

**The role of New Zealand endemic lizards in
Salmonella transmission in the takahē
translocation network**

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Abstract

The dynamics of disease in wildlife populations often involve a complex relationship between physiological, environmental and ecological variables. Defining the pattern of pathogen infection between primary hosts, sympatric species and their environments is crucial in the understanding of potential impacts a pathogen may have on its host species. Very little is known about pathogens of native, New Zealand wildlife. Understanding the role pathogens play in structuring communities is crucial in the conservation of threatened New Zealand wildlife species. *Salmonella* is an important pathogen of reptiles, birds and mammals, and Salmonellosis has caused significant mortality in wildlife around the world. Recently, *Salmonella* has been isolated from takahē from a private island in the takahē translocation network. Reptiles have been implicated as asymptomatic carriers and transmitters of *Salmonella*, and lizards from a private (undisclosed) island have been implicated in the transmission of *Salmonella* to takahē. To investigate the capacity for lizards to act as a *Salmonella* reservoir in the takahē translocation network, I examined the distribution, abundance and *Salmonella* prevalence of lizards within takahē territories on the private island and on Maud Island where takahē also reside. Additionally, I investigated the presence of *Salmonella* in soil and water samples from takahē territories on the private island, Maud Island and at the Burwood Bush takahē rearing facility. Lizard densities on the private island were estimated to be between 466-6020 lizards/ha, and 118-1528 lizards/ha on Maud Island. *Salmonella* serovars concurrent with those isolated from takahē were isolated from 2% of lizards, 25% of water and 50% of soil samples on the private island, indicating that lizards and the environment play an important role in transmission and maintenance of *Salmonella* to takahē. *Salmonella* was not isolated from lizards or environmental samples on Maud Island or at Burwood Bush, indicating prevalence too low to detect in this study or a *Salmonella* reservoir unique to the private island. Further investigation of *Salmonella* sources; serovars and seasonal patterns of *Salmonella* infection is needed to better inform takahē translocation actions on the private island. Additionally, further examination of *Salmonella* in lizards and the environment is necessary to assess the risk of *Salmonella* infection to takahē on Maud Island.

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Chapter One: General Introduction

Wildlife disease ecology

Infectious disease represents a substantial threat to endangered wildlife and has been implicated in significant declines of many species. For example, avian malaria and avian pox have been cited as one of the main drivers of species declines and extinctions of endemic Hawaiian avifauna (van Riper et al. 2002). Additionally, chitridiomycosis has caused the decline or extinction of over 1800 species of amphibians worldwide (Skerratt et al. 2007) and canine distemper has caused the decline of the endangered black-footed ferret in the United States (Williams et al. 1988), African wild dogs in Kenya and the Serengeti (Alexander & Appel 1994) and Caspian seals in the Caspian Sea (Kennedy et al. 2000). Despite numerous reports of the significant impacts disease can have on animal populations, historical conservation efforts have focused primarily on other drivers of species decline such as habitat loss and invasive species (Woodroffe 1999). However, wildlife disease has received increased attention in recent years, parallel to an apparent increase in disease emergence within threatened wildlife populations (Daszak et al. 2000). With the recognition of the important role infectious disease can play in structuring endangered wildlife communities, the need to develop methods to detect and manage disease threats has become apparent. Wildlife disease dynamics are influenced by complex interactions between host, pathogen and the environment and a combination of ecological and epidemiological perspectives could be key in understanding pathogen dynamics in wild life populations and communities (Holt et al. 2006; Lafferty & Gerber 2002).

Understanding disease dynamics is particularly crucial for the conservation and management of threatened species. Pathogens can reduce fitness in wild hosts through decreased survival, increased risk of predation and low reproductive output (Johnson et al. 2006; Smith et al. 2008), factors that could have a significant impact on already threatened wildlife populations. Additionally, the development of reintroduction as a conservation management strategy in recent years has increased the connectivity of threatened wild life populations, thus amplifying the risk of disease transmission throughout translocation networks (Hess 1996). Reintroduction is the movement of animals to re-establish

populations in their former natural range or to augment existing populations (Griffith et al. 1989) and in New Zealand alone there have been over 3000 official translocations of threatened animals for conservation purposes (Sherley et al. 2010). The inadvertent movement of any pathogens that are associated with translocated animals is inevitable and could pose a significant risk to animals already in residence at the site of translocation (Viggers et al. 1993). For example, avian malaria credited with the extinction and decline of endemic bird species in Hawaii is thought to have been co-introduced with domestic birds in the 1800s (van Riper et al. 2002). Conversely, immunologically naïve animals that are translocated could be exposed to novel pathogens present in their new environment, as evidenced by mortality in three endangered kakapo (*Strigops habroptilus*) when exposed to the pathogenic bacteria *Erysipelas rhusiopathiae* during translocation in New Zealand (Gartrell et al. 2005). Additionally, stress associated with translocation could lower a target animal's immune function, potentially allowing any pathogenic organisms to manifest as disease (Teixeira et al. 2007). There is now an understanding of the important role pathogens can play in the success of reintroduction programmes, and disease risk assessment, screening and quarantine procedures are now routinely carried out during animal translocations (McInnes et al. 2004). However, documentation on wildlife disease in New Zealand remains sparse and disease risk assessments largely target known pathogens. *Salmonella* is one such targeted pathogen. *Salmonella* is an important bacterial pathogen with over 2500 described serovars (Mermin et al. 2004). Many of these serovars are host generalist and can infect a wide range of birds, reptiles and mammals (McClelland et al. 2001). Salmonellosis has been implicated in the morbidity and mortality in wild animal populations worldwide (McClelland et al. 2001).

Salmonella in takahē

In 2012 *Salmonella houtenae* and *S. mississippi* were isolated from the flightless, New Zealand endemic rail, the takahē (*Porphyrio hochstetteri*) from a privately owned island (this island can not be identified for security reasons) (Grange pers. Comm. 2015). These serovars had not previously been isolated from

takahē despite routine disease screening for *Salmonella* species, indicating that the private island may harbour an environmental *Salmonella* reservoir. Additionally, one of the birds that had tested positive for *Salmonella mississippi* was moved to the Burwood Bush takahē rearing facility in Te Anau where it also tested positive for *S. Saintpaul* a week later. The isolation of *S. Saintpaul* from this bird could provide an indication that the takahē was infected with multiple *Salmonella* serovars from the private island; alternatively, it could indicate another source of infection at the Burwood Bush facility.

Takahē (*Porphyrio hochstetteri*) (Fig. 1) are endangered, flightless rails, endemic to New Zealand. Classified as Nationally Critical under the New Zealand Threat Classification System, the current population estimate stands at approximately 227 adult birds (Wickes et al. 2009). Due to habitat loss and pressure from introduced predators, small populations are now restricted to several offshore New Zealand islands, main land sanctuaries and one free-ranging population in Fiordland (Fig. 2).



Figure 1: Adult takahē, Maud Island, Pelorus Sound (Photo S.Rose).



Figure 2: Map of New Zealand showing free-ranging populations of takahē (Murchison Mountains, five offshore islands and one mainland sanctuary) and captive populations of takahē (Te Anau, Burwood and Pukahaha Mount Bruce) (Wickes et al. 2009). Location of the privately owned Southland Island not disclosed for security reasons.

The remaining takahē population is intensively managed and individuals are regularly moved between these sites to retain as much genetic diversity as possible in the larger population and ensure breeding success (Jamieson & Briskie 2006; Wickes et al. 2009). Birds are routinely screened for disease during the translocation process in order to mitigate disease outbreaks (McInnes et al. 2004), although disease detection is often limited due to inexact tests or difficult to detect diseases (Johnson-Delaney 1996). Birds selected for translocation are

often quarantined at the Burwood Bush takahē rearing facility in Te Anau before being moved to other sites, making the Burwood Bush site an important hub in the translocation network (Grange et al. 2014).

Although the *Salmonella* infected takahē did not exhibit signs of clinical disease, *Salmonella* infection can remain asymptomatic in a healthy host, only presenting symptoms at times of stress (Pocock et al. 2001). It is not known how this infection might affect the birds in future, or what effect it might have on takahē and other species present at other sites within the takahē translocation network. *Salmonella* species are particularly prevalent in wild avian populations and Salmonellosis been associated with serious illness and mortality in several bird species including wild turkeys in Texas (Rocke and Yuill 1987), free-living passerine birds (*Passeriformes*) in the United States (Brittingham and Temple 1986; Hall and Saito 2004), England and Wales (Lawson et al. 2010) and New Zealand (Ewen et al. 2007). Despite a high number of documented *Salmonella* outbreaks in avian populations, the transmission and impact of this bacterium on wild avian hosts is poorly understood (Ewen et al. 2012). Many *Salmonella* serovars do not display host specificity, resulting in interspecies transmission and communities containing a complex web of multiple host species and environmental sources (Pocock et al. 2001). Additionally, asymptomatic carriage of *Salmonella* by some hosts can result in the maintenance of *Salmonella* reservoirs in an ecological system (Pocock et al. 2001), further confounding interpretation of disease transmission pathways. Very little is known about the animal community and species interactions on the private island making identification of potential *Salmonella* reservoirs difficult. However, takahē on the private island have been observed consuming lizards, presenting a possible source of *Salmonella* infection (Grange pers. comm. 2015).

Salmonella in lizards and the environment

Salmonella is often considered a commensal component of lizard gut fauna (Scheelings et al. 2011) and the ability of lizards to carry *Salmonella* infection whilst remaining asymptomatic is well documented (Hall & Saito 2008; Onderka

& Finlayson 1985). Additionally, *Salmonella mississippi* and *S. houtenae* have recently been isolated from New Zealand endemic skinks and geckos (Baling et al. 2013; Middleton et al. 2011; Middleton et al. 2014), and lizards have been implicated as a possible reservoir for *S. Saintpaul* in sheep in New Zealand (McInnes 1971). However, a lack of fundamental ecological knowledge about lizard communities on the private island makes it difficult to understand the role they might play in *Salmonella* persistence and transmission of *Salmonella* to sympatric takahē. Furthermore, little is known about the ecology of lizards at other takahē translocation sites. For example, Maud Island in the Pelorus Sound is also host to a diverse community of skinks and geckos, and also hosts a population of takahē that are routinely moved throughout the takahē translocation network. Although *Salmonella* has not previously been isolated from lizards on Maud Island (Middleton et al. 2010), very little is known about lizard ecology at this site. Without basic ecological knowledge about the lizard communities within the takahē translocation network, it is difficult to assess the risk they might pose as *Salmonella* reservoirs to takahē. In addition to animal hosts, *Salmonella* has the capacity to survive for extended periods of time in the environment.

For example, *Salmonella* has been reported to maintain viability in soil for over 300 days (Holley et al. 2006), in fresh water for 115 days and in animal faeces for 30 months (Johnson & Delaney 1996). The capacity for *Salmonella* to survive independently of a host allows infectious species of *Salmonella* to further exploit transmission strategies by increasing the likelihood of contact with potential hosts whilst still viable (Holley et al. 2006). Artificial water feeders are provided for takahē on the private island, Maud Island and the Burwood bush takahē rearing facility. These water feeders are congregation sites for takahē and sympatric species, increasing opportunities for *Salmonella* transmission via inter and intraspecific contact. Additionally, build-up of faecal material from *Salmonella* infected animals in and around these water sources could potentially result in environmental *Salmonella* reservoirs in the soil and water.

Thesis aim and outline

The aim of this thesis was to investigate the ecology of lizard populations within the takahē translocation network and assess their role as potential *Salmonella* reservoirs for takahē. To do this, I investigated the ecology and *Salmonella* carriage of lizards living sympatrically with takahē on the private island, Maud Island and the Burwood Bush takahē rearing facility in Te Anau. In addition, I examined the prevalence of *Salmonella* in environmental sources within takahē territories at these three sites. The findings reported in this thesis will help to advance the understanding of disease processes occurring within the takahē translocation network and better inform takahē disease management strategies.

Chapters two and three are written as stand-alone manuscripts and as such, some repetition is inevitable. Chapter two describes the ecology of lizards within takahē territories on the private island and Maud Island with reference to the potential impacts these lizard populations could have on sympatric takahē. Chapter three describes *Salmonella* ecology in lizard populations and the environment within takahē territories on the private island, Maud Island and Burwood Bush takahē rearing facility.

Chapter four provides a synthesis of the main findings of this thesis and describes implications for the future management and conservation of lizards and takahē in addition to directions for future research.

***Chapter two: Are New Zealand endemic lizards
abundant enough to support a Salmonella
reservoir?***

2.1 Introduction

New Zealand is host to an extensive endemic reptile fauna comprised of over 100 lizard species from the families *Gekkonidae* and *Scincidae*, 60 of which are described, and the tuatara (*Sphenodon*) (Tingley et al. 2013). Due to habitat loss and pressure from introduced mammalian predators many New Zealand reptile populations have undergone significant decline in numbers and geographical range (Towns et al. 2001). Over one half of endemic New Zealand reptile species are now restricted entirely to predator free offshore islands (Towns & Daugherty 1994) and nearly 75% of all species are considered threatened (Hitchmough et al. 2013). New Zealand reptiles are largely considered cryptic species making detection and monitoring of these animals problematic (Hoare et al. 2001; Towns & Ferreira 2001), and monitoring lizard populations on remote offshore islands can prove logistically challenging to conservationists. As a result, there is dearth of knowledge regarding the demography, distribution, abundance and density of many New Zealand endemic reptile species.

Basic understanding of ecology is essential for the conservation of endemic New Zealand lizards and understanding distribution, habitat occupancy, activity periods and disease ecology, for example, are fundamental in informing conservation management and habitat restoration strategies (Nielson et al. 2006). For example, investigation of habitat use of the nationally threatened chevron skink (*Oligosoma homalonotum*) on Great Barrier Island, New Zealand found that stream habitats were of particular importance to this species (Nielson et al. 2006). This basic understanding of the chevron skink's resource use will ultimately inform management decisions such as suitable sites for translocation and habitat restoration of sites where the skinks occur. In addition, knowledge of the preferred habitat of the chevron skink enables targeted searching for this species in its known range (Nielson et al. 2006).

Baseline information of New Zealand lizard ecology can provide an indication of how introduced predators affect lizard populations, and how these lizard populations respond to subsequent management actions such as predator

eradication. For example, a study of the effects of the pacific rat, kiore (*Rattus exulans*) on an island population of Duvaucel's gecko (*Hoplodactylus duvaucelii*) prior to and after kiore eradication, provides empirical evidence of the importance of baseline ecological knowledge of lizard populations in New Zealand (Hoare et al 2007). The kiore is a known predator and resource competitor of lizards (McCallum 1986; Towns 1996), and its introduction to New Zealand resulted in widespread changes to survival and distribution of native fauna (Worthy & Holdaway 2002). Prior to kiore eradication, the population of *H. duvaucelii* on Ohinau Island was skewed towards larger individuals, and habitat use was largely restricted to coastal areas and high rocky outcrops (Hoare et al 2007). A survey of the *H. duvaucelii* population six months following kiore eradication found a change in habitat use, a significant increase in the number of smaller individuals captured and an increase in captures overall. The change in numbers, demographic structure and habitat use of *H. duvaucelii* subsequent to kiore eradication provide evidence that kiore were limiting juvenile recruitment to the population through predation, and changing the distribution of the geckos through a combination of predation and resource competition (Hoare et al. 2007).

Lizards and kiore coexisted on a privately owned island in Southland, New Zealand for many years until kiore were eradicated from the island in 1999 (this island is unable to be identified for security reasons and will be referred to as the "private island" for the purpose of this paper). However, without an existing inventory of lizard species on the private island and their demographic and geographic distributions prior to kiore eradication, it is impossible to know what effect kiore invasion and eradication has had on lizard populations. Comparison with other New Zealand islands where kiore have never been present suggests lizard diversity and abundance on the private island are likely to have suffered declines. Surveys of the lizard population on Lizard Island in the Hauraki Gulf found lizard detection rates were over 20 times lower after a kiore invasion of only one year (McCallum 1986). Middle and Green Islands in the Mercury island group avoided the introduction of mammalian predators and although they are comparatively small (13ha and 6ha respectively), the reptile fauna is comprised

of 10 and seven species of lizard respectively and the tuatara (Towns et al. 1990). Similarly, a mouse (*Mus musculus*) incursion was detected in 2013 on Maud Island in Pelorus Sound, New Zealand, and attempted eradication of the mice by aerial poison drop was carried out in 2014 (Phil Marsh *pers. comm.* 2014). Maud Island is an important refuge for many endangered species including six species of lizard. However, no official inventory of lizard species and their distribution had previously been carried out on Maud Island, and so effects of the mouse incursion and subsequent eradication on resident lizard populations remain unknown.

