The prevalence of *Salmonella* and the spatial distribution of its serovars amongst New Zealand's native lizards

Danielle Mary Rose Lea Middleton

A thesis presented in partial fulfillment of the requirements for the degree of

Master of Science
in
Zoology

at Massey University, Palmerston North,
New Zealand

2008

Abstract

This thesis considers the prevalence and spatial distribution of *Salmonella* serovars amongst wild endemic lizards on offshore islands around the coast of New Zealand. The mean test prevalence of faecal excretion of *Salmonella* was 4.7%. Skinks (*Scincidae*) were more likely (8.5%) to be carriers of *Salmonella* than geckos (1.6%). Each island was host to between one and three *Salmonella* serovars that were not found on any other islands in this study. Two exceptions were *Salmonella* Bousso and *Salmonella* Mana which were found on two islands within the same geographical area. Based on the findings of this study, different islands are likely to be hosts to different *Salmonella* serovars which could have implications for future translocations of native lizards.

I also assessed the prevalence and spatial distribution of faecal excretion of *Salmonella*, *Aeromonas* and *Hafnia alvei* within Mana Island. The prevalence of *Salmonella* on Mana Island was estimated at 5.8%. *Salmonella* was found predominantly in skinks (10.0%) and less often in geckos (4.1%). *H. alvei* was found at a prevalence of 1.9%. No *Aeromonas* species were cultured from any of the cloacal swabs, suggesting that the 95% confidence interval for the true prevalence is 0-3%. Each site sampled in this study was host to one or more unique serovar of *Salmonella* not found at any of the other sites. The results of this study indicate that *Salmonella* serovars may become established within populations of lizards and is not spread between them. This may be due to a lack of dispersal of lizards between sites, raising important considerations for the translocation of native lizards.

I investigated the prevalence of faecal excretion of *Salmonella*, *H. alvei* and *Aeromonas* by New Zealand native lizards from two captive populations. The mean prevalence of faecal excretion of *Salmonella* in the captive lizards sampled was 11.5%. There was a higher prevalence of *Salmonella* within captive population A (22.0%) than in population B (3.6%). No *Aeromonas* was cultured from any of the lizards. *H. alvei* was found at a prevalence of 5.2%. The prevalence of *Salmonella* and *H. alvei* was significantly higher in captive lizards than in wild populations. Captive lizards may, therefore, not be appropriate founders for new populations of wild lizards.

Finally I assessed the different efficiencies of two media and two temperatures in isolating six *Salmonella* serovars from a reptilian source. All serovars grew equally well at 37°C and 27°C. For most serovars XLD agar was the more successful media than MacConkey agar but the success of different culture media depended on the serovar being cultured. Because lizards are frequently host to a wide range of *Salmonella* serovars, screening samples using multiple microbiological methods is likely to give the best chance of isolating all *Salmonella* serovars present.

Acknowledgements

Within this thesis I wanted to incorporate my passion for conservation, lizards and microbiology. This was to prove to be a daunting task. Not only because much of New Zealand's lizard fauna are maintained on offshore islands where access is restricted and difficult but also due to the multidisciplinary nature of the topic. So whilst it is accepted that a thesis has a single author this project would not have been possible without the help of a great many people who helped in a vast array of ways.

To everyone who has contributed to this thesis by: answering my questions; asking me questions; setting me on the right path and occasionally the wrong path; sitting beside me and understanding; making me laugh (and cry); allowing me into your homes and lives and imparting your wisdom, thank-you. You have all contributed towards making this thesis what it is.

This thesis would not have been possible without the excellent assistance I received from a great many volunteers in the field. Together we have suffered in the heat and shivered till 3am in howling winds and driving rain when all the lizards seemed to be MIA. You all know who you are and thanks for making what could have been some very cold depressing nights more bearable.

A special thank you must go to my family as they have been the longest suffering of anyone involved in this project – which actually began long before I started university. Thank you for all your unconditional love and support; for instilling in me an interest in nature and conservation; for listening to all the ecology related conversations with interest and encouragement. I love you all. My mum requires special mention for her hands on help in this project. Not only was she one of my many field assistants but she has also spent a vast number of hours editing, entering data and offering stylistic advice, however, more important than any of that she has been there with soft words and a cup of tea when I needed it most. Thank you very much to my uncle Darryl for the long hours he has put in to finding missing commas and full stops within this thesis. I know that he gave up many weekends to ensure that this thesis was grammatically correct.

I gratefully acknowledge my supervisors Ed Minot and Brett Gartrell for their friendship; many hours of editing and advice and the invaluable weekly meetings. I also thank Hamish Mack for his help with the microbiological aspects of this project, for helping to make the long hours in the lab more bearable with his entertaining stories and the odd joke, but most of all for not complaining when I filled up all the incubators with my samples.

There are a great number of other people who have assisted me in many ways throughout this journey. The Department of Conservation (DoC) not only provided funding towards this project but also allowed me access to some of the most beautiful and important offshore islands that New Zealand has to offer. I am also very grateful to the Department for allowing me the use of their huts whilst I was on the islands. There are many people within the Department who require special mention but there are just too many of you to name. To everyone who has issued my permits or invited me onto your island homes, a special thank you. I also must thank the numerous Tangata Whenua who are kaitiaki of these islands; Ngati Koata, Ngati Toa, Ngati Hei, Ngati Whanaunga, Ngati Kuia and Te Ati Awa.

Finally, I must thank the Massey University Ecology Group for making life bearable over the last two years. A special thank you also belongs to Joel Bradley who has seen me at my worst but still stood by me and whose art work adorns the beginning of each chapter within this thesis.

This project was funded with a Massey University Masterate Scholarship and several smaller grants from New Zealand Federation of Graduate Women, New Zealand Biosecurity Award, J P Skipworth Scholarship and the Julie Alley Bursary.

This thesis is dedicated to my late grandmother who did not get to see this thesis in its completion, but whose love and support was a driving force behind it. Her spirit lives on through my desire for a sustainable future.

Preface

This thesis has been written as a series of self-contained chapters, which will form the basis of a number of papers to be submitted to peer-reviewed scientific journals. Each chapter is therefore written as a fully referenced self-contained paper, and investigates specific components of the spatial distribution and prevalence of *Salmonella* amongst New Zealand endemic lizards. Because of this, there is some overlap between chapters, but essentially they each provide new information towards different components of the spatial distribution and prevalence of *Salmonella* amongst New Zealand native lizards.

I conducted the fieldwork, statistical analyses and have written each chapter. My supervisors have contributed throughout the thesis with help during the fieldwork, analysis, and write-up stages of the study.

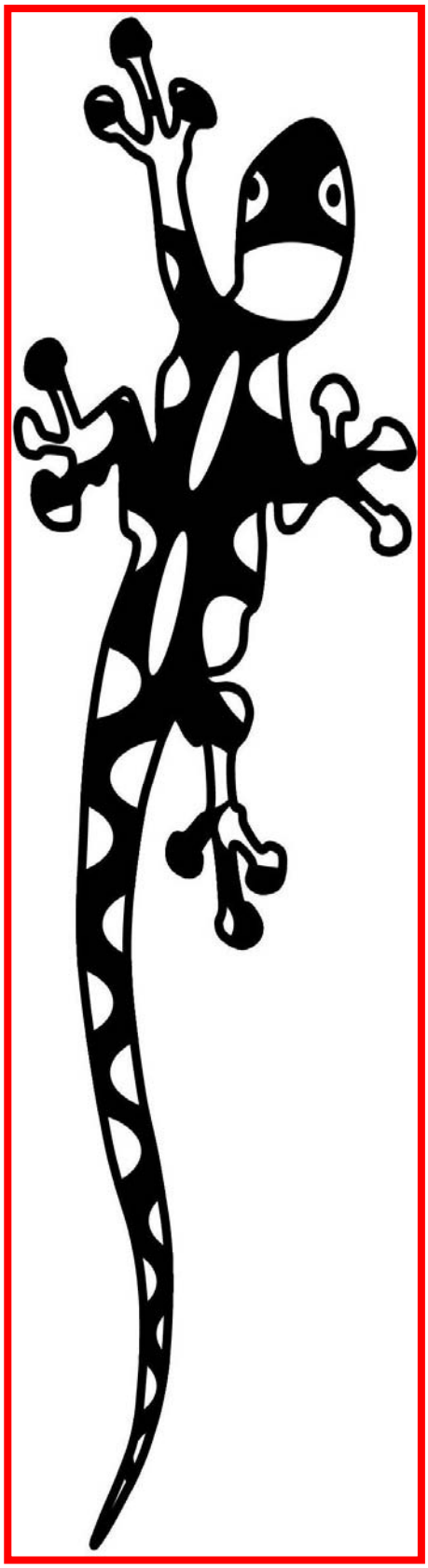
Contents

Chapter Two: *Salmonella* enterica in lizards of New Zealand's offshore islands

Chapter Three: A survey of potential pathogens in New Zealand native lizards from Mana Island, New Zealand

List of Figures

List of Tables



Chapter One:

General Introduction

General Introduction

1.1 Introduction

New Zealand's endemic reptile fauna is the most diverse of any temperate archipelago (Towns and Daugherty 1994). Only one species of lizard has successfully established in New Zealand from overseas; the Australian skink (*Lampropholis delicata*), all other species are endemic to New Zealand. New Zealand reptiles include two species of tuatara (*Sphenodon punctatus* and *Sphenodon guntheri*) and approximately 60 species of lizard (Daugherty et al 1994; Towns et al 2001; Towns and Ferreira 2001; Department of Conservation 2002). All endemic geckos and skinks give birth to live young, except *Oligosoma suteri*, more commonly known as the egg-laying skink (Gill and Whitaker 1996).

Since human colonisation of New Zealand there has been a rapid decline in both the abundance of lizards and the availability of suitable habitat (Daugherty et al 1994; Towns and Daugherty 1994; Towns et al 2001). In 2001 all free-living tuatara and 37% of endemic lizard species were confined to off-shore islands, the remainder are confined to small 'islands' of remnant habitat on the mainland (Towns and Daugherty 1994; Towns et al 2001).

Unfortunately, New Zealand also has more introduced mammalian predators than any other archipelago (Towns et al 1997; Towns et al 2001) and together with habitat loss this is described as being the driving force behind reptilian declines in New Zealand (Towns and Daugherty 1994). It is now considered that one-quarter of geckos and one-half of skinks require urgent conservation action (Towns et al 2001). The main strategy for the recovery of New Zealand reptiles is to establish populations on islands where introduced pests have been eradicated and on which extensive replanting programmes are undertaken in order to restore a suitable habitat (Towns 1999; Towns et al 2001).

Figure 1.1: Maud Island, extensive replanting has been undertaken on this island although its agricultural history is still evident in many places.

Unfortunately, the suitable habitat on these islands often occurs in patches that can only sustain small populations of lizards. They also restrict the ability for dispersal between populations of lizards, resulting in genetic deterioration within the population (Caughley and Gunn 1996). This raises the necessity of translocating lizards between islands or from captive-bred populations back into the wild in order to improve genetic diversity.

The translocation of rare and endangered animals has now become a common and widely accepted conservation technique, especially where the animals have previously been restricted to small fragmented habitats that limit dispersal (Woodford and Rossiter 1994). Throughout this thesis translocation is defined according to the IUCN (1987) definition – "The movement of living organisms from one area with free release in another". In many cases these translocations occur as part of a greater restoration programme aimed at restoring the natural ecosystems of islands and where possible should involve only species thought to have occurred in the area prior to human habitation (IUCN 1987).

The presence and possible spread of disease is an important consideration when translocating any animal to a new area (Woodford and Rossiter 1994). Few studies have been conducted in New Zealand on the prevalence and importance of commonly encountered pathogens in endemic reptiles. Two bacterial pathogens, *Salmonella* and *Aeromonas*, are commonly encountered in reptiles overseas and frequently cause invasive disease. However, no comprehensive study has been conducted on the prevalence of these organisms amongst New Zealand native reptiles. Little is known about the importance of another bacterial pathogen, *Hafnia alvei*, which was recently isolated from tuatara (*Sphenodon punctatus*) on Stephens Island (Gartrell et al 2007).

1.2 Importance of lizard conservation in New Zealand

Biological diversity, or biodiversity, has been defined as the "structural and functional variety of life forms at genetic, population, species, community and ecosystem levels" (Gaston 1998). Biodiversity should be maintained for many reasons, including on commercial, intrinsic and aesthetic grounds.

The extent of the role lizards play in New Zealand's ecosystems is only just beginning to be understood. Recent studies have found lizards are the primary seed dispersers and pollinators of many indigenous plant species (Wotton 2002; Olesen and Valido 2003). The fruit of New Zealand native divaricating shrubs is often located deep within the plant and on the underside of branches, apparently inhibiting access to the fruit by other vertebrate frugivores (Wotton 2002). Wotton (2002) found lizards played an important role in local or short distance seed dispersal of *Coprosma propinqua*.

Lizards are also of considerable cultural importance to the indigenous people of New Zealand. Maori feared lizards, known as ngārara, as they were seen as the earthly representatives of Whiro, the god of darkness, evil and death (Reed 1963a). It was believed that lizards crawled into the mouths of people while they were sleeping and gnawed at their insides, resulting in illness and death (Reed 1963b). *Naultinus elegans* (green gecko), named by the Maori people moko kākāriki, were thought to be the most evil of all the lizards. Reptiles are also represented as kaitiaki (guardians) and are often released near burial sites to watch over the dead (Orbell 1995). Carvings of lizards were

frequently used to guard the entrances of meeting houses (wharenui) to ensure the safety of both the building and those who used it.

1.3 Conservation status and ecology of New Zealand native lizards

Geckos and skinks are renowned for their colonising ability and are generally thought to have arrived in New Zealand by 'rafting' after the split from Gondwanaland 80 million years ago (Patterson 2000). New Zealand is now home to approximately 60 species of lizard (Towns et al 2001; Department of Conservation 2002) and this number continues to increase as further research is conducted in this area (Gill and Whitaker 1996). Of these 60 species many have not yet been formally named and, with one exception, are endemic to New Zealand (Department of Conservation 2002). The current status of lizards in New Zealand is such that around 30 species (50%) are threatened or endangered, a further 43% or 26 species are entirely or predominantly restricted to offshore islands (Daugherty et al 1994) and they all have complete protection under the Wildlife Act (Department of Conservation 2002).

