Linking science and management for effective long-term conservation:

A case study of black-fronted terns/tarapirohe (Chlidonias albostriatus)

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Abstract

Conservation biologists are facing a huge challenge. Ongoing, human-induced global change is leading to accelerating biodiversity loss, particularly in freshwater ecosystems where many highly mobile and migratory species live. To halt this decline, effective conservation strategies for threatened species are required. Such conservation strategies should take ecological and evolutionary processes into account, and ultimately aim to maintain genetic diversity and gene flow within a species. One little studied, highly mobile and threatened species is the black-fronted tern/tarapirohe (*Chlidonias albostriatus*). It is endemic to New Zealand and specialises in breeding on shingle bars in the braided river beds of the South Island, while migrating to the coast of all three main islands during winter. It is currently in decline, primarily due to habitat loss and predation by introduced predators. Effective conservation actions are urgently needed, but critical information has been lacking.

My thesis aim was to illustrate how genetic and ecological research can inform holistic conservation planning by investigating diversity, connectivity, demographic processes, and management actions in black-fronted terns. To be able to incorporate genetic considerations into management requires the development of appropriate molecular markers first. I developed 18 species-specific polymorphic microsatellite markers and mitochondrial control region primers as well as using universal primers to amplify the mitochondrial cytochrome b gene. I then evaluated the level of genetic diversity present and assessed geographic patterns of genetic and phenotypic divergence throughout the species’ breeding range based on microsatellite markers, mitochondrial DNA, and phenotypic data (weight, head-bill length, bill depth, wing length) with the aim of delineating conservation units within the species. Analyses showed that black-fronted terns have 1) relatively high levels of genetic diversity; 2) low genetic differentiation between breeding colonies; and 3) no genetic signature of isolation-by-distance; but, 4) a phenotypic signature of isolation-by-distance consisting of increasing body size with increasing latitude. Furthermore, I assessed the demographic history and the current status of the species using the genetic data set. My analyses provided evidence for an expansion during the last glaciation period and a recent human-induced decline, emphasising the effect of poor recruitment on the population. Lastly, I evaluated the effectiveness of creating clear (i.e. free of introduced vegetation) islands to create ‘safe’ nesting refugia to increase nesting success of black-fronted terns. Unexpectedly, I identified native southern black-backed gulls/karoro (*Larus dominicanus*) as primary predators of black-fronted tern nests, and nesting success was low, independent of island vegetation cover.
My results highlighted that it is critical to confirm the impact of current threats for species of conservation interest throughout their range. Based on my findings, I recommend that black-fronted terns are managed as a single metapopulation in one conservation unit to maintain gene flow throughout their range and conserve phenotypic variation and genetic diversity. To achieve the primary goal of increasing recruitment, catchments throughout the whole South Island should be protected and the specific impacts of different predator guilds evaluated to ensure that conservation interventions are achieving the desired outcomes.

This work demonstrates how genetic data can provide the basis to inform many conservation issues and decisions and how the gap between science and implementation can be bridged, so that genetic management can be included in long-term conservation strategies in highly mobile and migratory species.
For my family

At home and abroad
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CHAPTER 1

General introduction
ACHEIVING EFFECTIVE CONSERVATION OF BIODIVERSITY

Linking science and management for black-fronted tern conservation

Similar to emergency doctors, conservation biologists often need to respond rapidly to save species and communities. Although reactive conservation measures are important, they are not enough to slow down the global biodiversity loss in the long-term, particularly in the face of increasing environmental change (Smith and Bernatchez 2008). Conservation strategies need to ultimately take ecological and evolutionary processes into account to maintain the connectivity of populations, species, communities and also ecosystems in the long-term (Mace and Purvis 2008; Eizaguirre and Baltazar-Soares 2014). Successful conservation management therefore requires clear knowledge of the diversity within and among populations and the degree of connectivity between populations in order to maintain both diversity and connectivity (Haig et al. 2016). In addition, it is vital to assess past and present population demographic trends to determine the conservation status and evaluate potential extinction risk of a species (Luikart et al. 2010). It is also important to evaluate the effectiveness of management interventions through monitoring to ensure that threats within the habitat are addressed and conservation efforts lead to the desired outcome (Arlettaz et al. 2010; Gibbons, Wilson, and Green 2011). Failure to integrate short- and long-term goals into conservation efforts may lead to continuous “emergency room” scenarios (Sgrò, Lowe, and Hoffmann 2011).

Ecosystems that are especially vulnerable to biodiversity loss are those where diverse habitats and communities are concentrated (Myers et al. 2000; Seabloom, Dobson, and Stoms 2002). A prime example of such ecosystems are freshwater ecosystems, particularly gravel-bed braided rivers (Tockner et al. 2006). These ecosystems and the species specialised in living in them are disproportionately unprotected while being disproportionately affected by human activities and under increasing threat world-wide (Dudgeon et al. 2006; Hauer et al. 2016). New Zealand has not only an extremely large number of braided rivers, but sadly, also one of the highest proportions of endangered species for its land mass, with many being habitat specialists (Bell 1991). To counter biodiversity loss in freshwater ecosystems, it is critical to maintain the connectivity of populations, species, and communities (Ridley and Alexander 2016). My thesis aim was to highlight how genetic and ecological research can inform holistic conservation planning by investigating diversity, connectivity, demographic processes, and management actions in an endangered braided river specialist endemic to New Zealand — the black-fronted tern/tarapirohe (Chlidonias albostriatus).
THE IMPORTANCE OF EVOLUTIONARY PROCESSES IN CONSERVATION BIOLOGY

One major branch within biology is conservation biology. It is a multidisciplinary and integrative scientific discipline with the aim of providing principles and tools to preserve biological diversity on all three levels: genetic, species, and ecosystem diversity (Soulé 1985). It is often perceived as a crisis discipline focusing on immediate actions and short-term goals, although its primary objective is also to address the long-term viability of species and communities (Soulé 1985; Cook and Sgrò 2016). To counter the rapid human-induced biodiversity loss in the face of ongoing global change, conservation efforts need to incorporate evolutionary resilience into management strategies (Sgrò, Lowe, and Hoffmann 2011; Ceballos et al. 2015). Put simply, long-term goals of conserving species and communities can be achieved only if ecological and evolutionary processes are maintained (Mace and Purvis 2008; Smith and Bernatchez 2008).

One example of a key ecological process is dispersal, while the main evolutionary processes are mutation, selection, gene flow and genetic drift (i.e. random genetic change from one generation to the next due to sampling error).

Ecological and evolutionary processes are of course linked, but until recently, little attention was given to incorporating evolutionary processes into conservation management (Lankau et al. 2011; Smith et al. 2014; Cook and Sgrò 2016; Ralls et al. 2018). The reason for this disregard was either because the time-frame of evolution had been vastly overestimated or because genetic factors in the extinction of species were deemed negligible (Spielman, Brook, and Frankham 2004; Jamieson, Wallis, and Briskie 2006; Hendry, Nosil, and Rieseberg 2007). For conservation biologists, there are two major components of evolutionary processes that are of particular interest: 1) the genetic diversity present in populations; and 2) gene flow between them. Higher levels of genetic diversity provide greater evolutionary potential (e.g. greater disease resistance), which is necessary in the face of ongoing environmental change (Spielman et al. 2004; Sgrò, Lowe, and Hoffmann 2011). Gene flow between populations maintains levels of genetic diversity (Allendorf, Luikart, and Aitken 2013). Quantifying both of these components is crucial, so that effective long-term conservation actions can be designed to counter the loss of genetic diversity and actively manage gene flow (e.g. Ralls et al. 2018).

Population decline through for example habitat loss often leads to small, fragmented populations. As a consequence, genetic diversity is directly lost by the decrease in population size. Additionally, if the fragmentation also hinders gene flow between populations, the maintaining force of genetic diversity is lost as well (Coleman et al. 2013). Small populations are also more affected by genetic drift, which can result in even further loss of genetic diversity.
Chapter 1: General introduction

A further concern in small populations is inbreeding and ultimately inbreeding depression (i.e. lower fitness of individuals whose parents are closely related), which leads to a further decrease in viability of those populations (Keller and Waller 2002; Frankham et al. 2017). For example, the dramatic reduction of native forest in the South Island of New Zealand has led to fragmented and isolated populations of mōhua (*Mohoua ochrocephala*), an endemic forest bird species (O’Donnell 1996). The recent fragmentation and isolation of populations resulted in a significant loss of overall genetic diversity and an increase in genetic structure due to the lack of gene flow between populations (Tracy and Jamieson 2011). Artificial gene flow through reciprocal translocations has been instigated to avoid further loss of genetic diversity (Miskelly and Powlesland 2013; O’Donnell, pers. comm.).

Conservation strategies thus need to include goals to 1) maintain genetic diversity; 2) facilitate gene flow where appropriate; and 3) counter population decline (Frankham et al. 2017). If all three goals are addressed by the conservation strategy, species will have the maximum adaptive potential for future challenges such as climate change (Sgrò, Lowe, and Hoffmann 2011). A good starting point for conservation planning is to delineate pertinent conservation units (Allendorf, Luikart, and Aitken 2013; Frankham et al. 2017). A next logical step is to examine the demographic history and current status of the species (Ho and Shapiro 2011; Luikart et al. 2010). A look into the past allows researchers to learn about a species’ capacity to adapt to environmental change and to identify potential drivers of decline. By determining the contemporary effective population size (the size of a population that would experience the same amount of genetic drift), its extinction risk can be more accurately assessed (Luikart et al. 2010). Finally, management actions that target the maintenance of genetic diversity, facilitate appropriate gene flow where necessary and an increase in population size need to be developed, tested and put into place (Frankham et al. 2017). A quantitative assessment of the overall diversity present, genetic differentiation between populations, population sizes and trends, and potential management action can provide a sound basis for an effective long-term conservation strategy (Gibbons, Wilson, and Green 2011; Haig et al. 2016; Ralls et al. 2018).

**BRIDGING THE GAP FROM RESEARCH TO IMPLEMENTATION**

*Molecular markers and their utility*

There are a range of methods available to study genetic diversity in natural populations (Schlötterer 2004; Frankham 2010). Two major kinds of DNA exist, each of which have different
properties: 1) circular organelle DNA in the mitochondria of animals or chloroplasts of plants; and 2) nuclear DNA in chromosomes. The first does not usually undergo recombination, but is inherited from a single parent. In contrast, the second kind of DNA undergoes recombination and is inherited from both parents. For most goals in conservation biology, it is best to use molecular markers of both kinds of DNA to obtain a comprehensive picture of genetic differentiation and gene flow (Rubinoff and Holland 2005). As conservation biologists frequently work with non-model species, molecular markers often need to be developed first for a given species. Furthermore, limited resources mean that marker development should be cost-effective and that the resulting markers can be used on many individuals of the target species.

Two commonly used mitochondrial molecular markers in conservation genetic studies of animals are the protein coding cytochrome b gene and the non-coding sequence of the control region (d-loop). Cytochrome b has the advantage that it is very well studied, much of its evolutionary dynamics are understood, and universal primers are available (Johns and Avise 1998; Weir and Schluter 2008). The control region requires species-specific primers, but it is generally more variable than cytochrome b making it suitable for detecting finer genetic differences (Wan et al. 2004).

As nuclear DNA markers, microsatellites have become one of the most widely applied genetic markers in conservation studies and population genetics (Schlötterer 2004; Allendorf, Luikart, and Aitken 2013). Microsatellites are tandem repeats of short sequence motifs, that occur in high frequency throughout the genome. As nuclear DNA is biparentally inherited, each individual has two copies of the same microsatellite (alleles) and these may differ in length. For example, for microsatellite 1 (locus 1) the repeat motif is “AC” and individual A = “ACACACAC” = 4 repeats (allele A) and “ACACACACAC” = 5 repeats (allele B). Microsatellites are highly polymorphic as they mutate frequently (i.e. the number of repeats change due to slippage or proofreading errors during DNA replication; Ellegren 2004). In comparison to analysing sequence differences where the actual nucleotides are of interest, it is sufficient to compare differences in repeat length for microsatellite markers. Alleles are visualised using gel electrophoresis where the different DNA fragments are separated on the basis of length (Selkoe and Toonen 2006). When using fluorescent dye to identify each locus separately on the sequencing gel, multiple loci can be amplified in a single reaction tube simultaneously using polymerase chain reaction (PCR) (i.e. a multiplex; Schuelke 2000). Multiplex PCRs greatly reduces the cost of genotyping many individuals at many loci once the appropriate multiplex conditions are developed (Allendorf, Luikart, and Aitken 2013). It has been shown that sampling approximately 25 to 30 individuals per population is enough to accurately estimate the allele frequencies necessary for population...
genetic studies (Hale, Burg, and Steeves 2012). While the high mutation rate of microsatellite markers generates high levels of allelic diversity necessary for studies on ecological time scales, different mitochondrial DNA sequences have slower mutation rates that provide insights into much deeper time scales (Schlötterer 2004).

How do we delineate conservation units?

It is best to use an integrative approach using phenotypic and genetic data to delineate conservation units as both the adaptive and non-adaptive divergence of a species is encompassed (Crandall et al. 2000; Fraser and Bernatchez 2001). While phenotypic divergence might be more strongly driven by selection, genetic divergence is probably more driven by stochastic processes (Winker 2009). Simplified, one can think of genotypic and phenotypic data as unidimensional axes in a multidimensional space signifying the inherently complex process of divergence (Fig. 1.1; Moritz 2002; Winker 2009). For example, population divergence can follow more strongly the largely adaptive axis of phenotypic divergence (e.g. differences in bill morphology of Darwin’s finches (Geospiza fortis) depending on food sources; Fernando de León et al. 2010). Alternatively, population divergence can also follow more strongly the largely neutral axis of genotypic divergence (e.g. glaciation separating populations of kiwi (Apteryx spp.; Weir et al. 2016)). Both axes of divergence can eventually lead to such substantial divergence that different species are recognised (Fig. 1.1; Moritz 2002; Winker 2009). A comprehensive foundation and guidance to conservation management can be provided by integrating both data types and considering each carefully (Dussex et al. 2018a).

Similar to taxonomic studies, different phenotypic data can be used to delineate conservation units including morphology, behaviour, and vocalisation (Cracraft 1983). Morphological features are often the easiest to measure, particularly if individuals are caught and handled for other purposes. Using phenotypes to describe biological diversity has been
often criticised for hindering conservation by artificially inflating the number of conservation units or subspecies (e.g. Zink 2004). Using solely phenotypic features to delineate conservation units is particularly difficult if morphological differentiation is clinal (e.g. colouration in the barn owl (Tyto alba); Antoniazza et al. 2010; or body size of kākā (Nestor meridionalis); Dussex et al. 2015). However, in combination with genetic data, the mechanisms (i.e. evolutionary adaptation or phenotypic plasticity) underlying such phenotypic variation can be elucidated. Most importantly, such diversity is still of interest for conservation purposes when examined together with genetic data and both are given equal weight (Dussex et al. 2018a).

Advances in genetic technologies have long skewed the debate around defining units of conservation towards relying predominantly on genetic data (e.g. Moritz 1994). The need for using multiple molecular markers to make inferences on population divergence is now widely accepted and similarly the importance of other data types in providing a more complete picture has been increasingly recognised (Haig et al. 2016). For example, populations of kākā differ in size and plumage colouration between the South and North Island of New Zealand and were thus previously classified as different subspecies (Heather, Robertson, and Onley 2015). However, recent analysis of morphometric and genetic data showed a body size cline following Bergmann’s rule and no strong genetic differentiation within the remaining population of kākā (Dussex et al. 2015). This study identified a single genetic conservation unit for kākā, which — critical for conservation management — meant that translocations mimicking natural gene flow between islands are an option that should be preferred over long-distance translocations within the same island (Dussex et al. 2015).

**What is the population size and has it changed?**

Three related demographic components are of particular interest to conservation biologists. First, a look into the past can inform on baseline population levels prior to human impacts, identify potential drastic reduction in population size (bottlenecks) and sometimes can even pinpoint some of the drivers that potentially have led to a change in population size (Hoelzel 2010; Ho and Shapiro 2011; Younger et al. 2016). Secondly, determining the contemporary effective population size of a species provides a measure of current extinction risk (Luikart et al. 2010). The effective population size is the size of a population that would lose genetic diversity at the same rate as the observed population, assuming random mating, random variance in reproductive success, an even sex ratio, and the absence of selection or mutation (Fisher 1930; Wright 1931). Hence, if a population experiences a loss of genetic diversity through genetic drift or inbreeding at the same rate as an idealised population of 20, then its effective population size
is also 20, independent of census size. Particularly in long-lived species, population census size can be reasonably large and can mask high extinction risk, if only a few individuals are able to reproduce successfully and no information on effective population size is available (e.g. Taylor et al. 2017). And lastly, population census size is nevertheless important for classifying the conservation status of a species and assessing trends (Rodrigues et al. 2006).

Genetic-based approaches are proving to be increasingly useful in assessing demographic history, effective and census population size, as well as providing a way to monitor highly mobile and cryptic species (Schwartz, Luikart, and Waples 2007; Lopes and Boessenkool 2010; Luikart et al. 2010). For example, Dussex et al. (2014) used genetic data to investigate past and present population size changes and effects on population structure in the endangered New Zealand kea (*Nestor notabilis*). Their study showed that kea had been reduced in population size and range during glaciation events, and then expanded their range again after the last glacial maximum forming three distinct clusters. Although population size has been significantly reduced from historical levels, there is no evidence of a bottleneck due to human persecution in the last century (Dussex, Wegmann, and Robertson 2014). Kamath et al. (2015) used a long-term genetic data set to assess current population trends of the grizzly bear (*Ursus arctos*) population in the Greater Yellowstone ecosystem and evaluate the adequacy of conservation measures. Their work revealed a more than four-fold increase in effective population size between 1980 and 2000 and an overall increase in population size, which means that the isolated population is not in immediate danger of inbreeding depression and current conservation measures are aiding the recovery.

Unfortunately, such long-term data sets are not available for many species, but such studies highlight the usefulness of genetic markers in estimating effective population size and the value of continuous monitoring. For many highly mobile and cryptic species — such as seabirds, shorebirds or waders — there are no population size estimates available (Kirby et al. 2008; Paleczny et al. 2015). Although there is some uncertainty around the ratio of effective to population census size in wild populations, estimates based on genetic data can provide insight into current status and trends (Frankham 1995; Palstra and Ruzzante 2008; Lee, Engen, and Sæther 2011; Waples et al. 2013).

Which conservation measures are necessary and how do we implement them?

Fundamental knowledge of the number and size of conservation units, as well as past and present population trends, provide general guidance for how long-term conservation measures
should be implemented. However, they do not necessarily translate into a realistic pathway for conservation actions (Arlettaz et al. 2010; Habel et al. 2013). To develop that pathway, three factors are essential.

First, the fundamental knowledge needs to be integrated with existing information on threats and potential management tools (Sutherland et al. 2004; Hulme 2014). For example, such knowledge might stem from conservation management actions that have been successful for similar species or in similar ecosystems (e.g. creating artificial breeding habitat). Both, information from published articles in peer-reviewed journals and often undocumented, personal knowledge of managers or community groups, should be considered.

Secondly, clear short-term and long-term goals should be set. For instance, a short-term goal could be to increase reproductive success for key populations within a defined conservation unit, with a long-term goal to maintain gene flow throughout the range of a species by halting population decline. Developing a successful management tool that achieves both short-term and long-term conservation goals is often an iterative process (Gibbons, Wilson, and Green 2011).

Thirdly, monitoring is vital in evidence-based conservation management (Pullin and Knight 2003). For example, although creating artificial breeding habitat for a similar species has been enough to halt population decline, it might not be enough for the target species. The reason why a conservation action is not necessarily transferrable could be because other factors contributing to the population decline have not been addressed (e.g. a novel predator). If no monitoring occurs, the effectiveness of the management tool cannot be evaluated and the management cannot be adjusted accordingly (Margoluis et al. 2013). In order to determine if the threats are appropriately addressed and whether the conservation management is achieving the set short-term goal, monitoring needs to be undertaken.

Ultimately though, to achieve both short- and long-term goals, the translation from science to practice as well as into policy is necessary (Cook and Sgrò 2016; Ridley and Alexander 2016). For instance, a recent case study highlighted how connectivity based on the multi-disciplinary scientific study of gene flow and ecological interactions between organisms is incorporated into policy for achieving connectivity within rivers and watershed systems in the United States (Ridley and Alexander 2016). Freshwater ecosystems are home to a large number of specialists, under increasing threat worldwide, and an ecosystem where connectivity within and between species and communities is of particular importance (Dudgeon et al. 2006; Hauer et al. 2016). The case study by Ridley and Alexander (2016) could be used as a model in other countries on how
science, management, and policy can be linked to protect freshwater ecosystems and the species within it that are dependent on the connectivity of these ecosystems.
BRAIDED GRAVEL-BED RIVERS IN NEW ZEALAND

Worldwide, terrestrial ecosystems that are particularly threatened by biodiversity loss are those that create diverse habitats and communities, while also being located in lowland areas where human activities are more concentrated (Myers et al. 2000; Seabloom, Dobson, and Stoms 2002). New Zealand is no exception to that threat pattern in biodiversity loss. While about a third of its land area is protected for conservation purposes, the most irreplaceable and vulnerable native habitats most at risk of biodiversity loss are in lowland areas (Walker et al. 2006; Walker, Price, and Stephens 2008). Braided gravel-bed rivers are one example of a nationally and globally threatened, yet highly diverse ecosystem (Holdaway, Wiser, and Williams 2012).

Braided gravel-bed rivers are rivers that over some part of their length flow in multiple mobile channels across gravel floodplains (Bertoldi, Zanoni, and Tubino 2009). Such rivers are uncommon worldwide as they require variable discharge and a high level of erosion. Steep mountain ranges and wide plains downstream are the basis for the development of braided rivers as highly erodible bedrock and no constraints to lateral movement are present (Singh et al. 2017). They occur near the Rocky Mountains in North America, the Alps of Europe, the Andes of Patagonia, the Himalayas in Asia and in a disproportionately large number around the Southern Alps in New Zealand (Gray and Harding 2007; Hauer et al. 2016). The vast majority of braided rivers in New Zealand drain the eastern slopes of the Southern Alps (Caruso 2006). The Southern Alps and the braided rivers draining its ice fields began to form during a period of intensive tectonic uplift in the late Miocene and Pliocene, initiating rapid biological speciation (Craw et al. 2016). During Pleistocene glacial periods, the coastline and braid plain would have extended a further 70 km eastwards as sea-levels were lower than present day levels (Browne and Naish 2003). Today, braided rivers are home to many rare plant, fish, and invertebrate species, as well as a unique guild of endemic birds (O’Donnell and Moore 1983; Hughey 1985; Maloney et al. 1997; Heenan and Molloy 2004; Gray et al. 2006; Gray and Harding 2007; O’Donnell et al. 2016). Many threatened and endangered species are dependent for some or all of their life cycle on the diverse habitats that braided rivers create.

Braided rivers play an important role worldwide in sustaining biodiversity (Hauer et al. 2016). Yet they are also greatly affected by human infrastructure and activities as well as receiving poor legal protection on a national and global scale (Tockner and Stanford 2002; Williams et al. 2007; Hauer et al. 2016). In fact, in New Zealand only a very small proportion of braided rivers are within National Parks or reserves and most areas are Crown land administered by Land
Information New Zealand, which has no direct responsibility for natural heritage or indigenous wildlife (O’Donnell et al. 2016). New Zealand’s braided riverbed ecosystem has been classified as ‘Endangered’ using the International Union for Conservation of Nature (IUCN) red-list criteria as severe decline throughout more than 80% of its extant distribution has occurred (Holdaway, Wiser, and Williams 2012). This classification is mostly due to the severe spread of alien invasive plants (Williams and Wiser 2004; Brummer et al. 2016), which have far-reaching effects changing ecosystem processes by altering soil chemistry and thus plant community composition (Bellingham, Peltzer, and Walker 2005). Introduced plants also directly impact on available nesting habitat of birds, and indirectly upon their feeding habitat as the river braids are constrained into deeper channels (Maloney et al. 1999; Hicks et al. 2007; McClellan 2009; O’Donnell et al. 2016).

It is not only introduced plant species, but also introduced animal species, that have altered ecosystem functioning. Introduced mammals and fish prey upon braided river specialised wildlife (Sanders and Maloney 2002; Townsend 2003; Murphy et al. 2004; Jones, Moss, and Sanders 2005; Dowding, Elliott, and Murphy 2015). In addition, two avian predators of native wildlife, Australasian harriers/kāhu (Circus approximans) and Southern black-backed gulls/karoro (Larus dominicanus), are thought to have increased in population size over recent centuries (Heather, Robertson, and Onley 2015). This increase in abundance is attributed to the clearance of forest and creation of pastoral farmland around rivers in the last few centuries, abundance of new food sources such as introduced rabbits (Oryctolagus cuniculus), and lack of competition (Pierce and Maloney 1989; Waters, Fraser, and Hewitt 2013; Heather, Robertson, and Onley 2015).

Braided rivers are also under pressure from the growing demand of irrigation schemes and hydroelectric power generation for freshwater (Booker, Henderson, and Whitehead 2016). An over-allocation of water use consents exists in several catchments, particularly in the eastern part of the South Island (Booker 2018). Water abstraction, diversion, or impoundment alters river flows. Simultaneously, an intensification of agriculture has resulted in the conversion or modification of flood plains and river margins to irrigated or cultivated land, leading to poorer water quality (Grove et al. 2015; Ministry for the Environment and Stats NZ 2017). Altered flow patterns and poorer water quality have severe impacts on biodiversity, although not all impacts are clearly understood (Ministry for the Environment and Stats NZ 2017). Reduced flows favour the spread and establishment of invasive exotic plants and movement of introduced mammals in the riverbed, while also directly degrading habitat for native bird, fish and invertebrate species (Hughey 1985; Lessard et al. 2013; Pickerell 2015; Brummer et al. 2016).
The interaction of spreading invasive plants, increased predation pressure from introduced and native top predators, and reduced water flows in addition to other human activities, such as four-wheel driving, constitutes a serious threat to the persistence of New Zealand’s braided river ecosystem (O’Donnell et al. 2016).
BRAIDED RIVER SPECIALISTS — BLACK-FRONTED TERNs

From marshes to rivers

Part of the unique braided river ecosystem in New Zealand is the endemic black-fronted tern/tarapirohe (*Chlidonias albostriatus*; Fig. 1.2). The black-fronted tern is very distinctive in its breeding plumage with the black cap contrasted by a narrow white cheek stripe (Higgins and Davies 1996; Heather, Robertson, and Onley 2015). It is a small tern with slate grey body plumage, bright orange bill and short orange legs (Higgins and Davies 1996; Heather, Robertson, and Onley 2015). To Māori, it is a taonga (highly valued) species and eggs used to be harvested as a food source (Ngāi Tahu Claims Settlement Act 1998; Fyfe and Davis 2015).

The taxonomic classification of black-fronted terns provoked considerable confusion for a long time, placing them either with the ‘typical black-capped’ tern (genus *Sterna*) or with the ‘marsh’ terns (genus *Chlidonias*) (Lalas and Heather 1980). A phylogenetic analysis of terns placed them firmly within the marsh terns confirming that the slightly darker plumage and dispersion inland for breeding reflect more strongly their taxonomic affinity than the absence of marsh nesting (Bridge, Jones, and Baker 2005). So far, it is unresolved if black-fronted terns or whiskered terns (*C. hybridus*) are the most basal of the marsh terns, but the radiation of the clade is thought to have taken place around 5 million years ago at the beginning of the Pliocene (Bridge, Jones, and Baker 2005). It is the only tern species that is naturally range-restricted and one of four endangered species of the family Sternidae.
**Black-fronted tern ecology**

During the austral spring and summer (September–January), black-fronted terns breed in the braided riverbeds of the South Island of New Zealand, while migrating to the coast of all three main islands (South Island, North Island, and Stewart Island) during winter (Lalas 1979). Breeding occurs in loose colonies on shingle bars or islands in the riverbeds (Higgins and Davies 1996; Keedwell 2005). Before colonies are occupied, pairs form after intensive courtship displays, including synchronous aerial glides, sometimes with the exchange of prey items (Fig. 1.3; Lalas 1977). The pairs are thought to be monogamous (Higgins and Davies 1996). The nest consists of a shallow bowl in fine sand, on medium-sized shingle or between large boulders depending on the substrate of the river (A. Schlesselmann, pers. obs.). Generally, two eggs (range 1–4 eggs) are laid 1–2 days apart and are incubated by both adults for about 25 days (range 22–31 days) (Keedwell 2005). Nest formation between colonies is often asynchronous, but reasonably synchronous within colonies (Keedwell 2005). During daylight hours, black-fronted terns will attempt to repel intruders near breeding colonies by alarm calling and dive bombing, often in groups (Stead 1932; Higgins and Davies 1996; Keedwell 2005). Chicks leave the nest after a few days and fully fledge after approximately 30 days (Keedwell 2005). Overall, hatching and fledging success is low and highly variable among individual colonies (Keedwell 2005; Cruz et al. 2013; Bell 2017).

Little is currently known about black-fronted terns natal and breeding site fidelity and site tenacity. They are difficult to mark and follow using traditional methods; not many colour bands can be used as their legs are very short and the small size does not make them suitable for wing bands. Furthermore, they are highly mobile and live in areas that are hard to survey (O’Donnell and Hoare 2011). Keedwell (2005) banded 168 birds of which only nine were re-sighted at or within 20 km of their natal colony. Similarly, of 69 banded adults, only 31 were re-sighted in the same river, but not in the same colony (Keedwell 2005). Colony locations seem to vary between years and not all colony locations are re-used each year (Keedwell 2005; O’Donnell et al. 2016).

**Threats to black-fronted terns**

The threats faced by the braided river ecosystem as a whole are also affecting black-fronted terns. Indeed, it is predominantly the synergy between two common drivers of extinction that threaten many species worldwide, namely introduced predators and habitat loss or degradation (Brook, Sodhi, and Bradshaw 2008).
Introduced mammalian, as well as native avian, predators are a primary cause of mortality of black-fronted tern eggs, chicks and adults (Keedwell et al. 2002; Sanders and Maloney 2002; Keedwell 2003; Keedwell 2005; Steffens et al. 2012; Bell 2017). The main mammalian predators in the Mackenzie Basin and in the upper Clarence and Acheron Rivers are feral cats (Felis catus), ferrets (Mustela furo), stoats (M. erminea) and hedgehogs (Erinaceus europaeus occidentalis) (Sanders and Maloney 2002; Keedwell 2005; Bell 2017). In contrast, in the lowland Wairau River, Australasian harriers were identified as the main nest predator (Steffens et al. 2012). In addition, a single predator can cause nest failure of a whole breeding colony by directly preying upon nests, as well as causing the desertion of nearby nests (Keedwell 2005; O’Donnell, Sedgeley, and van Hal 2010). Thus, the species’ colonial nesting habit makes them vulnerable to catastrophic nesting failure.