Understanding New Zealand lizard ecology is not only important for the conservation of lizards but may be essential to the conservation of entire ecosystems. For example, the decline of *Daphne rodriguezii*, a perennial shrub endemic to the Balearic Islands in the Mediterranean, was attributed to the introduction of mammalian predators and subsequent extirpation of the frugivorous lizard *Podarcis lilfordi* (Traveset & Riera 2005). The lizard was the only disperser of *Daphne rodriguezii* seeds and in its absence; the threatened shrub is facing extinction (Traveset & Riera 2005). Fruit also constitutes an important dietary component for many New Zealand lizard species (Whitaker 1987). Analysis of gut content of *W. maculatus* at Turakirae Head in Wellington found up to 65% of animals had consumed fruit from *Coprosma* and *Muehlenbeckia* species while 86% of *O. grande* sampled in Macraes Flat, Otago contained seeds from *Muehlenbeckia* and *Hymenanthera* species, indicating that New Zealand lizards may play an important role in seed dispersal (Whitaker 1987). Additionally, *Dactylocnemis pacificus* have been observed feeding on the nectar from flowers of the New Zealand endemic pohutukawa tree (*Metrosideros excelsa*) in congregations as large as seven lizards per m² (Whitaker 1987). The geckos were also observed covered in pollen over 50 metres from the nearest pohutukawa tree, providing an indication of the important role geckos might play in pollinating endemic New Zealand plant species (Whitaker 1987). This evidence suggests that New Zealand lizards might play a key role in the reproductive strategies of New Zealand endemic plant species. Lizard communities on islands free of rats can have extremely high species diversity

(Towns 1990) and can attain densities of as many as 13 individuals/m² (Towns 1975). As a substantial component of island ecosystem biomass, lizards could have a significant impact on ecosystem functions.

In addition to providing ecosystem services, New Zealand lizards have been implicated in disease persistence and transmission in ecological systems. For example, *Salmonella* has been isolated from New Zealand endemic skinks and geckos on several offshore islands including Mana, Cuvier, Korapuki, Matiu and Stephens Islands (Middleton et al. 2010; Middleton et al; 2014). The potential for lizards to act as disease reservoirs on New Zealand's offshore islands may pose a threat to sympatric species.

A 2012 study revealed a potential connection between *Salmonella* infection in the endemic rail, the takahē (*Porphyrio hochstetteri*) from the private island, and contact with *Salmonella* infected lizards living sympatrically with these birds (Grange pers. comm. 2015). In addition to the private island, there are three mainland sites and four islands (Maud, Mana, Kapiti and Tiritirmatangi) throughout New Zealand that host takahē populations (Fig. 1). Considering the broad distribution of lizards throughout New Zealand (Tingley et al. 2013), it is extremely likely that takahē co-exist with lizard populations at all of these sites. Takahē are routinely moved throughout this translocation network (Grange et al. 2014) and any concomitant organisms are inevitably moved with them. If lizards at these sites represent a potential *Salmonella* reservoir, takahē who come into contact with them and become infected could pose a significant risk to the entire takahē population.

The private island is not managed by the Department of Conservation and has limited access. As a result, no scientific studies have been implemented to investigate lizard species, distribution and potential interactions with other species present there. Additionally, little is known about the lizard ecology on other islands in the takahē translocation network. This provides a clear example of an important gap in the knowledge concerning New Zealand endemic reptile species. Gaining fundamental ecological knowledge is critical to the conservation of any threatened species (Jenkins 1998), and information regarding health,

distribution and density of lizards species on the private island and other sites within the takahē translocation network may be crucial to decisions regarding the urgency and course of conservation management for sympatric takahē.



Figure 1: Map of takahē populations sites within New Zealand. Location of the private island not disclosed for security reasons.

The aim of this chapter is to provide basic information on the ecology of lizards within takahē territories on the private island and Maud Island by meeting the following objectives:

1. Investigate species composition of lizard communities within takahē territories on the private island and Maud Island.
2. Describe aspects of demography, morphology and distribution of lizards within takahē territories on the private island and Maud Island.

2.2 Methods

Study Sites

Private Island

The private island is an approximately 100-hectare landmass in Foveaux Strait, New Zealand). It has a history of more than 100 years of intensive grazing by sheep and remained inhabited by humans up until 1989. It was returned to Te Rūnanga o Ngāi Tahu as Māori freehold land in 1998 (Ngai Tahu Claims Settlement Act 1998, Deed of Settlement Section 13) and is now free of both permanent human residents and livestock. Historic grazing and land clearing has resulted in vegetation cover dominated by native and exotic grass pastures and low growing shrubs including Hebe, bracken fern (*Pteridium aquilinum* var *esculentum*), flax (*Phormium tenax*) and gorse (*Ulex europaeus*). Restoration of island vegetation is ongoing, with gorse removal and native planting regularly carried out.

The Pacific rat Kioie (*Rattus exulans*) was eradicated from the island between 1997 and 1998 by Maori stakeholders and the Department of Conservation (McClelland 2002) and it has subsequently remained free of mammalian predators. The island now supports numerous endemic species including takahē (*Porphyrio hochstetteri*), tokoeka kiwi (*Apteryx australis*) and Hooker's sea lions (*Phocarctos hookeri*), in addition to several transient sea bird colonies including little blue penguins (*Eudyptula minor*), yellow eyed penguins (*Megadyptes antipodes*) and Southern black back gulls (*Larus dominicanus*). Introduced passerines such as dunnocks (*Prunella modularis*), starlings (*Sturnus vulgaris*) and sparrows (*Passer domesticus*) also inhabit the island. No surveys of the lizard population on the private island have been undertaken outside of this study and so little is known about the existing lizard assemblage. However, lizards distributed throughout the Stewart Island archipelago might be expected to occur on the private island.

Species description of lizards in the Stewart Island Archipelago

Oligosoma notosaurus (“Southern Skink”) is a diurnal, viviparous skink, endemic to New Zealand (Patterson & Daugherty 1990). This species is distributed throughout the Stewart Island archipelago in Foveaux Strait, New Zealand including Stewart, Codfish and Betsy Islands and is found in sand dunes, grasslands, scrub and rocky areas at these sites (Patterson & Daugherty 1990; IUCN Red List 2014). Its current New Zealand conservation status is “not threatened” (Hitchmough et al. 2013).

Oligosoma nigriplantare polychroma (“Common Skink”) is a diurnal, viviparous skink, endemic to New Zealand. This species is distributed widely throughout both the North and South Island of New Zealand and several offshore islands and is commonly found in tussock and grasslands, scrub and rocky areas (Patterson & Daugherty 1990). Its current conservation status is “at risk/declining” (Hitchmough et al. 2013).

Oligosoma stenotis (“Small-eared Skink”) is a diurnal, viviparous skink, endemic to New Zealand. This species is only known to occur on Stewart Island and very little is known about its ecology (Towns & Elliot 1996). Its current conservation status is “naturally uncommon” (Hitchmough et al. 2013).

Oligosoma inconspicuum (“cryptic skink”) is a diurnal viviparous skink endemic to New Zealand. This species occurs throughout Otago and Southland and is commonly found in grass and tussockland, and rocky coastal sites (Whitaker et al. 2002). Its current conservation status is “at risk” (Hitchmough et al. 2013).

Oligosoma chloronoton (“green skink”) is a diurnal viviparous skink endemic to New Zealand. This species occurs from the south of Canterbury to the Stewart Island archipelago and is found in coastal and inland grass and tussocklands. Its current conservation status is “at risk” (Hitchmough et al. 2013).

Maud Island

Te Hoiere/Maud Island is a 309-hectare scientific reserve located in Pelorus Sound in Marlborough Sounds New Zealand (41° 01 'S, 173° 52 'E). The island has a long history of human modification including land clearing and intensive livestock grazing up until 1972. Only a small number of sheep are now retained in fenced areas on the island in order to provide grazing pasture for resident takahē. Re-vegetation efforts have resulted in a gradual reversion to young regenerating forest and shrubland dominated by whauwhaupaku (*Pseudopanax arboreus*), hebe, mahoe (*Melicactus ramiflorus*), tauhinu (*Ozothamnus leptophylla*), karamu (*Coprosma robusta*), bracken (*Pteridium* spp.), exotic pasture and approximately 25 hectares of native and pine remnant forest (Le Roux 2008).

Maud Island maintains a diverse, endemic avian fauna comprised of both land and sea birds. Takahē were first moved to the island in 1984 and a small population of up to 16 birds has been maintained there since (Department of Conservation, 2015). In addition to takahē, bell birds (*Anthornis melanura*), tui (*Prosthemadera novaeseelandiae*), the New Zealand falcon (*Falco novaseelandiae*), morepork (*Ninox novaseelandiae*), little blue penguins, black back (*Larus Marinus*) and red billed gulls (*Larus novaehollandiae*) and several species of cormorant maintain stable populations on the island. The island also supports its own species of frog, the Maud Island frog (*Leiopelma pakeka*) (Department of Conservation, 2015). The lizard community on Maud Island is comprised of the Stephen's island striped gecko (*Toropuku stephensi*), common gecko (*Woodworthia maculatus*), forest gecko (*Mokopirirakau granulatus*), brown skink (*Oligosoma zealandicum*), speckled skink (*Oligosoma infrapunctatum*) and common skink (*Oligosoma nigriplantare polychroma*) (Department of Conservation, 2015).

Species descriptions of lizard species on Maud Island

Toropuku stephensi ("Stephens Island gecko"; "striped gecko") is a nocturnal viviparous gecko endemic to New Zealand. It is found on Stephens Island, Maud

Island and the Marlborough Sounds area and the Coromandel Peninsula in habitats such as lowland and coastal shrub and forest (Whitaker et al. 1999). Its current conservation status is “nationally vulnerable” (Hitchmough et al. 2013).

Mokopirirakau granulatus (“forest gecko”) is a nocturnal viviparous gecko endemic to New Zealand. It is found in the northern half of the North Island and the Marlborough Sounds and Nelson in forest and shrubland. Its current conservation status is “at risk” (Hitchmough et al. 2013).

Oligosoma infrapunctatum (“speckled skink”) is a diurnal viviparous skink endemic to New Zealand. It has a patchy distribution throughout the North Island and occurs in Nelson and the Marlborough Sounds and is found in grass and tussocklands, and open forest (Whitaker & Lyall 2004). Its current conservation status is “at risk” (Hitchmough et al. 2013).

Oligosoma zelandicum (common name “Brown Skink”) is a diurnal, viviparous skink, endemic to New Zealand. This species is distributed over the south of the North Island from Wellington to Taranaki, and the north of the South Island in Marlborough, Nelson and Westland and is commonly found in shady, moist habitats with abundant vegetation cover (Towns & Elliot 1996). Its current conservation status is “at risk/declining” (Hitchmough et al. 2013).

Woodworthia maculatus (common name “Common Gecko”) is a nocturnal, viviparous gecko, endemic to New Zealand. This species is distributed widely over North and South Islands and is commonly found in low forest shrub and rocky coastal areas (Towns & Elliot 1996). Its current conservation status is “not threatened” (Hitchmough et al. 2013).

Oligosoma nigriplantare polychroma: See above for species description.

While Maud Island was previously classified as a predator free reserve, a mouse infestation was detected in late 2013 and efforts to control the outbreak are ongoing (Phil Marsh, pers. comm. 2014). Additionally, there are occasional

incursions of the omnivorous weka (*Gallirallus australis*) and carnivorous stoat (*Mustela erminea*) from the mainland, and trapping is routinely carried out to control these incursions.

Lizard survey

I carried out mark-recapture surveys of the lizard populations within accessible takahē territories on the private island and Maud Island. Each survey was carried out over five consecutive days during January 2014 on the private island and February 2014 on Maud Island. Accurate delineation of takahē territories is difficult as little is known about how far the takahē range on both the private island and Maud Island. However, staff at each of the islands determined the approximate extent of each territory using sightings of individual birds as a guide. The private island takahē population consists of approximately 20 birds at any one time (Phil Marsh pers. comm. 2014) and eight accessible takahē territories suitable for lizard pitfall placement were identified (*Fig. 1*). Six takahē territories suitable for lizard pitfall grid placement were identified on Maud Island (*Fig. 3*), as there are a comparatively small number of takahē with numbers ranging from eight to 16 birds at any one time (Phil Marsh pers. comm. 2014).

I used a random stratified design for the placement of pitfall traps within each territory. A random compass direction and distance of ten metres from each territory's water source were assigned for the starting points of transects. Three transect lines consisting of four pitfall traps each were allocated to each takahē territory (12 traps total) and traps were placed four metres apart to account for the naturally small home range size of New Zealand lizards (Towns & Elliot 1996; Coddington & Cree 1997). In cases where selected pit fall placement sites were inaccessible, traps were placed in the nearest suitable area. Additionally, because surveys were undertaken during the takahē breeding season, areas of undergrowth with potential takahē tracks were avoided to minimise disturbance to the birds and their chicks and reduce the likelihood of birds tampering with the traps. In the case of the private island, three traps from transect E were

flooded due to the high water table in this area. As a result, these traps and area directly surrounding them were excluded from all analyses.

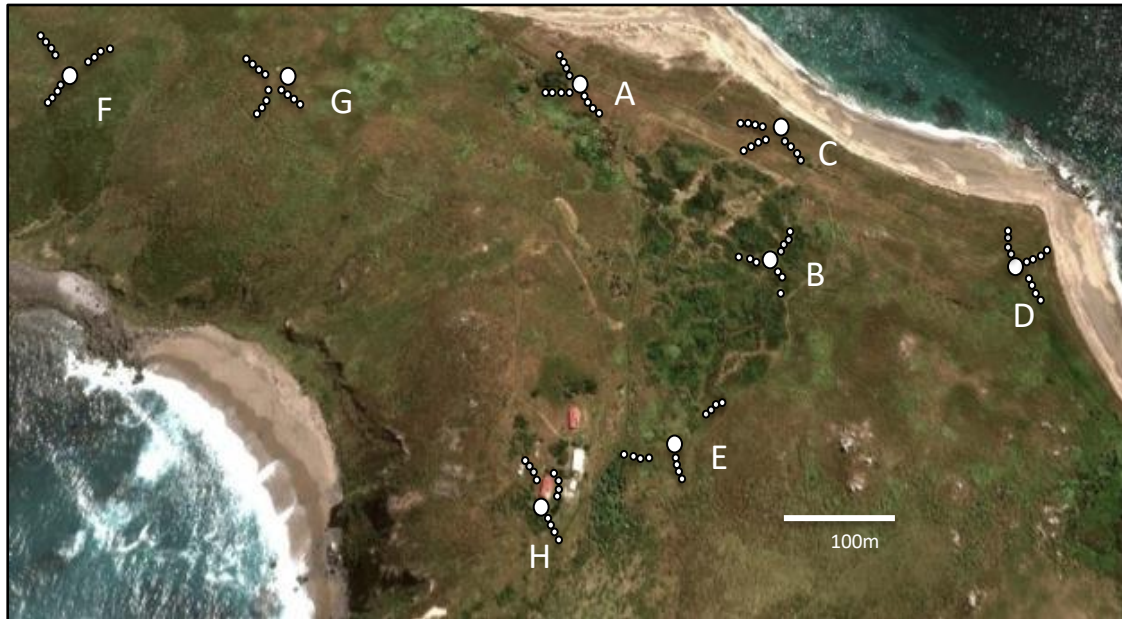


Figure 2: Map of pitfall transects on the private island. Large circles indicate takahē water sources within pitfall transects and small circles indicate pitfall trap placement. Large-scale map of the private island not provided to protect its location. Image from Google Earth ,2014.



Figure 3: Map of Maud Island with pitfall grid areas enlarged. Large circles indicate takahē water sources within pitfall grids and small circles indicate pitfall trap placement. Images from Google Earth, 2015.

