 2006). Since then, however, the Pliocene-Pleistocene transition (~2 Ma.) gave rise to the intensification of ice sheet coverage in the northern and southern hemispheres, the formation of the Isthmus of Panama and established the Benguela Current characteristics

present today (Marlow *et al.*, 2000; Krammer *et al.*, 2006). The establishment of the Benguela Current had a major impact on the fish fauna of the South African, Namibian and southern Angolan coasts and resulted in a marked change in the distribution and abundance of fishes in the region (Henriques, 2011).

The Benguela Current is a highly productive, cold-water upwelling regime that flows northward along the southwest coast of Africa between 15° S and 34° S (Siesser, 1980) and forms the eastern limb of the subtropical gyre (Nelson and Hutchings, 1983; McCutcheon and McCutcheon, 2003; Veitch *et al.,* 2006; Veitch, 2007). The Benguela Current extends from the Angola-Benguela Frontal Zone (ABFZ), over the western Agulhas Bank (WAB) to Cape Agulhas (Shannon *et al.*, 1983; Lutjeharms *et al.*, 1991), not to Cape Point as formerly proposed (Andrews and Hutchings, 1980). This current is unique when compared to the other eastern boundary currents as it is bounded by warm water current systems, the western boundary Agulhas Current in the south and the warm, tropical Angolan Current in the north, which create warm-temperate confluence zones at both borders (Shannon and Nelson, 1996; Veitch, 2007).

As an upwelling regime, the Benguela Current is rich in nitrate, silicate and phosphates. It has abundant phytoplankton and consequently rich fishing grounds (Siesser, 1980), although these characteristics are not uniform throughout its distribution. Dividing the Benguela into northern and southern subsystems is the perennial (Lutjeharms and Meeuwis, 1987) Lüderitz Upwelling Cell (27.5° S). The Lüderitz upwelling is the largest of its kind on earth (Demarcq and Dagorne, 2011), characterized by strong winds and high turbulence (Hutchings *et al.*, 2009), and compared to other upwelling cells, it exhibits cooler average sea surface temperatures and extends further offshore (Lutjeharms *et al.*, 1991). The magnitude of the Lüderitz Upwelling Cell makes it a quasi-physical barrier to marine species (Demarcq and Dagorne, 2011). The Benguela Current is therefore considered to be an important phylogeographic barrier separating previously connected populations (Floeter *et al.*, 2007; Helfman *et al.*, 2009; Hutchings *et al.*, 2009; Demarcq and Dagorne, 2011; Dudgeon *et al.*, 2012; Luiz *et al.*, 2012; Taylor and Hellberg, 2015), particularly of warmer water species (Henriques *et al.*, 2012, 2014a).

Several studies (**Table 1.1**) of various cephalopod and teleost species have shown diverse genetic and phenotypic responses to being isolated by the Benguela biogeographic barrier. Some species have been shown to have developed significant morphological changes but have limited genetic divergence. Others are characterized by deep genetic divergence, yet have shown limited morphological differentiation.

Table 1.1: Existing information on species differentiation, morphometric and genetic, across the Benguela barrier

Species	Biology	Reference	Morphology	Reference	Genetics	Reference
Blacktail seabream *Diplodus capensis* (Smith, 1844)	Significant biological divergence	Richardson, 2010	Morphological divergence	Richardson, 2010	Two distinct and divergent genetic clusters	Henriques, 2011
Geelbeck croaker *Atractoscion aequidens* (Cuvier, 1830)	Significant biological divergence	Henriques *et al.*, in press	Little morphological divergence	Henriques *et al.*, in press	Two independent stocks, evidence of cryptic speciation	Henriques *et al.*, 2014a
Leervis *Lichia amia* (Linnaeus, 1758)	Little biological divergence	Potts *et al.*, 2008	No data available		Two independent stocks	Henriques *et al.*, 2012
Common octopus *Octopus vulgaris* Cuvier 1797	No data available		Lack of morphological divergence	De Beer, 2014	Significant genetic divergence	De Beer, 2014
Cape Hope squid *Loligo reynaudii* d'Orbigny, 1845	No data available		Morphological divergence	Van der Vyver, 2014	Significant genetic divergence	Van der Vyver, 2014
Zebra sea bream *Diplodus cervinus* (Lowe, 1838)	Distinct biological divergence	Winkler, 2013	Morphological divergence	Winkler, 2013	Two independent stocks	Gwilliam, pers. comm.
Silver Kob *Argyrosomus inodorus* Griffiths and Heemstra, 1995	Little biological divergence	Griffiths, 1996; Kirchner, 1998; Kirchner and Voges, 1999	Little morphological divergence	Griffiths and Heemstra, 1995	Two independent stocks	Henriques *et al.*, 2014b

Species	Biology	Reference	Morphology	Reference	Genetics	Reference
Yellowfin tuna *Thunnus albacares* Bonnaterre, 1788	No data available		No data available		Atlantic vs. Indian Ocean stocks	Henriques, 2011
Sand steenbras *Lithognathus mormyrus* (Linnaeus, 1758)	No data available		Little morphological divergence	Kruger *et al.*, in prep	No evidence of independent stocks	Gwilliam, in prep
Steentjie *Spondyliosoma emarginatum* (Valenciennes, 1830)	No data available		Distinct morphological divergence	Kruger *et al.*, in prep	Significant genetic divergence	Gwilliam, in prep
Baardman *Umbrina canariensis* Valenciennes, 1843	No data available		No data available		Significant genetic divergence	Gwilliam, in prep
Streepie *Sarpa salpa* (Linnaeus, 1758)	No data available		Little morphological divergence	Kruger *et al.*, in prep	Significant genetic divergence	Gwilliam, in prep

Besides the formation of the Benguela Current, several other historical events (e.g. sea level variation and habitat change) and contemporary features (e.g. temperature, chlorophyll) have also shaped the biogeographic patterns of fishes in southern Africa. While these patterns are well described for South Africa (Lombard *et al.*, 2004), Africa (Whitfield, 2005; Potts *et al.*, 2015) and on a global scale (Ekman, 1953; Hedgpeth, 1957; Briggs, 1974a, 1995; Hayden *et al.*, 1984; Bailey, 1998; Longhurst, 1998a; Adey and Steneck, 2001; Spalding *et al.*, 2009), the spatial resolution provides limited information for southern Africa and west coasts.

The most relevant biogeographic studies for this thesis are those by Whitfield (2005), Spalding *et al.* (2009) and Potts *et al.* (2015). Spalding *et al.* (2009), based on the results of a hierarchical and nested model, identified 12 realms, 62 provinces and 232 ecoregions called the Marine Ecoregions of the World (MEOW). They defined the coastal oceans of southern Africa as wholly temperate. Whitfield (2005), in a review of the biogeography of southern African estuarine fauna, grouped sub-Saharan African estuaries into four broad zones, in which Angola consisted of tropical and sub-tropical zones, Namibia included sub-tropical, warm-temperate and cool-temperate zones, and South Africa was categorized into cool-temperate, warm temperate and sub-tropical. Unfortunately, this data may not be accurate due to the lack of estuaries along the west coast. For this reason, Potts *et al.* (2015) in a review of the impacts of climate change on coastal fishes in southern Africa used sea surface temperatures and the distribution of fish fauna to propose biogeographic regions for the coastal fauna. They identified cool-temperate (False Bay to Hentiesbaai), warm-temperate (Hentiesbaai to Namibe), sub-tropical (Namibe to Rio Longa) and tropical (Rio Longa northward) zones along the west coast of southern Africa (**Figure 1.1**).

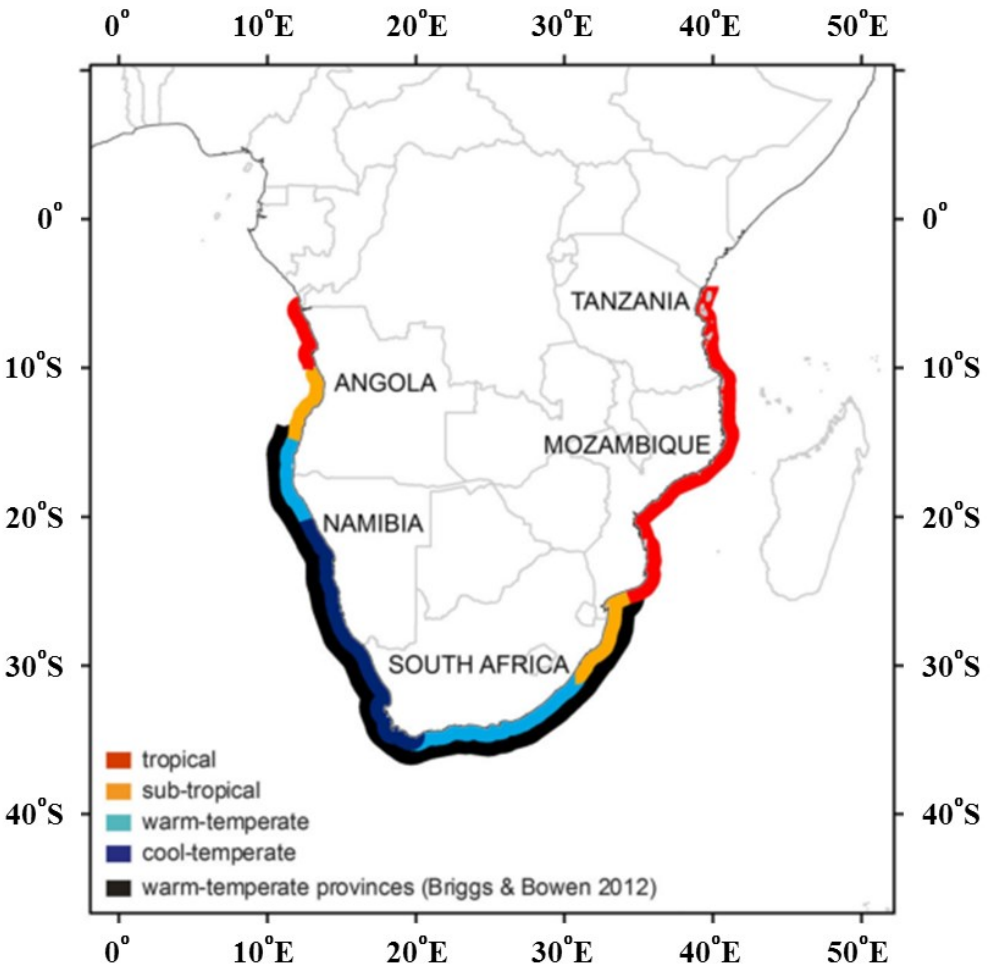

Figure 1.1: Biogeographic zonation of southern Africa as presented by Potts *et al.* (2015)

This isolation of warm temperate fish assemblages to the north and south of the Benguela Current, as well as the consideration of marine realms, provinces and ecoregions, provides a good model system for studying ecological speciation. The contrasting evolutionary response of different species in the frontal zones and the known temporal nature of the formation of the features of the current provide us with a natural laboratory to study allopatric speciation. Furthermore, this provides a unique opportunity to conduct systematic research to gain an understanding of evolutionary processes contributing to biodiversity.

Elasmobranchs should be more susceptible to the impacts of climate change as they would have to rely on the slow process of genetic mutation and evolution for adaptive responses. However, elasmobranchs have survived, relatively unchanged, and through many past climate events (e.g. glacials and interglacials) suggesting a conflicting inherent resilience to climate change. Elasmobranchs therefore provide ideal candidates for inclusion in studies that examine the contrasting evolutionary response of marine species to allopatry. Ecosystems such as the Benguela, where the temporal nature of the formation of an isolation barrier is

known, provide unique opportunities to conduct this kind of comparative research. In addition, the assessment of phenotypic plasticity and rate of evolutionary change in elasmobranchs will greatly assist scientists when making predictions on the likely responses of species to rapid environmental change.

Due to differences in life history traits, evolution of elasmobranchs and teleosts is predominantly dissimilar. Unlike many teleosts, shark species are characterized by slow growth rates, long lifespans, few offspring, late maturity (Smith *et al.*, 1998), low fecundity and high maternal investment, and possibly polyandry (Feldheim *et al.*, 2014). Without pelagic larvae to benefit from oceanic circulation, sharks rely solely on the juvenile and adult stages for dispersal (Duncan *et al.*, 2006); thus dispersal potential is dependent on adult vagility (Schultz *et al.*, 2008; Benavides *et al.*, 2011).

When it comes to intrapopulation differences in sharks, there are limited studies available quantifying life history traits and a complete absence of research describing morphological differences amongst populations. It also appears that life history comparisons are purely based on latitudinal variation and therefore temperature differences amongst populations. For instance, bonnethead sharks (*Sphyrna tiburo*) populations at the highest latitudes showed the largest asymptotic sizes, largest and oldest median size at maturity and largest near-term embryos (Parsons, 1993; Carlson and Parsons, 1997; Lombardi-Carlson *et al.*, 2003). Likewise, patterns of larger size at maturity with latitude were reported for the cloudy catshark, *Scyliorhinus torazame* (Horie and Tanaka, 2002), shortspine spurdog, *Squalus mitsukurii* (Taniuchi *et al.*, 1993) and the starspotted dogfish, *Mustelus manazo* (Yamaguchi *et al.*, 2000). Despite the fact that life history parameters of many marine fish stocks have been shown to contrast in response to environmental variation and interactions with the environment and genotype (Begg, 2005), comparisons of life history parameters with genotype and/or morphology are lacking, particularly for sharks.

The family Triakidae (hounds, tope, and whiskery sharks) is one of eight families making up the order Carcharhiniformes (López *et al.*, 2006). Sharks of the genus *Triakis* Müller and Henle, 1838, are small to medium size sharks largely inhabiting tropical and temperate coastal regions and feed on benthic crustaceans, cepahalopods and osteichthyes (López *et al.*, 2006). There are currently five recognized species of *Triakis* (Compagno, 1988) found in the world (**Figure 1.2**): *Triakis scyllium* Müller and Henle, 1839 (banded houndshark); *T. megalopterus*

(Smith, 1849) (spotted gully shark); *T. semifasciata* Girard, 1854 (leopard shark; *T. maculata* Kner and Steindachner, 1867 (spotted houndshark); *T. acutipinna* Kato, 1968 (sharpfin houndshark). Although *Scylliogaleus quecketti* Boulenger, 1902 (flapnose houndshark) is not currently classified as a species of *Triakis*, *T. megalopterus* is more closely related to *S. quecketti* than it is to all of the other members of *Triakis* (López *et al*., 2006; Naylor *et al*., 2012) and has therefore been included in the distribution map for comparison.

Figure 1.2: Distribution map of the five species from the genus *Triakis* and *Scylliogaleus quecketti*. Solid yellow regions for *Triakis megalopterus* indicate areas of known distribution, while the dashed red lines are indicative of dispersal uncertainty and/or low abundance.

The spotted gully shark, *Triakis megalopterus* (**Figure 1.3**), is found in the temperate continental waters of the western Indian and south-eastern Atlantic oceans, in southern Angola, Namibia and South Africa (Smale and Goosen, 1999; Compagno, 2009). This species of shark is a bottom dweller typically found in shallow (<50 m) subtidal water in sandy and rocky habitats (Bass *et al.*, 1975; Compagno, 1984a; Smale and Goosen, 1999). The body of *T. megalopterus* is commonly grey/bronze, with a lighter ventral side, and possesses small black spots frequently spread out over the body (Compagno *et al.*, 1989). As described by Smale and Goosen (1999), *T. megalopterus* can reach a total length (TOT) of 2075 mm, have a rotund head, broadly rounded snout, large mouth and pointed teeth. Crustaceans, cephalopods, Osteichthyes and small elasmobranchs appear to be the prey of choice for this species (Compagno, 1984a; Smale and Goosen, 1999). Booth *et al*. (2011) found that *T. megalopterus* live up to 25 years of age and mature at 11 and 15 years for males and females, respectively. The embryos of *T. megalopterus* obtain nourishment from their own yolk sacks making this species of shark ovoviviparous (Bass *et al.*, 1975; Compagno, 1984a). The

estimated gestation period is 19–21 months, with 5–15 pups (possibly 16) attaining an estimated size at birth of 420–450 mm (Smale and Goosen, 1999).

Figure 1.3: Spotted gully shark, *Triakis megalopterus*, caught at the Cunene River mouth in southern Angola (male, 2.6 kg, TOT = 79.5 cm)

Listed on the International Union for Conservation of Nature (IUCN) as near threatened, *T. megalopterus* does not yet qualify for a threatened status, although shark expert Leonard Compagno considers it to be threatened with extinction in the near future (Compagno, 2009). Due to their habitat preferences, large size at maturity, lengthy pregnancies, small litter sizes (Smale and Goosen, 1999), narrow distribution and small population sizes (Compagno *et al.*, 1989), this species can sustain only limited fishing pressure (Booth *et al.*, 2011) and is vulnerable to overexploitation by inshore recreational and commercial shark fisheries (Smale and Goosen, 1999). While *T. megalopterus* is legislated as a non-commercial species (i.e. it may not be marketed by commercial operators), it is commonly mistaken as the commercial species *Mustelus mustelus* (Booth *et al.*, 2011) and is a frequent bycatch in the South African demersal longline fisheries in Gaansbaai and False Bay (Compagno, 2009). According to Attwood and Farquhar (1999), *T. megalopterus* is also exploited by shore anglers between Walker Bay and Cape Hangklip in the Western Cape, South Africa. Although these recreational rock and surf anglers typically practise catch and release (C&R) on elasmobranchs, the high anaerobic activity, muscular fatigue and air exposure (Skomal, 2007) during the capture event results in physiological stress that has been shown to affect the feeding, growth, population size structure, reproductive potential (Cooke and Schramm, 2007) and consequently abundance (Stevens *et al.*, 2000) of targeted species. Unfortunately, little is known about the post-release mortality of sharks subject to C&R (Skomal, 2007) and one can therefore not exclude the impact of recreational angling when estimating fishing mortality.

T. megalopterus is a suitable model species to assess the impact of allopatry as it is a coastal species that is generally (no published migration data yet available) associated with decreased

vagility (Musick *et al.*, 2004) and is also characterized by: a longer (± 20 months; Smale and Goosen, 1999) than average (9–12 months; Helfman *et al*., 2009) gestation rate; distributive disjunction (absent and/or unknown distribution between Cape Point and central Namibia); absence of pelagic larvae due to its ovoviviparous reproductive strategy; and late maturity – full maturity is only reached at 78% of average maximum size (Smale and Goosen, 1999).

The aim of this study is to use the populations of *T. megalopterus* within and surrounding the natural laboratory provided by the Benguela Current (Angola, Namibia and South Africa), to gain a better understanding of allopatric speciation and the evolutionary processes contributing to biodiversity in elasmobranchs. As very little is known about the biology of *T. megalopterus,* particularly in Namibia and Angola, this thesis also aims to address this lack of knowledge by presenting comparative data on the genetics, morphology and life history of this species throughout its southern African distribution.

It is hypothesized that the development of the cold water of the Benguela Current has formed a biogeographic barrier to the distribution of *T. megalopterus* along the southern African coastline and that despite this isolation, little or no morphological and/or life history change has occurred between the populations.

The aims of the study are to improve our current understanding of allopatric speciation in elasmobranchs by means of a holistic approach using molecular, morphometric and life history characteristic comparisons. This will permit the description of potential threats to the conservation of this species and identify whether the species or its populations may be susceptible to rapid environmental change.

Thesis outline

To achieve the overall aim, this thesis adopts a holistic design approach containing several categories of analysis and is divided into four data oriented chapters:

Chapter 1: Provides a general introduction that outlines the scope of the project, an introduction to the study species and the general hypotheses, aims and objectives.

Chapter 2: Describes the marine environment and major oceanographic features within each sampling site and provides the general material and methods utilized throughout the study.

Chapter 3: Assesses the population genetics of *T. megalopterus* from four geographical locations using mitochondrial (mtDNA) and microsatellite (nDNA) markers. An examination of the patterns of gene flow, population connectivity/structure and the demographics of *T. megalopterus* can be used to gain a better understanding of elasmobranch evolution.

Chapter 4: Compares the body and teeth morphology of *T. megalopterus* from South Africa and southern Angola using traditional morphology and landmark methods. Since traditional morphology generally lacks geometric properties (Bookstein, 1982), a truss protocol was designed and specifically developed for elasmobranchs. This was considered necessary in view of the perceived slow evolutionary rate of elasmobranchs, which potentially makes morphological changes between populations difficult to detect using traditional morphological techniques.

Chapter 5: Examines differences in the diet of populations in dissimilar habitats; diet may be an important driver of morphological change. Therefore, this chapter provides a comparison of the feeding ecology of *T. megalopterus* between the southern Angola and South African populations. The feeding ecology is compared to the results from chapters three and four to determine if differences in the diet influenced morphology through the process of evolution or due to inherent phenotypic plasticity.

Chapter 6: Quantifies important life history traits such as growth rate, age and length at maturity for *T. megalopterus*. As each population is subject to different environmental conditions, the life history traits are compared between populations to assess whether various marine environments may affect the life history of this species.

Chapter 7: Provides a summary of results obtained and a general discussion drawing conclusions on the biology, evolution and likely impacts of climate change for *T. megalopterus*.

Chapter 2:
Site selection and general methods

As stated in the introduction (**Chapter 1**), the purpose of this thesis is to gain a better understanding of the biology of *Triakis megalopterus* while at the same time using this species as a case study to further understand allopatric speciation and the evolutionary processes contributing to biodiversity in elasmobranchs. In order to accomplish this, the sample site selection of *T. megalopterus* was based on the representation of different oceanographic regimes around the southern African coast. Sample sites were chosen on the coasts of southern Angola, northern Namibia, and the Western and Eastern Cape provinces of South Africa (**Figure 2.1**). All sample sites were situated within the temperate southern Africa realm (see **Chapter 1**; Spalding *et al.,* 2014). Southern Angola, northern Namibia, Western and Eastern Cape provinces were selected to test population parameters within and potential isolation between the four areas recognised as potentially separate zones. Whole specimens were collected from the southern Angola, Eastern Cape and Betty's Bay sample sites and used for the genetic, morphology, feeding and life history aspects of this thesis. Only fin clips were collected from Namibia and Cape Point for the genetic component of this thesis.

Study sites

Southern Angola

Whole specimens from southern Angola were collected between Baia dos Tigres (16° 36' 14.1" S 11° 49' 03.2" E) and the Cunene River mouth (17° 14' 33" S, 11° 44' 59" E). This warm-temperate zone (Potts *et al.*, 2015) forms part of the Namib ecoregion in the northern Benguela province (Spalding *et al.*, 2009) where the intertidal zone is dominated by sandy beaches, sandstone rocky outcrops and a continental shelf of approximately 36 km wide (Duarte *et al.*, 2005). In the Cunene River mouth and Baia dos Tigres, the sea floor on the continental shelf is lined by coarse sand and clay/silt ocean floors, respectively (Bianchi, 1992).

The marine conditions off Angola are influenced by both the Angola and Benguela Currents. The Angola Current is a warm, southward flowing (Bianchi, 1992; Kostianoy and Lutjeharms, 1999), narrow and stable current (Moroshkin *et al.*, 1970); it forms part of the Angola subsurface gyre (Lass *et al.*, 2000) driven by the South Equatorial counter current (13°

S; 4° E; McCutcheon and McCutcheon, 2003). The confluence of the Angola and Benguela currents gives rise to the Angola-Benguela Frontal Zone (ABFZ; 18° S), a permanent frontal system (Meeuwis and Lutjeharms, 1990; Veitch *et al.*, 2006) that demonstrates seasonal variability dependent on the strength of the contributing ocean currents (Kostianoy and Lutjeharms, 1999).

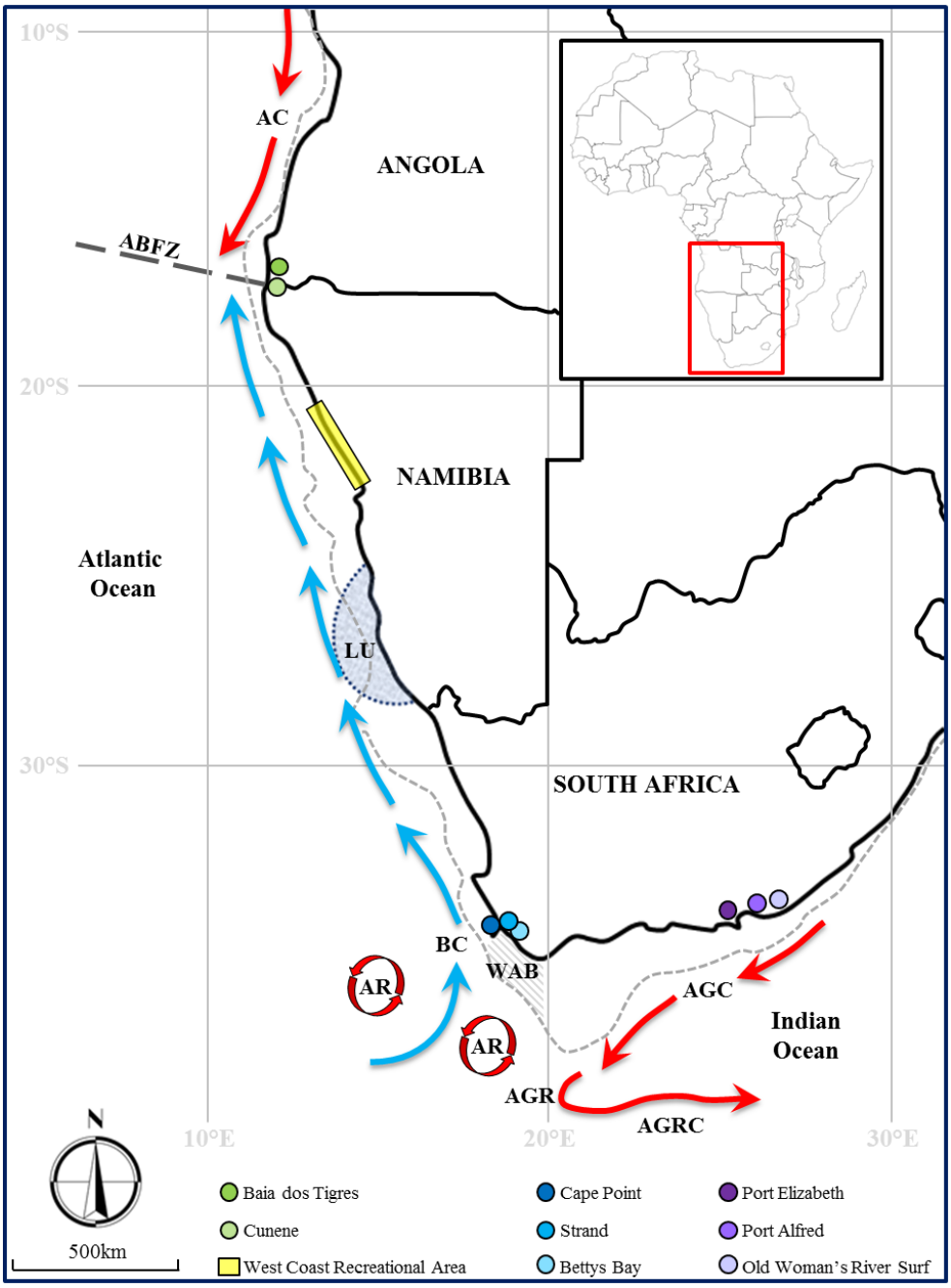

Figure 2.1: Map of sampling sites and oceanographic features in the oceans surrounding southern Africa; *AC = Angola Current, ABFZ = Angola-Benguela Frontal Zone, LU = Lüderitz Upwelling Cell, AR = Agulhas Rings, BC = Benguela Current, WAB = Western Agulhas Bank, AGR = Agulhas Retroflection, AGRC = Agulhas Return Current, AGC= Agulhas Current (adapted from Shannon et al., 2006; Coetzee et al., 2008; von der Heyden et al., 2011).*

During autumn/winter, the Benguela Current velocity is strongest and pushes the ABFZ further north (± 16° S), while in spring/summer, the ABFZ is placed further south (± 20° S) as a result of the higher velocity of the Angolan Current (Meeuwis and Lutjeharms, 1990; Kostianoy and Lutjeharms, 1999; Veitch *et al.*, 2006; Ekau and Verheye, 2010). The seasonal variation in current velocity has pronounced effects on regional productivity as winter is primarily influenced by the cool, nutrient rich water of the Benguela Current whereas in summer, the warm oligotrophic water of the Angolan Current governs the ABFZ (Hutchings *et al.*, 2009). This seasonal variability is reflected in the inshore water temperatures (Richardson, 2010), and, while Whitfield (2005) has characterized this region as a subtropical biogeographic zone, the thermal regime is more akin to a temperate zone. Also influencing the nutrient enrichment in southern Angola's marine environment is the Congo River discharge and shelf-break upwelling (Bianchi, 1992).

Northern/Central Namibia

Fin clips for molecular analyses were collected from throughout the West Coast Recreational Area (WRCA), a 200 km section of coastline between the northern boundary of the Namib Naukluft National Park (22° 38' 29" S, 14° 31' 34" E) and the Ugab River (21° 10' 55" S, 13° 38' 30" E) in Namibia. The WCRA falls within the cool-temperate zone (Potts *et al.*, 2015) in the northern Benguela subsystem (Lüderitz to Angola) and shares the same type of habitat as southern Angola: sandy beaches and rocky outcrops (Sakko, 1998). The Namibian marine environment falls within the cool-temperate zoogeographic province (Emanuel *et al.*, 1992) and is situated in the Namib ecoregion of the northern Benguela province (Spalding *et al.*, 2009)**.**

The northern/central Benguela subsystems are characterized by the seasonal movements of the Angola Current (known to penetrate as far as Walvis Bay; Moroshkin *et al*., 1970) and ABFZ, as well as low oxygen water bodies and year round coastal upwelling cells off Cape Frio and Lüderitz (Sakko, 1998). These upwelling cells are generally related to regions of narrower continental shelf and stronger southerly winds (Hutchings *et al.*, 2009). Regardless of the upwelling cell at Cape Frio, primary productivity in the northern subsystem is lower than in the central region. This is attributable to the intrusion of warm, nutrient-poor Angolan Current water during austral summers (Hutchings *et al.*, 2009). On its southward journey, the Angola Current plunges deeper to form the Benguela Poleward Undercurrent establishing the low oxygen water (LOW) boundary conditions for the northern/central Benguela systems (Veitch, 2007). The Central Benguela region (Namibia) is characterized by multifaceted

interactions between remotely forced shelf processes, biogeochemical carbon fluxes that control the LOW oscillations, and cyclical thermocline inconsistency (Monteiro *et al.*, 2006).

Western Cape, South Africa

Whole specimens were caught in Betty's Bay (34° 22' 22" S, 18° 51' 44" E) and Strand (34° 08' 38" S, 18° 51' 02" E), whereas only tissue samples for genetics studies were collected from Cape Point (34° 21' 23" S, 18° 29' 51" E). All Western Cape sample sites are located between Cape Point and Cape Agulhas and therefore inside the borders for the WAB (Coetzee *et al*., 2008). These sample sites all fall within the cool-temperate zone (Potts *et al.*, 2015) in the Agulhas province and the Agulhas Bank ecoregion (Spalding *et al.*, 2009). This section of the Agulhas Bank is unique because it is located within the meeting point of a western boundary shelf system, the EAB (Swart and Largier, 1987) and an eastern boundary shelf system, the west coast of southern Africa (Largier *et al.*, 1992). This makes the WAB the transition zone between the Agulhas and Benguela shelf systems and consequently, the hydrodynamic processes within this section of the continental shelf are influenced by both the Agulhas and Benguela Currents (Dufois and Rouault, 2012). The WAB and the EAB are characterized by upwelling and non-upwelling regimes respectively (Largier *et al.*, 1992). The circulation and stratification differences within the WAB and EAB are caused by wind forcing and their respective exposure to coast-parallel upwelling winds, and by oceanic forcing and their respective distance from the Agulhas Current, as well as the continental shelf characteristics of the eastern and western boundaries (Largier *et al.*, 1992). The two subdivisions are further distinguished from one another by the oxygen (Chapman and Shannon, 1987), nutrient (Chapman and Largier, 1989) and plankton (De Decker, 1984) distributions therein. The nearshore habitat in this region is characteristically rocky patches with large sandy sections and kelp forests (Turpie *et al.*, 2009).

Eastern Cape, South Africa

Sample collection took place in Port Elizabeth (34° 02' 42" S, 25° 36' 38" E), Port Alfred (33° 32' 28" S, 27° 02' 54" E) and Old Woman's River mouth (33° 29' 02" S, 27° 08' 55" E). As with the Western Cape sample sites, the Eastern Cape sample sites fall within the warm-temperate zone (Potts *et al.*, 2015) of the Agulhas province and Agulhas Bank ecoregion (Spalding *et al.*, 2009). The Agulhas Current (27° S to 40° S) governs the marine coastline in the Eastern Cape (Barange, 1994). This narrow, southward flowing Agulhas Current (Nelson and Hutchings, 1983), is one of the strongest currents in the world (McCutcheon and McCutcheon, 2003). The Agulhas Current is the western boundary current of the South Indian

Ocean, and extends the warm water from the subtropics (East Madagascar and Mozambique Currents) down the east coast of southern Africa (Gordon, 1985; Branch *et al.*, 2004). After flowing parallel to the eastern coastline of South Africa, the Agulhas Current begins to move offshore at the Agulhas Bank (±22° E) before making an abrupt anticyclonic turn known as the Agulhas Retroflection (Gordon, 1985). This retroflection continues east as the Agulhas Return Current and is characterized by substantial meandering caused by current instability and bottom topography (Quartly and Srokosz, 2015). Within this meandering, anticyclonic rings are shed and able to move far enough westward to form the foundation for inter-ocean heat and salt leakage (Hutson, 1980) from the Indian Ocean to the Southern Atlantic Ocean (Quartly and Srokosz, 2015). This part of the coastline is dominated by mixed sand and rocky reef surf zones (Hutchings and Clark, 2012).

General material and methods

Sampling took place over a three year period, beginning in November 2011 and ending in February 2013. Since there is no commercial fishery for this species, specimens were collected with the help of local fishermen. Local fishermen aided in the collection of small specimens from Old Woman's River and large specimens from offshore in Port Elizabeth; these specimens were frozen in blast freezers at the Department of Ichthyology and Fisheries Science (Rhodes University) and Bayworld (Port Elizabeth), respectively. Local fishermen also facilitated the collection of tissue samples from Namibia and Cape Point. Primarily, however, the sample collections were done by the author and research teams. The exact numbers of specimens used per analysis has been described in the relevant chapters.

Specimens of *T. megalopterus* were collected using standard angling techniques and sacrificed by severing the spinal cord. Mass was taken using a Salter Scale (50 kg, 200 g precision). Total length (TOT), fork length (FOR) and precaudal length (PRC) were measured, with a measuring tape, to the nearest 10 mm (**Figure 2.2**). Length measurements were made on a horizontal line, from the tip of the snout to the tip of the caudal fin, at full extension (Compagno, 1984a). All length measurements used in this thesis refer to TOT, unless otherwise specified.

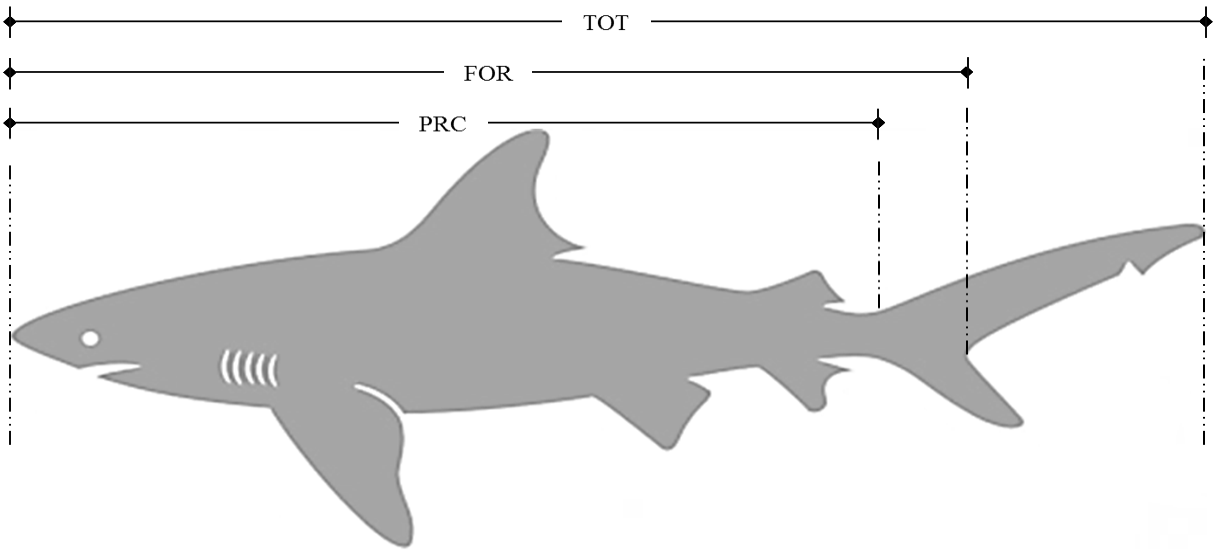

Figure 2.2: Illustration of the total length (TOT), fork length (FOR) and precaudal length (PRC) measured from each specimen according to the Compagno (1984a)

Muscle tissue was removed from below the dorsal fin using a sterilized blade and forceps before being stored in 70% ethanol for genetic analyses. All morphological measurements (**Chapter 4**) were obtained before specimens were dissected. After measurements were finalized, the jaws were removed, cleaned of excess tissue and dried for use in the feeding morphology component of the study.

Stomachs were removed by severing the base of the oesophagus and the intestine immediately anterior to the pylorus. Stomachs were weighed whole using a digital balance (500g, 0.1g precision). If the stomachs could not be assessed fresh, the entire contents were removed and weighed before removing the otoliths and cephalopod beaks, which were dried and stored in 70% ethanol, respectively. The rest of the stomach contents were stored in 10% neutral buffered formalin for later analysis.

The liver was removed, weighed and discarded. Stage of maturity was assessed using the macroscopic criteria developed by Bass *et al*. (1975); that is, each specimen was assigned a reproductive stage of: embryo, immature, adolescent, mature, or pregnant (for females). Gonads were removed and all components (eggs, oviducal glands and embryos) of the female reproductive system were weighed and measured (length and width). The number of eggs and embryos were also counted.

Three to five vertebrae were removed posterior to the skull and from below the dorsal fin. Vertebrae were separated, carefully cleaned of excess tissue and left to dry for later age analysis.

Chapter 3:
Genetic connectivity of *Triakis megalopterus* across its southern African distribution

Introduction

Evolution is the change in inherited traits of a population over successive generations driven by variations in biological form originating from the genotype and phenotype (Stearns, 1989). Understanding evolutionary change requires an understanding of population genetic structure and the biotic and abiotic forces governing the patterns of neutral and adaptive genetic divergence (Hemmer-Hansen *et al.*, 2007). Species are a cornerstone of biology, ecology and conservation (Petit and Excoffier, 2009) and due to growing concerns over threats to biodiversity, species delimitation is imperative (Wiens, 2007). The inability to effectively identify species, stocks and populations could potentially have major consequences for contemporary and future global biodiversity conservation, sustainability and fisheries management (Carvalho and Hauser, 1998; Ward, 2000).

Systematics can be considered to have two key objectives: to discover and describe species and to determine the phylogenetic relationships of these species (Wiens, 2007). In the past, species identification and delimitations have mainly been based on morphological differences (Wiens and Penkrot, 2002), but the advances of modern molecular techniques have strengthened traditional taxonomic species delimitation (Hebert and Gregory, 2005; Vogler and Monaghan, 2007). The population structure of species is, however, composed of two sections, demographic structure (biological features associated with life history) and genetic structure (population structure, mutation, selection and evolution (Sunnucks, 2000). The relationships between life history features (mating, birth/death rates) and the distribution of molecular genetic diversity is vital as changeability of life history features is generaly a good indicator of potential genetic sub-structuring patterns (Graves, 1998; Bargelloni *et al.*, 2005; Gaggiotti *et al.*, 2009; Galarza *et al.*, 2009). The subdivision of species into self-recruiting populations has important ecological and evolutionary consequences; for example, local recruitment facilitates local adaptation. As alleles favourable in a particular local environment can be selected for whilst not compromising their lower fitness in different environments (Sale *et al.*, 2006). This mechanism forms the basis of the ecological species concept, which

proposes that a species evolves as it becomes adapted to a particular set of resources (niche) in the environment (Ridley, 2003).

Chondrichthyans have existed for at least 450 million years (Hoenig and Gruber, 1990), thus demonstrating remarkable historic endurance (Grogan *et al.*, 2012). Life history traits such as slow growth, large adult size, late reproduction and few, well-formed young (Prince, 2005) are thought to contribute to the evolutionary rates that Martin and Palumbi (1993a) estimated to be six times slower than in mammals and up to eight times slower than in primates and ungulates (Martin *et al.*, 1992).

Species with wide distribution ranges, such as sharks (Dudgeon *et al.*, 2012), may frequent habitats with different ecological characteristics (coastal or oceanic, tropical or subtropical), and consequently distinct evolutionary units may develop (Mendonça *et al.*, 2013). That said, coastal species (e.g. *Triakis megalopterus*) are frequently reliant on near-shore habitats, which limits dispersal potential and is more likely to result in the subdivision of populations (Heupel *et al.*, 2007). Furthermore, near-shore species usually aggregate for natal philopatry, maturation and parturition (Simpfendorfer and Milward, 1993), all of which affect the level of population subdivision and genetic differentiation amongst geographic areas. When common to both sexes, behaviour such as philopatry contributes to the development of closed populations. Here reproduction and recruitment are more significant determinants of population dynamics than migration (Secor, 2002). For example, lemon sharks, *Negaprion brevirostris* (DiBattista *et al.*, 2008) and bull sharks, *Carcharhinus leucas* (Tillett *et al.*, 2012) exhibit female philopatry, while great white sharks, *Carcharodon carcharias* have been seen to exhibit male and female philopatry (Jorgensen *et al.*, 2010).

Evolutionary forces such as gene flow, mutation, genetic drift and natural selection govern population genetic structure (Dudgeon *et al.*, 2012; Ovenden *et al.*, 2013). Gene flow is an important evolutionary force in which genes are exchanged between populations. There are a number of factors affecting the rate of gene flow between different populations. One of the most significant factors is mobility: the more mobile an individual is, the greater its migratory potential. Unlike teleosts, sharks lack a larval stage which significantly limits their dispersal potential (Whitney *et al.*, 2012) and correspondingly, gene flow between populations (Musick *et al.*, 2004). Sharks do, however, reach large sizes and the increased vagility promotes dispersal during the adult phase (Musick *et al.*, 2004). Dispersal potential can also be sex-biased (Petit and Excoffier, 2009), which can be assessed by comparing patterns of genetic

diversity distribution presented in biparentally inherited nuclear markers (e.g. microsatellites) and maternally inherited mitochondrial markers (Veríssimo *et al.*, 2012).

An organism's ecology and the characteristics of the marine environment may also influence patterns of dispersal. Barriers may inhibit gene flow amongst even the most mobile species. Biogeographic barriers facilitate allopatric speciation, which may also lead to high levels of narrow endemism (Myers, 1997). Barriers to gene flow can be in the form of biogeographical obstructions (Glor and Warren, 2010; Dudgeon *et al.*, 2012), historical climatic fluctuations (Brown *et al.*, 1996; Hewitt, 2000; Janko *et al.*, 2007) and landmasses (Jackson, 2010). Oceanic characteristics such as wide ocean basins, temperature gradients (Spalding *et al.*, 2009), deep oceanic water (Briggs, 1974b), freshwater outflows (Floeter *et al.*, 2007), ocean currents (Gaylord and Gaines, 2000), frontal zones (Sournia, 1994; Reuschel *et al.*, 2010) and upwelling (Luiz *et al.*, 2012) are also considered barriers to gene flow. For instance, the genetic structure of the scalloped hammerhead shark, *Sphyrna lewini,* has been affected by the formation of the Isthmus of Panama landmass (Daly-Engel *et al.,* 2012), interglacial dispersal and Pleistocene divergence events as well as the decline in gene flow amongst ocean basins (Duncan *et al.*, 2006).

Various models/parameters of genetic differentiation have been proposed, such as:

The isolation by distance (IBD) model where interbreeding is restricted to small distances by the occurrence of short range of dispersal (Wright, 1943), which results in limited gene flow between populations (Jensen *et al.*, 2005), e.g. lemon sharks, *Negaprion brevirostris* (Schultz *et al.*, 2008).

A stepping stone model suggests that the exchange of genes is restricted between neighbouring populations (Kimura and Weiss, 1964), and genes tend to move or disperse a single step among population subunits every generation (Van Dyke, 2008), e.g. the scalloped hammerhead shark, *Sphyrna lewini* (Duncan *et al.*, 2006).

Clines (geographic gradient in the frequency of a gene) are caused by differential adaptation to ocean conditions, e.g. temperature, pH, salinity or depth (Teske *et al.*, 2011b) or by natural selection favouring a different form along the gradient (Ridley, 2003).

Founder effects are caused by the establishment of a population from a small number of individuals and/or genetic bottleneck where a population undergoes a drastic reduction in population size (Ayala, 1982; Pierce, 2010). Bottlenecks caused by historical climatic fluctuations have been reported in the narrownose smooth-hound shark, *Mustelus schmitti*

(Pereyra *et al.*, 2010), basking shark, *Cetorhinus maximus* (Hoelzel *et al.*, 2006) and bull sharks, *Carcharhinus leucas* (Tillett *et al.*, 2012).

Lastly, rapid genetic discontinuity results in limited gene flow across a biogeographic barrier where the barrier separates a species' distribution range (Slatkin, 1987), subsequently limiting gene flow for a long enough period for alleles in each population to drift to fixation of alternate alleles or mutual monophyly (Heupel and Heuter, 2002), e.g. the blacktip shark, *Carcharhinus limbatus*, whose distribution was interrupted by the Isthmus of Panama (Keeney and Heist, 2006).

A molecular marker is an easily identifiable piece of DNA that can be used to distinguish individuals, populations, or species. Bi-parentally inherited nuclear DNA (nDNA) and/or maternally inherited mitochondrial DNA (mtDNA) is used to determine demographic history and population genetic structure in sharks (Veríssimo *et al.*, 2010). Microsatellite markers are short segments of DNA that have a repeated sequence (simple sequence repeats; SSRs) of 1–6 nucleotides (Wyman and White, 1980), which are most frequently used in kinship (Goodnight and Queller, 1999) and population studies (Selkoe and Toonen, 2006). Microsatellites are abundant, randomly dispersed in high frequencies throughout the genome of most eukaryotes (Litt and Luty, 1989) and tandemly organized into uninterrupted, interrupted or compound loci, e.g. $(AG)_n$, $(GC)_nAT(GC)_n$, and $(GC)_n(AT)_n(GT)_n$, respectively (Tautz, 1989; O'Connell and Wright, 1997; Estoup and Cornuet, 1999; Anne, 2006; Liu, 2007). According to Selkoe and Toonen (2006), microsatellite markers are useful at a wide range of scales of analyses including parentage analysis, relatedness, inbreeding levels (FIS), genetic structure of subpopulations and populations, demographic history, gene flow between populations, phylogeographic studies and fine-scale phylogenies to the level of closely related species.

