

**THE CONSERVATION OF SOUTH AFRICAN WHITE SHARKS: POPULATION
NUMBERS, GENETIC DISTINCTIVENESS AND GLOBAL CONNECTIONS**

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DECLARATION

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ABSTRACT

The white shark (*Carcharodon carcharias*) is IUCN red listed as vulnerable, but the lack of basic biological information is arguably the biggest obstacle facing the conservation of the species. The aim of this project was to aid in the conservation of white sharks by producing various data sets that can be used in adaptive management. In doing so we estimated the South African white shark population number, their genetic connectivity along the coastline and investigate various behavioural aspects. We developed a categorization system to manage large photographic databases for individual identifications of white sharks by making use of dorsal fin images. The novel categorization system was developed by making use of 4398 photos taken over a 27 month period. A notches code method was produced and this proved to significantly reduce the search time associated to accurately identify individuals. From the photos we identified 426 individuals in the Gansbaai region of South Africa. By using a mark-recapture technique and the open population model POPAN, we estimated a range between 353 - 522 individuals (95% confidence). These data were confirmed by analyses of 14 polymorphic microsatellite markers for *C. carcharias* that revealed a contemporary effective population size (CNe) of 338 individuals (95% confidence, $P_{crit} = 0.01$). Both estimates are in the same range but considerably less than a previously published estimate (e.g. $N = 808$ to 1008) that relied on fin matching software (DARWIN) to automatically match the sharks' dorsal fin. Through software validation, we provided evidence that DARWIN failed to produce accurate estimates and the discrepancy in population numbers are most likely due to the inclusion of false negatives in the published literature. To determine whether the Gansbaai population forms a unique evolutionary unit, the phylogeography of white sharks along the South African coastline was investigated by making use of mtDNA and microsatellite markers. A total of 238 unique individuals were sampled originating from five aggregation sites. Four mtDNA haplotypes were found for the entire range. One common mtDNA haplotype was shared by 89% of the individuals sampled, and a second haplotype (13 bp different) was present in 10% of the remaining sharks. No phylogeographic structure was found among aggregation sites. This finding was supported by microsatellite analyses and both data sets show a remarkably low level of

genetic diversity ($h = 0.02$, $\pi = 0.0027$; $N_a = 7.6$, $H_o = 0.675$). The genetic results suggest that the South African population is the result of a founder event or a severe bottleneck in the recent past. These data were combined with published mtDNA data at the global level and results suggest that at the continental scale three distinct mtDNA clades occur. These are confined to the Mediterranean and Indo-Pacific Oceans (Clade 1), the Atlantic and Indian Oceans (Clade 2) and a single haplotype restricted to the waters of South Africa (Clade 3). These clades are probably the result of allopatric speciation associated with the closure of the Isthmus of Panama as confirmed by dating analyses. By combining the mark-recapture analyses and genetic techniques, permutations test revealed that during scavenging situations, sharks associate with conspecifics in a non-random structure (Mean of pairwise associations = 728, mean of permutation test = 597, $P = 0.00$). Analyses of the nature of such associations were not statistically significant, but provided some insights (e.g. partial sexual segregation and different class size groupings) indicative of a complex social system which may rather mirror that of marine mammals. Based on the results of this study South African white sharks require more protection than previously thought and a long term management plan is needed to secure the future survival of the species, this will need to take into account the low genetic diversity and to include constant assessments of the population numbers.

OPSOMMING

Die wit haai (*Carcharodon carcharias*) is deur die IUCN Rooi Lys gelys as kwesbaar, maar die gebrek aan basiese biologiese inligting is waarskynlik die grootste struikelblok wat die bewaring van die spesies in die gesig staar. Die doel van die projek was om te help met die bewaring van die wit haai deur verskei stelle data te genereer wat vir die aanpasbare bestuur van die spesie gebruik kan word. Deur dit te doen kon ons skat wat die Suid-Afrikaanse bevolkingsgrootte van die wit haai is, hulle genetiese verbinding langs die kus is, asook verskeie gedrags aspekte ondersoek. Deur gebruik te maak van fin-beelde het ons 'n groepeerings stelsel ontwikkel wat help om groot fotografiese databasise te orden vir individuele identifikasies van die wit haai. Die unieke kategoriseringstelsel is ontwikkel deur gebruik te maak van 4398 fotos wat geneem is oor 'n tydperk van 27 maande. 'n Kerwe kode metode was ontwikkel wat drasties gehelp het om tyd te bespaar tydens die akurate indentifikasie van individuele diere. 426 individuele haaie was geïdentifiseer in die Gansbaai streek van Suid Afrika. Deur gebruik te maak van 'n merk-en-hervang tegniek en die oop populasie model POPAN, het ons beraam dat daar tussen 353-522 individue tans is (95% akuraat). Hierdie data is bevestig deur die ontleding van 14 polimorfiese mikrosatelliet merkers vir *C. carcharias* wat 'n kontemporêre effektiewe bevolkingsgrootte (CNe) van 338 individue voorstel (95% vertroue, $P_{crit} = 0,01$). Beide berekininge is in dieselfde omtrek maar aansienlik minder as wat voorheen beraam is (b.v. $N = 808$ tot 1008). Die vorige beraming is gedoen deur te vertrou op die sagteware program (DARWIN) wat die haaie se dorsale-finpatroon outomaties soek en identifiseer. Deur die sagteware te toets het ons bewys dat DARWIN nie kon voldoen aan die vereiste om akkurate skattings te produseer nie en die verskil in bevolkingsgetalle is waarskynlik as gevolg van die insluiting van vals positiewes in die gepubliseerde literatuur. Om te bepaal of die Gansbaai bevolking 'n unieke evolusionêre eenheid vorm, was die filogeografie van die wit haaie langs die Suid-Afrikaanse kuslyn ondersoek deur gebruik te maak van mtDNA en mikrosatelliet merkers. 'n Totaal van 238 unieke individue is versamel afkomstig uit vyf verskillende streke. Slegs vier mtDNA haplotipes is gevind vir die hele Suid Afrikaanse verspreiding. Een algemene mtDNA haplotipe is gedeel deur 89% van die individue, en 'n tweede haplotipe (wat 13

bp verskillend was) was teenwoordig in net 10% van die oorblywende haaie. Geen filogeografiese struktuur is gevind langs die kuslyn nie. Hierdie bevinding word ondersteun deur die mikrosatelliet ontledings en beide stelle data toon 'n merkwaardig lae vlak van genetiese diversiteit ($h = 0,02$, $\pi = 0,0027$; $N_a = 7.6$, $H_o = 0,675$). Die genetiese resultate dui daarop dat die Suid-Afrikaanse bevolking moontlik die gevolg is van 'n stigter-gebeurtenis of 'n ernstige bottelnek in die onlangse verlede. Hierdie data is gekombineer met gepubliseerde mtDNA data op die globale vlak en resultate dui daarop dat op kontinentale skaal, drie afsonderlike mtDNA klades voorkom. Dit is beperk tot die Middellandse See en Indo-Stille Oseaan (klade 1), die Atlantiese en Indiese Oseane (klade 2) en 'n enkele haplotipe beperk tot die waters van Suid-Afrika (klade 3). Hierdie klades is waarskynlik die gevolg van allopatriese spesievorming wat verband hou met die sluiting van die Isthmus van Panama soos bevestig deur daterings wat gedoen is. Deur die merk-en-hervangmetode ontleding en genetiese tegnieke te kombineer, het permutasie toets getoon dat gedurende aas-voeding situasies, assosieer haaie met mekaar in 'n nie-ewekansige manier (gemiddelde van paarsgewyse verbindings = 728, gemiddeld van permutasie toets = 597, $P = 0,00$). Ontleding van die aard van sulke verwantskappe was nie statisties beduidend nie, maar verskaf 'n paar insigte (bv. gedeeltelike seksuele segregasie en verskillende klas grootte groepe assosieer met mekaar). Hierdie toon op 'n aanduiding van 'n komplekse sosiale stelsel wat weerspieël word in ander mariene soogdiere. Gebaseer op die resultate van hierdie studie, dui dit duidelik daarop dat die Suid-Afrikaanse wit haaie vereis meer beskerming as wat voorheen gedink is en 'n langtermyn bestuursplan is nodig om die voortbestaan van die spesie te verseker. Lae genetiese diversiteit moet in ag geneem word en die gereelde assessering van bevolkings-getalle moet in plek gestel word.

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CHAPTER 1

GENERAL INTRODUCTION ON THE BIOLOGY AND CONSERVATION OF THE WHITE SHARK *CARCHARODON CARCHARIAS*

1 Introduction

1.1 General biology of the white shark

The white shark (*Carcharodon carcharias* L.) is an elasmobranch at the top of the marine trophic web and has a nearly worldwide distribution (Pardini et al. 2001; Boustany et al. 2002; Bonfil 2005; Jorgensen et al. 2009; Gubili et al. 2010). In contrast to many marine fishes, white sharks are considered to reach sexual maturity slowly: males mature at ≥ 3.8 m TL (Mollet et al. 1996), and females at ≥ 4.5 m (Hubbell 1996). Maturity in the male can be determined by the increased size, mobility and calcification of the claspers (Pratt 1996). The sexual dimorphism between males and females, determined by the presence of the external organs of reproduction, is well visible externally and, most of the time, allows for gender recognition during the data collection (Pratt 1996).

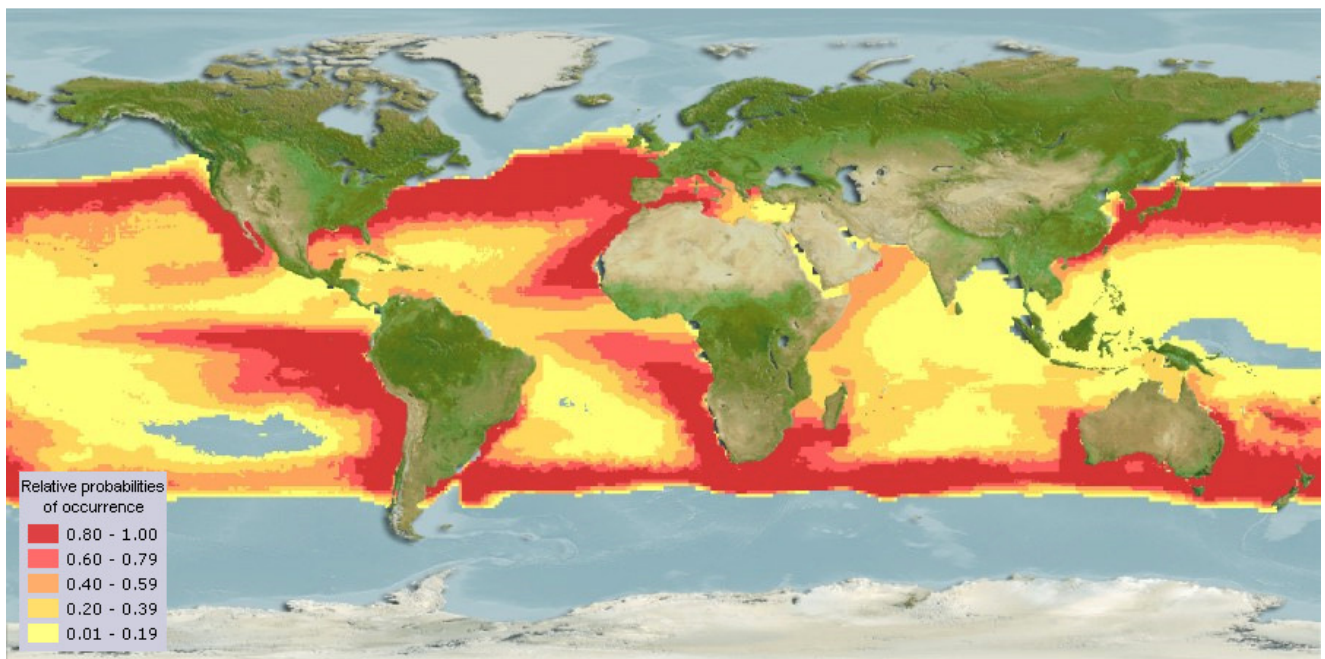


Figure 1.1 White shark global distributions, based on the relative probability of occurrence (taken from www.fishbase.org).

To determine the age of individual white sharks is more problematic. Specifically, their growth rate is difficult to estimate and possibly correlated with regional endothermy: as part of the family *Lamnidae*, white sharks can warm up regions of their body (from 8 to 15 °C higher than the water

temperature) thanks to a counter-current heat exchange system known as the *rete mirabile* (McCosker 1987). The regional endothermy causes variations in the distribution of the energy towards growth and thermoregulation, which ultimately results in a non-constant growth at different water temperatures (McCosker 1987; Goldman 1997; Domeier 2012).

From studies on their stomach contents and teeth ontogeny it appears that juveniles and adults white sharks do not compete for the same food resources, as they have different diets (Tricas & McCosker 1984; Klimley 1985; Compagno et al. 2001). Juveniles predate mostly on Teleostei and other elasmobranch species and adults supplement their diet (approximately the 20% of it) with more energy rewarding marine mammals (Tricas & McCosker 1984; Klimley 1985; Compagno et al. 2001; Laroche et al. 2008).

Despite the worldwide distribution, photographic identification and genetic analyses suggest that white sharks display high site fidelity around pinnipeds' colonies (or more generally around sites where food is readily available) and females tend to be philopatric (Klimley 1985; Pardini et al. 2001; Domeier & Nasby-Lucas 2006, 2006, 2008; Weng et al. 2007; Jorgensen et al. 2009; Gubili et al. 2010; Anderson et al. 2011; Blower et al. 2012; Kock et al. 2013). In the shorter term, however, satellite tagging provided evidence for short scale movements around the South African coastline as well as transoceanic return migration between South Africa and Australia (Bonfil 2005).

1.2 Conservation problems and challenges

Although considered as one of the most studied sharks in the world (Domeier 2012) a large degree of uncertainty exist regarding white shark population numbers (Cliff et al. 1996; Chapple et al. 2011; Blower et al. 2012; Towner et al. 2013), number of stocks worldwide, and the general population dynamics (Jorgensen et al. 2009; Blower et al. 2012). Since the species status is vulnerable by the International Union for Conservation of Nature (In: IUCN red list of threatened species. Version 2014.1. Available at www.iucnredlist.org), protected by the Convention on

International Trade in Endangered Species (CITES) and the Convention for Migratory Species (CMS) more data is critically needed to assist with the white shark conservation planning.

The estimation of population abundance (n) and effective population size (N_e ; the number of breeding individuals) can help to predict the extinction risk of populations (Luikart et al. 2010, see also chapter 3), but most of these parameters are based on model assumptions that, mostly for white sharks, have not been extensively studied. For example the lack of knowledge regarding growth rate, age of maturity, gestation time, frequency and longevity are all hampering the conservation efforts. Under these circumstances, a precautionary conservation approach has been adopted including legislative protection measures controlling the illegal catching of white sharks and the trade in their derivatives (e.g., fins, skin, jaws, and flesh). Countries affected include Australia, South Africa, Namibia, Malta, and California and the Atlantic states of the U.S.A. (Compagno et al. 1997). However, despite legal protection, there is still a huge demand for white shark body parts such as jaws, and fins (Compagno et al. 1997; Chapman et al. 2003; Worm et al. 2013). In addition many individual white sharks are killed on a constant basis by anti-shark nets and baited drumlines primarily employed for beachgoer protection (Cliff et al. 1989; Department of Fisheries Western Australia 2013). In Durban, South Africa, seven gill nets were deployed in 1952 (each 130 m long) and in the first year of operation 552 elasmobranchs were caught in these nets (<http://www.shark.co.za/CatchStatistics>). Since 1989, live sharks are released but it is estimated that only 12,5% of the sharks captured in anti-sharks nets survive (Cliff et al. 1989; Wetherbee et al. 1994; Department of Fisheries Western Australia 2013). Shark nets caught approximately 1063 white sharks from 1978 to 2008 (Peschak 2009). Without accurate knowledge on the population numbers, the long term survival of white sharks is at risk.

1.3 Extant population assessment by individual photographic identification

Over the last 15 years, photographic identification techniques have been used to study population ecology and life history of many species, particularly those under threat (Wursig et al. 1990; Langtimm et al. 2004; Chapple et al. 2011; Marshall & Pierce 2012). The method has been successfully adopted for several wild terrestrial and marine species (Wursig et al. 1990; Dufault & Whitehead 1995; Markowitz et al. 2003; Gamble et al. 2007). Photographic identification techniques have several advantages, as they are relatively cheap, non-invasive and allow the researcher to “re-sample” the investigated individuals several times, with limited interference into their natural activities (see also chapter 2 and chapter 3). The recent improvements of digital photography also allows improvements in that several high quality pictures can be taken and viewed instantaneously without additional printing expenses (Markowitz et al. 2003). Furthermore digital images are easier to store, compare and share in real time than printed photographs.

The white shark was one of the first elasmobranch species on which a photographic identification approach was implemented. Peter Klimley and Scot Anderson (Klimley et al. 1996) were the first to use photographic evidence to verify the movements of white sharks in Californian waters, after which numerous additional studies followed (Anderson & Goldman 1996; Domeier & Nasby-Lucas 2006; Anderson et al. 2011; Chapple et al. 2011; Towner et al. 2013). Different identification techniques have been employed on the white sharks: the most common is a dorsal fin photograph, but also alternative methods based upon the underwater photographs of the pigment patterns on the gill flaps, pelvic fins, and caudal fins have also been tested (Domeier & Nasby-Lucas 2006). Two recent studies, however, showed evidence that the pigmentation pattern can be subjected to temporal changes (Anderson et al. 2011; Robbins & Fox 2012), while the pattern on the rear of the dorsal fin can persist for at least 22 years (Anderson et al. 2011). The latter is thus regarded a more reliable permanent identification marker (Anderson et al. 2011).

Despite being able to provide extremely valuable information, photo identification also presents several challenges (Gamble et al. 2007) from weather-dependent data collection to the time consuming visual matching of the images (Marshall & Pierce 2012). Image analysis techniques

require that images should be comparable in terms of quality, size and observation angle. To date a standardization method for white shark photographic identification has not been developed, resulting in images from different datasets not being comparable to each other.

The selection of good-quality images to ensure the reliability of the data is one of the stepping stones of good photographic identification practices (Stenhouse 1985; Gowans & Whitehead 2001) and, in the absence of existing guidelines, some studies on white sharks might have been compromised by the poor quality of photographic images (Gubili et al. 2009; Chapple et al. 2011). The second challenge is data processing; working with large photographic catalogues is labour-intensive and without an optimal visual matching protocol, the results are susceptible to human error (Galton 1895; Kelly 2001; Van Tienhoven et al. 2007; Schofield et al. 2008; Martin-Smith 2011). Indeed the success of previous studies was confined to datasets with relatively low numbers of images (i.e. 321 images of Chapple et al. 2011). Recently there have been attempts to optimise data processing by identifying white sharks using software matching (Towner et al. 2013) originally developed for use with marine mammals (e.g. DARWIN, FINSCAN); however, in some other investigations this technique has resulted in unacceptably high levels of errors in matching images (Chapple et al. 2011).

To avoid some of these shortcomings, especially when dealing with large numbers of individuals, personal markings should rather be subjected to categorical coding (Galton 1895; Gill 1978; Gamble et al. 2007), in which a morphological pattern can be translated into a numerical code.. It is thus important to develop and validate a photo identification technique that incorporates coding of unique marks on white sharks' dorsal fins that will allow accurately identification over a temporal scale.

1.4 Historical and contemporary population genetics

To verify and test the results obtained from a photo identification technique, the addition of genetic data is hugely beneficial. From a conservation perspective, genetic data can provide a more robust picture about the population dynamics, site philopatry, effective population sizes, and metapopulation dynamics. By combining the genetic data with individual identification, some aspects of the behaviour of white sharks can also be investigated (Gubili et al. 2009). Along these lines, genetic techniques already provided insights into the process driving white shark biodiversity (Pardini et al. 2001; Jorgensen et al. 2009; Dudgeon et al. 2012; Blower et al. 2012; Ovenden 2014) and genetic markers have been used in other marine predators, to determine population structure between different localities, migration rate or movements among regions (Hoelzel et al. 2002, 2006; Natoli et al. 2005, 2006; Meekan et al. 2006; Castro et al. 2007). The maternally inherited DNA control region provides a more conservative picture if compared to the nuclear microsatellite DNA, but a combined analysis of the two markers can provide insights into sex biased dispersal and connectivity among populations at different scales (Awise 1994; Holsinger & Weir 2009; Dudgeon et al. 2012).

To date few genetic samples are available and often come from opportunistic sampling of sharks caught in fishing nets or stranded on beaches. Only 256 samples are available for genetic analyses worldwide, and originate from four publications: 52 from Australia and 43 from South Africa (Pardini et al. 2001), 59 from California (Jorgensen et al. 2009), five from the Mediterranean (Gubili et al. 2010) and 97 from Australia (Blower et al. 2012). From these, the comparison of the maternally inherited mitochondrial genome revealed two divergent genetic lineages within white sharks from Australia and from South Africa, suggesting female philopatry. Based on six microsatellites markers the authors suggested that only males contribute to gene flow across the Indian Ocean (Pardini et al. 2001), however, the gene flow contribution and the frequency of such migrations are unknown and the global connectivity between populations hasn't been fully investigated. Interestingly, South African and Australian fur seals (food source for white sharks) are also closely related and it is likely that the Australian fur seal population arose as a once-off

colonisation of seals from South Africa (Matthee et al. 2006). Mitochondrial DNA also clearly divides northeast Pacific and Australia-New Zealand white shark populations (Jorgensen et al. 2009) but it is unknown whether genetic divergence is maintained by limited gene flow, and future analyses of microsatellite and other nuclear loci will help determine the connection between these populations (Jorgensen et al. 2009). The migration across country borders and onto the high seas clearly also has international management and conservation implications for the white sharks.

Very little information however is available for white sharks along the South African coastline (Bonfil 2005; Johnson & Kock 2006). An acoustic tag study provided evidence of six main white sharks aggregation sites and identified them as False Bay, Gansbaai, Mossel Bay, Struisbaai, Port Elizabeth and Grootbrak, but claimed regular movement between these areas (Johnson & Kock 2006). Regardless of the huge migratory potential (Bonfil 2005), white sharks are suspected to be sensitive to thermal fronts (Gubili et al. 2010; Blower et al. 2012), present female (Pardini et al. 2001; Jorgensen et al. 2009; Gubili et al. 2010) and suspected male philopatry (Blower et al. 2012). It is thus a possibility that the South African aggregation sites correspond to different genetically identifiable sub-populations since they are located across an oceanographically diverse coastline.

The South African coastline extends from the mouth of the Orange River on the west coast to Kosi Bay on the east coast and can be divided into several biogeographic provinces with distinct temperature gradients and nutrient distributions (Hedgpeth 1957; Emanuel et al. 1992; Spalding et al. 2007). The three main regions are the cool-temperate west coast, the warm-temperate south coast, and the subtropical east coast (Stephenson & Stephenson 1972; Emanuel et al. 1992; Harrison 2002). The exact locations of the boundaries separating these provinces are not agreed upon and they probably overlap to some extent (Teske et al. 2006). Several previous genetic studies focusing on organisms around the South African coastline indicated that many marine rocky shore species exhibited high levels of genetic structure along the South African coastline (Fratini & Vannini 2002; Matthee et al. 2007; Zardi et al. 2007; von der Heyden et al. 2008; Teske et al. 2011), often corresponding to biogeographic provinces (Teske et al. 2011). Other species,

however, revealed a panmictic distribution (von der Heyden et al. 2007; Neethling et al. 2008). These phylogeographic studies, however, focused primarily on coastal species with data on offshore species poorly represented in both biodiversity and genetic studies (von der Heyden 2009; Teske et al. 2011).

1.5 White shark social structure

To date the only studies that investigated the white sharks' social dynamics provided some evidence of a seasonal sexual segregation (Jorgensen et al. 2009; Kock et al. 2013) and social interactions among individuals during scavenging situations (Sperone et al. 2010, 2012; Fallows et al. 2013). Behavioural studies revealed the existence of social behaviour (e.g. behavioural interactions) between similar sized conspecifics (Martin et al. 2005; Sperone et al. 2010) and empirical observations described white sharks passing by the same area in stable clans of two to six individuals (Martin & Martin 2006), with relatedness within a clan being unknown. When tissue samples from individuals are available, genetic techniques combined with photographic data collection can provide accurate estimates of the relatedness between free ranging individuals, and in turn can be an invaluable source of information when dealing with elusive marine organisms (Mann 2000; Krutzen et al. 2003; Möller et al. 2006). Genetic markers such as DNA microsatellites in combination with the analyses of pairwise associations have been previously used in marine mammals *Tursiops aduncus* (Möller et al. 2006) but also in elasmobranch species, to closely investigate their social structure (Guttridge et al. 2011). Understanding the role that a social structure play for white sharks can transform the current way we are managing this predator, as for species where social interaction exists, the removal of a single individual can have a number of drastic effects on the network structure (Krause et al. 2007; Croft et al. 2008).

1.6 Study rationale and objectives

The lack of basic information on the species is arguably one of the biggest obstacles in conservation planning, and studying white sharks is extremely challenging. Their high mobility combined with the large home range (Bonfil 2005; Jorgensen et al. 2009) makes even the easiest of the tasks, such as collecting simple photographic data a challenge on its own. Moreover the lack of basic guidelines when dealing with a photographic catalogue can be an additional challenge when trying to assess the population number or simply keeping track of the sampled individuals. A targeted collection of data (both photographic and genetic) from free ranging white sharks around the South African coastline has never been attempted but it could provide invaluable information for the adaptive management of white sharks stock. For example, the assessment of the number of stocks and or connectivity across different geopolitical regions can provide the baseline to sustainably manage the quotas of the white shark's primary food resources. These data can also be used to advise the local government upon the risks of implementing lethal shark protection measures on popular beaches.

Therefore in an attempt to provide data useful for the conservation of white sharks this project aims to firstly assess and validate non-lethal techniques (photographic and genetic) for data gathering, and then to utilize such data to answer specific questions regarding population size and genetic structure of white sharks. Part of this thesis is descriptive in nature (chapter 2 and 3) and where data is available to formulate hypotheses we have done so. Photographic identification data of white sharks are complemented with the genetic information of each known individual. The integration of the mark-recapture results with the genetic assessment of the contemporary effective population size (Huson & Bryant 2006; Portnoy et al. 2009; Dudgeon et al. 2012) will give a better perspective on the real population status and become one of the few studies where the two techniques are compared (Woods et al. 1999; Smith et al. 1999; Carbone et al. 2001; Waits et al. 2001; Ovenden 2014). Sampling around the entire coastline of South Africa was also performed to allow for an assessment of the population structure between aggregation sites and indicate the

level of genetic diversity currently found in Gansbaai, along the South African coastline and globally. Furthermore by placing the phylogeographic outcome of this study in a global perspective, the study contributes evidence of the evolutionary mechanisms that have shaped the South African population. Finally, the combination of the photographic identification with values of relatedness between individuals can provide insights of white sharks social structure.

To achieve the above objectives the project was divided into four chapters that are written up in the form of publications:

1. In chapter 2 we developed and validated a categorization system to manage reasonably large numbers of white sharks through assigning a personal code to each animal based upon the notches on the dorsal fin. This is a descriptive chapter where the novel notches code method is introduced;
2. In chapter 3 the photographic dataset was used for generating mark-recapture data, and these were compared to a genetic analysis on nuclear genetic markers. The combination of the two techniques was then used to estimate white shark population size at a local and national scale. Based on a recent published data set we hypothesised that wrong population numbers were estimated where automated software DARWIN was used. We tested this by verifying the software and to compare the published values to our estimates.
3. In chapter 4 we determined the movement and genetic connectivity between different geographic aggregations sites along the South African coastline and then placed this in a global perspective. In this chapter we hypothesized that white sharks along the South African coastline will show some genetic structure conforming to aggregation sites. In addition, we hypothesise that South African white sharks will form a unique genetic assemblage at the global scale and if so we set out to embark on an investigation to explain the mechanisms behind this.
4. In chapter 5 we give an insight of the hypothesis that a social structure exist in white shark. By making use of the individual identifications and the genetics of each individual we investigated if white sharks form non random pairwise associations and then we explored the nature of such associations in an attempt to explain the potential mechanisms of such social behaviour.

CHAPTER 2

A NOVEL CATEGORISATION SYSTEM TO ORGANIZE A LARGE PHOTO IDENTIFICATION DATABASE FOR WHITE SHARKS *CARCHARODON CARCHARIAS*

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2.1 Introduction

Due to difficulties associated with working in the marine environment, knowledge of the population dynamics of marine fauna is often limited. This is particularly so when dealing with large, migratory, marine species such as the white shark *Carcharodon carcharias*, which has an extensive range (Compagno et al. 2001; Boustany et al. 2002; Bonfil 2005; Domeier & Nasby-Lucas 2008; Jorgensen et al. 2009). The white shark is a top predator protected under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the Convention for Migratory Species (CMS) and is listed as vulnerable to extinction according to the International Union for the Conservation of Nature (In: IUCN 2013. IUCN red list of threatened species. Version 2013.2. Available at www.iucnredlist.org). Despite *C. carcharias* receiving international attention each year, many white sharks are killed as a consequence of a variety of anthropogenic activities, such as trade in their jaws, teeth and fins (Compagno et al. 1997, 2001) and protective beach nets (Cliff et al. 1989). With continued anthropogenic mortality, and gaps in the basic life-history information for this species (including population dynamics), future advances in its conservation and management may be severely hindered.

Individual identification (using photographs or genetic fingerprinting) of members of a vulnerable species can facilitate accurate assessments of population sizes, which are often a critical component of conservation. In addition, recent advances using genetic methods and isotope analyses can provide accurate insights into the biology, ecology and health status of individuals or species. Repeated recognition of individuals over temporal scales can lead to a better understanding of the results, and can provide baseline data for long-term management programs (e.g. see (Taberlet & Luikart 1999; Silver et al. 2004). In the marine environment, photo identification techniques have demonstrated the potential to provide data needed to determine certain life-history characteristics and aspects of the population ecology of threatened species (Wursig et al. 1990; Chapman et al. 2003; Schofield et al. 2008; Chapple et al. 2011). The estimation of population size based on photograph-derived mark-recapture seems to be a feasible approach for large marine animals (Langtimm et al. 2004; Meekan et al. 2006). The method allows

researchers to 're-sample' an individual several times (Hammond 1990; Chapple et al. 2011; Martin-Smith 2011) and to monitor the individuals over long periods (Markowitz et al. 2003; Domeier & Nasby-Lucas 2006; Schofield et al. 2008; Chapple et al. 2011; Martin-Smith 2011), which ultimately allows for the construction of a capture-mark-recapture dataset. Hence photo identification techniques are recommended as the preferred non-invasive sampling method for vulnerable organisms, specifically those with natural identification marks (Meekan et al. 2006; Domeier & Nasby-Lucas 2006; Schofield et al. 2008; Dulvy et al. 2008).

In the case of the white shark, its elusive nature and ability to migrate large distances (Boustany et al. 2002; Bonfil 2005) make it a notoriously difficult species for which to obtain accurate data useful for conservation and management. Traditionally, white sharks have been identified using dorsal fin photographs (Klimley et al. 1996; Gubili et al. 2009; Anderson et al. 2011; Chapple et al. 2011), because the trailing edge of the dorsal fin is analogous to a human fingerprint and can render an individual shark uniquely identifiable. Remarkably, recent studies have indicated that the trailing edge of the dorsal fin can remain informative. Anderson et al. (2011) reported three different sharks to retain the same morphology for 19, 20 and 22 years respectively, enabling the long term monitoring of individuals.

