Health risks associated with the conservation of kākāpō (*Strigops habroptilus*) on offshore islands

A thesis presented in partial fulfilment of the

requirements for the degree of

Doctor of Philosophy

In

Veterinary Science

at

Massey University, Manawatū,

New Zealand.

Chamindani Manjula Jayasinghe Meda Gedara

2021

Abstract

New Zealand has a long history of using translocations of native fauna for conservation and offshore islands have been regarded as isolated ecosystems for conservation purposes. Maintaining the biosecurity of these islands is a fundamental part of conservation management. Island translocation has protected the endemic New Zealand parrot kākāpō (*Strigops habroptilus*) from becoming extinct and resulted a viable population in three offshore islands outside its historical range. However, kākāpō have been affected with novel diseases arising in the island sanctuaries. The aim of this research was to investigate the health risk for kākāpō in offshore islands, focusing on two main diseases. Exudative cloacitis is a disease of the cloaca in kākāpō from Whenua Hou for which no aetiology has been identified. An epidemiological study was conducted to identify the factors associated with the initiation of this disease in kākāpō. Results suggested that the disease is unlikely to have an infectious origin and diseased birds were not geographically clustered within Whenua Hou but reflected the kākāpō distribution within the island. Analysing the pH, moisture and ammonium content of kakapo roost materials did not show any evidence for the cause of the disease in the cloaca. Disease incidence has not been affected by the annual maximum temperature and rainfall each year.

Erysipelas, an acute infection caused by the bacteria, *Erysipelothrix rhusiopathiae,* emerged as a small cluster of mortality in translocated kākāpō in 2004, and has subsequently been diagnosed as a sporadic cause of death for kākāpō and other endangered New Zealand fauna. This study reported the presence of *E. rhusiopathiae* in different seabird species in two islands Whenua Hou and Te Hauturu-o-Toi through a targeted survey. Whole genome sequencing revealed that there was a geographic difference in genomic diversity of *E. rhusiopathiae*, and phylogenetic evidence suggested seabirds as a possible reservoir of the organism for endangered native land birds. An *Erysipelothrix* spp. phylogenetically different from *E.*

rhusiopathiae was detected in Cook's petrels (*Pterodroma cookii*) and in kākāpō indicating the diversity in the genus *Erysipelothrix* in these island ecosystems.

The investigations in this thesis demonstrate that while island translocations have been instrumental in conserving endangered fauna in New Zealand, this conservation strategy does expose the translocated populations to novel health risks. Disease management should be a priority in managing endangered species and these results should help to guide management actions for new health issues as they arise.

Acknowledgements

Throughout this journey of PhD, I received a great deal of support and assistance from many people.

First, I would like to thank my main supervisor, Professor Brett Gartrell, whose expertise was invaluable in formulating the research question and methodology. Your feedback pushed me to sharpen my thinking and brought my work to a higher level.

I'm grateful to have a supportive team of co-supervisors, who were always willing to share their knowledge and experiences in their field of expertise. To Wendi Roe, Anne Midwinter, Emilie Vallee, and Charlotte Bolwell, thank you all for supporting and putting up with me for more than three years. I appreciate your patience and providing me with a supportive and caring environment to work in. To David Wilkinson, you are a great teacher for someone who had zero knowledge of genomic data analysis. Thank you for teaching me how to convert raw genomic data into something very interesting.

During this remarkable PhD journey, I was one of the luckiest ones to visit two beautiful islands in New Zealand. I believe it's a once-in-a-lifetime experience. I was able to witness the amazing works of the Kakapo Recovery Team, Department of Conservation, to protect unique New Zealand fauna. Thank you to Dr. Andrew Digby, Scientific manager, Deidre Vercoe, operations manager, and all the members of the Kakapo Recovery Team for their support with sample collection in Whenua Hou.

I would like to extend my sincere thanks to Richard Whale and the family and Chippy Wood for their great hospitality and support throughout our stay on the Te Hauturu-o-Toi. I appreciate the support provided by the iwi community at Manuhiri Kaitiaki Charitable Trust with sample collection in Te Hauturu-o-Toi.

To three of my wonderful field assistants, Ravina Khude, Ji Ye Ahn and Adrienne French, I was very lucky to have you as my supporters in the field. Your contribution was huge for the success of my study. Thank you for everything and the happy memories.

In addition, I must thank Laura West, Laurelle Jones, Stevie-Ray Cairns and Bryony Hitchcock, a group of volunteers from a weeding group who were working at the time on Te Hauturu-o-Toi for their huge support with sample collection. Because it was a great challenge sampling in Te Hauturu-o-Toi than I have imagined.

I would like to thank Ian Furkert, Senior Technician, and Lance Currie, Senior Technical Manager from School of Agriculture and Environment for allowing me to use their lab for my sample analysis and the immense support provided with analysing kakapo roost materials.

To Lynn Rogers, you always offered your helping hand when I needed and I'm forever grateful for your support provided during my lab work sessions.

I would like to thank Simon Verschaffelt for all the technical support, you were there to rescue me all the time when my computer was giving me a hard time.

I would like to express my gratitude to Debbie Hill and other staff, my fellow students of the School of Veterinary Science of Massey University for the collaboration over these years.

I highly appreciate the Massey University Doctoral scholarship and University Grant Commission Sri Lanka for the financial support provided during my PhD study. In addition, my thanks are extended to Massey University Research Fund (2017) and Massey Foundation Grant (2018) for providing me with funds to make my research possible.

My special thanks are expressed to my parents, husband and two brothers for always encouraging me to work hard towards my goals.

List of journal articles

Accepted

1. Jayasinghe, M., Midwinter, A., Roe, W., Vallee, E., Bolwell, C., Gartrell, B. 2021. Seabirds as possible reservoirs of *Erysipelothrix rhusiopathiae* on islands used for conservation translocations in New Zealand. Journal of Wildlife Diseases. *In press.*

Prepared

1. Jayasinghe, M., Vallee, E., Bolwell, C., Digby, A., Roe, W., Midwinter, A., Gartrell, B. 2021. An epidemiological investigation of exudative cloacitis in kākāpō (*Strigops habroptilus*) in Whenua Hou/Codfish Island. Preventive Veterinary Medicine

List of conference papers

1. Jayasinghe, C. M., Vallee, E., Bolwell, Midwinter, A., Roe, W., Gartrell, B. 2018. What are the health risks associated with the conservation of kākāpō (*Strigops habroptilus*) on offshore islands? Presented at the postgraduate colloquium of School of Veterinary Sciences, Massey University
2. Jayasinghe, C. M., Vallee, E., Bolwell, Midwinter, A., Roe, W., Gartrell, B. 2019. An epidemiological investigation of exudative cloacitis in kākāpō (*Strigops habroptilus*). Presented at the annual conference of the Australia and New Zealand College of Veterinary Sciences, Gold Coast, Australia.
3. Jayasinghe, M., Midwinter, A., Vallee, E., Roe, W., Bolwell, C., Gartrell, B. 2019. Prevalence of *Erysipelothrix rhusiopathiae* in islands used for conservation translocations, New Zealand. Presented at the joint conference of Asian Society of Conservation Medicine and Wildlife Disease Association- Asia Pacific section in Phnom Penh, Cambodia.
4. Jayasinghe, M., Midwinter, A., Vallee, E., Wendi, R., Bolwell, B., Gartrell, B. 2019. *E. rhusiopathiae* on islands used for conservation translocations, New Zealand. Presented at the New Zealand Microbiological Society annual conference, Palmerston North.
5. Jayasinghe, M., Midwinter, A., Wilkinson, D., Roe, W., Vallee, E., Bolwell., C., Gartrell, B. 2020. Genomic diversity of *Erysipelothrix* in islands using for conservation translocation, New Zealand. Presented (virtual) at the Wildbase Post-graduate research symposium.

Table of contents

List of figures

List of tables

Chapter 1
General Introduction

1.1. Kākāpō conservation and biology

kākāpō (*Strigops habroptilus*) are critically endangered flightless, nocturnal parrots endemic to New Zealand, with an estimated population of 205 adult birds as of March 2021 (Department of Conservation (DOC) 2021). It is the heaviest living parrot in the world and the body weight can vary between 1.6-3.6 kg in male and 0.9-1.9 kg in female birds showing a pronounced sexual dimorphism in body mass (Higgins, 1999). Plumage of kākāpō is moss green mottled with yellow and black. Kākāpō have an owl-like face with a conspicuous facial disc. They have a pale grey broad beak, short legs and large feet (Powlesland et al., 2006). All these features including diminished keel on the sternum, reduced wing muscles, and robust legs and neck are considered important adaptations for thriving in oceanic islands with abundant food and few predators (Powlesland et al., 2006).

Kākāpō are herbivores, feeding on leaves, fruits, seeds, leaf bases, stems, buds, and rhizomes of a wide range of plant species and fungi (Clout and Merton, 1998; Powlesland et al., 2006). They are selective feeders and prefer seasonally available food species. Their preference for different plant species in different islands during breeding and non-breeding years has been recorded (Powlesland et al., 2006). Analysis of kākāpō faecal samples from Rakiura/Stewart Island has shown that there are marked seasonal and annual variations in their diet. Kākāpō have special preference for the seasonal rimu (*Dacrydium cupressinum*) fruit and the high protein content in this fruit is considered as the trigger for their breeding on southern New Zealand islands. However, little is known about the natural diet of nestlings and fledglings (Powlesland et al., 2006).

Kākāpō breed only once in 2-5 years and are the only parrot known to have a “lek” breeding system (Powlesland et al., 1992; Elliott et al., 2001). Male birds create a track system along ridges and excavate and maintain shallow bowls connected by the tracks. At night, they make

a loud resonant sound known as “booming” and display competitively to attract female birds for mating. This deep “booming” is a low frequency sound amplified by inflated air sacs and can be heard up to 2 km away (Powlesland et al., 2006). Females lay 2-4 eggs in hollows in grounds or cavities in trees. As male birds make no contribution to incubation and chick rearing, females incubate and rear the young birds alone. Birds may walk for kilometres each night to forage and the nestlings are left unattended for several hours. Usually, one or two juveniles are raised to independence (Powlesland et al., 2006).

Kākāpō were once widespread and abundant across the New Zealand mainland and inhabited forest, scrubland and alpine habitats. The population decline of kākāpō began with human settlement and resulted in the local extinction. Several unique features possessed by kākāpō have made them vulnerable to anthropogenic changes occurring in their environment since New Zealand was colonised by humans. Kākāpō breed only once in 2-5 years (Elliott et al., 2001). Since only the female is responsible for incubation and chick rearing, nests are unattended for long periods at night and vulnerable to predation and chilling. Their nocturnal behaviour and flightless nature make adult birds vulnerable to predation. The kākāpō population declined primarily due to predation, and the combined effects of habitat loss and habitat modification (Powlesland et al., 2006; Bergner et al., 2016). During the 1970s and early 1980s, intensive research and management on kākāpō was started on two populations of birds from Fiordland and southern Rakiura of New Zealand (Clout and Merton, 1998; Elliott et al., 2001). By 1995 the kākāpō population size was 51 birds and they possibly suffered a severe population bottleneck (Powlesland et al., 2006; Robertson et al., 2006). Conservation attempts in early years faced many problems and translocations of kākāpō to potentially predator free islands were not successful. Elliott et al., (2006) reported that the egg hatching success was only 42% in these birds during 1995-2002.

Growth of the kākāpō population from 51 birds in 1995 to the current size of 205 adult birds is a direct result of the intensive management programmes over the last two decades. Three breeding populations are managed on three predator free offshore islands, Whenua Hou/Codfish Island, Te Hauturu-o-Toi/Little Barrier Island and Pukenui/Anchor Island (Fig 1.1). Currently, the largest breeding population is in Pukenui (45° 45′ 30″ S, 166° 31′ 0″ E) situated in the area of the Dusky Sound, New Zealand. These three populations currently require intensive management which include safety and health observations (Jansen, 2006; Clout, 2006) and regular supplementary feeding with a pelleted parrot food (Harrison's Adult Lifetime Coarse Pellets and Harrison's High Potency Coarse Pellets, Harrison's Bird Foods, Brentwood, TN, USA). Management of the kākāpō today is being guided by the Kākāpō Recovery Project (Neill, 2008). The current management plan outlines four key goals for the species; maximise recruitment in the kākāpō population, minimise the loss of genetic diversity in the population, secure, restore or maintain sufficient habitat to accommodate the expected increase in the population, maintain public awareness and stakeholder support for kākāpō conservation.

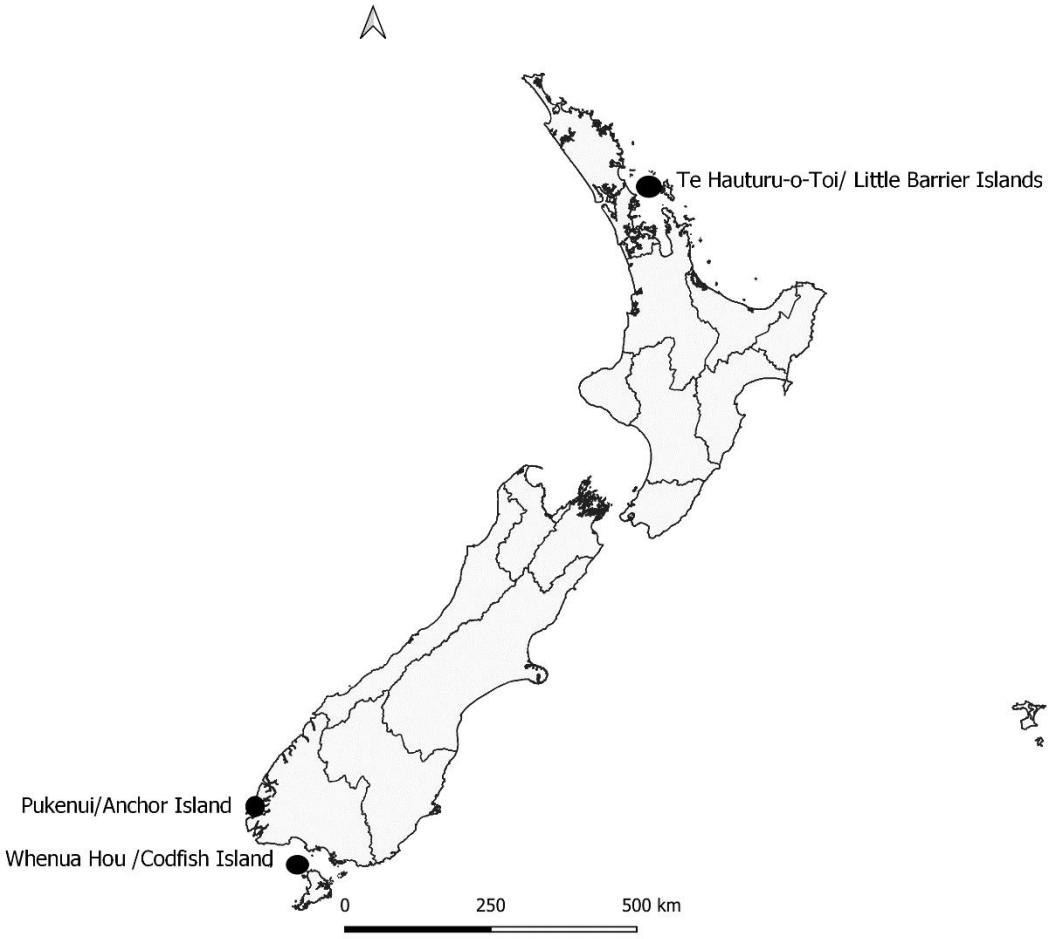


Figure 1. 1. Locations of the three breeding populations of kākāpō in three offshore islands in New Zealand.

1.2. Concept of species translocation

Species translocation is the deliberate movement of animals from one geographical location to another to augment an existing population or establishing, or re-establishing a disappeared population (Griffith et al., 1989; Kock et al., 2010; Seddon et al., 2012). Species translocations have been conducted for years as a conservation management tool in many parts of the world in response to an accelerated rate of species extinction in recent history and a reduction in overall biodiversity (Griffith et al., 1989). Conservation translocations are complex procedures, which involve time consuming and expensive monitoring procedures to maintain good

standards (IUCN 2013; Batson et al., 2015). A survey conducted to evaluate the animal translocations between 1973-1986 revealed that nearly 700 translocations occur per year of which about 7% are translocations of endangered or sensitive species (Griffith et al., 1989). Factors that may affect the success or failure of translocations include: the size of the founder population, the quality of the habitat, the genetic diversity among founders, any competitors, if the animals being transferred are wild caught or captive reared, and finally the conservation status of the species as threatened or not (Griffith et al., 1989).

1.2.1. Conservation translocation of New Zealand birds

Animal translocations in New Zealand have a long history and have been done for many reasons. Translocations have prevented the extinction of a number of endemic New Zealand bird species such as little spotted kiwi (*Apteryx owenii*), buff weka (*Gallirallus australis hectori*), black stilt (*Himantopus novaezelandiae*), kākāpō, South Island saddleback (*Philesturnus carunculatus*) and black robin (*Petroica traversi*). Habitat loss and predation by invasive mammalian species were the principle causes for population decline and extinction of many native bird species (Miskelly and Powlesland, 2013). Translocations have been crucially important for the management and restoration of threatened New Zealand birds. The earliest reported efforts to translocate native birds were by Richard Henry, the pioneer New Zealand conservationist. He tried to translocate a group of kākāpō, little spotted kiwi and South Island brown kiwi (*Apteryx australis*) to islands in Dusky and Breaksea Sounds during 1875-1915 to protect them from predators (Hill and Hill, 1987). Miskelly and Powlesland (2013) have reviewed conservation translocations events of New Zealand birds from 1863-2012 and reported that some of the translocations failed mainly due to the low number of founders in each translocation and little effort on predator control at the release site.

Success rates of the translocation efforts in New Zealand have varied for different bird species. Whitehead (*Mohoua albicilla*), yellowhead (*Mohoua ochrocephala*), South Island saddleback, takahē (*Porphyrio hochstetteri*) and red-crowned parakeet (*Cyanoramphus novaezelandiae*) are some of the bird species that have shown the highest success rates for species that have been translocated eight or more times (Miskelly and Powlesland, 2013). On the other hand, the lowest success rates have been recorded for bellbird (*Anthornis melanura*), North Island weka (*Gallirallus australis greyi*), South Island brown kiwi, brown teal (*Anas chlorotis*) and kākāpō. However, kākāpō translocations are considered as one of the examples that prevent extinction of critically endangered bird species through intervention (Miskelly and Powlesland, 2013). Armstrong et al., (1999), divided the factors determining the success of translocated populations in relation to two phases, factors that act during the establishment phase and those that affect the long-term dynamics of the population. Number of founders, the composition of the founder group, the methods and timing of the translocation, pre- and post-release training, and post-release management methods were considered important in determining the survival and behaviour of animals during the establishment phase. Importantly, for this study, pathogens have been identified as one factor that can affect the long-term dynamics of translocated populations (Armstrong et al.,1999).

1.2.2. New Zealand's conservation islands

Any land masses always surrounded by water off the mainland of New Zealand are recognised as offshore islands. These are important components of New Zealand's natural habitat and contain species endemic to specific archipelagos and support relict populations extinct in the mainland (Towns et al., 2012). There are 735 offshore islands that belong to New Zealand, extending from the subtropics to the sub-Antarctic region. Approximately, 250 of those islands

larger than 1 ha are maintained by the Department of Conservation (Parkes and Murphy, 2003). Terrestrial communities in offshore islands consist of large invertebrates, reptiles (tuatara, *Sphenodon punctatus* and lizards) and seabirds, especially the species that live in burrows (Towns and Ballantine, 1993). Many island ecosystems have been modified following human settlement and some islands were used as gardens to grow introduced tropical plants (Bellingham et al., 2010).

Polynesian and European settlement resulted in population declines and extinction of many native New Zealand wild animal species. Subsequently, translocation of threatened species to remote offshore islands came in to practice as a conservation management tool in New Zealand (Simberloff, 2002). Conservation goals have been expanded from individual species to ecosystems over the years considering the interactions between species and over terrestrial and marine ecosystems (Towns and Ballantine, 1993). Management of island ecosystems for conservation purposes includes species translocations, eradication of non-native mammals and public involvement. Islands have provided homes for some wild animal species in locations where the species may historically never have occurred. Many of these islands are home for a large number of sea birds and some non-native bird species (Towns and Ballantine, 1993). The significance of maintaining the biosecurity of island ecosystems has been identified as epidemic and endemic disease continue to occur in island populations of New Zealand birds (Gartrell et al., 2005; Ewen et al., 2007; Hale and Briskie, 2009), marine mammals (Castinel et al., 2007; Maloney et al., 2009) and reptiles (Gartrell et al., 2006; Middleton et al., 2014).

1.2.3. Conservation of seabirds: the importance of islands for seabird biodiversity

Seabirds represent 3.5% of all the bird species in the world with about 350 species in total and they are considered as indicators of ecological changes, both regionally and globally (Croxall et al., 2012). Seabirds forage at sea and breed on land while delivering marine derived nitrogen to terrestrial ecosystems. They are an important component of biodiversity in New Zealand, especially on island ecosystems. New Zealand is a breeding ground for 84 out of 140 seabird species recorded there. New Zealand is known as the "seabird capital" of the world and is the only breeding ground for 35 species (Taylor, 2000). New Zealand has the highest number of threatened seabird species, having more than double of any other country (Croxall et al., 2012). In New Zealand, all the sea bird taxa, except for southern black-backed gulls (*Larus dominicanus*), are protected by the Wildlife Act 1953 (Taylor, 2000).

Eighty-three percent of the world's sea bird species are threatened, and it is a challenge to ensure the survival and improve the status of the many seabird species which are already threatened with extinction. The commercial fishing industry poses one of the main threats to seabirds worldwide (Croxall et al., 2012). Marine and land pollution, invasive predators, habitat degradation and human disturbance are recognised as other significant threats for seabird survival. Some of the species are threatened by direct exploitation both at sea and ashore (Croxall et al., 2012). A lack of accurate population data interferes with prioritizing, planning and assessment of conservation actions for many seabird taxa (Rayner et al., 2007a). The most threatened orders of seabird in the world are the Sphenisciformes (penguins) and Procellariiformes (albatrosses/petrels). These two orders represent 43% of all seabirds and contain many pelagic species.

Islands support large breeding colonies for seabirds while some islands are used only as roosting grounds. Since they can gather in large colonies, a large amount of marine debris gets accumulated through the deposition of guano in coastal ecosystems (Otero et al., 2018). In addition to the possible enhancement of primary productivity, accumulation of these materials has caused important environmental changes in costal ecosystem. Chemical changes in soil composition, physical disturbances and alteration of competitive processes are examples of some of the major changes documented (Vidal et al., 2000; Sánchez-Piñero, 2000). New Zealand's offshore islands provide breeding grounds for a range of seabird species and potentially have an important influence on ecological processes of those ecosystems. Breeding habitats of some seabird species are restricted to island ecosystems since they have lost their habitat on the main islands. For example, breeding populations of Cook's petrel (*Pterodroma cookii*) is limited to islands in the southern and northern extremities of New Zealand (Rayner et al., 2007a). Controlling the effect of the mammalian predators has a positive influence on the population growth of some of the sea bird species. For examples. eradication of weka and Pacific rats (*Rattus exulans*) on Codfish Island in 1980 and 1998 has improved the population size of Cook's Petrels improving the world population status of the species (Rayner et al., 2008).

1.2.4. Potential new hazards for species translocated to islands

Re-introduction guidelines by the International Union for Conservation of Nature (IUCN) emphasize that animals should be translocated within their historical range, after ensuring that the previous causes for decline have been removed (IUCN 2013). However, species have been translocated to favourable habitats outside their historical range in increasing frequency (Ricciardi and Simberloff, 2009). New Zealand is the world pioneer in island translocations and has prevented the extinction of endemic birds, reptiles and invertebrates (Jones and Merton,

2011; Miskelly and Powlesland, 2013). Translocations resulted in the establishment of viable populations in new areas beyond their natural ranges (Miskelly and Powlesland, 2013). However, conservation translocations between geographic locations always carry some risks, but these risks must be weighed against those of extinction losses and ecosystem losses. Potential additional risks could be pathogens, alterations in environmental factors and interactions with the other species sharing the same ecosystem. Types of hazards or threats could be different from one time period to another creating new challenges for conservation managers. The concept of "emerging ecosystems" or "novel ecosystems" has been defined by some researchers to explain the environmental changes that may result from human induced changes in species composition and relative abundance which has not previously occurred within a given ecosystem (Seddon, 2010).

Diseases can impact the success of potentially expensive high-profile wildlife translocations. Many of these diseases are infectious and may pose a risk to both biodiversity and human health (Kock et al., 2010). The significance of disease management for conservation translocations has been well recognised and assessment of disease associated risks during translocation programmes is recommended (Woodford, 2001; Leighton, 2002; IUCN 2013). Translocated animals can infect other host animals at the site of destination, or they may be infected from pathogens acquired at the release site. Deem et al. (2001) suggested that the significance of diseases and parasites on wildlife populations may increase with accelerated impacts on habitat and landscape by human activities, especially in combination with increased numbers of translocations between bird populations. Avian malaria (*Plasmodium relictum*), possibly introduced through non-native birds, has reduced the range and numbers of many native birds in Hawaiian forests below 1500 m elevation (van Riper et al., 1986). In New Zealand, knowledge on wildlife diseases and their effects on the survival of wildlife have been

increased over the years (Derraik et al., 2008; Alley et al., 2010; Alley and Gartrell., 2019; Schoener et al., 2020).

The role of sea birds as disease reservoirs was evident with recent spreading of some infectious diseases and emergence of diseases in some regions of the world (Altizer et al., 2011; Fuller et al., 2012). For example, the occurrence of Lyme disease in north-eastern USA and Canada has recently increased and is connected to the movement of birds with infected ticks during the spring migration (McCoy et al., 2016). Seabirds normally fly long distances, and feed at sea. Most species are colonial breeders which means that they aggregate in large colonies to reproduce and stay in those locations for several months. These colonies with large numbers of susceptible animals and predictable seasonal occurrence makes them good hosts for pathogen transmission. In addition, seabirds are long living species which allows them to maintain and transmit pathogens for very long periods of time. Therefore, it is important to study the role of seabirds as disease reservoirs and carriers of parasites and other pathogens (McCoy et al., 2016). On the other hand, seabird behaviour of spending more time on feeding at the sea and returning to land only to breed makes it difficult to investigate the pathogens and their impact on seabirds (Weimerskirch, 2004). Pathogen spillover from more abundant seabirds to endangered island populations of native birds is a possibility in island ecosystems. In addition, the physical environment of the islands can be changed due to the burrowing and guano that seabirds bring to the island (Sánchez-Piñero, 2000).

1.3. The characteristics and history of the two study islands

1.3.1. Te Hauturu-o-Toi/Little Barrier Island

Te Hauturu-o-Toi is located 24 km off the eastern coast of northern New Zealand (36.1991°S, 175.0814°E) (Veitch, 2001). The New Zealand government acquired the island as a nature reserve in 1894. It is a highly eroded extinct volcanic island with an area of 28 km^2, has several central peaks while Mount Hauturu is the tallest, being 722 m in height. The island is deeply dissected by a series of steep ridges radiating from a central range towards the coast. The climate of the island varies as the lower slopes have a warm subtropical climate while the cloud forest communities at higher altitude experience a wet climate. An altitudinal gradient can be seen in the forest and the forest cover in higher altitude areas is considered intact. This island receives about twice as much rainfall in winter as in summer (Moorhouse, 1991).

Te Hauturu-o-Toi is one of the most important predator free nature reserves that serves as a refuge for over 350 species of plants and several species of threatened birds. For example, Te Hauturu-o-Toi has been important for the survival of the endemic hihi (*Notiomystis cincta*), and the island is also used for a breeding population of kākāpō reintroduced to the island in 2012. The first group of kākāpō were translocated to this island in 1982 and included 18 kākāpō from southern Rakiura and 4 birds that had been previously transferred to Maud Island (Moorhouse, 1991). Then the kākāpō were removed from the island in 1999 for a period of time and reintroduced in 2012 (DOC, 2017).

A population of critically endangered New Zealand storm petrel (*Fregetta maoriana*) was identified breeding on the island in 2013 (Flood, 2003). Te Hauturu-o-Toi is the most important breeding ground for endangered Cook's petrel with over 50,000 pairs of breeding in higher

altitude areas. Black petrel (*Procellaria parkinsoni*), little blue penguin (*Eudyptula minor*), grey faced petrel (*Pterodroma macroptera*) are other seabirds found on this island.

1.3.2. Whenua Hou/ Codfish Island

Whenua Hou is a nature reserve located in southern New Zealand (46°45 S, 167°38 E), 3 km from the north-west coast of Rakiura (Fig 1. 2). It was first classified as a scenic reserve in 1915 and upgraded to a status of a nature reserve in 1986. Whenua Hou has a land area of 14 km^2 and reaches an elevation of 250m close to the southern coast. The island is dominated by a large valley on the north-east side and steep coastal cliffs on the south-west (Sedgeley et al., 2006; Scott et al., 2009). It has a cold-temperate maritime climate; the temperature varies from 9.2°C to 18.8°C in summer and between -1.7°C and 12°C during winter (Sedgeley et al., 2006). Apart from Rakiura, Whenua Hou is the largest offshore island in this area which has the diverse range of habitats typical of the Rakiura.

Whenua Hou is historically important due to the early settlement between Māori and European people that existed at Sealers Bay during 1800 and 1850. This area is a recorded archaeological site on the island. Several other archaeologically important sites relating to occupations as early as the 13th century can be seen on the island. The island was identified as a potential location for native species in 1960 and measures were taken for island restoration including a restriction on unauthorized access in 1968 (DOC, 2012). New Zealand Department of Conservation recognized Whenua Hou as a high priority area for eradication of Pacific rat before 1995 and eradication was an essential step in ecological restoration of the island (McClelland, 2002). It has been free from weka since 1984, possums (*Trichosurus vulpecula*) since 1990, and rats since 1998 following successful eradication programmes.

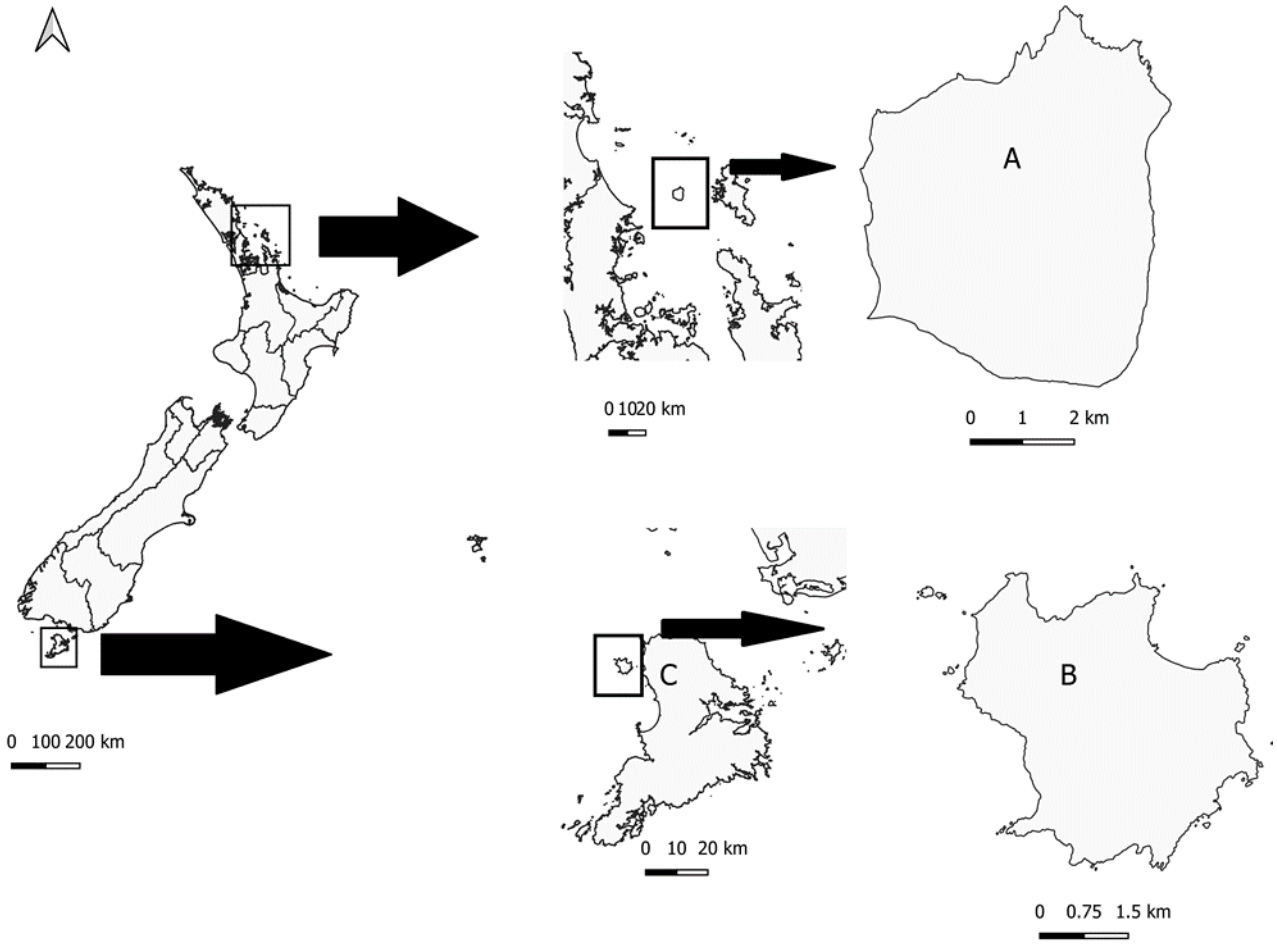


Figure 1. 2. Locations of, A) Te Hauturu-o-Toi/Little Barrier Island, B) Whenua Hou/Codfish Island, C) Rakiura/Stewart Island.

Types of vegetation cover on the island vary from dense mature podocarp forest to coastal scrub, dunes and cushion bog (Sedgeley et al., 2006; Scott et al., 2009). It is home to southern short-tailed bats (*Mystacina tuberculata tuberculata*), kaka (*Nestor meridionalis*), fernbirds (*Bowdleria punctata*), red-crowned parakeet, yellow-crowned parakeet (*Cyanoramphus auriceps*), Pacific black ducks (*Anas superciliosa*) and a breeding population of kākāpō. Whenua Hou was the main site for the conservation management of critically endangered kākāpō for a long time. In addition, it provides a home for translocated populations of endangered yellowhead and Campbell Island teal (*Anas nesiotis*). This offshore island is important as a breeding ground for several species of seabirds including some endemic species.