Black-fronted tern breeding and foraging habitat is being degraded by invasive weed species, such as crack willow (Salix fragilis) and broom (Cytisus scoparius), as they stabilise shingle islands hindering the natural island turnover and increasing channelisation (Hicks et al. 2007). Introduced vegetation in riverbeds also provides habitat (i.e. food sources, such as rabbits, and cover) for mammalian predators (Pascoe 1995; Maloney et al. 1999; Pickerell 2015). Water abstraction, diversion and impoundment for irrigation or hydroelectric power generation reduces water flow in rivers (Ministry for the Environment and Stats NZ 2017). Reduced water flow facilitates weed encroachment, reduces available foraging habitat for black-fronted terns, and increases the accessibility of colonies to mammalian predators (Bunn and Arthington 2002; Jowett, Richardson, and Bonnett 2005; Brummer et al. 2016).

These interacting factors cause black-fronted terns to be of particular conservation concern. They are currently classified as ‘Endangered’ by the IUCN, and ‘Nationally Endangered’ by the New Zealand Department of Conservation (DOC) (BirdLife International 2017; Robertson et al. 2017). The population has been estimated at fewer than 10,000 individuals, but precise numbers are not known (Keedwell 2002; O’Donnell and Hoare 2011). A meta-analysis of population trends based on braided river bird counts from 1962–2008 detected widespread declines, particularly on smaller rivers, and predicted an overall population reduction of 50% over the next three generations (O’Donnell and Hoare 2011). One of the key issues identified is that the current recruitment failure might be masking a future catastrophic population decline (Townsend et al. 2008; Robertson et al. 2017). Current conservation management focuses on predator control, at either a very localised or large landscape-level, and has been primarily undertaken in the Mackenzie Basin (Cruz et al. 2013; O’Donnell et al. 2016; but see Bell 2017). The success of these conservation measures has been mixed as either an increase in breeding
success was not demonstrable, or the level of predator control is not currently sustainable over multiple sites (Cruz et al. 2013; O’Donnell et al. 2016).
THESIS AIMS AND STRUCTURE

In this thesis, I use black-fronted terns (*Chlidonias albostriatus*) as a case study in the integration of genetic and ecological research for effective, holistic conservation planning. The thesis comprises this general introduction (Chapter 1), four chapters addressing different aspects of conservation biology, a final chapter synthesising the findings and an appendix.

In Chapter 2, I describe the isolation and characterisation of 18 novel microsatellite markers for black-fronted terns from next-generation sequencing data. These microsatellite markers provided the basis for the further investigation of genetic diversity, population structure and demographic dynamics.

In Chapter 3, I examine the population genetic and phenotypic diversity and structure of black-fronted terns across their complete range, using contemporary molecular and morphological data. Given that black-fronted terns are highly mobile, I expected high gene flow between breeding colonies. Based on the potential weak breeding site fidelity, I expected no differences in phenotypes throughout New Zealand despite its large latitudinal range. This study provides the basis for delineating conservation units for black-fronted terns, ensuring that adaptive and genetic diversity is captured and range-wide gene flow can be sustained.

In Chapter 4, I assess past demographic trends and the current population status of black-fronted terns based on range-wide contemporary molecular data. Specifically, I expected that population sizes were historically larger and population size expansion occurred during the last glaciation period. In addition, I wanted to test if black-fronted terns have already experienced a population bottleneck given that contractions in range and size have been reported.

In Chapter 5, I address management issues by testing a tool to reduce ecological risks (i.e. habitat loss through introduced weeds and predators) in a currently unmanaged lowland river site. Vegetation was cleared from seven islands in the lower Waitaki River using heavy machinery, with mammalian predator abundance and black-fronted tern nesting success monitored beforehand and afterwards. I expected that more mammalian predator species would be detected on the banks and vegetated islands compared to cleared islands. Additionally, I expected breeding success to be higher on cleared islands and the main predator to be feral cats or stoats.

In Chapter 6 — a synthesis of the entirety of my work — I apply my findings to give clear guidance for the short- and long-term recovery of black-fronted terns. I also discuss considerations for assessing connectivity and population trends of endangered species and
triailling management options for successful short- and long-term conservation strategies, particularly for highly mobile and migratory species.

In the Appendix, I present additional detail to some of the analyses as well as a short note describing the distribution of black-fronted tern colonies in a single season. This short note was the product of searching for black-fronted tern colonies in the spring 2014 to catch individuals and collect blood samples. It provides a baseline for future breeding colony surveys. The work has been published as:


Each of the data chapters is intended for publication as a stand-alone scientific paper. Consequently, some degree of repetition exists in each chapter, but I have attempted to keep it to a minimum and combined all references at the end of the thesis. As each publication will be co-authored, I have also used first person plural throughout the thesis. Nevertheless, the work presented in this thesis is largely my own. It benefitted from discussions and input of others. The specific input from others into each chapter is highlighted on the title pages of each chapter.
CHAPTER 2

Isolation and characterisation of 18 polymorphic microsatellite loci for black-fronted terns

A version of this chapter has been published as:


Bruce Robertson assisted with the design of the primers and commented on drafts. Department of Conservation staff and contractors assisted with some of the blood sample collection. The majority of samples were collected by Jamie Cooper and myself. Fiona Robertson assisted with DNA extractions and Tania King with optimisation procedures. I undertook the majority of lab work, analysis and manuscript preparation.
Abstract

Eighteen polymorphic microsatellite loci were isolated and characterised from the endangered black-fronted tern (*Chlidonias albostriatus*), a species endemic to New Zealand. The loci were initially tested on seven individuals spanning the entire breeding range and then for a larger data set of 345 samples covering 30 colonies from throughout the species’ range. The number of alleles per loci ranged from 4 to 24, and observed and expected heterozygosity ranged between 0.46 and 0.95 and 0.45 to 0.86, respectively. One locus showed significant heterozygote deficit and appears to be sex-linked. The similar annealing temperatures across loci and the wide fragment sizes allowed multiplex polymerase chain reaction and rapid multilocus genotyping. The microsatellite markers will be useful tools for further investigation into the genetic diversity, population structure and effective population size of this endangered species and for informing conservation management.
INTRODUCTION

The New Zealand endemic black-fronted terns (*Chlidonias albostriatus*) are nationally and internationally classified as ‘Endangered’ (Robertson et al. 2017; BirdLife International 2017). A recent meta-analysis of population trends in breeding colonies of black-fronted terns predicted a decline by approximately 50% over the next 30 years, with more severe reductions of around 90% on rivers with low flow (O’Donnell and Hoare 2011). The main threats are predation and ongoing habitat degradation and loss (Maloney et al. 1999; Keedwell et al. 2002; Keedwell 2005; Steffens et al. 2012). There is an urgent need to manage black-fronted tern populations and reverse the current population trends; however, critical information about the species’ biology, particularly the connection of breeding colonies within and between catchments, is currently lacking. Genetics provides a tool to assess population structure, connectivity and dispersal, which allows researchers to identify appropriate management units for conservation (Moritz 1994; Haig et al. 2011; Allendorf, Luikart, and Aitken 2013). We developed primers for 18 microsatellite markers for black-fronted terns to examine genetic diversity, population structure and effective population size. This information will allow conservation practitioners to instigate management at the appropriate locations and geographic scale.
Chapter 2: Microsatellites for black-fronted terns

**MATERIALS AND METHODS**

**Primer development**

Whole genomic DNA was extracted from one black-fronted tern individual using a standard phenol/chloroform extraction method. An Illumina TruSeq DNA PCR-Free library was prepared and 2 x 250 bp reads were generated using Illumina’s MiSeq. MSATCOMMANDER was used to detect di-, tri, and tetra-nucleotide microsatellite repeats and design appropriate primer pairs (Faircloth 2008). Forward primers were tagged with a M13 sequence (5’-TGTAAAACGACGGCCAGT-3’) at the 5’ end to facilitate the use of a universal fluorescent-labelled M13 primer (Schuelke 2000).

All primers were initially tested using seven black-fronted tern individuals spanning the breeding range. Polymorphic loci were then screened in a larger data set of 345 individuals from 30 breeding colonies. Loci were assigned to multiplex groups for polymerase chain reaction (PCR) according to allele size ranges (Table 2.1). Each 2 µl PCR reaction included 1 µl (10-20 ng) DNA (dried down), 1 µl of 2 x Type-it Multiplex PCR Master Mix (QIAGEN), 0.04 µM (per loci) of each M13-labeled, locus-specific forward primer, 0.16 µM (per loci) of each locus-specific reverse primer and 0.16 µM of universal M13 primer labeled with fluorescent dye (Applied Biosystems DS-33; 6-FAM, NED, PET or VIC). Thermocycling conditions were an initial 15 min denaturation at 95 °C, followed by eight cycles of 94 °C for 30 sec, 60 °C (touchdown 1 °C per cycle) for 90 sec and 72 °C for 60 sec, a further 25 cycles of 94 °C for 30 sec, 52 °C for 90 sec, 72 °C for 60 sec, and a final elongation step of 60 °C for 30 min. Electrophoresis of amplified PCR products was performed on an ABI 3730xl DNA Analyzer (Applied Biosystems) using GeneScan 500 LIZ as size standard. Alleles were subsequently scored using GENEMAPPER (Applied Biosystems).

**Tests for errors and diversity**

Tests for Hardy-Weinberg equilibrium and linkage disequilibrium for each population were performed using GENEPOP v. 4.4 (Rousset 2008), and both measures were adjusted for multiple pairwise comparisons using the sequential Bonferroni correction (Rice 1989). Furthermore, tests for the presence of null alleles and allelic dropout were conducted in MICRO-CHECKER (van Oosterhout et al. 2004). Genetic diversity based on number of alleles and expected and observed heterozygosities were calculated using GENALEX v. 6.5 for the species as a whole (Peakall and Smouse 2012).
Chapter 2: Microsatellites for black-fronted terns

Table 2.1 Characteristics of 18 microsatellite loci developed for the black-fronted tern. Na = number of alleles, $H_E$ = expected heterozygosity, $H_O$ = observed heterozygosity. All loci were characterised with 345 individuals sampled.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer Sequence (5’-3’)</th>
<th>Repeat Motif</th>
<th>Na</th>
<th>Size Range (bp)</th>
<th>$H_E/H_O$</th>
<th>Dye (Mix No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calbo 1</td>
<td>F: TGTAAAACGACGGCCAGTGACCATCCACGAAATCCTGCAATGAGTGAGTAGTCGAGTC</td>
<td>AC</td>
<td>11</td>
<td>136-160</td>
<td>0.787/0.838</td>
<td>NED (1)</td>
</tr>
<tr>
<td>Calbo 2</td>
<td>F: TGTAAAACGACGGCCAGTGGACTGCTCATCTAACCCTGCC</td>
<td>AAT</td>
<td>23</td>
<td>164-230</td>
<td>0.861/0.949</td>
<td>6-FAM (2)</td>
</tr>
<tr>
<td>Calbo 5</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>ATCC</td>
<td>11</td>
<td>167-207</td>
<td>0.822/0.874</td>
<td>PET (2)</td>
</tr>
<tr>
<td>Calbo 14*</td>
<td>F: TGTAAAACGACGGCCAGTGGACTGCTCATCTAATGCAATG</td>
<td>AGAT</td>
<td>14</td>
<td>168-224</td>
<td>0.777/0.461</td>
<td>6-FAM (1)</td>
</tr>
<tr>
<td>Calbo 15</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>AC</td>
<td>6</td>
<td>249-261</td>
<td>0.424/0.482</td>
<td>6-FAM (1)</td>
</tr>
<tr>
<td>Calbo 17</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>AC</td>
<td>10</td>
<td>210-228</td>
<td>0.780/0.836</td>
<td>VIC (1)</td>
</tr>
<tr>
<td>Calbo 19</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>AC</td>
<td>9</td>
<td>151-167</td>
<td>0.618/0.647</td>
<td>PET (2)</td>
</tr>
<tr>
<td>Calbo 22</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>ATCC</td>
<td>10</td>
<td>136-176</td>
<td>0.690/0.707</td>
<td>6-FAM (2)</td>
</tr>
<tr>
<td>Calbo 24</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>AG</td>
<td>6</td>
<td>251-261</td>
<td>0.659/0.680</td>
<td>PET (2)</td>
</tr>
<tr>
<td>Calbo 27</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>AG</td>
<td>7</td>
<td>214-228</td>
<td>0.527/0.536</td>
<td>VIC (2)</td>
</tr>
<tr>
<td>Calbo 28</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>AC</td>
<td>7</td>
<td>122-134</td>
<td>0.603/0.638</td>
<td>VIC (1)</td>
</tr>
<tr>
<td>Calbo 29</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>AC</td>
<td>7</td>
<td>170-188</td>
<td>0.587/0.576</td>
<td>VIC (2)</td>
</tr>
<tr>
<td>Calbo 31</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>AC</td>
<td>4</td>
<td>123-129</td>
<td>0.457/0.455</td>
<td>6-FAM (1)</td>
</tr>
<tr>
<td>Calbo 34</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>AC</td>
<td>5</td>
<td>130-138</td>
<td>0.445/0.467</td>
<td>PET (1)</td>
</tr>
<tr>
<td>Calbo 35</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>AAT</td>
<td>13</td>
<td>162-202</td>
<td>0.824/0.894</td>
<td>PET (1)</td>
</tr>
<tr>
<td>Calbo 39</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>AC</td>
<td>10</td>
<td>241-267</td>
<td>0.564/0.603</td>
<td>VIC (2)</td>
</tr>
<tr>
<td>Calbo 43</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>AAT</td>
<td>19</td>
<td>120-183</td>
<td>0.818/0.863</td>
<td>NED (2)</td>
</tr>
<tr>
<td>Calbo 48</td>
<td>F: TGTAAAACGACGGCCAGTGGCTCGAGTTCCTAATGCAATG</td>
<td>AC</td>
<td>4</td>
<td>255-261</td>
<td>0.543/0.584</td>
<td>PET (1)</td>
</tr>
</tbody>
</table>

*Locus appears to be sex-linked.

To investigate one locus that showed significant heterozygote deficit, we ran tests for Hardy-Weinberg equilibrium and linkage disequilibrium separately for each sex. Black-fronted terns show no obvious sexual differences in plumage, and it is not possible to confidently determine the sex of black-fronted terns solely based on external measurements due to overlap in body size (Chapter 3). Thus, to determine the sex of individuals we used a molecular sexing technique that tests for the detection of the female-specific W chromosome (Ellegren and Sheldon, 1997). We used the P8-Forward/M5-Reverse primer pair targeting the sex-specific versions of the chromo-helicase-DNA binding protein (CHD1; Griffiths et al., 1998; Bantock, Prys-Jones, and Lee...
2008) and included it in microsatellite multiplex mix 1 using the same thermocycling conditions and the same post-PCR procedures as for the microsatellite loci.

Technical failure or PCR anomalies can incorrectly class a female (ZW) as a male bird (ZZ), and hence either known-sex individuals or a second independent test is needed to verify sexes (Robertson and Gemmell 2006). For black-fronted terns, no known-sex individuals were available and another sex marker (2550F/2718R (Fridolfsson and Ellegren 1999)) performed poorly in a test run. We assumed that all pairs (n = 31) caught were monogamous heterosexual pairs and checked if each pair was assigned a female and male bird to test the error rate of the molecular sexing technique as well as blind-repeated the PCR amplification of 59 randomly chosen individuals (> 10% samples).
RESULTS AND DISCUSSION

Twenty-nine of the 48 loci amplified a product of expected size, with 24 loci being polymorphic. Of those 24 polymorphic loci, 18 amplified cleanly and consistently. A total of 176 alleles were identified across the 18 loci, with each locus having on average 9.8 alleles (range = 4-23 alleles; Table 2.1). Overall observed and expected heterozygosities ranged from 0.455 to 0.949 and 0.445 to 0.861, respectively. It was interesting to note that tri- and tetra-nucleotide repeat loci (e.g. Calbo 5) appear to exhibit higher levels of polymorphism ($Na$ and $H_e$) than di-nucleotide repeats (e.g. Calbo 27; Table 2.1). Generally, the degree of polymorphism is proportional to the underlying rate of mutation, with longer repeat lengths having higher mutation rates independent of the repeat motif (Ellegren 2004). One locus (Calbo 14) showed a significant heterozygote deficit (Table 2.1). Only 11 of 152 known females were heterozygous at this locus. Re-running the analysis with only male individuals removed the heterozygote deficit suggesting that this locus is sex-linked. We are confident that the molecular sexing technique was appropriate as all 31 pairs caught were heterosexual based on molecular sexing, no signs of polygamy were observed in the field and the repeat amplifications had a very low error rate of 0.0169. We did not detect a significant linkage disequilibrium among pairs of loci. Calbo 17, 28, 29 and 31 showed evidence of null alleles in one of the 30 populations each; however, no consistent evidence for null alleles was found. No evidence for large allele dropout was detected.

These specifically developed novel microsatellite markers are currently used together with mitochondrial DNA (cytochrome b and control region) in the population and conservation genetic study of black-fronted terns with the aim of assisting in the species’ conservation management (Chapters 3 and 4). Furthermore, these 18 markers may also be a useful resource in population genetic studies of other tern species, for which few microsatellite markers currently are available.
CHAPTER 3

Clinal variation in body size, despite low genetic differentiation of black-fronted terns, is important for conservation management.
Chapter 3: Delineating conservation units for black-fronted terns

ABSTRACT

Knowledge of genetic diversity and population structuring is vital to designing effective species conservation actions. Appropriate genetic management of threatened species can be instigated through basing the delineation of appropriate units of conservation on molecular markers and phenotypic data. The New Zealand endemic black-fronted tern (Chlidonias albostriatus), a specialist braided river bird, is classified as ‘Endangered’ due to populations being in decline. A range of threats, predominantly predation in combination with ongoing habitat degradation and loss, is causing this decline. As a highly mobile and cryptic species, basic information important to the species’ conservation is currently lacking. We utilised two mitochondrial (cytochrome b/control region) and 17 microsatellite markers, as well as morphological data (weight, head-bill length, bill depth, wing length) from the species’ whole breeding range, to evaluate the level of genetic diversity present, assess and compare geographic patterns of genetic and phenotypic divergence, and provide management recommendations. Genetic diversity within the species is high, showing no pattern of isolation-by-distance and little genetic divergence between breeding colonies. However, our data show morphological divergence in three traits consisting of increasing body size with increasing latitude. To maintain genetic diversity and gene flow throughout the whole range, we recommend that black-fronted terns are managed as a single genetic conservation unit. Importantly, to protect the metapopulation and facilitate natural colonisation and abandonment of breeding sites to occur from nearby colonies, we recommend targeting sites on a catchment level across the entire breeding range of the South Island, New Zealand.
INTRODUCTION

Successful long-term conservation takes ecological and evolutionary processes into account (Mace and Purvis 2008; Sgrò, Lowe, and Hoffmann 2011). A first step to designing successful conservation management actions for species of conservation concern is to delineate conservation units (Avise 1989; Frankham 2005; Frankham, Ballou, and Briscoe 2010; Frankham et al. 2017). These conservation units are ideally determined by integrating several kinds of information, including genetic and phenotypic data (Sæther et al. 2007; Allendorf, Luikart, and Aitken 2013; Dussex et al. 2018a). It has been particularly challenging to identify conservation units for highly mobile and migratory species with relatively large ranges (Martin et al. 2007; Runge et al. 2014; Paleczny et al. 2015; Runge et al. 2015). A case in point are seabirds and shorebirds which are increasingly threatened, yet often only little information is available to adequately plan conservation strategies (Kirby et al. 2008).

Many seabirds or shorebirds occur in geographically patchy distributions as they nest in discrete colonies at breeding sites. These breeding sites are linked by dispersal, which leads to regular abandonment (i.e. extinction) and occupation (i.e. colonization) of breeding sites (Spendelow et al. 1995; Akçakaya et al 2003; Martínez-Abraín et al. 2003; Oro 2003; Schippers et al. 2009; Catlin et al. 2016; García-Quismondo et al. 2018). While regular extinction and colonisation of patches is a typical aspect of metapopulations, such seabird or shorebird populations are not classical metapopulations (“Levins metapopulations”) as they are relatively rare for vertebrates (Harrison 1991; Hanski 1998; Fronholer et al. 2012). Nevertheless, seabird or shorebird populations still exhibit metapopulation dynamics that have important conservation implications (Hanski 1999; Szczys, Oswald, and Arnold 2018). To direct management to the right geographic location and scale, it is important to determine if there is a geographic pattern of population differentiation within the metapopulation consisting of either distinct population clusters or a cline.

For highly mobile species that occupy a large range, differential selection across the landscape and gene flow often result in clines (Frankham et al. 2017). One example of a cline, which is common in birds and mammals, is an increase in body size with increasing latitude (Ashton 2002; Meiri and Dayan 2003). This is also broadly known as Bergmann’s rule and presumed to be linked to the temperature budget of animals (Bergmann 1847, Salewski and Watt 2016). To make inferences about local adaptation and inform conservation management, a comparison of $P_{ST}$ (phenotypic divergence) with $F_{ST}$ (neutral genetic divergence) can be used (Saether et al. 2007; Cano et al. 2008; Leinonen et al. 2008; Brommer 2011). There are two
possible outcomes for this comparison if a cline in phenotypic divergence exists (Merilä & Crnokrak 2001; McKay and Latta 2002; Brommer 2001):

1. If $P_{ST} > F_{ST}$, divergence in phenotypic traits under study exceeds what is expected on the basis of drift, which is suggestive of natural selection favouring different phenotypes in different populations.

2. If $P_{ST} \approx F_{ST}$, the observed differentiation is most likely the result of genetic drift.

Because $P_{ST}$ is not able to measure quantitative divergence directly, the robustness of the inference of natural selection is dependent on the relationship between the extent by which phenotypic variation between populations is determined by additive genetic effects and the extent by which phenotypic variation within populations is determined by additive genetic effects (Brommer 2011). It is possible to assess the robustness of inference of local adaptation based on a $P_{ST}$-$F_{ST}$ comparison, but it is important to cautiously interpret results and rather consider them indicative only (Brommer et al. 2014). Nevertheless, it is possible to still gain some level of understanding on putative actions of natural selection (Antoniazza et al. 2010; Brommer et al. 2014; Dussex et al. 2015; Seeholzer and Brumfield 2018). Furthermore, the comparison of phenotypic and genetic divergence of populations enables a comprehensive delineation of conservation units and helps to ensure that effective long-term conservation can be achieved (Sgrò, Lowe, and Hoffmann 2011; Allendorf, Luikart and Aitken 2013; Dussex et al. 2018a).

Highly mobile and migratory species provide interesting case studies as potentially few barriers to gene flow and dispersal exist, yet they also often exhibit a high degree of philopatry and they occupy relatively large ranges over a large latitudinal range (Friesen, Burg, and McCoy 2007). The New Zealand endemic black-fronted tern/tarapirohe (Chlidonias albostriatus) is a good example of a species where little is known about the geographic pattern of population differentiation throughout its metapopulation of breeding colonies and conservation actions are urgent. Black-fronted terns are classified as ‘Nationally Endangered’ under the New Zealand Threat Classification System and ‘Endangered’ on the IUCN Red List (BirdLife International 2017; Robertson et al. 2017). Phylogenetic analysis has placed black-fronted terns within the marsh tern clade (genus Chlidonias), that radiated during the Pliocene ca. 5 million years ago (Bridge et al. 2005). They have specialised to breed on gravel bars within the braided rivers of the South Island of New Zealand, but migrate to the coast of all three main islands during the winter (Lalas 1979; Higgins and Davies 1996). The most recent estimate of total population size ranged between 7,000–10,000 individuals and populations are predicted to decline by approximately 50% over the next 30 years (Keedwell 2002; O’Donnell and Hoare 2011).
These declines are mainly thought to be the product of habitat loss and predation. Reduced river flows through water abstraction or impoundment potentially decrease foraging habitat availability (O’Donnell and Hoare 2011) and facilitate weed encroachment on nesting habitat (Maloney et al. 1999; Williams and Wiser 2004; Williams et al. 2007; Brummer et al. 2016). In addition, introduced mammals prey upon eggs, chicks and adults of nesting black-fronted terns and increased numbers of native avian predators reduce breeding success (Keedwell et al. 2002; Keedwell 2005; Steffens et al. 2012; Chapter 5). Only a very small proportion of the breeding habitat lies within national parks where some conservation management is in place, such as predator or weed control (O’Donnell and Hoare 2011; O’Donnell et al. 2016; Schlesselmann, Cooper, and Maloney 2017). Loose breeding colonies form in rivers in roughly similar areas annually, but exact locations can vary between years and site fidelity of pairs or colonies is not well-understood (Higgins and Davies 1996). Basic information on breeding biology, feeding ecology, and agents of decline exists, although this information mainly comes from breeding populations of a single region, the Mackenzie Basin (Lalas 1977; Keedwell 2002; Steffens et al. 2012). However, critical information across the species’ range important to the conservation of black-fronted terns, such as levels of genetic diversity, population structure and genetic connectivity between colonies, is still lacking.

Here, we utilise a combination of genetic and morphological data from extensive sampling from across the entire breeding range of black-fronted terns to identify conservation units for black-fronted terns. We evaluate both recent and historical divergence using mitochondrial and microsatellite marker data and assess range-wide morphological differentiation to inform future conservation management. Specifically, we aim to i) evaluate the level of black-fronted tern genetic diversity; ii) identify geographic patterns in genetic and phenotypic divergence between breeding colonies; iii) compare genetic and phenotypic divergence; and iv) provide management recommendations for the species conservation.
Chapter 3: Delineating conservation units for black-fronted terns

**MATERIALS AND METHODS**

*Sample collection and DNA extraction*

Black-fronted tern blood samples were collected from 31 breeding colonies from throughout their entire breeding range in the austral summer breeding seasons of 2013/14 and 2014/15 (Fig. 3.1). Colonies were located by searching river stretches on foot targeting areas of past colony records and, using binoculars, by following individuals that carried prey items back to colony or breeding site locations. A breeding site was defined as one or more pairs within a 1 km stretch of river. The stage of nesting was checked after locating a colony. Catching effort focused on adults on nests with at least two eggs (mean clutch size 1.9 ± 0.4 eggs; Keedwell 2005) to avoid any damage to females with developing eggs or risk desertion of nests in early incubation stages, and to avoid sampling relatives. If the colony was already at a stage where chicks had hatched, samples were collected from chicks ≥ 7 days old. Sampling was only undertaken in fine weather conditions and abandoned in cold (< 12°C) or hot (> 24°C) temperatures to minimise temperature stress to adults, chicks and eggs. Adults were caught with a custom-made remote electronically-triggered drop-trap or with a split-stick drop-trap carefully placed over the nest (Mills and Ryder 1979; Otago University ethics approval number 61/14). If it was evident that both partners were present, the trap was re-set to catch both adults. Chicks were caught by hand. Re-sampling of birds was prevented by banding each individual with a metal leg band (C-band) issued by the New Zealand Department of Conservation Banding Office.

Blood samples (< 20 µl) were obtained by brachial vein puncture with a sterile needle (26G x 1/2”). The blood was collected with a glass capillary tube and immediately transferred into 1 ml of Queens lysis buffer (10 mM Tris, 10 mM NaCl, 10 mM Na-EDTA, 1% n-lauroylsarcosine; pH 7.5; Seutin, White, and Boag 1991). DNA was extracted and purified from the blood using a standard 5% Chelex protocol (Walsh, Metzger, and Higuchi 1991).

Morphometric measurements of adult black-fronted terns were collected from 27 colonies at the same time as collecting blood samples in the austral summer breeding season of 2014/15 (Fig. 3.1). Weight, wing length, bill depth, and head-and-bill length were measured for female (n = 178) and male (n = 178) adults only. Wing length (to the nearest 0.1 mm) was measured using a stopped ruler from the leading edge of the wrist joint to the tip of the longest primary without flattening the wing chord. Bill depth (to the nearest 0.1 mm) was measured using SPI dial calipers (Swiss Precision Instruments, Inc.) from the top of the nare, and head and bill length (to the nearest 0.1 mm) from the back of the head to the bill tip.
**Microsatellite genotyping and molecular sexing**

All samples were genotyped at 17 microsatellite loci and sex was determined using DNA sexing (in two multiplexes; Chapter 2). Electrophoresis of amplified polymerase chain reaction (PCR) products was performed on an ABI 3730xl DNA Analyser (Applied Biosystems) using GeneScan™ 500 LIZ as a size standard and alleles were scored manually using GENEMAPPER V. 4.0 (Applied Biosystems). Samples failing to amplify for more than one locus were discarded. A total of 422 were used in subsequent analyses from 31 colonies spanning 9 broad regions (Marlborough, Tasman, Westland, Amuri Basin, Canterbury Plains, Canterbury High Country, Mackenzie Basin, Otago, Southland, Fig. 3.1).

![Diagram showing sampling locations](image)

**Figure 3.1** Black-fronted tern sampling locations (rivers) in the South Island of New Zealand. Numbers in parentheses represent numbers of samples analysed for microsatellites (listed first), mitochondrial DNA cytochrome b (listed second), mitochondrial DNA control region (listed third), and morphometric data. ‘-’ indicates that no samples were collected at a location.

**Mitochondrial DNA sequencing**

We sequenced a spatially representative subsample of individuals from across the breeding range (Fig. 3.1) for two molecular markers: cytochrome b gene \((n = 35)\) and the control region
(n = 64). We incrementally added individuals into the subset until further individuals did not reveal any further deep structuring.

To amplify and sequence the whole cytochrome b gene (1,143 base-pairs (bp)), we used the primers L14764 and H16064 (Sorenson et al. 1999). PCRs were performed in a 25 µl reaction containing 10-30 ng of DNA, 1 x PCR buffer, 1.5 mM MgCl₂, 200 µM of each dNTP, 0.5 units of Taq polymerase (Bioline USA, Inc, Randolph, MA, USA) and 1 pmol of each primer. The thermal cycling conditions were an initial 3 min denaturation at 94°C, followed by 10 cycles consisting of 20 s at 94°C, 25 sec at 60°C (touchdown by 1 °C per cycle), and 70 sec at 72°C, then 25 cycles of 20 s at 94°C, 25 sec at 50°C, and 70 sec at 72°C. All PCR products were purified using Acropep 96 filter plates (PALL Corporation) and sequencing was carried out with a BigDye v.3.1 sequencing kit (Applied Biosystems). Sequence products were purified using Sephadex-G50 gel filtration (Amersham Bioscience) and run on an ABI 3730xl DNA analyser.

