Exploring the murky world of the sevengill shark, *Notorynchus cepedianus*, in southern New Zealand

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Abstract

The broadnose sevengill shark, *Notorynchus cepedianus,* is an important marine apex predator found in temperate coastal regions worldwide. Despite being identified as an abundant, high trophic level species, there is a paucity of ecological research dedicated to sevengill sharks and their use of marine systems. In particular, there is a lack of specific research on the distribution and demography of sevengill shark populations.

This study represents the first systematic data on the seasonal distribution, habitat use and genetic structure of sevengill shark populations in Aotearoa, New Zealand. During 71 sampling trips conducted from July 2013 to May 2015, sharks were attracted to coastal sampling sites at Ōtākou (Otago Harbour) and Te Whaka ā Te Wera (Paterson Inlet) using chum.

Sharks were implanted with stainless steel dart tags (n = 55) and photographs of unique dorsal markings (n = 23 unique individuals) were obtained. At Ōtākou, a clear seasonal pattern of sevengill shark sightings emerged. Sharks were detected on 86% of survey trips in summer, whilst no sharks were detected in winter or spring. At Te Whaka ā Te Wera, sharks were sighted throughout all seasons, but a decline in shark encounters occurred during winter. Both male and female sevengill sharks were encountered, and individuals ranged between 1.5 m and 2 m total length.

Using a logistic regression model, water temperature was identified as a key predictor of sevengill shark presence at Ōtākou and Te Whaka ā Te Wera. In addition, location, cloud cover, and sea state were also identified as influential predictors.

On supplementary sampling trips, two individual sevengill sharks were re-sighted using recognition of tags, and three individuals were re-sighted using photo-ID. Long-term stability of natural marks and higher re-sight rates suggest photo-ID is an effective, less invasive alternative to physical tagging in sevengill sharks. Instances of tag shedding and bio-fouling further support photo-ID as a more robust means to studying sevengill shark demographics. Individual sevengill sharks showed some fidelity to coastal areas, but low re-sight rates suggest large population sizes, and/or high levels of migration among populations are occurring.

Phylogenetic relationships among sevengill shark populations were also explored, using tissue samples extracted from free-swimming sevengill sharks in conjunction with

previously collected samples. Mitochondrial DNA sequencing detected no differentiation in mtDNA COI (n = 41) and ND4 (n = 42) at a national scale. COI sequences also detected no genetic structure among sevengill shark populations from Argentina, Australia, and New Zealand. These results suggest that sevengill sharks in New Zealand display low breeding site fidelity, and high mobility among sites.

The findings of this study provide some of the first data to help comprehend the role of sevengill sharks in marine coastal systems in New Zealand. This information will be useful for current and future ecological assessments of sevengill shark populations, and the coastal communities of which they reside.

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I would like to dedicate this thesis to two water loving adventurers:

Louise Emma Jull, and Julie Laurenson.

Okea ururoatia

Fight like a shark, be tenacious in pursuit of your goals

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Chapter One

General Introduction

1.1 Elasmobranchii

Large sharks (Chondrichthyes, Elasmobranchii) are often apex predators in marine coastal communities (Myers et al., 2007; Williams et al., 2012). These animals may utilise wide spatial ranges, potentially contributing to the structure and function of multiple ecosystems (Williams et al., 2004; Wirsing et al., 2007). For this reason, gaining an understanding of spatio-temporal habitat use by large sharks is imperative for assessing ecosystem function in a range of distinct locations (Dill et al., 2003; Barnett et al., 2011). Such assessments can contribute to effective fisheries management and conservation strategies, and thus, to the longevity of important shark species and their habitats (Bonfil, 1997; Baum et al., 2003; Ferretti et al., 2010).

The class Chondrichthyes comprises all cartilaginous fish, including sharks, batoids and chimaeras (Enault et al., 2016; Weigmann, 2016). More than 1000 chondrichthyan species inhabit the Earth's oceans and the International Union for Conservation of Nature (IUCN) Red List estimates one in four of these are threatened due to overfishing and incidental take (Dulvy et al., 2014; Larson et al., 2015). Large, shallow water chondrichthyan species have been identified as the most at risk of extinction (Dulvy et al., 2014). Furthermore, within the dominant sub-class Elasmobranchii (sharks, skates, and rays), the proportion of threatened oceanic species is even greater (Dulvy et al., 2008).

1.2 Threats to Shark Populations

Anthropogenic pressures have been recognised as one of the most critical factors contributing to the decline of top oceanic predators worldwide (Myers & Worm, 2003; Jacques, 2010). Over-exploitation has resulted in declining shark populations on a global scale, in estuarine, open-ocean, deep sea, freshwater, and coastal habitats (Jardine et al., 2003; Dulvy et al., 2008; Simpfendorfer et al., 2011; Hoogenboom et al., 2015). Valuable shark products include fins, leather, cartilage, liver oil, and meat (Simpfendorfer et al., 2011). In addition to being targeted in commercial and recreational fisheries, sharks are regularly caught as bycatch in trawl, longline, purse seine, and gillnet fisheries (Baum et al.,

2003; Zeeberg et al., 2006). A main driver of shark fishing is to meet the international demand for shark fin soup, a traditional Chinese delicacy (Dulvy et al., 2014). As shark fins are typically worth more than shark meat, economic incentive drives fishers to discard carcasses at sea, whilst retaining the fins (Dulvy et al., 2008).

As well as the anthropogenic pressures described above, shark species in general display life history characteristics that further contribute to their vulnerability (Barker & Schluessel, 2005; Jacques, 2010). Slow growth rates, low fecundity and late maturity, in conjunction with complex breeding and feeding behaviours, mean shark populations often display little resilience to even modest exploitation (Stevens et al., 2000). Additionally, shark species may congregate or travel in groups, often segregated by sex or reproductive state (Graham & Roberts, 2007; Barnett et al., 2010a). These partitions can exacerbate the threat of exploitation, as cohorts of reproductively active sharks are depleted from a population (Barker & Schluessel, 2005).

Historically, despite extensive exploitation, oceanic shark catches have not been adequately reported in fisheries records (Barker & Schluessel, 2005; Dulvy et al., 2014). Though specific initiatives have been developed to improve catch and bycatch estimates in particular regions, generally, effective regulations for reporting catch rates are sparse (Barker & Schluessel, 2005; Dulvy et al., 2008). Many countries currently lack the expertise, resources, or political desire to implement effective strategies to conserve and manage vulnerable marine species, including sharks (Veitch et al., 2012; Dulvy et al., 2014). In New Zealand, 11 of 113 chondrichthyan species are managed under the current Quota Management System (Francis et al., 2014).

1.3 Role of Shark Populations in Coastal Ecosystems

The removal of apex predators from the Earth's oceans can be detrimental to the structure and biodiversity of multiple marine systems (Myers & Worm, 2005). High trophic level species exert both direct and indirect pressures on subordinate prey species, resulting in the functional disruption or collapse of entire communities upon their removal (Wirsing et al., 2007; Jacques, 2010). Referred to as "trophic cascades" (Williams et al., 2004; Heithaus et al., 2008), the depletion of apex predators and the subsequent top-down systemic effects have been observed in marine communities worldwide (Daskalov, 2002; Barker & Schluessel, 2005). Because of the potential for ecosystem wide ramifications, the global

decline in marine apex predator abundance is a growing concern (Baum et al., 2003; Heithaus et al., 2008).

In the Black Sea, a severe depletion of high trophic level predators was associated with a series of complex alterations to ecosystem dynamics and structure (Daskalov, 2002). Overfishing of predatory animals such as dolphins and mackerel in the 1970's, eventually resulted in an increase of planktivorous fish, and thus, decreased numbers of zooplankton, subsequently leading to high levels of phytoplankton and eutrophication (Daskalov, 2002). Similarly, in the north-west Atlantic, the overfishing of shark species has been indirectly linked to the collapse of a century-old scallop fishery, following the subsequent increase of cownose rays, *Rhinoptera bonasus* (Myers et al., 2007).

The worldwide decline of oceanic predators has prompted calls for improved conservation and management measures on a global scale (Graham & Roberts, 2007). To develop such measures, an understanding of the abundance, distribution, and interactions of high trophic level marine predators is required (Barnett et al., 2011). Effective conservation and management measures are underpinned by the validity of demographic and ecological assessments, which can be difficult to obtain due to the often elusive nature of marine predators (Graham & Roberts, 2007; Speed et al., 2010).

Recent studies have begun to address the complex roles of apex predators with large spatial distributions (Ferretti et al., 2010; Barnett & Semmens, 2012). For example, previously unexplored migratory behaviours of sharks have been determined through the development of technologies such as acoustic and satellite telemetry (e.g. Gibbons & Andrews, 2004; Hammerschlag et al., 2011), as well as molecular methods, such as genetic sequencing and stable isotope analysis (e.g. Abrantes & Barnett, 2011; Larson et al., 2015). Despite these developments, there is still a paucity of published quantitative studies on the population distribution and structure of large shark species worldwide (Heithaus et al., 2007; Barnett et al., 2010a).

A further aggravating factor is the negative preconceptions and fear of sharks held by the public, often as a result of sensationalised narratives of shark attacks portrayed in the media (Simpfendorfer et al., 2011; O'Bryhim & Parsons, 2015). Such attitudes have led to the implementation of shark attack prevention measures, and as such, have been identified as one of the greatest challenges facing shark research and conservation efforts (Muter et al., 2013; Ferretti et al., 2015). Preventative measures undertaken to protect humans from

shark attacks, such as nets, have proven detrimental to non-target marine animals including turtles, dolphins, and rays, which become entangled as bycatch (Simpfendorfer et al., 2011). Furthermore, shark culling, as implemented in areas of California and Western Australia, may cause irreparable damage to already vulnerable shark populations, often with negligible impact on public safety (Dulvy et al., 2014; Ferretti et al., 2015).

The combination of the exploitation, life history traits, systemic influences, and public perception of shark species, collectively contributes to the underlying vulnerability of shark populations worldwide (Dulvy et al., 2008; Jacques, 2010; Simpfendorfer et al., 2011).

1.4 Biology and Distribution of the Sevengill Shark

The broadnose sevengill shark, *Notorynchus cepedianus* (Peron 1807, Chondrichthyes: Hexanchidae; Fig. 1.1), is a relatively common shark species distributed throughout temperate coastal regions worldwide (Barnett et al., 2012; Larson et al., 2015). These predators are commonly found in shallow (< 200 m) coastal waters, bays and estuaries and are often associated with areas of high productivity, muddy or sandy shallows, rocky reef habitats, and kelp beds (Van Dykhuizen & Mollet, 1992; Barnett et al., 2012; Williams et al., 2012).

Figure 1.1: The broadnose sevengill shark, Notorynchus cepedianus. *Image: Quentin Bennett.*

To date, research on populations of the broadnose sevengill shark (hereafter sevengill shark), has been undertaken in four main regions of the world: western USA, Argentina, southern Africa, and Tasmania, Australia (Ebert, 1991, 1996; Crespi-Abril et al., 2003;

Barnett et al., 2012). Despite a basic understanding of the biology and life history characteristics of sevengill sharks, there remains a paucity of research on the abundance and distribution of sevengill shark populations (Williams et al., 2012; Dudgeon et al., 2015), particularly outside the focal regions. Sevengill sharks display highly migratory behaviour, a factor which can complicate demographic assessments, thus hindering effective management strategies (Williams et al., 2012; Stehfest et al., 2014). To date, only one published study devoted to sevengill shark population genetics exists worldwide (Larson et al., 2015). In addition, sevengill sharks are currently listed as data deficient in the IUCN List of Endangered Species (Fowler et al., 2005).

Sevengill sharks exhibit sexual dimorphism in total body length (TL), and variation in size at maturity (Barnett et al., 2010a; Awruch et al., 2014; Stehfest et al., 2014). For males, previous studies investigating the calcification and length of claspers determined the size at maturity to be 150-170 cm TL for sevengill sharks caught in Patagonian, Californian and South African waters (Ebert, 1989, 1996; Lucifora et al., 2005). A similar study conducted in Tasmania, however, found a larger size at maturity for male sevengill sharks, between 190-194 cm TL (Awruch et al., 2014). This disparity suggests that size at maturity may vary among locations (Awruch et al., 2014). In all populations studied to date, female sevengill sharks have been found to reach maturity at 210-224 cm TL (Lucifora et al., 2005; Awruch et al., 2014). Large females are expected to grow to a maximum of 300 cm TL (Barnett et al., 2011; Barnett et al., 2012).

Female sevengill sharks exhibit a lecitotrophic viviparous mode of reproduction (Musick & Ellis, 2005). Adult females appear to have a bi-annual reproductive cycle, giving birth approximately every two years, following a six to twelve month ovarian cycle, and separate twelve month gestation period (Ebert, 1989; Awruch et al., 2014). Specific bays in California and Argentina have been identified as important nursery areas for sevengill sharks (Ebert, 1985; Lucifora et al., 2005), though in coastal areas of Washington and south-east Tasmania, no such nursery areas have been located (Barnett et al., 2010b,c; Williams et al., 2012). Despite the slow-growing, late maturing nature of the species, sevengill sharks are considered highly fecund in comparison to other elasmobranchs (Ebert, 1996; Awruch et al., 2014).

Sevengill sharks consume a wide range of prey including gastropods, cephalopods, teleosts, chondrichthyans, and marine mammals (Ebert, 2002; Lucifora et al., 2005; Barnett

et al., 2010c), and display ontogenetic dietary shifts from invertebrates and small fish, through to shark species and marine mammals (Ebert, 2002). Whilst generally described as nocturnal feeders, sevengill sharks have also been observed opportunistically attacking prey encountered during the day (Barnett et al., 2010b). Sevengill sharks both scavenge and directly hunt prey, and it has been suggested they display pack-hunting behaviour to prey upon large animals, such as fur seals (Ebert, 1991). Given the diverse range of their diet, sevengill sharks have been identified as one of the most important apex predators found within temperate coastal marine systems worldwide (Ebert, 2002; Lucifora et al., 2005).

A meta-analysis of the diet of 149 shark species identified sevengill sharks as the highest trophic level species (n = 313 sevengill sharks, trophic level 4.7) from those sampled (Cortes, 1999). This result placed sevengill sharks above white sharks, *Carcharodon carcharias* (n = 259, trophic level 4.5), finding that the sevengill shark diet, on average, consisted of a greater percentage of higher trophic level prey (Cortes, 1999). These outcomes have since been referred to in more current literature (e.g. Ebert, 2002; Barnett et al., 2010c, Abrantes & Barnett, 2011). Though sevengill sharks may occupy the same trophic level as white sharks, stomach content analysis has revealed that white sharks may sometimes consume sevengill sharks (Dr Malcolm Francis, personal communication). In addition, orca, *Orcinas orca*, have been observed preying on sevengill sharks in the Bay of Islands, New Zealand (Dr Ingrid Visser, personal communication).

Sevengill sharks display seasonal use of coastal areas, which they migrate to for reasons such as feeding, mating, or pupping (Lucifora, 2005; Abrantes & Barnett, 2011; Williams et al., 2012). Furthermore, sevengill sharks can be one of the most abundant apex predators in coastal habitats (Ebert, 1989; Barnett et al., 2010a), thus significantly affecting the structure and function of coastal communities through their behaviour and interspecific interactions (Barnett et al., 2010c; Dudgeon et al., 2015). Investigating the temporal and spatial distribution of sevengill sharks populations is essential to begin to understand their influence within these systems (Barnett et al., 2010a).

Due to the slow growing, late maturing nature of the species, sevengill shark populations are considered highly vulnerable to fishing mortality (Smith et al. 1998; Barnett et al., 2012; Williams et al., 2012). Sevengill sharks are increasingly captured as bycatch in both recreational and commercial fisheries worldwide (Braccini et al., 2010). Whilst not specifically targeted by commercial fishers, their presence in areas where species such as

the gummy shark, *Mustelus antarticus*, are targeted, means that sevengill sharks are susceptible to mortality as bycatch (Barnett et al., 2010b; Stehfest et al., 2014). Sevengill sharks are also susceptible to exploitation by recreational fishers (Lucifora et al., 2005).

Despite being recognised as an important marine apex predator in coastal communities, very few studies have investigated the distribution, abundance and habitat use of sevengill sharks (Barnett et al., 2010a; Stehfest et al., 2014). This lack of information presents a significant gap in the understanding of both sevengill shark ecology, and their interspecies dynamics within coastal communities (Barnett & Semmens, 2012; Dudgeon et al., 2015). Coupled with the data deficient status of sevengill sharks under the IUCN Red List of Threatened Species, this paucity of information underlines the necessity for further investigation of sevengill shark populations worldwide.

1.5 Sevengill Sharks in New Zealand

Anecdotally, sevengill sharks are known to occur in bays, estuaries, and coastal areas of New Zealand, particularly during the warmer summer months of December to February. Traditionally, Māori used the teeth of the sevengill shark as blades, which they set into wood to make knives (Hutching, 2012). Although Cox and Francis (1997) recognised high abundances of sevengill sharks in the northern part of the country, to date, there have been no further studies of the species in New Zealand.

The current lack of biological, ecological and fisheries data on sevengill sharks in New Zealand leaves many questions unanswered concerning their population and conservation status, and the impacts of their presence in marine coastal areas. This study represents the first systematic data on the spatio-temporal distribution of sevengill sharks in New Zealand. Due to the unknown conservation status of sevengill shark populations in New Zealand, the least invasive field methods were developed and implemented in this research. With the increasing number of vulnerable shark populations worldwide, the need for developing non-destructive sampling methods is imperative (Barnett et al., 2010d).

Following methods developed to investigate the abundance and distribution of white sharks, physical tagging, photo identification (photo-ID), and genetic sampling were used to investigate the habitat use and occurrence of sevengill sharks at two locations in southern

New Zealand: Ōtākou[1] (Otago Harbour), Ōtepoti (Dunedin) and Te Whaka ā Te Wera (Paterson Inlet), Rakiura (Stewart Island).

1.6 Research Objectives

The objectives of this research were to:

1. Investigate the spatio-temporal habitat use of sevengill sharks in New Zealand.
2. Develop methods for investigating demographics of sevengill sharks in coastal waters.
3. Assess the genetic structure of sevengill shark populations in New Zealand, in relation to populations elsewhere in the southern Pacific Ocean.

1.7 Thesis Outline

Chapter Two describes the observed patterns of distribution of sevengill sharks in southern New Zealand, as a result of abiotic factors. This chapter investigates spatio-temporal habitat use by sevengill shark populations at Ōtākou and Te Whaka ā Te Wera, from 2013 to early 2015. The implications of this chapter will be crucial for establishing baseline data on southern New Zealand sevengill shark populations, which before this time, did not exist.

Chapter Three is dedicated to establishing photo identification (photo-ID) as a viable, minimally invasive method of distinguishing between individual sevengill sharks. Mark-recapture tagging using both photo-ID and stainless steel dart tags is used to investigate site fidelity, and the potential for future demographic studies of sevengill sharks at Ōtākou and Te Whaka ā Te Wera. Photo-ID methods for mark-recapture have not yet been documented in sevengill sharks, and this chapter will discuss the potential benefits and deficiencies of this technique.

Chapter Four investigates genetic structure among sevengill shark populations from various locations around the New Zealand coastline. Using fin clip samples extracted from

[1] Where possible, Māori place names have been used to encourage their use in the science fields within New Zealand.

sharks at several locations across New Zealand (samples taken in 2010), in addition to skin extractions taken during this study (2013 – early 2015), the mtDNA genes COI (cytochrome oxidase I) and ND4 (NADH dehydrogenase 4) are sequenced, compared, and aligned with sevengill shark populations from overseas to establish phylogenetic relationships. This data will give previously unexplored insight into the potential large scale movements and population mixing of sevengill shark populations within New Zealand and abroad.

2 Chapter Two

Distribution

2.1 Introduction

Understanding the relationship between a species and its environment is an important aspect of ecology (Hacohen-Domené et al., 2015). For mobile marine species in particular, there is an increasing interest in understanding the function of environmental conditions as drivers of population occurrence, abundance, and distribution (Elith et al., 2011). In mobile species, migration between habitats is commonly displayed as a response to both biotic and abiotic environmental influences, and at differing spatial and temporal scales, migration may be influenced by factors such as season, temperature, sex, or maturity (Elith et al., 2009; Williams et al., 2012).

Seasonal migration is recognised in a range of marine megafaunal species (e.g. Dudgeon et al., 2009, Couturier et al., 2012). For example, many species of marine mammals seasonally travel between polar, temperate, and tropical waters for feeding, mating, or breeding purposes (Stern, 2009). Humpback whales, *Megaptera novaeangliae*, migrate over 8000 km, travelling from Antarctica to wintering locations off the Pacific Coast of Central America (Rasmussen et al., 2007). On a reduced scale, populations of manatee, *Trichechus manatus*, travel shorter distances (median 280 km) spanning the Atlantic coast of the USA, between southern waters in the winter and northern waters in the summer, in response to seasonal changes in water temperature (Deutsch et al., 2015).

Many shark species also display predictable seasonal migrations (Williams et al., 2012). Basking sharks, *Cetorhinus maximus*, undertake extensive migrations to utilise peak productivity on continental shelf habitats (Sims et al., 2003). Tiger sharks, *Galeocerdo cuvier*, seasonally return to Shark Bay, Western Australia, coinciding with warmer water temperatures, and the occurrence of their target prey species, sea snakes, and dugongs, *Dugong dugon* (Heithaus, 2001).

White sharks demonstrate large scale seasonal migrations, from temperate waters around New Zealand during summer, to tropical Pacific waters during winter (Duffy et al., 2012). Evidence suggests this migration correlates with the winter occurrence of calving humpback whales in these tropical locations (Johnson & Wolman, 1984; Bonfil et al., 2009).

This example underlines how optimal habitat conditions and interspecific interactions are intrinsically linked to the seasonal behaviour of migratory animals; humpback whales utilise warm tropical waters to calve, whilst white sharks exploit this opportunity, preying on vulnerable new-borns.

2.1.1 Habitat Selection

Following migrations, many shark species enter coastal habitats for feeding, mating or parturition purposes (Speed et al. 2010). Aggregations of juvenile sharks that frequent habitats for extended periods of time, suggest that a particular location is a nursery area (Heupel et al., 2007). Dynamic coastal systems commonly have temporally varying environmental conditions, and generally support high levels of ecosystem diversity (Morin et al., 1992; Knip et al., 2010). A range of abiotic variables are considered to influence the use of coastal habitats by sharks, including water temperature, depth, salinity, turbidity, and dissolved oxygen (McCallister et al., 2013; Drymon et al., 2014). For example, juvenile lemon sharks, *Negaprion brevirostris*, have been shown to preferentially select coastal habitats with water temperatures above 30°C (Morrissey & Gruber, 1993). Additionally, biotic factors such as physiology, sex, and maturity of individual sharks, can interact with these abiotic variables, with consequent effects on distribution of populations (Yates et al., 2015). Identifying the mechanisms that contribute to habitat selection can be particularly difficult, especially in species with wide ranges and complex life histories (Scales et al., 2015).

Characterising spatio-temporal habitat use by shark species contributes knowledge to the complex and relatively poorly known field of shark ecology (Speed et al., 2010). This information is essential to the future conservation and management of not only shark species (Williams et al., 2004; Austin et al., 2006), but also the ecosystems of which they are key components (Myers et al., 2007; Heithaus et al., 2008).

2.1.2 Species Distribution Models

Species Distribution Models (SDMs) are numerical tools that combine observations of species occurrence or abundance with environmental assessments (Elith & Leathwick, 2009). These models are used to interpret the role of environmental conditions in driving population distributions (Hacohen-Domené et al., 2015), and may be useful in predicting the

likelihood of species' occurrence in areas where biological knowledge is limited (Robertson et al., 2003). SDMs can be constructed using a variety of methods, ranging from relatively simple regression models to complex non-linear models such as Generalized Additive Models (GAMs; Guisan et al., 2002) and Classification and Regression Trees (CART; Gey & Nedelec, 2005). SDMs have been applied in many population distribution analyses, of both large and small scale migratory shark species, and generally incorporate measurements of hydrographic variables with sighting or occurrence data (Dambach & Rödder, 2011; McCallister et al., 2013; Drymon et al., 2014).