Microsatellites are markers characterized by codominance (Jarne and Lagoda, 1996), hypervariability (Weber and Wong, 1993; Slatkin, 1995), locus-specificity (Portnoy and Heist, 2012) and Mendelian heritability, allowing distinguishability of heterozygote and homozygote individuals for the same locus (Selkoe and Toonen, 2006). Microsatellites also permit the analysis of genetic relationships at population (using allelic frequencies) and individual (through genotypes) levels (Selkoe and Toonen, 2006) and, due to their high mutation rates (Tautz, 1989), assessment of recent genetic changes in population structure (Hedrick, 2005).

Microsatellites are not without disadvantages. These kinds of markers need to be isolated *de novo* from most species being examined for the first time (Hoffman and Nichols, 2011), usually produce relatively few loci to work with (Glenn and Schable, 2005) and may be prone to amplification inaccuracies that lead to genotyping errors (Hoffman and Amos, 2005; Girard and Angers, 2008). These errors include null alleles (failure of an allele to amplify) and stuttering (multiple bandings of a single allele caused by slippage of *Taq*). Size homoplasy, where alleles of the same size but different lineages reduce the visible allelic diversity of populations, may also occur (Blankenship *et al.*, 2002; Epperson, 2005). Regardless of these limitations, microsatellite markers have been applied in elasmobranchs for genetic stock characterization, individual identification, discerning genetic mating systems, kinship, relatedness, sex-biased dispersal and philopatry (Dudgeon *et al.*, 2012; Portnoy and Heist, 2012).

Mitochondrial DNA are non-nuclear and make up a small portion (<1%) of the DNA of eukaryotic cells located within the mitochondria (Castro *et al.*, 1998). This type of DNA is intron free (Wan *et al.*, 2004) maternally inherited, not subjected to recombination and exhibits higher substitution rates compared to the nuclear genome (Avise *et al.*, 1987; Castro *et al.*, 1998). Due to its maternal inheritance, the effective population size of mtDNA is a quarter of that for nuclear genes (Avise *et al.*, 1987). However, the maternal inheritance of mtDNA is also its major drawback as it results in a quarter of the effective population size of the nDNA (Wan *et al.*, 2004). Hence, nDNA markers are more introgressive than mtDNA markers and, consequently, less diagnostic (Petit and Excoffier, 2009). Evolving at a rate of up to 10 times faster than nDNA, mtDNA is better for assessing relationships among species and populations that diverged within the past 5–10 Mya (Brown *et al.*, 1979). In marine species, mitochondrial markers are frequently used to investigate levels of population connectivity and genetic diversity (Bester-van der Merwe *et al.*, 2011), evolutionary history (Corrigan and Beheregaray, 2009) and phylogeographic patterns (Von der Heyden *et al.*, 2011).

Different mitochondrial genes have been used to assess numerous aspects of shark genetics. These include intra- and interspecies global phylogeography of scalloped hammerhead, *Sphyrna lewini* (Duncan *et al.*, 2006) and lemon sharks, *Negaprion* spp. (Schultz *et al.*, 2008), population structure of spiny dogfish, *Squalus acanthias* (Veríssimo *et al.*, 2010) and whale sharks, *Rhincodon typus* (Castro *et al.*, 2007), species interrelationships of sleeper sharks,

Somniosus spp. (Murray *et al*., 2008), and biogeographic patterns and molecular clock analysis of angel sharks, *Squatina* spp. (Stelbrink *et al.*, 2010).

Although the influence of the Benguela Current on the isolation of elasmobranchs has not yet been investigated, Henriques (2011) studied the genetic differentiation, population connectivity and evolutionary history of five coastal (*Diplodus capensis, Argyrosomus inodorus, Argyrosomus coronus, Atractoscion aequidens* and *Lichia amia*), and one pelagic (*Thunnus albacares*) teleost fish species across the Benguela Current. Results indicate that oceanic species display shallow population differentiation across the Atlantic and Indian Oceans, whereas coastal species range from shallow structuring (*Argyrosomus inodorus* and *Thunnus albacares*) to speciation events (*Atractoscion aequidens*, *Argyrosomus coronus* and *Argyrosomus japonicus*). All of the aforementioned genetic structure was found to be congruent with Benguela Current oceanographic features signifying a feasible vicariant barrier to dispersal of coastal fish species.

Although genetic studies exist for the phylogeny of Triakidae (López *et al.*, 2006) and the paternity (Nosal *et al.*, 2013) and genetic structure (Lewallen *et al.*, 2007) of leopard sharks, *Triakis semifasciata,* there is no molecular data available for the genetic connectivity of *T. megalopterus* across its southern African distribution. The aims of this chapter are to assess the patterns of gene flow, population connectivity, population structure, and demographics of *T. megalopterus* and to relate these findings to the hydrodynamics, biogeographic barriers and historical climate and coastal changes along the southern African coastline.

Material and methods

Sample collection and DNA extraction

For mtDNA Control Region (mtCR), a total of 86 individuals were randomly selected across four sample sites, the south-east Atlantic Ocean samples were collected from southern Angola (AN), northern/central Namibia (NA) and Western Cape (WC), and the south-west Indian Ocean samples from the Eastern Cape (EC). Total genomic DNA extraction was done using the Wizard® Genomic DNA Purification Kit (*Promega, USA*). Approximate concentration of extracted genomic DNA was assessed in a 1% Ethidium bromide (EtBr) stained agarose gel, using 100 bp KAPA Universal Ladder as a size and concentration reference. In order to confirm DNA concentration, all samples were quantified in a NanoDrop ND-1000 spectrophotometer v3.0.1 (NanoDrop®).

For microsatellite analysis, 130 samples were analysed which included the same 86 individuals from the mtCR analysis. Genetic samples were collected from four geographic locations across two oceanic regions, the south-east Atlantic and south-west Indian Ocean. The south-east Atlantic Ocean samples were collected from AN, NA and WC and the south-west Indian Ocean samples from the EC. Total genomic DNA was extracted from muscle tissue or fin clips using the standard cetyl trimethyl ammonium bromide (CTAB) method (Saghai-Maroof *et al.*, 1984). Quantification of extracted DNA was assessed using a NanoDrop ND-1000 spectrophotometer v3.0.1 (*NanoDrop*®). Thereafter, each sample was adjusted to a 10ng/µl working concentration and stored at -20 ºC.

Species identification

To ensure correct species identification, individuals were barcoded using the mitochondrial *COI* gene using primers *FishF1* and *FishR1* with recommended PCR conditions demarcated in Ward *et al.* (2005). The PCR amplicons were viewed on a 2% agarose gel stained with EtBr using a Promega 100 bp molecular size ladder. Sequencing was performed using the standard Sanger sequencing chemistry (BigDye® terminator v3.1 cycle sequencing kit; *Applied Biosystems*) and capillary electrophoresis conducted at the DNA sequencing unit of the Central Analytical Facility of Stellenbosch University. Sequences were compared to known *T. megalopterus* sequences using the Barcode of Life Data System (BOLD; Ratnasingham and Hebert, 2007).

mtCR sequencing and alignment

A fragment of the mtCR gene was analysed for population genetic structure and phylogeographic pattern inference of *T. megalopterus.* Polymerase chain reaction (PCR) was conducted using primers ElasmoCR15642F (5′- TTG GCT CCCAAA GCC AAR ATT CTG -3′) and ElasmoCR16638R (5′- CCC TCG TTT TWG GGG TTT TTC GAG -3′) according to the recommended PCR conditions outlined in Naylor *et al.* (2005). Success of PCR amplification of the target DNA region was assessed for the presence of single amplification product of the expected size against a known size standard (KAPA Universal Ladder) in a 1% agarose gel stained with EtBr. The PCR amplicons were sequenced at Macrogen, Korea. Sequences were aligned using Bioedit v7.0.9 (Hall, 1999) then manually corrected and trimmed to equal lengths using Mega v6.06 (Tamura *et al.*, 2013).

mtCR sequence analysis

Unique haplotypes were identified using Arlequin v3.5.1.2 (Excoffier and Lischer, 2010). Number of polymorphic sites (*S*), haplotype diversity (*h*), nucleotide diversity (π), and nucleotide composition were assessed using DnaSP v5.10.1 (Rozas *et al.*, 2010). The *h* is the probability of randomly choosing two different haplotypes from the one population, and π is the likelihood that two homologous base positions, from two different haplotypes of the same population, were different (Tillett *et al.*, 2012).

Connectivity between capture locations was subsequently assessed using *F*-statistics estimated in Arlequin v3.5.1.2 (Excoffier and Lischer, 2010) using pairwise F_{ST} values at 1000 bootstrap replicates. Genetic variability (*F*-statistics) is based on values that range from zero to one; zero indicates complete panmixia, one represents genetically distinct or isolated populations (Roesti *et al.*, 2012). In addition, average regional population pairwise differences were estimated by the average between populations, within population and the average pairwise differences. Arlequin was also used for analysis of molecular variance (AMOVA) whereby three types of pooling/grouping strategies were used: 1) according to sample sites (AN, NA, WC and EC), 2) coastline (AN, NA and South Africa) and 3) oceanic placement (Atlantic and Indian Oceans). Grouping by country/coastline also coincides with northern versus southern Benguela subsystems, while oceanic position represents possible transoceanic genetic structure.

To investigate the geographical distribution of haplotypes, a maximum-parsimony haplotype network was constructed in Network v4.6.1.2 (Fluxus Technology Ltd, 2015) using the median joining algorithm (Bandelt *et al.*, 1999). To test for divergence between lineages possibly associated with warmer (Betty's Bay and AN) and colder (Cape Point and NA) water, a second haplotype network was constructed excluding the EC population. Mega was again used to select the best fit model (Bootstrap, 1000 permutations) for phylogenetic inference. To display the genetic relationship between the study haplotypes, the Maximum Likelihood (ML) method based on the Tamura 3-parameter model (Tamura, 1992), was used.

Population demographics

Demographic analyses using the mtCR sequence data were performed in Arlequin. Mitochondrial DNA may be subject to purifying selection over short timescales, which can influence haplotype frequencies and bias estimates of population structure and demographic history (Avise, 2000). To account for this, deviations from selective neutrality (therefore

population expansion) were tested with Tajima's *D* (Tajima, 1989), Fu's F_S (Fu and Li, 1993) and Ewens–Watterson *F* (Slatkin, 1994) neutrality tests using 10 000 permutations and $\alpha = 0.05$. Summary statistics were computed to test if data conformed to the expectations of neutral (non-selective) evolution. Significant differences in Tajima's *D* were used to confirm deviation of population equilibrium (Tillett *et al.*, 2012) whereas positive and negative significant values for Tajima's *D* and Fu's F_S may be indicative of population bottlenecks or expansions, respectively (Ramos-Onsins and Rozas, 2002).

In order to investigate past demographic expansions in *T. megalopterus*, a mismatch distribution analysis was conducted in Arlequin to calculate the observed and simulated differences between sequences. Inferences of population size based on the frequency of pairwise differences among haplotypes were made using Harpending's raggedness index (H_{RI}) at 10 000 permutations following the expectations that a multi-modal (ragged) distribution proposes a stable population and a unimodal (smooth) distribution advocates a rapid population expansion (Slatkin and Hudson, 1991; Rogers and Harpending, 1992). Significance of deviations from the hypothesis of a past demographic expansion was assessed using the sum of squared differences (SSD) at 10 000 iterations.

Calculating the time since expansions and effective population sizes is highly dependent on mutation rate (μ). Unfortunately, mutation rates are currently not known for Triakidae or congeneric species. Previous studies have used average mutation rates gathered from a range of shark species (Martin *et al.*, 1992; Duncan *et al.*, 2006; Keeney and Heist, 2006; Schultz *et al.*, 2008). These average mutation rates have, however, been calculated using non-congeneric species having different life history traits. The rate of molecular evolution is associated with generation time further correlated with metabolic rate, generation time, body size and other physiological and life history variables (Martin and Palumbi, 1993a). Therefore, due to the absence of CR mutation rate estimates for *T. megalopterus* or closely related species and/or species with similar life histories, dating a population expansion event and estimating female effective population size (N_{ef}) was not considered for this study.

Microsatellite genotyping

Population structure and phylogeographic patterns were assessed based on 22 microsatellite markers (**Table 3.1**) using Polymerase Chain Reaction (PCR) amplification. These markers were previously developed for *Mustelus canis* (Giresi *et al.*, 2012), *Galeorhinus galeus* (Chabot and Nigenda, 2011) and *M. henlei* (Byrne and Avise, 2012; Chabot, 2012). In a

recent study, these markers were combined into four multiplex assays and successfully tested for cross-species amplification in 16 elasmobranch species (Maduna *et al.*, 2014). Each of these markers was tested in a subset of eight *T. megalopterus* individuals, two individuals from AN, NA, WC and EC respectively. Polymerase Chain Reaction amplifications were run according to the conditions specified by Maduna *et al.* (2014) using the GeneAmp® PCR System 2700. Amplicons were viewed on a 2% agarose gel stained with 0.05 ng/µl EtBr. A Promega 100 bp molecular size ladder was used for size determination.

Table 3.1: Primer sequence, motif, fluorescent tag (dye) and source of the 22 microsatellite markers tested for their use in *Triakis megalopterus*; * = TMeg1, ** = TMeg2 (dye labels from Maduna *et al*., 2014)

Locus	Primer sequence (5'-3')	Motif	References	Dye
*Mh1**	F: GGAGGAGGGAAGCCTATGG R: TCTCTGGCTCCATTCAGGG	$(AG)_n$	Chabot, 2012	VIC
Mh2	F: ACTACACTGCATATAAACAGGC R: TTTTCAGAGGGCATAACTCAC	$(GA)_n$	Byrne and Avise, 2012	VIC
Mh9	F: CAACCATCTTTACTACACTG R: GATGGACCTCACATTTAACAC	$(GA)_n$	Byrne and Avise, 2012	FAM
*Mh25**	F: TGCAATAACCGTTCTGCGTC R: TCACACCCGCAGTTAGATCC	$(CT)_n$	Chabot, 2012	FAM
Mca25	F: ACACACTTTCACGCACAAGC R: TCGCTCAAGTGAGACCAGAG	$(CA)_n(CT)_n$	Giresi *et al.*, 2012	PET
*McaB39**	F: GGACAGGCAGCATCTGTGTA R: CCCAGGGGGATTAGGATATT	$(CA)_nGAT(AC)_n$	Giresi *et al.*, 2012	NED
*McaB5***	F: TAATCGACACGCAGTCATCG R: AAGCTCCAATTCTCACTGTGC	$(GT)_n$	Giresi *et al.*, 2012	VIC
*McaB6***	F: AGGATAAATACACGCACACAGG R: TTTTTGTTTTGCAATCTCACG	$(CA)_n$	Giresi *et al.*, 2012	FAM
*McaB22***	F: TCCTCTCCAGGACAAACACAC R: TCCCACCTGCCATAGTAATTG	$(AC)_n$	Giresi *et al.*, 2012	NED
*McaB27***	F: ATCCAGTGGTTTTGAAATGC R: CCTCGTAGGTCTCGTC	$(GT)_n$	Giresi *et al.*, 2012	PET
*Mca33***	F: CATTTGAACCCCGACAGAAC R: TCCAAGTAAGGATGAGTGACACC	$(ATC)_n$	Giresi *et al.*, 2012	FAM
*McaB37***	F: TCTGCCTCTGTGTCTCATCC R: TTTCCATTTCCGACATAGGG	$(GT)_n$	Giresi *et al.*, 2012	NED
*Gg2**	F: TGGCTCAGTCCAGAAACCC R: CCCTATTCGAGAGGCCCAG	$(TG)_n$	Chabot and Nigenda, 2011	NED
*Gg3**	F: CCGTGACTGAAAGCAGCC	$(GATT)_n$	Chabot and Nigenda, 2011	PET

Locus	Primer sequence (5'-3')	Motif	References	Dye
	R: CCCTCAACCATGGCAAGTG			
Gg7	F: CTGTGGAACCAAACTCCAGC R: AGCTGGTCGAGGTGAATGC	$(AG)_n$	Chabot and Nigenda, 2011	NED
Gg11	F: AAGTTGCACGTTTCCCAGC R: TACTGCAGGACCGGTTTCC	$(TCCC)_n$	Chabot and Nigenda, 2011	NED
Gg12	F: TGTCAAACACCATCGCAGG R: TGCTCTGAAGTCTACAAGAATGG	$(TA)_n$	Chabot and Nigenda, 2011	FAM
Gg15	F: GGCTGAATGGTTTCCCAGC R: GCCTCCAACTTAGCATAGCC	$(GA)_n$	Chabot and Nigenda, 2011	FAM
Gg17	F: CCTGCTTGTGACAGTTACCC R: ACAGGCATCACCTCTGTGC	$(AC)_n$	Chabot and Nigenda, 2011	PET
*Gg18**	F: TCCACTTCAGGAAGGCCAG R: CAAAGCCAGGTGGTTCTCC	$(GA)_n$	Chabot and Nigenda, 2011	VIC
Gg22	F: TCCTGGGATGGCAACTTCG R: AGGCCACCCAACTATCCTG	$(GT)_n$	Chabot and Nigenda, 2011	FAM
Gg23	F: ACAGACCACAGGGCATGG R: TGCAGAGCAGGCTAGATGG	$(AC)_n$	Chabot and Nigenda, 2011	VIC

Of the 22 microsatellite markers used in *T. megalopterus*, 12 were successfully cross-amplified and selected for screening of genetic variation in *T. megalopterus.* To facilitate multiplex PCR, forward primers of the successful markers were fluorescently labelled with one of four fluorescent tags (FAM, VIC, PET, or NED). The 12 markers were separated into two multiplex assays, TMeg1 (Gg2, Gg3, Gg18, McaB39 Mh1 and Mh25) and TMeg2 (McaB5, McaB6, McaB22, McaB37, McaB27, and Mca33). The two multiplex assays were used to genotype all 140 *T. megalopterus* specimens using a Qiagen Multiplex PCR kit according to the manufacturer's recommendations. Fragment analysis was performed with the LIZ600 internal size standard using a forced-capillary 3730 instrument. Based on fragment size, genotypes were scored using GeneMapper v4.0 (Applied Biosystems, 2006).

Marker validity

Microsatellite loci were screened for genotyping errors due to null alleles (Shaw *et al.*, 1999), short allele dominance (allele dropout) and/or stuttering using MicroChecker v2.2.3 (Van Oosterhout *et al.*, 2004). Deviations from Hardy–Weinberg expectations of random mating within loci and across sample populations and linkage disequilibrium (i.e. non-random association of alleles between loci within samples) were tested applying the Inbreeding Coefficient (F_{IS}) calculated in Genepop v4.0 (Raymond and Rousset, 1995) using the Markov chain parameters of 500 batches and 10 000 iterations. In Arlequin, the Slatkin's exact test

was used to test for selective neutrality using the Ewens–Watterson infinite-alleles model at 10 000 permutations (Slatkin, 1995).

Genetic diversity and population differentiation

Measures of genetic diversity such as allelic richness (A_R), number of alleles (N_A), expected (H_E) and observed (H_O) heterozygosity and Shannon–Weaver Information Index (I), mean relatedness (r) within populations (Queller and Goodnight, 1989) with 1000 permutations and derived 95% confidence intervals and pairwise F_{ST} between sample sites were calculated using GenAlEx v6.5 (Peakall and Smouse, 2012). Relatedness (r) is the measure of biological relationship between individuals. The amount of relatedness ranges from zero (unrelated) to one (identical twins) where expected full siblings and half siblings will result in $r = 0.5$ and $r = 0.25$, respectively (Queller and Goodnight, 1989).

Arlequin was also used for analysis of molecular variance (AMOVA) whereby three types of pooling/grouping strategies were used: 1) according to sample sites (AN, NA, WC and EC), 2) coastline (AN, NA and South Africa) and 3) oceanic placement (Atlantic and Indian Oceans). To visualize the distribution of genetic variation, a factorial correspondence analysis (FCA) plot was created using Genetix v4.05 (Belkhir *et al.*, 2000). The IBD suggested by the FCA was analysed by means of a Mantel test (Mantel, 1967) implemented via GenAlEx v6.5 (Peakall and Smouse, 2012)

To detect the number of possible distinct genetic clusters (K) present in *T. megalopterus*, a Bayesian clustering admixture model with correlated allele frequencies was performed in Structure v2.3.4 using 10 replicates from $K = 1$ to $K = 6$, with 1 000 000 iterations. Structure output files were used to assess the most likely K value in Structure Harvester v0.3 (Earl and VonHoldt, 2012). Assignments of individuals to populations in Structure were calculated using Clumpp v1.1.2 (Jakobsson and Rosenberg, 2007) and the output visualized with Distruct v1.1 (Rosenberg, 2004).

Demographic history

Estimates of effective population size (N_E) were done using the linkage disequilibrium (LD) test (minimum allele frequency 0.02) and heterozygosity excess method in N_EEstimator v2.01 (Do *et al.*, 2014). As long term monitoring and historic data of *T. megalopterus* is unavailable, detecting past population bottlenecks needs to be done with methods that require only a single temporal sample (Cornuet and Luikart, 1996). For this reason, the possibilities of recent

bottlenecks were analysed using Bottleneck v1.2.02 (Piry *et al.*, 1999), a method that tests for temporary heterozygote excess caused by a bottleneck that is relative to that expected under the mutational drift equilibrium. Heterozygosity excess or deficiency was tested under three commonly accepted mutation models for microsatellite evolution, Infinite Alleles Model (IAM; Watterson, 1984), Stepwise Mutation Model (SPM; Chakraborty and Nei, 1977) and Two-Phase Mutation Model (TPM; Di Rienzo *et al.,* 1994). Bottleneck analysis made use of 1 000 replications at the 5% nominal level using a TPM composed of 70% SMM and 30% IAM and a variance of 30 (Piry *et al.*, 1999). The one-tailed Wilcoxon signed rank test was used to determine significance of the observed deviations.

Results and interpretation

Mitochondrial DNA (mtCR)

Based on the COI gene and the BOLD database, all specimens were positively identified as *T. megalopterus*. A 673bp fragment from the mtCR, was successfully amplified in a total of 86 *T. megalopterus* samples collected from four sample sites (AN, NA, WC and EC). Sequence alignment in Mega revealed nucleotide composition of 36.2% thymine, 21.2% cytosine, 29.7% adenine and 13.0% guanine. The Tamura 1992 model (T92; Tamura, 1992) was approximated to be the best fit to the data using the Bayesian Information Criterion (BIC) in Mega.

A total of six haplotypes were characterized by six polymorphic segregating sites (*S*) of which three were parsimony informative. Haplotype diversity (**Table 3.2**) varied substantially (h = 0.000–0.0524) between sample sites where the South African localities displayed the highest haplotype diversities as opposed to NA which presented zero haplotype diversity. Nucleotide diversity was zero in all sample sites besides WC (π = 0.001). Therefore WC showed the highest haplotype and nucleotide diversity.

Table 3.2: Summary of population statistics for *Triakis megalopterus* integrated overall mitochondrial Control Region haplotypes from all four sampling sites; *n = number of individuals, H = number of haplotypes, h = haplotype diversity, π = nucleotide diversity*

Population	*n*	*H*	*H*	*Π*
Angola	16	2	0.125	0.0004
Namibia	15	1	0.000	0.0000
Western Cape	22	4	0.524	0.0014
Eastern Cape	33	4	0.328	0.0006

Network analysis revealed a central haplotype, TMH1, with two independent lineages representing mainly SA and AN respectively. Haplotype divergence was low, differing by only one mutational step, except TMH1-TMH5 which diverged by two mutational steps (**Figure 3.1**).

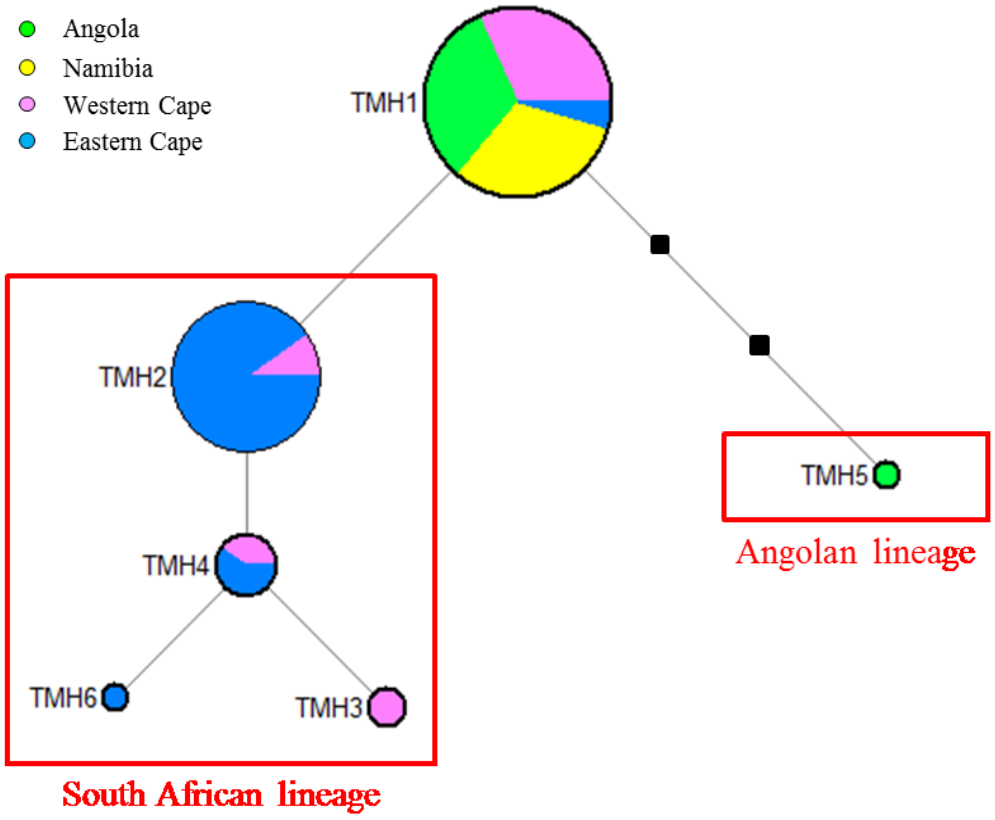

Figure 3.1: Median-joining network of haplotypes in mitochondrial Control Region for all *Triakis megalopterus* individuals. Node size is proportional to number of individuals sampled within the haplotype. Branch lengths correspond to one nucleotide substitution between haplotypes except where black squares represent unsampled ("missing") haplotypes.

A second haplotype network excluding the east coast samples (**Figure 3.2**) also showed the main and central haplotype, TMH1, and included representatives from both the proposed cold (Cape Point and NA) and warm (Betty's Bay and AN) water sample sites. Thereafter, the haplotype network showed clear separation between cold (Cape Point; TMH4) and warm (AN; TMH5) water lineages.

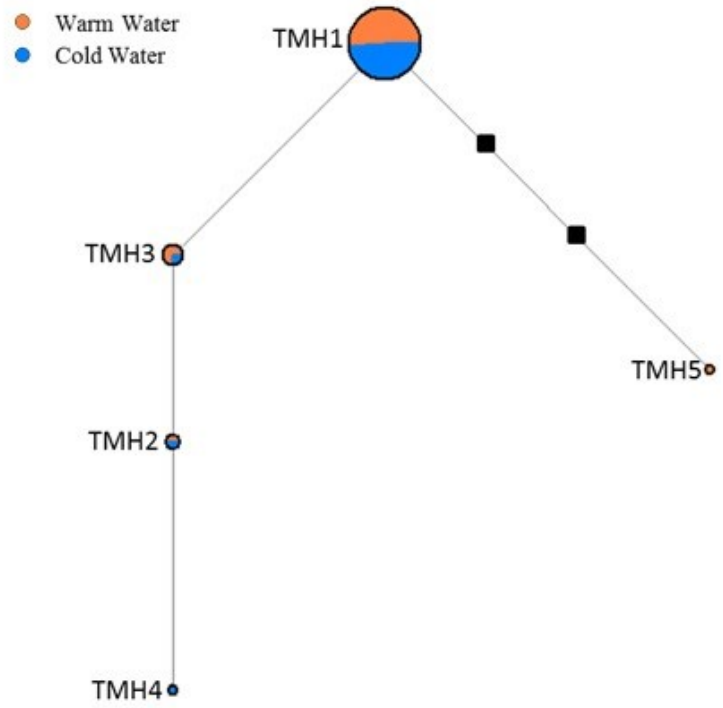

Figure 3.2: Median-joining network of warm and cold water habitats (excluding the Eastern Cape population) haplotypes in mtCR for *Triakis megalopterus*. Node size is proportional to number of individuals sampled within the haplotype. Branch lengths correspond to one nucleotide substitution between haplotypes except where black squares represent a double mutation between TMH1 and TMH5.

Phylogenetic reconstruction also revealed that the haplotypes were separated into two clades (**Figure 3.3**), consistent with the haplotype network in that separate clades were apparent for AN (TMH5) and the South African sample sites, WC (THM3) and EC (THM2, THM4 and THM6).

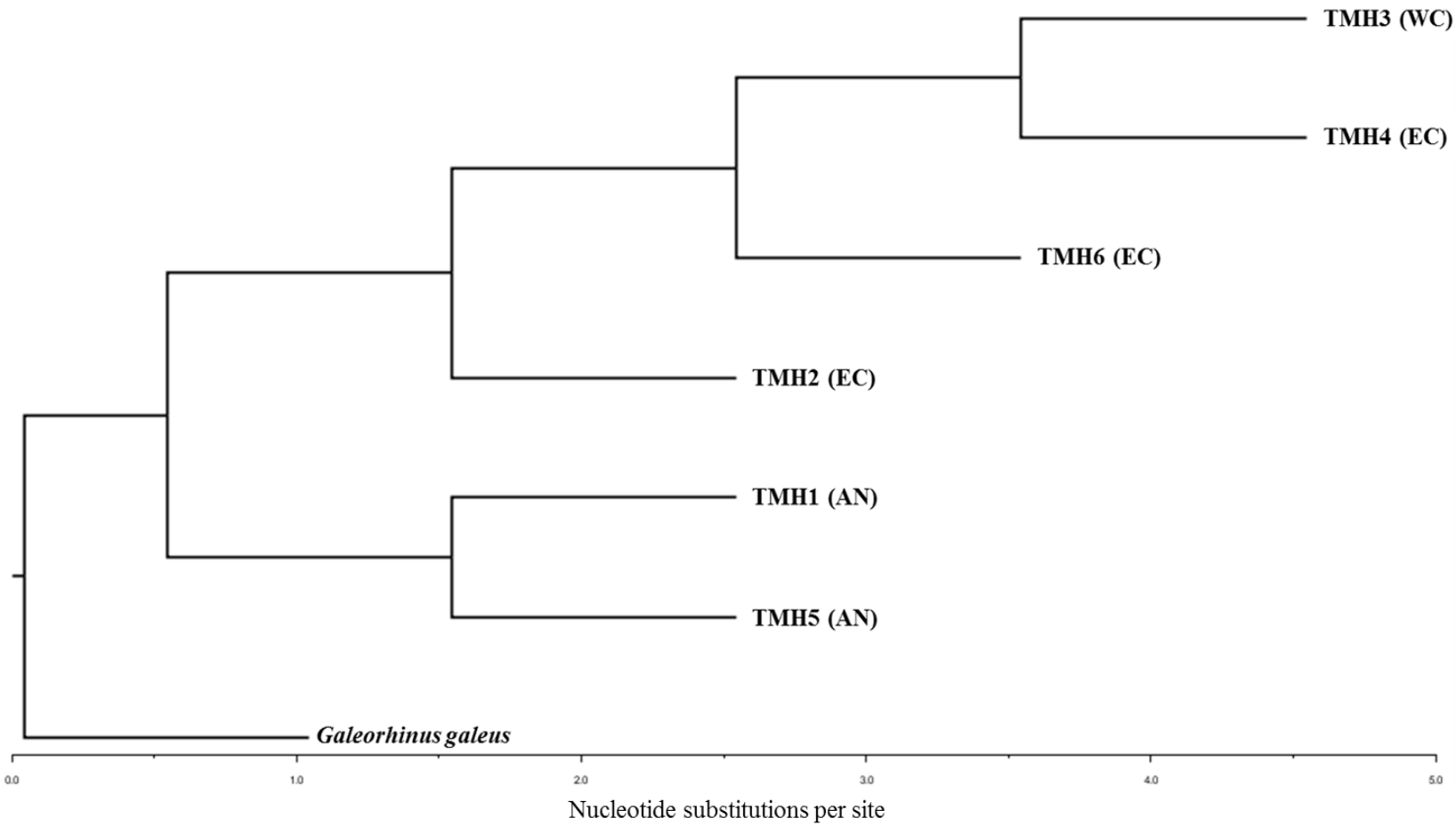

Figure 3.3: Molecular Phylogenetic tree of mitochondrial Control Region analysis for *Triakis megalopterus* using Maximum Likelihood (ML) molecular phylogenetic analysis with a bootstrap consensus tree inferred from 1000 replicates; *AN = Angola, WC = Western Cape, EC = Eastern Cape.*

mtCR population structure and phylogeographic patterns

Genetic differentiation ($F_{ST;}$ **Table 3.3)** was significant in all sample site comparisons except AN-NA (p = 0.991) which appears to be a homogeneous panmictic population. The largest genetic differences were amongst EC and all other sample sites (F_{ST} >0.380, p<0.01) revealing two diverse interoceanic groups, the Atlantic Ocean (AN-NA-WC) and Indian Ocean (EC), supporting trans-oceanic population structure. However, there was evidence of admixture (although non-significant) between the two South African sample sites, WC and EC (F_{ST} = 0.380, p<0.01) but lower levels of genetic differentiation between WC-AN (F_{ST} = 0.150, p = 0.027) and WC-NA (F_{ST} = 0.164, p = 0.045).

Table 3.3: Estimates of pairwise F_{ST} values from mitochondrial Control Region for *Triakis megalopterus* from four sample sites across southern Africa. Genetic distances and significance values are represented below and above the diagonal, respectively; *statistical significance at the 5% level is highlighted in bold.*

	Angola	**Namibia**	**Western Cape**	**Eastern Cape**
Angola	-	0.991	**0.027**	**0.000**
Namibia	-0.004	-	**0.045**	**0.000**
Western Cape	**0.150**	**0.164**	-	**0.000**
Eastern Cape	**0.717**	**0.762**	**0.380**	-

Average pairwise distances (**Figure 3.4**) between populations were highest and lowest between AN-EC and AN-NA, respectively. Within population differences were highest and lowest in WC and NA, respectively, correlated to the haplotype and nucleotide diversities for these populations. Nei's genetic distance showed the most distantly related sample sites were EC-AN and EC-NA, while the lowest genetic distance values were between WC-AN and WC-NA.

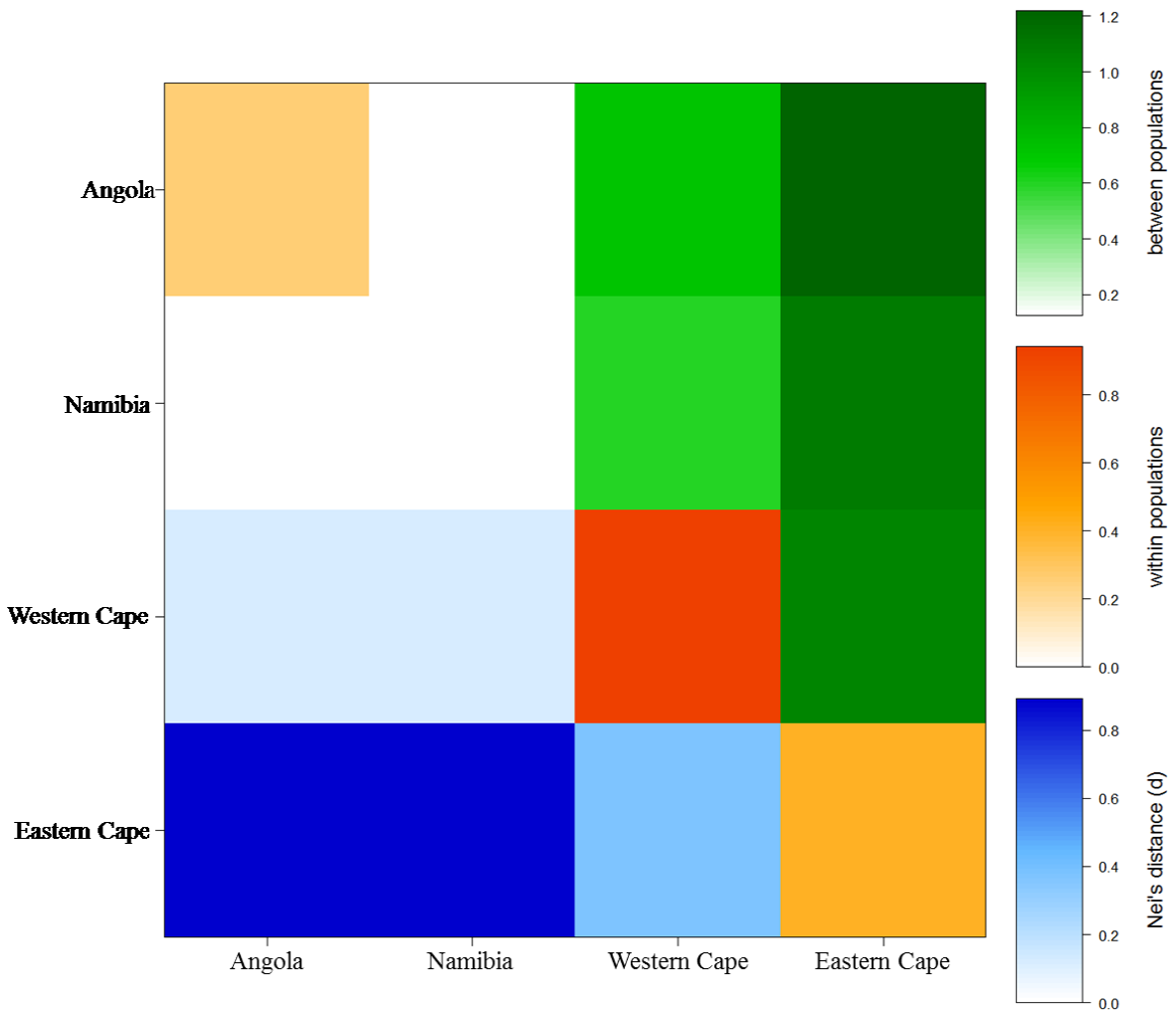

Figure 3.4: Average number of pairwise differences (π) for mitochondrial Control Region between sampled populations by means of three colour scales. Orange on diagonal represents π within populations; green above diagonal shows π_{xy} between pairs of populations and blue below diagonal gives the net number of nucleotide differences between populations.

Significant differences (p<0.05) were present within groups and within populations for the northern vs. southern Benguela and trans-oceanic grouping strategies (**Figure 3.5**). However, non-significant (p>0.05) differences were evident for the amongst group variables for the same pooling strategies, thus not supporting the cold vs. warm lineage hypothesis.

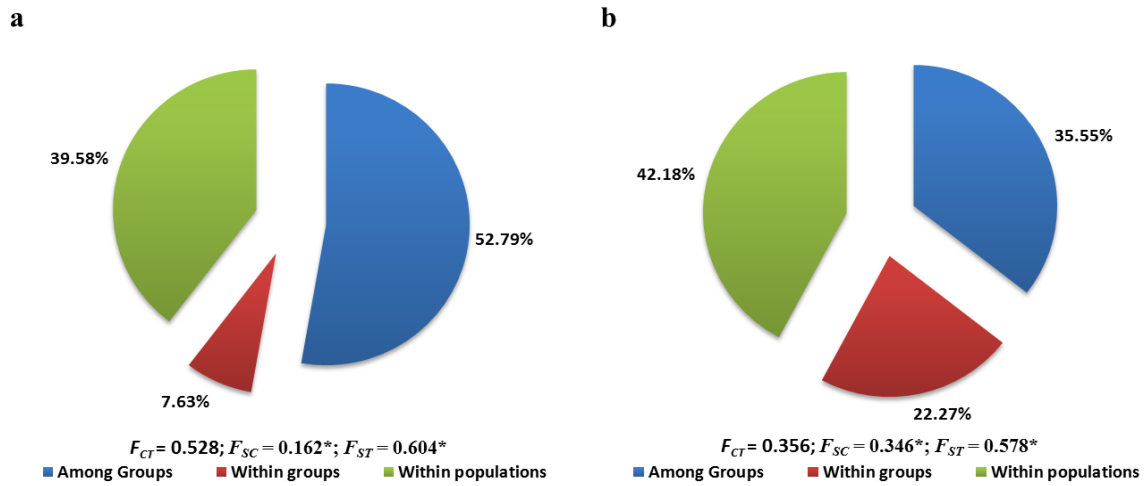

Figure 3.5: Analysis of molecular variance results for mitochondrial Control Region of (a) Atlantic (AN, NA and WC) vs. Indian (EC) Oceans and (b) northern (AN and NA) vs. southern (WC and EC) Benguela subsystems; *AN = Angola, NA = Namibia, WC = Western Cape and EC = Eastern Cape, * = significance at the 5% level.*

mtCR population demographics

Both Tajima's *(D* = -0.788, *p* = 0.244) and Fu's *(F_S* = -1.248, *p* = 0.289) statistics for neutrality were negative and non-significant indicating no excess of alleles that would be expected following a population expansion event.

Overall value for SSD (SSD = 0.007) was low and non-significant, *p* ($_{\text{Sim. SSD >= Obs. SSD}}$) = 0.116. This signified no deviations from expectations under the model of expansion and thus it was not possible to reject the null hypothesis of a past demographic expansion. Harpending's raggedness index (H_{RI} = 0.113) was also not statistically significant, *p* ($_{\text{Sim. HRI >= Obs. HRI}}$) = 0.050, an indication that the data has relatively good fit to a model of population expansion (Harpending, 1994; Schneider and Excoffier, 1999).

The mismatch distribution (**Figure 3.6**) exhibited a clear unimodal pattern of the genetic differences between pairs of individuals in a mismatch distribution considering all the analysed individuals. This is characteristic of populations that have undergone a demographic expansion in the past. This was reiterated by the mismatch analysis where the estimated ancestral population size (θ_0) of 0.000 increased dramatically to an actual population size (θ_1) of 99 999.00 with a τ value of 0.842. The large variance between θ_0 and θ_1 is an indication of population expansion.

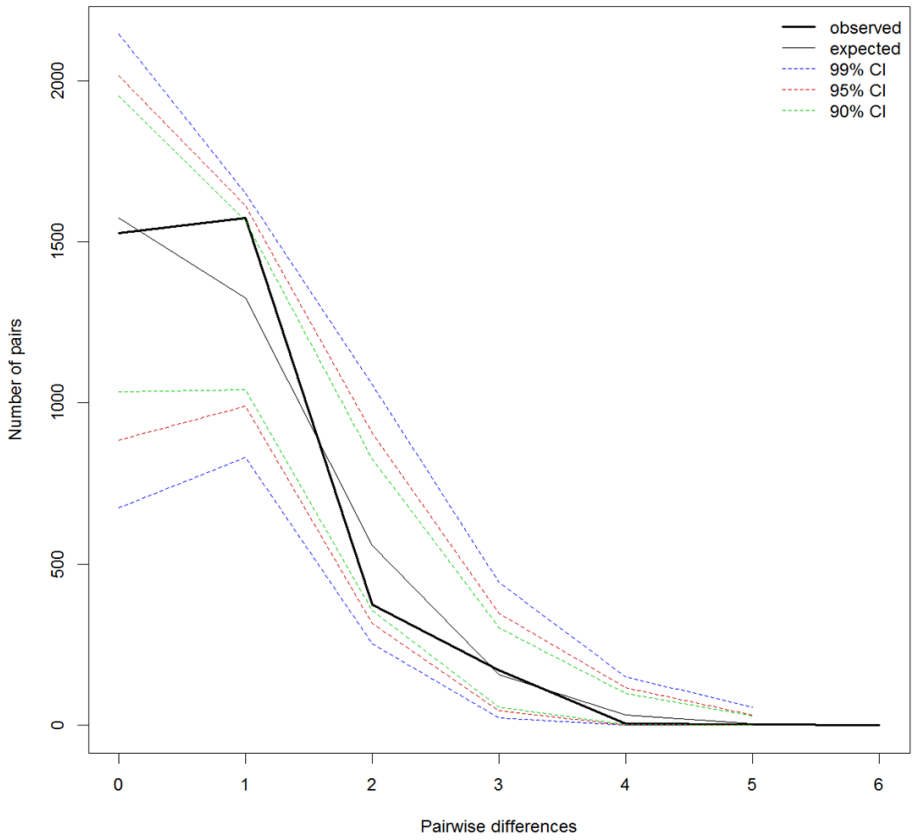

Figure 3.6: Mismatch distribution of demographic expansion based on the infinite allele model for mitochondrial Control Region indicating the observed (thick black line) and expected (thin black line) numbers of pairwise differences for *Triakis megalopterus*

Microsatellites (nDNA) marker validity

The genetic structure of *T. megalopterus* was assessed using a subset of six microsatellite markers (Gg2, Gg3, Mh1, Mh25, McaB22 and McaB37). The remaining markers (Gg18, McaB5, McaB6, McaB27, McaB39 and Mca33) were excluded from further analyses as they displayed genotyping errors and/or did not conform to Hardy–Weinberg equilibrium and selective neutrality. A total of 99 *T. megalopterus* individuals from four sample sites (AN, NA, WC and EC) across southern Africa were successfully genotyped.

nDNA genetic diversity and population differentiation

Mean within-population pairwise relatedness (*r)* for nDNA loci from four *T. megalopterus* sample sites showed all sample site means were within the upper and lower confidence levels indicating that individuals from all populations were sampled randomly. Values amongst populations were not exceptionally high and ranged from r = -0.115 in EC to r = 0042 in NA. Comparable genetic diversity was evident across all sample sites (**Figure 3.7**). The mean number of private alleles was highest in WC with two markers (Mh1 and Gg3; A_p = 0.333)

showing private alleles compared to a single marker in all other sample sites (A_p = 0.167). Genetic diversity levels were highest in EC (mean H_E = 0.578; mean A_R = 2.441; mean I = 1.020) and lowest in WC (mean H_E = 0.514; mean A_R = 2.328; mean I = 0.945).