To amplify the control region, we developed primers in three steps. Details for all primers used in the development and final amplification can be found in Table A.1 in the appendix. We first designed primers based on cytochrome b and 12S sequence of black-fronted terns (NCBI accession numbers: AY631295.1 and AY631331.1) in the cytochrome b and 12S genes (L-BFT-cytb12Sa (Table A.1) and H-BFT-cytB12S (Table A.1)). Using these primers, we obtained a ca. 2500 bp fragment from which we designed a further primer located in the ND6 gene, L-BFT-ND612s (Table A.1). In the second step, we used L-BFT-ND612s in conjunction with H-BFT-cytB12S to yield an approximately 1200 bp fragment. We experienced problems with consistent amplification with these primers and hence band-stabbed the fragment out of 1% agarose gel (TAE: 134 mM Tris, 74.9 mM boric acid, 2.55 mM EDTA pH 8.8) under blue light and purified using MEGA-QuickSpin Extraction kit (iNtRON Biotechnology). This fragment was then sequenced using two generic primers located in the more conserved second and third domain of the control region, Av510FDloop (Table A.1) and Av807DloopR (Table A.1). Based on the fragments obtained in combination with the reads of the Illumina TruSeq DNA PCR-Free library (Chapter 2), we used a custom blast search in GENEIOUS V. 6.0.6. (Biomatters Ltd., New Zealand) to assemble the whole black-fronted tern control region using little tern (Sternula albifrons; NCBI accession number: NC028176) as a reference. Lastly, we designed black-fronted tern specific primers for PCR amplification located just outside the control region, BFT-CR-ND6-1F (Table A.1) located in t-RNA-Glu and BFT-CR-12S_1R (Table A.1) located just at the t-RNA-Phe end of the 12S gene. In addition, we designed three additional internal primers (BFT-CR-F1, BFT-CR-R2, and BFT-CR-goosehairpin-F1; Table A.1) located in between the poly-C structure known as goose
hairpin and the microsatellite at the end of the third domain of the control region as we were not able to sequence through both structures using the amplification primers.

We amplified approximately 1000 bp of mitochondrial control region in a 25 µl reaction containing 10-30 ng of DNA, 1 x PCR buffer, 1.5 mM MgCl₂, 200 µm of each dNTP, 0.5 units of Taq polymerase (Bioline USA, Inc, Randolph, MA, USA) and 1 pmol of each primer. The thermal cycling conditions were an initial 5 min denaturation at 95 °C, followed by 10 cycles consisting of 20 s at 95 °C, 25 sec at 64 °C (touchdown by 1 °C per cycle), and 70 sec at 72°C, then 25 cycles of 20 sec at 95°C, 25 sec at 50°C, and a final extension for 70 sec at 72°C.

Purification and sequencing was carried out for the control region as described above for cytochrome b. We used a combination of all five primers to amplify and sequence all black-fronted tern samples (Table A.2), which yielded an 898 bp fragment.

Genetic diversity

Microsatellite Data
To check for genotyping errors, 13% of all samples were randomly selected and blind repeat-genotyped at all loci (Hoffman and Amos 2005). The error rate per allele was calculated as the number of incorrect alleles divided by the total number of alleles. MICRO-CHECKER v. 2.2.3. (van Oosterhout et al. 2004) was used to check for scoring error due to stuttering, large allele dropout, and null alleles. Tests for Hardy-Weinberg proportions (HWE) and linkage disequilibrium (LD) were carried out for each river in GENEPOP v. 4.4 (Rousset 2008) using the Markov chain Monte Carlo (MCMC) method with 10,000 dememorisations, 1,000 batches and 10,000 iterations. Significance levels were adjusted for multiple statistical tests using a sequential Bonferroni correction (Rice 1989). Levels of genetic diversity were calculated for different rivers, sampling regions and for the species overall. Expected (Hₑ) and observed heterozygosities (Hₒ) and mean number of alleles were calculated in GENALEX v. 6.5 (Peakall and Smouse 2012). Mean allelic diversity calculated as allelic richness, which corrects for sample size, and was calculated in FSTAT v. 2.9.3.2 (Goudet 2001) including breeding colonies with > 5 samples only. To compare phenotypic divergence with genetic differentiation, we also calculated overall Fₛᵗ with 95% confidence intervals using 15,000 bootstrap replicates in FSTAT v. 2.9.3.2 (Goudet 2001).

Mitochondrial Data
All sequences were imported into GENEIOUS v. 6.0.6. (Biomatters Ltd., New Zealand) for alignment and variable sites were confirmed by inspecting the chromatograms visually.
Cytochrome b sequences were translated to detect reading frame errors and stop codons. Single-site ambiguities were detected in six individuals at six different sites in the CR sequences. We attributed these ambiguities to single-site heteroplasmy for the following reasons (Moum and Bakke 2001). First, Charadriiformes are not known to have a duplicated nuclear copy of the control region (Hu et al. 2017). Second, primers were developed specifically for black-fronted terns and we started with a long fragment (> 2000 bp). Third, we only ever obtained one band in 1% agarose gels (TAE: 134 mM Tris, 74.9 mM boric acid, 2.55 mM EDTA pH 8.8) to check if the PCR was successful. And lastly, when we searched Genbank the resulting fragment only matched other mitochondrial sequences. These ambiguous sites were excluded from subsequent analyses.

We calculated the number of observed haplotypes ($H$), nucleotide diversity ($\pi$; average number of nucleotide differences per site in pairwise sequence comparisons; Nei 1987) and haplotype diversity ($hd$; probability that two randomly sampled alleles are different) in DNAsp 6 (Rozas et al. 2017).

*Genetic population structure analyses*

**Microsatellite Data**

To assess the spatial structure of genetic data, we employed two different Bayesian clustering methods (one non-spatial and one spatial) using *STRUCTURE* v. 2.3.4. (Falush, Stephens, and Pritchard 2003; Pritchard, Stephens, and Donnelly 2000) and *Tess* v. 2.3.1. (Chen et al. 2007). These methods assign individuals probabilistically to clusters ($K$) based on their individual multilocus genotypes by calculating membership coefficients ($Q$) per individual and cluster. All samples from 31 breeding colonies were included and priors chosen to accommodate for potential unbalanced sampling (Wang 2017). Both *STRUCTURE* and *Tess* employ a MCMC simulation; however, while *STRUCTURE* relies solely on genetic information from each individual, *Tess* explicitly uses both spatial (geographic coordinates) and genetic information to infer clusters (François and Durand 2010).

In *STRUCTURE*, clusters are estimated by maximising HWE while minimising LD. We tested for $K = 1$ to 9, with nine being the maximum number of broad geographical regions sampled. After initial exploratory runs, we employed a burn-in of 100,000 chains followed by 500,000 MCMC chains with 20 iterations for each number of $K$ without any prior population information (No LOCprior model). We used an admixture model, which allows each individual to draw fractions of their genome from multiple of the $K$ populations and a correlated allele frequency model.
which permits allele frequencies to be similar in different populations (Falush, Stephens, and Pritchard 2003). Both of these models were chosen as they enable more accurate assignments even in closely related populations (Falush, Stephens, and Pritchard 2003). Furthermore, we chose the population-specific ancestry prior and an initial ALPHA = 1/K (≈ 0.11) to allow for potential unbalanced sampling because only ten individuals from Westland were sampled in comparison to 81 individuals from the Mackenzie Basin and in most circumstances alternative prior values yield more accurate assignments (Wang 2017). Changes in ALPHA level were monitored closely to ensure the models converged as generally they will reach an equilibrium before the end of the burn-in phase (Pritchard, Wen, and Falush 2010).

In addition, we also analysed the data set including prior location information (LOCPRIOR model), that is the river section an individual was sampled from. Using LOCPRIOR models can be advantageous in cases where there is little information in the data set because clustering solutions that correlate with locations are preferred and they are still robust against detecting structure when there is none (Hubisz et al. 2009). All other settings remained the same as described above. Changes in ALPHA and r level were monitored to ensure models converged and locations were informative (Pritchard, Wen, and Falush 2010).

We used two different methods to determine the maximum number of parental populations $K_{\text{max}}$ that best fits the data. Firstly, we inferred the best value of $K_{\text{max}}$ by assessing the probability of obtaining the genotype data given each value of $K$ (Pritchard, Stephens, and Donnelly 2000). We therefore averaged the likelihood for each assumed $K$ across the 20 replicate runs with the highest mean likelihood value being indicative of $K_{\text{max}}$. Secondly, we also assessed $K_{\text{max}}$ by using the $\Delta K$ method (Evanno, Regnaut, and Goudet 2005) in STRUCTURE HARVESTER V. 0.6.94. (Earl and von Holdt 2012). This is an ad hoc statistic based on the rate of change in the log probability of the data between successive $K$ values and a local maximum denotes $K_{\text{max}}$ (Evanno, Regnaut, and Goudet 2005).

In TESS, we tested first for $K = 2$ to 9 and then extended the analysis to $K = 2$ to 20 because the initial analysis did not reach a plateau (see below). In both instances, we used an admixture model and a conditional auto-recessive (CAR) variance of 1.0, a spatial interaction parameter of 0.6, a mean distance scale parameter and a linear trend surface. This admixture model, similar to the admixture model in STRUCTURE, assumes that parts of individual genomes are drawn from multiple potentially unknown parental populations and arise through admixture of those populations (Durand et al. 2009). Furthermore, by incorporating spatial autocorrelation and trend surfaces, it enables detection of smooth clinal variation (Durand et al. 2009; François and
Durand 2010). The burn-in length was 10,000 sweeps, while the total number of sweeps was 50,000. A total of 50 iterations per $K$ were performed. We used breeding colony geographical coordinates and a pairwise distance matrix between individuals. To assess the best value of $K$, we considered the deviance information criterion (DIC) and the stability of the bar plots (Durand et al. 2009). We plotted the DIC values of all runs for each $K$ and the respective mean value against $K$ to determine the optimum cluster solution $K_{\text{max}}$, which is indicated by a low value of DIC and a plateau in the DIC curve (Durand et al. 2009). For both the STRUCTURE and TESS analyses, the iterated runs were averaged with CLUMPP v. 1.1.2. (Jakobsson and Rosenberg 2007) and visualised using DISTRUCT v. 1.1. (Rosenberg 2004).

To complement the Bayesian analyses, we conducted a discriminant analysis of principal components (DAPC) using the regions as prior population information (Jombart, Devillard, and Balloux 2010). DAPC partitions between- and within-cluster variation and employs principal components, which maximise the between variation while minimising the within variation, making it particularly suitable to identify clusters while also having the advantage of not relying on any particular population genetics model (Jombart, Devillard, and Balloux 2010). Cross-validation was carried out as part of the DAPC analysis to determine the number of principal components (PCs) to retain to achieve the highest predictive power by using 90% of the data as the training set and 10% as the validation set. This was implemented in R 3.3.3 (R Core Team 2017) using the adegenet 2.0.1 package (Jombart 2008).

We calculated pairwise $F_{ST}$ values for microsatellites between the different sampling locations (river sections) in ARLEQUIN v. 3.5.2.2 (Excoffier and Lischer 2010) after removing populations with small sample size ($n \leq 5$; Ophi n = 2; Ohau n = 1, Tasman n = 3; Mararoa n = 3). To investigate the degree of genetic differentiation among the sampling locations and test for differences, we used 1,000 permutations and adjusted significance levels for multiple comparisons using the sequential Bonferroni correction (Rice 1989). For comparison to pairwise $F_{ST}$ values, we also estimated Jost’s $D_{est}$ using SMOGD v. 1.2.5. (Jost 2008; Crawford 2010; Meirmans and Hedrick 2011). $D_{est}$ is calculated as the harmonic mean across all loci for each pairwise comparison.

In addition, we tested for isolation by distance (IBD) using the microsatellite markers in GENALEX v. 6.5 (Peakall and Smouse 2012) by performing a Mantel test (9,999 permutations) using pairwise linearised $F_{ST}$ values (Slatkin 1995). This was done using straight-line distances between all sampling locations and using waterway distances between a sub-sample of sampling
locations (Wairau, Grey, upper Waimakariri, Waitaki, Makarora, Clutha, Eglinton, Aparima rivers) as other tern species use waterways as flyways (Austin 1953).

To further investigate the low level of mitochondrial genetic diversity detected in the Mackenzie Basin (see Results) and to test whether this low diversity was due to family groups being sampled by chance, we examined the relatedness of all individuals after removing populations with small sample size ($n \leq 5$; Opihi $n = 2$; Ohau $n = 1$, Tasman $n = 3$; Mararoa $n = 3$) using the Queller & Goodnight (1989) relatedness coefficient.

Mitochondrial Data
We inferred a maximum-likelihood and Bayesian phylogeny for each of the mitochondrial sequence data sets (cytochrome $b$ and control region) to further examine the population structure of black-fronted tern breeding colonies. The AIC selection criterion in JMODELTEST v. 2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012) selected HKY + I as the most appropriate model of molecular evolution for both loci. Maximum-likelihood analyses were performed for each locus separately in PHYML v. 3.0 (Guindon et al. 2010) performing 1,000 bootstrap replicates.

The Bayesian phylogenies for each loci were obtained using the program MrBayes v. 3.2 (Ronquist et al. 2012) by using three cold and one heated MCMC chain, which were sampled every 200 generations over 200 million generations in two independent runs generating 100,000 trees each. The first 25% was discarded as burn-in and the remaining trees were used to construct a majority consensus tree. MCMC convergence was assessed by evaluating the standard deviations of the split frequencies and effective sample size values in MrBayes. Trees were rooted using the common tern (Sterna hirundo) as an outgroup (MF582632) as it was the closest relation for which we were able to attain sequences for both regions of interest.

In addition, we also investigated evolutionary and geographic relationships between haplotypes with median joining networks of cytochrome $b$ and the control region using POPART (Leigh and Bryant 2015).

Analysis of phenotypic differentiation across populations
To test if there was any evidence for a latitudinal cline in phenotypic variance, we constructed general linear mixed models using Bayesian MCMC methods. Head-bill length, bill depth, weight, and wing length were analysed separately as response variables and ‘Latitude’ and ‘Sex’ (to account for the variance between the sexes) were included as fixed effects as well as ‘River’
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(breeding colony origin) as a random effect (to account for population specific variance). Models were run for a total of 60,000 iterations with a sampling interval of 50 and a burn-in of 10,000 iterations. We also used uninformative priors to estimate the mode and 95% credible intervals of the posteriors and implemented all models in the package ‘MCMCglmm’ (Hadfield 2010). We used effect sizes and 95% confidence intervals to assess statistical significance of latitudinal cline and sexual size dimorphism (Nakagawa and Cuthill 2007).

Comparison of genetic and phenotypic divergence

To further compare the level of phenotypic divergence of traits across populations with genetic divergence, we used a $P_{ST} - F_{ST}$ comparison for the three traits for which a latitudinal cline was detected (head-bill length, bill depth, weight) (Leinonen et al. 2006; Brommer 2011). $P_{ST}$ is a proxy measure for $Q_{ST}$ (the quantitative genetic divergence (Spitze 1993)). Measuring $Q_{ST}$ based on additive genetic effects for wild bird species is incredibly challenging as common garden breeding experiments (i.e. taking individuals from throughout the range into captivity and raising offspring under controlled environmental conditions) are needed to determine to what degree genetic and environmental differences contribute to the observed phenotypic variation (Leinonen et al. 2008). However, the phenotypic divergence can be measured and is defined as:

$$P_{ST} = \frac{c/h^2(\sigma_b^2)}{c/h^2(\sigma_b^2) + 2\sigma_W^2}$$

Eq. 3.1

where $\sigma_b^2$ is the phenotypic variance between populations, $\sigma_W^2$ is the phenotypic variance within populations, and $h^2$ is the heritability (the proportion of phenotypic variance caused by additive genes). The scalar $c$ measures the degree to which phenotypic differences between populations are caused by genetic differences between populations rather than phenotypic plasticity in response to environmental differences or nonadditive genetic variance (the proportion of between-population variance due to additive genetic effects across populations; Pujol et al. 2008; Dussex et al. 2015; Seeholzer and Brumfield 2018). The degree to which $P_{ST}$ approximates $Q_{ST}$ relies on the value of $c/h^2$ and the true value for $c$ or $h^2$ is unknown (Brommer et al. 2014).

We initially estimated $P_{ST}$ for each phenotypic trait using the conservative assumption of $c = h^2$ as the ratio cannot be estimated directly from wild populations. To assess the robustness of our inference from the $P_{ST} - F_{ST}$ comparison, we then also estimated the critical value of $c/h^2$ for each trait for which $P_{ST}$ still exceeds $F_{ST}$ (Brommer 2011). The lower this critical value of $c/h^2$ is, the greater the proportion of phenotypic variance due to phenotypic plasticity in response to environmental conditions in different populations can be and $P_{ST}$ would still exceed $F_{ST}$. 

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Inferences of local adaptation should be nevertheless considered indicative only (Brommer et al. 2014). We calculated the critical value of $c/h^2$ as the value for which the lower 95% credible interval of $P_{ST}$ equals the upper 95% confidence interval of $F_{ST}$. This was obtained by replacing $P_{ST}$ in Equation 1 with the upper 95% confidence interval of $F_{ST}$ and solving for $c/h^2$, through which we obtained

$$\frac{c}{h^2} = \frac{2 \sigma_W^{2(\text{upper})} F_{ST(\text{upper})}}{\sigma_B^{2(\text{lower})} (1 - F_{ST(\text{upper})})}$$

Eq. 3.2

Global $P_{ST}$ values for head-bill length, bill depth, and weight were obtained through Bayesian linear mixed models with the phenotypic traits as response variable, ‘Sex’ as a fixed variable to account for variance due to sexual differences, and ‘River’ as a random variable. We again used 50,000 iterations with sampling every 50 iterations and a burn-in of 10,000 iterations and uninformative priors to estimate the mode and 95% credible intervals of the posteriors of between-population phenotypic variance ($\sigma_B^2$) and residual variance ($\sigma_W^2$). Both posteriors were used in Equation 3.1 to calculate $P_{ST}$ and together with upper 95% confidence intervals of $F_{ST}$ in Equation 3.2 to calculate critical $c/h^2$ values.

All phenotypic and $P_{ST}$-$F_{ST}$ analyses were carried out in R 3.3.3 (R Core Team 2017) and convergence of all models was visually assessed in R.
RESULTS

The overall level of missing data for the microsatellites was 1.09% with at most a single locus missing per individual. The mean genotyping error was negligible with 0.0136. Five loci (Calbo 1, 17, 28, 29 and 35) showed signs of null alleles in one population each, but no consistent evidence of null alleles was found. No evidence for the dropout of large alleles or stuttering was found. There was no consistent departure from Hardy-Weinberg proportions or linkage disequilibrium detected in any other loci (data not shown).

Genetic diversity

The average expected and observed heterozygosity across all microsatellite loci were 0.69 and 0.68, respectively (Table 3.1). Microsatellites showed that genetic diversity within each river and overall was relatively high, with the lowest level of allelic richness being detected in the northern-most river, the Wairau, and the highest level in the Orari River (Table 3.1). A lack of private alleles was also observed in the more northern rivers with one private allele being present in the Canterbury High Country (upper Ashburton River) and Canterbury Plains (lower Rakaia River), three in the Mackenzie Basin (one in the Cass and two in the Tekapo rivers), and five in the Southland region (one each in the Oreti and Mararoa rivers, three in the Aparima River). In comparison, we detected 13 and 14 haplotypes for the mitochondrial cytochrome b and control region, respectively (Table 3.1, Table A.3 and A.4). Diversity measures for both mitochondrial loci were similar for both cytochrome b \( (hd = 0.787 \text{ and } \pi = 0.0015) \) and the control region \( (hd = 0.760 \text{ and } \pi = 0.0022; \text{ Table 3.1}) \). The Mackenzie Basin showed lower diversity measures for both mitochondrial markers compared to other regions (cytochrome b: \( hd = 0.333 \text{ and } \pi = 0.0009; \) control region \( hd = 0.673 \text{ and } \pi = 0.0014; \text{ Table 3.1} \)). For microsatellite data, global \( F_{ST} \) was 0.010 (95% confidence intervals: 0.007-0.012).
Table 3.1 Genetic diversity at 17 microsatellite loci and at two mitochondrial loci (cytochrome b (1143 bp) and control region (898 bp)) of black-fronted tern breeding colonies in catchments and regions in the South Island, New Zealand.

<table>
<thead>
<tr>
<th>Region</th>
<th>River</th>
<th>N</th>
<th>Na</th>
<th>R</th>
<th>H_e</th>
<th>H_o</th>
<th>N</th>
<th>H</th>
<th>hd</th>
<th>π</th>
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<td>4.6</td>
<td>0.64</td>
<td>0.66</td>
<td>4</td>
<td>2</td>
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<td>-</td>
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<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
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<td>4.8</td>
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<td>-</td>
</tr>
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<td></td>
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<td>4.9</td>
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N = Number of individuals, Na = Mean number of alleles per locus, R = allelic richness based on a minimum 8 individuals for the regional and on 7 individuals for the river level, H_e = expected heterozygosity, H_o = observed heterozygosity, H = number of haplotypes, hd = haplotype diversity, π = nucleotide diversity.
Genetic population structure

Clustering and multivariate analyses show one single cluster

Both STRUCTURE analyses indicated very little population structuring. Both the with and without LOCPRIOR model showed the highest mean likelihood value for $K_{\text{max}} = 1$, followed by a decrease of mean likelihood values together with an increase in uncertainty as $K$ values increased (Fig. 3.2 a and b). The plateau in likelihood, which is normally indicative of $K_{\text{max}}$, was not observed (Pritchard, Wen, and Falush 2010). The $\Delta K$-method indicated $K_{\text{max}} = 2$ for the model without using prior location information (Fig. 3.2 c), while it indicated $K_{\text{max}} = 3$ for the LOCPRIOR model (Fig. 3.2 d). However, $\Delta K$ cannot be calculated for $K_{\text{max}} = 1$ (Evanno, Regnaut, and Goudet 2005) and for both models the magnitude of $\Delta K$ was very small. No consistent clustering of individuals into groups was observed for the model without LOCPRIOR ($K_{\text{max}} = 2$) and individual $Q$ membership values were low with an average assignment value of 0.50 to either cluster, indicating random assignment (Fig. 3.3 a). A slight gradient in group membership from north to south was observed in the LOCPRIOR model for $K_{\text{max}} = 2$, with individuals from the Wairau River having relatively high assignment values (> 0.82) to one cluster with steadily decreasing assignment values further south (Fig. 3.3 b). Most other individuals showed mixed membership and average $Q$ values only increased marginally compared to the no LOCPRIOR model to 0.56. Similar assignments were obtained with the $K_{\text{max}} = 3$ model, with one cluster comprising the individuals from the Wairau River in the north of the South Island, with an average assignment value of 0.75 and mostly admixed individuals further South with lower $Q$ values to any of the clusters (Fig. 3.3 c).

TESS also indicated $K_{\text{max}} = 1$ as the plot for the DIC values for different values of $K$ did not plateau (Fig. 3.4), even when $K$ was extended up to 20 (results not shown). The plot showed that DIC values decreased whilst variance around the mean DIC value increased with increasing values of $K$. TESS cannot test directly for $K_{\text{max}} = 1$, however inspecting $Q$ values of individuals for different values of $K$, most individuals were assigned with very high probability (> 0.9) to the same cluster.

The results of $K_{\text{max}} = 1$ from the Bayesian clustering analyses were also supported by the DAPC analysis. A large overlap between individuals from the different regions was observed with a very slight north to south gradient distinguishable (Fig. 3.5). We used 75 principal components (PCs) and 8 discriminant functions retaining 92.8% of the original variance. Cross-validation indicated low predictive power and high associated mean root squared error even with the optimal number of PCs retained. Hence correct assignment of individuals to regions of sample origin was low (62%) indicating admixed groups.
Chapter 3: Delineating conservation units for black-fronted terns

Figure 3.2 Diagnostic plots to determine the number of genetic clusters ($K_{\text{max}}$) present in black-fronted terns after implementing STRUCTURE by using the mean estimated likelihood of the data given each $K$ averaged across all 20 runs (± standard deviation) in a) and c); and the $\Delta K$ method using STRUCTURE HARVESTER in b) and d). The upper row corresponds to the model without prior information (No LOCPRIOR) and the lower row to the model using river section as prior information (LOCPRIOR).

Figure 3.3 Proportional membership ($Q$) of 422 black-fronted terns to genetic clusters ($K_{\text{max}}$) estimated using STRUCTURE and a) with no LOCPRIOR model and b) and c) with LOCPRIOR model. Vertical bars represent individuals and colours correspond to specific clusters. Individuals are ordered by sampling location (river section within the nine broad geographic regions) from north to south. LOCPRIOR model $K_{\text{max}} = 2$ is shown for comparison to the no LOCPRIOR model.
Chapter 3: Delineating conservation units for black-fronted terns

Figure 3.4 Diagnostic plot to determine the number of genetic clusters ($K_{\text{max}}$) for black-fronted terns after implementing TESS using the deviance information criterion (DIC). Averaged DIC values (across 50 runs) ± standard deviation plotted against the different number of $K$.

Figure 3.5 Discriminant analysis of principal components (DACP) for 17 black-fronted tern microsatellites. River sampling origin is indicated by colour and each number representing the centroid of the cluster corresponds in a north-south gradient to the following regions: 1) Marlborough 2) Tasman 3) Westland 4) Amuri Basin 5) Canterbury Plains 6) Canterbury High Country 7) Mackenzie Basin 8) Otago 9) Southland. Number of PCA eigenvalues (x-axis) in respect to cumulative variance (y-axis) captured shown on the bottom left inset. Number of DA eigenvalues (x-axis) against F-statistic (y-axis) shown in bottom right inset.
No significant population differentiation and isolation-by-distance

Pairwise $F_{ST}$ and Jost’s $D_{est}$ values show a low level of genetic differentiation between populations overall ($F_{ST}$: mean = 0.009, range = 0 – 0.061; $D_{est}$: mean = 0.006, range = 0 – 0.075; Table 3.2), with pairwise comparisons of $F_{ST}$ generally not being significantly different from zero. Apart from within Marlborough, differentiation was higher among regions compared to within regions, with the highest differentiation observed between southern and northern rivers or inland rivers. Similar patterns were observed using Jost’s $D_{est}$ values (Table 3.2).

Microsatellite markers showed no pattern of isolation-by-distance using a straight-line distance ($R^2 = 0.0006, P = 0.408$; Fig. 3.6 a). Using waterway distance as the geographic distance measure, the Mantel test was suggestive of a linear trend, although this trend was not statistically significant ($R^2 = 0.0337, P = 0.231$; Fig. 3.6 b).

**Figure 3.6** No observed relationship between a) straight-line geographic and genetic distance (Mantel test; $R^2 = 0.0006, P = 0.408$) and b) waterway-distance and genetic distance (Mantel test; $R^2 = 0.0337, P = 0.231$) using microsatellite data of black-fronted terns from breeding colonies from throughout the South Island, New Zealand.
### Table 3.2 Pairwise genetic differentiation among black-fronted tern breeding locations based on 17 microsatellite loci. $F_{ST}$ is shown below the diagonal and Jost’s $D_{est}$ above the diagonal. Bold values indicate statistically significant values from zero after strict Bonferroni corrections for multiple comparisons and shading indicates value with higher values in darker shades.

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<tr>
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<tr>
<td>U. Ahuriri</td>
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<td>0.008</td>
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<td>0.010</td>
<td>0.008</td>
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<td>0.008</td>
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<td>0.004</td>
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<td>0.004</td>
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<td>0.002</td>
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<td>0.008</td>
<td>0.002</td>
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<td>0.002</td>
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<tr>
<td>Oreti</td>
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<td>0.010</td>
<td>0.008</td>
<td>0.002</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.002</td>
<td>0.000</td>
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<tr>
<td>Aparima</td>
<td>0.008</td>
<td>0.010</td>
<td>0.008</td>
<td>0.002</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.002</td>
<td>0.000</td>
</tr>
</tbody>
</table>
Mitochondrial phylogenies and haplotype networks are shallow

Neither the cytochrome b phylogeny nor the control region phylogeny showed any significant clades. As the topologies for the trees for each molecular marker were similar between the maximum-likelihood and Bayesian method, only the Bayesian trees are presented (Fig. 3.7 and 3.8). Bayesian probabilities supported most clades reasonably well in comparison to maximum-likelihood bootstrap support values. The overall divergence between clades was very shallow for both molecular markers and employing both methods. The haplotype networks revealed a similar pattern as both were very shallow and showed a somewhat radial pattern (Fig. 3.9 a and b). Out of the 13 haplotypes identified for cytochrome b, one haplotype was very common and present throughout the South Island in 13 of the 31 sampled populations, while seven haplotypes were only detected in single populations showing a slight north-south cline along the South Island (Fig. 3.9 a and Table A.3). The control region showed a similar pattern, with the most common haplotype being present in 22 out of 31 catchments sampled and five haplotypes were unique to single catchments, again in a slight north-to-south cline (Fig. 3.9b and Table A.4). Multiple haplotypes were detected for the control region in the majority of catchments (71 %), with the exception of the Mackenzie Basin where only a single haplotype was found in five of the six catchments sampled (Fig. 3.9 b and Table A.4).
Chapter 3: Delineating conservation units for black-fronted terns

Figure 3.7 Bayesian mitochondrial DNA cytochrome b (1143 bp) phylogeny of black-fronted terns using MrBayes. Posterior probabilities are shown above the nodes and maximum-likelihood bootstrap support values (> 50) below. Scale depicts the distance between samples corresponding to 0.003 substitutions per site and the distance to the outgroup common tern (Sterna hirundo) is shortened for visual presentation of the intra-species relationships of black-fronted terns. Labels are sample name and the origin catchment. Colours show to which broad geographic region samples belong.
Chapter 3: Delineating conservation units for black-fronted terns

Figure 3.8 Bayesian mitochondrial DNA control region (898 bp) phylogeny of black-fronted terns using MrBayes. Posterior probabilities are shown above the nodes and maximum-likelihood bootstrap support values (> 50) below. Scale depicts the distance between samples corresponding to 0.003 substitutions per site and the distance to the outgroup common tern (Sterna hirundo) is shortened for visual presentation of the intra-species relationships of black-fronted terns. Labels are sample name and the origin catchment. Colours show to which broad geographic region samples belong.
Figure 3.9 Median joining haplotype networks and distribution of mitochondrial DNA haplotypes of black-fronted terns for a) cytochrome b and b) control region. Colours represent haplotype identity and size is proportional to the number of individuals with each haplotype. Black dots represent one base-pair change between sequences.
We detected significant latitudinal clines in three of four morphometric traits measured (Table 3.3; Fig. 3.10); head-bill length, bill depth and weight increased with increasing latitude. We also detected significant differences between the sexes for three of the morphometric traits measured (head-bill length, bill depth, and wing-length) as credible intervals did not include zero (Table 3.3; Fig. 3.10). However, while male black-fronted terns had on average significantly greater length measurements than females, they were not significantly heavier than females. \( P_{ST} \) exceeded \( F_{ST} \) in the three traits for which latitudinal clines were also detected (head-bill length, bill depth, and weight) suggesting a potential adaptive origin of clinal divergence (Table 3.4). Critical \( c/h^2 \) values for all traits were less than 1 indicating that natural selection may have shaped the variation of those three traits and were lower for weight (critical \( c/h^2 = 0.252 \)) and bill depth (Critical \( c/h^2 = 0.361 \)) compared to head-bill length (Critical \( c/h^2 = 0.656 \); Table 3.4).