In one example, SDMs were developed using sea-surface temperature, minimum depth, and salinity conditions, to investigate population distributions of white sharks (Dambach & Rödder, 2011). These models were used to predict future and past white shark migrations, and range shifts of northern hemisphere white sharks to higher latitudes over four decades (Dambach & Rödder, 2011). This research also demonstrated the use of SDMs to predict species' responses to environmental impacts, such as climate change (Elith & Leathwick, 2009; Dambach & Rödder, 2011).

On a smaller scale, a study in Mobile Bay, Alabama, used SDMs to examine the presence of juvenile bull sharks, *Carcharhinus leuca*, in response to estuary conditions, and found a combination of factors such as temperature, salinity and dissolved oxygen influenced distribution (Drymon et al., 2014). This study also detected spatial disparity in the presence of sharks between upper and lower bay areas, and recommended the consideration of multiple environmental parameters when developing SDMs for coastal shark distributions (Drymon et al., 2014).

SDMs were also used to model the effect of abiotic factors on habitat use by a range of shark species in north-east Florida (McCallister et al., 2013). Site, month, and bottom water temperature were revealed as the most important predictors of shark presence, with further hydrographic conditions such as depth, salinity and dissolved oxygen having a lesser influence (McCallister et al., 2013).

2.1.3 Seasonal Migration in Sevengill Sharks

Sevengill sharks are seasonally abundant in shallow, coastal, temperate habitats (Ebert, 1996; Lucifora et al., 2005; Williams et al., 2012). Studies conducted off the coasts of Patagonia (Lucifora et al., 2005), western USA (Ebert, 1989; Williams et al., 2012), and

Tasmania (Barnett et al., 2010a; Abrantes & Barnett, 2011; Stehfest et al., 2014) demonstrated a marked increase in abundance of sevengill sharks in coastal embayments and near shore areas during spring and summer, followed by near absences in winter.

Previous studies have identified water temperature as a key environmental cue responsible for initiating these seasonal migrations (Williams et al., 2012; Stehfest et al., 2014). In response to temperature changes, sevengill sharks undertake migrations for the purpose of breeding or feeding (Stehfest et al., 2014). On Patagonian (Lucifora et al., 2005) and Californian (Ebert, 1989) coasts, mating and parturition are considered the main drivers of migration in sevengill sharks. At sites on Washington (Williams et al., 2012) and Tasmanian (Barnett et al., 2010b,c) coasts, prey abundance is considered the main driver of seasonal occurrence; sevengill sharks enter coastal areas seeking seasonally abundant target prey species. In addition, evidence suggests sevengill sharks may frequent warm shallow embayments to enhance physiological performance relating to elevated core body temperatures (Williams et al., 2012). In shark species, warmer environments have been suggested to increase breeding and digestion efficiency, while minimising energy expenditure (Hight & Lowe, 2007).

Thus, water temperature may either serve as a cue to induce migratory behaviour, or augment physiological processes in sevengill sharks (Williams et al., 2012; Stehfest et al., 2014). These two explanations are not mutually exclusive. Optimal habitat conditions and interspecific interactions are, therefore, probably linked to the migration of sevengill sharks to coastal locations. To date, only a few such locations have been identified worldwide (Ebert, 1996; Williams et al., 2012).

In southern Tasmania, sevengill sharks also display sexual segregation in their migratory behaviour (Abrantes & Barnett, 2011). Movement and catch data indicate that the majority of females return to coastal areas in spring, with males appearing in late summer (Barnett et al., 2010a; Barnett et al., 2011). A study conducted in Willapa Bay, Washington, found males and small females frequented shallow boundaries of embayments, whilst larger females tended to frequent deeper central channels (Williams et al., 2012). In both southern Tasmania and Washington, some females remained resident in coastal embayments year-round (Barnett et al., 2010a; Williams et al., 2012). These intrapopulation differences in spatio-temporal habitat use can influence community dynamics and food webs (Matich et

al., 2011), and should therefore be taken into consideration when determining the functional role of sevengill sharks within coastal systems (Abrantes & Barnett, 2011).

2.1.4 Aims

Despite evidence identifying the important contribution of sevengill sharks in structuring multiple coastal ecosystems, there remains a paucity of data on the species' spatio-temporal habitat use and biology in many regions of the world (Ebert, 2002; Barnett et al., 2010a,c; Dudgeon et al., 2015). Although anecdotally sevengill sharks are reported to be seasonally abundant in coastal habitats of New Zealand, currently no published data exist to support such observations.

This study provides the first systematic data on the seasonal abundance of sevengill sharks in New Zealand. Specifically, it examines their occurrence in coastal habitats in response to abiotic environmental conditions at two locations.

2.2 Methods

2.2.1 Preliminary Research

Before the commencement of this study, preliminary research was conducted to determine the location of sevengill shark populations within New Zealand, as communicated by fishers and other informed individuals who had encountered sevengill sharks. This information was used to identify potential sites where field research could be conducted. In 2013, requests for sighting information were advertised to diving, fishing, and surfing clubs, as well as marine researchers from the southern regions of the South Island. Responses revealed a number of sevengill shark sightings, predominantly in the lower South Island, though details of sightings in northern regions were also received. This information was useful in exposing the potential whereabouts of sevengill shark populations, and used to initiate the design of field research for this project. Seventy two sevengill shark sightings were reported, forty seven of which occurred in Ōtepoti and Rakiura (Figure 2.1).

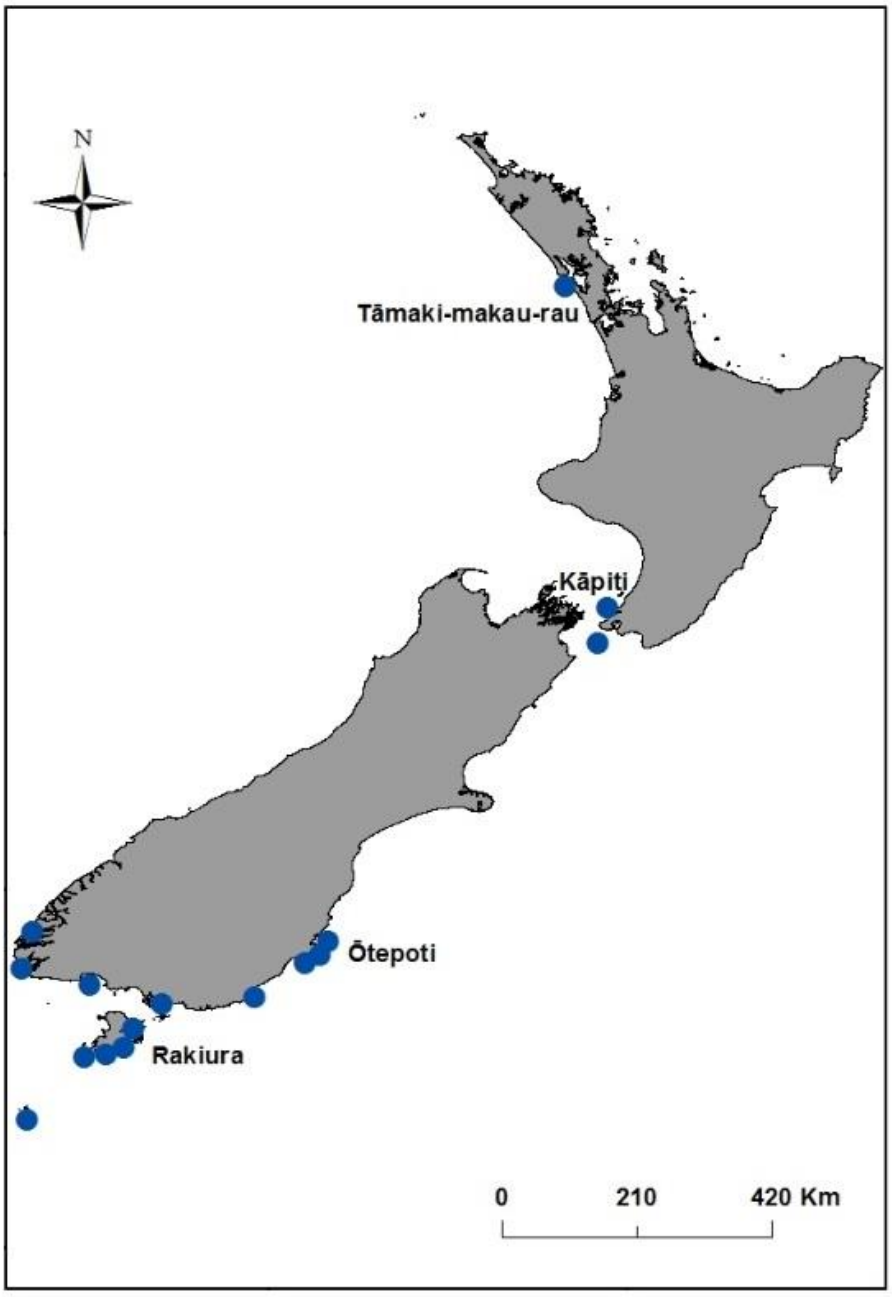


Figure 2.1: Sevengill shark sighting locations in New Zealand, as informed by water users.

2.2.2 Field Surveys

Given the lack of previously published data on sevengill sharks in New Zealand, a pilot study was conducted in 2013 to find study sites where the species could be reliably located, and develop logistically feasible sampling methods. During this time, ten exploratory survey trips were conducted at candidate study sites. These candidate sites were selected based on anecdotal evidence shared by local individuals who had encountered sevengill sharks, and practical considerations such as ease of access and avoiding interaction

with members of the public. Five of these exploratory trips took place at Ōtākou (Otago Harbour), Ōtepoti (Dunedin), between January and February 2013, whilst five took place at Te Whaka ā Te Wera (Paterson Inlet), Rakiura (Stewart Island), in November 2013.

Based on the highest encounter rate of sevengill sharks during the pilot study, five of the candidate sites were chosen as regular study locations: two sites at Ōtākou (Saint Leonards, Harington Point), and three sites at Te Whaka ā Te Wera (Ackers Point, Sawdust Bay, South West Arm; Figure 2.2).

Ōtākou is a naturally formed inlet of Ōtepoti consisting of a 21 km channel of water shaped by sporadic land indents, spanning from Dunedin City in the south-west, to the harbour mouth, adjoining the Pacific Ocean (Figure 2.2). Te Whaka ā Te Wera, located on the east of Rakiura, consists of a central 15 km channel, with three main divaricating arms (Figure 2.2).

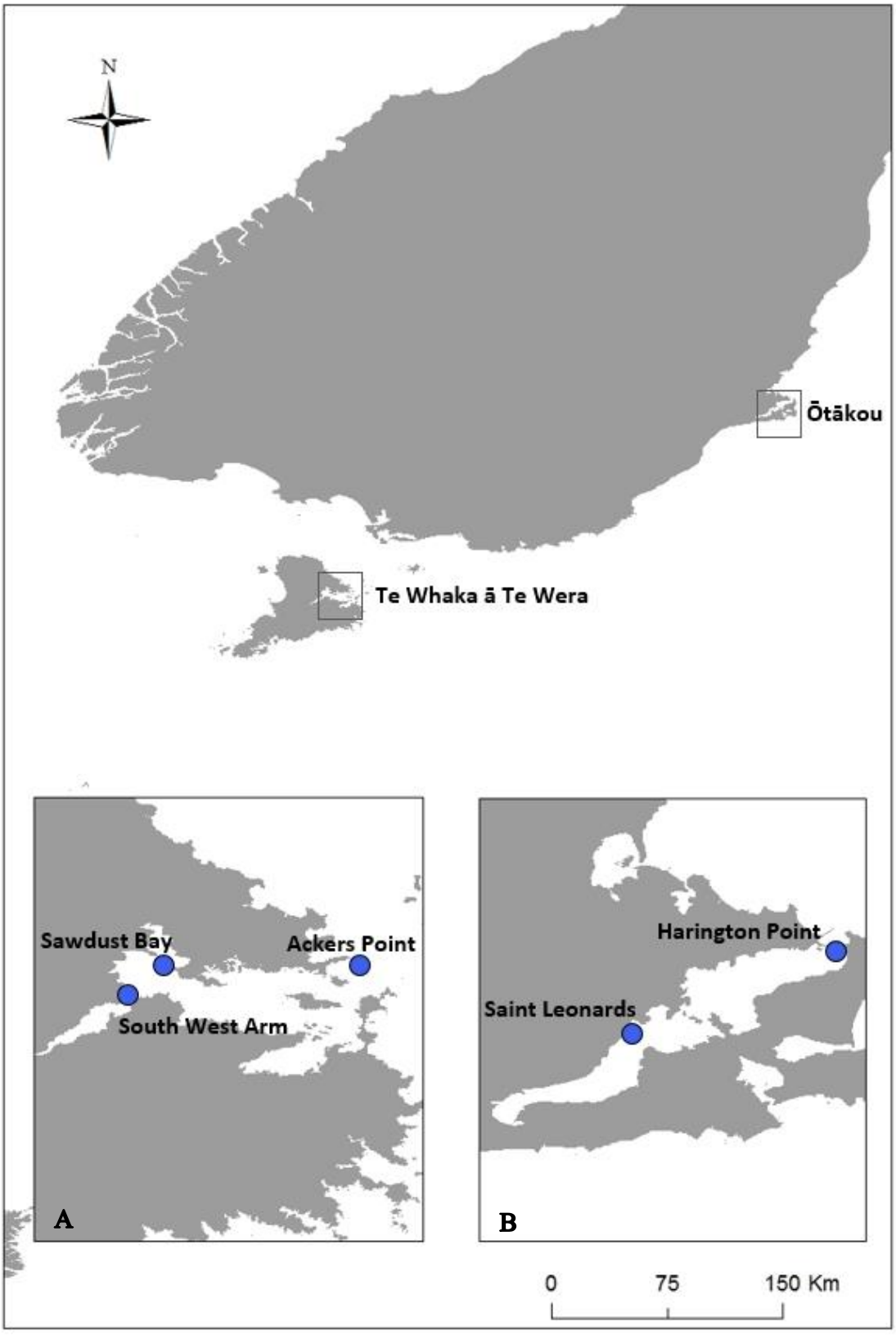


Figure 2.2: Map displaying the two study locations in southern New Zealand. Insets show the three study sites in Te Whaka ā Te Wera (A) and two study sites in Ōtākou (B).

At Te Whaka ā Te Wera, sampling was undertaken using a standardised protocol, on approximately three survey trips per month, during 11 months between December 2013 and May 2015. Uniform sampling effort across the three sites was attempted, but choice was dictated by weather and sea conditions. The research platform was the RV Naiad, a 6.3 m rigid hulled inflatable boat. On each sampling occasion, a number of variables were recorded on arrival at the sampling site (Table 2.1). Water visibility was originally included as an environmental variable, but was discontinued as visibility continually exceeded the depth from the sea surface to the sea floor. Swell height was not required as an environmental variable, as all sites did not experience waves greater than a height of 0.25 m.

Table 2.1: Variables measured at each survey trip at Ōtākou and Te Whaka ā Te Wera.

Variable	**Measurement Tool**	**Unit of Measurement**
Water Temperature	Thermometer, water surface	°C
Water Flow	Drogue	cm/s
Cloud Cover	Visual Observation	%
Sea State	Visual Observation	Beaufort Scale

Following the recording of the variables at the sampling site, sevengill sharks were attracted using chum and tuna oil, which was poured into the surrounding water, whilst the research vessel sat anchored at a depth of 3 - 6 m. Using a combination of fish and oils to attract sharks is a method known as chumming, that has been applied in a number of shark population studies (Soldo & Peirce, 2005; Bruce et al., 2006). The chum used was either blue cod (*Parapercis colias*), sole (*Peltorhamphus novaezeelandiae*) or miscellaneous minced fish. To entice a shark to ascend directly beside the research vessel, the head of a blue cod was tied to the end of a rope and used as a lure. Generally the shark could be led directly beside the boat, where information specific to the individual could then be collected. For each shark sighting, the time of arrival and duration of visibility in the vicinity of the

research vessel were recorded. Following this, the total length of the shark, from tip of the nose to the posterior tip of the caudal lobe, was estimated in comparison to a known distance marked on the research vessel's pontoon. Sex, determined by visual observation of the presence or absence of claspers (Stevens & McLoughlin, 1991) was also recorded, but only in the instance where an animal gave a clear sighting of the ventral side of the body. To distinguish among individuals in the field, sharks were identified using unique spots, scars, or fin nicks. In this way, individuals that returned to the research vessel, would not be mistaken as a new shark sighting. Survey trips took place at dawn and twilight, following, and at the early stages of peak sevengill shark activity, as observed in Tasmania and South Africa (Ebert, 1991; Barnett et al., 2010b). To maintain uniform sampling, survey trips were conducted within two hours from the time chumming began.

At Ōtākou, survey trips took place approximately every three weeks, from July 2013 to April 2015. Protocols were very similar to those applied at Te Whaka ā Te Wera, though due to ease of access, chumming at Ōtākou was conducted from the harbour shore. Total length of the shark was estimated in comparison to defined distances on a 3 m plastic pole, which was held in the water as the shark swam past. This technique has been applied to other large, mobile marine animals, such as white sharks (Strong et al., 1992).

2.2.3 Statistical Analysis

Though identified in preliminary research as likely encounter sites, no sevengill sharks were sighted at Harington Point, Ōtākou, or Ackers Point, Te Whaka ā Te Wera, over the 22 month research period. For this reason, data for these sites were omitted prior to statistical analysis, and the remaining sites were pooled within each location, resulting in the two distinct regions: Ōtākou and Te Whaka ā Te Wera. In addition, due to a scarceness of data, time of survey trip (dawn or twilight), was not considered a factor in any analyses. To compare the seasonal occurrence and mean number of sevengill sharks sighted in this study, the proportion of survey trips with sharks present was calculated, and the mean number of sharks present per trip, standardised to within two hours of when chumming began, was plotted against season at each location. Size and sex distributions, stratified by season, were plotted to investigate whether the demographic classes of sharks encountered varied with time of year. Due to difficulties associated with determining shark size and sex from land at Ōtākou, data from both Ōtākou and Te Whaka ā Te Wera were pooled together for this

analysis. A Pearson's Chi-square test was run to assess any bias in the sex ratio among seasons.

Additionally, a suite of logistic generalized linear models (GLMs) were constructed to test the effect of abiotic variables and sighting conditions on the probability of encountering sevengill sharks. GLMs are extensions of ordinary linear regressions, allowing for non-normal error distributions in the data, using a function to link predictors to a response variable (McCullagh & Nelder, 1983). In the case of a binary response, such as presence or absence of the species of interest, a logistic function is used (Guisan et al., 2002). In north-east Florida, researchers used this particular method to assess the factors affecting the presence and absence of 11 species of shark at two estuaries (McCallister et al., 2013).

For the current study, the response variable was sighting (1), or not sighting (0), a sevengill shark on each survey trip, standardised to within two hours of when chumming began. The explanatory variables included in the logistic models are listed in Table 2.1, with the addition of the categorical variables: "Location", classified as Ōtākou or Te Whaka ā Te Wera; "Season", classified as spring (September - November), summer (December - February), autumn (March - May), or winter (June - August); and "Chum Type", classified as blue cod, sole, or fish mix (miscellaneous minced fish). Sea State, although strictly ordinal, was included as a continuous variable in this analysis. These seven predictors were considered to potentially have influence on the likelihood of encountering a sevengill shark: water temperature and season have been recognised in a number of studies as influencing the distribution of shark species (Barnett et al., 2010a; Heithaus, 2001). Although water temperature and season are likely to be correlated, temperatures were quite different at Ōtākou and Te Whaka ā Te Wera, so both explanatory variables were included. Additional environmental factors such as water flow, sea state, and cloud cover, may affect sensory reception or detection of prey (Ebert, 1991; Hammerschlag et al., 2006; Robbins, 2007). The type of prey available and the location of the habitat may also influence the occurrence of shark species, as populations are more likely to inhabit areas that provide optimal conditions for survival (Knip et al., 2010).

All possible combinations of the seven explanatory variables were used to construct a set of competing models. Akaike's Information Criterion (AIC), was then used to select the best explanatory model from the suite of constructed models (Burnham & Anderson, 2002). AIC identifies the optimal model among a selection of competing models, accounting for

best fit and model parsimony (Bozdogan, 1987). Using AIC, Akaike weights can then be calculated, which can be interpreted as conditional probabilities for each model (Wagenmakers & Farrell, 2004). As the number of survey occasions was small in relation to the number of predictor variables (Gill et al., 2011), AIC values were corrected for small sample size (AICc). The best explanatory model was therefore selected based on the lowest AICc value (Burnham & Anderson, 2002). Akaike weights were calculated to support model selection (Wagenmakers & Farrell, 2004).

Box and whisker plots were then created to compare the spread of the explanatory predictors in the best-fit model between events that did or did not sight a sevengill shark.

All analyses were conducted using R programming language run under R Studio version 0.98.1091 (RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. URL http://www.rstudio.com/).

2.3 Results

In total, 71 survey trips were conducted in southern New Zealand between July 2013 and May 2015, with at least one sevengill shark encountered on 53% of trips. After a total of 26 survey trips at Ōtākou, a clear seasonal pattern of sevengill shark sightings emerged (Table 2.2). Sharks were detected on 86% of survey trips in summer (mean = 2.14 sharks per trip), while no sharks were detected in winter or spring, despite six and seven survey trips respectively (Table 2.2).

At Te Whaka ā Te Wera, sevengill sharks were sighted throughout all seasons, over a total of 45 survey trips (Table 2.2). Sharks were encountered on between 71% and 79% of trips between spring and autumn, whilst this rate declined to 33% in winter. The average number of sevengill sharks sighted per survey trip at Te Whaka ā Te Wera ranged from 2.36 in autumn to 3.29 in spring (Table 2.2, Figure 2.3).

Table 2.2: Total number of sampling trips per season, percentage of trips with at least one sevengill shark detected, and mean number of sharks sighted per trip, standardised to within two hours of when chumming began (± Standard Error), at Ōtākou and Te Whaka ā Te Wera, New Zealand, between July 2013 and May 2015.

		Spring	Summer	Autumn	Winter
Ōtākou	**Number of survey trips (n)**	**7**	**7**	**6**	**6**
	% of trips with sharks encountered	**0%**	**86%**	**33%**	**0%**
	Mean number of sharks sighted (± SE)	**0**	**2.14 ± 0.74**	**0.67 ± 0.82**	**0**
Te Whaka ā Te Wera	**Number of survey trips (n)**	**8**	**14**	**14**	**9**
	% of trips with sharks encountered	**75%**	**71%**	**79%**	**33%**
	Mean number of sharks sighted (± SE)	**3.29 ± 1.69**	**3.00 ± 0.82**	**2.36 ± 0.54**	**2.89 ± 1.68**

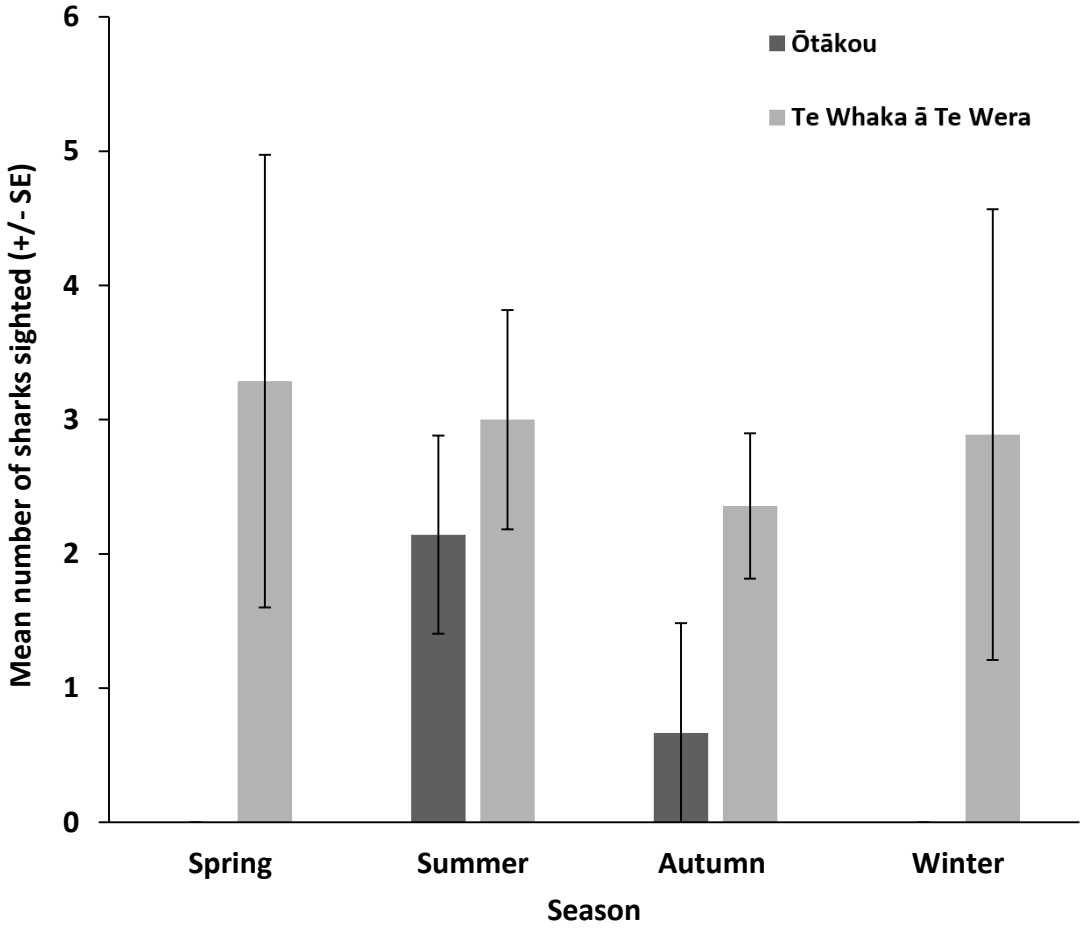


Figure 2.3: Mean number of sevengill sharks sighted per survey trip, standardised to within two hours of when chumming began, during the four seasons in Ōtākou and Te Whaka ā Te Wera (± SE).