Figure 1.2: Robust skink (*C. alani*) registered as Vu by the IUCN Red List

New Zealand lizards (Lacertilia) fall into four genera *Hoplodactylus* and *Naultinus* from the family *Gekkonidae* (geckos) and *Cyclodina* and *Oligosoma* from the family *Scincidae* (skinks) (Gill and Whitaker 1996; Towns et al 2001). Table 1.1 describes the habitat and distribution of New Zealand geckos (Gill and Whitaker 1996). Geckos belonging to the genus *Hoplodactylus*, comprising nine species, are generally nocturnal and grey-brown in colour (Gill and Whitaker 1996; Department of Conservation 2002). The diurnal green geckos generally belong to the *Naultinus* genus which comprises seven species (Gill and Whitaker 1996; Department of Conservation 2002). The habitat and distribution of New Zealand skinks is summarised in Table 1.2 (Gill and Whitaker 1996; Department of Conservation 2002). Those skinks in the genus *Cyclodina* generally have heavy bodies, short limbs and toes, a scaly lower-eyelid and are predominantly nocturnal (Teal 2006). Diurnal skinks belong to the genus *Oligosoma* and have long slender bodies, long limbs and digits, and a transparent palpebral disc in the centre of the lower eyelid (Teal 2006).

Twenty-one species or sub-species of gecko have been identified by the Department of Conservation as threatened, two of which are ranked as nationally critical. The Department of Conservation has identified that five skink species are in serious decline or worse. Two species of skink, grand skink (*Oligosoma grande*) and Otago skink (*Oligosoma otagense*), are nationally critical (Hitchmough et al 2005). Since European settlement, approximately 200 years ago, it has been suggested that the grand skinks have declined from a continuous distribution throughout Central Otago to just 8% of their former range (Whitaker and Loh 1995).

Table 1.1: Habitat and distribution of *Gekkonidae* species found wild in New Zealand.

Common Name	**Scientific Name**	**Habitat**	**Distribution**
Goldstripe gecko	*Hoplodactylus chrysosireticus*	Taranaki and Mana Island	Forest, scrub, coastal vegetation, farmland and gardens, often in New Zealand flax
Duvaucel's gecko	*Hoplodactylus duvauceli*	Forest, scrub, coastal vegetation and cliffs	Islands off the northeast coast of North Island and in Cook Strait
Forest gecko	*Hoplodactylus granulatus*	Forest and scrub	Mainland New Zealand and few offshore islands
Blackeyed gecko	*Hoplodactylus kahutarae*	Alpine bluffs and rocky outcrops	Marlborough and Nelson
Common gecko	*Hoplodactylus maculatus*	Forest, scrub and grassland	Throughout New Zealand.
Cloudy gecko	*Hoplodactylus nebulosus*	Forest and scrub	Stewart Island and associated islands
Pacific gecko	*Hoplodactylus pacificus*	Forest, scrub and grassland	North Island and most northern offshore islands.
Harlequin gecko	*Hoplodactylus rakiurae*	Wind-swept sub-alpine scrub with granite outcrops	Stewart Island
Striped gecko	*Hoplodactylus stephensi*	Coastal forest and scrub	Stephens and Maud Islands, Marlborough Sounds area
Common green gecko	*Naultinus elegans*	Forest and scrub commonly found in manuka and kanuka	*Punctatus* East Cape, Hawkes Bay and southern North Island. *Elegans*: rest of North Island & Great & Little Barrier Islands
Jewelled gecko	*Naultinus gemmeus*	Forest, scrub and tussock grasslands	Canterbury, Otago, Southland and Stewart Island
Northland green gecko	*Naultinus grayii*	Forest and scrub	Northland north of Whangaroa
Marlborough green gecko	*Naultinus manukanus*	Forest and scrub	Marlborough region including Sounds, Stephens & D'Urville Islands
Rough gecko	*Naultinus rudis*	Forest and scrub	Marlborough and Canterbury
Nelson green gecko	*Naultinus stellatus*	Forest and scrub	Nelson
West Coast green gecko	*Naultinus tuberculatus*	Forest and scrub	Westland

Table 1.2: Habitat and distribution of *Scincidae* species found wild in New Zealand.

Common name	**Scientific name**	**Habitat**	**Distribution**
Copper skink	*Cyclodina aenea*	Forest. Open or covered areas with adequate ground cover. Compost heaps, rock gardens	Widespread throughout North Island and its outliers only
Robust skink	*Cyclodina alani*	Low, coastal forests	Islands off north-eastern North Island
McGregor's skink	*Cyclodina macgregori*	Coastal forest and scrub	Offshore islands in the North Island only.
Marbled skink	*Cyclodina oliveri*	Coastal forest and scrub	Offshore islands: Poor Knights Islands and Alderman Island
Ornate skink	*Cyclodina ornate*	Forest and open areas with rock piles	North Island and its outliers only.
Whitaker's skink	*Cyclodina whitakeri*	Coastal forest and scrub	Islands off the north-eastern coast of North Island
Rainbow skink	*Lampropholis delicata*	Open areas and slightly wooded areas	North Island. Introduced species
Green skink	*Oligosoma chloronoton*	Tussock, grassland, scrub, boulder fields and coastal vegetation	Otago, Southland and Stewart Island
Fiordland skink	*Oligosoma acrinasum*	Rocky shorelines and boulder beaches	Islands off south-west Fiordland.
Three Kings skink	*Oligosoma fallai*	Coastal forest and scrub	Three Kings Islands
Grand skink	*Oligosoma grande*	Tussock grassland with rocky outcrops	Otago
Chevron skink	*Oligosoma homalonotum*	Stream margins in native forests	Great and Little Barrier Islands
Cryptic skink	*Oligosoma inconspicuum*	Herbs and shrub	Otago and Southland
Speckled skink	*Oligosoma infrapunctatum*	Open forest, scrub and tussock	Bay of Plenty to Nelson and Westland. Stephens and Mana Islands
Spotted skink	*Oligosoma lineoocellatum*	Grassy and scrubland areas	Eastern areas from the Hawkes Bay to Wellington. Eastern areas from Nelson to Canterbury and some offshore islands
Long-toed skink	*Oligosoma longipes*	Dry, rocky areas	Marlborough and Canterbury
McCann's skink	*Oligosoma maccanni*	Tussock and scrub	South Island. Canterbury to Southland
Small Scaled skink	*Oligosoma microlepis*	River beds and grassy areas with loose rocks	Taupo to Rangitikei River
Moko skink	*Oligosoma moco*	Open forest and scrub	Auckland, Bay of Plenty and islands off the north-eastern coast of North Island
Common skink	*Oligosoma nigriplantare*	Dry, open areas with low vegetation	*Polychroma*: Southern North Island, South Island and Stewart Island. *Nigriplantare*: Chatham Islands
Southern skink	*Oligosoma notosaurus*	Open, scrub and rocky areas	Stewart and Codfish Islands
Otago skink	*Oligosoma otagense*	Tussock grassland with rocky outcrops	Otago
Shore skink	*Oligosoma smithi*	Near shore line	Northern North Island and offshore islands
Small-eared skink	*Oligosoma stenotis*	Sub-alpine scrub	Stewart Island
Striped skink	*Oligosoma striatum*	Rotting logs in native forest	Central North Island, Great and Little Barrier Islands.
Egg-laying skink	*Oligosoma suteri*	Boulder or shingle beaches	Three Kings to Alderman Islands. Mainland North Island.
Scree skink	*Oligosoma waimatense*	Open rocky areas, steep gravel screes	Marlborough to Otago
Brown skink	*Oligosoma zelandicum*	Forest or shady, moist areas on farms or gardens	West of ranges from Taranaki to Wellington. Marlborough Sounds, Nelson and North Westland

1.4 Human impacts

New Zealand geckos and skinks are almost completely confined to offshore islands and protected areas of the mainland (Towns et al 2001). Their former range, however was certainly much larger - covering most of New Zealand from sea level to approximately 2200m. Both numbers of individuals and habitat availability have declined rapidly and this has been attributed primarily to predation and habitat loss and degradation following human colonisation (Towns et al 2001).

The arrival of Pacific rats (*Rattus exulans*) between 700 and 2000 years ago radically changed New Zealand's ecosystem (Craig et al 2000), sparking a reduction in reptile and other native species' abundance (Towns and Daugherty 1994; Towns et al 2001). With the arrival of Europeans and their mammalian species approximately 200 years ago the lizard population was further decimated (Craig et al 2000). A number of mammalian species were especially detrimental to geckos and skinks in New Zealand (Towns and Daugherty 1994). It is estimated that a single cat (*Felis catus*) can consume more than one thousand skinks annually (Patterson 2000). An excellent example of this was found in 1983 when a feral cat killed in Otago was found to have 14 undigested skinks in its stomach (Patterson 2000). Other important predators to New Zealand lizards include; mustelids (*Mustela furo, M. ermine,* and *M. nivalis*), mice (*Mus musculus*) and rats (*R. exulans, R. rattus,* and *R. norvegicus*) (Towns and Daugherty 1994).

Habitat loss and degradation due to agricultural practices, clearing and burning of forest and grasslands were also significant contributors to the decline of native lizards and still threaten remnant populations on the mainland today (Towns and Daugherty 1994). After human colonisation, fire and felling resulted in a reduction of the native forest in New Zealand from 78% of land area, to an estimated 23% by the 1980s (Clout and Saunders 1996). Remnant patches of suitable lizard habitat are now small and isolated, capable of sustaining only small populations of indigenous animals, raising problems such as demographic stochasticity and genetic deterioration (Caughley and Gunn 1996). The conservation of New Zealand lizards is based on assessing the threats discussed above and implementing strategies aimed at mitigating them. Species recovery plans,

defined by Towns et al (2002) as "a statement of intentions for conservation of particular species for defined periods", have been written for many of New Zealand's endemic lizards. Each of these plans outlines the conservation status, threats and past conservation efforts for the species involved (Towns et al 2002). Often these recovery plans have very little information regarding pathogenic threats to the species.

1.5 Translocation as a conservation tool

Further to the IUCN definition outlined earlier, a translocation is also defined by Griffith et al (1989) as "the intentional release of animals to the wild in an attempt to establish, re-establish, or augment a population". A translocation can of course only be considered a success if it results in a self-sustaining population (Griffith et al 1989), which is undeniably the goal of any translocation project. There are a number of factors that are likely to affect the success of a translocation project. These include; the rate of population increase, the number of founders, competition rates, presence of refugia and high genetic diversity amongst founders (Griffith et al 1989). Translocations of species back into their historical ranges have been demonstrated to be more successful than translocating species outside of their historical range (Griffith et al 1989).

In the past, translocations of terrestrial vertebrates have had limited success, which has brought into question their effectiveness as a conservation strategy (Nelson et al 2002). Historically, translocations have been perceived as isolated management strategies that generally occurred only under crisis conditions (Armstong and McLean 1996). Reptile translocations with extensive documentation and follow up have only recently become a management strategy in New Zealand, enabling a better evaluation of the causes of success or failure (Towns and Ferreira 2001; Nelson et al 2002).

Nearly 260 species re-introductions have occurred between 1960 and 2000 (Craig et al 2000). Compared to overseas re-introduction programmes, the number of founder individuals used in New Zealand is typically lower, while success rates often match or exceed those found overseas (Craig et al 2000). Thus, either New Zealand wildlife may be less susceptible to inbreeding depression, or wildlife managers in New Zealand are selecting destinations in which species will do well (Craig et al 2000). In New Zealand,

most species translocations are to remote mainland sites or islands to which the public are denied access (Craig et al 2000). There are exceptions, such as Tiritiri Matangi Island and Matiu/Somes Island, which are open to the public and are an easy ferry ride from the main centres of Auckland and Wellington respectively (Craig et al 2000).

With one-quarter of geckos and one-half of skinks now considered to require urgent conservation action, the Department of Conservation (DoC) has established management plans for a number of the more at-risk species (Towns et al 2001). The main strategy for the recovery of New Zealand native reptile species is to re-introduce them to islands where introduced pests have been eradicated (Towns et al 2001). There has been a substantial increase in numbers of some species of lizards since the establishment of these management plans. The increase is attributed mainly to captive breeding and translocation to predator-free offshore islands.

Island restorations and translocations serve several functions;

- translocations may be used to increase the genetic heterogeneity of small populations
- establishing small satellite populations may reduce the risk of species loss due to catastrophes or
- translocating species may help them to re-colonise areas that have been restored following human destruction (Towns and Daugherty 1994; Towns et al 2002).

The presence and possible spread of disease is an important consideration when translocating reptiles and all animals should be screened for disease before being introduced to a new population (Woodford and Rossiter 1994). Animals born and bred in a captive breeding facility or captured from a wild population will have acquired local infections and may be symptomless carriers of disease. If these animals are translocated to new areas, they may introduce pathogens to animals existing in the area. This could have a severe impact on the wild and domestic animals found around the release site (Woodford and Rossiter 1994). African horse sickness was introduced into Spain following the introduction of two wild-caught zebra from Namibia in 1987. This had a considerable impact on the horse (*Equus caballus*) population of the area (Woodford and Rossiter 1994). In a reptilian example, captive bred Mojave Desert tortoises (*Xerobates agassizii*) sourced from pet shops in California were released back

into the Mojave Desert. These reptiles are believed to have infected the wild tortoises with a fatal respiratory disease probably acquired in the pet shop (Jacobsen et al 1991). It is not just the possibility that translocated animals will carry diseases endemic to the area in which they were born that is of concern but also they will inevitably lack the acquired immunity to infections present at the release site (Woodford and Rossiter 1994). Arabian oryx (*Oryx leucoryx*) that were bred in captivity in the US succumbed to botulism when they were released in Oman. Botulism is enzootic among sheep and goats of Oman and the Arabian oryx lacked acquired immunity, as they were naïve to botulism (Woodford and Rossiter 1994).

A further concern is that the management of reptiles necessitates direct human contact. Thus, there is the possibility of disease being spread from the reptiles to human workers and vice versa. In the 1930s, muskrats (*Ondatra zibethicus*) were translocated into the Soviet Union for the fur trade. The local water voles (*Arvicola amphibious*) harboured tularaemia as an enzootic disease. Muskrats are highly susceptible to tularaemia and were sharing wetlands with the local water voles. Within the rapidly expanding population of muskrats a massive epizootic of the disease occurred and before long it affected the human muskrat trappers. This previously enzootic disease became epizootic among the naïve and rapidly increasing muskrat population and consequently became a serious health hazard to humans and wild animals in contact with the muskrats (Woodford and Rossiter 1994).