Common diving petrel (*Pelecanoides urinatrix*), South Georgian diving petrel (*Pelecanoides georgicus*), Cook's petrel, mottled petrel (*Pterodroma inexpectata*), and sooty shearwater (*Puffinus griseus*) are the burrowing seabirds found on this island. Diving petrel and prions (*Pachyptila* spp.) are limited to areas near the coast while Cook's petrel is a forest nesting species and burrows are distributed across large areas of slope and ridge-top habitat mostly at higher altitudes (Rayner et al., 2008).

1.4. Island disease ecology

Disease ecology is defined as the investigation of the interactions between hosts and pathogens in relation to their environment and evolution (Kirkpatrick and Altizer, 2010). These investigations try to understand the process of pathogen transmission and spread over space and time and the effect on host abundance (Hudson et al., 2002; Hawley and Altizer, 2011). The main aims of ecological investigations could be different based on the field of study, for example epidemiological studies focus on identifying risk factors for infectious and non-infectious diseases while parasitological studies are mainly aimed at parasite taxonomy and life cycles. The impact of a pathogen on a population is dependent on many factors including the pathogen virulence and the effect of the pathogen on host survival and reproduction. The level of impact of a disease can vary if the pathogen is affecting host survival, reproduction or both (Hudson et al., 2002). Pathogens that affect host reproduction can have severe negative impacts on host populations which can then influence population survival.

Island populations of wildlife species have a greater risk of extinction than mainland populations because of the small size and naïve nature of most populations (Atkinson et al., 1995). Island populations may also be exposed to a limited number of pathogens during their

evolutionary history compared to mainland species (Atkinson et al., 1995). Loss of genetic diversity in small, isolated populations can also compromise their ability to mount effective immune responses (Atkinson et al., 1995). This may manifest as inbreeding with consequent reduction of the diversity of immunogenetic sequences such as the major histocompatibility complex or toll-like receptors (Grueber et al., 2015; Jamieson, 2015; Grueber et al., 2017). As a result, the combined effects of evolution of island species in an isolated environment with long-term loss of genetic diversity often result in a compromised ability to respond to new pathogens.

Multihost pathogens can infect a range of host species and many such pathogens can be transmitted by multiple species (Woolhouse et al., 2001). This ecological generalism may affect the pathogenicity of such pathogens in alternative hosts compare to their primary host (Woolhouse et al., 2001). A generalist infection may spread between apparently isolated populations through their mutual contact with a more continuously distributed reservoir host. Multihost pathogens are important because they are ubiquitous and are more likely to cause emerging infections in both humans and domestic animals than single species pathogens (Woolhouse et al., 2001). Emerging infectious diseases in endemic and threatened wild animal populations can reduce their population size to a critical level leading to possible extinction. Examples include the impact of avian malaria and poxviruses on indigenous Hawaiian birds (Atkinson et al., 1995), the near eradication of black-footed ferret (*Mustela nigripes*) by canine distemper (Williams et al., 1988), the possible role of chytrid fungi and iridoviruses in declining amphibian populations worldwide (Skerrat et al., 2007; Tompkinson et al., 2015; Berger et al., 2016) and rabies and canine distemper in Ethiopian wolves (*Canis simensis*) (Laurenson et al., 2008).

1.4.1. Causes of disease/mortality of island kākāpō

Kākāpō disease and mortality have been found to have both infectious and non-infectious causes. There are several examples of diseases or mortality associated with infectious causes in kākāpō. A death of a captive reared chick resulted from aspiration pneumonia and enteritis, associated with a pure growth of *Klebsiella ozaenae* (Jakob-Hoff and Gartrell, 2012). Two other neonatal deaths have been due to bacterial aspiration pneumonia with acute, necrotizing, umbilical infection and septicaemia resulting in peracute multifocal hepatitis (Jakob-Hoff and Gartrell, 2012). Potter and Mackenzie (2008) documented that bacterial septicaemia was the most likely cause of death of seven hand reared kākāpō chicks; specific pathogens were not identified related to these cases.

Erysipelas, caused by *Erysipelothrix rhusiopathiae*, is an important infectious disease reported in kākāpō. An outbreak of erysipelas has killed three juveniles in a group of eighteen birds (Gartrell et al., 2005) while a sporadic infection has killed an adult bird. However, comprehensive investigations on infectious agents causing diseases or death in island kākāpō have not been done. A recent aspergillosis outbreak had a devastating impact on the kākāpō population killing nine birds which included two adult females and seven chicks/juveniles. In total, 21 kākāpō were affected and 12 birds have recovered after treatment (DOC, 2020).

Reported examples of non-infectious diseases in kākāpō are aflatoxicosis and heavy metal toxicosis. Aflatoxicosis was diagnosed in an adult kākāpō found dead in the wild with no reported signs of illness. This incident was probably related to a high level of aflatoxins found in some ingredients used for supplementary feeding (Alley et al., 2005). An adult kākāpō from Whenua Hou was diagnosed with lead toxicity, the most likely source for this exposure was the wire mesh used on the pens in which the bird had been temporally accommodated (Jakob-Hoff and Gartrell, 2012).

However, there are also some syndromes affecting kākāpō health for which a cause has not been found yet. In this thesis I have used two current health issues as examples, one of unknown aetiology (exudative cloacitis) one infectious (erysipelas) to illustrate the issues faced when translocating wildlife to non-native habitats.

1.5. Exudative cloacitis

1.5.1. Anatomy of the cloaca

The cloaca provides a common opening for the gastrointestinal tract, urinary tract and reproductive system in birds. Faecal matter and uric acid are excreted through the vent which is the external opening and closing of the vent is controlled by striated sphincter muscles. The cloaca is divided in to three compartments by the complete annular folds: coprodeum, urodeum and proctodeum (Fig 1. 3). The coprodeum, which is the most cranial division of the cloaca, is a continuation of the colon where faeces are stored. The urodeum is the middle segment which contains the openings of the ureters and the genital ducts. The short caudal part which ends in vent is the proctodeum. The opening in the dorsal wall leads to the cloacal bursa of Fabricius. Faecal contamination of the urodeum and proctodeum is prevented by the protuberance of the copro-urodeal folds through the vent during defaecation (Lumeij, 1994).

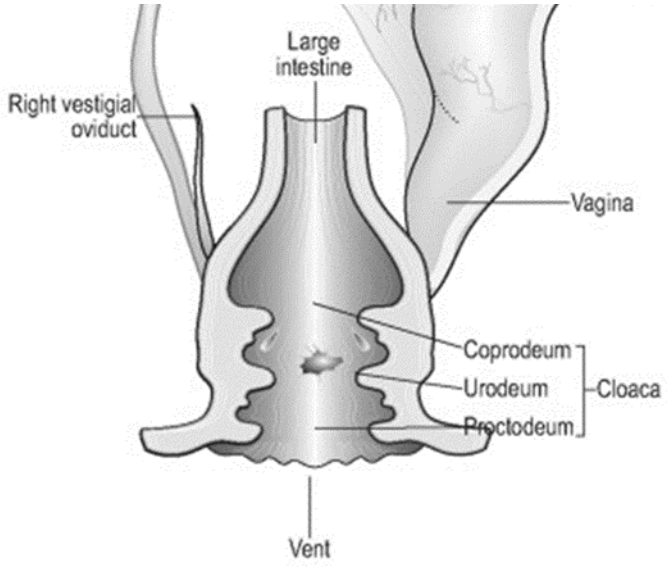


Figure 1.3. Ventral view of the cloaca and vent of a female bird. (Adapted from O'Malley 2005).

1.5.2. Vent gleet in poultry

Inflammation of the cloaca or cloacitis in poultry is commonly known as vent gleet. It is a chronic condition sporadically occurring in female birds during the reproductively active period and can occur in male birds occasionally. In birds with vent gleet, the cloacal mucosa may be covered with a yellow diphtheritic membrane and feathers and skin around the vent can be contaminated with urates and inflammatory exudate (Jorden, 1990; Lumeij, 1994). Clinical signs generally seen with cloacal disorders may include flatulence, tenesmus, soiled pericloacal feathers, protruding tissues from the cloaca and foul-smelling faeces (Lumeij, 1994). In ducks, scaring has been described as a complication of cloacitis which reduces the elasticity and diameter of the cloaca and may prevent egg laying in severe cases (Jorden, 1990). Vent gleet in laying ducks has been associated with drinking dirty water and laying of very large eggs (Lumeij, 1994). Use of local or systematic treatment with an appropriate antibiotic and treatment for the underlying cause is usually effective in treating the condition (Wakenell, 1996). In addition, increase water hygiene has also been recommended as a disease

management option. Control of obesity and the size of eggs through feed reduction programmes have helped to reduce the incidence (Wakenell, 1996).

1.5.3. Vent dermatitis/cloacitis in other bird species

It is believed that vent dermatitis or cloacitis is associated with multiple causes. It is frequently has diagnosed as a sequel to chronic oviductitis, enteritis, digestive and /or renal disorders, and rarely seen as a primary disease (Lumeij, 1994; Jorden 1990). In psittacine birds, cloacitis is seen often in birds with cloacal papillomatosis (Forbes, 2005). Trauma, surgery, or infectious diseases may occasionally result in cloacitis in other bird species (Forbes, 2005).

1.5.4. Infectious causes

Although, they can cause significant secondary pathology, bacteria are recognised as one of the uncommon causes for cloacitis in birds (Taylor, 2016). Abnormal adherence of bacteria to the coprodeal epithelium due to nutritional deficiencies or because of epithelial changes caused by papillomatous disease, or after trauma which might occur during egg laying has been suggested (Taylor, 2016). *Pseudomonas* and *E. coli* infections are also associated with cloacitis secondary to cloacal neoplasia, cloacolith, cloacal prolapses and excessive fat deposits around the vent (Lumeij, 1994). *Neisseria* and *Mycoplasma* spp. and *Candida albicans* have been associated with vent diseases in ganders (Beemer et al., 1973). Similar aetiologies have been suspected in cloacitis in drakes.

1.5.5. Environmental causes

Cloacitis in broiler breeders, is associated with cloacal prolapse and subsequent contamination with faecal materials (Aziz et al., 2010). In addition, the everted cloaca can be injured by objects and materials in the poultry house and subjected to secondary bacterial infections (Aziz et al., 2010).

1.5.6. Exudative cloacitis in kākāpō

In kākāpō, exudative cloacitis refers to an inflammation of the vent margin which is often ulcerated and covered in crusty exudate (Jakob-Hoff and Gartrell, 2011; White et al., 2015). This disease has been sporadically observed, only within the population of kākāpō on Whenua Hou since 2002 (Jakob-Hoff and Gartrell, 2011; White et al., 2015). Cloacitis can lead to reduced fertility in birds like other diseases and disorders of the cloaca and this could significantly affect the population growth of this critically endangered species. This condition can include ulceration and exudation of the muco-cutaneous margin of the cloaca but can also include inflammation and adherent caseous necrotic plaques within the cloaca. The disease is diagnosed by visual observation of the cloacal area. The condition is debilitating and is thought to contribute to both male and female infertility. An extensive pathological and microbiological investigation has failed to identify a consistent primary pathogenic organism involved (Jakob-Hoff et al., 2009; Jakob-Hoff and Gartrell, 2011). White at al., (2015) suggested that the presence of the bacteriophage known to infect enterobacteria in faecal material from a diseased kākāpō as a possible aetiology for this disease. However, only one diseased bird was sampled for the study, severely limiting the ability to support a link between this finding and presence of the disease.

1.6. **Erysipelas**

The disease erysipelas is caused by the Gram-positive bacillus, *Erysipelothrix rhusiopathiae* in diverse species of animals, wild or domestic, from mammals and birds to reptile and fish (Wood, 1992; Bricker and Saif, 1997; Chong et al., 2015). Swine erysipelas is the most prevalent and economically important disease caused by this organism. Disease in swine could be either sporadic cases of acute septicaemia, subacute cutaneous lesions or chronic arthritis (Opriessnig et al., 2010). It also causes economically important disease in commercial poultry, lambs and calves (Wang et al., 2010; Eriksson et al., 2009).

Erysipelas in birds is characterised by either acute, fulminating infections or more rarely chronic infections causing infertility in male birds and reduced egg production in females (Bricker and Saif, 1997). Susceptibility for infection after being exposed to this pathogen is believed to be different in bird species with turkeys being most susceptible (Milne et al., 1997). *E. rhusiopathiae* is a zoonotic pathogen; however, the infections in humans is occupationally related, affecting people in contact with infected animals, their products, contaminated soil, or water (Wang et al., 2010). Although the disease in domestic animals is controlled in most countries through effective management systems, the incidence of swine erysipelas in Japan, China and mid-western USA has increased considerably in recent years (Bender et al., 2011; To et al., 2012; Kwok et al., 2014). *E. rhusiopathiae* has re-emerged after years in Europe with the changes in poultry rearing systems like free range or perchery systems (To et al., 2012; Forde et al., 2016).

1.6.1. *Erysipelothrix rhusipathiae* infections in wild animals

The first case of erysipelas in wild birds was reported from a Hungarian zoological park in 1919 and the organism has been isolated from thrush (*Turdus* spp.), quail (*Coturnix* spp.) and parrots (Wolcott, 2007). Erysipelas is reported as individual cases or isolated clusters of cases in over 60 species of wild birds. This includes, little blue penguin, laughing kookaburra (*Dacelo novaeguineae*), rainbow lorikeets (*Trichoglossus moluccanus*), eclectus parrot (*Eclectus roratus*) and in the endangered Hawaiian crow (*Corvus hawaiiensis*) (Work et al., 1999; Boerner et al., 2004; Opriessnig et al., 2005; Galindo-Cardiel et al., 2012). However, only a few mass mortality events associated with *E. rhusiopathiae* have been reported in wild bird populations (Wolcott, 2007). It is estimated that the *Erysipelothrix* outbreak in Great Salt Lake in Utah, USA killed more than 10,000 eared grebes (*Podiceps nigricollis*) (Jensen and Cotter, 1976). A similar incidence at the same location affected eared grebes and California gulls (*Larus californicus*) in winter 2001 (Wolcott, 2007). An estimated 600 brown pelicans (*Pelecanus occidentalis*) died in another outbreak of erysipelas in California from October 1987 to March 1988 (Wolcott, 2007). A mass mortality event due to *E. rhusiopathiae* killed insectivorous little swift (*Apus affinis*) nesting in South Africa (Van vuuren and Brown, 1990). Erysipelas is one of the infectious diseases recorded in Southern Ocean albatrosses and probably caused outbreaks in mid-1980s (Weimerskirch, 2004).

Wood and Shuman (1981) recorded *E. rhusiopathiae* in many species of wild mammals which includes three species of shrew, four lagomorphs, 20 species of rodents, eight species of carnivores, five species of artiodactyls, three pinnipeds, four dolphins, and two species of primates. Infections with *E. rhusiopathiae* were later reported from brush-tailed phascogales (*Phascogale tapoatafa*) (Barker et al., 1981), bandicoots (*Isoodon macrouris*), bilby (*Macrotis lagotis*) (Eamens et al., 1988) and opossum (*Didelphis virginiana*) (Joachim et al., 1988);

swamp beavers or coypu (*Myocaster coypus*) (Kohler et al., 1987); white tailed deer (*Odocoileus virginianus*) (Bruner et al.,1984); moose (*Alces alces*) (Campbell et al. 1994); roe deer (*Capreolus capreolus*); Canadian Muskoxen (*Ovibos moschatus wardi*) (Kutz et al., 2015), and caribou (*Rangifer tarandus*). Significant mortality events in muskoxen in Canadian Arctic during the period of 2009-2013 is considered as an unusually severe presentation of this opportunistic pathogen in free living wild animals (Forde et al., 2016). Erysipelas is probably underdiagnosed in free living animal populations given the uncertainty of the epidemiology of this disease in the wild.

Captive cetaceans, particularly unvaccinated calves are the marine mammals most susceptible to *E. rhusiopathiae* and erysipelas in these species is manifested as acute septicaemia or multicentric dermatitis (Kinsel et al., 1997; Boerner et al., 2004). Poorly preserved dead fish have been considered as the main source of infections for captive marine mammals (Suer and Vedros, 1988, Higgins, 2000, Boerner et al., 2004). Other likely sources are opportunistic colonization of wounds and flying insects serving as vectors between contaminated areas and humans who then develop erysipeloid (Wood and Shuman, 1981). Kelp gulls (*Larus dominicanus*) which breed on coasts and islands through much of the southern hemisphere were suspected as the source of *E. rhusiopathiae* for wound infections in two southern right whale (*Eubalaena australis*) calves (Fiorito et al., 2016). Gulls may encounter *E. rhusiopathiae* through feeding in landfills where large amounts of waste from the fishing industry and slaughterhouses are dumped (Fiorito et al., 2016) and may act as a reservoir of this pathogen for the southern right whale. *E. rhusiopathiae* on the external mucous layer of fish can survive for long periods and can be a source of bacteria for birds (Wood, 1973). Erysipelas in hooded seals, harp seals and ringed seals has resulted cutaneous lesions similar to those found in swine ersipelas.In addition, *E. rhusiopathiae* was isolated from the tooth /gum margins of

asymptomatic northern fur seals (2/10) (*Callorhinus ursinus*) and northern elephant seals (2/10) (*Mirounga augustirostus*) (Suer and Vedros, 1988; Higgins et al., 2000).

Erysipelothix rhusiopathiae is commonly found in mucus or slime of fish and in fish products (Wood, 1973; Wang et al., 2010). Death caused by haemorrhagic septicaemia associated with *E. rhusiopathiae* was recently reported in Australian eels, *Anguilla reinhardtii* and *A. australis* (Chong et al., 2015). Serotypes and surface protective antigen types (Spa) of marine isolates of *E. rhusiopathiae* from fish and marine mammals were found to differ from terrestrial isolates of *Erysipelothrix* spp. (Shen et al., 2010; Opriessing et al., 2013). However, in an experimental study nearly 70% of the marine isolates tested have induced characteristic gross and microscopical skin lesions in pigs (Opriessing et al., 2013).

1.6.2. Epidemiology of *E. rhusiopathiae* in animals

E. rhusiopathiae is ubiquitous in the environment with a worldwide distribution and is believed to be able to survive in contaminated soil for weeks (Wood, 1992). Although the actual portal of entry in most animal groups has not been established, transmission of *E. rhusiopathiae* is thought to be either through the gastrointestinal tract by the intake of contaminated food or water or through breaks in the mucous membranes or skin (Bricker and Saif, 2013). *E. rhusiopathiae* is maintained in the environment because of the subsequent dissemination of the organism by asymptomatic carriers (Brooke and Riley, 1999). *E. rhusiopathiae* is resistant to desiccation and to various chemicals (Brooke and Riley, 1999). It can survive meat processing methods like smoking and pickling, as well as in frozen or chilled meat, dried blood, decaying carcasses, and fish meal (Reboli and Farrar, 1989). Fish meals or fish were suggested as the source of infection for some avian species. An outbreak has been reported in racing pigeons after ingestion of composted waste (Cousquer, 2005). There is no evidence to support vertical

or egg transmission of the bacteria. Control of the infections is challenging because of the organism's ubiquitous nature in the environment and the ability to survive asymptomatically within a reservoir host.

Erysipelothix species can be isolated from the cutaneous slime of both fresh and saltwater fish and can persist for long periods in marine locations (Wang et al., 2010). In addition, these bacteria may persist on insects, molluscs, and crustaceans (Brooke and Riley, 1999, Wang et al., 2010). Rodents were found to be infected with the organism and suggested as a possible source of infection for other animals (Brooke and Riley, 1999). Poultry red mite, *Dermanyssus gallinae* has been studied as a potential vector for *E. rhusiopathiae* and similar serotypes of the bacterium have been recorded from infected hens and red mites from the farms studied (Chirico et al., 2003). The role of vectors in the epidemiology of *E. rhusiopathiae* remains unclear; however, they may play an important role in the transmission cycle of the bacterium both due to the diverse host range of the bacterium and the sporadic nature of outbreaks.

The prevalence of erysipelas among commercial poultry and wild birds is not well understood. Two field studies reported the prevalence of antibodies to *E. rhusiopathiae* as 5.5% (200 samples) and 39.8% (545 samples) among poultry (Takahashi et al., 2000; Kurian et al., 2012). Stephenson & Berman (1978) estimated that 30-50% of healthy swine harbour *E. rhusiopathiae* in their tonsils making them the most important reservoir for other animals. Infected pigs can transmit the organism indirectly by contaminating soil, bedding, and water. As antibodies against *E. rhusiopathiae* are recorded in healthy cattle, they are considered to be a potential source of infection to other animals and humans (Hassanein et al., 2000). Shimazaki et al., (2005) reported 5% prevalence of antibodies against *E. rhusiopathiae* among a stray dog population in Japan.

Seroprevalence of antibodies against *Erysipelothrix* has been investigated in several species of wild animals. A serological study on Tasmanian wildlife showed positive results for antibodies against *E. rhusiopathiae* in 2.7% fallow deer (*Dama dama*) and 11.1% forest ravens (*Corvus tasmanicus*) (Munday et al., 1972). Prevalence of antibodies against *E. rhusiopathiae* in wild boar (*Sus scrofa*), Yezo deer (*Cervus nippon yesoensis*) and Japanese deer (*Cervus nippon*) in Japan was 66.7%, 3.6% and 23.1% respectively (Shimizu et al., 2016). However, two studies on European wild boar reported 5.3% and 5.0% prevalence of antibodies against *E. rhusiopathiae* (Vicente et al., 2002; Closa-Sebastià et al., 2011) while another study resulted in a comparatively higher prevalence (15%) in the same species (Boadella et al., 2011).

It is believed that more than 30 species of wild birds and 50 species of wild mammals can harbour *E. rhusiopathiae* and act as a reservoir in natural ecosystems (Wolcott, 2007). The source of infection is not clearly understood in many situations. Stress or various other factors which weaken the host defence mechanisms may lead to clinical disease in healthy carriers (Barker et al., 1981). In agricultural systems, rearing of animals in premises previously used for swine farming, and stress during the growing stages have been considered as predisposing factors for infections in most outbreaks that have occurred in domestic birds (Griffiths and Buller, 1991). In wild birds, extreme weather conditions during winter are suspected to predispose susceptible birds to infection with *E. rhusipathiae* (Wolcott, 2007).

1.6.3. Diagnosis of *E. rhusiopathiae* in animals

1.6.3.1. Clinical signs

Clinical signs of erysipelas are described mostly for commercial turkey flocks with the sudden loss of one or several birds. Some animals may be lethargic with an unsteady gait just before

death. Cutaneous lesions may be seen in some animals. In some animals with endocarditis, gradual emaciation, weakness, and signs of anaemia have been noticed (Bricker and Saif, 2013). In affected chickens, ducks, geese, pheasant and quail, the main clinical signs include general weakness, depression, diarrhoea, and sudden death (Bricker and Saif, 2013). Except occasional lethargy, there has been no specific clinical signs associated to *E. rhusiopathiae* infection in wild birds. The death of one or more birds would be the first clinical signs in acute septicaemia (Wolcott, 2007).

1.6.3.2. Pathology

Common gross pathological lesions described in turkey are generalised congestion; fat degeneration on the thigh; fat degeneration and haemorrhage in pericardial fat. Petechial haemorrhages were observed in abdominal fat and heart muscle. The liver, spleen and kidney were enlarged, friable and possibly mottled. Gastrointestinal lesions may include thickening and ulceration of the proventriculus and gizzard wall, small yellow nodules in the caecae, catarrhal or sanguino-catarrhal enteritis. In some birds, the only lesions could be slight catarrhal enteritis and petechial haemorrhages in the heart fat (Bricker and Saif, 2013).

Pulmonary haemorrhage, oedema of the lungs and intestine with clotted blood in bronchi, and mucosal petechiation of the proximal third of the small intestine with clotted blood in the lumen has been observed in little blue penguins that have died from erysipelas (Boerner et al., 2004). Gross lesions described in rainbow lorikeets (*Trichoglossus moluccanus*) and eclectus parrots (*Eclectus roratus*) include moderately atrophied adipose tissues, moderate to severe dehydration, severe hepatomegaly, a large area of dark red discoloration in the left ventricle, mild accumulations of yellowish transparent fluid in the coelomic and pericardial cavities, mild splenomegaly, and mild bilateral thyroid enlargement (Galindo-Cardiel et al., 2012). Gross

pathology of the kākāpō chicks that died from erysipelas, were congested, slightly swollen and mottled livers and congested and swollen spleens, with variable degrees of enteritis. All birds were in good body condition with good fat reserves and food still present in the crop (Gartrell et al., 2005).

1.6.3.3. Morphological and biochemical identification

Prominent histopathological features are the vascular changes with engorgement of blood vessels and sinusoidal channels in almost all organs (Bricker and Saif, 2013). Usually, aggregation of bacteria accompanied by fibrin thrombi are seen in capillaries, sinusoids and venules. Severe vascular impairment may be evident by oedema and haemorrhages in lungs and heart (Bricker and Saif, 2013). In acute cases of erysipelas, generalised parenchymal cell damages are usually obvious in liver, spleen and kidney. Diffuse congestion of the liver with focal infiltrations of lymphocytes and plasma cells around many blood vessels was observed in kākāpō chicks died from erysipelas (Gartrell et al., 2005). Aggregation of bacteria was seen within the cytoplasm of the majority of Kupffer cells and the cytoplasm of some endothelial cells. In addition, diffuse lymphoid hyperplasia and accumulation of histocytes filled with bacteria was seen in spleen. Similar bacteria were observed in multiple organs including kidney, heart, proventriculus and small intestines (Gartrell et al., 2005).

Most of the field cases of erysipelas have been diagnosed with necropsy examination in combination with identification of the causal organisms. Demonstration of the presence of clumps and segregated gram-positive, beaded, slender, and pleomorphic rods in smears prepared from liver, spleen, heart blood, or bone marrow provide a rapid tentative diagnosis. Isolation of the organism from birds that have died of the disease occurs more frequently than the isolation of the bacteria from sick birds that are killed (Bricker and Saif, 2013).

Packer's medium which contains sodium azide-crystal violet and the tryptose phosphate broth medium with 5% horse serum plus kanamycin, neomycin, vancomycin, and novobiocin are two inhibitory medias to isolate *E. rhusiopathiae* (Bricker and Saif, 2013). For primary isolation of the organism, inoculating the plates containing 5% blood agar and Packer's medium followed by incubation in an atmosphere of 5–10% CO2 or reduced oxygen is advised. *E. rhusiopathiae* selective media have been described by Shimoji et al. (1998) containing tryptic soy broth, ethidium bromide, and sodium azide.

Morphological and biochemical identification of *Erysipelothrix* species is based on Gram's stain, cultural morphology, motility, haemolytic characteristics and biochemical properties. Colony morphology is described as clear, circular and very small, with a diameter of 0.1–0.5 mm after 24 h incubation at 37° C, or 0.5–1.5mm after 48 h. They could be either smooth colonies with a bluish, transparent, and convex appearance or rough colonies which are larger and have a flat rough surface with irregular edges (Jones, 1986). Microscopically, smooth colonies contain small, slightly curved, slender rods with rounded ends while rough colonies have long filaments rods up to 60 µm or more, in chains. Alterations in pH and incubation temperature can result in morphological differences. *Erysipelothrix* species are relatively inactive biochemically and negative for catalase, oxidase, methyl red, indole, esculin, and nitrate. The majority of species produce hydrogen sulphide (H2S) as demonstrated in triple sugar iron (TSI) agar (Ewald, 1981; Reboli and Farrar, 1989). Acid is produced from glucose, fructose, galactose and lactose but not from maltose, xylose, and mannitol. The ability of *E. tonsillarum* to ferment sucrose differentiates it from *E. rhusiopathiae* (Wang et al., 2010).

Great serological, biochemical and antigenic variation has been demonstrated between the strains of *E. rhusiopathiae* (Takahashi et al., 1987). Categorising different isolates of *E. rhusiopathiae* strains is attempted using different tools. Serotyping is a method frequently used which differentiates various strains of *E. rhusiopathiae* based on the peptidoglycan antigens of

the bacterial cell wall. This technique involves testing for agglutination with specific antisera (Kucsera, 1973; Wang et al., 2010). Serotyping is important as a diagnostic technique in diagnosing *E. rhusiopathiae* infection (Pal et al., 2010; Forde et al., 2016). horizontal exchange of capsule-specific genes in many bacterial species reduces its use as a technique to detect evolutionary relatedness among isolates. In addition, immunogenic proteins, surface protective antigen (Spa) has been used recently to differentiate between *E. rhusiopathiae* strains (Jansen` et al., 2015).

1.6.4. Molecular identification and classification of *E. rhusiopathiae* isolates

Polymerase Chain Reaction (PCR) has been used to identify E. *rhusoipathiae* in tissue samples of swine, poultry and other animal species. A direct, rapid, sensitive method was developed to detect *Erysipelothrix* spp, by Makino et al., (1994) based on bacterial DNA sequence encoding its 16S ribosomal RNA. Shimoji et al., (1998) developed a broth cultivation-PCR technique for rapid diagnosis of erysipelas using primers to amplify a 937-bp DNA region from *E. rhusiopathiae*. A PCR technique designed by Takeshi et al. (1999), using 4 specific primer sets was able to identify *E. rhusiopathiae, E. tonsillarum*, and strains representing serotypes 13 and 18. A PCR technique has also been used to diagnose *E. rhusiopathiae* using the DNA extracted from formalin-fixed, paraffin-embedded liver tissues (Hennig et al., 2002) and for rapid diagnosis of *E. rhusiopathiae* in chicken blood (Harada et al., 2009). Restriction fragment length polymorphism analysis (Ahrne et al., 1995) and pulsed field gel electrophoresis (Okatani et al., 2001) are two molecular methods alternatives to PCR and DNA sequencing that have been used to diagnose and characterize *E. rhusiopathiae* isolates. In addition, the use of MALDI-TOF MS (Matrix-assisted Laser Desorption/Ionization with Time-of-flight Mass

Spectrometry) for diagnosis of *E. rhusiopathiae* in tissue samples, blood and tissue fluids has been demonstrated recently (Principe et al., 2016; Asimaki et al., 2017). This technique allows rapid and accurate identification of bacteria within few hours and it is increasingly being used for diagnosis of *E. rhusiopathiae* in clinical diagnosis and in research studies.

Several studies have reported findings from complete genome sequencing of different *E. rhusiopathiae* strains (Ogawa et al., 2011; Kwok et al., 2014; Tang et al., 2016). These studies recognised the *E. rhusiopathiae* genome as one of the smallest genomes in phylum *Firmicutes*. Complete genome sequencing of *E. rhusiopathiae* has revealed that it has features similar to other Gram-positive bacteria. This includes a complete set of peptidoglycan biosynthesis genes, two-component regulatory system and various virulence factors such as capsule and adhesins which are associated with the cell wall (Ogawa et al., 2011). However, gene reduction with lack of some DNA repair enzymes and genes to produce fatty acids and some amino acids is suggestive of a strong link with either the host or environment of this pathogen (Ogawa et al., 2011).

Investigation of global genomic diversity and the population structure of *E. rhusiopathiae* has been attempted using 83 newly sequenced isolates representing countries from North America, South America, Europe, Asia and Australia (Forde et al., 2016). This research suggested evidence for recombination of the bacterial genome throughout its evolutionary history. It also recognized three major clades *E. rhusiopathiae* across multiple continents and in livestock and wildlife host species (Forde et al., 2016). This study has not found consistent differences in gene content between isolates from different host species, geographic locations or clades suggesting limited host association of *E. rhusiopathiae*. In an experimental infection Opriessnig et al., (2013) has shown that multiple species are susceptible to the same strains of *E. rhusiopathiae.* These studies provide a useful baseline for the planned study of *E. rhusiopathiae* in New Zealand wildlife.

1.6.5. Erysipelas in kākāpō

In July 2004, an outbreak of erysipelas affected the success of translocation of a group of juvenile kākāpō from Whenua Hou to Chalky Island killing three of the nineteen birds translocated (Gartrell et al., 2005). Eighteen young birds and one adult bird were translocated in two batches, 5 days apart. Pre-translocation health screening performed included complete blood counts and faecal cultures for the bacterial species *Yersinia, Salmonella,* and *Campylobacter* (Brangenberg et al., 2003). After the translocation, all the birds were monitored using radio-transmitters. Following low levels of activities, three birds from both batches died within 72 hours of transport between two islands. Gross and histopathological and bacteriological diagnoses were similar in all three birds and confirmed bacterial septicaemia, and *E. rhusiopathiae* was isolated from liver, kidney, and spleen of all the birds (Gartrell et al., 2005).

This was the first incidence where *E. rhusiopathiae* was diagnosed in kākāpō and the first report that described the effect of an infectious disease on translocation of this critically endangered parrot (Gartrell at el., 2005). Sporadic infections have subsequently been diagnosed as a cause of death for kākāpō and other endangered New Zealand fauna on offshore islands. Seabirds were considered to be the most likely source of infection for the kākāpō as both Whenua Hou and Chalky Islands have a diverse fauna of marine and terrestrial birds. Ulnar bone marrow of the fifteen seabird carcasses recovered from Whenua Hou at the time of the kākāpō mortalities were subjected to bacteriological diagnosis and ten out of fifteen samples resulted in positive cultures of *E. rhusiopathiae.* Surveillance of local fauna and ectoparasites has been recommended in order to learn more about the epidemiology of the disease (Gartrell et al., 2005) and aid in securing these island sanctuaries for endangered species conservation

(Gartrell et al., 2005; Jakob-Hoff and Gartrell, 2012). However, to date these studies have not been performed.

After the outbreak, sixteen remaining kākāpō were recaptured and treated with antibiotics, and vaccination of all kākāpō except six adult males was initiated with a commercial killed bacterin used for turkeys. Vaccination was followed by boosters at 1 and 12 to 16 months. Serum antibody levels for *E. rhusiopathiae* were tested at the time of vaccination using an antibody enzyme-linked immunosorbent assay (ELISA). The results indicated that most of the adult birds had serum antibodies against *E. rhusiopathiae* (Livingston et al., 2013). Currently, the vaccine is only used for nestlings and hand reared chicks, although kākāpō in island ecosystems are exposed to *E. rhusiopathiae* in their natural environment (Jakob-Hoff and Gartrell, 2012). The epidemiology of *E. rhusiopathiae* in the wild is hardly known, although, the organism has been isolated from many species of wild animals and has caused outbreaks in several species (Wolcott, 2007). Understanding the epidemiology of this pathogen is important for preventing future outbreaks and for conservation of kākāpō.