Pit fall trap design

Pit fall traps used in this study conform to the guidelines outlined in the Department of Conservation document Herpetofauna: pitfall-trapping v 1.0. I used four litre, straight sided, white plastic buckets with drainage holes drilled in the bases (*Fig. 4*). Traps were dug in flush with the ground and edges packed with substrate to allow easy access for lizards. Bucket lids were secured in place over each trap using steel garden pegs to prevent flooding and reduce the risk of trapped lizards becoming desiccated at higher temperatures. Moistened sponges were placed in the bottom of each trap and checked daily to further reduce the risk of heat stress to lizards. A gap of two centimetres was left between the bucket and lid to allow access for lizards while excluding potential lizard predators such as takahē, kiwi and weka.



Figure 4: Lizard pitfall trap on the private island. Photo courtesy of Thomas Burns.

I recorded features of trap locations including GPS coordinates, vegetation type and height, and each trap was issued individual identification including territory and trap number. Pitfall traps were baited with pear and checked in the same sequence each day to ensure that a full 24-hour period passed between checks.

Lizards caught were held in gauze bags prior to processing to minimize handling and stress to the animals. Trap identity, species, sex, age group (juvenile, sub-adult, adult), snout-vent length (SVL), vent-tail length (VTL) and weight were recorded for each individual and the reproductive status of female lizards was determined via abdominal palpation. A unique identifying number was applied to the ventral surface of each individual using a non-toxic, xylene free artline® pen and unknown species were photographed for later identification. Lizards were then released adjacent to their capture trap location.

Statistical Analyses

Population number estimates

I used a weighted mean model to estimate population numbers of lizards on both the private and Maud Island (Begon 1979). This model is a modification of the simple Petersen estimate that allows for low numbers of captures and the use of data collected over several days.

Weighted mean model

$$\hat{N} = \frac{\sum m_i n_i}{(\sum M_i) + 1}$$

Where \hat{N} = estimation of the population mean,
 m_i = the number of individuals caught on day i ,
 M_i = the number of marks at risk on day i and
 n_i = the number of individuals caught on day i .

$$SE_{\hat{N}} = \hat{N} \sqrt{\frac{1}{\sum m_i + 1} + \frac{2}{(\sum m_i + 1)^2} + \frac{6}{(\sum m_i + 1)^3}}$$

The validity of the weighted mean model relies on the same set of assumptions as the Petersen estimate:

1. The population is closed and population size is constant over the sampling period. This means there are no births or deaths and no immigration or emigration in the population over the course of the study.
2. All identifying marks attributed to animals are permanent for the course of the study.
3. Identifying marks are not misread on subsequent captures.
4. Capture and marking of animals has no effect on catchability of individual animals or on the individual animal's chances of dying or emigrating.
5. Each individual animal in the population has an equal probability of being caught.

The majority of the above assumptions were met on both the private island and Maud Island. Both sampling periods were carried out over the relatively short period of five days, limiting the number of gains and losses to the population. Both islands are also geographically isolated, restricting immigration and emigration but some degree of lizard movement between traps is inevitable. However, the pitfall traps were placed four metres apart reflecting the home range size of many New Zealand lizard species (Towns & Elliot 1996), thus limiting movement of lizards outside of pitfall transects. Individual marks were deemed permanent for the course of the survey as indicated by lizards originally marked on the first day of the sampling period on the private island retaining their marks until recapture on the last day of the sampling period. All field team members were involved in the marking of captured lizards and interpretation of recaptured lizard's marks, reducing the probability of misidentification of marks. For the purpose of these analyses, I assumed that capture and marking of lizards did not affect their subsequent catchability as handling times were minimised

and the marking technique was non-invasive. However, there is the possibility that some individuals became “trap shy” after initial handling, making future recaptures less likely. Additionally, the assumption of equal catchability of individuals in a population is difficult to assess and may not have been met in this case. Patterns of lizard catchability may have been discernible if sufficient numbers were caught to analyse data in a sophisticated mark recapture program such as MARK. MARK has the capacity to include factors such as variable individual capture probabilities in the estimation of population sizes while simple models such as the weighted mean are not able to account for such variation.

Minimum numbers alive

$$MNA = \text{Captures}_{total} - \text{Recaptures}_{total}$$

MNA is a model commonly used to provide a rough indication of animal abundance and relies on the same assumptions as the weighted mean model (Hare 2012).

Sex ratios

I used chi square goodness of fit tests to examine whether sex ratios of lizard species captured differed from 1:1.

Density estimates

I estimated lizard density for the private island and Maud Island using both the population estimates obtained from the weighted mean model and those from MNA. Areas for density estimates were extrapolated out from the total area covered by pitfall transects, including an area of four metres radiating out from transect lines to account for spill over between lizard territories. Three pitfall traps in transect B were flooded at the beginning of the survey and so the area surrounding these traps was excluded from density estimates. I excluded the 25 hectares of forest from analysis of Maud Island densities due to the dissimilarity of this habitat to the habitats I was able to sample on the island. In contrast, the private island is dominated by pasture and low shrub, and as I was able to

sample in both these habitats, I included the entire island area in density analysis.

Effects of temperature and humidity on lizard capture rates

I used a linear regression to assess the effect that temperature had on lizard capture rates on the private island. Relative humidity data were not available for the private island or Maud Island during the relevant capture period and so the effects of temperature only on capture rates was assessed. Temperature data for the private island were obtained from the Stewart Island weather station and Maud Island data were obtained from the closest available weather station in Crail Bay, Pelorus Sound. Temperature data were obtained from the NIWA National Climate Database.

Effects of habitat on lizard capture rates

I conducted F tests to compare capture rates of lizards over habitat types. Different habitats were defined by the dominant height and structure of vegetation surrounding each pit fall trap and standardised to fit into three groups for ease of analysis. Pasture and native grasses over 10 centimetres in height were grouped together as “grass”, low-lying bush less than 1.5 metres in height including bracken, *Coprosma* spp., gorse and flax were grouped together as “shrub”. The remaining habitat grouping was defined as leaf litter and only occurred on Maud Island. I used chi square goodness of fit tests to assess whether habitat type had any effect on capture rate of males, females or juveniles.

2.3 Results

Capture Summaries and lizard morphological measurements

Private island: Three species of skink were captured on the private island. These were the Southern skink (*Oligosoma notosaurus*, Fig. 4) 91.5% of total captures, common skink (*Oligosoma nigriplantare polychroma*) 7.5% of total captures, and small-eared skink (*Oligosoma stenotis*, Fig. 5, identified by James Reardon DOC) 1.1% of total captures.



Figure 4: *Oligosoma notosaurus* captured on private island. From the top, lateral, dorsal and ventral views. Photos courtesy of Thomas Burns.



Figure 5: *Oligosoma stenotis* captured on private island. From the top, lateral, dorsal and ventral views. Photos courtesy of Thomas Burns.

A total of 176 skinks were captured with four recaptures. Of the adult and sub-adult *O. notosaurus* captures, males accounted for 33% and females 67%, while juveniles accounted for 14% of total *O. notosaurus* captures (Table 1).

Species	Male	Female	Juvenile (sex unknown)	Total captured
<i>Oligosoma notosaurus</i>	45	91	25	161
<i>Oligosoma stenotis</i>	1	1	0	2
<i>Oligosoma nigriplantare polychroma</i>	4	6	3	13
Total captures	50	98	28	176

Table 1: Summary of lizard captures from the private island.

A Chi square goodness of fit test found the sex ratio of adult and sub-adult *O. notosaurus* (49:100, male to female) was significantly different from the expected 1:1 ratio (X^2 (1, N=136)=15.559, $p<0.0001$). Of the adult and sub-adult *O. nigriplantare polychroma* captures, 40% were male and 60% female, while juveniles made up 23% of captures. The sex ratio in adult and sub-adult *O. nigriplantare polychroma* was not significantly different from the expected 1:1 ratio (X^2 (1, N=10)=0.400, $p=0.5271$). Two adult *O. stenotis* were captured, one male and one female. A summary of morphology and sex proportions of lizards captured on the private island is reported in *Table 2*.

Species	Sex	Proportion pregnant	Proportion of sex	Average SVL (mm) (SD)	Weight (g) (SD)	Total proportion of captures
<i>Oligosoma nigriplantare polychroma</i>	M		0.4	55.5 (3.32)	2.98 (0.84)	0.07
	F	0.67	0.6	60.2 (7.05)	3.58 (1.43)	
<i>Oligosoma notosaurus</i>	M		0.33	54.62 (4.76)	2.82 (0.74)	0.92
	F	0.4	0.67	57.83 (5.08)	3.56 (1.09)	
<i>Oligosoma stenotis</i>	M		0.5	64	3.3	0.01
	F	0	0.5	52	2.2	

Table 2: Summary of morphology, reproductive status and proportions of lizards captured on the private island.

Maud Island: Three species of lizard were captured on Maud Island, the brown skink (*Oligosoma zelandicum*) (*Fig. 6*) 47.5% of total captures, the common skink

(*Oligosoma nigriplantare polychroma*) (Fig. 6) 5%, and the common gecko (*Woodworthia maculatus*) (Fig. 7) 47.5% of total captures.



Figure 6: Left: *Oligosoma zelandicum*, right: *Oligosoma nigriplantare polychroma*. Photos courtesy of Steve Attwood, Auldwood Photography.



Figure 7: *Woodworthia maculatus*. Left photo courtesy of Thomas Burns, right photo S. Rose.

A total of 34 lizards were captured in pitfall traps. Ten lizards were captured by hand inside takahē territories but outside of pitfall transects and were therefore excluded from density estimates. There were no recaptures. A summary of hand captured lizards in reported in *Table 3* and a summary of morphology and sex proportions of lizards captured is reported in *Table 4*.

Species	Male	Female	Juvenile (sex unknown)	Total captured

<i>Oligosoma zelandicum</i>	3	9	2	14
<i>Woodworthia maculatus</i>	10	10	0	20
Total captures	13	19	2	34
Total proportion of captures	0.38	0.56	0.06	

Table 3: Summary of lizards captured in pitfall traps on Maud Island.

Species	Male	Female	Juvenile (sex unknown)	Total captured
<i>Oligosoma zelandicum</i>	0	7	0	7
<i>Woodworthia maculatus</i>	0	1	0	1
<i>Oligosoma nigriplantare polychroma</i>	0	2	0	2

Table 4: Summary of lizards caught by hand on Maud Island.

Of the adult *O. zelandicum*, males accounted for 13% of adult captures and females 87%, while juveniles accounted for 9.5% of total *O. zelandicum* captures. The sex ratio of adult and sub-adult *O. zelandicum* (18:100, male to female) was significantly different from the expected 1:1 ratio (X^2 (1, N=19)=8.895, $p=0.0029$). Of the adult and sub-adult *H. maculatus* captures, 48% were male and 52% female. The sex ratio in adult and sub-adult *H. maculatus* was not significantly different from the expected 1:1 ratio (X^2 (1, N=21)=0.048, $p=0.8273$). Two sub-adult *O. nigriplantare polychroma* were captured, both female. A summary of morphology and sex proportions of lizards captured in pitfall traps on Maud Island is reported in Table 5.

Species	Sex	Proportion pregnant	Proportion of sex	Average SVL (mm) (SD)	Weight (g) (SD)	Total proportion of captures
<i>Oligosoma zelandicum</i>	M		0.13	55.5 (6.36)	3.85 (0.07)	0.41
	F	0.63	0.87	62.38 (5.17)	6.18 (1.63)	
	M		0.5	68.6 (3.75)	9.28 (2.19)	0.59

**Woodworthia
maculatus**

F 0.8 0.5 67.9 (5.19) 8.67 (1.33)

Table 5: Summary of morphology, reproductive status and proportions of lizards captured on Maud Island.

Population and density estimates

Study site	Total area of all pitfall transects (m ²)	Weighted mean population estimate for all pitfall transects	MNA over all pitfall transects	Weighted mean density estimate per hectare	MNA density estimate per hectare
Private island	3776	2273 (SE 6819)	176	6020	466 (SD 230)
Maud Island	2880	440 (SE 7163)	34	1528	118 (SD 164)

Table 6: lizard population number and density estimates from weighted mean model and MNA for the private island and Maud Island.

Site	Pitfall transect area (m ²)	Lizards captured	Density (lizards/ha)
A	480	17	354
B	416	18	433
C	480	17	354
D	480	38	792
E	480	41	854
F	480	20	417
G	480	12	250
H	480	13	271
Mean		22	466

Table 7: Private island lizard densities per pitfall site based on MNA. Mean density per pitfall grid 446 (S.E. 3). Three pitfall traps were removed from site B due to flooding and the area of this transect reflects this.

Site	Pitfall transect area (m ²)	Lizards captured	Density (lizards/ha)
A	480	0	0
B	480	0	0
C	480	6	125
D	480	3	63
E	480	4	83

F	480	21	438
Mean		6	118

Table 8: Maud Island lizard densities per pitfall site based on MNA. Mean density per pitfall grid 118 (S.E. 67).

Effect of temperature on capture rates

There was a strong positive correlation between maximum daily air temperature on the day of sampling and lizard capture rates on the private island ($R^2 = 0.9$, $F(1,3) = 27.15$, $p = 0.014$), (Fig. 8). No significant association was found between maximum daily air temperature on the day of sampling and lizard capture rates on Maud Island ($R^2 = 0.570$, $F(1,3) = 3.98$, $p = 0.14$), (Fig. 9).

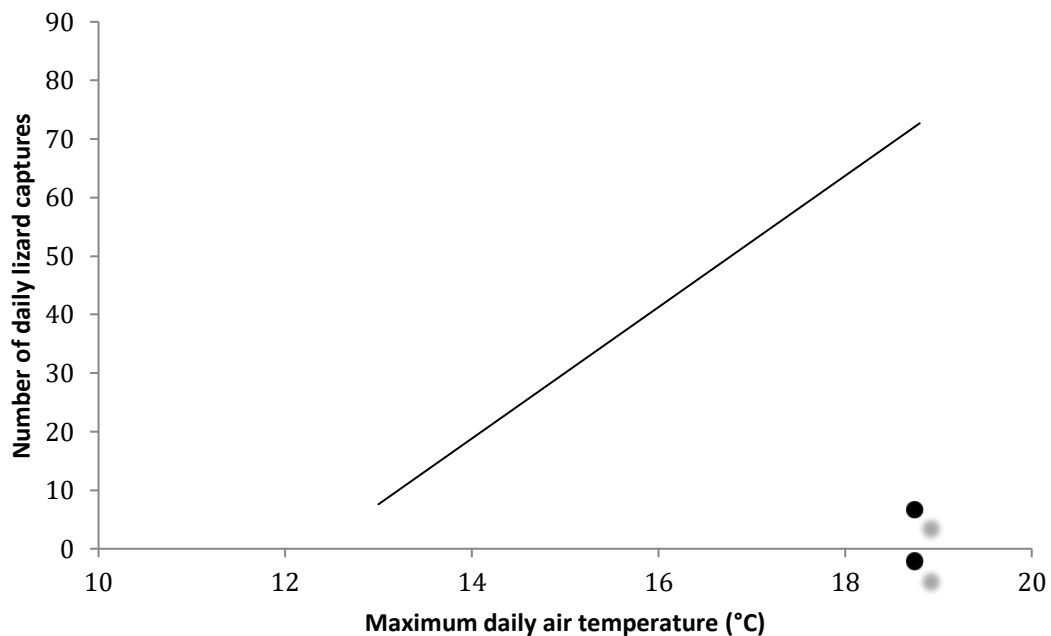


Figure 8: Linear regression showing the positive relationship between maximum daily air temperature and total number of daily lizard captures on the private island ($R^2 0.9$). Maximum air temperatures obtained from the Stewart Island weather station, NIWA National Climate Database.