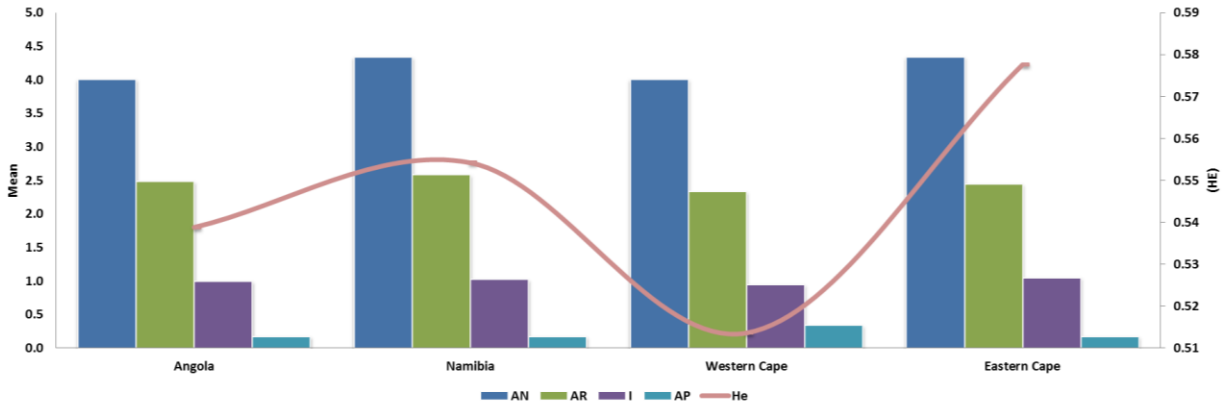

Figure 3.7: Mean genetic diversity estimates using microsatellite loci from *Triakis megalopterus*; A_N = number of alleles, A_R = allelic richness, I = information index, A_P = number of private alleles, H_E = heterozygosity.

Pairwise genotypic differentiation (**Table 3.4**) indicated highly significant ($p \leq 0.002$) population differentiation amongst four of the six population comparisons. All comparisons with EC were significantly different. Although AN and NA are neighbouring populations, both present within the Atlantic Ocean and in the northern Benguela subsystem, the AN-NA comparison revealed significant differences (F_{ST} = 0.039, p = 0.001). Interestingly, complete panmixia (F_{ST} = 0.000, p = 0.404) was apparent in AN-WC implying that there was gene flow between *T. megalopterus* from WC-AN and WC-NA but no significant gene flow between AN-NA.

Table 3.4: Microsatellite pairwise F_{ST} values for the four sample site comparisons of *Triakis megalopterus*. Shown above and below the diagonal line are the significant *(p)* and F_{ST} values, respectively; *statistical significance at the 5% level highlighted in bold.*

	Angola	**Namibia**	**Western Cape**	**Eastern Cape**
Angola		**0.001**	0.404	**0.001**
Namibia	**0.039**		0.060	**0.002**
Western Cape	0.000	0.017		**0.001**
Eastern Cape	**0.112**	**0.040**	**0.113**	

The hierarchical AMOVA (**Figure 3.8**) for the Atlantic (AN, NA and WC) vs. Indian Ocean (EC) geographic groupings showed trans-oceanic genetic structure with significant (p<0.05) differences amongst oceans (F_{CT} = 0.055) and within populations (F_{ST} = 0.024). Northern Benguela (AN and NA) vs. southern Benguela (WC and EC) grouping revealed significant (p<0.05) differences within groups (F_{SC} = 0.039) and within populations (F_{ST} = 0.073). Structure between the northern and southern Benguela subsystems (F_{CT} = -0.037) were non-significant (p>0.05).

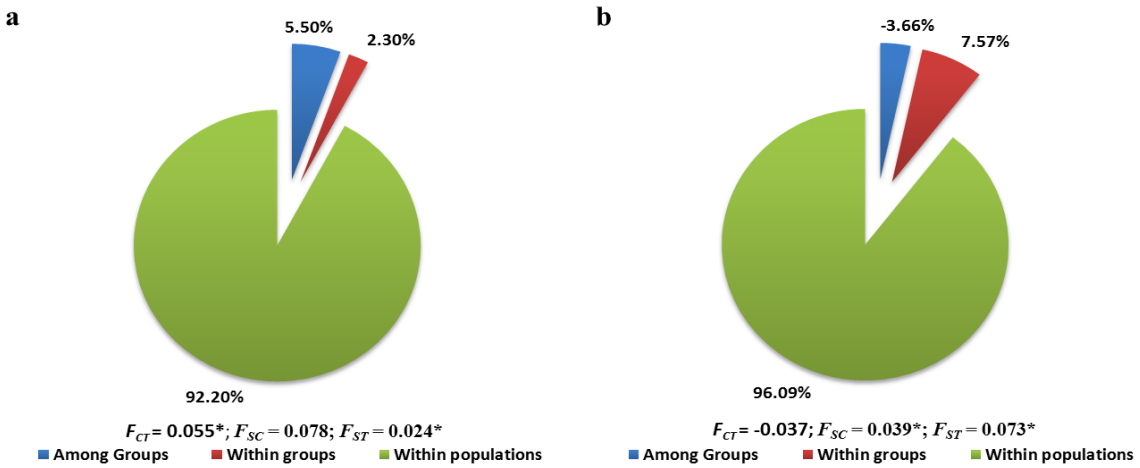

Figure 3.8: Analysis of molecular variance results from microsatellite data of *Triakis megalopterus* groupings of (a) Atlantic (AN, NA and WC) vs. Indian (EC) Oceans and (b) northern (AN and NA) vs. southern (WC and EC) Benguela subsystems; *AN = Angola, NA = Namibia, WC = Western Cape and EC = Eastern Cape, * = significance at the 5% level.*

Factorial Correspondence Analysis (FCA; **Figure 3.9**) did not identify distinct groupings for individuals from the different sample sites, neither from the different ocean regions or the northern and southern Benguela subsystems. However, the vast majority of AN (left) individuals seem to form a tighter cluster, while the other three locations (NA, WC, and EC) appear to be more overlapping and dispersed.

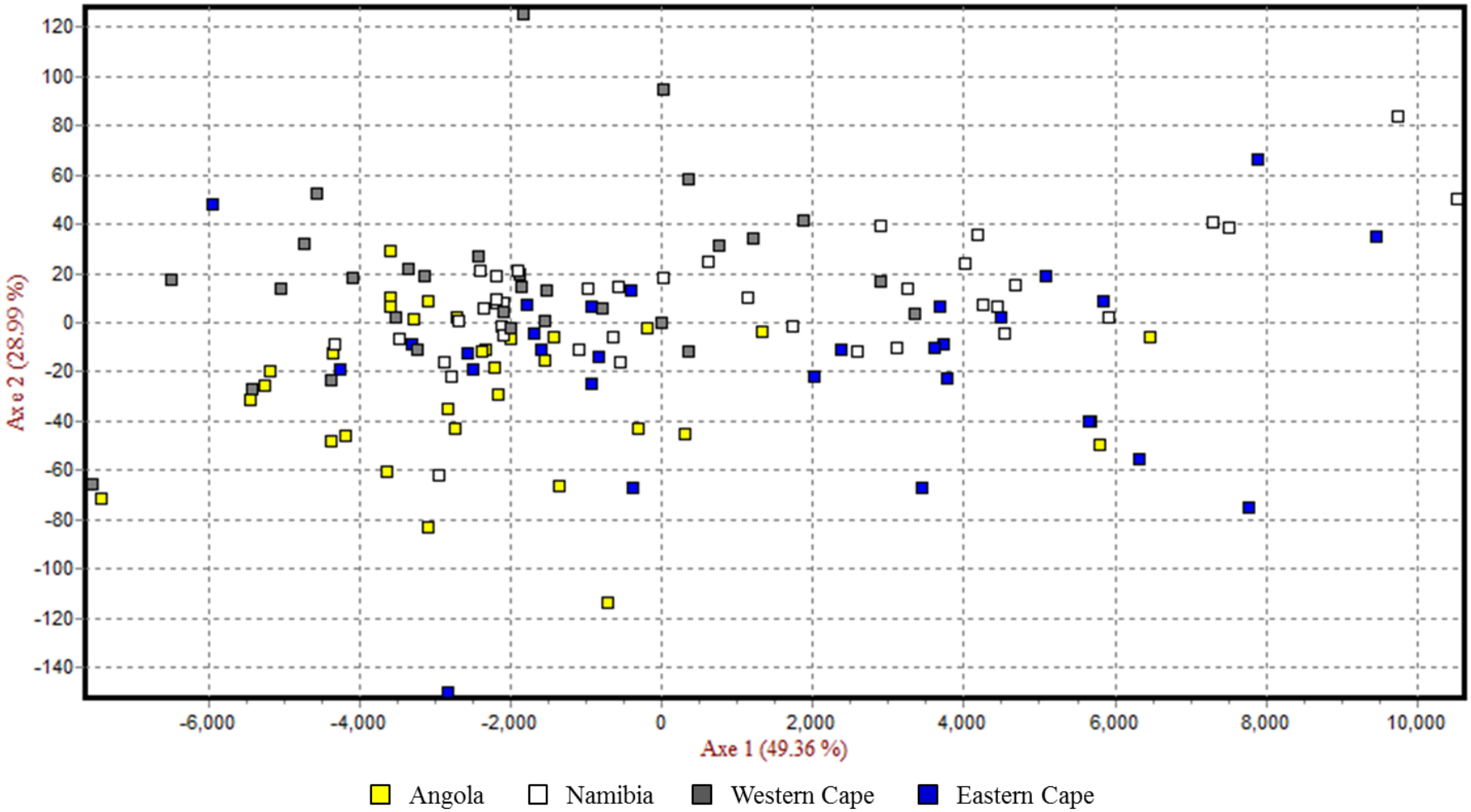

Figure 3.9: Factorial correspondence analysis plots of microsatellite loci of *Triakis megalopterus* from all sample sites.

A regression of genetic distance (F_{ST}) and geographical distance of the four sample sites of *T. megalopterus* (**Figure 3.10**) showed no significant correlation between the four sample sites (r^2 = -0.410, p = 0.419).

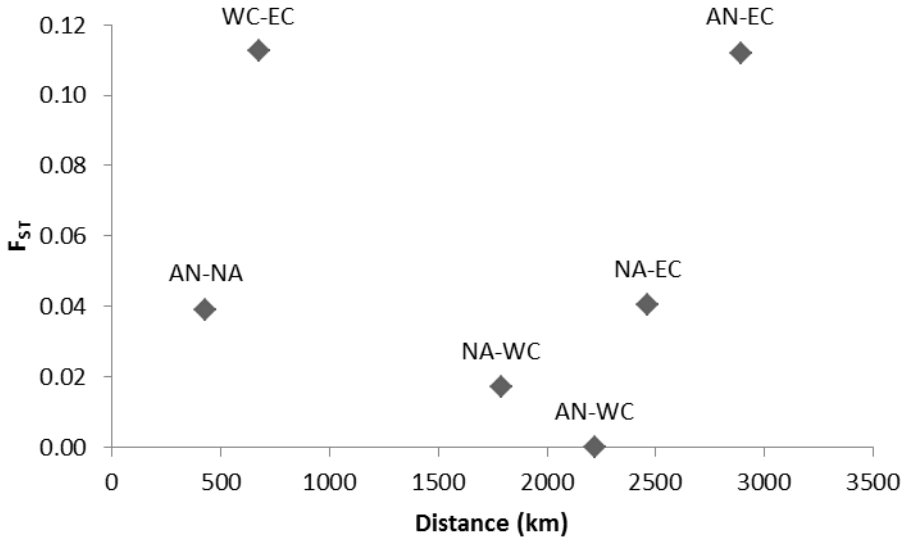

Figure 3.10: Isolation by distance of microsatellite data showing pairwise population F_{ST} vs. geographical distance amongst all four sample sites of *Triakis megalopterus*.

Bayesian clustering analysis in Structure indicated that the most likely number of clusters (K) for *T. megalopterus* was two (**Figure 3.11 a and b**) with AN, NA and WC representing one cluster and the other cluster comprising individuals only from the EC. This supported limited gene flow between the Atlantic and Indian Oceans. Furthermore, K = 3 (**Figure 3.11 c and d**) is shown to demonstrate clinal variation, gradual differences in allele frequencies across the four sample sites spanning the southern African coastline.

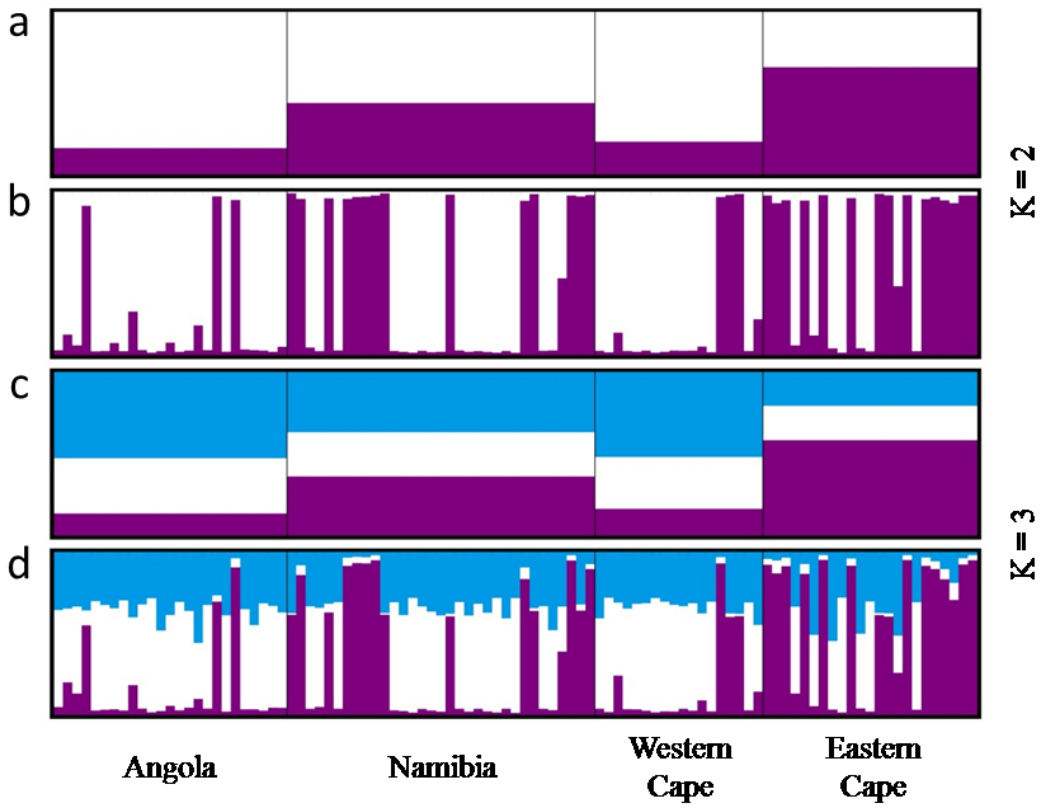

Figure 3.11: Genetic structure of microsatellite data for four *Triakis megalopterus* sample sites based on Bayesian clustering analyses, (a) K = 2 population Q-matrix; (b) K = 2 individual Q-matrix; (c) K = 3 population Q-matrix and (d) K = 3 individual Q-matrix.

nDNA demographic history

Estimates of contemporary effective population size (N_E; **Table 3.5**) indicated low (N_E <3.3) effective population sizes for all populations except AN (N_E = 26.9). Unfortunately, all confidence intervals contain infinity giving little power to make any inferences about N_E.

The bottleneck analyses showed a normal L-shaped distribution for all sample sites. Using the Wilcoxon signed-rank test, no significant (p>0.05) heterozygosity excess or deficiencies were apparent under the TPM or SMM across all the study sites. Lack of significance for heterozygosity deficiency and heterozygosity excess were indicative of no recent demographic expansion or bottleneck events.

Table 3.5: Microsatellite effective population size (Ne) estimates based on linkage disequilibrium and heterozygosity excess (Neb) amongst four sample sites of *Triakis megalopterus*

			TPM		SMM	
	N_e [95% CI]	N_{eb} [95% CI]	H_e deficiency	H_e excess	H_e deficiency	H_e excess
Angola	26.9 [6.6 - ∞]	∞ [2.8 - ∞]	0.410	0.633	0.213	0.820
Namibia	2.4 [1.7 – 4.4]	∞ [3.8 - ∞]	0.936	0.082	0.590	0.455
Western Cape	3.2 [1.8 – 11.2]	∞ [2.5 - ∞]	0.633	0.410	0.500	0.545
Eastern Cape	3.2 [2.1 – 8.5]	∞ [2.9 - ∞]	0.850	0.180	0.545	0.500

Discussion

As marine habitats have few barriers to physical dispersal, many marine species are known to range over very large distances (Lessios *et al.*, 1998; Waples, 1998). Despite this, marine taxa with potentially high dispersal abilities have been known to show abrupt discontinuity in their distributions (Teske *et al.*, 2005; Waters *et al.*, 2005). Due to the fact that the South Africa marine realm shows no hard barriers to dispersal, it is difficult to explain phylogeographic breaks by means of contemporary oceanography alone (Teske *et al.*, 2013). Historical processes such as geological changes and large scale climatic events can intensely effect the evolution of a species (Wilson, 2006; Hemmer-Hansen *et al.*, 2007; Pardiñas *et al.*, 2010). Therefore, historical events, such as those observed during the Pleistocene are most likely the cause of a reduction/break in gene flow amongst populations. The Earth's climate became cooler through the Tertiary, 65 Mya, with frequent oscillations increasing in intensity leading to the series of major ice ages of the Quaternary, 2.4 Mya to the present (Hewitt, 2000). Since the Mesozoic, several geophysical events have shaped marine biotas e.g., the emergence of the Isthmus of Panama, closure of the Tethys Seaway and the formation of the Benguela upwelling (Duda and Lessios, 2009).

In southern Africa, climate oscillations through the Pleistocene have altered coastal morphology (Teske *et al.*, 2011b). Although sea level fluctuations on the southern African

coast have been less than 3 m in the last 7 000 years (Ramsay, 1995; Baxter and Meadows, 1999; Compton, 2001), the Last Glacial Maximum (LGM), between 26 500 and 19 000 years ago (Clark *et al.*, 2009), saw sea level drops of approximately 120–140 m lower than present (Ramsay and Cooper, 2002). During the LGM, the continental shelf of the South African continent, known today as the Agulhas Bank, was left exposed resulting in the southernmost tip of Africa (Cape Agulhas) lying approximately 200 km south of where it is today (Teske *et al.*, 2013). According to von der Heyden *et al.* (2011), the drop in sea level and exposure of the Agulhas bank caused a range contraction, decline in available habitats and isolation of east and west coast populations. Studies have shown that during these cooler glacial periods, many temperate species experienced shifts in distribution and/or declines in population abundance (Hewitt, 2004).

The most prominent and constant result signified by the mtCR (F_{ST}) and nDNA (Bayesian clustering analysis) showed that EC was a separate population. This indicates that the population structure of *T. megalopterus* conform to a transoceanic arrangement, Atlantic (AN, NA, WC) versus Indian Ocean (EC) populations. This transoceanic pattern of population structure was also found in the yellowfin tuna, *Thunnus albacares* (Henriques, 2011). The similarities in the structure of these two species indicate that, much like *T. albacares*, *T. megalopterus* is able to withstand the colder waters of the Benguela Current and displays a larger distribution potential for genetic admixture. Much like *T. albacares*, *T. megalopterus* may also migrate preferentially within ocean basins.

Von der Heyden *et al.* (2011) proposed that the drop in sea levels and exposure of the Agulhas Bank during the LGM resulted in rocky shore habitat being replaced by sandy beaches in southern Africa, thus driving divergence of populations occupying isolated rocky shores. This occurrence has been noted in the Caribbean where a 90% decrease in reef habitat was recorded during the LGM (Bellwood and Wainwright, 2002). Von der Heyden's hypothesis was recently confirmed by Toms *et al.* (2014) who found that between Marine Isotope Stage 4 (MIS 4) and the LGM, the rocky shore refugia of the southwest and southeast coasts of southern Africa were disconnected by sandy shores for at least 40 000 years resulting in two lineages (west and east coast) of the clinid *Clinus cottoides* in southern Africa. As *T. megalopterus* in known to occupy rocky intertidal zones (Bass *et al.*, 1975; Smale and Goosen, 1999), it would appear that the exposure of the Agulhas Bank and reduced rocky shore habitat may have played a considerable role in the separation of the EC population. Phylogeographic breaks between adjacent populations, particularly when there is concordance in several species with different life histories (such as *C. cottoides and T.*

megalopterus), is influenced by the presence and duration of a historical geographic barrier (Kuo and Avise, 2005).

Further confirmation that the split between the Atlantic and Indian Ocean populations may be historic is the fact that the ORI tagging data show that *T. megalopterus* is uninterruptedly present from WC to EC and able to move between the two provinces (Dunlop and Mann, 2014). According to Irwin (2002), phylogeographic breaks are still possible between continuously distributed species, particularly if mean dispersal distances and/or population sizes are low. Although not much is known about the population sizes of *T. megalopterus*, the tagging data show that this species displays a high level of residency and/or philopatry as approximately 80% of the recaptures were within a 20 km radius. One specimen was recaptured within 7 km after 17.4 years. The movement of this species explains why there is evidence of contemporary gene flow between WC and EC despite the separation of the two populations in the Bayesian cluster analysis. Tagging and/or acoustic tracking that includes sex data will also assist in a more robust evaluation of *T. megalopterus* movement patterns and help to determine the extent of female philopatry and the dependence of this species on their specific ranges.

The Pleistocene has been hypothesized as a significant period for speciation (Thum and Harrison, 2009). This period is characterized by large-scale climatic changes which led to persistent variations in oceanic circulatory patterns, sea levels, productivity, SST (Hemmer-Hansen *et al.*, 2007) and reduction in suitable habitats (Toms *et al.*, 2014), all of which have prominently influenced the evolutionary history of living marine taxa (Liu *et al.*, 2007; Larmuseau *et al.*, 2009). During the Pleistocene, cooler glacial cycles initiated a contraction and shift of temperate species to lower latitudes after which, during interglacial (warming) periods, these species would recolonize resulting in population growth. This is known as the Expansion–Contraction model (Provan and Bennett, 2008). Postglacial expansion into new habitats has been suggested to be vital in the geographic distribution of population and species genomes (Teske *et al.*, 2013).

Throughout the aforementioned glacial periods, changes in the Agulhas Current were also evident. This current was cooler and weaker in summer months and believed to have ceased to flow in winter (Hutson, 1980). Consequently, Agulhas leakage was considerably reduced and essentially halted the mixing between the Indian and south Atlantic ocean waters (Franzese *et al.*, 2006) and the respective populations therein. These are all possible reasons for the isolation of the EC population from other sample sites in this study. Although EC may

have been isolated during these cold periods of the LGM, neither mtCR (non-significant Tajima's D) nor nDNA data (non-significant heterozygosity excess) showed evidence of historical or contemporary bottleneck events.

Population expansion, however, was evident by means of a unimodal mismatch distribution and a large increase from ancestral to actual population size. Although, due to the weak nature of observed patterns in the data, this population expansion may be speculative. However, if an population expansion did in fact take place, it can be hypothesized that the population expansion may have occurred in *T. megalopterus* after the LGM in a period where warming expanded populations from their glacial refugia (O'Brien *et al.*, 2013). During this period of warming, sea levels were again elevated increasing appropriate habitat ranges for various marine organisms (Marko *et al.*, 2010; Nance *et al.*, 2011), in turn causing population expansions (Peltier, 1988; Miller *et al.*, 1995; Teske *et al.*, 2006, 2011a). This was also hypothesized for *M. mustelus* (Maduna, 2014).

The nDNA F_{ST} data show a link between WC-NA and WC-AN but not AN-NA. As the NA population inhabits the colder waters of the Benguela Current and the AN population the warmer waters of the Angolan Current, it may be plausible that *T. megalopterus* consists of a warm and cold water lineage. The haplotype network of hypothesized warm versus cold clades shows evidence of warm and cold lineages. The warm clade comprised the population from AN and the warmer parts of the Western Cape, in this case, Betty's Bay. The cold clade originated from NA and the cooler waters of the Western Cape, in this instance, Cape Point. Again, a lack of mutation rate for *T. megalopterus* hinders the exact dating of when this split was most likely associated with the inception of the Benguela Current and/or historical glacial/interglacial episodes. A similar pattern has been observed in two species of kob (*Argyrosomus japonicus* and *A. coronus*) from southern Africa. According to Potts *et al.* (2013), these two species stemmed from a common ancestor around southern Africa. *Argyrosomus* spp. were split into north-eastern and south-eastern populations due to rapid warming at the equator during an interglacial period (3.78–1.68 Mya.). This split was followed by sympatric speciation of *Argyrosomus inodorus* from coastal *Argyrosomus* spp. (early–mid Pleistocene, 2.41–1.33 Mya.) when cool and warm water individuals started spawning in separate grounds. Consequently, the final speciation event, to form what are today known as *A. japonicus* and *A. coronus*, was caused by the allopatric isolation of the warm water lineage during the establishment of the cold Benguela Current (2.19–0.93 Mya.). As sharks are known to evolve much slower than many other animals (Martin *et al.*, 1992; Martin and Palumbi, 1993b), it may take many more years of uninterrupted isolation of the

warm and cold water clades before speciation would be evident in *T. megalopterus*. Future research is needed to determine an accurate mutation rate for *T. megalopterus,* which is imperative to precisely determine the demographic history of this species, its patterns of population stability, concentration or expansion, timing of population expansion events and accurate effective population sizes.

According to the global biogeographic patterns (see **Chapter 1**), the South African populations of WC and EC are both present within the Agulhas province and Agulhas Bank bioregion, while AN and NA are both located within the Benguela province and Namib ecoregions. As the biogeographical provinces are said to be constrained by boundaries, such as geochemical influences, hydrographic features and geomorphological features, and ecoregions by temperature regimes, nutrients, freshwater incursion, upwelling systems, ocean currents, bathymetry, coastal complexity, isolation and/or ice regimes (Spalding *et al.*, 2009), one would expect the genetics of species to represent these biogeographic patterns. Although the mtCR data (median-joining haplotype network and phylogenetic tree) showed a South Africa versus Angola split in populations, which conforms to a separation by biogeographic region, the nDNA exhibited two clades, separated by ocean, for *T. megalopterus*. It may therefore be possible that the distribution of this species was affected by biogeographic region before the exposure of the Agulhas Bank, which shaped the contemporary population structure of *T. megalopterus*.

Phylogeographic breaks have also been well documented to separate lineages associated with cool-temperate and warm-temperate biogeographic regions (Emanuel *et al.*, 1992; Turpie *et al.*, 2000; Evans *et al.*, 2004; Teske *et al.*, 2007b). Several molecular studies within South Africa have identified marine localities that act as barriers to gene flow, namely Cape Point, Cape Agulhas, Algoa Bay, the Wild Coast and the Mozambique border (Von der Heyden *et al.*, 2011). Should any of the aforementioned barriers have an effect on *T. megalopterus*, it would likely be Cape Agulhas, as this potential barrier is located between the sampling sites of the WC and EC. Cape Agulhas has been proven as a barrier to the dispersal of several marine species namely, abalone *Haliotis midae* (Evans *et al.*, 2004; Bester-van der Merwe *et al.*, 2011), various clinids (Von Der Heyden *et al.*, 2008), the caridean shrimp, *Palaemon peringueyi* (Teske *et al.*, 2007a), the mudprawn, *Upogebia africana* and the isopod, *Exosphaeroma hylecoetes* (Teske *et al.*, 2006). Cape Agulhas was also proposed to be the prominent barrier in the dispersal of the common smoothhound, *Mustelus mustelus* (Maduna, 2014). Interestingly, however, the ORI tagging data show that *T. megalopterus* is able to move between Cape Point, Cape Agulhas and Algoa Bay (Dunlop and Mann, 2014),

providing further evidence that the population structure of this species appears to be predominantly affected by historical events.

The conservation of genetic variation is a fundamental element of many species management programmes. Effective management of biological resources therefore lies in gaining adequate knowledge of the genetic variability both intra- and inter-populations because, ultimately, this is what permits the adaptation of a species to varying environments and their response to selection (O'Connell and Wright, 1997). Consequently, historic and contemporary patterns of population divergence have the potential to inform scientists of the outcome of historical interactions between a species and its surrounding environment (Grosberg and Cunningham, 2001). According to Gray (1997), a decrease in genetic diversity may be caused by environmental stress, species introduction and invasions, habitat degradation, fragmentation and loss and/or watersheds and physical alterations of coast. Fishing pressure (Smith *et al.*, 1991), the intermediate disturbance hypothesis (Connell *et al.*, 1978), global climate change (Pernetta, 1993) and bottlenecks (Landergott *et al.*, 2001) may also contribute to declines in genetic diversity. All of the selection pressures mentioned above, whether natural or human-related, are capable of shaping the heritable adaptations of a species, which in turn will alter its characteristics over time.

Compared to teleost species, it has been widely generalized that sharks have low genetic diversity (Smith, 1986; Dudgeon *et al.*, 2012; Portnoy and Heist, 2012). Although this is true for the majority of shark species, there are exceptions, e.g.: the whale shark, *Rhincodon typus* ($h = 0.97$, $\pi = 0.011$; Castro *et al.*, 2007); sandbar shark, *Carcharhinus plumbeus* ($h = 0.959$, $\pi = 0.00475$; Portnoy *et al.*, 2010) , ($h = 0.852$, $\pi = 0.0029$; Blower *et al.*, 2012); spiny dogfish, *Squalus acanthias* ($h = 0.735$, $\pi = 0.0029$; Veríssimo *et al.*, 2010); blacktip shark, *Carcharhinus limbatus* ($h = 0.843$, $\pi = 0.0041$; Keeney and Heist, 2006); and the basking shark, *Cetorhinus maximus* ($h = 0.720$, $\pi = 0.0013$; Hoelzel *et al.*, 2006).

Historical (mtCR) genetic diversity for *T. megalopterus* showed an overall low mtCR haplotype and nucleotide diversity ($h = 0.213$, $\pi = 0.0006$), particularly in the NA population where only one haplotype was present and zero diversity was recorded. Overall, haplotype and nucleotide diversity for *T. megalopterus* was slightly lower than recorded for the closely related *Mustelus schmitti* ($h = 0.226$, $\pi = 0.0015$; Pereyra *et al.*, 2010) but less than 50% of the diversity found for the common smoothhound, *Mustelus mustelus* ($h = 0.517$, $\pi = 0.00104$; Maduna, 2014) and the gummy shark, *Mustelus antarcticus* ($h = 0.534$, $\pi = 0.0014$; Gardner and Ward, 2002). Intermediate levels of genetic diversity were detected with nDNA loci

(mean H_E = 0.546), higher than that of mtCR diversity. This discrepancy can be partly explained by a weak correlation between nuclear and mtCR diversity, based on independent demographic processes imposed by historical events (e.g. Brunner *et al.*, 1998; Bernatchez *et al.*, 2002) and different mutation rates known to occasionally be higher in nDNA (e.g. Moritz *et al.*, 1987; Weber and Wong, 1993) resulting in a higher effective number of alleles at mutation-drift equilibrium (So *et al.*, 2006).

The greatest number of haplotypes found in *T. megalopterus* was four, which was found in the WC and EC populations. This is congruent with the greatest number of haplotypes found in the closely related leopard shark (*Triakis semifasciata*) from Los Angeles (Lewallen *et al.*, 2007). Although the lack of haplotype and nucleotide diversity in NA is alarming, it is not uncommon in shark populations. Several shark species have shown nucleotide and haplotype diversities of zero in certain populations: e.g., the ragged-tooth shark, *Carcharias taurus*, in Japan and Eastern Australia (Stow *et al.*, 2006; Ahonen *et al.*, 2009); scalloped hammerhead shark, *Sphyrna lewini*, in Thailand (Duncan *et al.*, 2006); lemon shark, *Negaprion brevirostris*, in Taiwan, French Polynesia and Pacific Mexico (Schultz *et al.*, 2008); zebra shark, *Stegostoma fasciatum*, in Indonesia, Borneo, Japan and South Africa (Dudgeon *et al.*, 2009); and the whitetip reef shark, *Triaenodon obesus*, in Hawaii, Cocos Island and Costa Rica (Whitney *et al.*, 2012).

The low genetic variation found for *T. megalopterus* may be best explained by processes such as especially low rate of molecular evolution and/or demographic events in the deep history of the species (Ahonen *et al.*, 2009). Stow *et al.* (2006) attributed the low variability and single haplotype in the ragged-tooth populations to historical processes such as sequential founder effects followed by isolation. Although there was no indication of a founder event for *T. megalopterus* (non-significant Tajima *D* and Fu F_S), and the NA and AN populations were panmictic according to mtCR, the contemporary (nDNA) data showed no mixing of these two populations. As mtDNA is maternally inherited, this may be an indication that NA females were migrating into the warmer waters of AN, specifically to the Cunene River mouth, to pup. Bull sharks (*Carcharhinus leucas*), for instance, are well known to make use of estuarine environments as nurseries (Heupel and Simpfendorfer, 2008). Although there is no evidence that *T. megalopterus* would migrate into estuaries or rivers, they may well make use of river mouths as nursery grounds as most of the juveniles caught in this study were found at the Cunene River and Old Woman's River (EC) mouths. This may be the reason that, although AN and NA were panmictic populations, NA presented a lower haplotype and nucleotide diversity compared to the AN population.

The contemporary oceanographic attributes of the Benguela upwelling system (Diester-Haass *et al.*, 1990; Krammer *et al.*, 2006) can act as both a soft or hard barrier to different species depending on their behavioural, morphological, ecological and/or physiological characteristics (Luiz *et al.*, 2012). This has been shown in several studies of various marine species: e.g., leerfish, *Lichia amia* (Henriques *et al.*, 2012); geelbeck croaker, *Atractoscion aequidens* (Henriques *et al.*, 2014a); blacktail seabream, *Diplodus capensis* (Henriques, 2011); silver kob, *Argyrosomus inodorus* (Henriques *et al.*, 2014b); and the zebra sea bream, *Diplodus cervinus* (Gwilliam, in prep). The Benguela does not appear to be a barrier to the dispersal of *T. megalopterus*, as contemporary data show gene flow between WC-NA and WC-AN. Historical data (mtCR), however, did not reveal the same pattern, as gene flow was not recorded between these same populations. As well as the warm and cold water clades mentioned previously, this discordance in the mtCR and nuclear markers may indicate the presence of male-mediated gene flow. This is not uncommon in shark species where the movement and reproductive mixing of females is inhibited by the need to return to coastal nursery areas for parturition (Feldheim *et al.,* 2014). Males, however, do not necessarily display the same level of fidelity, possibly indicative that male-mediated gene flow often occurs over a wider geographic area. Female philopatry and male-mediated gene flow have been recorded in several shark species: e.g. white sharks *Carcharodon carcharias* (Pardini *et al.*, 2001); shortfin mako, *Isurus oxyrinchus* (Schrey and Heist, 2003); blacktip shark *Carcharhinus limbatus* (Keeney *et al.*, 2005); and lemon sharks *Negaprion* spp. (Schultz *et al.*, 2008). Male-mediated gene flow in *T. megalopterus* may also be the reason for low genetic variation seen in the mtCR data for the NA specimens, as the contemporary data, which includes paternal inheritance, showed higher genetic diversity for NA individuals. Unfortunately, male-mediated gene flow in *T. megalopterus* cannot be confirmed by the ORI tagging data as the sex of neither tagged nor recaptured individuals was recorded.

Interbreeding between AN and WC appears to increase over time as mtCR data generally shows an SA vs. AN population structure (haplotype network, phylogenetic tree) whereas nDNA indicates a trans-oceanic structure (F_{ST}, Bayesian cluster analysis). Throughout the results, EC appears to be an isolated population although the mtCR F_{ST} values do show evidence (although non-significant) of admixture with WC. Although IBD was not evident, the data seem to be consistent with a stepping-stone model (Kimura and Weiss, 1964) whereby individuals are exchanged between neighbouring or adjacent populations. Under stepping stone gene flow, pairwise gene flow estimates are high for close populations, (AN-WC and WC-EC), but lower for more distant populations (AN-EC) where these distant

populations are linked via intermediate 'stepping stones' (Hellberg *et al.*, 2002; Hellberg, 2009), in this case the WC population. This makes sense as the WC populations are situated within the WAB Coetzee *et al*., 2008)**,** in the transition zone between the Agulhas and Benguela shelf systems, which means this section of the continental shelf is influenced by both the Agulhas and Benguela Currents (Dufois and Rouault, 2012).

Conclusions

Triakis megalopterus showed low to moderate levels of genetic diversity based on the haplotype and nucleotide frequencies, observed number of alleles, allelic richness and expected heterozygosity. Historical data (Median joining network and Phylogenetic analysis) showed a southern Benguela subsystem and northern Benguela subsystem genetic structure with evidence of an EC separation, whereas contemporary data (FCA, F_{ST} and Structure) showed a distinct trans-oceanic genetic structure. The separation of the EC clade seems to be predominantly due to the historical isolation of populations owing to the exposure of the Agulhas Bank during the Pleistocene and extended periods of a reduction of the reef habitat of *T. megalopterus* during the LGM. Global biogeographic patterns and well known barriers to gene flow (Cape Agulhas and Cape Point) do not seem to play a role in the population structure of *T. megalopterus*. This difference in structuring may also, however, be indicative of limited female dispersal due to philopatry and contemporary male-mediated gene flow amongst WC-NA-AN. Significant Harpending's raggedness index, unimodal mismatch distribution and a large increase from ancestral to actual population size indicates a population expansion in the demographic history of *T. megalopterus*. The hypothesis is that this expansion took place after the last glacial maximum when warmer conditions, higher sea levels and recolonizations were recorded. Cold (NA and Cape Point) and warm (AN and Betty's Bay) water lineages may be possible for *T. megalopterus* and would probably have split during the glacial and interglacial periods and/or the inception of the Benguela Current. Future genetic studies need to include additional sampling from Betty's Bay to Port Elizabeth to help detect if there is in fact a contemporary break in the dispersal ability of *T. megalopterus*. This could determine whether the separation of the EC population is in fact predominantly historical. Population genomics will also be beneficial to improve our understanding of microevolution and assist with a better understanding of the phylogenetic history and demography of this species.

Chapter 4:
Morphology of *Triakis megalopterus* using traditional and geometric techniques

Introduction

Adaptations to ecological niches and the constraints of demanding environments have become evident in sharks (Stevens, 1999a). Changes in several morphological characteristics, such as protrusible upper jaws, advanced sensory structures, specialized dentition and reproductive systems have shown that these animals are not as primitive as once thought (Tricas *et al.*, 2002).

Morphology is the quantitative study of biological form and structure of an organism (Bookstein, 1991; Webster and Sheets, 2010) which may be investigated quantitatively by using morphometrics and meristics (Turan, 2004). The study of morphology in fish is multidisciplinary and includes: the analyses of phylogeny (Morrison *et al.*, 2006); phenotypic plasticity (Gillespie and Fox, 2003); functional morphology (Dean *et al.*, 2006); ontogeny (Debowski *et al.*, 1999; Hard *et al.*, 1999); fish condition (Smith *et al.*, 2005); stock structure identification (Cadrin and Friedland, 1999; Moore and Bronte, 2001; Alfonso, 2004; Zimmerman *et al.*, 2006; Bronte and Moore, 2007; Shao *et al.*, 2007; Bagherian and Rahmani, 2009); estimation of biomass (Hockaday, 2000); and descriptions of new species (Teugels *et al.*, 2001; Welsh and Wood, 2008).

The phenotype of an organism comprises the observable physical characteristics, which are influenced both by its genotype and the environment (see **Chapter 3**; Ayala 1982). Although the genotype of an organism will remain the same throughout its life, a single genotype can exhibit variable phenotypes (Fordyce, 2006), this phenomenon is known as phenotypic plasticity. Phenotypic plasticity (Price *et al.*, 2003; Byers, 2008) can change the biochemistry, physiology, morphology, behaviour, life history (Price *et al*., 2003; Whitman and Agrawal 2009), development and phenology of organisms (West-Eberhard, 1989). Plasticity may be caused by the absence of gene flow between spatially or temporally isolated allopatric populations (genetic change), a reaction norm in which adaptation is caused by changing random developmental noise (Scheiner, 1993) and/or environmental conditions (Ayala, 1982; Bronte and Moore, 2007). Environmental conditions include physical and biological characteristics, such as nutrition/diet (Meyer, 2014), climate or stress (Badyaev, 2005), and/or external influences, such as prey behaviour, mates, competitors and/or predators (Fitzpatrick, 2012). At an organismal level, plasticity can lead to an evolutionary change in the tolerance

and/or avoidance ability or even prompt novel traits or extinction at population level (Badyaev, 2005) and is not only visual, but also includes developmental, physiological responses and/or behavioural flexibility (Fitzpatrick, 2012).

Plasticity can be adaptive or nonadaptive. Adaptive plasticity enhances the fitness of an organism (Hard *et al.*, 1999; Fitzpatrick, 2012) by enabling its establishment and persistence in a new environment, thereby placing populations close enough to a new phenotypic optimum (Ghalambor *et al.*, 2007). Consequently, phenotypic plasticity may be essential for the reproduction, survival and thus fitness of an organism (Robinson and Dukas, 1999). This type of plasticity includes immunity response, anti-predator behaviour, acclimatization, and/or life-history shifts (West-Eberhard, 1989; Schmid-Hempel, 2005). Non-adaptive plasticity occurs when an organism's reaction to its environment steers its phenotypic response further away from the favoured optimum (Ghalambor *et al.*, 2007) and does not enhance its fitness (Fitzpatrick, 2012). This type of plasticity makes organisms more vulnerable to abiotic factors (Miner and Stein, 1996) such as heat shock (Pigliucci *et al.*, 2006) or manipulation by parasites or pathogens (Kenyon and Hunter, 2007; Poinar and Yanoviak, 2008). It is, however, possible that an organism is able to produce the same phenotype, regardless of environmental variation, this is known as canalization (Stearns, 1989).

According to Webster and Sheets (2010), there are two general styles of morphometrics, the older traditional (linear measurements) and "newer" (~30 years) geometric (landmark, outline, truss based) methods. Due to the slow evolutionary rate of elasmobranchs, morphological changes between shark populations may be difficult to detect. Despite this, traditional morphological methods still dominate elasmobranch taxonomic studies regardless of the availability of innovative new methods such geometric morphology.

Linear based approaches to traditional morphology have several disadvantages. The main drawback is that the focus is on longitudinal measurements, while depth and breadth characteristics are limited (Humphries *et al.*, 1981). Correspondingly, there is pronounced repetition of linear data, while depth, breadth and diagonal dimensions are generally absent, thus specimen coverage is uneven (Strauss and Bookstein, 1982). The choice of landmark also plays a vital role as these points have the potential to be either "extremal" or "anatomical" (Moyers and Bookstein, 1979). Anatomical landmarks are true homologous points defined by biological characteristics (Jardine, 1969), e.g., tooth cusp or apex of the first dorsal fin. Extremal landmarks are inferred from geometry rather than biology and defined in terms of minimum or maximum distances, e.g., greatest body depth. This makes extremal landmarks ambiguous as they are not based on definitive structures (Moyers and Bookstein, 1979; Strauss and Bookstein, 1982; Parsons *et al.*, 2003).

Although a difficult feature to quantify, body shape is an important trait in morphometric studies and the omission of shape information may reduce statistical power when attempting to discriminate amongst samples (Parsons *et al.*, 2003). For this reason, methods that include both landmark and outline data were designed; this is known as geometric morphology (Adams *et al.*, 2004). In effect, landmark data are far better at analysing shape than traditional methods (Bagherian and Rahmani, 2009). Because they incorporate the geometry of an organism, geometric methods have made progress in solving limitations of traditional morphometric methods (Rohlf and Marcus, 1993; Adams *et al.*, 2004). Another major advantage of this type of morphology is the ability to instantly visualize the form of specimens (Webster and Sheets, 2010).

The study of geometry using landmark data has become mainstream (Richtsmeier *et al.*, 2002). Geometric morphology is a powerful tool for identifying morphological variation that uniformly incorporates the entire specimen, and since it takes shape into account (Cavalcanti *et al.*, 1999), it provides greater discriminatory power (Cadrin and Friedland, 1999). While an understanding of developmental patterns and the mechanisms driving phenotypic variation is vital for evolutionary research (Stearns, 1989), there is a paucity of whole body traditional and geometric morphometric studies on elasmobranchs. In the past, traditional techniques have been used to study the head and olfactory morphology of Carcharhinids and Sphyrnids (Kajiura, 2001; Kajiura *et al.*, 2005), the brain morphology of pelagic sharks (Lisney and Collin, 2006), caudal fin morphology of Lamniformes (Kim, 2010) and fin morphology as a means of species identification (Marshall, 2011). Only one study used traditional morphology on whole specimens to determine interspecific differences between blacktip, *Carcharhinus limbatus* (Valenciennes, 1841) and spinner sharks *C. brevipinna* (Müller and Henle, 1839; Siqueiros-Beltrones, 1990). Although digitizers have been used on elasmobranchs, studies to date do not incorporate whole specimens. These studies include, but are not limited to, the pectoral fins of white spotted bamboo sharks, *Chiloscyllium plagiosum* (Wilga and Lauder, 2001), fossil teeth of the great white, *Carcharodon carcharias* (Nyberg *et al.*, 2006), mako sharks, *Isurus* spp. (Whitenack and Gottfried, 2010), megalodon, *Carcharocles megalodon* (Pimiento et al., 2010) and the extinct weasel sharks, *Hemipristis serra* (Chandler *et al.*, 2006) and caudal fin shape in *Squalus acanthias* (Reiss and Bonnan, 2010). Naylor and Marcus (1994) also used video digitizing to classify 22 species of *Carcharhinus* from around the world.

While there is a lack of traditional morphometrics, there is a complete absence of geometric morphology on whole elasmobranch specimens. The deficiency of geometric data on whole specimens is justifiable as the size and shape of most elasmobranchs make it difficult, if not impossible, to accurately measure diagonal lines across the body. Accordingly, morphological

studies using digital photography and digitizers are also not typically feasible as most sharks, due to their cartilaginous skeletons (Tricas *et al.*, 2002), lose their shape on land. Thus far, there seems to be only one way to get around this issue. One can use flow tanks and measurements can be taken from video footage as did Wilga and Lauder (2000) using small leopard sharks. This, however, is only feasible for larger specimens that are housed in large (normally public) aquaria and alternative methods for developing geometric data on elasmobranchs are critical.

Not only does external morphology play an important role in morphometric studies, teeth are also intricately designed through evolution (Frazzetta, 1988). Chondrichthyan dentition has various classifications based on the form and function for prey capture and processing, e.g. dentition suited for clutching, tearing, cutting, crushing or grinding (Cappetta, 1987). Although variation in the teeth of same species of elasmobranchs is not uncommon (Shimada, 2002), tooth morphology may still differ between life stages (Reif, 1976) and/or sexes (Kajiura and Tricas, 1996). Previous studies seem to concentrate on functional tooth morphology (e.g. Ramsay and Wilga, 2007), while few look at how tooth morphology varies according to sex, ontogeny or prey composition.