2.3.1 Demographic Differences

Data from Ōtākou and Te Whaka ā Te Wera were pooled to investigate the total length (TL) and sex of sevengill sharks encountered in this study. TL was estimated for 61% (n = 86) of sighted sharks. Most sharks measured ranged between 1.5 m and 2 m TL. Overall the range of total lengths measured followed a normal distribution. The minimum length observed was 1.0 m, whilst the maximum length measured was 2.8 m. Similar size distributions were observed across all seasons (Figure 2.4).

Of the 86 measured sevengill sharks, 51% exposed the ventral side of the body clearly, allowing for sex classification using the presence or absence of claspers (e.g. Awruch et al., 2014). An additional eight animals were sexed, without approximation of TL. Similar frequencies of female and male sevengill sharks were observed across all seasons, with the

exception of spring, when more females were sighted (Figure 2.5). There was no evidence for a significantly biased sex ratio among seasons (χ^2 (3) = 2.84, p = 0.4175).

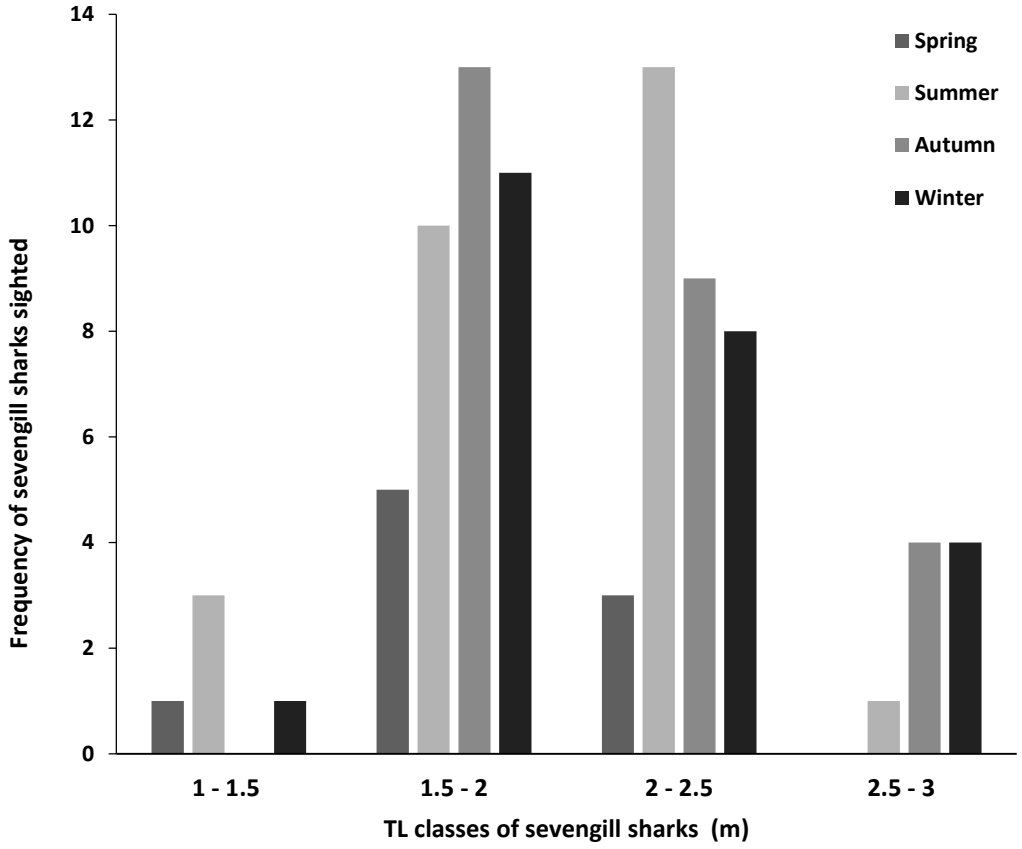


Figure 2.4: Frequency of sevengill sharks sighted by total length class and season, in Ōtākou and Te Whaka ā Te Wera.

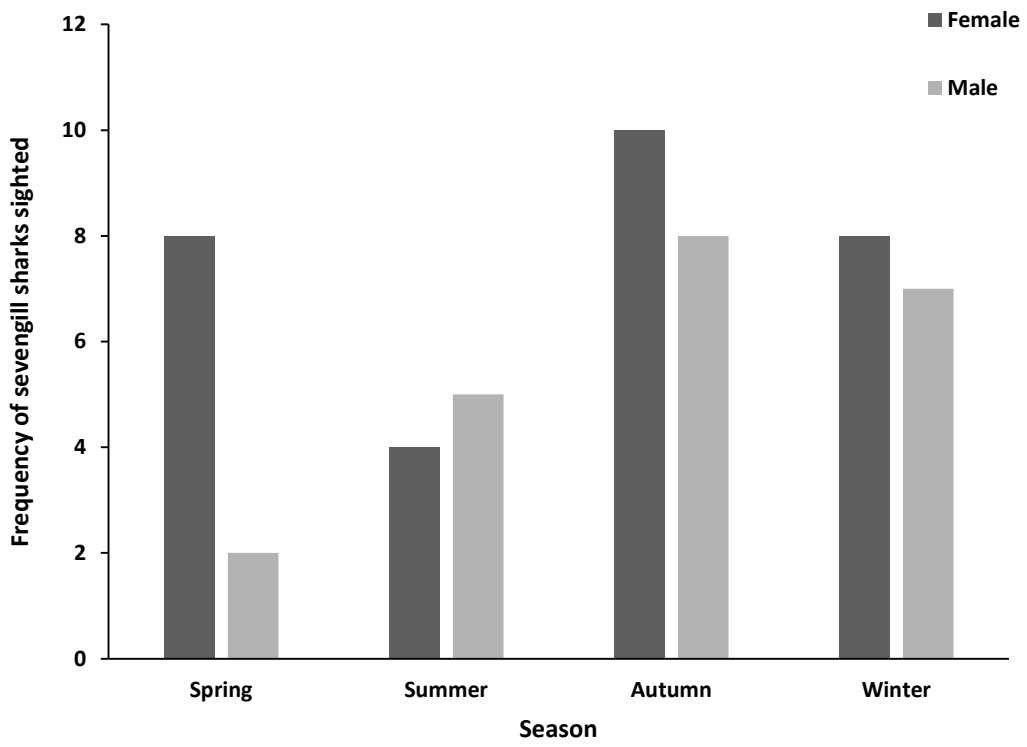


Figure 2.5: Frequency of sevengill sharks sighted by sex and season, in Ōtākou and Te Whaka ā Te Wera.

2.3.2 Model Selection

In the model selection process, location, water temperature (temp), cloud cover (CC), and sea state (SS) were included in the top four models and were therefore considered to be good predictors of encountering a sevengill shark. Furthermore, the best-fit model, indicated by the lowest AICc score (56.01; Model 1, Table 2.3), included only these four predictors. Based on the model weights (*wi*), Model 1 was the only model with any meaningful support. For example, the respective model weights suggest that Model 1 was 28 times more likely than Model 2. The low weights assigned to the remaining models suggest that season, water flow (WF) and chum type were not important predictors of encountering a sevengill shark.

The individual effects of each of the explanatory variables in the best model (Model 1) are displayed in Table 2.4. Location significantly affected the likelihood of encountering a sevengill shark. The likelihood of sighting a shark at Te Whaka ā Te Wera, was 12.8 times greater than the likelihood of sighting a shark at Ōtākou (Table 2.4). Water temperature had a highly significant, positive effect on encountering a sevengill shark. With every unit

increase in water temperature, the likelihood of sighting a shark was 1.67 times greater. Cloud Cover also had a positive, significant effect; with every unit increase in cloud cover, the likelihood of sighting a shark increased by a factor of 1.04. There was a significant negative effect of sea state, with every unit increase in sea state reducing the probability of encountering a shark by a factor of 0.52. Furthermore, no confidence intervals for the odds ratios contained 1, supporting the significance of these predictors in affecting the probability of sighting of a sevengill shark.

Table 2.3: Model-selection analysis. Models are ranked using AICc scores. Only the top eight models in the competing model set are shown. df: degrees of freedom; ΔAICc: difference in AICc score between ith model and best model in set; wi: Akaike weight of the ith model; CC: cloud cover; chum: chum type; SS: sea state; temp: water temperature; WF: water flow.

Model		**df**	**AICc**	**ΔAICc**	***wi***
1	location + temp + CC + SS	58	56.01	0	0.952
2	location + temp + CC + SS + season	55	62.66	6.65	0.034
3	location + temp + CC + SS + season + WF	54	65.37	9.36	0.009
4	location + temp + CC + SS + season + WF + chum	52	69.58	13.57	0.001
5	location + temp + CC + season + WF	55	69.61	13.60	0.001
6	temp + CC + SS + season	56	69.34	13.33	0.001
7	location + temp + SS + season + WF	55	70.22	14.21	0.001
8	location + CC + SS + season + WF	55	71.77	15.76	0.000

The most significant predictor of sighting a sevengill shark was identified as water temperature (Table 2.4). The model results and plots of raw data show that sevengill sharks were more likely to be encountered at warmer water temperatures. This pattern was evident in both locations, although more pronounced at Ōtākou where a wider range of temperatures was experienced (Figure 2.6). At Ōtākou, water temperatures ranged from as low as 4°C during winter sampling to 18°C in summer, with no sharks sighted below 12°C. In contrast, water temperatures at Te Whaka ā Te Wera ranged from 7°C to 14.5°C during sampling, with sharks sighted throughout the range of temperatures.

In relation to percentage cloud cover, the combined model results and box and whisker plots associate an increase in percentage cloud cover, with an increased likelihood of sighting a shark (Table 2.4, Figure 2.6). This pattern is most pronounced at Te Whaka ā Te

Wera, where most shark sightings were made at high values of percentage cloud cover (Figure 2.6).

A negative association was observed between sea state conditions and the likelihood of sighting a shark at Ōtākou and Te Whaka ā Te Wera (Table 2.4). Sevengill sharks were more likely to be sighted at lower levels on the Beaufort Scale (Table 2.4, Figure 2.6).

Table 2.4: Effects of the explanatory variables in the best model (Model 1).

	B(SE)	**z value**	**P >|z|**	**95% Confidence Interval for Odds Ratio**		
				Lower	**Odds Ratio**	**Upper**
(Intercept)	-8.83(2.48)	-3.56	0.0004			
Location: Te Whaka ā Te Wera	2.55(0.88)	2.908	0.004	2.62	12.8	87.33
Water Temperature	0.52(0.15)	3.448	0.0006	1.28	1.67	2.34
Cloud Cover	0.04(0.01)	2.48	0.0131	1.01	1.04	1.07
Sea State	-0.66(0.29)	-2.294	0.0218	0.27	0.52	0.86

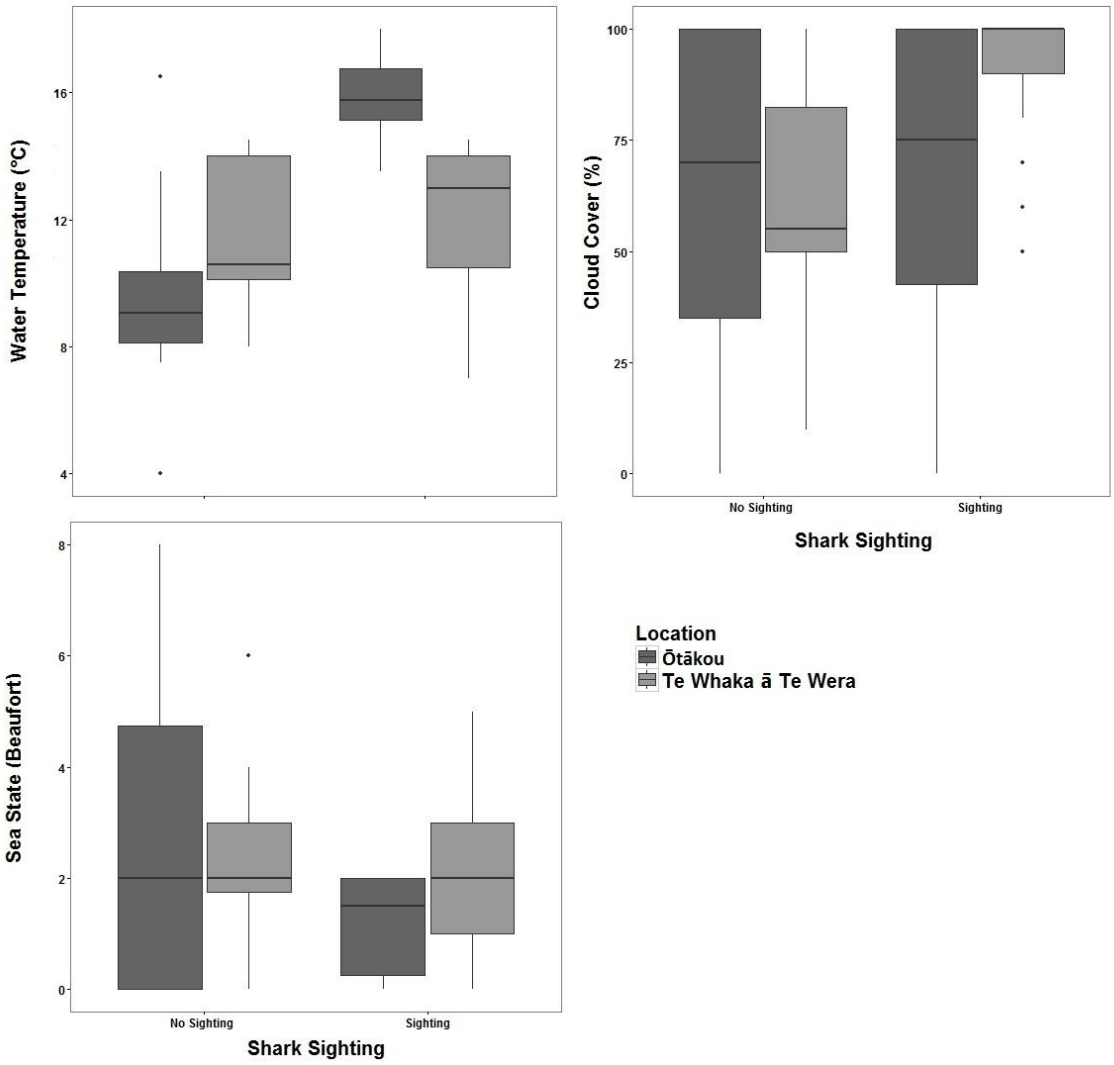


Figure 2.6: Box and whisker plots displaying median, interquartile range, range, and outliers of Water Temperature (℃), Cloud Cover (%), and Sea State (Beaufort Scale), when sevengill sharks were not sighted, or sighted in Ōtākou and Te Whaka ā Te Wera.

2.4 Discussion

Despite being recognised as one of the most important apex predators in temperate coastal communities (Last & Stevens, 2009), no studies have investigated the habitat use and movement behaviour of sevengill sharks in areas of New Zealand. The present study begins to address this knowledge gap, providing insight into the distribution of sevengill sharks in southern New Zealand.

The data on seasonal variation in occurrence from Ōtākou and Te Whaka ā Te Wera suggest that in southern New Zealand, sevengill sharks utilise coastal embayments at distinct times of the year. The clear seasonal pattern at Ōtākou, where sharks were sighted in spring

and summer, but not in autumn and winter, is similar to distribution patterns displayed by sevengill sharks in Patagonia (Lucifora et al., 2005), Western USA (Ebert, 1989; Williams et al., 2012), and Tasmania (Barnett et al., 2010a; Abrantes & Barnett, 2011; Stehfest et al., 2014). Despite the year-round presence of sevengill sharks at Te Whaka ā Te Wera, a decline in sightings was observed in winter.

These findings suggest that at specific times of the year, sevengill sharks in New Zealand migrate from coastal bays and estuaries. Large scale movements or migrations of apex predators, such as sevengill sharks (Abrantes & Barnett, 2011), have been attributed to a change in prey availability, reproductive opportunities, or environmental conditions (Kuhn et al., 2009; Knip et al., 2010; Speed et al., 2010). Assessing these distribution patterns and their drivers is essential to further understanding the ecology of the species, and may also be useful in estimating responses to anthropogenic impacts (Stehfest et al., 2014).

Combining the outcomes of seasonal occurrence with the species distribution modelling assisted in identifying key factors that are associated with the presence of sevengill sharks in southern New Zealand. Elsewhere, researchers have identified important coastal embayments used consistently by populations of sevengill sharks (Ebert, 1989; Lucifora et al., 2005; Barnett et al., 2010a,b; Williams et al., 2012), which are suggested to provide specific environmental conditions, allowing for particular life history processes to be carried out (Barnett et al., 2010c; Abrantes & Barnett, 2011).

In the top-ranked SDM, water temperature was identified as an important predictor of encountering a sevengill shark. Throughout the year, water temperature varied at Ōtākou and Te Whaka ā Te Wera, contributing to the likelihood of sighting a shark, and explaining the observed seasonal trends in the data. The relatively narrow range of water temperatures at Te Whaka ā Te Wera, compared to the broader range observed at Ōtākou, may explain the disparity in shark encounters between these locations during different times of the year.

Using acoustic and satellite tagging, researchers in Willapa Bay, Washington, and Derwent and Norfolk Bays, Tasmania, identified 11°C as the lowest water temperature threshold that determined the migration of sevengill sharks away from coastal sites (Williams et al., 2012; Stehfest et al., 2014). Additionally, a growth rate study reported the highest abundance of sevengill sharks in water temperatures between 12°C to 18°C (Van Dykhuizen & Mollet, 1992). Considering this information, there may be an optimal range of water temperatures for sevengill sharks in southern New Zealand. At Te Whaka ā Te

Wera, the majority of sharks were sighted at water temperatures between 11°C and 14°C, although there were sightings at temperature as low as 7°C. At Ōtākou, this optimal range appeared to be higher, with sharks sighted only between 13.5°C and 17°C.

In prior studies, water temperature has been identified as a cue to initiate shark migrations, driving populations to select or avoid particular habitats (Heupel & Simpfendorfer, 2008). The purpose of such migrations in sevengill sharks has been linked to specific behaviours, such as mating, parturition, and feeding (Ebert, 1989; Stehfest et al., 2014). Increased water temperatures may aid the efficiency of such biological processes, as a result of elevated core body temperatures (Williams et al., 2012).

On Patagonian (Lucifora et al., 2005) and Californian (Ebert, 1989) coasts, researchers observed the seasonal use of embayments by sevengill sharks, for the purpose of mating and parturition. In contrast, the seasonal occurrence of sevengill sharks in Willapa Bay, Washington, and both Derwent and Norfolk Bay, Tasmania, was attributed to the exploitation of seasonally abundant prey species such as gummy sharks, and spiny dogfish, *Squalus acanthias* (Barnett et al., 2010b,c; Williams et al., 2012). This information suggests that sevengill sharks use different habitats to carry out specific biological processes, and migrate between locations to perform them.

Identifying the biological purpose of sevengill shark habitat use in southern New Zealand was not the focal objective, nor within the scope of the current study. Some inferences can be made, however, by considering the distributions of the prey of the sevengill sharks at Ōtākou and Te Whaka ā Te Wera. At Ōtākou, a number of fish species seasonally migrate into the harbour, using shallow, protected areas as nurseries and feeding grounds (Boyd, 2008; James et al., 2010). Known populations of sevengill shark prey species, such as spiny dogfish, (Barnett et al., 2010c), and elephant fish, *Callorhinchus milli* (Crespi-Abril et al., 2003), occur at Ōtākou (Boyd, 2008), whilst other small shark species such as rig, *Mustelus lenticulatus,* and school shark, *Galeorhinus galeus,* occur on a seasonal basis for the purpose of parturition (Boyd, 2008). Flatfish, a further potential prey (Crespi-Abril et al., 2003), including speckled sole, *Peltorhamphus latus,* sand flounder, *Rhombosolea plebeia,* and greenback flounder, *Rhombosolea tapirina,* demonstrate peak abundances of juveniles during summer at Ōtākou (Roper & Jillett, 1981). At Te Whaka ā Te Wera, the narrower seasonal temperature range may mean that seasonal changes in prey abundance are less pronounced. For example, blue cod, one of the most abundant fish

species in the inlet, are present in high numbers year round (James et al., 2004). A more consistent supply of prey could explain why sharks are present throughout the year. As blue cod are endemic to New Zealand (Pankhurst & Conroy, 1987), no literature has yet described the species as sevengill shark prey, though their consumption is not unlikely given the wide range of known prey species consumed by sevengill sharks (Ebert, 2002). Furthermore, the consistent occurrence of sevengill sharks at Te Whaka ā Te Wera may be influenced by the presence of salmon farms, located at Big Glory Bay; a divaricating arm within the inlet. Anecdotally, sevengill sharks aggregate throughout all seasons around the two aquaculture facilities. The presence of spiny dogfish are also found at Te Whaka ā Te Wera, with high abundances occurring in autumn (James et al., 2004).

Diet studies may provide a useful technique to explore such predator-prey relationships (Barnett et al., 2010d) in southern New Zealand. In south-east Tasmania, stomach flushing and stable isotope sampling determined that local sevengill shark populations exploit seasonally abundant prey species (Barnett et al., 2010d; Abrantes & Barnett, 2011). These methods would provide greater insight into the predator-prey relationships of sevengill sharks at Ōtākou and Te Whaka ā Te Wera.

Cloud cover was also identified as a significant predictor when assessing the likelihood of sighting a sevengill shark, with sharks more likely to be sighted at higher cloud cover. This conclusion is supported by the predatory behaviour of sevengill sharks observed elsewhere. For example, in Humboldt Bay, California, sharks were observed foraging most actively during overcast days, or nocturnally (Ebert, 1991). In Luderitz Lagoon, Namibia, sevengill sharks displayed very little response to extensive chumming from shore during daylight hours, contrasting with very active approaches at night (Ebert, 1991). In addition, fine scale predator-prey studies conducted in south-east Tasmania, suggest that sevengill sharks are nocturnal feeders, that may opportunistically prey on animals during daytime encounters (Barnett et al. 2010a).

The negative trend associated with an increase in measured sea state and the likelihood of sighting sevengill sharks, may be interpreted in a number of ways. One explanation is that sharks did not alter their behaviour according to sea state, but the ability to detect the animals was affected. Incorporating quality underwater video equipment into future sampling methods would decrease this human sampling bias (MacCauley et al., 2012). Alternatively, the increasingly poor environmental conditions associated with higher sea state may have

affected the ability of sevengill sharks to detect the presence of food in disturbed water conditions. In white sharks, behavioural research suggests that abiotic factors that affect chemoreception and vision will likely influence predator-prey dynamics (Hammerschlag et al., 2006). Therefore, if factors such as water flow and water clarity were influenced by an increased sea state, the ability of sevengill sharks to detect the chum and bait may have been compromised. To clarify the effects of sea state, it is recommended that water clarity be included as a factor in future studies of distribution of sevengill sharks.