1.6 *Salmonella*

Herpetofauna have often been implicated as transmitters of *Salmonella* and reptile-associated salmonellosis is a significant threat to the health of humans, domestic animals and other wildlife (Mader 1996; Mermin et al 2004). To date, no extensive surveys have been conducted in New Zealand into the prevalence of *Salmonella* amongst endemic lizards.

1.6.1 *Salmonella* characteristics

Salmonella species are Gram-negative rods that belong to the phylum Proteobacteria (Madigan et al 2003; Haraga et al 2008). Proteobacteria are all Gram-negative and represent the majority of Gram-negative bacteria of medical, industrial and agricultural significance (Madigan et al 2003). *Salmonella* species are quite closely related to another genus of bacteria that commonly inhabit the intestinal tract of mammals, *Escherichia*. These genera show 50% homology by DNA:DNA hybridisation (Madigan et al 2003). However, unlike *Escherichia* which frequently inhabits the gastrointestinal tract of warm-blooded animals without resulting in disease, *Salmonella* is usually pathogenic to mammals (Madigan et al 2003). The genus *Salmonella* can be divided into two species, *Salmonella enterica* and *Salmonella bongori* (Centers for Disease Control and Prevention 2007). Members of the species of *Salmonella enterica* are much more common and we will only discuss *Salmonella enterica* throughout this thesis.

Salmonella serotypes have been designated according to the Kauffmann-White scheme which is the nomenclature maintained by the World Health Organisation (WHO) and is utilised by most of the world (Centers for Disease Control and Prevention 2007). Salmonella serotypes can be divided into six subspecies (Table 1.3) which are identified by biochemical and genetic tests (Centers for Disease Control and Prevention 2007). Under the Kauffmann-White scheme, serotypes belonging to subspecies I are named while subspecies II through VI are identified by formula (Centers for Disease Control and Prevention 2007). Some serovars belonging to subspecies II through VI were named prior to 1968, but these names are now obsolete and have been replaced with formulae (Centers for Disease Control and Prevention 2007).

The six subspecies of *Salmonella enterica* can be identified by Roman numerals or names (Table 1.3) (Centers for Disease Control and Prevention 2007). There are some inconsistencies in the Roman numeral numbering of these subspecies. This is due to the fact that subspecies IIIa and IIIb were historically considered a separate genus named *Arizonae* (Centers for Disease Control and Prevention 2007). It should also be noted that no *Salmonella* subspecies V exists. For some time *Salmonella bongori* was thought to be a subspecies of *Salmonella enterica* and hence was named *Salmonella enterica* subspecies V prior to determination of it as a separate species.

Table 1.3: Six subspecies of *Salmonella enterica* can be designated by names or Roman numerals

***Salmonella enterica* subspecies**	
I	*enterica*
II	*salamae*
IIIa	*arizonae*
IIIb	*diarizonae*
IV	*houtenae*
VI	*indica*

More than 2,300 *Salmonella* serovars are known to exist, however only a small proportion of these are regularly isolated from humans or animals (Mermin et al 2004). Typically, identification of *Salmonella* serovars involves a vast array of tests (Madigan et al 2003). Firstly, one must determine the presence of *Salmonella enterica* and identify the subspecies present. This is usually achieved through a variety of biochemical tests (Madigan et al 2003; Centers for Disease Control and Prevention 2007). Immunological characterisation of *Salmonella* is based on three cell surface antigens (Madigan et al 2003). The O somatic (cell wall) antigen; the H (flagella) antigen and the Vi (outer polysaccharide layer) antigen are all examined to determine the serovar of *Salmonella* (Madigan et al 2003). The more than 2,300 distinct serotypes of *Salmonella* each have different antigenic specificities in their O, H and Vi antigens (Madigan et al 2003). Perhaps surprisingly, there is little or no correlation between the antigenic type of *Salmonella* and the disease symptoms. Immunological typing of the bacterium allows tracing of a single strain which may be present in an epidemic (Madigan et al 2003).

All *Salmonella* serovars can be identified using a formula, however, as already mentioned, *Salmonella enterica* subspecies I are identified using a name (Centers for Disease Control and Prevention 2007). The typical format for a *Salmonella* serotype and the protocol followed in this thesis is:

Subspecies O antigens : Phase 1 H antigen : Phase 2 H antigen

e.g. IV 43: z4,z23: -

Salmonella enterica subspecies II, IIIa, IIIb, IV and VI are generally associated with cold-blooded vertebrates, whilst members of subspecies I are most frequently isolated from avian and mammalian hosts (Baumler et al 1998). To date, no comprehensive studies have been conducted on the capability of *Salmonella enterica* subspecies II, IIIa, IIIb, IV and VI to survive in the macrophages of poikilothermic animals (Baumler et al 1998). A number of authors have found that experimental oral exposure of reptiles with subspecies I, II or III results in no clinical signs of disease and no colonisation of organs other than the intestinal tract (Baumler et al 1998). This is in contrast to subspecies I serovars which frequently colonise internal organs and reticuloendothelial cells of their homeothermic hosts (Baumler et al 1998). For this reason it has been speculated that *Salmonella* serotypes evolved in the alimentary canal where they evolved from pathogens to commensal organisms (Baumler et al 1998).

Salmonella serovars have varying host specificities and virulences. The clinical manifestation of salmonellosis depends upon the virulence of the serotype, nature of the lesion and innate immunity of the host (Gopee et al 2000). *Salmonella enterica* serovar Typhi is known only to infect human hosts in contrast to *Salmonella enterica* serovar Typhimurium which can infect a range of hosts including humans, reptiles and mammals. The mechanisms through which *Salmonella* serovars infect their hosts and the reasons for varying host specificities and virulence is explained in detail by Haraga et al (2008). It is thought that differences in receptor sites on the cell walls of varying host species is the cause of varying virulence between hosts (Haraga et al 2008).

Despite the vast amount of ecological diversity seen in these bacteria all serovars are highly environmentally stable (Otokunefor et al 2003). It has been demonstrated that

Salmonella can survive for up to four weeks in tap water and wet sand, six weeks in direct contact with air and up to eight weeks when mixed with dry sand (Otokunefor et al 2003). *Salmonella* has also been isolated from six-month old reptile stool (Mermin et al 2004)

1.6.2 *Salmonella* prevalence and disease in reptiles

Herpetofauna have often been implicated as transmitters of *Salmonella* and there is a large body of literature pertaining to North American herpetofauna (Mader 1996; Mermin et al 2004). However, there is little information in the literature about the prevalence of *Salmonella* in New Zealand reptiles and the vast majority of reptile species have never been sampled. A recent study of tuatara (*Sphenodon punctatus*) from Stephens Island (Takapourewa) found no *Salmonella* in the 100 individuals sampled (Gartrell et al 2007).

Reported prevalence of *Salmonella* carriage in wild reptiles has varied significantly. Richards et al (2004) showed the frequency of isolation from essentially healthy reptiles in Virginia, USA to be 14% and Chambers and Hulse (2006) report a prevalence rate as high as 95%. Other studies have found a zero percent prevalence of *Salmonella* amongst free-living reptiles (Richards et al 2004). Gopee et al (2000) found that squamates had the highest prevalence of *Salmonella* when compared with other reptiles. *Salmonella* is not continually shed from the gastrointestinal tract. It is often shed intermittently as a result of stress on the animal (Richards et al 2004). This raises difficulties with screening of reptiles for *Salmonella* and repeated samples are often required in order to gain a true indication of the prevalence of this organism within a population (Gartrell et al 2007).

Studies overseas have found reptiles kept in captivity have much higher prevalences of *Salmonella* within the population than free-living reptiles (Otokunefor et al 2003; Awad-Masalmeh et al 2005; Ebani et al 2005; Nakadai et al 2005; Pasmans et al 2005). Studies of the intestinal carriage of *Salmonella* amongst captive reared reptiles in the USA found prevalences that ranged from 12% to more than 93% (Johnson-Delaney 1996; Holz and Middleton 2005). Comparisons among reptile owners further emphasises the high potential for the spread of this organism. A study of reptile

breeders in Europe showed that between breeders either no *Salmonella* was found in the collection or nearly all animals in the collection were positive for *Salmonella* (Geue and Loschner 2002). This could be an important consideration for translocations, especially if considering the formation of new wild populations from captive-bred reptiles.

Despite the often high rates of intestinal carriage of *Salmonella* in reptiles, they frequently show no clinical signs of the disease (Johnson-Delaney 1996; Geue and Loschner 2002). *Salmonella* serotypes are found naturally occurring in the gastrointestinal tract of lizards. Approximately 40% of all *Salmonella* serotypes have been cultured primarily from reptiles, but are rarely found in other animals or humans (Mermin et al 2004). *Salmonella* subspecies IIIa and IIIb are the most common subspecies isolated from reptiles (Mitchell 2006). Reptiles are frequently the hosts of several different *Salmonella* serovars simultaneously (Geue and Loschner 2002).

Reptiles may initially become infected with *Salmonella* via faeces which has contaminated food and water, insect vectors or soil that contains the organisms (Mermin et al 2004). Reptiles may also become infected before birth while in the ovary, oviduct or cloaca (Mermin et al 2004; Richards et al 2004). Long periods of stress, such as those which may be experienced in captivity or in transit, may cause asymptomatic carriers to begin to excrete the organism from the gastrointestinal tract (Richards et al 2004). The normal intestinal flora of reptiles and mammals generally inhibits the growth of *Salmonella* by producing volatile organic acids and blocking attachment sites required for *Salmonella* (Haraga et al 2008). Stresses such as water deprivation, changes in diet, antibiotic therapy, transportation and overcrowding can disrupt the normal intestinal flora, therefore allowing *Salmonella* to establish in the gut (Quinn et al 1994). In rare instances where *Salmonella* colonisation results in disease, reptiles may present with depression, anorexia, vomiting, lethargy, wasting, respiratory distress, abortion, nervous signs and sudden death (Twentyman 1999).

It has been suggested that there may be a geographical pattern of *Salmonella* serotypes in the wild (Twentyman 1999). If this suggestion is accurate, then the importance of screening prior to translocations is highlighted. If we neglect to screen reptiles for *Salmonella* prior to translocation, we risk introducing new strains to both reptiles and other native species. If lizard species are kept in close proximity while in captivity, it is

possible that species carrying *Salmonella* serotypes specific to one area may infect individuals previously naïve to that serotype (Twentyman 1999). Translocating these reptiles into the wild will risk further spread of the disease amongst lizards and other endemic fauna.

1.6.3 Disease outbreaks of *Salmonella* in other wildlife

The potential for cross infection from mammals and birds to reptiles and vice versa is high due to the environmental stability of *Salmonella*. In apparently healthy birds and mammals in the USA and Trinidad, the frequency of isolation of *Salmonella* has been found to be approximately 7% (Gopee et al 2000; Richards et al 2004). In New Zealand, sea-birds and house sparrows have been shown to excrete *Salmonella* (Robinson and Daniel 1968; Clark et al 2002; Tizard 2004).

Between 1998 and 2001 a significant outbreak of *Salmonella* Typhimurium DT160 occurred amongst birds and humans in New Zealand. This disease had not been recorded in humans or animals prior to 1998 (Alley et al 2002). The first isolation of *S.* Typhimurium DT160 was a human case occurring in Christchurch in November 1998 and by December 2000 the organism had spread throughout the country causing significant disease and a 20% hospitalisation rate (Alley et al 2002). By 2001, *S.* Typhimurium DT160 was responsible for 34% of all cases of human salmonellosis (Alley et al 2002). During mid 2000, mass mortalities involving several hundred birds were beginning to be noted in Christchurch and the Manawatu (Alley et al 2002). These mortalities predominantly involved house sparrows (*Passer domesticus*) along with small numbers of greenfinches (*Carduelis chloris*), goldfinches (*Carduelis carduelis*), chaffinches (*Fringilla coelebs*) and occasionally blackbirds (*Turdus merula*) and native silvereyes (*Zosterops lateralis*) (Alley et al 2002). Mortalities often occurred in suburban areas and particularly around garden feeding stations (Alley et al 2002). Kaka (*Nestor meridionalis*) were only affected at captive facilities in the North Island where the birds were in contact with sparrows or their droppings (Alley et al 2002).

Salmonellosis has also been identified in hihi (*Notiomystis cincta*) on Tiritiri Matangi (Ewen et al 2007). Whilst only nine bodies were found, it is suspected that the mortality rate could have been as high as 30%. Histopathological investigation of the birds found acute septicaemic lesions typical of *S.* Typhimurium infection and further analyses found it was a new strain, DT195 (Ewen et al 2007). Tiritiri Matangi is an open sanctuary and regularly visited by groups of tourists involved in conservation and management projects. It therefore seems likely that this outbreak in hihi resulted from human introduction of the disease (Ewen et al 2007).

In New Zealand, various *Salmonella* serovars have been isolated from rodents, birds and hedgehogs as well as native and imported reptiles (Clark et al 2002). There is significant evidence for the spread of these serovars between species. Black-backed gulls (*Larus dominicanus vetula*) have been implicated in the spread of *S.* Brandenburg amongst domesticated sheep, resulting in abortions (Clark et al 2002).

Salmonella infections of cattle and sheep have been of concern in New Zealand since 1948 and 1949 when the first cases of infection were reported (Clark et al 2002). Between 1948 and 1957 six *Salmonella* serovars were identified from 20 different species of animal and bird, including livestock, cats, dogs, rabbits and guinea pigs (Clark et al 2002). *S.* Typhimurium was the most common serovar isolated and was found in all 20 animal species (Clark et al 2002). *S.* Typhimurium has also been the cause of sporadic sheep abortions in New Zealand (Clark et al 2002).

The prevalence of *Salmonella* amongst native tuatara (*Sphenodon punctatus*) on Takapourewa (Stephens Island), New Zealand has been investigated and no *Salmonella* was identified in any of the individuals (Gartrell et al 2007). This could be very important if tuatara are naïve to *Salmonella* which is then introduced to the island through lizard translocations.