The magnitude to which environmental factors affect morphometric variation is not well understood (Hard *et al.*, 1999), and research on shark morphology is scarce. Studies on the plasticity of shark morphology are virtually non-existent and plasticity research in elasmobranchs has focused on trophic niche (*Rhizoprionodon terraenovae*; Drymon *et al.*, 2011), diel vertical migrations (*Lamna nasus;* Pade *et al.*, 2009), size at maturity (*Squalus mitsukirii;* Lucifora *et al.,* 1999) and behaviour (*Carcharhinus limbatus* and *Ginglymostoma cirratum*; Gardiner *et al,.* 2014).

The aims of this chapter are: 1) to assess the phenotypic variation of *T. megalopterus* between populations using traditional morphometric methods on full body and tooth morphology; 2) to develop a protocol that allows the use of landmark, outline and truss data to be a viable method for future morphometric studies of elasmobranchs; 3) to test the validity of the new geometric morphology protocol for detecting interspecific and interpopulation differences in *T. megalopterus* and 4) to describe the phenotypic plasticity of *T. megalopterus* and to investigate whether genetic variation (**Chapter 3**) is reflected in morphological traits.

Material and methods

A total of 120 *T. megalopterus* were measured, of which 43 were from Angola (29 F, 14 M), 33 from the Western Cape (24 F, 9 M) and 44 from the Eastern Cape (28 F, 16 M). Overall, the sex ratio was female biased (81 F, 39 M).

It was not always possible to work on fresh specimens, due to logistical constraints of remote study sites. Consequently, two methods of sample preservation (frozen and preserved) were used. In order to freeze specimens, freshly caught *T. megalopterus* were carefully placed into containers and positioned in a "natural" posture. The containers were then placed into a blast freezer until the morphometric analysis. For chemical preservation, freshly caught specimens were injected into the abdominal cavity with 10% formalin before the whole specimen was immersed into a 10% formalin solution. Specimens were left in the 10% formalin solution for a minimum of a month. Subsequently, the specimens were removed from the formalin and transferred into 10% and 50% ethanol solutions for three days each, respectively. If either frozen or preserved specimens appeared distorted or damaged, they were excluded from the analysis.

Traditional morphology

Measurements followed the methods from *Sharks of the world, FAO species catalogue* (**Figure 4.1**; Compagno, 1984a). In total, 98 linear measurements were taken from the left lateral aspect of each specimen (**Table 4.1**). Measurements <300 mm were made using digital callipers to the nearest 0.01 mm. Measurements >300 mm were made with a measuring tape to the nearest 0.1 mm.

Due to sampling limitations in Angola, P1R was not measured. Since accuracy could not be guaranteed for SOD, PDI, DPO, PDO, DAO and DAI, these measurements were removed from the analysis. An extra two measurements were included over and above those described in the FAO guide, MOL2 (width of the medial-bottom jaw) and PRC2 (tip of the snout to the end of the second dorsal base). In total, 92 traditional linear morphometric measurements were used to assess morphometric differences between populations of *T. megalopterus*.

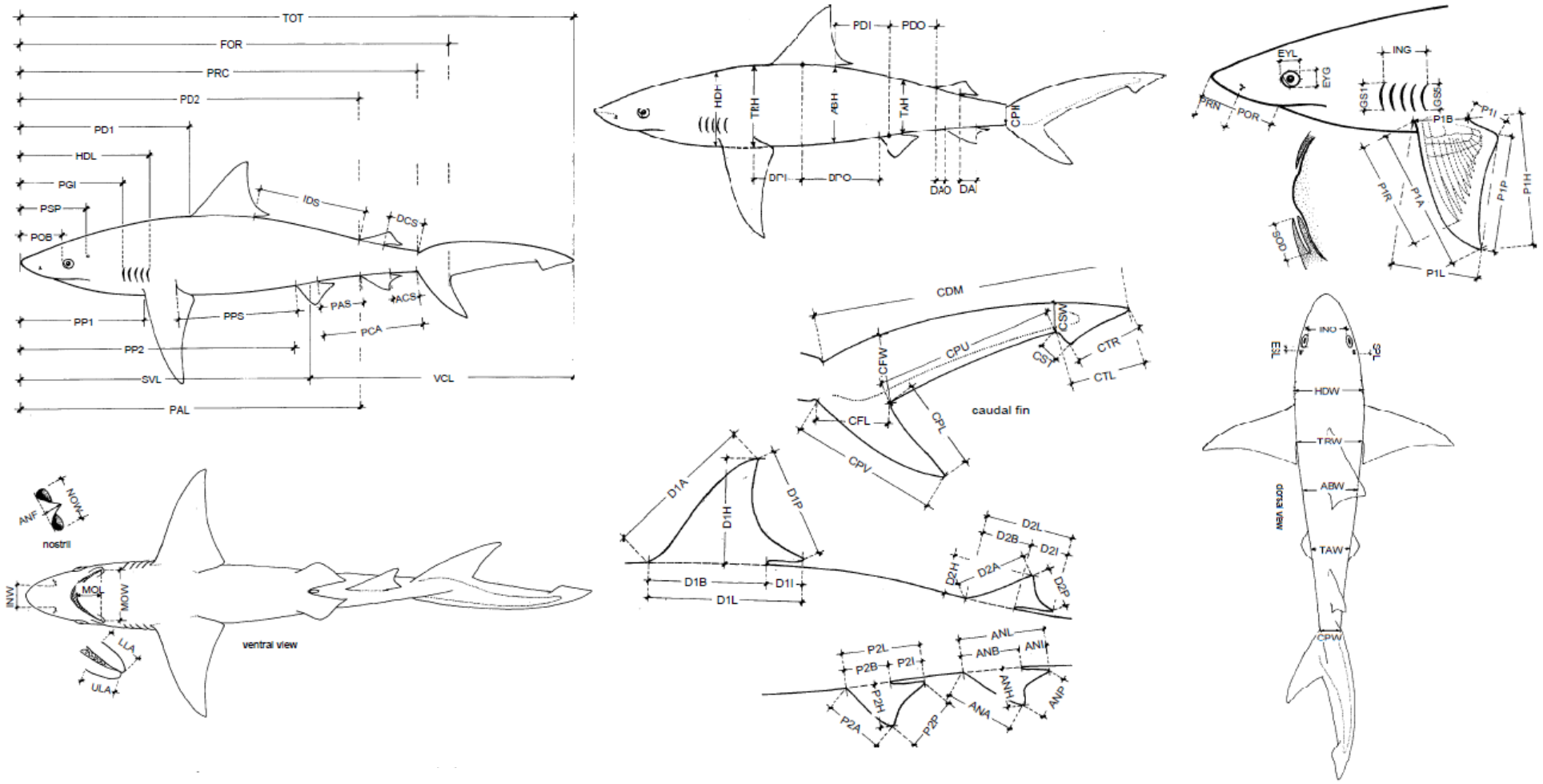

Figure 4.1: Morphological measurements taken from each whole specimen as described in the sharks of the world, FAO species catalogue (Compagno, 1984a).

Table 4.1: Variable names for abbreviation of morphology measurements as per the sharks of the world, FAO species catalogue (Compagno, 1984a)

Abbreviation	Variable name	Abbreviation	Variable name	Abbreviation	Variable name
ABH	Abdominal height	DAO	Second dorsal origin-anal origin	P2B	Pelvic base
ABW	Abdomen width	DCS	Dorsal-caudal space	P2H	Pelvic height
ACS	Anal-caudal space	DIB	First dorsal base	P2I	Pelvic inner margin length
ANA	Anal anterior margin	DIP	First dorsal posterior margin	P2L	Pelvic length
ANB	Anal base	DPO	First dorsal midpoint-pelvic origin	P2P	Pelvic posterior margin length
ANF	Anterior nasal flap length	ESL	Eye spiracle space	PAL	Preanal length
ANH	Anal height	EYH	Eye height	PAS	Pelvic-anal space
ANI	Anal inner margin	EYL	Eye length	PCA	Pelvic-caudal space
ANL	Anal length	FOR	Fork length	PD1	Pre-first dorsal length
ANP	Anal posterior margin	GS1	First gill slit height	PD2	Pre-second dorsal length
CDM	Dorsal caudal margin	GS2	Second gill slit height	PDI	Pelvic midpoint-first dorsal origin
CFL	Caudal fork length	GS3	Third gill slit height	PDO	Pelvic midpoint-second dorsal origin
CFW	Caudal fork width	GS4	Fourth gill slit height	PG1	Prebranchial length
CPH	Caudal peduncle height	GS5	Fifth gill slit height	POB	Preorbital length
CPL	Lower postventral caudal margin	HDH	Head height	POR	Preoral length
CPU	Upper postventral caudal margin	HDL	Head length	PP1	Prepectoral length
CPV	Preventral caudal margin	HDW	Head width	PP2	Prepelvic length
CPW	Caudal peduncle width	IDS	Interdorsal space	PPS	Prespiracular length
CST	Subterminal caudal margin one	ING	Intergill length	PRC1	Precaudal length
CSW	Subterminal caudal margin two	INO	Interorbital space	PRC2	Precaudal length two
CTL	Terminal caudal lobe	INW	Internarial width	PRN	Prenarial length
CTR	Terminal caudal margin	LLA	Lower labial furrow length	PSP	Prespiricular length
D1A	First dorsal anterior margin	MOL1	Mouth length one	SOD	Subocular pocket depth
D1H	First dorsal height	MOL2	Mouth length two	SPL	Spiracle length
D1I	First dorsal inner margin	MOW	Mouth width	SVL	Snout-vent length
D1L	First dorsal length	NOW	Nostril width	TAH	Tail height
D2A	Second dorsal anterior margin	P1A	Pectoral anterior margin	TAW	Tail width
D2B	Second dorsal base	P1B	Pectoral base	TOT	Total length
D2H	Second dorsal height	P1H	Pectoral height	TRH	Trunk height
D2I	Second dorsal inner margin	P1I	Pectoral inner margin	TRW	Trunk width
D2L	Second dorsal length	P1P	Pectoral posterior margin	ULA	Upper labial furrow length
D2P	Second dorsal posterior margin	P1R	Pectoral radial length	VCL	Ventral caudal length
DAI	Second dorsal origin-anal origin	P2A	Pelvic anterior margin		

Development of truss morphology method

The digital outline of each specimen was recreated from the traditional morphology measurements using AutoCAD 2011 design and drafting software. Sixteen measurements were used to re-create the left hand side, dorsal outline of specimens (**Figure 4.2 a**). Since right and left sides were assumed to be equal and the right side was mirrored from the left to complete the dorsal outline. A total of 13 landmarks (**Figure 4.2 b**) were assigned to the dorsal outline in order to create the truss network (from above).

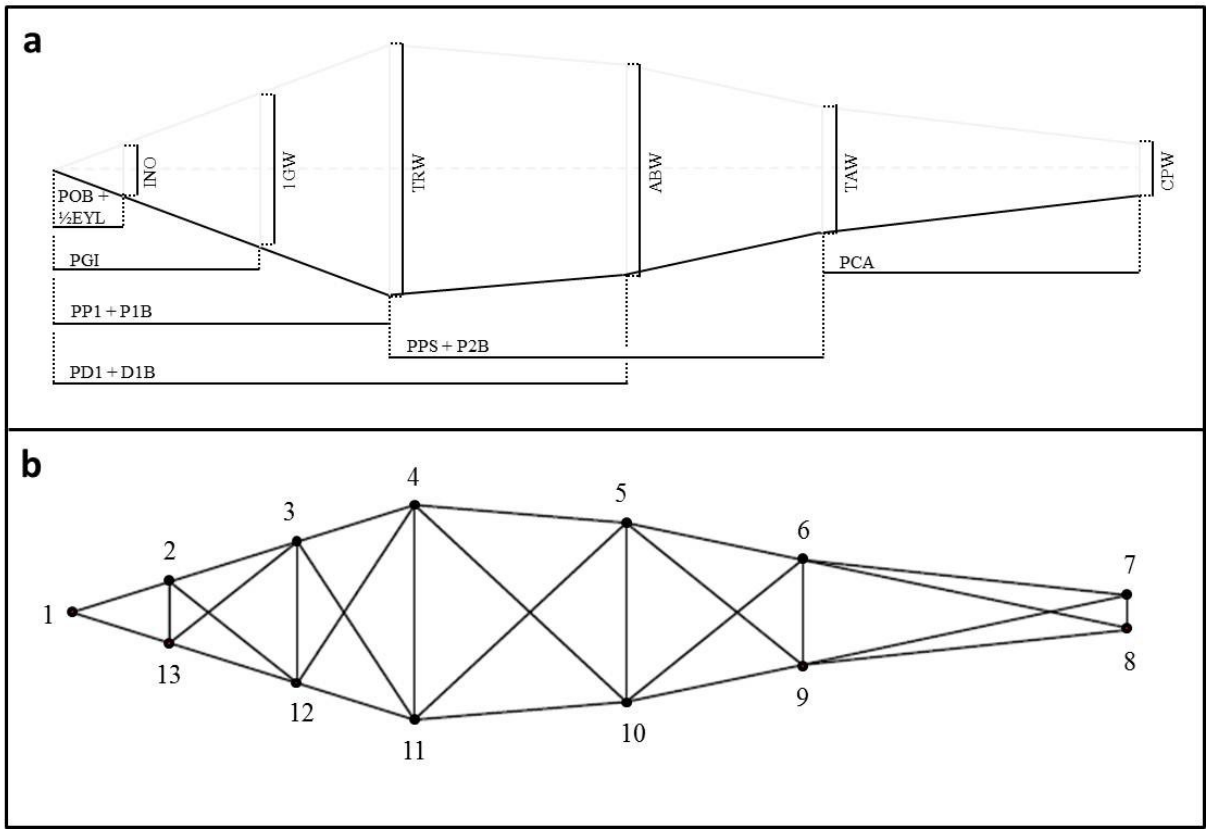

Figure 4.2: Truss development illustrating (a) the measurements used to recreate the left dorsal aspect of the truss outline, (b) complete truss diagram displaying the 13 landmarks; *PP1 = Prepectoral length, P1B = Pectoral base, POB = Preorbital length, EYL = Eye length, PPS = Pectoral-pelvic space, P2P = Pelvic posterior margin length, PCA = Pelvic-caudal space, INO = Interorbital space, IGW = First gill (head) width, TRW = Trunk width, ABW = Abdomen width, TAW = Tail width, CPW = Caudal peduncle width, PGI = Prebranchial length, PD1 = Pre-first dorsal length, D1B = First dorsal base*

Once the truss was drawn in AutoCAD, each line could be measured, using the software, to the nearest 0.01 mm. Seven length (L1–L7; **Figure 4.3 a**), five diagonal (D1–D5; **Figure 4.3 b**) and six vertical (V1–V6; **Figure 4.3 c**) measurements were taken per specimen. AutoCAD was also used to measure four angles (A1–A4; **Figure 4.3 d**).

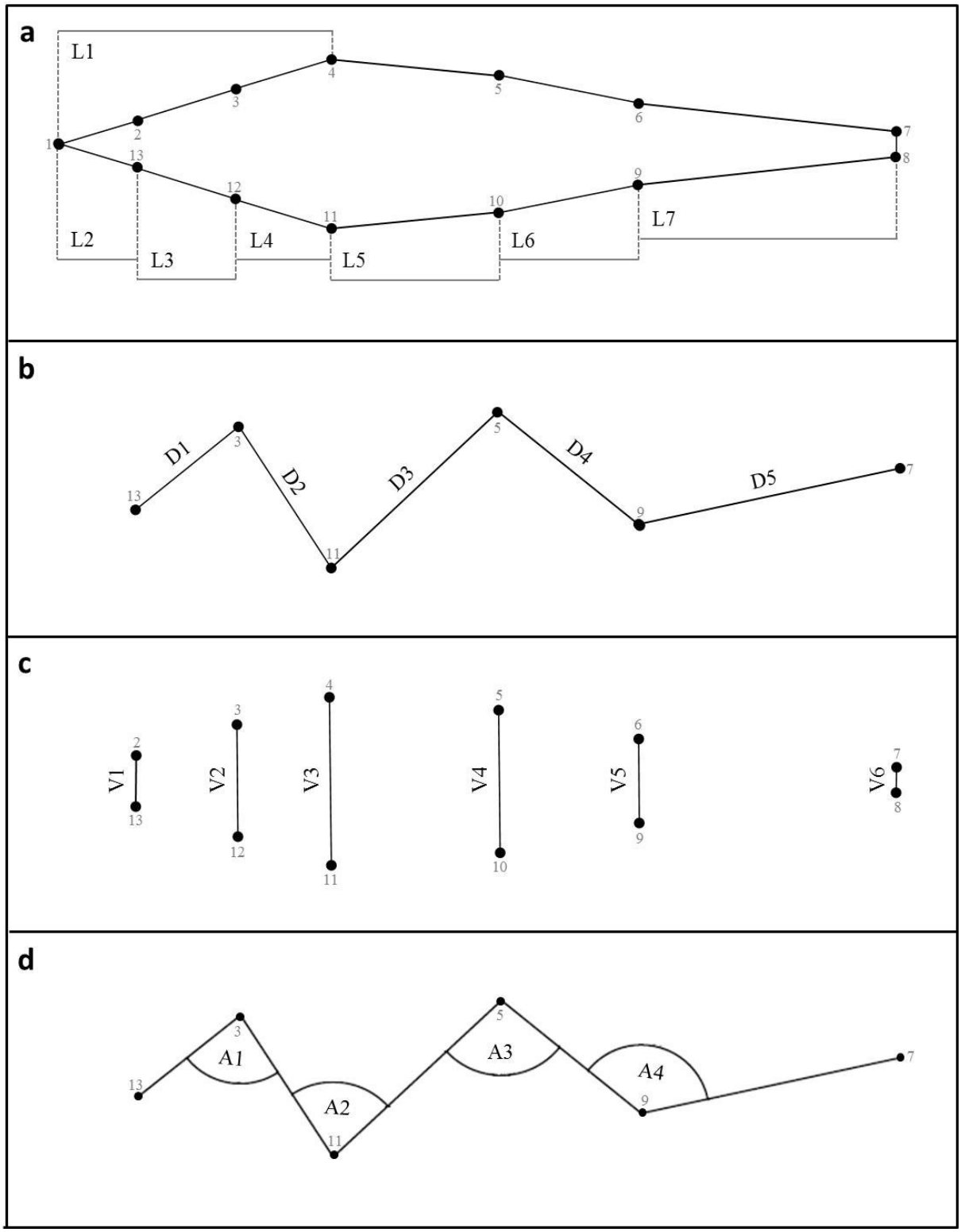

Figure 4.3: Truss development illustrating the (a) seven lengths (L1–L7), (b) five diagonals (D1–D5), (c) six vertical (V1–V6) and (d) four angles (A1–A4) measured by the truss system

The truss protocol was first trialled for its efficacy in discriminating between different species. The multispecies analysis compared 30 specimens evenly distributed over three species, *T. megalopterus* (spotted gully; SG), *Mustelus mustelus* (smooth hound; SH) and *Haploblepharus edwardsii* (puffadder shyshark; PA). The *M. mustelus* and *H. edwardsii*

specimens were all freshly measured. The *M. mustelus* specimens were collected from AN while the *H. edwardsii* were collected from Betty's Bay. Truss networks were then developed for the 109 *T. megalopterus* specimens to examine the interpopulation morphological variation for specimens from AN, EC and WC.

Tooth morphology

Variances in tooth morphology between the different regional populations (AN, WC and EC) were examined. The jaws were placed in hot water (~60–90 ^{o}C) for approximately five minutes or until the connective tissue was soft. The teeth were then extracted using forceps. Tooth form varied from the lateral-superior (**Figure 4.4 a**), medial-superior (**Figure 4.4 b**), lateral-inferior (**Figure 4.4 c**) and medial-inferior (**Figure 4.4 d**) jaw quadrants. Thus, three sequential teeth were removed from each of the four quadrants. To minimize bias due to polyphyodonty, teeth were removed from the third lingual row to avoid underdeveloped or worn/damaged teeth. This area was chosen as it was evident that the teeth were fully developed, yet still protected by a thin covering of connective tissue. From the three sequential teeth, the most pristine specimens were individually positioned onto microscope slides. Using a Leica DMC2900 microscope camera, photos of 80 sets of teeth were taken overall using a dissecting microscope (magnification 40 x). Photos were imported into SigmaScan Pro 5 software where landmarks to measure the maximum mesial width, maximum height, maximum distal width, and crown tip were identified and marked. The landmarks were connected with straight lines to yield a box truss of six length measurements, area and four angles (**Figure 4.4 e**). To add quantitative measurements of shape, two of SigmaScan's built in parameters, shape factor and compactness, were also incorporated into the analysis.

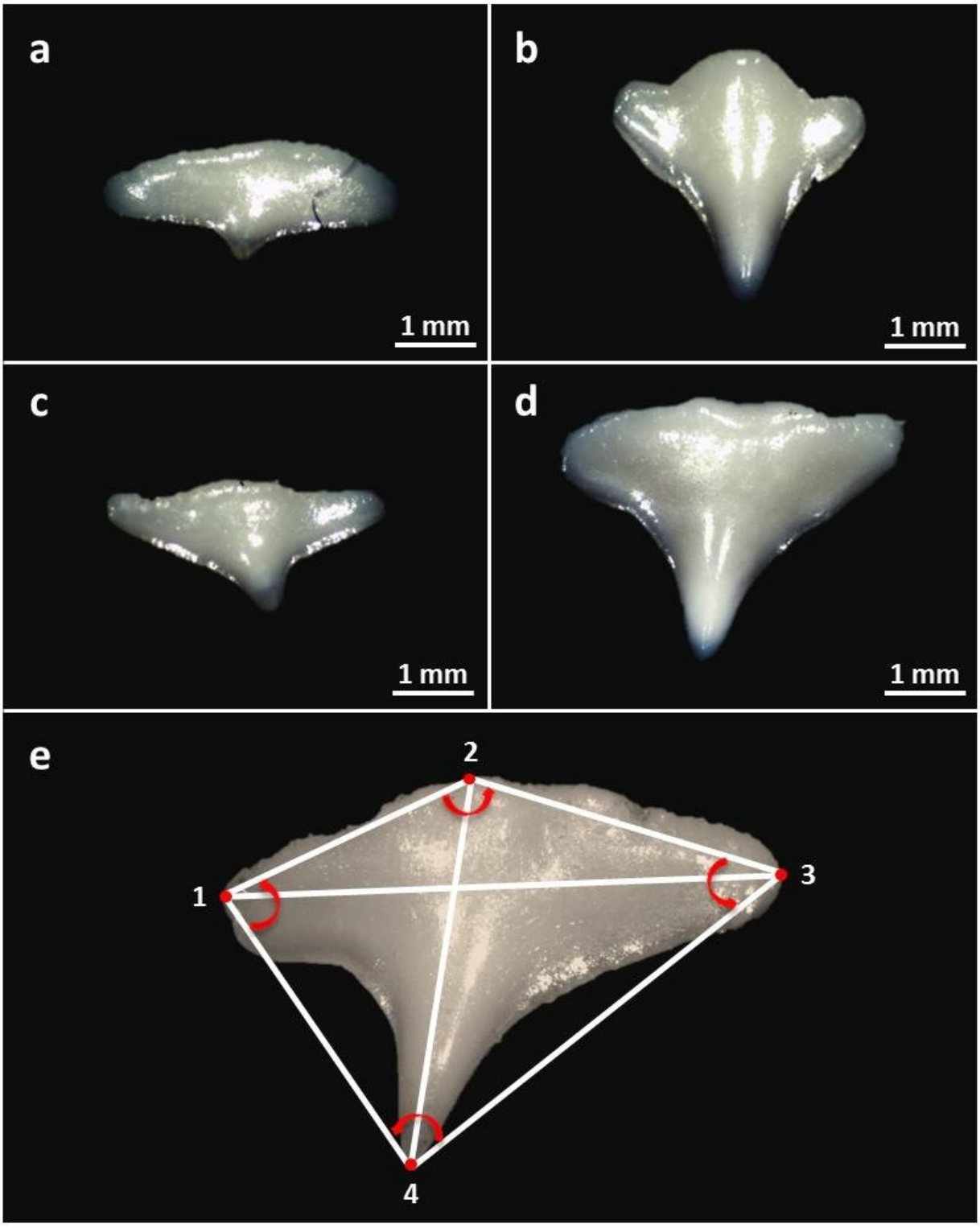

Figure 4.4: Lingual view of *T. megalopterus* teeth depicting (a) lateral superior, (b) medial-superior, (c) lateral-inferior and (d) medial-inferior and (e) the four landmarks and associated box truss used to infer morphological differences amongst size and locations; *1 = maximum mesial width, 2 = maximum height, 3 = maximum distal width, 4 = crown tip.*

Outlier detection

Extreme outliers (more than three times the inter-quartile range to the left or right of the first and third quartiles) were identified using box plots. Outliers were checked for data entry errors and corrected where possible. If an entire specimen was identified as an outlier, it was removed from the analysis.

Frozen versus preserved

Paired sample t-tests were run using IBM SPSS Statistics 20 (IBM Corporation, 2011) on ten randomly sampled specimens of fresh-frozen and fresh-preserved specimens subsets to test for significant (p<0.05) differences between the different preservation methods.

Transformation of absolute measurements

Since allometric shifts in growth are common amongst elasmobranch fishes (e.g. Lowry, 2005; Frisk and Miller, 2006; Lowry *et al.*, 2007; Reiss and Bonnan, 2010; Irschick and Hammerschlag, 2015) absolute morphological measurements were transformed to size-independent measurements before the final analysis. Although a popular choice when it comes to size correction in data, the use of ratios has been known to result in false approximations of shape differences (Brookstein *et al.*, 1985), to be indifferent to allometric variances (Parsons *et al.*, 2003) and to reduce statistical power (Atchley *et al.*, 1976). For this reason, all morphometric characters were adjusted to an overall mean total length of $\overline{TOT}$ = 1205 cm according to the following equation (Simon *et al.*, 2010):

$$\boldsymbol{Y'_{ij} = log\, Y_{ij} - \, b_j\, (log\, TOT_i - log\, \overline{TOT})}$$

where:

Y'_{ij} = is the adjusted value of character j for individual i

Y_{ij} = is the original value

b_j = is the pooled regression coefficient of log$Y$ on logTOT

TOT_i = is the total length of individual i

TOT = overall mean total length

The efficacy of size transformation was determined from the coefficient of determination (R^2) values of the logY' vs. logTOT regression.

General data analyses

Principal component analysis (PCA) aims to condense a large set of variables into a smaller set of "artificial" variables called principal components. By doing this, PCA reflects the covariance/correlation structure of the data by looking at the relationships amongst the morphometric measurements rather than extracting individual morphometric differences (Brookstein *et al.*, 1985). In this study, the aim was to assess the differences observed, between populations, for each variable. The best way to do this was to compare individual variable means amongst populations. Therefore, the analysis of variance (ANOVA) was selected to analyse the data in this chapter.

Variations amongst populations were tested using ANOVA after a Levene's median test confirmed equal/unequal variance. If variances were equal ($p > 0.01$), Fisher's least significant difference (LSD) procedure was used to identify homogenous groups. If variances were not equal ($p<0.01$), data were analysed using the Games-Howell posthoc test. All statistical analyses were done using Statistica v10 (StatSoft, Inc. 2011) and IBM SPSS Statistics v20 (IBM Corporation, 2011). Variables were considered to be significantly different if $p<0.02$ as these results showed no overlap at a 95% confidence interval making these variables completely distinguishable amongst the population comparisons. Because all morphometric measurements were adjusted to an overall mean total length, the effect of size differences between sexes should have been removed. Therefore, combined sex data were used for the initial analyses. However, when highly significant ($p<0.02$) differences were found between populations, separate ANOVAs were run for sex, using only the highly significantly different variables, to ensure the differences were not sex based. If significant differences were found between sexes within a population comparison, paired sample t-tests were run to determine exactly where the significance lay.

In order to gain some insight into whether intrapopulation differences were phenotypic responses to environmental differences or genotypic changes, the morphology data (traditional, truss and tooth) were analysed using a separate ANOVA where the six haplotypes identified in the mtCR analyses (TMH1–TMH6; see **Chapter 3**) were selected as the grouping variable (instead of site). Should significant differences be congruent in the morphology analyses grouped by site (related to phenotype) and by haplotype (related to genotype), this may indicate the presence of adaptive divergence.

Tooth shape

To assess tooth development, SHAPE 1.3 (Iwata and Ukai, 2002) was used to evaluate and illustrate the growth of the teeth of *T. megalopterus* from EC as this population had the best distribution of individuals in each size class. The SHAPE software is made up of four different applications. ChainCoder converts the uploaded images into black and white before extracting and delineating the information into chain code (Freeman, 1974). Chc2Nef uses the chain code data to calculate the elliptic Fourier descriptors (EFD; Kuhl and Giardina, 1982) from which a principle component analysis (PCA) is performed using PrinComp. Thereafter, the shape variations of the principle components are illustrated (Furuta *et al.*, 1995).

Results and interpretation

Outlier detection

Although extreme outliers were identified in the data, all were data entry errors which were corrected. No specimens were discarded due to outliers.

Frozen versus preserved

No significant differences were evident between fresh-frozen ($p = 0.067$) and fresh-preserved ($p = 0.083$) specimens. Frozen specimens appeared to wrinkle and soften when defrosted, thus losing a small amount rigidity whereas preserved specimens kept their shape well. Thus, preserving specimens appears to be the better method for sample preservation of whole elasmobranchs.

Transformation of absolute measurements

Adjusting absolute measurements to size-independent shape characters was successful in eliminating any variation resulting from allometric growth. Correlations determined by the coefficient of determination (R^2) values of the logY' vs. logTOT regression removed significance in the traditional ($p>0.311$), tooth ($p>0.994$), interspecific truss ($p>0.559$) and *T. megalopterus* truss ($p>0.445$) data.

Traditional morphology

Of the 92 variables analysed, 36 showed highly significant ($p<0.02$) differences between the populations (**Table 4.2**). Of the 36 variables that showed highly significant differences, only one of these variables, MOW, was significantly ($p = 0.001$) different between males and females. A paired-samples t-test conducted to compare MOW for AN-EC (the population comparison rendering significant differences amongst sample sites) showed that this variance

was attributed to a significant difference between females from AN (M = -3.000, SD = 6.367) and EC (M = 4.556, SD = 6.072); t(26) = -4.347, p<0.001. This result shows that females from EC have wider mouths compared to females from AN.

Eighty-six percent of the 36 highly significant differences were observed between AN and EC, 47.2% between AN and WC and 12.1% between EC and WC.

Compared to South African (WC and EC) populations, AN specimens had significantly shorter pectoral (P1A, P1P), first dorsal (D1H, D1P) and second dorsal (D2H, D2P) fins with a wider pelvic fin base (P2B). AN individuals also had significantly shorter fifth gill slit (GS5), smaller eyes (EYL, EYH) and spiracle (PSP), while the tail region was thicker (TAW, CPW) than that of WC and EC specimens.

Compared to EC, AN specimens had significantly shorter pectoral fins (P1H), second dorsal and anal inner margins (D2I, ANI) and pelvic fin lengths (P2L) with a wider second dorsal fin base (D2B) and increased abdominal and tail (ABH, TAH) heights. Individuals from AN also had shorter gill slits (G1–G5), smaller mouth widths (MOW) and anterior nasal flap lengths (ANF). The caudal fins of AN specimens had larger caudal fork width (CFW), lower postventral caudal margin (CPL) and terminal caudal margin (CTR).

Compared to EC, WC had smaller preventral caudal margin (CPV) and pectoral inner margin (P1I) and compared to AN, WC had a shorter second Precaudal length (PRC2) and larger Intergill length (ING). There were significant differences that all populations showed in the upper labial furrow length (ULA), where the smallest was in AN (-1.596) and largest in EC (1.550).

Of the 36 variables that showed highly significant differences in traditional morphology amongst sample sites, 13 of these variables showed significant (p<0.05) differences, between haplotypes (highlighted in grey in **Table 4.2**). All (100.0%) of the significantly different variables were present between AN and EC, 6.5% between AN and WC and only 1.1% between EC and WC. These variables primarily included characters associated with the gills, the posterior fins and eyes. Consequently, there is evidence that the morphological differences for *T. megalopterus* are not just phenotypic responses to the environment but may also be associated with different genetic types. Unfortunately, out of the 120 specimens used in the morphology analysis, only 58 of these specimens had the associated haplotype data. The relationship between the genotype and phenotype may have been more evident had all of the specimens in the morphological study been analysed for the mtCR.

Table 4.2: Results of ANOVAs for traditional morphology data of *T. megalopterus* from Angola (AN), Western Cape (WC) and Eastern Cape (EC); *F = Levene's F statistic, p = Levene's significance,* ***** *= significance at the 1% nominal level,* ****** *= significance at the 2% nominal level, grey highlight depicts where significant differences were evident between haplotypes, see Table 4.1 for list of abbreviations*

		Levene's Test for Equality of Variance		Least Significant Difference/Games-Howell			Population Means		
		F	***p***	**AN-EC**	**AN-WC**	**EC-WC**	**AN**	**WC**	**EC**
Body Lengths	HDL	3.068	0.050	0.659	0.124	0.254	1.818	-3.072	0.527
	SVL	7.453	**0.001***	0.097	0.032	0.357	-9.972	10.298	2.022
	PPS	15.882	**0.000***	0.028	0.046	0.648	-10.334	9.251	3.160
	PAS	5.076	**0.008***	0.083	0.556	0.922	4.247	-1.271	-3.198
	ACS	0.316	0.730	0.401	0.530	0.878	0.613	-0.226	-0.429
	PCA	5.121	**0.007***	0.020	0.913	0.246	4.555	2.265	-6.150
	VCL	5.540	**0.005***	0.412	0.966	0.427	4.126	1.998	-5.531
	IDS	4.225	0.017	0.246	0.446	0.751	1.865	-0.487	-1.457
	DCS	4.915	**0.009***	0.468	0.936	0.852	0.732	0.106	-0.795
Pectoral Fin	PP1	0.747	0.476	0.761	0.535	0.365	0.234	-1.916	1.208
	P1A	0.647	0.526	**0.000***	**0.000***	0.452	-7.294	3.008	4.873
	P1B	2.384	0.097	0.047	0.164	0.643	1.752	-0.546	-1.303
	P1I	3.036	0.052	0.043	0.216	**0.002***	-0.693	-3.138	3.030
	P1L	3.989	0.021	0.796	0.091	0.053	1.060	-3.684	1.727
	P1P	4.256	0.016	**0.000***	**0.000***	0.592	-8.558	5.684	4.101
	P1H	4.169	0.018	**0.000***	0.025	0.273	-5.359	1.189	4.346
Caudal Fin	PRC1	4.006	0.021	0.752	0.041	0.078	-5.209	10.717	-2.947
	PRC2	3.003	0.053	0.225	**0.001***	0.024	-11.488	17.492	-1.892
	CDM	4.773	0.010	0.284	0.480	0.773	-1.372	0.364	1.067
	CPV	3.282	0.041	0.012	0.402	**0.002***	-1.093	-2.538	2.972
	CPU	3.124	0.048	0.180	0.785	0.329	-0.863	-0.421	1.160
	CPL	7.445	**0.001***	**0.003***	0.029	0.917	-2.411	1.660	1.111
	CFW	3.248	0.042	**0.002***	0.017	0.639	-1.956	0.790	1.318
	CFL	3.395	0.037	0.049	0.518	0.234	-1.442	-0.346	1.668
	CST	4.085	0.019	0.245	0.112	0.008	0.043	-1.555	1.124
	CSW	6.470	**0.002***	0.995	0.773	0.783	0.196	-0.452	0.147
	CTR	4.913	**0.009***	**0.010***	0.591	0.466	-2.071	-0.091	2.093
	CTL	2.145	0.122	0.037	0.595	0.155	-1.293	-0.496	1.636
1st Dorsal Fin	PD1	0.652	0.523	0.417	0.047	0.209	-5.321	7.575	-0.481
	IDS	4.225	0.017	0.246	0.446	0.751	1.865	-0.487	-1.457
	D1L	7.519	**0.001***	0.403	0.723	0.998	-1.962	0.984	1.180
	D1A	6.679	**0.002***	0.114	0.465	0.949	-2.549	0.969	1.764
	DIB	6.013	**0.003***	0.056	0.981	0.341	1.996	1.387	-2.991
	D1H	2.006	0.139	**0.000***	**0.000***	0.185	-4.725	1.006	3.864
	D1I	1.038	0.357	**0.003***	0.736	**0.002***	-1.559	-2.117	3.111
	DIP	0.232	0.794	**0.000***	**0.000***	0.815	-8.384	5.151	4.330

		Levene's Test for Equality of Variance		Least Significant Difference/Games-Howell			Population Means		
		F	***p***	**AN-EC**	**AN-WC**	**EC-WC**	**AN**	**WC**	**EC**
2nd Dorsal Fin	PD2	0.628	0.536	0.882	0.317	0.253	-1.291	4.449	-2.075
	DCS	4.915	**0.009***	0.468	0.936	0.852	0.732	0.106	-0.795
	D2L	2.717	0.070	0.729	0.017	0.036	-1.885	3.941	-1.113
	D2A	2.976	0.055	0.028	0.025	0.833	-3.512	2.266	1.732
	D2B	2.390	0.096	**0.001***	0.374	0.025	2.983	1.083	-3.727
	D2H	4.020	0.020	**0.000***	**0.003***	0.137	-5.022	1.079	4.099
	D2I	5.632	**0.005***	**0.000***	0.390	0.241	-2.129	-0.196	2.228
	D2P	1.825	0.166	**0.002***	**0.000***	0.016	-7.143	7.828	1.109
Pelvic Fin	PP2	8.139	**0.000***	0.169	0.112	0.614	-8.104	7.992	1.926
	P2L	10.249	**0.000***	**0.008***	0.126	0.980	-3.407	1.670	2.077
	P2A	1.382	0.255	0.022	0.120	0.558	-2.931	0.833	2.240
	P2B	7.710	**0.001***	**0.000***	**0.000***	0.341	6.212	-5.197	-2.174
	P2H	1.521	0.223	0.159	0.617	0.416	-1.681	-0.321	1.884
	P2I	5.255	**0.007***	0.039	0.421	0.840	-3.231	0.936	2.456
	P2P	2.869	0.061	0.015	0.198	0.325	-2.877	0.249	2.625
Anal Fin	PAL	4.236	0.017	0.354	0.226	0.722	-7.936	6.904	2.578
	ANL	4.187	0.018	0.069	0.094	0.993	-2.438	1.350	1.370
	ANA	1.983	0.142	0.019	0.241	0.301	-2.523	0.096	2.394
	ANB	2.707	0.071	0.021	0.022	0.885	2.679	-1.650	-1.381
	ANH	1.786	0.172	0.091	0.784	0.065	-1.132	-1.751	2.420
	ANI	9.091	**0.000***	**0.010***	0.528	0.734	-1.326	0.138	1.192
	ANP	2.694	0.072	0.103	0.478	0.416	-1.427	-0.081	1.455
Gills	PGI	2.953	0.056	0.040	0.068	0.932	-2.548	1.321	1.499
	ING	1.526	0.222	0.025	**0.000***	0.010	4.325	-5.523	-0.084
	GS1	3.740	0.027	**0.001***	0.103	0.108	-1.782	-0.002	1.743
	GS2	2.404	0.095	**0.001***	0.017	0.488	-2.188	0.744	1.580
	GS3	2.934	0.057	**0.001***	0.007	0.584	-2.334	0.932	1.582
	GS4	3.117	0.048	**0.001***	0.008	0.540	-2.270	0.862	1.572
	GS5	5.069	**0.008***	**0.000***	**0.012****	0.854	-2.656	1.134	1.745
Mouth	POR	2.453	0.090	0.034	0.086	0.803	-1.129	0.497	0.731
	MOL1	2.700	0.071	0.326	0.303	0.053	0.037	0.798	-0.635
	MOL2	3.979	0.021	0.739	0.061	0.115	-0.598	1.187	-0.306
	MOW	2.673	0.073	**0.001***	0.050	0.246	-3.013	0.506	2.565
	ULA	2.551	0.082	**0.000***	**0.005***	**0.007***	-1.596	0.013	1.550
	LLA	4.486	0.013	0.013	0.785	0.039	-0.679	-0.481	1.024
Eyes, Spiracles & Nares	PSP	0.160	0.852	**0.000***	**0.000***	0.643	-2.608	1.740	1.244
	POB	1.306	0.275	0.408	0.270	0.734	-0.626	0.557	0.194
	PRN	6.128	**0.003***	0.138	0.797	0.672	-0.744	-0.049	0.764
	EYL	4.065	0.020	**0.000***	**0.004***	0.020	-1.686	0.110	1.566
	EYH	0.888	0.414	**0.000***	**0.000***	0.553	-1.939	1.307	0.915
	NOW	1.821	0.166	0.166	0.492	0.049	-0.128	-0.441	0.456
	INW	1.941	0.148	0.563	0.861	0.475	-0.086	-0.187	0.224
	ANF	2.764	0.067	**0.001***	0.014	0.509	-0.741	0.263	0.527
	INO	4.062	0.020	0.037	**0.001***	0.145	2.111	-2.241	-0.383

		Levene's Test for Equality of Variance		Least Significant Difference/Games-Howell			Population Means		
		F	***p***	**AN-EC**	**AN-WC**	**EC-WC**	**AN**	**WC**	**EC**
	SPL	2.977	0.055	0.144	0.340	0.686	-0.401	0.103	0.315
	ESL	0.891	0.413	0.483	0.618	0.249	0.052	0.312	-0.285
Widths	HDW	10.681	**0.000***	0.297	0.945	0.856	-2.110	-0.386	2.351
	TRW	6.193	**0.003***	0.194	0.809	0.878	-2.580	0.261	2.326
	ABW	9.479	**0.000***	0.988	0.926	0.831	-0.472	2.210	-1.196
	TAW	2.657	0.074	**0.001***	**0.000***	0.433	4.630	-3.472	-1.921
	CPW	0.320	0.727	**0.000***	**0.003***	0.054	1.939	-0.284	-1.682
Heights	HDH	9.174	**0.000***	0.067	0.147	0.717	5.243	-5.173	-1.244
	TRH	6.300	**0.003***	0.022	0.072	0.887	7.062	-5.234	-2.976
	ABH	5.295	**0.006***	**0.007***	0.123	0.985	6.444	-3.183	-3.911
	TAH	8.128	**0.000***	**0.000***	0.648	0.259	3.598	0.771	-4.095
	CPH	1.384	0.255	0.008	0.131	0.321	1.077	-0.146	-0.943

Truss morphology: Interspecific trials

From the 22 variables analysed for the ANOVA (**Table 4.3**), 14 variables showed highly significant (p<0.01) differences. These highly significant results showed no overlap at a 95% confidence interval making these variables completely distinguishable between *T. megalopterus* (spotted gully; SG), *Mustelus mustelus* (smooth hound; SH) and *Haploblepharus edwardsii* (puffadder shyshark; PA) specimens.

In comparison to the SG and PA, the SH had a significantly longer snout (L2) and tail section (L7, D5) and a shorter snout to gill length (L3), head diagonal (D1) and trunk length (D2, D3 and L5). The PA had a significantly wider body (V3, A2) compared to the SH and smaller trunk (A3) and tail (A4) widths compared to both SG and SH. The length between the gills and first dorsal (L4) was significantly longer in the SG compared to that of the SH. The first angle (A1) was significantly different for all three species with the largest angle found in the SG and smallest in the PA. These results suggest that SH have a more slender frame than SG and PA.

Table 4.3: Results of ANOVAs for interspecies truss data of *T. megalopterus* (spotted gully; SG), *Mustelus mustelus* (smooth hound; SH) and *Haploblepharus edwardsii* (puffadder shyshark; PA); *F = Levene's F statistic, p = Levene's significance, * = significance at the 1% nominal level, L1-L6 = lengths, V1-V6 = verticals, D1-D5 = diagonals, A1-A4 = angles*

	Levene's Test for Equality of Variance		Least Significant Difference/Games-Howell			Species Means		
	F	***p***	**SG-SH**	**SG-PA**	**SH-PA**	**SG**	**SH**	**PA**
L1	1.685	0.204	0.059	0.331	0.334	5.140	-5.129	-0.011
L2	3.029	0.065	**0.000***	0.569	**0.000***	-7.185	12.392	-5.207
L3	5.442	0.010	**0.002***	0.696	**0.006***	4.397	-7.417	3.020
L4	3.601	0.041	**0.002***	0.273	0.024	7.924	-10.111	2.187
L5	1.648	0.211	**0.006***	0.669	**0.002***	14.185	-35.651	21.467
L6	1.899	0.169	0.518	0.875	0.423	-2.686	7.940	-5.255
L7	0.150	0.861	**0.000***	0.253	**0.003***	-11.616	15.951	-4.335
V1	0.670	0.520	0.037	0.359	0.217	-3.206	3.546	-0.340
V2	0.747	0.483	0.652	0.375	0.186	-0.693	-2.821	3.514
V3	3.143	0.059	0.024	0.508	**0.005***	3.217	-10.216	6.999
V4	7.484	**0.003***	0.745	0.978	0.288	-2.868	3.943	-1.075
V5	3.732	0.037	0.085	0.702	0.172	-2.799	4.105	-1.306
V6	5.541	**0.010***	0.121	0.647	0.168	1.152	-1.389	0.237
D1	0.229	0.797	**0.001***	0.841	**0.002***	3.457	-6.371	2.914
D2	6.343	**0.006***	**0.004***	0.970	**0.000***	6.184	-11.380	5.196
D3	1.777	0.188	**0.004***	0.562	**0.001***	12.427	-33.418	20.992
D4	1.766	0.190	0.423	0.942	0.383	-3.639	8.372	-4.733
D5	0.119	0.888	**0.000***	0.254	**0.003***	-11.397	15.600	-4.202
A1	6.901	**0.004***	**0.008***	**0.000***	**0.007***	99.810	89.841	72.240
A2	0.564	0.575	0.086	0.028	**0.000***	94.664	88.116	103.210
A3	15.316	**0.000***	0.458	**0.000***	**0.000***	108.721	105.761	34.146
A4	13.972	**0.000***	0.983	**0.000***	**0.000***	133.900	134.385	44.003

Truss morphology: Intraspecies

Eight of the 22 truss variables were highly significant ($p<0.01$) between the three populations (**Table 4.4**). Compared to AN, WC specimens had longer snouts (L2) and smaller associated first angle (A1) caused by a shorter gill to first dorsal fin length (L4). Although L2 was significant at a higher 2% nominal level, this variable still showed no overlap of the 95% confidence intervals between AN-WC. Additionally, EC differed from AN with three vertical (width) measurements which suggest that specimens from the EC have a wider head (V1 and V2) and caudal (V6) region. Angolan specimens also had a significantly wider trunk region (V5) compared to both EC and WC. Within South Africa, only one highly significant variable

was detected between the EC and WC populations. Here, the second angle (A2) was larger in EC than it was in WC.