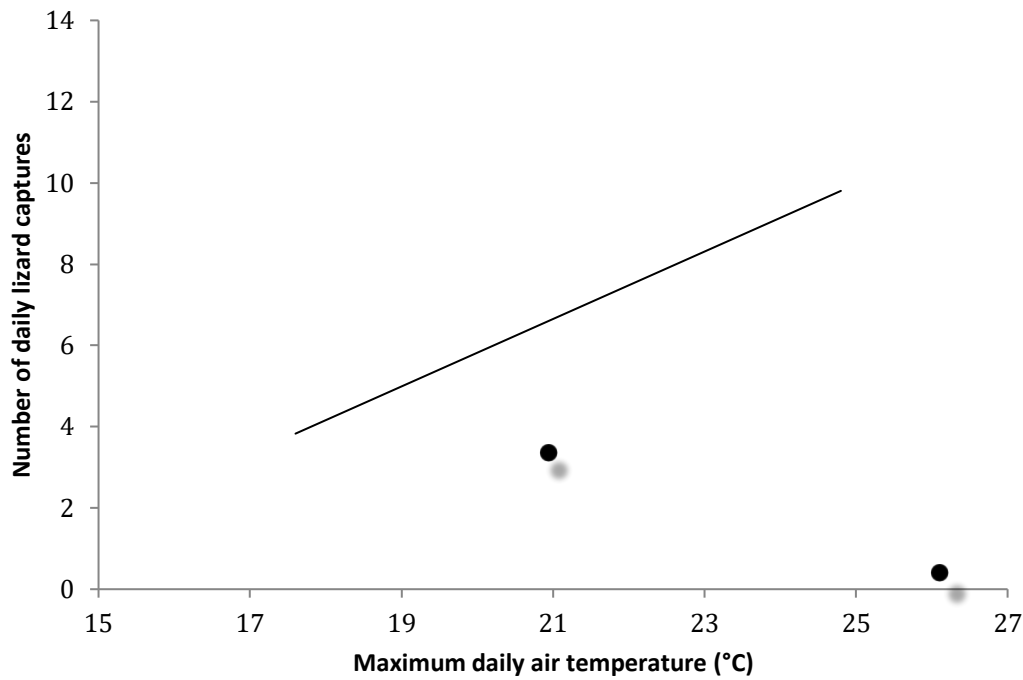


Figure 9: Linear regression showing a weakly positive relationship between maximum daily air temperature and total number of daily lizard captures on the Maud Island (R^2 0.570). Maximum air temperatures obtained from the Crail Bay weather station, NIWA National Climate Database.

Effect of vegetation type on lizard capture rates

Private island: There was no significant effect of vegetation type on total capture rates for the private island $F(1,91)=1.596$, $p=0.210$ (Fig. 10). Chi square goodness of fit tests showed no significant difference in female lizard catches according to vegetation type ($X^2(1, N=98)=0.163$, $p=0.6862$), male lizard captures according to vegetation type ($X^2(1, N=50)=0.320$, $p=0.5716$), or juvenile lizard captures according to vegetation type ($X^2(1, N=28)=0.143$, $p=0.7055$) (Fig. 11).

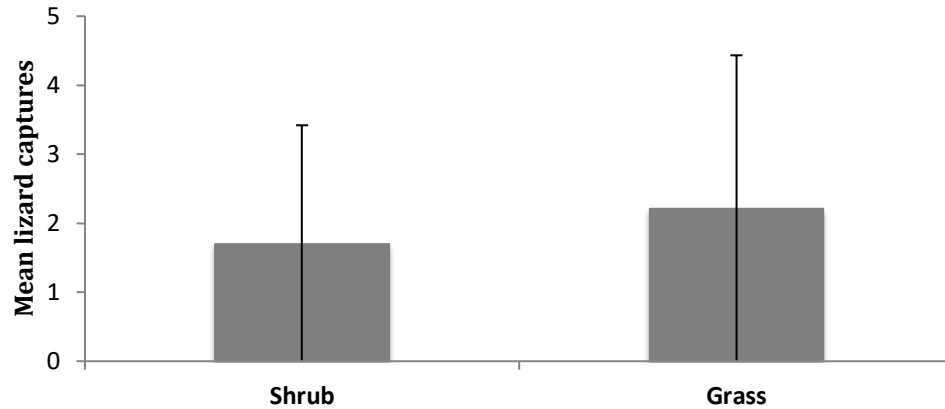


Figure 10: Mean lizard captures by vegetation type surrounding pitfall traps on the private island. Shrub N= 85, Grass N= 91 (+/- 1 SD). No significant difference in total lizard capture rates between habitats was found.

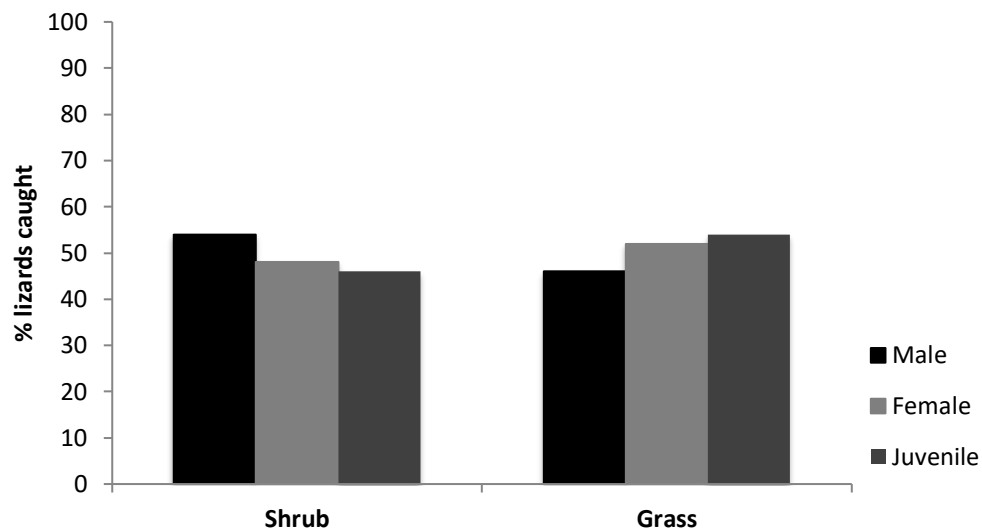


Figure 11: Percentage of male, female and juvenile captures in pitfall traps surrounded by each vegetation type on the private Island. Male N= 50, Female N= 98, Juvenile N= 28. There was no significant effect of vegetation type surrounding pitfall trap on age group trapped.

Maud Island: There was no significant effect of vegetation type on total capture rates for Maud Island $F(2,69)=0.704$, $p=0.498$ (Fig. 12). Chi square goodness of fit tests showed no significant difference in female lizard catches according to vegetation type ($\chi^2(1, N=17)=2.882$, $p=0.0896$) or male lizard captures according to vegetation type ($\chi^2(1, N=12)=3.000$, $p=0.0833$) (Fig. 13). Juveniles and leaf litter were excluded from Chi square analysis due to low values.

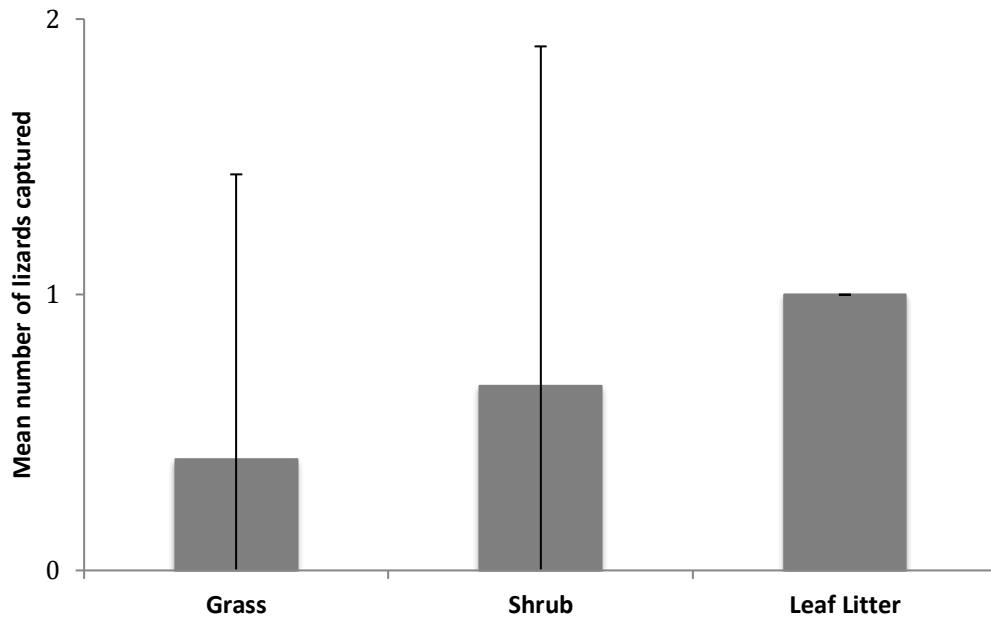


Figure 12: Mean lizard captures by vegetation type surrounding pitfall traps on Maud Island. Leaf litter N= 3, Shrub N= 8, Grass N=3 (+/- 1 SD). No significant difference in total lizard captures between habitats was found.

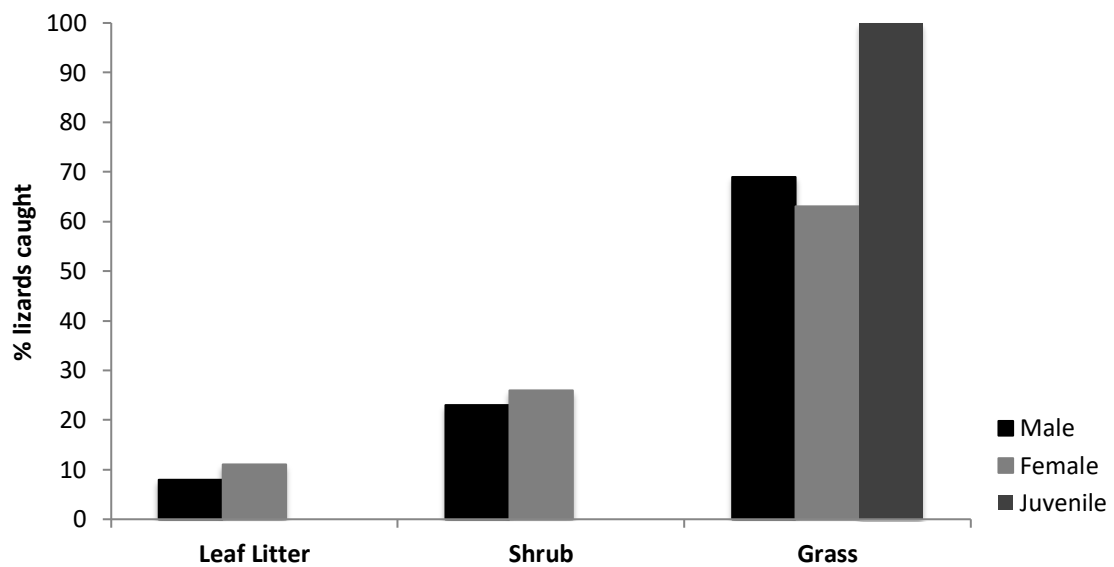


Figure 13: Percentage of male, female and juvenile captures in pitfall traps surrounded by each vegetation type on Maud Island. Male N= 13, Female N= 19, Juvenile N= 2. There was no significant effect of vegetation type surrounding pitfall trap on age group trapped.

2.4 Discussion

Population estimates

The weighted mean model estimated lizard densities to occur at as many as 6,020 individuals/ha and 1,528 individuals/ha on the private island and Maud Island respectively, while the MNA model produced much lower density estimates of 466/ha (SD 230) and 118/ha (SD 164). However, the potential violation of the assumption of equal catchability and the limitations of simple mark-recapture based models imply that the population numbers estimated in this study should be interpreted cautiously. Conservation objectives for the management of threatened animal species are often articulated in terms of population size (Lancia et al. 1994). However, population estimates for New Zealand lizard species are notoriously difficult due to their cryptic nature, unequal capture probabilities between individuals and territorial behaviour (Towns 1975). This individual heterogeneity within lizard communities can cause negative bias in commonly used estimators. For instance, estimates of gecko abundance can be severely underestimated due to their ability to escape pitfall traps. Additionally, small lizards may avoid traps (Towns & Elliot 1996), resulting in underestimation of juvenile and small species abundances.

Furthermore, the MNA model can produce biased population estimates with counts disproportionate to actual population size, as it is sensitive to the low catchability of unmarked individuals (Efford 1992). As a result, population numbers attained using this method are commonly an underestimation of actual numbers (Efford 1992). Conversely, population estimators that utilise mark-recapture, such as the weighted mean model, have a tendency to overestimate population numbers when recaptures are very low (Roff 1973), and standard errors for mark recapture models are strongly correlated with population estimates, resulting in often inaccurate and unreliable estimation of the true mean of a population (Begon 1979). More sophisticated models such as those available in mark-recapture software packages should be employed wherever possible to account for such variability. However, in cases where data are too deficient to employ more sophisticated methods of population estimation, a

choice must be made as to whether insufficient data should be discarded entirely or if less accurate results are better than no results (Begon 1975).

Taking these limitations into account, there are clear differences in lizard population estimates and community structure between the private island and Maud Island. The private island maintains a considerably higher density of lizards than Maud Island (*Tables 7; 8*). The discrepancy in lizard densities could be due to differences in habitat between the islands; the private island vegetation consists largely of exotic pasture and low scrub, while Maud Island has a comparatively high proportion of regenerating forest. Many *Oligosoma* species are diurnal and display basking behaviour and therefore, are most often associated with comparatively open habitats such as those present on the private island (Gill 1976; Patterson 1992; Newman 1994). The gradual reforestation of Maud Island may have resulted in range contractions of diurnal skinks, ultimately reducing the population size. Similarly, reforestation efforts on Mana Island, New Zealand (East et al. 1995) and at the mainland New Zealand site, Pukerua Bay (Towns & Elliot 1996) are predicted to significantly reduce range and abundance of *O. nigriplantare polychroma*.

Additionally, the recent mouse incursion on Maud Island could have the potential to reduce lizard numbers. Mice are known predators of New Zealand lizards (Tocher 2009) in addition to competing for the same resources (Reardon et al. 2012). For example, surveys of the McGregor's skink (*O. macgregori*) and *W. maculatus* populations on Mana Island demonstrated a significant increase in pitfall capture rates after mouse eradication (Newman 1994). However a survey of *O. aeneum* and *O. nigriplantare polychroma* populations post mouse eradication on Mana Island found no significant increase in these species (Newman 1994) It is unclear what effect the mice have had on the Maud Island lizard populations, as there is limited knowledge on lizard abundance and diversity prior to the incursion.

Although the private island appears to host a higher density of lizards, Maud Island has higher species diversity including three species of gecko. Notably,

gecko species were absent from the private island, or occurred at numbers too low to detect in this study, although geckos are able to escape from pit fall traps so may not have been detected using this method. There is anecdotal evidence that geckos existed on the private island historically but were extirpated when kiore reached high densities (Sandra Cooke, *pers. comm.* 2014). Kiore are more active at night and so nocturnal or crepuscular ground dwelling species of lizard such as *W. maculatus* are most at risk from predation (Whitaker 1973). There have been sightings of a “green gecko” in a stand of trees on the northwest side of the private island (Sandra Cooke *pers. comm.*) but a search of this area during the day and spot light search during the night revealed no sign of any geckos. However, a more extensive survey of the private island is needed to better assess the species present.

Additionally, bias can be present in simple density indices as they are unable to account for variation in individual detectability and variable density over different habitats (Hare 2012). Although the private island vegetation is relatively homogeneous, Maud Island consists of a patchwork of different habitats, making extrapolation of density estimates to large areas difficult. However, despite this potential for bias, lizard densities for both the private island and Maud Island are similar to density estimates of other New Zealand sites. For example, a survey of the *O. nigriplantare polychroma* population at Birdlings Flat, Canterbury, estimated a density of 200-400 lizards/ha (Freeman 1997). In addition, a survey of lizard species present at Pukerua Bay estimated *Oligosoma* species densities of between 656 and 4919 lizards/ha, regardless of lizard predators present at this site (Towns & Elliot 1996). Similar lizard density estimates at other New Zealand sites suggest that lizard densities estimated in this study are possible and even likely.