1.7. Objectives of the thesis

What are the health risks associated with the conservation of kākāpō (*Strigops habroptilus*) on offshore islands? To answer that question, three objectives were established that focus on the two most important health problems affecting kākāpō in offshore islands: exudative cloacitis and erysipelas. The first objective was to perform an epidemiological study to investigate the environmental factors contributing to the initiation of cloacitis on Whenua Hou (Chapter 2). It was anticipated that this initial data mining study would highlight gaps in the data that need to be more fully investigated on the island, and suggest more targeted pathological, environmental

and/or microbiological investigations. This project is part of a wider research effort to investigate exudative cloacitis in kākāpō. Given the similarities of this disease seen in kākāpō to the vent gleet seen in poultry, this project will focus on an epidemiological approach to the disease.

The second objective was to perform targeted surveys of pelagic and coastal seabirds that visit two of New Zealand's offshore islands used for kākāpō conservation with the goal of determining the prevalence of *Erysipelothrix rhusiopathiae* (Chapter 3). The third objective was to use whole genome sequencing to investigate the reservoirs and diversity of *Erysipelothrix rhusiopathiae* in some threatened New Zealand birds (Chapter 4). A better understanding of the factors affecting the health and wellbeing of the kākāpō may provide essential information for their conservation and management in offshore islands.

This thesis is presented as a series of 5 chapters as an introduction, three research chapters and a general discussion. Three research chapters will be presented as separate manuscripts prepared for publication in peer reviewed journals.

Chapter 2

An epidemiological investigation of exudative cloacitis in kākāpō (*Strigops habroptilus*) on Whenua Hou/Codfish Island

2.1. Introduction

Disease may pose a significant threat for the survival of endangered wildlife populations, reducing their population size to a critical level (Van Riper III et al., 1986; Berger et al., 1998; Daszak et al., 1999). Wildlife disease investigation has become an important component in the management of free-ranging wildlife in many countries, sometimes for the benefit of human or domestic animal health (Morner et al., 2002), but increasingly for the conservation management of the affected wildlife. These investigations can help to detect ongoing processes that affect both individual and population health and to confirm the presence or absence of certain disease-causing agents (Morner et al., 2002). However, there is a scarcity of information on the causes, history and epidemiology of many important wildlife diseases (Morner et al., 2002; Wobeser, 2007). Unless there are significant morbidity or mortality events, it is not easy to identify the occurrence of disease in wild animal populations and some diseases may go unnoticed despite having a significant impact on fitness or reproductive success.

The kākāpō (*Strigops habroptilus*) is a critically endangered flightless, large (1.5-4 kg), nocturnal parrot endemic to New Zealand with an estimated total population of 205 adult birds as at March 2021 (DOC, 2021). They were widespread and abundant across the New Zealand mainland before human settlement (Brunner, 1848; Scarlett, 1979: Reischek, 1884; Bergner et al., 2016; Dussex et al., 2018) but are now locally extinct from the two main islands of New Zealand (Higgins, 1999). Kākāpō are unique parrots having a lek breeding system where male birds defend small, clustered breeding territories and they breed only once in 2-5 years (Elliott et al., 2001). The kākāpō population declined primarily since European settlement of New Zealand due to predation, and combined effects of habitat loss and habitat modification (Bergner et al., 2016). During 1970s and early 1980s, intensive research and conservation

management of kākāpō was started from two remnant populations of birds found from Fiordland and southern Rakiura of New Zealand (Clout and Merton, 1998; Elliott et al., 2001).

As a result of intensive management programmes over the last two decades, the kākāpō population has been increased from 51 birds in 1995 (Powlesland et al., 2006) to its current size, and breeding populations are managed on three predator-free offshore islands, Whenua Hou/Codfish Island, Te Hauturu-o-Toi /Little Barrier Island and Pukenui/Anchor Island. These three populations currently require intensive management which include safety and health observations (Jansen, 2006; Clout, 2006) and regular supplementary feeding with a pelleted parrot food (Harrison's Adult Lifetime Coarse Pellets and Harrison's High Potency Coarse Pellets, Harrison's Bird Foods, Brentwood, TN, USA). Management of the kākāpō is guided by the Kākāpō Recovery Project (Neill, 2008).

In recent years, a disease called exudative cloacitis has emerged in the kākāpō held on Whenua Hou and the first case was identified in 2002 (Jakob-Hoff and Gartrell, 2011; White et al., 2011). In kākāpō, exudative cloacitis refers to an inflammation of the vent margin and/or cloaca which is often ulcerated and covered in crusty exudate (Jakob-Hoff and Gartrell, 2011; White et al., 2015). The disease is diagnosed by the clinical signs of dermatitis in the vent and cloacal area. The condition is debilitating and can lead to reduce fertility in birds like the other diseases and disorders of cloaca, and this could significantly affect the population growth, affecting the conservation of this critically endangered species.

The factors responsible for initiating exudative cloacitis in kākāpō are not known despite extensive pathological and microbiological investigations (Jakob-Hoff et al., 2009; Jakob-Hoff and Gartrell, 2012). The disease is geographically limited to Whenua Hou and this support the hypothesis that the aetiology of the exudative cloacitis may be due to some as yet unidentified

environmental factors in the kākāpō habitat. A similar condition seen in poultry called vent gleet, is associated with irritation of the vent by old, heavily faecally-contaminated litter and/or diarrhoea. It is suggested that this condition in poultry is often not involved with any primary pathogen but is instead associated with a combination of infectious and non-infectious causes such as bacterial infections, mycotic infections, neoplasia, cloacolith, prolapse, and excessive fat deposits around the vent (Crosta et al., 2003).

It is possible that the exudative cloacitis in kākāpō is associated with some island-specific factors that lead to irritation, inflammation and ulceration of the muco-cutaneous border of the cloaca. The initiating causes of the exudative cloacitis may be masked by secondary infection and inflammation associated with the contact of faeces, urine and urates on ulcerated mucosa. Identification of possible contributing causes is critical in deciding on treatment and prevention of this condition in kākāpō.

The description of disease frequency in individuals, and in time and space in different populations, is a key method used in epidemiology to investigate a disease outbreak (Wobeser, 2007). The aim of this research was to perform an epidemiological study to investigate the environmental factors contributing to the initiation of exudative cloacitis in kākāpō by 1) producing and describing a case series, 2) mapping the geographic distribution of exudative cloacitis cases, 3) investigating the chemical characteristics of kākāpō roosting sites and 4) assessing the effects of climatic factors on the incidence of exudative cloacitis each year.

2.2. Materials and Methods

2.2.1. Case series

Kākāpō health data was obtained from a surveillance programme managed by the Kākāpō Recovery Team, Department of Conservation (DOC), New Zealand. This data collection contained all the data collected during annual transmitter changes, health checks and any other opportunistic observations made on individual kākāpō during field visits. Usually, health examinations of kākāpō were performed once a year, individuals with a history of exudative cloacitis have been examined four times per year in many years. In 2017, all the kākāpō were examined four times per year for possible signs of exudative cloacitis. Variables included in the dataset were the ID of the kākāpō, sex, age, date of observation, location data as easting and northing (NZTM- New Zealand Transverse Mercator) and exudative cloacitis status at the time of observation. The exudative cloacitis case definition used for the surveillance data was the presence of inflammation, ulcerations of the mucocutaneous margins of the cloaca and/or accumulation of exudates around and within the cloaca (Jakob-Hoff and Gartrell, 2011). A grading scheme to categorise exudative cloacitis in kākāpō has been developed by the DOC. Depending on the severity of the lesion on the cloaca and/or the vent, cases have been graded as mild, moderate and severe (Fig 2.1). All new kākāpō cases (incident cases) diagnosed with exudative cloacitis between 2002 and 2017 were selected for the case series. Descriptive statistical analysis was done using R version 3.4.1 (RStudio Team, 2016) to identify the trend of disease occurrence over the years and to describe the birds diagnosed with the disease by age, sex, location and if they belonged to the founder population or not.

Island populations of kākāpō are comprised of individuals from the founder population which included the last birds found from Fiordland and Rakiura and the birds hatched on offshore islands. Between 1987 and 1997, 30 kākāpō from Rakiura have been translocated to Whenua

Hou. The survival rate of those kākāpō was as high as 98 % per year and 26 out of original 30 birds were known to survive to 1997 (Clout and Merton, 1998). At the time of this study, 23 kākāpō on Whenua Hou were from the founder population. The age descriptor for older kākāpō of unknown age have been calculated assuming a minimum of 10 years old on the date of their first discovery. Age groups as chicks (0-149 days), juveniles (150 days -5 years) and adults (over 5 years) have been determined by considering the average age of fledging and sexual maturity of kākāpō on offshore islands (Farrimond et al., 2006b; Eason et al., 2006).

2.2.2. Spatial distribution and investigation of clustering of the kākāpō exudative cloacitis

The distribution of exudative cloacitis cases was mapped for the period 2002-2017, within Whenua Hou and used to describe the pattern of the disease. Point maps were developed in R version 3.4.1 (RStudio Team, 2016) and QGIS (Quantum GIS) version 3.8.1(QGIS Development Team) using the geographic locations of the individual kākāpō on Whenua Hou each year for diseased and healthy birds. Point maps were created using packages rgdal (Bivand et al., 2019a), ggmap (Kahle and Wickham, 2013), ggplot2 (Wickham, 2016), epiR (Stevenson et al., 2013), ClassInt (Bivand et al., 2007), RcolorBrewer (Neuwirth, 2014), plyr (Wickham, 2011), rgeos (Bivand et al., 2019c), sp (Bivand et al., 2013) , maptools (Bivand et al., 2019b), raster (Hijmans and van Etten, 2012), spatstat (Baddeley et al., 2015), and scales (Wickham, 2017) for R.

Severity score for the lesions on the vent

Grade 1 -normal may be clean or have a layer of feacal contamination which is easily washed off.
Grade 2 - (Mild) Mild ulceration or abscesses with or without waxy exudate. Exudate is firmly attached to skin, causing it to break the skin beneath and pull away
Grade 3 –(Moderate) Moderate to large ulcers or abscesses (one or more) and or raised waxy exudate (with or without crusting), firmly attached at some point to the vent skin.
Grade 4 –(Severe) raised hard crust covering most or all of vent. Waxy exudate beneath, firmly attached to skin which may be broken and pulling away. Deep wounds may cause bleeding and scabbing. Usually a lot of faecal contamination, secondary infection and smelly.

Severity score for the lesions on the cloaca

Grade 1- normal healthy cloaca
Grade 2 (mild)- include mildly inflamed cases and
Grade 3 (moderate)- include those birds with moderate inflammation and/or ulceration or exudate (attached to the mucosa), generally extending from the vent, but mostly confined to the muco-cutaneous junction.
Grade 4 -(severe) cases are inflamed at the vent, possibly with bleeding or friable tissues, with or without fissures, ulceration or exudate

Figure 2.1. A picture showing the kākāpō exudative cloacitis severity score from 1-4 developed by the DOC based on the severity of the lesion on the vent and cloaca.

To analyse the spatial point pattern and identify a possible spatial clustering of cases, the K-function was calculated for both case and healthy birds. The difference between these two K-functions was examined as it provides an effective summary of spatial dependence over a large distance (Diggle, 1995; Carpenter, 2001; Bohm et al., 2008; Mountrakis and Gunson, 2009). The K-function of a point pattern is defined as the expected number of further points within a distance 'h' of an arbitrary point, divided by the overall density of points (Ripley, 1976). The K-function analysis was performed using packages fields (Nychka et al., 2017), SpatialEpi (Chen et al., 2018) and gridExtra (Auguie and Antonov, 2017) for R. To provide 95% confidence intervals for this function, Monte Carlo simulations of the Poisson point pattern process were used (Harrison, 2010).

2.2.3. Investigation of chemical characteristics of kākāpō roosting sites

To investigate the environmental characteristics of the kākāpō roosting sites in Whenua Hou, three chemical parameters of the topsoil layer of the roosting sites were examined. In addition, the same chemical parameters were analysed from the topsoil layer of the general areas where kākāpō roosts were not found on Whenua Hou and Te Hauturu-o-Toi as control samples. Sample collections were conducted from August 2017 to July 2018 in Whenua Hou and during October 2018 in Te Hauturu-o-Toi. Samples were transported in polyethylene bags and stored in 4^{o}C until analysed to determine pH, ammonia, and moisture contents. Prior to analysis, each sample was mixed thoroughly and sieved using a 2 mm sieve.

In Whenua Hou, 49 soil samples were collected from the kākāpō roosting sites, which included six samples from the roosts of diseased kākāpō at the time of collection. Twenty-seven and 32 soil samples were collected from the general areas of the Whenua Hou and Te Hauturu-o-Toi, respectively (Fig 2.3). Moisture content of the soil samples was measured gravimetrically, after oven drying a known amount of sample to a constant weight for 12 hours at 105ºC and calculating the weight loss of the original sample after drying (Stumpy and Binkley, 1993). The pH of the roost materials was measured in deionized water using a pH meter (HANNA Instrument, HI 2211 pH/ORP meter). Ten grams of soil was mixed with 25 ml of deionized water and the pH of the solution was measured after 12 hours. Exchangeable ammonia (NH_4) that can be extracted at room temperature with a neutral potassium (K) salt solution is measured. Ammonia was extracted by shaking with 2.0M KCl as described in Maynard and Kalra (1993) and Keeney and Nelson (1982). The amount of ammonium in the extract was determined by colourimetric techniques (Maynard and Kalra, 1993). Box plots were made to visualise the distribution of moisture content (%), pH and ammonium (ppm) in kākāpō roost and general areas of the Whenua Hou and general areas of the Whenua Hou and Te Hauturu-o-Toi using the R packages ggplot2 (Wickham, 2016) and EnvStats (Millard, 2013).

The mean and confidence intervals for the pH, moisture content and ammonia were calculated as a summary statistic in R version 3.4.1 (R Studio Team, 2016). Soil moisture content, ammonia and pH comparisons were made by Welch's t-test in R version 3.4.1.

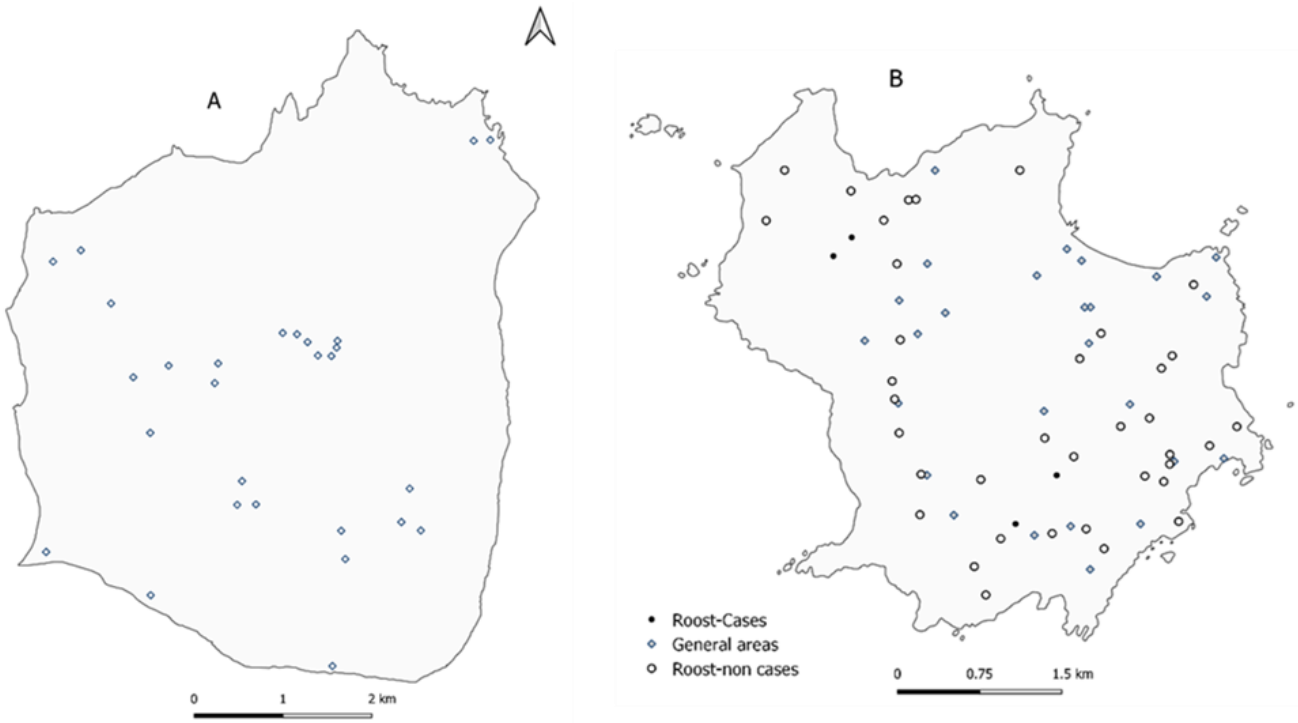


Figure 2. 3. Sample collection sites to evaluate the environmental characteristics (pH, moisture and ammonia) in kākāpō roosting sites and general areas of A) Te Hauturu-o-Toi and B) Whenua Hou.

2.2.4. Investigation of the association of annual climatic factors with the occurrence of exudative cloacitis

Climate data for Whenua Hou could not be collected for the time period of 2002-2017 because the island had no functioning weather station for that period. Therefore, the data recorded from the nearest located weather station on Stewart Island was used for this analysis. Available data for wind direction, wind speed, wind run, relative humidity, rainfall, dry temperature, wet temperature, annual maximum and minimum temperatures of Rakiura (1987-1993 and 2002-2017) and for Whenua Hou (from 1987-1993) were obtained from New Zealand's National Climate Database (National Institute of Water and Atmospheric Research

(NIWA)). Prior to extracting the relevant weather data for Rakiura, correlation between the available weather data of Whenua Hou (from 1987-1993) with that of the Rakiura was examined through examination of scatterplots and a Pearson correlation.

Correlation between the data from Rakiura and available data from Whenua Hou were analysed using library readr (Wickham, 2018) in R. In order to examine the effect of climate on the number of exudative cloacitis cases observed each year, a negative binomial regression analysis was performed in R version 3.4.1 using the MASS package (Venables and Ripley, 2002). The number of exudative cloacitis cases per year was used as the outcome variable, and the population at risk as an offset. Climate variables that had a Pearson's correlation of 0.85 or more between measurements on Rakiura and Whenua Hou were retained, and their Rakiura annual average used as independent variables in the model. Incidence risk ratio was calculated by exponentiating the coefficient and the boundaries of the confidence interval.

2.2. Results

2.2.1. Case series

There were differences in the number of kākāpō that had undergone health examination in Whenua Hou in each year (Fig 2 .4). Exudative cloacitis was diagnosed in 22 kākāpō over the period 2002-2017, and all cases occurred on Whenua Hou, which included 12 male and 10 female birds. No cases of exudative cloacitis were recorded on any other site used to hold kākāpō. The affected birds belonged to all age groups; 13 adults, 7 juveniles and 2 chicks. Nine out of 22 cases were from the older founder birds that had established the population of kākāpō on Whenua Hou. Occurrence of slow progressing sporadic cases of different severity were

observed over time, with an increased number of cases detected during the years 2015-2016.

No cases were detected in some years during the study period (Fig 2.4 and Fig 2.5).

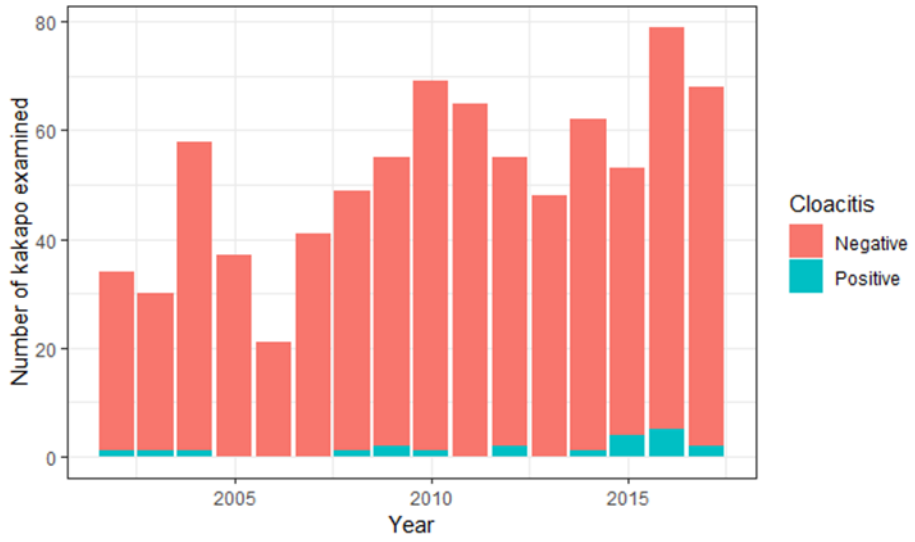


Figure. 2. 4. Number of kākāpō on Whenua Hou examined for exudative cloacitis from 2002-2017 and the number of birds found with and without the disease each year.

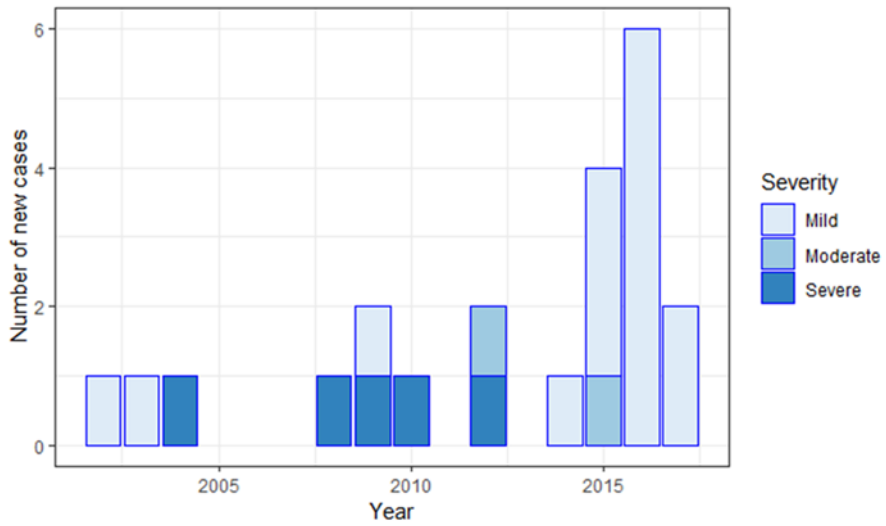


Figure 2. 5. Number of new cases of exudative cloacitis in kākāpō observed with varying degree of severity in Whenua Hou from 2002-2017.

2.3.2. Spatial distribution of exudative cloacitis of kākāpō (2002-2017)

As shown on the point map, kākāpō with exudative cloacitis were distributed throughout Whenua Hou (Fig 2.6) and the disease was recorded in all areas of the island where the kākāpō population is distributed. The observed K patterns (Fig 2.7) of the distribution of diseased and healthy kākāpō were compared to the theoretical K curve under complete spatial randomness. According to these two graphs, both the diseased and the healthy population of kākāpō are clustered within all distances. Kākāpō with exudative cloacitis are less clustered, or more spread out, than the healthy kākāpō population (Fig 2.8).

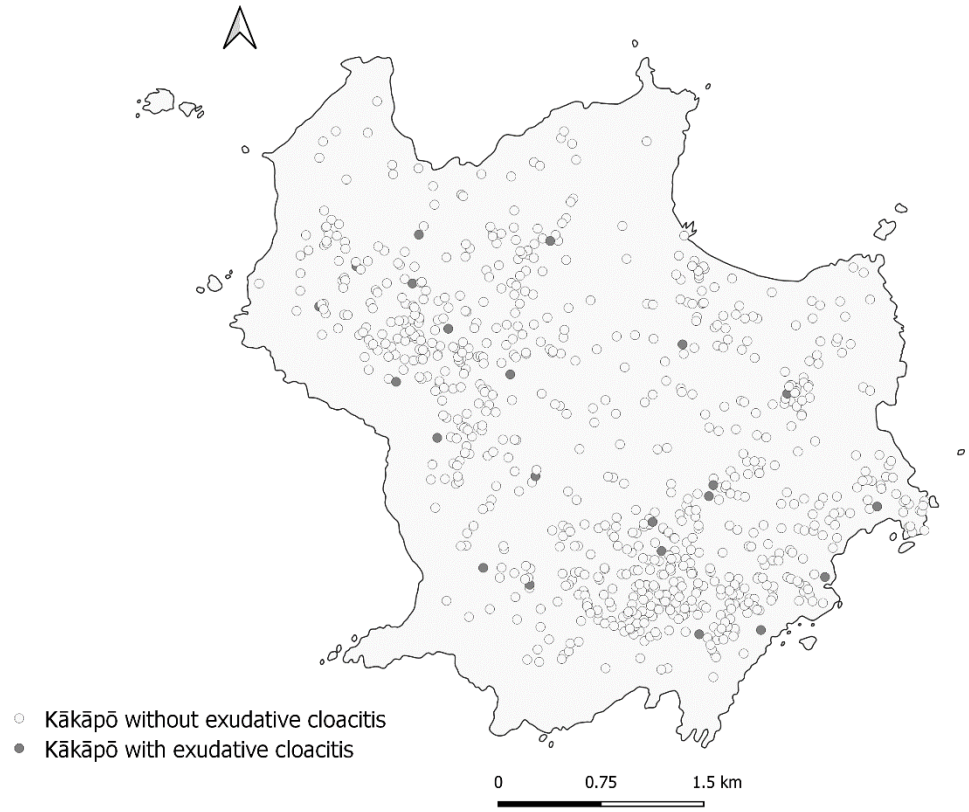


Figure 2. 6. Spatial distribution of kākāpō and cases of exudative cloacitis in Whenua Hou over the years from 2002-2017.

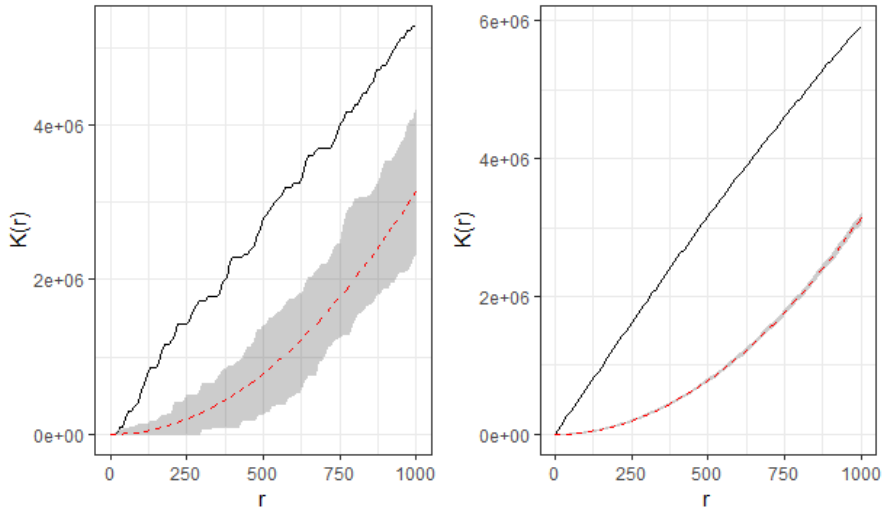


Figure 2. 7. K function graphs illustrating the spatial distribution pattern of the kākāpō without (left) and with (right) exudative cloacitis in Whenua Hou, 2002-2017. Observed spatial pattern is indicated by the solid line and the expected pattern under Complete Spatial Randomness (CSR) is indicated by the red dashed line. The shaded area indicates the 95% confidence envelope.

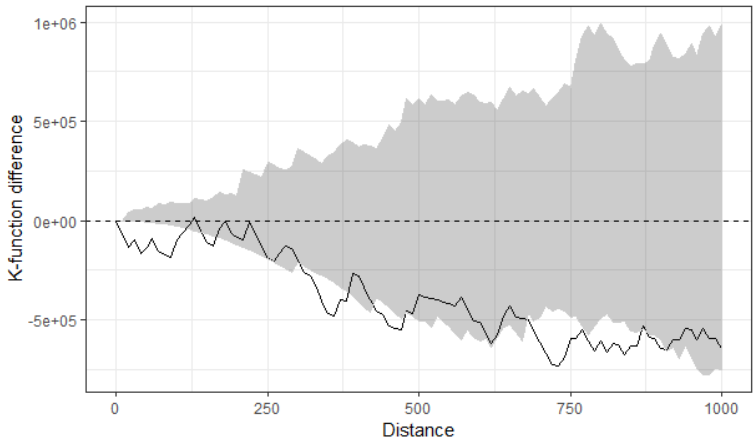


Figure 2. 8. K function graph illustrating the difference between spatial distribution patterns of kākāpō with and without exudative cloacitis in Whenua Hou, 2002-2017. Horizontal line (dashed line) indicates the null hypothesis (no difference in spatial distribution pattern) and the solid line indicates the difference between observed spatial patterns. The shaded area represents the 95% confidence envelope.

2.3.3. Chemical characteristics of kākāpō roosting sites

All three chemical parameters examined, showed a range of distribution in kākāpō roosts and general areas of the two islands (Fig 2.9 & 2.10). The mean ammonium content of the few kākāpō roost samples of diseased birds (138.98, CI -163.22-441.19) was higher than the mean ammonium content of the roost samples without cases on Whenua Hou (32.38, CI 6.83-57.92). This difference was due to the higher ammonium contents of two samples from the cases. However, this difference was not statistically significant (p=0.384). A statistical comparison for moisture and pH were not performed due to the low sample size.

The mean soil pH of the roost samples (5.29, CI 5.00-5.58) was lower (p=0.026) (ie. more acidic) compared to the general areas (5.92, CI 5.48-6.36). The mean moisture content of the roost sites (64.23, CI 59.53-68.93) was significantly lower than that of the general areas (p=0.031). However, mean ammonium levels of the roost (32.38, CI 6.83-57.92) and general areas (23.43, CI 9.66-37.20) of Whenua Hou were not significantly different (p=0.244). The soil analysis from Te Hauturu-o-Toi's general areas, where exudative cloacitis has not yet occurred, showed very strong evidence (p=0.0001) that the pH was more acidic, and moisture content was lower (p=0.0014) while there was weak evidence (p=0.041) that the ammonium content was lower in the substrate samples from Te Hauturu-o-Toi in comparison to the general samples from Whenua Hou.

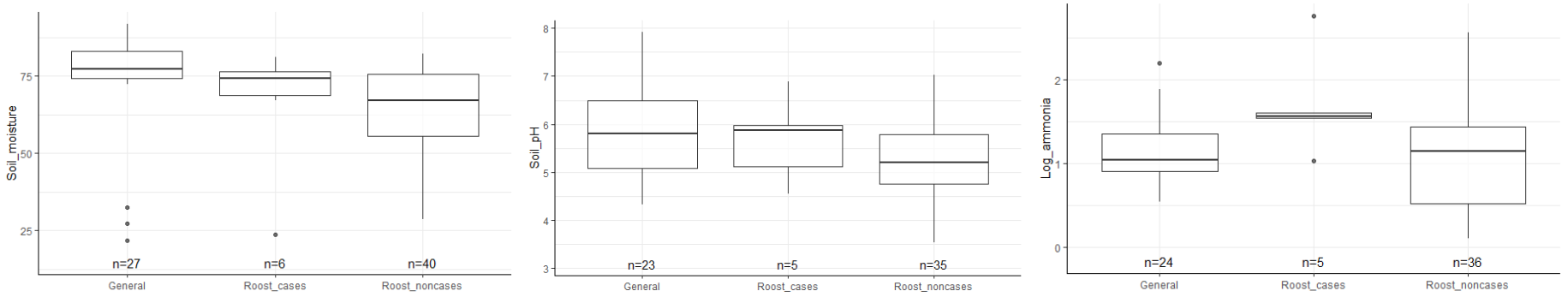


Figure 2. 9. Box plots showing the moisture content (%), pH and ammonium (ppm) in kākāpō roost with and without the disease exudative cloacitis and in general areas of Whenua Hou.

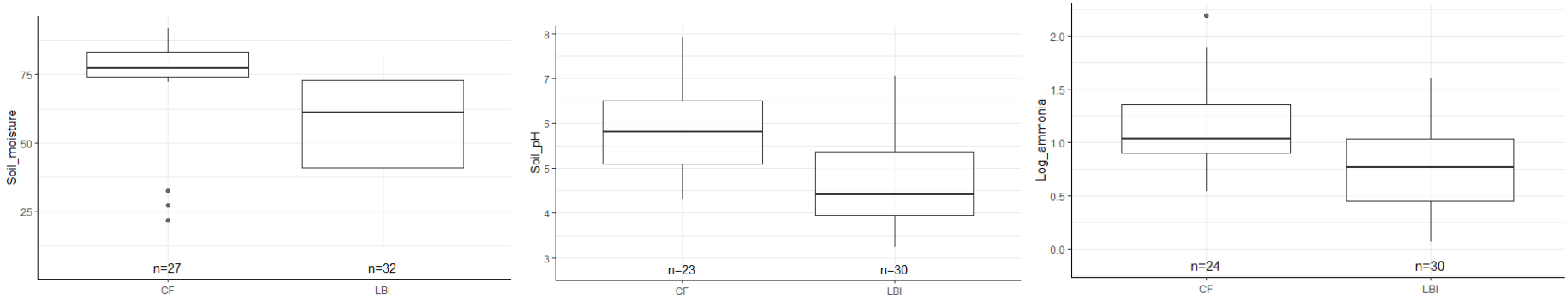


Figure 2. 10. Box plots showing the moisture content (%), pH and ammonium (ppm) in general areas of the Whenua Hou (CF) and Te Hauturu-o-Toi (LBI)

2.3.4. Association of annual climatic factors with the occurrence of exudative cloacitis

There was a strong positive correlation between the annual maximum temperature and rainfall for Whenua Hou (between 1987-1993) and those of Rakiura with corelation coefficients of 0.88 and 0.86, respectively) (Appendix 1). Therefore, weather data for Rakiura for the period of 2002-2017 was used to investigate the effect of annual maximum temperature and rainfall for Whenua Hou. Incidence risk ratio for annual maximum temperature and rainfall were 1.14 and 1.00, indicating that every increase of temperature by 1 degree the incidence increases by 14%. Therefore, neither temperature nor rainfall were significantly associated with the number of exudative cloacitis cases in kākāpō on Whenua Hou each year (Fig 2.11).