Table 4.4: Results of ANOVAs from intraspecies truss data of interspecies truss data of *T. megalopterus* from Angola (AN), Western Cape (WC) and Eastern Cape (EC); *F = Levene's F statistic, p = Levene's significance, * = significance at the 1% nominal level, L1-L6 = lengths, V1-V6 = verticals, D1-D5 = diagonals, A1-A4 = angles*

	Levene's Test for Equality of Variance		Least Significant Difference (LSD)/Games-Howell			Population Means		
	F	***P***	**AN-EC**	**AN-WC**	**EC-WC**	**AN**	**WC**	**EC**
L1	0.489	0.615	0.476	0.019	0.076	3.623	-5.569	1.066
L2	12.117	**0.000***	0.037	**0.013****	0.643	-4.401	2.708	1.630
L3	7.168	**0.001***	0.972	0.798	0.821	0.880	-1.295	0.217
L4	1.186	0.309	0.025	**0.000***	0.097	7.221	-6.994	-0.835
L5	3.013	0.053	0.165	0.012	0.184	-8.768	9.400	0.360
L6	2.124	0.125	0.637	0.501	0.247	0.141	-4.638	3.229
L7	7.238	**0.001***	0.110	0.941	0.444	3.792	1.846	-4.417
V1	9.953	**0.000***	**0.005***	0.062	1.000	-3.719	1.792	1.735
V2	9.389	**0.000***	**0.014***	0.119	0.981	-5.867	3.222	2.453
V3	7.961	**0.001***	0.099	0.680	0.885	-3.918	0.515	2.818
V4	9.858	**0.000***	0.901	0.804	0.912	-2.318	2.560	0.042
V5	3.300	0.041	**0.003***	**0.000***	0.316	4.602	-3.361	-1.323
V6	0.609	0.546	**0.002***	0.021	0.561	1.775	-0.522	-1.069
D1	6.982	**0.001***	0.545	0.811	0.981	-1.673	0.480	1.015
D2	5.767	**0.004***	0.846	0.654	0.386	0.374	-2.801	1.715
D3	5.459	**0.006***	0.218	0.074	0.514	-9.075	8.469	1.281
D4	2.720	0.070	0.845	0.287	0.193	1.520	-5.464	2.702
D5	7.791	**0.001***	0.080	0.887	0.484	4.181	1.464	-4.459
A1	0.020	0.980	0.111	**0.001***	0.065	95.671	88.644	92.478
A2	0.136	0.873	0.442	0.032	**0.003***	93.720	89.298	95.167
A3	0.420	0.658	0.022	0.999	0.027	109.937	109.935	113.497
A4	0.022	0.978	0.331	0.885	0.430	135.049	135.276	136.459

Of the eight variables that showed highly significant differences in the intraspecies truss morphology, only one of these variables, V2 (the width of the head at the sight of the gills), was significantly (p<0.001) different between sexes. A paired-samples t-test conducted to compare V2 for AN-EC (the population comparison rendering significant differences amongst sample site) showed there was a significant difference in the female V2 from AN (M = 125.167, SD = 35.749) and EC (M = 51.179, SD = 18.742); t(25) = 4.248, p<0.001. This

result conflicts with the significant difference in MOW from the same populations. Female *T. megalopterus* from AN exhibit a smaller mouth width, but larger head width at the first gill slit.

None of the highly significant variables were significantly different amongst haplotype.

Ontogenetic shifts on tooth shape

The shape analysis (**Figure 4.5**) of the EC sample subset showed the teeth of *T. megalopterus* increase in area and width with ontogeny. Small specimens had dorsoventrally flattened and more molariform lateral teeth, while the teeth of larger specimens were broader at their base and lengthened to produce a single cusp. This cusp was more evident in the teeth positioned in the medial parts of the jaw.

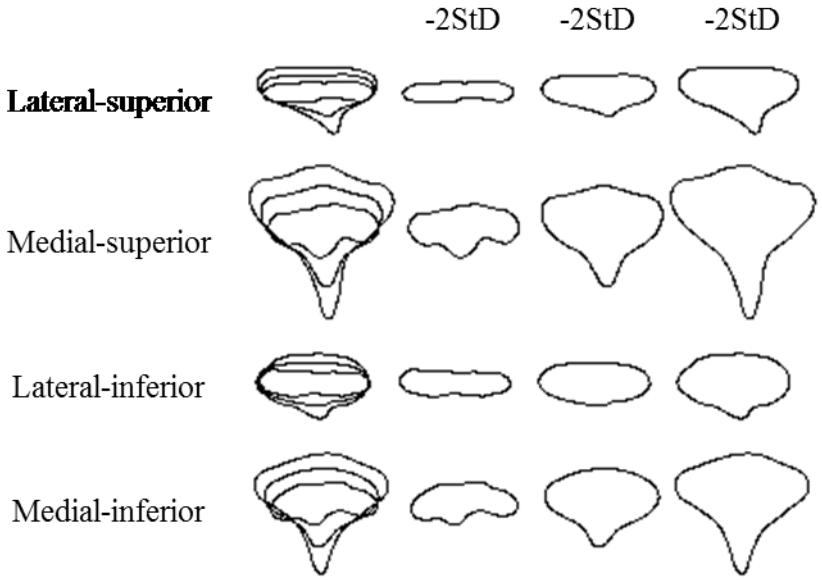

Figure 4.5: Shape variation of *Triakis megalopterus* teeth from Eastern Cape. Superimposed outlines in the first column represent all of the shape variations per tooth type.

Tooth morphology

The tooth morphology analysis revealed comparable results for all four teeth from the three populations (**Table 4.5**). Of the 52 variables analysed, only one variable (D3) for the medial-superior teeth showed a highly significant (p<0.01) difference between AN and EC. This indicated the EC specimens have a wider base for their medial-superior teeth than the AN specimens.

The only significant variable in the tooth data (D3, medial superior), was not significantly different between sex or between haplotype.

Table 4.5: Results of ANOVAs of tooth data for *T. megalopterus* from Angola (AN), Western Cape (WC) and Eastern Cape (EC); F = Levene's F statistic, p = Levene's significance, * = significance at the 1% nominal level

		Levene's Test for Equality of Variance		Least Significant Difference (LSD)/Games-Howell			Population Means		
		F	***P***	**AN-EC**	**AN-WC**	**EC-WC**	**AN**	**EC**	**WC**
Lateral Superior	W	0.483	0.619	0.188	0.491	0.559	0.657	0.705	0.739
	L	0.567	0.570	0.184	0.131	0.684	-0.396	-0.231	-0.267
	A1	5.844	**0.004***	0.779	0.831	0.980	4.763	4.813	4.820
	A2	1.125	0.330	0.508	0.725	0.776	3.790	3.757	3.734
	A3	2.682	0.075	0.999	0.713	0.655	5.030	5.022	5.030
	A4	4.299	0.017	0.862	0.123	0.094	4.082	4.407	4.114
	D1	0.929	0.399	0.132	0.357	0.611	0.155	0.234	0.270
	D2	1.223	0.300	0.226	0.342	0.880	-0.031	0.041	0.051
	D3	1.316	0.274	0.085	0.134	0.966	-0.004	0.114	0.117
	D4	0.689	0.505	0.552	0.606	0.210	-0.005	-0.043	0.034
	S	1.925	0.153	0.696	0.237	0.311	-0.886	-0.815	-0.865
	A	0.912	0.406	0.155	0.203	0.987	-0.434	-0.240	-0.241
	C	1.808	0.171	0.699	0.225	0.291	3.417	3.345	3.397
Medial-Superior	W	1.775	0.176	0.213	0.867	0.257	0.778	0.786	0.829
	L	0.027	0.973	0.043	0.806	0.061	0.611	0.627	0.727
	A1	3.443	0.037	0.141	0.449	0.503	4.354	4.320	4.294
	A2	1.766	0.178	0.197	0.753	0.314	4.316	4.330	4.369
	A3	1.646	0.199	0.196	0.698	0.357	4.890	4.879	4.858
	A4	6.696	**0.002***	0.699	0.914	0.404	4.464	4.516	4.347
	D1	0.351	0.705	0.165	0.830	0.082	0.614	0.604	0.676
	D2	0.147	0.863	0.076	0.685	0.157	0.548	0.571	0.636
	D3	1.051	0.355	**0.008***	0.158	0.228	0.143	0.209	0.255
	D4	0.330	0.720	0.710	0.361	0.135	0.207	0.160	0.223
	S	0.353	0.703	0.120	0.400	0.510	-0.473	-0.455	-0.443
	A	0.200	0.819	0.044	0.745	0.076	0.701	0.731	0.868
	C	0.426	0.654	0.137	0.456	0.483	3.003	2.987	2.975
Lateral Inferior	W	0.273	0.762	0.586	0.433	0.128	0.637	0.682	0.608
	L	3.434	0.037	0.345	0.703	0.143	-0.347	-0.316	-0.416
	A1	3.813	0.026	0.439	0.381	0.817	4.879	4.912	4.905
	A2	1.365	0.261	0.146	0.319	0.720	3.750	3.685	3.665
	A3	2.198	0.118	0.176	0.677	0.342	4.939	4.947	4.963
	A4	2.153	0.123	0.173	0.805	0.080	4.515	4.558	4.307
	D1	0.689	0.505	0.853	0.200	0.082	0.001	0.077	-0.008
	D2	0.901	0.410	0.490	0.599	0.172	0.046	0.078	0.008
	D3	0.230	0.795	0.923	0.184	0.089	-0.005	0.078	-0.010
	D4	0.596	0.553	0.202	0.897	0.129	0.024	0.032	-0.050
	S	3.933	0.024	0.414	0.763	0.609	-0.804	-0.818	-0.839

		Levene's Test for Equality of Variance		Least Significant Difference (LSD)/Games-Howell			Population Means		
		F	***P***	**AN-EC**	**AN-WC**	**EC-WC**	**AN**	**EC**	**WC**
	A	1.780	0.175	0.347	0.522	0.077	-0.374	-0.296	-0.475
	C	3.540	0.034	0.414	0.779	0.591	3.335	3.349	3.370
Medial-Inferior	W	0.320	0.727	0.381	0.894	0.273	1.011	1.005	1.042
	L	0.989	0.376	0.064	0.398	0.327	0.706	0.752	0.797
	A1	1.610	0.206	0.737	0.979	0.744	4.417	4.416	4.408
	A2	2.248	0.112	0.086	0.193	0.783	4.168	4.229	4.240
	A3	0.139	0.870	0.461	0.868	0.556	4.898	4.894	4.883
	A4	1.582	0.212	0.170	0.600	0.399	4.622	4.526	4.397
	D1	0.444	0.643	0.063	0.266	0.509	0.635	0.686	0.711
	D2	0.241	0.787	0.607	0.927	0.509	0.775	0.770	0.802
	D3	4.343	0.016	0.651	0.054	0.065	0.438	0.343	0.419
	D4	0.038	0.963	0.041	0.092	0.868	0.359	0.449	0.457
	S	2.676	0.075	0.660	0.643	0.304	-0.462	-0.471	-0.455
	A	0.996	0.374	0.338	0.591	0.096	1.092	1.055	1.149
	C	3.189	0.047	0.705	0.623	0.319	2.991	3.001	2.985

Discussion

The morphology and biomechanics of sharks range from slender and flexible in benthic species to more streamlined and stiff-bodied in pelagic species (Shadwick and Goldbogen, 2012). Cartilage is living tissue that is able to change its shape in response to primary stresses (Carter and Beaupré, 2007). As sharks possess a purely cartilaginous skeleton (Cailliet *et al.*, 1983b), interspecies adaptation may occur when members of a population become better suited to their environments, increasing their chances of survival and/or improving their fitness (Futuyma, 2009). These types of morphological differences play an integral role in the ecological performance, and thus survival, of species and populations (Liem, 1990).

From the sample site descriptions (see **Chapter 2**), it is clear that all three populations of *T. megalopterus* analysed for this chapter are exposed to very different environmental factors, which include ocean currents, temperature, oxygen and habitat. The genetic results (see **Chapter 3**) also show population structure for this species. Thus, we may expect that the morphology of *T. megalopterus* will show differences amongst populations. It is, however, possible that phenotypic variation is not linked to the genetics of a species. This occurs when the phenotypic expression is dependent on environmental influences, and stabilizing selection favours the same phenotype (Vogt *et al.*, 2008).

Adjusting the morphometric measurements to an overall mean total length was successful in removing the effect of allometry and sexual dimorphism from the analyses. Only two variables from the traditional and truss morphology revealed significant differences between females from AN and EC. The mouth width in EC was significantly larger than in AN, while the head width (at the site of the gills) was significantly larger in AN compared to EC. This is a contradictory result that may have been caused by a sampling error since one would expect a wider head in the population that shows a wider mouth. As female sharks attain larger sizes, it is no surprise that they would have larger mouths as they will be able to take on larger prey than the males. In *G. cuvier,* for example, females had a higher prey diversity than the males (Simpfendorfer *et al.*, 2001). Larger mouths in females from EC may be indicative of their more durophagous diet, whereas AN specimens feed on more soft bodied prey such as teleosts and cephalopods (see **Chapter 5**). For instance, cephalopods are easily manipulated in the mouth allowing smaller predators to ingest larger octopus as prey.

Few significant differences were apparent between WC-AN (ING, INO) and WC-EC (P1I, CPV). The majority of the morphological differences were found between AN-SA and AN-EC indicating that AN specimens appear to group out from the other locations. Generally, compared to the South African populations, AN specimens appear to have smaller fins (pectoral, 1st and 2nd dorsal), wider fin bases (pelvic, 2nd dorsal), shorter gill slits, smaller eyes and spiracles, wider tail regions and larger caudal fork, smaller post ventral and terminal caudal margins. Many of these differences produce a more streamlined body for AN specimens. Unfortunately, functional morphology was beyond the scope of this thesis. For this reason, although differences amongst populations can be seen from the statistics, the reason for these differences cannot be accurately explained, only hypothesized. In order to properly quantify the mechanical and evolutionary association amongst the behaviour, dynamics and functional form of the sharks, all of the significant morphological differences in this chapter require further investigation into their physiology and functional morphology.

Largely, theory states that the function of the pectoral fins is to generate lift to balance the movement generated by the heterocercal tail (Alexander, 1965; Simons, 1970; Thomson and Simanek, 1977). However, in *T. semifasciata*, the pectoral fins are critical for manoeuvrability, not for generating lift (Wilga and Lauder, 2000). Correspondingly, the dorsal fins are used for stabilization and to assist in sudden turns (Lingham-Soliar, 2005a). Therefore, it may be probable that the combination of shorter fins and larger fin bases, serves to increase the rigidity of fins offering less manoeuvrability (Helfman *et al.*, 2009).

Consequently, sharks from AN may have less need for manoeuvrability, possibly due to feeding differences (feeding in the open water column) and/or as an adaptation to their local habitats conditions. The smaller fins (pectoral and dorsal) in AN specimens may also aid in streamlining the shark by minimizing inertial and viscous drag (Helfman *et al.*, 2009) and permitting higher speeds (Bushnell and Moore, 1991), an adaptation found in active swimming species. Streamlined bodies are consistent with ram feeders (Webb, 1977, 1984) making the predator more successful in attacks on elusive, fast prey such as teleosts or cephalopods (Webb, 1982; Moody *et al.*, 1983).

Compared to the SA populations, AN possess a larger tail region (anterior to the caudal fin) that is both laterally and dorsoventrally greater. A study of free swimming leopard sharks, *Triakis semifasciata,* shows the movement of the tail surface deflects water ventrally and posteriorly, generating an anterodorsally directed reaction force with both lift and thrust components (Wilga and Lauder, 2000). The propulsion of the caudal fin is generated by both red and white axial muscles. The red muscle has a smaller diameter, is aerobic and active at slower, continuous swimming speeds, while the white muscle is larger in diameter, generally anaerobic and active for brief bursts of speed or fast-starts possibly geared to capturing fast swimming prey or to avoid predators (Flammang, 2010; Shadwick and Goldbogen, 2012). Generally, red muscle only varies in position and does not increase in quantity between species (e.g. *Prionace glauca, Triakis semifasciata, Isurus oxyrinchus, Carcharodon carcharias, Lamna ditropis, Alopias vulpinus;* Bernal *et al.,* 2003). Similarly, in the caudal peduncle in the white shark, *Carcharodon carcharias*, there is an increase in the epaxial and hypaxial musculature combined with a dense layer of collagen fibre-reinforced adipose tissue, vital to efficient oscillatory motions of the caudal fin (Lingham-Soliar, 2005b). The thicker tail region in AN may be attributed to an increase in white muscle mass, possibly indicative of a morphological adaptation to brief bursts of speed. Therefore, the larger diameter caudal region may be related to the behaviour of the dominant prey species which are the relatively mobile *Diplodus sargus capensis, Sardinella aurita, Atractoscion aequidens* and *Pomadasys olivaceum* in AN compared with the sedentary *Jasus lalandii* in the WC and the predominantly crustacean based diet in the EC.

Smaller eye height and widths were recorded in *T. megalopterus* from AN compared to SA. Although there are not much data available on differences in eye size amongst elasmobranch populations, there are significant differences in eye size amongst elasmobranch species. For instance, in a study of the residual axial eye diameter of 46 elasmobranchs, the residuals

ranged from 1.604 to 0.821 for the pelagic big-eye thresher (*A. superciliosus*) and the benthic and coastal coffin ray (*Hypnos monopterygius*), respectively. Generally, larger eyes, which imply that the shark will rely heavily on vision (Warrant and Locket, 2004), are commonly found in species of sharks that feed on more active and mobile prey (Lisney and Collin, 2007). Large eyes also mean longer focal length, which aids in resolution and sight sensitivity (Lisney and Collin, 2007). For these reasons, the eyes of most pelagic species are generally large and well-developed (Fritsches *et al.*, 2003), e.g. bigeye thresher shark, *A. superciliosus*, silky shark, *Carcharhinus falciformis*, blue shark, *Prionace glauca* and crocodile shark, *Pseudocarcharias kamoharai* (Lisney and Collin, 2006). The larger eyes in the SA populations of *T. megalopterus* may be an adaptation to hunting prey that requires higher resolution and sight sensitivity.

Interestingly, the spiracles and all of the gill slits were shorter in AN compared to the SA populations. As both of these features have to do with the respiration and thus oxygen extraction (Stevens, 1999b), shorter gill slits and smaller spiracles in AN may be an adaptation to more oxygenated waters or waters with a more stable oxygen supply. Smaller gills and spiracles may also be an indication of a generally less active population that does not require the relatively large gill surface areas that more active specimens would. For instance, fast swimming mackerel have a 50 times larger relative gill surface area compared to slow swimming, bottom dwelling goosefish (Schmidt-Nielsen, 1997). Unfortunately, the respiratory system and gill structure was beyond the scope of this study. It is therefore difficult to accurately explain the shorter gill slits and smaller spiracles in AN compared to EC and further investigation is required.

Morphological differences may be genetic, environmental (phenotypic) or usually both (Swain *et al.*, 2013). Adaptations to environmental changes or varying environments take place through either phenotypic plasticity, a reversible change to the morphological phenotypes, independent of genotype or genetic adaptation, or a change in phenotype caused by changes in allelic composition by means of selection (Dillon *et al*., 2014). Theories on adaptive phenotypic plasticity predict that given genetic variation (evident in **Chapter 3**), selection will favour adaptive plasticity (different phenotypes) when populations inhabit different environments and are subjected to varying conditions (Ghalambor *et al.*, 2007). We know that the three populations of *T. megalopterus* studied occupy different habitats which are controlled by different ocean currents, varying temperatures, oxygen and salinity. Therefore it is not impossible that some, if not all, of the morphological differences seen may

be adaptive (e.g. smaller fins, wider fin bases and larger tail area in AN specimens) placing populations close to a new phenotypic optimum (Ghalambor *et al.*, 2007) that is essential for the survival (Robinson and Dukas, 1999) of *T. megalopterus*.

The variation in the traditional morphology, present between SA and AN was consistent with the mtCR haplotype network and phylogenetic tree which showed an SA vs. AN genetic structure. Although the mtCR F_{ST} showed a transoceanic structure, there was evidence of admixture between WC and EC. This admixture is confirmed by the *T. megalopterus* tagging data, which clearly showed movement between WC and EC (Dunlop and Mann, 2014). The variation in the traditional morphology that was present between AN and EC specimens is consistent with mtCR F_{ST} and the transoceanic structure (AN-NA-WC vs. EC) reported from the nDNA Bayesian clustering analysis. From this overview of the morphology versus genetics, the general trend is that AN and EC are the most divergent populations, both phenotypically and genotypically, while the South African (SA) populations show a large amount of similarities. Therefore, the morphological analysis supports the distinction of the EC and AN populations and it can be hypothesized that these morphological changes began to slowly occur after the isolation of *T. megalopterus* populations during the Pleistocene (see **Chapter 3**).

To properly assess the underlying causation of morphological variation in *T. megalopterus*, resolution of the relationship between morphological features and environmental variation is required. This can be done by means of "common garden experiments" in which captive individuals from different environments are subjected to the same controlled environmental conditions (Trip *et al.*, 2008; Dudgeon *et al.*, 2012). This, however, was not possible in the current study. Alternatively, in an attempt to assess whether the morphological variation found amongst populations of *T. megalopterus* was evolution or adaptation, the morphology data were analysed using the six haplotypes from mtCR as the grouping variable. The haplotype ANOVA shows that 38.9% (14/36) of the variation (second dorsal fin, gill slit lengths, eye size, ULA and CFW) is congruent between phenotype and genotype. The results of the ANOVA's run using the six haplotypes confirmed this distinction and gave evidence that the morphological differences found amongst populations of *T. megalopterus* may not be strictly phenotypic and may possibly represent positive selection and adaptive divergence.

Unfortunately, both mtDNA and microsatellites were neutral markers. This means that gene variants detected using these markers do not have a direct effect on fitness (Holderegger *et al.*,

2006). Therefore, this type of genetic variation is selectively neutral and tells us nothing about the adaptive or evolutionary potential of a population or a species (Holderegger et al., 2006). In order to properly test whether the morphological differences are genotypic, population genomics needs to be undertaken using multi-locus data sets from multiple populations to identify non-neutral or outlier loci by contrasting patterns of population divergence among genetic regions (Nosil and Buerkle, 2010). Population genomics were beyond the scope of this study but will be very beneficial in the future to aid in the understanding of shark evolution and their adaptability potentials to different habitats, environmental conditions and global warming. For this reason, although morphological differences are apparent, one can only speculate as to what the causes of the differences may be.

Despite the teeth playing an integral role in shark feeding, the principal focus on functional feeding studies have concentrated on the head morphology, muscle and jaw function and cranial components (e.g. Summers *et al.*, 2004; Dean *et al.*, 2006; Huber *et al.*, 2006; Lowry *et al.*, 2007). Very little information is available on tooth morphology and its function for sharks. The teeth of *T. megalopterus* form a plate-like dentition where the teeth in the front of the jaw have a molar-like base rising into a sharp cusp. This plate like dentition is consistent with a crushing feeding mechanism and a durophagous diet (Moss, 1977), while the cusps pierce the flesh of soft prey enabling the shark to grasp and manipulate teleosts and molluscs (Ramsay and Wilga, 2007). This dentition is consistent with the feeding study by Smale and Goosen (1999) who state that *Triakis megalopterus* from the Eastern Cape of South Africa feeds on crustaceans, cephalopods, teleosts and small elasmobranchs. The teeth of *T. megalopterus* grow and change shape during ontogeny, most likely caused by a change in the feeding habits of this species through ontogeny (see **Chapter 5**).

In the subset analysis of EC dentition, small *T. megalopterus* have dorsoventrally flattened and more molariform lateral teeth. These lateral teeth have no cusps, only small, coarse serrations which would be more suited for a durophagous diet where crushing or grinding of hard-bodied prey is required. As *T. megalopterus* grow, so do their teeth grow and change shape. There is an increase in the area and width of the teeth from small to large specimens where the medial-inferior and medial-superior broaden at their base and lengthen to produce a single cusp. The development of this cusp may enable larger specimens to grasp/clutch and manipulate softer-bodied prey to inhibit their escape (Ramsay and Wilga, 2007). Therefore, the development of this cusp may be indicative of a diet expansion to include softer-bodied prey species with increasing size. In one of the few statistical studies to incorporate feeding

and dental morphology throughout ontogeny (Powter *et al.*, 2010), the Port Jackson shark, *Heterodontus portusjacksoni*, showed a similar, although opposite pattern to that observed for *T. megalopterus*. Juvenile *H. portusjacksoni* have the sharp, cuspidate anterior teeth needed to grasp soft bodied benthic invertebrates, while the adults had more molariform teeth suited to their durophagous diet.

Different biotic environments could result in differences in food composition as prey diversity and abundance varies with location (Yamaguchi and Taniuchi, 2000). Consequently, varied feeding habits in diverse habitats may result in different tooth morphology amongst populations. In this study, however, there was only a single variable (medial-superior base width) that differed significantly between AN and EC. Although this result suggests that the diet of *T. megalopterus* may be similar in the AN, WC and EC, this was not the case (see **Chapter 5**). Another explanation for the similar tooth morphology of the three populations may be that *T. megalopterus* implements feeding mechanisms that do not require biting, e.g. ram or suction feeding (Motta *et al.*, 1997).

During ram feeding the predator engulfs its prey whole or seizes it in its jaws (Motta and Wilga, 2001) thereby not making use of its teeth unless there is some degree of prey manipulation. For instance, Wilga and Motta (2000) state that the bonnethead shark, *Sphyrna tiburo*, ram feed on benthic prey by approaching prey with mouth wide open, depressing their mandible and engulfing the prey, after which this species implements prey manipulation by a combination of lateral headshakes and crushing of the prey. Conversely, during suction feeding the predator expands the volume of its oral cavity and/or throat causing a decrease in pressure inside the mouth/throat resulting in prey being pulled into the mouth (Motta and Wilga, 2001). The nurse shark, *Ginglymostoma cirratum*, is an obligate suction feeder that doesn't require the additional help of prey manipulation techniques as its suction power alone can dismember prey (Matott *et al.*, 2005).

Male elasmobranchs are known to bite the fins and flanks of females during copulation in order to coerce females into and/or stabilize the female during mating (Byrne and Avise, 2012). Therefore, sexual dental dimorphism may be apparent in elasmobranchs where it would be theoretically advantageous for males to develop sharp, pointed teeth during the mating season to enhance the grip efficiency and ultimately to increase male reproductive success (Kajiura and Tricas, 1996). There was, however, no evidence of sexual dental dimorphism in *T. megalopterus* as both sexes developed sharp cusps at larger sizes. There was

also no physical evidence, such as lacerations or mating scars on females. This may suggest that this species may not implement these courtship/mating behaviours. More likely, the males may still grip the females; their teeth, however, are just not large or sharp enough to cause severe lacerations. Mature female *T. megalopterus* may also possess thicker skin to accommodate the aggressive mating behaviour of males. For instance, the dermis of mature female blue sharks, *Prionace glauca,* is twice as thick as that of males, which means it is seldom that the males teeth penetrate the females dermis and cause damage to the musculature (Pratt, 1979). In this study, the skin thickness was not measured for *T. megalopterus*. It might, however, be useful to do so in any future studies.

The truss system was effective in recognizing differences amongst *Triakis megalopterus, Haploblepharus edwardsii* and *Mustelus mustelus*. The results from the truss analysis of these three species revealed that *M. mustelus* has a more slender body whilst *T. megalopterus* and *H. edwardsii* were more similar due to their flatter, stouter frames. *T. megalopterus* and *M. mustelus*, which both belong to the subfamily Triakinae, appear to be morphologically similar to the untrained eye and are often mistaken for one another by fishers (Booth *et al.*, 2011); the truss system, however, was successful in separating these species.

The truss was also successful in detecting differences between populations of *T. megalopterus* that were not apparent in the traditional morphology. For instance, in the traditional morphology, compared to AN, WC had a larger POB though this variable did not differe significantly. However, in the snout length in the truss, L2 (POB + ½EYL) was significantly different between AN and WC. The traditional morphology showed that AN had a significantly larger caudal region, while the truss morphology also identified that specimens from EC have a wider head (V1 and V2) and caudal (V6) region.

Dorsal truss networks are generally used on dorsoventrally flattened species, such as sand sharks, rays, skates (e.g. Orlando *et al.*, 2015) and lobster (e.g. Cadrin and Friedland, 1999). Ventral truss networks are generally used on laterally flattened species, for instance, many teleosts (e.g. Hockaday, 2000; Turan, 2004; Shao *et al.*, 2007; Bagherian and Rahmani, 2009). With the exception of flatfish (e.g. sandsharks and rays), sharks display depth and width which means both dorsal and lateral truss systems are possible. In this study, the truss was restricted to a dorsal view though an analysis based on the lateral view is also possible with sharks and may be more beneficial as one can include landmarks relating to the insertion points of all fins. Correspondingly, the choice and number of landmarks used in the analysis

was limited as the specimens had already been dissected, this may have influenced the statistical power of the analysis (Parsons *et al.*, 2003). Although the truss did reveal some differences between populations, the separation could have been better with the freedom to develop the truss from scratch by choosing the most relevant landmarks.

By reconstructing the morphological measurements into a truss system one overcomes the main drawbacks of traditional morphology. The truss method is not dominated by redundant measurements along a single axis, which provides a more complete characterization of shape. This method makes use of longitudinal, diagonal, breadth and width measurements, encompasses systematic coverage of the entire specimen without repetition of linear measurements and makes use of anatomical rather than extremal landmarks by basing landmarks on definitive biological structures. By reconstructing the outline of specimens in AutoCAD, one also has the key advantages of visualizing the form of specimens and early recognition of measurement errors as the reconstruction will not be successful with errors in measurements. Most importantly, reconstructing specimens in AutoCAD means one does not need to worry about specimens losing shape on a planar surface due to their cartilaginous skeletons not being able to withhold their weight. The use of AutoCAD also reduces the amount of software one needs to use for analysis. Once the specimen is reconstructed, distances (length measurements), angles and landmark coordinates can be computed. Specimens can also be analysed in both two and three dimensional aspects. Therefore, the proposed truss morphology protocol will be very beneficial for protected species where lethal morphology methods are not an option. This methodology will also be a useful addition to traditional morphology for a holistic approach to morphological analyses.

In order to understand exactly why morphological intrapopulation differences exist in *T. megalopterus,* future work on the functional morphology of the species will be beneficial and assist in understanding shark evolution and their adaptability potentials to different habitats, environmental conditions and global warming. Information such as prey detection and capture capabilities is also needed for a more comprehensive understanding of the functional feeding and therefore feeding strategies of this species.

Conclusions

Specimens from AN appear to be more streamlined than those from SA, an indication of an adaptation to local environmental conditions and/or feeding differences. Morphological

differentiation in *T. megalopterus* reflected the population structure found in both the mtCR and nDNA results. As *T. megalopterus* has shown differences in phenotype amongst populations, which appear to correspond with the genetic data, it is apparent that the phenotype of this species is influenced both by its genotype and the environment. The dentition of *T. megalopterus* appears to be consistent with a durophagous diet that also includes soft bodied prey (e.g. teleosts and cephalopods). The teeth of *T. megalopterus* show a broader base and longer cusp in larger specimens, possibly indicative of a dietary shift during ontogeny. There is no evidence of sexual dental dimorphism and virtually no difference amongst populations, despite differences in feeding amongst *T. megalopterus* populations, particularly in the WC. The truss network was effective in separating species as well as identifying intraspecific morphological differences that were not detected using traditional morphometrics. With additional testing, this methodology may very well be suitable for non-lethal studies which will allow for morphological studies on protected species.

Chapter 5:
Comparison of the diet of *Triakis megalopterus* from three biogeographic zones in southern Africa

Introduction

Successful foraging by elasmobranchs is essential to sustain bodily functions such as growth and reproduction (Ernest *et al.*, 2003). Understanding the feeding habits of various species provides essential information about their natural history, role in ecosystems (Braga *et al.*, 2012), resource partitioning and habitat quality (Guedes and Araújo, 2008), habitat selection (Sims, 2003), trophic ecology (Cortés, 1999) and evolutionary specialization (Wilga *et al.*, 2007).

Allopatric populations inhabit different geographic regions (Ayala, 1982) where they are subject to contrasting environmental conditions, habitat, resource availability, exploitation and predation. These factors are known as selective pressures and have the ability to alter the phenotype of individuals by changes to basic biological parameters (Bakun, 2010), fitness and behaviour (Shiu and Borevitz, 2008). Since these types of selective pressures are the driving force of natural selection and evolution (Schaffner and Sabeti, 2008), feeding can have a major impact on the phenotypic and genotypic expression in populations.

Besides geographic dietary variations, it is not uncommon for animals to shift their diets with ontogeny. This switch often corresponds with changes in morphology, as shown by the teleost, *Rutilus rutilus* (Hjelm *et al.*, 2003). According to Motta and Wilga (2001), an ecomorphological association between diet, feeding behaviour, and dental morphology also exists in sharks. In the family Heterodontidae (horn sharks), for example, the shape of the teeth and number of cusps change with ontogeny (Motta and Wilga, 2001). Dentition of the great white shark also changes when its diet shifts from predominantly fish to marine mammals (at length >3 m; McCosker, 1985). Several other shark species have similarly displayed morphological change related to dietary ontogenetic shift and their role in the ecosystem: e.g. tiger shark (*Galeocerdo cuvier*; Lowe *et al.*, 1996); starspotted-dogfish (*Mustelus manazo;* Yamaguchi and Taniuchi, 2000); sevengill cow shark (*Notorynchus cepedianus;* Ebert, 2002); lemon sharks (*Negaprion brevirostris*; Newman *et al.*, 2012); sandbar shark (*Carcharhinus plumbeus;* Ellis and Musick, 2006); and the Atlantic sharpnose

shark (*Rhizoprionodon terraenovae;* Hoffmayer and Parsons, 2003; Bethea *et al.*, 2004, 2006).

Epigenetic inheritance will also allow for morphological changes between populations. Epigenetic inheritance is a phenotypic change that allows an organism to adjust its morphology and/or behaviour to suit different circumstances (Ehlinger, 1990) and to maximize their payoff in some kind of fitness (Schoener, 1971). This is possible when offspring transmit traits to the next generation that were not characteristic of the parent; this is called trans-generational developmental/phenotypic plasticity (Çabej, 2011). There is a little known about epigenetic inheritance of characters associated with feeding in elasmobranchs.

Feeding ecology in sharks can be investigated in various ways: gut content analysis of deceased specimens (Braccini and Perez, 2005; Ebert and Ebert, 2005); non-lethal techniques such as stomach flushing (Kao 2000; Liao *et al*., 2001); DNA analysis of prey (Jarman and Wilson, 2004; King *et al.*, 2008; Barnett *et al.*, 2010); the analysis of tissue by means of organochlorine and/or stable-isotope (Fisk *et al.*, 2002; Herman *et al.*, 2005; Estrada *et al.*, 2006) and quantitative fatty acid signatures (Iverson *et al.*, 2004; Herman *et al.*, 2005); and visual/observation feeding analysis (Van Dykhuizen and Mollet, 1992).

Each of these methods comes with its own particular bias. Dietary studies, in general, will be biased in favour of durable parts (e.g. cephalopod beaks, otoliths, vertebrae) attributable to differential rates of digestion (Tollit *et al.*, 1997). Non-lethal methods do not guarantee full recovery of all prey items (Foster, 1997) and, depending on particular method used, prey retrieval success is dependent on species and specimen/prey size (Kamler and Pope, 2001). While DNA analysis of prey will increase the rate of data accumulation by enabling the identification of typically unidentifiable fragments of prey (flesh, bone, spine rays etc.), the success of this method is, however, expensive, time consuming and dependent on matching an unknown sequence to a reference sequence (Dunn *et al.*, 2010) within a databank such as Barcode Of Life Database (BOLD). When using stable isotopes, isotopic ratios are affected by preservation techniques (Rau *et al.*, 2003), nutritive stress changes (Bond and Jones, 2009), rate of metabolism (Sears *et al.*, 2009) and even latitudinal gradients (Quillfeldt *et al.*, 2005). Fatty acid composition of the inner fat layer is generally believed to be more metabolically active and thus, reflects diet better (Olsen and Grahl-Nielsen, 2003). Hence, this method is hindered by the difficulty of sampling the predator's inner fat layer. Selective metabolism and biosynthesis by the predator also needs to be quantitatively accounted for by

forming calibration factors from captive feeding studies (Iverson *et al.*, 2004). Visual observation techniques are generally difficult with large and/or pelagic species that need to be observed in open waters (Herman *et al.*, 2005).

Novel methods, such as stable isotopes, have advantages such as the ability to assess assimilated and not just ingested prey (Estrada *et al.*, 2006), to distinguish between inshore and offshore feeding patterns (France, 1995) and to evaluate long-term feeding behaviours (Post, 2002; Peterson and Fry, 2015). Unfortunately, these modern methods are often not comparable with studies done before their inception. For this reason, when historical data are available for a study species, the use of commensurable methods is of more benefit for comparative purposes.

Besides the confounding influence of different methods, dietary comparisons between marine populations are complicated by a variety of extrinsic and intrinsic factors. Extrinsic factors include prey availability and/or risk of predation (Perry and Pianka, 1997). The higher the species diversity in a marine ecosystem, the greater the number of potential prey species available to predators (Petchey, 2000). The latitudinal diversity gradient states that species richness increases towards the equator (Mittlebach, 1986), which is significantly correlated with mean sea surface temperature (Roy *et al.*, 1998). The theory behind this is known as the temperature hypothesis, which suggests that increased temperature and therefore metabolism, supports greater speciation rates, culminating in higher diversity (Rohde, 1992). In a study of 11 567 species across 13 taxa, sea surface temperature was the only ecological predictor correlated to the diversity across all taxa thereby supporting this hypothesis (Tittensor *et al.*, 2010).

Seasonality, particularly in temperate regions, leads to fluctuations in temperature, dissolved oxygen and salinity (Abrahams *et al.*, 2007). This may, in turn, lead to changes in prey abundance and thus predator feeding behaviour (Pulliam, 1974). For example, leopard sharks (*Triakis semifasciata*) are seasonally abundant in Humboldt Bay (Ebert and Ebert, 2005) and Elkhorn Slough, California, during spring, summer and autumn (Yoklavich *et al.*, 1991). The absence of this species during winter has been attributed to their prey availability, reproduction, temperature and salinity variations (Hopkins and Cech, 1994; Carlisle and Starr, 2009). For these reasons, dietary data need to be collected during all seasons to gain a comprehensive understanding of the dietary differences amongst populations and in different seasons.

Intrinsic factors such as age (size), sex, reproduction, epigenetic inheritance, dietary preference and nutritional requirements can affect feeding ecology (Perry and Pianka, 1997). An ontogenetic dietary shift is a change in the feeding of a species that coincides with its growth (Lowe *et al.*, 1996; Kamura and Hashimoto, 2004) due to variations in prey capture success rates (Norton, 1991) and feeding behaviour (Ferry-Graham *et al.*, 2002) of growing organisms. Body size is one of the key characteristics that determines food acquisition (Lucifora *et al.*, 2009). Larger predators will have larger mouths and increased energy requirements (Cohen *et al.*, 1993). Engen and Stenseth (1989), stated that larger sharks are able to feed on bigger prey; larger sharks tend to have greater nutritional requirements as maturity and reproductive processes increase their energy demand (Robbins, 1983). Larger and older sharks may also just be more experienced hunters allowing them to pursue and feed on species that are more difficult to catch (Rutz *et al.*, 2006). Evidence of dietary ontogenetic shifts have been described in a number of shark species, including: *Triakis semifasciata* and *Chiloscyllium plagiosum* (Lowry, 2005; Lowry *et al.*, 2007); *Galeocerdo cuvier* (Lowe *et al.*, 1996; Simpfendorfer *et al.*, 2001); *Carcharodon carcharias* (Estrada *et al.*, 2006); *Notorynchus cepedianus* (Ebert, 2002)*; Carcharhinus plumbeus* (Ellis, 2003; McElroy *et al.*, 2006)*; Rhizoprionodon terraenovae* (Hoffmayer and Parsons, 2003; Bethea *et al.*, 2004); *Carcharhinus limbatus* (Bethea *et al.*, 2004), *Squalus megalops* (Braccini *et al.*, 2005), *Sphyrna tiburo* (Bethea *et al.*, 2007); and *Hexanchus griseus* (Andrews *et al.*, 2010). As dietary ontogenetic shifts appear relatively common in elasmobranchs, similar size classes should be used when comparing the diets of sharks from different populations.

Sharks are generally considered to be asynchronous opportunistic predators, feeding on the most abundant prey item available to them (Motta and Wilga, 2001). Although, more recently, with the use of newer techniques such as stable isotopes, there is evidence of specialization (e.g. bull sharks, *Carcharhinus leucas* Müller and Henle, 1839), although this is not ubiquitous and is dependent on spatial overlap, competition, food-predation risk trade-offs and resource availability (Matich *et al.*, 2011). Sharks will find, catch and eat to maximize calorie intake with minimal energy loss, according to the optimal foraging theory (OFT; MacArthur and Pianka, 2014). According to this theory, predators will give preference to prey that yields more energy per unit of handling time; lesser value prey will not feature in a predator's diet when a greater abundance of higher value prey is available (Pulliam, 1974). Generally, teleosts and cephalopods are the most common prey items in the diet of elasmobranchs (Wetherbee *et al.*, 2004). Small sharks associated with reef systems generally prey on invertebrates such as crabs, shrimps, squids, and small fishes, while the larger reef

dwelling sharks prey on larger bony fishes and molluscs (Tricas *et al.*, 2002). Amongst the triakids, the leopard shark is a ram-suction (Ferry-Graham, 1998), opportunistic, generalist feeder preying on a wide variety of prey including benthic invertebrates (Ackerman, 1971; Talent, 1976; Kao, 2000). Occasionally, elasmobranchs are also included in the diet of this species (Ackerman, 1971; Talent, 1976; Ebert and Ebert, 2005). The diets of leopard sharks in California differ between locations. In Humboldt Bay, juvenile leopard sharks consume fish eggs, while adults shift their diets to feed on crustaceans (Ebert and Ebert, 2005). According to Talent (1976), this same species in Elkhorn Slough shows a dietary shift from predominantly crabs (<800 mm) to worms and clams (>800 mm). Over two decades later, however, another study in Elkhorn Slough reported that the ontogenetic dietary shift previously recorded was no longer evident (Kao, 2000). Only one analogous study could be found, on *T. scyllium* in Japan (Kamura and Hashimoto, 2004). Results show this species to feed on infaunal and epifaunal benthos where smaller (<700 mm) sharks favour the smaller prey such as innkeeper worms and shrimp, while larger (>701 mm) sharks only fed on larger prey such as octopus.

To date, only one study has examined the feeding habits of *Triakis megalopterus* and this was done in the Eastern Cape of South Africa (Smale and Goosen, 1999). According to these authors, *T. megalopterus* appears to be a nocturnal feeder, with a diet consisting of crustaceans, cephalopods, teleosts and small elasmobranchs. There is presently no information available on the diet of *T. megalopterus* in the rest of South Africa (i.e. southern and Western Cape) or in southern Angola. The lack of feeding studies limits the knowledge of influences on the trophic dynamics of marine ecosystems (Cortés and Gruber, 1990). The data collected for this study provide a unique opportunity to examine the differences in feeding ecology of an elasmobranch across three biogeographic zones, in two countries, spanning the Atlantic and Indian oceans, the Angolan, Benguela and Agulhas ocean currents and both warm and cool temperate regions.

The objectives of this chapter are to 1) describe the diet of *T. megalopterus* from South African (Western and Eastern Cape) and southern Angola populations and examine ontogenetic shifts and seasonal variability; 2) to investigate medium-term temporal changes in the diet of *T. megalopterus* in the Eastern Cape and relate this information to the phenotypic and genotypic differences between the three populations.

Material and methods

Data collection and diet description

A total of 119 *T. megalopterus* stomachs were analysed from AN (n = 40), WC (n = 33) and EC (n = 46) between November 2011 and February 2013. Eviscerated mass of the sharks were recorded to the nearest 0.1 g. Stomachs were removed by severing the oesophagus and the start of the spiral valve intestine. Where possible, stomach contents were analysed fresh. If stomachs had to be preserved for later analysis, the otoliths in the contents were removed, dried, and stored in Eppendorf tubes. Beaks were cleaned of excess tissue and stored in 70% ethanol. The remainder of the contents was then stored in 10% formalin. Freshly processed stomachs were weighed whole then cut open and the contents removed. Bait was immediately removed and excluded from subsequent analysis. Prey were sorted, enumerated and identified to the lowest taxonomic level possible. If prey items were not whole, the numerical estimates were based on countable parts, such as claws and legs for crustaceans, otoliths for fishes, and beaks for cephalopods. Stomach contents were sieved to drain excess fluid, and weighed to the nearest 0.01 g. Unidentifiable matter was weighed separately. All prey items were also measured. Total length (TL) for teleosts, carapace width (CW) and length (CL) for crustaceans and mantle width (MW) and length (ML) for cephalopods, henceforth termed as "prey size".

Diet quantification

Prey items were quantified using a range of common indices (Hyslop, 1980). These included percent frequency of occurrence (%O), which is the number of stomachs containing a specific prey item divided by the total number of stomachs containing prey. This index reflects the proportion of predators utilizing a prey resource, or the homogeneity of the hunting method (Cortés, 1997). Occurrence does not however give any information on the number or quantity of prey nor does it consider digestion rates of prey items. Percent frequency of occurrence (%O) was calculated as:

$$\%\boldsymbol{O_i} = \frac{\boldsymbol{J_i}}{\boldsymbol{P}} \times \mathbf{100}$$

where:

J_i = number of fish containing prey item i

P = number of fish with food in their stomach.

Percent abundance or number (%N) is the total number of prey items within each category divided by the total number of individual prey items. Although this index generally provides information on the feeding behaviour of a population (Zacharia, 2004), no allowance is made for the size differences between food items. Percent number (%N) was calculated as:

$$\%\boldsymbol{N_i} = \frac{\boldsymbol{N_i}}{\sum_{\boldsymbol{i1}}^{\boldsymbol{Q}} \boldsymbol{N_i}}$$

where:

N_i = number of food category *i*

The percent gravimetric index (%W) is the weight of a prey category divided by the total weight of all prey items. This method generally provides information on the nutritional importance of the different dietary items (Zacharia, 2004), however, digestion makes this method difficult, as prey items are not always whole. Percentage weight was calculated as:

$$\%\boldsymbol{W_i} = \frac{\boldsymbol{W_i}}{\sum_{\boldsymbol{i1}}^{\boldsymbol{Q}} \boldsymbol{W_i}}$$

where:

W_i = number of food category *i*

Each of these three measures (%O, %N and %W) provides a different insight into the feeding habits of the organism in question (Cortés, 1997). When considered separately, these three indices reflect a bias toward highly abundant prey (%O), small/digested prey (%N) or infrequent, large, non-digested prey (%W). Factors such as prey type, meal size, and evacuation rates (Bush and Holland, 2002) may also obscure prey importance.