Despite being able to provide extremely valuable information, photo identification also presents challenges (Gamble et al. 2007). In the field, the behaviour of white sharks is dependent on sea conditions (e.g. swell, temperature and visibility). During periods of high swells, the animals tend to stay deeper in the water column and further from the vessels. In conditions of low visibility, they typically increase their speed when reacting to the bait, making photography more challenging. Even in optimal sea conditions, individual differences in behavioural habits are observed, with some sharks staying submerged more often than others (SA and MR, pers. obs.). In the laboratory, the first challenge pertains to the standardisation of the images, as seen with other biometric photo identification systems such as fingerprint- (Galton 1895) or human iris analysis. Image analysis techniques require that, to maximise accuracy, images should be comparable in terms of quality, size and observation angle. At the matching stage, it is important to

discard poor-quality images because the potential for incorrect assignment of individuals is much higher in cases where markings are either incorrectly orientated or not easily visible (Arzoumanian et al. 2005; Speed et al. 2008; Marshall & Pierce 2012). Several studies based on photo identification have underlined the importance of using good-quality images to ensure the reliability of the data (Stenhouse 1985; Gowans & Whitehead 2001). Potentially, some studies on white sharks might have been compromised by the poor quality of photographic images used, and successes may have been confined to studies where sample sizes were relatively low (Gubili et al. 2009; Chapple et al. 2011). The second challenge is data processing; working with large photographic catalogues is labour-intensive and the matching process (visual comparison of the images) is susceptible to human error (Galton 1895; Kelly 2001; Van Tienhoven et al. 2007; Schofield et al. 2008; Martin-Smith 2011). Recently there have been attempts to optimise data processing by identifying white sharks using software originally developed for use with marine mammals (e.g. DARWIN, FINSCAN); however, to date this has resulted in unacceptably high levels of error in matching images (Chapple et al. 2011, see also chapter 3).

To address the problems highlighted above, only high-quality standardised photographs should be used to score the individual markings and, when dealing with a large quantity of images and individuals, the images should be subjected to careful storage, labelling (Marshall & Pierce 2012) and categorical coding (Gill 1978; Gamble et al. 2007; Schofield et al. 2008). The first objective of the present study was to develop and test a novel categorization system, known as the 'notches code'¹, which focuses on the most reliable marks present on white shark dorsal fins. The second objective was to demonstrate that the notches code can be used as a tool to quickly and accurately identify a large number of white sharks.

2.2 Material and methods

From April to July 2009 and February 2010 to December 2011 data were collected (weather permitting) during the daily white sharks cage-diving eco-tourism activities (Laroche et al. 2007) of

¹ The notches code has been referred to as the 'Rutzen Method (RM)' in O'Connell et al. (2012)

the local operator 'Shark Diving Unlimited'. Data were collected on a total of 314 days (sampling occasions) in the proximity of the Dyer Island Nature Reserve (Kleinbaai, Gansbaai, South Africa, Figure 2.1a). The area is approximately 25 km² and includes two main white shark aggregation sites, one in close proximity to Dyer Island and Geyser Rock (34°40.61' S, 019°23.93' E) and one inshore, adjacent to the beach of Walker Bay Nature Reserve (34°37.95' S, 019°25.98' E; Figure 2.1b).

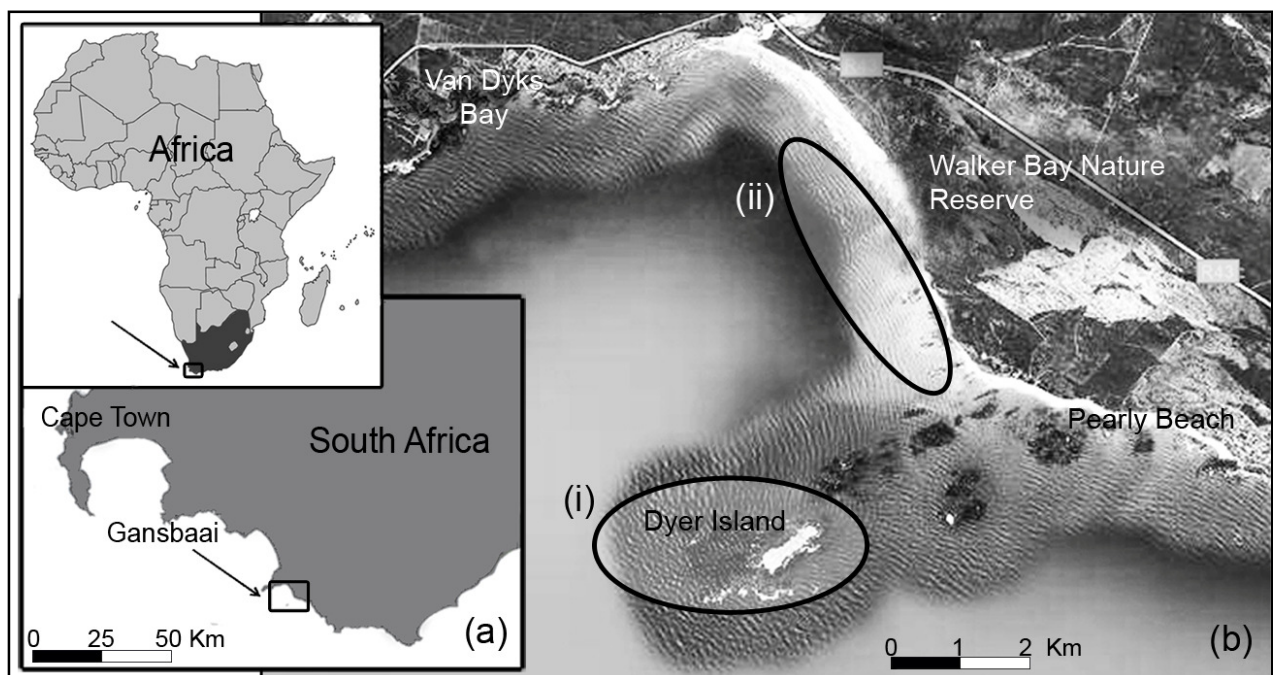


Figure 2.1: Sampling area: (a) Kleinbaai, Gansbaai, South Africa; (b) Main white shark aggregation sites in the area: (i) offshore area, in the proximity of Dyer Island Nature Reserve, (ii) inshore area, off the beach of Walker Bay Nature Reserve.

During sampling days, sharks were attracted to the boat by using olfactory cues provided by a natural fish chum. Once close to the boat, they were lured to the surface using a tuna head attached to a buoy. This maximized the potential for taking good-quality, high-definition photographs of the dorsal fin, which becomes fully exposed above the surface of the water. During this process, the shark cage-diving permit conditions (permit numbers RES2010/71 and

RES2011/55), as prescribed by the Department of Environmental Affairs, were strictly followed and no animal was fed intentionally during the entire study period.

2.2.1 The notches code: photo identification criteria, coding and analysis

The notches code is a categorisation method specifically designed to build and organize a large, user-friendly database of dorsal fin photographs that enables search time to identify re-sighted individuals to be minimised.

To ensure high quality images, the dorsal fin photographs of white sharks were taken in RAW format with a Canon® EOS 300D (8.2 megapixel) or Canon® EOS 40D (10.1 megapixel) SLR digital camera. The cameras were fitted with 55–200 mm zoom lenses (Tamron® Di-II) and polarising filters, to capture as much detail as possible and to minimize reflection and glare. Good quality light was needed to optimise the sharpness of the pictures. The exposure of the complete dorsal fin was an important criterion to ensure the standardisation of the images for subsequent matches. In addition, due to the extremely flexible nature of the dorsal fin, the only photographs used for photo identification were of fins that were not bent and were perpendicular to the sea surface and the camera lens (Figure 2.2a). Dorsal fin photographs that did not meet these criteria were not incorporated in the reference database (Figure 2.3).

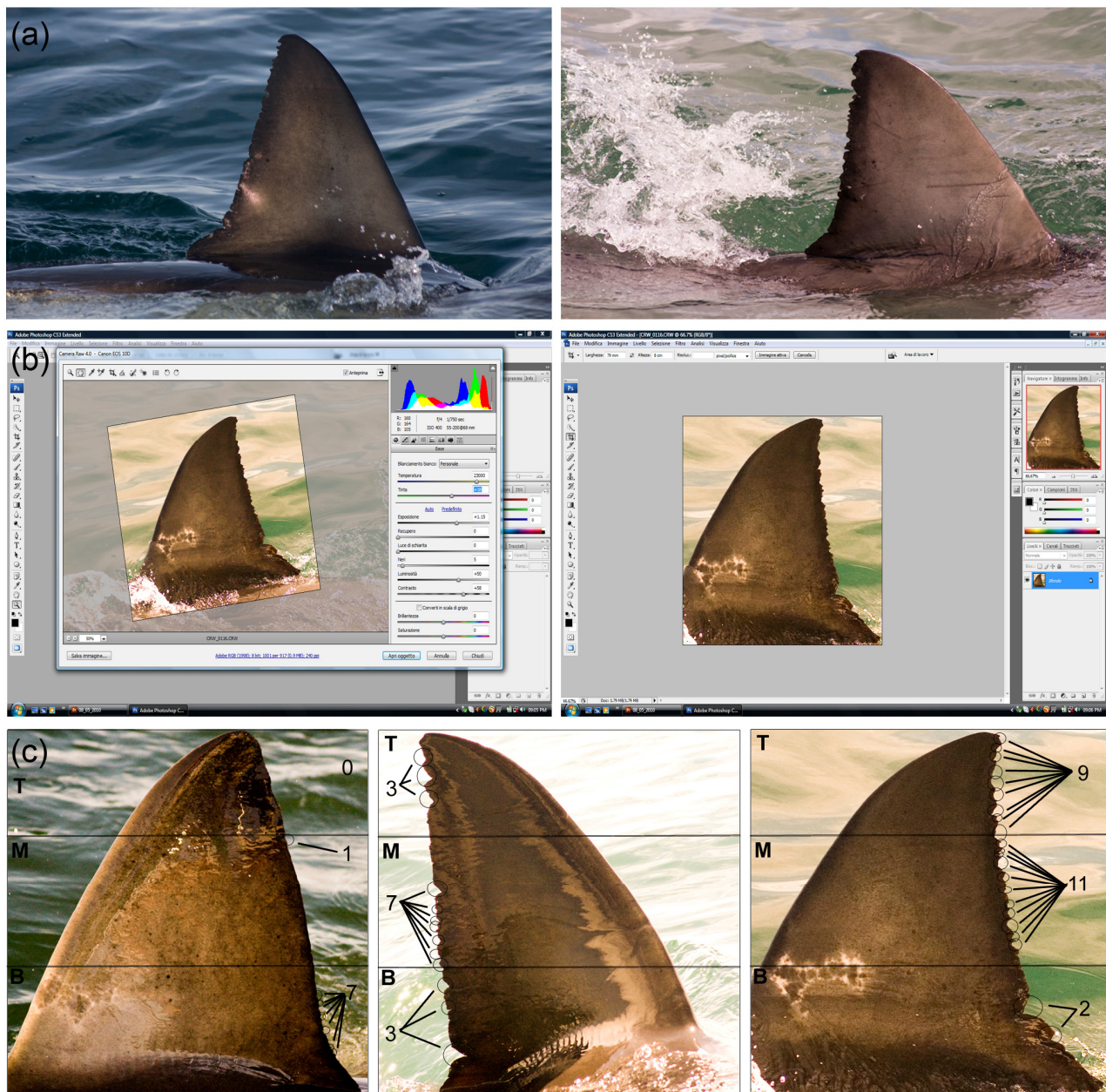


Figure 2.2: (a) Examples of photo identification images of white sharks at Kleinbaai, South Africa, taken perpendicularly to the sea surface; (b) enhanced and resized; (c) examples of notches selected for counting (notches code: left C_000107; centre C_030703; right C_091102).

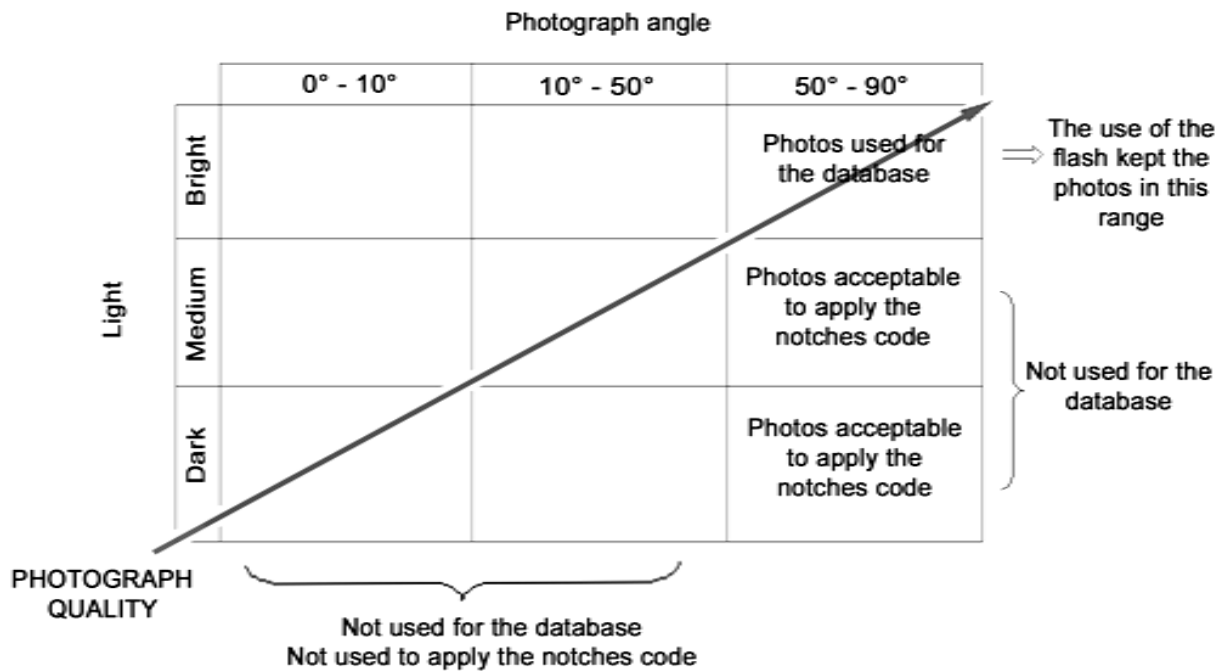


Figure 2.3: Strict criteria adopted to select dorsal fin photographs for applying the notches code categorization system and for inclusion in the identification database. The quality of the photographs is based on the light and the angle of the dorsal fin, relative to the sea surface and camera lens. The best photographs are those taken in good light conditions, and when the dorsal fin is perpendicular to the sea-surface and camera lens.

Useable photographs were edited manually using Adobe® Photoshop CS3 (© 2007). To simplify the process associated with the visual comparison between photographs, images were edited to enhance contrast of the trailing edge of the dorsal fin against the background, and other natural marks, such as black spots or white patches were emphasised (Figure 2.2b). Once edited, the photographs were tightly cropped around the dorsal fin and then resized to 8.0×7.9 cm to avoid mismatches due to size biases (Figure 2.2b). A standardised grid with three equal-sized sections was then superimposed over the photograph that divided the dorsal fin into three regions: top, middle, and bottom (Figure 2.2c). Within each region, the quantities of 'principal notches' were recorded, creating a six digit code (C_...), with each pair of digits reflecting the quantity of notches within a grid region (Figure 2.2c). In the present study the principal notches were chosen by their relative sizes: for a fin with multiple notches ranging in size between 0.5 cm and 3 cm deep, only

the biggest (>1 cm) were counted, but in instances where fins contained very few notches >0.5 cm, all notches were counted. For example, a fin with three (03) notches at the top, five (05) in the middle and two (02) at the bottom would have a notches code of 'C_030502'.

2.2.2 Photo identification database and the matching process

The notches code was used as a unique barcode to identify individuals in a reference database. The reference database included either one or two (right and left side of the dorsal fin) high-quality photographs for each shark. Each shark in the reference database was named using the notches code, which was used to organize the photographs according to the unique notch patterns for each animal. Additional photographs (sometimes of lower quality) of a given individual were stored in a separate folder - i.e. one folder per identified shark - of a larger database (referred to as the full database), to keep track of the re-sightings of each individual.

To compare a new photograph with those in the reference database, the new photograph, with its associated code, was compared visually with the photographs in the database that had similar codes. However, because the base of the dorsal fin was sometimes distorted by shark movement or was partially obscured by water drops, most of the emphasis on coding and visual matching was placed on the two upper regions (*T* – top and *M* – middle) of the dorsal fin (Figure 2.2c). The visual comparison began with photographs with the same *T* code (see Figure 2.2c). For example, all photographs with three notches in the top section were compared with all those that had a different code for the *M* region. If there was no obvious match between two sharks with the same *T* number, the process was extended to a wider range of potential *T* codes (1 or 2 values above or below; known as the 'range check'). For example, a shark coded as C_030506 would be compared with all sharks beginning with C_02 and C_04, eventually broadening the search to the entire reference database.

The range check helped to prevent errors due to the subjective choice of principal notches and the rare occurrence of fresh cuts on the dorsal fin. If the range check (extended to the entire

database) did not yield a re-sight (i.e. match), the individual was considered unique and was added to the reference database. In all instances, once a match was made using the notches code, it was further validated by comparing other identification features present on the dorsal fin, such as white patches or black spots (Anderson et al. 2011; Chapple et al. 2011).

2.2.3 Accuracy and validation of the categorisation method

Data collection and the application of the notches code method were performed over the entire study period by the same observer. Our study design allowed us to assess the importance of using only high quality photographs in the reference database. Pictures taken between April 2009 and the beginning of April 2010 were initially all included in the reference database, irrespective of the strict quality criteria outlined above. From the beginning of April 2010, only high-quality photographs meeting the criteria were used. To verify that the reference database contained only unique sharks, a second researcher compared all the photographs in the database to determine the number of false negatives (i.e. a shark included more than once as different individuals).

To assess whether the notches code method can be used efficiently by other researchers to quickly and reliably match dorsal fins in a given database, an experiment was conducted with six additional people: one trained researcher, familiar with the method (she had used it about 20 times before this experiment) but independent of its development, and five untrained researchers (each received a 30 min briefing on how the method works). The six individuals were each asked to match the same 20 fin photographs, randomly chosen from different white sharks, known to be in a reference database. Two reference databases with the same images were created for the purpose of the experiment, with the difference being that in one database the images had not been coded and organized, and in the other they had. First, they were asked to use manual searching techniques in the reference database of non-organized and non-coded fins. Then they were asked to repeat the experiment by first coding the 20 fins using the notches method and then searching the reference database where the fins had been organized by the authors using the notches code. All six researchers were allowed to score the principle notches based on their own perceptions and

no limitation on notch depth was prescribed. The 20 photographs provided were not the same as those in the reference database so that matches would be made between sharks and not between identical photographs. The search time required to match a photograph using a non-organized database was compared with the time taken to find the same individual in an organised database of coded images. As a control, it is important to realize that the coded database (reference collection) was not re-coded. The time taken to match photographs, with or without the notches code method, was tested for both small and large datasets: four people (of whom one had previous experience with the method) worked with the full reference database ($n = 426$ photographs), while two conducted their experiment using a smaller database ($n = 130$ photographs).

2.3 Results

From 4398 usable photographs, a total of 426 white sharks was individually identified in the proximity of the Dyer Island Nature Reserve. The reference database of white sharks created in the present study contained 636 dorsal fin photographs, however, because images of both sides of the dorsal fin were obtained in the case of some sharks.

In 2011 the number of resighted sharks (from previous years) exceeded the number of newly identified individuals (Figure 2.4): 47 animals identified in 2009 were resighted in 2010 and, of 227 sharks identified in 2011, 127 had been identified previously between 2009 and 2010.

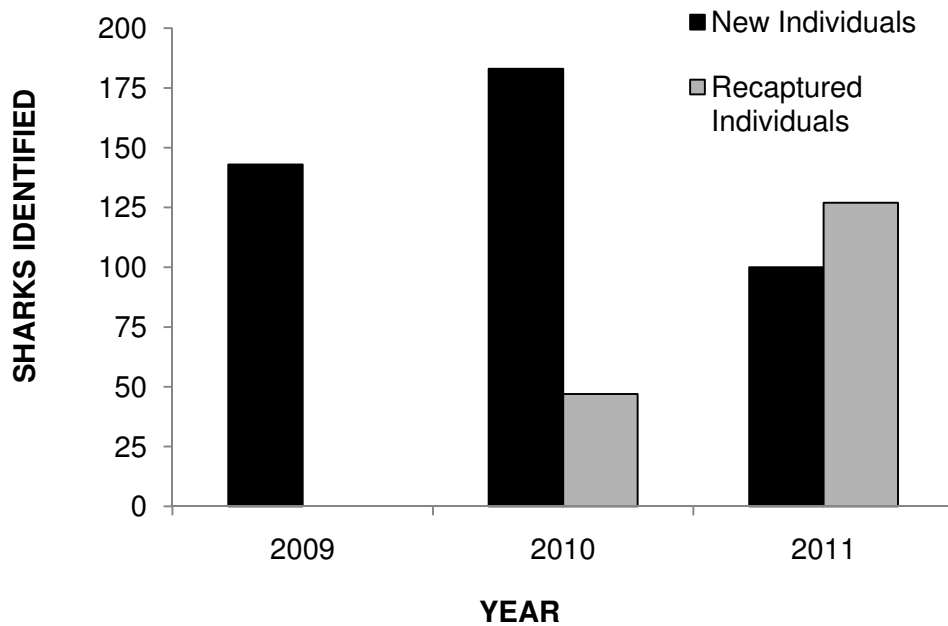


Figure 2.4: The annual number of white sharks sighted for the first time (new individuals), and those resighted (or recaptured, i.e. already included in the database from previous years).

Although this was a short-term study, there were few obvious changes to the notches in resighted animals. There was a noticeable change in one individual (C_040701; sampled in March and again in May 2010) due to the addition of a new cut in the *T* region of the fin. This individual remained easily identifiable, however, when subjected to the range check as outlined above. Besides a slight change in notch pattern, two sharks (C_050101 and C_040102) exhibited some modification in natural white marks during the study period, providing some evidence that such marks are temporally less stable than notches (Figure 2.5).

(a) C_050101



(b) C_040102



Figure 2.5: Example of retention of notches on the posterior edge of the dorsal fins, and of changes in the white natural marks in two different white sharks: (a) C_050101, over a three year period and (b) C_040102, over a two year period, at Kleinbaai, Gansbaai, South Africa.

The inclusion in the reference database of photographs not meeting the prerequisite criteria for quality resulted in a large number of false negatives, as indicated by an independent visual comparison of all the images in the full database. For the period April 2009–April 2010, 48 (20.34%) in a total of 236 individuals were found to have been categorised incorrectly as new and unique. After implementing the stricter criteria (April 2010–December 2011), only 1.21% of the categorised individuals were false negatives, with only three duplicate, misidentified sharks in a total of 247 individuals (Figure 2.6).

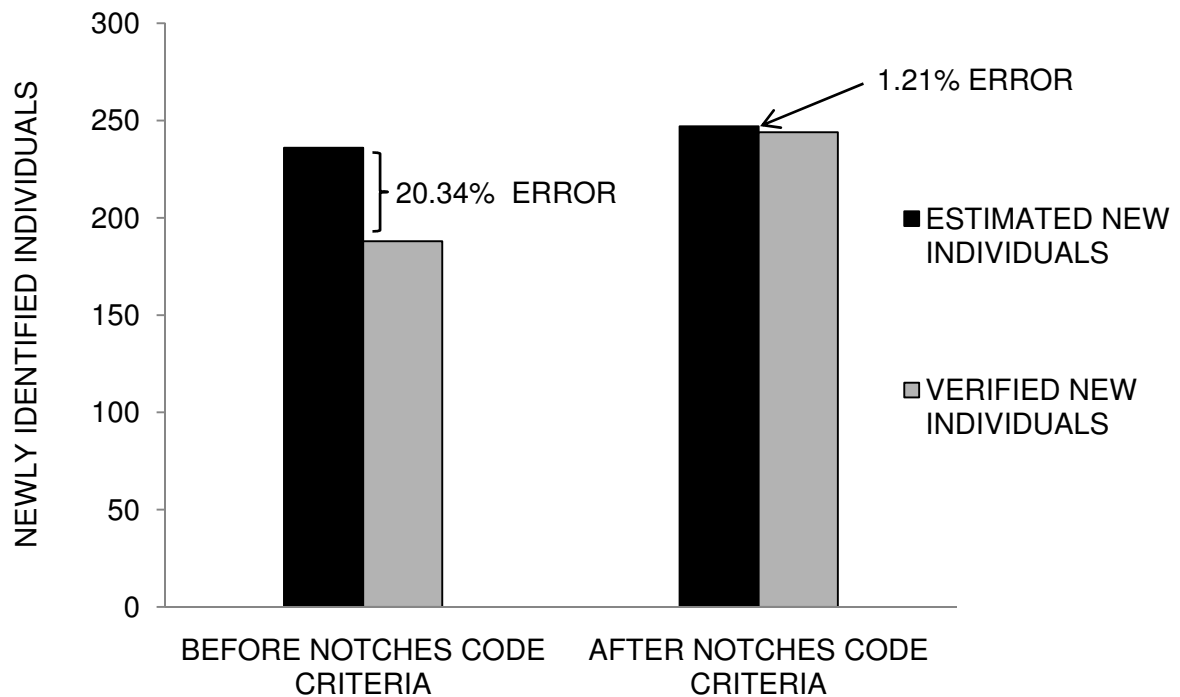


Figure 2.6: Percentage of false negatives in the photographic identification of white sharks before (20.34%) and after (1.21%) the adoption of the strict notches code criteria (see Fig 2.3).

During the validation experiment each of the six people successfully matched the dorsal fins with the correct sharks in the reference database (with and without the coding system). The selection of principal notches on the dorsal fins, although subjective (Figure 2.7a), had almost no effect on the efficiency of the notches method. As predicted, the *T* section of the photograph yielded the least variation among different researchers (Figure 2.7b).

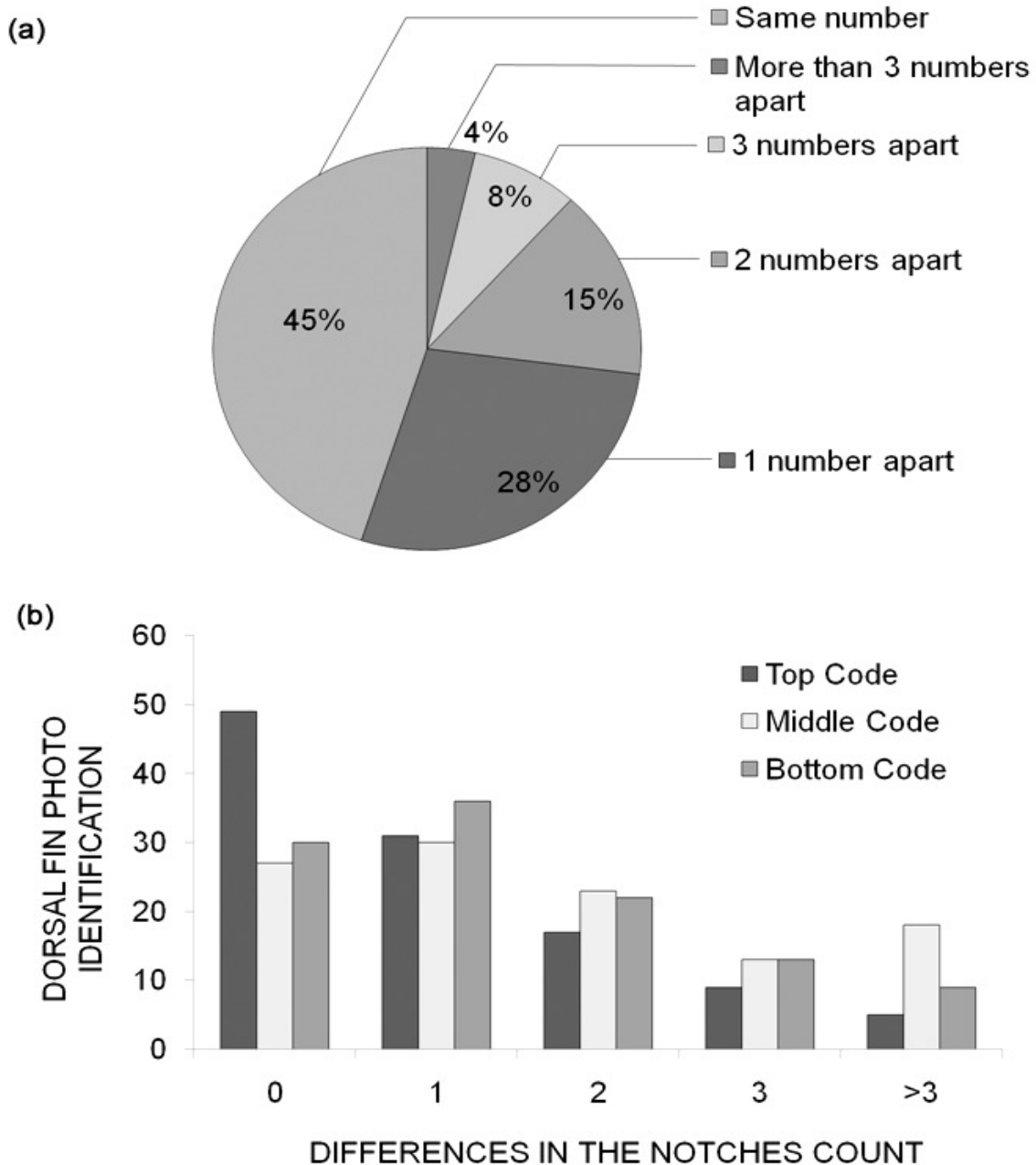


Figure 2.7: Differences in counts of principal notches by six people examining the same photographs of dorsal fins. Variations in counts of notches in (a) the *T* (top) region of the fins and (b) each of the three regions ($n = 110$).

For a researcher trained in the notches code technique (20 matching sessions), the average time to match a fin in a non-organized database of 426 photographs was 4.42 min (SD 1.79); with the coding system it took an average of 2.08 min (SD 2.34). The average time taken by the three

untrained researchers was 6.43 min (SD 8.62) in the first trial and 5.95 min (SD 7.91) using the coding system. These data suggest that the notches code method outperformed the traditional method in both instances but that the success of the method increased with experience. When a smaller reference database was used ($n = 130$ photographs), the two untrained researchers needed an average of 1.87 min (SD 2.18) to find the match in the non-organized database and 2.03 min (SD 2.37) using the coding system. The trial conducted with the non-organized databases showed a correlation between the time taken to find a match and the position of the shark in the database, while the time taken to match a fin using the notches code system was correlated with the ability to code the shark consistently (Figure 2.8).