Coefficients:

	Estimate	Std. Error	Pr(>|z|)	95% Conf. Interval
(Intercept)	-2.5063820	5.7193460	0.661	-13.7161 - 8.7035
DMax_tem	-0.1294734	0.3139192	0.680	-1.2304 - 0.4858
A_rain	0.0006708	0.0012862	0.602	-0.0018 - 0.0032

Exponentiated the coefficient and the boundaries of the confidence interval to calculate the In cidence Risk Ratio (IRR)

Coefficients:

	IRR	95% Conf. Interval
(Intercept)	12.2605	905,370.9- 6,023.9
DMax_tem	1.1382	- 3.4225 – 1.6254
A_rain	1.0007	-1.0018 - 0.9968

Table 1.1. Results of the negative binomial regression with annual maximum temperature and annual rainfall of Rakiura as predictive variables and the number of cases of kākāpō exudative cloacitis per year (from 2002-2017) as the dependent variable, with an offset for population size.

2.4. Discussion

This study provides the first description and investigation of the outbreak of exudative cloacitis in kākāpō between the years 2002 and 2017. The results confirm that the disease was geographically restricted to Whenua Hou, but that within the island there was no evidence of spatial clustering, with disease occurrence mirroring kākāpō distribution on the island. While there was evidence of temporal variation in case occurrence between years, with some years having no new cases identified, there was no evidence of temporal clustering of the new cases. The progression of exudative cloacitis has been slow over the years and the pattern appears to be sporadic. The small increase in the number of cases detected in 2015 and 2016 could be either a true increase in the incidence or represent observational bias in rangers detecting more cases as they increased the frequency of clinical examinations per year and had increased awareness about the disease at this point. There was also no evidence for an age or sex predilection since both sexes and a mixed group of adults and juveniles were affected by the disease. These epidemiological descriptors do not support the hypothesis that exudative cloacitis is a transmissible infectious disease, nor do they support the hypothesis of a geographically constrained environmental hazard within Whenua Hou that is affecting the kākāpō. In summary those results suggest an initiating cause for exudative cloacitis that is constrained to Whenua Hou, and, within the limits of our data, affects birds independently of age, sex and founder status.

Space-time interactions are increasingly used in the study of human, livestock and wildlife disease investigations to identify disease distribution pattern, risk factors and to estimate the scale at which infectious hosts pose a risk to others (Bohm et al., 2008; Xu et al., 2012). The spatial relationship between the distribution of the diseased population and the distribution of population at risk should be investigated when studying disease distribution in wildlife

populations (Fortin et al., 2010). Although there was spatial clustering in both the diseased and the healthy populations, the distribution of the diseased kākāpō was a reflection of the population at risk.

In general, kākāpō are solitary and live in individual home ranges which are usually overlapped during most of the year (Higgins, 1999; Powlesland et al., 2006). Kākāpō home ranges sizes were found to be different on their offshore islands around New Zealand (Moorhouse and Powlesland, 1991; Powlesland et al., 2006). On Whenua Hou with a dense population of kākāpō, the mean home range size recorded for adult females and newly fledged juveniles was approximately 15 ha (Farrimond et al., 2006a). However, all these home range calculations are for remnant kākāpō populations and population density of kākāpō in these locations are probably lower than what would have occurred in their former habitats before they become endangered. On Whenua Hou, habitat selection of breeding and non-breeding adult female kākāpō were found to be dependent on the distribution patterns of different types of vegetation. Research on habitat selection by kākāpō has shown that they are not distributed randomly across the landscape, but they rather prefer some habitats more commonly than the others (Moorhouse, 1985; Walsh et al., 2006). Home range areas used by majority of breeding and non-breeding female kākāpō contained tree species preferred by kākāpō such as rata, rimu and totara (Wilson, 2004; Whitehead, 2007). Therefore, the distribution pattern of birds with exudative cloacitis on Whenua Hou could represent the pattern of their preferred habitats on the island.

Kākāpō usually roost in a shady area on the ground or natural cavities such as tree logs within their home range. Some roost sites have been used repeatedly or irregularly over many weeks or even years by some individuals (Higgins, 1999; Powlesland et al., 2006). All the chemical parameters of the roost sites and other general areas of the two islands examined showed high variability in their distribution. In general, the soil was mostly acidic in all the samples studied

and there was a difference between the pH levels detected in roost and general areas of Whenua Hou. Low pH levels have been detected in the New Zealand offshore island, Takapourewa/Stephens Island, located in Cook Strait with dense populations of seabirds (Mulder and Keall, 2001). Nitrification of ammonium in bird faeces is one of the mechanisms suspected to be contributing to the soil acidity (Mulder and Keall, 2001) and previous investigations have documented this as one of the effects of numerous seabirds on the islands (Fukami et al., 2006; Otero et al., 2018). Both Whenua Hou and Te Hauturu-o-Toi support large breeding colonies of several species of seabirds and the acidic pH on both islands could be the effects of the guano of these birds on the islands' soil. Within the kākāpō roost sites, the higher acidity of the substrate is likely to represent faecal and urate contamination of the roost by the kākāpō rather than from seabirds, although some roost sharing does occur (Powlesland et al., 2006). Exudative cloacitis has not yet been diagnosed in the currently largest population of kākāpō which inhabits Pukenui. This island is situated in the area of the Dusky Sound and the information about the presence of seabird colonies on the island is very limited. There are some reports mentioning colonies of sooty shearwater, mottled petrel and broad-billed prions (*Pachyptila vittata*) on islands in Dusky Sound (Miskelly, 2017). The substrate characteristics of this island and the kākāpō roost sites there should be investigated to compare with Whenua Hou and Te Hauturu-o-Toi to see if the lack of seabirds might be a one possible explanation for the absence of the disease on this island.

On Whenua Hou, there was no difference between the mean ammonium content in kākāpō roost compared to that of the general areas. Uric acid and small amounts of ammonia in bird excreta is one of the main ways that ammonia is added to the soil. The uric acid is mineralised readily to NH_4 and available in soil for the terrestrial plants. Volatilisation is estimated to releases a large amount of this soil ammonium to the atmosphere (Riddick et al., 2012; Riddick et al., 2014). It is not clear if the high ammonium content in the roost could have initiated the

initial irritation around the cloacal mucosa as occurs in poultry or whether the ammonium level of the roost soil is elevated because the diseased birds were using the same roost repeatedly. For example, the increased ammonia seen in the roost sites may be due to a difference in diet between affected and unaffected birds resulting in increased nitrogenous wastes being excreted by diseased birds. The ammonia levels of the roost sites are an important chemical parameter to investigate through prospective sample collection in future studies of this disease.

Usually, poultry are exposed to 50 ppm level of ammonia in poultry house atmosphere, but in poorly ventilated housing this level can go as high as 200 ppm which affects the health status of poultry (Carlile, 1984; Ferguson et al.,1998). High levels of ammonia in poultry houses are recognised as a cause for keratoconjunctivitis, respiratory infections caused by mucosal damage, and blisters on the breast (Carlile, 1984; Ferguson et al., 1998). In this study, ammonia levels in the soil of kākāpō roosts and general areas of the Whenua Hou and Te Hauturu-o-Toi were lower than the level of ammonia that the poultry are usually exposed to in poultry houses. However, the mean ammonia levels of the kākāpō roost with diseased kākāpō are higher than the critical level of ammonia in poultry houses. According to this finding and the higher level of ammonia in kākāpō roosts compared to the substrate in general areas of Whenua Hou, this might suggest ammonia levels in kākāpō roosts as a predisposing factor for the initiation of exudative cloacitis. There were also significant differences in all the chemical properties of the substrate from general areas of the Whenua Hou and Te Hauturu-o-Toi. However, it is not clear if any of these three environmental chemical properties of the soil are implicated in the presence of exudative cloacitis in birds on Whenua Hou but not on Te Hauturu-o-Toi.

If an environmental factor is important in the initiation of exudative cloacitis in kākāpō, the clinical signs of disease would most probably start as a skin irritation followed by ulceration and inflammation with subsequent secondary infections. This is difficult to confirm in kākāpō due to the necessarily sporadic monitoring of the birds in their island habitats. It has been

believed that a combination of wet litter, high ammonia content and other chemical factors are responsible for the vent and leg dermatitis of broiler chickens (Wang et al., 1998). Experimental studies on the effect of ammonia, pH and moisture on the incidence of foot pad dermatitis on broiler chicken have demonstrated that only the moisture content of the litter has an effect on the disease incidence, not the pH or the ammonia with a correlation between high moisture content (%) and the incidence of foot pad dermatitis (Martland, 1984; Wang et al., 1998). The moisture content of the kākāpō roosts varied between 24-80% in our study, and the mean moisture content was significantly lower than that of the general areas of the island. In relation to exudative cloacitis in kākāpō, there was no clear evidence for the effects of moisture or ammonium on the initiation of the disease. However, both the ammonia and moisture content of the kākāpō roosts are important chemical factors to prospectively evaluate with the occurrence of disease in future.

We were not able to demonstrate any evidence for an effect of the annual maximum temperature and rainfall on the incidence of exudative cloacitis each year. Temperature, rainfall and other climatic factors can affect disease incidence, distribution and severity, and the likely impacts of climate change have been discussed in length, especially related to vector borne diseases and emerging diseases (Dazek et al., 2000; Harvell et al., 2002; Rosenthal, 2009; Gallana et al., 2013). With regards to the incidence of exudative cloacitis in kākāpō, temperature and the annual rainfall could affect the availability of food resources, which are important for the reproductive success, growth of the birds and development of immunity against diseases. However, in this study, our data is limited as the weather data were obtained from the neighbouring Rakiura to represent the weather in Whenua Hou in relation to the incidence of exudative cloacitis in kākāpō. We recommend the reinstatement of weather stations on all of the islands used for kākāpō conservation in order to assess the effects of

changing weather patterns in the local climate on this vulnerable species. In addition, looking at maximum annual temperature and rainfall is a very broad scale approach to looking at climatic effects on the disease. But in this study, the scale at which the relationship was studied between the climatic factors and the number of cases detected was limited by the temporal resolution of the case detection. Looking into short term climatic fluctuations however would be difficult due to the low population size and slow progression of the disease.

The results of the study highlight the gaps in the data that need to be collected to allow a more complete investigation of the epidemiology of exudative cloacitis in kākāpō in Whenua Hou. Kākāpō are one of the most intensively monitored free living birds in the world. Individual birds on their island locations are monitored by telemetry and data are collected and recorded accordingly. However, this study was conducted retrospectively and the initial data collection on kākāpō management was not focused on investigation of diseases or disease surveillance. In wildlife populations, basic demographic characteristics are often unknown making it difficult to design and implement a disease surveillance technique (Tompkins et al., 2015). In relation to the epidemiological study on exudative cloacitis, gaps in the data on kākāpō home ranges in Whenua Hou, environmental characteristics of the roosting sites of the initially affected birds, information on kākāpō diet, contact with other species including use of burrows and their guano, climatic data of the island over the years, other habitat characteristics (soil types, vegetation types); early nesting environment; parentage of the founders; and/or individual health parameters may have limited our ability to identify the key risk factors for the initiation of the disease. Identification of these key risk factors would allow more targeted pathological, environmental and/or microbiological investigations of the exudative cloacitis.

In conclusion, this study revealed no evidence of temporal or spatial clustering and no evidence of sex or age effect on the initiation of exudative cloacitis in kākāpō on Whenua Hou. However, further investigation of environmental soil and roost characteristics, such as the ammonia and

moisture content of kākāpō roosting sites, is recommended in prospective studies when new cases are diagnosed.

Chapter 3

Seabirds as possible reservoirs of *Erysipelothrix rhusiopathiae* on islands used for conservation translocations in New Zealand

3.1. Introduction

Erysipelas is the infectious disease caused by the pathogenic bacteria *Erysipelothrix rhusiopathiae* in a wide range of mammals, birds, some reptiles and fish (Bricker and Saif, 2013; Chong et al., 2015). *E. rhusiopathiae* is a Gram-positive bacillus, ubiquitous in nature and considered an opportunistic pathogen in diverse species of animals (Wood, 1992). The organism can be found in the tonsils or the intestinal tract of healthy carrier animals (Brooke and Riley, 1999). It has been suggested that *E. rhusiopathiae* can multiply in alkaline soils during warm weather, and it can survive up to maximum of a 35 days in soil under various conditions (Wood, 1973). Transmission of *E. rhusiopathiae* is thought to be through the gastrointestinal tract following ingestion of contaminated food or water or through breaks in the mucous membranes or skin (Bricker and Saif, 2013). Asymptomatic carriage by animals with subsequent dissemination into the environment and other animals has been suggested as an important mechanism for maintenance of *E. rhusiopathiae* in nature (Brooke and Riley, 1999). Although the main route of shedding has not been established, *E. rhusiopathiae* could be entering to the environment through infected carcass and through saliva, droppings of live birds. *E. rhusiopathiae* has been isolated most frequently from cecal tonsils, liver, large intestine, heart and blood of carrier birds (Bricker and Saif, 2013). Erysipelas in birds is characterised by either acute, fulminating infections or more rarely chronic infections causing infertility in male birds and reduced egg production in females (Bricker and Saif, 2013).

In July 2004, an outbreak of erysipelas affected the success of a translocation of juvenile kakāpō (*Strigops habroptilus*) from Whenua Hou to Chalky Island killing three of the nineteen birds translocated (Gartrell et al., 2005). Translocation of juvenile birds to islands where breeding success has been lower is one of the management practices used by the Kākāpō Recovery team to enhance the chances of successful reproduction in adult birds (Cresswell,

1996; Clout and Merton, 1998). This was the first time that an infectious disease affected a translocation event in kākāpō and the first time that erysipelas was reported in this critically endangered New Zealand parrot (Gartrell at el., 2005). Subsequently, sporadic deaths due to erysipelas have been seen in takahē (*Porphyrio hochstetteri*) (McLelland et al., 2011), kākāpō from two islands studied, and kiwi (*Apteryx* spp.) from another location indicating the persistence of this bacterium in New Zealand ecosystems.

Many species of wild birds and mammals have been reported to carry *E. rhusiopathiae* and act as reservoirs in natural ecosystems (Wolcott, 2007). This bacterium has been isolated from many species of domestic and wild birds of different geographic regions of the world (Jensen and Cotter, 1976; Van Vuuren and Brown, 1990; Griffith and Buller, 1991; Work et al., 1999; Boerner et al., 2004; Weimerskirch, 2004; Opriessnig et al., 2005; Gartrell et al., 2005; Bricker and Saif, 2013; Kutz et al., 2015), but the prevalence in wild bird populations is unknown. Surveillance of local fauna and ectoparasites (Chirico et al., 2003; Eriksson et al., 2009) has been recommended in order to learn more about the epidemiology of the disease (Gartrell et al., 2005). During the erysipelas outbreak in 2004, seabirds were considered to be the most likely source of infection for the kākāpō as both the source and the destination islands have a diverse fauna of marine and terrestrial birds (Gartrell et al., 2005). Ulnar bone marrow of the fifteen seabird carcasses recovered from Whenua Hou at the time of mortality was subjected to bacteriological culture, and. ten out of fifteen samples were positive for *E. rhusiopathiae* (Gartrell et al., 2005).

The objective of the study was to perform a survey of pelagic and coastal seabirds in two offshore islands used for conservation of native New Zealand fauna; Whenua Hou and Te Hauturu-o-Toi) to estimate the prevalence of *E. rhusiopathiae.* These two islands are the home for several translocated native bird species threatened with extinction, including kākāpō. Both

Whenua Hou and Te Hauturu-o-Toi are home to large populations of nesting seabirds (Rayner et al., 2007a, 2008; Sagar et al., 2015; Scott et al., 2009).

3.2. Methods

3.2.1. Study locations

Study samples were collected from two offshore islands, Te Hauturu-o-Toi (36.1991°S, 175.0814°E) and Whenua Hou (46.7833°S, 167.6333°E). These two predator free islands are located approximately 1,000 km apart (Taylor, 2000).

3.2.2. Time of sampling

Sampling of dead and live seabirds on Whenua Hou and Te Hauturu-o-Toi was conducted during the Austral spring and early summer of 2018, from 15th October to 1st November and from 21st November to 6th December, respectively.

Samples were collected under a Massey University animal ethics permit MUAEC Protocol 17/51 and Department of Conservation, New Zealand permit 65400-DOA and 65402-FAU for dead and live seabird sampling from the two nature reserves.

3.2.3. Dead seabird sampling

On both islands dead sea birds were sampled from seabird colonies and along the tracks outside the colonies. On Whenua Hou, large mottled petrel (*Pterodroma inexpectata*) colonies sampled were distributed near the summit area (250 m). Cook's petrel (*Pterodroma cookii*) colonies on this island have a patchy distribution within the forest area. Sooty shearwater (*Puffinus griseus*) colonies were found near the coastal side in southwest and in eastern side of the island. Some of the sooty shearwater colonies were observed closer to Cook's petrel colonies. On Te

Hauturu-o-Toi, dead birds were sampled from two low altitude colonies, two high altitude colonies and along the tracks outside the colony areas (Fig 3.1). Black petrel (*Procellaria parkinsoni*) colonies were observed a few meters above the higher altitude Cook's petrel colonies.

Opportunistic or convenience sampling was practiced in collecting seabird samples in the current study since there was no prior data on this disease on particular species or spillover event. On Whenua Hou and Te Hauturu-o-Toi, seabirds found dead were sampled and one long bone, preferably an ulna, was collected and stored in thick zip-lock plastic bags until transported to the laboratory. Species of the dead birds were identified by checking the colour of the plumage and using skeletal and bill morphometrics.

3.2.4. Live seabird sampling

Live bird sampling was carried out in mottled petrel colonies in Whenua Hou and in Cook's petrel colonies in Te Hauturu-o-Toi. Birds were caught inside their burrows and restrained by controlling their head, wings and feet. Thick gloves and towels were used for handling and restraining the birds. Birds were checked for identification bands and 0.2-0.3ml of blood was collected from the cutaneous ulnar vein of each bird. If the birds were not banded, they were microchipped using Allflex® Minichip. Collected samples were stored in CaEDTA anticoagulant tubes (Sarstedt Inc SC Micro tube 1.3ml K3 EDTA®) at 4^0C until transported to the lab. Storage time for collected blood samples ranged from 3-6 days.

3.2.5. Isolation and Identification of *E. rhusiopathiae*

Laboratory isolation and identification of *E. rhusiopathiae* was conducted in [m]EpiLab Hopkirk Institute, Massey University, New Zealand. Bone marrow from the collected bones was cultured in Brain Heart Infusion broth (Fort Richard Laboratories, Auckland, New Zealand) and incubated at 37°C for 48 hours. The broth was sub-cultured on Brain Heart Infusion agar with kanamycin and vancomycin (Fort Richard Laboratories, Auckland, New Zealand) and on Columbia Horse Blood agar (Fort Richard Laboratories, Auckland, New Zealand) and incubated at 37°C with 5% CO_2. Cultures were checked for colonies showing morphology typical of *E. rhusiopathiae* (tiny or small, colourless or grey, circular colonies) in 24 hrs and 48 hrs intervals. All the cultures similar to *E. rhusiopathiae* on Brain Heart Infusion or blood agar were subjected to a catalase test, which detects the enzyme catalase that converts hydrogen peroxide into water and gaseous oxygen (Markey et al., 2013). All the catalase negative isolates were sent for confirmation by MALDI-TOF MS (Matrix-assisted Laser Desorption/Ionization with Time-of-flight Mass Spectrometry).

3.2.6. MALDI – TOF MS (Matrix-assisted Laser Desorption/Ionization with Time-of-flight Mass Spectrometry).

A protein profile of bacteria can be obtained directly from the colonies and analysed by the MALDI – TOF MS. It allows rapid and accurate identification of bacteria within a few hours based on their molecular size and electrical charge. MALDI – TOF MS has been recognized as a reliable tool to identify bacteria in routine practice as well as for identification of rare bacterial pathogens (Holland et al., 1996; Bizzini et al., 2010; Seng et al., 2013).

Bacterial colony from a pure culture was homogenised with 300 µL of sterile water and then mixed with 900 µL of 100% ethanol and stored in -20°C until further analysis. At the time of analysis, samples were further processed and identified by MALDI – TOF MS in the MALDI Biotyper 3.0 (Bruker Daltonics) as described by the manufacturer (Stępień-Pyśniak et al., 2017) using the laboratories of Food Assurance at the Fonterra Research and Development Centre. In the MALDI Biotyper system, the reliability of identification of the bacterial isolates was expressed in points. An isolate is considered identified correctly to the species level if the score (log) was between 2.0-3.00. A score (log) between 1.70 -1.99 indicated identification to genus level while < 1.70 indicated no organism identification was possible.

3.2.7. DNA extraction from blood samples and PCR

DNA was extracted from the seabird blood samples using QIAamp® DNA Blood Mini Kit (Qiagen, Valencia, CA, and USA) following the manufactures recommendations. Extracted DNA was subjected to PCR using *Erysipelothrix* genus specific primers MO101 (5'AGATGCCATAGAAACTGGTA3') and M0102 (5'CTGTATCCGCCATAACTA3') described earlier (Makino et al., 1994). The primers are based on the DNA sequence coding for 16s rRNA gene *Erysipelothrix* spp.

The PCR method described by Makino et al. (1994) was used with some modifications to the PCR reaction mixture. Sensoquest Labcycler Thermocycler (Sensoquest, Gottingen, Germany) was used for the PCR process using 20µl of reaction mixture containing, HOT FIREPol® Blend Master Mix; 4µl (each), 50 pmol of oligonucleotide primers ;1 µl (each), water; 13 µl (each) and 2 µl of various sample DNA. The process followed denaturation (at 94°C for 1.0 min), annealing (at 54°C for 2 min) and extension (at 72°C for 2 min) and amplification was repeated for 30 cycles. The samples were heated at 94°C for 2 min prior to cycling and an

additional extension step was performed at 72°C for 7 min. The amplified product was electrophoresed at 110v on 1% (w/v) agarose gels in Tris borate buffer and bands were photographed under UV light.

3.2.8. Statistical analysis

Apparent prevalence estimates and the 95% confidence intervals (Wilson method) for *E. rhusiopathiae* on the two islands and in different species of seabirds were calculated in R version 3.4.1 (RStudio Team, 2016) using the package 'prevalence' (v 0.4.0).

3.3. Results

3.3.1. Dead seabird sampling

In total, 86 dead seabirds were collected on Whenua Hou over three weeks. Most of the birds sampled were mottled petrels (36/86) followed by sooty shearwaters (34/86) and Cook's petrels (4/86). Twelve out of 86 bodies found were extremely decomposed and could not be identified. On Te Hauturu-o-Toi, 44 dead seabirds were collected during the study period. Cook's petrels were the most commonly collected (37/44) bird species on Te Hauturu-o-Toi and a single sample was collected from a black petrel, a little blue penguin (*Eudyptula minor*) and an Australasian gannet (*Morus serrator*). Four out of 44 bodies from Te Hauturu-o-Toi could not be identified and listed as unknown (Appendix 2 & Appendix 3).

3.3.2. Live Bird sampling

A total of 50 blood samples were collected from mottled petrels on Whenua Hou and 18 blood samples were collected from Cook's petrels on Te Hauturu-o-Toi. DNA was extracted from all 68 blood samples and subjected to PCR with *Erysipelothrix* spp. primers.

3.3.3. Prevalence of *E. rhusiopathiae* in Whenua Hou and Te Hauturu-o-Toi

E. rhusiopathiae was identified though MALDI-TOF-MS from 3 of 86 dead seabirds on Whenua Hou (3.5%, 95% CI 1.1%-9.7%) and 5 out of 44 on Te Hauturu-o-Toi (11.4%, 95% CI 4.9%-23.9%). In Whenua Hou, positive samples were found in two sooty shearwater (5.9%, 95% CI 1.6%-19.6%) and a mottled petrel (2.8%,95% CI 0.4%-13.8%). In Te Hauturu-o-Toi, all the birds found to be positive for *E. rhusiopathiae* were Cook's petrels (for a prevalence of 13.5%, 95% CI 5.9%-27.9%). In total, 8 seabirds belonging to three species were infected with this bacterium in both study locations (Table 3.1).

Out of 68 blood samples, one mottled petrel (1/50) was positive for *Erysipelothrix spp*. specific primers in PCR showing a prevalence of 2.0% (CI 0.36%- 10.5%).

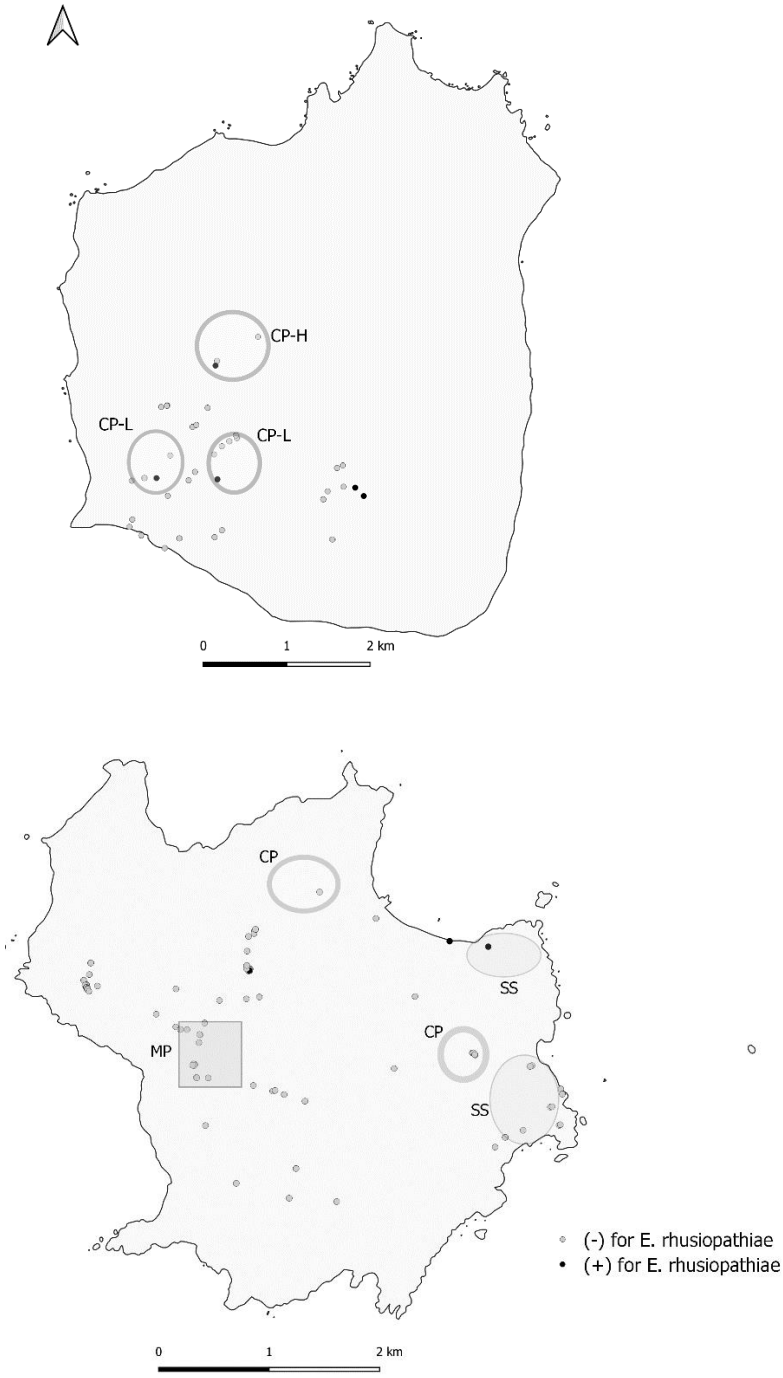


Figure 3. 1. A Maps showing the areas of dead seabird collection on Te Hauturu-o-Toi (top) and Whenua Hou (below) with dots corresponding to the collection location of positive and negative samples. On Te Hauturu-o-Toi, circled areas are indicating low altitude (CP-L) and high altitude (CP-H) breeding areas of Cook's Petrels sampled for the study. On Whenua Hou circled and squared areas are indicating breeding areas of Sooty shearwater (SS), Mottled Petrel (MP) and Cook's Petrels (CP) sampled for the study. Boundaries of the colonies are not exact.

Table 3. 1. The number of dead seabirds of different species sampled from and Te Hauturu-o-Toi and Whenua Hou during October- December 2018 and the prevalence of *E. rhusiopathiae* in each species with 95% Confidence Intervals. Cook's petrel (*Pterodroma cookii*), black petrel (*Procellaria parkinsoni*), little blue penguin (*Eudyptula minor*), Australasian gannet (*Morus serrator*), sooty shearwater (*Puffinus griseus*), mottled petrel (*Pterodroma inexpectata*)

Seabird species collected from two islands	No. of dead seabirds sampled	No. of dead seabirds positive for E. rhusiopathiae	Prevalence of E. rhusiopathiae	95% Confidence Intervals
Te Hauturu-o-Toi				
Cook's Petrels	37	5	13.5%	5.9-27.9%
Black Petrels	1	0	0	0-7.3%
Little Blue Penguin	1	0	0	0-7.3%
Australasian Gannet	1	0	0	0-7.3%
Other /Unknown species	4	0	0	0-4.0%
Whenua Hou				
Cook's Petrels	4	0	0	0%-4.0%
Sooty Shearwater	34	2	5.9%	1.6% -19.0%
Mottled Petrels	36	1	2.8%	0.4% -14.1%
Other/Unknown species	12	0	0	0%-1.8%

3.4. Discussion

Erysipelas is a sporadic cause of death in threatened species in New Zealand. Our study confirmed that burrowing seabirds inhabiting two of the offshore islands used for the conservation of kākāpō and other species are a possible source of *E. rhusiopathiae* within these island environments. While the prevalence of this bacterium in dead seabirds on Te Hauturu-o-Toi (11.4%) was comparatively higher than the overall prevalence on Whenua Hou (3.5%), *E. rhusiopathiae* was isolated from two seabird species on Whenua Hou, including the numerous sooty shearwaters. Sooty Shearwaters are one of the most widely distributed seabirds in the world and they breed in large dense colonies on small islands in the south Pacific and south Atlantic Oceans, mainly around New Zealand, the Falkland Islands, Tierra del Fuego,

and in the Auckland Islands and Phillip Island off Norfolk Island (Sagar, 2013). They are used as a food source for humans in Australia and New Zealand, with the New Zealand harvest estimated at 250,000 birds per year; in New Zealand sooty shearwaters are important seabirds both culturally and ecologically. Considering the public health significance, the implications of our study may extend beyond our two study sites.

We also detected *E. rhusiopathiae* in mottled petrel and Cook's petrel. Mottled petrels only breed on the Snares, Whenua Hou and Big South Cape Island, with small colonies on other islands around Rakiura and in Fiordland in Southern New Zealand (Sagar, 2013). Te Hauturu-o-Toi and Whenua Hou are the only breeding grounds for Cook's petrels (Taylor, 2000). We detected *E. rhusiopathiae* in Cook's petrels from Te Hauturu-o-Toi, but not in those from Whenua Hou. However, the number of Cook's petrels sampled in Whenua Hou was lower, and more samples would be needed to study the presence of this bacterium in that island.

The species-specific prevalence was higher in Cook's petrels (13.5%) compared to the sooty shearwater (5.9%) and mottled petrels (2.8%). However, Cook's petrels represent the bulk of the dead bird sample (84.0%) from Te Hauturu-o-Toi. Given this species bias in our opportunistic sampling, and that our study was limited to only two islands, we cannot make any accurate conclusions about the wider prevalence of *E. rhusiopathiae* in seabirds, or the diversity of species affected. However, our results suggest wider studies are warranted, not only to understand the role of the seabirds for island conservation management, but also to understand the effect of this infection on the seabird species.

In acute and lethal infections, *E. rhusiopathiae* bacteria will be present in high numbers in bone marrow (and in blood), but the birds only survive in this state for 2-3 days (Bricker and Saif, 2013). To understand the role of seabirds as reservoirs, we need to be able to identify live birds that are carrying non-lethal infections. These birds are likely to have far lower numbers of

organisms in their blood, therefore examination of blood samples from live birds was expected to produce lower prevalence estimates than the bone marrow samples from dead birds and indeed, only 1/50 mottled petrel was positive. To understand the role and prevalence of live birds that are carrying this infection, there is a need to validate sensitive techniques that can detect and quantify low numbers of organisms in blood. Culturing the blood prior to PCR may improve the sensitivity of the assay used in this study. Alternatively, serological investigations could be used as the next step to assess the extent of current or previous infections of *E. rhusiopathiae* in these free-living seabird populations.

The pathogen prevalence in the host species is one of the major determinants of spillover risk between species (Plowright et al., 2017). We found *E. rhusiopathiae* in burrowing seabird species in New Zealand island ecosystem, prevalence appearing to vary between species. These birds share the same environment with endangered native birds and there is evidence of direct contact between those species (Powlesland et al., 2006) providing possible routes for pathogen spillover. On Whenua Hou, close encounters between Cook's petrels and kākāpō have been recorded, with birds sharing the same nesting burrow on one occasion while in another record a Cook's petrel was killed by an aggressive female kākāpō during the breeding season (Powlesland et al., 2006). Further studies are required to confirm if seabirds act as reservoirs for *E. rhusiopathiae*, whether spillover is occurring between the species and, if so, the exact mechanism of transmission.

The transmission pathways of *E. rhusiopathiae* under natural conditions are not well understood; widely accepted hypotheses include transmission through ingestion, or via skin lesions or mucous membranes when bacteria are present in the environment. Our current hypothesis is that *E. rhusiopathiae* may be shed from decomposing infected seabirds' bodies, contaminating the soil, which may then be ingested by other species during preening or feeding. The organism can survive in damp soil for weeks to months (Reboli and Farrar, 1989) and

environmental studies of the persistence of the organism on the islands could provide evidence to support this hypothesis. Close proximity between different species in these island ecosystems may provide other opportunities for pathogen transmission between species.

Our finding of a potential reservoir of *E. rhusiopathiae* in these offshore islands should be considered when evaluating the need to vaccinate at-risk species (Livingston et al., 2013) such as kākāpō and takahē. This is particularly important for translocations of young birds, which are likely to be more susceptible (Benskin et al., 2009).