The total length measurements of sevengill sharks observed in this study ranged between 1.3 m and 2.8 m. In Anegada Bay, Argentina, researchers used the presence of juvenile sevengill sharks caught in a recreational fishery, as an indicator that the embayment was used as a nursery area (Lucifora et al., 2005). The total length at maturity for male sevengill sharks has been determined as 150 – 180 cm, and for females, between 220 - 250 cm (Ebert, 2002; Lucifora et al., 2005). In Tasmania, applying the same premise, researchers concluded that sevengill sharks did not use sampled coastal embayments as nurseries or pupping areas (Barnett et al., 2010a). Sevengill sharks are one of the most highly fecund shark species; reproductive studies have observed from 59 to 107 mature oocyctes in individual reproductive females (Ebert, 1996; Lucifora et al., 2005). Given the required energy consumption for growth by juvenile sevengill sharks, and the relatively high abundance of neonates and juvenile sevengill sharks expected in nursery and pupping areas, a lack of encounters would suggest that these animals were not present within the study area (Barnett et al., 2010a; Ebert, 2002). The absence of smaller sevengill sharks (< 0.8 m; Barnett et al. 2010d) therefore suggests that Ōtākou and Te Whaka ā Te Wera were not utilised as parturition or nursery areas. In addition, no sevengill sharks were sighted with fresh wounds, which are used as an indicator of active mating (Ebert, 1996). An alternative explanation to the absence of smaller sized sharks encountered in this study, could be that the chumming methods used were not effective in attracting smaller individuals.

There was no significant difference in the sex ratio of sevengill sharks sighted throughout this study. In contrast, in south-east Tasmania, female sevengill sharks have been observed to withstand colder water temperatures, potentially even remaining in coastal embayments year round, whilst males migrate to other areas (Stehfest et al., 2014). In shark species, disparity in sex ratios has been attributed to reproductive requirements (Sims et al., 2001), and large female sevengill sharks have been observed concentrating in deeper channels, whilst smaller females and males utilise shallower areas on the periphery of bays

(Williams et al., 2012). Observations such as these suggest that sexual dimorphism drives specific responses to seasonality, or thermal tolerance (Stehfest et al., 2014). The size, or sex, of a sevengill shark may, therefore, play a role in determining its distribution (Lucifora et al., 2005; Stehfest et al., 2014). The absence of sex specific distribution patterns in this study may suggest that a more comprehensive sampling design is required. To investigate potential habitat partitioning (Speed et al., 2010), chumming should take place in the deeper channels of Ōtākou and Te Whaka ā Te Wera, as well as the shallow peripheral areas.

2.4.1 Considerations

Chumming proved successful for attracting sevengill sharks, allowing data on individual identity, length and sex to be gathered. However, as chumming was conducted from the research vessel at Te Whaka ā Te Wera and the shore at Ōtākou, some bias may have been introduced to the study. This consideration is a form of gear bias (Robson & Regier, 1964), whereby the fishing technique applied may affect the cohort of fish encountered (Kohler & Turner, 2001; McCallister et al., 2013). At Ōtākou, visualising the length, and presence of claspers of individual sevengill sharks was made more difficult by conducting surveys from shore. To minimise any potential bias in future studies, it is recommended that survey trips at Ōtākou also be conducted from a research vessel.

It is also possible that this study only captured the cohort of sevengill sharks that broke the surface following arrival to the chummed area. Researchers conducting longline fishing surveys of sevengill sharks in Tasmania, identified the possibility that smaller sized sharks were not caught by their fishing equipment, or that they avoided the area due to the presence of larger individuals (Barnett et al., 2010a). However, in the case of this study water visibility often meant sharks below the water's surface were also sighted, none of which were of juvenile length (< 0.8m TL; Barnett et al. 2010d), implying smaller class individuals were not present in these areas.

Chumming was not intended to provide an estimate of the total number of sharks at sampling sites, simply to make first attempts to describe the population, and investigate seasonal variation and relative abundance. As sevengill sharks are typically associated with demersal feeding during daylight hours (Barnett et al., 2010b), luring the animals to the surface for sampling would not give an accurate estimate of the total number of sharks in the area. Chumming was chosen over fishing techniques, such as long-lining, to reduce the

risk of injury or mortality experienced by the animals. Although under-water video was trialled to assess shark presence at Ōtākou and Te Whaka ā Te Wera, water turbidity and movement at Ōtākou meant this method was unsuccessful.

2.5 Conclusions

Sevengill sharks were monitored at two locations in southern New Zealand. The species displayed a strong seasonal pattern of occurrence at Ōtākou, with sharks only encountered in spring and summer. In contrast, sevengill sharks were sighted year round at Te Whaka ā Te Wera. Species distribution modelling suggested that the seasonal trends in sevengill shark distribution are most likely due to variation in water temperatures, as observed in populations elsewhere (Ebert, 1991; Barnett et al., 2010a). The range of temperatures observed at Ōtākou were wider than those observed at Te Whaka ā Te Wera, which may account for variation in sevengill shark distributions between the two locations. Foraging opportunities may be a reason why sevengill sharks at Ōtākou and Te Whaka ā Te Wera use these coastal habitats. In addition, there is no evidence to suggest that these locations are being used as nursery areas for sevengill sharks.

3 Chapter Three

Habitat Use and Photo Identification

3.1 Introduction

Reliable assessments of population demographics are essential for the effective management and conservation of animal species (Pine et al., 2003, Castro & Rosa, 2005). Many ecological studies use recognition of individual animals (Gibbons & Andrews, 2004) to achieve such assessments by monitoring identified animals over time (Augé et al., 2014). Through the tracking or re-sighting of individuals, information such as habitat use, distribution, survivorship, and feeding and breeding behaviours can be discerned (Block et al., 2003; Gibbons & Andrews, 2004; Heupel et al., 2007). Furthermore, estimates of population size, density, and growth can also be determined (Alexander et al., 1997; Graham & Roberts, 2007). For taxa that are elusive or poorly studied, as well as developing an understanding of the ecology of a species, individual animal recognition can help establish valuable baseline demographic estimates (Couturier et al., 2012). In addition, reliable abundance and distribution assessments can be particularly useful for evaluating the impacts of anthropogenic threats, and the effectiveness of mitigating actions (Chapple et al., 2011).

Individual animal recognition has been used in a number of ecological studies, utilising a range of species, techniques, and environments (Kohler & Turner, 2001; Gibbons & Andrews, 2004). Individuals can be recognised using natural marks, artificial tags, or a combination of both (Seber, 1982). Natural marks take advantage of the physical features of an animal; distinctions such as skin patterns and scars characterise individuals (Castro & Rosa, 2005; Auger-Méthé & Whitehead, 2007; Karki et al., 2015). Tags, however, typically consist of objects affixed externally, or implanted inside an animal's body (Kohler & Turner, 2001). On a molecular level, genetic differences and radioactive isotope tagging can also be used to distinguish among individual animals (Griffin, 1952; Stevick et al., 2001).

Photographic identification (photo-ID) of natural marks is a non-invasive recognition technique (Graham & Roberts, 2007), which uses photographs as evidence of animal sightings (Barker & Williamson, 2010; Marshall & Pierce, 2012). Typically, specific areas displaying distinguishing features on an animal's body are targeted, with researchers observing differences among individuals (Castro & Rosa, 2005; MacCauley et al., 2012).

These natural marks are only useful if they readily enable individuals to be reliably distinguished repeatedly (Auger-Méthé & Whitehead, 2007). This technique is often applied to species that are sensitive to population disturbance, or are dangerous to encounter at close-range (Graham & Roberts, 2007; Karki et al., 2015). For example, in Otago, New Zealand, researchers developed population size and distribution estimates of endangered Hector's dolphins, *Cephalorhynchus hectori,* following photo-ID surveys from small research vessels (Turek et al., 2013). Researchers used nicks and notches in the dorsal fin to identify individual dolphins (Turek et al., 2013), minimising physical disturbance to the animals and their natural behaviours. Similarly, in Chitwan National Park, Nepal, population estimates of wild tigers, *Panthera tigris*, were constructed following the use of camera stations to capture images of individual animals, which were distinguishable through their unique stripe patterns (Karki et al., 2015).

Whilst advantageous as a minimally invasive technique, the validity of photo-ID relies on the longevity of naturally occurring marks (Auger-Méthé & Whitehead, 2007). Identifying features arising from natural pigmentations, such as the spots of a whale shark, *Rhincodon typus*, or stripes of a tiger (Arzoumanian et al., 2005; Karki et al., 2015), may be more reliable than those features acquired over an animal's lifetime, such as scars or wounds (Auger-Méthé & Whitehead, 2007; Marshall & Pierce, 2012). Whilst scars or wounds may be distinctive features initially, there is potential for them to heal or alter in appearance through time (Gibbons & Andrews, 2004; Kitchen-Wheeler, 2010). In some species, however, acquired marks may be the only source of natural feature present to characterise an animal. This is the case for studies on most dolphin species, where researchers use dorsal fin nicks to identify individuals (Bejder et al., 2006; Turek et al., 2013). Without validating the longevity of naturally occurring marks, estimates of population parameters may be compromised by inaccurate identification (Marshall & Pierce, 2012). Ventral patterns of the manta ray, *Manta alfredi*, have been verified for up to 30 years (Homma et al., 1999), whilst individual whale sharks have been re-sighted up to 12 years apart (Meekan et al., 2006).

A further consideration for photo-ID techniques is the risk of misidentification of individual animals, which is typically exacerbated by the use of poor quality images (Stevick et al., 2001; Arzoumanian et al., 2005). As a result, researchers often construct quality criteria for images when applying photo-ID, to limit the likelihood of generating such inaccuracies (Stevick et al., 2001). In such cases, a number of images may be rejected prior to analysis. For example, in a photo-ID study of whale sharks at Ningaloo Reef, 28% of

photographs were rejected due to poor image clarity, composition, or angle (Meekan et al., 2006).

In contrast to natural marks, artificial tagging consists of objects affixed externally or implanted within individual animals (Kohler & Turner, 2001). Forms of tagging can include simple numbered tags, implanted chips, dyes, mutilation, and branding (Kohler & Turner, 2001). Advanced tags may also include the capability to store information, track animal movements, and document environmental conditions (Gibbons & Andrews, 2004; Robertson et al., 2011). In general, as affixing or implanting tags is at least somewhat invasive, tagging studies often require ethical approval (Wilson & Mcmahon, 2006; Yates et al., 2015).

Complications such as tag fouling and shedding, site infection, and unnecessary stress experienced by animals should be considered when implementing tagging studies (Marshall & Pierce, 2012). These issues have the potential to influence animal behaviour, survival, and reproduction thus compromising estimates of demographic parameters (Mereu et al., 2014; Best et al., 2015). In addition, tag-related complications can affect reliable recognition of an animal, such that the assumptions of analytical methods are violated (Kohler & Turner, 2001; Haddon et al., 2008). Whilst introducing a greater level of invasiveness, and thus stress experienced by an animal, tagging can provide comprehensive occurrence, distribution, and environmental information that could not otherwise be obtained through reliance on natural marks (Kohler & Turner, 2001; Pine et al., 2003). Such data are particularly useful for elusive species (Kohler & Turner, 2001; Dudgeon et al., 2015), such as the brown kiwi, *Apteryx mantelli*. In Rarewarewa, New Zealand, the population growth of the brown kiwi was investigated using two forms of tags; uniquely numbered leg bands, and subcutaneous transponders (Robertson et al., 2011).

3.1.1 Demographic Studies Using Individual Animal Recognition

In mobile marine species, population demographics are often difficult to obtain due to the nature of sampling in aquatic environments (Kohler & Turner, 2001; Newsome et al., 2010) and the limited knowledge of species' habitat use or distributions (Tyberghein et al., 2012). A number of demographic estimates are based upon fisheries data, and are thus limited by fishing effort (Bonfil, 1997; Baum et al., 2003). Estimates attained in this way may inadequately represent whole populations, particularly for large migratory species

(Graham & Roberts, 2007). Studies that implement individual animal recognition provide an alternate means to estimate demographic parameters of large marine species (Pine et al., 2003; Barnett et al., 2010b). For shark species that associate with shallow habitats or coasts, estimates of population size are often calculated using the re-sighting of natural marks, or tags (Knip et al., 2010; MacCauley et al., 2012). Using a technique known as "mark-recapture", researchers use the capture histories of individuals to estimate demographic parameters of the focal population (Lettink & Armstrong, 2003; Dudgeon et al., 2008).

Individual animal recognition has been applied to elasmobranch species, providing valuable information on demographic parameters including abundance, survivorship, fine-scale movement, and distribution patterns (Kohler & Turner, 2001; Castro & Rosa, 2005). Many species can be identified by their natural pigmentation, which researchers utilise to perform photo-ID and mark-recapture (Marshall & Pierce, 2012). Using the pattern of spots on the skin of whale sharks, researchers identified 95 individual animals over a ten year period at the island of Utila, in the western Caribbean Sea (Fox et al., 2013). Following the recognition of individuals, researchers could then assess the length, sex, and residency status of identified sharks (Fox et al., 2013). Similar studies have also been applied to zebra sharks, *Stegostoma fasciatum* (Dudgeon et al., 2008), white sharks (Graham & Roberts, 2007), and in various manta ray species (Kitchen-Wheeler, 2010; Ari, 2014). After capturing photographs of sighted animals, researchers may use computer aided spot-matching algorithms to assist in identification of individuals, as seen in studies applied to grey nurse sharks, *Carcharias taurus* (Van Tienhoven et al., 2007), and whale sharks (Arzoumanian et al., 2005).

Similarly, individual animal recognition using tagging has been used to gather demographic information on elasmobranch species (Pine et al., 2003; Hammerschlag et al., 2011). A collaborative study conducted off the Atlantic coast of North America, used scientists and volunteers to deploy individually distinct tags on 2459 mako sharks, *Isurus oxyrinchus,* over a 28 year period (Casey & Kohler, 1992). This study investigated the sex, length, distribution and recapture rate of mako sharks, using the tags as identifiers in the event of a recapture. In total, 9.4% of individuals were recaptured, from 16 different countries, with a maximum time at liberty of 8.2 years (Casey & Kohler, 1992). In Puget Sound, Washington, sixgill sharks, *Hexanchus griseus,* were implanted with acoustic tags, and tracked over two seasons (Andrews et al., 2007). In general, individual animals in this study displayed fidelity to tagging sites, indicative of a localised population; an outcome

with important implications for the future management of sixgill sharks in Puget Sound (Casey & Kohler, 1992).

3.1.2 Demographic Studies in Sevengill Sharks

3.1.2.1 Tagging

Studies in Tasmania and Washington have estimated abundance, survival, and site fidelity of sevengill sharks using re-sights of tagged individuals (Lucifora et al., 2005; Barnett et al., 2010a; Dudgeon et al., 2015). To estimate the abundance and apparent survival of sevengill sharks in Norfolk Bay, Tasmania, researchers used recapture data from individual sharks tagged with conventional tags, and acoustic transmitting tags (Dudgeon et al., 2015). Between 2006 and 2009, 263 sevengill sharks were tagged with conventional tags, whilst 25 were affixed with acoustic tags (Barnett et al., 2010a; Barnett et al., 2011; Dudgeon et al., 2015). In the third year of sampling, the median abundance of sevengill sharks in Norfolk Bay was estimated as 562, with an apparent annual survival rate of 0.86 (Dudgeon et al., 2015). Furthermore, sevengill sharks affixed with acoustic tags displayed site fidelity to coastal areas in Tasmania over multiple years, with a number of individuals returning to their original tagging location (Barnett et al., 2011). In Willapa Bay, Washington, acoustic tagged sevengill sharks displayed similar behaviour, with high instances of site fidelity detected among individuals (Williams et al., 2012). In contrast, a satellite telemetry study in coastal Tasmania showed that sevengill sharks undertook large scale migrations of up to 1000 km from tagging locations (Barnett et al., 2011; Stehfest et al., 2014).

3.1.2.2 Natural Marks

Sevengill sharks are typically deep grey in colour on the dorsal side, with numerous irregular blotches and spots extending over both the dorsal, and lighter-coloured ventral side of the animal (Ayres, 1855; Daniel, 1934; Ebert, 1985), potentially allowing for individual identification. In a series of experiments in Melbourne Aquarium, Australia, researchers used these unique blotches and spots, as well as variation in fin and body shapes, to identify individual sharks (Daly et al., 2007). In coastal regions of California, researchers recognised differences in the skin colour of sevengill sharks from different locations, though no further studies have been performed to support these observations (Ebert, 1985). To date, there are

no studies in the published literature which have explored the viability of photo identification of sevengill sharks.

Figure 3.1: Dorsal skin patterns of a sevengill shark, showing irregular blotches and spots.

3.1.3 Aims

To date virtually nothing is known about demographics of sevengill sharks in New Zealand. Given the utility of mark-recapture studies for investigating population size, survival rates, fecundity and movements of coastal shark species, it is highly desirable to develop a method for individual recognition of sevengill sharks. Therefore, the aim of this research was to establish a robust method of individual recognition of sevengill sharks in New Zealand. Furthermore, this research aimed to determine the viability of photo-ID as a less invasive alternative to tagging of sevengill sharks at Ōtākou and Te Whaka ā Te Wera, New Zealand. Site-fidelity of sevengill shark populations at Ōtākou and Te Whaka ā Te Wera, would also be explored using these developed methods.

The outcomes of this research will provide a useful assessment of the habitat use of sevengill shark populations in southern New Zealand, and demonstrate the feasibility of applying minimally-invasive photo-ID techniques to sevengill shark populations elsewhere.

3.2 Methods

At two sites in Ōtākou (Saint Leonards, Harington Point), and three sites at Te Whaka ā Te Wera (Ackers Point, Sawdust Bay, South West Arm; see Figure 2.2), sevengill sharks were attracted using chum and tuna oil. At Te Whaka ā Te Wera, sampling was undertaken on approximately three days per month, between December 2013 and May 2015. Chumming took place from the research vessel, RV Naiad, a 6.3 m rigid hulled inflatable boat. At Ōtākou, chumming took place approximately every three weeks, from July 2013 to April 2015, but due to ease of access, sampling was conducted from the harbour shore. Chumming took place at dawn and twilight, following, and at the early stages of the suggested peak in sevengill shark activity (Ebert, 1991; Barnett et al., 2010b). Once attracted, sharks were lured to the surface using a blue cod bait tied to the end of a rope, or encased within a net bag. For each shark sighting, an estimate of length and sex was attempted, reliant on a clear sighting of the individual. For full details of the survey methods see Chapter Two.

3.2.1 Tagging and Photo-ID

Following the arrival of a shark, an attempt would be made to photograph the animal's left, right, dorsal, and ventral sides. Obvious markings, such as disfigured fins or scars, were also photographed and sketched into a field notebook (e.g. Castro & Rosa, 2005). Photographs were taken using both a Nikon DSLR camera (Nikon D90, AF Nikkor 35-80mm lens) from above the surface, and a GoPro (HERO) camera held beneath the water's surface if conditions permitted.

If the shark was not already tagged, an attempt was then made to deploy a fish tag on each shark using a specially designed tagging pole. Similar to the tagging of free swimming white sharks in Australia (Bruce et al., 2006), sevengill sharks were tagged using negatively buoyant, 17 cm long Hallprint stainless steel dart tags (Figure 3.2). As the shark swam at the surface of the water, tags were inserted into the dorsal muscular area at the base of the dorsal fin. Each tag displayed a unique code number, colour combination, and either the presence or absence of three black bands (eg. Blue-green 05 |||). This was to ensure that on any future encounter, individual sevengill sharks could be easily identified. On subsequent sampling trips, the re-sighting of tagged sevengill sharks was recorded. An attempt to photograph all sharks was made, regardless of whether the shark had a tag.

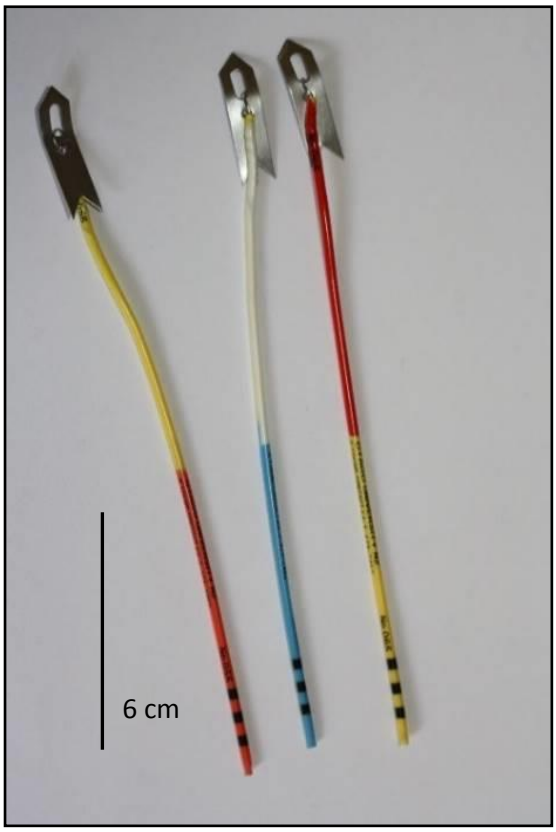


Figure 3.2: Hallprint Stainless Steel Dart Tags used to identify tagged sevengill sharks.

Figure 3.3: Appearance of a tagged sevengill shark, from the research vessel, Naiad, in Te Whaka ā Te Wera.

3.2.2 Public Engagement

In an attempt to gain more information about tagged sharks, posters were produced encouraging local water users to report any sevengill shark encounters. In elasmobranch population studies, collaborating with the public to increase the likelihood of re-sighting individuals is a commonly applied technique that is also beneficial for increasing awareness about the study species (Barker & Williamson, 2010; Marshall & Pierce, 2012).

3.2.3 Image Quality

Prior to analysis, all photographs of natural marks were assessed for quality criteria. During this process, the dorsal side of the head emerged as the most efficient region to distinguish between individuals a ambiguity resulting from photographing different sides of the same animal was avoided. Difficulties associated with individual recognition, as a result of photographing alternate sides of an animal, are not uncommon in photo-ID studies (Stevick et al., 2001). The head of the sevengill shark was also the most visible area of the animal during sampling, so focussing on this region simplified methods for future sampling

occasions. Considering this, if images did not meet the following criteria, they were disregarded and not included in further photo-ID analysis.

1) The image was in sharp focus with no blur, light reflection or artefacts in the target region.

2) Photographs clearly depicted the dorsal region of the shark, from the tip of the nose to the line of the seventh gill slits, with markings plainly visible.

Figure 3.4 shows examples of the quality criteria. Images A and C meet both criteria and would be accepted for further analysis. Image B fails to meet criteria 1, and D fails to meet criteria 2; therefore both would be discarded.