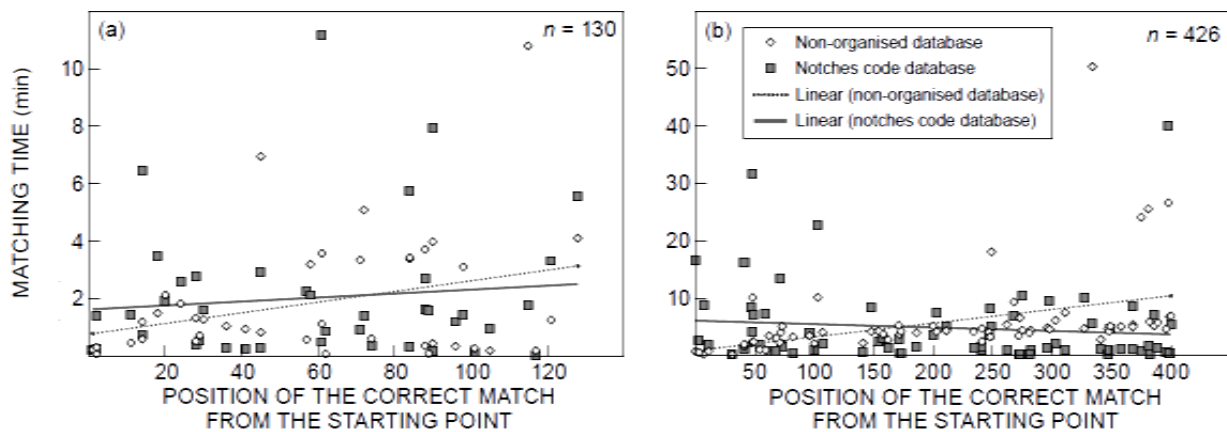


Figure 2.8: Comparison between the time taken to match a photograph in a non-organized database (♦) and in a database organized using the notches code (■). The visual matching of 20 dorsal fin images was performed by (a) two people using a reference database containing 130 identified sharks and (b) four people using the complete reference database developed in this study (426 identified sharks).

2.4 Discussion

Human error is one of the biggest problems associated with photo identification based on visual matching, and it is likely to increase proportionally with the number of images. The notches code categorisation system proposed here proved to be a rapid and accurate codification method for analysing and organising a large database of individuals (426 sharks) for purposes of individual recognition. The benefit of using the system is greatest when working with a large number of

individuals (>180) and over an extended period. Using a non-organised database, the time taken to find a correct match increased linearly with the number of individuals. The notches code system, despite the subjectivity associated with notch identification, yielded consistent results between researchers. For 96% of the time, notch counts varied by three or less. It is probable that the difference between trained and untrained researchers in the time needed to find a match is explained by the ability to perform the 'range check'. The subjectivity associated with counting the principal notches had a negligible effect on the performance of the notches code method. In addition, the validation experiment demonstrated differences between researchers in the time required to visually match dorsal fins with those in a given reference database. One of the researchers required an average of 15.16 min (SD 13.11) to find the correct match in the non-organized database, and (despite allocating the correct notch codes) an average of 10.76 min (SD 10.50) using the organised database. Excluding the search times of this individual, the average time required for matching using the large non-organized database was 4.22 min (SD 2.5), while the time required when making use of the notches code dropped to 2.88 min (SD 2.8). This indicates that this system reaches its full potential after some training.

The large number of white shark individuals in the reference database (all observed within a small geographic area), allowed the identification of individuals observed more than 29 months apart, and it is encouraging to note that use of the notches code could be successfully used and reduced the search time of all six independent researchers. Thus it is reasonable to conclude that the reference database, organised according to the notches code, that was produced in the present study can be utilized as a baseline database for future white shark research in the region.

This study provided some insights regarding the stability of surface markings on the dorsal fins of white sharks, information that is fundamental to the development of a future recognition program. In the present study, 127 sharks that were entered into the reference database during 2009 and 2010 were re-sighted during 2011, indicating that the marks are reliable identification tools over this time period. Although the period was short, these findings are consistent with the suggestion of Anderson et al. (2011) that dorsal fin notches are stable markers. Natural white

marks proved to be less stable over time than the fin notches (see also Dufault & Whitehead 1995; Robbins & Fox 2012). In particular, shark C_050101, which was identified initially on 14 June 2009 and was easily re-identified using the dorsal fin notches on 6 March 2010 (265 days later), and again on 20 March 2011 (379 days later), showed clear changes in its white marks over time. The risk associated with changes in natural white marks is the potential to misidentify a given individual, which, for population estimate studies based on mark-recapture analysis, is comparable to 'losing the mark', which would violate the first assumption of mark-recapture models (Sutherland 2006).

When compared to a physical tagging technique, photo identification of white sharks provides a cheaper and non-invasive alternative (Marshall & Pierce 2012) that, if applied consistently, can allow for accurate studies of population dynamics. The number of white sharks that converge at Gansbaai is amongst the largest known in South Africa. However, individual white sharks can also migrate large distances (Bonfil 2005), and animals from the Gansbaai area have been shown to move to other South African bays, such as Mossel Bay to the east and False Bay to the west (Laroche et al. 2007). Consequently, unless there is a reliable, centralised photo identification database, the movements of white sharks may lead to the same individuals being counted on multiple occasions at different localities, resulting in an overestimation of the overall abundance. Standardising the method and accuracy of data collection is the first step toward the development of a national and even an international photo identification database for white sharks.

CHAPTER 3

AN INTEGRATED MARK-RECAPTURE AND GENETIC APPROACH TO ESTIMATE THE POPULATION SIZE OF WHITE SHARK IN SOUTH AFRICA

3.1 Introduction

The ultimate success of adaptive management in conservation is to gather reliable information that can be used to inform decision makers (Keith et al. 2011). Population abundance (n) and effective population size (N_e) are among the most important parameters in wildlife management and conservation, because they can help to predict the extinction risk of populations (Luikart et al. 2010). Estimating population sizes accurately is problematic for a variety of reasons, mostly when dealing with elusive and cryptic species, as the hypergeometric models to estimate population size (also called mark-recapture models) are based on a series of assumptions that are rarely met in the field. Also, when the information available on the target species are not sufficient, the model to be selected is itself a challenge [see as an example the close vs open population model debate in Burgess et al. (2014) revision of Chapple et al. (2011)]. Specifically, the following assumptions must be met during the data collection: homogenous sampling and re-sampling of all individuals, tagging method must not affect subsequent catchability, no tags are lost and random sampling has to be performed in a representative area related to the distribution of the population (Sutherland 2006).

To meet some of the above assumptions non-invasive methods, such as photo identification techniques, became increasingly popular, mostly because digital cameras make this more feasible. Photo identification proved to offer unique opportunities to study the population ecology and life history of species, particularly those under serious threat (Wursig et al. 1990; Whitehouse & Hall-Martin 2000). It allows researchers to “re-sample” an individual several times without interfering with its natural activities for extended time periods (Carbone et al. 2001; Jackson et al. 2006; Kelly et al. 2008; Schofield et al. 2008; Chapple et al. 2011; Marshall & Pierce 2012; Andreotti et al. 2014). Therefore studies on cryptic species such as jaguars, *Panthera onca* (Wallace et al. 2003; Silver et al. 2004), snow leopard, *Panthera uncial* (Jackson et al. 2006) tigers, *Panthera tigris* (Carbone et al. 2001) and cougar, *Puma concolor* (Kelly et al. 2008) relied on hidden cameras in combination with baited-traps to capture-recapture the individuals. Unfortunately, despite the attempts of sampling without disturbing the natural population dynamics

it is virtually impossible to predict each individual subsequent behaviour after approaching the baited-traps, making the method not 100% reliable (see for example Burgess et al. 2014).

To overcome the criticisms associated with mark-recapture, recent studies on large vertebrates used molecular fingerprinting of hair samples and/or faecal DNA, as an alternative to “mark-recapture” datasets (Taberlet et al. 1999; Woods et al. 1999; Mowat & Strobeck 2000), specifically for estimating the population census size (N_c). Traditional capture-mark-recapture studies require multiple sampling sessions while DNA-based N_c estimates can be obtained from a single sampling session. One-sample N_c estimation simply infer “re-capture” from a multilocus genotype (i.e. individual) captured two or more times in the same sampling section (Luikart et al. 2010), but, despite the promises, these studies also underline several pitfalls associated with the genetic approach: these include un-quantified genotyping error rate, or biases related to mixed samples (Taberlet et al. 1999; Mills et al. 2000; Waits et al. 2001; McKelvey & Schwartz 2004; Roon et al. 2005). Congruence among studies based on different data types (with different assumptions) can be regarded as stronger support for a particular hypothesis. Hence, a combination of photographic identification and genetic methods should thus ideally be used.

Population census size (N_c) and effective population size (N_e) are crucial parameters that influence population viability, wildlife management decisions and conservation planning (Luikart et al. 2010). Population census size is a broad term that in most studies refers to the number of adults in a study area or population. This is estimated specifically to avoid the inclusion of thousands of offspring in fecund species that have no chance to reach sexual maturity (Sutherland 2006; Luikart et al. 2010). Nevertheless the definition of population census size can vary between studies, and it is thus strongly recommended to report the definition of population size and what age or stage-classes are counted in N_c estimations (Luikart et al. 2010). Since white sharks have a long life span (Calliet et al. 1985), coupled to low fecundity and low estimated natural mortality (Smith et al. 1998), the present study define the census (or abundance) as the maximum number of individuals identified, after reaching saturation (Sutherland 2006). The population estimate N , based on mark-recapture analyses of photographic identifications, is defined as the estimate of all

the individuals in the study area and if the assumptions for the model are met, it ideally doesn't differ from N_c .

N_e (the genetic based, effective population size) is defined as the size of an ideal population that has the same rate of change of allele frequencies and heterozygosity as the observed population (Fisher 1930; Wright 1931). Demographic estimators, however, tend to overestimate the true N_e as they seldomly include all the factors, such as variance in reproductive success (but see Saura et al. (2008) for an exception). In addition, DNA-based N_e estimates are generally challenging to compare across different studies, because they have been applied to many different measures of genetic change (Crow & Denniston 1988). The two most used concepts of N_e are the inbreeding N_e (N_{el}), concerned with the loss of heterozygosity, and the variance N_e (N_{eV}), concerned with change in allele frequencies through time (see a review in Luikart et al. 2010).

The specific N_e measured in the present study is the coalescent effective size (CN_e) also known as contemporary effective population size. Whereas other forms of N_e (N_{eV} and N_{el}) include only a single measure of the rate of genetic drift (variance in allele frequencies) or inbreeding (heterozygosity) (Wakeley & Sargsyan 2009), CN_e arguably considers all aspects of genetic change. The DNA-based contemporary effective population size CN_e approximates the mean number of breeding individuals contributing offspring per generation (Huson & Bryant 2006; Portnoy et al. 2009; Dudgeon et al. 2012), which has direct relevance to conservation (Luikart et al. 2010). CN_e can be calculated on the amount of pairwise linkage disequilibrium between microsatellite loci. This method is considered more powerful than the temporal method for early detection of a bottleneck or fragmentation (Luikart et al. 2010), and this is entirely dependent on sample size and loci number (Waples & Do 2010). CN_e can be estimated by the newly developed program Ne Estimator Version 2 (Do et al. 2014), following a similar genetic study performed on Australian white shark population (Blower et al. 2012), ultimately allowing for comparison across the two studies.

The main advantage of DNA microsatellite analysis over the photographic mark-recapture method is in the lower amount of time and effort to obtain statistically sufficient data, while

providing equally valuable estimates on the population status (Hoelzel et al. 2002, 2006; Luikart et al. 2010; Blower et al. 2012). Irrespective the value of using both techniques, the congruency between the mark-recapture and the genetic methods, and the accuracy of each, hasn't been extensively explored (Waples & Do 2010). When compared to other census techniques, the combinations of the two methods on the same population have been attempted few times on land animals (Mowat & Strobeck 2000; Mowat & Paetkau 2002) and in the marine environment (Schwartz et al. 1998; Smith et al. 1999; Stevick et al. 2001; Portnoy et al. 2009).

The white shark, *Carcharodon carcharias*, has a circumglobal distribution (Compagno et al. 2001; Boustany et al. 2002; Bonfil 2005; Domeier & Nasby-Lucas 2008; Jorgensen et al. 2009), and is a vulnerable apex marine predator (IUCN Red List, Category VU A1cd+2cd). The elusive nature of the species limits the collection of baseline information, critically needed for the long-term survival of the taxon. To date only two population estimate studies on white sharks were published for South Africa: Cliff et al. (1996) and Towner et al. (2013). The first white shark population estimate was performed from 1989 to 1993 by tagging the sharks with a stainless steel head and a plastic streamer. This study only included sharks caught by commercial line fisherman and in the Kwazulu Natal Shark Board nets (Cliff et al. 1996). During the five year study, 73 sharks were tagged, of which six were recaptured; a modified Lincoln-Peterson, closed estimation model, was used to calculate the white shark population size of the South African coast for each year, which were then used to provided a mean estimate of $N = 1279$ (Cliff et al. 1996). Sampling a large number of individuals is likely to improve the accuracy of a population estimate (Sutherland 2006) and it can be argued that the opportunistic capture-recapture effort performed for the first population estimate of South Africa coastline could have been biased by the low number of recaptures ($m_i = 6$). More recently, by making use of the unique trailing edge of the dorsal fin of white sharks (Anderson et al. 2011; Burgess et al. 2014; chapter 2), Towner et al. (2013) suggested that in the Gansbaai region, the estimated total number of sharks ranged between 808-1008 individuals. However the result of this study relied on the software DARWIN (Stanley 1995), designed to automatically match dolphin's dorsal fins. Hence, Towner et al. (2013) did not take into

account previous criticism associated with the usage of this software when analysing white sharks dorsal fins (Chapple et al. 2011), and failed to provide a validation of both the software's accuracy and the presence of false negatives in their dataset. It is, however, critically important to first verify the accuracy of the software used, since the error associated with false negatives (e.g. failing to re-match an individual and duplicating it in the dataset as two different animals) is comparable to "losing a mark" (violating the first assumption of mark-recapture models). By not excluding duplicates in the photographic dataset (e.g. false negatives) the population is also likely to be overestimated by the mark-recapture models (Sutherland 2006), therefore as part of the study we validated, with our photographic dataset, the accuracy of the software DARWIN for matching white sharks dorsal fins.

A quote by John Shepard (from Hilborn 2002) well exemplifies the challenges associated with estimating the population number of a marine species: "*Counting fish is like counting trees, except they are invisible and they keep moving*", nevertheless for a vulnerable species such as white shark, it's vitally important to provide a strong baseline for future evaluations of the population fluctuations. The greatest improvement in population size estimation can be achieved by using genetic estimates of demographic movement, and to estimate effective population size, to inform our capture mark recapture models (Luikart et al. 2010). Therefore, in the present study, we combined photographic identification and genetic techniques, on free ranging white sharks occurring in South Africa, to strengthen the population estimate results. The photographic identification was used to determine the census (or abundance, defined as the maximum number of individuals identified, after reaching saturation; Sutherland 2006), and to subsequently re-evaluate Towner et al. (2013) population size estimate N of the white sharks in the region of Gansbaai (N , defined as the estimate of all the individuals in the study area).

The white shark is known to be a highly migratory species (Pardini et al. 2001; Bonfil 2005; Jorgensen et al. 2009) making the definition of stock number and management units of extreme importance for improving the conservation efforts already in place for this species (Moritz 1994; Palsbøll et al. 2007). Hence, specifically to provide both a local and a national estimate of the

population, and to understand whether the population estimate for the region of Gansbaai can be representative for the entire coastline, tissues biopsies were collected from free ranging individuals in Gansbaai as well as in four additional known white shark aggregation spots around the coastline. The genetic techniques utilized for this study followed Blower et al. (2012) to estimate the contemporary effective population size (CN_e - mean number of breeding individuals contributing offspring per generation) (Portnoy et al. 2009; Dudgeon et al. 2012; Blower et al. 2012; Ovenden 2014).

Inferring N_e from the census size (or vice versa) would be possible only if their ratio (N_e/N) remains stable over time. Unfortunately little is known about the stability of such ratios which is expected to be population specific and only the accumulation of new information will help build future guidelines (Luikart et al. 2010). The magnitude of both the population estimate (N) and the effective population size (CN_e) and their ratio can give the unprecedented opportunity of correlating the two in future studies. Another use of understanding the N_e/N ratio is in predicting how management actions could increase N_e to maintain genetic variation (Cooper et al. 2010). Therefore, the results of this study can provide powerful insights into the status of the species, essential for recommending future conservation measures.

3.2 Materials and Methods

3.2.1 Data collection

Between April 2009 and April 2014 photographic data and biopsy samples were collected by attracting white sharks close to a vessel with natural fish chum (Department of Environmental Affairs of South Africa Permit numbers: RES2009/18, RES2010/71, RES2011/55, RES2012/38, RES2013/41, RES2014/39). The baited condition of this study exposed all individuals attracted (whether captured by the camera or not) to the same treatment, comparable to “setting the trap open and baited”: a practice used to eliminate “trap happy” bias, which can result in an underestimate of the population numbers (Sutherland 2006). The photographs used for the mark recapture analyses were collected during 298 days of sampling over 28 months of study in

Gansbaai (34° 40'614" S, 019° 23'934" E). To provide both a local and a national estimate of the population, tissues biopsies were collected from free ranging individuals in Gansbaai but also in four additional known white shark aggregation spots around the coastline: False Bay (34° 08'036" S, 018° 34'930" E), Struisbaai (34° 35'403"S, 020° 24'786"E), Mossel Bay (34° 08'985"S, 022° 07'220" E) and Algoa Bay (33° 44'753"S, 026°13' 523" E). The latter was performed to test the difference between the local Gansbaai population number and those occurring at five different aggregation sites, to determine if the Gansbaai population can be used as a representative of white sharks along the entire coastline.

3.2.2 Mark-recapture data

Dorsal fin pictures were taken in RAW format with high resolution cameras following Andreotti et al. (2014). The sex and total length (TL) of each individual was documented by visually recording the presence or absence of claspers at the posterior portion the pelfic fins of the individuals and by comparing the size of the sharks with a fixed size length [i.e. the cage of the cage-diving vessel, see also Leurs et al. (2014)]. Photo-identification data collected during the study period was stored in a separate folder (referred as "the full database" in chapter 2) per individual shark, named after the date of first capture (i.e. for all the sharks captured on the 5th of March the two first numbers of their folders will be "0305...". To separate each shark, the third number will indicate the sequence in which the animal approached the vessel: the folder for shark #4 will then be "030504"). All subsequent photographs of the same shark obtained a re-sighting code (RS) based on the "month/day" of re-capture (i.e. the photographs of the shark 030504, re-captured on 11 April, will be named as: 030504_RS 0411). The use of a storage system, together with the creation of a document summarizing all the additional morphological information (length and sex) allowed the user to double check the match of the re-captured shark, since new visual matches could be visually compared to the suit of all the previous photos of the same shark.

The final dataset of photo identification was double checked manually for duplicates (e.g. false negatives: same shark catalogued as two different individuals), before the mark-recapture analyses (see dataset details in chapter 3). To minimize human error in the data analyses, an *ad hoc* algorithm was created in Python to extrapolate the recapture data directly from the storage folders of each shark. The algorithm generated a binomial re-capture history matrix (1: captured; 0: non-captured) of all the sharks (Y-axis) captured in each sampling occasion (X-axis). The re-capture history matrix generated by the *ad hoc algorithm* was manually double checked by two of the authors.

The relatively small number of captures per capture occasion (averaging 4-5 sharks daily), for a prolonged period of time (298 days) can potentially inflate the estimates of the population numbers (Holmberg et al. 2008), therefore the time intervals of the capture-re captures history matrix were collapsed from daily to monthly time intervals. Two history matrixes for re-captures were generated: a) per month (27 capture occasions) and b) per winter season (3 capture occasions between May and July each year). From these the population sizes (N) were estimated using mark-recapture models.

To assess which model was more appropriate to estimate population abundance, the history matrix was subjected to the Close Test. Close Test uses the Otis (Otis et al. 1978) and Stanley and Burnham (Stanley & Burnham 1999) closure tests to detect closure violation in mark-recapture data sets (Stanley & Richards 2005). The Otis et al. (1978) test was developed under a null model allowing for heterogeneity in capture probabilities under closure, the Stanley and Burnham (1999) under a null model allowing for time-specific variation in capture probabilities under closure (Stanley & Richards 2005). The results of both tests suggested that the population is not closed, with the latter indicating that there were both losses and additions to the population, therefore the open population model POPAN, an improved variant of the Jolly-Seber model for open populations, provided in MARK, version 6.1 (White & Burnham 1999), was ultimately chosen to obtain an estimate of white shark abundance. Prior to applying the POPAN model, the following assumptions were made: (1) that the marking remained constant during the sampling period

(Anderson et al. 2011) (2) photographic process did not affect the catchability of individuals (Chapple et al. 2011) (3) since white sharks have a long life span (Calliet et al. 1985), coupled to low fecundity and low estimated natural mortality (Smith et al. 1998), we can assume that the population numbers did not change sufficiently to influence the estimate during the three year study period.

The POPAN model estimates the population size (N); the apparent survival rate (Φ); the probability of entry into the population (β); and the capture probability (p). Different variations of the model were applied to both the capture-recapture matrix (e.g. history matrix) of 27 month encounter occasions and the 3 winter seasons encounters, by setting Φ , β and p either constant (.) or time dependent (t). Link functions were specified as the Logit link for Φ and p and the multinomial Logit (MLogit) link for β (GC White, Program MARK Help files). Selection of the most parsimonious model was based on Akaike's Information Criterion with correction for finite sampling sizes (AICc). Goodness of fit (GoF) of the data under the open population model assumptions was tested using tests 2 and 3 in RELEASE GOF (available in MARK).

3.2.3 Genetic data

During 115 successful sampling occasions, a total of 302 tissues biopsies were collected from 233 different white sharks (confirmed using photo identification techniques). Biopsy samples were taken with a 2.53 m long pole equipped with a sterilized biopsy sampler from a small region at the base of the dorsal fin, and stored in 80% alcohol on board of the vessel. In the laboratory a small portion of each tissue sample was air dried from the alcohol and extracted with the DNeasy Blood and Tissue Kit (Qiagen) extraction kit, following the Qiagen manufacturer protocol. Fourteen microsatellites markers: Cca1419, Cca83, Cca1536, Cca1273, Ccar1, Cca711, Cca1072, Ccar6.27x, Cca1466, Cca1276, Cca1226, lox10, Ccar9, Ccar13 (Pardini et al. 2000; Gubili et al. 2010; O'Leary et al. 2013) were amplified in four multiplex reactions with the following conditions: 10 μ l reaction mix with 1 μ l template DNA, 5 μ l Kapa2G™ Fast Multiplex PCR Kit (Kapa Biosystems) and 1 μ l of each primer (10nmol). The PCR cycling included denaturation at 94°C for 3 min, followed by 30 cycles of 15 sec at 94°C, 30 sec at T_m primer's dependent and 1 min at

72°C, the final extension was performed for 7 min at 72°C (Appendix I). Once the microsatellites protocol was optimized, the PCR runs were performed in panels of 96 amplifications, with fluorescently labelled markers (6-FAM, VIC, NED, PET, see also Appendix I). To ensure consistency in the scoring of the microsatellites across different runs, the alleles were trimmed and scored by making use of an internal size marker (Liz).

Genotype scoring was performed in Geneious Version 5.6.5 (Copyright © 2005-2012 Biomatters Ltd.). Assessment of amplification errors, such as large allele drop out, stuttering and null alleles was conducted in Microchecker Version 2.2.3 (Van Oosterhout et al. 2004). The software SHAZA Version 1.0 (Macbeth et al. 2011) was used to assess duplicated samples, which were eliminated from subsequent analyses.

To prevent bias of the results by sampling overlapping generations (Waples & Do 2010), we included only samples from non sexually mature individuals. Following Blower et al. (2012), the estimate of CNe was performed from the amount of pairwise linkage disequilibrium between microsatellite loci (Hill 1981; Waples 2006) with the program Ne estimator version 2.0 (Do et al. 2014). A random mating, over monogamy was assumed as this is the most likely system for white sharks (see also Blower et al. 2012). The software Ne estimator Version 2 (Do et al. 2014) provides a range of CNe estimates to choose from, depending on the optimal allele frequency exclusion criterion P_{crit} (Waples & Do 2010). The software provides a point estimate and a confidence interval with a 95% accuracy, based on the number of loci and the number of samples used for the analysis (Waples 2006; Waples & Do 2010; Blower et al. 2012; Do et al. 2014). Due to the large sample size (>100) and the 14 nuclear markers, we could obtain acceptable estimates of CNe with an optimal allele frequency exclusion criterion P_{crit} of 0.01 (Waples & Do 2010).

3.2.4 Validation of DARWIN for white sharks photo identifications

DARWIN [Version 2.22 – (Stanley 1995)] allows for comparison (“Fin Matching”) of traces (lines automatically drawn around the edge of the dorsal fin by the function “Fin Trace”) to match a new fin photograph with one existing in the database. “Fin Matching” ranks all database photographs by

probability of match; if it succeeds to match a new trace precisely with an existing fin trace in the database, that match will be ranked in the first position (Rank = 1).

To validate the accuracy of DARWIN, 426 individual high quality white shark photo identifications (Andreotti et al. 2014), derived from 4398 pictures, were used to test-match 122 additional images from 53 unique sharks. To optimize matching success, the edges of the dorsal fin traces were manually adjusted when “Fin Trace” failed to accurately detect the notches in the fin. The trial comprised matching the 426 traced images with: (1) 50 duplicate photographs copied from the database as the control (the same photographs were expected to 100% match with themselves, Rank = 1) (2) 50 random white sharks photographs taken from the entire collection of 4398 pictures (3) 72 photographs of three repeatedly sampled white sharks. The validation of DARWIN was based on the null hypothesis that the rank of the correct matching image from the database would be equal to one. To be less stringent, we also allowed a match within the top 20 ranked positions. A paired t-test was used to quantify how much the means of the DARWIN result (e.g. rank obtained) against the correct result (e.g. Rank = 1) differ among each other. Likewise, to allow for minor failure, we also allowed for a hypothesized difference of 20, compared to the real mean difference between the two.

3.3 Results

3.3.1 Mark-recapture

A total of 426 (n) individual white sharks were identified in Gansbaai (Andreotti et al. 2014). The sex ratio (males:females) was 1:1.09, with 329 non reproductive individuals (juveniles and sub adults, < 3.5m) and 86 adults (> 3.5m). Photographs of the same shark collected on the same day (i.e. both sides of the dorsal fin) were systematically named with a progressive number between brackets [i.e. if the first photograph was named 020509, all the subsequent photographs of the same shark, collected the same day, were named 020509 (2), 020509 (3) etc] and subsequently excluded from the mark-recapture analyses, reducing the number of “informative photographs” from 4398 to 1307. Three false negatives were found and excluded after manual checking (see

chapter 3). A total of 58% of the sharks in the database were re-sampled (877 re-sighting / 1304 total capture events) and the number of recaptured events spanned from 2 to 18 (Figure 3.1 and see Appendix II).

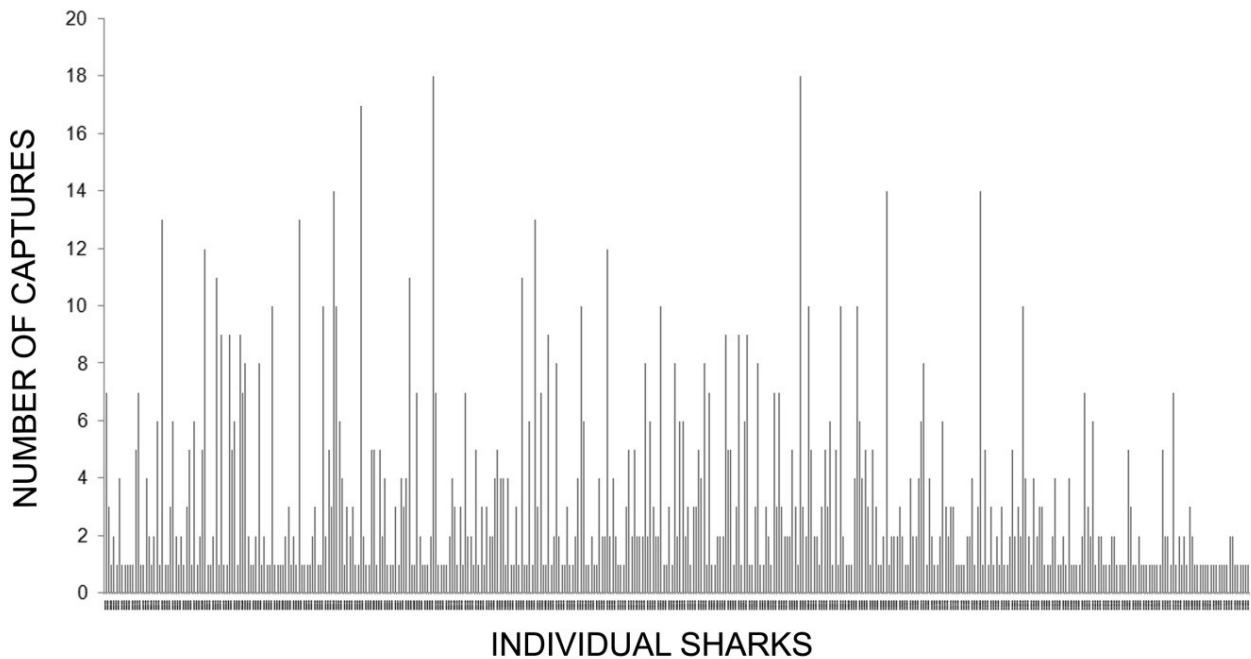


Figure 3.1: Captures of each individual shark (N=426) included in the dataset. The individuals are order by the first date of capture (left to right, from 2009 to 2011).

Forty seven sharks identified in 2009 were recaptured in 2010 and 128 sharks from previous years were recaptured in 2011. The latter exceed the number of newly identified sharks for the same year. Saturation of new sightings occurred once 400 individuals were sampled (Figure 3.2) indicating a sufficient amount of sampling effort (Sutherland 2006).