**Figure 3.10** Phenotypic variation in relation to latitude and sex of four morphometric traits of black-fronted terns reveals significant latitudinal clines in three traits measured: a) head-bill length, b) bill depth, and c) weight as well as differences between sexes in three traits a) head-bill length, b) bill depth and d) wing length. Females are shown by open circles and dashed line; males by filled circles and solid line.
Chapter 3: Delineating conservation units for black-fronted terns

Table 3.3 Summary results of Bayesian linear mixed models of four different phenotypic traits of black-fronted terns. Mode and 95% credible intervals (CIs) of the variables ‘Sex’ and ‘Latitude’ (latitudinal location of breeding colony) on the different phenotypic traits. Values in bold indicate that CI for these values do not include zero and have a significant effect on the phenotypic trait.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Variable</th>
<th>Mode</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head-bill length</td>
<td>(Intercept)</td>
<td>63.480</td>
<td>63.178</td>
<td>63.831</td>
</tr>
<tr>
<td></td>
<td>Sex (Male)</td>
<td>2.160</td>
<td>1.789</td>
<td>2.481</td>
</tr>
<tr>
<td></td>
<td>Latitude</td>
<td>0.456</td>
<td>0.235</td>
<td>0.714</td>
</tr>
<tr>
<td>Bill depth</td>
<td>(Intercept)</td>
<td>7.267</td>
<td>7.178</td>
<td>7.347</td>
</tr>
<tr>
<td></td>
<td>Sex (Male)</td>
<td>0.368</td>
<td>0.285</td>
<td>0.459</td>
</tr>
<tr>
<td></td>
<td>Latitude</td>
<td>0.104</td>
<td>0.033</td>
<td>0.168</td>
</tr>
<tr>
<td>Weight</td>
<td>(Intercept)</td>
<td>94.156</td>
<td>92.917</td>
<td>95.274</td>
</tr>
<tr>
<td></td>
<td>Sex (Male)</td>
<td>-0.586</td>
<td>-1.761</td>
<td>0.485</td>
</tr>
<tr>
<td></td>
<td>Latitude</td>
<td>1.836</td>
<td>0.830</td>
<td>2.780</td>
</tr>
<tr>
<td>Wing length</td>
<td>(Intercept)</td>
<td>247.098</td>
<td>246.159</td>
<td>247.905</td>
</tr>
<tr>
<td></td>
<td>Sex (Male)</td>
<td>4.162</td>
<td>3.041</td>
<td>5.417</td>
</tr>
<tr>
<td></td>
<td>Latitude</td>
<td>0.083</td>
<td>-0.544</td>
<td>0.678</td>
</tr>
</tbody>
</table>

The variable ‘Latitude’ was centred, so that the intercept indicates the size of the phenotypic trait in the centre of the breeding range of black-fronted terns.

Table 3.4 Results of $P_{ST}$-$F_{ST}$ comparison for head-bill length, bill depth, and weight of black-fronted terns. Modes (and 95% credible intervals) of between-population ($\sigma_B^2$), within-population ($\sigma_W^2$). Bolded values indicate significant divergence as lower credible interval of $P_{ST}$ exceed the upper confidence interval of $F_{ST}$. Lower values indicate a more robust inference of local adaptation in cases where it is not possible to measure this value directly.

<table>
<thead>
<tr>
<th>Trait</th>
<th>$\sigma_B^2$</th>
<th>$\sigma_W^2$</th>
<th>$P_{ST}$ $^{a)}$</th>
<th>Critical $c/h^2$ $^{b)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head-bill length</td>
<td>0.482 (0.119-0.950)</td>
<td>2.763 (2.370-3.216)</td>
<td>0.080 (0.024-0.129)</td>
<td>0.656</td>
</tr>
<tr>
<td>Bill depth</td>
<td>0.039 (0.013-0.074)</td>
<td>0.167 (0.143-0.193)</td>
<td>0.105 (0.043-0.162)</td>
<td>0.361</td>
</tr>
<tr>
<td>Weight</td>
<td>9.052 (3.054-16.110)</td>
<td>27.400 (22.980-31.660)</td>
<td>0.142 (0.062-0.203)</td>
<td>0.252</td>
</tr>
</tbody>
</table>

$^{a)}$ $P_{ST}$ values for each trait were obtained through Equation 3.1 with a null assumption of $c/h^2 = 1$. $P_{ST}$ were compared to global $F_{ST}$ values of microsatellites estimated at 0.010 (95% confidence intervals: 0.007-0.012).

$^{b)}$ The critical value of $c/h^2$ is the value for which the lower credible interval of $P_{ST}$ would equal the upper confidence interval of $F_{ST}$ and was obtained using Equation 3.2.
DISCUSSION

This is the first extensive genetic and morphometric study of endangered black-fronted terns and of any tern species across their entire breeding range. Analyses based on polymorphic microsatellite loci and of mitochondrial cytochrome b gene and the control region show relatively high levels of genetic diversity across the range of black-fronted terns. We detected no significant recent or historical population structuring based on genetic data, although we found evidence for a latitudinal cline in morphometric traits.

High levels of genetic diversity

Our study shows that black-fronted terns have so far maintained a high level of nuclear and mitochondrial genetic diversity despite continuing population declines (O’Donnell and Hoare 2011). The level of genetic diversity is similar to other threatened New Zealand avian species such as kea, (*Nestor notabilis*; Dussex, Wegmann, and Robertson 2014) and blue duck/whio (*Hymenolaimus malacorhynchos*; Grosser et al. 2017). Moreover, it is also similarly high to other *Chlidonias* species (Dayton et al. 2017; Szczys et al. 2017). For example, Eurasian whiskered terns (*C. hybrida hybrida*) are thought to currently experience a range expansion and show an $H_e$ of 0.59 (6 microsatellite loci; $n = 78$) as well as an average haplotype diversity of 0.62 and average nucleotide diversity of 0.0017 for the partial cytochrome b-gene (467 bp; $n = 74$; Dayton et al. 2017). Black-fronted terns are thought to have bred historically on the Volcanic Plateau in the North Island and also to have occurred on the Snares Islands (Higgins and Davies 1996). Given the documented decline in range and population size, historical and ancient genetic diversity could potentially have been even greater than our data indicate.

Low levels of genetic differentiation

Strong phylogeographic structuring is expected in species with limited dispersal abilities, distributions that include barriers to gene flow such as mountain ranges, or that exhibit philopatry (Allendorf, Luikart, and Aitken 2013). In birds, phylogeographic structure is typically weak given the generally greater ability for dispersal and expected only at large geographic scales (Crochet 2000). In New Zealand birds, a number of phylogeographic patterns have emerged in species breeding only in the South Island, including deep north-south splits along the length of the South Island (e.g. rock wren/piwauwau (*Xenicus gilviventris*); Weston and Robertson 2015), a shallow distinction between extremes of the range (e.g. kea; Dussex,
Chapter 3: Delineating conservation units for black-fronted terns

Wegmann, and Robertson 2014) or no genetic structure throughout the South Island (e.g. yellowhead/mohua (Mohoua ochrocephala); Tracy and Jamieson 2011).

Population genetic studies of other terns have detected population structuring when either large land masses, oceans or behavioural differences divided populations in their breeding and/or wintering ranges. For example, sooty terns (Sterna fuscata) are a circumtropical species that exhibit strong differentiation between the Atlantic and Indo-Pacific breeding colonies (Avise et al. 2000). Globally distributed Caspian terns (Hydropogne caspia) show differentiation on a continent scale in North America, where colonies on the Pacific Coast differed from colonies east of the Rocky Mountains, and colonies in central Canada to colonies in the Great Lakes area (Boutilier et al. 2014). Differentiation between breeding sites of Eurasian black terns (C. niger niger) is most likely linked to separate post-nuptial staging sites and migration routes during winter migration (Szczys et al. 2017).

In contrast to other New Zealand avian species and various tern species globally, our genetic analyses of mitochondrial and nuclear markers indicated one panmictic population with no significant population structuring within black-fronted tern breeding colonies along the length of the South Island of New Zealand. We did not detect a pattern of isolation-by-distance for microsatellite markers using straight-line distance, albeit a suggestion of a trend using waterway distance. This is probably best explained by the overall very low pairwise genetic distances between the breeding colonies. Similar to other New Zealand endemic species such as kea or rock wren, we detected a slight north-south cline in allelic richness and in the distribution of private alleles in our microsatellite data (Dussex, Wegmann, and Robertson 2014; Weston and Robertson 2015). However, this pattern was not mirrored in the mitochondrial markers where some river-specific haplotypes were detected (e.g. haplotype 4 in Wairau River, haplotype 10 in lower Rangitata River, haplotype 13 in Hunter River) throughout the whole breeding range and no cline in diversity was obvious. Overall, the shallow phylogeny and star-like pattern of the haplotype network suggest past population expansion as seen in other New Zealand bird species (Trewick et al. 2017).

The high genetic connectivity of breeding colonies of black-fronted terns within the South Island is perhaps not surprising, given that they are highly mobile and very capable flyers. Indeed, black-fronted terns have been observed at sea 35 km from the nearest land, as well as at high altitudes (1,860 m above sea level; Latham 1981; Child 1986). Although other Chlidonias and Sterna species show a strong degree of site fidelity (Lebreton et al. 2003; Becker et al. 2008; van der Winden and van Horssen 2008), it is unknown if this is also true for black-fronted terns.
as philopatry varies between species (Palestis 2014). One banding study followed 69 black-fronted terns in the Mackenzie Basin, of which 31 were re-sighted: 6 in the same colony, 18 in the same river and 7 individuals in a different river ca. 20 km away (Keedwell 2005). Migration routes and non-breeding distributions are assumed to be overlapping, but data are lacking to confirm this.

To further investigate the low level of mitochondrial genetic diversity detected in the Mackenzie Basin compared to the other regions, we tested whether this low diversity was due to family groups being sampled by chance through examining the relatedness of individuals within breeding sites and ensuring that predominantly adults \((n = 8)\) rather than chicks \((n = 3)\) were sequenced for the mitochondrial markers. No overall elevated levels of relatedness were detected in the Mackenzie Basin (Fig. A.1). Given this, one possible explanation for the lower level of mitochondrial diversity in the Mackenzie Basin is that possibly there has been a lack of dispersal into the basin. Many of the rivers in the basin were glaciated during the last Ōtiran glaciation (14,500–75,000 years ago) and as glaciers receded the area would have been recolonised more recently relative to the lowland rivers (Golledge et al. 2012; Williams et al. 2015). Although other rivers in the Canterbury High Country would have been also covered in ice, it would have been to a lesser extent and these may have been colonised more easily from directly further downstream (Golledge et al. 2012).

If glaciation is behind the low variation in mitochondrial DNA, there are two potential explanations for the discrepancy between mitochondrial and microsatellite genetic diversity patterns: sex-biased dispersal or difference between sampled time scales of the markers. First, as mitochondrial DNA is only maternally inherited in comparison to bi-parentally inherited microsatellite markers, lower haplotype diversity might still be evident despite a lack of any pattern in microsatellite markers, if male black-fronted terns disperse at a higher rate than females (Allendorf, Luikart, and Aitken 2013). Generally though, females are the dispersing sex in birds (Greenwood 1980; Clarke, Sæther, and Røskaft 1997; Paradis et al. 1998), but examples for male-biased dispersal nevertheless exist, such as the Siberian jay \((Perisoreus infaustus;\) Li and Merilä 2010). Second, as the mutation rate of microsatellites is much faster compared to mitochondrial markers, microsatellites enable inferences to be made on more recent processes, while mitochondrial markers provide insights into processes of the more distant past (Selkoe and Toonen 2006; Zink and Barrowclough 2008). This would mean that the historical lack of gene flow into the Mackenzie Basin due to glaciation is still reflected in the mitochondrial markers, but not in microsatellite markers as the signature has been removed through recombination. Research on natal dispersal and philopatric behaviour of black-fronted terns and potential
differences between sexes would aid our understanding of black-fronted tern behaviour and likely demographic consequences (e.g. Lebreton et al. 2003; Bracey et al. 2018).

Future studies of black-fronted terns could benefit from a genomic approach, such as using large numbers of single nucleotide polymorphisms (SNPs) generated through restriction-site associated DNA-sequencing (RAD-seq; Baird et al. 2008) or similar technologies (Elshire et al. 2011). For example, subtle population structure was discovered using this approach in emperor penguins (Aptenodytes forsteri), which breed in colonies all around Antarctica, and in other migratory and highly mobile seabird species, such as white-chinned petrels (Procellaria aequinoctialis) for which other genetic approaches lacked precision (Rexer-Huber 2017; Younger et al. 2017). However, other studies have not always found further or finer population structure using such genomic approaches and a balance between cost and extra information gained needs to be achieved for many conservation issues. For example, a comparison of a microsatellite panel consisting of 15 markers with a SNP array of 5,568 loci and RAD-seq in Atlantic salmon (Salmo salar) showed that the spatial structure recovered between populations was highly similar independent of the marker type used (Bradbury et al. 2015).

**Phenotypic body size cline**

Our study confirmed range-wide sexual size dimorphism in black-fronted terns in three external traits (except weight) with significant overlap. This overlap originates from the presence of a latitudinal cline with individuals being larger and heavier at breeding colonies at higher latitudes. A correlation of increasing body size with increasing latitude (as a proxy for temperature gradients) are common for birds, although often more pronounced in sedentary species compared to migratory ones (Ashton 2002; Meiri and Dayan 2003). Similar clines have been reported in other New Zealand avian species such as kākā (Dussex et al. 2015), New Zealand falcon/kārearea (Falco novaeseelandiae; Trewick and Olley 2016), and bellbird/korimako (Anthornis melanura; Bartle and Sagar 1987), little penguins/kororā (Eudyptula minor; Grosser, Scofield, and Waters 2017) as well as from endemic invertebrates (e.g. giraffe weevils/tūwhaitara (Lasiorhynchus barbicornis; Painting, Buckley, and Holwell 2014)). Bergmann (1847) was one of the first to observe this pattern. His hypothesis was that species living in colder regions are larger compared to those in warmer regions due to the relationship between heat loss and surface area-to-volume ratio. This is referred to as Bergmann’s rule, although often it is used in a broader sense referring only to the pattern in body size variation and not to a direct test of underlying processes (Salewski and Watt 2017). In a broad sense, black-fronted terns
conform to Bergmann’s rule, however we did not include any thermoregulatory mechanism to test for Bergmann’s rule in a strict sense.

The inference drawn from the $P_{ST} - F_{ST}$ comparison rests on the critical value of $c/h^2$. If even for low critical $c/h^2$ values $P_{ST}$ still exceeds $F_{ST}$, then inferences of local adaptation are still valid even if the proportion of additive genetic between-population variance over total between-population variance (i.e. $c$) is much lower than the heritability $h^2$ (Brommer 2011). Although in this way the robustness of the comparison can be assessed, as the actual value of the quantities ($c$ and $h^2$) remains unknown, any inferences on local adaptation should be considered indicative only (Brommer et al. 2014; Seeholzer and Brumfield 2018). Given that it is impossible to measure $c$ and $h^2$ directly for black-fronted terns, comparison to other studies of bird species showing a latitudinal cline provide a mean of comparison for appropriate critical $c/h^2$ values. Both, Brommer et al. (2014) studying latitudinal clines of house sparrows (*Passer domesticus*) in Finland and Dussex et al. (2015) investigating latitudinal clines in kākā in New Zealand, obtained a critical $c/h^2$ for weight of around 0.1. In contrast, Bertrand et al (2016) studying Réunion white-eyes (*Zosterops borbonicus*) and Seeholzer and Brumfield (2018) investigating the body size cline of line-cheeked spinetail (*Cranioleuca antisiensis*) in the Andes recovered a critical $c/h^2$ value for weight of around 0.5. In our study of black-fronted terns, we recovered a critical $c/h^2$ value for weight of 0.252, which is in between those of our other studies. Brommer (2011) recommends that critical $c/h^2$ value would need to be low (<0.20) to have a basis for drawing inferences on local adaptation. Hence, although our results from the $P_{ST} - F_{ST}$ comparison are suggestive of natural selection, they do not rule out phenotypic plasticity due to environmental, epistatic or dominance effects (Whitlock 1999; Pujol et al. 2008).

As a point in case, the observed phenotypic differentiation could also have entirely resulted through differences in environmental factors (including temperature) and resources also being latitudinally structured. For example, black-fronted terns in the Eglinton River were recorded regularly feeding on skinks (O’Donnell and Hoare 2009), compared to black-fronted terns in Tasman, Ohau, Ahuriri, and Waitaki rivers that only occasionally consumed skinks and mostly fed on aerial insects and emerging nymphs from the water surface (Lalas 1977). Hence, individuals in the south are larger potentially due to higher habitat quality and lower population density in the southern rivers (O’Donnell and Hoare 2011).

Lastly, our study of phenotypic traits could have benefitted in two ways. Firstly, we only measured four external traits to avoid handling birds for extended periods of times and chose traits that were easily measurable. The addition of further skeletal traits (e.g. tarsus length),
which are little influenced by seasonality or local food resources, could be beneficial (Brommer et al. 2014). Secondly, measurements were undertaken by multiple observers throughout the range and we cannot exclude the possibility of some of the variation being attributed to differences between measurements taken by different observers (although this also should be accounted for by the random effect in the model). Our study was carried out though within a single breeding season (over 85 days) and birds were sampled throughout this period in the whole South Island, thereby minimising seasonal effects. In addition, weight, which is generally not biased by measurement error through different observers (Goodenough et al. 2010), showed the largest $P_{ST}$ value and lowest critical $c/h^2$ value.

Phenotypic variance along a geographic gradient in the absence of neutral genetic divergence has been detected in other species (Sæther et al. 2007; Antoniazza et al. 2010; Brommer et al. 2014; Dussex et al. 2015; Bertrand et al. 2016; Seeholzer and Brumfield 2018). Our study and these examples suggest that, particularly for highly mobile species, it is not enough to rely on neutral genetic markers alone for identifying appropriate conservation units, because important ecological differentiation can be missed (Crandall et al. 2000; Fraser and Bernatchez 2001; McKay and Latta 2002). $P_{ST}$-$F_{ST}$ comparisons provide valuable insights and some level of understanding on putative actions of natural selection in wild species (Leinonen et al. 2008). This is particularly important for conservation biologists aiming at maintaining functional as well as adaptive genetic diversity (McKay and Latta 2002; Frankham 2010; Allendorf, Luikart, and Aitken 2013).

**Conservation implications**

There is no specific New Zealand Department of Conservation recovery plan available for black-fronted terns. Management and research priorities have been identified for the braided river ecosystem as a whole, focusing on direct habitat protection in the form of introduced predator and weed control, as well as through appropriate statutory processes (O’Donnell et al. 2016). Predator control and/or weed control aimed at protecting black-fronted terns or a range of braided river species is in place in the Eglinton, Ohau, Tasman, upper Rangitata, Ashley and Clarence rivers (O’Donnell and Hoare 2011; Cruz et al. 2013; Monks and O’Donnell 2013; O’Donnell et al. 2016; Bell 2017). However, current management sites include none of the larger, lower braided rivers, such as the lower Rangitata River. One key area in need of research is determining which sites should be prioritised for conservation management action to achieve long-term survival of key braided river species, such as black-fronted terns (O’Donnell et al. 2016).

This research addresses the knowledge gap regarding the required scale and location of management actions for black-fronted terns. Given that no distinct breaks through genetic and phenotypic analyses were identified, conservation management should focus on catchments throughout the South Island. Studies of other colony-breeding seabirds or shorebirds highlight the importance of protecting occupied and empty patches and several subpopulations within the metapopulation (Martinez-Abrain et al. 2003; Catlin et al. 2016). These secure sites should target areas of past colony records, aim to protect multiple potential colony sites, and cover their entire breeding range (O’Donnell and Hoare 2011; Schleselmann, Cooper, and Maloney 2017).
Black-fronted tern populations expanded during glaciation and experienced a human-induced decline.

Bruce Robertson provided fruitful discussions and advised on data analysis. Nicolas Dussex advised on the ABC and Bayesian skyline analysis. Helpful advice also came from Stefanie Grosser and Robin Waples on aspects of data analysis. I carried out sample collection, laboratory work, and data analysis. This chapter is intended for publication in a suitable international journal.
ABSTRACT

Understanding a species’ past and present population size is vital for assessing conservation status and to guide future management. Highly mobile and migratory species are some of the most threatened yet hardest to monitor groups. Due to their high mobility and specific habitat requirements, they also often have complex past population dynamics. We used mitochondrial DNA (cytochrome b gene/control region) sequences and 17 nuclear microsatellite markers to investigate past and present population size of the endangered black-fronted tern/tarapirohe (Chlidonias albostriatus), a threatened species endemic to New Zealand. We hypothesised i) that black-fronted terns expanded in population size during the last glacial period due to favourable habitat conditions; and ii) that subsequently they have undergone a human-induced decline. Furthermore, we also wanted to determine historical effective population size ($N_e$), and contemporary effective numbers of breeders ($N_b$) and $N_b$, and census size ($N_C$), to assess extinction risk and inform conservation management. Our results confirmed our hypotheses, indicating that the black-fronted tern population expanded between 33,000–70,000 years ago, earlier than reported for other New Zealand species. The black-fronted tern population has subsequently decreased substantially. We estimated contemporary $N_b$ to be $\sim$700 individuals. Estimates of $N_e$ varied depending on the method used. $N_e$ based on a conversion of $N_b$ and two life history traits was $\sim$700 individuals, while $N_e$ based on mixed-aged samples was $\sim$3,000 individuals. Compared to our estimated historical $N_e$, contemporary $N_e$ was only about a quarter or less. Estimates of historical and contemporary $N_C$ varied depending on the ratio of $N_e/N_C$ used, but contemporary size is likely still to be between 5,000–10,000 mature individuals. Furthermore, we discovered a low ratio of $N_b/N_e$ which is probably driven by $N_b$ being more sensitive to the large recruitment failure of individuals, large differences in reproductive success between same-age and same-sex individuals, and the ongoing overall population decline. This highlights that in relatively long-lived species, $N_b$ rather than $N_C$ is the more appropriate measure for assessment of the population status. We recommend ongoing genetic monitoring of black-fronted terns based on $N_b$ and that conservation management be instigated to prevent further decline and irreversible impacts.
INTRODUCTION

Understanding past and present population trends can provide useful guidance for long-term conservation management of wildlife species (Bonebrake et al. 2010; Hoelzel 2010; Luikart et al. 2010; Allendorf, Luikart, and Aitken 2013). Past demographic processes shape present distribution patterns, population structure, and affect the overall genetic composition of populations (Hewitt 2000; Hewitt 2004). Hence, knowing how a species responded to past climatic changes can indicate adaptive capacities, niche requirements, and potential future responses (Younger et al. 2016; Kozma et al. 2018). Information on the historical population size of a species can help managers set realistic conservation goals (Bonebrake et al. 2010). A further vital piece of information for conservation managers is the current status of a species (i.e. its effective and its census population sizes), so that trends can be assessed and appropriate management responses implemented (Luikart et al. 2010).

Glaciation events had profound effects on the distribution and population size of many species in temperate regions (Hewitt 1999; Hewitt 2000; Stewart et al. 2010). In the Northern Hemisphere, glaciation was extensive, forcing many species to shift their range or to contract into refugia (Stewart et al. 2010; Lindgren et al. 2016). In the oceanic Southern Hemisphere, glaciation was less extensive, yet still resulted in a variety of species’ responses to the cooling climate (Fraser et al. 2012). New Zealand provides a particularly interesting setting in the Southern Hemisphere as both continental and island-like patterns of biogeography can be observed (Wallis and Trewick 2009). During the last glacial period (Ōtira glaciation 73,000–14,000 years ago), large glacial fields throughout the middle of the Southern Alps separated the southern part of the South Island from the northern area (Williams et al. 2015). The lowland areas were dominated by dry grasslands with some shrubs and small forest pockets (Golledge et al. 2012; Lorrey and Bostock 2017; Wood et al. 2017). With globally lowered sea levels, New Zealand’s coastline extended beyond its present day limits by about 70 km providing more habitat to specialists of dry grasslands (Browne and Naish 2003; Cutler et al. 2003; Siddall et al. 2003).

Humans have also significantly altered environments. New Zealand was uninhabited by humans until the first Polynesian settlers arrived in the 13th century, followed by a wave of European colonisation beginning in the 19th century (Wilmshurst et al. 2008). Humans had direct and indirect impacts on New Zealand’s biodiversity as they changed the landscape through burning and clear-felling, and also by introducing exotic animals and plants (King 1984). Many
native species were vulnerable to predation and habitat loss, and have declined (Dowding and Murphy 2001; Robertson et al. 2017).

The demographic history of a population leaves a signature in the genome of its modern representatives and hence genetic tools can be used to infer historical patterns of population expansion or decline (Charlesworth 2009; Ho and Shapiro 2011). For example, population expansion out of glacial refugia after the last glacial maximum has been inferred for many of New Zealand’s forest bird species based on genetic analysis (Murphy, Flux, and Double 2006; Goldberg, Trewick, and Powlesland 2011; Dussex et al. 2015). Furthermore, genetics can also be used to assess more recent population dynamics due to human impacts. For instance, the pre-human population size of New Zealand short-tailed bats/pekapeka (*Mystacina tuberculata*) was assessed in this way and displayed a drastic population decline after human arrival to New Zealand due to deforestation and the impact of introduced species on bat populations (Lloyd 2003).

Many species endemic to New Zealand are now extinct or in decline due to the direct or indirect impacts of humans (Robertson et al. 2017). The assessment of contemporary population size is key to evaluating the status of species of conservation concern. As populations decline in size, demographic and genetic stochasticity, along with environmental deterministic factors such as habitat degradation, lead to an increased risk of extinction (Allendorf, Luikart, and Aitken 2013). Two parameters — population census size ($N_C$) and effective population size ($N_e$) — are of interest to conservation biologists, because they can help predict population extinction risk (Luikart et al. 2010). The census population size is generally defined as the number of adults (Nunney and Elam 1994). Effective population size is defined as the size of an ‘ideal population’ that would lose genetic diversity at the same rate of the observed population (Fisher 1930; Wright 1931). An ‘ideal population’ has random mating, random variance in reproductive success, an even sex ratio, and no selection or mutation occurring. Hence, for an ideal population, $N_e$ would equal $N_C$, but in natural populations $N_e$ is often far smaller than $N_C$ as, for example, reproductive success among individuals varies greatly (Nunney and Elam 1994; Frankham 1995; but see Waples et al. 2013).

For threatened species, determining $N_e$ and $N_C$ is critical to assess extinction risk and hence to prioritise species through threat classifications such as the IUCN Red List (Rodrigues et al. 2006). Two guidelines for $N_e$ have been suggested as critical thresholds for species conservation: $N_e > 100$ individuals is necessary to avoid short-term negative impacts in the next five generations (i.e. inbreeding depression); and $N_e > 1,000$ is necessary for long-term survival (i.e.
maintaining evolutionary potential) (Frankham, Bradshaw, and Brook 2014). The IUCN Red List classifies species predominantly on $N_e$ and recent population trends (Rodrigues et al. 2006). Both, $N_e$ and $N_c$, can be estimated through genetic tools (Luikart et al. 2010).

Migratory and highly mobile species, such as waders or shorebirds, provide an interesting challenge to conservation managers. Globally, many marine and freshwater bird species are in decline, although data is often scarce (Croxall et al. 2012; Paleczny et al. 2015). In addition, although these species are highly mobile, past climatic changes will likely have had differing effects on populations depending on the species’ biology and habitat requirement (Kraaijeveld and Nieboer 2000; Verkuil et al. 2012). New Zealand has the greatest diversity of seabirds in the world, with a large number of them being endemic (Taylor 2000; Paleczny et al. 2015). Black-fronted terns/tarapirohe (Chlidonias albostriatus) are one of New Zealand’s endemic, yet little studied, species, and are currently classified as internationally and nationally ‘Endangered’ (BirdLife International 2017; Robertson et al. 2017).

In the IUCN classification, the black-fronted tern population is estimated to lie in the broad category of having between 2,500–9,999 mature individuals experiencing a predicted decline of $\geq 50\%$ in population size over the next three generations as well as a decline in range based on observations (O’Donnell and Hoare 2011; BirdLife International 2017). The New Zealand National Threat Classification assessment employed similar criteria, estimating the population at between 1,000–5,000 mature individuals, with a predicted decline of 50–70% over next three generations (O’Donnell and Hoare 2011; Robertson et al. 2017). The associated qualifiers with this classification are ‘data poor’, ‘sparse’, ‘recruitment failure’ and ‘conservation dependent’ (Robertson et al. 2017). ‘Sparse’ refers to populations occurring only in small and scattered populations, as they breed in colonies in the braided rivers of the South Island and migrate to the coast over the winter (Lalas 1979; Townsend et al. 2008; Schlesselmann, Cooper, and Maloney 2017). Recruitment failure has been observed in different populations (Keedwell 2005; Cruz et al. 2013; Bell 2017; Chapter 5) and it is expected to most likely cause a very skewed age structure and the potential for future catastrophic declines (Townsend et al. 2008). Hence, ‘conservation dependent’ means that it is assumed that the species will move to a higher threat category without management.

The aim of this study was to investigate the past and present population size of black-fronted terns to inform conservation management of this endangered species. Specifically, i) we hypothesised that in contrast to many other New Zealand forest birds, black-fronted terns experienced a historical population expansion during the Ōtiran glaciation due to favourable
habitat conditions; however ii) we also hypothesised that black-fronted terns have since experienced a human-induced decline similar to that of other New Zealand shore birds; iii) we predicted that current effective population size is below conservation critical thresholds; and iv) we aimed to provide an additional estimate of past and contemporary census size of black-fronted terns to inform on the conservation status of the species.
MATERIALS AND METHODS

Sample collection and DNA extraction

We collected blood samples \((n = 589)\) from chick and adult black-fronted terns from throughout their breeding range as described in Chapter 3 (see Figure 3.1). Blood samples (< 20 µl) were obtained by brachial vein puncture with a sterile needle (26G x 1/2”). The blood was collected with a glass capillary tube and immediately transferred into 1 ml of Queens lysis buffer (10 mM Tris, 10 mM NaCl, 10 mM Na-EDTA, 1% n-lauroylsarcosine; pH 7.5) (Seutin, White, and Boag 1991). DNA was extracted and purified from the blood using a standard 5% Chelex protocol (Walsh, Metzger, and Higuchi 1991).