1.6.4 Reptile-associated salmonellosis in humans

The zoonotic potential of *Salmonella* has been widely investigated (Mader 1996; Otokunefor et al 2003; Mermin et al 2004). While most *Salmonella* infections amongst humans are caused by the consumption of contaminated meat, poultry or eggs, there

have been investigations into infections that have occurred after direct or indirect contact with reptiles (Mermin et al 2004). Forty percent of the greater than 2,300 *Salmonella* serotypes have been cultured predominantly from reptiles. It has been estimated that annually in the US 74,000 cases of *Salmonella* infections are associated with reptiles (Mermin et al 2004). The increasing popularity of maintaining exotic reptiles as pets is likely to have a significant influence on this number (Bauwens et al 2006). Reptile-associated salmonellosis is more likely to involve infants than any other *Salmonella* infection, it is also more likely to be associated with invasive disease and lead to hospitalisation (Mermin et al 2004). Due to the stability of *Salmonella* in the environment, direct contact with reptiles is not required for transmission of the disease. In New Zealand wild geckos and skinks inhabiting the kitchens of homes in Otago resulted in an outbreak of salmonellosis caused by *Salmonella* Saintpaul. A wooden barrier at a Komodo dragon (*Varanus komodoensis*) exhibit was linked to an outbreak of salmonellosis in children, displaying the ease of transmission through an environmental surface (Friedman et al 1998).

In New Zealand, there was an average of 1,706 cases of human salmonellosis reported per year during the six-year period of 1995 – 2001. Of these, 176 cases per year resulted in hospitalisation and for the total six-year period 12 deaths were attributed to salmonellosis (Thornley et al 2002). Salmonellosis in humans generally results in self-limiting diarrhoea. In infants, elderly or immuno-compromised individuals however, serious sequelae may occur, including sepsis, meningitis and death (Mermin et al 2004).

1.6.5 *Salmonella* culture techniques

1.6.5.1 Isolation media

There are two enrichment broths commonly used for *Salmonella* isolation from reptilian sources, Muller-Kaufmann tetrathionate and selenite F (Harvey and Price 1983; Mitchell 2006). The enrichment broth Rappaport is commonly used in the isolation of *Salmonella* from poultry, although previous studies have found that only three of the 11 strains of *Salmonella* in subgenus III (the most common isolates from reptiles) were

subcultured from Rappaport (Vassilliadis 1968). Rappaport is therefore not commonly used to isolate *Salmonella* from a reptilian source.

The recovery of *Salmonella* serotypes can be affected by the type of enrichment broth (Bager and Petersen 1991). For example previous studies have shown that certain *Salmonella* serovars may be inhibited in tetrathionate broth if the inoculum is small (Van Schothorst et al 1977) and selenite F has been indicated as toxic to *Salmonella* Cholerae-suis and other serovars (Smith 1952; Bauwens et al 2006). There has also been the suggestion that certain serovars may be recovered more easily from one enrichment medium rather than another (Harvey and Price 1976). A number of studies have found selenite F broth to be the better enrichment broth for identification of reptile associated *Salmonella* serovars (Harvey and Price 1983; Kodjo et al 1997). In contrast to this however, Koopman and Janssen (1973) found all enrichment media equally likely to isolate *Salmonella* from reptile faeces.

A number of different selective agars are used for *Salmonella* isolation. Mitchell (2006) has found xylose-lysine deoxycholate (XLD) agar to have a high degree of success in the isolation of *Salmonella* from squamates and chelonians.

The variable sensitivities of the numerous microbiological techniques used for the isolation of *Salmonella*, combined with the intermittent faecal shedding of *Salmonella* by symptomless carriers, raises a problem for epidemiological investigations of *Salmonella* in reptiles (Bager and Petersen 1991).

1.6.5.2 Effects of isolation temperature

In recent years, there has been a substantial amount of research into the effect of temperature on the isolation of *Salmonella*. The majority of this research has considered the elevation of temperature from 37°C to 43°C (Van Schothorst et al 1977). Many of these studies have found that there is an increase in the number of *Salmonella* isolations from foods, faeces and feeds when incubation temperature is increased (Harvey and Price 1968; Carlson and Snoeyenbos 1972; Van Schothorst et al 1977). Other studies have found that some *Salmonella* serotypes are not able to reproduce at

43°C (Carlson and Snoeyenbos 1974). Reptilian body temperatures are said to range between 18°C and 32°C (Rossi 2006).

To the best of our knowledge, no studies have considered the effects of *Salmonella* isolation at temperatures closer to the normal reptilian body temperature.

1.7 Hafnia alvei

Hafnia alvei is a potentially zoonotic organism found in mammals, birds, fish, soil, water, sewage and foods, as well as reptiles (Janda and Abbott 2006). Very little is known about *H. alvei* and the role it plays in both human and veterinary disease (Janda and Abbott 2006).

1.7.1 Characteristics of *Hafnia alvei*

Hafnia alvei is a motile, facultatively anaerobic, Gram-negative bacillus (Proietti et al 2004) and is a member of the family Enterobacteriacea (Janda and Abbott 2006). This species occurs ubiquitously in the environment, particularly in soil, sewage and water, but is only rarely considered to be pathogenic (Proietti et al 2004). *H. alvei* was originally assigned to the genus *Enterobacter*, however a DNA hybridisation study found that this organism had only a 20% binding ratio with *Ent. cloacae* (Okada and Gordon 2003). Two genetic clusters of *H. alvei* are known to exist and these clusters differ in their host distribution, degree of genetic diversity and biochemical characteristics (Okada and Gordon 2003).

1.7.2 *Hafnia alvei* disease amongst reptiles

There have been no previous systematic studies into the prevalence of *H. alvei* in reptiles in New Zealand and limited information from overseas; thus we know little about the risk of transmission of *H. alvei* from reptiles to humans and other wildlife. A study of tuatara (*Sphenodon punctatus*) in New Zealand found *H. alvei* at a prevalence of 30% amongst the population on Stephens Island (Gartrell et al 2007). There is very little information available in the literature pertaining to the prevalence of *H. alvei* in

reptiles both in New Zealand and overseas. Okada and Gordon (2003) studied the genetic and ecological structure of *H. alvei* in Australia and found 54 reptiles positive for this organism, including snakes, geckos and skinks. Okada and Gordon (2003) also found that reptiles in Australia were equally likely to be positive for *H. alvei* type 1 or type 2.

1.7.3 *Hafnia alvei* disease amongst other wildlife

The gastrointestinal tracts of mammals appear to be a common ecological niche of hafniae and this does not appear to be a new relationship (Janda and Abbott 2006). In Michigan and Ohio, USA, paleomicrobiological investigations of intestinal mass samples and sediment collected from 12,000-year-old mastodon remains found *H. alvei* within the samples (Janda and Abbott 2006). In a systematic study of 642 Australian mammals, *H. alvei* was found to be the third most common enteric bacteria, following *Escherichia coli* and *Enterobacteria cloacae* (Janda and Abbott 2006).

Hafnia alvei has been isolated from avian species at prevalences of between 3 – 16%. *H. alvei* is most commonly isolated from birds of prey, including turkey vultures (*Cathartes aura*), owls (*Strigiformes* spp) and falcons (*Falconidae* spp) (Janda and Abbott 2006). An outbreak of *H. alvei* occurred amongst laying hens in Spain (Janda and Abbott 2006). Symptoms of this outbreak included loss of appetite, decreased egg productivity, catarrhal enteritis, and fulminant septicaemia (Janda and Abbott 2006). At necropsy, histopathological features included hepatosplenomegaly, multifocal necrotising hepatitis, and splenitis (Janda and Abbott 2006). A further outbreak occurred in 2004 amongst pullets in Italy (Proietti et al 2004), and the results from autopsy were very similar to those identified in the Spanish chickens (Janda and Abbott 2006).

Hafnia alvei has been found to cause haemorrhagic septicaemia in rainbow trout and laying hens, equine abortion, pneumonic infections in goats and mastitis in cows. It has also been isolated from the gastrointestinal tracts of bees and in honey (Janda and Abbott 2006).

1.7.4 Human cases of *Hafnia alvei*

H. alvei has been isolated from many human food sources including cows' milk, honey, goats' cheese and fish (Quinn et al 1994; Janda and Abbott 2006). It is also thought to be ubiquitous in the environment and hence, an opportunistic pathogen (Quinn et al 1994; Mader 1996), although a possible association exists with gastroenteritis, septicaemia and urinary infections in humans (Proietti et al 2004; Janda and Abbott 2006). Hafniae have also been recovered from respiratory secretions - once again these are rarely if ever pathogenic.

Hafnia alvei is unquestionably an opportunistic pathogen in debilitated hosts (Janda et al 2002).

1.8 Aeromonas

Aeromonas is ubiquitous in aquatic habitats and is frequently isolated from and the cause of disease in reptiles overseas, particularly in North America (Cooper and Jackson 1981; Mader 1996).

1.8.1 Characteristics of *Aeromonas*

Members of the species *Aeromonas* are heterotrophic, Gram-negative rods, belonging to the phylum Proteobacteria (Madigan et al 2003). *Aeromonas* species are commonly found in aquatic environments (Mader 1996), including chlorinated drinking water (Glunder and Siegmann 1989). *Aeromonas hydrophila* is the most common of the *Aeromonas* species (Madigan et al 2003).

1.8.2 *Aeromonas* disease in reptiles

Aeromonas is known to cause significant disease in reptiles. Stomatitis (mouth rot) is possibly the best known disease of reptiles and *Aeromonas* is often isolated from the infection site (Cooper and Jackson 1981). This disease generally occurs secondary to stress that may result from overcrowding, poor nutrition or low temperatures.

Aeromonas is also one of the organisms most commonly associated with septicaemic diseases of reptiles (Cooper and Jackson 1981). Septicaemia can result from *Aeromonas* entering a wound or it can follow a localised infection (Cooper and Jackson 1981). As with salmonellosis, certain stressors can result in septicaemia among captive reptiles with no underlying factors (Cooper and Jackson 1981). Also, as with *Salmonella*, *Aeromonas* is often an opportunistic pathogen that is frequently cultured from clinically healthy reptiles (Mader 1996).

Aeromonas has been isolated from many of the largely aquatic reptiles such as Nile crocodiles (*Crocodylus niloticus*) (Turutoglu et al 2005), anacondas (*Euncectes murinus*) (Miller et al 2004) and sea turtles (*Caretta caretta*, *Chelonia myads* and *Dermochelys coriaceae*) (Oros et al 2005). The prevalence of *Aeromonas* amongst Nile crocodiles has been shown to be as high as 90% (Madsen 1996).

1.8.3 Aeromonas in other wildlife

Aeromonas species have been isolated from a range of avian carriers including house sparrows (*Passer domesticus*) and owls (*Tyto alba* and *Asio otus*) (Needham et al 1979; Glunder and Siegmann 1989). Glunder and Siegmann (1989) found that aquatic birds, such as gulls and storks, were more likely to be *Aeromonas* carriers than terrestrial birds. The prevalence rates amongst aquatic and terrestrial birds in this study were found to be 18.5% and 3.4% respectively (Glunder and Siegmann 1989). This is likely to be a reflection of the aquatic nature of this organism. A carnivorous diet also appears to pre-dispose birds to infection by *Aeromonas* (Glunder and Siegmann 1989). This was demonstrated by Shane et al (1984) who found 13% of raptors to be positive for faecal contamination of *Aeromonas* compared to only 2% of non-carnivorous birds.

Aeromonas is thought to be an opportunistic pathogen within avian species and it has been shown that avian species are more likely to be carriers of this organism during the winter months, suggesting some association with seasonal climatic stress factors (Shane et al 1984).

1.8.4 *Aeromonas* in humans

Aeromonas is found as a common bacteria in lakes, ponds and water that reptiles, amphibians and fishes inhabit and infection by *Aeromonas* in humans can be caused by contamination of open wounds from these water sources (Mader 1996). Other potential sources of contamination include meats, fish, raw milk and milk products (Glunder and Siegmann 1989). Human aeromoniasis symptoms include wound infections, septicaemia and self-limiting diarrhoea (Quinn et al 1994). *Aeromonas* is generally considered to be an opportunistic pathogen and therefore is of concern only for people with lowered immunity, the elderly and young children. *Aeromonas* infection rates do appear to be seasonal, with peak infection rates occurring in summer (Glunder and Siegmann 1989). This may be a reflection of the ecological requirements of *Aeromonas* or the behavioural patterns of humans, which sees them frequenting rivers and lakes, where they are most likely to be in contact with this organism, more often during the summer months.

1.8.5 Microbiological isolation of *Aeromonas*

A number of researchers suggest the use of blood agar in the isolation of *Aeromonas* (Glunder and Siegmann 1989). Mishra et al (1987) found that at least three selective media were required in order to obtain 100% detection of *Aeromonas*. The use of non-selective media enables the growth of species which may be sensitive to some selective agents (Glunder and Siegmann 1989).

Further research is needed into the prevalence of *Salmonella*, *H. alvei* and *Aeromonas* amongst herpetofauna due to their possible effect on humans and other wild animals. It is also important that we have at least a basic knowledge of the distribution of these diseases in order to minimise the risks to humans, reptiles and other wildlife involved in the translocation process.

1.9 Study sites

The study sites used in this research (Table 1.4) can be divided into two broad groups: islands which have had lizard translocations in the past and islands upon which the lizard fauna are endemic and have not been re-introduced from elsewhere.

Table 1.4: Islands on which lizards were sampled for *Salmonella* in this study

Islands with a history of lizard translocations		**Islands without a history of lizard translocations**	
Island	Location	Island	Location
Korapuki Island	Coromandel	Cuvier Island	Coromandel
Mana Island	Wellington	Little Barrier Island	Hauraki Gulf
Matiu/Somes Island	Wellington	Stephens Island	Marlborough Sounds
Maud Island	Marlborough Sounds		
Motuopao Island	Northland		

1.9.1 Korapuki Island

Korapuki Island (36°38′S, 175°52′E) is an 18-hectare crown-owned nature reserve in the Mercury Islands group (Towns 2002). It is a highly modified island, once inhabited by Pacific rats (*Rattus exulans*) and rabbits (*Oryctolagus cuniculus*) (Towns 2002). In 1986 a programme of poisoning was begun to remove the rats from the island. This was followed by an eradication of the rabbits (by shooting) in 1987 (Towns 1994). Fires approximately fifty years ago decimated large portions of the island's natural vegetation and this was further influenced by rabbit browsing (Towns 1994). Consequently, the islands vegetation is composed largely of regenerating pohutukawa (*Metrosideros excelsa*) over a subcanopy of mahoe (*Melicytus ramiflorus*) (Towns 1994). The island is now moderately inhabited by sea-birds and their burrows. There are currently nine species of lizard on Korapuki Island (Table 1.5) (Towns 2002). Four of these lizard species were re-introduced from Green Island and Middle Island (Towns and Ferreira 2001). Tuatara are absent from Korapuki Island at present, however skeletal remains indicate they were once present on the island (Towns 1994).