Species, sex ratios and age group proportions

Three species of skink were detected on the private island in this study, *Oligosoma notosaurus*, *O. nigriplantare polychroma* and *O. stenotis*. *Oligosoma nigriplantare polychroma* is distributed throughout New Zealand and *O.*

notosaurus is common in Stewart Island archipelago. However, identification of *Oligosoma stenotis* on the private island is significant. *Oligosoma stenotis* is previously known only from Stewart Island, where they were originally mistaken for *Leiopisma nigriplantare polychroma* (now *Oligosoma*) due to their similar morphology (Patterson & Daugherty 1994). The discovery of this range extension emphasises the paucity of knowledge of New Zealand lizard species and their range and distribution.

Oligosoma notosaurus captured on the private island and *O. zelandicum* captured on Maud Island exhibited female biased sex ratios. These sex ratios may represent a real bias in *O. notosaurus* and *O. zelandicum* populations. Stochastic processes acting on small populations can cause sex bias (Flannagan 2000), but this is not uncommon in New Zealand lizard populations with female biased sex ratios found in natural populations of *Woodworthia chrysosireticus* (Flannagan 2000), *Oligosoma nigriplantare polychroma* (Freeman 1997), *O. whitakeri* and *O. alani* (Towns & Ferreira 2001). Female biased sex ratios in lizards are not always detrimental to population persistence. For example, selection of female biased groups of lizards for translocation is a common management technique in New Zealand as it increases the probability of reproductive success in introduced populations (Towns & Ferreira 2001). Alternatively, the sex bias encountered in this study may be an artefact of female specific behaviours such as the increase in basking behaviour associated with pregnancy in skinks (Schwarzkopf & Shine 1991). A high proportion of females sampled in this study were pregnant and if pitfall grids were situated near basking areas it is likely that a higher proportion of females would encounter traps due to their increased basking activity.

In addition to biased sex ratios, both the private island and Maud Island had disproportionately low captures of juvenile lizards to adult lizards (0.16 and 0.06 of total captures respectively). This may suggest a real age group bias in the populations due to low survivorship of juveniles but could also be explained by trap avoidance by smaller lizards, a higher proportion of basking females in trapping areas or a combination of these factors.

Site fidelity

Three of the *O. notosaurus* recaptured on the private island were found in the traps they had originally been caught in. This result may be indicative of strong site fidelity. Site fidelity is common in New Zealand lizard species. For example, a mark recapture survey at Pukerua Bay found that *O. zelandicum*, *O. nigriplantare* and *W. maculatus* were rarely recaptured more than four metres from their original capture site (Towns & Elliot 1996), and a survey of *O. grande* and *O. otagenese* individuals in Otago found strong site fidelity with the majority of individuals moving less than 9 metres from where they were originally sighted (Coddington & Cree 1997). In contrast, one adult male on the private island travelled 142 metres from the original trapping site in one day but it is not uncommon for lizard species that typically display strong site fidelity to travel long distances. For example, an *O. aenea* individual was observed 64 metres from its original capture site at Pukerua Bay (Towns & Elliot 1996) and an *O. grande* was recorded 139m from where it was original sighted (Coddington & Cree 1997). The recapture data from the private island might suggest that lizard species at this site do display strong site fidelity but will move out of their home range, perhaps in search of mating or resource opportunities. Alternatively, handling during sample could result in trap aversion and influence dispersal behaviour. Lizard movement over long distances has implications for the transmission of disease between lizard populations and sympatric species. Strong site fidelity might restrict the spread of infectious disease such as *Salmonella* to lizard populations and other species within a small area. However, if infected lizards commonly travel long distances in search of new territory or mating opportunities, the risk of disease transmission to lizards, other sympatric species and environmental sources may extend to neighbouring areas. The risk of disease transmission by infected lizards over large areas may be highest during summer when mate-searching behaviour occurs most frequently. Further investigation of lizard site fidelity on the private island is necessary to determine if a seasonal pattern of long distance movement exists.

Morphology

Average SVL measurements of skink species found on both the private island and Maud Island are roughly comparable to previously recorded measurements from other predator-free island sites within New Zealand and marginally larger than the same species found at mainland sites (*Table 6*).

Species	Study site	min-max SVL/mm	Island SVL comparison	Mainland comparison
<i>O. nigriplantare polychroma</i>	private island	51-70	77mm maximum, Stewart Island (Patterson & Daugherty 1990)	58mm ave, Birdlings Flat (Freeman 1997)
<i>O. notosaurus</i>	private island	42-70	75.5mm maximum, Stewart and Codfish Islands (Patterson & Daugherty 1990)	Not known on the mainland
<i>O. stenotis</i>	private island	52-64	74.5mm maximum, Stewart Island (Patterson & Daugherty 1994)	Not known on the mainland
<i>O. zelandicum</i>	Maud Island	51-72	data unavailable	54.1mm maximum SVL, Manawatu (Gill 1976)
<i>W. maculatus</i>	Maud Island	58-74	66.2mm average, Mana Island (Monks & Rose unpub. data 2013)	51.1mm ave SVL, Pukerua Bay (Monks & Rose unpub. data 2013)

Table 6: Comparison of lizard SVL from other island and mainland sites.

Effect of temperature on capture rates

Lizard capture rates on the private island were positively correlated with the maximum air temperature on the day of capture. In contrast, there was not a strong correlation between lizard capture rates and air temperature on the day of capture on Maud Island. Emergence behaviour of diurnal skinks is extremely weather dependent due to their heliothermic physiology (Roughton & Seddon 2006; Coddington & Cree 1992). Higher air temperatures provide better basking opportunities and hence, skinks are more active, and more likely to be captured under these conditions. Lizards may be more responsive to temperature fluctuations on the private island due to its generally cooler climate and may exploit warmer weather more quickly than lizards on Maud Island where

temperatures are higher. Conversely, the capture rates of nocturnal lizard species such as *W. maculatus* can be negatively associated with air temperature (Hare 2012). As a result of this temperature associated capture variability, variation in temperature over lizard sampling periods can greatly influence population estimates and this variation should be accounted for when estimating lizard population numbers. However, lizard recapture rates were too low in this study to apply more sophisticated population models to account for temperature variability and as a result, population estimates reported should be interpreted cautiously.

Effect of vegetation type on lizard capture rates

There was no significant effect of vegetation type on lizard capture rates on either the private island or Maud Island. Analyses of capture numbers according to vegetation type were averaged over all lizard species in this study as accurate, species-specific analyses of lizard habitat preference were not feasible for most species due to low sample sizes and recapture rates. However, *Oligosoma notosaurus* was found in approximately equal numbers in grass (81 lizards) and shrub (80 lizards) habitats. Habitat type plays a large role in lizard species densities and distribution as many species have very different ecological requirements (See species descriptions section). However, variation in density and distribution of different lizard species may also be attributed to factors such as resource competition, moisture and territoriality (Freeman 1996). Therefore, it is likely that habitat preference does have an effect on lizard density and distribution on the private island and Maud Island but the sample size in this study was too low to detect any trends.

The information gained from this study provides valuable insights in the distribution, density and diversity of lizard population occurring within takahē territories on the private island and Maud Island. Lizard population and density estimates reported here provide an indication of how lizards might play an important role in island ecosystem functions. Investigation of habitat occupancy provides a starting point for informing decisions regarding the effects of

vegetation restoration on lizard populations on the private island and Maud Island. Additionally, this study constitutes the first lizard inventory for the private island and the discovery of a range extension of *Oligosoma stenotis* will help to inform future management decisions regarding this naturally uncommon species (Hitchmough et al. 2013). Finally, the density of skinks discovered in this study provide a clear indication that lizards are abundant on the private island and Maud Island and have the potential to act as a *Salmonella* reservoir at these sites. Although *Salmonella* has not yet been detected on Maud Island, the translocation of *Salmonella* infected takahē to this site is a very real threat both to resident takahē and lizard populations.

***Chapter 3: Are skinks the Salmonella reservoir in
the takahē translocation network?***

3.1 Introduction

Pathogens play an important role in the ecology of their wild host species, potentially affecting health, reproductive output and even survival of infected organisms (Holt et al. 2003). However, the role that pathogens play in structuring wildlife populations and communities is sporadically documented (Griffith et al. 1993). The dynamics of disease in wildlife populations often involve a complex relationship between physiological, environmental and ecological variables (Holt et al. 2003), rendering elucidation of pathogen transmission pathways problematic. Not only do pathogens affect primary host species, non-specific pathogens can spill over to sympatric species, creating the potential for increased host availability (Anderson et al. 1986; Smith et al. 2009). Additionally, many pathogens are able to survive outside of a living host for extended periods of time (Flint 1987; Holley et al. 2006), increasing the likelihood of contact with new hosts and thus increasing pathogen persistence in wildlife communities. Defining the pattern of pathogen infection between primary hosts, sympatric species and their environments is crucial in the understanding of potential impacts a pathogen may have on its host species (Cunningham 1996). In the current climate where species are increasingly threatened by decline, understanding the role pathogens play in structuring wildlife communities is imperative to conservation and management efforts.

The translocation of animals has become a common technique in conservation management (Woodford 1993). Groups of animals are commonly moved to predator free offshore or mainland islands in an effort to conserve wildlife threatened by habitat loss and introduced predators (Hitchmough et al. 2010). For example, in New Zealand alone, over 3,000 official translocations of native terrestrial species have been carried out for conservation purposes since 1894 (Sherley et al. 2010). However, the movement of any concomitant organisms with species targeted for translocation is inevitable and co-introduced pathogens have the potential to negatively impact both the managed species and sympatric species at the translocation site (Cunningham 1996). A translocated animal may carry a pathogen with no apparent negative effects but transmission of the pathogen to immunologically naïve hosts at the site of translocation may result

in disease (Viggers et al. 1993). For example, the introduction of domestic avian stock infected with avian pox to Hawaii in the 1800s caused widespread disease and extinction in the endemic avifauna already present at the site of translocation (van Riper et al. 2002).

Conversely, translocated animals may be exposed to foreign pathogens in their new environment (Viggers et al. 1993). For example 19 kakapo (*Strigops habroptilus*), an endangered flightless parrot endemic to New Zealand, were translocated to Chalky Island from Codfish Island in 2004, all birds having undergone routine disease screening for *Salmonella*, *Yersinia* and *Campylobacter* prior to translocation (Gartrell et al. 2005). Three of the birds died within 72 hours of transfer from *Erysipelothrix rhusiopathiae*, an infection potentially transmitted to the kakapo from sea birds in residence at the site of translocation. Additionally, the physiological stress associated with capture and translocation of animals may diminish immune competence and hence resistance to disease, potentially allowing pathogens to cause disease in infected animals involved in the translocation process (Teixeira et al. 2007). Very little is known about pathogens of native species, or sources of infection in New Zealand wildlife populations. I investigated environmental factors that may influence the transmission and prevalence of *Salmonella* in the New Zealand endemic bird, the takahē.

Takahē (Porphyrio hochstetteri)

The takahē is a critically threatened species with only 227 adult birds remaining. Small, geographically isolated populations of takahē now persist solely on predator free offshore islands and mainland sanctuaries (*Fig. 1*) and as a result, translocation is routinely employed to maintain genetic diversity and ensure breeding success (Jamieson & Briskie 2006).



Figure 1: Map of New Zealand showing free-ranging populations of takahē (Murchison Mountains, five offshore islands and one mainland sanctuary) and captive populations of takahē (Te Anau, Burwood and Pukaha Mount Bruce) (Wickes et al. 2009). Location of the privately owned Southland Island not disclosed for security reasons.

Birds are routinely screened for the presence of pathogens during translocation to mitigate disease outbreaks (McInnes et al. 2004). Additionally, takahē selected for translocation are quarantined at the Burwood Bush takahē rearing facility in Te Anau before being moved to other sites, making the Burwood Bush site an important hub in the translocation network (Grange et al. 2014).

In an investigation of disease in the takahē translocation network, *Salmonella mississippi* and *Salmonella houtenae* were isolated for the first time from the takahē population on a privately owned southland Island during disease screening (this island is unable to be identified for security reasons) (Grange pers. comm. 2015). An infected bird was moved from the private island to the Burwood Bush takahē rearing facility for quarantine prior to translocation. Subsequently, *Salmonella Saintpaul* was isolated from this bird, which had tested positive for *S. mississippi* a week earlier. *Salmonella Saintpaul*, *S. mississippi* and *S. houtenae* had not been isolated previously from takahē during routine disease screening for translocation. The isolation of novel *Salmonella* serovars from the private island takahē population is indicative of the presence of a *Salmonella* reservoir on the private island. Additionally, the isolation of *Salmonella Saintpaul* from the bird after having been moved to Burwood Bush could indicate another source of *Salmonella* at the rearing facility.

Salmonella

Comprised of two species (*Salmonella enterica* and *S. bongori*), *Salmonella* bacteria currently have over 2500 described serovars (Mermin et al. 2004). While some serovars such as *S. typhi* display host specificity, infecting only humans, others are host generalists and are capable of infecting a wide range of species (McClelland et al. 2001). Therefore, *Salmonella* are considered important pathogens for both humans and animals (Woodward et al. 1997; Mermin et al. 2004), with many serovars possessing the propensity for interspecies transmission.

Salmonella transmission most often occurs through the faecal-oral route entering the host via the gastrointestinal tract (Gopinath et al., 2012). Once the gut has been colonized, the bacterium then translocates to the host's systemic tissues and can be shed either persistently or intermittently via the faeces (Gopinath et al. 2012). Salmonellosis is defined by symptoms ranging from gastroenteritis, bacteremia and even death (Acheson & Hohmann, 2001). However, infected hosts may or may not develop clinical disease, with

pathogenesis often reliant on host immunosuppression (Acheson & Hohmann, 2001; Tizard, 2004). Because *Salmonella* does not always eventuate in clinical symptoms, healthy carriers can act as *Salmonella* reservoirs, playing an important role in the transmission of this bacterium (Giovannini *et al.*, 2013).

One of the serovars isolated from takahe on the private island (*Salmonella mississippi*) had previously been isolated from reptiles in New Zealand (Middleton *et al.* 2014) and *Salmonella Saintpaul* is also commonly a reptile-associated serovar (De Hamel & McInnes 1971). Additionally, takahē on the private island had been observed hunting and consuming skinks. Direct contact with infected lizards through consumption or indirect contact with lizard faeces could result in transmission of *Salmonella* to takahē, further indicating that the lizard population on the private island may provide a potential *Salmonella* reservoir.

Salmonella is now known to be a common component of reptile intestinal gut flora (Kingsley *et al.* 2000; Warwick *et al.* 2001) with at least 40% of all *Salmonella* serovars cultured predominantly from reptiles (Mermin *et al.* 2004). Many lizards carrying *Salmonella* remain asymptomatic (Kingsley *et al.* 2000; Onderka & Finlayson 1985), as *Salmonella* is commonly restricted to the gastrointestinal tract of reptiles with no extraintestinal spread (Sheelings *et al.* 2011). However, conditions of physiological stress can cause the bacterium to manifest as clinical disease (Sheelings *et al.* 2011).

Little is known about the pathogenicity of *Salmonella* in free-living New Zealand lizards (Middleton *et al.* 2010). However, surveys of *Salmonella* prevalence in New Zealand lizard populations (Middleton *et al.* 2010; 2014) found no loss of individual body condition associated with *Salmonella* carriage, indicative of an asymptomatic carrier state. The persistence of virulent pathogens in an environment is often attributed to the presence of asymptomatic carriers such as reptiles (McCallum 2012). If a pathogen causes mortality in its target host, it will eventually extinguish itself when the host population falls below a certain level (McCallum 2012). However, a pathogen can persist in an animal community by

utilising healthy carriers as vectors (McCallum 2012; Viggers et al. 1993). The capacity for reptiles to harbour *Salmonella* as part of their intestinal flora without showing signs of clinical disease could increase the likelihood of endemic lizards acting as a competent *Salmonella* reservoir on the private island.