Microsatellite genotyping

All samples were genotyped at the 17 microsatellite loci specifically developed for black-fronted terns, excluding the potentially sex-linked locus (see Chapter 2; Table 2.1). Polymerase chain reaction conditions are described in Chapter 2 and electrophoresis and scoring of alleles is described in Chapter 3. Samples failing to amplify for more than one locus were automatically discarded. This yielded 422 samples in total consisting of 104 of chicks and 318 samples of adults. To check for genotyping error, 13% of all samples were randomly blind-repeat genotyped at all loci and the error rate calculated as the number of incorrect alleles divided by the total number of alleles (Hoffman and Amos 2005). Tests for scoring error due to stuttering, large allele dropout, and null alleles were conducted in MICRO-CHECKER v. 2.2.3. (van Oosterhout et al. 2004). In addition, tests for Hardy-Weinberg proportions and linkage disequilibrium were carried out for each river in GENEPOP v. 4.4 (Rousset 2008) using the Markov chain Monte Carlo (MCMC) method with 10,000 dememorizations, 1,000 batches and 10,000 iterations. We used a sequential Bonferroni correction to adjust the significance levels for multiple statistical tests (Rice 1989).

Mitochondrial DNA sequencing

We sequenced 35 individuals from across the range of black-fronted terns for the whole cytochrome b gene (1,143 base pairs (bp)) using the primers L14764 and H16064 (Sorenson et al. 1999) as described in Chapter 3.

In addition, we sequenced 64 individuals (using the same birds as for cytochrome b where possible) for an 898 bp fragment of the mitochondrial control region. Primers BFT-CR-ND6-1F, BFT-CR-12S-1R together with the internal primers BFT-CR-F1, BFT-CR-R2, and BFT-CR-
goosehairpin-F1 (Tables A.1 and A.2) were used for amplification and sequencing as described in Chapter 3.

All sequences were aligned in GENEIOUS V. 6.0.6. (Biomatters Ltd., New Zealand) and variable sites were confirmed by inspecting the chromatograms visually. Cytochrome b sequences were translated to detect reading frame errors and stop codons. We detected single-site ambiguities in six individuals at six different sites in the control region sequences, which we attributed to single-site heteroplasmy (see Chapter 3). Ambiguous sites were excluded from subsequent analyses.

**Sampling scheme and bias**

Methods for assessing demographic history and estimating effective population size are susceptible to bias if population substructure exists and one panmictic population is not being studied (Städler et al. 2009; Luikart et al. 2010; Neel et al. 2013; Grant 2015). Prior analysis (Chapter 3) did not reveal any substructure, immigration, or signs of inbreeding, and we therefore analysed samples from the whole breeding range together. In addition, for all analyses that took generation time into account, we assumed generation time to be 10 years for black-fronted terns (Keedwell 2002; O’Donnell and Hoare 2011).

**Analyses of demographic history**

We undertook several analyses to investigate the demographic history of black-fronted terns: Neutrality statistics together with sequence mismatch analysis; extended Bayesian skyline plots; and approximate Bayesian computation analysis.

**Neutrality statistics and sequence mismatch analysis**

We first calculated Tajima’s $D$ and Fu’s $F_S$ statistics for cytochrome b and control region mitochondrial DNA (mtDNA) (Tajima 1989; Fu 1997). Negative values of these statistics are an indication of population expansion whereas positive values are an indication of reduction in population size. We also conducted a mismatch analysis (MMA) generating mismatch distributions (i.e. the distribution of the number of observed nucleotide differences between pairs of haplotypes) for the cytochrome b and the control region data sets separately. The shape of the distribution is expected to be unimodal in populations that have undergone recent population expansion, and ragged or multimodal when the population has been more stable (Rogers and Harpending 1992; Slatkin and Hudson 1991). Goodness of fit to the expected model of sudden population expansion was tested using the sum of squared deviations (SSD) and
raggedness indices were computed. All analyses were performed in **ARLEQUIN v. 3.5.2.2** (Excoffier and Lischer 2010) with 1,000 permutations to test for significance.

Based on the MMA, the time since population expansion (t) can be calculated through

$$ t = \frac{\tau}{2u} $$

Eq. 4.1

where \( \tau \) (tau) is the mode of the unimodal mismatch distribution and \( u \) is the cumulative (across the sequence) probability of substitution (Rogers 1995; Rogers and Harpending 1992; Schenekar and Weiss 2011). This probability of substitution can be obtained through

$$ u = N \times \mu $$

Eq. 4.2

where \( N \) is the number of nucleotides and \( \mu \) is the mutations per site per generation. We calculated \( \tau \) in **ARLEQUIN v. 3.5.2.2** (Excoffier and Lischer 2010) and estimated 95% confidence intervals (CIs) using 1,000 permutations. Since the MMA for the control region was slightly ragged (see Results) and the CIs for \( \tau \) included zero, the control region data was not used to calculate the time since population expansion as results would have been meaningless. For cytochrome b, we used the published divergence rate for Charadriiformes of 2.61% per million years (Weir and Schluter 2008), which is twice the substitution rate per nucleotide (i.e. 0.01305 substitutions/site/million years).

**Extended Bayesian Skyline Plot**

Secondly, we used an Extended Bayesian Skyline Plot (EBSP) coalescent method in **BEAST v. 2.4.8** to investigate changes in population size through time (Heled and Drummond 2008; Bouckaert et al. 2014). ‘Skyline plots’ are nonparametric methods to estimate demographic history from sequence data or estimated genealogy (Pybus, Rambaut, and Harvey 2000). The EBSP is a piecewise-linear model without restrictions on possible demographic models (e.g. exponential growth, linear decline, etc.), which allows population size to change continuously along each modelled interval (Pybus, Rambaut, and Harvey 2000; Heled and Drummond 2008; Ho and Shapiro 2011). In addition to its flexibility in modelling demographic history, it also has the advantage of allowing simultaneous analysis of multiple loci. We analysed the cytochrome b and control region data set together to maximise sample sizes from throughout the breeding range and the number of site polymorphisms by including longer DNA sequences (Grant 2015). We used an HKY model for cytochrome b and an HKY+G model of molecular evolution for the control region data set as first selected by the AIC selection criterion in **JMODELTEST v. 2.1.10** (Guindon and Gascuel 2003; Darriba et al. 2012). For cytochrome b, we used a strict clock with a rate of 1.305 x 10^8 substitutions per site per year based on the divergence rate for Charadriiformes of
2.61% per million years again (Weir and Schluter 2008). In the absence of published estimates of substitution rates of Chlidonias and great variation among different Charadriiformes species (Crochet and Desmarais 2000), we estimated the substitution rate in BEAST. We used a prior of $7.4 \times 10^{-8}$ substitutions per site per year based on the published divergence rate of the control region for dunlins (Calidris alpina; Order: Charadriiformes) of 14.8% per million years (Wenink et al. 1996). The analyses were run for 50 million generations with a 10% burn-in and sampling every 1,000 generations. We used TRACER v.1.7 (Rambaut and Drummond 2007) to visualise the sampling trace and check that the effective sampling size values (ESS) > 200 to confirm convergence. We visualised the resulting ESBP in R v. 3.3.3. (R Core Team 2017). As our analysis was based on mitochondrial data, which is only maternally inherited, and a mutation rate in the unit of mutations/site/year, the population size parameter on the y-axis represents female effective population size ($N_e$) x generation time.

**ABC analysis**

We used an approximate Bayesian computation (ABC) approach (Beaumont, Zhang, and Balding 2002) implemented in DIYABC v. 2.1.0 (Cornuet et al. 2014) to test for different demographic models and specifically test for a population bottleneck since the arrival of humans to New Zealand. For many complex models, it is difficult to calculate the specific model likelihoods needed for model-based inference (Beaumont, Zhang, and Balding 2002). The difficulty of calculating likelihoods is circumvented in ABC by using massive simulations. The posterior probabilities of different models and/or posterior distributions of demographic parameters under a given model are determined by comparing the observed data set (i.e. summary statistics of multilocus genotypes and sequences) with a large number of simulated data sets (Beaumont 2010). A recent simulation-based evaluation of DIYABC approaches showed that the precision of capturing detailed demographic history tends to be poor when many complex competing models are evaluated (Cabrera and Palsbøll 2017). The resulting recommendation was to rather focus upon a few demographic events with large effect sizes (Cabrera and Palsbøll 2017). Hence, we limited our comparison to three general models: 1) constant population size through time; 2) glacial expansion occurring 1,000–10,000 generations ago (i.e. 10,000–100,000 years ago); 3) glacial expansion and recent bottleneck occurring 1–100 generations ago (i.e. 10–1,000 years ago; Fig. 4.1).

We tested these models using both microsatellite and mtDNA data (control region) together with a subset of samples for which all data were available ($n = 61$) using wide priors (Table 4.3). As summary statistics for microsatellite data, we used: 1) the mean number of alleles across loci;
Chapter 4: Past population expansion and recent decline of black-fronted terns

2) mean gene diversity across loci (Nei 1987); 3) mean allele size variance across loci; and 4) mean M index across loci (Garza and Williamson 2001; Excoffier, Estoup, and Cornuet 2005). As a mutation model for microsatellite data, we used a generalised stepwise mutation model (GSM) with a mean rate uniformly distributed between $1.00 \times 10^{-6}$ and $1.00 \times 10^{-3}$ substitutions/generations (Estoup, Jarne, and Cornuet 2002). As summary statistics for mtDNA data, we used: 1) the number of distinct haplotypes; 2) the number of segregating sites; 3) mean pairwise difference; and 4) Tajima’s $D$ statistic. We used an HKY substitution model (Hasegawa, Kishino, and Yano 1985) for mtDNA data with a mean rate uniformly distributed between $1.00 \times 10^{-8}$ and $1.00 \times 10^{-7}$ substitutions/generations based on the information described above.

For each model, we generated one million data sets. We calculated Euclidean distances between the observed and normalised simulated summary statistics using the weighted local linear regression method of Beaumont, Zhang, and Balding (2002). We then estimated and compared the posterior probabilities of the different demographic models using a logistic regression of each scenario probability on the deviations of simulated and observed summary statistics (‘logistic approach’) (Fagundes et al. 2007; Cornuet et al. 2014). Point estimates and 95% confidence intervals were calculated. We retained the 10,000 simulated data sets (1%) with the smallest Euclidean distance to calculate posterior parameter distributions. Confidence in the model choice was assessed by generating 500 pseudo-observed data sets and estimating type I and type II errors using the logistic regression approach (Cornuet et al. 2014).

![Figure 4.1 Three alternative demographic models for black-fronted terns evaluated in DIYABC. The areas of figures represent changes in population size through time. Most recent events are shown at the bottom of the figure, with time of changes in population size ($t_1$ and $t_2$) measured in generations and not shown to scale. $N_{cont}$ = contemporary population size; $N_{pre-human}$ = population size before humans arrived in New Zealand; and $N_{glacial}$ = population size during the Ōtiran glaciation.](image-url)

Figure 4.1 Three alternative demographic models for black-fronted terns evaluated in DIYABC. The areas of figures represent changes in population size through time. Most recent events are shown at the bottom of the figure, with time of changes in population size ($t_1$ and $t_2$) measured in generations and not shown to scale. $N_{cont}$ = contemporary population size; $N_{pre-human}$ = population size before humans arrived in New Zealand; and $N_{glacial}$ = population size during the Ōtiran glaciation.
**Population size estimates**

**Historical (long-term) effective population size**

We estimated the historical effective population size \( N_e \) based on the microsatellite and mitochondrial data sets. We used the average expected heterozygosity of the microsatellite loci to calculate historical \( N_e \) under the stepwise mutation model (SMM) (Lehmann et al. 1998). It is currently not fully resolved how microsatellites evolve (Ellegren 2004; Bhargava and Fuentes 2010). The SMM assumes that microsatellite allele length has equal probability to increase or decrease by one repeat unit (Ohta and Kimura 1973; Kimura and Ohta 1978). However, mutation events sometimes involve more than one repeat unit (Di Rienzo et al. 1994). The SMM is nevertheless an appropriate fit, as the majority of mutation events are single step events rather than multiple units (Ellegren 2004; Selkoe and Toonen 2006; Bhargava and Fuentes 2010). Long-term effective population size was calculated under the SMM with the following equation:

\[
N_e = \frac{1}{(1 - H_e)^2} - 1} / 8\mu
\]

where \( H_e \) is the unbiased expected heterozygosity (Nei 1987) and \( \mu \) is the mutation rate. We calculated the unbiased \( H_e \) for all loci to obtain a mean estimate and standard error. As microsatellite mutation rates can differ among loci, alleles and also among species, we calculated long-term \( N_e \) for two different mutation rates: a faster rate of \( 1 \times 10^{-4} \) and a slower rate of \( 1 \times 10^{-5} \) substitutions/generation (Ellegren 2004).

We estimated long-term female \( N_e \) from the mitochondrial sequences using Watterson’s theta (\( \theta \)) estimator (Watterson 1975) in the following equation:

\[
N_e = \theta / 4u
\]

where \( \theta \) was calculated in ARLEQUIN v. 3.5.2.2 (Excoffier and Lischer 2010) and \( u \) is obtained through Eq. 4.2. For the control region, we based the divergence rate on the estimate obtained in the BEAST analysis as no divergence rates for black-fronted terns or closely related species are published (see above). Assuming an even sex ratio in black-fronted terns, we doubled this estimate for comparison to the estimates from nuclear data.

**Contemporary effective number of breeders and population size**

Black-fronted terns are an iteroparous species (i.e. a species that reproduces multiple times during its life time and therefore has overlapping generations), but all single-sample methods
for estimating effective population size (unless detailed information on age class and sex is available) use models that assume populations with discrete, nonoverlapping generations (Waples 2005; Wang 2009). These models can still be applied to iteroparous species with overlapping generations and two related quantities can be estimated: effective population size (\(N_e\)) per generation and effective number of breeders (\(N_b\)) in one reproductive cycle (Waples, Antao, and Luikart 2014).

We first applied the widely used bias-corrected linkage disequilibrium (LD) method (Hill 1981; Waples and Do 2008) implemented in the program NESTimator v. 2 (Do et al. 2014) to estimate \(N_b\) as well as \(N_e\). The principle upon which the LD method is based is that as \(N_e\) decreases, genetic drift acting on individuals leads to non-random associations among alleles at different loci, i.e. gametic disequilibrium or linkage disequilibrium (Waples 1991; Luikart et al. 2010). We used 0.02 as a critical cut-off value for the frequency of rare alleles, which provides a reasonable balance between precision and bias (Waples and Do 2010). We specified the mating system as monogamous and used a jack-knife method for estimating 95% CIs (Keedwell 2002; Cabot and Nisbet 2013).

First, we estimated \(N_b\) only using samples from individual cohorts, i.e. chicks. To obtain acceptable sample size and coverage of the whole breeding population, we pooled the samples of two consecutive breeding seasons, the chick cohorts of the 2013/14 (\(n = 35\)) and 2014/15 (\(n = 69\)), based on assuming a minimum reproductive age of 2 years to ensure no parent-offspring pair exist within the two consecutive cohorts (Keedwell 2002). This has been shown to provide an adequate estimate of \(N_b\) (Robinson and Moyer 2012; Waples, Antao, and Luikart 2014). Waples, Antao, and Luikart (2014) suggested the following bias correction of \(N_b\) based on extensive simulation of species with different life tables:

\[
\hat{N}_{b(Adj)} = \frac{\text{raw~}N_b}{1.103 - 0.245 \times \log(AL)}
\]

where the two life history traits used are adult life span (\(AL\); the maximum number of years in which an individual can reproduce) and the age at maturity (\(\alpha\); the youngest age with non-zero fecundity). For black-fronted terns, we assumed \(AL = 25\) and \(\alpha = 2\) following Keedwell (2002).

Next, using the adjusted \(N_b\) estimate, we calculated \(N_e\) using a conversion based on the same life history traits (Waples, Antao, and Luikart 2014):

\[
\hat{N}_{e(Adj)} = \frac{\hat{N}_{b(Adj)}}{0.485 + 0.758 \times \log(AL)}
\]
In addition, we also estimated $N_e$ using the mixed-age adult sample ($n = 318$), as well as the mixed-age adult and chick samples ($n = 422$), for comparison of estimates obtained from $N_b$ and with long-term $N_e$. As LD from mixed-aged samples for populations with overlapping generations is generally overestimated, $N_e$ is underestimated (Robinson and Moyer 2012; Waples, Antao, and Luikart 2014).

Lastly, we also used the sibship assignment (SA) method implemented in COLONY v. 2.0.6.4 for the same pooled chick cohorts (described above) to obtain a second estimate of $N_b$ for comparison (Wang 2009; Jones and Wang 2010). The SA method is based on the principle that a smaller population contains a higher proportion of siblings (Wang 2009). The frequencies of two offspring drawn at random from the population being siblings (i.e. sharing the same one or two parents) are estimated based on the multilocus genotypes. These sibship frequencies are used to estimate $N_b$ through a predictive equation (Wang 2009). We defined the mating system as female and male monogamous (i.e. neither sex was expected to have extra-pair copulations or switch partners within the breeding season; Keedwell 2002; Cabot and Nisbet 2013). We employed a medium length of run and a full likelihood method, scaled sibship assignment, but did not update allele frequencies and used a weak prior.

Simulations have shown that LD is the most accurate method for populations with no immigration (Gilbert and Whitlock 2015). Furthermore, LD and SA methods have also been shown to be the most precise methods for larger $N_e$ (> 500 individuals), using a moderate number of loci ($n = 17$), and are reasonably robust to different sampling properties (i.e. sample size ratio to true $N_e$) (Kamath et al. 2015; Wang 2016).

Population census size
Based on the genetic estimates of historical and current $N_b$ and $N_e$ estimates, we calculated census population size $N_C$ for both time frames. Ratios of $N_e$ to $N_C$ in wild populations can vary significantly (Frankham 1995; Palstra and Ruzzante 2008), because the estimation of $N_e$ or $N_b$ is based on a number of assumptions (e.g. no variance in reproductive success, stable population size, equal sex ratio, which are often not met in wild populations; Allendorf, Luikart, and Aitken 2013). We estimated the historical and current number of breeders and overall census population size using three ratios: 1) the median value of wild populations of 0.11 (Frankham 1995); 2) the median value of populations under conservation concern of 0.37 (Palstra and Ruzzante 2008); and 3) a value of 0.5 for populations with long generation times (Lee, Engen, and Sæther 2011).
RESULTS

Genetic data quality

Previous studies using the microsatellite data set did not detect consistent departures from Hardy-Weinberg proportions, linkage disequilibrium or signs of null alleles, stuttering, or large allele dropouts (Chapter 2 and 3). Mean genotyping error was low with 0.0136 and the overall level of missing data was 1.09% with only ever a single locus missing per individual (Chapter 3).

Demographic history

Neutrality statistics and mismatch analyses

The neutrality statistics and sequence mismatch analyses provided some evidence of population expansion. For cytochrome b, both neutrality statistics were significantly negative, whereas for the control region only Fu’s $F_s$ was significantly negative indicating population expansion (Table 4.1). Similarly, the mismatch distributions showed a reasonable fit between the observed number of pairwise differences and expected frequencies of haplotypes under a rapid population expansion model (Fig. 4.2; Table 4.2). The distribution was clearly unimodal based on cytochrome b data (Fig. 4.2 a), but it was slightly ragged based on the control region data (Fig. 4.2 b). Nevertheless, SSD and raggedness indices were non-significant for both markers supporting historical rapid population expansion. Based solely on the cytochrome b data and using $\tau = 2.135$ (CI: 0.396–5.557), we estimated the timing of the purported population expansion at 71,561 years ago (CI: 13,274–186,275 years).

![Mismatch distribution in black-fronted terns using two different mitochondrial DNA markers](image)

Figure 4.2 Mismatch distribution in black-fronted terns using two different mitochondrial DNA markers a) Cytochrome b and b) Control region. Bars represent the observed frequencies of pairwise differences and the dashed line represents the expected frequencies under a model of sudden population expansion.
Extended Bayesian Skyline Plot

In addition, we found support for a moderate population expansion of black-fronted terns based on the coalescent analysis in BEAST (Fig. 4.3). Despite the relatively large posterior density intervals (HPDs) in the skyline plot, we found an increase in population size ∼55,000 years ago (Fig. 4.3). However, we were unable to reject constant population size as an alternative demographic scenario, because HPDs of the number of population changes also included zero.

**Table 4.1** Results of black-fronted tern past demographic analyses. *P*-values in bold indicate significant neutrality statistics supporting population expansion.

<table>
<thead>
<tr>
<th></th>
<th>Tajima’s <em>D</em></th>
<th><em>p</em>-value</th>
<th>Fu’s <em>F</em>&lt;sub&gt;S&lt;/sub&gt;</th>
<th><em>p</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cytochrome b</em></td>
<td>-1.555</td>
<td><strong>0.031</strong></td>
<td>-27.344</td>
<td>0</td>
</tr>
<tr>
<td><em>Control region</em></td>
<td>-0.958</td>
<td>0.174</td>
<td>-27.305</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 4.2** Indices of population expansion in black-fronted tern populations of mismatch distributions. Squared sum of deviances (SSD), raggedness index (RI) and associated *p*-values. *P*-values in bold support population expansion.

<table>
<thead>
<tr>
<th></th>
<th>SSD</th>
<th><em>p</em>-value</th>
<th>RI</th>
<th><em>p</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cytochrome b</em></td>
<td>0.012</td>
<td><strong>0.171</strong></td>
<td>0.053</td>
<td><strong>0.219</strong></td>
</tr>
<tr>
<td><em>Control region</em></td>
<td>0.026</td>
<td><strong>0.172</strong></td>
<td>0.100</td>
<td><strong>0.127</strong></td>
</tr>
</tbody>
</table>

**Figure 4.3** Demographic history of black-fronted terns over the last 85,000 years based on an extended Bayesian skyline plot based on cytochrome b (1143 bp) and control region (898 bp) sequences from throughout their range. The female effective population size (*N*<sub>ef</sub>) x generation length (τ) of 10 years is shown on the y-axis and time in years before present on the x-axis. The solid line indicates the median estimate and the blue block represent the 95% highest posterior density interval.
ABC analysis

When testing for different demographic models using the ABC approach, the ‘glacial expansion and recent population bottleneck’ was strongly supported with a posterior probability of 97.6%. The model of ‘glacial expansion’ had a posterior probability of less than 3%, while the model of ‘constant population size’ received the least support with less 1% posterior probability. The population size was estimated at \(~15,100\) individuals (95% HPD: 2,790–26,400) expanding to \(~106,000\) individuals (95% HPD: 54,600–483,000) before human arrival and then contracting to \(~28,900\) individuals (95% HPD: 8,470–29,800) (Table 4.3). However, no clear mode was observed for the last estimate and hence it should be interpreted with caution. The timing of the initial expansion was estimated at \(~33,300\) years ago (95% HPD: 15,700–97,100), and the contraction at \(~778\) years ago (95% HPD: 31.2–781) (Table 4.3). Again, there was no clear mode discernible for the last estimate and its precision should be taken with caution. Type I and Type II error rates were 0.18 and 0.14, respectively.

Table 4.3 Prior and posterior distributions of parameters for the demographic model (‘glacial expansion and recent population bottleneck’) that obtained the highest posterior probability when comparing different scenarios for black-fronted terns. The timings of events are measured in generations assuming a generation time of 10 years for black-fronted terns. HPD = highest posterior density.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Prior</th>
<th>Posterior mode</th>
<th>5% HPD</th>
<th>95% HPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N_{\text{contemporary}})</td>
<td>Uniform (1,000–30,000)</td>
<td>28,900</td>
<td>8,470a</td>
<td>29,800a</td>
</tr>
<tr>
<td>(N_{\text{pre-human}})</td>
<td>Uniform (10,000–500,000)</td>
<td>106,000</td>
<td>54,600</td>
<td>483,000</td>
</tr>
<tr>
<td>(N_{\text{glacial}})</td>
<td>Uniform (1,000–30,000)</td>
<td>15,100</td>
<td>2,790</td>
<td>26,400</td>
</tr>
<tr>
<td>(t_1)</td>
<td>Uniform (1–80)</td>
<td>77.80</td>
<td>3.12b</td>
<td>78.10b</td>
</tr>
<tr>
<td>(t_2)</td>
<td>Uniform (1,000–10,000)</td>
<td>3,330</td>
<td>1,570</td>
<td>9,710</td>
</tr>
</tbody>
</table>

Conditions: \(N_{\text{pre-human}} > N_{\text{contemporary}}\); \(N_{\text{pre-human}} > N_{\text{glacial}}\); \(N_{\text{contemporary}} > N_{\text{glacial}}\)

a) HPD may not be useful—posterior density did not reach low levels near the upper or lower limit of the prior
b) HPD may incorrect due to multiple peaks

Population size estimates

Historical effective population size

Mean historical effective population size of black-fronted terns varied depending on the method and mutation rate (Table 4.4). Assuming an even sex ratio, historical population size based on the control region was estimated at 16,686 adults (95% CI: 10,566–22,806), and 22,634 adults based on cytochrome b (95% CI: 13,832–31,436) (Table 4.4). The estimate of the control region was more precise, presumably as the sample size was larger. Historical population size based on microsatellite data and the faster mutation rate of \(1 \times 10^{-4}\) substitutions/generation was estimated at 13,264 adults (95% CI: 10,457–17,375) (Table 4.4). Thus, this estimate based on nuclear data had a similar range to that of the estimates based on mitochondrial data.
Chapter 4: Past population expansion and recent decline of black-fronted terns

### Table 4.4 Historical effective population size (\(N_e\)) estimates of the global black-fronted tern population based on microsatellite loci (\(n = 17\)) and two mitochondrial markers (cytochrome b (1143 bp) and control region (898 bp)). Estimates of the microsatellite data are based on the stepwise mutation model (SMM) using two different mutation rates (\(\mu\)) and mean unbiased expected heterozygosity (\(H_e\)). Estimates based on the mitochondrial data are obtained using Watterson’s theta estimator (\(\theta\)).

<table>
<thead>
<tr>
<th>Data set</th>
<th>(\mu)</th>
<th>(H_e)</th>
<th>(\theta)</th>
<th>Historical (N_e)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microsatellites</strong></td>
<td>1.00 x 10^{-4} &amp; 0.693 (0.654 - 0.732) &amp; - &amp; 13,264 (10,457 - 17,375)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.00 x 10^{-5} &amp; 0.693 (0.654 - 0.732) &amp; - &amp; 132,641 (104,568 - 173,746)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cytochrome b</strong></td>
<td>1.3105 x 10^{-8} &amp; - &amp; 3.376 (2.063 - 4.689) &amp; 22,634 (13,832 - 31,436)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control region</strong></td>
<td>1.976 x 10^{-8} &amp; - &amp; 2.961 (1.875 - 4.047) &amp; 16,686 (10,566 - 22,806)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **a)** Assuming an even sex ratio, these estimates have been doubled as they are based on only maternally inherited mitochondrial markers and otherwise only reflect female effective population size.
- **b)** The mutation rate (\(\mu\)) x sequence length x generation time (10 years) is based on the published rate for Charadriiformes of 2.61% divergence/mya (Weir and Schluter 2008).
- **c)** The mutation rate (\(\mu\)) x sequence length x generation time (10 years) is based on the mode of the rate obtained from the BEAST analysis.

### Contemporary effective number of breeders and population size

Estimates of the current effective number of breeders were congruent between the three different methods (SA \(N_b\), LD \(N_b\), and corrected LD \(N_b(adj2)\)). Mean estimates were relatively low ranging between 630 to 880 adults (Table 4.5). Precision was highest for the SA method (95% CI: 449–1,006 individuals). The estimate of current \(N_e\) calculated using the corrected estimate of LD \(N_b\) and the two life-history traits (age at maturity and adult life span) was only a quarter of the estimates obtained using mixed-age adults or all samples (mean \(N_e(adj2)\) 668 adults compared to mean LD \(N_e\) based on adult samples of 2,889 and mean LD \(N_e\) using all samples of 3,216 individuals; Table 4.5). While the two latter estimates again were similar in mean \(N_e\), precision was improved using all samples, as confidence intervals were much narrower.

### Table 4.5 Contemporary effective number of breeders (\(N_b\)) and effective population size (\(N_e\)) estimates (lower and upper 95% confidence intervals) for the global black-fronted tern population using different single sample estimators. SA = Sibship assignment; LD = Linkage disequilibrium. Details on the bias correction of \(N_b\) and the conversion to \(N_e\) are described in the methods.

<table>
<thead>
<tr>
<th>Dataset/Details</th>
<th>(N)</th>
<th>Method</th>
<th>Contemporary (N_b) or (N_e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicks (2013/14 &amp; 2014/15 cohort)</td>
<td>104</td>
<td>SA (N_b)</td>
<td>630 (449–1,006)</td>
</tr>
<tr>
<td>Chicks (2013/14 &amp; 2014/15 cohort)</td>
<td>104</td>
<td>LD (N_b)(raw)</td>
<td>734 (381–4,229)</td>
</tr>
<tr>
<td>Waples et al. (2014) bias correction</td>
<td>104</td>
<td>LD (N_b(adj2))</td>
<td>880 (456–5,070)</td>
</tr>
<tr>
<td>Waples et al. (2014) conversion based on LD (N_b(adj2))</td>
<td>104</td>
<td>LD (N_e(adj2))</td>
<td>668 (347–3,851)</td>
</tr>
<tr>
<td>Mixed-age adults</td>
<td>318</td>
<td>LD (N_e)</td>
<td>2,889 (1,449–29,709)</td>
</tr>
<tr>
<td>All samples</td>
<td>422</td>
<td>LD (N_e)</td>
<td>3,216 (1,827–10,611)</td>
</tr>
</tbody>
</table>
Population census size

Estimates of historical and contemporary population census size depended on the method and conversion ratio used, but mean estimates suggested $N_e$ was over four times larger in the past compared to contemporary estimates of mixed-age adults or all samples (Figure 4.4; Table B.1). However, confidence intervals around those contemporary estimates were large and overlapped with some of the historical estimates (Table B.1). Mean population size estimates based on $N_e$ estimated from mixed-age adults or all samples and converted with a ratio of 0.11 for wild populations are 2.5–3 times the current published estimate of a total population size of ca. 10,000 individuals (Fig. 4.4). Hence, using the more conservative conversion ratio of 0.37, the mean contemporary population size ranges between 7,807 to 8,692 individuals compared to a mean of 35,849 to 358,488 individuals in the past (Fig. 4.4; Table B.1). Applying the conversion ratio of 0.37 to $N_b$ produces mean census estimates between 1,703 to 2,378 adults (Fig. 4.4; Table B.1).