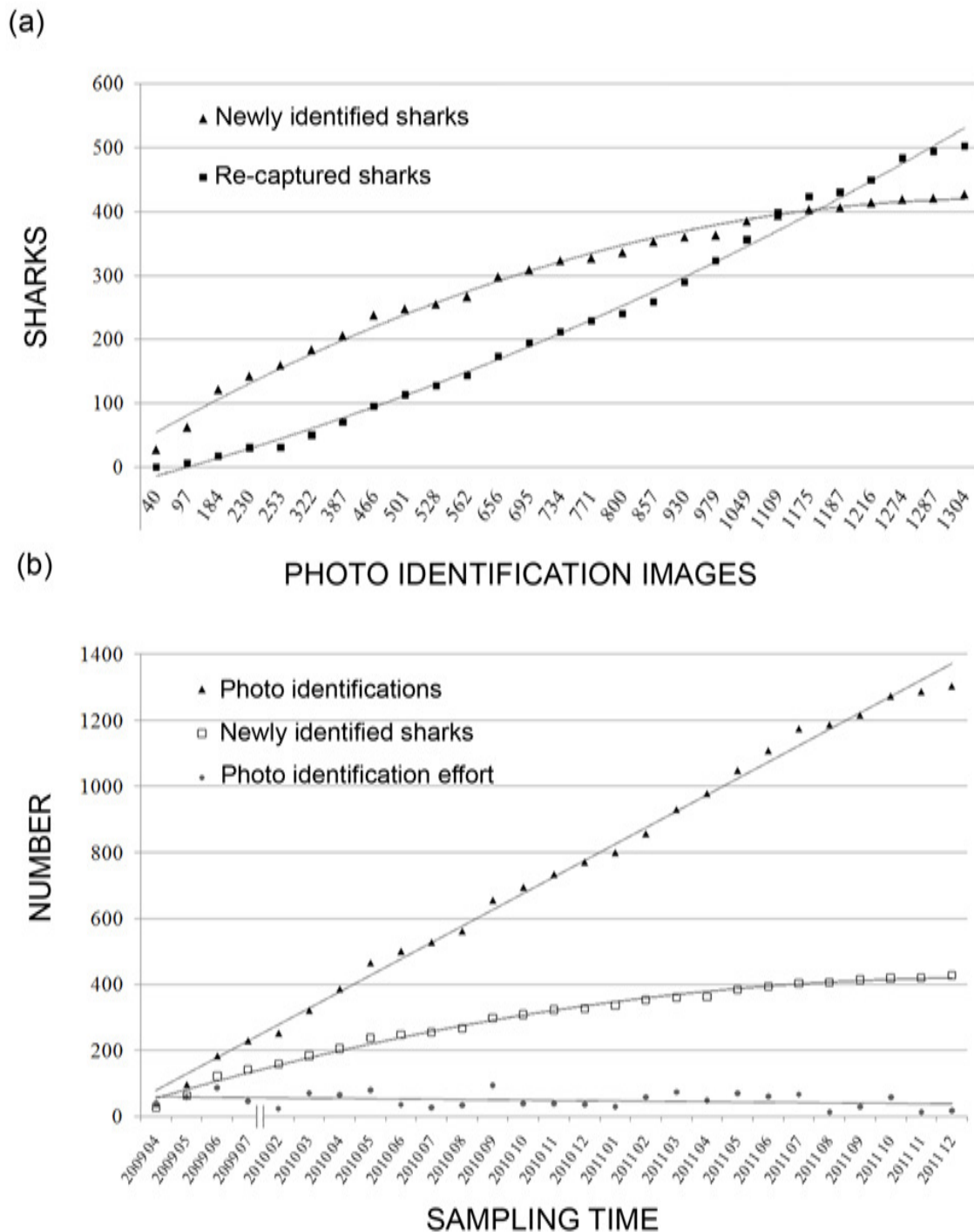


Figure 3.2 (a) Inverse relationship between newly sighted and re-captured individuals.
(b) Cumulative curves of photo identifications, newly identified sharks and photo identification effort in terms of days of sampling.

RELEASE GOF tests 2 and 3, suggest that the monthly capture history matrix was consistent with the open population model assumptions ($\chi^2 = 251.9$; $P = 0.00$). Irrespective of the history matrix used (per month or per winter season) the most parsimonious model for the POPAN variants, provided in MARK, version 6.1 (White & Burnham 1999) was when the capture probability (ρ) was time dependant, and the survival rate (Φ) and the probability of entry into the population (β) were kept constant. As expected, the population estimate derived from the larger monthly capture history matrix ($N = 426$), showed a narrow interval ranging between $N = 429$ and $N = 431$ ($\chi^2 = 0.66 \times 10^6$), while the same estimate from the winter season ($N = 255$), ranged between $N = 353$ and $N = 522$ ($\chi^2 = 0.34 \times 10^3$) (Table 3.1).

Table 3.1 Selected parameter estimates and model selection criteria for four model variants in the POPAN model for the two capture history matrixes. N-hat = population estimate and N-interval = 95% confidence limits; Φ = survival rate, β = probability of entry into the population; ρ = capture probability; (.) is constant; (t) is time dependent; models are sorted according to ascending AICc.

| Sampling Interval | Name | AICc | Delta AICc | AICc Weight | Model Likelihood | Number of parameters | Deviance | N-hat | N-interval |
|-----------------------------|------------------------------|----------|------------|-------------|------------------|----------------------|----------|-----------------|----------------|
| Monthly (N=27) | $\{\Phi(.)\rho(t)\beta(.)\}$ | 3963.86 | 0 | 1 | 1 | 28 | 0 | 430 ± 1 | 429-431 |
| | $\{\Phi(t)\rho(t)\beta(t)\}$ | 39961.08 | 35997.21 | 0 | 0 | 79 | 35710.3 | 3161 ± 150 | 2867-3455 |
| | $\{\Phi(t)\rho(.)\beta(t)\}$ | 40177.58 | 36213.71 | 0 | 0 | 53 | 35987.16 | 2715 ± 131 | 2457-2972 |
| Winter seasons (N=3) | $\{\Phi(.)\rho(t)\beta(.)\}$ | 347.55 | 0 | 0.51 | 1 | 6 | 0 | 438 ± 42 | 353-522 |
| | $\{\Phi(t)\rho(.)\beta(t)\}$ | 347.75 | 0.2 | 0.28 | 0.9 | 6 | 0 | 471 ± 45 | 382-531 |
| | $\{\Phi(.)\rho(t)\beta(t)\}$ | 349.31 | 1.73 | 0.21 | 0.4 | 7 | 0 | 408 ± 36 | 336-479 |

3.3.2 Genetic population estimate

Likelihood-based genotype matching by SHAZA Version 1.0 (Macbeth et al. 2011) identified 60 duplicated samples among all the tissue biopsies taken. These duplicate samples were indicated blindly with SHAZA Version 1.0 (Macbeth et al. 2011) and subsequently re-confirmed by photo identification (Figure 3.3).

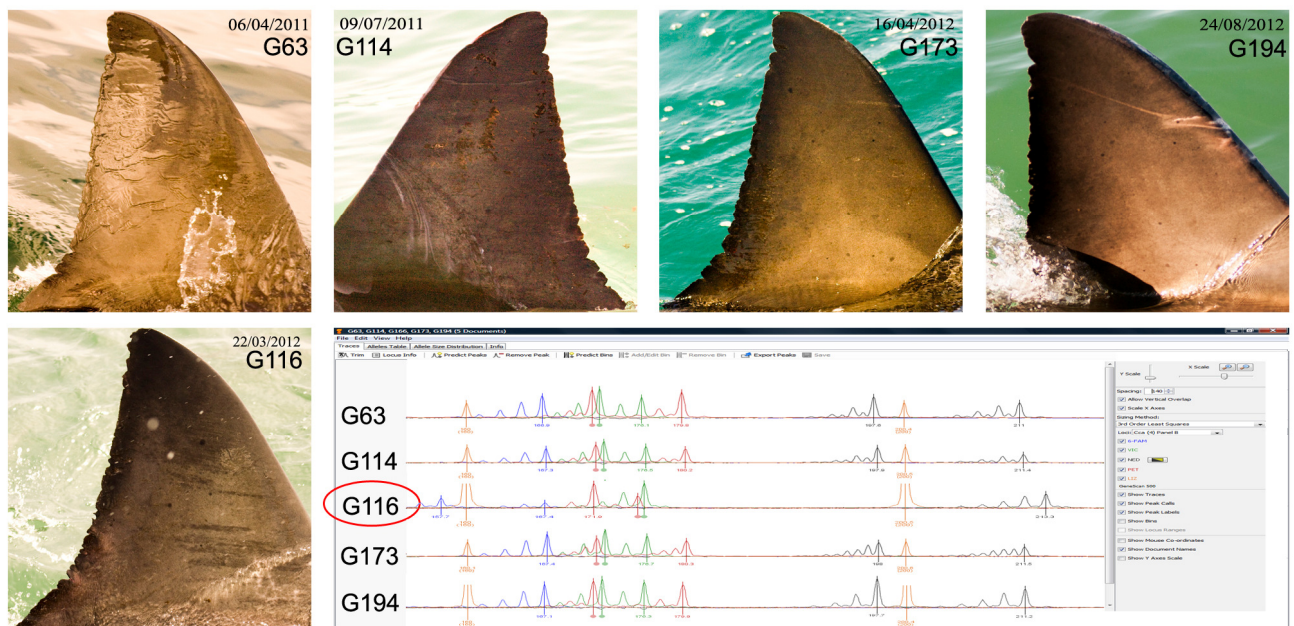


Figure 3.3 Correspondence between photo identification and genetic fingerprint of a shark (C_050606B) sampled in four different occasions across two years. Each genetic sample has been uniquely coded (G63, G114, G173 and G194) to allow for blind scoring of duplicates using a genetic fingerprint. The sample G116 belongs to a different individual (C_040705), as confirmed by the genetic profile and the different notches pattern of the dorsal fin.

As such, these individuals were removed from all further analyses, leaving 233 sharks for genotyping, and to avoid overlapping generations we selected only 147 non-sexually mature individuals (TL smaller than 3.5m), of which 102 were sampled in Gansbaai. In the dataset there was no evidence for stuttering, large allele drop out or null alleles. Overall, all loci were under Hardy-Weinberg equilibrium and no evidence of linkage was detected. However, two loci (Cca1072 and Ccar9) had homozygote excess and were double checked for consistencies in scoring. Estimates of CNe, for Gansbaai (N = 102) obtained a point estimate of 338 (95% CI = 204 – 858).

For the entire coastline, and P_{crit} of 0.01, a point estimate of 336 (95% CI = 229 – 592, $n = 147$) resulted, with an estimated number of contemporary breeders of 50 (95% CI = 11 – ∞).

3.3.3 Validation program DARWIN for white sharks photo identifications

“Fin Trace” failed to automatically draw an accurate edge around the dorsal fin and manual optimization was needed in each instance (Figure 3.4). After adjusting the edge, 86% of the control pictures (e.g. photographs copied from the database) were correctly matched by DARWIN (Rank = 1); an additional 12% ranked in the top 20. In the validation trial, DARWIN correctly matched 20% of the random photos taken from the entire collection with the images in the database, and an additional 20% could be found in the first 20 images. The remaining 60% ranked lower than 20, with some matches as low as position 234 (Figure 3.4). The trial conducted with photographs of the same three re-sighted individuals showed similar results (Figure 3.5). The average difference (95% confidence), between first rank and the software’s match is 66.34. The paired t-test rejected the null hypothesis that DARWIN can successfully match a white shark dorsal fin image in the upper range of up to 20 ranked images ($t\text{-stat}=5.73$, $P = 3.72 \times 10^{-8}$).

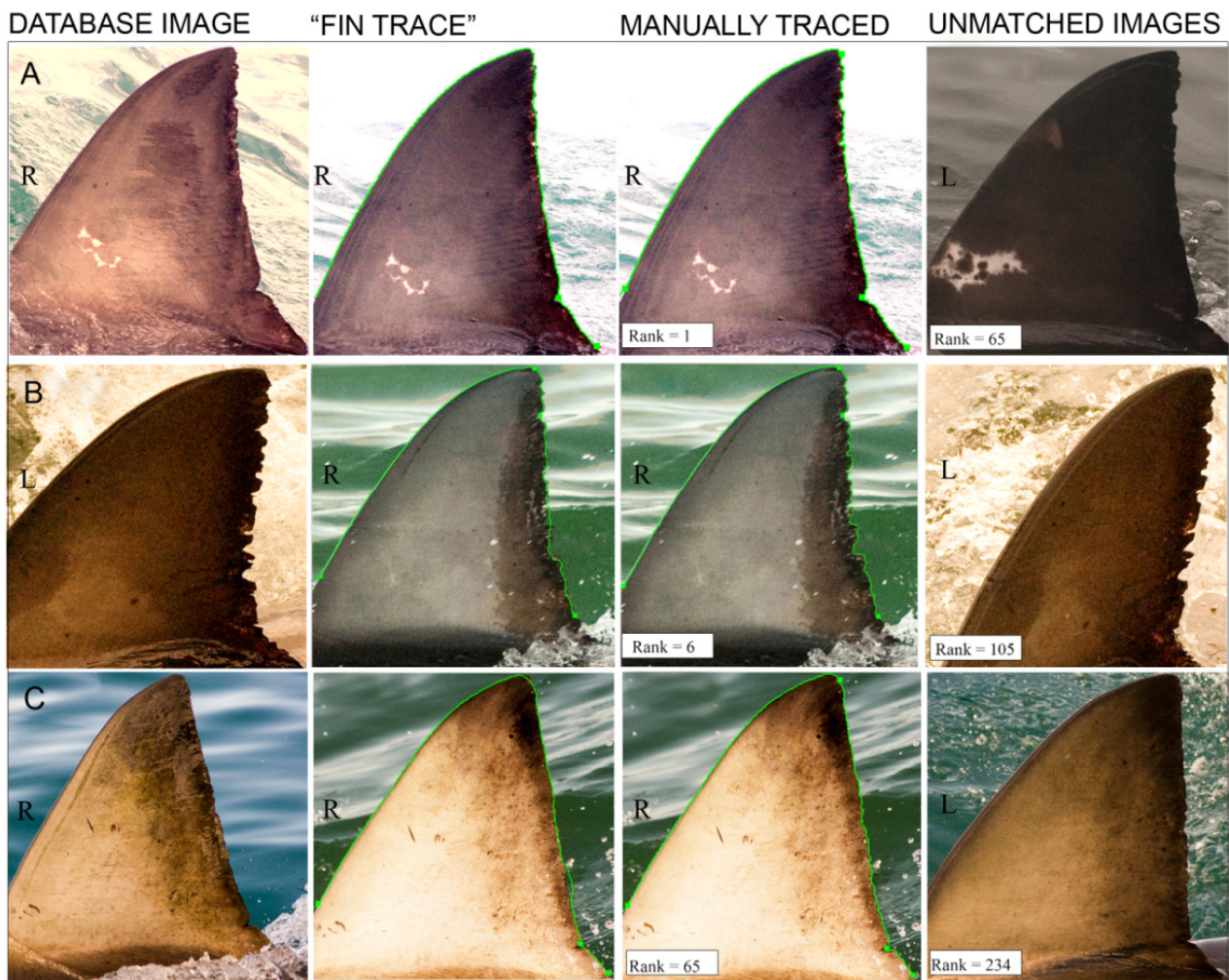


Figure 3.4 Example of photo identification for three different white sharks (one row for each shark). Due to DARWIN's inherent prerequisite to work with fins from the left side only, some of the photographs were rotated horizontally (the true side of the fin is indicated as R = right, L = left, on the image). Procedures flow from the left: (1) image from the database; (2) image to be matched showing the line traced automatically by the software "Fin Trace"; (3) images with the line manually re-traced to allow for better comparison within "Fin Matching"; (4) examples of an image that could not be matched by the software. Row (a) is an example of a matched image (Ranked=1); Row (b) Example of a correct match found in the first 20 ranked images (Ranked=6); Row (c) example of an unmatched image (Ranked=65). The Rank assigned by the software to the correctly matched image is indicated on the photograph.

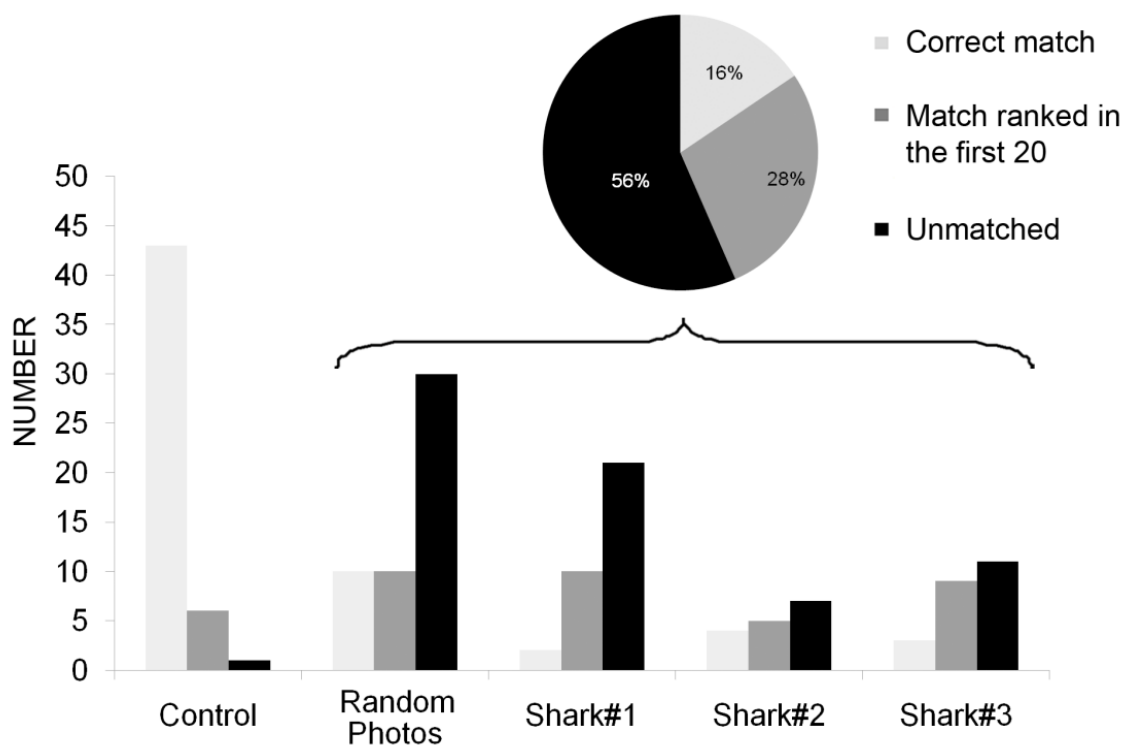


Figure 3.5 DARWIN results showing the number of matched images for each trial conducted. The pie chart indicates the percentage of each score.

3.4 Discussion

The most remarkable result of our study is the striking similarity between the estimates derived from the genetic (95% CI = 229 – 592) and the mark recapture (95% CI = 353 - 522) methods. These values also correspond very well to the census number of individuals previously reported (Andreotti et al. 2014). The adult census based on photographic identification was 86 adults (> 3.5m), 36 individuals higher than the estimated number of breeders in the population $N_b = 50$ (Do et al. 2014). The number of adults in the census is, as expected, higher than the true N_b , as it can't include all the factors, such as variance in reproductive success (Luikart et al. 2010).

When compared to other marine fishes (Hoarau et al. 2005), this is unexpected since the census size (n) of a fish population is usually vastly different from the mean number of breeding individuals contributing offspring per generation CNe (Hill 1981; Hoarau et al. 2005; Waples 2006; Waples & Do 2010). More specifically a recent review on the ratios of $CNe : n$ (census size) in marine teleosts revealed that generally CNe is two to six orders of magnitude lower than the census size

(Hauser & Carvalho 2008). However, in sharks it is to be expected that there should be more similarity between these estimates, given the unique slow mode of reproduction. Indeed, congruence between effective population size and the census size has been reported for sandbar sharks *C. plumbeus* (Portnoy et al. 2009), grey nurse shark *C. taurus* (Ahonen et al. 2009), zebra sharks *Stegostoma fasciatum* (Ovenden 2014) and reef manta rays *Manta alfredi* (Ovenden 2014). In this context, our findings support the notion that more congruence is to be expected in marine species with high or constant lifetime survivorship and low fecundities (Luikart et al. 2010).

Despite the suggestions that the notches on the dorsal fin are stable for at least 22 years (Anderson et al. 2011), an often discussed limitation of mark recapture studies (based on photographic identifications), is that, unlike a fingerprint, the pattern on white sharks dorsal fin may change over time (Robbins & Fox 2012; Marshall & Pierce 2012; Towner et al. 2013; Burgess et al. 2014). These wrong assignments will result in false negatives and an over-estimate of the population (Burgess et al. 2014). A previous study combining genetics and photo identification revealed that the photo-identification method was only 85% accurate at identifying individuals over a five year period (Gubili et al. 2009). Conversely the results of our study, conducted over a longer period (6 years: 2009-2014) provide solid evidences of stability in the notches pattern. In fact, the genotype comparison of 60 identified individuals sampled in more than one occasion confirmed in each instance the accuracy of photo identification (Figure 3.3).

The difference from previous studies might be that we selected only high quality photographs (Andreotti et al. 2014) and we used only the trailing edge of the dorsal fin for matching individuals, as white marks proved to change over time (Robbins & Fox 2012; Andreotti et al. 2014, see chapter 2, Figure 2.5). Another limitation of mark-recapture studies in general is the possibility of missing some individuals due to differences of behavioural patterns among individuals (Sperone et al. 2010; Delaney et al. 2012; Jacoby et al. 2014) or weather conditions (Marshall & Pierce 2012; Andreotti et al. 2014). It is thus very enlightening that our white shark population estimate is congruent when the mark-recapture analysis are compared to the genetic results. The information derived from the photographic identification study can indicate that most of the assumptions for

estimating CNe were met. Specifically: equal sex ratio could be confirmed for our population (1:1.09) and overlapping generations, which can potentially bias the linkage-disequilibrium CNe estimate (Luikart et al. 2010), could be excluded by knowing in advance the size of the sharks and therefore selecting only non-sexually mature sharks. The biggest advantage of integrating the mark-recapture technique with molecular markers is that, when using the Linkage Disequilibrium method, it is very unlikely to mistake a population with moderately small Ne for one with large Ne (Waples & Do 2010), which makes the combination of the two techniques preferable when dealing with elusive and difficult to sample species.

In our study, the mark recapture census shows clear saturation of new sightings occurring at $n = 400$ (Figure 3.2b). The saturation results support the fact that white shark are known to show site philopatry (Pardini et al. 2001; Hueter et al. 2005; Jorgensen et al. 2009; Gubili et al. 2010; chapter 4) and the majority of individuals are thus re-sighted even up to three years after the first capture (249 of the identified white sharks were re-sighted 877 times). The distribution of the most captured individuals spreads throughout the dataset (Figure 3.1), showing a great re-capture rate of individuals captured in the first year, an indicator of the method's ability to re-capture the individuals over long period of time. Our genetic and mark recapture data, however, is in sharp contrast to the recent study by Towner et al. (2013). The Towner study was similarly performed in Ganbaai (South Africa), with photographic data collected during the same years of our study. The only difference from our data collection was that Towner et al. (2013) used the software DARWIN (Stanley 1995) for matching and cataloguing the identified white sharks. As Towner et al. (2013) failed to provide a validation of the software's accuracy (and no mention of the rankings were given), the discrepancy between the results are more than likely due to the inclusion of false negatives in their dataset (also see Sutherland 2006). This hypothesis is confirmed by the DARWIN validation trial we performed that indicated that DARWIN significantly failed to correctly match the white sharks fin images ($t\text{-stat}=5.73$, $P < 0.000$, Figure 3.4 and 3.5). Another line of evidence in support of our hypothesis for false negatives can be found in the fact that, contrary to

our dataset (Figure 3.2), the curve of newly identified sharks in the Towner et al. (2013) study never reached saturation (see Figure 2 in Towner et al. 2013).

Of course, all these data points should be seen in context of the limitations of each method, and the assumption that the white sharks sampled in Gansbaai over a 4 year period is representative of the entire coastline. It is, however, alarming that different methods broadly concur on a low number of surviving white sharks. From a conservation viewpoint it is important to consider that the genetic analyses suggest that there are only 50 breeders in the current population. This value is commonly regarded as the lowest limit before inbreeding depression becomes a serious concern (Franklin & Frankham 1998; Frankham et al. 2010; Dudgeon et al. 2012). The CNe point estimates by including individuals from the entire coastline (N = 147) or just from Gansbaai (N = 102), were remarkably close: (Coastline CNe = 336, 95% CI = 229 – 592, Gansbaai CNe = 338, 95% CI = 204 – 858). The only difference is in the confidence interval, as accuracy of the linkage-disequilibrium method increases proportionally to the number of samples and markers used: with less samples (e.g. Gansbaai alone) it is expected for the confidence interval to become broader (Waples & Do 2010; Dudgeon et al. 2012; Do et al. 2014). If these values are correct, it seems reasonable to suggest that sharks sampled at Gansbaai over a 2.5 year period is more than likely representative of the entire South African population. This is further confirmed by the mtDNA and microsatellite data presented later (chapter 4) and by previous acoustic tagging studies (Bonfil 2005) and also photo identification evidence (see chapter 4 and Appendix III), all suggesting that white shark individuals disperse freely between aggregations sites (see <http://www.ocearch.org/#SharkTracker>).

Despite the fact that white sharks have been protected in South Africa since 1994, it appears that the population didn't recover since the first population estimate study conducted in 1996 (Cliff et al. 1996), which indicated the presence of 1279 in the region of Kwazulu-Natal (South Africa). This study was based on a very small sample, but our study indicates that the situation is even more severe. The low estimates from both the photographic mark-recapture and genetic techniques could be representing a drastic decline in the population number over the last 30 years, due to food

resource depletions (Stevens et al. 2000; Anticamara et al. 2011), increased pollution and or anthropogenic mortality (Cliff et al. 1989; Compagno et al. 1997; Chapman et al. 2003; Worm et al. 2013). The genetic CNe estimates approximates the number breeders that, from the previous generation, contributed to the current population status. The number of current breeders as reported by Ne estimator Version 2 (Do et al. 2014) was $N_b = 50$, which gives a ratio between population and breeders of 10:1. Transferring this ratio to the previous white shark generation, as calculated by CNe in our study, it would indicate that the previous generation of white sharks counted approximately 3000 individuals and declined to 500 in only one generation. Considering that between 1978 and 2008 approximately 1063 great white sharks (*C. carcharias*) were killed in the nets of Kwazulu Natal Shark Board alone (Peschak, 2009), this estimate does not seem too farfetched. To conclude, the result of our study are defining a gloomy picture for South African white sharks and the survival of this population can be seriously compromised if management measures are not improved in the short term.

CHAPTER 4

THE EFFECT OF FEMALE SITE PHILOPATRY, FOUNDER EVENTS AND LONG DISTANCE MIGRATION ON THE POPULATION GENETICS OF WHITE SHARKS, *CARCHARODON CARCHARIAS*

4.1 Introduction

The white shark (*Carcharodon carcharias* L.) is one of oldest shark lineages with an evolutionary history dating back to ~about 14 Ma (Gottfried & Fordyce 2001). This species has a nearly worldwide distribution, with individual sharks having the potential for dispersing large distances (Bonfil 2005). These long-distance dispersers however, do not seem to contribute greatly towards genetic exchange between geographically distant populations (Pardini et al. 2001; Jorgensen et al. 2009), as significant genetic differentiation has been documented between white sharks sampled in South Africa and Australia (Pardini et al. 2001) and on a finer scale, also among Australian sub-populations (Blower et al. 2012). At the global scale, Gubili et al. (2010) suggested the pattern is generally maintained through natal female philopatry, but noted that in some instances long distance dispersals may lead to founder events. These findings suggest a hypothesis of a global genetically structured white shark population.

In the marine environment, direct observations of migrations are extremely challenging, making population genetic tools among the most used to define management units (Moritz 1994) and stock structure in fishes (Ovenden 1990). To date, most of the studies focused on stock assessment of commercially exploited fishes, with relatively less attention given to elasmobranchs species (Dudgeon et al. 2009, 2012). Contrary to marine teleostes and invertebrates, elasmobranchs lack pelagic larvae, making coastal and pelagic movements of adults and sub-adults the only sensible contributors towards dispersal and ultimately geneflow (Heist 2004). Particularly relevant to the current study, the reproduction cycle and strategy of sharks can vary greatly across species (Compagno et al. 2001) and so can the range of dispersal, which generally corresponds to the global genetic structure. One such example can be seen in the whale shark, *Rhincodon typus*, as the species has a high dispersal potential, corresponding to the lack of global genetic structure (Castro et al. 2007; Vignaud et al. 2014) with the most common haplotype globally distributed across oceans basins. Contrastingly, the more residential angel shark, *Squatina californica*, showed large levels of differentiation between closely sampled sites (Gaida 1997). Ovenden et al. (2009) investigated the population structure of four species of ground sharks (Carchariniiformes),

and found a lack of structure between eastern and western Australian waters and documented population genetic structure between Indonesian and Australian waters for two of the four species. Genetic population structure of three oviparous Chondrichthyan species: the thornback ray, *Raja clavata* (Chevolot et al. 2006), the thorny skate, *Amblyraja radiata* (Chevolot et al. 2007) and the zebra shark, *Stegostoma fasciatum* (Dudgeon et al. 2009) surprisingly didn't support natal philopatry, as contrasting patterns of genetic subdivision were found over comparable spatial scales. In contrast, the genetic differentiation in species with viviparous reproduction such as white sharks, has been attributed to females displaying natal philopatry to pupping grounds and nursery areas (Blower et al. 2012) as was similarly observed in black tip sharks *Archarhinus limbatus*, (Keeney et al. 2005), and scalloped hammerhead *Sphyrna lewini* (Duncan et al. 2006). The presence of stable pupping grounds and nursery areas can be of great interest for the management of a vulnerable species, as once protected from overexploitation it could promote the constant recruitment and replenishment of a population (Bansemer & Bennett 2009).

White sharks are considered vulnerable in the red list of the International Union for the Conservation of Nature (IUCN, Category VU A1cd+2cd), are included in the Appendix II of the Convention on International Trade in Endangered Species (CITES) and in the Convention for Migratory Species (CMS). Despite this, the species is continuously subjected to anthropogenic mortality associated with fishing, the illegal trade of their body parts (primarily jaws and fins) and anti-shark nets (Stevens et al. 2000; Pardini et al. 2001; Dulvy et al. 2008). Effective conservation of white sharks is difficult to achieve for a number of reasons. Firstly, the global white shark population does not appear to function as a metapopulation (Pardini et al. 2001; Gubili et al. 2010) and local extinctions of genetically divergent populations can thus have serious implications for the conservation of the species (Ryman et al. 1995; Allendorf et al. 2008; Pinsky & Palumbi 2014). Secondly, the elusive nature of white sharks make them less well studied in terms of life history features and thirdly, their large distribution range makes concerted conservation efforts problematic since individuals occupying different aggregation sites are particularly vulnerable to different geopolitical influences (Kellert 1985; Favre 1989; Jorgensen et al. 2009; Dudgeon et al. 2012).

Lastly, the species is on the forefront of human / shark conflict and in some instances extreme measures such as baited drum lines and shark-nets are implemented to reduce this conflict (Wetherbee et al. 1994; Department of Fisheries Western Australia 2013).

The South African coastline represents one of the major aggregation areas for white sharks in the world (Compagno et al. 1997; Dulvy et al. 2008). Anecdotal evidence suggests that the species occurs along the entire coastline, and acoustic tags have provided evidence of five aggregation sites: False Bay, Gansbaai, Struisbaai-De Hoop, Mossel Bay and Algoa Bay (Figure 4.1) (Johnson & Kock 2006). Dispersal analyses suggest that these aggregation sites can be seen as one single panmictic population (Bonfil 2005). The coastline, however, is heterogeneous in nature, with distinct temperature gradients and nutrient distributions that in concert result in the recognition of at least five biogeographic provinces (Hedgpeth 1957; Emanuel et al. 1992; Spalding et al. 2007).

Previous genetic studies indicated that several continuously distributed species show disjoint genetic patterns corresponding to the biogeographic provinces (Teske et al. 2011). However, these phylogeographic studies focused primarily on coastal species with data for offshore species poorly represented in both biodiversity and genetic studies (von der Heyden 2009; Teske et al. 2011). The genetic dispersal of large pelagic species are also generally not influenced by thermal fronts (Grant & Bowen 1998; Baker et al. 2006; Teske et al. 2011), but in the case of the white shark, both thermal fronts (Gubili et al 2010) and female site philopatry (Pardini et al. 2001; Jorgensen et al. 2009; Gubili et al. 2010; Blower et al. 2012) have been identified as limiting dispersal. It is thus a possibility that the South African aggregation sites correspond to different genetically identifiable sub-population units.

To gain a better understanding of the regional genetic structure of white sharks along the South African coastline, we embarked on a phylogeographic investigation to test whether South African white sharks are indeed genetically structured. Further, given the global reduction of shark's populations (Worm et al. 2013) it is likely that members of the species have been facing similar declines in population size. To assess the influence of this population decline on the

genetics of remaining white sharks, we characterised their levels of genetic diversity and compared this with other fish and shark species, both in South Africa and worldwide. Our data also provided an opportunity to extend the current knowledge on global white shark connectivity (Gubili et al. 2010). To address the latter, we analysed our data in a global context by comparing the South African lineages with other global populations and used these data to interpret both the local and global evolutionary history of this apex predator.