There is limited literature available on the prevalence of *Salmonella* in the wild reptiles of New Zealand, but routine disease screening of lizards for translocation within the Hauraki Gulf, New Zealand revealed faecal excretion of *Salmonella enterica* subspecies IV 40:g,t:- in three skink species (*Oligosoma smithi*, *O. moco* and *O. aeneum*) and one common gecko (*Hoplodactylus maculatus*) (Baling et al. 2013). This was the first record of *Salmonella* subspecies IV in New Zealand reptiles, emphasising the lack of baseline information available on *Salmonella* infection in New Zealand lizards. In addition, a survey carried out by Middleton et al. (2010) examined the prevalence of *Salmonella* in endemic lizards on eight offshore New Zealand islands, finding the mean test prevalence of *Salmonella* in lizard populations on the islands to be between 0-9.9%. A total of 11 *Salmonella* serovars were isolated from lizard populations on five of the eight islands including 29 from sub species I (subsp. *enterica*), and five from subspecies IV (subsp. *houtenae*). This study found that the majority of *Salmonella* was isolated from skinks (family *Scincidae*). Additionally, *Salmonella* was isolated from 6.5% of skinks from the *Oligosoma* genus in a survey of the lizard population on Stephens Island, New Zealand (Middleton et al. 2014). In contrast, two surveys of *Salmonella* prevalence in a population of the endemic tuatara (*Sphenodon punctatus*), found a *Salmonella* prevalence of zero (Gartrell et al. 2007; Middleton et al. 2014).

Similarly to takahē, many endemic New Zealand lizard species are now restricted to predator free offshore islands (Towns & Daugherty 1994). All five islands where takahē populations are managed (Private, Mana, Tiritiri Matangi, Kapiti and Maud Islands) are also residence to free-living endemic lizard populations (New Zealand Herpetological Society, 2014) potentially acting as *Salmonella* reservoirs. In the 2010 Middleton study, lizards were surveyed for faecal excretion of *Salmonella* on two of the islands that are also inhabited by takahē

populations, Mana Island off the west coast of Wellington and Maud Island in the Pelorus Sound. *Salmonella* Mana was isolated from one skink (*Oligosoma nigriplantare*) and one gecko (*Hoplodactylus maculatus*) on Mana Island. The same serovar had been isolated in takahē on Mana Island for the first time in 1998 (ESR 2010). The isolation of a concurrent *Salmonella* serovar in the lizard and takahē populations on Mana Island indicates that further investigation of the potential for lizards to act as *Salmonella* reservoirs in the takahē translocation network is warranted. Conversely, no lizards tested positive for *Salmonella* serovars on Maud Island. However, a maximum possible *Salmonella* prevalence of 22.0% was estimated for Maud Island indicating that *Salmonella* might still occur at a lower prevalence than detectable by sample sizes used in the study (Middleton et al. 2010).

Although the carrier state remains the principal foundation for *Salmonella* transmission between species (Gopee et al. 2006), it can persist in the environment for extended periods of time. While *Salmonella* typically achieves optimal growth at between 35-37° C, serovars have been reported to grow in temperatures as low as 5.5° C (Matches & Liston 1968) allowing it to survive in range of environments and climates. The capacity for *Salmonella* to survive independently of a host allows infectious species of *Salmonella* to further exploit transmission strategies by increasing the likelihood of contact with potential hosts whilst still viable (Holley et al. 2006).

Salmonella has been reported to maintain viability after over 300 days in soil under ideal conditions (Holley et al., 2006) and evidence of *Salmonella* survival in the New Zealand environment is found in the estimate of *Salmonella* Saintpaul prevalence of 8.4% from 50 soil samples taken from Stephens Island (Middleton et al. 2014). Takahē may be particularly vulnerable to contact with environmental sources of *Salmonella*, as they are primarily grass and tussock grazers. In winter months they often rely on underground rhizomes to supplement their diet (Mills et al. 1980), increasing the probability of coming into contact with *Salmonella* residing in the soil.

In addition, long-term survival of *Salmonella* in water sources is well documented (Bjergbaek & Roslev, 2005; Jacobsen & Bech, 2012). *Salmonella* has been shown to survive in pond water for up to 115 days and in animal faeces for 30 months (Johnson-Delaney 1996). Water sources within takahē territories on the private island are most likely used by sympatric wildlife, and faecal contamination of these water sources could result in transmission of *Salmonella* to takahē. Although intensive management of takahē means that water sources are provided and replenished within takahē territories, the private island is only accessible by helicopter, limiting opportunities for cleaning and refilling these water sources.

Salmonella infection has been linked with mortality in several bird species worldwide. Over 68,000 wild bird deaths were attributed to *Salmonella* between 1985 and 2004 in the United States (Hall & Saito 2004), while a seasonal pattern of mass *Salmonella*-induced death is seen annually in the finch population throughout Britain (Robinson et al. 2010). More recently, there have been reports of *Salmonella* related mortality in New Zealand's wild bird community. In 2000 an unusually high number of sparrow (*Passer domesticus*) mortalities occurred throughout the country (Alley et al. 2002), in addition to sporadic mortality in other passerines including the native silver eye (*Zosterops lateralis*) (Alley et al. 2002). Additionally, a 2006 outbreak of *Salmonella* caused mortality in as much as 30% of an island population of hihi (*Notiomystis cincta*), a New Zealand endemic passerine (Ewen et al. 2007). Notably, hihi are highly endangered and limited to small sanctuary populations (Ewen et al. 2007), emphasising the potential risk that *Salmonella* infection poses to threatened bird species such as the takahē.

The infected takahē from the private island exhibited no obvious clinical signs of disease from *Salmonella* infection. However, because the serovars have not previously been isolated from takahē, the ultimate physiological effects of infection are difficult to predict. Additionally, takahē from the private island represent an important source of birds for contribution to the takahē translocation network. The introduction of *Salmonella* infected birds to

immunologically naïve takahē populations through translocation could pose significant health risks to the larger takahē population. Therefore, understanding the dynamics and extent of *Salmonella* infection and transmission within the takahē translocation network is crucial to the management and conservation of takahē.

The aims of this chapter are to:

1. Investigate the prevalence of *Salmonella* in the lizard populations, soil and water sources within takahē territories on the private island, Maud Island and the Burwood Bush takahe rearing facility.
2. Assess whether lizard densities or environmental variables can predict *Salmonella* presence within takahē territories.
3. Determine whether any *Salmonella* serovars found in this study concur with those isolated from takahē.

3.2 Methods

Collection of samples

Lizard samples

All lizards were sampled from within takahē territories on the private island and Maud Island during the Southern hemisphere summer in January 2013 and February 2013 respectively. These territories were delineated with the aid of Department of Conservation staff and island caretakers. I collected cloacal swabs from 50 lizards from the private island and 30 lizards from Maud Island to screen for *Salmonella* species. No lizards were detected for sampling inside quarantine pens at the Burwood Bush takahē rearing facility (Phil Marsh pers. comm., 2014). I primarily sampled lizards caught in pitfall traps (see Chapter Two), however I also sampled hand-caught lizards on Maud Island where pitfall-caught lizard numbers were low. Cloacal swabs were collected using Minitip swabs and stored for transport in individual tubes containing Aimes agar gel with charcoal (Copan Diagnostics Inc., 2175 Sampson Ave, Suite 124, Corona, CA92879, USA). Cloacal swabs were refrigerated at 4°C for a maximum of nine days prior to culture. Lizard density of each site sampled was determined from total captures (Chapter Two) and assigned the value of high (38-41 lizards), medium (17-20 lizards) or low (12-13 lizards).

Environmental samples and variables

I selected sites for environmental sampling within each takahē territory for both their proximity to water sources and takahē feeders. This was to maximise the likelihood of sampling areas where takahē traffic had recently been high and hence, detection of takahē associated *Salmonella* would be enhanced. I categorised each water source frequented by takahē within a given territory as either natural or artificial. Artificial water sources on the islands are rainwater-fed tank and trough systems, which are topped up manually by island staff intermittently. Water sources were classified as natural if they consisted of a stream or pond fed by a natural stream. I collected 500mls of water in sterile

containers directly from the main water source in each territory to culture for *Salmonella* species.

Soil samples were collected from areas directly adjacent to takahē water samples by rubbing a sterile swab over the surface of a 50cm² patch of soil directly underneath each takahē-feeding hopper. Swabs were then transferred directly to 400mls of sterile buffered peptone water for storage and transport. Substrate type was divided into the two main groups present in takahē territories on the private island, “hydric” if the predominate soil in a takahē territory had poor drainage and was saturated with water at the time of sampling, or “non-hydric” if well aerated with relatively good drainage. Water and soil samples were collected in this manner from the six quarantine pens and two brood pens at Burwood Bush takahē rearing unit. Environmental samples were collected on the last day of each trip and refrigerated to minimise sample degradation prior to culture.

Culture

Enteric bacteria typically attain ideal growth when incubated at 37° C but there is the possibility that some reptile associated serovars of *Salmonella* have adapted to the lower body temperature consistent with cold-adapted reptiles (Bronikowski et al. 2001). To allow for the cooler body temperature of lizards, each sample was incubated at 28° C and 37° C.

Cloacal swabs were chopped into 2 ml Phosphate Buffered Saline (PBS) and agitated using a vortex mixer. The PBS solution (500 µL), was then inoculated into 20 ml Buffered peptone water (BPW) and incubated at both 28° C and 37° C for 24 hours. All samples were initially incubated in non-selective pre enrichment media (BPW) to allow revival and proliferation of any bacteria present. Water samples were centrifuged and the resulting pellet re-suspended in 20 ml BPW for incubation at both temperatures, while soil samples were split into two and incubated in their existing BPW.

I then used two selective enrichment broths, Rappaport-Vassiliadis soya peptone (RVS) and Tetrathionate/iodine (TET) for all samples, to improve isolation of potential *Salmonella* species. Pre-enrichment culture was inoculated into the selective enrichment broths and RVS broth samples were then incubated at both 42° C and 28° C for 24 hours while the TET broth samples were incubated at 37° C and 28° C.

After incubation, the RVS and TET were streaked onto selective growth media Xylose Lysine Deoxycholate (XLD) and Brilliant Green Modified (BGM) agar plates for subcultivation. These samples were incubated at 37° C and 28° C for 18-24 hours.

Typical *Salmonella* colonies grow on XLD agar with well-defined black centres from H₂S production and a pale yellow or red transparent halo (Fig. 2). Suspect colonies were identified visually for subculture onto MacConkey agar, and then incubated at their respective temperatures for 18-24 hours.



Figure 2: Examples of typical suspected *Salmonella* colonies selected for subculture. *Salmonella* colonies on XLD typically present with well-defined black centres with transparent red or yellow halos (Photo S. Rose).

Confirmation

Triple Sugar Iron agar (TSI) and Lysine Iron Agar (LIA) slants were inoculated with grey colonies from the MacConkey agar and incubated at their respective temperatures for 18-24 hours. TSI presumptive positive cultures presented with alkaline slopes (red), acid butts (yellow) and with or without H₂S production (blackening). LIA presumptive positive cultures presented with alkaline slopes (purple) and alkaline butts (purple). Any LIA cultures with alkaline butts were considered potential *Salmonella* positive samples regardless of TSI reaction.

An oxidase strip test was then used to establish whether samples were of gram-negative bacteria and hence, likely to be Enterobacteria. A positive control of *Salmonella typhimurium* culture was used to confirm test efficacy. All oxidase negative samples proceeded to biochemical identification.

All presumptive *Salmonella* positive cultures were subject to broad scale serotyping using polyvalent flagella (poly-H) and polyvalent somatic (poly-O) slide agglutination tests to determine presence of *Salmonella*. Distinct clumping or agglutination of either polyvalent antiserum after 60 seconds of the test commencement was considered a positive antigen-antibody interaction. Positive isolates were then subcultured onto blood agar and incubated at their initial culture temperatures for 18-24 hours before further biochemical testing using the RapID ONE® system (Thermo Fisher Scientific, 12076 Santa Fe Drive, Lenexa, KS 14428, USA).

Suspensions were prepared from blood agar colonies and RapID® inoculation fluid and turbidity adjusted to the number two McFarland standard. After inoculation, test strips were incubated at 37° C for four hours before interpretation. *Salmonella* positive samples were subcultured to Dorset egg slopes and incubated at their initial culture temperatures for 18-24 hours. Samples were then sent to Environmental Science and Research in Wallaceville, Upper Hutt for definitive typing.

Statistical analyses

Prevalence

True prevalence of *Salmonella* in the lizard population from the private island was calculated using methods defined in Thrusfield (2005) using values for point and test prevalence. Values for test sensitivity and specificity were obtained from a similar *Salmonella* isolation technique and assumed to be 50% and 98% respectively (Middleton et al. 2010; Bager and Petersen 1991).

Point Prevalence

$$P = \frac{\text{number of individuals having disease at a particular point in time}}{\text{number of individuals at risk of disease at that point in time}}$$

True Prevalence

$$\hat{p} = \frac{p^t + \beta - 1}{\alpha + \beta - 1}$$

where \hat{p} = true prevalence,

p^t = apparent prevalence

β = specificity and

α = sensitivity

Values of sensitivity and specificity were obtained from a similar study by Bager & Petersen (1991) and were assumed to be 50% and 98% respectively.

Test prevalence

$$p^t = (P \times \alpha) + ((1 - P) \times (1 - \beta))$$

Variance

$$\frac{(\rho^t(1 - \rho^t))}{(\eta(\alpha + \beta - 1)^2)}$$

where η = population size estimated from chapter two.

95% Confidence Interval

$$\hat{\rho} \pm 1.96\sqrt{variance}$$

The Detection of Disease programme of online epidemiology software WinEpi (Working in Epidemiology, Facultad de Veterinaria, Universidad de Zaragoza, Spain) was used to estimate prevalence of *Salmonella* in the lizard population on Maud Island. This function allows the estimation of maximum possible prevalence of a pathogen in a population where no positive samples are obtained. For this analysis I used population size estimates from the minimum number alive and weighted means models calculated in chapter two of between 44 and 440 individuals per hectare respectively.

Environmental Variables

Fisher's Exact Test was used to evaluate the relationship between environmental variables and *Salmonella* positive soil, water and lizard samples taken from each takahē territory on the private island. The test included lizard density, substrate type and water source type at each site as explanatory variables and *Salmonella* positive or negative results as the dependent variable (*Table 1*). The analysis was performed in SPSS (SPSS Inc., Chicago IL).

3.3 Results

Salmonella on Southland Island

Of the 50 cloacal swabs taken from the private island lizards, *Salmonella houtenae* ser. 40: g,t:- was isolated from one skink (*Oligosoma notosaurus*) (Fig. 4). Point estimate of the true prevalence of *Salmonella* carriage in the lizard population was calculated to be 2% and true prevalence between 2 and 11.8% (95% C.I.). *Salmonella houtenae* ser. 40: g,t:- was isolated from 25% of water samples, one artificial and the other from a natural stream, while *Salmonella mississippi* was isolated from 50% of soil samples (Fig. 5). Both *Salmonella houtenae* and *S. mississippi* were isolated from takahē territory F water and soil samples respectively (Fig. 6). Fisher's Exact Test showed no significant association between *Salmonella* positive lizard samples and lizard density per takahē territory ($p=0.500$), between *Salmonella* positive soil samples and substrate type within takahē territories ($p=0.786$) or between *Salmonella* positive water samples and water source type found in the takahē territories ($p=0.643$).



Figure 4: *Oligosoma notosaurus* (Southland skink) on the private island. *Salmonella houtenae* was isolated from this species (Photo by Thomas Burns).

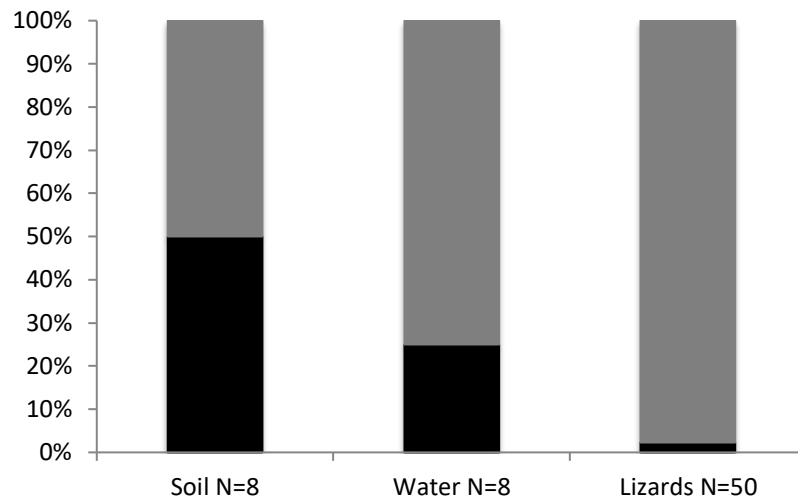


Figure 5: *Salmonella* positive samples from Southland Island. Percentage of samples positive for *Salmonella* isolates indicated in black, negative samples in grey. All positive soil samples contained *S. mississippi*, and positive water and lizard samples contained *S. houtenae*.