1.9.2 Mana Island

Mana Island (41°6′S,174°48′E) is a 217-ha scientific reserve that lies 2.5km from the mainland of the North Island at its closest point (Timmins et al 1987; Miskelly 1999; Wotton 2002; Miskelly and Taylor 2004). Mana Island's classification as a scientific reserve allows for visitors and during summer months day visitors are very common on the island (Miskelly 1999; Miskelly and Taylor 2004).

There has been a history of Maori and European occupation of Mana Island (Timmins et al 1987; Miskelly 1999). From 1832 to 1986 the island was farmed, resulting in extensive modification of the landscape (Timmins et al 1987; Wotton 2002; Miskelly and Taylor 2004). As a result, the indigenous vegetation was confined almost entirely to the cliffs and a small catchment on the flats (Miskelly 1999). An extensive replanting programme has been undertaken as part of the island restoration plan (Wotton 2002; Miskelly and Taylor 2004).

Aside from stock animals kept on the island, mice were the only mammals to inhabit the island and these were removed in 1989 (Newman 1994; Miskelly and Taylor 2004). Mana Island is currently home to 10 species of lizard, including a number of reintroduced species (Table 1.5) (Miskelly 1999; Armstong 2008).

1.9.3 Matiu/Somes Island

Matiu/Somes Island (41°16′S, 174°52′E) is unique amongst New Zealand's offshore islands (Watts and Gibbs 2002). It is a 25-hectare scientific and historic reserve owned by the Crown (Bull 2000; Watts and Gibbs 2002). Unlike many of New Zealand's offshore islands, Matiu/Somes has hundreds of summer visitors arriving via the hourly ferry from Wellington. This island has had a chequered history. From the early 1870s until 1920 Matiu/Somes was used as a human quarantine station (McGeorge 2004). Also, during World War I and most of World War II Matiu/Somes was used as an internment camp for suspected alien enemies thought to pose a security risk (McGeorge 2004). The island was also used as an outpost during this time for defending

Wellington Harbour from potential invasion. The gun emplacements still remain on the island today (McGeorge 2004).

The original vegetation on the island was cleared before 1870, and from 1889 to 1995 the island was primarily a pastural ecosystem due to the island's role as an agricultural quarantine station run by the Ministry of Fisheries and Agriculture (Bull 2000; Watts and Gibbs 2002). A few patches of remnant bush remain, but these are isolated to areas on or near the coastal cliffs and shoreline (Watts and Gibbs 2002).

Ship rats and other pests were removed from the island in the late 1980s (McGeorge 2004). Nowadays the only remaining mammals on the island are a few sheep used to maintain the pasture at low levels. Consequently, the lizard population has flourished and there are now seven species of lizard inhabiting the island (Table 1.5). Forest geckos (*H. granulatus)* and ornate skinks (*C. ornata*) were re-introduced to the island as part of the restoration plan (Armstong 2008).

1.9.4 Maud Island (Te Hoiere)

Located in Marlborough's Pelorus Sound, Maud Island (40°59′S, 174°03′E) covers approximately 309 hectares and is a designated scientific reserve (Bell et al 2004). Over 100 years of farming has left Maud Island highly modified and only a small 16-hectare remnant of native bush remains. This remnant was fenced off in 1965 to exclude stock and became the only remaining source of the Maud Island frog (*Leiopelma pakeka*). Many lizards also took refuge in this section of bush (Bell et al 2004; McGeorge 2004). Maud Island was privately owned for many years but in 1975 it was bought by the Crown and since then an extensive replanting and restoration programme has been undertaken (Bell et al 2004; McGeorge 2004). Whilst this island is essentially free from predators, its close proximity to the mainland (900 metres) makes stoats an ongoing problem. Trapping is continually conducted on the island and the coastal mainland to minimise the likelihood of re-invasions. A small flock of sheep are the only introduced mammals that currently inhabit the island.

Maud Island is now home to five species of lizard including the speckled skink (*O. infrapunctatum*), which is surviving well on the island following the introduction of 40 individuals from Stephens Island in 2005 (Cash, Bill. pers, comm.) (Table 1.5)

1.9.5 Motuopao Island

Motuopao Island (34°28′S, 172°38′E) sits just 200m to the north of Cape Maria Van Diemen and was home to the first lighthouse of the far north from 1879-1941. This 30-hectare island is now registered as a scientific reserve and has become home to many native birds and reptiles since the removal of rats in 1990 (Department of Conservation 2008)

Five species of lizard currently inhabit Motuopao Island, including the Matapia Island gecko (*Hoplodactylus* sp.) and the robust skink, which were both introduced to the island in 1997 from Matapia Island (Table 1.5) (Armstong 2008).

1.9.6 Cuvier Island (Repanga)

The 481-ha Cuvier Island (36°26′S, 175°46′E) was named after the French naturalist Baron Cuvier by the French navigator D'Urville. Maori people are thought to have occupied Cuvier Island in the early 19th Century. Cuvier Island became a lighthouse reserve during the late 1880s and about a quarter of the island was turned into farm land. Cattle, sheep and wandering goats were introduced in the late 1800s. By the late 1950s the island's natural vegetation had been decimated to form open park land, saddleback, red-crowned kakariki, pied tit and tui were all killed by cats and kiore, and tuatara were reduced to just seven individuals (Merton 1972).

The restoration of Cuvier Island began with the eradication of goats in 1961. Feral cats were removed three years later. In 1970, domestic cats were banned from the island and finally in 1993 Pacific rats were eradicated. The island is now a scientific reserve and the vegetation is recovering well together with the natural fauna (Department of Conservation 1996). Tuatara have recovered well and there are self-sustaining populations of five species of reptile (Armstong 2008) (Table 1.6).

1.9.7 Little Barrier Island (Hauturu)

Little Barrier Island (36°12′S, 175°08′E) was New Zealand's first Nature Reserve, established in 1894 (Galbreath 1993). It is also one of New Zealand's largest offshore islands, covering approximately 2,817-hectares. Cats and kiore were the only mammalian predators known to inhabit the island and these were removed in 1981 and 2004 respectively (Galbreath 1993; Little Barrier Island Supporters' Trust 2008). Little Barrier is now home to twelve species of lizard (Towns, D.R. pers. comm) (Table 1.6).

1.9.8 Stephens Island (Takapourewa)

Located at the northern tip of the Marlborough Sounds in the Cook Strait, Stephens Island (40°35'S 173°55'E) is a 150-hectare nature reserve (East et al 1995; Hare and Cree 2005). Stephens Island was once covered in dense native forest, however the arrival of the lighthouse keepers and their families in 1892 is thought to have resulted in a loss of approximately 90% of the native forest cover (East et al 1995).

Famous for the somewhat exaggerated tale of the lighthouse keeper's cat, which was reputed to be responsible for the extinction of the Stephens Island wren (*Xenicus lyalli*), cats were the only mammalian predator to have inhabited the island (Hare and Cree 2005). Goats and sheep were common on the island during its inhabitation by the lighthouse keepers and their families (East et al 1995; Hare and Cree 2005). This resulted in further decimation of the lizard habitat (Hare and Cree 2005). Stephens Island is now home to seven species of lizard (Armstong 2008)(Table 1.6)

Table 1.5: The distribution of lizards on New Zealand's offshore islands and the origin and date the founder population was translocated.

	Korapuki (18- ha)	**Mana (217-ha)**	**Matiu/Somes (25-ha)**	**Maud (320-ha)**	**Motuopao (30-ha)**
Sphenodon	*s*		G		
Geckos					
H. chrysosireticus		+			
H. duvaucelii	+	North Brother 1997			
H. granulatus			Captivity 2005		
H. maculatus	+	+	+	+	
N. elegans		Wellington 1998/99	+		
H. stephensi				+	
Hoplodactylus sp*					Matapia 1997
Skinks					
O. smithi	+				+
O. moco	+				+
C. aenea	+	+	+		+
O. suteri	Green 1992				
C. ornata			Wellington 2006		
C. oliveri	Green 1992-93				
C. whitakeri	Middle 1988-90				
C. macgregori		+			
O. zelandicum		+		+	
O. nigriplantare		+	+	+	
O. infrapunctatum		Stephens		Stephens 2005	
O. lineoocellatum		Matiu/Somes 1998	+		
C. alani	Green 1992-93				Matapia 1997

s, subfossil remains only (Towns 2002); +, present on the island; G, *S. guntheri*; *, Matapia Island gecko

Table 1.6: Presence of lizards on New Zealand offshore islands included in this study that do not have a history of lizard translocations.

	Cuvier (170-ha)	**Little Barrier (2817-ha)**	**Stephens (150-ha)**
Sphenodon	+P	+P	+P
Geckos			
H. duvauceli		+	
H. granulatus		+	
H. maculatus	+	+	+
H. pacificus	+	+	
H. stephensi			+
N. elegans		+	
N. manukanus			+
Skinks			
C. aenea		+	
C. oliveri		+	
C. ornata		+	
O. homalonotum		+	
O. infrapunctatum			+
O. lineoocellatum			+
O. moco	+	+	
O. nigriplantare			+
O. smithi	+	+	
O. striatum		+	
O. suteri	+		
O. zelandicum			+

+, present on the island; P, *Sphenodon punctatus*

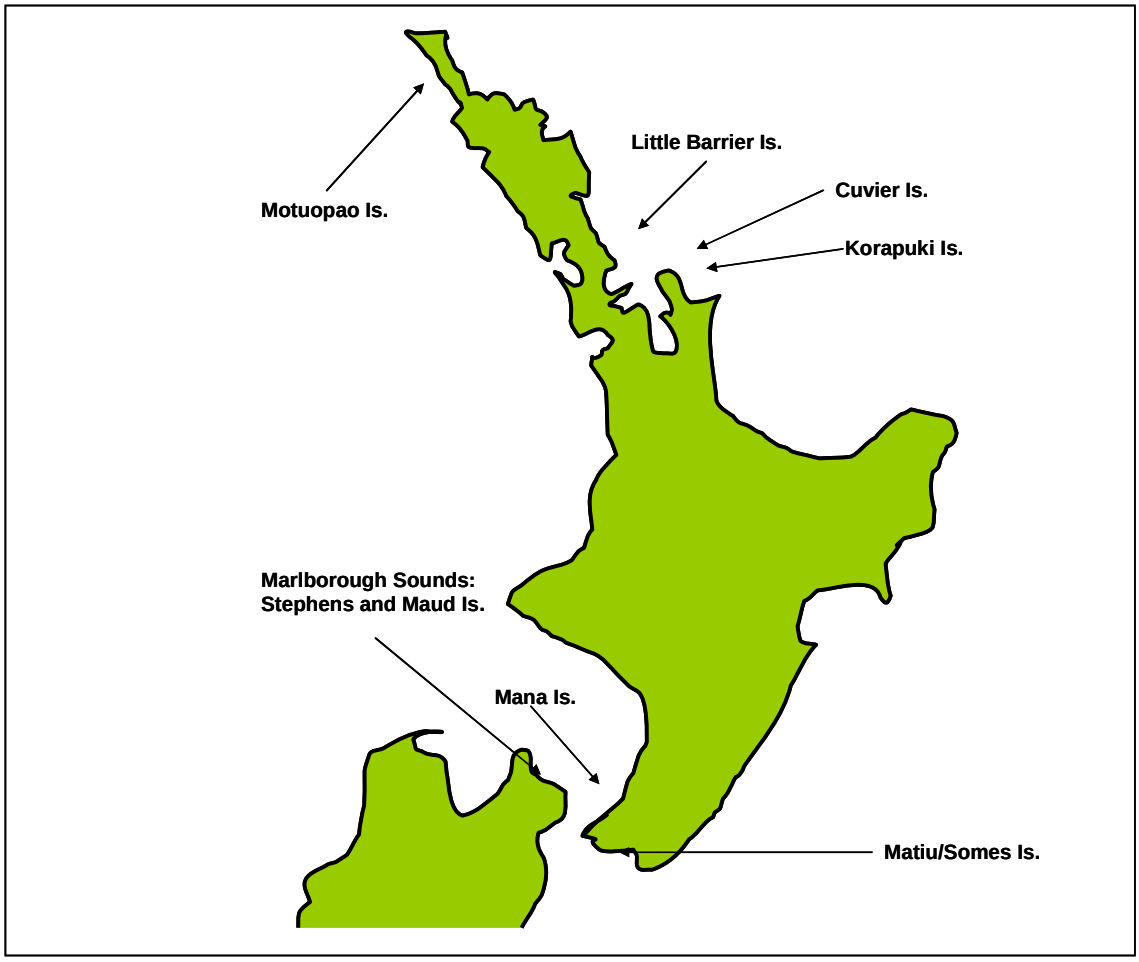


Figure 1.3: North Island and upper South Island showing location of islands surveyed in this study

1.10 Thesis aims and organisation

Management strategies set in place to sustain populations of endemic New Zealand lizards should be constantly evaluated. Before any truly effective conservation strategies can be carried out, information is required on the incidence and significance of disease amongst species. This study examines whether common bacterial pathogens known to occur in reptiles are found within New Zealand endemic reptiles and whether there is a geographical distribution of these organisms. In New Zealand, the translocation of lizards is often used as a conservation technique to enhance existing populations of lizards or to start new populations. At present little is known about the distribution of common diseases amongst endemic New Zealand lizards, so we are unable to predict the risks, if any, involved in this translocation procedure.

Chapter Two describes the prevalence and distribution of *Salmonella* serovars on eight of New Zealand's offshore islands using presence-absence data. Analysis considers the effects of species, sex, age and location on the prevalence of *Salmonella*. As all populations on these islands are isolated, this information will provide an understanding of *Salmonella* distribution that should be used in management decisions for endemic lizard conservation in the future and should result in a modification of the translocation guidelines for these species.