The prevalence of *E. rhusiopathiae* in wild populations of nesting seabirds in New Zealand has not been assessed. Previously, *E. rhusiopathiae* was found as a cause of extensive chick mortality in Indian yellow nosed albatross (*Thalassarche carteri*) on Amsterdam Island in the southern Indian Ocean (Weimerskirch, 2004), resulting in a low breeding success and population decline (Jaeger et al., 2018). Additionally, *E. rhusiopathiae* has been detected through PCR in oro-pharyngeal swabs (1/21) of Amsterdam albatross (*Diomedea amsterdamensis*) chicks and adult sooty albatross (1/30) (*Phoebetria fusca*) and in cloacal swabs (3/30) of adult Northern rockhopper penguins (*Eudyptes moseleyi*) on Amsterdam Island (Jaeger et al., 2018).

Our study describes the presence of *E. rhusiopathiae* in dead Cook's petrels from Te Hauturu-o-Toi, an important place for the conservation of threatened New Zealand birds. Jaeger's (2018) finding that erysipelas was a cause of mortality, low breeding success and possible population decline in the Indian yellow nosed albatross (*Thalassarche carteri*) may indicate the erysipelas is having a wider effect in seabird population dynamics than previously recognized. At present, the occurrence *E. rhusiopathiae* in seabirds in these island ecosystems are not monitored and the effect of this bacterium on the seabird population health is not known. Our results suggest that the effects of this bacterium on seabird breeding and survival are

worthy of further study, particularly for threatened seabird species. There may be multiplication and increased transmission of the pathogen within seabirds during their breeding season, as many gather in large colonies for several months. Longitudinal studies of the seabird populations are difficult due to their pelagic movements, however a focussed study over the breeding season could elucidate the dynamics of *E. rhusiopathiae* infection in the seabirds at this time.

Investigation of the genetic structure of *E. rhusiopathiae* on the two islands studied and in different seabird species could improve our understanding of the possible sources or origin of this pathogen in island ecosystem, whether one or several introduction events has occurred, and might identify the epidemiological networks of the bacteria. Such information is important for the assessment of transmission risk between local and introduced translocated populations, or between marine and terrestrial birds. Our results confirm that burrowing seabirds are possible reservoirs of *E. rhusiopathiae* on both islands studied and may be the source of spillover to other translocated species on the islands. This should be considered in the conservation management practice of translocation of endangered New Zealand birds to these islands and should inform vaccination and health screening strategies.

Chapter 4

Genomic diversity of *Erysipelothrix* on islands using for conservation translocation, New Zealand

4.1. Introduction

Pathogen or disease control is recognised as an important component in maintaining population viability in threatened wild animal populations (Thompson et al., 2010). However, insufficient knowledge on disease epidemiology and host-pathogen relationships has often limited the ability to manage pathogenic threats, hindering the conservation of threatened species (Sainsbury and Vaughan-Higgins, 2012). Epidemiological investigations are essential components of disease control and conservation management strategies in wild animal populations as they provide information pertaining to the risks of pathogen transmission between vulnerable populations.

Genomic investigations of pathogens provide novel insight into epidemiology and infectious disease ecology. Whole-genome sequencing (WGS) can now replace more classical typing methods, providing high discriminatory power to distinguish between pathogens. High-resolution genomic data have been used to understand pathogen evolution, outbreak dynamics, cross-species transmission, and the spatial distributions of pathogens at local and global levels (Benton et al., 2015). The increasing availability and ever-reducing costs of WGS have made their use possible in routine epidemiological investigations (Bertelli and Greub, 2013). Genomic techniques have been used extensively to understand infectious disease epidemiology, particularly in human clinical research. However, their use in the field of wildlife disease investigation is relatively new (Benton et al., 2015). Molecular epidemiology and the risks posed by wild animals as environmental reservoirs for zoonotic bacterial pathogens (Kovanen et al., 2019) as well as transmission dynamics of bacterial pathogens among wildlife and domestic livestock (Kamath et al., 2016; Crispell et al., 2019) have been studied using WGS. However, records on the use of genomic techniques to investigate pathogens in endangered wildlife are rare (Grange et al., 2016; Grange et al., 2017).

Erysipelothrix rhusiopathiae, the causative agent of the diseases erysipelas in animals and erysipeloid in humans (Reboli and Farrar, 1989; Brooke and Riely, 1999) is the most important pathogenic bacterium belonging to the genus *Erysipelothrix.* Animal mortality as a result of erysipelas has challenged conservation efforts in wild animal populations in several ecosystems throughout the world (Weimerskirch, 2004; Gartrell et al., 2005; Kutz et al., 2015; Jaeger et al., 2018). In New Zealand, *E. rhusiopathiae* has been identified as a threat for the conservation of critically endangered kākāpō (*Strigops habroptilus*) in offshore islands (Gartrell et al., 2005) and has caused deaths in other threatened bird species such as takahē (*Porphyrio hochstetteri*) (McLelland et al.2011) and kiwi (*Apteryx* spp.). This Gram-positive bacterium has a diverse cell morphology, remarkable chemical tolerance, wide range of hosts and a broad geographic distribution (Kwok et al., 2014). Prevention and control of the disease erysipelas has often been challenging because *E. rhusiopathiae* is ubiquitous in nature and both domestic and wild animal reservoirs can act as asymptomatic carriers (Kwok et al., 2014). Identification of reservoirs of *E. rhusiopathiae* in island ecosystems in New Zealand is important as most of these offshore islands are used as source and destination sites for conservation translocations.

Twenty-three serovars of *E. rhusiopathiae* have been identified to date (To and Nagai, 2007). However, the use of serotyping is questionable in epidemiological investigations because of the considerable variations in morphology and pathogenicity between isolates (Kwok et al., 2014). Recent studies have investigated the genomic diversity and population structure of *E. rhusiopathiae* based on pulsed-field gel electrophoresis (PFGE) profiles, multi locus sequence typing (MLST) and WGS (Janssen et al., 2015; Forde et al., 2016; Ogawa et al., 2017; Forde et el., 2020). However, most of the isolates used in these studies are derived principally from swine and poultry. Molecular typing methods such as PFGE or MLST only offer limited resolution for strain typing. Lack of discriminatory power has limited the ability of these

techniques to distinguish between closely related strains (Bertelli and Greub, 2013; Benton et al., 2015).

The objective of this study was to use WGS to investigate the seabird reservoirs and diversity of *E. rhusiopathiae* in two New Zealand offshore islands important for the conservation of kākāpō; Whenua Hou and Te Hauturu-o-Toi. These predator-free offshore islands are safe refuges for the conservation of threatened New Zealand birds including kākāpō. Here, we have used WGS to characterise the genetic diversity of *E. rhusiopathiae* among nesting seabirds in two offshore islands and in three species of endangered native land bird species. Findings from this study will support the management practices for high-risk conservation interventions such as translocations of endangered wildlife populations in New Zealand offshore islands.

4.2. Methods

4.2.1. Sample selection

In this study, fifteen *Erysipelothrix* isolates identified as *E. rhusiopathiae* through Matrix-Assisted Laser Desorption/Ionization with Time-of-Flight Mass Spectrometry (MALDI – TOF MS) were analysed by WGS. Isolates used were derived from nesting seabirds found dead in Whenua Hou and Te Hauturu-o-Toi and from three species of endangered native land bird species. Seabird isolates included nine from Cook's petrels (*Pterodroma cookii*), one isolate from a sooty shearwater (*Puffinus griseus*) and one isolate from a mottled petrel (*Pterodroma inexpectata*). The causes of death of the seabirds were unknown. Four *E. rhusiopathiae* isolates previously been collected opportunistically from samples of endangered native land bird species were provided by the Kākāpō Recovery Team (DOC, New Zealand) Wildbase

Pathology (Massey University, New Zealand) and Veterinary Pathology New Zealand. These opportunistic isolates included two from takahē, one isolate from a kākāpō and one isolate from a North Island brown kiwi (*Apteryx mantelli*) (Table 4.1). The disease erysipelas was the cause of death for the sampled takahē and North Island brown kiwi, while the kākāpō was found dead and the cause of death was not determined.

All the isolates were subcultured on Brain Heart Infusion agar (Fort Richard Laboratories, Auckland, New Zealand) and incubated in 37°C with 5% CO_2 for 24-48 hrs. Morphologically *Erysipelothrix* were grey – colourless, small circular colonies. A single colony was re-streaked to obtain a clonal population for storage in nutrient broth (Oxoid, Hampshire, UK) with 15% glycerol at -80°C and DNA extraction.

4.2.2. DNA extraction and genome sequencing

Genomic DNA was extracted using a Qiagen DNeasy blood and tissue kit (Qiagen GmbH, Germany) according to the manufacturer's instructions for Gram-positive bacteria. The quantity of extracted DNA was evaluated using Qubit® dsDNA HS Assay Kits (Life Technologies, Oregon, USA). DNA was stored in -20°C before preparing libraries for sequencing. DNA library preparation was performed using the Nextera XT v2 kit (Illumina, San Diego, CA) according to the manufacturer's instructions. WGS was conducted at Massey University Genome Services on a MiSeq sequencer (Illumina, Inc.), which generated 150 base pair, paired end reads.

4.2.3. Bioinformatic Analyses

The quality of the sequence data was assessed using FastQC (version 0.11.8) (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and sequence reads were quality

trimmed using the program Trimmomatic version 0.39 (Bolger et al., 2014). Then the sequences were assembled using Skesa version 2.3.0 (Souvorov et al., 2018) and annotated with Prokka version 1.14.6 (Seemann, 2014). Genomic statistics were extracted from the assembled genomes using Seqkit version 0.13.2 (Shen et al., 2016).

Table 4. 1. Origin and the host species of *Erysipelothrix* isolates from New Zealand wild bird species used for whole genome sequencing. Kākāpō (*Strigops habroptilus*), North Island brown kiwi (*Apteryx mantelli*), mottled petrel (*Pterodroma inexpectata*), sooty shearwater (*Puffinus griseus*), takahē (*Porphyrio hochstetteri*), Cook's petrel (*Pterodroma cookii*).

Isolate	Host Species	Sample Type	Origin of the isolate
EK002	Kākāpō	Bone marrow	Whenua Hou
EK003	North Island brown kiwi	Joint fluid	
EK055a2	Mottled petrel	Bone marrow	Whenua Hou
EK110	Sooty shearwater	Bone marrow	Whenua Hou
EK144b	Takahē	Bone marrow	Burwood Takahē Centre*
EK144c	Takahē	Bone marrow	Burwood Takahē Centre*
EK176a	Cook's petrel	Bone marrow	Te Hauturu-o-Toi
EK177a	Cook's petrel	Bone marrow	Te Hauturu-o-Toi
EK177b	Cook's petrel	Bone marrow	Te Hauturu-o-Toi
EK182a	Cook's petrel	Bone marrow	Te Hauturu-o-Toi
EK182b	Cook's petrel	Bone marrow	Te Hauturu-o-Toi
EK204a	Cook's petrel	Bone marrow	Te Hauturu-o-Toi
EK204b	Cook's petrel	Bone marrow	Te Hauturu-o-Toi
EK205a	Cook's petrel	Bone marrow	Te Hauturu-o-Toi
EK205b	Cook's petrel	Bone marrow	Te Hauturu-o-Toi

*Appendix 4

4.2.4. Core-Genome Alignment and Phylogenetics

Genome sequences of 11 *E. rhusiopathiae* strains (of which nine complete genomes and two draft genomes) were obtained from the GenBank® database (www.ncbi.nlm.nih.gov/genbank/) of the National Centre for Biotechnology Information (NCBI) in August and September 2019 (Table 4.2). Comparative analysis of the genomes, pan-genome assessment and visualisation was performed using the program Roary version 3.11.2

(Page et al., 2015). A pie chart was made to illustrate the pan-genome results in R version 3.4.1 (RStudio Team, 2016) following the guidelines from https://www.r-graph-gallery.com/pie-plot.html using the package RColorBrewer version 1.1-2 (Neuwirth, 2014).

Phylogenetic analyses were performed in the Mega-X (Molecular Evolutionary Genetics Analysis) software package (https://www.megasoftware.net/) (Kumar et al., 2018). A maximum likelihood tree was built using a general time reversible model and bootstrap method to test the phylogeny with 100 bootstrap replicates. Phandango, an interactive genome visualiser (Hadfield et al., 2017) was used to visualise the results. We used Scoary version 1.4.0 (Brynildsrud et al., 2016) to quantify associations between pan-genome gene content and available metadata.

From all the genomes used in this study, 16S rRNA gene sequences were extracted using barrnap version 0.9 and aligned and a neighbour-joining tree was constructed using a single 16S rRNA gene from each isolate in Geneious prime software. The nucleotide-level genomic similarities between the genomes were identified by calculating the ANI (Average Nucleotide Identities) (Rodriguez-R and Konstantinidis 2016). Pairwise Single Nucleotide Polymorphism (SNP) distance between the genomes calculated from a FASTA sequence alignment (https://github.com/tseemann/snp-dists#readme).

Table 4.2. Metadata of the *E. rhusiopathiae* genomes used for the comparative analysis of the genomes. Pig (*Sus scrofa domesticus*), Rough-tooth dolphin (*Steno bredanensis*)

Strain	**Country of Origin**	**Host**	**Sample**	**Year of Isolation**	**GenBank accession**
ATCC19414	USA	Human	Skin	2011	GG700723
Fujisawa	Japan	Pig		2011	AP012027
SY1027	China	Pig		2013	NC_021354
WH13013	China	Pig	Spleen	2013	NZ_CP017116
ML101	China	Pig		2018	CP029804
RU		Pig		2016	
ZJ	China	Pig		2016	SAMN12347781
NCTC 7999	France	Unknown		1900/1949	ERS1487938
NCTC 8163	UK	Pig	Spleen	1950	NZ_LR134439
Kc-sb-R1[1]	South Korea	Rough-tooth dolphin		2018	CP033601.1
GXBY-1[2]	China	Pig		2012	CP014861.1

[1] Strain name was modified as KcsbR to be used in the data analysis
[2] Strain name was modified as GXBY to be used in the data analysis

4.3. Results

4.3.1. Whole Genome Sequencing and assembly of *Erysipelothrix* spp.

In this study, WGS data from 15 New Zealand wild-bird *Erysipelothrix* isolates (11 from seabirds and four from three species of endangered native land birds) were used to assemble draft genomes. Genome sizes of the isolates were in the range of 1,720,018 bp and 1,790,484 bp (1.7-1.8 million base pairs), and the average G+C ratio was 36.36% (Table.3).

4.3.2. Comparative genomic analysis

Pan-genome analysis with 11 *E. rhusiopathiae* genomes available in the GenBank and 15 *Erysipelothrix* spp. (identified as *E. rhusiopathiae* using MALDI-TOF MS) genomes from the present study identified, 1,371 core genes, 69 soft core genes (present between 95-99% strains),

565 shell genes (present between 15-95% strains), 782 cloud genes (present between 0-15% strains) and a total pan genome of 2,782 genes (Fig 4.1). Pan genomic view showing the presence and absence of genes in all the genomes analysed in the present study are shown in Fig 4.2.

Table 4.3. General features of the 15 *Erysipelothrix* draft genomes from seabirds and endangered New Zealand native birds.

Isolate	**Genome Size (BP)**	**GC content (%)**	**N50**	**Contigs**
EK002	1,725,092	36.67	79671	55
EK003	1,779169	36.22	66425	64
EK055a2	1,740,293	36.33	76448	64
EK110	1,751,332	36.28	66891	63
EK144b	1,783,754	36.31	111863	37
EK144c	1,790,484	36.36	111864	47
EK176a	1,720,216	36.65	79842	48
EK177a	1,720,499	36.65	79842	46
EK177b	1,720,018	36.65	139592	49
EK182a	1,777,298	36.20	139592	38
EK182b	1,777,939	36.20	269215	28
EK204a	1,772,510	36.23	269206	27
EK204b	1,772,952	36.22	269206	27
EK205a	1,772,983	36.22	280318	26
EK205b	1,775,294	36.22	258128	28

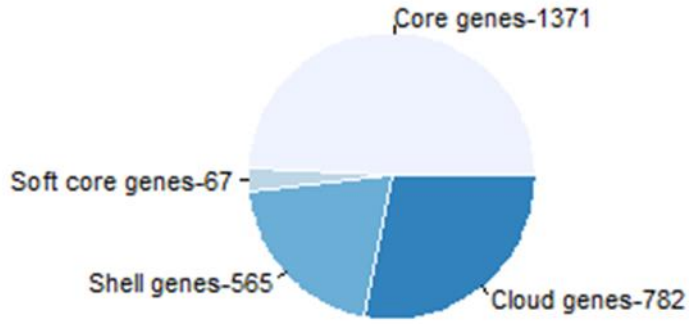


Figure 4. 1. Pie chart showing pan-genome summary statistics with number of core and accessory genes of *Erysipelothrix* spp.

4.3.3. Genomic diversity of *Erysipelothrix* from different seabird species and threatened native birds

Phylogenetic analyses of conserved genetic content between *Erysipelothrix* in this study grouped the isolates in two genetically distinct clusters (Fig 4.3). *E. rhusiopathiae* isolates from two islands are in different clusters, corresponding to the two geographical origins of the isolates. Within Te Hauturu-o-Toi, isolates from the same genomic cluster are distributed in different Cook's petrel colonies. *E. rhusiopathiae* isolates from takahē were most closely related (6177-6180 SNPs) to the isolates from Cook's petrels from Te Hauturu-o-Toi while the kiwi *E. rhusiopathiae* was in close identity with isolates from sooty shearwater (7574 SNPs) and mottled petrel (5541 SNPs) from Whenua Hou.

According to16S rRNA phylogeny, EK002, EK176a, KcsbR1, EK177a, and EK177b have branched off from all the other isolates used in this study (Fig 4.4). Out of 15 isolates from New Zealand wild birds, 11 shared 99% ANI similarities to the *E. rhusiopathiae* reference stain Fujisawa (GenBank Acc. No. AP012027; Table 2), confirming that these isolates belong to this bacterial species, while 4 (EK002, EK176a, EK177a and EK177b) only shared 92% ANI similarity to available reference genomes (Fig 4.5). The threshold criteria for ANI-similarity between members of the same bacterial species is 95% (Jain et al., 2018), thus these results

suggest that these four atypical isolates (3-Cook's petrels, 1- kākāpō) from the present study hence can be considered as members of a new, previously undescribed *Erysipelothrix* sp.

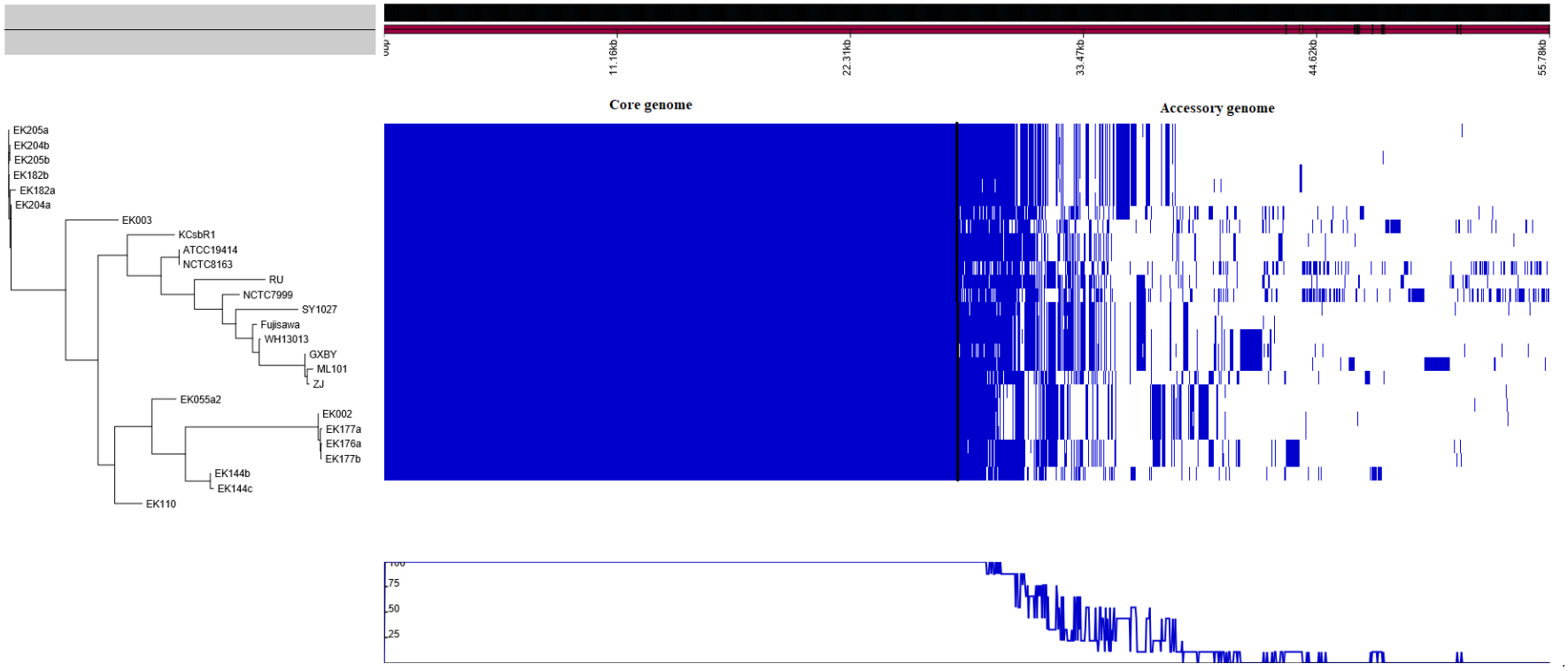


Figure 4. 2. Linearised pan-genomic view of 26 *Erysipelothrix* isolates. The middle panel displays presence (blue) or absence (white) of blocks relative to genes and contigs in the pan-genome and metadata.

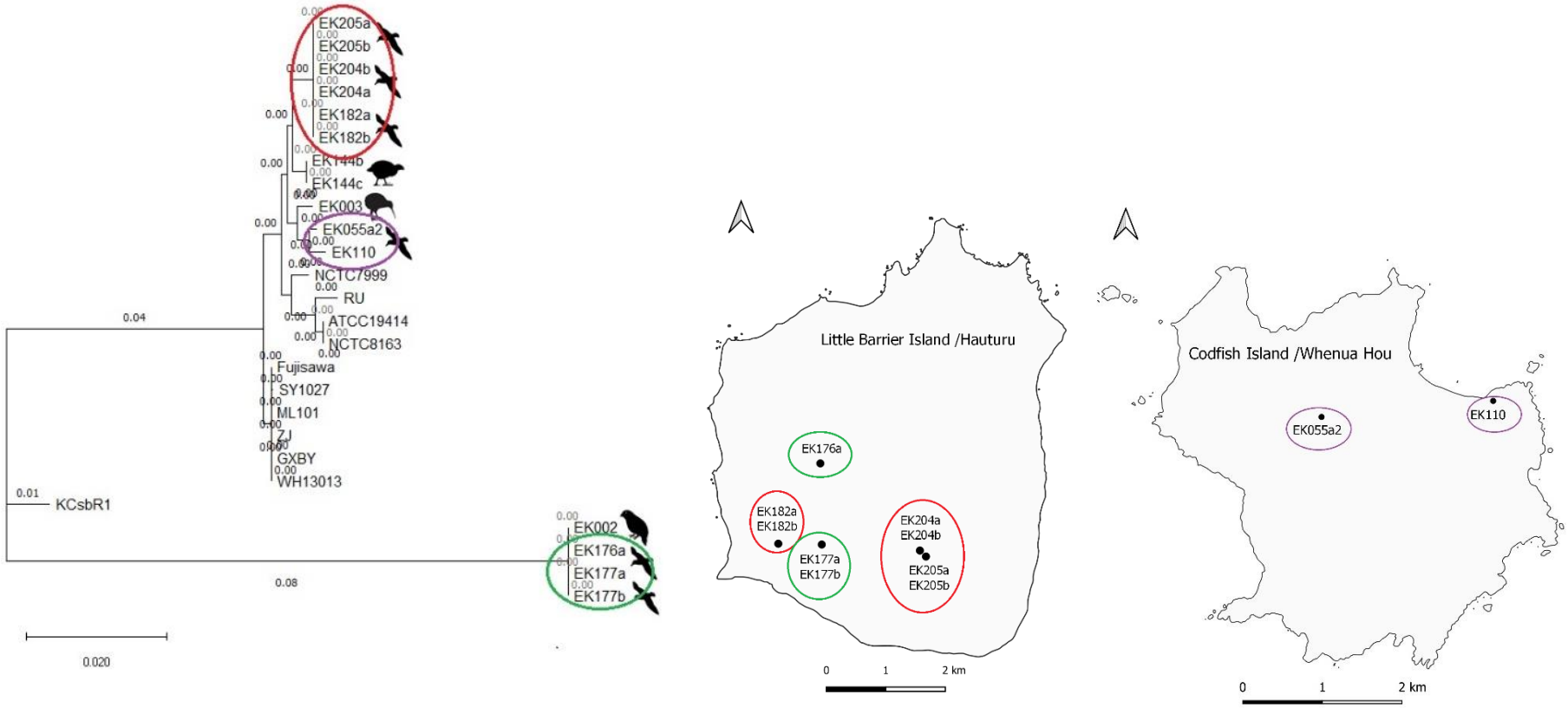


Figure 4.3. Genomic diversity of *Erysipelothrix* isolates from three seabird species (Cook's petrel, mottled petrel, sooty shearwater) from Whenua Hou and Te Hauturu-o-Toi and three species of endangered New Zealand land bird species (takahē, North Island brown kiwi and kākāpō). The maximum likelihood tree is constructed based on the SNP variation and using available reference genomes (NCTC7999, RU, ATCC19414, NCTC8163, Fujisawa, SY1027, ML101, ZJ, GXBY-1, WH13013, Kc-sb-R).

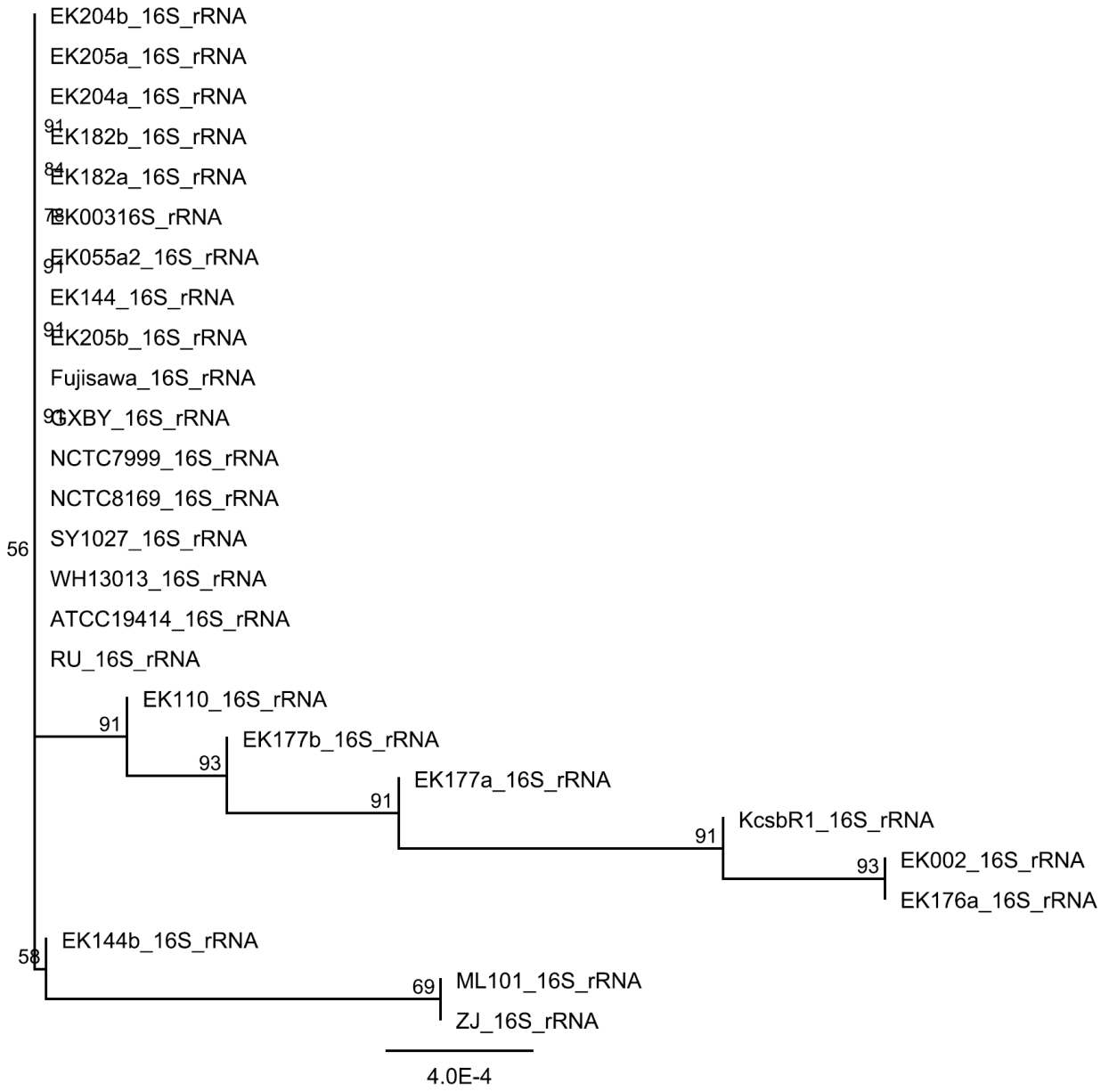


Figure 4.4. Phylogenetic tree of *Erysipelothrix* species based on 16S rRNA generated using the neighbour-joining method in Geneious prime software

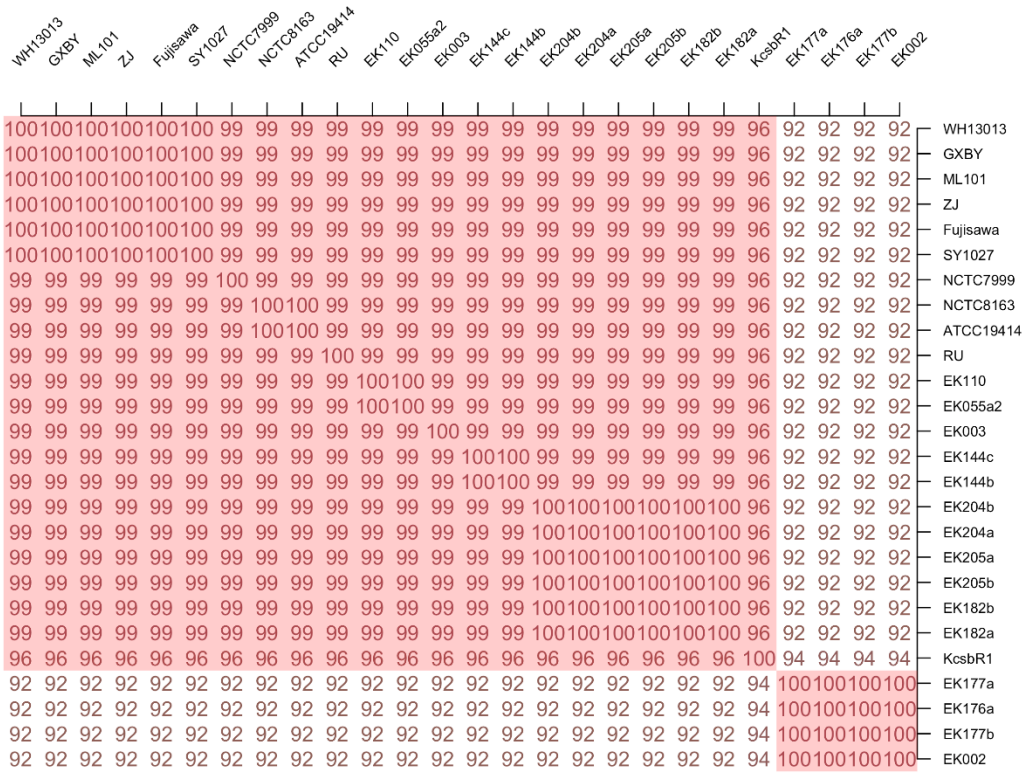


Figure 4. 5. A matrix showing the Average Nucleotide Identities (ANI), a measure of nucleotide level genomic similarities between the coding regions of the *Erysipelothrix* spp. genomes used in the study.

4.4. Discussion

This study was designed to describe the genomic characteristics and the molecular epidemiology of an opportunistic pathogen *E. rhusiopathiae* in two New Zealand offshore islands that are used for conservation purposes. Sporadic infections associated with *E. rhusiopathiae* have been shown to threaten the conservation of endangered New Zealand land birds over the years, but the reservoirs of this bacterium in their isolated ecosystems were not known. The present study demonstrates the diversity of *Erysipelothrix* spp. present in bird species studied in two offshore islands and the numerous seabirds nesting on these islands are

one possible source of infection of *E. rhusiopathiae* for endangered native land bird species such as kākāpō, takahē and kiwi.

All the isolates from New Zealand wild birds used in the present study were initially identified as *E. rhusiopathiae* based only on MALDI-TOF MS. We have used WGS to analyse those isolates as it provides increased resolution and differentiates between closely related species. Phylogenetic analysis based on whole genome and 16S rRNA genes revealed a distinct clustering of four isolates from rest of the group indicating that those genomes belong to a different group. This was supported by average genomic relatedness showing only 92% ANI sharing by this group with all the other isolates except KcsbR1. The average nucleotide identity of all orthologous genes shared between any two genomes are recognized as ANI. Two organisms are considered to belong to the same species when they show ≥95% ANI (Jain et al., 2018). Therefore, those isolates derived from Cook's petrels from Te Hauturu-o-Toi and a kākāpō from Whenua Hou can thus be considered as a separate *Erysipelothrix* sp. Although, MALDI-TOF MS could identify microorganisms rapidly and reliably from the primary selective isolation medium, it is limited with the ability to identify closely related species (Wieser et al., 2012). Therefore, in the initial MALDI-TOF analysis, both *E. rhusiopathiae* and *Erysipelothrix* sp. have been identified as *E. rhusiopathiae.*

Prior studies have recorded *E. rhusiopathiae* as one of the smallest genomes within the phylum Firmicutes with 1.8 million base pairs (MB) and 36.5% average G+C content (Kwok et al., 2014; Ogawa et al., 2011; Tang et al., 2016). The findings from this study are consistent with the previous studies and the size of the *E. rhusiopathiae* genome from New Zealand wild bird species varied between 1.75-1.8 million base pairs and showed low genomic G+C contents of 36.25%. The genome size of the novel *Erysipelothrix* sp. was 1.72 million base pairs and the average G+C content was 36.65%.