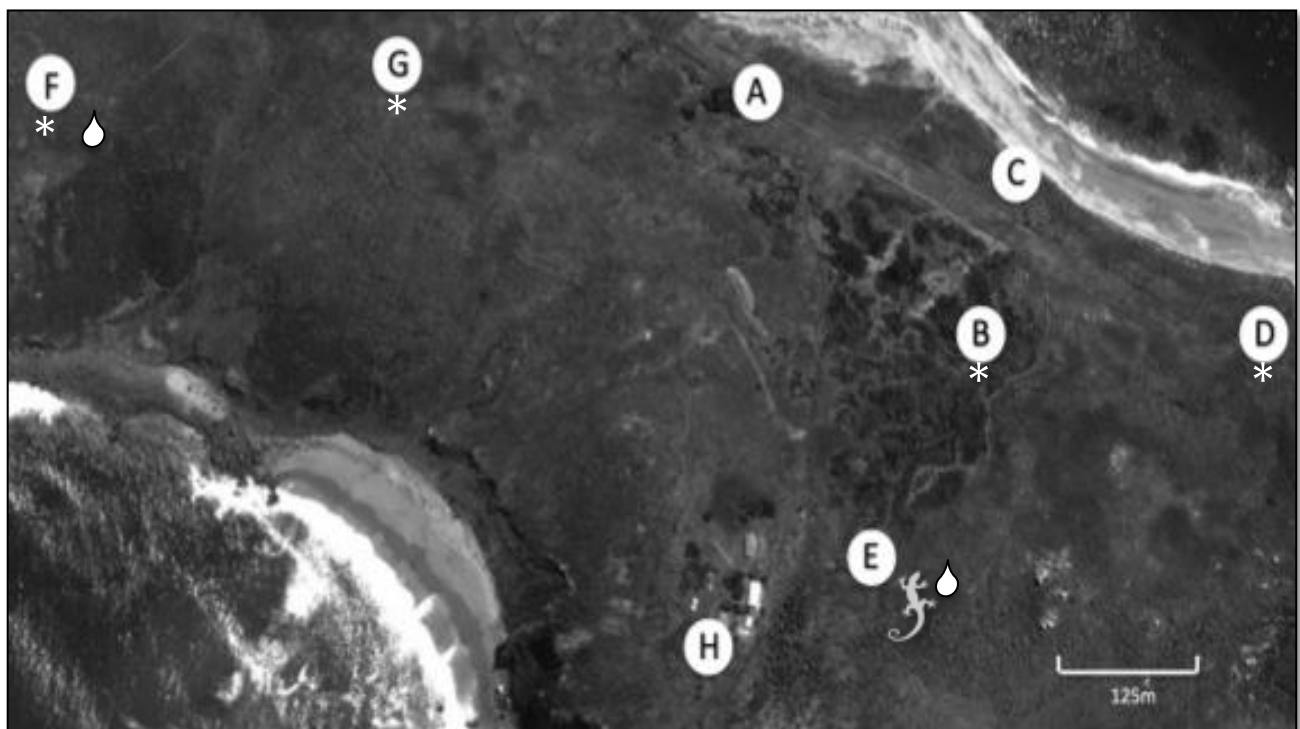


Figure 6: Map of *Salmonella* positive samples on the private island (Google Maps 2014). Site location not disclosed and partial map presented for security reasons. Letters indicate locations of water sources within each territory in addition to location of water and soil samples taken. An asterisk next to a letter indicates *Salmonella* was found in the soil in this territory, a water drop indicates *Salmonella* was found in the water source of the territory and a lizard indicates *Salmonella* in a lizard sampled in the territory.




Site	Substrate	Water Source	Lizard density	<i>Salmonella</i>
A	Non-hydric	artificial	medium	none
B	Hydric	natural	medium	*
C	Non-hydric	natural	medium	none
D	Non-hydric	artificial	high	*
E	Hydric	natural	high	 
F	Non-hydric	artificial	medium	* 
G	Non-hydric	artificial	low	*
H	Non-hydric	artificial	low	none

Table 1: Classification of water and soil types sampled, lizard densities and *Salmonella* found within takahē territories on the private island. An asterisk indicates *S. mississippi* isolated from soil in the respective territory, a water drop indicates *S. houtenae* isolated from the water and a lizard indicates *S. houtenae* isolated from a lizard.

Salmonella on Maud Island and Burwood Bush

The lizard population on Maud Island had a *Salmonella* prevalence of zero. Additionally, all water and soil samples taken from Maud Island tested negative for *Salmonella* isolates. Statistical analyses of Maud Island lizard population estimates using WinEpi produced an estimate of maximum possible prevalence of lizard *Salmonella* carriage of between 6.82% and 9.5% at the 95% confidence level (based on minimum number alive and the highest confidence limit from the weighted means model respectively. Reported in Chapter Two). All environmental samples taken from the Burwood Bush takahē rearing facility tested negative for *Salmonella* isolates.

3.4 Discussion

Salmonella in lizards

Salmonella was isolated from the cloacal swab of an adult *Oligosoma notosaurus* sampled within takahē territory E on the private island. None of the lizards sampled on Maud Island tested positive for *Salmonella* isolates. A true prevalence of *Salmonella* carriage of 7-8.2% was estimated for the lizard population on the private island while a maximum possible prevalence of 5.88-9.49% was calculated for the lizard population on Maud Island.

These results correspond to previous findings where *Salmonella* prevalence of between 0 and 9% was estimated in wild lizard populations on offshore, New Zealand islands (Middleton et al. 2014; Middleton et al. 2010). However, the intermittent excretion of *Salmonella* commonly observed in infected reptiles can result in an underestimate of *Salmonella* prevalence (Scheelings et al. 2011). Intermittent shedding of a bacterium increases the probability of obtaining false negatives and limits the probability of obtaining positive samples from infected animals (Pfleger et al. 2003) suggesting that the *Salmonella* prevalence in New Zealand lizards may approach the high end of estimates.

In addition, a degree of seasonality to the prevalence of *Salmonella* in lizards on the private island is likely with greater prevalence occurring in summer. A significant reduction in *Salmonella* carriage and excretion within free-living reptile populations has been observed in colder months (Kourany & Telford 1981) and this reduction may be attributed in part to colder temperatures creating sub-optimal growth conditions for bacteria. Enteric bacteria such as *Salmonella* achieve optimal growth between 35 and 37° C (Bronikowski et al. 2001) but the body temperature of ectothermic hosts such as lizards can vary significantly depending on environmental temperatures (Werner & Whitaker 1978). For example, body temperatures of gravid *Oligosoma maccani* (McCann's skink) in Otago, New Zealand were found to fluctuate from between 7.2 - 32.7° C depending on environmental temperatures (Hare et al. 2009).

Previous studies (Baling et al. 2013; Middleton et al. 2014; Middleton et al. 2010) provide a clear indication that endemic lizards have the capacity to harbour *Salmonella* in New Zealand environments, however each of these studies were carried out at sites off the coast of the North Island of New Zealand or at the northernmost tip of the South Island. The private island is at a significantly more southern latitude than these sites with mean air temperatures as much as 7° C cooler in winter months (NIWA National Climate Database). Therefore, temperature-dependent bacteria such as *Salmonella* may be subject to differential survival in the private island climate.

The highest mean air temperatures on the private island occur from December to March and temperatures during sampling in this study ranged from 11-14° C (NIWA Climate Database), indicating that *Salmonella* prevalence in the lizard population likely reaches a maximum during this time. However, the mean annual air temperature on the Southland Island for 2014 was 7° C and fell as low as 1° C in winter (NIWA Climate Database). This temperature pattern suggests that lizards on the private island are seldom, if ever able to attain a body temperature high enough for optimal bacterial growth to occur, potentially reducing *Salmonella* prevalence in the lizard population to an undetectable level in colder weather.

Additionally, as with the Middleton et al (2010) survey, *Salmonella* was not isolated from the lizard population on Maud Island. Failure to isolate *Salmonella* serovars from lizards within takahē territories on Maud Island in this study may be indicative of a sample size too small to detect a potentially low *Salmonella* prevalence. Similarly, the Middleton et al. (2010) study concluded that a larger sample size might be required to identify *Salmonella* on Maud Island. Furthermore, the microbiological method used to isolate *Salmonella* is estimated to have a test sensitivity of only 50% (Middleton et al. 2010; Bager & Peterson 1991), which suggests that 50% of individuals carrying *Salmonella* may not be identified using this method.

The serovar isolated from the private island skink, *Salmonella houtenae* (IV) ser. 40: g,t:-, is concurrent with the serovar isolated from two of the eight water sources sampled within takahē territories (E and F) in this study in addition to the serovar isolated from a takahē from the same island in 2012 (Grange *pers. comm.* 2015). *Salmonella houtenae* is typically a reptile-associated serovar (Schröter et al. 2004; Willis et al. 2002) and so this congruity is suggestive of *Salmonella* transmission among lizards, water sources and takahē on the island. However, formal identification of *Salmonella* serovars isolated from takahē, lizards and environmental samples from the Southland Island through whole genome sequencing would confirm sequence similarity of the samples more definitively.

Despite the seemingly low *Salmonella* prevalence in the lizard population, estimates of lizard population numbers within takahē territories on the private island are indicative of as many as one lizard per square metre (Chapter Two). Fewer than twenty adult takahē reside on the private island at any one time (Phil Marsh *pers. comm.* 2014) and although *Salmonella* may not persist in the takahē population alone, a high density of alternative hosts such as lizards may aid in pathogen persistence and interspecific transmission within the system (Begon 2008). In contrast, estimates of the lizard population numbers within takahē territories on Maud Island suggest a much lower density of approximately one lizard per seven-square metres. It is possible that this number is under the required species abundance threshold to enable *Salmonella* species to persist at a detectable level within the lizard population and hence, the risk of transmission to is takahē negligible.

Salmonella in other sympatric animals

Each takahē territory on the private island has a central water source in the form of a natural stream or pond as in territories B, C and E or rain fed water feeders as in territories A, D, F, G and H, which are supplemented by island caretakers intermittently (*Fig 6*). As access to the private island is limited and there are no permanent caretakers in residence, artificial water feeders can evaporate during

drier months eliminating them as potential water sources. This may put takahē at higher risk of disease transmission due to a higher density of sympatric species utilising common water resources during summer months. *Salmonella* contamination of water sources has been shown to increase significantly with the number of animals exploiting them. For example, a survey of *Salmonella* in cattle water troughs found that contamination increased asymptotically as the number of starlings (*Sturnus vulgaris*) using the troughs increased (Carlson et al. 2011). Subsequent control of the starling population resulted in a substantial decline of *Salmonella* in the water troughs (Carlson et al. 2011^b). Additionally, high densities of birds at garden feeders have been implicated in outbreaks of *Salmonella typhimurium* DT40 in the United Kingdom (Carlson et al. 2011). The private island is host to several species of passerines (*Passeriformes*) including starlings and sparrows (*Passer domesticus*), both of which have been implicated in *Salmonella* outbreaks (Carlson et al. 2011; Connolly et al. 2006). It is highly probable that these species also share water sources with takahē, providing another potential source of faecal contamination. *Salmonella* was isolated from the natural stream in territory E on the private island. This stream may be one of the only water sources available to takahē during dry months and any animals utilising it may be at higher risk of *Salmonella* infection. In addition, takahē may be forced to travel through adjacent territories searching for water during drier weather. This behaviour could aid in the intraspecific transmission of *Salmonella* in the takahē population on private island by increasing contact opportunities between individual birds and contamination of water sources and soil from the faeces of already infected takahē.

The *Oligosoma notosaurus* that *Salmonella* was isolated from in this study is from the family *Scincidae*, which was found to have the highest prevalence of *Salmonella* serovars in the Middleton 2010 study. In addition, this study found that lizards sampled from coastal zones had a much higher prevalence (P^T 11.5%) than those caught in bush or open terrain habitats (P^T 2.5-3.3%) (Middleton 2010). Correspondingly, *O. notosaurus* is commonly found in coastal habitats (Chapple 2010) suggesting that ecology and habitat choice of lizard populations may play a role in *Salmonella* prevalence.

Salmonella carriage in lizards and takahē in coastal environments on the private island could be a result of seabirds and marine mammals living in close proximity (Middleton et al. 2010). A survey of sub Antarctic wildlife found a *Salmonella* prevalence of 22% in fur seals (*Arctocephalus forsteri*) and 7% in Gentoo penguins (*Pygoscelis papua*) (Palmgren et al. 2000), and a survey of Antarctic wildlife found *Salmonella* prevalence as high as 8.9% in Antarctic seabird species and 5.6% in Weddel seals (*Leptonychotes weddelli*) (Vigo et al. 2011). Additionally, large congregations of Herring gulls (*Larus argentatus*) have been implicated as vectors of *Salmonella* to sheep and cattle in the United Kingdom (Tizard 2004). Periodic visitation by New Zealand fur seals and seasonal congregations of seabirds are common on the private island and so transmission of *Salmonella* to takahē and lizards could be occurring through faecal contamination of the environment and food sources by these visiting animals.

Until recently, research has focussed on simple host-parasite systems such as single host-single parasite (Collinge & Ray 2006; Ostfeld 2010). In reality many host parasite interactions are far more complex (Holt et al. 2003) and the presence of multiple host species within an ecosystem has the potential to greatly increase pathogen persistence (Begon 2008). In order to persist, a pathogen requires a threshold abundance of individual hosts, but if multiple, susceptible hosts are available in a system, then even if the hosts occur under the required abundance threshold the pathogen can persist through interspecific transmission (Begon 2008; Holt et al. 2003). The presence of the same serovar of *Salmonella* in both takahē and lizards on the private island may be an indication of a complex system involving multiple potential host species including marine mammals, seabirds and passerines, rendering the definitive interpretation of transmission pathways problematic.

Salmonella in soil and water

Salmonella mississippi was isolated from four of the eight soil samples and *Salmonella houtenae* was isolated from two of the eight water samples taken

from takahē territories on the Southland island. There was no significant association found between water source type and *Salmonella* detection in water samples, substrate type and *Salmonella* detection in soil samples or lizard density and *Salmonella* detection in environmental (water or soil) samples taken from the private island. However, more intensive sampling from throughout the takahē territories may be necessary to accurately determine environmental predictors of *Salmonella* occurrence on the private island. Analyses of the correlation between soil type and *Salmonella* prevalence in broiler chicken farms found that a higher proportion of hydric or “waterlogged” soil increased the probability of *Salmonella* detection (Volkova et al. 2009). Several sites on the private island are dominated by hydric soils, potentially increasing survival of *Salmonella* in waterlogged areas.

The presence of *Salmonella houtenae* in water samples taken from the Southland Island provides an indication that takahē water sources might provide a potential *Salmonella* reservoir. Fresh water sources have long been implicated in *Salmonella* transmission and persistence (Baudart et al. 2000). For example, a survey of surface waters in Georgia, United States found that 79% of all samples contained *Salmonella* serovars with significantly higher concentrations of *Salmonella* in addition to significantly higher serovar diversity in summer months (Haley et al. 2009). Additionally, over 40 *Salmonella* serovars were isolated from natural aquatic systems within a Mediterranean coastal watershed (Baudart et al. 2000). Takahē water troughs on the private island were cleaned and refilled just four days prior to sampling. *Salmonella* survival is greatly reduced in clean water (Murray 1991) and it is likely that cleaning the water troughs reduced the amount of bacteria present, subsequently reducing the capability of culture methods to detect *Salmonella*. Artificial water sources on private island are usually cleaned infrequently due to limited access to the island and the subsequent build-up of organic matter may provide a high nutrient content for *Salmonella* survival and growth in the water troughs under normal conditions (Murray 1991). Cleaning the water troughs may have masked a higher prevalence of *Salmonella* in the artificial water sources than what was ultimately detected. In addition, time taken to transport soil and water samples

from the private island, Maud Island and Burwood Bush to the laboratory for culture could have resulted in sample degradation. Transport of soil samples in pre-enrichment media and refrigeration of soil and water samples prior to culture would have helped to negate this effect but it is not known how much, if any, *Salmonella* was lost during this process.