4.2 Materials and methods

4.2.1 Areas and method of sampling

From October 2010 to November 2013 photographic identifications and tissues biopsies were collected from five South African white shark aggregation spots (Figure 4.1): False Bay (34° 08'036" S, 018° 34'930" E), Gansbaai (34° 40'614" S, 019° 23'934" E), Struisbaai / De Hoop (34° 35'403"S, 020° 24'786"E), Mossel Bay (34° 08'985"S, 022° 07'220" E) and Algoa Bay (33° 44'753"S, 026°13' 523" E). During 115 successful sampling occasions a total of 302 tissues biopsies were collected from free-ranging white sharks (Table 4.1).

Table 4.1 Sampling sites and number (N) of *C. carcharias* mitochondrial DNA control region

| Region | Location | Sampling Area | N | Sample type | GeneBank Acc. Nr. |
|----------------------|---------------|--------------------|-----|--------------------|----------------------------------|
| Western Indian Ocean | South Africa | False Bay | 12 | Tissue | KP058665 - KP058902 ^a |
| | | Gansbaai | 224 | Tissue | |
| | | Struisbaai/De Hoop | 18 | Tissue | |
| | | Mossel Bay | 29 | Tissue | |
| | | Algoa Bay | 12 | Tissue | |
| Eastern Indian Ocean | New Zealand | | 4 | mtDNA CR sequences | AY026196-AY026224 ^b |
| | Australia | | 12 | mtDNA CR sequences | |
| | Australia | | 18 | mtDNA CR sequences | HQ414073-HQ414086 ^c |
| Northeast Pacific | California | | 20 | mtDNA CR sequences | GU002302-GU002321 ^d |
| Northwest Atlantic | Florida | | 2 | mtDNA CR sequences | HQ540294-HQ540298 ^e |
| Mediterranean | Mediterranean | | 2 | mtDNA CR sequences | |

^a This study; ^b Pardini et al., 2001; ^c Blower et al., 2012; ^d Gubili et al., 2010.

Sharks were sampled from the boat of Shark Diving Unlimited; biopsies were taken from a small region at the base of the dorsal fin, with a 2.53 m long pole, equipped with a sterilized biopsy sampler (Department of Environmental Affairs of South Africa Permit numbers RES2010/71, RES 2011/55, RES 2012/38 and RES 2013/41). After collection, samples were stored in 80% ethanol. Between each sample collection the equipment was sterilized with ethanol over a flame. To avoid as much as possible duplicate sampling in the field, all individuals were identified using a photo identification method as described in Andreotti et al. (2014) before the samples were taken. Sharks that could be visually recognized as previously sampled were avoided. The duplication in sequencing the mtDNA of the same individual twice was further avoided through using microsatellite fingerprinting (also see chapter 3 and Appendix I). To obtain a global evolutionary perspective on the South African white shark population, mtDNA sequences from individuals sampled outside of South Africa were downloaded from GenBank and included in a comparative analysis (Table 4.1).

4.2.2 Laboratory Procedures

A small portion from each tissue sample was cut, air-dried and extracted with the DNeasy Blood and Tissue Kit (Qiagen) extraction kit, following the manufacturer protocol. The mtDNA control region was amplified using the polymerase chain reaction (PCR) and published GWSMT1F and GWSMT1R primers and protocol as outlined in Blower et al. (2012). All PCR products were visually inspected and the amplified products were analysed on an automated sequencer (ABI 3730 XL DNA Analyzer, Applied Biosystems) using the GWSMT1F primer for sequencing. Ten percent of the PCR products were sequenced with both the forward and reverse primer to check for potential sequencing errors. To gain a clearer perspective on the hypothesis of female philopatry and the possibility of male biased dispersal we also included 14 microsatellites markers: Cca1419, Cca83, Cca1536, Cca1273, Ccar1, Cca711, Cca1072, Ccar6.27x, Cca1466, Cca1276, Cca1226, lox10, Ccar9, Ccar13 (Pardini et al. 2000; Gubili et al. 2010; O'Leary et al. 2013). These markers were amplified in a multiplex fashion as described in chapter 3 (see also Appendix I).

4.2.3 Sequences analysis of the mtDNA control region

All sequences were manually edited and aligned in Geneious version 5.6.5 (Copyright © 2005-2012 Biomatters Ltd). In order to compare with sequences downloaded from GenBank, sequences were trimmed to 839bp (Pardini et al. 2001; Jorgensen et al. 2009; Gubili et al. 2010; Blower et al. 2012). Aligned sequences were exported to DNAsp V5.10.01 (Copyright © 1999-2009 Universitat de Barcelona) for the calculations of haplotype (h) and nucleotide (π) diversity, and subsequently compared with the published h and π values from other sharks ($N=5$), marine mammals ($N=4$) and teleosts ($N=23$) species (Table 4.2).

All South African sampling sites were defined *a priori* by aggregation site to test for fine scale population differentiation using an analysis of molecular variance: False Bay ($N=12$), Gansbaai ($N=175$), Struisbaai ($N=15$), Mossel Bay ($N=27$) and Algoa Bay ($N=9$). Population pairwise ϕ_{st} values were calculated in Arlequin Version 3.5.1.2 (Excoffier & Lischer 2010) with statistical significance achieved after 10 000 permutations. To illustrate the evolutionary

relationships among haplotypes at local and global levels, a statistical parsimony haplotype network was generated using the program TCS (Version 1.2; (Clement et al. 2000). To avoid possible duplication in sampling the Pardini et al. (2001) samples originating from South Africa (Pardini et al. 2001) were not included in analyses. Furthermore, a NeighbourNet network was constructed from all the haplotypes using SplitsTree v4.10 (Bryant & Moulton 2004). Estimation of the most suitable nucleotide substitution model was performed in Modeltest Version 3.7 (Posada & Crandall 1998), using the BIC criteria. The substitution model identified was subsequently used in BEAST (Drummond & Rambaut 2007), in order to estimate time since most recent common ancestor, a proxy for time since divergence. The porbeagle shark, *Lamna nasus* (GenBank accession number: GU266740), a close sister taxon to *C. carcharias*, was used as outgroup (Gubili et al. 2010). Simulations of divergence time between white sharks populations were performed assuming a constant population size as tree prior, with a strict molecular clock enforced (as within the same species no rate variation is expected, see also Drummond & Rambaut 2007; Henriques et al. 2014), running for 1 000 000 MCMC steps. The dating analysis was carried out using two separate rates of divergence per million years calculated for the white shark control region (Gubili et al. 2010): a fast 1.19% rate, calibrated with the rise of Panama Isthmus (3.5Ma, Coates et al. 1992), and a slow 0.74% rate, calibrated with the separation of the Sunda-Sahul shelves (5Ma; Haq et al. 1987). Convergence and performance of runs were assessed in TRACER Version 1.5 (Drummond & Rambaut 2007).

Table 4.2 List of species, nucleotide (π) and haplotype (h) diversity and reference used to plot marine organism diversity value in Figure 4.3.

| SPECIES | Common name | π | h | REFERENCE |
|--|-------------------------|-------|-------|----------------------------------|
| Sharks | | | | |
| <i>Charcharodon carcharias</i> (SA) | White shark | 0.003 | 0.021 | (This study) |
| <i>Charcharodon carcharias</i> (AU) | White shark | 0.007 | 0.868 | (Blower et al. 2012) |
| <i>Carcharodon carcharias</i> (CA) | White shark | 0.001 | 0.660 | (Jorgensen et al. 2009) |
| <i>Cetorhinus maximus</i> | Basking shark | 0.001 | 0.720 | (Hoelzel et al. 2006) |
| <i>Carcharhinus limbatus</i> | Blacktip shark | 0.002 | 0.805 | (Keeney et al. 2005) |
| <i>Carcharias taurus</i> | Sand tiger shark | 0.003 | 0.717 | (Stow et al. 2006) |
| <i>Sphyrna lewini</i> | Scalloped hammerhead | 0.013 | 0.800 | (Duncan et al. 2006) |
| <i>Carcharinus leucas</i> | Bull shark | 0.003 | 0.760 | (Karl et al. 2011) |
| Marine Mammals | | | | |
| <i>Physeter macrocephalus</i> | Sperm whale | 0.002 | 0.860 | (Lyrholm et al. 1996) |
| <i>Orcinus orca</i> | Killer whale | 0.005 | 0.874 | (Hoelzel et al. 2002) |
| <i>Tursiops truncatus</i> | Bottlenose dolphin | 0.013 | 0.420 | (Natoli et al. 2005) |
| <i>Delphinus delphis</i> | Common dolphin | 0.012 | 0.853 | (Natoli et al. 2006) |
| Marine Teleostei (SA) | | | | |
| <i>Clinus cottoides</i> | Bluntnose klipfish | 0.003 | 0.660 | (von der Heyden et al. 2008) |
| <i>Hippocampus capensis</i> | Cape seahorse | 0.004 | 0.780 | (Teske et al. 2003) |
| <i>Merluccius capensis</i> | Shallow-water Cape hake | 0.006 | 0.880 | (von der Heyden et al. 2007) |
| <i>Merluccius paradoxus</i> | Deep-water Cape hake | 0.001 | 0.530 | (von der Heyden et al. 2010) |
| <i>Rhabdosargus holubii</i> | Cape stumpnose | 0.006 | 0.910 | (Oosthuizen 2007) |
| <i>Caffrogobius caffer</i> | Banded goby | 0.002 | 0.960 | (Neethling et al. 2008) |
| <i>Argyrosomus japonicus</i> | Dusky kob | 0.009 | 0.960 | (Klopper 2005) |
| Marine Teleostei | | | | |
| <i>Merluccius australis</i> | Southern hake | 0.002 | 0.470 | (Machado-Schiaffino et al. 2009) |
| <i>Lophius piscatorius</i> | Anglerfish | 0.008 | 0.770 | (Charrier et al. 2006) |
| <i>Lophius budegassa</i> | Anglerfish | 0.009 | 0.880 | (Charrier et al. 2006) |
| <i>Cynoscion acoupa</i> | Grey snapper | 0.003 | 0.890 | (Rodrigues et al. 2008) |
| <i>Girella punctata</i> | Largescale blackfish | 0.009 | 0.910 | (Umino et al. 2009) |
| <i>Acanthocybium solandri</i> | Barracuda | 0.006 | 0.920 | (Theisen et al. 2008) |
| <i>Lateolabrax japonicus</i> | Japanese seabass | 0.003 | 0.960 | (Liu et al. 2006) |
| <i>Trachurus trachurus</i> | Atlantic mackerel | 0.009 | 0.960 | (Comesaña et al. 2008) |
| <i>Lateolabrax maculatus</i> | Seabass | 0.012 | 0.960 | (Liu et al. 2006) |
| <i>Ocyurus chrysurus</i> | Yellowtail snapper | 0.019 | 0.960 | (Vasconcellos et al. 2008) |
| <i>Aphanopus carbo</i> | Black scabbardfish | 0.019 | 0.970 | (Stefanni & Knutsen 2007) |
| <i>Helicolenus dactylopterus</i> | Blackbelly rosefish | 0.005 | 0.980 | (Aboim et al. 2005) |
| <i>Scomber scombrus</i> | Atlantic mackerel | 0.029 | 0.990 | (Nesbo et al. 2000) |
| <i>Pagrus pagrus</i> | Red porgy | 0.011 | 0.990 | (Ball et al. 2007) |
| <i>Centropyge spp.</i> | Dwarf angelfish | 0.022 | 0.990 | (Bowen et al. 2006) |
| <i>Latris lineata</i> | Striped trumpeter | 0.040 | 0.990 | (Tracey et al. 2007) |

4.2.4 Genotype analyses of nuclear markers

Genotype scoring was performed in Geneious Version 5.6.5 (Copyright © 2005-2012 Biomatters Ltd.). Assessment of amplification errors, such as large allele drop out, stuttering and null alleles was conducted in Microchecker Version 2.2.3 (Van Oosterhout et al. 2004). Due to the large sample size and the migratory nature of the species, it is possible to re-sample the same individuals in different sampling events. As such, the software SHAZA Version 1.0 (Macbeth et al. 2011) was used to assess duplicated samples, which were eliminated from all analyses (duplicates were also excluded from the mtDNA data set discussed above).

To verify the statistical power of the results, given the number of samples and genetic markers, the algorithm implemented in Powsim Version 4.1 (Ryman & Palm 2006) was applied. This software evaluates the minimum number of samples per population necessary to detect a given structure (F_{st}), based on the effective population size (N_e) and number of generations passed (t). Given the unequal sample size among aggregation sites and in order to test if the dataset had enough power to detect $F_{st} \geq 0.02$, simulations were run with $N_e = 500$ and sample size in POP#1 = 20, by varying the sample size of POP#2 from 5 to 100, for a $t +$ (Ryman & Palm 2006). Statistical significance was assessed based on the proportion of chi-squared tests that gave a result \geq than expected.

Inference of population panmixia (Hardy Weinberg equilibrium) and linkage disequilibrium was determined in GENEPOP (Rousset 2008). Genetic diversity was estimated as expected and observed heterozygosity (H_e and H_o), number of alleles per locus, allelic richness, and inbreeding levels (F_{IS}) in Arlequin. Finally, potential population structure was evaluated using Wright's F-statistic, F_{st} implemented in the AMOVA analyses of Arlequin Version 3.5.1.2 (100 000 permutations). *A priori* populations were defined based on the outcome of the mtDNA pattern and also based on the sampling regime used (aggregation sites). To test for potential differences in allele frequencies due to temporal variation and sex, the genetic divergence was also estimated for gender (males = 85 and females = 112) and size classes (juveniles <2.7m = 64, sub adults 2.8m –

3.5m = 73, and adults >3.6m = 80 (see Andreotti et al. 2014 for further details). Furthermore the program Structure (Pritchard et al. 2000) was used to visualize the presence of population structure by making use of the multilocus genotype of each individual. Structure (Pritchard et al. 2000) was run with the admixture model, but without *a priori* assumptions, with K ranging from 2 to 5 and 5000 iterations. The samples were visually organized firstly by sampling sites and then by mtDNA haplotypes.

4.3 Results

4.3.1 MtDNA control region

Forward and reverse sequences resulted in identical reads with no evidence of conflicting base calls; sequences were generated for 238 unique sharks (GenBank Accession No. KP058665 - KP058902). Of the 839bp analysed, 15 positions were variable, with 14 parsimony informative sites. Only four unique haplotypes were detected, with no clear geographic pattern observed (Figure 4.1).

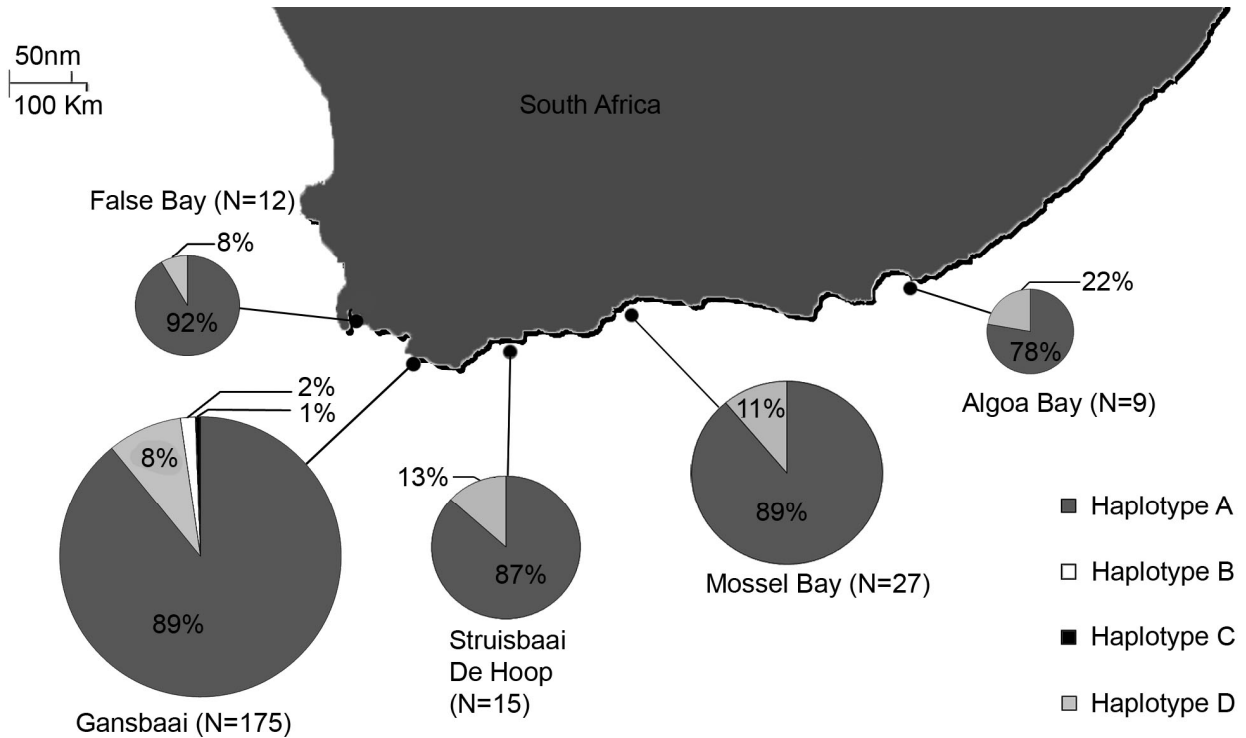


Figure 4.1: White shark mtDNA haplotype distribution along the South African coastline. The five aggregations sites from which samples were collected are: False Bay, Gansbaai, Struisbaai / De Hoop, Mossel Bay and Algoa Bay.

The majority of the 238 individuals (~89%; N=211) share a single haplotype A, closely related to haplotype B (shared by ~1 %; N=3 of the individuals) and haplotype C (represented by a single individual). The fourth haplotype, D, was represented by ~10% (N=23) of the sampled individuals but was remarkably distant from the other three haplotypes. The two divergent lineages differed by 13 transitions and could not be connected in the haplotype network using a 95% confidence interval (Figure 4.2).

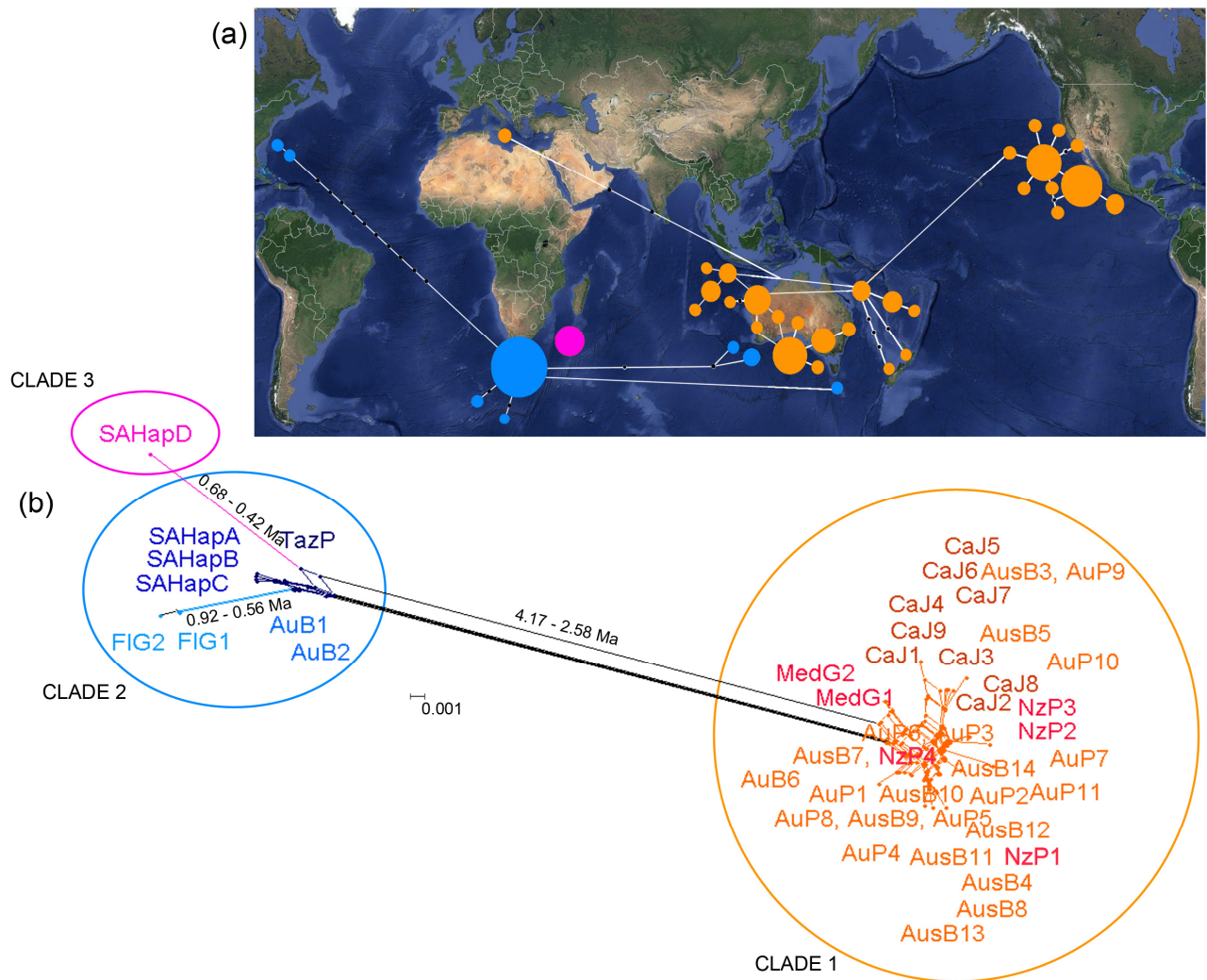


Figure 4.2: (a) Parsimony haplotype network based on the mtDNA analyses of the samples collected around the South African coastline for this study and sequences downloaded from Genebank. The colours represent the affiliation with each of the three global Clades that could not be connected by 95% certainty. (b) NeighbourNet network of *C. carcharias* mitochondrial control region haplotypes, with coalescence times estimated in BEAST v1.8.0 indicated on the branches. From left to right: SAHap – South Africa; Fl – Florida; Taz – Tasmania; Au – Australia; Nz – New Zealand; Med – Mediterranean; Ca – California. The capital letter after the location's name indicates the first letter of the author who published the sequences: P- (Pardini et al. 2001); J - (Jorgensen et al. 2009); B - (Blower et al. 2012); G - (Gubili et al. 2010).

Pairwise ϕ_{st} values (10 000 permutations) among the five sampled South African aggregation sites showed no significant genetic variation ($\phi_{st} = 0.018$, $P=0.71$). The haplotype (h), and to a lesser degree, nucleotide diversity (π) for white sharks in South Africa was comparatively low when compared to populations globally ($h = 0.00200$ and $\pi = 0.00352$; Table 4.3; Figure 4.3).

Table 4.3 Diversity indexes at the mtDNA control region among *C. carcharias* sequences. See Table 4.1 for the reference to all the sequences downloaded from GenBank.

| Location | Number of sequences | Number of Haplotypes | Nucleotide Diversity (π) | Haplotypic diversity (h) | Reference |
|--------------|---------------------|----------------------|--------------------------------|--------------------------|-----------------------|
| GLOBAL | 412 | 36 | 0.02331 ± 0.00158 | 0.0720 ± 0.0230 | this study + GenBank |
| South Africa | 238 | 4 | 0.00276 ± 0.00076 | $0.0205 \pm 0.0330^*$ | this study |
| Australia | 94 | 12 | 0.00672 ± 0.00153 | 0.8680 ± 0.0150 | Blower et al. 2012 |
| California | 59 | 10 | 0.00109 ± 0.00081 | 0.6600 ± 0.0400 | Jorgensen et al. 2009 |

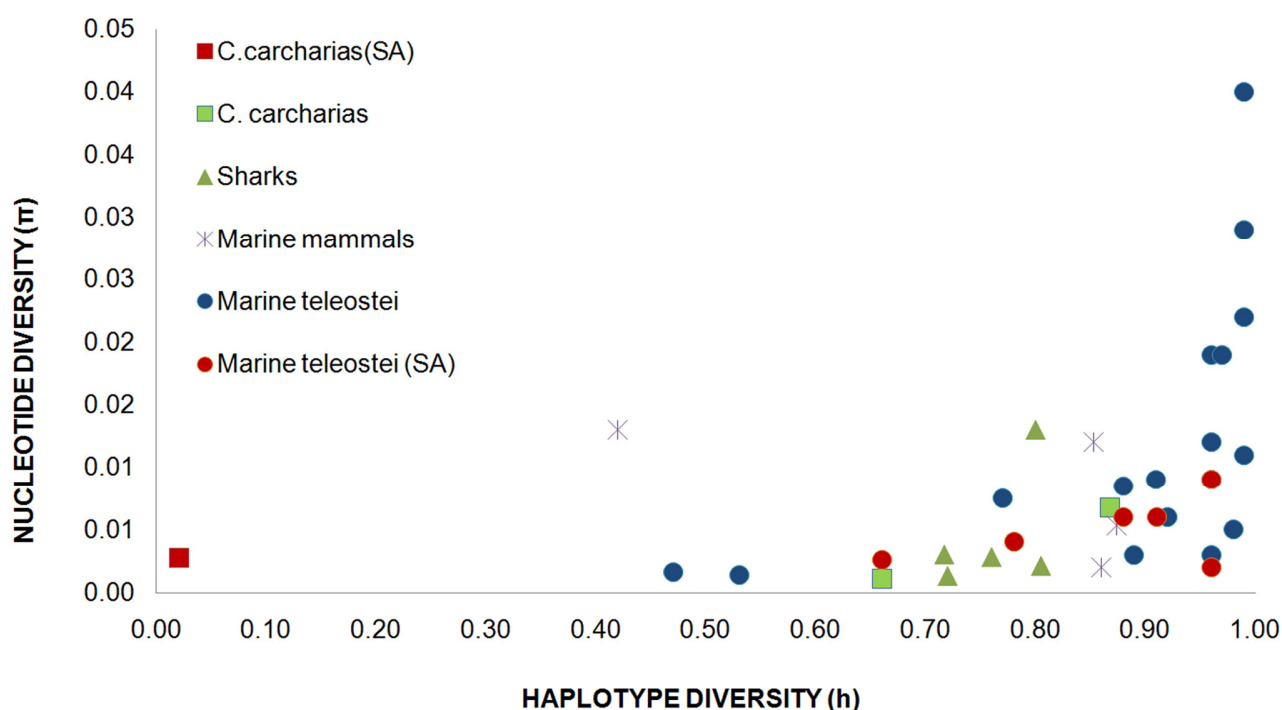


Figure 4.3 Graph showing mtDNA nucleotide and haplotype diversity for a range of marine organism (see Table 4.2 for species and references). South African species are indicated in red, white sharks are indicated by “■”. The two green squares represent respectively (from left to right) Californian and Australian white sharks populations.

At a global scale a strong geographic pattern was observed among distinct haplogroups (Figure 4.2). Overall, three clades were obtained which could not be connected with 95% confidence in the haplotype networks (Figure 4.2a); the distant relationships among these clades

are similarly reflected by the NeighbourNet network (Figure 4.2b). The first haplogroup (clade 1, Figure 4.2b) included individuals from Australia, New Zealand, the Mediterranean and California; the second haplogroup included the most common (88.66%) South African haplotype A, which had evolutionary connections with Florida and in low frequency to Australia (clade 2, Figure 4.2b). The last white shark lineage was represented by haplotype D detected only in South Africa (clade 3, Figure 4.2b). The NeighbourNet network, revealed a high genetic differentiation between clade 1 and the remainder of the white shark lineages and also suggested a closer evolutionary relationship between haplotype D and clade 2 (Figure 4.2b).

Modeltest Version 3.7 suggested the HKY substitution model as the most likely model of nucleotide substitution. Estimates of time since most recent common ancestor varied with the divergence rate used: when the 1.9% divergence per Myr was enforced, the separation between clade 1 and 2 occurred approximately 2.58 ± 0.13 Ma, the South African population diverged from the North West Atlantic population (e.g. Florida) 0.56 ± 0.02 Ma, while the haplotype D found in South Africa diverged from clade 2 approximately 0.42 ± 0.02 Ma. The more conservative nucleotide divergence rate of 0.74% per Myr overall indicated an older divergence: clade 1 and 2 diverged 4.17 ± 0.20 Ma, the South African population diverged from the North West Atlantic population around 0.92 ± 0.04 Ma and haplotype D diverged from clade 2 approximately 0.68 ± 0.03 Ma (Figure 4.2b).

4.3.2 Nuclear markers

Two hundred and ninety three sharks were successfully genotyped at 14 microsatellite loci. Likelihood-based genotype matching by SHAZA Version 1.0 (Macbeth et al. 2011) identified 60 duplicated samples in the dataset, further confirmed by photo identification (see Appendix I). As such, these individuals were removed from all further analyses, leaving 233 genotypes for analysis. There was no evidence of stuttering, large allele drop out or null alleles. Overall, all loci were under Hardy-Weinberg equilibrium and no evidence of linkage was detected. However, two loci (Cca1072 and Ccar9) had homozygote excess and were double checked for consistencies in scoring. The

observed heterozygosity (H_o) per locus ranged from $H_o = 0.446 - 1.000$ and expected heterozygosity (H_e) per locus ranged between $H_e = 0.358 - 0.808$ (Table 4.4, see also Appendix IV *per location*).

Table 4.4 Genetic diversity at the 14 microsatellite loci sourced from this study. N – number of successfully genotyped individuals per locus; Na – number of alleles at each locus; AF – allele frequencies; H_o – observed heterozygosity, H_e – expected heterozygosity and F_{IS} – inbreeding coefficient (see additional information in Appendix IV). Loci with homozygote excess are indicated with a *.

| Locus | N | Na | AF | H_o | H_e | F_{IS} |
|-------------------------|-----|----------|--------------|--------------|--------------|--------------|
| Cca1419 | 233 | 3 | 0.006 | 1.000 | 0.526 | -0.906 |
| Cca83 | 233 | 7 | 0.015 | 0.803 | 0.789 | -0.017 |
| Cca1536 | 233 | 7 | 0.015 | 0.850 | 0.808 | -0.051 |
| Cca1273 | 233 | 2 | 0.004 | 0.446 | 0.446 | 0.000 |
| Ccar1 | 233 | 6 | 0.013 | 0.742 | 0.691 | -0.075 |
| Cca711 | 233 | 9 | 0.019 | 0.562 | 0.556 | -0.036 |
| Cca1072* | 233 | 8 | 0.017 | 0.695 | 0.807 | 0.139 |
| Ccar627.x | 233 | 3 | 0.006 | 0.489 | 0.464 | -0.054 |
| Cca1466 | 226 | 3 | 0.007 | 0.456 | 0.358 | -0.274 |
| Cca1276 | 226 | 16 | 0.035 | 0.916 | 0.867 | -0.057 |
| Cca1226 | 227 | 5 | 0.009 | 0.299 | 0.322 | 0.069 |
| lox10* | 214 | 5 | 0.012 | 0.673 | 0.677 | 0.006 |
| Ccar9 | 213 | 15 | 0.035 | 0.770 | 0.828 | 0.070 |
| Ccar13 | 197 | 9 | 0.023 | 0.701 | 0.607 | -0.060 |
| Average all loci | | 7 | 0.016 | 0.672 | 0.625 | -0.07 |

Results obtained from Powsim indicated that a minimum of 15 samples per population were enough to detect F_{st} as low as 0.02 with 90.2% confidence. When testing our dataset (sample size and microsatellite markers) the current sample size was sufficient to estimate F_{st} ranging from 0.01 to 0.05, with 100% confidence (Chi-square = 1.00; Fisher's exact tests = 1.00).