Currently, five bacterial species belonging to genus *Erysipelothrix* have been described as *E. rhusiopathiae, E. tonsillarum, E. inopinata, E. larvae* and *E. piscisicarius* sp. nov (Opriessnig et al., 2020; Pomaranski et al., 2020). *E. rhusiopathiae* and *E. tonsillarum* are the two major species in this genus and these isolates are found in mammals, birds, and fish (Takahashi et al., 2008). *E. rhusiopathiae* is usually associated with highly pathogenic infections (Takahashi et al., 2008; Opriessnig et al., 2010; Chong et al., 2015; Forde et al., 2016a) whereas *E. tonsillarum* has little aetiological significance for many species (Takahashi et al., 1987; Takahashi et al., 1993). *E. inopinata* and *E. larvae* were isolated from a vegetable peptone broth (Verbarg et al., 2004) and the larvae of rhinoceros beetle, *Trypoxylus dichotomus* (Bang et al., 2015) respectively. *E. piscisicarius* sp. nov is a novel *Erysipelothrix* sp. causing disease in ornamental fish that is different from disease caused by *E. rhusiopathiae* (Opriessnig et al., 2020; Pomaranski et al., 2020). Genomic analysis has shown a distinct difference between *E. piscisicarius* sp. nov and *E. rhusiopathiae* from healthy fish skin, marine mammals and terrestrial animals (Pomaranski et al., 2018; Pomaranski et al., 2020).

The pan-genome analysis with 26 genomes representing different hosts and environments in this study has revealed that *E. rhusiopathiae* is genetically diverse. The pan-genome is the sum of all genes in all the isolates which make up the core and accessory genomes. The core genome is composed of those genes that are present in all isolates and contributes to the functions probably related to the basic biology and phenotypes of the bacterial species. The accessory genome contains genes present in some but not in all the strains studied. It also includes the genes that are specific to strains. The accessory genome contributes to the diversity of the species and some of the functions that are not essential but are specific to strains such as antibiotic resistance (Tettelin et al., 2008; Mira et al., 2010). In the present study, gene content between the isolates varies and the variations are higher between the *E. rhusiopathiae* and new *Erysipelothrix* sp. Those genomic variations could correlate with different phenotypes of the

species, and the differences might attribute to the variations in characteristics such as virulence between the new species and *E. rhusiopathiae.*

However, this pan-genome analysis may be limited in terms of capturing the *E. rhusiopathiae* diversity because of the availability of comparatively low number of *E. rhusiopathiae* genomes in NCBI database and the limited diversity in the host species previously reported. The majority of genomes from NCBI database are derived from swine isolates and all the genomes from our study were from avian species. On geographic scales, the published genomes are sourced from different areas of the world, while all our genomes are from New Zealand. It is possible to expect potential additional genetic variations due to *E. rhusiopathiae'*s wide host and environmental range. A greater variety of isolates from multiple host species representing different ecological niches would be required to achieve higher-resolution evolutionary insights.

Variations in the genetic diversity of *E. rhusiopathiae* have been observed in different studies. Ogawa et al., (2017) examined the genetic relatedness among the *E. rhusiopathiae* in pigs from Japan and China using Pulsed-field gel electrophoresis analysis and revealed that there was no clearly noticeable difference between the isolates. However, a recent study using WGS has reported a high diversity of *E. rhusiopathiae* strains in British pigs (Forde et el., 2020). High MLST diversity has been observed in 165 *E. rhusiopathiae* isolates predominantly from avian hosts from different geographic locations (Janssen et al., 2015). There was no indication of geographic or host specificity of the isolates suggesting that the mammals and birds may be infected by phylogenetically similar strains either through a common reservoir or indirect transmission from one species to the other (Janssen et al., 2015).

The current study revealed a difference in genomic diversity of *E. rhusiopathiae* from the two geographic locations studied. However, the isolates from the same individual did not differ by

SNPs. Within the phylogeny derived from these results, *E. rhusiopathiae* from mottled petrel and sooty shearwater from Whenua Hou have grouped in a separate cluster from the *E. rhusiopathiae* of Cook's petrel from Te Hauturu-o-Toi. This may suggest that specific *E. rhusiopathiae* strains can have limited geographical distributions. In previous studies, host or ecological predilection of *E. rhusiopathiae* has been identified but not the host specificity (Forde et al., 2016b). However, clustering of *E. rhusiopathiae* isolates according to the host species has been observed, as isolates from marine mammals from aquaria all over the world has clustered within a one clade. (Forde et al., 2016b). The host and geographic clustering observed in our study can be explained as specific host adaptation that has resulted from independent evolution of the species over multiple times during its evolutionary history, as suggested by Söderlund et al., (2020). However, it would be worth further examining this host and geographic clustering observed in our study by using more isolates from different host species representing different geographic areas.

This study is the first one to report the genomes of *E. rhusiopathiae* from three species of burrowing seabirds: Cook's petrels, sooty shearwater and mottled petrels and two species of endangered native land bird species, takahē and North Island brown kiwi. This study has also reported the presence of a new *Erysipelothrix* sp. in New Zealand wild birds, which has not been previously described. This proposed new bacterial species was negative for catalase and phenotypically similar to *E. rhusiopathiae* on blood agar and brain heart infusion agar. As our knowledge on *Erysipelothrix* sp. is limited, further investigations on morphological, physiological, and biochemical profiles, *spa* isoforms and virulence factors could be used to characterise and explore the pathogenicity of *Erysipelothrix* sp. isolates from the present study. In addition, MALDI-TOF protein spectra could be used to compare the distinct protein profiles of new *Erysipelothrix* sp. with type stains of other species of genus *Erysipelothrix* (Koziel et al., 2014).

The cause of death for both the takahē and kiwi used in this study have been confirmed as erysipelas. Given the phylogenetic relatedness of *Erysipelothrix* spp., seabirds can be considered as a reservoir for of both *E. rhusiopathiae* and potentially the new *Erysipelothrix* spp. in endangered native land bird species managed in island ecosystems. Cross-species transmission has been evident in previous studies with *E. rhusiopathiae* isolates from wildlife and domestic host species (Forde et al., 2016b). *E. rhusiopathiae* has been shown to affect the survival of some seabird species such as the critically endangered Amsterdam albatross (*Diomedea amsterdamensis*) (Jaeger et al., 2018). Although mottled petrels and Cook's petrels are listed as 'at risk' in terms of their conservation status, the clinical significance of *E. rhusiopathiae* on those seabirds is not known. Further investigations on the pathogenicity of *E. rhusiopathiae* within seabirds are needed to understand the effect of this bacteria on these populations.

In conclusion, the genomic diversity of *Erysipelothrix* spp. in two offshore islands, important for the conservation of endangered native land bird species, was investigated in this study using WGS. We identified a proposed new *Erysipelothrix* sp. that has not been described earlier, indicating that more than one species of *Erysipelothrix* is present New Zealand birds. The results suggest a high genomic diversity within this genus. *Erysipelothrix rhusiopathiae* strains were different between the bird species and between the two islands investigated. This study provides evidence to support the hypothesis that seabirds are a possible reservoir of *Erysipelothrix* spp. for endangered native land bird species sharing the island habitat. Although, the study was limited by the number of *E. rhusiopathiae* isolates recovered from endangered native land birds, it has highlighted the importance of this pathogen for the conservation of New Zealand native birds.

Chapter 5

General discussion

5.1. Overview of the key findings

The principal aim of this thesis was to explore the health risks for kākāpō (*Strigops habroptilus*) on offshore islands focusing on two major health problems affecting this critically endangered New Zealand parrot. While focussed specifically on kākāpō and their diseases, there are wider implications for the use of offshore islands in biodiversity conservation. Specifically, translocating wildlife to these areas may expose them to novel environmental and habitat changes that may result in disease, and that seabirds may weaken the biosecurity of these offshore refuges by being reservoirs of pathogens.

Chapter 2 covered an epidemiological investigation on exudative cloacitis, a disease affecting cloaca and the vent of the kākāpō. I was able to present a case series and map the geographical distribution of exudative cloacitis cases over the years (from 2002-2017). In addition, this study measured and compared pH, moisture and ammonium from kākāpō roosts and analysed any association of the climatic factors with the occurrence of new cases in Whenua Hou, within the limits of the available meteorological data. Those investigations revealed that there was no gender or age group preference for birds affected with exudative cloacitis and there was no spatial clustering of the diseased birds. That is, the disease distribution reflected the normal kākāpō distribution within the island. My analysis determined there was no evidence of a relationship between the selected chemical characteristics of the substrate of roosting sites with the occurrence of new cases of exudative cloacitis. However, it was evident that the roost ammonium and moisture content would be worth further examining with future cases. In terms of climatic data analysis there was no association of the available annual maximum temperature and rainfall data with the disease incidence each year. However, this analysis was limited by the lack of climatic data reporting from Whenua Hou and the study highlighted the value of replacing a functioning weather station on the island.

Chapter 3 described a survey conducted to determine the prevalence of *E. rhusiopathiae* in seabirds in two offshore islands which are important for the conservation of endangered New Zealand birds. Within the framework of disease surveillance, surveys like this provide an early estimation of the percentage of animals infected in a population especially when the reservoirs of infection are not completely known (Haydon et al., 2002; Guberti et al., 2014). The study provided new information on which species of seabirds were infected with *Erysipelothrix* species and allowed an estimation of the prevalence of the infection at the time.

Chapter 4 further described the prevalence and the genomic diversity of *Erysipelothrix* spp. in burrowing seabirds in Whenua Hou and Te Hauturu-o-Toi. Whole genome sequencing revealed the genetic diversity of *Erysipelothrix* spp. isolated from seabirds breeding on two selected islands and in endangered New Zealand land birds. This study revealed the presence of a new *Erysipelothrix* sp. phylogenetically distinct from *E. rhusiopathiae* in Cook's petrel (*Pterodroma cookii*) from Te Hauturu-o-Toi and in a kākāpō from Whenua Hou. Phylogenetic analysis revealed that there is a geographic differentiation of the *E. rhusiopathiae* isolates from the two islands studied.

5.2. Reservoirs of infections

Considering the phylogenetic evidence that *E. rhusiopathiae* isolates from takahē (*Porphyrio hochstetteri*) and kiwi (*Apteryx* spp.) have clustered with seabird isolates, I have gathered further evidence that suggests burrowing seabirds from both islands could act as reservoirs of *E. rhusiopathiae* for kākāpō and other endangered species conserved on these islands. For the purposes of this study, the endangered kākāpō would be defined as the target population and the seabirds would be the potential disease reservoir. However, what species is labelled as the

target population depends on the focus of study. For example, seabirds such as the Cook's petrel are also threatened, and the recovery of *E. rhusiopathiae* from dead seabirds suggests this organism may play a role in mortality of these birds as well.

Practical indicators to identify a reservoir of infection include epidemiological evidence of association, evidence of natural infection in nontarget populations, genetic or antigenic characteristics and intervention studies (Haydon et al., 2002). However, Hallmaier-Wacker et al., (2017) have suggested to use the term 'disease reservoir' only if evidence is available for maintenance and a feasible transmission route of the pathogen to the target species. Therefore, a combined investigative approach is needed with methods based on species-specific assays and technically sound field investigations to understand pathogen dynamics within a natural reservoir of an infection. In this thesis, I have uncovered good evidence that the pathogen is maintained in the seabirds, but further study is required to fully understand any possible transmission routes between the seabirds and the kākāpō, or other endangered native land bird species managed on offshore islands.

My study has shown genetic evidence for abundant seabirds nesting on island ecosystem acting as a potential reservoir of *Erysipelothrix* spp. for endangered wildlife sharing those ecosystems. Seroprevalence of *Erysipelothrix* antibodies in seabird population could be assessed as a next step in confirming the circulation of the bacterium in those populations. For example, a high prevalence of antibodies against *E. rhusiopathiae* have been associated with die offs and population decline of muskoxen (*Ovibos moschatus*) in Canada and Alaska (Mavrot et al., 2020). Ideally, longitudinal studies with multiple sampling events are needed to confirm the persistence and the maintenance of the pathogen in the reservoir host.

5.3. Pathogen spillover

Circulation of the pathogen within the reservoir host is a requirement for disease spillover events to occur (Glennon et al., 2019). The pathogen prevalence in the reservoir host species is one of the major determinants of spillover risk between species (Plowright et al., 2017). We have demonstrated the presence of *Erysipelothrix* sp. including the pathogenic *E. rhusiopathiae* in burrowing seabird species in New Zealand island ecosystem with a higher prevalence in some species. These birds share the same environment with endangered native land birds and there is evidence of direct contact between those species (Powlesland et al., 2006) providing possible routes for pathogen spillover between the species.

Since many animal species can act as asymptomatic carriers of *E. rhusiopathiae,* environmental contamination and exposure to this pathogen could be common for animals. It can be assumed that host susceptibility, environmental factors and the virulence of the bacterial strain are affecting the possibility of an infected bird showing the clinical signs of disease following pathogen transmission. The pathway of transmission of *E. rhusiopathiae* from infected seabirds to endangered native land birds was not determined in this research. As I have mentioned earlier, one hypothesis is that *E. rhusiopathiae* may be shed from decomposing seabird bodies, contaminating the soil which may then be ingested by other species during preening or feeding. I recommend conducting an environmental study to investigate the persistence and the potential transmission pathways of this pathogen between the bird species.

The effects of *Erysipelothrix* on seabird population health have not been studied in great detail, however, they may be at high risk from infection as they feed exclusively on marine prey. *E. rhusiopathiae* can survive on the external mucus layer of fish for long periods (Wood, 1973). *E. rhusiopathiae* could potentially be a pathogen that affects the survival and conservation of threatened seabird species worldwide as shown by a study on mortality in yellow nosed

albatross (*Thalassarche carteri*) (Jaeger et al., 2018). There may be multiplication and increased transmission of the pathogen within seabirds as many gathers in large colonies during their breeding season for several months. Longitudinal studies of the seabird populations are difficult due to their pelagic movements, however a focussed study over the breeding season could elucidate the dynamics of *E. rhusiopathiae* infection in the seabirds at this time.

5.4. Methodological limitations

As with any research conducted on wild populations, there were some unavoidable limitations to this study. For example, in Chapter 3, I described a survey conducted to determine the prevalence of *E. rhusiopathiae* in seabirds in two selected islands. These kinds of targeted studies are traditionally conducted as cross-sectional surveys taking a representative sample from the population (Cross et al., 2009; Cowled et al., 2012). Obtaining samples that are large enough and representative of the population of interest is a difficulty in epidemiological studies of wildlife populations (Courchamp et al., 2000; Nusser et al., 2008). Although random sampling in time and space has been recommended, opportunities for random sampling are rare due to the logistic challenges and non-random distribution of hosts in the study of wildlife diseases (Becker et al., 2019). On the other hand, disease distribution is rarely random. Opportunistic or convenience sampling was practiced in collecting seabird samples in the current study since there were no prior data on this disease in particular species or spillover events. Some areas of the islands were not accessible for sampling due to the nature of the terrain and management restrictions. Some of the areas were overrepresented due to the presence of known seabird colonies. However, based on the findings from this study, a

stratified random sampling design could be possible for future longitudinal studies, within the limits imposed by the pelagic lifestyle of the birds.

We were only able to calculate the apparent prevalence of *E. rhusiopathiae* because of the lack of details on test sensitivity and specificity for the MALDI-TOF MS and PCR using *Erysipelothrix* spp. specific primers to estimate the true prevalence. To calculate the true prevalence of *Erysipelothrix* sp. the above diagnostic tests must be validated for different wild bird species in this study. Most diagnostic tests that are developed for domestic animals are used often for wild animals without being validated for species concerned due to the logistical difficulties of performing this in wild populations (Breed et al., 2009).

In chapter 3, all the *Erysipelothrix* isolates were identified as *E. rhusiopathiae* using MALDI-TOF MS. However, four of those isolates were recognised as genetically distinct from *E. rhusiopathiae* in phylogenetic analysis. The ability of MALDI-TOF MS to accurately identify organisms directly from primary selective isolation medium and the rapidity of identification are major advantages over traditional identification methods. However, as MALDI-TOF MS uses ribosomal protein spectra to identify bacterial species, this technique is not able to differentiate between isolates with very similar sequences (Wieser et al., 2012). Nucleotide sequences between two *Erysipelothrix* species, *E. rhusiopathiae* and *E. tonsillarum* are very similar (99.8%) with only three nucleotide differences between them (Kiuchi et al., 2000). However, MALDI-TOF MS will identify both as *E. rhusiopathiae* (Eriksson et al., 2014). The number of spectra available in the database MALDI-TOF MS are limited and only the organisms already in the database are able to be identified (Eriksson et al., 2014). Therefore, identification of new organisms depends on the availability of peptide mass fingerprints of the type strains of specific genera or species or subspecies (Church et al., 2020). The similarity of ribosomal protein spectra of the *E. rhusiopathiae* with the novel *Erysipelothrix* sp. in my study caused both species to be initially identified as *E. rhusiopathiae*., the limitations of MALDI-

TOF were offset by further DNA sequencing, and this approach is recommended for future microbiological surveys using MALDI-TOF MS.

5.5. Implications for conservation management

The epidemiological study in chapter 2 highlighted the gaps in the data that would have provided for a more complete epidemiological investigation. I was able to make recommendations for the collection of additional data with new cases of exudative cloacitis in future that may assist in better understanding the aetiology of this disease.

One important objective of wildlife health research is to identify risk factors for disease and then, where possible, modify these population risk factors to indirectly manage wildlife diseases (Ryser-Degiorgis, 2013). Based on the findings from the epidemiological investigation, I have suggested to evaluate the moisture and ammonium content of the substrate from kākāpō roosts as close in time to initiation of the disease as possible with future cases. The knowledge gaps highlighted in my study will facilitate a more detailed epidemiological study in future. The targeted survey of *E. rhusiopathiae* in seabirds provided baseline information that will facilitate targeted surveillance. Disease surveillance is the systematic collection of health-related data for analysis, interpretation and action in a predefined way and it aims to improve the health within populations (Toma et al., 1999; Ryser-Degiorgis, 2013; Walton et al., 2016). Greater rates of interactions between humans and wildlife have made disease surveillance increasingly important as evidenced by the emergence of SARS-CoV, MERS-CoV and SARS-CoV-2 from wildlife populations (da Silva et al., 2021). Disease surveillance has become a necessary component in identification of potential threats to animal and human health (Daszak et al., 2001; Jones et al., 2008; Artois et al., 2012). I recommend

that seabird mortalities on these offshore islands be routinely investigated by post-mortem examination, including microbiological and/or molecular testing for *Erysipelothrix* spp.

Understanding the reservoir of infections allows better control or sometimes elimination of infections by applying disease control measures towards the reservoir. This study has provided information on the prevalence of *E. rhusiopathiae* and phylogenetic evidence that supports seabirds as a reservoir of this pathogen for endangered native land birds managed on offshore islands. When planning translocations of endangered species and associated disease screening, the presence of seabirds on islands used as source and destination sites for translocated wildlife should be considered important. These findings are also important to consider in vaccination strategies used to control erysipelas in endangered wildlife. I recommend that endangered species held on offshore islands with large seabird colonies should be vaccinated for erysipelas where feasible. Finally, the wider effects of *E. rhusiopathiae* on seabirds should be considered regarding conservation of these birds already threatened with commercial fishing industry, marine and land pollution, invasive predators and habitat degradation (Croxall et al., 2012).

5.6. The health risks for kākāpō on offshore islands

The key advantages of island ecosystems for translocated animals are the lack of introduced mammalian predators, limited conflict with human development and interactions, reduced exposure to domestic animals and the diseases they carry and the ability to use quarantine protocols for entry to the islands to manage disease and pest exposure. However, potential crowding as the endangered population grows and concentration and interaction of birds around focal areas on the island such as feeding stations or watering points are major disadvantages of these habitats. Overcrowding on islands may lead to a limited ability for

individuals to move out of less desirable habitat to avoid conflict. In addition, large numbers of seabirds also using the islands as habitat may act as potential disease reservoirs (Altizer et al., 2011; Fuller et al., 2012) or modifiers of the environment through their urates (Otero et al., 2018). This type of environment modification could possibly contribute to the soil ammonium content of the island ecosystem as discussed in the Chapter 2.

Island habitats have potential health implications for the translocated birds. Those health problems could be associated with exposure to new environmental threats (as hypothesised as a potential cause for exudative cloacitis) or new pathogens (as demonstrated by *E. rhusiopathiae* spillover events). Disease is a difficult conservation challenge to manage for small, fragmented populations, and island ecosystems may be particularly vulnerable to novel disease issues.

The management of disease should be considered as an essential component of the conservation management plans of endangered species. Management of the health of wildlife on island habitats needs to include surveillance for new health issues such as these and adaptive management to address new issues as they arise as is currently being implemented by the Kākāpō Recovery Team.

Chapter 6

Bibliography

Ahrne, S., Stenstrom, I., Jensen, N., Pettersson, B., Uhlen, M., & Molin, G. (1995). Classification of Eysipelothrix Strains on the Basis of Restriction Fragment Length Polymorphisms. *International Journal of Systematic Bacteriology, 45*(2), 382-385

Alley, M. R. (2002). Avian wildlife diseases in New Zealand: current issues and achievements. *New Zealand Veterinary Journal, 50*(sup3), 118-120.

Alley, M. R., Castro, I., & Hunter, J. E. (1999). Aspergillosis in hihi (*Notiomystis cincta*) on Mokoia Island. *New Zealand Veterinary Journal, 47*(3), 88-91.

Alley, M. R., Hale, K. A., Cash, W., Ha, H. J., & Howe, L. (2010). Concurrent avian malaria and avipox virus infection in translocated South Island saddlebacks (*Philesturnus carunculatus carunculatus*). *New Zealand Veterinary Journal, 58*(4), 218-223.

Alley, M., & Gartrell, B. (2019). Wildlife diseases in New Zealand: recent findings and future challenges. *New Zealand veterinary journal, 67*(1), 1-11.

Alley, M., Gartrell, B., & Morgan, K. (2005). Aflatoxicosis in a kakapo. *Kokako, 12*(29)

Altizer, S., Bartel, R., & Han, B. A. (2011). Animal Migration and Infectious Disease Risk. *Science, 331*(6015), 296-302.

Armstrong, D. P., Castro, I., & Griffiths, R. (2007). Using adaptive management to determine requirements of re-introduced populations: the case of the New Zealand hihi. *Journal of Applied Ecology, 44*(5), 953-962.

Armstrong, D. P., Castro, I., Alley, J. C., Feenstra, B., & Perrott, J. K. (1999). Mortality and behaviour of hihi, an endangered New Zealand honeyeater, in the establishment phase following translocation. *Biological Conservation, 89*(3), 329-339.

Artois, M., Ben Jebara, K., Warns-Petit, E., & Leighton, F. (2012). National wildlife health surveillance systems. Paper presented at the Animal health and biodiversity–preparing for the future (Compend OIE Global Conf Wildl).

Asimaki, E., Nolte, O., Overesch, G., & Strahm, C. (2017). A dangerous hobby? *Erysipelothrix rhusiopathiae* bacteremia most probably acquired from freshwater aquarium fish handling. *Infection, 45*(4), 557-562.

Atkinson, C. T., Woods, K. L., Dusek, R. J., Sileo, L. S., & Iko, W. M. (1995). Wildlife disease and conservation in Hawaii: Pathogenicity of avian malaria (*Plasmodium relictum*) in experimentally infected Iiwi (*Vestiaria coccinea*). *Parasitology, 111*(S1),

Auguie, B., & Antonov, A. (2017). Miscellaneous Functions for ``Grid'' Graphics. R package version 2.3.

Aziz, T., Martin, M., Barnes, H., & Fletcher, O. (2010). Cloacitis: an emerging problem in broiler breeder hens. *World Poultry, 26*(8), 12-13.

Baddeley, A., Rubak, E., &Turner, R. (2015). Spatial Point Patterns: Methodology and Applications with R. Chapman and Hall/CRC Press, London.

Barker, I.K., P.L. Carbonell, and A.J. Bradley. (1981). Cytomegalovirus infection of the prostate in the dasyurid marsupials *Phascogale tapoatafa* and *Antechinus stuartii*. *Journal of Wildlife Diseases* 17:433–441.

Batson, W. G., Gordon, I. J., Fletcher, D. B., Manning, A. D., & Hayward, M. (2015). Review: Translocation tactics: a framework to support the IUCN Guidelines for wildlife translocations and improve the quality of applied methods. *Journal of Applied Ecology, 52*(6),

Becker, D. J., Washburne, A. D., Faust, C. L., Pulliam, J. R., Mordecai, E. A., Lloyd-Smith, J. O., & Plowright, R. K. (2019). Dynamic and integrative approaches to

understanding pathogen spillover. *Philosophical Transactions of the Royal Society B* 374:2019001420190014. http://doi.org/10.1098/rstb.2019.0014

Beemer, A. M., Kuttin, E. S., & Katz, Z. (1973). Epidemic Venereal Disease Due to *Candida albicans* in Geese in Israel. *Avian Diseases, 17*(3), 639-649.

Bellingham, P. J., Towns, D. R., Cameron, E. K., Davis, J. J., Wardle, D. A., Wilmshurst, J. M., & Mulder, C. P. H. (2010). New Zealand island restoration: seabirds, predators, and the importance of history. *New Zealand Journal of Ecology, 34*(1), 115-136.

Benskin, C. M. H., Wilson, K., Jones, K., & Hartley, I. R. (2009). Bacterial pathogens in wild birds: a review of the frequency and effects of infection. *Biological Reviews, 84*(3), 349-373.

Berger, L., Roberts, A. A., Voyles, J., Longcore, J. E., Murray, K. A., & Skerratt, L. F. (2016). History and recent progress on chytridiomycosis in amphibians. *Fungal Ecology, 19*, 89-99.

Berger, L., Speare, R., Daszak, P., Green, D. E., Cunningham, A. A., Goggin, C. L., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K. R., Hines, H.B., Lips, K.R., & Marantelli, G. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences, 95*(15), 9031-9036. https://doi.org/10.1073/pnas.95.15.9031

Bergner, L., Dussex, N., Jamieson, I., & Robertson, B. (2016). European Colonization, Not Polynesian Arrival, Impacted Population Size and Genetic Diversity in the Critically Endangered New Zealand Kākāpō. *The Journal of Heredity 107*(7), 593 - 602.

Bivand, R., Keitt, T., Rowlingson, B., Pebesma, E., Sumner, M., Hijmans, R., Rouault, E., Warmerdam, F., Ooms, J., & Rundel, C. (2019a). rgdal: Bindings for the 'Geospatial' data abstraction library. R package version 1.4-8. L

Bivand, R., Lewin-Koh, N., Pebesma, E., Archer, E., Baddeley, A., Bearman, N., Bibiko, H., Brey, S., Callahan, J., Carrillo, G., Dray, S., Forrest, D., Friendly, M., Giraudoux, P., Golicher, D., Rubio, V.G., Hausmann, P., Hufthammer, K.O., Jagger, T., Johnson, K., Luque, S., MacQueen, D., Niccolai, A., Pebesma, E., Lamigueiro, O.P., Plunkett, E., Short, T., Snow, G., Stabler, B., Stokely, M., & Turner, R. (2019b). maptools: Tools for handling spatial objects. R package version 0.9-9. http://maptools.r-forge.r-project.org/

Bivand, R., Ono, H., Dunlap, R., Stigler, M., Denney, B., & Hernangómez, D. (2007). ClassInt: Choose univariate class intervals. R package version 0.1-7

Bivand, R., Rundel, C., Pebesma, E., Stuetz, R., Hufthammer, K.O., Giraudoux, P., Davis, M., & Santilli, S. (2019c). rgeos: Interface to geometry engine - open source ('GEOS'). R package version 0.5-2. https://r-forge.r-project.org/projects/rgeos/

Bivand, R.S., Pebesma, E., & Gomez-Rubio, V. (2013). Applied spatial data analysis with R, Second edition. Springer, NY. https://asdar-book.org/.

Bizzini, A., Durussel, C., Bille, J., Greub, G., & Prod'hom, G. (2010). Performance of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. *Journal of Clinical Microbiology, 48*(5), 1549–1554.

Bo¨hm, M., Palphramand, K. L., Newton-Cross, G., Hutchings, M. R., & White, P. C. L. (2008). The spatial distribution of badgers, setts and latrines: the risk for intra-specific and badger-livestock disease transmission. *Ecography, 31*, 525-537.

Boadella, M., Ruiz-Fons, J. F., Vicente, J., Martin, M., Segales, J., & Gortazar, C. (2012). Seroprevalence evolution of selected pathogens in Iberian wild boar. *Transboundary and Emerging Diseases, 59*(5), 395-404.

Boerner, L., Nevis, K. R., Hinckley, L. S., Weber, E. S., & Frasca, S. (2004). *Erysipelothrix* septicemia in a little blue penguin (*Eudyptula minor*). *Journal of Veterinary Diagnostic Investigation, 16*, 145–149.

Brangenberg, N., McInnes, C., Connolly, J. H., & Rogers, L. E. (2003). Absence of Salmonella and Campylobacter species in faecal and cloacal swab samples from kakapo (*Strigops habroptilus*) on Codfish Island, New Zealand. *Journal of Avian Medicine and Surgery, 17*(4), 203-205. 10.1647/2003-023

Breed, A. C., Plowright, R. K., Hayman, D. T., Knobel, D. L., Molenaar, F. M., Gardner–Roberts, D., Cleaveland, S., Haydon, D. T., Kock , R. A., Andrew A. Cunningham, A. A., Sainsbury, A. W., & Delahay., R.J. (2009). Disease management in endangered mammals. In *Management of Disease in Wild Mammals*. Springer, pp 215-239.

Bricker, J. M., & Saif, Y. M. (2013). Erysipelas. In Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL, & N. VL (Eds.), *Diseases of Poultry.* Blackwell Publishing Ames, pp 971-1053

Brooke, C. J., & Riley, T. V. (1999). *Erysipelothrix rhusiopathiae*: bacteriology, epidemiology and clinical manifestations of an occupational pathogen. *Journal of Medical Microbiology, 48*, 789-799.

Bruner, J. A., Griffith, R. W., Greve, J. H., & Wood, R. L. (1984). *Erysipelothrix rhusiopathiae* serotype 5 isolated from a white-tailed deer in Iowa. *Journal of Wildlife Diseases, 20*(3), 235-236.

Brunner, T. (1848). Journal of an expedition to the interior of the Middle Island, New Zealand, 1846-1848. In Taylor N. M. (Eds.), Early travellers in New Zealand. Clarendon Press, Oxford, pp 257-320.

Carlile, F. S. (1984). Ammonia in Poultry Houses: A Literature Review. *World's Poultry Science Journal, 40*(2), 99-113.

Carpenter, T. E. (2001). Methods to investigate spatial and temporal clustering in veterinary epidemiology. *Preventive Veterinary Medicine, 48*, 303-320.

Castinel, A., Pomroy, B., & Grinberg, A. (2007). Hookworm infection and *Klebsiella pneumoniae* epidemics in New Zealand sea lion pups. *Veterinary Microbiology, 125*(3-4), 388-389.

Chen, C., Kim, A.Y., Ross, M., & Wakefield, J. (2018). SpatialEpi: Methods and data for spatial epidemiology. R package version 1.2.3.

Chirico, J., Eriksson, H., Fossum, O., & Jansson, D. (2003). The poultry red mite, *Dermanyssus gallinae*, a potential vector of *Erysipelothrix rhusiopathiae* causing erysipelas in hens. *Medical and Veterinary Entomology, 17*, 232–234.

Chong, R. S., Shinwari, M. W., Amigh, M. J., Aravena-Roman, M., & Riley, T. V. (2015). First report of *Erysipelothrix rhusiopathiae*-associated septicaemia and histologic changes in cultured Australian eels, *Anguilla reinhardtii* (Steindachner, 1867) and *A. australis* (Richardson, 1841). *Journal of Fish Diseases, 38*(9), 839-847.

Church, D. L., Cerutti, L., Gürtler, A., Griener, T., Zelazny, A., & Emler, S. (2020). Performance and application of 16S rRNA gene cycle sequencing for routine identification of bacteria in the clinical microbiology laboratory. *Clinical Microbiology Reviews, 33*(4)

Closa-Sebastià, F., Casas-Díaz, E., Cuenca, R., Lavín, S., Mentaberre, G., & Marco, I. (2011). Antibodies to selected pathogens in wild boar (*Sus scrofa*) from Catalonia (NE Spain). *European Journal of Wildlife Research, 57*(4), 977-981.

Clout, M. N. (2006). A celebration of kakapo: progress in the conservation of an enigmatic parrot. *Notornis, 53*(1), 1-2

Clout, M. N., & Merton, D. V. (1998). Saving the Kakapo: the conservation of the world's most peculiar parrot. *Bird Conservation International* (8), 281-296.

Courchamp, F., Say, L., & Pontier, D. (2000). Detection, identification, and correction of a bias in an epidemiological study. *Journal of Wildlife Diseases, 36*(1), 71-78.

Cousquer, G. (2005). Erysipelas outbreak in racing pigeons following ingestion of compost. *The Veterinary Record* (156), 656.

Cowled, B. D., Ward, M. P., Laffan, S. W., Galea, F., Garner, M. G., MacDonald, A. J., Ian Marsh,Petra Muellner,Katherine Negus,Sumaiya Quasim,Andrew P. Woolnough . . . Quasim, S. (2012). Integrating survey and molecular approaches to better understand wildlife disease ecology. *PLoS One, 7*(10), e46310. https://doi.org/10.1371/journal.pone.0046310

Cross, P. C., Drewe, J., Patrek, V., Pearce, G., Samuel, M. D., & Delahay, R. J. (2009). Wildlife population structure and parasite transmission: implications for disease management. In *Management of Disease in Wild Mammals.* Springer. Pp 9-29

Crosta, L., Gerlach, H., Habilb., Bu ¨rkle, M., & Timossi, L. (2003). Physiology, diagnosis, and diseases of the avian reproductive tract. *Veterinary Clinics of North America: Exotic Animal Practice, 6,* 57–83.

Croxall, J. P., Butchart, S. H. M., Lascelles, B. E. N., Stattersfield, A. J., Sullivan, B. E. N., Symes, A., & Taylor, P. (2012). Seabird conservation status, threats and priority actions: a global assessment. *Bird Conservation International, 22*(01), 1-34.

Cunningham, A. A. (1996). Disease risks of wildlife translocations. *Conservation Biology, 10*(2), 349-353.

da Silva, P. G., Mesquita, J. R., Nascimento, M. d. S. J., & Ferreira, V. A. M. (2020). Viral, host and environmental factors that favour anthropozoonotic spillover of coronaviruses: An opinionated review, focusing on SARS-CoV, MERS-CoV and SARS-CoV-2. *Science of the Total Environment*, 141483.