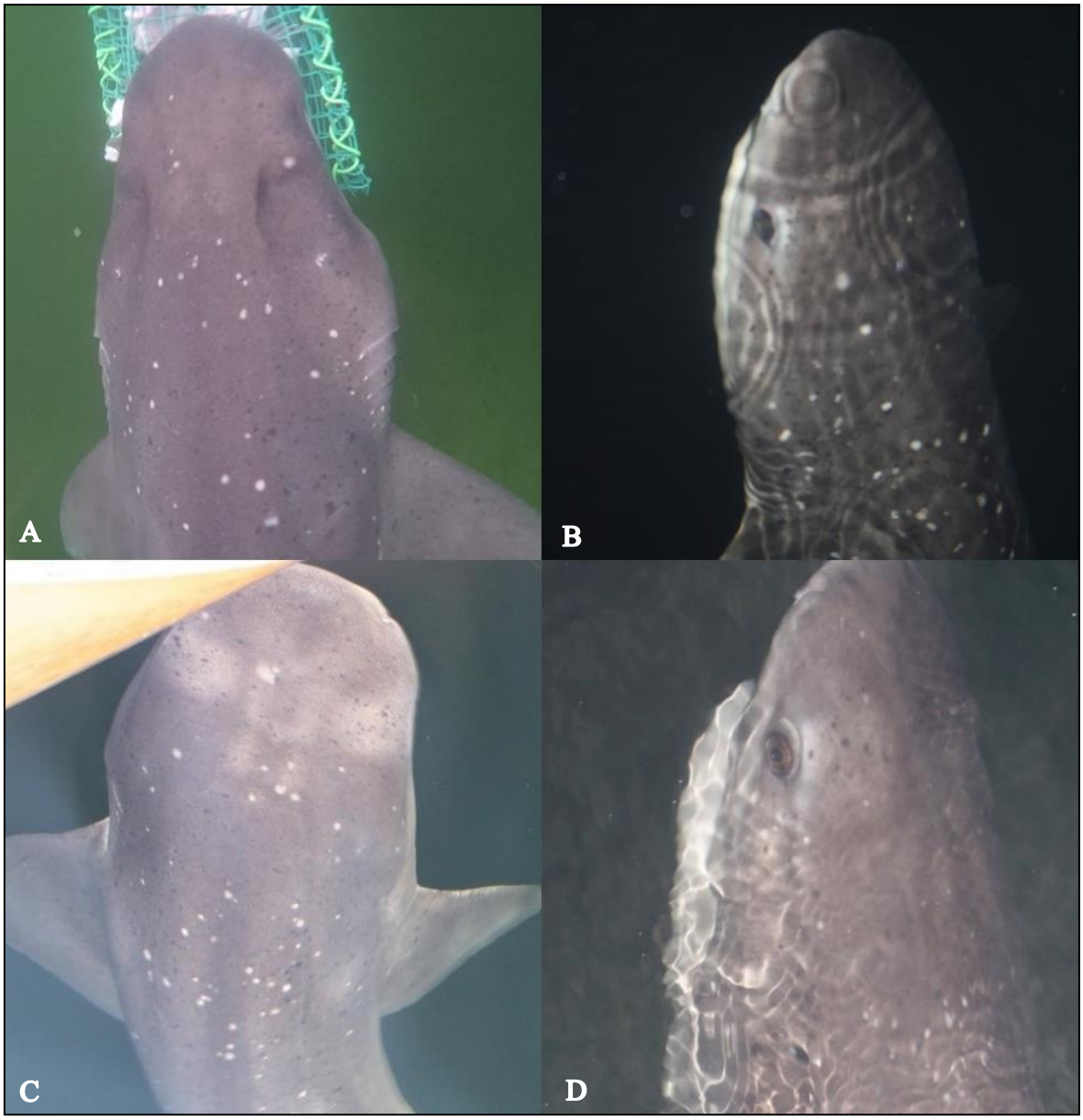


Figure 3.4: Examples of suitable images to either meet or not meet the outlined photo-ID quality criteria for sevengill shark photo-ID. Clockwise from the left: Image A - meets Criteria 1, Image B - does not meet Criteria 1, Image C - meets Criteria 2, Image D - does not meet Criteria 2.

3.2.4 Pattern Permanence

Due to the short duration of this study, the ability to establish permanency of natural marks in sevengill sharks was limited. Therefore, photographs of two resident sevengill sharks at the National Aquarium of New Zealand, in Napier, were used to investigate the stability of natural skin-patterns over time, comparing images captured in 2010 and 2015. The images were kindly supplied by a local enthusiast, Quentin Bennett. In order to make

fair comparisons between images, two regions on the dorsum of each animal were chosen and compared.

3.3 Results

From a total of 71 survey trips between July 2013 and May 2015, sevengill sharks were encountered on 38 trips. During these 38 trips there was a total of 141 encounters with sharks.

*Table 3.1: Tagged, Photo-ID and re-encountered sevengill sharks in Te Whaka ā Te Wera, New Zealand, between February 2014, and March 2015 (*tag 15 Red-Green, located on Moeraki Beach, Ōtākou). Check marks (✓) indicate the means of initial tagging, and the means of recognition used upon re-encounters.*

Shark Information			**Initial Tagging**			**Re-encounter 1**			**Re-encounter 2**		
Location Encountered	**Shark ID**	**Tag ID**	**Date**	**Tag**	**Photo-ID**	**Date**	**Tag**	**Photo-ID**	**Date**	**Tag**	**Photo-ID**
Te Whaka ā Te Wera	T25	35 Red-White	20 Feb 2014	✓		21 Feb 2014	✓				
Te Whaka ā Te Wera	T58	29 Yellow-Red	3 Apr 2014		✓	22 Jul 2014		✓	23 Jul 2014	✓	✓
Te Whaka ā Te Wera	T40	63 Blue-Red ‖‖	5 Apr 2014	✓		5 Apr 2014	✓				
Te Whaka ā Te Wera	T01	23 Green-Orange	22 Jul 2014	✓	✓	6 Nov 2014	✓	✓	11 Mar 2015		✓
Te Whaka ā Te Wera	T75	62 White-Red ‖‖	5 Nov 2014		✓	6 Nov 2014	✓	✓			
Te Whaka ā Te Wera*	T80	15 Red-Green	6 Nov 2014	✓		25 Mar 2015	✓				

3.3.1 Tagging

At Ōtākou, sevengill sharks were encountered on 8 out of 26 survey trips. From 19 encountered sharks, two were tagged, with no re-sights observed. At Te Whaka ā Te Wera, sevengill sharks were encountered on 30 out of 45 survey trips. From 122 encountered sharks, 53 individuals were tagged, of which two (3.7%) were clearly re-sighted. These two

tag re-sights took place during the same three day research period, either on the evening of the same day, or the following morning (Table 3.1).

A further two tagged sevengill sharks were re-encountered on subsequent sampling trips, but due to tag deficiencies, these encounters were not considered explicit tag re-sights (Table 3.1; Figure 3.5, Figure 3.7). The first individual, T80, was tagged on the 6th of November 2014, at Te Whaka ā Te Wera (15 Red-Green; Table 3.1). On the 25th of March 2015, a member of the public found the tag, unattached, and washed up on Moeraki Beach, approximately 300 km north of Te Whaka ā Te Wera (Figure 3.5). The shark was not located. The second re-encountered individual, T01 (23 Green-Orange; Table 3.1), displayed evidence of tag bio-fouling, but was also recognised using photo-ID, and is thus described in Section 3.3.2 (Figure 3.7).

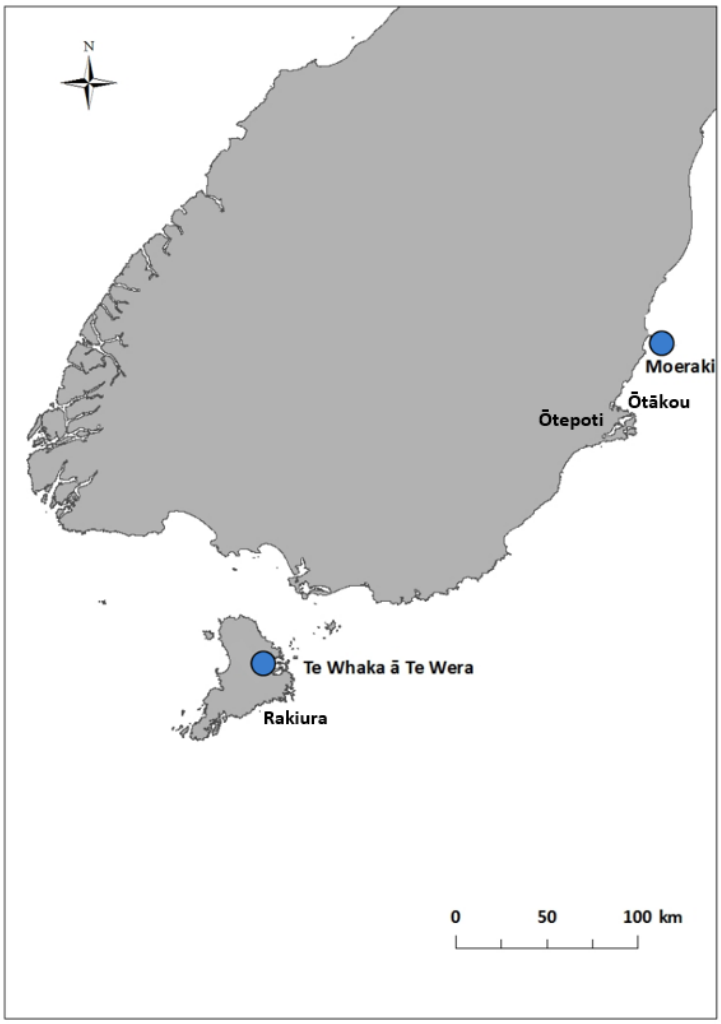


Figure 3.5: Original tagging location of the sevengill shark, T80, tagged on the 6th of November, 2014, in Te Whaka ā Te Wera, and the location of the recovered tag, on the 25th of March, 2015, at Moeraki Beach.

3.3.2 Photo-ID

An attempt was made to photograph every sevengill shark that was encountered throughout this study, however, due to failure to meet the image quality criteria, 87% (150 total) of photographs were omitted prior to photo-ID analysis. Thus, using natural marks and photo-ID, 23 individual sharks were identified at Ōtākou and Te Whaka ā Te Wera. These sharks all displayed distinct, unique marks that could be utilised for recognition of individuals. Two of these sharks were photographed at Ōtākou, with no re-sights on any future survey. At Te Whaka ā Te Wera, 21 sevengill sharks were photographed, and three (14.3%) were re-sighted, one of which (shark ID T57) occurred only during the same three day sampling period (Table 3.1).

Two sharks were successfully re-sighted in different sampling periods using photo-ID (Table 3.1). T58, was first photographed on the 3rd of April 2014, then re-sighted on the 22nd of July, 2014 (Figure 3.6), and then again on 23rd July 2014 (Table 3.1), a total of 111 days at liberty.

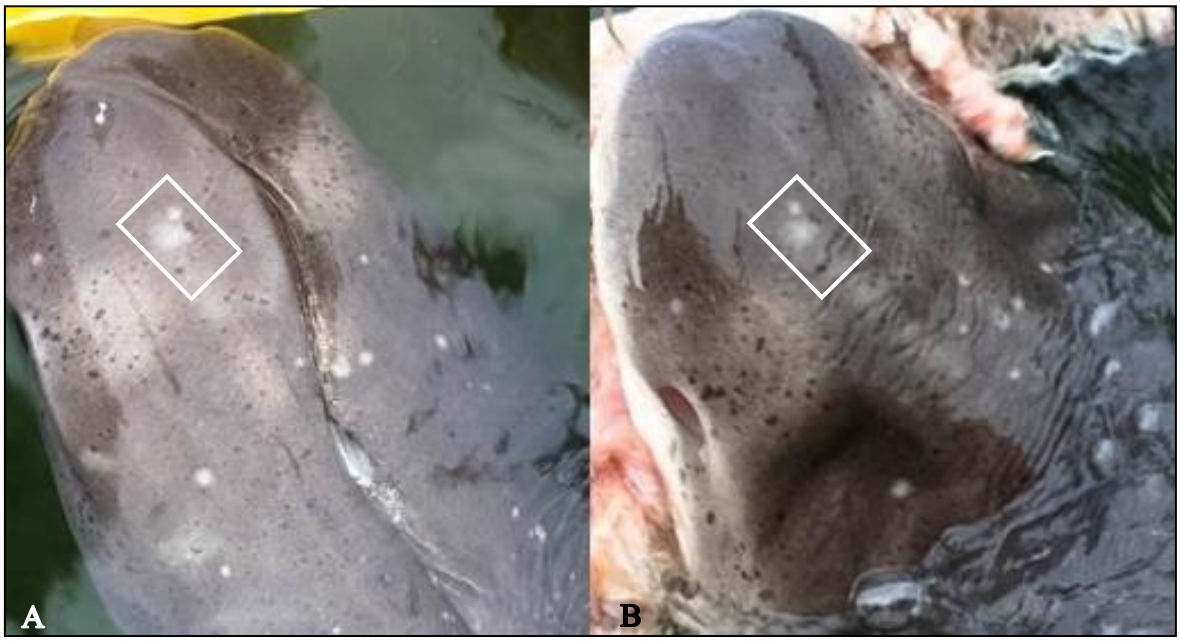


Figure 3.6: T58, photographed on the 3rd of April, 2014 (Image A), and re-sighted 22nd of July, 2014 (Image B) in Te Whaka ā Te Wera.

The shark T01 was tagged and first photographed on the 22nd of July 2014 at Te Whaka ā Te Wera (23 Green-Orange; Table 3.1; Figure 3.8). On the 6th of November, 2014, the shark was re-sighted in Te Whaka ā Te Wera, and re-photographed. Although the tag was visible on this occasion, it was fouled with what appeared to be green algae and hence could not be read correctly (Figure 3.7). Following this, on the 11th of March 2015, T01 was photographed on a third occasion (Figure 3.8), by a chance encounter on a wharf at Te Whaka ā Te Wera (9 km from the original tagging location), where a member of the public had fished the animal from the water. On this occasion the tag was absent from the animal. In addition, over the three encounters of the shark, T01 displayed healing of a dorsal fin wound (Figure 3.7, Figure 3.8: Image C, Image D).

Figure 3.7: T01, photographed on the 6th of November 2014 in Te Whaka ā Te Wera. Image displays bio-fouling of the tag; compromising identification.

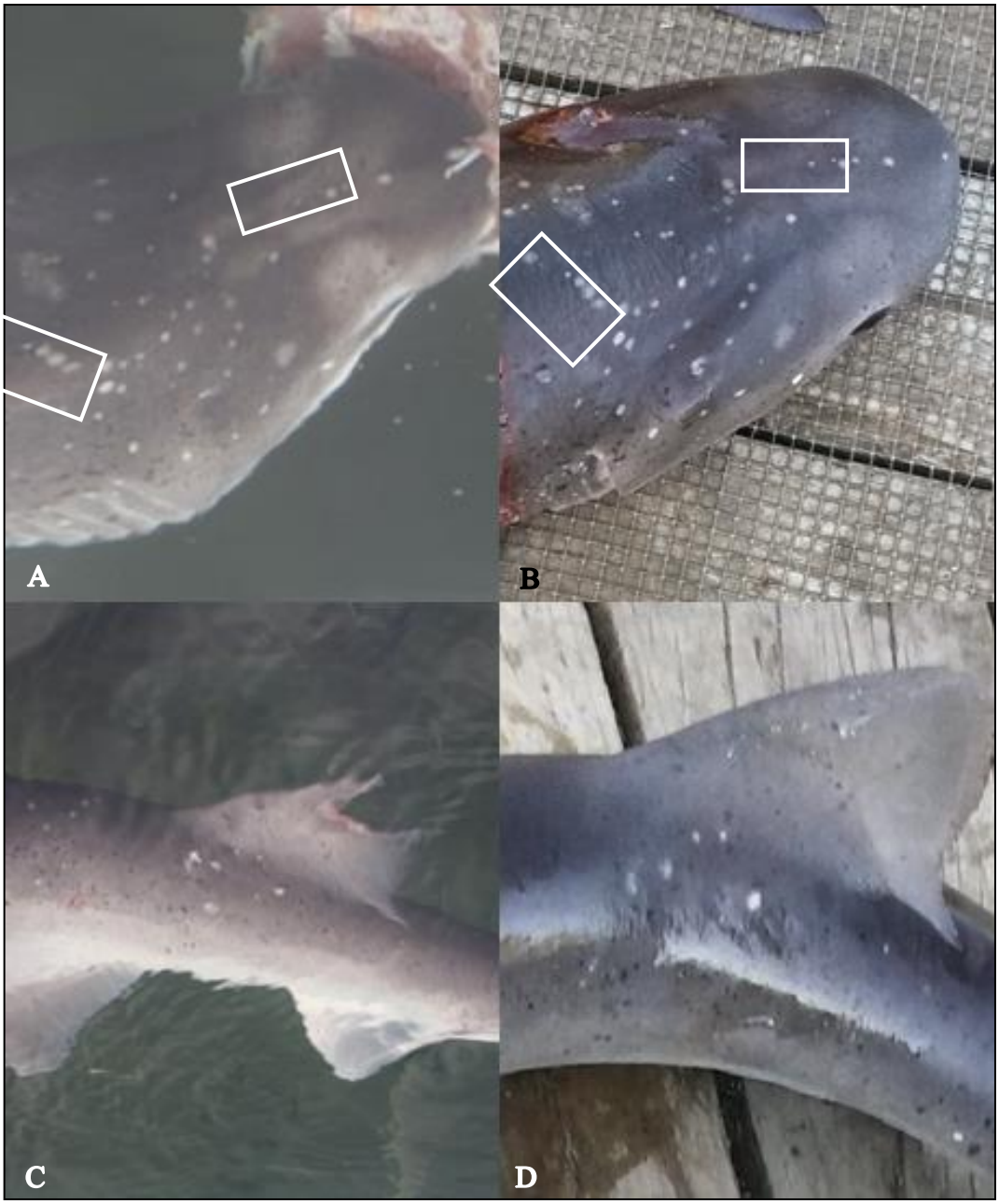


Figure 3.8: T01, photographed on the 22nd of July 2014 (Image A, Image C) and 11th of March 2015 (Image B, Image D), in Te Whaka ā Te Wera. Image C and Image D display the extent of dorsal fin healing between July 2014 and March 2015.

3.3.3 Pattern Permanence

Between 2010 and 2015, the two individual sevengill sharks photographed in the National Aquarium of New Zealand, displayed no visual change in natural skin patterns. The permanence of these patterns is demonstrated in Figure 3.9. Close inspection of the skin patterns suggests that the black and white spots on the dorsal surface of both sharks were persistent over the five year period between photographs.

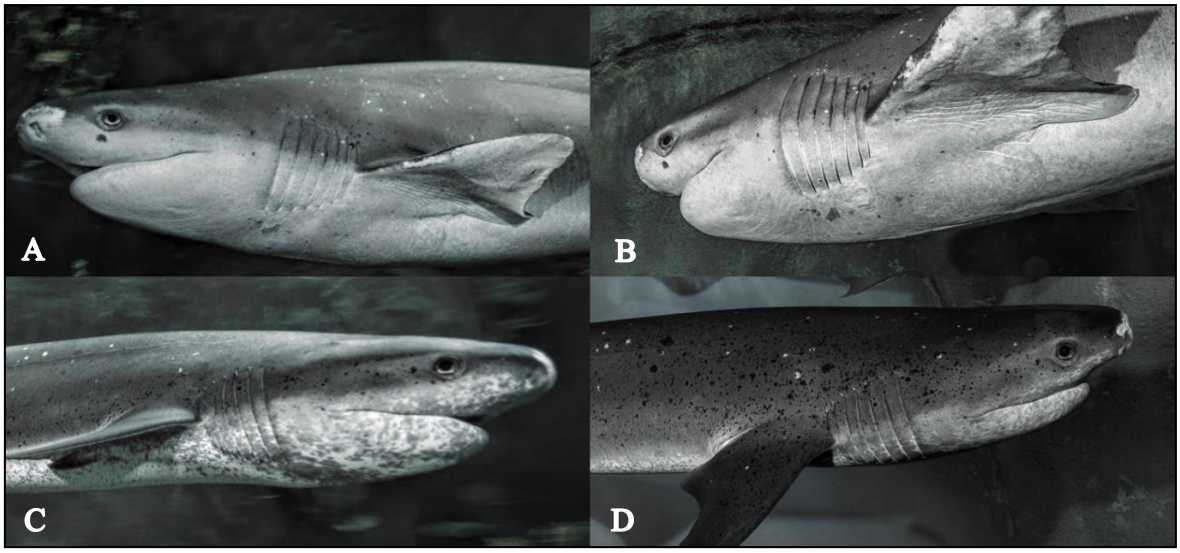


Figure 3.9: Sevengill sharks in the National Aquarium of New Zealand, Napier. Image A and Image B display Spot, photographed in 2010 and 2015, respectively. Image C and Image D display Speckles, photographed in 2010 and 2015, respectively. Photographer: Quentin Bennett.

3.4 Discussion

During this study, despite tagging more than 50 sevengill sharks with dart tags, only two (3.7%) sound re-sights were observed. Low re-sight or recapture rates are not uncommon in shark tagging studies, though identifying the cause of such outcomes can prove to be challenging (Kohler & Turner, 2001). In a review of 52 shark tagging publications, Kohler & Turner (2001) found that the recapture rate was less than 5% in more than half of the studies. In shark species, modest tag re-sight rates can be a result of a variety of factors, such as tag shedding, tag bio-fouling, mortality, migration, fishing pressure, and incorrect individual identification due to human error (Pepperell, 1990; Schwarz & Arnason, 1990; Kohler & Turner, 2001).

Tag shedding has been identified as one of the most critical influences on tag recapture rates (McFarlane et al., 1990), with the potential to introduce severe bias to demographic estimates (Cowen & Schwarz, 2006). In shark species, rates of tag loss have been found to vary with tag type, capture method, and tag positioning or placement (Kohler & Turner, 2001; Gibbons & Andrews, 2004). Often, to account for tag loss in demographic studies, researchers perform "double tagging", whereby two alternate methods of tagging, or two tags, are deployed on the same individual (Cowen & Schwarz, 2006). Based on the presence of tagging scars, researchers in Tasmania estimated an 18% shed rate of conventional tags

by sevengill sharks in one study, and a 16% shed rate based on conventional and acoustic tags in a further study (Dudgeon et al., 2015). In the latter case, the surgically inserted acoustic tags were retained in all of the recaptured animals (Dudgeon et al., 2015).

In the current study, evidence from re-sights of the sevengill shark T01, demonstrated conclusively that tag loss could occur in this population. Unfortunately, due to the low number of re-sights over the course of the study, the rate of tag loss could not be accurately estimated. In this case, it is possible that a combination of the tag type and tag placement contributed to the likelihood of tag loss. As described above, surgically inserted tags were more successfully retained on sevengill sharks in a Tasmanian study, in comparison to externally placed conventional tags (Dudgeon et al., 2015), as used in this study. Furthermore, although less invasive than hooking, or capture, deploying tags on free swimming sharks has been reported as increasing the likelihood of tag shedding (Hammerschlag et al., 2011); probably due to the reduced probability of a successful tag insertion. A further consideration is deliberate removal of tags by the animals themselves; lemon sharks have been observed scraping their bodies against the sea floor, presumably in an attempt to remove inserted tags (Hammerschlag et al., 2011).

The capture history of shark T01 at Te Whaka ā Te Wera also demonstrated the occurrence of tag bio-fouling. Though still intact, the re-sighted tag was unable to be identified in the free swimming shark four months after initial tagging, due to an accumulation of biofilm which prevented tag recognition. Similarly, in south-east Queensland, researchers observed heavy fouling of dart tags only a few months after the initial tagging of zebra sharks (Dudgeon et al., 2008). Bio-fouling and tag loss have serious implications for mark-recapture studies, as they impair the ability for researchers to recognise individuals upon re-sighting an animal (Cowen & Schwarz, 2006; Dudgeon et al., 2008).

As an alternative to tagging, the use of natural marks and photo-ID has become increasingly common in studies of elasmobranch populations (Castro & Rosa, 2005; Barker & Williamson, 2010). These methods avoid some of the complications traditionally associated with tags, such as shedding, or bio-fouling (Marshall & Pierce, 2012). Using photo-ID, though a smaller number of sevengill sharks were originally identified, the re-sight rate was higher for sharks using natural marks, than sharks marked with dart tags in this study. Photo-ID of natural marks has been recognised as an effective non-invasive tool

for demographic studies of marine populations, particularly for species that repeatedly aggregate in certain areas (Marshall & Pierce, 2012; Hoogenboom et al., 2015). In this study, the recognisable marks present on the head region of each animal were unique to the individual, allowing for successful identification of 23 sevengill sharks, and re-sights of three of these animals (14.3%).

The use of strict photo-ID quality criteria is essential for minimising bias in elasmobranch demographic studies (Marshall & Pierce, 2012). This criteria reduces human error and subjectivity, ensuring the most robust results possible (Marshall & Pierce, 2012). In the current study, whilst the quality criteria disallowed the inclusion of a large number of images in photo-ID analysis, more confidence can be placed in the final results. The large proportion of discarded images (87%) reflects the difficulties associated with photographing mobile marine animals, and the stringent adherence to the photo-ID quality criteria. As survey trips were conducted at dawn and dusk, light conditions were often poor, contributing further to the challenges of obtaining quality photographs. Furthermore, as observed in this study, photographers become more skilled over time, therefore decreasing the likelihood of capturing images that do not meet the photo-ID quality criteria. It is suggested that this bias be accounted for in future by using experienced photographers from the beginning of the study.