Thus it is suggested that a compound index of all the above indices be used, the most popular of these being the index of relative prey importance (IRI; Pinkas *et al*., 1971):

$$\boldsymbol{IRI_i} = (\%\boldsymbol{N_i} + \%\boldsymbol{W_i})\,\%\boldsymbol{O_i}$$

The IRI value was converted into a percentage, for ease of comparison among food types, using the following equation (Cortés, 1997):

$$\%IRI = \frac{100\ IRI_i}{\sum_{i=1}^{n} IRI_i}$$

where:

n = total number of food categories at a given taxonomic level.

There is, however, much controversy regarding the accuracy of these types of compound indices. Not only do they use average values from different measures, they also combine the variation and errors associated with them (Hyslop, 1980). Thus, IRI has solely been included in this thesis to enable direct comparison with other studies.

Ontogenetic shift

Sharks from which samples were collected were divided into three size classes: small (<999 mm), medium (1000–1399 mm) and large (>1400 mm) following Smale and Goosen (1999). The N%, %W, %O and %IRI for each prey category and size class was calculated. The %N data were standardized, square root transformed and subjected to Bray–Curtis similarity analyses (Bray and Curtis, 1957), group averaged clustering and multidimensional scaling (MDS; Clarke and Warwick, 2001) ordination using Primer v6. The MDS was included as the data are not forced into a hierarchy as with the cluster analysis, thus MDS is less constraining (Shepard, 1980). For the MDS, a minimum stress of 0.01 and 25 restarts were used and agglomerative cluster analysis based on the Bray–Curtis similarity coefficients was calculated. Significant differences of size classes were tested using the one-way analysis of similarity (ANOSIM) in Primer v6. Differences between sites were considered significant at $p<0.05$. The extent of significant differences was determined by the *R*-statistic (Clarke and Green, 1988), which ranges between zero (no similarity) and one (100% similarity), thus the *R* value indicates no similarity and 100% resemblance respectively. A one-way analysis of variance (ANOVA) was done in IBM SPSS Statistics 20 to test for significant differences in the %N of prey families between size classes. When the ANOVA indicated significant differences (*T. megalopterus* 0.05), a Tukey HSD post hoc test was performed. All of the data were analysed by the family each prey species belonged to.

Diet seasonality

Sampling in AN only took place in summer (November/December 2011) and winter (June/July 2012). For this reason, and for the purpose of direct comparison, all sampling localities were only analysed using summer (December–February) and winter (March–August) for the seasonal analysis. The %N, %W, %O and %IRI for each prey category, in each season (summer and winter), was calculated. Data for %N was standardized, square root transformed and subjected to Bray–Curtis similarity analyses (Bray and Curtis, 1957), group averaged clustering and two-dimensional non-metric Multidimensional Scaling (MDS; Clarke and Warwick, 2001) ordination. Independent t-tests were used to test the seasonal differences within each locality. Again, all of the data were analysed according to the family each prey species belonged to.

Results and interpretation

Of the 119 stomachs that were analysed, nine were empty. Only one small (470 mm) individual was caught in WC. Unfortunately, this was one of the specimens that had an empty stomach. For this reason, small sharks in WC could not be included in the analyses.

All populations of *T. megalopterus* preyed upon teleosts, crustaceans and molluscs. A total of 19 prey species belonging to seven families of teleost, eight families of crustaceans, one family of elasmobranch and three mollusc families were observed (**Table 5.1**). However, this may be an underestimate as there was a large amount of digested and unidentifiable prey in the stomachs. The elasmobranchs' stomach contents included egg cases of *Haploblepharus* spp., as well as miscellaneous items such as a shell (*Urosalpinx subsinuatus*), small black stone, barnacle (*Tetraclita serrata*), and pieces of aquatic plant (Phaeophyceae) were also present. These miscellaneous items were considered accidental ingestions, thus not included in the analyses. From WC stomach contents, besides one *Octopus vulgaris* beak, no digested and/or unidentifiable matter was present and all prey was whole and easily identifiable.

Ontogenetic change

There were significant differences in the mean size of teleost (ANOVA, $F(2.27) = 29.12$, $p<0.01$) and crustacean (ANOVA, $F(2.27) = 12.44$, $p<0.01$) in the diet of small, medium and large *T. megalopterus* (**Figure 5.1**). A Tukey post-hoc test revealed that teleosts in the diet of the smaller size class were significantly smaller than in the medium ($p<0.01$) and large ($p<0.01$) size classes. Crustaceans were significantly different when comparing small and

large classes (Tukey post-hoc test, $p<0.01$). There were no significant difference in the size of the molluscan prey between the size classes (ANOVA, $F(2.27) = 0.68$, $p = 0.54$).

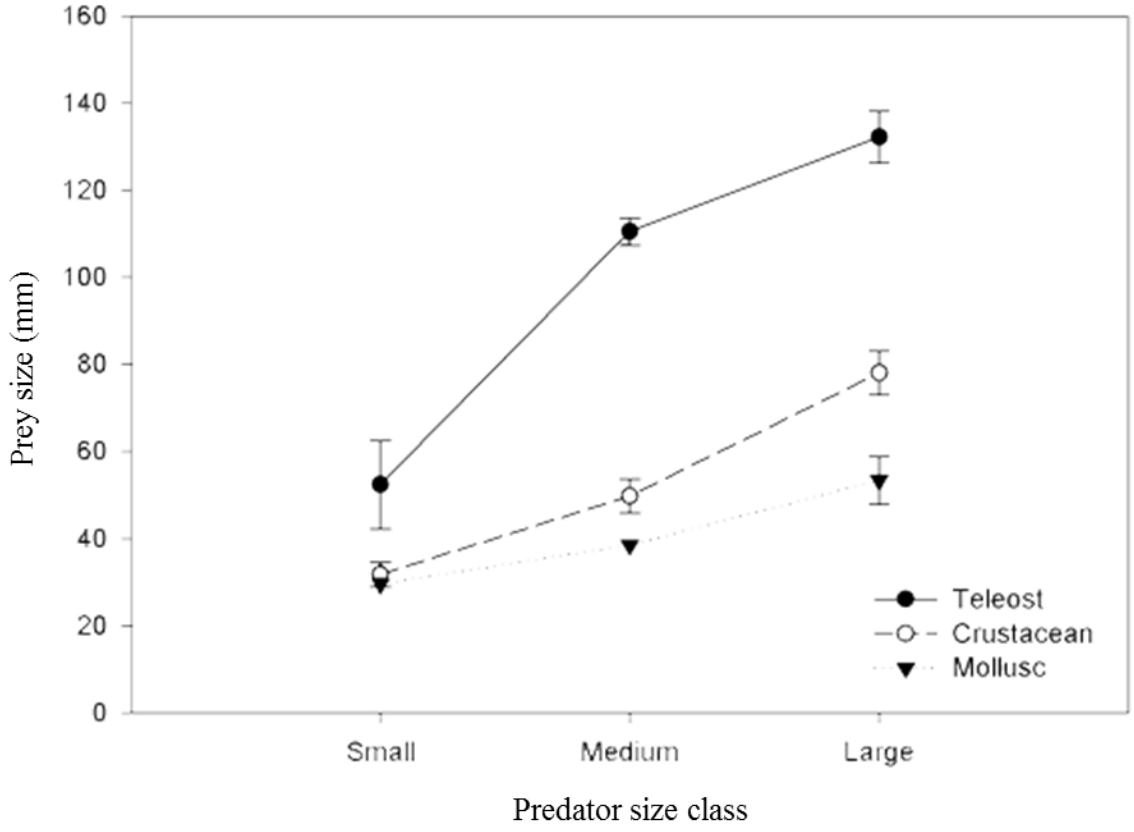

Figure 5.1: Relation between predator size class and prey size (teleost = TL, crustacean = CW and mollusc = MW); *TL = total length, CW = carapace width, ML = mantle length, error bars represent standard error*

Prey diversity for all size classes (**Table 5.1**) increased closer to equator, from WC to EC and AN with three, seven and 17 species present, respectively (excluding miscellaneous items). Stomach contents from WC contained no unidentifiable material. Both EC and AN had extensively digested material present. Highest quantities of unidentifiable material were in EC, from the small and large (%N = 6.67) to medium (%N = 7.14) specimens. Angolan specimens showed unidentifiable material of between %N = 3.57 and 6.06 for medium and large specimens, respectively.

Although teleosts dominated the diet of all size classes of *T. megalopterus* in AN, they were absent in the diet of EC-small, EC-medium and all WC specimens. The importance of teleosts decreased in the diet of *T. megalopterus* with size, while the importance of crustaceans (AN-medium, %N = 4.36) and molluscs (AN-large, %N = 19.12) increased. Although all size

classes of *T. megalopterus* in Angola fed on *Sardinella aurita*, it was less important in the diet of the larger individuals. The smallest sharks (AN-small) fed mainly on the small *Parablennius pilicornis* (%N = 17.1), while the largest sharks (AN-large) fed on the bigger and noxious *Galeichthys feliceps* (%N = 16.2). The only teleost identified in the stomach contents of *T. megalopterus* from EC was *Galeichthys* sp. which was observed in the diet of the large sharks. Elasmobranch (*Squalus* sp.) egg cases (%N =2.0) were also found in the diets of large *T. megalopterus*, but only in the large AN specimens.

Crustaceans were present in the diet of *T. megalopterus* from all size classes and at all locations, although they were most dominant in both South African EC and WC populations. The Cape rock lobster (*Jasus lalandii*) dominated the diet of WC individuals (%N >98.1), while the only other crustacean identified from the WC was one crab, *Plagusia chabrus* (%N = 1.9). The number of crustaceans ingested decreased with the size of the EC specimens (small %N = 86.7, medium %N = 64.3, large %N = 46.7). In EC, *P. chabrus* dominated the crustacean prey category for all size classes, whereas *Metacarcinus magister* was the most favoured crustacean for AN-small (%N = 4.9) and AN-medium (%N = 14.3), while AN-large *T. megalopterus* consumed more *Ovalipes trimaculates* (%N = 4.0).

Molluscs were present in the diet of *T. megalopterus* from all three populations. In AN, the common octopus (*Octopus vulgaris)* was found in the stomach contents of all size classes though the most were found in the large individuals (%N = 26.3). Octopus were only present in the large size class from WC (%N = 1.9) and EC (%N = 6.7). Abalone (*Haliotis midae*) flesh was found in the stomach contents of EC-medium (%N = 14.3) and EC-large (%N = 6.7). No abalone was found in WC or AN specimens. Squid (*Loligo reynaudii*) were only found in AN-medium (%N = 3.6) and AN-large (%N = 3.0) specimens.

Table 5.1: Diet quantification indices (%N, %W, %O and %IRI) for small, medium and large *T. megalopterus* from Western Cape, Eastern Cape and Angola; *%N = percent number, %W = percent weight, %O = percent frequency of occurrence, %IRI = percent index of relative importance*

	Western Cape								Eastern Cape												Angola							
	Medium				Large				Small				Medium				Large				Small				Medium				Large			
	%N	%W	%O	%IRI	%N	%W	%O	%IRI	%N	%W	%O	%IRI	%N	%W	%O	%IRI	%N	%W	%O	%IRI	%N	%W	%O	%IRI	%N	%W	%O	%IRI	%N	%W	%O	%IRI
Teleost																	20.00	31.82	21.43	24.51	70.73	65.37	45.45	74.59	67.86	53.83	60.00	88.84	49.49	34.41	40.54	34.52
Ariidae																																
Galeichthys sp.																	13.33	10.24	14.29	15.33									16.16	22.78	16.22	21.98
Sparidae																																
Diplodus sargus capensis																													3.03	0.07	4.05	0.44
Clupeidae																																
Sardinella aurita																					14.63	20.88	9.09	11.49	7.14	10.77	13.33	5.10	7.07	6.94	5.41	2.64
Bleniidae																																
Parablennius pilicornis																					17.07	17.04	9.09	11.04								
Sciaenidae																																
Atractoscion aequidens																													1.01	0.01	1.35	0.05
Haemulidae																																
Pomadasys olivaceum																													1.01	0.00	1.35	0.05
Ammodytidae																																
Ammodytes sp.																					2.44	0.25	4.55	0.44	3.57	3.41	6.67	0.99	1.01	0.04	1.35	0.05
Unidentified Teleost																	6.67	21.58	7.14	9.18	36.59	27.20	22.73	51.62	57.14	39.66	40.00	82.75	20.20	4.58	10.81	9.32
Elasmobranchs																													2.02	0.08	2.70	0.20
Squalidae																																
Squalus sp.																													2.02	0.08	2.70	0.20
Crustaceans	100.00	100.00	100.00	100.00	98.11	99.87	96.43	99.96	86.67	97.87	83.78	99.12	64.29	87.55	44.44	89.56	46.67	59.49	42.86	61.83	17.07	23.72	36.36	17.93	21.43	25.27	20.00	6.65	14.14	10.76	18.92	3.08
Polybiidae																																
Ovalipes trimaculatus																									3.57	21.11	6.67	3.52	4.04	4.28	5.41	1.57
Squillidae																																
Pterygosquilla sp.																													2.02	0.89	2.70	0.27
Cancridae																																
Metacarcinus magister																					4.88	1.05	9.09	1.92	14.29	3.65	6.67	2.56	1.01	1.22	1.35	0.10
Scyllaridae																																
Scyllarides elisabethae																	6.67	27.67	7.14	11.16												
Caridea									4.44	0.71	5.41	0.24													3.57	0.51	6.67	0.58	1.01	0.00	1.35	0.05
Xanthidae																																
Atergatis roseus																													2.02	1.94	2.70	0.37
Plagusiidae																																
Plagusia chabrus					1.89	0.58	3.57	0.05	73.33	92.69	67.57	98.08	64.29	87.55	44.44	89.56	13.33	9.46	7.14	7.41	7.32	7.27	18.18	9.45								
Palinuridae																																
Jasus lalandii	100.00	100.00	100.00	100.00	96.23	99.28	92.86	99.91	2.22	1.02	2.70	0.08					20.00	22.03	21.43	40.99									1.01	1.06	1.35	0.10
Unidentified Crustacean									6.67	3.46	8.11	0.72					6.67	0.33	7.14	2.27	4.88	15.40	9.09	6.56					3.03	1.37	4.05	0.62
Molluscs					1.89	0.13	3.57	0.04					14.29	8.32	22.22	6.67	13.33	6.49	14.29	6.44	7.32	8.27	9.09	5.05	7.14	13.06	13.33	2.88	26.26	51.35	27.03	59.44
Cephalopods																																
Octopodidae																																
Octopus vulgaris					1.89	0.13	3.57	0.04									6.67	0.00	7.14	2.17	7.32	8.27	9.09	5.05	3.57	0.00	6.67	0.51	23.23	50.40	22.97	58.88
Loliginidae																																
Loligo vulgaris reynaudii																									3.57	13.06	6.67	2.37	3.03	0.95	4.05	0.56
Gastropods																																
Haliotidae																																
Haliotis midae													14.29	8.32	22.22	6.67	6.67	6.49	7.14	4.28												
Miscellaneous									13.33	2.13	16.22	0.88	21.43	4.13	33.33	3.77	20.00	2.20	21.43	7.22	4.88	2.64	9.09	2.43	3.57	7.84	6.67	1.63	8.08	3.40	10.81	2.76
Unidentified material									6.67	2.01	8.11	0.61	7.14	3.86	11.11	1.62	6.67	1.97	7.14	2.81	4.88	2.64	9.09	2.43	3.57	7.84	6.67	1.63	6.06	3.36	8.11	2.66
Tetraclitidae																																
Tetraclita serrata													7.14	0.00	11.11	1.05	6.67	0.00	7.14	2.17									1.01	0.00	1.35	0.05
Muricidae																																
Urosalpinx subsinuatus																													1.01	0.04	1.35	0.05
Black Stone									2.22	0.12	2.70	0.06																				
Phaeophyceae									4.44	0.00	5.41	0.21	7.14	0.27	11.11	1.09	6.67	0.23	7.14	2.24												

Regional differences

Dietary differences were clearly evident in terms of regions and size as depicted in the Bray–Curtis similarity matrix-based cluster analysis (**Figure 5.2 a**) and MDS plot (**Figure 5.2 b**) using %N. Differences in the diet of *T. megalopterus* were overall statistically significant in all three populations and their size classes (ANOSIM, R = -0.32, $p = 0.94$).

Cluster analysis of %N indicated an 18.2% similarity between WC and EC and 13.3% similarity between the South African and AN populations. The highest similarity was in WC-medium and WC-large (87.0%), where both size classes predominantly fed on *J. lalandii*. In EC, the diet of small and medium specimens was 62.1% similar with large quantities of *P. chabrus* included in the diet of both of these size classes. The diets of EC-large specimens, however, were only 36.5% similar to the combined small and medium classes, indicative of the diet expansion of large individuals to include teleosts and *O. vulgaris*. The diets of AN specimens were comparable in all size classes with a similarity of AN-large (50.8%) compared to small and medium (59.0%), as small, medium and large specimens all preyed upon all prey categories with only the prey diversity increasing.

Additional cluster analyses, where the three size classes were individually compared amongst locations, revealed that small specimens from EC and AN (no data for WC-small was available) showed a 24.1% similarity. Though both populations of small specimens fed on Plagusiidae, the quantity thereof was tenfold larger in EC (%N = 73.3) than in AN (%N = 7.3). There was zero similarity amongst all three sample sites for medium specimens as AN, WC and EC fed on unidentified teleosts (%N = 57.1), Palinuridae (%N = 100.0) and Plagusiidae (%N = 64.3), respectively. Large individuals from AN and EC grouped together with 40.7%, whereas both of these populations only showed a 24.4% similarity with WC.

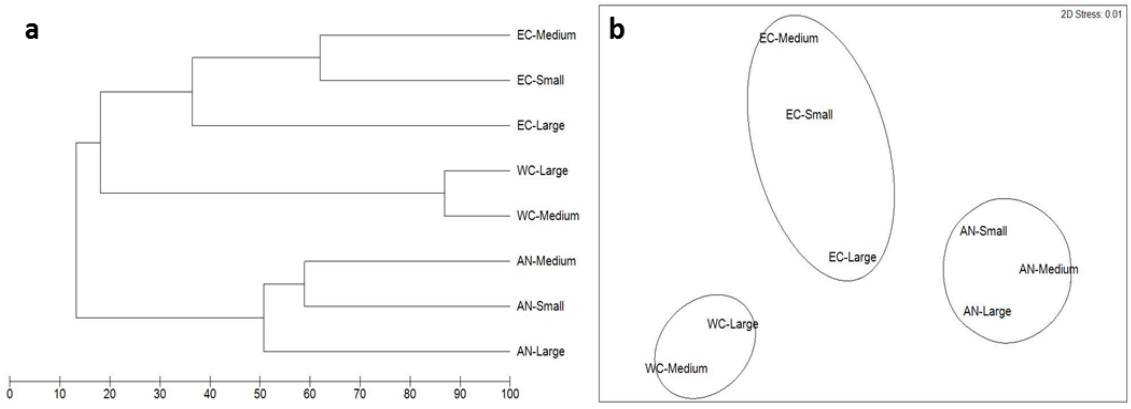

Figure 5.2: Size class analysis for the %N of prey family similarity displaying the (a) Bray–Curtis similarity matrix-based cluster analysis and (b) a two dimensional representation of the MDS plot depicting a 30% resemblance level; *%N = percent number, AN = Angola, EC = Eastern Cape, WC = Western Cape, MDS = multidimensional scaling.*

Diet seasonality

There were no seasonal differences in the diet of *T. megalopterus* in WC (**Table 5.2**), *J. lalandii* dominated the diet all year (%N >95.5). Seasonal changes were, however, present in EC and AN diets. In EC, no teleosts or molluscs were consumed in winter. Crustacean ingestion, however, increased from summer (%N = 64.4) to winter (%N = 86.2) mainly due to the increased intake of *Plagusia chabrus* (%N = 21.3%) and in the winter only Caridea (%N = 6.9) were consumed. Several prey items including *J. lalandii* (%N = 8.9), *Galeichthys sp.* (%N = 4.4), *Scyllarides elisabethae* (%N = 2.2), *O. vulgaris* (%N = 2.2), and *H. midae* (%N = 6.7) were only present in the diet of *T. megalopterus* in summer.

In AN, *T. megalopterus* fed on teleosts, crustaceans and molluscs in similar quantities in both summer and winter. The largest change in diet of AN specimens was a %N = 9.83 increase in molluscs intake during summer. Unlike in EC, *Galeichthys sp.* were present in AN during both seasons, although their numbers doubled in summer (%N = 11.1). Both *P. pilicornis* (%N = 13.5) and *Ammodytes sp.* (%N = 5.8) were only present in summer diets of *T. megalopterus*, while *Atractoscion aequidens* (%N = 0.9), *Diplodus capensis* (%N = 2.56) and *Pomadasys olivaceum* (%N = 0.9) were only present in the winter diets of AN specimens.

Table 5.2: Diet quantification indices (%N, %W, %O and %IRI) separated by summer and winter from individuals of WC, EC and AN; *%N = percent number, %W = percent weight, %O = percent frequency of occurrence, %IRI = percent index of relative importance*

	Western Cape								Eastern Cape								Angola							
	Summer				Winter				Summer				Winter				Summer				Winter			
	%N	%W	%O	%IRI	%N	%W	%O	%IRI	%N	%W	%O	%IRI	%N	%W	%O	%IRI	%N	%W	%O	%IRI	%N	%W	%O	%IRI
Teleost									**4.44**	**16.63**	**5.88**	**1.31**					**57.26**	**54.07**	**46.75**	**61.22**	**57.69**	**22.07**	**39.39**	**32.83**
Ariidae																								
Galeichthys sp.									2.22	4.76	2.94	0.43					11.11	28.42	11.69	17.00	5.77	12.47	9.09	8.79
Sparidae																								
Diplodus sargus capensis																	2.56	0.12	3.90	0.39				
Clupeidae																								
Sardinella aurita																	6.84	9.40	7.79	4.65	13.46	6.46	6.06	6.40
Bleniidae																								
Parablennius pilicornis																					13.46	1.47	6.06	4.79
Sciaenidae																								
Atractoscion aequidens																	0.85	0.01	1.30	0.04				
Haemulidae																								
Pomadasys olivaceum																	0.85	0.00	1.30	0.04				
Ammodytidae																								
Ammodytes sp.																					5.77	0.58	9.09	3.06
Unidentified Teleost									2.22	11.87	2.94	0.88					35.04	16.10	20.78	39.10	19.23	1.10	9.09	9.79
Elasmobranchs																					**3.85**	**0.13**	**6.06**	**1.28**
Squalidae																								
Squalus sp.																					3.85	0.13	6.06	1.28
Crustaceans	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**64.44**	**74.33**	**58.82**	**91.58**	**86.21**	**99.53**	**83.33**	**99.22**	**15.38**	**14.60**	**19.48**	**2.97**	**19.23**	**10.47**	**27.27**	**14.89**
Polybiidae																								
Ovalipes trimaculatus																	0.85	2.02	1.30	0.14	7.69	8.35	12.12	10.31
Squillidae																								
Pterygosquilla sp.																	1.71	1.65	2.60	0.32				
Cancridae																								
Metacarcinus magister																	5.13	2.83	3.90	1.14	1.92	0.09	3.03	0.32
Scyllaridae																								
Scyllarides elisabethae									2.22	15.23	2.94	1.09												
Caridea													6.90	1.38	8.33	0.61	1.71	0.08	2.60	0.17				
Xanthidae																								
Atergatis roseus																	1.71	3.60	2.60	0.51				
Plagusiidae																								
Plagusia chabrus	4.55	1.81	10.00	0.36					51.11	46.48	41.18	85.03	72.41	93.43	66.67	97.75	1.71	0.28	2.60	0.19	3.85	0.37	3.03	0.68
Palinuridae																								
Jasus lalandii	95.45	98.19	90.00	99.64	100.00	100.00	100.00	100.00	8.89	12.45	11.76	5.31					0.85	1.97	1.30	0.14				
Unidentified Crustacean									2.22	0.18	2.94	0.15	6.90	4.73	8.33	0.86	1.71	2.17	2.60	0.37	5.77	1.66	9.09	3.58
Molluscs									**8.89**	**6.00**	**11.76**	**2.50**					**21.37**	**25.49**	**24.68**	**31.85**	**11.54**	**65.56**	**15.15**	**48.60**
Cephalopods																								
Octopodidae																								
Octopus vulgaris									2.22	0.00	2.94	0.14					18.80	21.67	20.78	30.94	9.62	65.56	12.12	48.29
Loliginidae																								
Loligo vulgaris reynaudii																	2.56	3.82	3.90	0.92	1.92	0.00	3.03	0.31
Gastropods																								
Haliotidae																								
Haliotis midae									6.67	6.00	8.82	2.37												
Miscellaneous									**22.22**	**3.03**	**23.53**	**4.60**	**13.79**	**0.47**	**16.67**	**0.78**	**5.98**	**5.84**	**9.09**	**3.95**	**7.69**	**1.76**	**12.12**	**2.41**
Unidentified material									8.89	2.82	11.76	2.92	3.45	0.23	4.17	0.14	5.98	5.84	9.09	3.95	3.85	1.70	6.06	1.78
Tetraclitidae																								
Tetraclita serrata									8.89	0.00	5.88	1.11									1.92	0.00	3.03	0.31
Muricidae																								
Urosalpinx subsinuatus																					1.92	0.06	3.03	0.32
Black Stone													3.45	0.23	4.17	0.14								
Phaeophyceae									4.44	0.21	5.88	0.58	6.90	0.00	8.33	0.51								

Seasonal differences in the feeding of *T. megalopterus* showed significant separation of all populations and seasons (ANOSIM, R = -0.4, p = 1.0). The seasonal cluster analysis (**Figure 5.3 a**) showed the similarity of summer and winter diets of *T. megalopterus* were 52.0%, 59.5% and 89.2% for EC, AN and WC, respectively. The MDS plot (**Figure 5.3 b**) clearly showed a split between all three populations at the 25% resemblance level. This, as with the ontogeny, showed the differences to be more related to location rather than season.

Only one specimen of *T. megalopterus* was obtained from the WC in winter, its stomach contents, which comprised *J. lalandii,* were similar to those captured in spring (n = 12) and autumn (n = 7). Therefore, although limited in data availability, it is unlikely that the diet during winter would change in this region. All specimens caught within the summer and winter months fed predominantly on *J. lalandii*, with one *P. chabrus* present in the stomach contents of a summer-sampled specimen. Only one WC specimen was found to have ingested an octopus (*O. vulgaris*); this specimen was caught in spring and therefore not included in the seasonal analysis. The largest seasonal change was in EC, where no teleosts or molluscs were found in the winter diet.

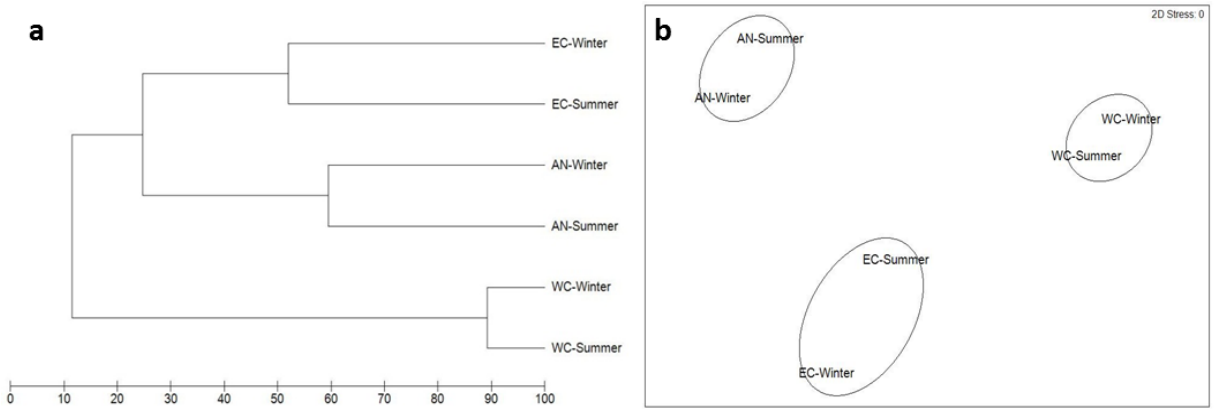

Figure 5.3: Seasonal analysis for %N of prey family similarity displaying the (a) Bray–Curtis similarity matrix-based cluster analysis and (b) a two dimensional representation of the MDS plot depicting a resemblance level of 25%; *%N = percent number, AN = Angola, EC = Eastern Cape, WC = Western Cape, MDS = multidimensional scaling*

Discussion

All populations of *T. megalopterus* seem to be benthic foragers, predominantly feeding on teleosts, crustaceans and molluscs. Stomach contents of specimens caught by day (AN and EC) had digested matter, which was not found in specimens caught at night (WC); this and the presence of nocturnal prey in stomach contents shows *T. megalopterus* is most likely a

predominantly nocturnal feeder. Although the warmer water in AN and EC may cause faster digestion rates (Kao, 2000), Smale and Goosen (1999) also suggested the EC population of *T. megalopterus* feed predominantly at night.

From a prey diversity perspective, Smale and Goosen (1999) identified 34 prey species in the diet of *T. megalopterus* in the EC. Their study was, however, conducted over a broader geographic range (Cape St. Francis to Coffee Bay), over a 12 year period and included an analysis of 110 stomachs. In this study, specimens were collected over a two year period and only 10 prey species were recorded in an analysis of 46 stomachs. Comparison of these two studies supports the suggestion that a higher diversity of prey items will be found in studies with larger sample sizes (Wetherbee *et al.*, 2004). Although Smale and Goosen (1999) found that *T. megalopterus* fed on elasmobranchs (*Haploblepharus fuscus* and *Rhinobatos* sp.), none were found in the stomach contents of EC specimens in this study. Nevertheless, both studies showed patterns consistent with a generalist benthic feeder with teleosts, crustaceans and molluscs the dominant prey items.

The diets of *T. megalopterus* from AN and EC comprised teleosts, crustaceans and molluscs. In the WC, however, these sharks prey almost exclusively on Cape rock lobster (*J. lalandii*). This is not surprising as this rock lobster is also nocturnal (Fielder, 1965) and occupies the same reef habitat (Booth and Phillips, 1994). The Cape rock lobster is also highly abundant in Betty's Bay where the WC sampling site was located. This is a consequence of increased population sizes of *J. lalandii* along the southern and eastern coasts since the early 1990s, which saw this species shift its distribution range eastwards to the east of Cape Hangklip (Tarr *et al.*, 1996; Turpie *et al.*, 2003; Cockcroft *et al.*, 2008; Blamey and Branch, 2012). The incursion of lobster into the Cape Hangklip area initiated a regime shift whereby the ecosystem, previously dominated by coralline algae and herbivores, is now dominated by lobster and macroalgae (Blamey *et al.*, 2010). The increase in numbers of *J. lalandii* is thought to be a consequence of the overexploitation of its main predators, reef fish and the Cape fur seal (*Arctocephalus pusillus*). The exploitation of the cape fur seal population began in the 18th century (Shaughnessy, 1984) and resulted in the population decreasing to less than 100 000 individuals (Shaughnessy and Butterworth, 1981). Since 1993, however, the Cape fur seal populations have been relatively stable with a population size of approximately 1.7 million (Kirkman, 2010) and at a status of least concern (Hofmeyr and Gales, 2008). Although the seal population has recovered, it does not appear that they have managed to control the lobster population. This is perhaps due to the eastward shift of the lobsters, while the bulk of the seal population remains on the west coast (David, 1989). The size of the

lobster population has had a profound effect on the sea urchin and juvenile abalone populations. Due to predation by the Cape rock lobster, the sea urchin populace collapsed in Betty's Bay and Mudge Point, South Africa in 1994 (Tarr *et al.*, 1996). As juvenile abalone shelter under sea urchins (Tarr, 1995), the collapse of the sea urchin populations thus resulted in a high mortality rate of juvenile abalone (Tarr *et al.*, 1996). Thus, despite the fact abalone do occur in the WC, none were found in the stomach contents of WC *T. megalopterus*, unlike the EC individuals. This pattern of reduction of predators (*A. pusillus* and reef fish), abundance of *J. lalandii* and consequent depletion of prey populations, is a prime example of the top-down effect predators have on their ecosystems (Posey *et al.*, 2002).

Approximately 80% of all Cape rock lobsters found in the stomach contents of *T. megalopterus* sharks were light in colour and had soft exoskeletons. This indicates that the lobsters had recently undergone ecdysis (Cockcroft and Goosen, 1995), making them immobile and more vulnerable to predation (Stein, 2013). This is called condition-dependent risk-taking (Wirsing and Ripple, 2010). In an attempt to maximize energy gain (Pulliam, 1974), predators are selective consumers. They find, capture and ingest prey that will allow them to gain maximum calories while saving time and consequently energy (MacArthur and Pianka, 2014). Accordingly, the high abundance, episodic vulnerability and slower movements of the Cape rock lobster make it easy prey, ensuring optimal foraging by the predator. This supports predictions of the OFT, in which preference is given to prey yielding more energy per unit of handling time (Pulliam, 1974). Fish are generally able to adjust their diet choices in response to prey abundance (Dill, 1983). The shift in diet to the most plentiful prey in the system indicates a flexible foraging tactic (Newman, 2003) in *T. megalopterus* as has been found in other shark species such as lemon sharks, *Negaprion brevirostris* (Newman *et al.*, 2012). This flexibility in the diet is a precise prediction of the OFT (Hugie and Dill, 1994) and its Basic Prey Model (BPM), which states a predator should choose its prey by its profitability especially when the prey is highly abundant (Gill, 2003), and a predator should concentrate on this prey instead of broadening its diet (Dill, 1983). An energetically beneficial prey species is one that provides the maximum amount of energy gain with the minimum amount of energy loss (Ferry-Graham, 1998).

The diets of *T. megalopterus* were the most diverse in AN, followed by the EC and WC. Geographical differences in shark diets have been observed for many species, including lemon shark, *N. brevirostris* (Cortés and Gruber, 1990); blue shark, *Prionace glizuca* (McCord and Campana, 2003); starspotted smoothhound, *Mustelus manazo* (Yamaguchi and Taniuchi, 2000); Atlantic sharpnose shark, *Rhizoprionodon terraenovae* (Bethea *et al.*, 2006);

and sandbar shark, *Carcharhinus plumbeus* (Ellis and Musick, 2006). These studies have highlighted various factors contributing to this trend, factors that all emphasize differences in the living biotic environment (Yamaguchi and Taniuchi, 2000), e.g. habitat differences (Bethea *et al.*, 2007), relative prey abundance (McCord and Campana, 2003) and increased species richness toward the tropics (Rohde, 1992). According to Reusch (2014), the ocean environment is partitioned by latitudinal and longitudinal gradients caused by combinations of numerous abiotic factors (i.e. light, turbulence, oxygen, pressure, temperature) that are affected by seasons and the diurnal cycle. All of these factors will affect species diversity and thus prey availability in different ecosystems. Because AN is closest to the equator and WC is furthest away, this pattern of species richness conforms to the latitudinal diversity gradient theory (Roy *et al.*, 1998; Mittelbach *et al.*, 2007; Ekau and Verheye, 2010; Sanders, 2014). The temperature hypothesis of the latitudinal diversity gradient (Tittensor *et al.*, 2010) suggests that the slow metabolic rates associated with lower temperatures leads to lower speciation rates and thus lower species diversity (Rohde, 1992). With mean annual sea surface temperatures of 20.4 ºC for AN (Richardson, 2010), 18.0 ºC for EC (Karczmarski *et al*., 1999) and 16.5 ºC for WC (Dufois and Rouault, 2012), these results also support the temperature hypothesis. Furthermore, Angola has a poorly developed economy and as a result, the absolute biodiversity loss has been relatively small in comparison to its natural resource base (Biggs *et al.*, 2008). There is also a distinct seasonal signal in AN as the warm Angola Current migrates southward into the study region during summer.

All three populations of *T. megalopterus* displayed different prey preferences. Differences between AN and South African populations (EC and WC) correspond with the marine ecoregions of the world that separate areas of dissimilar species composition (Spalding *et al.*, 2009). These ecoregions show that the AN sample site falls within the Benguela-Namib province, while the WC and EC sample sites are situated in the Agulhas Bank province. Furthermore, separating South Africa and Angola is the Lüderitz Upwelling Cell (Fennel, 1999), which clearly forms an environmental barrier that isolates Angolan species.

The Agulhas Bank is an approximately 116 000 km^2 extension of the South African coastal plain (Hutchings *et al.*, 2002). Though both the WC and EC sample sites are situated on the western and eastern sides of the Agulhas bank, respectively, EC had double the prey diversity of WC, which latter shows a distinct decrease in marine biodiversity. This is probably because of the warmer, more productive and well oxygenated water associated with the EC region, compared with the cold, low oxygen and low productivity (Hutchings *et al.*, 2009) waters characteristic of the WC study area. Many taxa show a trend of increasing species richness

from the west to east coasts of South Africa (Branch and Griffiths, 1988; Bustamante *et al.*, 1997; Awad *et al.*, 2002). According to Watson *et al.* (2005) in their contribution to the Millennium Ecosystem Assessment, overexploitation has the highest impact on the biodiversity of marine ecosystems. Overfishing in the 20th century severely depleted South African linefish populations (Griffiths, 2000), which is evident from the 80% drop in commercial catch per unit effort (CPUE) already reported over a decade ago in the Western Cape between Cape Hangklip and Walker Bay (Attwood and Farquhar, 1999). Although the east coast of South Africa has fewer and smaller commercial fisheries than that of the west coast, the east coast has a high human population density resulting in overexploitation of coastal fish as the recreational and subsistence fishers take advantage of inshore resources (Griffiths *et al.*, 2010).

A clear ontogenetic shift was apparent in the EC samples. However, as the small (Old Woman's River surf), medium (Port Alfred) and large (Port Elizabeth) sharks were captured in different locations, these differences may be a reflection of local habitat variability causing size segregation. This separation of size classes was not evident in southern Angola where *T. megalopterus* of all sizes were caught at the same locality off Cunene River mouth. The differences in size segregation amongst different populations most likely have to do with predator avoidance. The reproductive success in the shelf waters of southern Africa is hindered by strong winds, ocean currents and short term variability. This may mean the smaller *T. megalopterus* are located around the more productive upwelling of Cape Agulhas, a known nursery ground for numerous warm temperate species (Hutchings *et al.*, 2002). Juvenile *T. megalopterus* have also been reported in the Transkei (Bass *et al.*, 1975) and Gansbaai. Nursery grounds also offer better habitat for small sharks to avoid predation by larger sharks such as great whites and cow sharks. This may be why, during the two years of sampling for this study, only one juvenile *T. megalopterus* of 470 cm was caught on the Strand (WC) reefs. The next smallest individual captured was 1216 cm, suggesting that individuals between 500 and 1200 cm may be absent in False Bay and Betty's Bay.

It is also possible that smaller individuals are outcompeted by the larger individuals in the WC. The substantially reduced prey diversity in this region and the lack of teleost diversity between Cape Hangklip and Walker Bay (Attwood and Farquhar, 1999) may have increased competition for food resources. The dynamics of competition and simultaneous obtainability of resources may evoke a relocation response (Pittman and McAlpine, 2003). Furthermore, a decline in resource renewal rates can cause complete size-segregation in which a larger fish/cooler water pattern will emerge as per capita resource levels fall (Hughes and Grand, 2000). This may be why only larger specimens are found in Betty's Bay, Western Cape and

why smaller specimens were perhaps more abundant in the warmer and more species rich waters closer to Cape Agulhas. Salmon sharks, *Lamna ditropis*, exhibited this larger fish/cooler water distribution pattern off Japan and the southern Kuril Islands where smaller specimens are found, whereas larger sharks are found in the western and central Bering Sea (Nagasawa, 1998). This pattern, however, is not always the case as large white sharks are known to frequent the tropics, while juveniles frequent temperate coastlines.

For most fishes, prey size increases with predator size (Juanes, 1994; Scharf *et al*., 2000); larger prey are energetically beneficial because they provide a higher yield on energy investment (Labropoulou *et al.*, 1999). The increase in prey size in this study could also be attributed to varying habitat choice with growth that results in differences in prey availability. Juvenile lemon sharks, for instance, select warm, shallow water with underlying structure to provide shelter and predator avoidance (Morrissey and Gruber, 1993). Such habitat choice would explain why small *T. megalopterus* feed primarily on small, benthic organisms such as small crabs and fish (Blenniidae). The growth of *T. megalopterus* shows a trophic niche expansion of larger-sized prey and (except in WC) variety of prey species. This indicates that *T. megalopterus* is selecting its prey by type (prey category) and size.

Kamura and Hashimoto (2004) found that *Triakis* spp*.,* improved their hunting capability as they grew and that the larger sharks captured larger and more mobile prey. This may be due to a relationship between the size of a predator and its swimming speed (Lowe *et al.*, 1996), size of mouth gape (Ferry-Graham, 1998), tooth size, stomach volume (Wetherbee *et al.*, 2004), and visual acuity (Dalu *et al.*, 2013). These factors may have contributed to the increased hunting capability, which allows large sharks from AN and EC to feed on slow but spined, noxious prey such as sea barbel (Rutz *et al.*, 2006) and faster or more difficult to catch species such as squid and octopus. The increase in squid prey may also be attributed to the aggregatory habits of squid during spawning (Smale *et al.*, 2001). Furthermore, the increased strength of the larger *T. megalopterus* allows them to pull abalone off of rocks (EC) and subdue octopus (EC and AN). Although octopus were not found in the smaller sharks from EC, they were found in all three size categories from AN. This may attributable to the greater abundance of octopus in AN compared to EC (De Beer and Potts, 2013). The sizes of the molluscs were not dependent on the size class of *T. megalopterus*. Cephalopods do not possess bony skeletons and are therefore not rigid. Thus, as seen with squid (Smale and Compagno, 1997), molluscs are easily manipulated in the mouth allowing smaller predators to ingest larger octopus as prey. Feeding on cephalopods is therefore not reliant on gape size for small mouthed predators such as *T. megalopterus*.

Seasonal variation in the feeding habits of elasmobranchs is most likely due to changes in the abundance and distribution of prey (Braccini and Perez, 2005). According to Macpherson (2003), geographical boundaries in the world's oceans occur alongside oceanographic processes (e.g. currents and upwelling) that are categorized by temperature, salinity and productivity changes (Longhurst, 1998a). Despite environmental changes in the WC between seasons, there was limited evidence for corresponding dietary variation, with the Cape rock lobster remaining dominant throughout the year. This is most likely due to its abundance throughout the year and due to the general lack of teleost prey in the WC. Although not significant, *T. megalopterus* consumed fewer teleosts and more crustaceans and molluscs in winter months in AN and the EC. The reductions in teleosts in the diets are most likely a reflection of the seasonal abundance of this prey in each region. Baremore *et al.* (2010) found a similar pattern in Atlantic angel sharks (*Squatina dumeril*) where medium and large size specimens showed seasonal-related differences in their diets by feeding predominantly on squid in autumn and teleosts in spring. These authors proposed that this variation was due to a seasonal change in the demersal fish community, natural variation in the diet and/or a broadening of niche breadth with season.

Because they lack cutting teeth, *T. megalopterus* do not bite off pieces of prey: their dentition is suitable for grasping and/or crushing prey. Whole prey items in stomachs generally showed no sign of bite marks. The only evidence that teeth were used in the feeding strategy of *T. megalopterus* was the fact that some of the (non-moulting) Cape rock lobster antennae were severed at the base of their heads. This may be attributed to the bite force of the plate-like teeth that severed the antennae when the mouth closes. The absence of bite marks on whole prey items indicates that *T. megalopterus* makes use of a suction feeding technique which may also include manipulation of prey. This corresponds to the plate-like tooth morphology described by Tricas *et al.* (2002) who found that the teeth in the front of the jaw are rounded with a molar-like base rising into a sharp point for gripping. Although the back of the jaw contains flatter more molariform teeth, which would be suitable for crushing, prey does not appear crushed, suggesting the teeth are used more for manipulation of prey. This agrees with the results found in the tooth morphology analyses of *T. megalopterus* (see **Chapter 4**).

The results from this chapter show that the teeth grow and change shape during ontogeny, which is consistent with the ontogenetic feeding changes of this species. In the subset analysis of dentition of sharks from EC, small *T. megalopterus* have dorsoventrally flattened and more molariform lateral teeth. These lateral teeth have no cusps, only small, coarse serrations. As the small specimens feed predominantly on crustaceans, these teeth are well suited for crushing or grinding of hard-bodied prey, therefore well suited to their durophagous diet. The

teeth of *T. megalopterus* grow and change shape as the shark grows. This is evident in dentition of EC specimens which show an increase in the area and width of the teeth from small to large specimens. Generally, as *T. megalopterus* grows, it expands its diet to include teleosts and molluscs. With this expansion to softer-bodied prey species, the medial-inferior and medial-superior broaden at their base and lengthen to produce a single cusp. This cusp enables larger specimens to grasp/clutch and manipulate softer-bodied prey in order to inhibit their escape (Ramsay and Wilga, 2007). Medium and large sharks do, however, still exhibit durophagy. These cusps would typically be damaged trying to grip hard exoskeletons. When harder prey items such as lobster are ingested, the possibility of breaking the sharp cusps increases unless they have just moulted. To counteract this, Chondrichthyans are able to depress and rotate these cusps inwards leaving the broader labial face of the teeth to form a plate like grasping and/or crushing surface (Ramsay and Wilga, 2007).

Although the ontogenetic change of the teeth and feeding correlate, the tooth morphology and the feeding of the three different populations does not. The feeding showed differences between all three populations whereas the tooth morphology indicated the EC specimens teeth were both smaller in length, width and area compared to AN and WC. The longer teeth in AN specimens would allow for a longer cusp enabling these specimens to grasp/clutch and manipulate teleosts (Ramsay and Wilga, 2007). The shorter cusp observed in the EC specimens is more suited for crushing or grinding of hard bodied prey required for their durophagous diet. Interestingly, although WC consumed only crustaceans, their teeth were similar to those of AN specimens. This may be because the WC individuals were not grasping/manipulating prey as were the other two populations. All prey in WC stomach contents was whole indicating this population employs a suction feeding method, thus not using their teeth. Therefore their teeth do not need to evolve to suit their method of feeding. Alternatively, as the increased population sizes of *J. lalandii* east of Cape Hangklip only occurred in the 1990s (Tarr *et al.*, 1996; Turpie *et al.*, 2003; Cockcroft *et al.*, 2008; Blamey and Branch, 2012), the altered prey preference in this population may not have occurred long enough ago to evolve the tooth shape.