**Figure 4.4** Historical and contemporary census population size of black-fronted terns using three different published $N_e/N_c$ conversion ratios: 0.11 (Frankham 1995); 0.37 (Palstra and Ruzzante 2008); and 0.5 (Lee, Engen, and Sæther 2011). Details on the different $N_e$ and $N_b$ estimates can be found in Table 4.4 and 4.5. The red dashed line shows the contemporary published population size estimate (O’Donnell & Hoare 2011). Confidence intervals (CIs) were shortened for ease of representation (double lines through CIs). Full details are presented in Table B.1.
DISCUSSION

Our work revealed that the population size of black-fronted terns most likely expanded already during glacial periods in the late Pleistocene based on our analyses of mitochondrial and nuclear markers. A subsequent, human-induced decline in population size was evident, which was highlighted by the low number of effective breeders. The disparity between effective number of breeders and effective population size emphasises the need for management targeting reproductive failure.

Evidence of past population expansion

All analyses of the demographic history of black-fronted terns provided some evidence for past population expansion during the Ōtiran glaciation. The mismatch analysis indicated this expansion occurred earlier than suggested by the other analyses, about 70,000 years ago. In comparison, the Bayesian skyline plot and ABC analysis showed a population increase to have occurred around 55,000 and 33,000 years ago, respectively. In historical demography analyses of many bird species, the timing of expansions calculated through MMA tend to be earlier than those obtained through Bayesian skyline analyses (Grant 2015).

The first estimate coincides with the interstadial period just after the height of the Ōtiran glaciation between 72,000–62,000 years ago. During this period, there were the greatest glacial advances caused by wet and cold conditions (Schaefer et al. 2015; Williams et al. 2015). The other estimates date the population expansion during the interstadial period before the last glacial maximum (LGM) from 32,000–18,000 years ago. Even during this interstadial, some glacial advances occurred (Williams et al. 2015). New Zealand’s coastline and the braided river plains extended beyond its present day limits by about 70 km during the Ōtiran glacial period as sea levels were lower (Browne and Naish 2003). Dry grasslands with some shrubs and small forest pockets dominated the lowland landscape (Golledge et al. 2012; Lorrey and Bostock 2017; Wood et al. 2017). Our population expansion scenarios for black-fronted terns date much earlier than inferred expansions for other New Zealand species such as kea (*Nestor notabilis*; Dussex, Wegmann, and Robertson 2014), or rock wren/piwauwau (*Xenicus gilviventris*; Weston and Robertson 2015). These estimates seem plausible though, given that black-fronted terns would have benefited from open dry grasslands and are not as dependent on glacial refugia (i.e. forest cover) as other New Zealand species. For example, black-fronted terns have been observed systematically preying upon lizards over grasslands in the Eglinton Valley, one of two locations...
for which a recent positive population trend has been estimated (O’Donnell and Hoare 2009, 2011; Monks and O’Donnell 2013).

Many tern species breed in tropical or subtropical regions, and the majority have more widespread distributions than black-fronted terns (Cabot and Nisbet 2013). Other tern species experienced population expansion during the late Pleistocene and also responded to the formation of new areas of habitat under different climatic conditions. For example, Peck and Congdon (2004) used control region sequences of sooty tern (Onychoprion fuscata) from the Indo-Atlantic and Southwestern Pacific to infer historical processes and better understand genetic population structure. Their work showed that sooty tern populations expanded in the late Pleistocene globally, but the expansion into the Southwestern Pacific occurred more recently, after the LGM (Peck and Congdon 2004). This later expansion is presumably linked to the creation of areas of habitat as sea levels increased and coral reefs formed.

There are some important limitations to be aware of when inferring the historical demography of a species. First, the unique genealogical history of a gene influences the outcome of any demographic analysis due to the randomness in genetic transmission and mutational history (Rosenberg and Nordborg 2002). It is possible to account for this randomness among loci by using multiple sequences of different markers (Heled and Drummond 2008). In our case, although we included multiple marker sequences, both sequences stemmed from the mitochondria, which is inherited maternally without recombination. It should therefore be treated as a single locus (Ho and Shapiro 2011).

Second, estimating the timing of historical events accurately is highly dependent on the mutation rate chosen. In the absence of a black-fronted tern-specific mutation rate for any locus, we based our estimate on the general divergence rate of Charadriiformes for cytochrome b. We estimated mutation rate of the control region and obtained a rate that was on the lower end of the range for birds (Ruokonen and Kvist 2002). Slow rates of evolution in the control region have also been reported for the closely related gulls (Crochet and Desmarais 2000), so our estimate seems plausible. As mutation rates based on divergence rates will be slower (Ho et al. 2008, 2011; Emerson and Hickerson 2015; Grant 2015), our estimate could be an overestimation of the timing of population expansion. This would mean that black-fronted terns could have expanded in population size actually only after the last LGM, similar to other New Zealand species (e.g. Dussex, Wegmann, and Robertson 2014; Weston and Robertson 2015; Grosser et al. 2017). However, in contrast to those other species, we did not find any evidence of past contraction during the LGM such as genetic structuring (Chapter 3). In addition, we intentionally
used a wide prior in the ABC analysis, including before and after the last LGM as possible timing for when expansion occurred. The ABC analysis suggests a later population expansion than mismatch or skyline analyses, but still during the LGM.

Finally, small sample sizes (< 100 individuals) often lead to an underestimation of a population expansion (Grant 2015). In our analyses, HPDs were very wide leading to low precision. For future analyses, including nuclear genes and more samples might improve not only the precision, but also the accuracy of timing and magnitude of historical demographic events of black-fronted terns. Furthermore, the more recent historical demography could also be elucidated by incorporating historical museum and subfossil samples. For example, prehistorical anthropogenic impact in the form of range retraction and dramatic population reduction on the Otago lineage of another New Zealand endemic seabird species, the Stewart Island shag (Leucocarbo chalconotus), has been demonstrated through Bayesian modelling based on control region sequences from contemporary, historical and subfossil samples (Rawlence et al. 2015). Similarly, Bergner et al. (2016) analysed microsatellite marker and control region sequence data of contemporary and museum specimens of kākāpō (Strigops habroptilus) to determine that the decline in population size occurred only after European colonisation in New Zealand in the 19th century. These results have now been confirmed using the whole mitogenome of contemporary and museum specimens of kākāpō (Dussex et al. 2018b)

**Evidence of human-induced decline**

**Historical estimates compared to contemporary estimates**

Declines in population size and range of black-fronted terns are thought to have occurred since the arrival of humans (Stead 1932; Oliver 1955). Our analyses confirm this human-induced population decline. We obtained the strongest support for the ‘glacial expansion and recent bottleneck’ model in our ABC analysis. Although it was not possible to estimate the timing and contemporary population size precisely, the analysis provided a general insight and confirmation of the demographic history of black-fronted terns. Furthermore, the historical effective population size estimates based on microsatellite and mitochondrial data were at least four times or even higher than current contemporary $N_e$ estimates (~3,000 individuals).

A recent simulation study to evaluate the performance of ABC analyses based on contemporary genetic data yielded two important considerations (Cabrera and Palsbøll 2017). First, increasing the amount of contemporary genetic data does not significantly improve the
precision and accuracy of timing and population size change estimates. Second, it is not possible to distinguish between very similar models, but general insight is nevertheless provided. Including genetic data from museum specimens or subfossils could potentially improve the accuracy of overall estimates, but might not be able to estimate the timing of the bottleneck with higher precision. This has been done for other New Zealand species such as kākāpō (Bergner et al. 2016; Dussex et al. 2018b). But even in the most recent kākāpō study using whole mitogenomes, it has been difficult to distinguish between two competing timings of bottlenecks (Dussex et al. 2018b). While the population decline experienced by kākāpō was very severe and numbers are currently increasing (Powlesland, Merton, and Cockrem 2006), black-fronted terns are still in decline and rates of decline are more likely to increase in the future (O’Donnell and Hoare 2011). Given this, it might not be possible to accurately estimate the timing of the bottleneck for black-fronted terns.

The accurate estimation of historical $N_e$ largely depends on the mutation rate employed. We used two commonly reported mutation rates for microsatellite markers ($1 \times 10^{-4}$ and $1 \times 10^{-5}$; Ellegren 2004), which provide estimates of historical $N_e$ that differ in order of magnitude. The true historical $N_e$ is most likely some value in between. Similarly, contemporary estimates of $N_e$ are sensitive to the method used in estimating them. The LD method was developed for semelparous species (i.e. species that only reproduce once and therefore have non-overlapping generations), but can be used for iteroparous species (Waples, Antao, and Luikart 2014). It is sensitive to the number of cohorts sampled in mixed-age adult samples and will be more accurate and precise, when more individuals and age cohorts are sampled (Waples, Antao, and Luikart 2014). In our study, confidence intervals for contemporary $N_e$ are wide and some even overlap with historical estimates. Estimates based on single-consecutive cohorts of black-fronted terns are more precise and highlight the alarming status of the species and low number of breeders.

**Differences in estimates of $N_e$**

Waples et al. (2014) showed that in iteroparous species the estimation of contemporary $N_e$ based on mixed-age samples is always downwardly biased and that this bias is stronger if the number of cohorts included does not fully represent generation length. Hence, the estimates of contemporary $N_e$ of black-fronted terns based on mixed-age adult samples or all samples are most likely an underestimate of the true $N_e$. Surprisingly though, the estimate of $N_e$ based on the bias-adjusted estimate of $N_b$ is only about a quarter of those mixed-aged samples, although
in the simulations of Waples et al. (2014), this was always the most accurate estimate. There are three possible reasons for this finding.

First, if females skip one or multiple breeding cycles, it reduces $N_b$ and increases $N_e$, although this effect should be minimal for long-lived species with low-batch fecundity (Waples and Antao 2014). It is assumed that all black-fronted tern adults attempt to breed every year (Keedwell 2002). However, black-fronted terns have low productivity in many rivers due to predation by introduced mammals or native birds and habitat loss or degradation (Keedwell 2005; Cruz et al. 2013; Bell 2017; Chapter 5). Hence, many females will end up involuntarily “skipping” breeding cycles.

Second, the suggested conversion of $N_b$ to $N_e$ estimates assumes that individuals of the same sex and age will have identical reproductive success (Waples, Antao, and Luikart 2014). This will rarely hold for black-fronted terns in the wild due to the stochastic nature of the predominant impact of predation on breeding success (Keedwell 2005; Cruz et al. 2013; Chapter 5). The violation of this assumption will lead to a stronger downward bias in $N_b$ compared to $N_e$ (Lee, Engen, and Sæther 2011; Waples, Antao, and Luikart 2014). The reason for this is that the sampling window for $N_b$ is much smaller (i.e. only the chicks of one season or of two consecutive seasons). For $N_e$, sampling of any age group is possible and as almost all individuals attempt to nest, it is easier to obtain samples of most adults of a colony, but not necessarily of chicks.

Third, a further assumption is that the population of interest is of constant size and stable age structure (Waples and Antao 2014). Black-fronted terns are in decline and an overall population decline of about 50% over the next 25 years was predicted based on data from 10 years ago or more (O’Donnell and Hoare 2011). A recent strong population decline due to poor recruitment, which might result in a skew in age structure, will be more strongly reflected in $N_b$ than $N_e$ (Robertson et al. 2017). This is because $N_b$ only reflects that generation of breeders and not multiple generations of the past (Luikart et al. 2010). Most likely a combination of these three reasons will have affected the estimate of $N_b$ and hence the resulting $N_e$ estimate based on it. Importantly, the comparison of the different $N_e$ estimates emphasises how the poor recruitment is leading to an increased loss of genetic diversity manifested by the small number of effective breeders of black-fronted terns. In general, our analyses also highlight how the relative longevity of a species can potentially mask population declines in an iteroparous species when only $N_e$ is estimated and not compared to $N_b$ of single or consecutive cohorts.
Effective number of breeders and population size compared to census numbers

The minimum number of breeding adults in the 2014/15 breeding season was estimated to be 2,612 individuals (Schlesselmann, Cooper, and Maloney 2017). This estimate was derived from estimating the total number of breeders at each colony found throughout the South Island \((n = 31)\) in a single season. However, it did not cover the full length of many rivers and hence it is a minimum estimate (Schlesselmann, Cooper, and Maloney 2017). The mean census size of breeders obtained by the different \(N_e\) methods ranged from 5,727–8,000 adults using 0.11 as the conversion ratio, 1,703–2,378 adults using the intermediate ratio of 0.37, and 1,260–1,760 adults using 0.5 as conversion ratio.

Based on past braided river bird counts up to 2008, the black-fronted tern population size was estimated at around 10,000 individuals (O’Donnell and Hoare 2011), similar to an earlier estimate by Keedwell (2002). The estimate by O’Donnell and Hoare (2011) is based on standardised counts of black-fronted terns from braided river bed surveys over a large number of rivers \((n = 84)\), but numbers were summed over multiple years as counts were not carried out in each river every year (O’Donnell and Hoare 2011). The mean population census size obtained by the different \(N_e\) methods ranged from 6,073–29,236 adults using 0.11 as conversion ratio, 1,805–8,692 adults using the intermediate ratio of 0.37, and 1,336–6,432 adults using 0.5 as conversion ratio.

Comparing the estimates determined using the different conversion ratios to the other published census size estimates, the intermediate ratio of 0.37 appears the most realistic conversion ratio for black-fronted terns. Using the conversion ratio of 0.37 leads to a mean historical population size of black-fronted terns of 35,849–358,488 adults. When using \(N_e\)-derived historical population size estimates to inform species recovery, it is important to take into consideration that the carrying capacity of the environment can change over time. Anthropogenic impacts, such as climate change, water abstraction and lower water quality may decrease primary productivity of the environments and lead to declines of primary prey (Jaeger and Cherel 2011; Larned et al. 2016). In addition, available breeding habitat has been invaded by introduced vegetation (Williams and Wiser 2004; Brummer et al. 2016). However, given the evidence for other direct impacts such as predation significantly reducing productivity and the sparse nature of breeding populations (Keedwell 2005; Steffens et al. 2012; Cruz et al. 2013; Bell 2017; Schlesselmann, Cooper, and Maloney 2017), this seems highly unlikely. In addition, not all species respond in the same manner to environmental changes. For example, the vastly different responses to past climatic change by two sympatric Antarctic predators — population increase by emperor penguins (\textit{Aptenodytes forsteri}) and no population change by Weddell seals
— indicate the critical role that adaptive capacities and fine-scale niche differences can have (Younger et al. 2016). As black-fronted terns currently still maintain high levels of genetic diversity and low genetic differentiation between breeding colonies, are able to respond to changes in the environment quickly (Chapter 3 and 5), and show a high flexibility in their diet (Lalas 1977; O’Donnell and Hoare 2009), there is no reason to believe that a lower carrying capacity will hinder population growth and conservation management.

**Conservation implications**

Under its current New Zealand national threat classification, the black-fronted tern population is estimated to number between 1,000–5,000 mature individuals (Robertson et al. 2017), while the estimated population size applied for the IUCN Red List of Threatened Species ranges between 2,500–9,999 mature individuals (BirdLife International 2017). The estimates of the number of breeding adults based on genetic data ($N_b$ converted to $N_C$) reported here are closer to the national threat classification estimate. In comparison, the estimates of population size based on genetic data ($N_e$ converted to $N_C$) correspond to the larger IUCN estimate bounds. Our analyses suggest that black-fronted terns were much more abundant in the past than they are today and emphasise how recent anthropogenic impacts are playing a key role in their sharp decline. Current estimates place the $N_e$ of black-fronted terns above the recently revised guiding principle of a $N_e > 100$ individuals to avoid short-term negative impacts in the next five generations (i.e. inbreeding depression; Frankham, Bradshaw, and Brook 2014). However, the black-fronted tern population is not far above the recommended threshold $N_e > 1,000$ for long-term survival (i.e. maintaining evolutionary potential) using the mixed-aged samples (Frankham, Bradshaw, and Brook 2014). The black-fronted tern population is even below this threshold using the bias-adjusted $N_e$ estimate.

This low effective population size of the global black-fronted tern population means that if conservation management is not instigated with some urgency, adaptive potential critical for future environmental change will be lost (Waples 2002; Frankham 2005). Conservation measures need to target the whole metapopulation and should not be restricted to just one area (Chapter 3). The primary aim should be to improve recruitment. This requires identifying all factors impacting on successful recruitment in different river systems — natural and human-modified systems in upland and lowland regions — and developing management tools accordingly (Chapter 5). In addition, regular monitoring of effective population size, ideally $N_b$ should be instigated. Genetic monitoring provides a valuable tool for wildlife managers to assess if conservation management is aiding the recovery of threatened species and to continually
assess extinction risk (Schwartz, Luikart, and Waples 2007). This could be achieved by sampling black-fronted tern chicks over one or two consecutive breeding seasons every five years and using the existing microsatellite markers (Chapter 2) to estimate $N_0$ using the SA method. One example of the successful application of this approach involves the Yellowstone grizzly bear ($Ursus arctos$) population, where genetic monitoring showed that conservation management has been effective and the population has increased (Kamath et al. 2015). This study demonstrates how genetic data can be used to investigate past and present population size and how multiple independent estimators of genetically effective population size can be used to complement traditional ecological estimators of abundance for species that are highly mobile and otherwise difficult to monitor.
CHAPTER 5

Clearing islands as refugia for black-fronted tern breeding colonies in braided rivers

A version of this chapter has been published as:


Bruce Robertson, Colin O’Donnell, and Jo Monks assisted with experiment design, statistical analysis and commented on drafts. Paul Eddy, Brad Edwards, Jamie Cooper, Andrew Philpott, Bruce Scarlett, Sanjay Thakur, and Tom Waterhouse assisted with habitat creation and monitoring work in the river. Georgina Pickerell and Craig Gillies helped with the identification of ambiguous footprints. Tim Jowett and Matt Schofield provided statistical advice. Ken Miller assisted with the drawing of some figures. I planned and carried out the field work, undertook data analysis and preparation of the manuscript.
Chapter 5: Habitat creation for black-fronted terns

**ABSTRACT**

Black-fronted terns/tarapirohe (*Chlidonias albostriatus*) are highly adapted to nesting on clear shingle areas of the braided rivers in the South Island, New Zealand. They are nationally and internationally classified as ‘Endangered’. Ongoing threats, primarily an interaction of predation and habitat degradation or loss, have resulted in population decline. Conservation management in the form of control of introduced mammalian predators has proven partially successful. Using the lower Waitaki River as a case study, we cleared vegetation from seven islands creating potential refugia from mammalian predators and providing high quality bare gravel breeding habitat. We: i) determined the mammalian predators present on river banks, vegetated islands and cleared islands; ii) assessed the nesting success of black-fronted terns and primary causes of nest failure; iii) identified the predator species at nests using remote cameras; and iv) compared the nesting success on cleared and vegetated islands. Fewer mammalian predators were detected on islands compared to adjacent riverbanks: mustelids (*Mustela* spp.) occurred on approximately half of the vegetated islands, but only mice (*Mus musculus*) were detected once on one of the cleared islands. Black-fronted terns established three colonies on islands immediately after the clearing of vegetation, but nesting success in the lower Waitaki River was low overall (50.5–56.4% of nests contained at least one egg that hatched) and the primary cause of nest failure was predation before and after clearing islands. The main predators of nests (62.5% of predation events) were southern black-backed gulls/karoro (*Larus dominicanus*). There was no overall difference in nesting successes of colonies between cleared and vegetated islands, presumably because gulls depredated tern eggs irrespective of vegetation cover around target nests. Nesting success depended on the timing and size of the colony, with earlier established nests and nests in larger colonies being more successful. Artificially created nesting habitat can play a critical role for conservation, particularly on lowland rivers in New Zealand, and we recommend control of avian predators be considered.
INTRODUCTION

Black-fronted terns/tarapirohe (*Chlidonias albostriatus*) are endemic to New Zealand and only breed in the braided rivers of the South Island and migrate to the coast during winter (Higgins and Davies 1996; Schlesselmann, Cooper, and Maloney 2017). They form loose breeding colonies sometimes re-using the same breeding site in catchments (Keedwell 2005; Bell 2017). Similar to other marsh terns (genus *Chlidonias*), they specialise on using unstable and ephemeral breeding habitat, and are able to respond to changes in the environment quickly (Higgins and Davies 1996). They are currently classified nationally and internationally as ‘Endangered’ as population declines of around 50% over the next 30 years are predicted (O’Donnell and Hoare 2011; BirdLife International 2017; Robertson et al. 2017).

Their decline in population size is thought to be the product of multiple threats, primarily associated with habitat loss and increased risk of predation leading to low recruitment. An increasing demand for water abstraction for irrigation and diversion, and impoundment for hydroelectric power generation, has led to significantly reduced water flows in many braided rivers (Ministry for the Environment & Stats NZ 2017). Invasive alien plant species now dominate braided river beds, making areas unavailable to breeding black-fronted terns and other braided river avian specialists such as wrybill/ngutu pare (*Anarhynchus frontalis*) and black-billed gulls/tarāpukea (*Larus bulleri*) (Maloney et al. 1999; Williams and Wiser 2004; McClellan 2009; O’Donnell et al. 2016). Introduced mammalian predators (e.g. feral cats (*Felis catus*), stoats (*Mustela erminea*), ferrets (*M. furo*) and hedgehogs (*Erinaceus europaeus*)) are a primary cause of mortality in adults, chicks, and eggs (Sanders and Maloney 2002; Keedwell et al. 2002; Cruz et al. 2013; Bell 2017). In addition, native avian predators like the Australasian harrier/kāhu (*Circus approximans*) and the southern black-backed gull/karoro (*Larus dominicanus*) have increased in abundance since the arrival of Europeans in New Zealand in the 19th century (Turbott 1967). These species prey upon eggs and chicks of native braided river birds (Sanders and Maloney 2002; Steffens et al. 2012). The colonial nesting habit also makes black-fronted terns more vulnerable to localised events, e.g. catastrophic failure of nests often occurs due to flooding of a whole colony site or predation events of a few nests can lead to abandonment of the entire colony (Keedwell 2005; O’Donnell, Sedgeley, and van Hal 2010).

Reduced water flows, invasive plants and introduced predators are interacting threats that put further pressure on black-fronted tern populations. For example, lower river flows stabilise islands and increase channelisation, facilitating alien plant species encroachment and establishment, as well as increasing mammalian access to areas where black-fronted terns nest.
(Hicks et al. 2008; Pickerell 2015; Brummer et al. 2016). Furthermore, introduced vegetation provides habitat and cover for mammals (Norbury and Heyward 2008; Pickerell 2015). Effective methods for improving environmental conditions for black-fronted terns are needed (O’Donnell et al. 2016). However, these multiple threats, combined with colonial nesting with variable site fidelity of black-fronted terns, pose a significant challenge for conservation managers seeking to improve the nesting success of black-fronted terns, and reverse their population declines.

Conservation management of black-fronted terns has focused mostly on large-scale or localised control of introduced mammalian predators. Sustained year-round predator control on a landscape level on the Tasman River has been only partly successful (Cruz et al. 2013). Localised, intensive predator control on the Ōhau River successfully protected a single site (Anderson 2014). However, this level of localised control cannot be applied to most rivers because black-fronted tern colony locations generally vary depending on the availability of habitat at different sites (Keedwell 2005; O’Donnell et al. 2016). One study on the Tekapo River (Maloney et al. 1999), and anecdotal evidence from the Eglinton and lower Waitaki Rivers, have shown that black-fronted terns respond to vegetation clearance by using the resulting bare gravel for roosting and nesting. Nevertheless, we still do not have a good understanding in New Zealand of how vegetation clearance affects nesting success of black-fronted terns (Maloney et al. 1999; O’Donnell et al. 2016). Vegetation clearance has been used in Europe, North America, and Asia to improve the nesting success of other tern species dependent on open areas for nesting (e.g. Jenniges and Plettner 2008; Fujita et al. 2009; Tinbergen and Heemskerk 2016).

In New Zealand, braided river islands have the potential to act as refugia for black-fronted terns as water can act as a barrier to the movement of some mammalian species for example feral cats (Pickerell 2015). Detection rates of mammals are lower on braided river islands compared to adjacent banks (Pierce 1987; Pickerell 2015), and this can lead to greater nesting success of some bird species such as black-billed gulls or banded dotterels (Charadrius bicinctus) on islands (Rebergen et al. 1998; McClellan 2009; Pickerell 2015). Pickerell (2015) modelled the probability of mammalian predators being present on islands in the Rangitata River and concluded that islands smaller than 3.5 ha, clear of vegetation, more than 20 m from the mainland or nearest predator source, and separated by a channel with a discharge of more than 6 m$^3$s$^{-1}$ would provide the best sites for breeding bird species.

We tested whether clearing islands of vegetation improves the nesting success of black-fronted terns, particularly for lowland rivers which are the most invaded by alien plant species,
but also still harbour significant breeding populations of black-fronted terns (Brummer et al. 2016; O’Donnell and Hoare 2009; O’Donnell et al. 2016; Schlesselmann, Cooper, and Maloney 2017). Using the lower Waitaki River as a case study, we addressed the following questions: i) Which mammalian species are present on vegetated and cleared islands, and adjacent riverbanks?; ii) What is the nesting success of black-fronted terns and primary causes of nest failure? iii) Which species depredate nests?; and iv) Do cleared islands attract black-fronted tern breeding colonies and if so, is nesting success higher on cleared compared to vegetated islands?
Chapter 5: Habitat creation for black-fronted terns

Materials and Methods

Study area and data collection

The study took place on the Waitaki River, South Island, New Zealand (44°46′ S 170°31′ E) in the centre of the black-fronted tern breeding range (Schlesselmann, Cooper, and Maloney 2017). The Waitaki River is one of New Zealand’s major braided rivers as well as one of the country’s main sources of hydroelectric power. The 70 km section of the river, between the last hydroelectric dam and the sea, is called the lower Waitaki and it is braided for all but the first 5 km downstream of the dam (Hicks et al. 2008). The flow of the lower Waitaki River is controlled responding to energy demands, resulting in a reduced flood regime and mostly steadier river flows compared to the spikes in flow caused by spring floods and snow melt in other braided rivers (Tal et al. 2004). The lower Waitaki River is considered to be of national and international importance for braided river birds (O’Donnell and Moore 1983; O’Donnell 2000; Forest & Bird 2016) and it holds approximately 10% of the global black-fronted tern breeding population (O’Donnell and Hoare 2011). Invasive woody weed species, such as gorse (*Ulex europoeus*), Scotch broom (*Cytisus scoparius*), blackberry (*Rubus fruticosus* agg.), sweet briar (*Rosa rubiginosa*) and willow (*Salix* spp.), as well as annuals such as lupins (*Lupinus polyphyllus* and *L. arboreus*), sweet clover (*Melilotus albus*) and Californian poppy (*Eschscholzia californica*) dominate the vegetation on the riverbed. Herbicide spraying of approximately 300-ha sections of the river in the central braidplain is carried out annually by Environment Canterbury (P. Eddy, pers. comm.). The central braidplain of the Waitaki River has nevertheless reduced in width from approximately 2 km prior to the construction of dams in 1935 to approximately 0.5 km (Hicks et al. 2008). No comprehensive mammal pest control is carried out, but possums (*Trichosurus vulpecula*) and ferrets are controlled infrequently (G. May, pers. comm.). This was last undertaken on the northern bank of the lower Waitaki River between March and June 2016.

In this study, monitoring of predators and breeding of black-fronted terns occurred on a 15 km stretch of river from 1 km upstream of the SH 83 Kurow Bridge to the confluence of the Penticotico Stream on the true left of the Waitaki River (Fig. 1). Data were collected throughout the breeding season of black-fronted terns (October–January) in two phases: a pilot study before vegetation clearance occurred (2015/16), followed by a more extensive study post-vegetation clearance on islands (2016/17). Seven islands were cleared of all vegetation in April and May 2016 using a 2006 Komatsu D65 PX bulldozer (blade width 4 m). Initial trials showed that clearing vegetation on existing islands and covering it with gravel material from that island was faster and more cost-efficient than using riverbed gravel material to build up islands. Sites were
therefore chosen based on the size of pre-existing islands (0.3–0.5 ha), accessibility by the bulldozer, and their height above average flow (i.e. available gravel material). Between 0.4 to 2.0 ha (average 0.94 ha) was cleared from each site (approximately 1 km apart). The cleared islands were built up to a height of approximately 0.5 m above the mean watermark to protect islands from flooding. Channels (minimum width 14 m) separated each island from the adjacent riverbank or from other vegetated islands in the river. Channel flows around the islands were not measured and as river flow varied throughout the breeding season, discharge through channels surrounding those islands varied also.

![Figure 5.1 Study area on the lower Waitaki River, South Island, New Zealand. Locations of tracking tunnels and black-fronted tern colonies for the 2015/16 and 2016/17 breeding seasons are shown. Diamond symbols after the location name and breeding season indicate whether it was on a cleared (white) or vegetated (grey) island.](image)

**Mammalian predator monitoring**

We surveyed the presence of mammalian predators at two sites each on the southern and northern banks, with each site being about 10–12 km apart or separated by the main channel of the Waitaki River. The sites were representative of the river margins dominated by introduced woody vegetation and were selected partly based on their accessibility. At each site, a 0.8 km transect consisting of five large tracking tunnels (that allowed entry by predators as large as cats) was established at 200 m intervals parallel to the river (distance to the river between 19 and 280
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m with an average of 124 m) (following Pickerell et al. 2014). Transects were placed in vegetation to maximise the chance of predator detection (Cameron et al. 2005; Recio et al. 2010, 2013). Each tunnel was checked and re-baited with fresh rabbit (*Oryctolagus cuniculus*) meat every 10 days for a total of 70 days per tern breeding season.

We also monitored the presence of mammalian predators on 13 vegetated islands in both seasons and on seven islands after they were cleared in 2016/17. One tracking tunnel was placed on each island. As islands were generally small (range: 0.18–4.75 ha, average: 1.70 ha), it was assumed that apart from mice (*Mus musculus*) any mammal on the island would encounter the device (King 2005). Distance to the nearest riverbank ranged between 32 and 270 m (average of 91 m) for the vegetated islands and between 20 and 47 m (average of 36 m) for cleared islands. Depending on the water flow, islands could be connected to other vegetated islands on the river, but never to the banks of the river. We checked and re-baited each tracking tunnel with fresh rabbit meat every 12–15 days (average of 13.75 days) on vegetated islands in the 2015/16 season, and every 5–10 days (average of 7.25 days) for vegetated and cleared islands in the 2016/17 season. As access to islands was limited by jet boat availability, intervals between monitoring differed slightly between years.

Footprints on tracking cards were identified to species level where possible. Because ferret, stoat and weasel (*M. nivalis*) footprints overlap in size depending on sex and age of animals (Ratz 1997), we classified all of these footprints as mustelids. We calculated mean tracking rates over the entire period that the tracking tunnels were used, and minimum and maximum tracking rates of each interval between checks of tracking tunnels.