In Chapter Three, I look at the prevalence of three common pathogenic bacteria of reptiles amongst lizards on Mana Island, New Zealand. These pathogens are *Salmonella, Hafnia alvei and Aeromonas.* Once again we considered the effect of variables such as age, sex, location and species on the detection probability of these organisms. Information obtained in this study provides a general idea of the importance of these bacteria amongst New Zealand lizards and should be used by wildlife managers in the future to determine the health risks to a lizard during translocation.

Chapter Four describes the prevalence of *Salmonella, H. alvei and Aeromonas* amongst the lizards held by two captive breeders in the North Island of New Zealand. The prevalence found in these captive lizards is compared with those found in the wild. This comparison helps to assess the risks involved in translocating reptiles from captive

facilities. Captive reptiles may have an increased likelihood of contracting disease, both from their human handlers and domesticated animals.

Chapter Five compares microbiological isolation techniques of five *Salmonella* serovars, commonly found amongst lizards in this study, to determine whether microbiological isolation is better achieved by inoculation of selenite F broth plated onto xylose lysine decarboxylase (XLD) agar or inoculation of Muller Kauffmann tetrathionate broth plated onto MacConkey agar. I also analysed the effects of temperature on the isolation of five *Salmonella* serovars through isolation at both 37°C and 27°C. I envision that this analysis will improve knowledge of the best microbiological techniques for isolation of certain reptile-associated *Salmonella* serovars.

Chapter 6 outlines a summary of the research and recommendations for future research. I also provide a summary of conservation management strategies based on minimising the risk of transferring serovars of *Salmonella* and other diseases between islands and populations of lizard.

References

Alley MR, Connolly JH, Fenwick SG, Mackereth GF, Leyland MJ, Rogers LE, Haycock M, Nicol C, Reed CEM. An epidemic of salmonellosis caused by *Salmonella* Typhimurium DT160 in wild birds and humans in New Zealand. *New Zealand Veterinary Journal* 50, 170-6, 2002

Armstong DP, McLean LG. New Zealand translocations: theory and practice. *Pacific Conservation Biology* 2, 39-54, 1996

Armstong DP. 3 Jan. 2008. Reintroduction projects in New Zealand. http://www.massey.ac.nz/~darmstro/nz_projects.htm. 12 Feb 2008

Awad-Masalmeh M, Romanik G, Herkal C. The occurrence of salmonellae in reptiles kept privately or in zoos in Austria. *Tierarztliche Umschau* 60, 70-4, 2005

Bager F, Petersen J. Sensitivity and specificity of different methods for the isolation of *Salmonella* from pigs. *Acta Veterinaria Scandinavica* 32, 473-81, 1991

Baumler AJ, Tsolis RM, Ficht TA, Adams LG. Evolution of host adaptation in *Salmonella enterica*. *Infection and Immunity* 66, 4579-87, 1998

Bauwens L, Vercammen F, Bertrand S, Collard JM, De Ceuster S. Isolation of *Salmonella* from environmental samples collected in the reptile department of Antwerp Zoo using different selective methods. *Journal of Applied Bacteriology* 101, 284-9, 2006

Bell BD, Pledger S, Dewhurst PL. The fate of a population of the endemic frog *Leiopelma pakeka* (*Anura*: *Leiopelmatidae*) translocated to restored habitat on Maud Island, New Zealand. *New Zealand Journal of Zoology* 31, 123-31, 2004

Bull L. Fidelity and breeding success of the blue penguin *Eudyptula minor* on Matiu-Somes Island, Wellington, New Zealand. *New Zealand Journal of Zoology* 27, 291-8, 2000

Carlson VL, Snoeyenbos GH. Relationship of population kinetics of *Salmonella* Typhimurium and cultural methodology. *American Journal of Veterinary Research* 33, 177-84, 1972

Carlson VL, Snoeyenbos GH. Comparitive efficacies of selenite and tetrathionate enrichment broths for isolation of *Salmonella* serotypes. *American Journal of Veterinary Research* 35, 711-8, 1974

Caughley G, Gunn A. Treatment of declines. In: *Conservation Biology in Theory and Practice*. Pp 273-308. Blackwell Science, Cambridge, 1996

Centers for Disease Control and Prevention. *Salmonella* surveillance: Annual summary, 2005. US Department of Health and Human Services, CDC, Atlanta, Georgia, 2007

Chambers DL, Hulse AC. *Salmonella* serovars in the Herpetofauna of Indiana County Pennsylvania. *Applied and Environmental Microbiology* 72, 3771-3, 2006

Clark RG, Robinson RA, Alley MR, Nicol CM, Hathaway SC, Marchant RM. *Salmonella* in animals in New Zealand: the past to the future. *New Zealand Veterinary Journal* 50, 57-60, 2002

Clout MN, Saunders AJ. Conservation and ecological restoration in New Zealand. *Pacific Conservation Biology* 2, 91-8, 1996

Cooper JE, Jackson OF. Diseases of the Reptilia, Academic Press Inc, London, 1981

Craig J, Anderson S, Clout M, Creese B, Mitchell N, Ogden J, Roberts M, Ussher G. Conservation issues in New Zealand. *Annual Review of Ecology and Systematics* 31, 61-78, 2000

Daugherty CH, Patterson GB, Hitchmough RA. Taxonomic and conservation review of the New Zealand herpetofauna. *New Zealand Journal of Zoology* 21, 317-23, 1994

Department of Conservation. The Penguin guide to New Zealand wildlife: native and introduced birds, mammals, reptiles and amphibians, Penguin Books (NZ) Ltd, Auckland, 2002

Department of Conservation. Motuopao Lighthouse. http://www.doc.govt.nz. 13 March 2008.

East KT, East MR, Daugherty CH. Ecological restoration and habitat relationships of reptiles on Stephens Island, New Zealand. *New Zealand Journal of Zoology* 22, 249-61, 1995

Ebani VV, Cerri D, Fratini F, Meille N, Valentini P, Andreani E. *Salmonella* enterica isolates from faeces of domestic reptiles and a study of their antimicrobial in vitro sensitivity. *Research in Veterinary Science* 78, 117-21, 2005

Ewen JG, Thorogood R, Nicol C, Armstrong DP, Alley M. *Salmonella* Typhimurium in hihi, New Zealand. *Emerging Infectious Diseases* 13, 788-90, 2007

Friedman CR, Torigian C, Shillam PJ, Hoffman RE, Heltzel D, Beebe JL, Malcolm G, DeWitt WE, Hutwagner L, Griffin PM. An outbreak of salmonellosis among children attending a reptile exhibit at a zoo. *Journal of Pediatrics* 132, 802-7, 1998

Galbreath RA. Working for Wildlife: a history of the New Zealand Wildlife Service, Bridget Williams Books and Historical Branch, Dept. of Internal Affairs, Wellington, NZ, 1993

Gartrell BD, Youl JM, King CM, Bolotovski I, McDonald WL, Nelson NJ. Failure to detect *Salmonella* species in a population of wild tuatara (*Sphenodon punctatus*). *New Zealand Veterinary Journal* 55, 134-6, 2007

Gaston KJ. Biodiversity. In: Sutherland WJ (ed) *Conservation science and action*. Pp 1-19. Blackwell Science Ltd, Victoria, Australia, 1998

Geue L, Loschner U. *Salmonella enterica* in reptiles of German and Austrian origin. *Veterinary Microbiology* 84, 79-91, 2002

Gill B, Whitaker T. New Zealand Frogs and Reptiles, David Bateman Limited, Auckland, 1996

Glunder G, Siegmann O. Occurrence of *Aeromonas hydrophila* in wild birds. *Avian Pathology* 18, 685-95, 1989

Gopee NV, Adesiyun AA, Caesar K. Retrospective and longitudinal study of salmonellosis in captive wildlife in Trinidad. *Journal of Wildlife Diseases* 36, 284-93, 2000

Griffith B, Scott MJ, Carpenter JW, Reed C. Translocation as a species conservation tool: Status and strategy. *Science* 245, 477-80, 1989

Haraga A, Ohlson MB, Miller SI. Salmonellae interplay with host cells. *Nature Reviews Microbiology* 6, 53-66, 2008

Hare KM, Cree A. Natural history of *Hoplodactylus stephensi* (Reptilia: Gekkonidae) on Stephens Island, Cook Strait, New Zealand. *New Zealand Journal of Ecology* 29, 137-42, 2005

Harvey RWS, Price TH. Elevated temperature incubation of enrichment media for isolation of *Salmonellas* from heavily contaminated materials. *Journal of Hygiene Cambridge* 66, 377, 1968

Harvey RWS, Price TH. Isolation of *Salmonellas* from sewage-polluted river water using selenite F and Muller-Kauffmann tetrathionate. *Journal of Hygiene Cambridge* 77, 333, 1976

Harvey RWS, Price TH. *Salmonella* isolation from reptilian faeces: A discussion of appropriate cultural techniques. *Journal of Hygiene Cambridge* 91, 25-32, 1983

Hitchmough RA, Bull L, Cromarty PL. New Zealand Threat Classification Systems Lists. Science and Technical Publishings, Department of Conservation, Wellington, 2005

Holz PH, Middleton DR. The effect of feeding a prebiotic on *Salmonella* excretion in carpet pythons, *Morelia spilota*, and scrub pythons, *Morelia amethystine*. *Journal of Herpetological Medicine and Surgery* 15, 4-6, 2005

IUCN. The IUCN position statement on translocation of living organisms. IUCN, Gland, Switzerland, 1987

Jacobsen ER, Gaskin JM, Brown MB, Harris RK, Gardiner CH, Lapointe JL, Adams HP, Reggiardo C. Chronic upper respiratory-tract disease of free-ranging desert tortoises (*Xerobates agassizii*). *Journal of Wildlife Diseases* 27, 296-316, 1991

Janda JM, Abbott SL, Khashe S, Probert W. Phenotypic and genotypic properties of the genus *Hafnia*. *Journal of Medical Microbiology* 51, 575-80, 2002

Janda JM, Abbott SL. The Genus *Hafnia*: from Soup to Nuts. *Clinical Microbiological Reviews* 19, 12-8, 2006

Johnson-Delaney CA. Reptile zoonoses and threats to public health. In: Mader DR (ed) *Reptile Medicine and Surgery*. Pp 20-32. WB Saunders, Philadelphia, USA, 1996

Kodjo A, Villard L, Prave M, Ray S, Grezel D, Lacheretz A, Bonneau M, Richard Y. Isolation and identification of *Salmonella* species from chelonians using combined selective media, serotyping and ribotyping. *Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health* 44, 625-9, 1997

Koopman JP, Janssen FGJ. Occurrence of *Salmonellas* and lactose-negative Arizonas in reptiles in Netherlands, and a comparison of three enrichment methods used in their isolation. *Journal of Hygiene* 71, 363-71, 1973

Little Barrier Island Supporters' Trust. Hauturu: Little Barrier Island. www.littlebarrierisland.org.nz, Auckland, NZ, 2008

Mader DR. Reptile Medicine and Surgery, W.B. Saunders Company, Philadelphia, 1996

Madigan MT, Martinko JM, Parker J. Prokaryotic diversity: Bacteria. In: Snavely SL (ed) *Brock Biology of Microorganisms*. Pp 354-78. Pearson Education, Inc, New Jersey, 2003

Madsen M. Prevalence and serovar distribution of *Salmonella* in fresh and frozen meat from captive Nile crocodiles (*Crocodylus niloticus*). *International Journal of Food Microbiology* 29, 111-8, 1996

McGeorge P. New Zealand's Islands, David Bateman Ltd, Auckland, NZ, 2004

Mermin J, Hutwagner L, Vugia D, Shallow S, Daily P, Bender J, Koehler J, Marcus R, Angulo FJ. Reptiles, amphibians, and human *Salmonella* infection: A population-based, case-control study. *Clinical Infectious Diseases* 38, S253-S61, 2004

Merton DV. Cuvier - an island restored. *Forest and Bird* 184, 7-9, 1972

Miller DL, Radi ZA, Stiver SL, Thornhill TD. Cutaneous and pulmonary mycosis in green anacondas (*Euncectes murinus*). *Journal of Zoo and Wildlife Medicine* 35, 557-61, 2004

Mishra S, Nair GB, Bhadra RK, Sikder SN, Pal SC. Comparison of selective media for primary isolation of *Aeromonas* species from human and animal feces. *Journal of Clinical Microbiology* 25, 2040-3, 1987

Miskelly C. Mana Island Ecological Restoration Plan. Department of Conservation, Wellington, 1999

Miskelly CM, Taylor GA. Establishment of a colony of Common Diving Petrels (*Pelecanoides urinatrix*) by chick transfers and acoustic attraction. *Emu* 104, 205-11, 2004

Mitchell MA. *Salmonella*: Diagnostic methods for reptiles. In: Mader DR (ed) *Reptile Medicine and Surgery*. Pp 900-1. Saunders Elsevier, Missouri, USA, 2006

Nakadai A, Kuroki T, Kato Y, Suzuki R, Yamai S, Yaginuma C, Shiotani R, Yamanouchi A, Hayashidani H. Prevalence of *Salmonella* spp. in pet reptiles in Japan. *Journal of Veterinary Medical Science* 67, 97-101, 2005

Needham JR, Kirkwood JK, Cooper JE. A survey of aerobic bacteria in droppings of captive birds of prey. *Research in Veterinary Science* 27, 125-6, 1979

Nelson NJ, Keall SN, Brown D, Daugherty CH. Establishing a new wild population of tuatara (*Sphenodon guntheri*). *Conservation Biology* 16, 887-94, 2002

Newman DG. Effects of a mouse, *Mus musculus*, eradication program and habitat change on lizard populations of Mana Island, New Zealand, with special reference to McGregor skink, *Cyclodina macgregori*. *New Zealand Journal of Zoology* 21, 443-56, 1994

Okada S, Gordon DM. Genetic and ecological structure of *Hafnia alvei* in Australia. *Systematic and Applied Microbiology* 26, 585-94, 2003

Olesen JM, Valido A. Lizards as pollinators and seed dispersers: an island phenomenon. *Trends in Ecology and Evolution* 18, 177-81, 2003

Orbell M. The illustrated encyclopedia of Maori myth and legend, Canterbury University Press, Christchurch, NZ, 1995

Oros J, Torrent A, Calabuig P, Deniz S. Diseases and causes of mortality among sea turtles stranded in the Canary Islands, Spain (1998-2001). *Diseases of Aquatic Organisms* 63, 13-24, 2005