There was no evidence of significant population differentiation among aggregation sites ($F_{st} = 0.0014$, $P = 0.38$), among age classes (adults: $F_{st} = 0.0017$, $P = 0.29$; sub adults: $F_{st} = 0.0051$, P

= 0.51; juveniles: $F_{st} = 0.0096$, $P = 0.71$) or when populations were subdivided according to gender ($F_{st} = 0.0004$, $P = 0.42$) (Table 4.5). Similarly, when the data was partitioned according to the observed four haplotypes, no significant differentiation was observed ($F_{st} = 0.0020$, $P = 0.12$).

Table 4.5 Percentage of variation and F_{st} values for the nuclear data. N – number of successfully genotyped samples; Ng – number of groups in which the population was divided (5 aggregation sites and 4 haplotypes); %Var – percentage of variation between groups (aggregation sites and mtDNA haplotypes); F_{st} – population pairwise values and statistical significance of F_{st} (P value).

| AGGREGATION SITES (South Africa Coastline) | N | Ng | %Var | F_{st} | P |
|---|----------|-----------|-------------|----------------------------|----------|
| All sharks | 233 | 5 | 0.14 | 0.0014 | 0.38 |
| Juveniles | 64 | 5 | 0.96 | 0.0096 | 0.71 |
| Sub-adults | 83 | 5 | 0.52 | 0.0051 | 0.51 |
| Adults | 84 | 5 | 0.17 | 0.0017 | 0.29 |
| Males | 85 | 5 | 0.37 | 0.0030 | 0.34 |
| Females | 112 | 5 | 0.12 | 0.0010 | 0.26 |
| Adult Males | 31 | 5 | 3.58 | 0.0350 | 0.89 |
| Adult Females | 29 | 5 | 3.58 | 0.0350 | 0.89 |
| mtDNA HAPLOTYPES | | | | | |
| All sharks | 233 | 4 | 0.20 | 0.0020 | 0.12 |
| Males | 85 | 4 | 0.48 | 0.0040 | 0.48 |
| Females | 112 | 4 | 0.80 | 0.0070 | 0.49 |

The visualization of the multilocus genotype performed with the program Structure (Pritchard et al. 2000) confirmed the lack of structure between aggregation sites and the admixture between individuals from different mtDNA lineages (Figure 4.4).

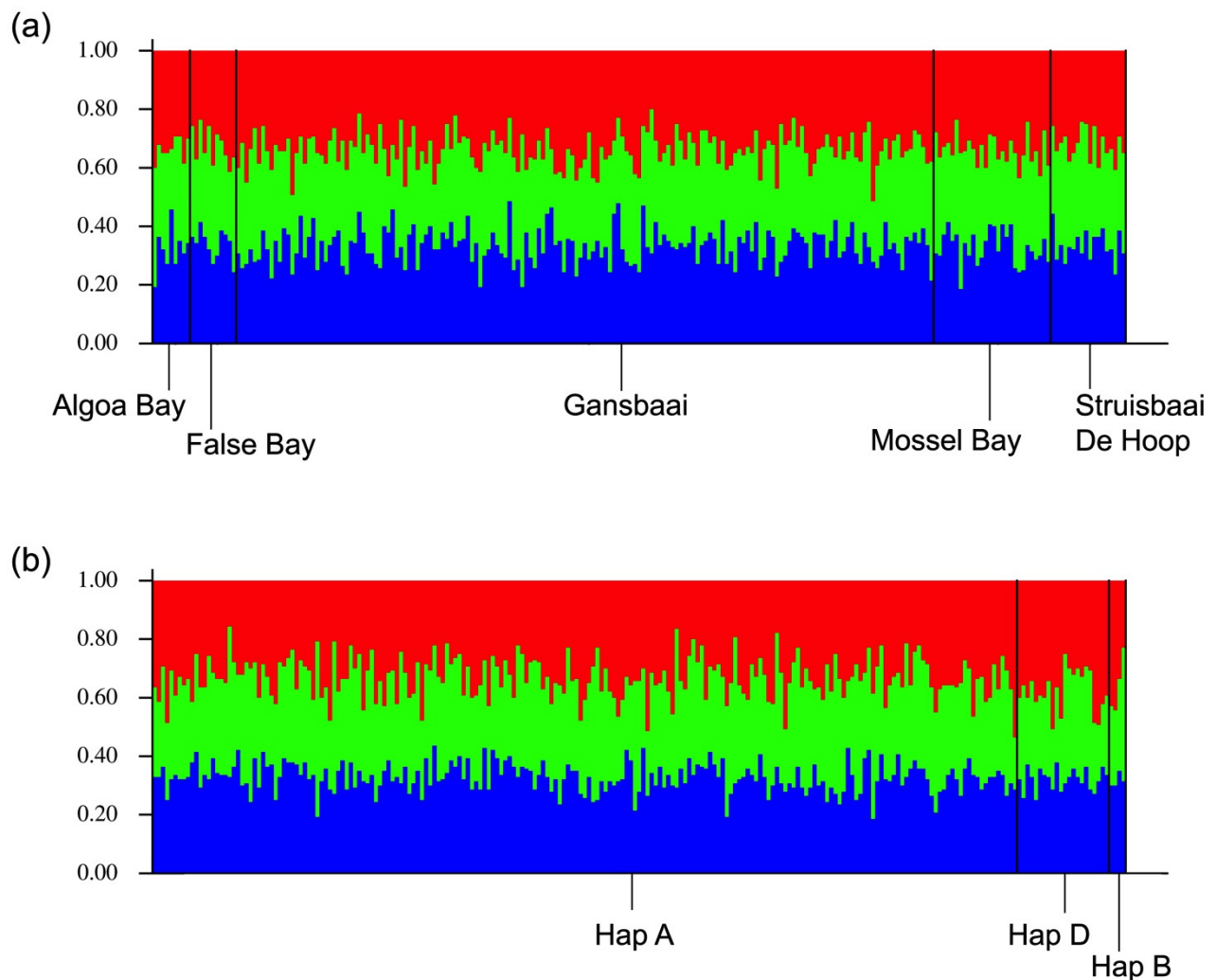


Figure 4.4 Structure plots of individual white sharks by making use of multilocus genotypes (see text for details). (a) White sharks genotypes (N = 233) organized by sampling location. (b) White sharks genotypes (N = 232, as haplotype C was represented by only one individual) organized by the three main mtDNA haplotypes.

4.4 Discussion

This study represents the first attempt of testing for patterns of gene flow using mtDNA and microsatellites among white sharks aggregating along the South African coastline as well as using mtDNA to test for global phylogeography. This region is characterized by different biogeographical barriers known to have contributed to the separation and isolation of numerous marine species (Teske et al. 2006, 2011; Matthee et al. 2007; von der Heyden et al. 2008, 2010; von der Heyden

2009; Henriques et al. 2012, 2014). However, our data fails to support the hypothesis of substructure within South African white sharks (or across biogeographical transitions) and the lack of genetic partitioning was evident regardless of the genetic marker used (mtDNA $\phi_{st} = 0.018$, $P=0.71$; nDNA $F_{st} = 0.0014$, $P = 0.38$). These results suggest that white sharks along the South African coastline belong to a single, interbreeding population, with no evidence for site fidelity that could contribute significantly towards genetic structure (Figure 4.4a). This finding is further supported by previous acoustic tagging studies (Bonfil 2005), as well as evidence from photo identification (see Appendix I) and population estimates for Gansbaai versus the entire coastline (chapter 3), all suggesting that white shark individuals disperse freely between aggregations sites (see <http://www.ocearch.org/#SharkTracker>, and Appendix III). It is, however, interesting to note that for the nuclear DNA data, male sharks showed a lower pairwise differentiation ($F_{st} = 0.0040$ $P = 0.48$) than that observed for females ($F_{st} = 0.0070$ $P = 0.49$). This observation, although not supported with significance, suggests that in our sample females are probably more philopatric than males, as already suggested several time in the past (Pardini et al. 2001; Feldheim et al. 2004; Jorgensen et al. 2009; Dudgeon et al. 2012).

The comparison of genetic diversity with that of other regional studies (Jorgensen et al. 2009; Blower et al. 2012), revealed striking differences for South African white sharks. The detection and frequency distribution of merely four haplotypes for 238 individuals sampled represents the lowest site specific haplotypic diversity ever detected globally for this species ($h = 0.02$; Figure 4.3).

Moreover, the frequency distribution of these haplotypes also resulted in a very low nucleotide diversity ($\pi = 0.0027$; Figure 4.3). However, one of the four haplotypes was exceedingly divergent from the remaining haplotypes and could not be connected in a single haplotype network with other South African haplotypes (also see Gubili et al. 2010, Haplotype GW45). This implies the presence of two mtDNA lineages for white sharks in South Africa.

Our results showing low genetic diversity differed from previously published data on South African white sharks, where seven haplotypes were described for a much smaller sample size (Pardini et

al. 2001). A plausible explanation is that the present study utilized more accurate automated sequencing technologies than the manual sequencing techniques employed by Pardini et al. (2001). The only difference between the current trimmed data set and the published data for South African sharks is the presence of a transversion (T to G) at position 832 in the Pardini (2001) data. This single change resulted in the detection of an additional 3 haplotypes in their data set. If this change is not included due to the suspected error [given that the chance of errors in manual sequencing is greater at the end / beginning of the read; also see (Khurshid & Beck 1993)] our data sets are totally congruent. Irrespectively, some aspects of the microsatellite analyses appear to corroborate the observed low levels of genetic variation for mtDNA. Particularly, when compared to the genetic diversity levels observed in the Australian population (Blower et al. 2012), it is clear that the South African white sharks exhibit significantly lower diversity values. Although comparisons between microsatellite studies are not usually straightforward, both the Australian and the present study used the same five microsatellite loci (Ccar1, Ccar627.x, lox10, Ccar9, Ccar13). When only these markers are considered, the average number of alleles and observed heterozygosity were also lower for the South African population ($N_a = 7.6$, $H_o = 0.675$), compared to sharks sampled in Australia ($N_a = 8.0$, $H_o = 0.729$). Furthermore, the observed genetic diversity levels in white shark's mtDNA were consistently lower than those reported for other marine teleosts, not only in the South African region, but also across the world (von der Heyden et al. 2010). Possible explanations for this low genetic diversity could be the occurrence of a recent founder event (Gubili et al 2010), a population bottleneck or a selective sweep (Hudson et al. 1987; Galtier et al. 2000; Kim 2004).

To gain a better perspective on the reasons for the genetic picture observed in the South African population (low genetic diversity and two very divergent haplogroups) the data needs to be integrated into a global picture (Grant & Bowen 1998). Explaining the two divergent mtDNA lineages along a single coastline is challenging. Since marked mtDNA differences between haplogroups have previously been detected amongst continental populations (Pardini et al. 2001; Blower et al. 2012), the presence of the two divergent South African haplogroups could point to two

independent founder events from a population or populations not yet sampled (Grant & Bowen 1998). Alternatively, it can be attributed to a single founder event followed by historical vicariance (two populations surviving in two refugia followed by subsequent post-bottleneck expansion; also see Hoelzel et al. 2002). Irrespective of the reasons for finding the two haplogroups in the same geographic region, the microsatellite nuclear DNA do not indicate genetic isolation between individuals carrying the rarer haplotype D, and the individuals carrying the remainder of the mtDNA haplotypes ($\phi_{st} = 0.0020$, $P = 0.12$), raising interesting questions regarding the evolutionary and biogeographic history of these white sharks.

At a broader, continental scale, observed mtDNA structure appeared to be congruent with previous suggestions that white sharks show strong female site philopatry (Pardini et al. 2001; Jorgensen et al. 2009; Gubili et al. 2010; Blower et al. 2012). The three distinct mtDNA clades/lineages are confined to 1) the Mediterranean Sea, the Pacific and Indian Oceans (clade 1, Figure 4.2 b); 2) Atlantic and Indian Oceans (clade 2, Figure 4.2b); 3) a single haplotype (D) confined to the waters of South Africa. This phylogeographic pattern could result from different evolutionary mechanisms, such as isolation by distance (Jorgensen et al. 2009), infrequent long distance dispersal (Pardini et al. 2001; Bonfil 2005; Gubili et al. 2010), founder events (Gubili et al. 2010) and biogeographical vicariant barriers (Gubili et al. 2010). The long distance dispersal hypothesis is supported by the presence of closely related haplotypes shared between California and Australia, while a founder event may have been responsible for the closer evolutionary relationship between the Mediterranean and Australian populations (Gubili et al. 2010), and also between the South African and Florida populations as similar patterns of population dispersal/isolation have been documented for pelagic teleosts such as Atlantic bluefin tuna (*Thunnus thynnus*) and swordfish (*Xiphias gladius*) (Alvarado Bremer et al. 2005).

The White shark (*Carcharodon carcharias*) is the third largest migratory Chondrichthyan, after the whale shark *Rhincodon typus* and the basking shark *Cetorhinus maximus* (Compagno et al. 2001). When compared to the aforementioned large globally distributed and highly migratory species, *C. carcharias* shows an atypical phylogeographic structure based on its mtDNA.

An extensive study on whale shark revealed the absence of geographical clustering of lineages and the most common haplotype was distributed globally (Castro et al. 2007; Vignaud et al. 2014). The absence of population structure across the Indian and Pacific basins indicates that oceanic expanses and land barriers in Southeast Asia are not impediments to whale shark historical dispersal (Castro et al. 2007). Another study on basking sharks found just six haplotypes defined by five variable sites and no significant differentiation between ocean basins (Hoelzel et al. 2006). Whale sharks, basking sharks and white sharks have similar reproduction strategies as they give birth to live young, while their diet and food resources differ, suggesting that life strategies do not seem to influence the historical structure and connectivity. Whale sharks and basking sharks rely on a planktonic diet while white shark is a top apex predator feeding on large pelagic fishes and marine mammals (McCosker 1987; Compagno et al. 2001).

It has been suggested that the distribution of prey species might shape the predator's genetic structure across connected regions. Specifically in bottlenose dolphins it has been observed how oceanographic feature may serve as a barrier to the movement of some prey species, and perhaps in this way define local populations of their predators (Natoli et al. 2005). Would that be true for white sharks, the founder events described in our study may be linked to cascade events on prey availability in the region: noticeably it has been shown that the Atlantic Ocean Atlantic bluefin tuna (*Thunnus thynnus*) and swordfish (*Xiphias gladius*) showed similar pattern of dispersal, particularly the Western Atlantic bioregion appears to be as a genetic niche periodically re-colonizing the East Atlantic coast (Alvarado Bremer et al. 2005). Similarly, South African and Australian fur seals (a food source for white sharks) are also closely related and it is likely that the Australian fur seal population arose from a once-off colonisation of seals from South Africa (Matthee et al. 2006), which could justify the presence of the link between South African mtDNA haplotype and southern Australia. In a world subjected to strong climate changes the increase or decrease of the water temperature and the depletion of food resources can be enough to compromise the connection between populations and to drive them to extinction (Grant & Bowen 1998, 2006). Due to their greater migration potential predators have been shown to be more susceptible than other species

to the loss of corridors (Gilbert et al. 1998; Haag et al. 2010) and the dispersal potential of white sharks (Bonfil 2005) and their prey potentially makes the connections between those ocean basins of the greatest importance for their conservation.

South Africa is a transition zone between ocean basins and it will be interesting to know in advance whether the extinction of the local South African white shark population will one day be replenished by individuals from the North West Atlantic, or whether the presence of the unique haplotypes found in South Africa come from a neighbour country not yet sampled.

Estimates of time since most recent common ancestor, using a faster and a more conserved molecular clock calibration, suggest that the two main continental clades (1 and 2) diverged between 4.17 and 2.58 Ma, spanning the Pliocene-Pleistocene transition period. The early Pliocene (6 - 3.5 Ma) was characterized by large scale changes in oceanographic patterns including a rapid biogenic bloom, both in the Indian and in the Pacific Oceans (Dickens & Owen 1999), and the closure of the Sunda-Sahul shelves (5 Ma) and the Isthmus of Panama (3.5 Ma) (Haq et al. 1987). The Indo-Pacific Ocean is considered to be a biodiversity hotspot (Dickens & Owen 1999) and based on haplotype diversity, the centre of white shark genetic diversity is also found in the Indo- and North-West Pacific Oceans (Clade 1). There is comparatively much less diversity observed in the Atlantic and South Indian Oceans (Clade 2). Therefore, due to genetic diversity levels and estimates of time since most recent common ancestor, it is reasonable to argue that this region may have served as a refugia, and later as a source population, where white sharks persisted during the large scale oceanographic changes associated with the early to middle Pliocene (Broecker & Denton 1989; Dickens & Owen 1999; Rommerskirchen et al. 2011; Cronin 2013). Conversely, the second lineage appears to have persisted somewhere in the Atlantic Ocean, with uncertainty regarding the geographic origin. By utilising both the slow and faster molecular clock calibration the ancestral Indo-Pacific population appears to have diverged between 4.17 and 2.58 Ma. If this holds it seems that the closure of the Isthmus of Panama (~ 3.5 Ma, see Coates et al., 1992) appears to have contributed to the divergence of the Indo- and North-West Pacific (Clade 1) and Eastern Atlantic populations (Clade 2, Haplotype D). Since the South African

coastline was severely exposed to periodic cooling and sea level changes during glaciations events [with concurrent collapses in primary productivity; (Grant & Bowen 2006; Henriques et al. 2014)], clade 2 more than likely originated in the northern Atlantic and then dispersed southwards to give rise to the South African lineages. Interestingly, the presence of white sharks in the Atlantic during the Pliocene, and their later expansion to the more temperate southern waters is supported by fossil evidence (Cione et al. 2012). In this case, the South African population represents a founder event from North West Atlantic at approximately 0.56 Ma. The recent colonization of the South African coastline from the northern hemisphere is in line with previous studies in marine teleosts (Grant & Bowen 2006), which would also explain the low haplotypic diversity observed in the region. In total this is suggesting the presence of a metapopulation with serial extinction-re colonization scenarios. Low genetic diversity in South Africa could be maintained through repeated population crashes in the South Hemisphere (Grant & Bowen 2006; von der Heyden et al. 2010) as a result of ten major episodes of global cooling (Broecker & Denton 1989; Lehman & Keigwin 1992; Grant & Bowen 2006; Cronin 2013; Henriques et al. 2014).

The complex evolutionary history of *C. carcharodon* requires careful consideration in the context of the conservation of this species. The actual extent of white shark population decline has not been established, but given the high levels of anthropogenic mortality (Stevens et al. 2000; Pardini et al. 2001; Dulvy et al. 2008; Worm et al. 2013), far-reaching measures are needed to ensure the survival of this species. Genetic diversity of South African white sharks compared with 32 other marine species (Table 5) showed similar levels to the endangered Black Sea bottlenose dolphin, *Tursiops truncatus* (Natoli et al. 2005; Birkun 2012) (Figure 4.3). The present findings suggest that South African white sharks represent the remnants of a single or two founder events at least half a million years ago, and the distribution of haplotypes at a global scale suggest that they represent the intermediate population connecting the East Atlantic population with the Indo-Pacific population (as confirmed by the presence of few haplotypes in Australia closely linked to the South African haplotype A; Figure 4.2). Given the low level of genetic diversity present in South African white sharks, further population declines could invariably reduce the evolutionary potential

and adaptive capacity of this already vulnerable population (Smith et al. 1991; Ryman et al. 1995; Grant & Bowen 1998; Hoelzel et al. 2006). Since white sharks are apex predators, they experience similar pressures to that reported for large marine mammals (Musick 1999; Daly-Engel et al. 2012) such as killer whale, *Orcinus orca* (Hoelzel et al. 2002) or some particularly vulnerable species of dolphin such as Hector's dolphin (*Cephalorhynchus hectori*) (Pichler & Baker 2000), which suffered a similar recent decline in the trend of mtDNA diversity. As such, the findings here presented suggest that, although panmictic, the white shark population off South Africa has a complex evolutionary history, deeply linked with past environmental changes. In consequence, this white shark population appears to be more vulnerable than previously recognised, demanding increased efforts in a long term management plan and further research for improving conservation success.

CHAPTER 5

FIRST INSIGHT IN THE SOCIAL STRUCTURE OF A “SOLITARY” PREDATOR:

**APPLICATION OF SOCIAL NETWORK AND GENETIC ANALYSES PROVIDE EVIDENCE OF NON-RANDOM
ASSOCIATIONS IN WHITE SHARK (*C. CARCHARIAS*)**

5.1 Introduction

A large number of animal species spend part or all of their life in groups (Pulliam & Caraco 1984). Group living strategically improves both individual fitness and the spatiotemporal dynamics of a population (Thornhill 1983; Taylor 1986; Parrish & Hamner 1997). In predatory species the individuals can form mutual associations with conspecifics for increasing the feeding intake (Hamilton 1964; Jacoby et al. 2012), defending a hunting ground, for improving the individual fitness by altruistic behaviour among family members (ref. Hamilton 1971) or for increasing information exchange (Slater & Halliday 1994).

An important distinction has to be drawn between 'groups' or 'associations', determined by limited food resources or specific habitat requirements (Jacoby et al. 2012), and 'social groups', a behavioural adaptation exhibited at different levels across species, determined by the degree of association between individuals during different activities (Slater & Halliday 1994). In several species displaying non-random patterns of associations, the social structure is determined by characteristics such as body size, sex, relatedness, colour and parasite load (Krause & Ruxton 2002; Ward & Hart 2003). Kin selection theory (Hamilton 1964; Eshel & Motro 1981) also suggests that individuals should preferentially associate and cooperate with kin whenever the inclusive benefits outweigh the costs. However, studies on marine mammals suggest that kinship relations are not always a prerequisite for social cluster membership (Mann 2000; Möller et al. 2006). The cost-benefit ratio to live in a social group is extremely difficult to determine for most species (Slater & Halliday 1994) and the situation become even more challenging when dealing with elusive marine predators such as sharks (Guttridge et al. 2011; Jacoby et al. 2012).

Sharks and rays are frequently observed in groupings (Jacoby et al. 2012). They are characterized by a high brain mass to body mass ratio (Northcutt 1977) and have elevated numbers of neurons and synapses (Yopak 2014). The latter has been put forward as an indication of their potential to develop and maintain complex social behaviours, such as dominance hierarchies and stable social bonds (Dunbar & Shultz 2007; Guttridge et al. 2009, 2011; Jacoby et

al. 2012). However, despite the importance of including information on their social structure for conservation management purposes (Tarlow & Blumstein 2007; Wey et al. 2008), very few studies investigated social groupings in elasmobranchs (see a review in Jacoby et al. 2012) and even fewer studies attempted to quantify structures recovered (Guttridge et al. 2009, 2011).

An emerging field of research for studying social grouping and animal association is network analyses (Krause et al. 2007, 2009; Croft et al. 2008; Wey et al. 2008; Franks et al. 2009). The method has been extensively used for analysing human social behaviour and only in recent years has become popular among behavioural ecologists (Croft et al. 2008). The main advantage of network analyses is that it provides the means to visualize the structure of the interactions (edges) between different individuals (nodes) and to analyse the global property of the system based on a variety of dyadic interactions (Croft et al. 2008). Moreover, such interactions can be weighted so that individuals connected by a stronger dyadic mutual bond can be identified. Social network analysis has been used in guppies, *Poecilia reticulata* (Croft et al. 2004), but also in lemon sharks *Negaprion brevirostris* (Guttridge et al. 2009, 2011) to generate and test assumptions about their social structure. Specifically Croft et al. (2004) suggested that free-ranging female guppies show social preferences for stable partners and co-operation during predator inspection. Juvenile lemon sharks were observed to show active partner preference explained by body length, possibly correlated to relatedness and the presence of leading individuals in the social system (Guttridge et al. 2011). However the immediate causes of social behaviour and the functions underlying aggregation in sharks remain relatively unexplored, especially in the wild (Jacoby et al. 2012).

Despite the reputation of being a solitary predator, previous studies suggested that some form of social organization exists in white sharks and the possibility of applying social network analyses on free ranging white sharks in the wild is just as fascinating as it is challenging. The white shark, *Carcharodon carcharias*, is a large migratory marine predator protected through the Convention on International Trade in Endangered Species (CITES) and the Convention for Migratory Species (CMS). White sharks are also red listed as “vulnerable” on the International Union for the Conservation of Nature (IUCN, Category VU A1cd+2cd) due to rapid stock declines

(Dulvy et al. 2008). Despite being one of the most studied shark species in the world (Domeier 2012), very few studies provided insights in their fine scale social structure (see Domeier & Nasby-Lucas 2008; Jorgensen et al. 2009; Kock et al. 2013). This leaves a significant information gap when assessing the conservation status of the species. Nonetheless, evidence exists of seasonal sexual segregations (Jorgensen et al. 2009; Kock et al. 2013), as well as hierarchical social interactions among individuals during scavenging situations (Sperone et al. 2010, 2012; Fallows et al. 2013). Behavioural studies revealed the existence of behavioural interactions between similar sized conspecifics (Martin et al. 2005; Sperone et al. 2010) and empirical observations described white sharks passing by the same area in stable clans of two to six individuals (Martin & Martin 2006). However, the relatedness among the “clan” members remains unknown.

Photographic identification techniques have largely been adopted to reveal the complex community structuring in a number of free ranging marine species (Gowans & Whitehead 2001; Meekan et al. 2006; Domeier & Nasby-Lucas 2006, 2006; Möller et al. 2006; Hoelzel et al. 2007), and it has been suggested for this technique to be used in combination with social network analyses as the key to enable a more detailed understanding of shark aggregation events (Jacoby et al. 2012). To address the lack of detailed knowledge on the social behaviour of white sharks, we employed the non-invasive photo identification techniques as outlined in chapter 2 (Andreotti et al. 2014). This technique allowed for the generation of a large temporal data set originating from one of the aggregation sites, Gansbaai, South Africa. This data set was supplemented with genetic data that were also sampled using a non-invasive method (chapter 3). Using a molecular approach is the only method for calculating relatedness between observed clan members previously reported to exist (Croft et al. 2006, 2008; Krause et al. 2007; Guttridge et al. 2009, 2011; Jacoby et al. 2012).

This is the first study to quantify the association patterns of free ranging white sharks visiting the area of Gansbaai (South Africa). Specifically by using network and genetic analyses we aim to investigate whether white sharks aggregate randomly during a scavenging situation or if stable pairwise associations can be identified over time; secondly we aim to explore the nature of

the associations to determine if it is based on phenotypic traits such as sex, size or relatedness. Similarly to Croft et al. (2006) we wanted to explore ‘How stable are individuals associations through time?’ and ‘Who interacts with whom?’.

5.2 Materials and methods

5.2.1 Study site

The collection of photos and biopsy samples were conducted in the proximity of the Dyer Island Nature Reserve (Kleinbaai, Gansbaai, South Africa - 34° 40'614" S, 019° 23'934" E). Data were collected on a daily basis (weather permitting) from 2009 to 2014. The sharks were attracted to the vessels provided by Shark Diving Unlimited by creating an olfactory cue with natural fish chum that simulates an opportunistic scavenging environment (Laroche et al. 2007; Fallows et al. 2013).

Sharks were firstly photographed and then biopsied with a 2.53 m long pole equipped with a sterilized biopsy sampler. After collection, samples were stored in 80% ethanol. Between each sample collection the equipment was sterilized with ethanol over a flame. The study was conducted following the guidelines set out by the Department of Environmental Affairs of South Africa (Permit numbers RES2010/71, RES 2011/55, RES 2012/38, RES 2013/41 and RES 2014/39).

5.2.2 Analyses of the associations

The total length (TL) of each individual was established by estimating the straight line distance between the rostrum and the upper caudal fin (Mollet et al. 1996) and measured against the known length of the dive-cage (3.7 m). To standardize the technique, the same three expert observers were used throughout the study period. White sharks have a slow growth rate and long life span (Calliet et al. 1985), therefore during the three years in which the photographic identifications were collected, it is assumed that few individuals could have change their size-class. Size classes were categorized as juveniles $\leq 2.7\text{m}$; sub adults 2.8m – 3.6m; and adults $\geq 3.7\text{m}$ (Calliet et al. 1985). Additionally, sex was determined by the presence/absence of the claspers, when visible. High

quality photographs of the dorsal fins were taken following the guidelines described in Andreotti et al. (2014). Although several thousands of photographs were taken, only 4398 were of sufficient quality for use. The difficulties associated with the matching and cataloguing of a large amount of photo identifications (Marshall & Pierce 2012) have been overcome by adopting the categorization system validated in Andreotti et al. (2014) and the final dataset excluded false negatives (chapter 3). The history of re-captures matrix was generated with the photographs collected from 2009 to 2011 by an *ad hoc* algorithm created using Python (McKinney 2012) to extrapolate the data directly from the photos catalogued over time for each shark (see Andreotti et al. 2014).

5.2.3 Randomization methods

To assess ‘How stable are individual associations through time?’ repeated pairwise associations of individual sharks were tested for departure from randomness by permuting the occurrence of the individuals within the sampling periods. Specifically we tested if the occurrences of two sharks captured together in more than one sampling occasion could be attributed to a random event. For the purposes of this study we clustered the sharks’ captures from a daily interval to a weekly interval. The choice of the weekly time interval was dictated by the average number of days the sharks stay in the Gansbaai area (Andreotti et al. 2014), therefore sharks observed within the same week form a single distinct capture. Knowing that white sharks are non-residential in the area of study, re-sampling the same pair of sharks in different weeks was assumed to be an indication of long-term ties between the two individuals. To prevent a bias of variation in shark abundance over different sampling weeks, the elements of the matrix were permuted by keeping the sum of each row and column numbers constant. Tests were performed with an algorithm created using Python (McKinney 2012), and a two tailed test ($\alpha = 0.05$). Gaussian curve of the results was obtained and plotted after 2000 permutations. A χ^2 test was used to quantify the significance of the observed results from the permutation runs.

Social network analyses

To assess ‘Who interacts with whom?’ the mark-recapture data (2009-2011) was used to generate a history of recaptures. The history of recapture matrix (e.g. row = shark sightings/ column = time of capture) was transformed in a 2-values association matrix between individuals (e.g. shark/shark), with the program UCINET (Borgatti et al. 2002). The association matrix in itself is a representation of a social network, as individuals are assumed to be in association if they are seen in the same group multiple times (Krause et al. 2009).

With UCINET (Borgatti et al. 2002) the association network was built with the NetDraw function and this was done to visualize the links between pairs of sharks that were captured at least twice in the dataset. From the original network (connecting all the sharks seen together), a sub-network was generated visualizing only the sharks that paired in three or more separate sampling occasions. The latter was performed to provide a better indicator of long term association between paired individuals (also see Croft et al. 2006; Krause et al. 2007). Each shark (node) was assigned two variables (size and sex), which is represented in the network by the size and colour (blue = males; pink = females) of the nodes.

The following assumptions of divergence from randomness were made by visually observing the network: (1) sharks are partially sexually segregated and (2) mixed size groups are formed more often than same-size groups. These hypotheses were tested against the null hypothesis of “association by chance” with a χ^2 test between observed frequencies of associations and expected frequencies of associations. To count the number of associations between individuals, the matrix was manually analyzed and filtered by sex and size class with Microsoft Excel, and the expected value was calculated based on the real number of sharks in each category (see Table 5.1).