Daszak, P., Berger, L., Cunningham, A. A., Hyatt, A. D., Green, & D. E., Speare, R. (1999). Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases*, *5*(6), 735.

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2001). Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta tropica*, *78*(2), 103-116.

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2003). Infectious disease and amphibian population declines. Diversity and Distributions, 9(2), 141-150.

Deem, S. L., Karesh, W. B., & Weisman, W. (2001). Putting theory into practice: Wildlife health in conservation. *Conservation Biology*, *15*(5), 1224-1233.

Derraik, J. G., Tompkins, D. M., Alley, M. R., Holder, P., & Atkinson, T. (2008). Epidemiology of an avian malaria outbreak in a native bird species (*Mohoua ochrocephala*) in New Zealand. *Journal of Royal Society of New Zealand*, *38(4)*, 237-242.

DOC. 2012. https://www.doc.govt.nz/about-us/our-policies-and-plans/statutory-plans/statutory-plan-publications/conservation-management-strategies/stewart-island-rakiura/section-one/part-two-places/2_3-codfish-island-whenua-hou-place/

DOC. 2021.https://www.doc.govt.nz/nature/native-animals/birds/birds-a-z/kakapo/

Devleesschauwer, B., Torgerson, P., Charlier, J., Levecke, B., Praet, N., Roelandt, S., Smit, S., Dorny, P., Berkvens, D., & Speybroeck, N. 2014. prevalence: Tools for prevalence assessment studies. R package version 0.4.0. http://cran.r-project.org/package=prevalence

Diggle, P. J., Chetwynd, A. G., Haggkvist, R., & Morris, S. E. (1995). Second-order analysis of space-time clustering. *Statistical Methods in Medical Research*, *4*, 124-136.

Dussex, N., Von Seth, J., Robertson, B. C., & Dalén, L. (2018). Full mitogenomes in the critically endangered kākāpō reveal major post-glacial and anthropogenic effects on neutral genetic diversity. *Genes*, *9*(4), 220.

Eamens, G.J., M.J. Turner, and R.E. Catt. 1988. Serotypes of *Erysipelothrix rhusiopathiae* in Australian pigs, small ruminants, poultry, and captive wild birds and mammals. *Australian Veterinary Journal* 65:249–252.

Eason, D. K., Elliott, G. P., Merton, D. V., Jansen, P. W., & Moorhouse, R. J. (2006). Breeding biology of kākāpō (*Strigops habroptilus*) on offshore island sanctuaries, 1990-2002. *Notornis*, *53*(1), 27-36.

Eliott, P. G., Merton, D. V., & Jansen, P. W. (2001). Intensive management of critically endangered species: the kakapo. *Biological Conservation* (99), 121-133.

Elliott, G. P., Eason, D. K., Jansen, P. W., Merton, D. V., Harper, G. A., & Moorhouse, R. J. (2006). Productivity of kakapo (*Strigops habroptilus*) on offshore island refuges. *Notornis, 53*(1), 138-142.

Eriksson, H., Bagge, E., Baverud, V., Fellstrom, C., & Jansson, D. S. (2014). *Erysipelothrix rhusiopathiae* contamination in the poultry house environment during erysipelas outbreaks in organic laying hen flocks. *Avian Pathology, 43*(3), 231-237.

Eriksson, H., Jansson, D. S., Johansson, K. E., Baverud, V., Chirico, J., & Aspan, A. (2009). Characterization of *Erysipelothrix rhusiopathiae* isolates from poultry, pigs, emus, the poultry red mite and other animals. *Veterinary Microbiology, 137*(1-2), 98-104.

Ersbøll, A. K., & Ersbøll, B. K. (2009). Simulation of the K-function in the analysis of spatial clustering for non-randomly distributed locations—Exemplified by bovine virus diarrhoea virus (BVDV) infection in Denmark. *Preventive Veterinary Medicine*, *91*(1), 64-71.

Ewald, F.W., 1981. The genus Erysipelothrix. In: Starr, MP, Stolp, H, Truper, HG, Balows, A, Schlegel, HG. (Eds.), The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria. Spring-Verlag, New York, pp. 1688–1700.

Ewen, G. J., Thorogood, R., Nicol, C., Armstrong, D. P., & Alley, M. (2007). *Salmonella typhimurium* in Hihi, New Zealand. *Emerging Infectious Diseases, 13*(5)

Ewen, J. G., Armstrong, D. P., Empson, R., Jack, S., Makan, T., McInnes, K., Parker, K. A., Richardson, K., & Alley, M. (2012). Parasite management in translocations: lessons from a threatened New Zealand bird. *Oryx, 46*(03), 446-456.

Farrimond, M., Clout, M. N., & Elliott, G. P. (2006a). Home range size of kākāpō (*Strigops habroptilus*) on Codfish Island. *Notornis 53*(1), 150-152.

Farrimond, M., Elliott, G. P., & Clout, M. N. (2006b). Growth and fledging of kākāpō. *Notornis, 53*(1), 112-115.

Ferguson, N., Gates, R. S., Taraba, J., Cantor, A., Pescatore, A., Straw, M., Ford, M.J., & Burnham, D. (1998). The effect of dietary protein and phosphorus on ammonia concentration and litter composition in broilers. *Poultry Science, 77*(8), 1085-1093.

Fiorito, C. D., Bentancor, A., Lombardo, D., & Bertellotti, M. (2016). *Erysipelothrix rhusiopathiae* isolated from gull-inflicted wounds in southern right whale calves. *Diseases of Aquatic Organisms, 121*(1), 67-73.

Flood, B. 2003. The New Zealand storm-petrel is not extinct. Birding World 16:479-482.

Forde, T., Biek, R., Zadoks, R., Workentine, M. L., De Buck, J., Kutz, S., Opriessnig, T., Trewby, H., van der Meer, F., & Orsel, K. (2016). Genomic analysis of the multi-host pathogen *Erysipelothrix rhusiopathiae* reveals extensive recombination as well as the existence of three generalist clades with wide geographic distribution. *BMC Genomics, 17*, 461.

Fortin, M.J., Dale, M.R.T., & Bertazzon, S. (2010). Spatial analysis of wildlife distribution and disease spread, in: Cushman, S.A., Huettmann, F. (Eds.), Spatial complexity, informatics, and wildlife conservation. Springer, Tokyo, pp. 255-272.

Frobes, N. A. (2005). Soft tissue surgery. InHarcourt-Brown, N & Chitty, J. (Eds.), BSAVA Manual of psittacine birds.British small animal veterinary association, pp 107-120.

Fukami, T., Wardle, D. A., Bellingham, P. J., Mulder, C. P., Towns, D. R., Yeates, G. W., Bonner, K. I., Durrett, M.S., Grant-Hoffman, M.N., & Williamson, W. M. (2006). Above- and below-ground impacts of introduced predators in seabird-dominated island ecosystems. *Ecology Letters*, *9*(12), 1299-1307.

Fuller, T., Bensch, S., Muller, I., Novembre, J., Perez-Tris, J., Ricklefs, R. E., Smith, T. B., & Waldenstrom, J. (2012). The ecology of emerging infectious diseases in migratory birds: an assessment of the role of climate change and priorities for future research. *Ecohealth*, *9*(1), 80-88.

Galindo-Cardiel, I., Opriessnig, T., Molina, L., & Juan-Salles, C. (2012). Outbreak of mortality in psittacine birds in a mixed-species aviary associated with *Erysipelothrix rhusiopathiae* infection. *Veterinary Pathology*, *49*(3), 498-502.

Gallana, M., Ryser-Degiorgis, M.-P., Wahli, T., & Segner, H. (2013). Climate change and infectious diseases of wildlife: altered interactions between pathogens, vectors and hosts. *Current Zoology*, *59*(3), 427-437.

Gartrell, B. D., Alley, M. R., Mack, H., Donald, J., McInnes, K., & Jansen, P. (2005). Erysipelas in the critically endangered kakapo (*Strigops habroptilus*). *Avian Pathology*, *34*(5), 383-387.

Gartrell, B. D., Jillings, E., Adlington, B. A., Mack, H., & Nelson, N. J. (2006). Health screening for a translocation of captive-reared tuatara (Sphenodon punctatus) to an island refuge. *New Zealand Veterinary Journal*, *54*(6), 344-349.

Glennon, E. E., Becker, D. J., Peel, A. J., Garnier, R., Suu-Ire, R. D., Gibson, L., Hayman, D. T. S., Wood, J. L. N., Cunningham, A. A., Plowright, R. K. & Restif, O. (2019). What is stirring in the reservoir? Modelling mechanisms of henipavirus circulation in fruit bat hosts. *Philosophical Transactions of the Royal Society B, 374*(1782), 20190021.

Grange, Z. L., Biggs, P. J., Rose, S. P., Gartrell, B. D., Nelson, N. J., & French, N. P. (2017). Genomic epidemiology and management of Salmonella in island ecosystems used for takahe conservation. *Microbial Ecology*, 74(3), 735-744.

Grange, Z. L., Gartrell, B. D., Biggs, P. J., Nelson, N. J., Anderson, M., & French, N. P. (2016). Microbial genomics of a host-associated commensal bacterium in fragmented populations of endangered takahe. *Microbial Ecology*, 71(4), 1020-1029.

Griffith, R. W., Scott, J. M., Carpenter, J. W., & Reed, C. (1989). Translocation as a species conservation tool: Status and strategy. *Science, New Series, 245*(4917), 477-480.

Griffiths, G., & Buller, N. (1991). *Erysipelothrix rhusiopathiae* infection in semi-intensively farmed emus. *Australian veterinary journal, 68*(3), 121.

Groeschel, M., Forde, T., Turvey, S., Joffe, A. M., Hui, C., Naidu, P., Mavrot, F., Kutz, S., Singh, A. E. (2019). An unusual case of *Erysipelothrix rhusiopathiae* prosthetic joint infection from the Canadian Arctic: whole genome sequencing unable to identify a zoonotic source. *BMC infectious diseases, 19*(1), 282.

Grogan, L. F., Berger, L., Rose, K., Grillo, V., Cashins, S. D., & Skerratt, L. F. (2014). Surveillance for emerging biodiversity diseases of wildlife. *PLoS Pathogens, 10*(5), e1004015.

Grueber, C. E., Knafler, G. J., King, T. M., Senior, A. M., Grosser, S., Robertson, B., Weston, K. A., Brekke, P., Harris, C. L. W., & Jamieson, I. G. (2015). Toll-like receptor diversity in 10 threatened bird species: relationship with microsatellite heterozygosity. *Conservation Genetics, 16*(3), 595-611. 10.1007/s10592-014-0685-x

Grueber, C. E., Sutton, J. T., Heber, S., Briskie, J. V., Jamieson, I. G., & Robertson, B. C. (2017). Reciprocal translocation of small numbers of inbred individuals rescues immunogenetic diversity. *Molecular Ecology, 26*(10), 2660-2673.

Guberti, V., Stancampiano, L., & Ferrari, N. (2014). Surveillance, monitoring and surveys of wildlife diseases: a public health and conservation approach. *Hystrix, the Italian Journal of Mammalogy, 25*(1), 3-8.

Hale, K. A., & Briskie, J. V. (2009). Rapid recovery of an island population of the threatened South Island Saddleback *Philesturnus c. carunculatus* after a pathogen outbreak. *Bird Conservation International, 19*(03), 239.

Hallmaier-Wacker, L. K., Munster, V. J., & Knauf, S. (2017). Disease reservoirs: from conceptual frameworks to applicable criteria: disease reservoir criteria. *Emerging Microbes and Infections, 6*(1), 1-5.

Harada, K., Uchiyama, M., Hoshi, T., & Takahashi, T. (2009). Comparison of three DNA extraction methods for detection of *Erysipelothrix rhusiopathiae* in chicken blood by polymerase chain reaction. *Journal of Veterinary Diagnostic Investigation, 21*, 354–358.

Harrison, R. L. (2010). Introduction to Monte Carlo Simulation. *AIP Conference Proceedings*,

Hartley, M., & Sainsbury, A. (2017). Methods of disease risk analysis in wildlife translocations for conservation purposes. *Ecohealth, 14*(Suppl 1), 16-29.

Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S., & Samuel, M. D. (2002). Climate warming and disease risks for terrestrial and marine biota. *Science, 296*(5576), 2158-2162.

Harwood, J., & Hall, A. (1990). Mass mortality in marine mammals: Its implications for population dynamics and genetics. *Trends in Ecology & Evolution, 5*(8), 254-257. https://doi.org/10.1016/0169-5347(90)90066-M

Hassanein, R., Sawada, T., Kataoka, Y., Ali, M., Itoh, K., & Suzuki, Y. (2000). Prevalence of *Erysipelothrix* Species in Healthy, Slaughtered Cattle. *Journal of Veterinary Epidemiology, 4*(71-79).

Hawley, D. M., & Altizer, S. M. (2011). Disease ecology meets ecological immunology: understanding the links between organismal immunity and infection dynamics in natural populations. *Functional Ecology, 25*(1),

Haydon, D. T., Cleaveland, S., Taylor, L. H., & Laurenson, M. K. (2002). Identifying reservoirs of infection: a conceptual and practical challenge. *Emerging Infectious Diseases, 8*(12), 1468-1473.

Hennig, G. E., Goebel, H. D., Fabis, J. J., & Khan, M. I. (2002). Diagnosis by Polymerase Chain Reaction of erysipelas septicemia in a flock of Ring-Necked pheasants. *Avian Diseases, 46*(2), 509-514.

Higgins, P. J. (1999). Handbook of Australian, New Zealand and Antarrctic birds; parrots to dollarbird (Vol. 4). Oxford University Press: Melbourne

Higgins, R. (2000). Bacteria and fungi of marine mammals: A review. *Canadian Veterinary Journal, 41*, 105-116.

Hijmans, R.J., & van Etten, J. (2012). raster: Geographic analysis and modelling with raster data. R package version 2.0-12. http://CRAN.R-project.org/package=raster

Hill, S., & Hill, J. (1987). Richard Henry of Resolution Island. *Dunedin, John McIndoe*

Holland, R. D., Wilkes, J. G., Ralli, F., Sutherland, J. B., Persons, C. C., Voorhees, K. J., & Lay, J. O. (1996). Rapid Identification of Intact Whole Bacteria Based on Spectral

Patterns using Matrix-assisted Laser Desorption/Ionization with Time-of-flight Mass Spectrometry. *Rapid Communications in Mass Spectrometry, 10*(10), 1227-1232.

Hooson, S., & Jamieson, I. G. (2003). Breeding biology of the South Island saddleback (*Philesturnus carunculatus carunculatus*, Callaeatidae). *Notornis, 50*(4), 191-200.

Hudson, P. J., Rizzoli, A., Grenfell, B. T., Heesterbeek, H., & Dobson, A. P. (2002). In *The Ecology of Wildlife Diseases*. Oxford University Press, Oxford.

IUCN (1998) Guidelines for Reintroductions. IUCN/Species Survival Commission Reintroduction Specialist Group, Gland, Switzerland.

IUCN/SSC. (2013). Guidelines for reintroductions and other conservation translocations. In *Version 1.0* (pp. viiii + 57 pp). Gland, Switzerland: IUCN Species Survival Commission.

Jacob-Hoff, R., & Gartrell, B. (2012). Veterinary Care of Kakapo. In Fowler, M. (Ed.), *Fowler's Zoo and Wild Animal Medicine Current Therapy*. Saint Louis: W.B. Saunders, pp 304-311.

Jaeger, A., Lebarbenchon, C., Bourret, V., Bastien, M., Lagadec, E., Thiebot, J. B., Boulinier, T., Delord K., Barbraud, C., Marteau, C., Dellagi, K., Tortosa, P.,Weimerskirch, H. (2018). Avian cholera outbreaks threaten seabird species on Amsterdam Island. *PLoS One, 13*(5), e0197291. 10.1371/journal.pone.0197291

Jakob-Hoff, R. M., Potter, J. S., & Shaw, S. D., 2009. Traumatic exudative cloacitis in a kākāpō. *Strigops habroptilus*. *Kākāpō 16*, 51-52.

Jamieson, I. G. (2015). Significance of population genetics for managing small natural and reintroduced populations in New Zealand. *New Zealand Journal of Ecology, 39*(1)

Jansen, P. W. (2006). Kakapo recovery: the basis of decision-making. *Notornis, 53*(1), 184.

Janssen, T., Voss, M., Kuhl, M., Semmler, T., Philipp, H. C., & Ewers, C. (2015). A combinational approach of multilocus sequence typing and other molecular typing

methods in unravelling the epidemiology of *Erysipelothrix rhusiopathiae* strains from poultry and mammals. *Veterinary Research, 46(1)*, 84.

Jensen, W. I., & Cotter, S. E. (1976). An Outbreak of Erysipelas in eared grebes (Podiceps nigricollis). *Journal of Wildlife Diseases, 12*(4), 583-586.

Joachim, G. L., & Marie, C. L. (1988). Characterization of *Erysipelothrix rhusiopathiae* isolated from an opossum (*Dideiphis virginiana*) with septicemia. *Journal of Wildlife Diseases, 24*(3), 557-559.

Jones, C.G. & Merton, D.V. (2011) A tale of two islands: the rescue and recovery of endemic birds in New Zealand and Mauritius. In Reintroduction Biology: integrating science and management, eds J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon, Chapter 2. Wiley-Blackwell, Oxford, UK.

Jones, D., 1986. Genus Erysipelothrix Rosenbach 367[al]. In: Sneath, P.H., Mair, N.S., Sharpe, M.E. (Eds.), Bergey's Manual of Systematic Bacteriology, vol. 2. Williams and Wilkins, Baltimore, pp. 1245–1249.

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature, 451*(7181), 990-993.

Jorden, F. T. W. (1990). Poultry Diseases. In (3 ed.): Baillière Tindall.

Kahle, D., & Wickham, H. (2013). ggmap: Spatial visualization with ggplot2. The R Journal, 5(1), 144-161. URL

Kākāpō recovery. 2020. https://www.doc.govt.nz/our-work/kakapo-recovery/

Keeney, D. R., & Nelson, D. W. (1982). Nitrogen in organic forms. In: Page, A.L. (Eds.), Methods of soil analysis. Part 2, Agronomy No. 9, American Society of Agronomy, Madison, WI, pp. 643-698.

Kinsel, M. J., Boehm, J. R., Harris, B., & Murnane, R. D. (1997). Fatal *Erysipelothrix rhusiopathiae* septicemia in a captive Pacific White-Sided Dolphin. *Journal of Zoo and Wildlife Medicine, 28*(4), 494-497.

Kirkpatrick, A., & Altizer, S. (2010). How do climate, evolution, and free-living hosts interact to determine the dynamics of pathogens and the burden of disease. *Nature Education Knowledge*, 1, 13.

Kock, R., Woodford, M., & Rossiter, P. (2010). Disease risks associated with the translocation of wildlife. *Revue scientifique et technique, 29*(2), 329-350.

Kohler, B., B. Wendland, U. Tornow, and M. Michael. 1987. The occurrence of bacterial infections in swamp beavers (*Myocaster coypus* Molina, 1782): 2. *Erysipelothrix rhusiopathiae* infections. Arch Exp Veterinarmed,41(3):442-446.

Kucsera, G. (1973). Proposal for standardization of the designations used for serotypes of *Erysipelothrix rhusiopathiae* (Migula) Buchanan. *International Journal of Systematic and Evolutionary Microbiology, 23*(2), 184-188.

Kurian, A., Neumann, E. J., Hall, W. F., & Marks, D. (2012). Serological survey of exposure to *Erysipelothrix rhusiopathiae* in poultry in New Zealand. *New Zealand Veterinary Journal, 60*(2), 106-109.

Kutz, S., Bollinger, T., Branigan, M., Checkley, S., Davison, T., Dumond, M., Elkin, B., Forde, T., Hutchins, W., Niptanatiak, A., Orsel, K. (2015). *Erysipelothrix rhusiopathiae* associated with recent widespread muskox mortalities in the Canadian Arctic. *Canadian Veterinary Journal, 56*, 260-263

Kwok, A. H. Y., Li, Y., Jiang, J., Jiang, P., & Leung, F. C. (2014). Complete genome assembly and characterization of an outbreak strain of the causative agent of swine erysipelas – *Erysipelothrix rhusiopathiae* SY1027. *BMC Microbiology, 14*(176)

Laurance, W. F. (2008). Global warming and amphibian extinctions in eastern Australia. *Austral Ecology, 33*(1), 1-9.

Leighton, F. A. (2002). Health risk assessments of the translocations of wild animals. *Revue scientifique et technique (International Office of Epizootics), 21*(1), 187-195.

Livingston, M., Fidler, A., Mellor, D., de Kloet, S., Eason, D., Elliott, G., & Moorhouse, R. (2013). Prevalence of IgY antibodies against *Erysipelothrix rhusiopathiae* in a critically endangered parrot (kakapo, *Strigops habroptilus*) and associated responses to vaccination. *Avian Pathology, 42*(5), 502-507.

Lloyd, B.D., & Powlesland, R.G. (1994). The decline of kākāpō *Strigops habroptilus* and attempts at conservation by translocation. *Biological Conservation, 69*(1),75-85.

Lloyd-Smith, J. O., George, D., Pepin, K. M., Pitzer, V. E., Pulliam, J. R., Dobson, A. P., Hudson, P.J., Grenfell, B. T. (2009). Epidemic dynamics at the human-animal interface. *science, 326*(5958), 1362-1367.

Lovegrove, T. G. (1996). Island releases of saddlebacks *Philesturnus carunculatus* in New Zealand. *Biological conservation, 77*(2-3), 151-157.

Lumeij, J. T. (1994). Gastroenetrology. In. Ritchie, B.W., Harrison, G.J., & Harrison, L.R. (Eds.), *Avian Medicine: Principles and Applications* (pp. 482-514): Wingers Publishing

Makino, S., Okada, Y., Maruyama, T., Ishikawa, K., Takahashi, T., Nakamura, M., Ezaki, T.,Morita, H. (1994). Direct and rapid detection of *Erysipelothrix rhusiopathiae* DNA in animals by PCR. *Journal of Clinical Microbiology, 32*(6), 1526-1531.

Maloney, A., Chilvers, B. L., Haley, M., Muller, C. G., Roe, W., & Debski, I. (2009). Distribution, pup production and mortality of New Zealand sea lion (*Phocarctos hookeri*) on Campbell Island / Motu Ihupuku, 2008. *New Zealand Journal of Ecology, 33*(2), 97-105.

Markey, B., Leonard, F., Archambault, M., Cullinane, A., & Maguire, D. (2013). Clinical veterinary microbiology e-book: Elsevier Health Sciences

Martland, M. (1984). Wet litter as a cause of plantar pododermatitis, leading to foot ulceration and lameness in fattening turkeys. *Avian Pathology, 13*(2), 241-252.

Mavrot, F., Orsel, K., Hutchins, W., Adams, L. G., Beckmen, K., Blake, J. E., Checkley, S.L., Davison, T., Francesco, J. D., Elkin, B., Leclerc, L., Angela Schneider, A., Tomaselli, M.,Elkin, B. (2020). Novel insights into serodiagnosis and epidemiology of *Erysipelothrix rhusiopathiae,* a newly recognized pathogen in muskoxen (*Ovibos moschatus*). *PLoS One, 15*(4), e0231724.

Maynard, D. G., & Kalra, Y. P. (1993). Nitrate and exchangeable ammonium Nitrogen, in: Carter, M.R. (Eds.), Soil Sampling and Methods of Analysis, Canadian Society of Soil Science, Lewis Publishers. pp. 25-28.

McClelland, P. J. (2002). Eradication of pacific rats (*Rattus exulans*) from Whenua Hou Nature Reserve (Codfish Island), Putauhinu and Rarotoka Islands. In C.R. Veitch & M.N. Clout (Eds.), *Turning the tide: the eradication of invasive species*. IUCN SSC Invasive Species Specialist Group IUCN: Gland, Switzerland and Cambridge, UK

McCoy, K. D., Dietrich, M., Jaeger, A., Wilkinson, D. A., Bastien, M., Lagadec, E., Boulinier, T., Pascalis, H., Tortosa, P., Le Corre, M., Dellagi, K., & Lebarbenchon, C. (2016). The role of seabirds of the Iles Eparses as reservoirs and disseminators of parasites and pathogens. *Acta Oecologica, 72*, 98-109.

McLelland, J., Gartrell, B., & Roe, W. (2011). A retrospective study of post-mortem examination findings in takahe (*Porphyrio hochstetteri*). *New Zealand Veterinary Journal, 59*(4), 160-165.

Middleton, D. M., La Flamme, A. C., Gartrell, B. D., & Nelson, N. J. (2014). Reptile reservoirs and seasonal variation in the environmental presence of Salmonella in an island ecosystem, Stephens Island, New Zealand. *Journal of Wildlife Diseases, 50*(3),

Millard, S. P. (2013). EnvStats: An R package for environmental statistics. Springer, New York. ISBN 978-1-4614-8455-4, http://www.springer.com.

Milne, E. M., Windsor, R. S., Rogerson, F., & Pennycott, T. W. (1997). Concurrent infection with enteric protozoa and *Erysipelothrix rhusiopathiae* in chicken and pheasant flocks. *The Veterinary Record, 141*, 340-341.

Miskelly, C. (2017). Seabird discoveries in remote southern Fiordland. Retrieved from https://blog.tepapa.govt.nz/2017/12/05/seabird-discoveries-in-remote-southern-fiordland/

Miskelly, C. M., & Powlesland, R. G. (2013). Conservation translocations of NZ birds, 1863-2012. *Notornis, 60*(1), 3-28.

Moorhouse, R. J. (1985). Ecology of Kākāpō *(Strigops habroptilus,* Gray 1845*)* liberated on Little Barrier Island (Hauturu). (MSc thesis), University of Auckland, New Zealand.

Moorhouse, R. J. (1991). Aspects of the Ecology of Kakapo *Strigops habroptilus* Liberated on Little Barrier Island (Hauturu), New Zealand. *Biological Conservation, 56*, 349-365.

Mörner, T., & Beasley, V. (2012). Monitoring for diseases in wildlife populations. *Ecology and Animal Health, Ecosystem Health and Sustainable Agriculture, 2*, 186-190.

Morner, T., Obendorf, D. L., Artois, M., & Woodford, M. H. (2002). Surveillance and monitoring of wildlife diseases. *Revue Scientifique et Technique de l'OIE, 21*(1), 67-76. doi: 10.20506/rst.21.1.1321

Mountrakis, G., & Gunson, K. (2009). Multi-scale spatiotemporal analyses of moose–vehicle collisions: a case study in northern Vermont. *International Journal of Geographical Information Science, 23*(11), 1389-1412. doi: 10.1080/13658810802406132

Mulder, C. P., & Keall, S. N. (2001). Burrowing seabirds and reptiles: impacts on seeds, seedlings and soils in an island forest in New Zealand. *Oecologia, 127*(3), 350-360.

Munday, B. L. (1972). A serological study of some infectious diseases of Tasmanian wildlife. *Journal of Wildlife Diseases, 8*(2), 169-175.

Neill, E. (2008). Kakapo recovery plan, 2006–2016. *Wellington, Department of Conservation*

Neuwirth, E. (2014). RcolorBrewer: ColorBrewer Palettes. R package version 1.1-2

Newman, J., Scott, D., Bragg, C., McKechnie, S., Moller, H., & Fletcher, D. (2009). Estimating regional population size and annual harvest intensity of the sooty shearwater in New Zealand. *New Zealand Journal of Zoology, 36*(3), 307-323.

Nusser, S. M., Clark, W. R., Otis, D. L., & Huang, L. (2008). Sampling considerations for disease surveillance in wildlife populations. *The Journal of Wildlife Management, 72*(1), 52-60.

Nychka, D., Furrer, R., Paige, J., & Sain, S. (2017). fields: Tools for spatial data. doi: 10.5065/D6W957CT, R package version 10.3, https://github.com/NCAR/Fields

Ogawa, Y., Ooka, T., Shi, F., Ogura, Y., Nakayama, K., Hayashi, T., & Shimoji, Y. (2011). The genome of *Erysipelothrix rhusiopathiae*, the causative agent of swine erysipelas, reveals new insights into the evolution of firmicutes and the organism's intracellular adaptations. *Journal of Bacteriology, 193*(12), 2959-2971.

Okatani, A. T., Uto, T., Taniguchi, T., Horisaka, T., Horikita, T., Kaneko, K., & Hayashidani, H. (2001). Pulsed-field gel electrophoresis in differentiation of Erysipelothrix species strains. *Journal of Clinical Microbiology, 39*(11), 4032-4036.

O'Malley, B. (2005). Clinical anatomy and physiology of exotic species: structure and function of mammals, birds, reptiles, and amphibians: Elsevier Saunders.

Opriessnig, T., Bender, J. S., & Halbur, P. G. (2010). Development and validation of an immunohistochemical method for rapid diagnosis of swine erysipelas in formalin-fixed, paraffin-embedded tissue samples. *Journal of Veterinary Diagnostic Investigation, 22*(1), 86-90.

Opriessnig, T., Shen, H., Bender, J., Boehm, J., & Halbur, P. (2013). *Erysipelothrix rhusiopathiae* isolates recovered from fish, a harbour seal (*Phoca vitulina*) and the marine environment are capable of inducing characteristic cutaneous lesions in pigs. *Journal of Comparative Pathology, 148*(4), 365-372.

Opriessnig, T., Vance, R. K., & Halbur, P. G. (2005). *Erysipelothrix rhusiopathiae* septicemia in a Laughing kookaburra (*Dacelo novaeguineae*). *Journal of Veterinary Diagnostic Investigation, 17*, 497–499

Otero, X. L., De La Peña-Lastra, S., Pérez-Alberti, A., Ferreira, T. O., & Huerta-Diaz, M. A. (2018). Seabird colonies as important global drivers in the nitrogen and phosphorus cycles. *Nature Communications*, *9*(1), 246.

Pal, N., Bender, J. S., & Opriessnig, T. (2010). Rapid detection and differentiation of *Erysipelothrix* spp. by a novel multiplex real-time PCR assay. *Journal of Applied Microbiology*, *108*(3), 1083-1093.

Parkes, J., & Murphy, E. (2003). Management of introduced mammals in New Zealand. *New Zealand Journal of Zoology, 30*(4), 335-359.

Plowright, R. K., Parrish, C. R., McCallum, H., Hudson, P. J., Ko, A. I., Graham, A. L., & Lloyd-Smith, J. O. (2017). Pathways to zoonotic spillover. *Nature Reviews Microbiology*, *15*(8), 502-510.

Potter, J., McKenzie, J. (2008). Report on kakapo chick illness in (Unpublished report to the New Zealand Department of Conservation)

Power, A. G., & Mitchell, C. E. (2004). Pathogen spillover in disease epidemics. *The American Naturalist, 164*(S5), S79-S89.

Powlesland, R. G., Lloyd, B. D., Best, H. A., & Merton, D. V. (1992). Breeding biology of the Kakapo *Strigops habroptilus* on Stewart Island, New Zealand. *Ibis, 134*(4), 361-373.

Powlesland, R. G., Merton, D. V., & Cockrem, J. F. (2006). A parrot apart: the natural history of the kakapo (*Strigops habroptilus*), and the context of its conservation management *Notornis, 53*(1), 3 - 26.

Principe, L., Bracco, S., Mauri, C., Tonolo, S., Pini, B., & Luzzaro, F. (2016). *Erysipelothrix rhusiopathiae* bacteremia without endocarditis: Rapid identification from positive blood culture by MALDI-TOF Mass Spectrometry. A case report and literature review. Infectious Disease Report, 8(1), 6368. 10.4081/idr.2016.6368

QGIS Development Team (2018). QGIS Geographic Information System. Open Source Geospatial Foundation Project.

Rayner, M.J., Clout, M.N., Stamp, R.K., Imber, M.J., Brunton, D.H., & Hauber, M.E. (2007b). Predictive habitat modelling for the population census of a burrowing seabird: A study of the endangered Cook's petrel. *Biological Conservation* 138, 235-247.

Rayner, M. J., Hauber, M. E., & Clout, M. N. (2007a). Breeding habitat of the Cook's Petrel (*Pterodroma cookii*) on Little Barrier Island (Hauturu): implications for the conservation of a New Zealalnd endemic. *Emu, 107*(1), 59.

Rayner, M. J., Parker, K. A., & Imber, M. J. (2008). Population census of Cook's Petrel *Pterodroma cookii* breeding on Codfish Island (New Zealand) and the global conservation status of the species. *Bird Conservation International, 18*(03)

Reboli, A. C., & Farrar, W. E. (1989). *Erysipelothrix rhusiopathiae*: an occupational pathogen. *Clinical Microbiology. Reviews, 2*, 354-359.

Reischek, A. (1884). Notes on New Zealand ornithology. Transactions of the New Zealand Institute. *17*,187-198.

Ricciardi, A., & Simberloff, D. (2009). Assisted colonization is not a viable conservation strategy. *Trends in Ecology and Evolution, 24*(5), 248-253.

Riddick, S. N., Blackall, T. D., Dragosits, U., Daunt, F., Braban, C. F., Tang, Y. S., MacFarlane, W., Taylor, S., Wanless, S., & Sutton, M. A. (2014). Measurement of ammonia emissions from tropical seabird colonies. *Atmospheric Environment, 89*, 35-42.

Riddick, S. N., Dragosits, U., Blackall, T. D., Daunt, F., Wanless, S., & Sutton, M. A. (2012). The global distribution of ammonia emissions from seabird colonies. *Atmospheric Environment, 55*, 319-327.

Ripley, B. D. (1976). The second-order analysis of stationary point processes. *Journal of Applied Probability*, *13*(2), 255-266.

Rosenthal, J. (2009). Climate change and the geographic distribution of infectious diseases. *Ecohealth*, *6*(4), 489-495.

RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL.

Ryser-Degiorgis, M.-P. (2013). Wildlife health investigations: needs, challenges and recommendations. *BMC veterinary research, 9*(1), 223.

Sagar, P. (2013). Sooty shearwater. *New Zealand Birds Online. www. nzbirdsonline. org. nz*

Sagar, R. L., Leseberg, A., Hunt, K., Nakagawa, K., Dunphy, B., & Rayner, M. J. (2015). Optimising translocation efforts of mottled petrels (*Pterodroma inexpectata*): growth, provisioning, meal size and the efficacy of an artificial diet for chicks. *Emu-Austral Ornithology*, *115*(2), 137-145.