Based on the different feeding habit of *T. megalopterus* from WC, it is plausible that their morphology (both body and teeth) may be divergent from the other two groups (AN and EC). However, the greatest morphological variation was found between the *T. megalopterus* populations from the southern (WC and EC) and northern (AN) Benguela subsystems (**Chapter 4**). This pattern corresponded to the feeding analysis when one examines the broad classification of prey categories. Here the diet of the AN individuals was dominated by teleosts, while the diets of the South African specimens were dominated by crustaceans. The

AN *T. megalopterus* generally have shorter fins (pectoral, 1st and 2nd dorsal), wider fin bases (pelvic, 2nd dorsal), shorter gill slits, smaller eyes and spiracles, wider tail regions and larger caudal fork, and lower post ventral and terminal caudal margins. Smaller fins found in AN individuals will also help streamline the shark, reducing drag and permitting higher speeds (Bushnell and Moore, 1991), while the wider tail region aids in propulsion (Weihs, 1981). All of these traits point to these sharks being better suited to hunt teleosts in the open water column. Indeed, the dominant teleost prey (*Galeichthys feliceps* and *Sardinella aurita*) in the diets of AN individuals primarily occupy the sandy benthic and pelagic habitats, which suggests that speed would be favoured over manoeuvrability. In contrast to the teleost prey in Angola, the crustaceans that were important in the diet of *T. megalopterus* from the EC and WC are primarily reef associated. As pectoral fins are used for manoeuvrability (Wilga and Lauder, 2000, 2001, 2002), larger fins would be more suited to the fine-tuned manoeuvrability required to locate and catch these prey items.

Although *T. megalopterus* is a nocturnal feeder and probably relies more heavily on non-visual senses than on sight, eye size differences were noted amongst populations. In relation to feeding, one would expect AN to have larger eyes than those of the South African populations as larger eyes are generally found in sharks that prey on more active and mobile prey (Lisney and Collin, 2007). This, however, was not the case. As a larger eye may improve the resolution and sensitivity of sight, it is possible that the eyes of South African *T. megalopterus* may have become larger to improve the detection of cryptic crustaceans in rocky reef habitats. However, as *T. megalopterus* are nocturnal feeders, eyesight probably plays a smaller role in feeding

Conclusions

With a diet primarily consisting of crustaceans, teleosts and molluscs, *T. megalopterus* appears to be a benthic forager. The primary feeding mechanism used by *T. megalopterus* is suction feeding with some prey manipulation. This species predominantly occupies reef systems, but also hunts over sandy bottoms and in the kelp forests east of Cape Hangklip. It would seem that the South African specimens are morphologically better suited to hunt in more high relief habitats and may have evolved from a primarily suction based feeding to also prying prey off of rocky reefs and in kelp forests. The extreme difference in feeding of WC individuals shows that *T. megalopterus* is able to employ flexible foraging tactics in order to feed on highly abundant prey.

The size of prey and the diversity of prey species generally increased with ontogeny. This may be associated with a relationship between predator size and an expansion of habitat, increase in foraging success, hunting capability, gape size and change in the feeding related morphology of the predator. A positive relationship between the body morphology and teleost prey preference of AN specimens is evident in shorter fins, more streamlined body and larger and more powerful tail and caudal fin. This shows the ability of predators to evolve in order to accommodate the change in diet.

The highest and lowest prey diversity in the Angola-Benguela Front and the Western Cape respectively, conforms to the theory of a latitudinal diversity gradient (species richness increases closer to the equator) as well as the related temperature hypothesis (latitudinal gradient is directly related to temperature change). The high biodiversity in southern Angola also reflects that characteristics of this ecosystem are consonant with the fundamental triad of ecological processes (enrichment, concentration and retention) that lead to propagative habitats and high species richness. It is therefore not surprising that the feeding of *T. megalopterus* is affected more by location than size or season. Thus biodiversity, environmental factors and oceanography (e.g. Lüderitz Upwelling Cell), have more of a direct impact on habitat and prey populations, and therefore the feeding ecology of this species. Although feeding biology is now better understood for *T. megalopterus*, information such as prey detection and capture capabilities is still needed for a more comprehensive understanding of feeding strategy of this species. Further analysis with larger sample sizes is also required to properly assess ontogenetic and seasonal feeding differences as well as ecomorphological studies to assess how different feeding habitats affect the morphology of this species.

Chapter 6:
Age, growth and reproduction of *Triakis megalopterus* from three biogeographic zones of southern Africa

Introduction

Studies on life history seek to explain the link between adaptive responses to ecological influences (density-dependent versus density-independent) and an organism's fitness as varying demographic responses are elicited by variable spatial and temporal scales (Roff, 1993; Reznick *et al.*, 2002; Winemiller, 2005). The most recognised life history theory is that of r and K-selection (Pianka 1970). Sharks are generally recognised as K-selected species as a result of being long lived, showing late maturity, having small reproductive effort and bearing few, large young (Stearns, 1976), having large adult size, long gestation periods, iteroparity, precocial offspring (Cortés, 2000) and high survival rates of all age classes (Camhi *et al.*, 1998). In populations that are regulated by density-dependent factors (e.g. cannibalism and competition), higher fitness is expected in K-selected species than in species demonstrating the opposite suite of r-selected traits (Winemiller, 2005).

Sharks are all recognised to be K-selected species, despite the large interspecies differences in life history traits (Smith *et al*., 1998). For instance, the age at maturity varies from 2–3 years in the female grey smoothhound shark, *Mustelus californicus* (Yudin and Cailliet, 1990) to approximately 35.5 years in the spiny dog fish, *Squalus acanthias* (Saunders and McFarlane, 1993). Fecundity (*F*) varies from $F = 2$ in the sand tiger shark, *Carcharias taurus,* to $F =$ 82-95 in the sevengill cowsharks, *Notorynchus cepedianus* (Smith *et al.*, 1998). Gestation periods in sharks generally average 9–12 months (Helfman *et al.*, 2009), but have been reported as low as 4.5–5 months in the bonnethead shark, *Sphyrna tiburo* (Manire *et al.*, 1995) and as long as 3.5 years in the basking shark, *Cetorhinus maximus* (Parker and Stott, 1965). Although many studies are available for single shark species within limited regions, very little research has focused on intraspecific life history traits and how these traits are affected by spatial patterns (Cope, 2006). This is peculiar as a single species can/will occupy different habitats and therefore be subject to the effects of diverse ecosystems and the different environments therein (Jones *et al.*, 2002), and generally, there is a difference in life history traits between different areas (Cope, 2006).

The life history traits of species can be affected by their environment (Cope, 2006). As the *T. megalopterus* sampled in this study occupy different oceans, ocean currents and biogeographic zones, intrapopulation differences in life history traits should be evident. For example, life history theory predicts that stability in environments promotes slow development, late maturity, smaller reproductive effort, fewer young and long life, while fluctuating environments may result in shorter life spans, faster development, earlier maturation, semelparity, and larger reproductive effort, including higher number of young (Stearns, 1976). The trade-off between the size and number of offspring can also be influenced by environmental conditions (Southwood, 1988) and resource availability (Pianka, 1970). Large body size offers fitness benefits (Roff, 1993; Blanckenhorn, 2000) such as access to a wider variety of prey (Juanes *et al.*, 1994), reduced vulnerability to predators (Parker, 1971), superior competitive capabilities (Fausch and White, 1981) and improved ability to withstand extreme environments (Henderson *et al.*, 1988) and/or disease (West and Larkin, 1987). Larger size also permits larger offspring (Chambers and Leggett, 1996; Green and McCormick, 2005) and/or larger productive output (Morris, 1996; Sogard *et al.*, 2008), which may contribute to the survival success.

Lombardi-Carlson *et al.* (2003) found that biological characteristics are generally affected by different ecological environments found at different latitudes. These differences are said to be caused by a physiological and/or genetic response to different environmental factors (Levins, 1989; Conover, 1990). In general, fish grow faster and mature earlier at low latitudes (Lombardi-Carlson *et al.*, 2003) while attaining larger sizes at high latitudes (Bergmann's Rule; Mayr, 1956). Size plays a crucial role in intraspecies research as most life history trait variation, including growth, age at maturity, offspring size, and fecundity, is correlated with body size (Holden, 1973; Brander, 1981). Latitudinal variation in life history traits has been recorded in sharks. For instance, in studies of adult bonnethead sharks, *Sphyrna tiburo,* sampled from three populations in the eastern Gulf of Mexico, the population of highest latitude showed the largest asymptotic sizes, largest and oldest median size at maturity and largest near-term embryos (Parsons, 1993; Carlson and Parsons, 1997; Lombardi-Carlson *et al.*, 2003). Similarly, patterns of larger size at maturity with latitude were reported for the cloudy catshark, *Scyliorhinus torazame* (Horie and Tanaka, 2002), shortspine spurdog, *Squalus mitsukurii* (Taniuchi *et al.*, 1993) and the starspotted dogfish, *Mustelus manazo* (Yamaguchi *et al.*, 2000). Fishing pressure may also affect life history traits amongst populations, particularly in sharks due to their K-selected traits and the direct relationship between stock size and recruitment (Holden, 1977). Exploitation has the potential to truncate

age/size classes and this in turn, has the potential to reduce the size and age at sexual maturity (Longhurst, 1998b; Rochet, 2000).

Age information is considered to be the most valuable of the life history variables in fisheries research (Hilborn and Walters, 2013). Correctly determining the age of elasmobranchs is important in understanding the population dynamics of exploited species and essential for estimating longevity, maturity, mortality and growth rates by species and area (Serra-Pereira *et al*., 2008; Booth *et al*., 2011). The main driving force for life-history change is the necessity for species to optimize reproductive capability. Subsequently, life-history aspects such as age, growth and size at maturity are shaped by natural selection geared to maximum reproductive success (Thresher, 1984).

The common ageing structures used for teleosts, such as scales, otoliths, or bones, are not applicable for elasmobranch ageing (Cailliet *et al.*, 1983a). Elasmobranchs are most commonly aged by means of the growth zones in vertebral centra which are either viewed in their natural state or enhanced by various methods, e.g. grinding and polishing (Smith, 1984); electron microprobe analysis (Cailliet and Radtke, 1987); x-radiography (Urist, 1961; Applegate, 1967); x-ray spectrometry (Jones and Geen, 1977); oil clearing (Cailliet *et al.*, 1983b); silver nitrate (Haskell, 1948; Stevens, 1975); alizarin red S (LaMarca, 1966); cobalt nitrate (Hoenig and Brown, 1988); alcohol immersion (Richards *et al.*, 1963); xylene impregnation (Daiber, 1960); or histology (Ishiyama, 1951).

Vertebral ageing, however, is not without its problems. Due to developmental variances (Officer *et al.*, 1996), different sections of the vertebral column may render dissimilar growth increment counts (Natanson and Cailliet, 1990). As increment formation can be influenced by environmental change (Geffen, 1992), it is critical to validate the rate of growth band deposition, and several techniques can be used to do so. In the past, the majority of elasmobranch ageing studies assumed that a single annual vertebral band pair was deposited annually throughout an individual's lifetime (Stevens, 1975), e.g., the lemon shark, *Negaprion brevirostris* (Gruber and Stout, 1983; Brown and Gruber, 1988), neonate sharpnose *Rhizoprionodon terraenovae*, sandbar *C. plumbeus*, (Branstetter, 1987a) and the bonnethead shark, *Sphyrna tiburo* (Parsons, 1993). There are, however, species that deposit two band pairs annually, e.g., the shortfin mako, *Isurus oxyrinchus* (Pratt and Casey, 1983), sand tiger, *Carcharias taurus* (Branstetter and Musick, 1994) and basking shark, *Cetorhinus maximus*, (Parker and Stott, 1965). Furthermore, additional difficulties in ageing elasmobranchs have been documented for the Pacific angel shark, *Squatina californica*. This species is born with

6–7 band pairs (Natanson, 1984), their band deposition is not annual (Natanson and Cailliet, 1990); band deposition appears to be caused by vertebral column strengthening and shows no time scale relationship (Natanson, 1984), and fewer bands are deposited in older, larger fish (Natanson and Cailliet, 1990), possibly due to termination of band development in reproductively active individuals (Natanson, 1993). Intraspecies differences have also been noted in elasmobranchs. For instance, two different studies on the scalloped hammerhead, *Sphyrna lewini*, showed a single annual band deposition in sharks from North Carolina and the north-eastern Gulf of Mexico (Schwartz, 1983), but a double band deposition in north-eastern Taiwan (Chen *et al.*, 1990). These issues highlight the necessity for both inter- and intraspecies validation.

Three methods of validation are typically used: marginal increment analysis (MIA); carbon dating; and the most common and reliable method of validation (Campana, 2001), mark and recapture of tagged sharks injected with a fluorescent marker such as oxytetracycline antibiotics (OTC) (Geffen, 1992). As recaptured, OTC injected *T. megalopterus* were available for this study, and because OTC is a common validation method in elasmobranchs, this was the chosen validation method for this study. When sharks are injected with OTC, it is absorbed and deposited at sites of active growth causing a fluorescent mark on the vertebra that illuminates under ultraviolet light. Subsequently, the growth (band deposition) observed after the OTC marking can be attributed to deposition over the time period the shark was at liberty. This allows the derivation of growth and deposition rates. Previous life history studies on *Triakis* spp. have reported an annual growth band deposition rate for OTC validated *T. semifasciata* (Smith, 1984; Kusher *et al.*, 1992) and MIA validated *Furgaleus macki* (Simpfendorfer *et al.*, 2000). Age validation is not yet available for the closely related *scylliogaleus quecketti*.

Reproductive studies of *T. semifasciata* showed an annual reproductive cycle, 10–12 month gestation period (Smith, 1984; Ebert, 2003), fecundity of approximately 1–37 embryos per female (Ebert and Ebert, 2005) and a commencement of reproduction at 17 years of age with fecundity increasing with age (Ackerman, 1971; Talent, 1985; Kusher *et al.*, 1992). Gestation periods for other Triakids are generally 9–12 months: e.g. soupfin shark, *Galeorhinus galeus* (Ripley, 1946); smooth-hound shark, *M. mustelus* (Smale and Compagno, 1997; Saïdi *et al.*, 2008); brown smooth-hound, *M. henlei* (Ebert, 2003); blackspotted smooth-hound, *M. punctulatus* (Saïdi *et al.*, 2009); and the gummy shark, *M. antarcticus* (Lenanton *et al.*, 1990). Shorter gestation times (7–9 months) have, however, been recorded in the whiskery shark, *F. macki* (Simpfendorfer and Unsworth, 1998).

Thus far, only two publications are available for the ageing and reproduction of *T. megalopterus*, both of which concentrate on specimens from the Eastern Cape, South Africa. Booth *et al.* (2011) OTC validated a single band pair annual deposition rate for *T. megalopterus* with a maximum age of 25 years and an 11 and 15 year age at maturity for males and females, respectively. Smale and Goosen (1999) states that *T. megalopterus* exhibits aplacental viviparity with a female reproductive cycle of 2–3 years, a gestation period of 19–21 months, a 9.7 mean number of embryos per pregnancy and a birth size of ~420–450 mm TOT. Furthermore, these authors found that *T. megalopterus* reached 50% maturity at TOT = 1320 mm in males and TOT = 1450 mm in females with their largest specimens analysed being a male of TOT = 1520 mm and a female of TOT = 2075.

The aims of this chapter are to 1) assess the life history traits, including growth rate, age, maturity and mortality of *T. megalopterus* from AN, WC and EC; 2) assess whether the life history traits are affected by habitat and environmental factors and 3) compare the life history of the three southern African *T. megalopterus* populations.

Material and methods

A total of 40 males and 81 females were collected from Angola, the Western Cape and the Eastern Cape using hook and line techniques (**Table 6.1**). All specimens were used for the reproductive analyses; however, vertebrae were not available for ageing one female from Angola, one female from the Western Cape and two males from the Eastern Cape. Due to size bias of small specimens in the EC population, the age data (n = 87) from Booth *et al.* (2011) was included in the age and growth analyses. This data was made up of 63 females and 26 males. Unfortunately, maturity data was not available from this previous study and was therefore not included in the maturity analyses.

Table 6.1: Sample numbers and sex allocation from all sample sites. Numbers in brackets illustrate the number of fish used in the age analyses.

Sample site	Males	Females	Combined sex
Angola	11(11)	29 (28)	40 (39)
Western Cape	9 (9)	24 (23)	33 (32)
Eastern Cape	20 (44)	28 (91)	48 (135)
Total	40 (64)	81(142)	121 (208)

Maturity was assessed using macroscopic methods developed by Bass *et al.* (1975). Male specimens were assigned to one of three reproductive stages: (1) juvenile, (short, soft claspers, threadlike testes, testis not developed), (2) adolescent (claspers partially calcified, testis developed, and no sperm in the seminal vesicle) or (3) mature (claspers calcified, sperm in the seminal vesicle). Females were assigned to one of four reproductive stages categorized as: (1) Juvenile (thin, thread-like uteri, no obviously developed ova in ovary, inconspicuous oviducal glands and), (2) adolescent (partly distended uteri), (3) mature (widened and fully flaccid uteri, enlarged ODG) or (4) pregnant (embryos present).

Three measurements were taken from the male claspers, the outer length (CLO), inner length (CLI) and width (CBW; Compagno, 1984b). For the females, the diameters of ovarian eggs, length and widths of the oviducal glands and maximum width of the uterus were measured. The absence or presence of uterine eggs and embryos was recorded along with the number, length, weight and, where possible, sex for all embryos. Embryo number, per left or right uterus, was recorded. The mean number and length of each litter member was calculated.

To assess the potential function of the liver as an energy source during pregnancy, the hepatosomatic index (HSI) of pregnant females was plotted against the mean embryo length (TOT). The HSI was defined as the ratio of liver weight to body weight, calculated as:

$$\mathbf{HSI} = \frac{\boldsymbol{LW}}{\boldsymbol{BW}} \times \mathbf{100}$$

where:

LW = liver weight in grams
BW = total body weight in grams.

Vertebrae preparation and reading

Five consecutive vertebrae from under the first dorsal fin were removed. Vertebra were separated and soaked in 4.5% sodium hypochlorite for up to 45 minutes (Booth *et al.*, 2011), rinsed under running water and dried for future analysis (Officer *et al.*, 1996). Cleaned vertebrae were embedded in polyester casting resin and sectioned to a thickness of 0.6 mm longitudinally through the focus of the centrum using a twin blade diamond bladed saw (Booth *et al.*, 2011). Sections were mounted onto glass slides using DPX mountant medium. Annual growth bands were defined as a distinct narrow opaque zone comparative to a broader

adjacent translucent zone in the corpus calcareum (Goosen and Smale, 1997). A dissecting microscope with transmitted white light was used to count growth bands.

All vertebrae were aged three times, each time by a different reader, without prior knowledge of the specimen's location, length or sex. If counts differed amongst readers, the resolution criteria implemented by Richardson (2010) was used. If two out of three counts were equal, that count was accepted as the age of that specimen. If the three counts resulted in a consecutive sequence of age (e.g. 1, 2 and 3), the median was accepted as the age of that specimen. If two of the readings differed by two counts, the average of the two closest readings was accepted (e.g. 1, 3 and 7 was accepted as 2).

Between reader estimates

The consistency of growth zone counts was assessed by calculating the index of average percentage error (APE; Beamish and Fournier, 1981) as:

$$\boldsymbol{IAPE = \frac{1}{n}\sum_{j=1}^{n}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{\left|X_{ij} - \bar{X}_j\right|}{\bar{X}_j}\right] \times 100}$$

where:

n	=	number of sharks
R	=	number of readings
X_{ij}	=	age determined for the *j*th shark and the *i*th reading
X_j	=	mean age calculated for the *j*th shark

An IAPE <10% was considered acceptable (Goosen and Smale, 1997).

Validation of growth zone deposition rate

A total of four *T. megalopterus* that had been tagged and injected with OTC were recaptured in the De Hoop Nature Reserve (information on individuals shown in **Table 6.3**) by the Department of Environmental Affairs, Oceans and Coast, Marine Biodiversity and Coastal Research. The vertebrae were stored in the dark to minimize deterioration of OTC fluorescence, prepared for examination as described above and photographed using transmitted white and ultra violet light in order to view the location of the OTC band in

relation to the opaque growth zones. The number of visible growth zones between the OTC marking and the vertebral edge were counted and related to the time at liberty.

Growth model

The growth of *T. megalopterus* was estimated by fitting the three parameter von Bertalanffy growth function (VBGF; Ricker, 1975) to observed length-at-age data. The VBGF is described by the equation:

$$\boldsymbol{L(t) = L_{\infty}\left(1 - e^{-k(t-t_o)}\right)}$$

where:

L_t = predicted age-at-length t

L_{∞} = theoretical asymptotic length

k = growth coefficient

t_o = age-at-zero length

Length at maturity

In order to assess the length at 50% maturity, specimens were separated into 100 mm length classes. The percentage of sexually mature sharks (PM_i) by length (l_i) and age was fitted with a logistic ogive of the form:

$$\boldsymbol{PM_i = \frac{1}{\left(1 + e^{-(l_i - l_{50})/\delta}\right)}}$$

where:

PM_i = proportion of mature sharks in the ith length (or age) class,

li = ith length class

$l50$ = mean length at 50% maturity

δ = width of the logistic ogive

Since the sample sizes of males was low and there was an uneven spread of maturity stage classes in EC and WC, sexes were pooled for the maturity ogives for each population.

Mortality rate estimates

Total mortality rate (Z) was estimated using the method of Hoenig (1983) by:

$$\boldsymbol{Z = \exp(1.44 - 0.982 \ln t_{max})}$$

where:

t_{max} = the age of the oldest fish per population

Natural mortality (M) was estimated using the method of Pauly (1980) by:

$$\boldsymbol{M = \exp(-0.0152 - 0.279 \ln L_{\infty} + 0.6543 \ln k + 0.463 \ln T)}$$

where:

L_{∞} and k = Von Bertalanffy growth model parameters (see **Table 6.4**)

T = the mean water temperature per population

The mean water temperatures (T) used for AN, WC and EC were 20.4 ºC (Richardson, 2010), 16.5 ºC (Dufois and Rouault, 2012) and 16 ºC (Smale and Goosen, 1999), respectively. Although the water temperature for EC should be higher than that for WC (18.0 ºC; Karczmarski *et al*., 1999), 16 ºC was used for direct comparison to the study by Smale and Goosen (1999).

The fishing mortality rate (F) was calculated by subtraction ($F = Z - M$).

Statistical analyses

Modelling of growth parameters followed the methods described by Potts *et al.* (2010). Model parameters were estimated using a downhill simplex search routine (Nelder and Mead, 1964), comprising a non-linear minimization routine to acquire model parameter estimations. Model fits were attained by minimizing the negative normal log-likelihood for the observed and predicted lengths-at-age. Bartlett's test for their homoscedascity and a non-parametric, one-sample runs test for residual randomness was applied to compare the models fit. A conditioned parametric bootstrap resampling method (Efron, 1982), with 1 000 iterations, was used to estimate variance. From the bootstrap data, 95% confidence intervals and standard errors were constructed using the percentile method described by Buckland (1984). A

likelihood ratio test (LRT; Cerrato, 1990) was used to compare growth model parameters amongst populations. Due to the low sample sizes and the lack of significant differences in the growth pattern between male and female *T. megalopterus* (Booth *et al.*, 2011), sexes were combined for the growth models. Paired sample t-tests were run to see if significant differences were apparent between the age estimates all population comparisons using IBM SPSS v20.0 (IBM Corporation, 2011)

Results and interpretation

Size range and sex ratio

Size ranges for the current study (**Figure 6.1**) were similar in AN (687 mm and 1830 mm TOT; ($\overline{TOT}$) = 1407 mm) and WC (470–1750 mm TOT; $\overline{TOT}$ = 1509 mm). Although EC had a comparable size range (396–1670 mm TOT), the majority of specimens collected were small, and the mean total length ($\overline{TOT}$ = 861 mm) was small compared with the other populations. Large (>1400 mm) specimens dominated the AN (66.7%) and WC (84.4%) samples. In contrast, EC was dominated by small (<999 mm) specimens making up 69.6% of the total sample size for this population. However, with the inclusion of these data from Booth *et al.* (2011), *T. megalopterus* was better represented in all size classes (353 mm and 1830 mm TOT; ($\overline{TOT}$ = 1061 mm; **Figure 6.2**). These data included six smaller (<396 mm) specimens ranging between 353–379 mm TOT and a largest male (1520 mm TOT) and female (1746 mm TOT) for the population.

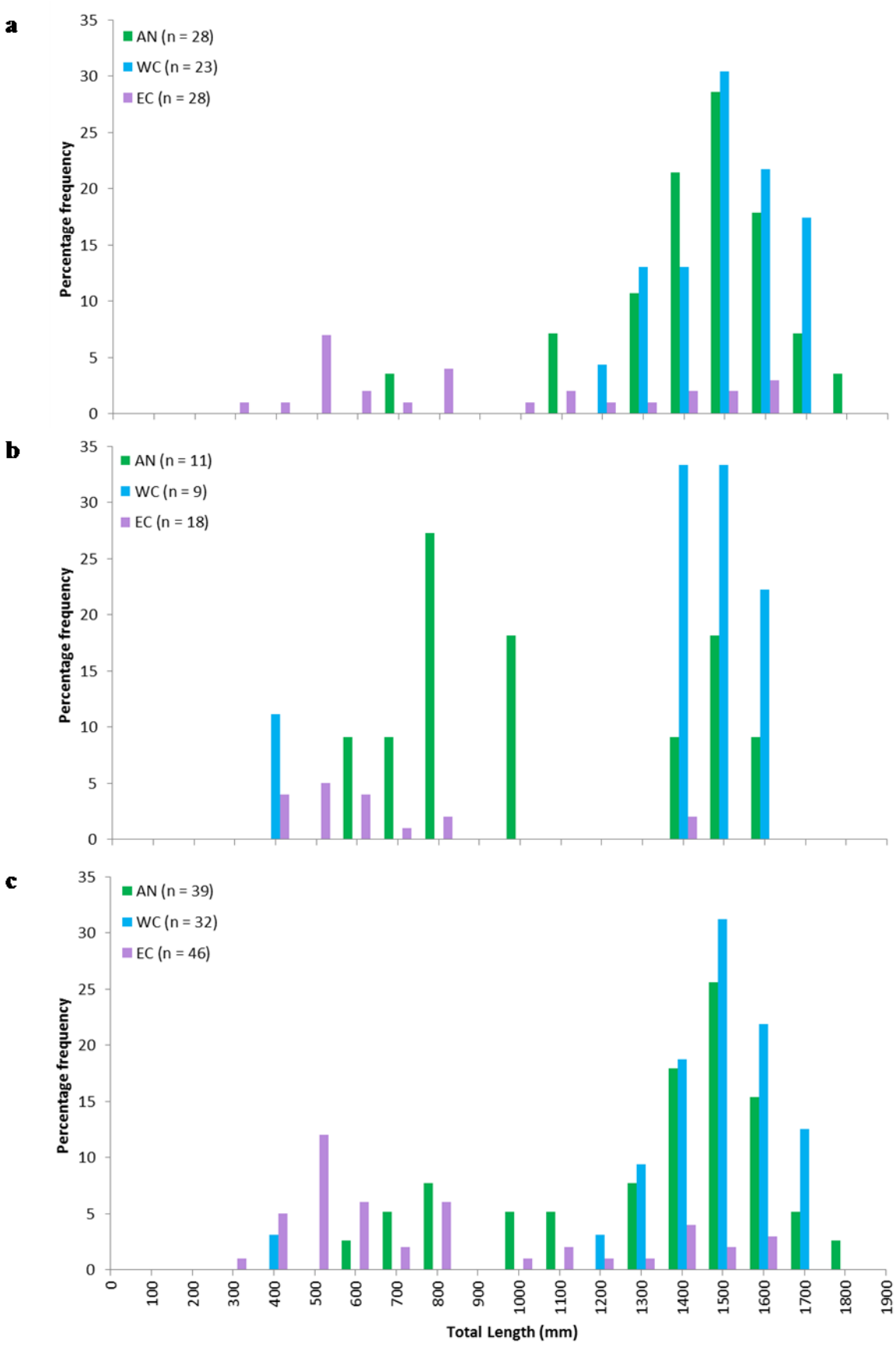

Figure 6.1: Length frequency histogram of *Triakis megalopterus*, showing (a) females, (b) males and (c) combined sexes from Angola (AN), Western Cape (WC) and Eastern Cape (EC).

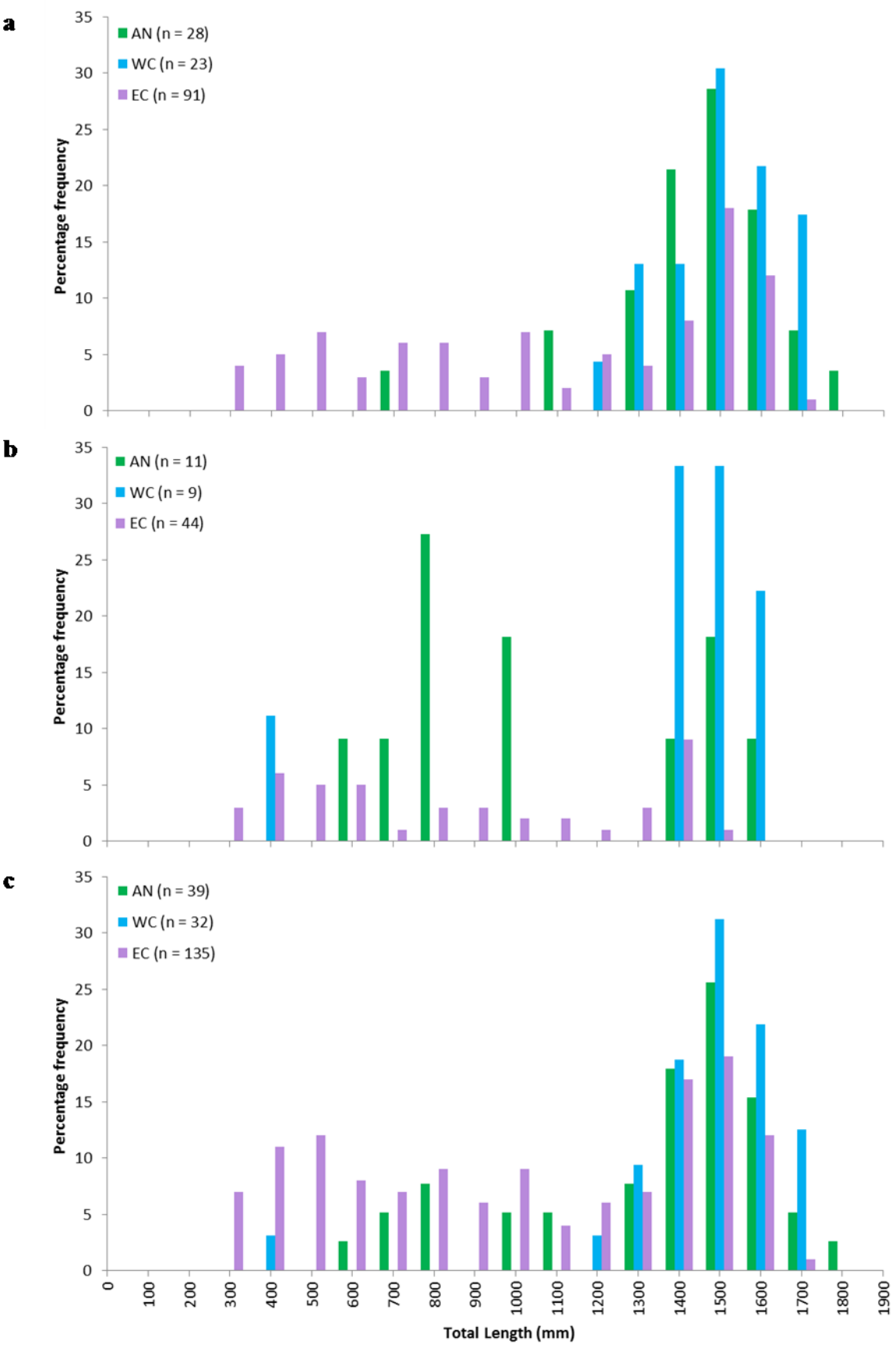

Figure 6.2: Length frequency histogram of *Triakis megalopterus*, including the data from Booth *et al.* (2011) showing (a) females, (b) males and (c) combined sexes from Angola (AN), Western Cape (WC) and Eastern Cape (EC).

The relationship between TOT and weight, for each population (AN, WC and EC), were best described by power functions and comparable amongst all populations. Power function equations are given in **Figure 6.3**. As the weight of the aged specimens was not available from Booth *et al.* (2011), this weight to TOT relationship only represents the current data.

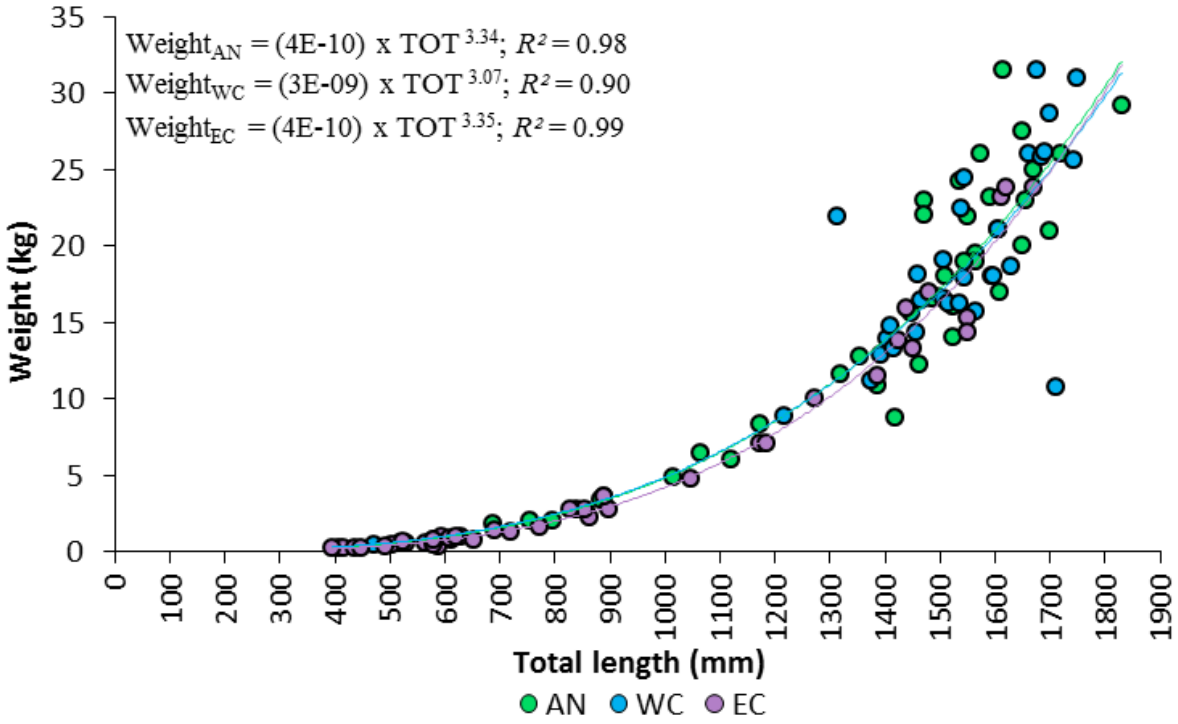

Figure 6.3: Relationship between total length and weight of *Triakis megalopterus* from southern Africa; *AN = Angola, WC = Western Cape, EC = Eastern Cape.*

The male:female (M:F) sex ratio was skewed towards females in all populations (**Table 6.2**). The AN (M1:F2.6) and WC (M1:F2.7) populations had similar sex ratios. In EC, however, more males were present, resulting in a more comparable and less female biased sex ratio (M1:F1.4). In EC there was a large number of small specimens captured which included 18 males and 16 females <900 mm TOT. The smallest free swimming individuals in each population were a 621 mm TOT female in AN, 470 mm TOT male in WC and a 369 mm TOT female in EC. The WC and EC neonates still had very evident umbilical scars indicating recent parturition. According to neonate size, WC appears to have a larger birth size than EC. It was not possible to estimate birth size from AN as neonates were not available in this population. Expectedly, the largest specimens in all populations were female. The largest specimen captured was from AN (1830 mm TOT).

The male skewed sex ratio decreased (M2:F1) with the inclusion of the data from Booth *et al.* (2011) in EC. This data increased the mean TOT for males ($\overline{TOT}$ = 941) and females ($\overline{TOT}$ =

1061). Furthermore, smaller minimum and larger maximum sizes were evident for both males and females.

Table 6.2: Sex ratio and number, mean, minimum and maximum size of male and female *Triakis megalopterus* captured in southern Africa; *Eastern Cape*[2] *= Booth* et al. *(2011) data* included

Location	Male : Female ratio	Males (mm TOT)				Females (mm TOT)			
		n	Mean	Min	Max	n	Mean	Min	Max
Angola	1:2.6	11	1341	687	1610	29	1407	621	1830
Western Cape	1:2.7	9	1509	470	1625	24	1551	1216	1750
Eastern Cape	1:1.4	20	747	406	1450	28	861	396	1670
Eastern Cape[2]	1:2	44	941	354	1520	89	1061	353	1746

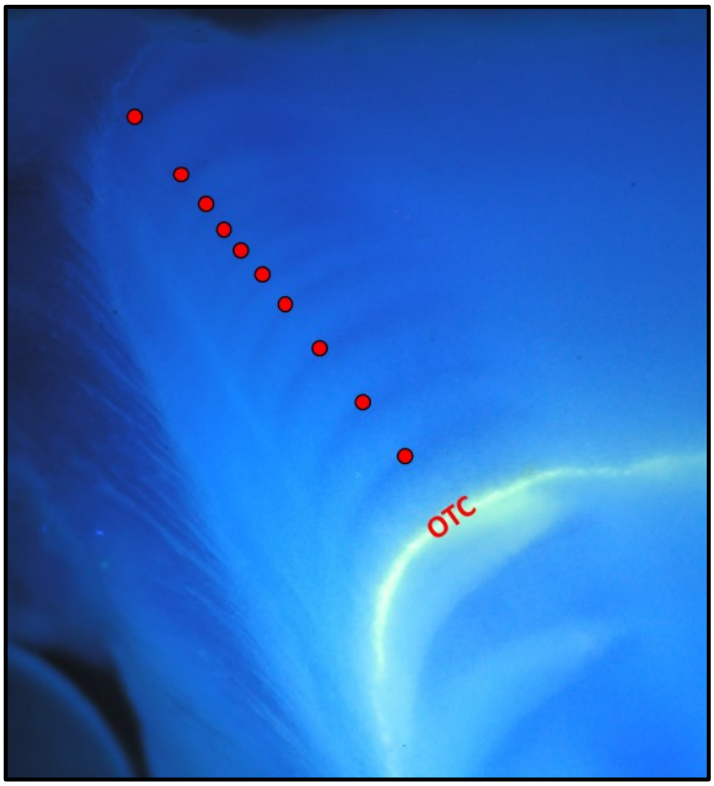

Figure 6.4: An example of a sectioned vertebra of a 22 year-old, 1566 mm TOT, *Triakis megalopterus* tagged and injected with oxytetracycline hydrochloride 10 years before recapture.

Age validation, growth and maturity

The time at liberty for OTC marked fish ranged from zero to 12 years (**Table 6.3**). Unfortunately, out of the four tagged, OTC injected and recaptured *T. megalopterus* used in this study, only two of these specimens had the data for the date on which the shark was tagged. The first was at liberty for 12 years. During that time it grew 205 mm. The second specimen was at liberty for eight years and grew 140 mm during that time. In both cases the number of opaque bands coincided with the number of years at liberty (e.g. in **Figure 6.4**).

Table 6.3: Information on the *Triakis megalopterus* individuals used in the chemical age validation experiment conducted in the De Hoop Nature Reserve, Western Cape (*NK = unknown*)

Capture → / recapture locations	Tagging date	Recapture date	Tagging length (mm TOT)	Recapture length (mm TOT)	Years at liberty	Zones distal to OTC	Total Age
NK → Lekkerwater	NK	06/05/2004	NK	1500	0	0 (793)	16
Koppie Alleen	24/10/1996	06/10/2014	1235	1440	12	12 (616)	15
Lekkerwater	09/5/1997	26/05/2005	1420	1560	8	8 (812)	19
NK → Lekkerwater	NK	06/10/2014	NK	1566	10	10 (082)	22

Age estimates were accepted for 117 (97%) of the 121 vertebrae analysed. There was a 26% agreement on all age assessments, 24% agreement between age readings within one year, 42% within two years and 8% agreement between readings within three years. Band pair counts were considered to be reasonably precise with an estimated IAPE of 7.86%, a satisfactory percentage within the acceptable limit (<10%) for use in ageing results for population analysis (Powers, 1983).

The youngest specimens were a male from WC (470 mm TOT) and a female from EC (396 mm TOT), both specimens were aged at zero years. The maximum ages were 24 years in AN (1830 mm TOT), 27 years in WC (1750 mm TOT) and 30 years in EC (1612 mm TOT). The oldest sharks in each population were mature females.

Predicted asymptotic length (L_∞) was smallest in AN (1696.7 mm TOT), followed by the WC (1828.2 mm TOT) and the EC (1962.7 mm TOT; **Table 6.4**). These differences were, however, not significant (LRT = 1.33; p = 0.24). There were significant differences in the Brody growth coefficient (LRT = 33.57; $p = 7.94 \times 10^{-31}$), with the AN population attaining

their theoretical maximum size (L_∞) at the fastest rate (K = 0.17 year^{-1}) followed by WC (K = 0.09 year^{-1}) and then EC (K = 0.06 year^{-1}).

Table 6.4: Life-history parameter estimates for combined sex *Triakis megalopterus* from Angola (AN), Western Cape (WC) and Eastern Cape (EC); size reported was TOT (mm).

Parameter	AN	WC	EC
Sample size	39	32	133
Theoretical maximum size (L_∞)	1696.74	1828.20	1962.67
Growth coefficient (K)	0.17	0.09	0.06
Theoretical age (years) at zero length (t_o)	0.45	-2.80	-3.23
Median size at maturity (L_{50})	1274.0	1425.0	1160.0
Observed maximum size	1830.0	1750.0	1746.0
Observed maximum age	24	27	30

The growth rates of *Triakis megalopterus* individuals from different populations are illustrated in **Figure 6.5.** The comparatively rapid growth of the AN population was most evident between the ages of six and 22 years (**Figure 6.5a**). Unfortunately, due to lack of specimens between zero and 4 years in the AN sample, there was no information available for early growth estimates. Similarly the lack of fish between the ages of zero and 12 years in the WC (**Figure 6.5b)** meant that the early growth of this population could not be well described (as evidenced by the broad confidence intervals).

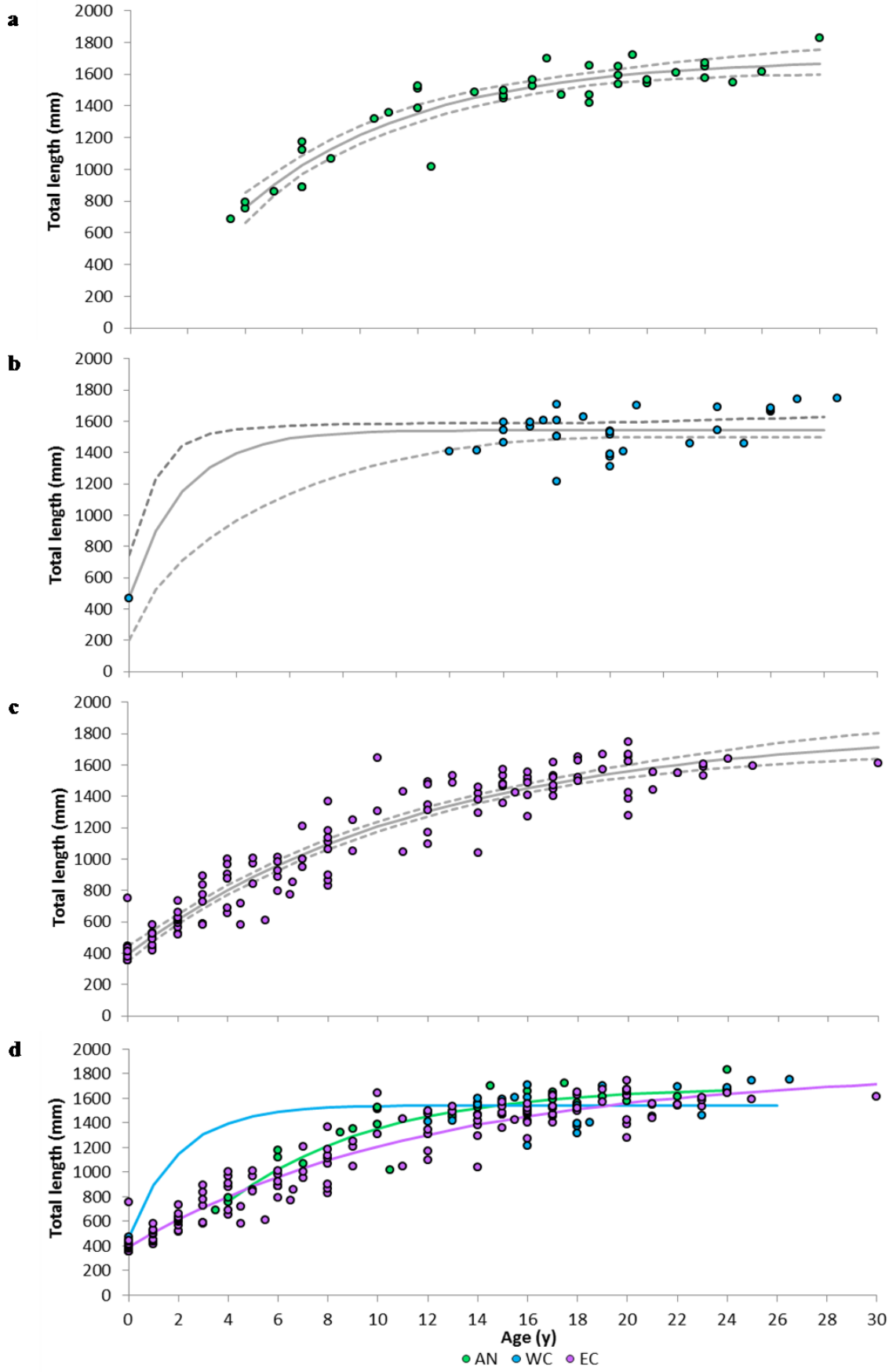

Figure 6.5: Von Bertalanffy growth function (with 95% confidence intervals) fitted to observed length-at-age data for *Triakis megalopterus* from (a) Angola, (b) Western Cape, (c) Eastern Cape and (d) all populations combined; *AN = Angola, WC = Western Cape, EC = Eastern Cape.*

Natural mortality (*M*) was highest for AN (0.143 yr^{-1}) followed by the WC (0.114 yr^{-1}) and EC (0.069 yr^{-1}). Conversely, fishing mortality (*F*) was highest for the EC (0.078 yr^{-1}) and lowest for AN (0.043 yr^{-1}).

Table 6.5: Mortality estimates for *Triakis megalopterus* from Angola (AN), Western Cape (WC) and Eastern Cape (EC)

Mortality Parameter	**AN**	**WC**	**EC**
Total mortality (*Z*)	0.19	0.17	0.15
Natural mortality (*M*)	0.14	0.11	0.07
Fishing mortality (*F*)	0.04	0.07	0.08

Size at sexual maturity

The smallest mature (1415 mm TOT) and largest immature (1410 mm TOT) males were both caught in WC (**Table 6.6**). The largest immature (899 mm) and smallest mature (1452 mm) males from EC are most likely under- and overestimations, respectively, as no adolescents and only two mature males were captured in this population. There was only a 5 mm difference in size between the smallest mature (1415 mm) and largest immature (1410 mm) males from WC. The smallest mature (1272 mm) and largest immature (1392 mm) females were captured in EC and WC, respectively.