A useful outcome resulting from this study was the evidence for longevity of naturally occurring marks in sevengill sharks. One individual in the wild was identified approximately eight months after initial tagging (T01), by comparing photographs captured on both occasions. In addition, the spot patterns and colouration observed in the two individuals at the National Aquarium of New Zealand were seen to be stable between 2010 and 2015. Permanent marks are a key assumption for many mark-recapture analyses (Jolly, 1982; Auger-Méthé & Whitehead, 2007). The documented re-growth of the dorsal fin observed in T01, suggests that alternate natural marks such as scars and wounds should not be used as identifiers in sevengill sharks, as they are not consistent in their appearance over time. This outcome is supported by observations in nurse sharks and manta rays, where significant healing of scars and missing tissue occurred over a short time span (Pratt & Carrier, 2001; Kitchen-Wheeler, 2010). Albeit at low rates, the small number of re-sights observed in this study provide the first evidence for site fidelity of sevengill sharks in New Zealand. Elsewhere, sevengill shark populations have displayed high levels of fidelity to coastal areas (Barnett et al., 2010a; Barnett et al., 2011). In Tasmania, sevengill sharks displayed strong

seasonal site fidelity over a number of seasons (Barnett et al., 2010a; Barnett et al., 2011; Awruch et al., 2014; Stehfest et al., 2014). Similarly, in Willapa Bay, Washington, 70-90% of sharks returned to their tagging location over the two summers following tagging events (Williams et al., 2012).

In animal tagging studies, low recapture rates can be indicative of highly abundant populations, or of a population containing migratory individuals (Dudgeon et al., 2015). Thus, at Ōtākou and Te Whaka ā Te Wera, the low re-sight rates of both naturally marked and tagged sevengill sharks, could suggest that the populations are very large and mobile. The discovery of the tag belonging to T80, approximately 150 km north of its original tagging location at Te Whaka ā Te Wera, provides evidence that this migratory behaviour is plausible. In studies elsewhere, migrations of sevengill sharks have been documented exceeding distances of 1800 km (Williams et al., 2012). Using pop-up archival satellite tags in coastal areas of southern Tasmania, researchers observed northern migrations of all tagged male sevengill sharks to areas of warmer water temperatures, whilst most female sharks remained close to their original tagging location. Two of the five female sharks did, however, move out to the southern edge of the Tasmanian Shelf before returning to the coastal area (Stehfest et al., 2014). In north-west USA, sex-specific migrations were also detected, but with the opposite pattern, with females undertaking long distance coastal movements to warmer waters (Williams et al., 2012). In the current study, T80, a male sevengill shark, was tagged in late spring at Te Whaka ā Te Wera, followed by a migration north where its tag was presumably shed sometime during the summer months at Moeraki Beach. Sex-specific differences in migratory behaviour of sevengill sharks have been attributed to factors such as thermal tolerance, reproduction requirements, and spatial resource partitioning (Springer, 1967; Williams et al., 2012) and can have important implications when devising future strategies for population management (Stehfest et al., 2014).

The paucity of re-sights observed in this study meant estimating population demographics was not possible. Furthermore, tag loss and bio-fouling directly violate crucial mark-recapture assumptions (Lettink & Armstrong, 2003; Cowen & Schwarz, 2006). To account for tag loss, researchers often perform double tagging, where a second tagging technique is used as an alternate identification source (Cowen & Schwarz, 2006). In the current study, though both tagging and photo-ID were carried out, a combination of small sample sizes and demographic factors lead to a small number of re-sights, compromising

the ability to draw firm conclusions from the data. Higher intensity or effort aimed at gaining photo-ID data would be required before attempting mark-recapture analyses. In addition, to increase the probability of recapture, under-water video techniques could be developed.

A number of beneficial outcomes did develop from the current study, which will assist in the design of future research into sevengill shark demography. Foremost, the use of photo-ID of natural marks for individual recognition of sevengill sharks is a viable technique that could be used in future studies. Using photographs of the head region is an effective means to identify individuals and reduce stress on sharks through traditional tagging. This method also has the potential to develop community engagement and awareness; a small number of citizen-science projects elsewhere use photo-ID to identify individual sevengill sharks, using images uploaded to public websites (Ocean Sanctuaries' Sevengill Shark Sightings, n.d.; Save our Seas Foundation, 2016).

Researchers have successfully used acoustic tagging of sevengill sharks on the coasts of USA and Tasmania, to investigate the abundance, survival, site fidelity, and fine-scale spatial use of local populations (Barnett et al., 2010b; Williams et al., 2012; Dudgeon et al., 2015). Acoustic tagging can improve the precision of demographic estimates that would otherwise be devised from sevengill shark encounters (Dudgeon et al., 2015). Acoustic tagging would therefore be a suitable supplementary technique to the use of natural marks, and would provide the opportunity for a more extensive assessment of photo-ID and the feasibility of its application in sevengill shark populations. Furthermore, to investigate the potential long-distance migrations of sevengill sharks as identified in the current study, satellite tagging could be considered. Satellite tags were successfully used on sevengill sharks from coastal areas of Tasmania, demonstrating both localised and long-distance movements of individuals (Barnett et al., 2011; Stehfest et al., 2014). In addition, advanced tagging techniques can provide ecological information, such as water temperatures, diel movement patterns, and water column use (Barnett et al., 2010b).

3.5 Conclusions

The results of this study contribute to the ecological understanding of sevengill shark populations in southern New Zealand, whilst also highlighting some of the difficulties associated with conducting tagging and photo-ID studies on sharks. Photo-ID was demonstrated as a valid technique for individual recognition of sevengill sharks, but does

require refinement. With development, it may successfully stand as a less-invasive alternative to traditional tagging. Re-sights suggest that sevengill sharks at Ōtākou and Te Whaka ā Te Wera display some philopatric behaviour. In addition, re-sights also indicate that sevengill shark populations are large, with individuals potentially undertaking long-distance transits. Ultimately, this study has helped to identify the methods required to fully comprehend the demography and behaviour of sevengill shark populations at Ōtākou and Te Whaka ā Te Wera.

4 Chapter Four

Genetic Connectivity in Shark Species

4.1 Introduction

4.1.1 Population Genetic Diversity

Genetic diversity is a fundamental concept in conservation and evolutionary biology, which allows for the adaptation and resilience of populations to environmental change (Frankham, 1996; Larson et al., 2011). Particularly for vulnerable species, an understanding of genetic diversity and connectivity among populations is crucial to the development of sufficient conservation measures (Reed & Frankham, 2003).

Researchers assess genetic structure and gene flow among populations by sequencing genomic regions, or markers, of extracted DNA (deoxyribonucleic acid; Tero et al., 2003; Hale et al., 2012). This technique is also used for species identification and to assess taxonomic relationships (e.g. Ward et al., 2005; Mabragana et al., 2011). Markers are positioned at specific loci throughout the genome and are flanked by primers, which are used to visualise target regions (Vignal et al., 2002). By comparing variations within markers of individual animals, the likelihood of gene flow, and thus the mixing potential between two populations, can be assessed (Lowe & Allendorf, 2010).

In northern Finland, genetic variation among seven populations of plant, *Silene tatarica* was investigated using nuclear markers, finding low levels of gene flow between clusters from different sites along the Oulankajoki River (Tero et al., 2003). In addition to variation among sites, no apparent within site diversity was detected, suggesting the presence of seven sub-populations, with gene flow occurring through a few long-distance dispersal events (Tero et al., 2003). In south-west France, researchers identified two genetic cohorts among a single population of roe deer, sampled from a 55 x 40 km hilly region (Coulon et al., 2006). This study concluded that landscape features which inhibit distribution, can influence population differentiation (Coulon et al., 2006).

4.1.2 Elasmobranch Genetic Connectivity

Since the first study of elasmobranchs utilising genetics (Smith, 1986), advancements in technology and affordability, and a demand for conservation management and stock assessments, have resulted in rapid development and accessibility of genetic techniques (Dudgeon et al., 2012). Many genetic based studies have used specific mitochondrial and nuclear DNA markers to examine gene flow, and thus, the migratory status of mobile shark populations (Table 4.1).

Migrations by shark populations are often complex (Castro et al., 2007), and can be attributed to reproduction, or seasonal shifts in prey distribution and abundance (Springer, 1967; Knip et al., 2010; Speed et al., 2010). Assessing the migratory status of shark populations has traditionally proven difficult, due to the high mobility, and undefined distribution range of many species (Bonfil et al., 2005; Newsome et al., 2010). With the development of sophisticated telemetric techniques such as acoustic and satellite tagging, investigating shark movements has become easier, but high associated costs and limited baseline distribution data often restricts their application (Gibbons & Andrews, 2004). A cost-effective, and viable alternative to assessing the migratory status of shark species is the characterisation of population genetics (Dudgeon et al., 2012). By sequencing and comparing variation of selected genomic regions, the likelihood of connectivity among sampled populations can be discerned (Lowe & Allendorf, 2010). Furthermore, using only small amounts of animal tissue, additional demographic information such as kinship, abundance, evolutionary relationships, and parenthood, can be ascertained for shark populations via genetic analyses (Dudgeon et al., 2012).

4.1.3 Genetic Mixing Potential of Shark Species

In general, large, highly mobile shark species such as basking sharks and whale sharks, display low genetic diversity on a global scale; indicative of gene flow among worldwide populations (Duncan et al., 2006; Hoelzel et al., 2006; Castro et al., 2007). Smaller shark species that may be incapable of larger scale, or trans-oceanic migrations, such as grey nurse sharks and zebra sharks, typically consist of distinct regional, or localised populations and therefore display greater genetic diversity on a global scale (Dudgeon et al., 2008; Ahonen et al., 2009; Table 4.1). Exceptions to these patterns occur when migratory species are prevented from population mixing due to oceanic barriers, such as deep water trenches,

strong currents, or land masses (Duncan et al., 2006; Dudgeon et al., 2009). Furthermore, when typically large scale, migratory animals exhibit philopatric behaviour (returning and re-using specific areas over time), genetic structure among conspecifics may develop (Duncan et al., 2006). An example of such behaviour can be observed in populations of white sharks in Australia (Blower et al., 2012). Whilst capable of extensive trans-oceanic migrations (Jorgensen et al., 2009), white shark populations from eastern and south-western coasts of Australia displayed site fidelity to reproductive areas, resulting in distinct genetic structure between the two populations (Blower et al., 2012; Table 4.1). Similarly, juvenile bull sharks sampled in northern Australia, displayed high levels of genetic diversity among sites revealed by analysis of mtDNA sequences (Tillett et al., 2012; Table 4.1). Despite being capable of movements exceeding 1500 km (Brunnschweiler et al., 2010; Carlson et al., 2010), this study found evidence to support reproductive philopatry of female bull sharks, influencing the diversification in juvenile mtDNA sequences sampled from different areas (Tillett et al., 2012).

Long-distance movement potential does not, therefore, assure lack of genetic structure, or diversity, among shark populations (Jorgensen et al., 2009). These examples show that migration ability does not necessarily predict genetic diversity among shark populations (Eckert & Stewart, 2001; Wilson et al., 2006; Tillet et al., 2012). This observation underlines further the complexities of evaluating the connectivity, and therefore migratory status of shark species (Castro et al., 2007). Factors such as species-specific migratory and homing behaviours, cohorts sampled (e.g. juveniles versus adults), and genomic regions assessed, can also influence the detected levels of genetic structure among sampled shark populations (Hueter et al., 2004; Dudgeon et al., 2012; Tillett et al., 2012).

Identifying the genetic mixing potential of shark populations has important implications for species conservation (Blower et al., 2012). Foremost, genetically isolated populations are more susceptible to detrimental effects following population decline than those that are open to genetic mixing (Blower et al., 2012). Thus, localised shark populations are less likely to recover from declines caused by targeted fishing, bycatch, or alternate means of anthropogenic pressure (Andrews et al., 2007; Knip et al., 2010). Naturally, populations that have experienced bottlenecks, or events that severely reduce the size of a population, are also susceptible to these adverse effects (Cristescu et al., 2010).

4.1.4 Nuclear versus Mitochondrial DNA

The function of genetic sequencing can differ between mitochondrial DNA (mtDNA) and nuclear DNA, due to the structure and mode of inheritance of the genetic material (Springer et al., 2001). Mitochondrial DNA is located in the mitochondria of eukaryotic organisms, consisting of maternally inherited, haploid genes (Palumbi & Baker, 1994). In contrast, nuclear DNA is found in the nucleus of eukaryotic cells, is inherited bi-parentally, and is thus diploid (Vignal et al., 2002; Dudgeon et al., 2012). In comparison to nuclear DNA, variation in mtDNA occurs solely through genetic mutation, allowing for a robust assessment of maternal ancestry and genetic relationships within, or between species (Brown et al., 1979; Spies et al., 2006) . However, a lack of recombination of genetic material from parent to offspring means that detecting genetic structure among mtDNA sequences can be difficult (Palumbi & Baker, 1994). In nuclear DNA, genetic mutations occur at a slower rate, but further variation is introduced to a population through a recombination of genetic material from parent to offspring (Dudgeon et al., 2012; Portnoy & Heist, 2012). Furthermore, nuclear DNA sequencing also allows for the assessment of bi-parental genetic relationships, which can be useful, especially in species which display complex social behaviours, such as sexual segregation (Palumbi & Baker, 1994; Sims et al., 2001).

Table 4.1: A selection of genetic based shark population studies and their major findings.

Species Name	Common Name	Location	No. sampling locations	Findings	Genes	Implications	Source
Notorynchus cepedianus	Sevengill shark	Western USA	2	Low to moderate genetic diversity between populations. High relatedness of individuals within populations.	Microsatellite loci: SG13, SG24, SG25, SG27, SG28, SG30, SG31	Two distinct populations with some genetic mixing. No significant historical bottlenecks.	Larson et al., 2015
Hexanchus griseus	Sixgill shark	Western USA	1	Moderate genetic diversity.	Microsatellite loci: SG05, SG10, SG11, SG13, SG24, SG25, SG27, SG28, SG32, SG33	One intermixing population with no significant historical bottlenecks.	Larson et al., 2011
Carcharhinus leuca	Bull shark	Northern Australia	13	High genetic diversity among juveniles.	Mitochondrial loci: ND4, Control Region.	Female reproductive philopatry.	Tillett et al., 2012
Carcharias taurus	Grey nurse shark	Global	6	Low genetic diversity within populations, but significant genetic structure when comparing populations.	Mitochondrial loci: Control Region.	Distinct regional populations.	Ahonen et al., 2009
Carcharodon carcharias	White shark	Australia	2	Genetic structure between eastern and south-western coasts.	Mitochondrial loci: Control Region.	Female reproductive philopatry.	Blower et al., 2012
Cetorhinus maximus	Basking shark	Global	5	Low genetic diversity with no significant differentiation between ocean basins.	Mitochondrial loci: Control Region	Migration among basins.	Hoelzel et al., 2006
Rhincodon typus	Whale shark	Global	10	Genetic diversity between Atlantic and Indo-Pacific populations. Absence of population structure across Indian and Pacific Basins.	Mitochondrial loci: Control Region	Mixing across Indian and Pacific Basins, but not Atlantic and Indo-Pacific.	Castro et al., 2007
Stegostoma fasciatum	Zebra shark	Indo-West Pacific	13	High genetic diversity between regions and at fine scale levels.	Mitochondrial loci: ND4.	Distinct localised populations.	Dudgeon et al., 2009

4.1.5 Sevengill Shark Connectivity

Tagging studies have revealed that sevengill shark populations display seasonal migratory behaviour, utilising and returning to coastal bays and estuaries during summer and winter months (Barnett et al., 2010a; Lucifora et al., 2005; Williams et al., 2012). In some cases, the distances covered exceeded 1800 km (Williams et al., 2012), introducing the potential for mixing between geographically distinct habitats, and thus, genetic exchange (Karl et al., 2011). Despite this mixing potential, only one published study is solely dedicated to investigating variation in genetic structure and diversity among sevengill shark populations (Larson et al., 2015; Table 4.1). This very recent paper used nuclear microsatellite markers, originally developed for the bluntnose sixgill shark (*Hexanchus griseus*; Larson et al., 2011), to detect two genetically distinct populations of sevengill sharks in western USA; one at Willapa Bay, Washington, and the other at San Francisco Bay, California (Larson et al., 2015). Aside from this study, sequencing of sevengill shark mtDNA regions has been reported in a small number of studies (Stoner et al., 2003; Ward et al., 2005, 2008; Tanaka et al., 2013), though the focal objectives of these papers were to facilitate DNA barcoding and species identification.

4.1.6 Aims

In New Zealand, although sevengill shark tissue samples have previously been collected for an international study (unpublished data), no published literature exists to assess the genetic structure of local sevengill shark populations. Therefore, the aim of this research was to provide the first evaluation of the genetic diversity of New Zealand sevengill sharks. This information will provide insight to the ecological role of sevengill sharks in coastal habitats, and has implications for the future management and conservation of the species.

4.2 Methods

4.2.1 Tissue Collection

Sevengill shark tissue samples from Ōtākou and Te Whaka ā Te Wera (N = 17), were obtained during field collections between 2013 and 2015 (Figure 4.1). Sevengill sharks were attracted to a research vessel or coastal site, as part of the sampling trips described in Chapter Two. Sampling trips took place at dawn and dusk, using chum to entice sharks.

Tissue extractions were performed using a 1.4 m wooden pole, with a custom made 1 cm long stainless steel scraper fastened to one end. As a shark swam near the research vessel, the collection pole was deployed, using the sharp end to scrape a small amount (~ 5 mm) of skin tissue from the dorsal side of the animal. Sharks were not hooked or restrained at any time. In addition, tissue samples from the dorsal fin were opportunistically extracted from deceased sevengill sharks: the first washed up on Ōtaki Beach, Kapiti Coast (December 2013; Figure 4.1), and the second washed up on Victory Beach, Otago (March 2015; Figure 4.1). All tissue samples were stored in 100% ethanol for later analysis.

In addition to the above tissue collections, sevengill shark fin-clip samples (N = 35), were provided from Craig Thorburn, who was involved in a study conducted throughout New Zealand in 2010, as a collaboration intended to assess the global genetic structure of sevengill shark populations (unpublished data). These samples were collected at eight different locations including Rakiura and Ōtākou (Figure 4.1), and were stored in 100% ethanol.

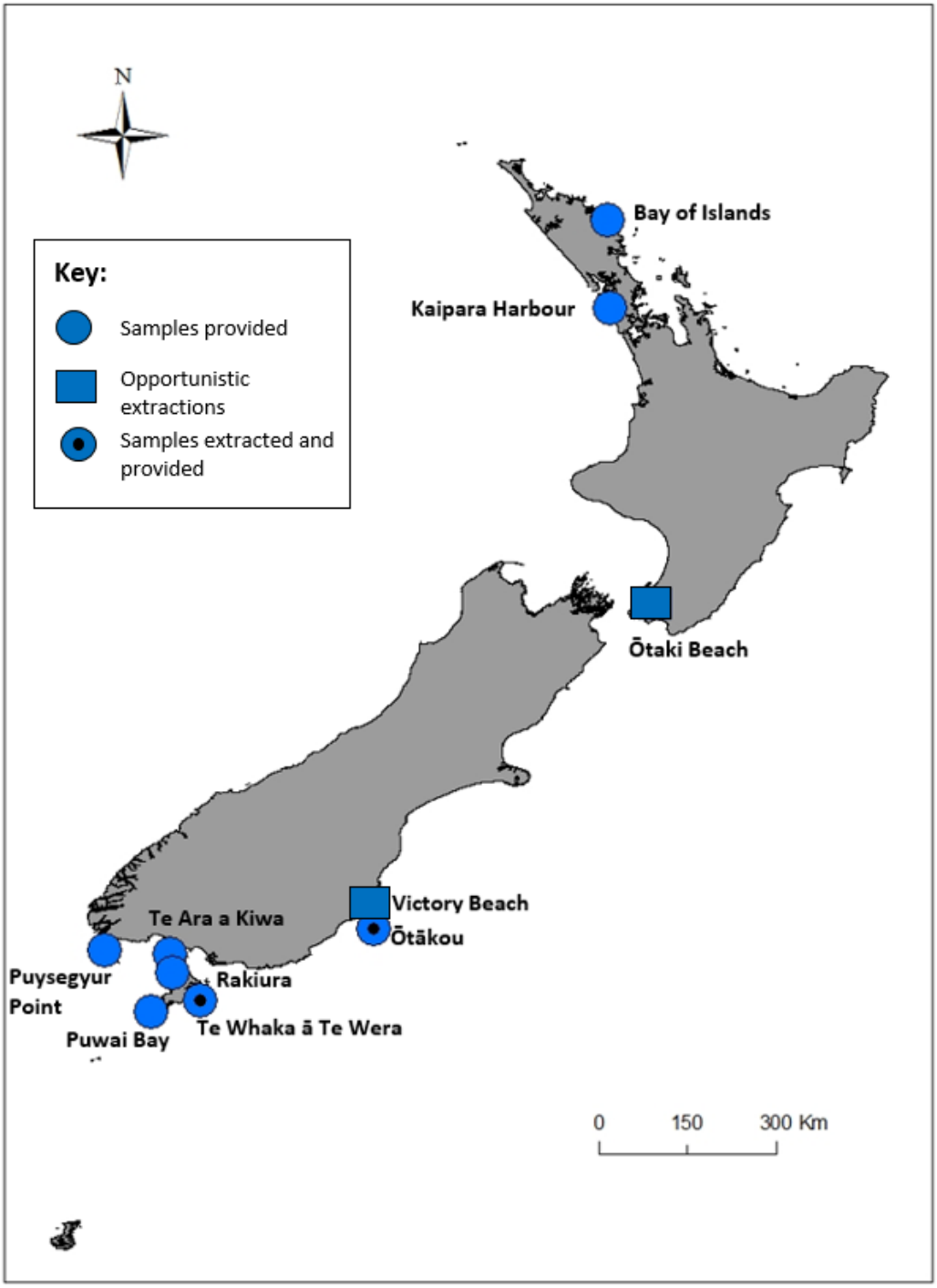


Figure 4.1: Collection locations of sevengill shark tissue samples 2010-2015, New Zealand.

4.2.2 Genetic Markers

Nuclear microsatellite markers were initially chosen for sequencing (following Larson, 2011). However, following the first round of sampling, this method was abandoned due to resulting null alleles in each of the sample products (N = 10). Null alleles are detected

through failed amplification of sample products during the polymerase chain reaction (PCR; Section 4.2.3). For this reason, mtDNA genomic regions were then explored.

Whilst the Control Region of the mtDNA is often sequenced in shark population genetic studies (Hoelzel et al., 2006; Ahonen et al., 2009; Blower et al., 2012), it was not used in the current research as it was suggested that genetic structure was unlikely to be detected (Dr Christine Testerman, personal communication). Therefore, genetic sequencing using two mtDNA genes: cytochrome oxidase I (COI), and NADH dehydrogenase 4 (ND4) was pursued.

4.2.3 Mitochondrial DNA Amplification and Sequencing

DNA was extracted[2] from tissue samples (N = 52) using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA). Following extractions, the COI and ND4 genes were amplified using the polymerase chain reaction (PCR) and sequenced. Total reaction volumes of 25 µl were prepared for each sample, which included 12.5 µl of 2x MyTaq Mix (Bioline Inc., USA), 1.25 µl of forward and reverse (10 µM) primers, 7.5 µl of PCR water, and 2.5 µl (1-50 ng) of DNA sample. These solutions were then placed in a MJ Research PTC-225 Peltier Thermal Cycler under the following conditions:

COI:

An initial denaturation for 3 minutes at 95 °C, followed by 35 cycles of denaturation for 15 seconds at 95 °C, annealing for 15 seconds at 48 °C, and an extension of 15 seconds at 72 °C. These cycles were followed by a final extension of 5 minutes at 72 °C.

ND4:

An initial denaturation for 5 minutes at 95 °C, followed by 30 cycles of denaturation for 15 seconds at 95 °C, annealing for 30 seconds at 56 °C, and an extension of 60 seconds at 72 °C. These cycles were followed by a final extension of 7 minutes at 72 °C.

[2] See Appendix B for DNA extraction protocol.

The PCR products were then purified using SAP-EXO enzymes by adding 2.5 µl of SAP and 0.625 µl EXO to each sample. Following this, samples were placed in the MJ Research PTC-225 Peltier Thermal Cycler for 30 minutes at 37°C, then 15 minutes at 80°C. The PCR products were then run on gel electrophoresis to assess amplification success. Before submitting for sequencing, 1 µl of PCR product was diluted in 50 µl of PCR water; 1 µl of this solution was then added to 1 µl forward (3.2 µM) primer and 3 µl of water, to bring to a 5 µl volume. The 5 µl solution was sequenced at the Otago University Genetic Analysis Service using the BigDye Terminator Version 3.1 Ready Reaction Cycle Sequencing Kit.