Otokunefor TV, Kindzeka BI, Ibiteye IO, Osuji GU, Obi FO, Jack AWK. *Salmonella* in gut and droppings of three pest lizards in Nigeria. *World Journal of Microbiology & Biotechnology* 19, 545-8, 2003

Pasmans F, Martel A, Boyen F, Vandekerchove D, Wybo I, Van Immerseel F, Heyndrickx M, Collard JM, Ducatelle R, Haesebrouck F. Characterization of *Salmonella* isolates from captive lizards. *Veterinary Microbiology* 110, 285-91, 2005

Patterson GB. Skinks on the edge. *New Zealand Geographic* 47, 60-81, 2000

Proietti PC, Passamonti F, Franciosini MP, Asdrubali G. *Hafnia alvei* infection in pullets in Italy. *Avian Pathology* 33, 200-4, 2004

Quinn PJ, Carter ME, Markey B, Carter GR. Clinical Veterinary Microbiology, Wolfe Publishing, London, 1994

Reed AW. Treasury of Maori folklore, C. Tinling and Co. Ltd, London, UK, 1963a

Reed AW. An illustrated encyclopedia of Maori life, Literary Productions Ltd, Auckland, NZ, 1963b

Richards JM, Brown JD, Kelly TR, Fountain AL, Sleeman JM. Absence of detectable *Salmonella* cloacal shedding in free-living reptiles on admission to the Wildlife Center of Virginia. *Journal of Zoo and Wildlife Medicine* 35, 562-3, 2004

Robinson RA, Daniel MJ. The significance of *Salmonella* isolates from wild birds and rats in New Zealand. *New Zealand Veterinary Journal* 16, 53-5, 1968

Rossi JV. General husbandry and management. In: Mader DR (ed) *Reptile Medicine and Surgery*. Saunders Elsevier, Missouri, USA, 2006

Shane SM, Harrington KS, Montrose MS, Roebuck RG. The occurrence of *Aeromonas* hydrophila in avian diagnostic submissions. *Avian diseases* 28, 804-7, 1984

Smith HW. The evaluation of culture media for the isolation of salmonellae from faeces. *Journal of Hygiene* 50, 21-36, 1952

Teal R. The future of indigenous fauna on private land: a case study of the habitat use of the small-scaled skink (*Oligosoma microlepis*). Massey University, Palmerston North, New Zealand, 2006

Thornley C, Baker M, Nicol C. The rising incidence of *Salmonella* infection in New Zealand 1995-2001. *New Zealand Public Health Report* 9, 25-8, 2002

Timmins SM, Atkinson IAE, Ogle CC. Conservation opportunities on a highly modified island: Mana Island, Wellington, New Zealand. *New Zealand Journal of Ecology* 10, 57-65, 1987

Tizard I. Salmonellosis in wild birds. *Seminars in Avian and Exotic Pet Medicine* 13, 11-3, 2004

Towns DR. The role of ecological restoration in the conservation of Whitaker's skink (*Cyclodina whitakeri*), a rare New Zealand lizard (Lacertilia: Scincidae). *New Zealand Journal of Zoology* 21, 457-71, 1994

Towns DR, Daugherty CH. Patterns of range contractions and extinctions in the New Zealand herpetofauna following human colonisation. *New Zealand Journal of Zoology* 21, 325-39, 1994

Towns DR, Simberloff D, Atkinson IAE. Restoration of New Zealand islands: redressing the effects of introduced species. *Pacific Conservation Biology* 3, 99-124, 1997

Towns DR. *Cyclodina* spp. recovery plan. Department of Conservation, Wellington, New Zealand, 1999

Towns DR, Daugherty CH, Cree A. Raising the prospects for a forgotten fauna: a review of 10 years of conservation effort for New Zealand reptiles. *Biological Conservation* 99, 3-16, 2001

Towns DR, Ferreira SM. Conservation of New Zealand lizards (Lacertilia : Scincidae) by translocation of small populations. *Biological Conservation* 98, 211-22, 2001

Towns DR. Korapuki Island as a case study for restoration of insular ecosystems in New Zealand. *Journal of Biogeography* 29, 593-607, 2002

Towns DR, Neilson KA, Whitaker AH. North Island *Oligosoma* spp. skink recovery plan 2000-2012, Department of Conservation, Wellington, 2002

Turutoglu H, Ercelik S, Corlu M. *Aeromonas* hydrophila-associated skin lesions and septicaemia in a Nile crocodile (*Crocodylus niloticus*). *Journal of the South African Veterinary Association-Tydskrif Van Die Suid-Afrikaanse Veterinere Vereniging* 76, 40-2, 2005

Twentyman C. Diseases in New Zealand reptiles. *Surveillance* 26, 3-5, 1999

Van Schothorst M, Van Leusden FM, Jeunink J, deDreu J. Studies on the multiplication of *Salmonella* in various enrichment media at different incubation temperatures. *Journal of Applied Bacteriology* 42, 157-63, 1977

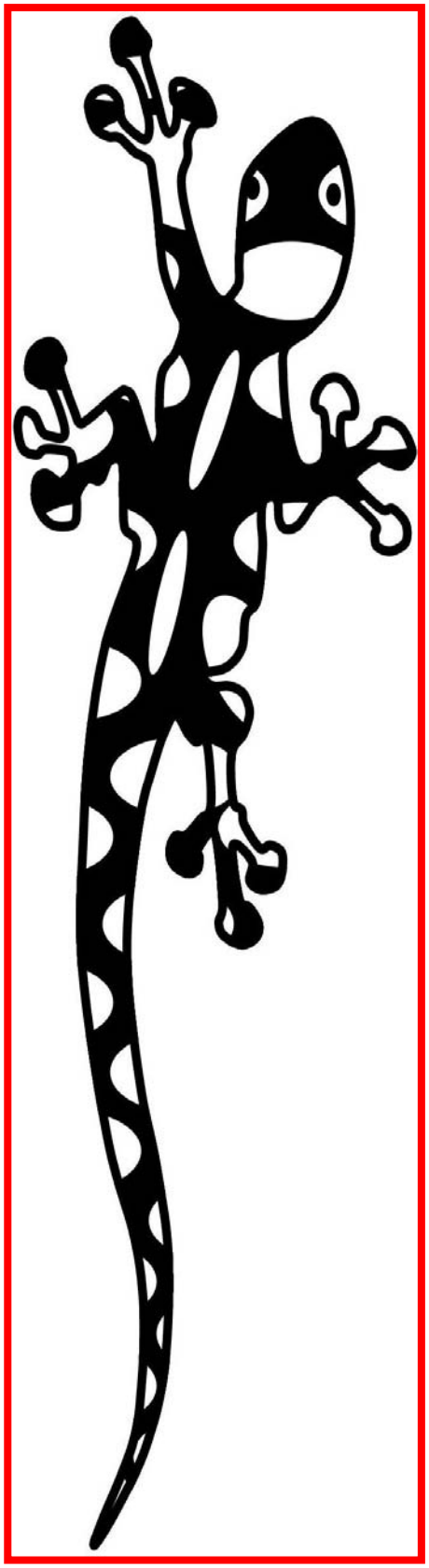
Vassilliadis P. *Shigella, Salmonella* cholerae-suis and arizona in Rappaport's medium. *Journal of Applied Bacteriology* 31, 1968

Watts CH, Gibbs GW. Revegetation and its effects on the ground-dwelling beetle fauna of Matiu/Somes Island, New Zealand. *Restoration Ecology* 10, 96-106, 2002

Whitaker AH, Loh G. Otago skink and Grand skink recovery plan (*Leiolopisma otagense* and *L.grande*). New Zealand Department of Conservation, Wellington, New Zealand, 1995

Woodford MH, Rossiter PB. Disease risks associated with wildlife translocation projects. In: Olney PJS, Mace GM, Feistner ATC (eds). *Creative Conservation: Interactive management of wild and captive animals*. Pp 179-99. Chapman and Hall, London, 1994

Wotton DM. Effectiveness of the common gecko (*Hoplodactylus maculatus*) as a seed disperser on Mana Island, New Zealand. *New Zealand Journal of Botany* 40, 639-47, 2002



Chapter Two:

Salmonella enterica in lizards of New Zealand's offshore islands

Salmonella enterica in lizards of New Zealand's offshore islands

Abstract

This study aimed to: assess the prevalence of faecal excretion of *Salmonella* by wild endemic lizards on offshore islands around the coast of New Zealand; determine whether there is a geographical distribution of *Salmonella* serovars amongst New Zealand native lizards and whether this distribution is affected by previous translocations. Cloacal swabs were obtained from 703 lizards on eight islands off the coast of New Zealand and these were cultured specifically for *Salmonella* using four aerobic enrichment and culture methods at two incubation temperatures. The distribution of *Salmonella* serovars found on these islands was compared, to determine whether a spatial distribution of *Salmonella* serovars exists amongst endemic New Zealand lizards. Faecal excretion of *Salmonella* was not found in all wild lizard populations sampled. The mean test prevalence of faecal excretion of *Salmonella* in all the lizards sampled was 4.7%. Skinks (*Scincidae*) were more likely (8.5%) to be carriers of *Salmonella enterica* than geckos (1.6%). Islands which were host to *Salmonella* had between one and three *Salmonella* serotypes that were not found on any other islands in this study. Two exceptions were *Salmonella* Bousso and *Salmonella* Mana which were found on two islands within the same geographical area. Infection with *Salmonella* serotypes found in this study did not have an adverse effect on body condition. The spatial distribution of *Salmonella* serotypes found across these eight islands is an important consideration for translocations. The lizards that were positive for *Salmonella* excretion showed no clinical signs of the disease, suggesting that they were asymptomatic carriers of the organism. Translocated reptiles should continue to be screened for *Salmonella* in order to prevent infecting wild populations.

Keywords: Bacteria, disease prevalence, lizard, reptile, *Salmonella*, serotypes, Squamata, translocation, wildlife, zoonosis.

2.1 Introduction

The presence and possible spread of disease is an important consideration when translocating animals outside of their natal home range (Woodford and Rossiter 1994). Animals born in one area may have acquired immunity to endemic diseases and may become symptomless carriers of pathogenic organisms. This poses a threat when animals are translocated outside of their normal home range. Individuals introduced from captivity or other wild populations can introduce disease to animals at the release site, which may have been previously naïve to the disease. Similarly, individuals born in one area will not have developed immunity to diseases found at the release site and may therefore be at risk after release (Woodford and Rossiter 1994).

Approximately half of New Zealand's lizard species are registered as threatened or endangered (Daugherty et al 1994). The Department of Conservation (DoC) has introduced recovery plans for many of these threatened lizards (Daugherty et al 1994; Towns et al 2001). These plans frequently involve translocating lizards to offshore islands where introduced pests have been eradicated and where habitat restoration programmes have been undertaken in order to provide refuges for returning native species (Towns 1999). Many of these islands are already host to some of New Zealand's rarest endemic lizards (Towns 1999). Disease screening is recommended prior to the translocation of any species to prevent the spread of disease to naïve wild populations (Woodford and Rossiter 1994; Gartrell et al 2006). Because of many factors, such as lack of funding, limitations of diagnostic testing and lack of a basic biomedical knowledge of the species involved, disease screening is not carried out as intensively or as accurately as is necessary to minimize the spread of disease (Woodford and Rossiter 1994; Gartrell et al 2006).

There are no previous studies reporting the prevalence of *Salmonella* within our wild populations of geckos and skinks in New Zealand. Recent studies have found an absence of *Salmonella* excretion amongst wild and captive tuatara (*Sphenodon punctatus*) (Gartrell et al 2006; Gartrell et al 2007). Of the more than 2,300 *Salmonella* serovars over 40% are primarily cultured from reptiles (Mermin et al 2004). *Salmonella* is frequently isolated from birds in New Zealand and overseas (Robinson and Daniel

1968; Clark et al 2002; Tizard 2004). Recently *Salmonella* Typhimurium DT160 resulted in the mortality of many sparrows (*Passer domesticus*) in New Zealand (Alley et al 2002). Shortly after the first isolation of this organism from sparrows it began causing significant disease in humans, resulting in a 20% hospitalisation rate (Alley et al 2002). This highlights the zoonotic potential of many *Salmonella* serovars. Reptiles can also act as sources of *Salmonella* serovars, potentially infecting other wildlife, domestic animals and humans within close proximity (Chambers and Hulse 2006). A significant outbreak of salmonellosis caused by *Salmonella* Saintpaul occurred in Otago and was linked to wild geckos and skinks inhabiting kitchens (de Hamel and McInnes 1971). Similarly, wildlife, domestic animals and humans may infect lizards with other *Salmonella* serovars to which they have previously been naïve.

Within New Zealand it has been suggested that there is a spatial distribution of *Salmonella* (Twentyman 1999) and, if this is the case then it is likely that lizards will have acquired immunity to serovars found at their natal site, but would not have this immunity to serovars introduced from other geographical areas. Obtaining baseline knowledge of the *Salmonella* serovars present on islands would enable us to identify risks involved in the translocation process both to lizards and other wildlife present at the release site. This is especially important as these offshore islands are home to some of New Zealand's most endangered lizards as well as tuatara and often extremely rare amphibians.

Salmonella is a Gram negative bacillus belonging to the Enterobacteriacae group. It is considered to be a generalist pathogen, able to cross host species barriers and is frequently isolated from humans, birds, mammals and reptiles (Mermin et al 2004; Haraga et al 2008). It is also a highly environmentally stable organism found to survive for up to four weeks in tap water and wet sand, six weeks in direct contact with air, up to eight weeks when mixed with dry sand and up to six months in dried reptile stool (Otokunefor et al 2003; Mermin et al 2004). It is widely accepted that lizards constitute a reservoir of *Salmonella* from which humans and other mammals can contract salmonellosis (de Jong et al 2005; Pasmans et al 2005; Chambers and Hulse 2006). It is now also clear that many of the serovars carried by lizards show the same characteristics in terms of genetic structure, antimicrobial resistance and invasion of human intestinal epithelial cells compared to serovars typically isolated from mammalian sources

(Pasmans et al 2005). *Salmonella* serovars belonging to subspecies I account for the vast majority of human salmonellosis infections (Pasmans et al 2005). Pasmans et al (2005) also found that common reptile associated serovars belonging to subspecies I were more efficient at invading homeothermic hosts than reptile-associated serovars belonging to subspecies II, IIIb and IV.