5.2.3 Genetic methods

Laboratory Procedures

A small portion of tissue was cut from each sample, air-dried and digested overnight in a DNA extraction buffer (containing Proteinase K 10mg/ml) as supplied by the manufacturer (Qiagen P/L

DNEasy extraction kit). Fourteen microsatellite markers were used to fingerprint each individual: Cca1419, Cca83, Cca1536, Cca1273, Ccar1, Cca711, Cca1072, Ccar6.27x, Cca1466, Cca1276, Cca1226, Iox10, Ccar9, Ccar13 (Pardini et al. 2000; Gubili et al. 2010; O'Leary et al. 2013). These markers were amplified in four multiplex reactions (see details in chapter 3). To ensure consistency in the scoring of the microsatellites, DNA from one control individual was added each time alleles were scored in consecutive runs.

Genotype analyses

Genotype scoring was performed in Geneious Version 5.6.5 (Copyright © 2005-2012 Biomatters Ltd.). Assessment of amplification errors, such as large allele drop out, stuttering, and null alleles was conducted in Microchecker Version 2.2.3 (Van Oosterhout et al. 2004). Due to the large sample size and the migratory nature of the species, it is possible to re-sample the same individuals in different sampling events. As such, the software SHAZA Version 1.0 (Macbeth et al. 2011) was used to assess duplicated samples, which were eliminated from all analyses.

The analyses of relatedness was performed with the program MI-relate (Kalinowski et al. 2006) on all the sharks genotyped, and subsequently on only the individuals captured at least twice. MI-relate (Kalinowski et al. 2006) is designed for microsatellite data, can accommodate null alleles and it uses simulations to determine which relationships are consistent with genotype data. The program MI-relate have been extensively used for similar studies (Gehring et al. 2003; Costello et al. 2008; Marucco et al. 2009; Rutledge et al. 2010) and generates an association matrix (e.g. shark/shark) with values that range from 0: not-related to 1: same individual. MI-relate evaluates the relatedness (R) between each shark pair, pending on their genotypes, and classifies their relationship as follows: unrelated ($R = 0$), half-sibling ($R \sim 0.2$), full-sibling ($R \sim 0.3$), and parent-offspring ($R = 0.5$).

5.2.4 Merged association and relatedness

By using only the genotyped sharks captured twice, the relatedness matrix and the association matrix of the same individuals could be compared. A series of preliminary analyses were

conducted by simply counting the occurrences of pairwise sharks observed together and attempting to match this with genetic relatedness. Firstly between individuals seen together at least once and related, and subsequently by filtering pairs of sharks seen together more than once and closely related ($R > 0.1$). The Mantel Z-test and the Dietz test (Dietz 1983) were performed with the program SOCRPROG (Whitehead 2009), to determine if the dyadic values of one association measure are significantly correlated with those of another (Whitehead 2009). Finally, the Mantel Z-test and the Dietz tests were performed by sorting the two association matrixes per sex and size classes in order to determine if, as an example, female sharks associating together are significantly more related than associating male sharks.

5.3 Results

5.3.1 Analyses of the associations

From 4398 photographs, collected during 298 days at sea (distributed over 93 weeks), 426 individual white sharks were identified. The sex ratio (males:females) was 1:1.09, with a normal size class distribution (117 juveniles, 212 sub adults and 86 adults). From these, 230 individuals were captured in more than one sampling occasion (e.g. week) from 2 to 18 weeks apart (see chapter 3, Figure 3.1). The observed number of associations between sharks were 9624, of which 1457 occurred over more than one sampling occasion (from 2 to 6 occurrences).

The repeated pairwise association between individual sharks was significantly different from a random mean (real associations = 728, permutation test mean = 597, $P = 0.00$) indicating a non-random association between the sharks (Figure 5.1).

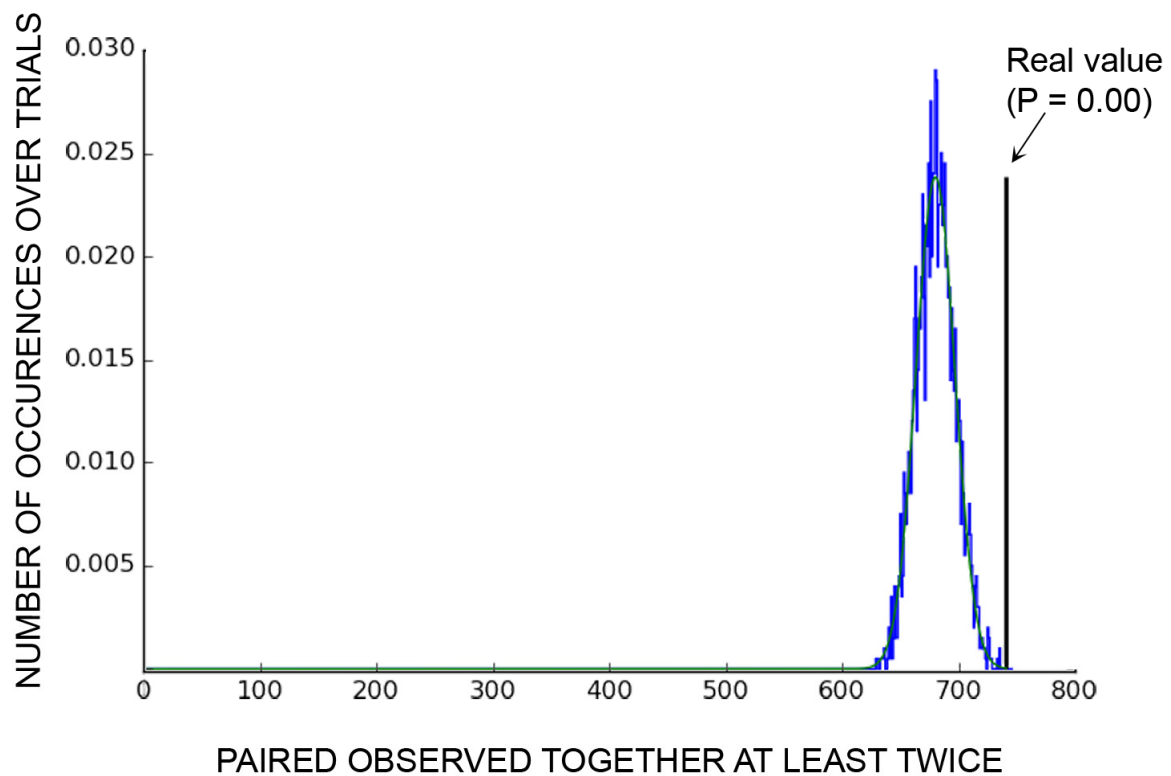


Figure 5.1 Permutation test results generated by 2000 simulations of the history of the re-capture matrix. The observed pairwise association are indicated by the black vertical line on the right.

A sub-network generated with the NetDraw option of SOCPROG (Whitehead 2009) used only the sharks sighted together in more than 3 occasions and this reduced the number of nodes (e.g. sharks) from 230 to 67 (Figure 5.2).

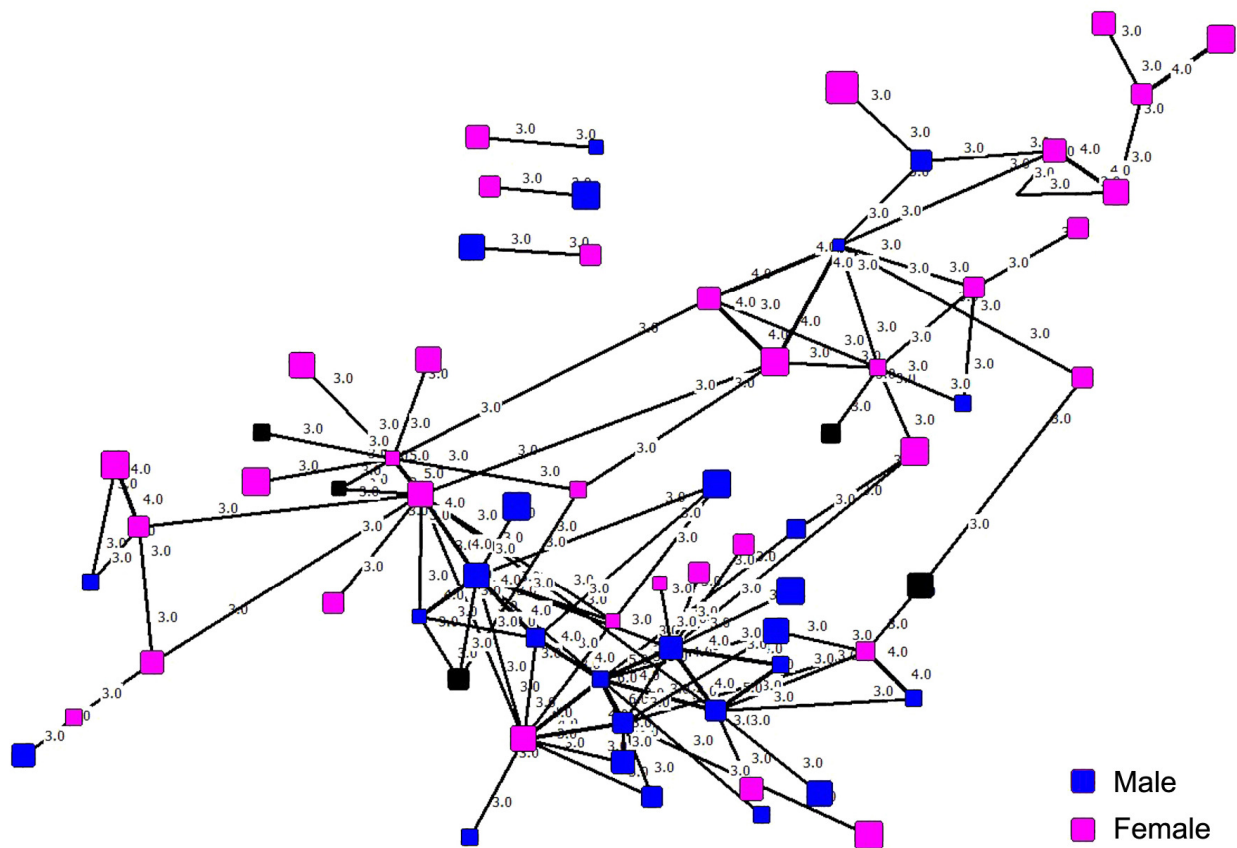


Figure 5.2 Sub-network of the sharks associated in 3 or more sampling occasions. Each node represents a shark: females are pink, males are blue and unknown sex are black. The size of the node represents the size of the shark.

The network indicates partial sexual segregation and also indicate that mixed size groups are formed more often than same-size groups. The separate analyses of difference genders and size classes, although not significant ($P > 0.05$, Figure 5.3, Figure 5.4), tend to confirm the pattern observed in the network representation (Table 5.1). Specifically there is also evidence of a higher frequency of associations between juveniles (J) and sub adults (SA) and a lower occurrence of pairing adults (A) sharks, than what is expected by chance alone.

Table 5.1 Analyses of the associations between sharks (F: females, M: males, J: juveniles, SA: sub adults, A: adults). N: number of shark; A: number of associations; A>1: number of occurrences in which paired sharks associated in more than one sampling occasion; Fo: Observed frequency of association; Fe: expected frequency of association; χ^2 : chi square values; P: P values of the χ^2 test.

| | N | A | A > 1 | Fo | Fe | χ^2 | P |
|------------------|------------|-------------|-------------|-------------|-------------|----------|------|
| BY GENDER | | | | | | | |
| Tot | 194 | 7402 | 1249 | - | - | - | - |
| F-F | 104 | 2353 | 392 | 0.31 | 0.27 | 0.01 | 0.93 |
| M-M | 90 | 1585 | 292 | 0.23 | 0.23 | 0.00 | 0.99 |
| F-M | 194 | 3464 | 565 | 0.45 | 0.50 | 0.00 | 0.95 |
| BY SIZE | | | | | | | |
| Tot | 230 | 9624 | 1457 | - | - | - | - |
| J-A | 106 | 2010 | 122 | 0.08 | 0.15 | 0.03 | 0.99 |
| J-J | 65 | 878 | 134 | 0.09 | 0.09 | 0.00 | 1.00 |
| J-SA | 189 | 6844 | 488 | 0.33 | 0.27 | 0.02 | 0.99 |
| SA-SA | 124 | 2813 | 478 | 0.33 | 0.18 | 0.12 | 0.94 |
| SA-A | 165 | 4686 | 224 | 0.15 | 0.24 | 0.03 | 0.98 |
| A-A | 41 | 226 | 8 | 0.01 | 0.06 | 0.05 | 0.98 |

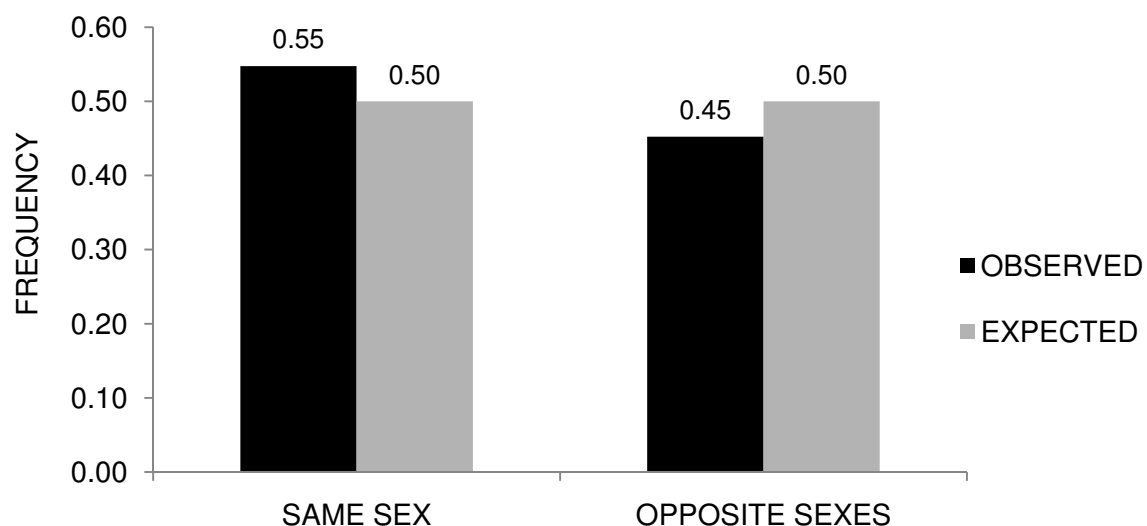


Figure 5.3 Frequency and expected number of associations observed in the dataset.

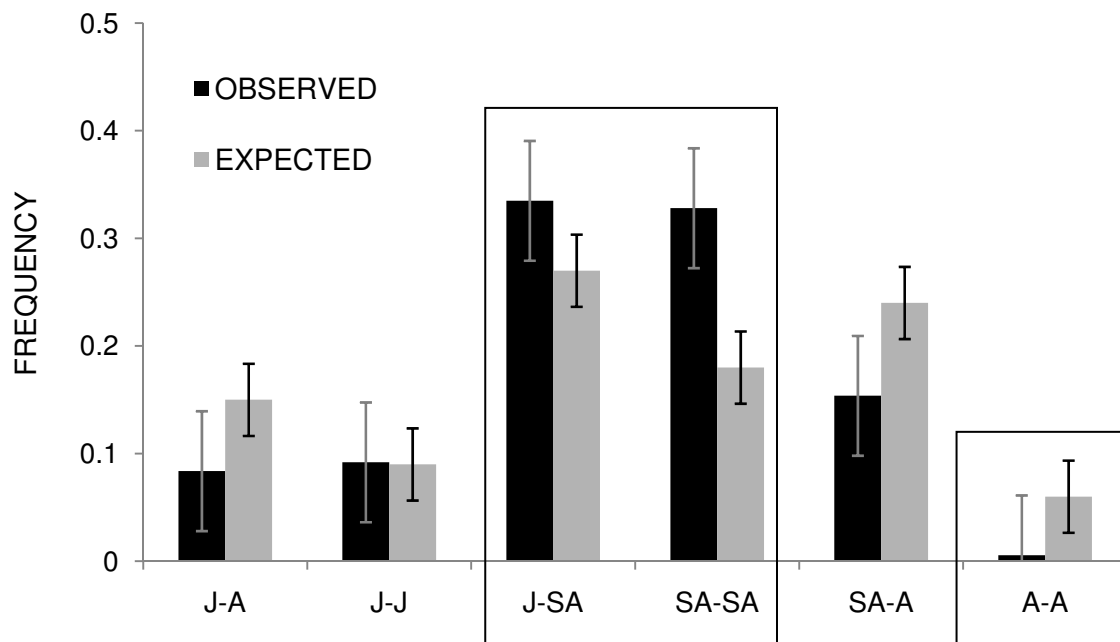


Figure 5.4 Frequency of association between paired of sharks grouped by size classes: J = juveniles, SA = sub adults and A = adults.

5.3.2 Genotype analyses

During the study period 215 tissues biopsies were collected in Gansbaai from free ranging white sharks. Likelihood-based genotype matching by SHAZA Version 1.0 (Macbeth et al. 2011) identified 49 duplicated samples in the Gansbaai dataset, confirmed in each instance by the photo identification. These individuals were removed from all further analyses, leaving 166 genotyped sharks. Overall, all 14 loci were under Hardy-Weinberg equilibrium and no evidence of linkage was detected. Two loci (Cca1072 and Ccar9) were identified to have homozygote excess, and these were double checked for consistencies in scoring. They were indicated as null in the program ML-relate (Kalinowski et al. 2006).

From the dataset of genotyped sharks we selected only the individuals that were photographed from 2009 to 2011 at least over two sampling occasions (e.g. weeks), reducing the number of sharks from 166 to 96. The results indicated 1290 relatedness (R) associations in the dataset, of which 715 were half sibling relationship ($R > 0.1$); 451 were full siblings ($R > 0.2$) and 40 were parent-offspring associations ($R = 0.5$)

5.3.3 Merged association and relatedness

The preliminary analyses conducted by simply counting the occurrences of pairwise sharks observed together and their genetic relatedness indicate an equal distribution of related and non-related sharks in the pairs observed together. When increasing the association (>1) or relatedness (>0.1) strength the trend is in favour of non-relatedness between associated individuals (Figure 5.5).

The result of the Mantel Z and the Dietz (Dietz 1983) permutation tests do not indicate any significant correlation between associated and related sharks or any trend when analyzing associations by sex or by size classes respectively (Table 5.2).

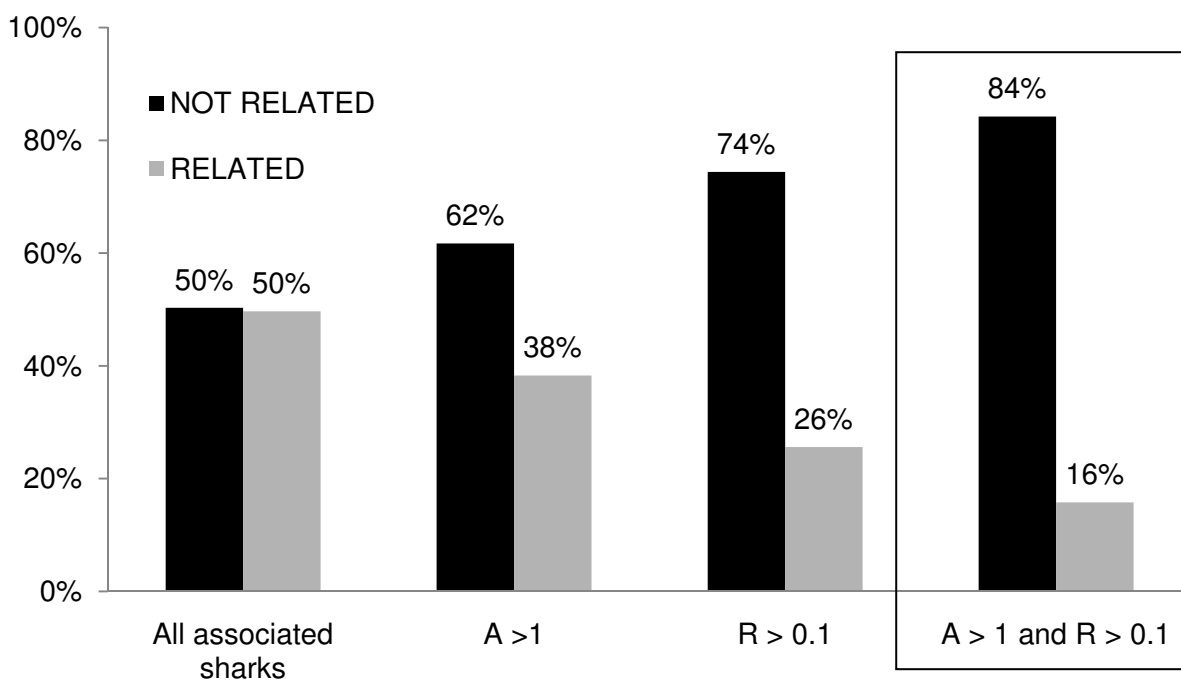


Figure 5.5 Correlation between pairwise associations (A) and relatedness (R) between 96 white sharks. The association matrix were filtered for individuals seen together more than once ($A > 1$), more closely related ($R > 0.1$) and for a combination of the two ($A > 1$ and $R > 0.1$).

Table 5.2 Results of the Mantel Z and Dietz tests to test for significant correlation between associations and relatedness dyadic values. The matrixes were filtered to analyze the associations between separate genders and size classes. (F: females, M: males, J: juveniles, SA: sub adults, A: adults). N: number of sharks; P values for the Mantel Z (P_M) and P values for the Dietz tests (P_D).

| | N | Mantel Z | P_M | Dietz | P_D |
|------------------|----------|----------------------|----------------------|----------------------|----------------------|
| All sharks | 96 | 0.00 | 0.49 | 0.00 | 0.50 |
| BY GENDER | | | | | |
| F-F | 46 | 0.00 | 0.49 | 0.00 | 0.45 |
| M-F | 82 | 0.00 | 0.45 | 0.00 | 0.43 |
| F-M | 82 | 0.00 | 0.11 | 0.00 | 0.12 |
| M-M | 36 | 0.00 | 0.51 | 0.00 | 0.53 |
| BY SIZE | | | | | |
| J-J | 25 | 4.38e ⁻¹² | 0.48 | 8.78e ⁻¹² | 0.52 |
| SA-SA | 51 | 0.00 | 0.48 | 0 | 0.48 |
| A-A | 18 | 0.00 | 0.49 | 0 | 0.50 |
| J-SA | 76 | 5.49e ⁻¹³ | 0.40 | 1.10e ⁻¹² | 0.40 |
| J-A | 43 | 0.00 | 0.62 | 0 | 0.51 |
| SA-A | 69 | 0.00 | 0.51 | 0 | 0.42 |

5.4 Discussion

The most striking result to emerge from our study is the quantitative evidence of non-random associations resulting from possible social interactions in white sharks. This finding corroborates previous observations, based on behavioural data, upon behavioural interactions, hierarchies and presence of stable clans of individuals in the species (Martin & Martin 2006; Sperone et al. 2010; Fallows et al. 2013).

The statistical analysis of the permutation test (Figure 5.1) clearly indicates that white sharks were actively associating with conspecifics in a non-random structure. In some instances this association lasted across the entire study period. Similar formations of lasting group associations in sharks have been demonstrated for juvenile lemon sharks (Guttridge et al. 2009, 2011; Jacoby et al. 2012), and other than simply increasing the foraging efficiency, optimal fitness strategies may also exist. Over time co-occurrence of particular individuals occurs in many aquatic taxa (Croft et al. 2004, 2006, 2008). These associative patterns are often linked to the evolution of cooperation and may also have implications relating to the flow of information through a population and social learning (Slater & Halliday 1994; Croft et al. 2008; Guttridge et al. 2009).

White sharks have never been observed hunting in packs, yet as a predator with a large potential to disperse (Bonfil 2005; Jorgensen et al. 2009), maintaining social groups could be an

efficient mechanism for transferring information, particularly if the groups are composed by few adults passing the information to the less experienced individuals (Klimley & Holloway 1999; Riccioni et al. 2010). For example the location of high value food sources (Sih et al. 2009) or recognition of potential threats (M. Rutzen, personal observation) may play a role. The interaction of white sharks with one another in a scavenging situation has been described both around a cage diving vessel (Sperone et al. 2010) and around a whale carcass (Fallows et al. 2013). From these, white sharks seem capable of social recognition and organization since they maintain a clear size-based pecking order during such situations (Sperone et al. 2010; Fallows et al. 2013). Our study provides evidence of long term associations between individuals, and suggests that the formation of the social groups follow a trend linked to size classes (e.g. stable frequency of juveniles-sub adults and adults rather than size segregated groups) and individual recognition. Due to the higher occurrence of associations between sub adults, and the low associations between adults alone, it appears that white sharks form fission-fusion social dynamics, that changes over the lifespan of an individual (as have been observed in other large marine vertebrates such as bottlenose dolphins, *Tursiops truncatus*; Mann 2000). Thus the groups do not appear to be size segregated as they include some juveniles, sub adults and generally few adults (Figure 5.2 and 5.4). With few exceptions, the occurrences of long term association between adults alone in our dataset are very rare, which can be attributed to either the small sample size (only eight adults were re-captured together more than once) or the possibility that a social hierarchy exists within small groups and it is controlled by one or two dominant adults.

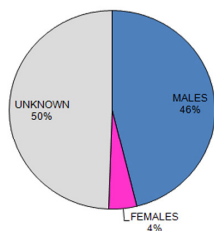
To complicate the matter we cannot rule out the possibility that white sharks have distinct personalities (Klimley et al. 2001; Burgess et al. 2014; Jacoby et al. 2014), with bolder individuals approaching the boat and breaking the surface (allowing for the photographic identification) more often than shyer ones (Delaney et al. 2012). This limitation of the photographic technique in our study was partially solved by grouping the sharks in one week captures, so that the possibility of capturing all the sharks in one group was increased. On the other hand grouping the sharks by the observed residence time, could have generated biased associations. To account for this, the same

dataset should be re-analyzed by grouping the sharks that appeared together within the same hour (information that can be retrieved from the photographs metadata) and relatedness between those animals checked for correlation. By doing so the number of ties between individuals will increase, and may provide a more robust picture for the analysis of the social network. Another limitation pertaining the data collection is the attribution of the size class to an individual shark. The categorization of an individual as juvenile or sub adult depends from the size (TL) estimated in the first day of capture. A recent photogrammetry study on white sharks indicated that visual TL estimates, when compared to the laser measurement, were underestimated 63% of the time, with a mean difference of 42.62 ± 32.0 cm between the two (Leurs et al. 2014). It is thus likely that some individuals categorized as sub adults were in fact adults. However, shark's length for this study was estimated always by the same three observers which at least guarantee some constancy across the measurements. The future employment of laser photogrammetry on the same population could allow for a re-assessment of the individual's original size, based on their individual growth rate. Another limitation of the size-class attribution is the growth of the individuals across different years of study. The growth rate and therefore age-length relationship of white sharks is currently unknown and more likely non-constant (see chapter 1), therefore a juvenile - sub adult association in 2009 could have become a sub adult - adult association without being detected in the analyses. However the dataset could be re-analyzed considering each sampling occasion as independent, were each of the daily observed individuals will be analyzed based uniquely on their size class, similar to Sperone et al. (2010).

The behavioural aspect of white shark associations, and for how long the groups are retained after the scavenging situation, is beyond the scope of our study. Tagging studies performed on white sharks suggested extended periods of sexual segregation can occur in this species (Weng et al. 2007; Jorgensen et al. 2009; Domeier 2012; Kock et al. 2013), and several hypotheses have been put forward to explain the underlying causes of sexual segregation. Geographic segregation of the sexes is a common phenomenon in sharks (see Wearmouth & Sims 2008 for review); females and males can differ in energy budget or females might avoid males

during gestation (Jacoby et al. 2012). The network analyses of our study seem to confirm the tendency of sharks to mostly associate with individuals of the same gender. Additionally, during the spring season (October-November 2011) photographic identifications and underwater photographs were collected inshore and offshore the area of Gansbaai. Offshore 91 white sharks were observed: 42 males and four females (for 45 sharks the sex couldn't be determined), giving a percentage of 46% males and 4% females; inshore 45 white sharks were observed: one mature male, 40 females (for four sharks the sex couldn't be determined), giving a 2% males and 89% females ratio (Figure 5.6). Therefore it appears that during the spring season gender segregation in white sharks become even more evident, a behavioural pattern that was also described in the white shark population offshore California (Jorgensen et al. 2009) and in grey nurse shark, *Carcharias taurus* in Queensland, Australia (Bansemer & Bennett 2009).

OFFSHORE SEX DISTRIBUTION
OCTOBER - NOVEMBER 2011



INSHORE SEX DISTRIBUTION
OCTOBER - NOVEMBER 2011

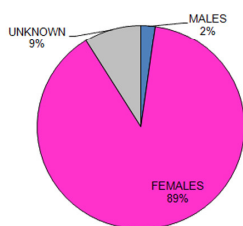


Figure 5.6 Offshore and inshore sex ratio of white sharks during 2011 spring season in Gansbaai. The sex of the sharks was confirmed from underwater photographs (on the right) by the presence or absence of the claspers.

The comparison between the dyadic values of association and relatedness didn't show a significant correlation, and only 16% of the strong associations (> 1) are correlated with

relatedness. The permutations tests conducted on our dataset confirmed the lack of correlation between the dyadic values (Table 5.2), but also showed a lack of statistic significance in our dataset (all P values > 0.05). The reason for this can be due to the small sample sizes (sharks were grouped in one week intervals diminishing the strength of the association values: if two sharks were sighted together 7 times in a week, the association counts as one) or in a limitation of the resolving power of the nuclear markers used.

When exploring the relatedness of elasmobranchs, female and male polyandry are common scenarios (Feldheim et al. 2004; Portnoy et al. 2007; Di Battista et al. 2008; Larson et al. 2011). The possibility of white shark males fathering the same brood hasn't been explored, but this possibility will cause half siblings and siblings to be familiarly equally connected during their early development despite the difference in genetic relatedness. This option will lead to other unexplored questions: "Do white sharks recognize kin?" and "How can white sharks recognized kin?". White shark individuals present unique phenotypic characters, but the connection of their genotype with phenotype characteristics hasn't been investigated. Therefore, at this stage, we can only speculate on the two different scenario that white sharks can recognize each by visual cues (Blaustein 1983) or, as seen in other species of fish, by utilizing chemical recognition (Waldman 1988; Mehlig et al. 2008).

Microsatellites in elasmobranchs species proved to have a slower mutational rate and level of polymorphism than for other species (Pardini et al. 2000, 2000; O'Leary et al. 2013). Furthermore the level of polymorphism in South Africa white shark is particularly low, when compared with other populations around the world (chapter 4). Although comparisons between microsatellite studies are not usually straightforward, the comparison of the average number of alleles and observed heterozygosity for the same 5 loci (Ccar1, Ccar627.x, lox10, Ccar9, Ccar13) are lower for the southern Africa population ($N_a = 7.6$, $H_o = 0.675$), than for the Australian population ($N_a = 8.0$, $H_o = 0.729$). The low level of variation at nuclear level might have biased the relatedness values (Schlötterer & Pemberton 1998; Selkoe & Toonen 2006), making the use of 14 nuclear loci not sufficient to determine the fine scale relationships between individuals. To address

this issue more nuclear markers should be used to re-evaluate the relatedness of our dataset. Additionally increasing the sampling size will also improve the statistical power of the analyses.