Table 6.6: Size, largest immature and smallest mature range of *Triakis megalopterus* from Angola, Western Cape and Eastern Cape. All measurements are given in mm TOT.

	Angola	**Western Cape**	**Eastern Cape**
Males			
Largest immature	1066	1410	899
Smallest mature	1420	1415	1425
Females			
Largest immature	1355	1392	1386
Smallest mature	1320	1312	1272

Length-at-50% maturity for males and females combined was estimated to be 1274 mm in AN, 1425 mm in WC and 1160 mm in EC (**Figure 6.6**). The maturity ogive was narrow in WC and EC specimens with sharks maturing within 300 mm TOT. Maturity in AN was however represented by a wider ogive where sharks matured within 850 mm TOT.

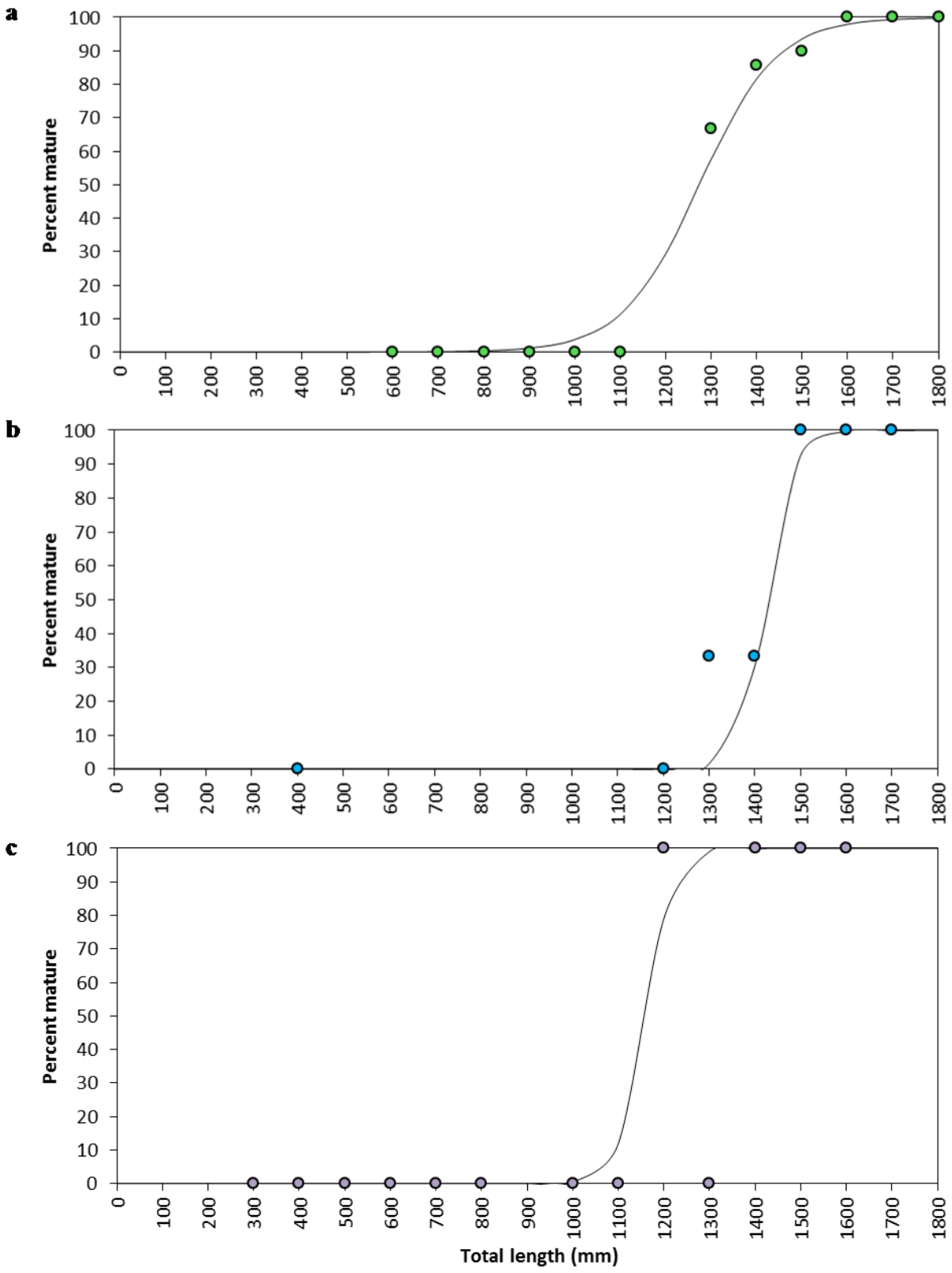

Figure 6.6: Logistic ogive for the combined sex maturation pattern of *Triakis megalopterus* from (a) Angola, (b) Western Cape and (c) Eastern Cape showing the percent maturity at total length (mm).

General reproductive characteristics

Mean clasper lengths ($\overline{CLI}$) were shortest in EC ($\overline{CLI} = 56.18$ mm), 70.5% shorter than WC ($\overline{CLI} = 190.23$ mm) and 57.0% shorter than AN ($\overline{CLI} = 130.51$ mm). The shortest claspers (20.7 mm CLI) were measured on a male captured in EC (406 mm TOT), the longest claspers (235.0 mm CLI) were measured on a 1525 mm TOT male from AN. Interestingly, the base of the claspers were already calcified in one small (857 mm TOT) male specimen from AN. Correspondingly, the CLI (101.21 mm) of this specimen was 41% larger than the $\overline{CLI}$ of all juveniles combined (42.29 mm TOT).

Of the 50 mature females sampled, 12 were pregnant with a total of 96 embryos (**Table 6.7**). The average litter size was eight embryos. The smallest mean embryo lengths were observed in two females caught in EC in February 2013. The largest mean embryo total lengths were recovered from pregnant females from AN ($\overline{TOT} = 437.45$) in July 2012 and from EC ($\overline{TOT} = 388.17$) in February 2013. These embryos were all still attached to yolk sacs (20–25 mm), although the sacs were flaccid and resorption was evident. This pregnant female from AN had a litter size of 11 in which the embryo total lengths ranged from TOT = 404–459 mm. The pregnant female from EC, however, had a litter size of six smallest and largest pups measuring 280 mm and 495 mm TOT, respectively. The WC specimens had the smallest (n = 2; $\overline{TOT} = 225$ mm) and largest (n = 12; $\overline{TOT} = 210$ mm) litter sizes. These belonged to pregnant females of comparable lengths (1740 mm and 1750 mm TOT).

The two smallest, free swimming, aged zero specimens from EC were captured in September and October indicating parturition in August/September for this population. The small, aged zero shark in WC was captured in February, and although this was only one specimen, may suggest a 3–6 month earlier commencement of parturition compared to EC. The youngest specimen from AN was 4 years of age. However, a heavily pregnant female, collected in July, contained embryos with a mean total length of 437.50 mm. Since the embryos were still attached to their yolk sacs it is possible that parturition may occur at a similar time to the EC (August–September).

The smallest neonate (396 mm TOT) and the largest embryo (495 mm TOT) recorded for the EC in this study; size at birth is estimated between 396 and 500 mm TOT. The smallest in the WC (470 mm TOT) and the largest embryo in AN (459 mm TOT) suggest (despite the low sample size) that the birth size may be fairly similar for all populations.

Table 6.7: Summary of information on the pregnant female *T. megalopterus* captured in Angola, Western Cape and Eastern Cape showing the month of capture, sex, total number, size range (total length, TOT) and mean TOT of embryos, per litter, as well as whether embryos were present in the left or right uterus

Sample Site	Pregnant female total length	Month	Total embryos (n)	Female embryos (n)	Male embryos (n)	Embryos in left uterus (n)	Embryos in right uterus (n)	Smallest Embryo (mm TL)	Largest Embryo (mm TL)	Mean embryo length (mm TL)
Angola	1650	December	6	3	3	4	2	156	251	196
Angola	1700	December	5	2	3	4	1	312	325	319
Angola	1590	December	10	8	2	5	5	322	323	323
Angola	1655	December	8	2	6	4	4	266	320	298
Angola	1614	July	11	5	6	5	6	404	459	437
Angola	1565	July	8	3	5	3	5	217	237	226
Western Cape	1740	May	12	8	4	6	6	205	215	210
Western Cape	1750	June	2	2	0	0	2	225	225	225
Eastern Cape	1612	February	6	3	3	1	5	280	495	388
Eastern Cape	1620	February	9	4	5	4	5	350	370	361
Eastern Cape	1550	February	10	-	-	5	5	84	98	82
Eastern cape	1670	February	9	-	-	4	3	76	101	93

Discussion

Interpopulation differences in *T. megalopterus* are evident from the life history analyses. In AN, sharks grow 2–3 times faster and have shorter lifespans (max age = 25 years) when compared to the EC and WC populations. Maximum size captured was smallest in EC, although this population displayed the largest L_{∞}. Size at maturity was also the smallest in the EC, which coincides with the highest fishing mortality. Although data was limited, parturition in WC appeared to commence 3–6 months earlier than in AN and EC.

Populations are dynamic entities whose characteristics and structure vary in response to numerous factors, such as their physical environment, evolutionary histories of habitat and species, human impacts, biological assemblages (Trip *et al.*, 2008), food availability (Childress, 1995) exploitation, environmental change/stressors (Planque *et al.*, 2010) and predation (Hutchings and Baum, 2005). Due to the numerous factors that have an effect on life history and the way they are intertwined with one another, establishing the causes of demographic variation is a challenging task.

Not only does *T. megalopterus* occupy coastal waters, this species also lives within different benthic habitats in different biogeographic zones with varying thermal regimes (see **Chapter 2**): all factors that have the potential to influence life history traits. For instance, lower temperatures (e.g. WC and EC) generally elicit earlier maturity (Lombardi-Carlson *et al.*, 2003), larger body size (Kozlowski *et al.*, 2004), and increased longevity (Beverton, 1992). Correspondingly, stable environments (e.g. AN) generally prompt later maturity and increased longevity (Stearns, 1976). Growth is the main factor that determines life history characteristics (Stearns, 1992). However, the growth of fishes is a very complex process dependent on many variables, such as prey abundance and temperature as well as populations genetic properties. Most sharks are ectotherms, thus, water temperature is thought to be one of the main physical factors affecting them (Speed *et al.*, 2012). Generally, it can be assumed that higher temperatures and food abundance favour faster growth rates and this has been used to explain the variation in the growth between areas (Magnussen, 2007). The AN sample site is situated in southern Angola, the most productive, pristine and unexploited (F = 0.043) of Angola's fishing zones (Beckensteiner, 2013). Temperatures in AN are approximately 5 ºC more than recorded in WC and EC (Richardson, 2010), and food abundance appears to be highest in this region (see **Chapter 5**). Warmer waters and increased foraging effort will lead to an increase in metabolism and ultimately an increase in growth. Faster growth rates in

warmer water are not unusual in elasmobranchs. For example, juvenile tiger sharks (*Galeocerdo cuvieri*) had faster growth rates in the warmer water of the Gulf of Mexico compared to the Atlantic Ocean off Virginia (Branstetter, 1987b).

Besides temperature, sharks may grow faster in environments with high risk of predation (Freitas *et al.*, 2006). While the relative abundance of predatory species in AN was not quantified, the coastal habitat, which comprised interspersed sand and low profile reef, provided limited refuge from predators. In comparison, the EC and WC coasts offer better refuge in high profile reef and kelp habitats. Therefore the faster growth in AN may be a compensatory mechanism (Freitas *et al.*, 2006) to reduce the amount of predation.

Latitude has been found to influence the life-history traits of various animal groups (Ray, 1960; Sinervo, 1990; Iverson *et al.*, 1993; Stergiou, 1999; Morrison and Hero, 2003). The latitudinal gradient theory predicts that populations will adapt and evolve to specific environmental conditions or stressors to maximize fitness (Frisk and Miller, 2006) in response to the temperature, seasonality and productivity changes associated with latitude (Trip *et al.*, 2008). Thus, populations at lower latitudes generally exhibit faster growth. This was the case in this study where AN, the population of lowest latitude, had a growth rate of 2–3 times larger than in the EC and WC, which two populations are located at a similar latitude. A latitudinal pattern of growth has been reported for other sharks such as scyliorhinids (Flammang *et al.*, 2008) and the bonnethead shark, *Sphyrna tiburo* (Parsons, 1993).

The lower temperature and larger L_∞ in the South African populations, are consistent with the temperature-size rule, which states that lower temperatures at higher latitudes should produce a cline of increasing body size with increasing latitude (Kozlowski *et al.*, 2004). According to Blackburn *et al.* (2008), larger body sizes are found at higher latitudes (and lower temperatures) as large animals have an advantage of better heat conservation because of their higher surface area to volume ratios. The same author also states that larger animals are more resistant to starvation and are more likely to survive the scarcity of resources in high latitude environments. Indeed a latitudinally-related increase in body size has been attributed to the requirement of energy reserves for the season of low resource availability in several shark species, e.g. the shortspine spurdog, *Squalus mitsukurii* (Taniuchi *et al.*, 1993), bonnethead, *Sphyrna tiburo* (Parsons, 1993; Carlson and Parsons, 1997), cloudy catshark, *Scyliorhinus torazame* (Horie and Tanaka, 2002) and the angular angel shark, *Squatina guggenheim* (Colonello *et al.*, 2007), and this may well be the case for the WC population, in particular.

Variations in length at maturity among geographic regions also have been reported for sharks and linked to temperature differences correlated to latitude (Yamaguchi *et al.*, 2000; Lombardi-Carlson *et al.*, 2003). A latitudinal increase in size at maturity has been reported for the angular angel shark, *Squatina guggenheim* (Colonello *et al.*, 2007) and the sliteye shark, *Loxodon macrorhinus* (Stevens and McLoughlin, 1991). Despite the low samples sizes, the results from this study suggest that individuals in the EC reach L_{50} at the smallest size (1160 mm TOT), followed by AN (1274 mm TOT) and WC (1425 mm TOT). It is possible that the earlier maturation in AN compared to WC may be attributed to the compound interest hypothesis which proposes that ectotherms mature earlier in warmer environments (Partridge and French, 1996; Fischer and Fiedler, 2002). As both AN and EC are warm-temperate environments (Potts *et al.*, 2015), one would expect similar size at maturity if temperature was the only influencing factor. However, this was not the case and suggests that other factors may play a role in the early maturation in EC. Unfortunately, the small sample sizes, female biased sex ratios and uneven representation of size classes, made the estimations of separate sex maturity estimates impossible and may very well have caused inaccuracies in the estimations for combined sex maturity. According to (Cortés, 2000), males generally mature at a smaller size and age (normally two years earlier) than females. This was confirmed by Smale and Goosen (1999) who identified a 130 mm differences in the size at 50% maturity for male (1320 mm) and female (1450 mm) *T. megalopterus*. This suggests that, unless the proportion of males and females were similar in each of the three populations (which they were not), the maturity ogives in this study are biased. This may also explain some of the results as males were overrepresented in the EC population. Nevertheless, the smaller size at maturity may also be an indication of exploitation in EC which will be explored further below.

While the low sample sizes are acknowledged, it was interesting that two females captured in EC (1610 mm; 30 yr) and WC (1750 mm; 27 yr) were older that the oldest age recorded by Booth *et al.* (2011) in the EC. Since one of the scientists (M. Smale) interpreting the vertebrae worked on both datasets, it is unlikely that this is a result of interpretation error. The maximum age recorded for the AN population was a 1830 mm TOT female of 24 years. Interestingly, the maximum ages recorded for this study were directly opposite to what would be expected according to the latitudinal gradient. According to this theory, lower latitudes should produce increased longevity (Beverton, 1992). However, in this study, the lowest maximum age was recorded in AN and the highest in EC. When looking at the longevity (maximum age) trends in this study, longevity from the Atlantic to the Indian Ocean

increased, with AN (Atlantic Ocean) tending to be shorter lived than EC (Indian Ocean) and WC being the intermediary population. The same pattern was seen in coral reef fish between the Indian (lower longitude) and Pacific (higher longitude) oceans, where Indian Ocean populations tended to be shorter lived than their Pacific Ocean counterparts (Trip *et al.*, 2008). The maximum age, combined with the previously discussed L_{∞} and L_{50} of *T. megalopterus* populations, indicates that longevity in AN may be a trade-off for faster growth and earlier maturity. A similar pattern was observed in the blue shark, *Prionace glauca*, in the North Atlantic Ocean (Skomal and Natanson, 2002).

Low latitude populations generally have extended seasonal windows for spawning compared to their low latitude counterparts (Srinivasan and Jones, 2006), which may result in differences in the time of parturition in the same species. The earlier, summer parturition in WC may be an adaptive response to size-selective winter mortality as this population is the only *T. megalopterus* population that is found in a cool-temperate environment. Parturition in the warmer summer waters may mitigate against the susceptibility of small sharks to cold conditions (Conover, 1990).

Based on the available information, size at birth for all populations of *T. megalopterus* is estimated between 396–500 mm TOT. This estimate is larger than previously reported (300–320 mm TOT) by Compagno (1984b) and has a wider range than the 420–450 mm TOT estimate by Smale and Goosen (1999). Unfortunately, estimates for WC were based on a single neonate and estimates for AN were based on a single litter of pups. So although we can be certain that birth size for AN and WC does fall within the estimates for EC, it may be possible that the ranges will be different. As parturition occurs earlier in WC it is possible that they may produce larger offspring in order to minimize winter mortality. This pattern has been found in *S. tiburo* where bigger neonates are born later at higher latitudes (Lombardi-Carlson *et al.*, 2003).

Due to their low reproductive rate and the direct relationship between stock size and recruitment (Holden, 1977), sharks are extremely susceptible to overfishing (Joung *et al.*, 2008). Due to their habitat preferences, large size at maturity, lengthy gestation, small litter sizes (Smale and Goosen, 1999), narrow distribution and population sizes (Compagno *et al.*, 1989), *T. megalpterus* has been listed as near threatened by the International Union for Conservation of Nature (IUCN) and is considered to be threatened with extinction in the near future (Compagno, 2009). Exploitation of *T. megalopterus* is apparent in the fishing mortality

values where all populations show fishing pressure greater than the level of 0.02 that according to Booth *et al.* (2011) is the fishing mortality rate required for a stable population. It is not surprising that fishing pressure was highest in the SA populations. Although legislated as a non-commercial species and proven to sustain very limited fishing pressure (Booth *et al.*, 2011), *T. megalopterus* is frequently mistaken in commercial fisheries as the commercial species *Mustelus mustelus* (Booth *et al.*, 2011). This species is also a frequent bycatch in the South African demersal longline fisheries (Compagno, 2009) and is exploited by recreational anglers (Attwood and Farquhar, 1999). Over exploitation has been shown to affect the feeding, growth, population size structure, reproductive potential (Cooke and Schramm, 2007) and consequently abundance (Stevens *et al.*, 2000) of targeted species. One of the most immediate and obvious effects of fishing is a reduction in the size of fish in the population because older/larger fish are preferentially eliminated from the population (Beckensteiner, 2013). Therefore, size-differential fishing results in a decline in maximum length in exploited populations (Planque *et al.*, 2010).

Another sign of exploitation is a smaller/younger age at maturity in populations exhibiting high mortalities (Promislow and Harvey, 1990). Despite the high L_∞ in the EC and WC, the average maximum size of sharks caught was 82 mm TOT smaller than in AN, with EC having the earliest maturity of all populations. Therefore, the highest fishing mortality, smallest maximum size and smallest size at maturity were all recorded in EC – all signs that this population has been affected by the greatest fishing pressure. Since this population was also identified to be a separate clade (**Chapter 3**) and divergent in terms of their morphology (**Chapter 4**), it is possible that exploitation may have driven evolutionary changes in EC (see Law, 2000; Frisk, 2010).

One of the caveats in many ageing studies (including elasmobranchs) is to stress the accuracy of age validation (Beamish and Fournier, 1981; Campana, 2001). Previously, Booth *et al.* (2011) examined 23 OTC marked vertebra, concluding that a single opaque and translucent band pair was deposited annually up to at least 25 years of age in EC. In this study, *T. megalopterus* displayed annular growth zone formation, depositing one band pair per year, validated at eight and 12 years for De Hoop Nature Reserve in the WC. This finding is also similar to the closely related *T. semifasciata* (Smith, 1984; Kusher *et al.*, 1992; Smith *et al.*, 2003). Unfortunately, as yet, no validation is available for AN. As it is possible for the same species from different areas to show differences in band formation periodicity (Schwartz, 1983; Chen *et al.*, 1990), the ageing of the AN population may be biased. While it is unlikely

that the AN population will lay down more than one opaque zone each year, validation of this is critical before this population's life history parameters can be fully accepted.

Due to difficulties associated with samples sizes, vertebral preparation and inter reader variability, growth is one of the most difficult traits to measure accurately (Cailliet and Tanaka, 1990). In this study, however, methods were kept standard meaning that if any methodological problems were encountered, these would have had an effect of all populations, simultaneously, and not caused bias or outliers in a single population.

If the life history of these *T. megalopterus* populations were influenced by genetic factors, one would expect the most divergent life history from the EC population. Indeed the AN and EC populations were the most divergent with regards to life history. This pattern also conforms to a transoceanic arrangement, Atlantic (AN, WC) versus Indian Ocean (EC) populations, and reflects the results of the mtCR and nDNA analysis (see **Chapter 3**). This separation is hypothesized to be caused by the historical split of EC population during the LGM when a drop in sea level and exposure of the Agulhas Bank caused rocky shore habitat to be replaced by sandy beaches in southern Africa. This was the driving force for the divergence of populations occupying isolated rocky shores. It may be that both genetic and phenotypic plasticity influence the varying life history of these populations, but the very divergent life history of the EC population may suggest that genetic factors play a role.

The published ORI tagging data show *T. megalopterus* to be uninterruptedly present from WC to EC and able to move between the two provinces (Dunlop and Mann, 2014). This admixture of WC and EC could be contemporary as a result of postglacial expansion into new habitats (Teske *et al.*, 2013). This recolonization, however, appears to have not been for long enough to prompt a genetic or life history response in *T. megalopterus* from EC. Thus, this population, although migrating to and from WC, still shows significantly different life history parameters to WC. Where there are similarities in the life histories (asymptotic length, longevity and growth) of EC and WC, these may be attributed to the observed genetic connectivity between these regions. Contemporary tagging data also show that there is no clear distributional break in connectivity between the WC to EC populations (Oceanographic Research Institute, unpublished data).

Of great concern is the well documented global decline and extinction risk of marine predator populations (Pauly *et al.*, 2002; Dulvy *et al.*, 2008) and the impacts that these extinctions will pose on marine ecosystems (Stevens *et al.*, 2000). Life history dictates fundamental susceptibility to extinction risk and these types of studies are essential for the conservation and management of all species (Winemiller, 2005; Joung *et al.*, 2008). This study shows the importance of assessing the entire geographical range of a species before accurate management decisions can be made. Although small sample sizes may have led to weak predictive powers in this study, the results herein do show the importance of quantifying spatial patterns in intraspecific life history traits, which according to Cope (2006), may allow for responsible management of regionally data-poor species.

Conclusions

Parturition in the cool-temperate WC appeared to commence 3–6 months earlier than in warm temperate AN and EC populations. The earlier, summer parturition in WC may be an adaptive response to size-selective winter mortality that mitigates against the susceptibility of small sharks to cold conditions. The birth size estimates recorded for *T. megalopterus* in this study are larger and have a wider range than previously documented, with embryo sizes ranging from 396–500 mm TOT.

Longevity of *T. megalopterus* is lengthier than previously noted, particularly in WC (27 years) and EC (30 years), five years longer than previous estimates (25 years) for the EC population. Interestingly, longevity conforms to a longitudinal, rather than a latitudinal, gradient. In the Atlantic Ocean (AN), *T. megalopterus* are shorter lived than in the Indian Ocean (EC) with WC being the intermediary population. The lowest longevity in AN (24 years) may be a trade-off for the faster growth and earlier maturity in AN. There was a strong latitudinal gradient in body size between AN and WC: AN reached smaller asymptotic size compared to WC. This conforms to the temperature-size rule where populations at higher latitudes, thus lower temperatures, should produce larger body sizes. EC reached L_{50} at the smallest size, followed by AN, then WC. The earlier maturation in AN compared to WC is consistent with the compound interest hypothesis, which states that ectotherms mature earlier in warmer (in this case AN) environments. The earliest maturation in EC, however, did not follow this hypothesis and appears rather to be directly correlated with the highest fishing mortality recorded for this population. Latitudinal gradients, thus water temperature, seemed to have the largest impact the growth of *T. megalopterus*, particularly between AN and WC

populations. In the warmer waters of AN, sharks grew at a higher a rate and matured at an earlier size compared to WC. The faster growth, smaller L_{∞} and L_{50} for AN shows this population lives a faster life attributable to warmer environments, but growth was also accelerated due to increased prey abundance and predator avoidance. Both SA populations showed larger L_{∞} in cooler waters which may be due to heat conservation and starvation resistance hypothesis.

As mentioned, the EC population seems to suffer the greatest fishing pressure as the highest fishing mortality, smallest maximum size and smallest size at maturity were all recorded in EC. Although EC did not generally conform to latitudinal gradients and showed more characteristics of an exploited population, this conforms to the genetic data which states that EC is a separate clade. This may be an indication of fisheries-induced evolution.

With the lack of validation for AN, and assuming annular growth bands as reported in EC and WC, it may be possible that this population has been aged incorrectly. Therefore interpretation of this age data needs to be provisional and validation is needed for AN to confirm whether age estimates are in fact correct for this population. Further analysis with larger sample sizes and a better spread of individuals over all maturity stages is also required to assess differences in the size at maturity of males and females within and amongst all populations.

Chapter 7:
General discussion

The formation of the cold Benguela Current led to a marked change in the distribution and abundance of fishes along the west coast of southern Africa. It essentially formed a barrier to gene flow between the warm-temperate fishes, which were restricted to either side of the Benguela Current (Henriques, 2011). With a known time of formation (~2 Mya), the Benguela Region has provided us with a natural laboratory in which to examine evolutionary patterns across multiple taxa and to gather much needed information on elasmobranch evolution and adaptation.

Elasmobranchs have existed for millions of years (Hoenig and Gruber, 1990) and clearly demonstrate a remarkably conservative morphology (Grogan *et al.*, 2012). With this seemingly slow rate of phenotypic change and evolution, elasmobranchs should provide a baseline against which other marine organisms can be compared. However, despite the warm temperate distribution of *T. megalopterus*, the results of this study suggest that the formation of the Benguela Current and its associated cold water upwelling systems may not have been the only vicariant barrier to the distribution of the species.

Vicariance

In the absence of obvious physical barriers, such as land bridges and offshore islands, processes shaping population structure along the South African coastline are poorly understood (Toms *et al.*, 2014). Previous studies within the same system showed that the Benguela Current was the primary cause of isolation amongst teleosts: e.g. the blacktail seabream, *Diplodus capensis* (Henriques, 2011); geelbeck croaker, *Atractoscion aequidens* (Henriques *et al.*, 2014a); leervis, *Lichia amia* (Henriques *et al.*, 2012); zebra sea bream, *Diplodus cervinus* (Gwilliam, pers. comm.); silver Kob, *Argyrosomus inodorus* (Henriques *et al.*, 2014b); baardman, *Umbrina canariensis* and steentjie, *Spondyliosoma emarginatum* (Gwilliam, in prep); as well as a cephalopod, e.g., the common octopus, *Octopus vulgaris* (De Beer, 2014). The northern versus southern Benguela subsystem structure evident in the species mentioned above is not obviously apparent in the *T. megalopterus* genetic structure. However, the mtCR data (median-joining haplotype network and phylogenetic tree) show a South Africa versus Angola split in populations. The F_{ST} values are significantly different

between the AN-WC (p = 0.027) and WC-NA (p = 0.045) population comparisons. Furthermore, AN-NA (p = 0.991) appears to be an homogeneous panmictic population, and although the WC-EC comparison is also significantly different, there is evidence of admixture between these two South African sample sites (F_{ST} = 0.380, p<0.01). This suggests that the formation of the Benguela Current may have played a role in the isolation of *T. megalopterus* populations of the northern (AN and NA) and southern (WC and EC) Benguela subsystems. Nevertheless, the mtCR F_{ST} does show the EC population to be significantly different to all other populations. This is confirmed in the nDNA (Bayesian clustering analysis) data which show that the contemporary population structure of *T. megalopterus* conforms to a transoceanic arrangement of Atlantic (AN, NA, WC) versus Indian Ocean (EC) populations. Without a clear and consistent pattern of southern versus northern Benguela population structure of *T. megalopterus*, it is apparent that there is another contributing factor to the genetic arrangement of this species.

The most plausible explanation for the consistent transoceanic arrangement of *T. megalopterus* may be climate oscillations through the Pleistocene Epoch. At that time, the coastal morphology was altered (Teske *et al.*, 2011b) by the exposure of the Agulhas Bank (Teske *et al.*, 2013) and the rocky shore habitat was reduced (Von der Heyden *et al.*, 2011; Toms *et al.*, 2014). As *T. megalopterus* is a reef associated species, it is possible that this may have resulted in the isolation of east and west coast populations. Interestingly, although this genetic structure did not agree with the findings of previous studies on teleost species separated by the Benguela Current, this pattern is congruent with the two genetically divergent lineages of Bluntnose klipfish, *Clinus cottoides* (Toms *et al.*, 2014). Although one would expect vast differences in genetic structure between a klipfish and a shark, both of these species have two things in common. Firstly, both species occupy rocky reef habitats. Secondly, both are viviparous. Two important characteristics: viviparity has been said to reduce dispersal potential, particularly in organisms utilizing rocky shore habitats (Von der Heyden *et al.*, 2008). Therefore, the exposure of the Agulhas Bank and the corresponding increase of predominantly sandy beaches would have acted much like a land bridge abruptly blocking marine circulation between the Atlantic and Indian Oceans. The rocky shore refugia of the southwest and southeast coasts of southern Africa were disconnected by sandy beaches for at least 40 000 years (Toms *et al.*, 2014). This equates to approximately 2424 generations when calculating the generation time (age at maturity/fishing mortality + 1). This appears to be a sufficient time frame to play a considerable role in the isolation and consequent divergence of the EC population.

This transoceanic pattern of population structure was also found in the yellowfin tuna, *Thunnus albacares* (Henriques, 2011). Much like *T. albacares*, *T. megalopterus* is able to withstand the colder waters of the Benguela Current and displays a larger distribution potential for genetic admixture compared to the previously mentioned teleost and cephalopod species, despite their larval stages that should theoretically increase their distribution potential. Furthermore, as with *T. albacares*, *T. megalopterus* may also migrate preferentially within ocean basins. The Cape Hope squid, *Loligo reynaudii* also showed morphometric (Van der Vyver, 2014) and genetic (Stonier, 2012) divergence between the south coast (central and EAB), west coast (WAB and west coast) and southern Angola. Isolation by distance (IBD) played a major role in the level of genetic flow between the sampled spawning aggregations, although some genetic flow between all of the groups is still occurring. The divergence of *L. reynaudii* populations is said to be caused by IBD as squid do not all necessarily move very large distances, despite their larval stages that should theoretically allow for increased dispersal potentials. Therefore, the transoceanic divergence of this species may also have been impacted by both the inception of the Benguela Current and the exposure of the Agulhas Bank, much like *T. megalopterus*.

The general patterns in other characteristics (morphology and life history) is congruent with the genetic structure of *T. megalopterus* populations, where EC is a separate clade and AN and EC are the most divergent populations. There does, however, appear to be interactions between the environment and underlying genetic architecture of this species that have affected the morphology and life history. The findings of this thesis suggest that several environmental factors may have played a role in the morphological and life history divergence between the populations since their isolation. These include sea temperature, prey availability, exploitation and habitat.

Temperature

Mean sea temperature was possibly one of the most noticeable differences in the habitats of the three populations (AN = 20.4 ºC, WC = 16.5 ºC, EC = 18.0 ºC). Indeed it appeared that this factor played a role in shaping the life history parameters of *T. megalopterus*, with higher growth rates observed in warmer temperatures. Faster growth rates generally have an impact on other life history parameters, such as size at maturity, which was lower in the populations where growth was the fastest. These trends conformed to the latitudinal gradient theory where species tend to grow faster and mature earlier in warmer waters.

Faster growth, smaller L_∞ and L_{50} for AN shows that in this population, *T. megalopterus* live faster lives attributable to warmer environments but growth is also accentuated due to increased prey abundance and predator avoidance. Bergmann's rule states that larger adult size is expected in colder environments (Partridge and French, 1996; Blackburn *et al.*, 2008) and in this case both of the SA populations had larger L_∞ (and maximum observed sizes) than the AN population. Blackburn *et al.* (2008) attributed larger size in cool environments to the requirements for heat conservation and resistance to starvation. While the results of this study seem to correlate with Bergmann's rule, the reduced sample sizes obtained in this study may also have had an influence, and the outcome (in terms of maximum size) may have been coincidental. However, besides maximum size, temperature may have also influenced other life history parameters, such as maturation and parturition. Here sharks in the cool temperate WC habitat matured later, while their parturition occurred earlier in the year. The earlier parturition most likely could be attributed to an adaptive response to size-selective winter mortality: parturition in warmer summer waters may mitigate against the susceptibility of small sharks to cold conditions (i.e., the tendency of small fish to die more readily than large fish; Conover, 1990). Unfortunately, there does not appear to be any published information of this phenomenon occurring in sharks.

The feeding of *T. megalopterus* was indirectly influenced by temperature through its impact on prey diversity. The temperature hypothesis suggests that increased temperature and therefore metabolism, supports greater speciation rates, culminating in higher diversity (Rohde, 1992). Therefore, species richness (and thus prey diversity) tends to increase closer to the equator with increasing temperature. The highest prey diversity in the Angola-Benguela Front and lowest prey diversity in WC conform to the aforementioned temperature hypothesis.

Exploitation

Exploitation may also have a profound effect on a population, principally in the life history as demographic traits such as growth and reproduction change under fishing pressure (Rodhouse *et al.*, 1998). However, several recent studies have also highlighted the influence of exploitation on the evolution of fish species (e.g. Conover and Munch, 2002; Heino and Godo, 2002; Grift *et al.*, 2003; De Roos *et al.*, 2006; Kuparinen and Merilä, 2007; Mollet *et al.*, 2007; Swain *et al.*, 2007; Enberg *et al.*, 2009). The potential impacts of exploitation were observed in the EC population, which is subject to the highest exploitation ($F = 0.78\ yr^{-1}$).

This is not surprising as *T. megalopterus* in the EC are exploited by the inshore recreational and the commercial shark fisheries (Smale and Goosen, 1999). Here the *T. megalopterus* population matured at 1160 mm TOT, which is 114 mm smaller than in AN and 265 mm smaller than in WC. The earlier onset of maturity in the EC may provide this population with some resilience to the impacts of exploitation by increasing the lifetime reproductive potential of individuals (Geraghty, 2013). This has been observed in *Carcharhinus obscurus* and *C. plumbeus*, which appear to mature at younger ages in south-east Australian waters compared to New South Wales (Geraghty, 2013).

Theoretically, exploitation should promote faster growth by reducing biomass, which decreases pressures of intraspecific competition (Rodhouse *et al.*, 1998; Law, 2000). However, exploitation can also remove the fastest growers first, and the consequence of this may be a fisheries-induced evolutionary response towards slower growth. The results of this study may hint towards a fishery induced evolutionary response. But ultimately, the comparisons may also be flawed due to the low samples sizes, and other potential confounding factors (such as prey availability) and further investigation is required.

Exploitation not only directly affects the life history of a population, it may also indirectly affect the feeding of a population when prey species are the focal point of the exploitation. While the diets of *T. megalopterus* from AN and EC consisted of teleosts, crustaceans and molluscs, in the WC these sharks fed almost exclusively on Cape rock lobster (*J. lalandii*). While the latitudinal and temperature hypotheses may have played a role in this result, (ie decreased prey diversity), a trophic cascade has been documented in the WC (Griffiths, 2000). This cascade could be ascribed in particular, to the overfishing of the WC linefish populations. Griffiths (2000) documented an 80% drop in commercial catch per unit effort (CPUE). This kind of decline was already reported over a decade ago in the Western Cape (Attwood and Farquhar, 1999) and suggests that many of the teleost prey are no longer readily available to the WC population. The second factor influencing the trophic cascade was the overexploitation of the Cape fur seal (*Arctocephalus pusillus*) in the Cape Hangklip area. This resulted in an increase in the quantity of their dominant prey species, the Cape rock lobster, *Jasus lalandii* (Tarr *et al.*, 1996; Turpie *et al.*, 2003; Cockcroft *et al.*, 2008; Blamey and Branch, 2012). The differences in feeding of *T. megalopterus* in the three populations suggest that this species has a broad trophic adaptability (defined as the ability of an organism to take advantage of the most profitable prey source at a particular time; Gerking, 2014). This is afforded to them by a mouth, teeth and jaw arrangement which favours generalist feeding.

They are also able to employ flexible foraging tactics, which include fast swimming to capture pelagic prey (as is the case for the AN population) and the ability to navigate high relief areas to capture crustaceans in rocky habitats (WC population). A morphology that supports broad trophic adaptability could be one of the key features that have allowed elasmobranchs to remain relatively unchanged through time.

Habitat

Different geographic regions are subject to contrasting selective pressures that have the ability to alter the phenotype of individuals by changing basic biological parameters (Bakun, 2010), fitness and behaviour (Shiu and Borevitz, 2008). We know that *T. megalopterus* occupy different habitats throughout their southern African distribution (see Chapter 2). Coarse sand and clay/silt ocean floors in AN (Bianchi, 1992), rocky reefs with large sandy sections and kelp forests (Turpie *et al*., 2009) in WC and mixed sand and rocky reef surf zones (Hutchings and Clark, 2012) in EC. These different habitats appeared to have had an effect on the morphology of *T. megalopterus* although the main driver of these may be related to optimizing the feeding success on the prey associated with these habitats. Compared to SA populations, AN sharks possess smaller fins (pectoral, 1st and 2nd dorsal), wider fin bases (pelvic, 2nd dorsal) and wider tail regions, all of which point to a more streamlined body that appears to be adapted for faster, but less manoeuvrable swimming. Therefore, in relation to their more open, sandy and less "reefy" environments, AN sharks are better suited to hunt more elusive, fast prey such as teleosts or cephalopods in the open water column. In contrast, the South African populations are morphologically better suited to hunt in more high relief habitats such as rocky reefs and in kelp forests. As of yet, there appears to be no evidence of other studies that have documented intrapopulation significant divergence of morphological features associated with prey capture between shark populations.

Although these morphological differences appear to be an adaptation to the local environment (i.e. phenotypic plasticity), this thesis provided some evidence there may be a genetic mechanism influencing these changes. When traditional morphometric variables were analysed using haplotype as a grouping variable, there were significant morphological differences between fish with the unique AN haplotype (TMH5) and the others. Individuals with the TMH5 haplotype had a smaller second dorsal fin, smaller gill slits and eyes and larger dorsal base and caudle peduncle height. This discrete morphology therefore correlates with the presence of two independent evolutionary lineages and suggests that there may be

directional selection on the above mentioned morphological characters in the AN population. Unfortunately, there is limited evidence for selection in elasmobranch populations. This study however suggests that given contrasting selective pressures (mainly associated with habitat, and diet), the morphology of sharks may diverge and that this divergence may be a result of genetic selection. In this species, it is estimated that these changes were prevalent after an isolation of approximately 2424 generations (see above calculation). However, since there are two or more mutational steps between the AN and other haplotypes, these evolutionary changes may have occurred earlier, and relatively rapidly.

Truss protocol

Landmark morphological methods have been used extensively in teleost taxonomy (Richtsmeier *et al.*, 2002). Landmark methods are a powerful tool for identifying morphological variation that uniformly incorporates the entire specimen (Cavalcanti *et al.*, 1999) and provides greater discriminatory power (Cadrin and Friedland, 1999). Correspondingly, unlike traditional techniques, landmark methods also have the advantage of incorporating depth and breadth characteristics (Humphries *et al.*, 1981) and make use of anatomical rather than extremal landmarks, where true homologous points defined by biological characteristics are used (Jardine, 1969).

Due to the slow molecular evolutionary rate of elasmobranchs (Martin *et al.*, 1992; Martin and Palumbi, 1993b; Martin, 1995), morphological changes between shark populations may be difficult to detect. This is why it is imperative to incorporate all possible morphometric methods to increase the likelihoods of detecting intrapopulation differences in sharks. The deficiency of geometric data on whole specimens is, however, justifiable as the sheer size and shape of elasmobranchs in general make it difficult, if not impossible, to accurately measure diagonal lines across the body. The use of digital photography and digitizers are not feasible on larger specimens as their cartilaginous skeletons lose shape on land. While recording video footage from flow tanks is possible with smaller sharks (e.g. Wilga and Lauder, 2000), this method is also not feasible on larger specimens. The method proposed in this thesis makes use of approximately 12 measurements taken from a specimen, after which the outline of the sample is reconstructed in AutoCAD. With the use of AutoCAD, there are key advantages of instantly visualizing the form of specimens and recognizing measurement errors as the reconstruction will not be successful with errors in measurements. The truss was successful in detecting differences between populations of *T. megalopterus* and even managed to detect

differences that were overlooked by the traditional morphology (e.g. snout length between AN and WC). The truss morphology also identified that specimens from EC have a wider head (V1 and V2) and caudal (V6) region compared to AN (see **Chapter 4**).

Because this thesis shows that *T. megalopterus* is susceptible to exploitation and is already showing the effects thereof, this and other protected species would benefit greatly from non-lethal taxonomic methods. For this the novel method of morphological analyses developed in this study can be of great benefit to protected species. This truss network can use as little as 12 measurements to recreate the outline of a shark using architectural software (AutoCAD). On average, when working with a preserved specimen, 102 measurements took approximately 25 minutes to complete. This equates to approximately 14.7 seconds per measurement. If the truss network requires only 12 measurements, this could potentially be completed on a shark that is placed into tonic immobility within 3 minutes. Thus, with further refinement, this technique could be breakthrough in the field of elasmobranch geometric morphometrics and morphological analyses, particularly for protected species where lethal measures are not an option. An additional benefit of the proposed truss method is that it can be used through the conversion of existing morphological datasets. This will allow the reanalysis of existing datasets and may provide valuable additional morphological information for taxonomic research or population delineation. In the future, this protocol may also be useful in fisheries management as a means of population identification. When combined with genetic analysis this new proposed truss analysis could provide methods for non-lethal evolutionary and taxonomic studies on sharks that may also contribute to our understanding of their adaptive and non-adaptive plasticity.

Shortcomings

According to the genetics of *T. megalopterus*, EC is a separate clade. Unfortunately, due to funding constraints, there was a large interval between sampling sites (Betty's Bay and Port Elizabeth), which prevented the identification of a potential break or overlap in lineages in this area. Therefore, a better geographic representation of *T. megalopterus*, particularly around Cape Agulhas, would have been beneficial for the genetic and morphometric chapters of this thesis.

There is ambiguity in describing the genetic versus plastic and adaptive versus nonadaptive variations amongst populations of *T. megalopterus*. However, without the inclusion of studies

such as population genomics, where specific genes pertinent to recent and ongoing differentiation are identified, a more accurate description of plasticity, adaptation and /or evolution was not possible. Similarly, climate-associated shifts in genotype and/or phenotype can only be postulated as it is difficult to pin point the exact environmental factors that cause a particular genotypic/phenotypic change.

Small sample sizes and an uneven spread of sex and size classes hampered the assessment of ontogenetic and seasonal feeding differences. Also, sexes were pooled for the maturity ogives and growth models for each population due to low sample sizes, female biased sex ratios and an uneven spread of maturity stage classes. This is not ideal, as the growth and maturity of *T. megalopterus* differs between sexes (Booth *et al.*, 2011). Also, with the lack of validation for AN and assuming annular growth bands as reported in EC and WC, it may be possible that this population has been aged incorrectly. Therefore interpretation of these age data needs to be provisional and validation needs to be undertaken in *T. megalopterus* from AN to confirm whether age estimates are in fact correct for this population.

Future research

To build on the results of this thesis and gain a better understanding of shark evolution and phenotypic plasticity, accurately determining the genetic versus plastic changes in *T. megalopterus* is essential. Population genomics will be highly beneficial to gain a better understanding of microevolution and the evolutionary processes that affect genomes. This type of analysis will not only facilitate the identification of adaptive molecular variation but also increase the estimation accuracy of important parameters such as phylogenetic relationships, population size and migration rates (Luikart *et al.*, 2003). Determining an accurate mutation rate for *T. megalopterus* will also permit a more precise description of the demographic history of this species as well as patterns of population stability, dating population expansion events and accurate effective population sizes. Information such as prey detection and capture capabilities is still needed for a more comprehensive understanding of feeding strategy of *T. megalopterus*. Studies on the ecomorphology of *T. megalopterus* will also be beneficial to give us a better understanding of the relationship between the ecological role of an individual and its morphological adaptations.

Conclusion

Unlike many other warm-temperate coastal marine species, *T. megalopterus* populations appeared to retain some connectivity across the cold Benguela Current system. This suggests that the adults of this species (which are the most mobile life history stage) are cold water tolerant and able to pass through the cold water barrier associated with the Lüderitz Upwelling Cell. Genetic isolation was however evident between the EC and AN populations, which suggested that historical climate changes (including sea level change) associated with the Pleistocene Epoch were responsible for a historical Atlantic/ Indian ocean isolation.

Despite the fact that sharks are thought to evolve at a slower rate than other animals (Martin *et al.*, 1992; Martin and Palumbi, 1993b), this study provided some evidence to suggest that genetic and morphological divergence may occur at equivalent rates to certain teleost species, particularly when exposed to selective pressures associated with feeding. While there was some evidence for adaptive evolution (e.g. the more streamline body and smaller gill slits of AN specimens and fisheries-induced evolution in EC), there was also evidence for phenotypic plasticity (e.g. larger tail regions and trunk height and width in AN). Therefore, it is possible that sharks are able to adapt to their environments and display a level of phenotypic plasticity, despite their "apparent slow evolutionary potentials".

The ability to evaluate fish stocks with minimal data is becoming increasingly important (Cope, 2006). By linking genetic data, which reveals intraspecific population substructure, with other information (e.g. morphology, feeding and life history), one can assess localized adaptations and plasticity in a population, which is imperative in species management (Avise, 2000; Roff, 2002). Although, the predictive power of this study may currently be weak due to the reduced sample sizes, the results of this thesis highlight the importance of incorporating a broad range of information through the distribution range of elasmobranch species. Failure to do that may result in the poor definitions of the "stock" and ultimately in unsuitable management recommendations for the species.

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