4.2.4 Data Analysis

Sequences were edited and aligned using MEGA Genetic Software (version 6.06). COI sequences (N = 41) were trimmed to 548 base pairs, and aligned with one sample each from USA, Argentina, and Australia, retrieved from GenBank (Accession Numbers: Hastings & Burton, USA - GU440425; Mabragana, Argentina - EU074507; Tanaka, Japan - AB560489; Ward, Australia - DQ108326). ND4 sequences (N = 42) were trimmed to 594 base pairs and also aligned with one sample each from USA, Argentina, and Australia, retrieved from GenBank (Accession Number: Tanaka, Japan - AB560489). Maximum likelihood phylogeny trees were then constructed using the Tamura-Nei sequencing evolution model (TN93; Tamura & Nei, 1993), as this model was deemed appropriate when running the model selection application within MEGA. The TN93 model is the most general sequence evolution model, accounting for different evolutionary rates in purine to purine, and pyrimidine to pyrimidine transitions (Suchard et al., 2001). Sequences from the genome of the sixgill shark, were used as an outgroup for these analyses (Accession Number: Tanaka, Japan - AB560490). In phylogenetics, outgroups are a related animal group, providing a reference to assist in examining evolutionary relationships (Farris, 1982). Bootstrapping of 1000 replications was used to test accuracy of phylogenies and pairwise distances calculated among sequences, using the TN93 model (Tamura & Nei, 1993) in MEGA. The resulting percentage strength of support for a given clade was written at each node of the produced phylogenetic trees. The degree of divergence between clades is indicated by the scale bar (Gregory, 2008).

4.3 Results

The sequencing results for both COI and ND4 mtDNA genes revealed no genetic structure among sevengill shark populations sampled within New Zealand (Figure 4.2, Figure 4.3).

Thirty eight genetic samples were processed for both COI and ND4 genes. In some cases, the PCR products were not successfully amplified, meaning these samples were not submitted for sequencing (COI = 2 samples failed to amplify, ND4 = 5 samples failed to amplify). An additional five samples were successfully sequenced for the COI gene, and an additional nine samples were sequenced for the ND4 gene.

For all sevengill sharks sampled in New Zealand, only a single COI haplotype was detected. Furthermore, this haplotype was shared in Tasmanian and Argentinian sevengill shark populations (Figure 4.2). Both COI and ND4 mtDNA sequences revealed diversity between New Zealand and Japanese populations of sevengill sharks, displaying branching from the southern hemisphere populations (Figure 4.2, Figure 4.3). COI mtDNA sequences revealed a genetic distance[3] of 0.006 between southern (New Zealand, Australia, Argentina) and northern (Japan, USA) hemisphere sevengill shark populations, whilst ND4 mtDNA revealed a genetic distance[4] of 0.010 between New Zealand and Japan sevengill shark populations.

[3] See Appendix C for pairwise distance matrix.
[4] See Appendix D for pairwise distance matrix.

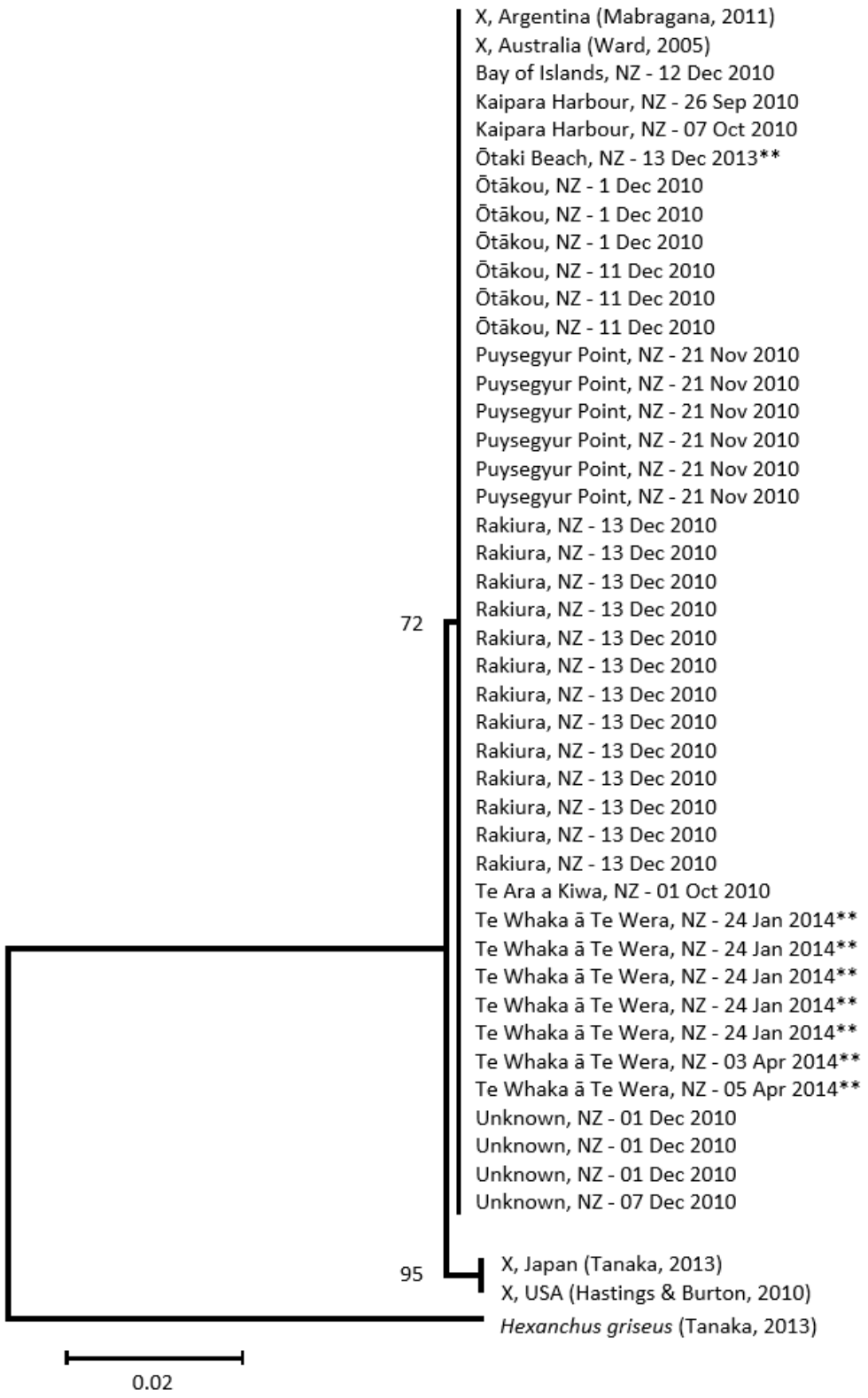


*Figure 4.2: COI mtDNA Maximum Likelihood Phylogeny Tree (n = 46), displaying sevengill shark individuals sampled from 2010 - 2014 (outgroup sixgill shark, Hexanchus griseus, 2013). Calculated bootstrap values align with associated branches. Samples collected in this research indicated by: **, sequences retrieved from GenBank preceded by: X.*

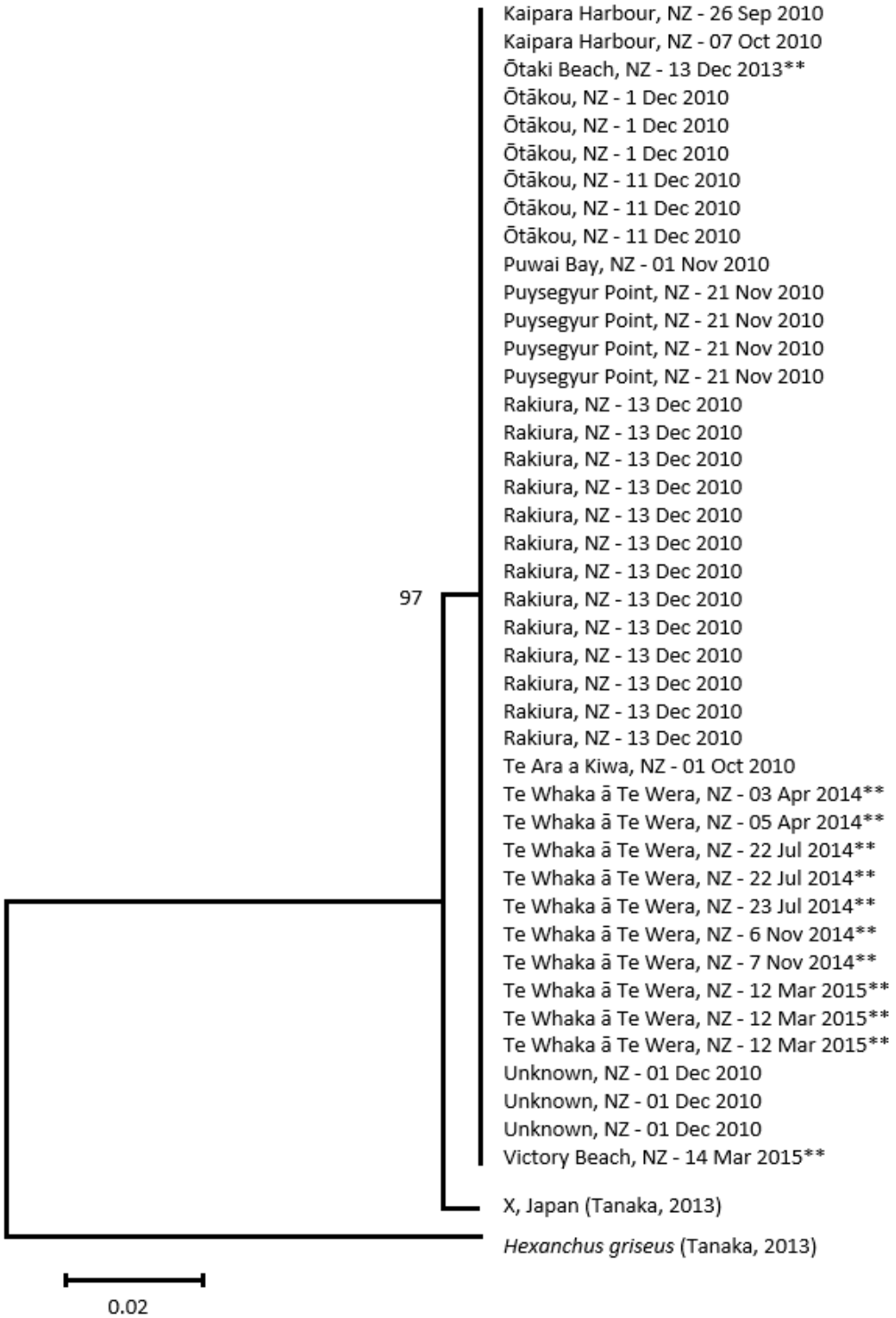


*Figure 4.3: ND4 mtDNA Maximum Likelihood Phylogeny Tree (n = 44), displaying sevengill shark individuals sampled from 2010 - 2015 (outgroup sixgill shark, Hexanchus griseus, 2013). Calculated bootstrap values align with associated branches. Samples collected in this research indicated by: **, sequences retrieved from GenBank preceded by: X.*

4.4 Discussion

Investigating genetic diversity and connectivity among populations is fundamental to the understanding of species' ecology, and thus contributes to the development of appropriate conservation measures (Blower et al., 2012). To avoid a loss of genetic variation, sustainable management should be based on a knowledge of population genetic structure (Laikre et al., 2005). In vulnerable species, such as many elasmobranchs, population genetic analyses can inform researchers of appropriate scales of management (Dudgeon et al., 2012). Populations that display limited genetic mixing are less resilient to the effects of population decline, and as such, would benefit from localised conservation measures (Blower et al., 2012).

4.4.1 Gene Flow among New Zealand Populations

In the current study, the fact that only a single haplotype for each of the COI and ND4 mtDNA genes was detected among New Zealand sevengill shark populations suggests that gene flow, and therefore migrations between distant locations within the country, is likely. Acoustic tagging studies conducted in north-east Pacific Ocean coastal embayments in the USA, revealed that sevengill sharks are capable of migrating large distances, throughout continental shelf and coastal environments (Williams et al., 2012). On average, tagged animals ranged between 20 to 240 km from original tagging locations, though some individuals covered distances up to at least 1800 km (Williams et al., 2012). Similarly, pop-up satellite archival tags (PSATs) revealed sevengill sharks tagged in Derwent Estuary and Norfolk Bay, Tasmania, covered distances of up to 880 km during coastal migrations (Stehfest et al., 2014). Large scale movements by sevengill sharks in New Zealand is thus, entirely reasonable. Furthermore, only a small number of long-distance migrants per generation are required to prevent the detection of genetic diversity between geographically distant populations (Veríssimo et al., 2010)**.**

The lack of genetic structure detected among New Zealand sevengill shark populations may also suggest that the mtDNA markers used in this study were too conservative. One study conducted on the bat fly, *Trichobius major,* found no variation in COI and ND4 mtDNA sequences among samples from caves widespread throughout Kansas, Oklahoma, and Texas, USA (Wilson et al., 2007). This particular study suggested that the COI and ND4 genes were not suitable genetic markers to detect intraspecific variation in the bat fly

(Wilson et al., 2007). Furthermore, shark species in general display low rates of genetic evolution (Larson et al., 2011), which may account for the absence of diversity found among the COI and ND4 sequences.

In zebra sharks, however, researchers discovered a number of mtDNA ND4 haplotypes, from sampled populations in the Indo-West Pacific (Dudgeon et al., 2009). These populations were separated into two mixing sub-populations divided by the oceanic barrier of the Timor Trench. Like the sevengill shark, zebra sharks are a medium sized chondrichthyan species (Dudgeon et al., 2009; Williams et al., 2012). This study demonstrated that examining variation in ND4 could be effectively used to reveal genetic structure within populations (Dudgeon et al., 2009), at a similar geographic scale to that used in the current study. This provides evidence that the lack of structure detected in the current study potentially reflects a well-mixed population of sevengill sharks within the southern hemisphere, rather than a failure of the method to detect this structure should it be present.

4.4.2 Philopatric Behaviours

A number of shark population genetic studies suggest that detected levels of genetic diversity are strongly influenced by philopatric behaviours, reflecting minimal gene flow (Ahonen et al., 2009; Portnoy & Heist, 2012). For example, species may undertake migrations to feed, and during this phase, conspecifics may be well mixed, with high rates of gene flow (Blower et al., 2012). In contrast, if individuals are sampled upon returning to frequented areas, low levels of gene flow may be detected amongst sampled populations (Blower et al., 2012). Such philopatric behaviour has been displayed in species such as lemon sharks and bull sharks (Schultz et al., 2008; Tillett et al., 2012).

Worldwide, only one published study has investigated genetic structure among populations of sevengill sharks (Larson et al., 2015). Using nuclear DNA markers researchers identified that despite some mixing, sevengill sharks at Willapa Bay, and San Francisco Bay, western USA, were genetically distinct, utilising separate breeding grounds, which they travelled to in related or same-sex groups. This observed connectivity, but diverse genetic structure, between the two populations, is indicative of philopatric behaviour (Williams et al., 2012; Larson et al., 2015). Acoustic tracking of sevengill sharks in Willapa Bay revealed strong site fidelity to the area, with individual sharks returning over three years

(Williams et al., 2012; Larson et al., 2015). This breeding site fidelity was not exhibited by sevengill shark populations in this study.

Differences arising from the use of mtDNA versus nuclear sequencing techniques may account for some of the disparity (Palumbi & Baker, 1994) between the results of the current study, and the research undertaken in western USA. Microsatellite sequencing provides a different perspective on population distributions, reproduction, kinship, and individual identification than simple mtDNA sequencing (Dudgeon et al., 2012; Portnoy & Heist, 2012). Microsatellite sequences consist of short, repeated fragments of DNA, and are one of the most prevalent forms of nuclear markers used in elasmobranch studies (Portnoy & Heist, 2012). As nuclear DNA is diploid (genes are inherited bi-parentally) however, developing primers to visualise nuclear markers can be somewhat difficult (Hueter et al., 2004; Portnoy & Heist, 2012). In the current study, attempts to perform sequencing using microsatellite markers as described for sixgill sharks (from Larson, 2011) were attempted, but this method was abandoned following a number of resulting null alleles. Furthermore, designing microsatellite primers specific to sevengill sharks was beyond the scope of this project. In contrast, mtDNA is maternally inherited, and therefore haploid, resulting in single strand sequences or haplotypes, allowing for simplified analyses (Dudgeon et al., 2012; Portnoy & Heist, 2012). Mitochondrial DNA is often used as a simple barcode sequence to detect genetic variation, both between, and within shark species (Ward et al., 2005; Tillett et al., 2012). Despite ongoing advances in sequencing technology, the rate of mtDNA evolution in elasmobranch species appears to be very slow, and thus, genetic variation in shark species can be difficult to detect (Martin et al., 1992; Portnoy & Heist, 2012). For this reason, additional techniques such as tagging or photo-ID of natural marks are often applied as a supplementary approach to investigate connectivity of shark populations (Dudgeon et al., 2012). Commonly, the non-coding Control Region of the mtDNA is used to assess inter-specific and intra-specific structure in elasmobranchs (Hoelzel et al., 2006, Castro et al., 2007). Using this method, high levels of genetic connectivity have been detected among populations of crocodile shark, *Pseudocarcharias kamoharai*, between the Atlantic and south-west Indian Oceans (Ferrette et al., 2015), and spiny dogfish, in Atlantic and South Pacific ocean populations (Veríssimo et al., 2010). In the current study, prior discussions with a colleague from Australia suggested that investigating the Control Region was unlikely to show genetic structure and is often difficult to optimise (Dr Christine Testerman, personal communication). Thus, sequencing of the Control Region was not pursued.

4.4.3 Trans-oceanic Gene Flow

A single COI mtDNA haplotype among New Zealand, Australian, and Argentinian populations of sevengill sharks provides evidence of trans-oceanic movements among southern hemisphere sevengill sharks on an evolutionary scale. Disparity between these southern hemisphere populations, and those from Japan and USA, may be due to undefined barriers that developed sometime in the past, impeding genetic flow. Among shark populations, oceanic barriers appear to have a very strong influence on genetic structure, and in some cases, a stronger influence than philopatric behaviours (Duncan et al., 2006; Schultz et al., 2008).

In grey nurse sharks, strong differentiation between mtDNA markers from northern and southern hemisphere populations led researchers to suggest that warm equatorial waters may be a significant barrier to grey nurse shark migration, and thus, genetic flow (Ahonen et al., 2009). Despite the presence of shallow coastal regions that would allow connectivity to occur, both genetic and tagging studies have observed isolation between northern and southern hemisphere populations (Lucifora et al., 2003). In sevengill sharks, this same equatorial barrier may explain the genetic disparity between populations from Japan and USA, and those from New Zealand, Australia, and Argentina. Furthermore, a worldwide population genetic study of whale sharks, indicates that thermal tolerances influence distribution of whale shark populations (Castro et al., 2007).

In shark species, oceanic barriers in the form of deep-water trenches, strong currents, and land masses may also restrict trans-oceanic mixing (Dudgeon et al., 2009). Highly mobile, coastal shark species often display genetic structure across ocean basins; hammerhead sharks, *Sphyrna lewini*, and lemon sharks, both exhibit genetic diversity between Atlantic and Pacific Ocean populations (Duncan et al., 2006; Schultz et al., 2008). In contrast, however, researchers found no structure among populations of basking shark sampled from worldwide oceanic basins (Hoelzel et al., 2006; Portnoy & Heist, 2012).

4.4.4 Implications

The outcomes of this study may contribute to the understanding of sevengill shark population or evolutionary biology in New Zealand. Although ecological and fine-scale population patterns could not be discerned, this study does provide a baseline understanding

that can now be investigated further using more extensive techniques. DNA sequencing using microsatellite markers (e.g. Larson 2015), could build on these results, and provide further insight into the genetic structure of sevengill sharks in New Zealand and abroad.

4.5 Conclusions

In conclusion, this study suggests that New Zealand sevengill sharks display genetic connectivity among New Zealand populations, and abroad to southern hemisphere populations in Australia and Argentina. Incorporating a multi-disciplinary approach, with the inclusion of supplementary techniques such as nuclear DNA sequencing, acoustic and telemetric tagging, or photo-ID studies, would complement this research (Dudgeon et al., 2012).

5 Chapter Five

Discussion

5.1 Structuring Multiple Systems

Apex predators, such as many large shark species, have a fundamental role in regulating marine systems by exerting "top-down" predation pressure and consuming lower trophic level species (Block et al., 2011; Espinoza et al., 2014). Due to the high diversity of their diet (Cortes, 1999; Ebert, 2002; Barnett et al., 2012), sevengill sharks have therefore been identified as one of the most important apex predators in temperate coastal locations worldwide (Last & Stevens, 2009; Barnett et al., 2012). In the context of the current study, the migratory and philopatric behaviours displayed by sevengill sharks at Ōtākou and Te Whaka ā Te Wera, coupled with the lack of genetic structure detected among New Zealand sevengill sharks, suggest that individuals have the potential to frequent multiple habitats, and therefore, influence a number of geographically distinct ecosystems throughout New Zealand.

5.2 Mobility and Mixing

The seasonal occurrence of sevengill sharks at Ōtākou is not dissimilar to spatio-temporal distributions of sevengill shark populations observed in other countries. Studies conducted in western USA, Argentina, southern Africa, and Tasmania have all reported the seasonal occurrence of sevengill sharks at coastal habitats (Ebert, 1991, 1996; Crespi-Abril et al., 2003; Barnett et al., 2010b). The absence of smaller sized individuals (< 0.8m TL; Barnett et al. 2010d), and the seasonal occurrence of potential prey species (Boyd, 2008; James et al., 2010) at Ōtākou and Te Whaka ā Te Wera, suggest that sevengill sharks use these habitats to capitalise on foraging opportunities. This idea aligns with the behaviour of populations in Washington (Williams et al., 2012) and Tasmania (Barnett et al., 2010b,c), where prey abundance has been recognised as the predominant driver of sevengill shark migrations into coastal areas.

The most important predictor of encountering a sevengill shark at Ōtākou and Te Whaka ā Te Wera was identified as water temperature. Studies elsewhere suggest that water temperature is the cue to initiate sevengill shark seasonal migrations (Stehfest et al., 2014). In other shark species, increased water temperatures have been predicted to assist with the

efficacy of physiological processes such as digestion and reproduction (Hight & Lowe, 2007; Williams et al., 2012). Considering this information, sevengill sharks at Ōtākou and Te Whaka ā Te Wera may display temporal distributions as a response to changes in water temperature, and to consume seasonally abundant prey under physiologically favourable conditions.

Water temperature may also influence the global distribution of sevengill shark populations, as reflected by the structure of COI mtDNA between the northern and southern hemispheres. Thermal tolerances have also been shown to influence the distribution of other shark species (Castro et al., 2007; Ahonen et al., 2009). As observed in grey nurse sharks, equatorial water temperatures may serve as a physiological barrier to migration and gene flow (Ahonen et al., 2009). At Te Whaka ā Te Wera, the majority of sevengill sharks were sighted at water temperatures between 11°C and 14°C, and at Ōtākou, sharks were sighted only between 13.5°C and 17°C. In addition, a growth rate study identified 12°C to 18°C as the water temperature range at which sevengill sharks were most abundant (Van Dykhuizen & Mollet, 1992).

5.3 Low Re-sight Rates

The low re-sight rates of identified sevengill sharks at Ōtākou and Te Whaka ā Te Wera can be interpreted in multiple ways. In mark-recapture studies, low re-encounter rates may indicate that sample populations are abundant, or contain highly migratory individuals (Dudgeon et al., 2015). Thus, the modest number of re-sighted sevengill sharks at Ōtākou and Te Whaka ā Te Wera, may reflect large populations of mobile individuals. Relative to other shark species, sevengill sharks are highly fecund (Ebert, 1996; Awruch et al., 2014), and have been recognised as one of the most abundant predators found in coastal habitats (Ebert, 1989; Barnett et al., 2010b).