To conclude, despite the lack of significance in the fine scale analyses of this study, the non-random associations between pairs of sharks provides a new insight in the biology of this predator and has important implications regarding the effective long term management of this endangered species (see a review in Jacoby et al. 2012). Understanding the role played by the social structure for the species, can transform the current way we are managing this predator, as for species where social interaction exists, the removal of a single individual can drastically compromise the network structure (Krause et al. 2007; Croft et al. 2008). As an example, mostly for small populations, the removal of an individual linking two groups causes the subdivision in sub-networks, with important consequences for the transmission of information (Krause et al. 2007; Croft et al. 2008). The white shark population in South Africa is estimated to currently contain approximately 500 individuals (chapter 3) and the presence of few reproductive individuals and low genetic diversity (chapter 4) can be exacerbated by the possibility that even fewer individuals are actively reproducing, due to social hierarchies. Therefore similarly to other top predators, such as wolves (as also suggested by Martin & Martin 2006), the elimination of a single key individual (Krause et al. 2007) can severely affect the reproductive potential of the whole species (Slater & Halliday 1994; Gehring et al. 2003). Our study is far from solving the mystery surrounding white sharks social dynamics and structures, but it provides the first dataset of which individuals have been recorded together, with an attempt to test kin selection. From this baseline, future studies can be planned to expand our understanding, and improve conservation and management measures for white sharks.

CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS

This project aimed to answer specific questions regarding South African white sharks, *C. carcharias*. Specifically the aim was to contribute towards the conservation of the species, (1) by developing an accurate categorization system to manage large photographic identification databases of white sharks dorsal fins; (2) by estimating the population number; (3) by assessing the genetic connectivity along the coastline and (4) by providing an insight of their social structure.

Human error is one of the biggest problems associated with photographic identification based on visual matching, and it is likely to increase proportionally with the number of images (Marshall & Pierce 2012). The notches code categorisation system developed in this study minimized the time taken to find a correct match and yielded consistent results between researchers. However, the biggest contribution of the categorization system were the guidelines provided to ensure the quality and repeatability of future studies in the field. Specifically, standardizing the photographic identifications method will aid in the fast comparisons of populations from different datasets, thereby allowing non-invasive investigations into white sharks migrations and short-term residence patterns. The manual analyses of 4398 photographs indicated that, once over 400 individuals are identified, the time required to manually match the images and cataloguing them (e.g. renaming, storing and updating the history of re-capture matrix) is very time consuming and becomes non-practical. Therefore to automate the matching and storing of shark dorsal fin photographs the future aim is to develop user friendly accurate software for matching and storing the images across a large data base (a project currently conducted in close collaboration with the Department of Applied Mathematics at Stellenbosch University). The ultimate goal of this newly developed software will be to merge fin recognition to a database system that will include genetic characteristics of each individual. The software will store the information available for each shark and indicate which information is missing, to help sampling new individuals and to prevent double sampling. Conversely, if a shark's dorsal fin gets damaged (and the individual has been genotyped before) a genetic sample can be collected to identify the shark and update the photographic database. Ultimately the comparison of photo-identifications and genotypes across different locations will give an unprecedented insight in the population dynamics of this vulnerable

predator and this software can be adapted and expanded and utilized for the long management of other species of sharks or marine mammals (as suggested in chapter 3).

Indeed the continued application of photographic identification in the first three years resulted in the saturation of the curve of new sightings, once 400 individuals were catalogued. As the population number accuracy increases when a large part of the population is sampled (Sutherland 2006), mark-recapture models could be applied to estimate the population size. The open population model, POPAN suggested ranges between 353-522 individuals (95% confidence), a strikingly low number, when compared to the 1279 estimate conducted in 1996 (Cliff et al. 1996). Due to recent criticisms on the usage of mark-recapture techniques on white sharks (see chapter 3 and Burgess et al. 2014), the integration of this estimate with genetic techniques proved to be fundamental to provide a more complete picture of the South African population. Genetic markers (14 microsatellites) analysis allowed for the determination of the contemporary effective population size (CNe), which approximates the mean number of breeding individuals contributing offspring per generation (Huson & Bryant 2006; Portnoy et al. 2009; Dudgeon et al. 2012) and have direct relevance to conservation (Luikart et al. 2010). Estimates of CNe, for South Africa coastline resulted in a point estimate of 336 (95% CI = 229 – 592, $n = 147$, $P_{crit} = 0.01$), with an estimated number of contemporary breeders of 50 (95% CI = 11 – ∞).

The confidence interval in the estimated number of breeders from 11 to $-\infty$, indicates that the program Ne estimator could not compute one of the tails (Do et al. 2014), showing a lack of statistical support for this result. The same parameter was re-estimated with the program COLONY (Jones & Wang 2010), which indicated a similar result of $N_b = 48$ (95% confidence) and yet, the program COLONY presents some limitations in the choice of the analyses model, as it requires information and assumptions regarding the number of potential breeders, which are not currently available for the species investigated. Arguably, for assessing with better certainty the current number of breeders in the population, it will be necessary to add more nuclear markers to the analyses.

On the other hand the striking similarity between the estimates derived from the genetic (229 – 592) and the mark recapture (353 - 522) methods was to be expected, given the lifetime survivorship and low fecundities of the species (Ahonen et al. 2009; Portnoy et al. 2009; Dudgeon et al. 2012; Ovenden 2014). Furthermore the point estimate based on samples collected around the entire coastline ($CNe = 336$) and from samples collected only in Gansbaai ($CNe = 338$) were extremely close, which allow to consider the mark-recapture estimate performed in Gansbaai as representative of the total population along the South African coastline. These findings are further supported by the F statistic results based on both mtDNA and microsatellites markers showing no genetic differentiation between the five aggregation sites of white sharks along the South African coastline (chapter 4).

The most surprising result regarding white shark's genetic structure in South Africa was the remarkably low level of genetic diversity of both data sets ($h = 0.02$, $\pi = 0.0027$; $N_a = 7.6$, $H_o = 0.675$), suggesting the occurrence of a founder event, or a severe population bottleneck in the recent past. Haplotype and nucleotide diversity of South African white sharks compared with 32 other marine species (chapter 4, Figure 4.3) showed a remarkably low value that has serious conservation implications. At a global level the phylogeographic pattern suggest that South African white sharks represent the remnants of a founder event at least half a million years ago, and the distribution of haplotypes at a global scale indicate that they represent the intermediate population connecting the East Atlantic population with the Indo-Pacific population.

White sharks proved to form non random pairwise associations during scavenging situations (chapter 5), which is supporting previous studies about their sociality (Sperone et al. 2010; Jacoby et al. 2012; Fallows et al. 2013). Finer scale analyses of the nature of such associations were not statistically significant (chapter 5), but provided some insight about the patterns. Specifically the social network analysis described a partial sexual segregation in the species and associations between different-sized individuals to be more frequent than size segregating groups, mostly between juveniles and sub adults. Those patterns indicate a complexity in their social system, which mirrors those of marine mammals (Mann 2000).

One of the key components of adaptive management is the utilization of reliable information and reliable instruments (Keith et al. 2011), preferably regarding the forces that influence the abundance of species. These forces are summarized by three concepts: demographic stochasticity, social dysfunction or behaviour, and genetic deterioration (Soulé & Simberloff 1986). These three forces are strictly connected and the deterioration in one of them will invariably pass on the other two (Hamilton 1964; Avise 1994; Slater & Halliday 1994). In fact small populations will always have less genetic variation than larger ones, detectable by a loss of the population heterozygosity (Avise 1994). In many species lower heterozygosity levels can also result in a general loss of individual fitness, increases in the mortality rate which may be exacerbated during environmental changes (Simberloff & Abele 1982; Soulé & Simberloff 1986). To minimize population extinction due to low heterozygosity levels, a common solution is the introduction of new individuals from other isolated reserves to increase the population size and recover the genetic richness (Gilbert et al. 1998; Haag et al. 2010) and, for ensuring the connections between isolated areas, the sites must be connected by corridors (Gilbert et al. 1998). Talking about corridors in the marine environment, mostly when dealing with a large predator such as the white sharks seems futile, but from an oceanic perspective the change of a current, the increase or decrease of the water temperature and the depletion of food resources can be enough to compromise the connection between populations and to drive them to extinction (Grant & Bowen 1998, 2006). Due to their greater migration potential predators proved to be more susceptible than other species from the loss of corridors (Gilbert et al. 1998; Haag et al. 2010) and the dispersal potential of white sharks (Bonfil 2005) potentially makes the connections between those ocean basins of the greatest importance for their conservation. Genetic evidence already showed that white sharks from the North West and South East Atlantic share a close common ancestor (see chapter 4) and that other species' gene flow is currently going in a west to east direction (Matthee et al. 2006), therefore the possible ingression of individuals from the North West Atlantic will be of the utmost importance for the survival of the South African population. Unfortunately, to protect a predator it is necessary to maintain the environment healthy and productive enough to sustain their presence, and the pressure on white sharks' main food resources (e.g. other elasmobranches and large teleostes)

are rarely taken into consideration (Worm et al. 2013). Specifically the huge increase in fishing effort around South Africa over the last 20 years, mostly on the West Coast, could have diminish white sharks' reproductive potential but also caused a barrier between distant white sharks populations by simply reducing their food availability (Stevens et al. 2000; Anticamara et al. 2011). Finally the SLOSS debate (see a review in Simberloff & Abele 1982) for white shark can only realistically go in the direction of protecting "several small" areas, rather than a "single large" one, as the single large one will have to be extended across three Oceans, while protection measures can successfully be implemented on a National scale.

The new aspects of the biology of this predator underlined in our study has important implications for its effective long term management (Krause et al. 2007; Croft et al. 2008; Jacoby et al. 2012). The legalization of anti-shark nets and baited drumlines (see chapter 1) off the costs of South Africa and Australia are justified by human safety and by the false belief that removing few individuals out of the sharks population won't cause any long term damage (Cliff et al. 1989; Department of Fisheries Western Australia 2013). Specifically, in 2014 the guidelines required fishermen to kill and dispose of all sharks that were captured and measured to be greater than or equal to 3 m (Department of Fisheries Western Australia 2013). The target killing of the reproductive individuals, mostly in the light of a possible social structure, can only be detrimental for a species that is already on the edge of extinction. On the other hand several research projects are focusing on the implementation of eco-friendly repellent technologies to minimize anthropogenic mortality on the marine species (Brill et al. 2009; Robbins et al. 2011; O'Connell et al. 2014a). As an example the Sharksafe Barrier is a newly developed eco-friendly technology, based on bio mimicry and magnetic repellent properties, to prevent shark-beachgoers interaction (O'Connell et al. 2012, 2014b, see also supplementary materials).

Based on the result of this study there are several critical actions that should be considered to prevent further exploitations of South African white sharks, and specifically: (1) greater attention should be given to study white shark' main food resources, in order to ensure the sustainable utilization of the latter; (2) international collaborations with countries currently commercially

harvesting white sharks should be enforced, to support the local development of eco-tourism activities; (3) harmful beach protection measures should be substituted by eco-friendly alternatives, in order to eliminate the constant depletion of the population and finally (4) there should be implemented regional and international monitoring programmes of white shark populations, by using photographic identification and genetic techniques.

To conclude, the results of this study provide new insights into the dynamics of the white shark population occurring along the South African coastline and provided important information relevant to the conservation of the species. Nevertheless the questions answered in this study are far less than the questions raised by it. Assessing the population size and genetic structure are fundamental steps forward, but the lack of information about geographical areas of reproduction prevents proper conservation actions. For example, it is critical to protect nurseries and mating sites. Also, the lack of information pertaining white sharks longevity and growth rate makes it difficult to predict their extinction rate. The female lineages between South Africa and Australia are distinct, but without a population comparison with nuclear markers, we can't evaluate the extent of the male mediated gene flow between the two populations as suggested by Pardini et al. (2001) or alternatively if the two should be regarded as separate Evolutionary Significant Units (Ryder 1986; Moritz 1994) or management units (Palsbøll et al. 2007). Finally, the social structure insights are a fascinating piece of the puzzle that will require more detailed analyses and increased sampling effort before it could be significantly advanced.

The low population number (chapter 3), combined with the low levels of haplotypic and nucleotide diversity (chapter 4) and the presence of non-random association (chapter 5) describe South African white sharks as a population on the edge of the extinction (Luikart et al. 2010). To minimize population extinction due to low heterozygosity levels, a common solution is the introduction of new individuals from other isolated reserves to increase the population size and recover the genetic richness (Gilbert et al. 1998; Haag et al. 2010) and, for ensuring the connections between isolated areas, the sites must be connected by corridors (Gilbert et al. 1998). Arguably South Africa could be a corridor to connect the Indian Ocean and the Atlantic populations

(clade 1 and 2) and it is the only area in which the third maternal lineage is currently found (clade 3). Therefore, based on the results of this study, to maintain the environment in a healthy status and to improve the protection of white shark and their food resources in South Africa would be of the greatest importance for the worldwide conservation of this species.

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APPENDIX I Microsatellites primers details, multiplex panels and annealing temperature.

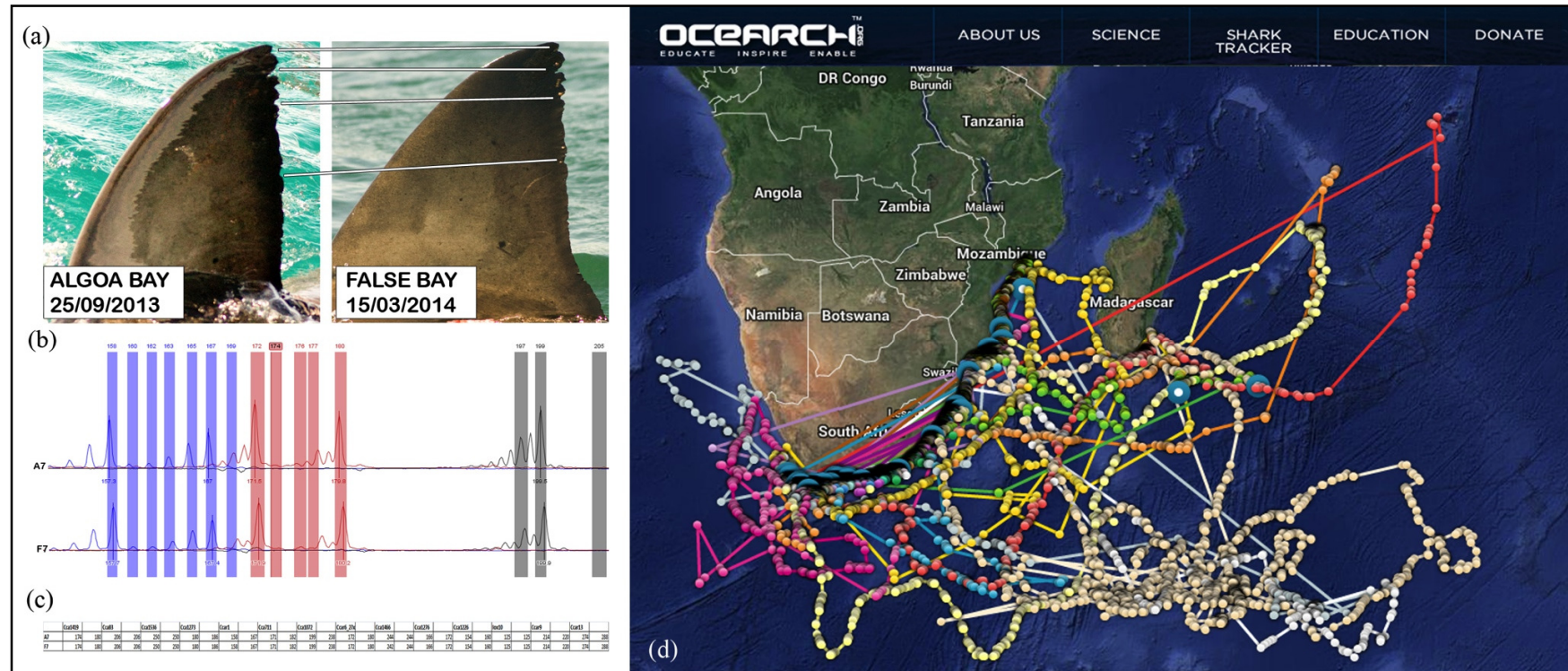
| MULTIPLEX PANEL | LOCUS | PRIMERS | Repeat | Annealing Temperature (°C) | Dyes | Range |
|-----------------|-----------|---|--|-----------------------------|-------|---------|
| A | | | | 50.0 | | |
| | CCa1419 | ATTATCGCATTGGGGGATTT GCAGCAGTCACTCTTGGGTA | [TG] ₁₃ | | 6-FAM | 150-190 |
| | CCa83 | CCTTAAAAGCACAGAACAAAGATAAA GGGGATTTACAGAGAGCATCC | [TAGA] ₁₆ | | VIC | 185-220 |
| | CCa1536 | ACTCCGGATTGGTGCTATTG TTGGTCCTCCTTTGCTGAAT | [TG] ₁₉ | | NED | 230-270 |
| Simplex | | | | 55.0 | | |
| | CCa1273 | TGTTTTTGCCTTTTATCCTTGAA CCAGAAACCCACCCAAGTAA | [TG] ₁₀ | | PET | 165-195 |
| B | | | | 60.0 | | |
| | Ccar1 | GCAGAGGTTGGGAAAGAGTT GCTATTCCAGTGACACTCTCC | [AC] ₂₂ | | 6-FAM | 155-170 |
| | CCa711 | GATGATTTTGCATGTCACTTTGA CTTTGCCTAATATTATTGAGAGAT | [CA] ₁₉ | | VIC | 165-195 |
| | CCa1072 | CCCTGTGTTCTTGCACAATG CCATTGAAGCCCTGTGAAGT | [AC] ₂₆ | | NED | 190-240 |
| | Ccar6.27x | GAGCATGTGTGGGAGCGAAAG TGGGACGATTCTGCCATTCTCTC | NA | | PET | 165-186 |
| C | | | | 60.6 | | |
| | CCa1466 | ATGTGTGCAAGCAAGTCTGC GCATAACACCCCCACAGAAG | [TG] ₉ [TG] ₁₁ | | 6-FAM | 230-250 |
| | CCa1276 | CCTAGCATTATGGTCAACATCAG GGTCACTTTCAACTTGAGCAAA | [TG] ₂₁ | | VIC | 155-195 |
| | CCa1226 | CTCTGGTTTCCTCCCAAGGT CAGGAGATGGGCACTACACA | [TG] ₁₂ | | PET | 150-170 |
| D | | | | 63.2 | | |
| | lox10 | AGGAAATTAGGTGGGGAGGCAG GCCAAATAGATTCTGTCCTTGACCC | [GA] ₁₈ | | 6-FAM | 110-130 |
| | Ccar9 | AATGGGTTGTGATGGGAGTTT CAAGTGGAAGTCAAGCAGGTT | [TG] ₂₃ | | NED | 200-247 |
| | Ccar13 | GCTGAGTGCTGGCTGACCT TATCCAGTTACCATCTCCAAAAA | [TG] ₄ TT[TG] ₉ TT[TG] ₃ TTTT[TG] ₂₃ | | PET | 265-300 |

APPENDIX II

Photographic identification sampling effort of each month (2009-2011): from the left to the right is indicated (1) the month of sampling or capture occasion (i); (2) the number of sampling days each month (3) the number of photo identifications collected (excluding multiple photographs of the same shark collected on the same day) and (4) the total number of identified sharks (n_i). The last two columns indicate, within the identified sharks, how many were newly identified (u_i) and how many were sharks previously included in the database (m_i).

| Sampling Effort | | | Identified Sharks | | |
|-----------------------|---------------|-------------|-------------------|------------|------------|
| Capture Occasions (i) | Sampling Days | Photo ID | n_i | u_i | m_i |
| 2009 04 | 17 | 40 | 28 | 28 | 0 |
| 2009 05 | 21 | 57 | 41 | 35 | 6 |
| 2009 06 | 16 | 87 | 70 | 58 | 12 |
| 2009 07 | 10 | 46 | 34 | 21 | 13 |
| 2010 02 | 5 | 23 | 18 | 17 | 1 |
| 2010 03 | 19 | 69 | 43 | 24 | 19 |
| 2010 04 | 14 | 65 | 42 | 22 | 20 |
| 2010 05 | 13 | 79 | 57 | 32 | 25 |
| 2010 06 | 8 | 35 | 28 | 10 | 18 |
| 2010 07 | 10 | 27 | 21 | 7 | 14 |
| 2010 08 | 8 | 34 | 28 | 12 | 16 |
| 2010 09 | 13 | 94 | 61 | 31 | 30 |
| 2010 10 | 14 | 39 | 32 | 11 | 21 |
| 2010 11 | 12 | 39 | 31 | 14 | 17 |
| 2010 12 | 12 | 37 | 21 | 4 | 17 |
| 2011 01 | 13 | 29 | 20 | 9 | 11 |
| 2011 02 | 11 | 57 | 36 | 17 | 19 |
| 2011 03 | 11 | 73 | 38 | 7 | 31 |
| 2011 04 | 11 | 49 | 36 | 3 | 33 |
| 2011 05 | 10 | 70 | 55 | 22 | 33 |
| 2011 06 | 9 | 60 | 51 | 9 | 42 |
| 2011 07 | 12 | 66 | 34 | 9 | 25 |
| 2011 08 | 5 | 12 | 10 | 3 | 7 |
| 2011 09 | 7 | 29 | 27 | 8 | 19 |
| 2011 10 | 10 | 58 | 39 | 5 | 34 |
| 2011 11 | 2 | 13 | 13 | 2 | 11 |
| 2011 12 | 5 | 17 | 14 | 6 | 8 |
| TOT | 298 | 1304 | 928 | 426 | 502 |

APPENDIX III: Photographic (confirmed by genetic fingerprint) and satellite telemetry evidences of the white shark's dispersal potential around southern Africa coastline.



Correspondence between photo identification and genetic fingerprint of a white shark (3.7m Female) double sampled at the two furthest sampling location of this study (e.g. Algoa Bay and False Bay). (a) Dorsal fin photo identification; (b) Example of microsatellites score with the software Geneious version 5.6.5 (Copyright © 2005-2012 Biomatters Ltd.) of the two samples belonging to the same shark; (c) Genotype identity between the two samples; (d) satellite telemetry tracking of white sharks around southern Africa coastline, from the website: <http://www.ocearch.org/#SharkTracker>.

APPENDIX IV Genetic diversity at the 14 microsatellite loci sourced from this study in each sampling site. n – number of successfully genotyped individuals per locus; Na – number of alleles at each locus; Ho – observed heterozygosity; He – expected heterozygosity; AR – allelic richness; F_{IS} – Inbreeding coefficient ;

| Locus | | Algoa Bay | False Bay | Gansbaai | Struisbaai De Hoop | Mossel Bay | Overall |
|----------------|----------------------------|-----------|-----------|----------|-----------------------|------------|---------------|
| Cca1419 | n | 9 | 11 | 167 | 18 | 28 | 233 |
| | Na | 3 | 3 | 3 | 3 | 3 | 3 |
| | Ho | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| | He | 0.582 | 0.567 | 0.519 | 0.586 | 0.527 | 0.526 |
| | AR | 3 | 3 | 3 | 3 | 3 | 3 |
| | F_{IS} | -0.800 | -0.833 | -0.933 | -0.744 | -0.931 | -0.906 |
| Cca83 | n | 9 | 11 | 167 | 18 | 28 | 233 |
| | Na | 5 | 5 | 7 | 6 | 7 | 7 |
| | Ho | 1.000 | 0.818 | 0.784 | 0.889 | 0.786 | 0.803 |
| | He | 0.752 | 0.792 | 0.794 | 0.706 | 0.820 | 0.789 |
| | AR | 4 | 4 | 8 | 7 | 8 | 6.2 |
| | F_{IS} | -0.358 | -0.034 | 0.012 | -0.268 | 0.043 | -0.017 |
| Cca1536 | n | 9 | 11 | 167 | 18 | 28 | 233 |
| | Na | 5 | 7 | 7 | 6 | 7 | 7 |
| | Ho | 0.889 | 0.818 | 0.850 | 0.944 | 0.786 | 0.850 |
| | He | 0.719 | 0.840 | 0.807 | 0.783 | 0.825 | 0.808 |
| | AR | 11 | 11 | 11 | 11 | 11 | 11 |
| | F_{IS} | -0.255 | 0.027 | -0.053 | -0.214 | 0.048 | -0.051 |
| Cca1273 | n | 9 | 11 | 167 | 18 | 28 | 233 |
| | Na | 2 | 2 | 2 | 2 | 2 | 2 |
| | Ho | 0.333 | 0.364 | 0.419 | 0.611 | 0.571 | 0.446 |
| | He | 0.503 | 0.416 | 0.443 | 0.437 | 0.486 | 0.446 |
| | AR | 3 | 3 | 3 | 3 | 3 | 3 |
| | F_{IS} | 0.351 | 0.130 | 0.054 | -0.417 | -0.180 | 0 |
| Ccar1 | n | 9 | 11 | 167 | 18 | 28 | 233 |
| | Na | 4 | 4 | 6 | 4 | 5 | 6 |
| | Ho | 0.889 | 0.909 | 0.743 | 0.778 | 0.607 | 0.742 |
| | He | 0.732 | 0.710 | 0.696 | 0.624 | 0.700 | 0.691 |
| | AR | 5 | 5 | 5 | 5 | 5 | 5 |
| | F_{IS} | -0.231 | -0.299 | -0.067 | -0.256 | 0.135 | -0.075 |
| Cca711 | n | 9 | 11 | 167 | 18 | 28 | 233 |
| | Na | 5 | 5 | 7 | 6 | 5 | 9 |
| | Ho | 0.556 | 0.727 | 0.563 | 0.611 | 0.464 | 0.562 |
| | He | 0.641 | 0.680 | 0.540 | 0.638 | 0.534 | 0.556 |
| | AR | 7 | 7 | 10 | 10 | 10 | 8.8 |
| | F_{IS} | 0.111 | -0.143 | -0.050 | -0.067 | 0.091 | -0.036 |
| Cca1072 | n | 9 | 11 | 167 | 18 | 28 | 233 |
| | Na | 5 | 8 | 8 | 7 | 8 | 8 |
| | Ho | 0.556 | 0.727 | 0.689 | 0.722 | 0.750 | 0.695 |

| | | | | | | | |
|------------------|-----------------|--------|--------|--------|--------|--------|---------------|
| | He | 0.667 | 0.857 | 0.815 | 0.775 | 0.810 | 0.807 |
| | AR | 20 | 20 | 20 | 20 | 20 | 20 |
| | F _{IS} | 0.175 | 0.158 | 0.155 | 0.069 | 0.076 | 0.139 |
| Ccar627.x | n | 9 | 11 | 167 | 18 | 28 | 233 |
| | Na | 3 | 2 | 3 | 3 | 3 | 3 |
| | Ho | 0.667 | 0.182 | 0.461 | 0.611 | 0.643 | 0.489 |
| | He | 0.621 | 0.173 | 0.452 | 0.538 | 0.515 | 0.464 |
| | AR | 4 | 4 | 4 | 4 | 4 | 4 |
| | F _{IS} | -0.079 | -0.053 | -0.020 | -0.140 | -0.254 | -0.054 |
| Cca1466 | n | 9 | 11 | 162 | 18 | 26 | 226 |
| | Na | 2 | 2 | 3 | 2 | 3 | 3 |
| | Ho | 0.333 | 0.636 | 0.457 | 0.278 | 0.538 | 0.456 |
| | He | 0.294 | 0.455 | 0.358 | 0.246 | 0.419 | 0.358 |
| | AR | 1 | 1 | 2 | 1 | 2 | 1.4 |
| | F _{IS} | -0.143 | -0.429 | -0.276 | -0.133 | -0.292 | -0.274 |
| Cca1276 | n | 9 | 11 | 163 | 18 | 25 | 226 |
| | Na | 7 | 6 | 16 | 10 | 9 | 16 |
| | Ho | 0.778 | 1.000 | 0.933 | 0.833 | 0.880 | 0.916 |
| | He | 0.843 | 0.836 | 0.874 | 0.870 | 0.856 | 0.867 |
| | AR | 13 | 5 | 15 | 14 | 14 | 12.2 |
| | F _{IS} | 0.082 | -0.209 | -0.067 | 0.043 | -0.029 | -0.057 |
| Cca1226 | n | 9 | 11 | 163 | 18 | 26 | 227 |
| | Na | 3 | 2 | 5 | 4 | 4 | 5 |
| | Ho | 0.556 | 0.455 | 0.264 | 0.333 | 0.346 | 0.299 |
| | He | 0.464 | 0.455 | 0.291 | 0.340 | 0.389 | 0.322 |
| | AR | 5 | 3 | 5 | 5 | 5 | 4.6 |
| | F _{IS} | -0.212 | 0.000 | 0.094 | 0.019 | 0.112 | 0.069 |
| Iox10 | n | 8 | 10 | 155 | 15 | 26 | 214 |
| | Na | 4 | 4 | 5 | 5 | 5 | 5 |
| | Ho | 0.750 | 0.500 | 0.671 | 0.867 | 0.615 | 0.673 |
| | He | 0.742 | 0.647 | 0.673 | 0.786 | 0.626 | 0.677 |
| | AR | 4 | 4 | 5 | 5 | 5 | 4.6 |
| | F _{IS} | -0.012 | 0.237 | 0.003 | -0.106 | 0.017 | 0.006 |
| Ccar9 | n | 8 | 9 | 156 | 15 | 25 | 213 |
| | Na | 7 | 8 | 15 | 10 | 12 | 15 |
| | Ho | 0.750 | 0.889 | 0.750 | 0.733 | 0.880 | 0.770 |
| | He | 0.800 | 0.797 | 0.831 | 0.862 | 0.832 | 0.828 |
| | AR | 11 | 12 | 19 | 13 | 14 | 13.8 |
| | F _{IS} | 0.067 | -0.123 | 0.098 | 0.154 | -0.059 | 0.07 |
| Ccar13 | n | 8 | 8 | 148 | 12 | 21 | 197 |
| | Na | 3 | 3 | 3 | 3 | 3 | 9 |
| | Ho | 0.750 | 0.750 | 0.696 | 0.667 | 0.714 | 0.701 |
| | He | 0.667 | 0.633 | 0.595 | 0.627 | 0.664 | 0.607 |
| | AR | 2 | 2 | 2 | 2 | 2 | 2 |
| | F _{IS} | 0.056 | -0.012 | -0.061 | -0.126 | -0.071 | -0.06 |

SUPPLEMENTARY MATERIALS: Additional contribution to published articles

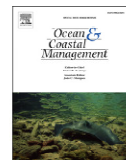
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The use of permanent magnets to reduce elasmobranch encounter with a simulated beach net. 2. The great white shark (*Carcharodon carcharias*)



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ABSTRACT

Beach nets are preventative devices that are utilized to minimize the potential interaction between a beachgoer and a predatory shark. One species, the great white shark (*Carcharodon carcharias*), the focal species for the present study and a protected species in South African waters, is often killed in beach nets within the KwaZulu-Natal (KZN) region. To address the issue of *C. carcharias* capture in beach nets and to

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Effects of the Sharksafe barrier on white shark (*Carcharodon carcharias*) behavior and its implications for future conservation technologies



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ABSTRACT

The white shark (*Carcharodon carcharias*) is an apex predator and is a protected species that suffers from several sources of anthropogenic mortality, such as shark nets. Shark nets are devices used to minimize the interaction between beachgoers and potentially dangerous sharks; however, these nets have negatively impacted local and migratory shark populations, in addition to killing substantial quantities of other marine organisms. To