Sánchez-Piñero, F., & Polis, G. A. (2000). Bottom-up dynamics of allochthonous input: direct and indirect effects of seabirds on islands. Ecology, 81(11), 3117-3132

Scarlett, R. J. (1979). Avifauna and man, Anderson, A. (Eds.), Birds of a feather. New Zealand Archaeological Association: monograph 11, pp. 76-82

Schoener, E., Tompkins, D., Parker, K., Howe, L., & Castro, I. (2020). Presence and diversity of mixed avian *Plasmodium* spp. infections in introduced birds whose distribution overlapped with threatened New Zealand endemic birds. *New Zealand Veterinary Journal*, 68(2), 101-106.

Scott, D., Moller, H., Fletcher, D., Newman, J., Aryal, J., Bragg, C., & Charleton, K. (2009). Predictive habitat modelling to estimate petrel breeding colony sizes: Sooty shearwaters (*Puffinus griseus*) and mottled petrels (*Pterodroma inexpectata*) on Whenua Hou Island. *New Zealand Journal of Zoology*, *36*(3), 291-306.

Seddon, P. J. (2010). From reintroduction to assisted colonization: Moving along the conservation translocation spectrum. *Restoration Ecology, 18*(6), 796-802.

Seddon, P. J., Strauss, W. M., & Innes, J. (2012). Animal translocations: What are they and why do we do them? In Ewen, J. G., Armstrong, D.P., Parker, K.A & Seddon, P.J. (Eds.), *Reintroduction Biology: Intergrating Science and Management*. West Sussex: Wiley-Blackwell, pp 1-32.

Sedgeley, J.A. (2001). Winter activity in the tree-roosting lesser short-tailed bats, *Mystacina tuberculata*, in a cold temperate climate in New Zealand. *Acta Chiropterologica*, *3*, 179-195.

Sedgeley, J.A. (2006). Roost site selection by lesser short-tailed bats (*Mystacina tuberculata*) in mixed podocarp-hardwood forest, Whenua Hou/Codfish Island, New Zealand. *New Zealand Journal of Zoology. 33*:(2), 97-111.

Seng, P., Abat, C., Rolain, J. M., Colson, P., Lagier, J. C., Gouriet, F., Fournier, P. E., Drancourt, M., La Scola, B., Raoulta, D. (2013). Identification of rare pathogenic bacteria in a clinical microbiology laboratory: Impact of matrix-assisted Laser Desorption Ionization– Time of Flight Mass Spectrometry. *Journal of Clinical Microbiology*, 2182-2194.

Shen, H. G., Bender, J. S., & Opriessnig, T. (2010). Identification of surface protective antigen (spa) types in *Erysipelothrix* reference strains and diagnostic samples by spa multiplex real-time and conventional PCR assays. *Journal of Applied Microbiology, 109*(4), 1227-1233.

Shimazaki, Y., Gamoh, K., Imada, Y., Makie, H., Kanzaki, M., & Takahashi, T. (2005). Detection of antibodies to *Erysipelothrix* in stray dogs in Japan. *Acta veterinaria Scandinavica, 46*(3).

Shimizu, T., Okamoto, C., Aoki, H., Harada, K., Kataoka, Y., Ono, F., Kadohira, M., Takai, S. (2016). Serological surveillance for antibodies against Erysipelothrix species in wild boar and deer in Japan. *Japanese Journal of Veterinary Research, 64*(1), 91-94.

Shimoji, Y., Mori, Y., Hyakutake, K., Sekizaki, T., & Yokomizo, Y. (1998). Use of an enrichment broth cultivation-PCR combination assay for rapid diagnosis of swine Erysipelas. *Journal of Clinical Microbiology, 36*(1), 86–89.

Simberloff, D. (2002). Today Tiritiri Matangi, tomorrow the world! Are we aiming too low in invasives control? In Veitch, C.R., & Clout, M.N. (Eds.), *Turning the Tide: The Eradication of Invasive Species*. Gland, Switzerland: IUCN, pp 4-12.

Skerratt, L. F., Berger, L., Speare, R., Cashins, S., McDonald, K. R., Phillott, A. D., Harry Bryan Hines, H. B., & Kenyon, N. (2007). Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth, 4*(2), 125-134.

Stephenson, B. M., Flood, R., Thomas, B., & Saville, S. (2008). Rediscovery of the New Zealand storm petrel (*Pealeornis maoriana* Mathews 1932): two sightings that revised our knowledge of storm petrels. *Notornis, 55*, 77-83.

Stephenson, E. H., & Berman, D. T. (1978). Isolation of *Erysipelothrix rhusiopathiae* from tonsils of apparently normal swine by two methods. *American Journal of Veterinary Research, 39*(1), 187-188.

Stępień-Pyśniak, D., Hauschild, T., Różański, P., & Marek, A. (2017). MALDI-TOF mass spectrometry as a useful tool for identification of *Enterococcus* spp. from wild birds and differentiation of closely related species. *Journal of Microbiology and Biotechnology, 27*(6), 1128-1137.

Stevenson, M., Nunes, T., Sanchez, J., Thornton, R., Reiczigel, J., Robison-Cox, J., & Sebastiani, P. (2013). epiR: An R package for the analysis of epidemiological data. R package version 0.9-43

Stumpy, L. M., & Binkley, D. (1993). Relationships between litter quality and nitrogen availability in Rocky Mountain forests. *Canadian Journal of Forest Research, 23*, 492-502.

Suer, L. D., & Vedros, N. A. (1988). *Erysipelothrix rhusiopathiae*. I. Isolation and characterization from pinnipeds and bitelabrasion wounds in humans. *Diseases of Aquatic Organisms, 5*, 1-5.

Takahashi, T., Fujisawa, T., Benno, Y., Tamura, Y., Sawada, T., Suzuki, S., Muramatsu, M., & Mitsuoka, T. (1987). *Erysipelothrix tonsillarum* sp. nov. isolated from tonsils of

apparently healthy pigs. *International Journal of Systematic and Evolutionary Microbiology, 37*(2), 166-168.

Takahashi, T., Takagi, M., Yamamoto, K., & Nakamura, M. (2000). A Serological survey on erysipelas in chickens by growth agglutination test. *Journal of Veterinary Medicine., 47*, 797-799.

Takeshi, K., Makino, S., Ikeda, T. T., N., Nakashiro, A., Nakanishi, K., Ogamuwa, K., Katoh, Y., Sunagawa, H., & Ohyama, T. (1999). Direct and rapid detection by PCR of *Erysipelothrix* sp. DNAs prepared from bacterial strains and animal tissues. *Journal of Clinical Microbiology, 37*(12), 4093–4098.

Taylor, J. D. (2000). Action Plan for Seabird Conservation in New Zealand.

Taylor, W. M. (2016). In B. L. Speer (Ed.), Current Therapy in Avian Medicine and Surgery W.B. Saunders, pp. 329-344: https://doi.org/10.1016/B978-1-4557-4671-2.00016-1

To, H., Sato, H., Tazumi, A., Tsutsumi, N., Nagai, S., Iwata, A., & Nagano, T. (2012). Characterization of *Erysipelothrix rhusiopathiae* strains isolated from recent swine erysipelas outbreaks in Japan. *Journal of Veterinary Medical Science, 74*(7), 949-

Tompkins, D.M., Carver, S., Jones, M.E., Krkosek, M., & Skerratt, L.F. (2015). Emerging infectious diseases of wildlife: a critical perspective. *Trends in Parasitology, 31* (4), 149-159.

Towns, D. R., & Ballantine, W. J. (1993). Conservation and restoration of New Zealand island ecosystem. *Trends in Ecology and Evolution, 8*(12), 452-457.

Towns, D. R., Bellingham, P. J., Mulder, C. P., & Lyver, P. O. B. (2012). A research strategy for biodiversity conservation on New Zealand's offshore islands. *New Zealand Journal of Ecology*, 1-20.

Van Riper III, C., Van Ripe, S. G., Golf, M. L., & Laird, M. (1986). The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecological Monograph, 56*(4), 327-344.

Van Vuuren, M., & Brown, J. M. M. (1990). Septicaemic *Erysipelothrix rhusiopathiae* infcetion in the little swift (*Apus affinis*). *Journal of South African Veterinary Association, 61*(4), 170-171.

Veitch, C. R. (2001). The eradication of feral cats (Felis catus) from little Barrier Island, New Zealand. *New Zealand journal of Zoology*, 28(1), 1-12.

Venables, W.N., & Ripley, B.D. (2002). Modern Applied Statistics with S, Fourth edition. Springer, New York. ISBN 0-387-95457-0, http://www.stats.ox.ac.uk/pub/MASS4.

Vicente, J., Leon-Vizcaino, L., Gortazar, C., Jose Cubero, M., Gonzalez, M., & Martin-Atance, P. (2002). Antibodies to selected viral and bacterial pathogens in European wild boars from southcentral Spain. *Journal of Wildlife Diseases, 38*(3), 649-652. doi: 10.7589/0090-3558-38.3.649

Vidal, E., Médail, F., Tatoni, T., & Bonnet, V. (2000). Seabirds drive plant species turnover on small Mediterranean islands at the expense of native taxa. *Oecologia,* 122(3), 427-434.

Wakenell, P. S. (1996). Obstetrics and reproduction of backyard poultry. *Seminars in Avian and Exotic Pet Medicine, 15*(4), 199-204.

Walsh, J. E., Wilson, K.-J., & Elliott, G. P. (2006). Seasonal changes in home range size and habitat selection by kākāpō (*Strigops habroptilus*) on Maud Island. *Notornis, 53*, 143-149.

Walton, L., Marion, G., Davidson, R. S., White, P. C., Smith, L. A., Gavier-Widen, D., Yon, L., Hannant, D., & Hutchings, M. R. (2016). The ecology of wildlife disease

surveillance: demographic and prevalence fluctuations undermine surveillance. *Journal of Applied Ecology, 53*(5), 1460-1469.

Wang, G., Ekstrand, C., & Svedberg, J. (1998). Wet litter and perches as risk factors for the development of foot pad dermatitis in floor-housed hens. *British Poultry Science, 39*(2), 191-197.

Wang, Q., Chang, B. J., & Riley, T. V. (2010). Review: *Erysipelothrix rhusiopathiae*. *Veterinary Microbiology, 140*, 405-417. 10.1016/j.vetmic.2009.08.012

Weimerskirch, H. (2004). Diseases threaten Southern Ocean albatrosses. *Polar Biology, 27*(6), 374-379.

White, D. J., Hall, R. J., Jakob-Hoff, R., Wang, J., Jackson, & B., Tompkins, D. M. (2015). Exudative cloacitis in the kākāpō (*Strigops habroptilus*) potentially linked to *Escherichia coli* infection. *New Zealand Veterinary Journal, 63*(3), 167-170.

Whitehead, J. K. (2007). Breeding success of adult female kākāpō (*Strigops habroptilus*) on Codfish Island (Whenua Hou): correlations with foraging home ranges and habitat selection. (MSc Thesis), Lincoln University, New Zealand.

Wickham H. (2017). scales: Scale functions for visualization. R package version 0.5.0.

Wickham, H. (2011). "The split-apply-combine strategy for data analysis." *Journal of Statistical Software, 40*(1), 1-29.

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4.

Wickham, H., Hester, J., Francois, R., Jylänki, J., & Jørgensen M. (2018). reader: Read rectangular text data. R package version 1.3.1.

Wieser, A., Schneider, L., Jung, J., & Schubert, S. (2012). MALDI-TOF MS in microbiological diagnostics-identification of microorganisms and beyond (mini review). *Applied Microbiology and Biotechnology, 93*(3), 965-974.

Williams, E. S., Thome, E. T., Appel, M. J. G., & Belitsky, D. W. (1988). Canine distemper in Black - footed ferrets (*Mustela nigripes*) from Wyoming. *Journal of Wildlife Diseases, 24*(3), 385-398.

Wilson, D. (2004). Diet of kākāpō in breeding and non-breeding years on Codfish Island (Whenua Hou) and Stewart Island. *Department of Conservation Science Internal Series, 182*,5-18.

Wobeser G. A. (2006). Essentials of diseases in wild animals. Blackwell Publishing, Iowa.

Wobeser, G. A. (2007). Investigation and management of disease in wild animals, Springer Science & Business Media.

Wolcott, M. J. (2007). Erysipelas. In Thomas, N.J., Hunter, B.D., & Atkinson, C.T. (Eds.), *Infectious Diseases of Wild Birds* Blackwell Publishing.

Wood, R. L. (1973). Survival of *Erysipelothrix rhusiopathiae* in soil under various environmental conditions. *Cornell Veterinarian, 63*, 390–410.

Wood, R. L. (1992). Erysipelas. In Leman, A. D., Straw,B.E., Mengeling, W.L., D'Allaire, S& Taylor, D.J. (Eds.), *Diseases of Swine* (7 ed., pp. 475-486). Ames, IA: Iowa State University Press.

Wood, R.L., and R.D. Shuman. (1981). *Erysipelothrix* infection. In Davis, J.W., Karstad, L.H., & Trainer, D.O. Infectious diseases of wild mammals, ed., Ames: Iowa State University Press, pp. 297–305.

Woodford, M. H. (2001). Quarantine and health screening protocols for wildlife prior to translocation and release into the wild. *Conservation Breeding Specialist Group, IUCN Species Survival Commission, Gland, Switzerland, OIE, Paris, France, Care for the Wild, Horsham, UK, and the European Association of Zoo and Wildlife Veterinarians, Liebefeld-Serne, Switzerland.*

Woolhouse, M. E. J., Taylor, L. H., & Haydon, D. T. (2001). Population biology of multihost Pathogens. *Science, 292*(5519), 1109-1112.

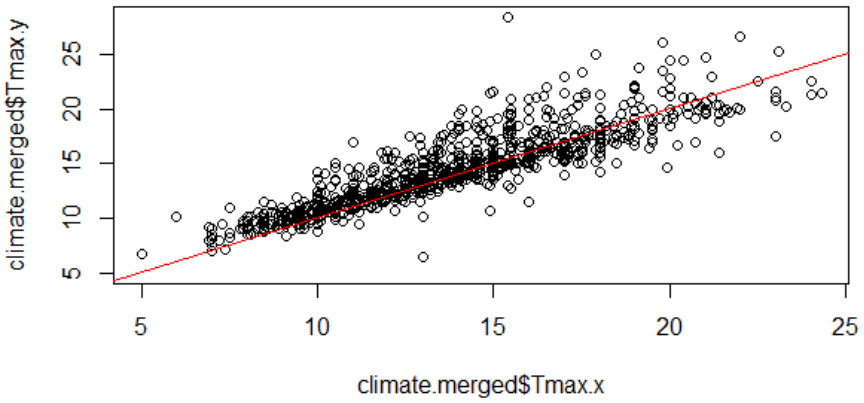
Work, T. M., Ball, D., & Wolcott, M. (1999). Erysipelas in a free-ranging Hawaiian Crow (*Corvus hawaiiensis*). *Avian Diseases, 43*(2), 338-341.

Xu, B., Marguerite, M., Stallknecht, D.E., Hodlerd, T.W., & Parker, K.C. (2012). Spatial and spatial–temporal clustering analysis of haemorrhagic disease in white-tailed deer in the south-eastern USA: 1980–2003. *Preventive Veterinary Medicine, 106*, 339– 347.

Appendix 1

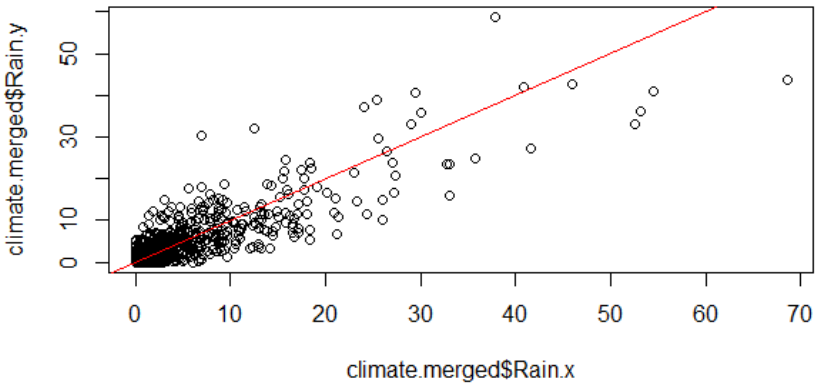
Chapter 2 supplementary information

The strong positive correlation between the rainfall and annual maximum temperature for Whenua Hou (between 1987-1993) and those of Stewart Island (0.88 and 0.86, respectively).



cor(climate.merged$Tmax.x, climate.merged$Tmax.y, method='pearson', use="pairwise.complete.obs")

[1] 0.8748608 (Correlation coefficient)



cor(climate.merged$Rain.x, climate.merged$Rain.y, method='pearson', use="pairwise.complete.obs")

[1] 0.8562482 (Correlation coefficient)

Appendix 2

Chapter 3 supplementary information

Seabird sample collection data from Te Hauturu-o-Toi

Sample_ID	Date	Track	Sample_type	Species	Northing	Easting
EK179	12/03/2018	Valley	Blood	Cook's petrel	5989970	1785389
EK180	12/03/2018	Valley	Blood	Cook's petrel	5989742	1685409
EK181	12/03/2018	Valley	Blood	Cook's petrel	5989975	1785392
EK184	12/04/2018	Valley	Blood	Cook's petrel	5989996	1785418
EK185	12/05/2018	Thumb	Blood	Cook's petrel	5991533	1785920
EK186	12/05/2018	Thumb	Blood	Cook's petrel	5991532	1785925
EK187	12/05/2018	Thumb	Blood	Cook's petrel	5991535	1785920
EK188	12/05/2018	Thumb	Blood	Cook's petrel	5991529	1785932
EK189	12/05/2018	Thumb	Blood	Cook's petrel	5991539	1785929
EK190	12/05/2018	Thumb	Blood	Cook's petrel	5991535	1785925
EK191	12/05/2018	Thumb	Blood	Cook's petrel	5991536	1785933
EK192	12/05/2018	Thumb	Blood	Cook's petrel	5991543	1785927
EK193	12/05/2018	Thumb	Blood	Cook's petrel	5991571	1785959
EK194	12/05/2018	Thumb	Blood	Cook's petrel	5991565	1785962
EK195	12/05/2018	Thumb	Blood	Cook's petrel	5991582	1785973
EK196	12/05/2018	Thumb	Blood	Cook's petrel	5991587	1785981
EK197	12/05/2018	Thumb	Blood	Cook's petrel	5991597	1785995
EK198	12/05/2018	Thumb	Blood	Cook's petrel	5991569	1785944
EK145	11/21/2018	Shag	Bone	Unknown	5989475	1785552
EK147	11/21/2018	Beach	Bone	Black petrel		
EK148	11/22/2018	Waipawa	Bone	Unknown	5991054	1785334
EK149	11/22/2018	Waipawa	Bone	Cook's petrel	5991071	1785406
EK150	11/22/2018	Waipawa	Bone	Cook's petrel	5991068	1785403
EK151	11/22/2018		Bone	Cook's petrel		
EK152	11/23/2018	Beach	Bone	Unknown	5989510	1785095
EK153	11/23/2018	Beach	Bone	Australasian gannet	5989358	1785377
EK155	11/26/2018	Thumb	Bone	Cook's petrel	5990471	1785442
EK156	11/26/2018	Thumb	Bone	Cook's petrel	5990814	1785709
EK157	11/26/2018	Thumb	Bone	Cook's petrel	5990838	1785755
EK158	11/26/2018	Thumb	Bone	Cook's petrel	5991043	1785891
EK159	11/27/2018	Hamilton	Bone	Unknown	5990680	1786242
EK160	11/27/2018	Valley	Bone	Cook's petrel	5990713	1786231
EK161	11/27/2018	Valley	Bone	Cook's petrel	5990642	1786152
EK162	11/27/2018	Valley	Bone	Cook's petrel	5990581	1786062
EK163	11/27/2018	Valley	Bone	Cook's petrel	5990483	1785967
EK164	11/27/2018	Valley	Bone	Cook's petrel	5990274	1785738
EK165	11/27/2018	Valley	Bone	Cook's petrel	5990172	1785663
EK166	11/27/2018	Valley	Bone	Cook's petrel	5989987	1785412
EK167	11/28/2018	Memorial	Bone	Cook's petrel	5989489	1785974
EK168	11/28/2018	Memorial	Bone	Cook's petrel	5989573	1786063

EK169	11/28/2018		Bone	Cook's petrel		
EK170	11/28/2018		Bone	Cook's petrel		
EK171	11/30/2018	Beach	Bone	Little Blue Penguin	5989613	1784951
EK172	11/30/2018	Hamilton	Bone	Cook's petrel	5990183	1786009
EK173	11/30/2018	Valley	Bone	Cook's petrel		
EK174	12/02/2018	Thumb	Bone	Cook's petrel	5991895	1786496
EK175	12/02/2018	Thumb	Bone	Cook's petrel	5991605	1786003
EK176	12/02/2018	Thumb	Bone	Cook's petrel	5991550	1785985
EK177	12/02/2018	Thumb	Bone	Cook's petrel		
EK178	12/02/2018		Bone	Cook's petrel	5990168	1784981
EK182	12/03/2108	Thumb	Bone	Cook's petrel	5990199	1785278
EK183	12/03/2018	Thumb	Bone	Cook's petrel	5990200	1785132
EK199	12/05/2018	Thumb	Bone	Cook's petrel	5989702	1784988
EK200	12/05/2018	Track 20	Bone	Cook's petrel	5989944	1787278
EK201	12/05/2018	Track 20	Bone	Cook's petrel	5989944	1787278
EK202	12/05/2018	Track 20	Bone	Cook's petrel	5990041	1787330
EK203	12/05/2018	Track 20	Bone	Cook's petrel	5990095	1787520
EK204	12/05/2018	Track 20	Bone	Cook's petrel	5990084	1787661
EK205	12/05/2018	Track 20	Bone	Cook's petrel	5989984	1787763
EK206	12/05/2018	Track 20	Bone	Cook's petrel	5989462	1787387
EK207	12/05/2018	Track 20	Bone	Cook's petrel	5990351	1787512
EK208	12/05/2018	Track 20	Bone	Cook's petrel	5990320	1787443

Appendix 3

Chapter 3 supplementary information

seabird sample collection data from Whenua Hou

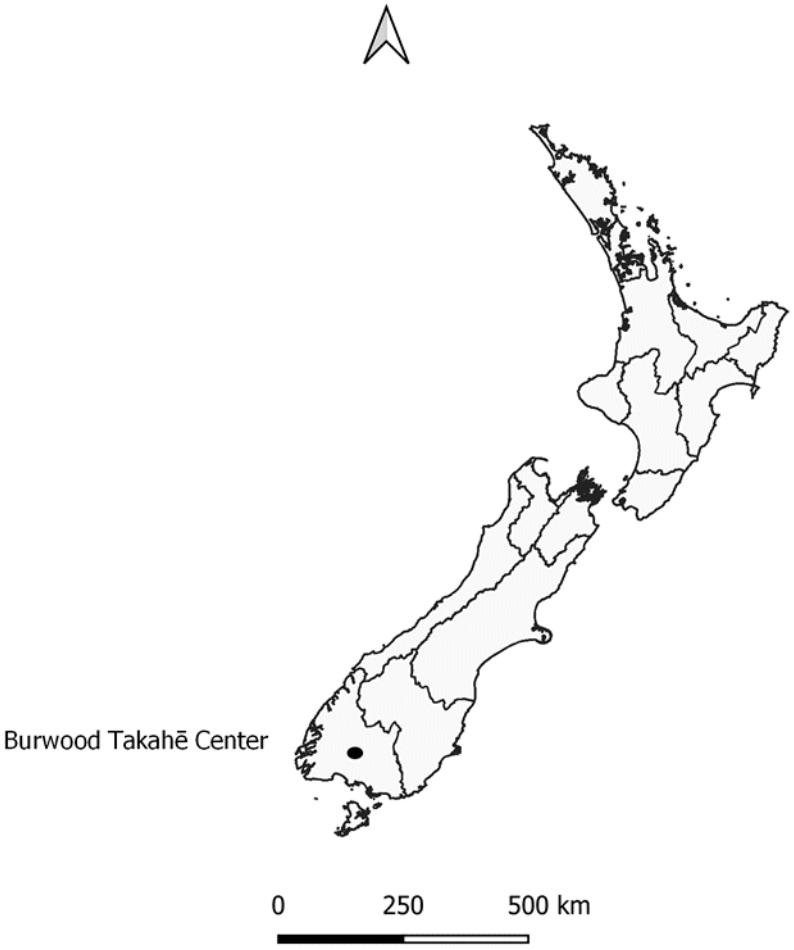
Sample ID	Date	Track	Sample_type	Species	Easting	Northing
EK005	15/10/2018	Loop	Bone	Unknown	1189284.5	4805219.5
EK006	15/10/2018	Loop	Bone	Unknown	1189564.4	4804696.3
EK007	15/10/2018	Loop	Bone	Unknown	1190106.0	4804830.0
EK008	15/10/2018	Loop	Bone	Unknown	1190106.0	4804830.0
EK009	16/10/2018	Mudwiggle	Bone	Sooty	1190995.0	4805737.0
EK010	16/10/2018	Miro	Bone	Cook's	1191185.6	4806389.6
EK011	18/10/2018	Rimu	Bone	Cook's	1191704.8	4805878.1
EK012	18/10/2018	Rimu	Bone	Cook's	1191714.8	4805878.2
EK013	18/10/2018	Rimu	Bone	Cook's	1191730.9	4805860.2
EK014	18/10/2018	South Bay	Bone	Sooty	1191911.5	4805024.3
EK015	18/10/2018	South Bay	Bone	Sooty	1192003.4	4805112.7
EK016	18/10/2018	South Bay	Bone	Sooty	1192004.4	4805113.7
EK017	18/10/2018	South Bay	Bone	Sooty	1192003.4	4805112.7
EK018	18/10/2018	South Bay	Bone	Sooty	1192164.5	4805177.2
EK019	18/10/2018	South Bay	Bone	Sooty	1192491.9	4805222.2
EK020	18/10/2018	South Bay	Bone	Sooty	1192502.9	4805228.2
EK021	18/10/2018	Huhugrab	Bone	Sooty	1192412.3	4805387.2
EK022	19/10/2018	South Bay	Bone	Unknown	1192246.0	4805763.4
EK023	19/10/2018	South Bay	Bone	Unknown	1192229.0	4805756.4
EK024	19/10/2018	South Bay	Bone	Sooty	1192508.1	4805550.8
EK025	19/10/2018	South Bay	Bone	Sooty	1192521.2	4805504.7
EK027	19/10/2018	Rimu	Bone	Sooty	1191726.9	4805863.2
EK028	20/10/2018	Summit track	Bone	Unknown	1189773.2	4806384.8
EK029	20/10/2018	Summit track	Bone	Sooty	1189656.0	4806368.4
EK030	20/10/2018	Summit track	Bone	Sooty	1189277.0	4806149.0
EK031	20/10/2018	Summit upper	Bone	Mottled	1189236.2	4806041.7
EK032	20/10/2018	Summit upper	Bone	Mottled	1189231.2	4806044.7
EK033	20/10/2018	Loop	Bone	Mottled	1189187.8	4805774.1
EK034	20/10/2018	Loop	Bone	Mottled	1189176.8	4805778.1
EK035	20/10/2018	Loop	Bone	Mottled	1189179.9	4805764.1
EK036	20/10/2018	Loop	Bone	Mottled	1189167.8	4805766.1
EK037	20/10/2018	Loop	Bone	Mottled	1189202.2	4805656.0
EK038	20/10/2018	Acessor	Bone	Mottled	1189206.2	4805652.0
EK039	20/10/2018	Acessor	Bone	Mottled	1189310.4	4805652.3
EK040	20/10/2018	Acessor	Bone	Mottled	1189310.4	4805652.3
EK041	20/10/2018	Acessor	Bone	Mottled	1189719.3	4805583.3
EK042	20/10/2018	Acessor	Bone	Mottled	1189894.7	4805531.6
EK043	20/10/2018	Acessor	Bone	Mottled	1189915.7	4805537.7
EK044	20/10/2018	Acessor	Bone	Mottled	1189999.0	4805500.9
EK045	20/10/2018	Valley	Bone	Sooty	1190187.4	4805441.3

EK046	22/10/2018	NorthWest Bay	Bone	Unknown	1190832.1	4807096.8
EK047	22/10/2018	NorthWest Bay	Bone	Mottled	1189411.7	4806353.7
EK048	22/10/2018	NorthWest Bay	Bone	Mottled	1190319.5	4807336.9
EK049	23/10/2018	Observation Rock track	Bone	Mottled	1189675.4	4806620.9
EK050	23/10/2018	Observation Rock track	Bone	Mottled	1189682.4	4806628.9
EK051	23/10/2018	Observation Rock track	Bone	Sooty	1189687.4	4806623.9
EK052	23/10/2018	Observation Rock track	Bone	Mottled	1189687.4	4806623.9
EK053	23/10/2018	Observation Rock track	Bone	Mottled	1189688.4	4806629.0
EK054	23/10/2018	Observation Rock track	Bone	Sooty	1189689.4	4806637.0
EK055	23/10/2018	Observation Rock track	Bone	Mottled	1189690.4	4806639.0
EK056	23/10/2018	Observation Rock track	Bone	Sooty	1189683.4	4806638.0
EK057	23/10/2018	Observation Rock track	Bone	Mottled	1189681.4	4806625.9
EK058	23/10/2018	Observation Rock track	Bone	Sooty	1189657.3	4806641.9
EK059	23/10/2018	Equinox	Bone	Mottled	1189016.7	4806458.8
EK060	23/10/2018	North hut	Bone	Sooty	1188243.8	4806693.2
EK061	23/10/2018	North hut	Bone	Mottled	1188245.8	4806694.2
EK062	23/10/2018	North hut	Bone	Mottled	1188231.0	4806588.9
EK063	23/10/2018	North hut	Bone	Mottled	1188181.1	4806536.7
EK064	23/10/2018	North hut	Bone	Unknown	1188206.2	4806496.7
EK065	23/10/2018	North hut	Bone	Mottled	1188206.2	4806496.7
EK066	23/10/2018	North hut	Bone	Mottled	1188206.2	4806491.7
EK067	23/10/2018	North hut	Bone	Mottled	1188193.2	4806490.7
EK068	23/10/2018	North hut	Bone	Mottled	1188204.3	4806471.7
EK069	23/10/2018	North hut	Bone	Sooty	1188206.3	4806470.7
EK070	23/10/2018	North hut	Bone	Sooty	1188217.3	4806464.7
EK071	23/10/2018	North hut	Bone	Sooty	1188211.3	4806457.7
EK072	23/10/2018	North hut	Bone	Sooty	1188214.4	4806452.7
EK073	23/10/2018	North hut	Bone	Unknown	1188227.4	4806436.7
EK075	23/10/2018	North hut	Bone	Sooty	1188838.0	4806229.0
EK076	23/10/2018	North hut	Bone	Sooty	1189015.6	4806114.3
EK077	23/10/2018	North hut	Bone	Sooty	1189057.8	4806091.3
EK078	23/10/2018	North hut	Bone	Unknown	1189116.9	4806088.5
EK108	26/10/2018	Summit track	Bone	Mottled	1189225.4	4805971.6
EK110	27/10/2018	Alpanose	Bone	Sooty	1191850	4806841
EK111	27/10/2018	Sayler's Bay Beach	Bone	Sooty	1191498.7	4806891.3
EK132	30/10/2018	Loop	Bone	Mottled	1190475.4	4804529.5
EK133	30/10/2018	Loop	Bone	Unknown	1190043.6	4804561.4
EK134	31/10/2018	Observation Rock track	Bone	Mottled	1189729.5	4806983.7

EK135	31/10/2018	Observation Rock track	Bone	Mottled		
EK136	31/10/2018	Observation Rock track	Bone	Mottled	1189674.5	4806934.4
EK137	31/10/2018	Observation Rock track	Bone	Mottled	1189725.6	4806959.6
EK138	31/10/2018	Observation Rock track	Bone	Sooty	1189738.5	4806998.7
EK139	31/10/2018	Observation Rock track	Bone	Sooty	1189661.9	4806801.2
EK140	31/10/2018	Observation Rock track	Bone	Sooty	1189659.2	4806670.9
EK0141	23/10/2018	North hut	Bone	Mottled	1188305.4	4806485.0
EK0142	19/10/2018	South Bay	Bone	Sooty	1192429.4	4805391.3

Appendix 4

Chapter 4 supplementary information



Appendix 5

Statements of contributions

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Chamindani Manjula Jayasinghe Meda Gedara
Name/title of Primary Supervisor:	Prof. Brett Gartrell
Name of Research Output and full reference:	
Jayasinghe, M., Midwinter, A., Roe, W., Vallee, E., Bolwell, C., Gartrell, B.2021. Seabirds as possible reservoirs of Erysipelothrix musiopathiae on islands used for conservation translocations. In New Zealand. Journal of Wildlife Diseases, 57(3).	
In which Chapter is the Manuscript /Published work:	Chapter 3
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	75%
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
Study design, Data collection, Data analysis, Writing and editing the manuscript	
For manuscripts intended for publication please indicate target journal:	
Candidate's Signature:	Manjula Jayasinghe Digitally signed by Manjula Jayasinghe Date: 2021.05.19 05:24:01 +12'00'
Date:	19/05/2021
Primary Supervisor's Signature:	Gartrell, Brett Digitally signed by Gartrell, Brett Date: 2021.05.28 10:38:52 +12'00'
Date:	28/05/2021

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)

GRS Version 4– January 2019

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Chamindani Manjula Jayasinghe Meda Gedara
Name/title of Primary Supervisor:	Prof. Brett Gartrell
Name of Research Output and full reference:	
Jayasinghe, M., Vallee, E., Bolwell, C., Digby, A., Roe, W., Midwinter, A., Gartrell, B. An epidemiological investigation of exudative cloacitis in kakapo (Strigops habroptilus) in Whenua Hou/ Codfish Island.	
In which Chapter is the Manuscript /Published work:	Chapter 2
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	75%
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
Study design, Data collection (laboratory analysis),Data analysis, Writing and editing the manuscript	
For manuscripts intended for publication please indicate target journal:	
Preventive Veterinary Medicine	
Candidate's Signature:	Manjula Jayasinghe Digitally signed by Manjula Jayasinghe Date: 2021.05.19 06:33:01 +12'00'
Date:	19/05/2021
Primary Supervisor's Signature:	Gartrell, Brett Digitally signed by Gartrell, Brett Date: 2021.05.28 10:37:57 +12'00'
Date:	28/05/2021

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)