*Monitoring of breeding colonies and determining causes of nest failure*

Each season, systematic searches for black-fronted tern colonies were carried out by observing birds either from the riverbanks or from on the river using a jetboat. Once a colony was located, systematic searches for nests were carried out. All nests were marked with a small rock cairn (10–15 cm tall) approximately 1 m upstream of the nest. We searched for additional nests from the second visit onwards; however, only 30 nests were monitored at a time per colony to minimise disturbance and stress to breeding birds. The status of the nests was assessed by regular walk-through checks on islands at the same intervals as tracking tunnel checks. In addition, remote cameras (Ltl Acorn Ltl-5310) were used in the 2016/17 breeding season to determine the identity of the predator in case of nest failure due to predation. Between two and seven cameras were set up simultaneously in a colony (colony-size dependent) with the aim of
at least 20% of the walk-through monitored nests being additionally monitored with a camera. Cameras were spread throughout the colony to increase the chance of capturing predation events if they occurred, but no other criteria were used in choosing nests to monitor with a camera. Cameras were attached to a wooden stand with a small solar panel at the top of the stand (LtAcorn Ltl-Sun Solar Charger) and placed 1.5–2.0 m from the nest at a height of 0.3 m, ensuring that nest contents were clearly visible. All cameras were motion-triggered and either took still photographs or short 10–30 s videos and were set to operate 24 hours per day. Memory cards were changed during each visit and cameras moved if chicks had hatched and left the immediate vicinity of the nest or the nest had failed. Camera footage showed that incubating adults returned to the nest within 5 min of researchers leaving the colony.

Nest outcomes were classified into the following categories: (1) Successful – if one or more eggs hatched and at least one chick was observed in or close to the nest bowl (black-fronted tern chicks are precocial and leave their nest, which is why fledging success is difficult to measure accurately); (2) Failed due to predation – no adults were present as the nest was approached, the nest bowl was either empty or contained damaged eggs, and no sign of chicks was observed; (3) Failed due to flooding – high watermark, flattened vegetation or didymo (Didymosphenia geminata) fragments around the nests and/or discoloured eggs; (4) Failed due to desertion – nest was unattended and eggs were cold; and (5) Unknown – nest outcome was unclear.

Statistical analyses

To assess the influence of vegetation clearance and island creation on nest success rates, we calculated the probability of success of nests in colonies on vegetated and newly cleared islands in 2016/17. We limited our analysis to the incubation and egg-laying periods using three different methods. We first used logistic regression of apparent hatching success (AHS), which treats each nesting attempt as a Bernoulli trial with nest fate being the response variable (1 = success, i.e. at least one egg hatched; 0 = failure). When birds are nesting on islands and in colonies, where nests are highly detectable and mortality events can often be catastrophic, AHS is more accurate as it has no assumption about the rate of mortality of nests (Johnson and Shaffer 1990). However as it is possible to miss early-stage nesting attempts by observational monitoring, AHS can lead to a bias in estimating hatching success (Mayfield 1961, 1975). Therefore, we also used a logistic regression of conditional hatching success (CHS) and a logistic-exposure model (Log-Exp; Shaffer 2004). Both latter methods treat each observation interval as a Bernoulli trial with the response being the number of successful observation intervals at a nest (1 = nest survived, 0 = nest failed) and the number of Bernoulli trials being equal to the number
of observation intervals. In this way, hatching success is adjusted for nests which have been found at a later stage and were exposed to less risk of failing during the observation period compared to early-stage nests. If all nests are found at the same stage early in the incubation period and it is possible to also find inactive nests, AHS will be as accurate as CHS and Log-Exp.

The conditional hatching success method is ‘conditional’ on when the monitoring commenced and only compares the risk of failure that was observed, whereas Log-Exp also takes into account that the probability of surviving an interval is dependent on the interval length (Shaffer 2004). The logistic-exposure model is equivalent to other logistic regression Mayfield estimators (e.g. Aebischer 1999; Dinsmore et al. 2002), but has the advantage that it does not assume the daily survival rate is constant. If a nest succeeded or failed between nest visits, and we could not determine the exact date through camera footage, we assumed that the nest was active for half of that interval (mid-way assumption; Mayfield 1961). As visitation rates varied in this study between 5 and 10 days, this assumption can lead to some bias and the method works best with short visitation rates (Shaffer 2004).

Variation in hatching success using the different methods was explored with generalised linear mixed models (GLMMs) with a binomial error term and a logit link (for the AHS and CHS approaches) or logistic-exposure link containing an exponent 1/t with t being the number of observation days for each interval (for the Log-Exp approach) and with different combinations of Colony Size (to accommodate potential survival benefits to nests of larger colonies as a result of diluted predation risk and communal antipredator defence), Timing (to accommodate seasonal variation in nest success), and Vegetation (to assess the effect of clearing vegetation on nesting success) as fixed terms, and Colony (to account for the non-independence of the fate of nests within colonies) as a random term (Table 5.1). We decided against including Mammalian presence (to account for increased risk of failure of nests in colonies where mammals are detected) as a fixed term, as we only detected mustelids (known predators of eggs, chicks, and adult black-fronted terns) on one vegetated island with a black-fronted tern colony present twice during the breeding season (see Results), which also would be captured by the random effect.

All continuous input variables were centred and standardised following Schielzeth (2010) to compare effect sizes of variables directly. We tested for collinearity of model terms using the global model (VIF = 1.05–1.38; Freckleton 2011). We ranked all models for each analysis using Akaike’s Information Criterion corrected for small sample size (AICc; Burnham and Anderson
As our focus was on the particular effect of vegetation on islands, we included vegetation in all our candidate models and did not average across multiple models (Cade 2015; Banner and Higgs 2016). All analyses were carried out in R v. 3.3.3 (R Core Team 2017).

Table 5.1 Response and explanatory variables, and model structures used in analyses of nest success probabilities of black-fronted terns on the lower Waitaki River 2016/17. The number of nests or monitoring intervals on vegetated and cleared islands in each analysis is in parentheses.

<table>
<thead>
<tr>
<th>a) Apparent Hatch Success (AHS):</th>
<th>Model response: Successful nests (Vegetated n = 108; Cleared n = 77)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution (link): Binomial (logit)</td>
<td>Type</td>
</tr>
<tr>
<td>Response</td>
<td>Hatching success</td>
</tr>
<tr>
<td>Explanatory (Fixed)</td>
<td>Vegetation</td>
</tr>
<tr>
<td></td>
<td>Timing</td>
</tr>
<tr>
<td></td>
<td>Colony size</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Explanatory (Random)</td>
<td>Colony</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>b) Conditional Hatch Success (CHS):</th>
<th>Model response: Successful nest intervals (Vegetated n = 347; Cleared n = 216)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution (link): Binomial (logit)</td>
<td>Type</td>
</tr>
<tr>
<td>Response</td>
<td>Hatching success</td>
</tr>
<tr>
<td>Explanatory (Fixed)</td>
<td>Vegetation</td>
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<td></td>
<td>Timing</td>
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<td>Colony size</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Explanatory (Random)</td>
<td>Colony</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>c) Logistic-Exposure (Log-Exp):</th>
<th>Model response: Successful nest intervals (Vegetated n = 240; Cleared = 136)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution (link): Binomial (Logistic-exposure)</td>
<td>Type</td>
</tr>
<tr>
<td>Response</td>
<td>Hatching success</td>
</tr>
<tr>
<td>Explanatory (Fixed)</td>
<td>Vegetation</td>
</tr>
<tr>
<td></td>
<td>Timing</td>
</tr>
<tr>
<td></td>
<td>Colony size</td>
</tr>
<tr>
<td>Explanatory (Random)</td>
<td>Colony</td>
</tr>
</tbody>
</table>
RESULTS

Mammalian predators

Cats, possums, hedgehogs, mustelids, and mice were detected on the banks of the lower Waitaki River in both breeding seasons, but no rats (*Rattus* spp.) were ever detected (Fig. 5.2 a, b). Possums, mustelids and mice were detected on vegetated islands, but no cats, hedgehogs or rats (Fig. 5.2 a, b). In both years, we detected mustelids at some point during the breeding season on about half of the vegetated islands (2015/16: 53.8%; 2016/17: 46.2%). On cleared islands, the only species detected were mice on one island (Fig. 5.2 b).

![Figure 5.2](image1.png)

**Figure 5.2** Mean tracking rates (footprints per tunnel, circle) and minimum and maximum tracking rates (lines) per time period (10 days) of mammalian predators during the black-fronted tern breeding season of a) 2015/2016 and b) 2016/2017 on the lower Waitaki River. Banks refer to 20 tunnels in four transects operated for 70 days on the southern and northern banks of the lower Waitaki River. Vegetated islands refer to tracking tunnels operated on 13 vegetated islands for 60 days and 64 days in 2015/16 and 2016/17, respectively. Cleared islands refers to tracking tunnels operated on seven cleared islands for 59 days.
Nesting success and causes of failure

In 2015/16, we monitored 78 nests in four colonies on four vegetated islands. In 2016/17, we monitored 108 nests in three colonies on three vegetated islands, and 77 nests in three colonies on three cleared islands (two colonies established at the Kurow Creek site independently, Fig. 5.1). In both years, about half of the nests hatched at least one chick (Table 5.2, Table C.1). The main reason for nest failure in both seasons was predation, followed by flooding and desertion (Table 5.2). In 2016/17, most nest successes occurred in October and November, while later in the breeding season (December) almost all nests failed primarily due to predation (Fig. 5.3 a).

Table 5.2 Black-fronted tern egg survival and causes for failed hatching on the lower Waitaki River in 2015/16 and 2016/17. Successful nests refer to nests that had at least one chick hatch. The percentage of total nests monitored is presented in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>2015/16</th>
<th>2016/17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nests monitored</td>
<td>78</td>
<td>188</td>
</tr>
<tr>
<td>Eggs laid</td>
<td>148</td>
<td>353</td>
</tr>
<tr>
<td>Successful nests</td>
<td>44 (56.4%)</td>
<td>95 (50.5%)</td>
</tr>
<tr>
<td>Nests failed (Total)</td>
<td>31 (39.7%)</td>
<td>90 (47.9%)</td>
</tr>
<tr>
<td>Nests preyed upon</td>
<td>17 (21.8%)</td>
<td>68 (36.1%)</td>
</tr>
<tr>
<td>Nests deserted</td>
<td>2 (2.6%)</td>
<td>12 (6.4%)</td>
</tr>
<tr>
<td>Nests flooded</td>
<td>12 (15.4%)</td>
<td>4 (2.1%)</td>
</tr>
<tr>
<td>Infertile or failed hatch</td>
<td>0</td>
<td>6 (3.2%)</td>
</tr>
<tr>
<td>Unknown outcome</td>
<td>3 (3.8%)</td>
<td>3 (1.6%)</td>
</tr>
<tr>
<td>Total number of nests estimated</td>
<td>112</td>
<td>302</td>
</tr>
</tbody>
</table>

Identity of nest predators

A total of 56 nests (30.3% of total nests and 37.2% of nests per colony) was monitored with remote cameras in addition to the walk-through nest checks in 2016/17 (Table 5.3). Eighteen of these nests were successful and 32 nests were depredated, primarily by an avian predator (n = 20), the southern black-backed gull (Table 5.3, Fig. 5.3 b, c and 5.4). Predation by southern black-backed gulls occurred throughout daylight hours and although black-fronted terns were often observed mobbing the intruder, they were unable to deter the much larger species. The ten nests for which the predator identity could not be confirmed at videoed nests were probably also preyed upon by southern black-backed gulls as all failures occurred in December in colonies where all other nests failed due to southern black-backed gull predation. Only one predation event by a stoat was observed in late December, where the stoat killed the incubating adult at
Chapter 5: Habitat creation for black-fronted terns

Figure 5.3 Timeline of outcomes of black-fronted tern nests monitored on the lower Waitaki River in 2016/17 on a) both island types monitored by cameras and walkthrough checks (n = 185), b) cleared islands monitored with cameras (n = 24), and c) vegetated islands monitored with cameras (n = 32). Successful nests are nests that hatched at least one chick. Other reasons for failure (see Table 5.2) include desertion, flooding, infertility, and in case of b) and c) predation by an unidentified predator or stoat (see Table 5.3).
night (1:45 a.m. New Zealand Standard Time), but did not prey upon the eggs. The nest was deserted by the partner the next morning. Again, of the camera-monitored nests on cleared islands (n = 24) and on vegetated islands (n = 32), most successes were observed early in the season and most failures, and particularly predation events, late in the season (Fig. 5.3 b, c).

Table 5.3 Outcome of black-fronted tern nests monitored with remote cameras on the lower Waitaki River in 2016/17. Successful nests refer to nests that had at least one chick hatch. The percentage of total nests monitored with cameras is presented in parentheses.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number of nests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nests monitored with cameras</td>
<td>56</td>
</tr>
<tr>
<td>Successful nests</td>
<td>18 (32.1%)</td>
</tr>
<tr>
<td>Nests failed (Total)</td>
<td>38 (67.9%)</td>
</tr>
<tr>
<td>Nests preyed upon by:</td>
<td></td>
</tr>
<tr>
<td>southern black-backed gull (Larus dominicanus)</td>
<td>20 (35.7%)</td>
</tr>
<tr>
<td>black-billed gull (Larus bulleri)</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>stoat (Mustela erminea)</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>unknown species</td>
<td>10 (17.9%)</td>
</tr>
<tr>
<td>Flooded</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>Infertile</td>
<td>3 (5.4%)</td>
</tr>
<tr>
<td>Deserted (cause unknown)</td>
<td>2 (3.6%)</td>
</tr>
</tbody>
</table>

Figure 5.4 Southern black-backed gull captured on remote camera preying upon a black-fronted tern nest containing two eggs on the lower Waitaki River in 2016/17.
Comparison of nesting success between cleared and vegetated islands

Black-fronted terns used cleared islands for breeding, roosting and feeding in the season immediately following island creation. Three breeding colonies established on two cleared islands and none of these islands were used while they were vegetated in the previous season (Fig. 5.1).

All models of nesting success were reasonably congruent (Fig. 5.5). Nesting success probability did not differ between vegetated and cleared islands as confidence intervals for Vegetation included zero in all models (Fig. 5.5). However, a slightly negative trend of vegetation on hatching success was observed using the AHS model compared to a slight positive trend in the other models. Black-fronted tern nest success probability decreased from ~90% to 60% within the breeding season until the end of December (Fig. 5.5, 5.6 a). In the CHS and Log-Exp model, the probability of a black-fronted tern nests being successful increased by ~30% with an increased colony size, up to 200 individuals (Fig. 5.6 b), but not in the AHS model as the confidence interval of the coefficient estimate included zero (Fig. 5.5).

Figure 5.5 Coefficient estimates with 95% confidence intervals for binomial models of apparent hatching success (AHS), conditional hatching success (CHS) and logistic-exposure (Log-Exp) scaled hatching success of black-fronted tern nests on the lower Waitaki River in 2016/17.
For all methods of estimating nesting success, models including all covariates had the lowest AICc values and the highest AICc weight (Table C.2). The random effect Colony identity explained zero variance (likely due to the other factors capturing the majority of differences between colonies) in the case of AHS, but explained 0.097 and 0.172 variance (logit) in the CHS and Log-Exp model, respectively (Table C.2).

Figure 5.6 Average marginal predicted probability of black-fronted tern nesting success using estimates of the CHS model on the lower Waitaki River (i.e. nest having at least one egg hatch) on vegetated islands (solid blue line with shaded 95% confidence interval) and on cleared islands (dashed red line with shaded 95% confidence interval) in relation to a) time of the season the nest was active and b) colony size.
**DISCUSSION**

*Mammalian predator presence*

We detected seven mammalian predator species on the lower Waitaki River apart from rats. Most importantly, in both seasons we detected mustelids on over half of the vegetated islands, but none on cleared islands. The nesting success of black-fronted terns was low in both years with predation being the primary cause of failure. Surprisingly, video cameras showed that the primary predators of black-fronted tern nests were southern black-backed gulls rather than mammals. Black-fronted terns did establish three breeding colonies on cleared islands immediately after their creation, but nesting success did not differ between vegetated and cleared islands.

Consistent with previous studies of mammalian predators in braided rivers, we detected fewer mammalian species and far fewer occurrences on islands compared to riverbanks, and even fewer mammalian predators on cleared islands compared to vegetated ones (Pierce 1987; Pickerell 2015). This was despite cleared islands being closer on average to the riverbanks where more mammals occurred (Fig. 5.2). On vegetated islands, mammalian predators can either be visitors or residents depending on the size of the island and availability of food sources year-round (Pickerell 2015). In our study, mustelids were often detected only once on a vegetated island, potentially indicating visiting individuals, particularly given the very good swimming abilities of stoats (King et al. 2014). Use of vegetated islands by mustelids poses a threat to black-fronted tern colonies on vegetated islands. Although these results are limited to the lower Waitaki River, and to one breeding season after clearing islands, they indicate that clearing islands is potentially useful for indirectly managing mammals in larger, lowland river systems, but further research using video cameras as well as tracking tunnels would be required to increase our understanding of detecting cryptic species in open environments (Smith and Weston 2017).

*Nesting success and causes nest failure*

This is the first study where the nesting success of black-fronted terns and causes of nest failure were investigated in a lowland river and nesting success of colonies on cleared and vegetated islands was compared. Overall, we found hatching success to be similarly low as in the upper river systems and nest failure mostly caused by predation (Keedwell 2005; Cruz et al. 2013; Bell 2017). In upland rivers, mammals have been reported as the primary nest predator (Keedwell 2005; Cruz et al. 2013; Bell 2017). On the Wairau River, also a lowland river, Australasian harriers
caused more than half of the 19 videoed nest failures (Steffens et al. 2012). To our surprise, although mammalian predators were present in the environment, southern black-backed gulls were the major predator of nests in the lower Waitaki River rather than mammals. Native avian predators are able to prey upon eggs and chicks of braided river birds (Sanders and Maloney 2002). In other parts of the world, specifically the impact of large gulls (Larus spp.) on tern productivity has been documented (e.g. Becker 1995; Devault et al. 2005; Donehower et al. 2007).

The decline in nesting success later in the season is most likely explained by the sudden rise in predation by southern black-backed gulls in December. A similar pattern (although involving a different predator species) has been recorded in the upper Clarence catchment and for other tern species (Arnold, Hatch, and Nisbet 2004; Bell 2017). For most bird species, it is thought that early breeding results in higher reproductive success due to potentially more or higher quality resources (e.g. more available breeding habitat, lower predation pressure, more feeding territories) being available early in the season, and birds in higher body condition and with more breeding experience nesting earlier (Perrins 1996; Price, Kirkpatrick and Arnold 1988). We also found that black-fronted tern nests in larger colonies on the lower Waitaki River had a higher probability of being successful. This colony size effect has also been reported for black-fronted terns nesting on the upper Clarence River, but not from the Mackenzie Basin (Keedwell 2005; Bell 2017). Larger breeding colonies can provide protection against aerial predators in other tern species elsewhere in the world (Hernández-Matías et al. 2003). A size or density effect has also been reported for two other New Zealand braided river specialists. Breeding success of black-billed gulls increased in larger colonies and banded dotterel nests at higher density have higher nest survival (Norbury and Heyward 2008; McClellan 2009). This might also be the case for black-fronted terns, but we currently do not know if the increase in predation by southern black-backed gulls late in the breeding season is related to the tern breeding cycle (e.g. targeting chicks, or colonies of smaller size, less experienced breeders later in the season), the gull breeding cycle (e.g. energy demands of chicks), and if it is the specialisation by one or a few gulls or the whole colony (Yorio and Quintana 1995; Guillemette and Brousseau 2001; Donehower et al. 2007). Predation occurred on both island types equally. In comparison to mammalian predators, southern black-backed gulls are not dependent on vegetation for cover and it is therefore not surprising that vegetation clearance had no effect.

Each of the different nest success estimators have some bias. While AHS enables comparison to other studies (Keedwell 2005; Bell 2017), it provides an overly simplistic model of hatching success. The other two estimators are based on an unbalanced sample of nest observations from
vegetated and cleared islands. For example, while AHS indicated a slight trend for vegetation clearance being beneficial for hatching success, the opposite was observed for the other two estimates. This is most likely an artefact due to only limited data being available from cleared islands as the first chicks were observed hatching in the large colony on a cleared island within a week of monitoring. Our study would have benefitted from more monitoring visits, particularly earlier in the season and recording hatching success of all nests of colonies would have provided a more balanced sample of both nesting habitats. Our access to colonies depended on the availability of a jetboat and we aimed to minimise disturbance within a breeding colony limiting our monitoring to a sample to 30 nests at a time. Advances in technology, particularly video cameras with increased memory capacity, will aid in overcoming these challenges and trade-offs between collecting data and disturbance involved in monitoring black-fronted terns, particularly on large, lowland rivers. The actual estimate of productivity (number of fledglings per nest) for the lower Waitaki River will be substantially lower than the hatching success as a high degree of mortality occurs pre- and post-fledging (Keedwell 2003, 2005; Cruz et al. 2013). Nevertheless, we add critical information on the hatching success of colonies in a lowland river where a large proportion of black-fronted terns breed. Different pressures are at play in lowland compared to upland rivers such as the degree of weed invasion and different impacts of predator species (Williams and Wiser 2004; O’Donnell and Hoare 2011; Steffens et al. 2012). Future studies should aim to estimate productivity, so that this information is also available from a lowland river environment.

Conclusion

In summary, this study provides further evidence that the conservation concern over this species is warranted (O’Donnell et al. 2016; Robertson et al. 2017; Wright 2017). It documents the large impact of a native avian predator on black-fronted tern nesting success on the lower Waitaki River and highlights the different impacts of predators affecting black-fronted tern colonies in lowland versus high-country rivers. Cleared islands are readily used by black-fronted terns for roosting, feeding and breeding. We present some evidence that cleared islands can provide habitat that is safe from flooding and have lower mammalian predation pressure. Artificially creating habitat to mimic natural habitat can play a critical role in population recovery of terns (Schippers et al. 2009; Pakanen et al. 2014). We recommend that the possible effects of avian predators and potential specialisation be considered (Sanz-Aguilar et al. 2009) and monitoring of black-fronted terns on lowland rivers be continued, extending it to include different life-stages, on cleared and vegetated islands.
CHAPTER 6

Synthesis and implications
My research considered how to incorporate ecological and evolutionary processes into conservation planning, focusing on an endangered, braided river specialist — the black-fronted tern/tarapirohe (*Chlidonias albostriatus*; Chapter 1). Specifically, my goals were to 1) determine the degree of genetic connectivity between breeding populations and variation within and among populations; 2) assess past and present population demographic trends and extinction risk of the population; and 3) evaluate the effectiveness of a possible management action. This work provides the knowledge basis for effective long-term conservation.

To achieve my goals, I first developed molecular markers specifically for black-fronted terns to determine genetic diversity and connectivity between breeding colonies (Chapters 2 and 3). Using phenotypic and genotypic data from throughout the whole breeding range, I showed that black-fronted terns have 1) high levels of genetic diversity; 2) low genetic differentiation between breeding colonies; 3) no genetic signature of isolation-by-distance; however, 4) a phenotypic signature of isolation-by-distance consisting of increasing body size with increasing latitude as predicted by Bergmann’s rule (Chapter 3). This suggested that while there is very little genetic differentiation, there is substantial morphometric differentiation between populations. Hence, black-fronted tern conservation managers should regard the whole South Island of New Zealand, as a single conservation unit consisting of one metapopulation of breeding colonies. Furthermore, I provided evidence for population expansion during the last glaciation indicating that black-fronted terns were not restricted to refugia (Chapter 4). Demographic analyses also revealed evidence for a recent human-induced decline as suggested by the low number of effective breeders and highlighting the effect of poor recruitment on the population. I then evaluated the effectiveness of creating clear (i.e. free of vegetation) islands as ‘safe’ nesting habitat, particularly in lowland rivers, with a view to increase nesting success of black-fronted terns (Chapter 5). Surprisingly, although exotic mammalian predators were effectively excluded from created islands, native southern black-backed gulls/karoro (*Larus dominicanus*) were the primary predators of black-fronted tern nests. Consequently, nesting success was low independent of vegetation cover on islands. My work highlighted that removing one guild of predators does not necessarily mean that a threatened species is protected, as predator-prey relationships are often complex. Therefore, it is critical to confirm the impact of current threats for species of conservation interest throughout their range. Monitoring and adjustment of management strategies is vital to ascertain that conservation interventions are achieving the desired outcomes.
ACHIEVING SUCCESSFUL LONG-TERM CONSERVATION

Genetic data form a basis for informing many conservation issues and decisions (Fig. 6.1; top left and right; Allendorf, Luikart, and Aitken 2013). Implementing this information into management actions is a key step and requires bridging from science to conservation management (Fig. 6.1; centre; Frankham et al. 2017). Well-structured monitoring programmes are necessary to evaluate effectiveness and the need for adjustment of management strategies (Pullin and Knight 2003). Including genetic management in conservation strategies helps facilitate successful long-term conservation in the face of ongoing global change (Fig. 6.1; bottom; Sgrò, Lowe, and Hoffmann 2011; Smith et al. 2014; Cook and Sgrò 2016; Ralls et al. 2018).

Figure 6.1 A schematic diagram of how conservation management actions can be informed by utilising genetic data to 1) determine where and at what scale management should happen (top left: delineating conservation units); and 2) assess how a species responded to change in the past and evaluate the current status of the species (top right: population size and trends). This information can then feed into management actions (centre), where well-structured monitoring programs allow conservation biologists to ascertain the need for adjustment to achieve the goal of maintaining gene flow and thus genetic diversity within a species (bottom).
Genetic data as a base to inform conservation issues and decisions

Highly mobile and migratory species with widespread breeding distributions, such as waders or shorebirds, often exist in metapopulations (Hanski 1999). The way these metapopulations are linked depends on past and contemporary processes. Understanding the connectivity between populations is of direct importance to conservation management (Esler 2000; Drechsler et al. 2003). The genetic signature in populations can inform conservation biologists about the historical processes shaping contemporary patterns (Allendorf, Luikart, and Aitken 2013). At the same time, molecular approaches provide a way to infer contemporary genetic population connectivity and structure for such highly mobile species on a range-wide scale (Frankham 2010; but also see Lowe and Allendorf (2010) for limitations).

Black-fronted terns are a prime example of a highly mobile species about which little is known. Black-fronted terns were most likely not restricted to refugia during the Ōtiran glaciation (117,000–14,000 years ago; Williams et al. 2015), but experienced a population expansion most likely due to an abundance of favourable tundra-like habitat (Chapter 4). Population expansions are also thought to have occurred in Arctic breeding species in the Northern Hemisphere associated with more extended tundra habitat during glacial periods (Kraaijeveld and Nieboer 2000; Rönkä et al. 2012). Interestingly, population expansion has been inferred to have occurred only after the last glacial maximum for the temperate, inland breeding Eurasian black tern (C. niger niger) (Szczys et al. 2017), but not for the Eurasian whiskered tern (C. hybrida hybrida) despite their similar breeding habitat requirements (Dayton et al. 2017). In the Southern Hemisphere, genetic analysis has provided evidence for both glacial and post-glacial expansion in the sooty tern (Sterna fuscata), which breeds in temperate and tropical regions: populations of the Atlantic and Indo-pacific oceans expanded much earlier than southwest Pacific populations (Peck and Congdon 2004).

To the best of my knowledge, neither the demographic history of other Southern Hemisphere tern species, nor of other braided river specialists have yet been investigated. Many forest birds (e.g. kōkako (Callaeas cinereai; Murphy, Flux, and Double 2006); wood pigeon/kererū (Hemiphaga novaseelandiae; Goldberg, Trewick, and Powlesland 2011); kākā (Nestor meridionalis; Dussex et al. 2015)) endemic to New Zealand were restricted to one or multiple glacial refugia where forest persisted. Based on my study of black-fronted terns, it seems plausible that other endemic braided river birds (e.g. wrybill/ngutu parore (Anarhynchus frontalis) or black-billed gull/tarāpuka (Larus bulleri)) also experienced population expansions during the Ōtiran glaciation and were likely not restricted to refugia. Nevertheless, looking at
the Northern Hemisphere, taxa responded to glacial cycles differently despite apparent habitat requirements being similar. This therefore represents an interesting gap in knowledge for New Zealand and the Southern Hemisphere as a whole. New Zealand provides excellent opportunities for phylogeographic studies as both island-like and continental processes have been observed (Wallis and Trewick 2009; Craw et al. 2016). The methods described in my thesis could be applied to a range of other braided river species, yielding a fuller picture of the range of species’ responses to historical climatic changes. This would potentially improve our ability to forecast species’ responses to future climate change (e.g. Younger et al. 2016) and reveal more about habitat niche requirements.

As with many other highly mobile species living in challenging environments, regular monitoring data and demographic estimates for black-fronted terns are scarce (O’Donnell and Hoare 2011; Paleczny et al. 2015). I estimated the contemporary global census and effective population size of black-fronted terns confirming a sharp human-induced decline in population size (Chapter 4). New Zealand’s avifauna in general and particularly other Charadriiformes are threatened by recently introduced species (Holdaway 1999; Dowding and Murphy 2001; Cruz et al. 2013). Given that black-fronted tern populations expanded rather than contracted during the Ōtiran glaciation, it is not surprising that contemporary breeding populations show little genetic differentiation (Chapter 3). The species therefore should be managed in a single conservation unit. As black-fronted terns regularly colonise new or once existing breeding sites in braided rivers while other sites are abandoned (Keedwell 2005; Chapter 5), conservation management should aim to protect this natural aspect of the metapopulation and aim to protect whole catchments to halt the population decline (Schippers et al. 2009). The cline in body size together with a cline of private haplotypes and alleles should be considered in designing conservation strategies. Black-fronted terns are likely to exhibit some degree of philopatry. Both dispersal and philopatry have important consequences for population dynamics at local and regional scales (Palestis 2014).