The discovery of the shed tag T80, and the lack of genetic structure detected among New Zealand sevengill shark populations, provides evidence that migrations are likely to be occurring. Research conducted elsewhere has also detected long-distance migrations in sevengill sharks (Williams et al., 2012; Larson et al., 2015). As revealed through acoustic tagging and nuclear DNA sequencing, sevengill sharks residing in embayments in north-east USA, travel large distances to alternate coastal locations, or across continental shelf areas (Williams et al., 2012; Larson et al., 2015). In addition, a study using pop-up satellite

archival tags (PSATs) in Tasmania, revealed that sevengill sharks migrated distances of up to 880 km between coastal locations (Stehfest et al., 2014). Such migrations have been attributed to environmental conditions, changes in prey availability, or reproductive opportunities (Kuhn et al., 2009; Knip et al., 2010; Speed et al., 2010).

5.4 Site Fidelity

Philopatric behaviour or site fidelity, may also influence the spatio-temporal distribution of sevengill sharks (Barnett et al., 2011). In coastal areas of Tasmania, and in Willapa Bay, Washington, acoustic tagged sevengill sharks displayed high levels of site fidelity, with individuals returning to their original tagging locations over a number of years (Barnett et al., 2011; Williams et al., 2012). At Te Whaka ā Te Wera, re-sightings of individual sharks, although few in number, indicate that individuals display some level of fidelity to the area. This behaviour can increase foraging efficiency and success, due to spatial familiarity and knowledge of local prey (Barnett et al., 2011; Williams et al., 2012).

The duration of this study, coupled with the low re-sight rates, meant that detection of long-term site fidelity was not possible. However, re-sighting of T01 over an eight month period suggests that medium term residency does occur. In contrast, the lack of genetic structure detected among sevengill shark populations showed no explicit support of philopatric behaviour in New Zealand sevengill sharks. However, whilst the genetic results indicate that mixing between New Zealand sevengill shark populations is apparent, they do not rule out the possibility of individual animals returning to selected habitats over time. Detecting social behaviours such as philopatry can be challenging using genetic analyses, and often supplementary methods such as tagging and mark-recapture assist in identifying these patterns (Castro et al., 2007; Dudgeon et al., 2012).

5.5 Photo-ID

This is the first study to demonstrate that photo-ID is a viable method for individual recognition of sevengill sharks. The re-sighting of T01 demonstrated that this technique could be used to repeatedly identify individuals over a period of eight months. In addition, the stability of naturally occurring marks of sevengill sharks at the National Aquarium of New Zealand, over a period of five years, demonstrate the potential for long-term individual recognition.

Photo-ID was established as a practical, less-invasive alternative to tagging in sevengill sharks. Whilst tagging was also used to successfully recognise individuals, the associated complications such as tag shedding and bio-fouling, suggest this technique is unreliable long-term. These complications are likely to have contributed to the small number of re-sights observed over the course of this study.

Photo-ID shows great promise as a technique for future sevengill shark demographic and ecological studies. Research on a number of other elasmobranch species already utilise such methods (Graham & Roberts, 2007; Ari, 2014). Development of sound under-water video techniques to identify individual animals is likely to improve the re-capture probability of sevengill sharks in these types of demographic studies.

5.6 Management

Assessing the spatio-temporal habit use of shark species is important for the development of informed management and conservation strategies (Bonfil, 1997; Baum et al., 2003). Such strategies have the potential to impact not only shark species, but also the ecosystems they are associated with (Williams et al., 2004; Heithaus et al., 2008). Even with informed assessments, however, designing appropriate management and conservation strategies can be difficult, due to complex migratory behaviours displayed by some shark species (Castro et al., 2007; Ferretti et al., 2010). These behaviours may be associated with environmental conditions, prey availability, breeding requirements, site fidelity, or habitat partitioning (Speed et al., 2010). To date, appropriate regulations to protect many shark populations have failed to be implemented, largely due to a lack of scientific data (Hammerschlag et al., 2011).

Marine protected areas may facilitate population conservation of large marine predators, especially for species that display residency or fidelity to coastal areas (Heithaus et al., 2012). Furthermore, to account for seasonal aggregations, partial closures may be implemented at coastal areas during peak immigration periods (Speed et al., 2010). Following ecological assessments, researchers have identified the need for protected areas at shallow embayments in California, due to human exploitation of seasonally abundant leopard sharks, *Triakis semifasciata* (Hight & Lowe, 2007). Similar observations have been made on populations of spiny dogfish and zebra shark, again due to human exploitation of predictable aggregations (Dudgeon et al., 2008). Additional restrictions such as reduced size

or catch limits, can also protect shark species at particular life history phases (Speed et al., 2010). More research, especially long term, is essential to developing successful management and conservation of coastal shark species (Speed et al., 2010).

The cumulative outcomes of the current research indicate that sevengill sharks in New Zealand potentially form a large, well-mixed population. This has particular implications for management, as populations with wider distributions are often less vulnerable to localised impacts (Knip et al., 2010). However, the fact that no smaller sized individuals were encountered at Ōtākou and Te Whaka ā Te Wera, suggests that there is likely some ontogenetic segregation of habitat use among New Zealand sevengill sharks.

In order to assess the management status of New Zealand sevengill sharks, a greater understanding of the location and use of mating and nursing areas is required. At present, the results of the current study identify Ōtākou as an important seasonal foraging habitat, and Te Whaka ā Te Wera as an important year-round foraging habitat for sevengill sharks. Without investigation of other coastal habitats, however, the relative importance of these particular locations is not yet clear.

5.7 Future Research

As discussed in Chapter Two, future sevengill shark research should implement a more comprehensive sampling design. More specifically, sampling methods across all locations should be consistent to avoid the possibility of introducing gear bias. The use of a vessel at both Ōtākou and Te Whaka ā Te Wera, may allow for a comparison of size and sex distributions between locations. A more comprehensive investigation of spatio-temporal distributions, and potential behaviours such as habitat partitioning, could be implemented by conducting sampling trips at both deep channels, and peripheral sites of embayments.

Advanced tagging techniques, such as acoustic and satellite tagging, may provide additional insight into the spatio-temporal distribution of shark species, but associated costs, animal stress resulting from capture, tag deployment and recovery should be considered (Kohler & Turner, 2001; Hammerschlag et al., 2011). A less invasive course of action could take advantage of tissue samples, to investigate sevengill shark population distribution and predator-prey relationships using advanced molecular techniques (Abrantes & Barnett, 2011; Larson et al., 2015). Genetic sequencing using nuclear DNA, and stable isotope sampling using $\delta^{13}C$ and $\delta^{15}N$, have both been successfully applied to sevengill shark

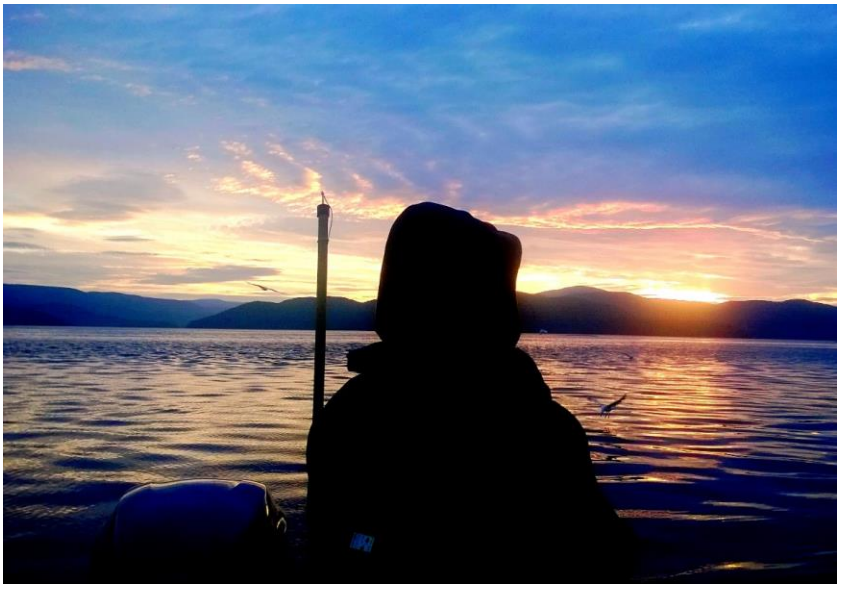
populations in north-west USA, and south-east Tasmania (Abrantes & Barnett, 2011; Larson et al., 2015). These techniques could be applied to the extracted tissue samples used in the current study, offering further insight into the ecology of New Zealand sevengill sharks.

Population monitoring using photo-ID could be applied to other coastal areas where sevengill sharks are found, as well as a continuing work at Ōtākou and Te Whaka ā Te Wera. As described in the current study, this method can be successfully applied to monitor population demographics and movements of sevengill sharks, with no physical harm to the individual animal. The long-term potential of photo-ID also means that this technique may be used to estimate demographic parameters such as survival and abundance, with greater precision than tagging. Te Whaka ā Te Wera would therefore be an ideal site to develop a long-term photo-ID project on sevengill sharks.

5.8 Conclusions

This study achieved the first investigation of spatio-temporal distribution and population connectivity of sevengill sharks in New Zealand, whilst using minimally invasive field techniques. As well as providing insight to the ecology of New Zealand sevengill sharks, this research identifies the potential for these animals to influence a number of coastal ecosystems throughout New Zealand.

There remains a vast amount to be understood about sevengill shark populations in New Zealand, and it is hoped that supplementary research will build upon the findings of the current study. As such, future research and further comprehension of these predators stands to benefit the health of the species themselves, and the habitats of which they frequent.



Appendix A: Table of recognised sevengill sharks at Ōtākou and Te Whaka ā Te Wera, detailing dates of initial identification and re-encounters.

Shark Information					Initial Tagging		
	Shark ID	Location	Shark Name	Tag ID	Date	Tag	Photo -ID
1	T03	Te Whaka ā Te Wera	Wanderer	56 Red-Yellow|||	11 Dec 2013	✓	
2	T04	Te Whaka ā Te Wera	Irish	52 White-Green |||	12 Dec 2013	✓	
3	T06	Te Whaka ā Te Wera	Arghy	45 Orange-Blue |||	13 Dec 2013	✓	
4	O03	Ōtākou	Aunty Passie	39 Yellow |||	29 Jan 2014	✓	
5	O05	Ōtākou	Beyonce		29 Jan 2014		✓
6	O04	Ōtākou	Magic Mike	55 Orange-Yellow |||	29 Jan 2014	✓	
7	T20	Te Whaka ā Te Wera	Buffalo Bill	35 Red-White	20 Feb 2014	✓	
8	T21	Te Whaka ā Te Wera	Gerald	27 Blue-Red	20 Feb 2014	✓	
9	T23	Te Whaka ā Te Wera	Ed Sheeran	30 Red-Orange	21 Feb 2014	✓	
10	T27	Te Whaka ā Te Wera	Good day	12 Blue-Green	21 Feb 2014	✓	
11	T24	Te Whaka ā Te Wera	Halfmoon	10 Red-Blue	21 Feb 2014	✓	
12	T29	Te Whaka ā Te Wera	Hulk	02 Green	21 Feb 2014	✓	
13	T30	Te Whaka ā Te Wera	Niggly	05 Red	21 Feb 2014	✓	
14	T26	Te Whaka ā Te Wera	Red	04 Red	21 Feb 2014	✓	
15	T28	Te Whaka ā Te Wera	Yolo	06 White	21 Feb 2014	✓	
16	O07	Ōtākou	Steveo		28 Mar 2014		✓
17	T58	Te Whaka ā Te Wera	Hercelise	29 Yellow-Red	3 Apr 2014		✓
18	T36	Te Whaka ā Te Wera	Americano	46 Red-Blue |||	4 Apr 2014	✓	
19	T35	Te Whaka ā Te Wera	Mini Hulk	38 Green |||	4 Apr 2014	✓	
20	T40	Te Whaka ā Te Wera	QingMing	63 Blue-Red |||	5 Apr 2014	✓	
21	T41	Te Whaka ā Te Wera	Bless	17 Blue-Yellow	5 Apr 2014	✓	
22	T39	Te Whaka ā Te Wera	Herc-Reggie	34 Yellow-White	5 Apr 2014	✓	
23	T45	Te Whaka ā Te Wera	Kale	64 Green-Red |||	5 Apr 2014	✓	
24	T44	Te Whaka ā Te Wera	LMP	70 Yellow-White |||	5 Apr 2014	✓	
25	T42	Te Whaka ā Te Wera	White Choc	42 White |||	5 Apr 2014	✓	
26	T56	Te Whaka ā Te Wera	Bastille	08 Yellow- Blue	5 Jun 2014	✓	
27	T55	Te Whaka ā Te Wera	Biddy	33 Green-White	5 Jun 2014	✓	✓
28	T57	Te Whaka ā Te Wera	Fathead	01 Blue	5 Jun 2014	✓	✓
29	T54	Te Whaka ā Te Wera	Girl	16 White-Green	5 Jun 2014	✓	

	Shark ID	Location	Shark Name	Tag ID	Date	Tag	Photo -ID
30	T50	Te Whaka ā Te Wera	Haley-Jane	22 Blue-Orange	5 Jun 2014	✓	
31	T48	Te Whaka ā Te Wera	Junecrown	13 Yellow-Green	5 Jun 2014	✓	
32	T51	Te Whaka ā Te Wera	Laura explorer	68 Blue-White |||	5 Jun 2014	✓	
33	T53	Te Whaka ā Te Wera	Lil B	28 Green-Red	5 Jun 2014	✓	
34	T52	Te Whaka ā Te Wera	Sunny Ōtaki	03 Yellow	5 Jun 2014	✓	
35	T01	Te Whaka ā Te Wera	Tom in town	23 Green-Orange	22 Jul 2014	✓	
36	T63	Te Whaka ā Te Wera	James Bond	07 Green-Blue	22 Jul 2014	✓	✓
37	T70	Te Whaka ā Te Wera	Katherine	18 Green-Yellow	22 Jul 2014	✓	
38	T69	Te Whaka ā Te Wera	Matariki	20 Red-Yellow	22 Jul 2014	✓	
39	T65	Te Whaka ā Te Wera	Naiad	25 Red-Orange	22 Jul 2014	✓	
40	T66	Te Whaka ā Te Wera	Pup	31 White-Red	22 Jul 2014	✓	
41	T64	Te Whaka ā Te Wera	Soreal	03 Blue-White	22 Jul 2014	✓	
42	T59	Te Whaka ā Te Wera	Whaea		22 Jul 2014		✓
43	T67	Te Whaka ā Te Wera	Whiti te ra	24 Yellow-Orange	22 Jul 2014	✓	
44	T72	Te Whaka ā Te Wera	Spook	21 White-Yellow	23 Jul 2014	✓	✓
45	T75	Te Whaka ā Te Wera	Will.i.am	62 White-Red |||	5 Nov 2014		✓
46	T76	Te Whaka ā Te Wera	Sean	11 White-Blue	5 Nov 2014	✓	
47	T80	Te Whaka ā Te Wera	Plot	15 Red-Green	6 Nov 2014	✓	
48	T91	Te Whaka ā Te Wera	Alice	57 White-Yellow |||	6 Nov 2014	✓	
49	T88	Te Whaka ā Te Wera	Bertha		6 Nov 2014		✓
50	T86	Te Whaka ā Te Wera	Big Boy		6 Nov 2014		✓
51	T92	Te Whaka ā Te Wera	Desmondo	69 Green-White |||	6 Nov 2014	✓	
52	T87	Te Whaka ā Te Wera	Grabyoney	58 Blue-Orange |||	6 Nov 2014	✓	
53	T90	Te Whaka ā Te Wera	Junior	44 Yellow-Blue |||	6 Nov 2014	✓	
54	T82	Te Whaka ā Te Wera	Marta		6 Nov 2014		✓
55	T93	Te Whaka ā Te Wera	Random		6 Nov 2014		✓
56	T79	Te Whaka ā Te Wera	Treason	14 Orange-Green	6 Nov 2014	✓	
57	T83	Te Whaka ā Te Wera	Whiskey	19 Orange-Yellow	6 Nov 2014	✓	
58	T100	Te Whaka ā Te Wera	Five	47 White-Blue |||	11 Mar 2015	✓	✓
59	T99	Te Whaka ā Te Wera	Four		11 Mar 2015		✓
60	T102	Te Whaka ā Te Wera	Gianna	41 Red |||	11 Mar 2015	✓	
61	T97	Te Whaka ā Te Wera	Lady	72 White-Red |||	11 Mar 2015	✓	

	Shark ID	Location	Shark Name	Tag ID	Date	Tag	Photo -ID
62	T96	Te Whaka ā Te Wera	Rich Froning		11 Mar 2015		✓
63	T98	Te Whaka ā Te Wera	Three		11 Mar 2015		✓
64	T105	Te Whaka ā Te Wera	Rua	51 Red-Green |||	12 Mar 2015	✓	✓
65	T104	Te Whaka ā Te Wera	Tahi		12 Mar 2015		✓
66	T103	Te Whaka ā Te Wera	Kore		13 Mar 2015		✓
67	T106	Te Whaka ā Te Wera	Pete	65 Yellow-Red |||	5 May 2015	✓	✓
68	T108	Te Whaka ā Te Wera	Ann-Marie		6 May 2015		✓

Appendix B: Sevengill shark mtDNA Extraction Protocol using DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA)

1. Cell Lysis:

   Aliquot 180 µl of Buffer ATL into a 1.5 ml microcentrifuge tube. To this, add ≤ 25 mg of sevengill shark tissue, then grind using a plastic pestle. Add 20 µl of Proteinase K, followed by overnight incubation at 56°C.

2. RNA Elimination and DNA Isolation:

   Vortex and spin down samples, then add 20 µl of 10 mg/ml of RNASE solution and incubate at room temperature for 3 minutes. Add 200 µl of Buffer AL. Mix thoroughly by vortexing and incubate at 56°C for 10 minutes.

3. Centrifugation and Elution:

   Add 200 µl of ethanol (96-100%). Mix thoroughly by vortexing, then pipet the mixture into a DNeasy Mini Spin Column placed in a 2 ml collection tube. Centrifuge at 8000 rpm for 1 minute. Discard the flow through and collection tube, then place the spin column in a new 2 ml collection tube. Add 500 µl of Buffer AW1, followed by a further 1 minute of centrifugation at 8000 rpm. Discard the flow through and collection tube. Place the spin column in a new 2 ml collection tube, add 500 µl of Buffer AW2, and centrifuge for 14 000 rpm for 3 minutes. Discard the flow through and collection tube. Transfer the spin column to a new 1.5 ml or 2 ml microcentrifuge tube. Elute the DNA by adding 100 µl Buffer AE to the centre of the spin column membrane. Centrifuge for 1 minute at 8000 rpm, then place 2 µl of sample on Nanodrop to assess DNA concentration.

Appendix C: COI mtDNA Pairwise Distance Matrix (n=46), displaying sevengill shark individuals sampled from 2010 - 2014 (outgroup sixgill shark, *Hexanchus griseus*, 2013). Samples collected in this research indicated by: **, sequences retrieved from GenBank preceded by: X.

Bay of Islands, NZ - 12 Dec 2010																																												
Kaipara Harbour, NZ - 26 Sep 2010	0.000																																											
Kaipara Harbour, NZ - 07 Oct 2010	0.000	0.000																																										
Ōtaki Beach, NZ - 13 Dec 2013**	0.000	0.000	0.000																																									
Ōtākou, NZ - 1 Dec 2010	0.000	0.000	0.000	0.000																																								
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Puysegyur Point, NZ - 21 Nov 2010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																																		
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Rakiura, NZ - 13 Dec 2010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																												
Rakiura, NZ - 13 Dec 2010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																											
Rakiura, NZ - 13 Dec 2010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																										
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Rakiura, NZ - 13 Dec 2010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																			
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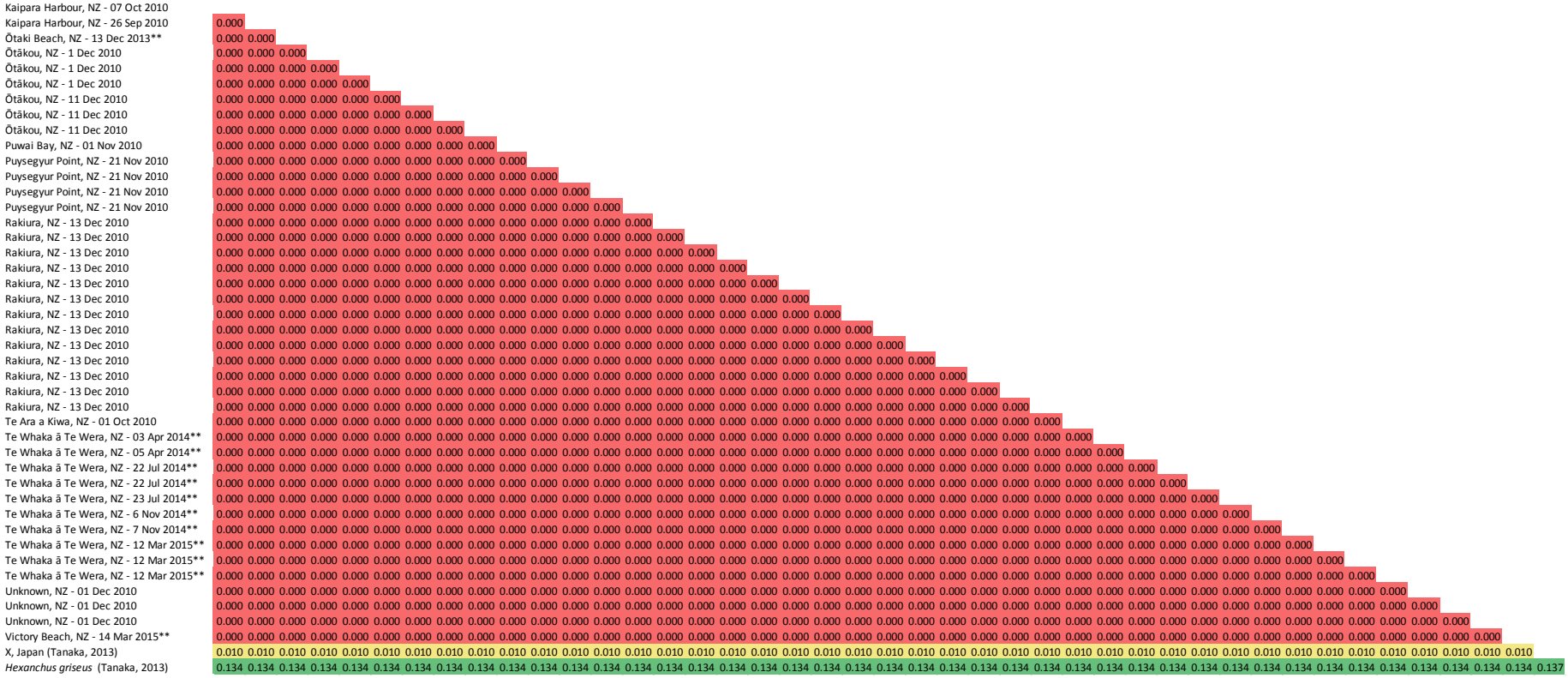
Rakiura, NZ - 13 Dec 2010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																
Te Ara a Kiwa, NZ – 01 Oct 2010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000															
Te Whaka ā Te Wera , NZ - 24 Jan 2014**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000														
Te Whaka ā Te Wera , NZ - 24 Jan 2014**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000													
Te Whaka ā Te Wera , NZ - 24 Jan 2014**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000												
Te Whaka ā Te Wera , NZ - 24 Jan 2014**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000											
Te Whaka ā Te Wera , NZ - 24 Jan 2014**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000										
Te Whaka ā Te Wera , NZ - 03 Apr 2014**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000									
Te Whaka ā Te Wera , NZ - 05 Apr 2014**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000								
Unknown, NZ - 01 Dec 2010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000							
Unknown, NZ - 01 Dec 2010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000						
Unknown, NZ - 01 Dec 2010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000					
Unknown, NZ - 07 Dec 2010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
X, Australia (Ward, 2005)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
X, Argentina (Mabragana, 2011)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
X, Japan (Tanaka, 2013)	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	
X, USA (Hastings & Burton, 2010)	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	
Hexanchus griseus (Tanaka, 2013)	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.107	0.107

Appendix D ND4 mtDNA Pairwise Distance Matrix (n = 44), displaying sevengill shark individuals sampled from 2010 - 2015 (outgroup sixgill shark, *Hexanchus griseus*, 2013). Samples collected in this research indicated by: **, sequences retrieved from GenBank preceded by: X.



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