In contrast to the private island, *Salmonella* was not isolated from soil or water samples taken from either Maud Island or the Burwood Bush takahē rearing facility. It is particularly interesting that *Salmonella* was not isolated from environmental samples at Burwood Bush considering it is one of the main translocation hubs in the takahē translocation network (Grange et al. 2014) and frequently houses takahē from several populations including *Salmonella* positive birds from the private island. However, failure to detect *Salmonella* from samples taken from Maud Island and Burwood Bush are not necessarily indicative of zero *Salmonella* prevalence at these sites. As with the lizard surveys in this study, prevalence of *Salmonella* in the soil and water sources at Burwood Bush and Maud Island might occur at such a low frequency that a much larger sample pool than was feasible to process within the context of this study is necessary to detect it. Additionally, Maud Island and the Burwood Bush takahē rearing facility house permanent staff that clean and refill water stations regularly, reducing build-up of organic material and potentially limiting survival of any *Salmonella* present. Free access to permanent water sources on Maud Island and at Burwood Bush may also reduce the need for takahē to cross into neighbouring territories in search of resources, thus reducing the risk of intraspecific transmission of pathogens. Furthermore, takahē feeding stations on Maud Island and at Burwood Bush are moved frequently, reducing concentration of takahē faecal material in any one area. This may hinder bacterial build up in the soil surrounding feeders, reducing *Salmonella* persistence in areas of high takahē traffic.

In addition to regular cleaning and movement of takahē feeders and water troughs, both Maud Island and Burwood Bush have strict quarantine procedures in place for human visitors prior to site access; this might prevent transmission

of pathogens from other animals and sites via contaminated field gear. In contrast, quarantine procedures prior to human access of the private island are limited. Additionally, examination of *Salmonella typhimurium* in the endemic passerine the hihi (*Notiomystis cincta*) on Tiritiri Matangi Island in New Zealand using pulsed field gel electrophoresis found that the serovar isolated from infected birds was virtually indistinguishable from that isolated from a human patient in New Zealand (Ewen et al. 2007). Tiritiri Matangi is an isolated island reserve, preventing movement of hihi to other areas and it receives over 30,000 human visitors each year indicating a possibility that *Salmonella* was introduced to the island via a human carrier. The potential for human introduction of *Salmonella* to the private island cannot be ruled out.

Salmonella infects a wide range of hosts with many serovars transmissible between species, which indicates a lack of specialised host adaptations (Foltz 1969). This lack of host specialisation may provide *Salmonella* bacteria with an increased capacity to survive a lifestyle of continued cycling through animal hosts and the environment (Foltz 1969). *Salmonella* has been reported to survive in soil for up to 332 days (Holley et al. 2006) and its ability to survive in aquatic environments is well documented (Jacobsen & Bech 2012). Although excretion into the environment exposes *Salmonella* to survival challenges such as variable temperature, pH and nutrient availability, *Salmonella* has evolved to cope with these challenges (Leyer & Johnson 1992; Matches & Liston 1968), enabling it to survive independently of animal hosts for extended periods of time thus increasing transmission opportunities via contact with new hosts. This ability of *Salmonella* to persist in the environment independently of a host greatly increases the potential for persistence of environmental reservoirs of this pathogen. Isolation of *Salmonella* from both water and soil samples from takahē territories in this study provides an indication that environmental sources play an important role in *Salmonella* persistence on the Southland Island.

While it is clear that a *Salmonella* reservoir or reservoirs exist on the private island, elucidation of transmission pathways is difficult. Lizards on the private island clearly occur at densities high enough to maintain a *Salmonella* reservoir (See Chapter two) but the presence of multiple other hosts such as passerines, sea birds and marine mammals indicates that several sources might be involved in *Salmonella* transmission to takahē. *Salmonella* serovars isolated from lizard, soil and water samples appear to concur with those isolated from takahē indicating a common source of infection and transmission on the private island. However, further investigation such as comparison of whole genome sequences of isolates is needed to confirm the relationship of these *Salmonella* isolates and clarify *Salmonella* transmission pathways between takahē, lizards and the environment.

***Chapter Four: Lizards play a role in Salmonella
persistence and in transmission to takahé***

4.1 Introduction

New Zealand endemic lizard species have been implicated in the transmission of *Salmonella* to the sympatric takahē population on a private island in the takahē translocation network. Takahē are routinely translocated between several offshore islands for conservation purposes and *Salmonella* infected takahē from the private island may pose a risk to other takahē in the translocation network. I investigated the ecology of lizard species within takahē territories on the private island in addition to Maud Island, which is residence to another important population of takahē. Species, distribution, density, morphology, habitat preference and *Salmonella* prevalence of lizards on the private island and Maud Island were investigated to assess the potential risk of *Salmonella* transmission from lizards to takahē. In addition, I investigated the presence of *Salmonella* in soil and water within takahē territories on the private island and Maud Island, and in quarantine and brood pens at the Burwood Bush takahē rearing facility. Soil and water samples were cultured for *Salmonella* in order to identify potential environmental *Salmonella* reservoirs in takahē territories.

Summary of major findings

Chapter Two

To investigate the ecology of lizards within takahē territories on the private island and Maud Island I carried out mark recapture surveys over five days at each island between January and February 2014. I detected three species of skink during the private island survey, *Oligosoma notosaurus*, *O. nigriplantare polychroma* and *O. stenotis*. Analyses of the lizard population on the private island found that densities are potentially as high as 6,000 lizards per hectare, with *O. notosaurus* accounting for 91.5% of total captures, *O. nigriplantare polychroma* 7.5% and *O. stenotis* 1.1%. No geckos were detected on the private island. The finding of *O. stenotis* on the private island is particularly important as this species was previously thought to be restricted to Stewart Island. I detected three species of lizard during the mark recapture survey on Maud Island, *O. zelandicum*, *O. nigriplantare polychroma* and *Woodworthia maculatus*. *Oligosoma*

zelandicum and *W. maculatus* represented 47.5% of total captures each and *O. nigriplantare polychroma* 5%. Three other lizard species, *Oligosoma infrapunctatum*, *Toropuku stephensi* and *Mokopirirakau granulatus* are present on Maud Island but were not detected during this survey. Estimates of lizard densities within takahē territories were between 466 and 6020 lizards per hectare on the private island and between 118 and 1528 lizards per hectare on Maud Island; the high range of estimates is due to low recapture numbers and the subsequent overestimation of population size by the weighted mean model. These densities provide an indication that lizards could aid in pathogen persistence at these sites and act as a potential disease reservoir for takahē.

Chapter Three

In order to investigate the role of lizards as *Salmonella* reservoirs in the takahē translocation network, cloacal swabs were taken from skinks and geckos found within takahē territories on the private island and Maud Island and cultured for *Salmonella*. Soil and water samples from within takahē territories on the private island and Maud Island were also tested for *Salmonella*, in addition to soil and water samples taken from quarantine and brooding pens at the Burwood Bush takahē rearing unit. *Salmonella houtenae* was isolated from one skink and two of eight water samples, and *S. mississippi* was isolated from four of eight soil samples from the private island. Prevalence of *Salmonella* in the lizard population of the private island was calculated to be between 2 and 11.8%. No *Salmonella* was isolated from Maud Island or Burwood Bush but a maximum possible *Salmonella* prevalence of between 5.9 and 9.5% was calculated for the lizard population on Maud Island. The serovars isolated from lizards, soil and water on the private island are concurrent with those isolated from takahē there. It appears that the private island maintains a persistent *Salmonella* reservoir but it remains unclear what its source might be. It is likely that there is a complex *Salmonella* transmission cycle occurring between takahē, lizards, the environment and other sympatric species such as sea birds and marine mammals on the private island.

4.2 Discussion

The ecology of endemic New Zealand lizards on the private island and Maud Island, and their potential to act as a *Salmonella* reservoir in the takahē translocation network are inextricably linked. For example, *Salmonella* carriage of between 2-11.8% in the lizard population on the private island may sound minimal until combined with lizard density estimates. Even at the lowest density estimate of 466 lizards per hectare, the number of *Salmonella* infected lizards on the private island could be as many as 55 individuals per hectare, and almost 700 infected individuals per hectare if the highest lizard density is considered. If takahē on the private island are regularly supplementing their diets with lizard prey then it is likely that they will eventually consume *Salmonella* infected lizards. *Salmonella* prevalence in New Zealand lizards has been found to be highest in lizards from the family Scincidae (Middleton et al. 2010), the most commonly occurring lizard family on both the private island and Maud Island.

Additionally, habitat preference of lizards within the takahē translocation network might affect *Salmonella* prevalence rates in the lizard population. Lizards with a preference for open habitats might achieve greater body temperatures than those found in shaded habitats, thus providing more optimal temperatures for any concomitant *Salmonella* species to proliferate (Kourany & Telford 1981) and lizard populations in coastal environments might harbour a higher prevalence of *Salmonella* carriage than lizards found inland (Middleton 2010) due to exposure to sea birds and marine mammals which are known *Salmonella* carriers (Palmgren et al. 2000; Tizard 2004). Furthermore, evidence of lizard movement outside of the expected four-metre home range on the private island might provide insight into how *Salmonella* is transmitted to other lizards, the environment or takahē in neighbouring territories. The area of takahē territories is often restricted by the limited habitat available to them on islands where they reside (Baber & Craig 2003). As a result, takahē territories are often overlapping, increasing the risk of lizards and takahē spilling over into neighbouring territories and increasing pathogen transmission.

Alternatively, *Salmonella* infection in lizards and takahē on the private island could originate in other sympatric species. Passerines are ubiquitous on the private island and sea birds and marine mammals are frequent visitors. Takahē and lizards may have direct or indirect contact with sympatric species, thus providing multiple opportunities for pathogen transmission.

Conservation Implications

This study represents the first survey of lizard populations carried out on the private island, and one of few undertaken on Maud Island, highlighting the apparent lack of knowledge regarding lizard ecology in New Zealand. The high density of lizards estimated in this study provide an indication that lizards might play a crucial role in both island's ecosystem functioning. Without fundamental ecological knowledge about New Zealand lizard ecology and their role in the ecological community, it is not known how management actions might affect lizard populations, or what effect changes in lizard populations might have on sympatric flora and fauna. For example, re-vegetation efforts on both the private island and Maud Island could cause range constrictions and reduced carrying capacity of lizard populations with a preference for more open habitat. The resulting decrease in lizard density could reduce or change patterns of pollination and seed dispersal (Traveset & Riera 2005) and diminish an important source of prey for sympatric species (Fox 1977; Van Winkle & Ji 2012). The potential effects of re-vegetation actions on lizard fauna, and carry on effects to sympatric plant and animal species should be considered during vegetation restoration planning stages.

Additionally, information on the species distribution, densities and morphology of lizard communities on Maud Island is severely lacking. Without this basic knowledge, the effects of the recent mouse incursion on the lizard population on Maud Island cannot be fully understood. A group of *Toropuku stephensi* were removed from Maud Island to protect them from the impact of mouse invasion (Chris Birmingham *pers. comm.* 2014). However, without an existing estimate of the *T. stephensi* population size on Maud Island, the benefit of this management

action cannot be fully assessed. Regular monitoring of the lizard population using a variety of trapping methods to target all species should be undertaken on Maud Island in order to better inform management decisions regarding the lizard fauna. In addition to Maud Island, the discovery of *Oligosoma stenotis* on the private island outside of its previously known range further emphasises the dearth of knowledge regarding New Zealand endemic lizard species. Development of a comprehensive New Zealand lizard species inventory including lizard distributions is essential for informing management of species. Without knowledge of where threatened and rare lizard species occur, management decisions regarding their conservation are likely to be ineffective (Hitchmough et al. 2013).

The high densities of lizards on both the private island and Maud Island indicate that lizards could play a vital role in pathogen persistence and transmission in the takahē translocation network. Pathogens such as *Salmonella* could persist in the lizard population when other hosts such as takahē are not abundant or maintain a pathogen during seasons where seabird colonies or marine mammals are absent. Furthermore, understanding the seasonal patterns associated with *Salmonella* prevalence in lizards and the environment within takahē territories on the private island may be crucial in mitigating disease transmission risks to takahē and in timing takahē translocations to and from the island.

Future Directions

Lizards

In order to better understand lizard ecology on the private island and Maud Island a more comprehensive survey of species presence and their distribution is necessary. Trapping using multiple methods may be needed to detect all lizard species present, particularly geckos that can escape the pitfall traps used in this study. Additionally, further analyses of species-specific habitat use will inform pitfall placements to better detect lizard species and improve re-vegetation planning in terms of meeting habitat requirements for all lizard species. Long

term, annual monitoring of lizards using mark recapture methods is needed to assess how re-vegetation on the private island and reforestation and pest control on Maud Island are affecting the distribution and abundance of lizard species residing at these sites.

Salmonella

While it is clear that a *Salmonella* reservoir or reservoirs exist on the private island, elucidation of transmission pathways could not be determined in this study and further research is needed to address this problem. The *Salmonella* serovars isolated from lizard, soil and water samples appear to concur with those isolated from takahē on the private island but more thorough examination of isolates is required to confirm this. Whole genome sequencing of *Salmonella* isolated from lizards, the environment and takahē from the private island are required to assess the degree of relatedness between isolates. Although serotyping used in this study is the accepted method of *Salmonella* serotype identification, it is limited in that it can't identify evolutionary relationships of strains within the same or between different serotypes (Selander et al. 1990). Multi-host pathogens such as *Salmonella* often display a pattern of rapid host adaptations resulting in what are sometimes major genetic changes, confounding elucidation of evolutionary relationships (Beltran et al. 1988). Analysis of whole genome sequences has emerged as a tool to measure relatedness between different strains of the same *Salmonella* serotype (Sukhnanand et al. 2005). Using whole genome sequencing to understand how related *Salmonella* isolates from lizards and the environment in this study are to *Salmonella* isolated from takahē could provide clearer insight into *Salmonella* transmission pathways on the private island. Additionally, methods for detection of *Salmonella* remain imperfect and development of alternative, more effective methods is necessary to advance *Salmonella* detection. The culture method used to detect *Salmonella* in this study has a sensitivity of 50% (Bager & Peterson 1990), indicating that as many as 50% of *Salmonella* positive samples remain undetected.

In addition, further investigation of *Salmonella* prevalence and serovars present in takahē, lizards, other sympatric species and soil and water is required to determine potential *Salmonella* reservoirs and aid in the understanding of disease transmission on the private island. Surveying takahē and sympatric species on the private island for *Salmonella* carriage over different seasons may provide an indication of when *Salmonella* prevalence in the wildlife community is highest, and how it cycles through individuals and the community, therefore informing decisions on timing of takahē translocations to and from the island. In addition, further sampling of lizard populations and the environment is needed to understand the potential prevalence of *Salmonella* on Maud Island and Burwood Bush and associated risks of *Salmonella* transmission to and from takahē at these sites.

Although it is difficult to isolate the sources of *Salmonella* infection, implementation of management actions might mitigate *Salmonella* transmission in takahē from the private island and throughout the wider takahē translocation network. I therefore recommend the following actions, although implementation is entirely subject to resources available to the Department of Conservation takahē management team and caretakers on the private island.

1. More frequent cleaning and filling of artificial water sources on the private island may reduce build up and survival of *Salmonella* whilst reducing the need for takahē to cross territories in search of water. This may limit ingestion of *Salmonella* infected water by takahē and sympatric species and reduce transmission of *Salmonella* among takahē territories.
2. Frequent movement of takahē feeding hoppers to prevent high concentrations of faecal material from building up, limiting the potential for *Salmonella* reservoirs in the soil.
3. Quarantine and treat *Salmonella* infected takahē on the private island prior to moving them through the translocation network to prevent *Salmonella* transmission to other birds and sites. Alternatively, develop a

quarantine unit on the mainland separate to the Burwood Bush facility specifically for private island takahē.

This study provides vital insight into the transmission of *Salmonella* and its maintenance in the takahē translocation network. Findings reported here will inform future decisions regarding takahē translocations and disease screening, and ultimately contribute to the conservation of this threatened species. Furthermore, this study supplies fundamental ecological knowledge about the lizard fauna on the private island and Maud Island and will aid in the preservation and management of New Zealand endemic lizard species.

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