Metapopulations of other tern and colonial nesting bird species are affected by Allee effects (i.e. positive density dependence; e.g. Serrano, Oro, and Ursu 2005). In fact, black-fronted tern nesting success increased with increasing size of the colony (Chapter 5). Simulations have shown that Allee effects in colonial nesting seabirds cause increased isolation between breeding colonies as dispersal distances are much shorter than without Allee effects (Schippers et al. 2011). These Allee effects have two consequences: 1) recolonisation of areas can be very slow and, therefore, 2) metapopulation recovery is also slow (Schippers et al. 2011). Recent research into metapopulation dynamics of roseate terns (Sterna dougallii) in the Northwest Atlantic
Ocean suggested that regional persistence of colonies is maintained by the larger populations (García-Quismondo et al. 2018). Declines in common tern (Sterna hirundo) populations in the German Wadden Sea has also seen the splitting of colonies and the abandonment of formerly successful breeding sites (Szostek and Becker 2012). Declines in common tern populations are also observed across eastern North America (Morris, Pekarik, and Moore 2012; Wilson et al. 2014; Palestis and Hines 2015). A genetic study of the North American metapopulation of common terns showed that asymmetrical dispersal rates from inland to the coast (but not vice-versa) have increased, substantially contributing to the greater decline of inland populations (Szczys, Oswald, and Arnold 2018). Allee effects could also affect the black-fronted tern metapopulation and a ‘tipping point’ could be reached where the rate of population decline accelerates once larger colonies have decreased in size and the breeding range is more fragmented. While my research on black-fronted terns has shed some light on the genetic connectivity between breeding colonies, there are still open questions about the demographic connectivity between breeding and wintering sites along the coast of all main islands, as well as threats encountered by black-fronted terns in those wintering areas (Fig. 6.2). Conservation action should take the dynamic nature of the species distribution in space and time into account, while at the same time aiming to focus research efforts on understanding the migratory connectivity (Runge et al. 2014; Bracey et al. 2018).

Figure 6.2 The annual cycle of black-fronted terns and the current state of knowledge. Spring and summer months when black-fronted terns are breeding in the braided rivers of the South Island shown in light green, and remaining months where little is known about key aspects of their ecology in blue colour. Key future research questions associated with stages of their annual cycle highlighted,
Conserving mobile species represents a huge challenge (Martin et al. 2007; Runge et al. 2015). My research on black-fronted terns together with studies of other tern species stress the importance of addressing the whole species as a network of individual populations when planning conservation actions for mobile species. Given the likelihood of Allee effects, achieving the recovery of the species is much easier when the loss of individual populations in the network is avoided (Schippers et al. 2011). The estimates of effective population size and number of effective breeders for black-fronted terns (Chapter 4) put forward a strong argument that widespread conservation action needs to be instigated with urgency as long-term recovery is still achievable (Frankham, Bradshaw, and Brook 2014). The discrepancy between different methods of estimating effective population size led to the conclusion that, in relatively long-lived, iteroparous species with poor recruitment and declining population size, the effective population size can mask the actual extent of the decline. The effective number of breeders is therefore not only the more precise, but also more accurate measure for the rate of loss of genetic diversity in the population. In addition, the effective population size estimates also emphasise that recruitment failure of black-fronted terns is of principal concern.

Often the impacts of threats are not affecting individual populations homogenously, but differ among populations. In the case of black-fronted terns, mammalian predators had been identified as the main reason for nest failure and low nesting success primarily based on research in an upland region, the Mackenzie Basin (Sanders and Maloney 2002; Keedwell 2005; Cruz et al. 2013). Through my research, it has become evident that identifying the predator guild can be more complex than anticipated and controlling one part of the predator guild does not necessarily increase nesting success (Chapter 5). Without clearly stating the goal of the management intervention, and monitoring outcomes, adjustment of the management action would not be possible (Gibbons, Wilson, and Green 2011). The impact of large gulls (Larus spp.) on tern productivity has been documented in other parts of the world (Becker 1995; Yorio and Quintana 1995; Whittam and Leonard 1999; Guillemette and Brousseau 2001; Hernández-Matías, Jover, and Ruiz 2003; Devault et al. 2005; Donehower et al. 2007). In the Northern Hemisphere, lethal control of large gull populations and targeted control of specialised individuals has been undertaken to protect endangered shorebirds (Sanz-Aguilar et al. 2009; Scopel and Diamond 2017). Lowland rivers in New Zealand harbour significant populations of black-fronted terns and other braided river specialists (O’Donnell and Hoare 2011; O’Donnell et al. 2016; Schlesselmann, Cooper, and Maloney 2017). To protect these populations and conserve metapopulations, the differing impacts of multiple threats need to be considered.
Chapter 6: Synthesis and implications

The importance of genetic management in conservation strategies for metapopulations

Conservation strategies should be designed to maximise future genetic diversity and adaptive potential of species to achieve successful long-term conservation (Mace and Purvis 2008; Sgrò, Lowe, and Hoffmann 2011; Eizaguirre and Baltazar-Soares 2014). This requires information on genetic diversity and the active management of it (Smith et al. 2014; Ralls et al. 2018). Although in principle the importance of evolutionary principles and processes for long-term successful conservation outcomes has been recognised, there is still a lack of integration of these into management practices and policies (Jamieson et al. 2008; Pierson et al. 2016; Cook and Sgrò 2016). One issue that has been identified in New Zealand regarding this lack of uptake is a communication barrier and the need for better engagement between scientists, conservation managers, and policy planners (Taylor, Dussex, and van Heezik 2017). However, steps forward are being made. In the United States, an ambiguous court ruling meant that streams and wetlands were only protected if a ‘significant nexus’ existed within the whole watershed (Alexander 2015). This led to the extensive collaboration between scientists and policymakers defining a ‘significant nexus’ by considering the ecological and evolutionary processes of plant and animal species dependent on such habitat and thereby achieving the protection of watershed connectivity in the long term (Alexander 2015; Hauer et al. 2016; Ridley and Alexander 2016).

The braided river ecosystem in New Zealand is under increasing threat (Ministry for the Environment and Stats NZ 2017; Wright 2017). Yet, little of the ecosystem is actively managed and the loss of diversity within it seems imminent (O’Donnell et al. 2016). Building on this research of black-fronted terns, ecological and evolutionary processes of other braided river specialists could be studied and successfully protected by focusing management on the appropriate scale and addressing the actual impacts. The cooperative engagement of scientists, managers, and policymakers could likewise achieve the connectivity of populations, species, and communities in New Zealand’s braided rivers.
IMPLICATIONS FOR CONSERVATION MANAGEMENT OF BLACK-FRONTED TERNs

Based on the results of my thesis, my specific recommendations for the conservation management of black-fronted terns include:

1) To maintain genetic diversity and genetic connectivity between black-fronted tern populations, conservation management should consider the whole South Island as one conservation unit and target conservation actions on a catchment-scale throughout the South Island.

2) Recruitment failure is of primary concern leading to a small effective number of breeders ($N_b \sim 700$ adults) and therefore a loss of genetic diversity at an increased rate. Management actions should seek to improve breeding success, including hatching and fledging success as well as post-fledging survival (Keedwell 2003; Keedwell 2005; Cruz et al. 2013). To ensure long-term survival, I suggest a minimum target for the effective number of breeders be set to $N_b \sim 1,316$ adults, which equates to an effective population size of $N_e \sim 1,000$ adults (Frankham, Bradshaw, and Brook 2014) based on the conversion of Waples, Antao, and Luikart (2014).

3) Impacts on nesting success of black-fronted terns differ between areas and outcome monitoring of management interventions is necessary to establish whether goals set are achieved. Further testing of combinations of habitat protection by reducing water abstraction, habitat creation and predator control is necessary.

4) Monitoring demographic and spatial trends is crucial to assess the status of black-fronted terns. This includes braided river bird surveys (O’Donnell and Hoare 2011) or species-specific range surveys (Schlesselmann, Cooper, and Maloney 2017). These surveys should be paired with regular genetic monitoring (e.g. every 5 years) to assess the effective number of breeders (Schwartz, Luikart, and Waples 2007; Kamath et al. 2015). Sampling chicks in consecutive seasons throughout the whole breeding range and estimating the effective number of breeders will provide the most precise estimate of extinction risk in the short- and long-term. Together, braided river bird counts, range surveys, and genetic monitoring will enable ongoing assessment of progress towards conservation goals.

5) The migratory connectivity between breeding and wintering sites of black-fronted terns is little understood. Further research on black-fronted terns should focus on whether breeding and wintering sites of colonies overlap and whether there are any impacts on
black-fronted terns in wintering sites to ensure that protection of the species throughout its whole range and life cycle can be achieved (Runge et al. 2015).
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This thesis would have not been possible without the encouragement, guidance, support and friendship of many people. I would like to thank my supervisors and advisors Bruce Robertson, Phil Seddon, Colin O’Donnell (Department of Conservation), and Jo Monks (Department of Conservation) for their time, advice and support. I wish to particularly thank Bruce Robertson, my primary supervisor for being an inspiration to me. Bruce supported me constantly through major challenges, always had an open door for any questions or discussions of ideas (and was very patient with someone without prior genetics experience), and gave me the freedom and trust to work in different conservation programmes throughout this PhD. Jo Monks has been an amazing mentor to me and I would like to thank her for her encouragement, support, advice, and guidance in the last five years, particularly through some rough times. It would be great to see more women like her in science! Discussions with Colin O’Donnell have always been thought-provoking, and his knowledge and expertise in braided river ecology (as well as so much else of New Zealand’s ecology) has given me something to aspire to. Thank you to Colin for reminding me that a PhD is not the end, but rather the start of something.

This research has been a team effort, from collecting blood samples to exporting high resolution graphs or providing a place to stay. I want to thank all the people that helped with locating breeding colonies and collected blood samples for me in the Mackenzie Basin and Marlborough. Thank you to the Webb family for supporting me throughout the fieldwork and giving me a base. I would like to specifically thank Fiona Robertson for making the introduction into the lab so easy and helping with DNA extractions. I would like to also thank Tania King for her enthusiasm and help with anything PCR or analysis related. I have learnt so much troubleshooting with you, thank you. Thank you to Martyn Kennedy for having an open door for questions, discussions, and your good explanations. Nicolas Dussex has given me advice throughout this PhD and I am very grateful that you always took the time and were happy to answer my questions.

Working in the lower Waitaki River has been a great experience and I would like to thank all the people that helped with the work — from the planning to the execution and monitoring. Mike Bell gave pointers on having cameras not fog up or trigger too often. Brett Dann (Jetboating NZ) volunteered his time and jetboat in the first season, thank you. Andy Philpot did the excellent bulldozer work and kept me up to date. It was a real pleasure to work with you. Brad Edwards and Tom Waterhouse from the Department of Conservation helped with keeping tabs on tern nests and predator prints.
I wish to particularly thank Jamie Cooper for sharing the 14,000 km road trip in the name of terns and coming back to work with me in the Waitaki River. Thank you for your kindness and friendship. I certainly will never forget to expect kārearea hitting my head at high speed at any moment, to embrace the feeling (or rather the lack of) in my feet from crossing icy cold river channels, to appreciate the slight differences between German and Kiwi communication, particularly in rising water levels, and to watch out for your loaf of bread (maybe). The laughter in the face of catastrophe kept me sane.

Thank you to the people in the Zoology Department that made logistics and everything else that comes with running a big project easier: Kim Garrett, Ronda Keen, Vivienne McNaughton, Ken Miller, Wendy Shanks, Esther Sibbald, and Jo Ward. I also would like to thank my Zoology office mates for their company and advice, particularly Leida dos Santos and Javiera Cisternas Tirapegui. Thank you to Georgina Pickerell for being enthusiastic about braided rivers and always there for discussions from setting up field gear, to footprints and modelling. I would like to thank Matt Larcombe for being a calming force. I am also grateful that Glenda Rees allowed me to use two of her beautiful photographs of black-fronted terns.

A big thank you to my friends. I wish to thank Becky Cameron for taking me surfing, being a great companion on any adventure, and making me feel at home. I would like to thank Hayley Ricardo for taking me away on breaks for coffees or to gigs. It has been great to meet you. I am also glad I met Dani Nicholson who is an amazing inspiration on how to live life to the fullest. I am thankful to have had fellow surfer Simone Langhans two offices down from me in the last six months — keeping me excited to work hard, so that I can enjoy the next bit of swell, and making me laugh so much. To keep this short, thank you to all the amazing people that have come on the many climbing, surfing, tramping, and mini mountaineering adventures. It has been brilliant! And of course, a massive thank you to my friends in Münster, particularly Aline Reinhard and Franziska Klauer, and long-time friends Mareike Fehling, Swantje Fehling, and Michael Lambertus for being there despite the distance. I also wish to mention the great people I met at the Landesbund für Vogelschutz, that all have been encouraging along the way and provided good discussions for forming my endeavours into conservation biology.

I would like to thank my mum and dad for always supporting my curiosity to explore. Thank you for always having an open ear and open arms. Your support carries me through. I also want to thank my brother Gregor for being a great adventure buddy and getting me back skiing. Another adventure is overdue.
Thank you, Sanjay Thakur. You have been incredibly kind and caring. You encouraged me and supported when I needed it most. You have been so understanding and I certainly would not be here without you. Thank you for making sure that I get a regular dose of Fiordland life putting everything into perspective. Thank you for the daily conversations, discussions, and laughs. Thank you for having an interest in my work, coming out into the field with me (and solving my camera problems) and proofreading. Und danke, dass du mit mir deutsch sprichst.

This work was undertaken in consultation with Te Runanga Ngāi Tahu and was funded by the University of Otago, Department of Conservation, and Environment Canterbury. All procedures performed in this study were in accordance with the ethical standards of the institutions (Reference Number AEC No. 61/14).
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Table A.1 Details of the primers used in the development and amplification of the control region of black-fronted terns.

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Table A.2 Origin of black-fronted tern samples and primer pairs used for PCR and sequencing of the mitochondrial control region.
Table A.3 Haplotype frequencies per river of the 13 haplotypes identified for the mitochondrial cytochrome b-gene for each of the 31 black-fronted tern populations sampled.

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Table A.4 Haplotype frequencies per river of the 14 haplotypes identified for the mitochondrial control region for each of the 31 black-fronted tern populations sampled.

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Figure A.1 Mean pairwise genetic relatedness (± 95% confidence intervals) estimates based on microsatellite data for 27 black-fronted tern breeding colonies (only colonies with \( n \geq 5 \)) from throughout the South Island, New Zealand. Values and confidence intervals were determined using 10,000 bootstraps. The overall index ranges between 1 and -1, with values of \( r \) approximating 0.5 for parent-offspring or full siblings, 0.25 for half-siblings, and 0 for unrelated individuals. Note that individuals of three breeding colonies (from the Wairau, Buller and Clutha rivers) have slightly elevated relatedness coefficients, but none from the Mackenzie Basin.
Appendix B

Table B.1 Estimates of historical and contemporary black-fronted tern population census size based on three different Ne/NC conversion ratios: 0.11 (Frankham 1995); 0.37 (Palstra and Ruzzante 2008), and 0.5 (Lee, Engen, and Sæther 2011). Conversions are based on \( N_0 \) and \( N_e \) estimates shown in Table 4.4 and 4.5. In brackets, 95% confidence intervals.

<table>
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<th>Time frame</th>
<th>Method</th>
<th>Dataset/Details</th>
<th>( \frac{N_e}{N_0} ) ratio</th>
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<td>( \mu = 1 \times 10^{-4} )</td>
<td>(95,061–157,951)</td>
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<td>( \mu = 1.3105 \times 10^{-4} )</td>
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<td>( \mu = 1.976 \times 10^{-4} )</td>
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<td>LD ( \hat{N}_c )</td>
<td>All samples</td>
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Literature cited


Palstra FP, Ruzzante DE 2008. Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? Molecular Ecology 17: 3428–3447.
Appendix C

Table C.1 Black-fronted tern nest survival on vegetated and cleared islands in the lower Waitaki River in 2016/17. Successful nests refer to nests that had at least one chick hatch. The percentage of total nests monitored is presented in parentheses.

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<td>Nests monitored</td>
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<tr>
<td>Successful nests</td>
<td>48 (44.4%)</td>
<td>47 (61.0%)</td>
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<tr>
<td>Nests failed (Total)</td>
<td>60 (52.8%)</td>
<td>30 (39.0%)</td>
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<tr>
<td>Nests preyed upon</td>
<td>40 (37.0%)</td>
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<td>Nests deserted</td>
<td>9 (8.3%)</td>
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<td>Nests flooded</td>
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<tr>
<td>Infertile or failed hatch</td>
<td>4 (3.7%)</td>
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<tr>
<td>Unknown outcome</td>
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<tr>
<td>Total number of nests estimated</td>
<td>126</td>
<td>176</td>
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</table>
Table C.2 Comparison of different models using a) apparent hatching success, b) conditional hatching success, c) logistic-exposure to explore variation in hatching success of black-fronted terns. All continuous variables are centred and scaled for effect size comparison.

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<th>Timing</th>
<th>K</th>
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<th>∆ AICc</th>
<th>AICc ω</th>
<th>Cum. ω</th>
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**a** Intercept constant, as colony intercept explained zero variance and was thus removed from the model; **b** only observations included where exposure > 0, i.e. the nest observed was observed and at risk of failing; K = the number of parameters in a model; ∆ AICc = delta AICc, i.e. the difference in AICc values between a given model and the best-supported model; AICc ω = Akaike weight; Cum. ω = cumulative weight of models; LL = log likelihood of model.
Appendix D

Single season colony records of black-fronted terns (*Chlidonias albostriatus*) spanning their entire breeding range

Black-fronted terns / tarapirohe (*Chlidonias albostriatus*) are braided river specialists, which are endemic to New Zealand (Higgins and Davies 1996). They breed in the braided riverbeds of the South Island from as early as September through to January, and migrate to the coast of all three main islands for the remainder of the year (Lalas 1979; Higgins and Davies 1996). Breeding occurs in loose colonies ranging from a few pairs to over 400 nests on shingle bars or islands in the riverbeds (Keedwell 2005; Department of Conservation (DOC) unpubl. data). The incubation period is ~25 days with chicks leaving the nest after a few days and fully fledging after 30 days (Lalas 1977; Keedwell 2005). Overall hatching and fledging success is low and highly variable among individual colonies (Keedwell 2005; Cruz et al. 2013).

The main threats to black-fronted terns are predation, as well as on-going habitat degradation and loss (Maloney et al. 1999; Keedwell et al. 2002; Sanders and Maloney 2002; Keedwell 2005; Duncan et al. 2010; Steffens et al. 2012, O’Donnell et al. 2016). A recent meta-analysis of trends in breeding populations of black-fronted terns predicted a decline of about 50% over the next 30 years with more severe reductions of around 90% on rivers with relatively low mean flows (< 30 m³s⁻¹; O’Donnell and Hoare 2011). Currently black-fronted terns are nationally and internationally classified as ‘Endangered’ (BirdLife International 2012; Robertson et al. 2013). This classification is based on rapid and ongoing population reduction at some wintering and breeding sites due to recruitment failure, the small overall population size and the sparse nature of colonies across their distribution (BirdLife International 2012; Robertson et al. 2013). The most recent total population estimates are variable and range from 6,000 to 10,000 individuals (Keedwell 2002; O’Donnell and Hoare 2011).

Information on black-fronted tern populations comes mostly from walk-through counts along braided river beds. However, not much information is published on the distribution and size of breeding colonies on a nation-wide scale. In walk-through counts, only a few rivers are surveyed each year due to the challenging environment and need for skilled observers (O’Donnell and Hoare 2011; DOC unpubl. data). Overall there are reports of black-fronted terns from 61 rivers.
in the South Island, with major populations (> 200 birds) on only 13 rivers in most recent counts covering the period from 1988 to 2008 (O’Donnell and Hoare 2011). These rivers are the Ahuriri, Aparima, Hurunui, Mararoa, Upper Ohau, Oreti, Rakaia, Rangitata, Tekapo, Waiau (Canterbury), Waimakariri, Wairau, and Waitaki (O’Donnell and Hoare 2011). Fidelity to breeding sites and rivers is not thoroughly understood and some locations are only used once, others intermittently, and some are used each year (Higgins and Davies 1996; Keedwell 2005).

We searched 28 rivers spanning the entire known breeding range, including those with records of previous major populations for black-fronted tern colonies, as part of a genetic study. Observations of the location and size of these colonies made during a single breeding season spanning their entire known breeding range are reported here, with the aim of providing baseline information for future surveys and research into black-fronted tern colonies.

We searched for breeding colonies during the period from 9 October 2014 to 7 January 2015. A colony was considered to be one or more pairs within a 1 km stretch of river with clearly discernible nest scrapes, eggs, and/or chicks present. These were located by searching river stretches on foot targeting areas of past colony records (DOC unpubl. data) and by following feeding birds back to colony locations using binoculars from a distance. Colonies were very conspicuous when a section of river was searched. The size of each colony was estimated by counting flying birds. Searches within rivers were generally abandoned once a colony was detected as the main aim was to confirm the presence of breeding on a river. Thus, this paper reports only on the minimum number of colonies per river.

We located a total of 44 colonies with nest scrapes, eggs, and/or chicks present in the 28 rivers that we searched (Fig. D.1). We assumed that breeding attempts occurred in the Dart River on an inaccessible island we could see from an island nearby and based on the behaviour of adult terns vigorously dive-bombing Southern black-backed gulls/karoro (Larus dominicanus). In addition, while a colony location was not found in the Upper Rakaia River, we observed fledglings on an adjacent farm paddock and therefore assumed successful raising of fledglings occurred on that river (see Table D.S1 for further details). Colonies were found throughout the South Island spanning from 46°S to 41°S in latitude and from 26 m to 610 m in altitude above sea level. The most remarkable discovery was a colony in the Grey River, only 25 km from the ocean on the West Coast. No previous breeding record of black-fronted terns existed from this site and so far west from the main divide (Higgins and Davies 1996; O’Donnell and Hoare 2011).
Appendix D

Colony size varied from a single breeding pair up to 300 pairs, with the mean size being 61 (± 15 se; median = 40) pairs per colony. We found the largest colonies in the Upper Ohau (600 individuals), Wairau (200 individuals), lower Rangitata (150 individuals), Clutha (100 individuals), Tekapo (100 individuals) and lower Waitaki (100 individuals) rivers. The largest number of fledglings (35 individuals in one colony and 20 roosting further upstream) was also observed in the Waitaki River; although this might reflect the timing of visits as many other colonies were surveyed earlier in the breeding season.

![Location and size of black-fronted tern (Chlidonias albostriatus) breeding colonies in the 2014 season (Oct 2014–Jan 2015).](image)

Previous reports of black-fronted tern populations have also shown strongholds in Canterbury and Marlborough (Oliver 1955; Lalas 1979; O’Donnell and Hoare 2011), which is also
where the majority of braided rivers are located (Caruso 2006). Based on the classification used by O’Donnell and Hoare (2011), all of the rivers in which we observed the larger colonies have high mean flows (≥ 100 m$^3$s$^{-1}$), apart from the Upper Ohau colony. However, in the case of the Upper Ohau, this area is subject to intensive predator control (Anderson 2013a, 2013b, 2014). Overall, we observed 2,612 breeding black-fronted terns in the subsample of known tern breeding rivers that we surveyed.

The timing for finding colonies is crucial as colony size can fluctuate quite rapidly (Keedwell 2005). When we re-visited some colony locations, we observed either drastic declines or total failure in the span of 14 days due to predation and/or disturbance. If those colonies had been found at a later stage, they would have provided different estimates of numbers. In other cases, we were not able to search a river until late in the breeding season (mid-December) due to logistical constraints and adverse weather. In the upper Rakaia River, for example, we observed 14 adult birds with six fledglings feeding in adjacent farmland, but it was not possible to determine the original location and size of that breeding colony (Table D.S1). Monitoring of tern colonies in the Ohau river has also shown how the number of adult terns in colonies fluctuates between weekly counts (Keedwell 2005; Anderson 2013a, 2013b, 2014).

It is not well understood in which way black-fronted tern individuals and/or entire colonies move within and between catchments within the same year. There is a possibility that some colonies counted later in the season contained birds that we may have recorded earlier in the breeding season in a nearby catchment. However, we caught and banded a total of 417 adult terns (20%) during our visits and did not recapture any of these birds in other rivers later in the season.

In conclusion, this paper reports the first breeding record of black-fronted terns far west of the Main Divide and provides insights into the distribution and sizes of breeding colonies within a single breeding season. The surveys provide contemporary data to compare with historical counts and a baseline for future research into colony locations and size.

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**Literature cited**


### Table D.S1 Overview of rivers searched for black-fronted tern (*Chlidonias albostriatus*) breeding colonies. \(N\) = Number of individuals.

<table>
<thead>
<tr>
<th>River</th>
<th>Description of colony location</th>
<th>Northing (NZTM)</th>
<th>Easting (NZTM)</th>
<th>Date</th>
<th>(N)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahuriri</td>
<td>0.5 km downstream of SH 8 bridge</td>
<td>5071445</td>
<td>1361023</td>
<td>16-Nov-14</td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td>Ahuriri</td>
<td>Ben Avon</td>
<td>5084059</td>
<td>1331208</td>
<td>5-Dec-14</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Aparima</td>
<td>Shaws Trees Rd</td>
<td>4886514</td>
<td>1221309</td>
<td>13-Dec-14</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Ashburton</td>
<td>Wakanui School Rd</td>
<td>5128049</td>
<td>1502344</td>
<td>30-Nov-14</td>
<td>70</td>
<td>Colony declined due to predation/disturbance. 18-Dec-2014 only 12 birds</td>
</tr>
<tr>
<td>Ashburton</td>
<td>1 km downstream of Buicks bridge</td>
<td>5171919</td>
<td>1452244</td>
<td>8-Dec-14</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>Ashburton</td>
<td>Entrance at border of conservation land</td>
<td>5170319</td>
<td>1452858</td>
<td>17-Dec-14</td>
<td>60</td>
<td>-</td>
</tr>
<tr>
<td>Buller</td>
<td>Downstream of Howard confluence</td>
<td>5381865</td>
<td>1573231</td>
<td>14-Nov-14</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>Cass</td>
<td>Downstream from Cass bridge</td>
<td>5138227</td>
<td>1399753</td>
<td>22-Nov-14</td>
<td>40</td>
<td>Very scattered colony from 500 m below bridge to almost the delta</td>
</tr>
<tr>
<td>Clutha</td>
<td>Grover's Island</td>
<td>4948963</td>
<td>1313064</td>
<td>14-Dec-14</td>
<td>100</td>
<td>On same island as black-billed gull colony</td>
</tr>
<tr>
<td>Dart</td>
<td>Humes Rd</td>
<td>5032945</td>
<td>1230719</td>
<td>8-Dec-14</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>Eglinton</td>
<td>Walker Creek</td>
<td>4994033</td>
<td>1204315</td>
<td>11-Dec-14</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>Grey</td>
<td>Brandy Jack Creek</td>
<td>5315493</td>
<td>1483684</td>
<td>3-Dec-14</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>Hunter</td>
<td>Upstream of Cotters Ck confluence</td>
<td>5100218</td>
<td>1320866</td>
<td>7-Jan-15</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Hunter</td>
<td>Lake Delta</td>
<td>5090686</td>
<td>1317534</td>
<td>7-Jan-15</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Hurunui</td>
<td>Downstream of SH1 bridge</td>
<td>5250610</td>
<td>1608784</td>
<td>10-Nov-14</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>Hurunui</td>
<td>Just upstream of SH 7 bridge</td>
<td>5253451</td>
<td>1580968</td>
<td>6-Dec-14</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Makarora</td>
<td>Wilkin Rd</td>
<td>5092229</td>
<td>1295935</td>
<td>17-Nov-14</td>
<td>40</td>
<td>Next to black-billed gull colony</td>
</tr>
<tr>
<td>Manuherikia</td>
<td>Above Fall's Dam</td>
<td>5028943</td>
<td>1356375</td>
<td>29-Oct-14</td>
<td>30</td>
<td>Birds dive-bombing/warning on 29-Oct-2014, scrapes (nest bowls) seen. Colony not active on 18-Nov-2014</td>
</tr>
<tr>
<td>Mararoa</td>
<td>1 km upstream of Mararoa Downs Station</td>
<td>4949984</td>
<td>1209785</td>
<td>24-Oct-14</td>
<td>4</td>
<td>Next to small black-billed gull colony</td>
</tr>
<tr>
<td>Mararoa</td>
<td>Little Hill</td>
<td>4957833</td>
<td>1214440</td>
<td>24-Oct-14</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>Maruia</td>
<td>Between Kowhai Downs and Rocky Hill</td>
<td>5320082</td>
<td>1534697</td>
<td>1-Dec-14</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>River</td>
<td>Description of colony location</td>
<td>Northing (NZTM)</td>
<td>Easting (NZTM)</td>
<td>Date</td>
<td>N</td>
<td>Notes</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------------------------------------</td>
<td>-----------------</td>
<td>----------------</td>
<td>------------</td>
<td>-----</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>Matakitaki</td>
<td>5 km downstream of Matakitaki Station</td>
<td>534993</td>
<td>1552755</td>
<td>1-Dec-14</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Ohau</td>
<td>Ohau Tern Island</td>
<td>5094245</td>
<td>1360988</td>
<td>1-Nov-14</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>Opiti</td>
<td>Opati Pleasant Point Rd Bridge</td>
<td>5098690</td>
<td>1451811</td>
<td>17-Dec-14</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Oreti</td>
<td>SH 97 Bridge Mosburn</td>
<td>5132112</td>
<td>1455574</td>
<td>21-Dec-14</td>
<td>50</td>
<td>Next to black-billed gull colony</td>
</tr>
<tr>
<td>Oreti</td>
<td>Quarry Rd</td>
<td>4932780</td>
<td>1229908</td>
<td>25-Oct-14</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Rakia</td>
<td>Dobbs Ford angler access</td>
<td>4933054</td>
<td>1228150</td>
<td>5-Nov-14</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Rakia</td>
<td></td>
<td>4934881</td>
<td>1353232</td>
<td>20-Nov-14</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Rakia</td>
<td></td>
<td>514881</td>
<td>1382532</td>
<td>15-Dec-14</td>
<td>14 adults and six fledglings observed in field</td>
<td></td>
</tr>
<tr>
<td>Ranga</td>
<td>Old Main Sh Rd</td>
<td>516346</td>
<td>1477419</td>
<td>17-Oct-14</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Ranga</td>
<td>Confluence of Potts River</td>
<td>5170777</td>
<td>1431312</td>
<td>2-Nov-14</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Ranga</td>
<td>Confluence of Potts River</td>
<td>516133</td>
<td>1438275</td>
<td>5-Dec-14</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Ranga</td>
<td>Forest Creek</td>
<td>5148125</td>
<td>1369537</td>
<td>17-Dec-14</td>
<td>86</td>
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<td>Ranga</td>
<td></td>
<td>5091732</td>
<td>1385582</td>
<td>16-Dec-14</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Ranga</td>
<td></td>
<td>5112669</td>
<td>1395043</td>
<td>23-Nov-14</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Ranga</td>
<td></td>
<td>5023953</td>
<td>1386288</td>
<td>23-Nov-14</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Waitaki</td>
<td>0.5 km upstream of Kurow (SH82) bridge</td>
<td>5044787</td>
<td>1399697</td>
<td>28-Nov-14</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Waitaki</td>
<td></td>
<td>5044797</td>
<td>1406582</td>
<td>24-Nov-14</td>
<td>100</td>
<td>Flock of 30 fledglings observed near colony</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2,612</td>
<td></td>
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