Although *Salmonella* infection in reptiles is often limited to non-symptomatic intestinal carriage, it frequently results in clinical disease in humans and other animals (Johnson-Delaney 1996; Chambers and Hulse 2006). When disease does occur in reptiles, it may result in septicaemia, pneumonia, coelomitis, abscessation, granulomatous inflammation and death (Johnson-Delaney 1996).

This study aims to:

1. assess the prevalence of faecal excretion of *Salmonella* by wild endemic lizards on offshore islands around the coast of New Zealand
2. determine whether there is a geographical distribution of *Salmonella* serovars amongst New Zealand native lizards and whether this distribution has been affected by previous translocations
3. determine whether species, sex or age of the lizards has an effect on the likelihood of *Salmonella* excretion.

2.2 Materials and Methods

All procedures involving the use of animals were approved by the Massey University Animal Ethics Committee (MUAEC 07/31), Palmerston North, New Zealand. All islands accessed in this study are Crown owned. Access to the islands and procedures involving the use of lizards were approved by the Department of Conservation (DoC) and local iwi.

2.2.1 Study sites

I sampled 703 lizards from eight islands (Figure 2.1) across two southern hemisphere summers. The study sites used in this research can be divided into two broad groups: islands which have had lizard translocations in the past and islands upon which the lizard fauna are endemic and have not been re-introduced from elsewhere (Table 2.1). Table 2.2 shows the reintroduction history of these islands, including species introduced and the source of these individuals.

Table 2.1: Islands on which lizards were sampled for *Salmonella* in this study and dates of survey

Islands with a history of lizard translocations			**Islands without a history of lizard translocations**		
Island	Location	Sampling dates	Island	Location	
Korapuki Island	Auckland/ Coromandel	December 2007	Cuvier Island	Auckland/ Coromandel	October 2007
Mana Island	Wellington	March 2006	Little Barrier Island	Auckland/ Coromandel	November 2007
Matiu/Somes Island	Wellington	January 2008	Stephens Island	Marlborough Sounds	February 2007
Maud Island	Marlborough Sounds	March 2008			
Motuopao Island	Northland	October 2007			

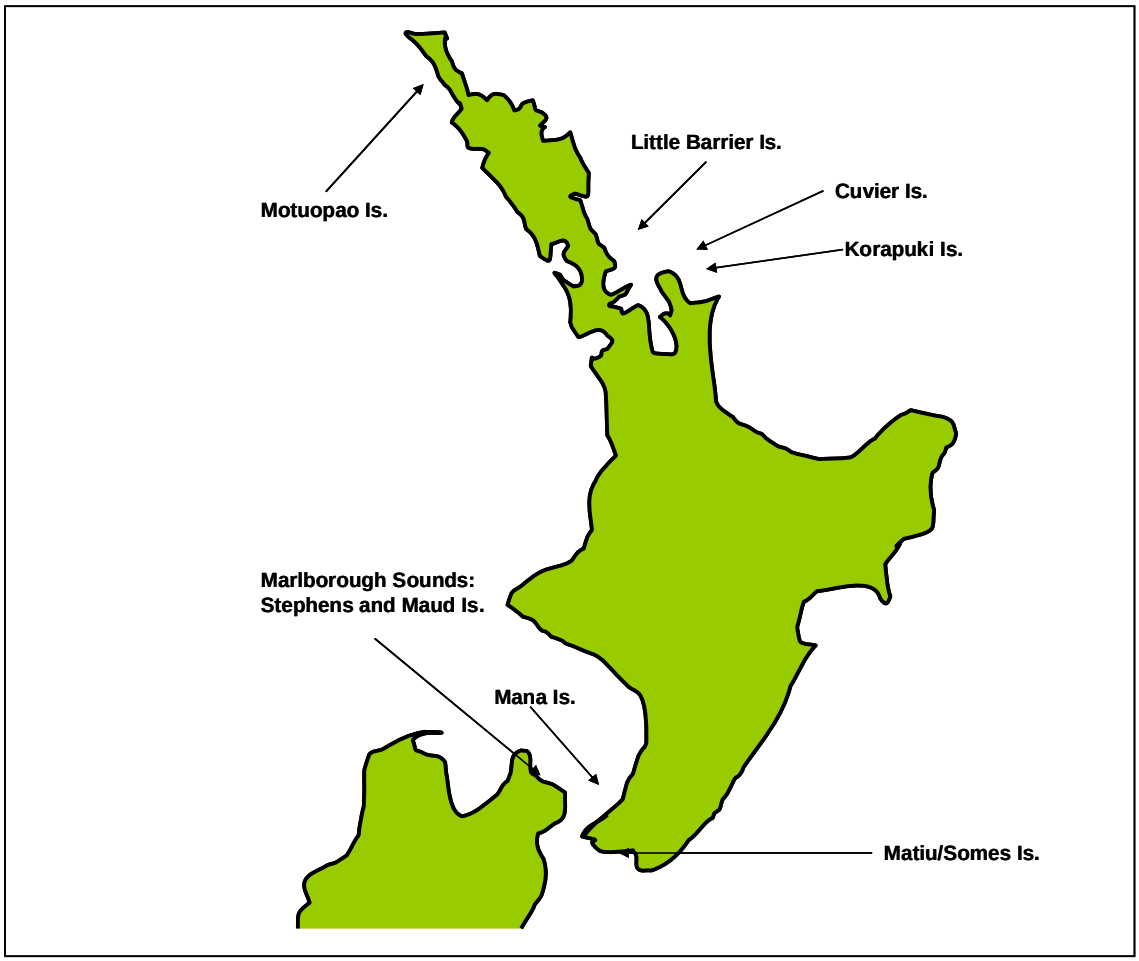


Figure 2.1: Location of study sites throughout the North Island and upper South Island of New Zealand

2.2.2 Field methods

Lizards from the families *Scincidae* and *Gekkonidae* were captured by hand or in pitfall traps and manually restrained for cloacal sampling. The Minitip cloacal swabs were stored in individual polypropylene tubes containing Aimes agar gel with charcoal (Copan Diagnostics Inc, 2175 Sampson Ave, Suite 124, Corona, CA 92879, USA) and stored on ice for up to five hours, followed by refrigeration on the island at 4-6°C for up to 13 days until transport to the laboratory where they were stored at 4°C until culturing.

For each individual, a measurement of weight and snout-vent length (SVL) was made. Lizards were measured along their abdomen from the tip of their jaw to their cloacal opening (Figure 2.2). This measurement was made to an accuracy of ±0.5mm. Weight was determined using Pesola scales with an accuracy of ±0.2g. This information was used to determine whether *Salmonella* excreting individuals had lower body weights

when compared with uninfected individuals. The species and sex of each individual was also recorded.

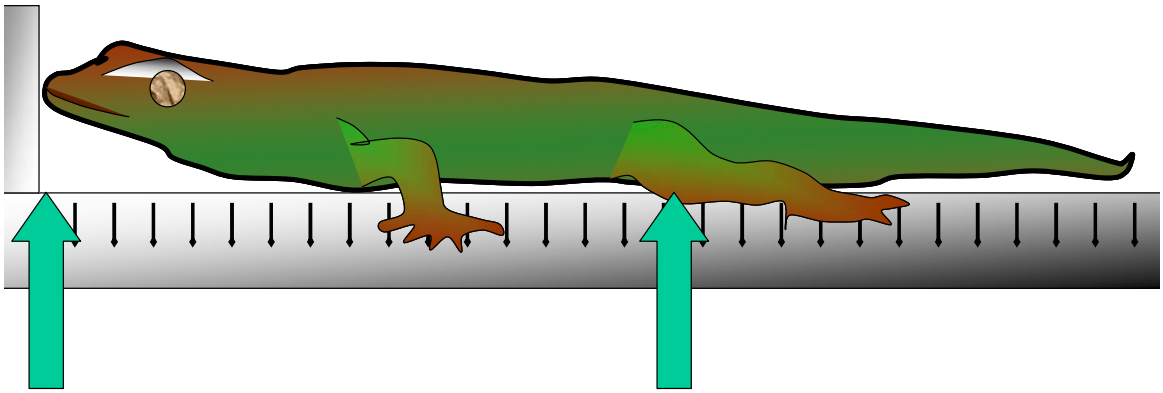


Figure 2.2: Obtaining an SVL measurement

Table 2.2: The history of lizard translocations to islands investigated in this study (based on data from Armstrong (2008) and Cash, B. pers. comm)

Island	Species	Source	Number of individuals	Date of release
Korapuki Island	*C. alani*	Green Island	7	November 1992 – March 1993
Korapuki Island	*C. oliveri*	Green Island	18	November 1992 - March 1993
Korapuki Island	*C. whitakeri*	Middle Island	28	February 1988 - March 1990
Korapuki Island	*O. suteri*	Green Island	30	November 1992 - March 1993
Mana Island	*H. duvaucelii*	North Brother Island	21, 18	February 1998, November 1998
Mana Island	*N. elegans*	Wellington	12	February 1998, November 1998, May 1999
Mana Island	*O. infrapunctatum*	Stephens Island	49	Unknown
Mana Island	*O. lineoocellatum*	Matiu/Somes Island	50	February 1998
Matiu/Somes Island	*H. granulatus*	Captivity	25	April 2005
Matiu/Somes Island	*C. ornata*	Wellington	26	November 2006
Maud Island	*O. infrapunctatum*	Stephens Island	40	January 2005
Motuopao Island	Matapia Island gecko	Matapia Island	41	April 1997
Motuopao Island	*C. alani*	Matapia Island	30	April 1997

2.2.3 Laboratory isolation of *Salmonella*

Salmonella isolation was achieved by inoculating selenite F enrichment broths (SFB, Fort Richard Laboratories, Otahuhu, Auckland, NZ) and tetrathionate enrichment broths (TB, Fort Richard Laboratories). Duplicates were made of each broth and incubated at 35-37°C and 25-27°C for 24 hours. The selenite broths were sub-cultured onto xylose lysine deoxycholate (XLD) plates (Fort Richard Laboratories) and tetrathionate broths were sub-cultured onto MacConkey (MC) agar plates (Fort Richard Laboratories), which were incubated in normal atmosphere at 35-37ºC and 25-27°C for a further 24 hours. Suspect colonies found with any method were used to inoculate triple sugar iron (TSI) agar slopes, urea slopes and lysine decarboxylase tubes (Fort Richard Laboratories). These were incubated overnight at both 37ºC and 27°C and results noted. Colonies that resulted in glucose fermentation and hydrogen sulphide production

on TSI slopes, positive lysine decarboxylase tests and acid (negative) urease tests were considered to be consistent with *Salmonella*. Each organism suspected of being *Salmonella* was tested for; fermentation of glucose, mannitol and xylose, hydrolysis of o-nitrophenyl-β-d-galactopyranoside (ONPG), indole production, acetoin production, citrate utilization, production of indolepyruvate and ornithine decarboxylase (Quinn et al 1994).

Samples which were found to be positive for *Salmonella* based on the tests outlined above were sent to Environmental Science and Research Services (ESR) for confirmation and serotyping. Serotyping was undertaken by antigenic determination, identifying the somatic 'O' and flagella 'H' antigens present.

2.2.4 Statistical analysis

The prevalence rates of *Salmonella* were calculated using the formulae described below (Thrusfield 2005) and making the assumptions that the *Salmonella* culture was 50% sensitive and 98% specific (Bager and Petersen 1991). Ninety five percent confidence intervals are reported.

The significance of the geographic distribution of *Salmonella* serovars and the distribution of *Salmonella* prevalence between islands, species and sexes were tested using a chi-square analysis.

The effect of *Salmonella* excretion on body condition of the lizards was tested using a multiple regression.

True Prevalence

$P = (P^T + \text{specificity} -1) / (\text{sensitivity} + \text{specificity} -1)$

Where P^T = test prevalence
and P = true prevalence

Variance

$\text{Variance} = [P^T(1-P^T)] / [n(\text{sensitivity} + \text{specificity} -1)^2]$

n = sample size

95% Confidence interval

$95\%\ CI = P \pm 1.96 \sqrt{\text{variance}}$

Statistical analysis was applied to data collected to detect the 95% confidence range for the maximum possible prevalence of *Salmonella* on islands where I found no lizards positive for *Salmonella* excretion (Cameron and Baldock 1998). The software used was the Detection of Disease component of WinEpiScope 2.0 (Facultad de Veterinaria Zaragoza, Spain; Wageningen University, The Netherlands; and University of Edinburgh, UK). The population size of lizards on Little Barrier and Maud Islands was estimated at 10,000 individuals. The population size of lizards on Motuopao Island was estimated at 1,000 individuals.

2.3 Results

Not all reptile populations sampled showed faecal excretion of *Salmonella.* The mean test prevalence of faecal excretion of *Salmonella* in the 703 lizards sampled was 4.7% and the true prevalence was in the range 2.3% - 8.8% at the 95% confidence interval (CI).

Salmonella serovars were found predominantly in *Oligosoma* (P^T=10.3%) and *Cyclodina* (P^T=4.9%) skinks and occasionally in *Hoplodactylus* geckos (P^T=1.6%). No *Salmonella* was found in the 14 *Naultinus* geckos sampled. Proportionately more *Hoplodactylus* (n = 373), *Oligosoma* (n=213) and *Cyclodina* skinks (n=103) species were sampled than the 14 *Naultinus* geckos.

In this study I found that skinks (P^T=8.5%, n = 316) were more likely to be carriers of *Salmonella enterica* than geckos (P^T=1.6%, n =387) (χ^2 = 19.14, p=0.001, DF =1). Twenty-two *Oligosoma* skinks (n=213) were positive for *Salmonella* but this was not a significantly higher prevalence than in the 5 *Cyclodina* skinks (n=103) (χ^2 =2.662, p=0.103, DF=1). Six *Hoplodactylus* geckos (n=373) were found to be positive for *Salmonella* excretion, however the sample size of *Naultinus* geckos (n=14) was too small to determine whether *Hoplodactylus* spp have higher excretion rates of *Salmonella*.

The prevalences of *Salmonella* on the eight islands studied ranged from 0 – 9.9% (Table 2.3). This was a statistically significant difference (χ^2 =18.3, DF=7, p=0.011).

All serovars isolated in this study belong to two subspecies. Members of subspecies I (n=29) were most commonly found in this study followed by subspecies IV (n=5), no other serovars were isolated in this study. Eleven serovars of *Salmonella* were isolated from New Zealand endemic lizards in this study (Table 2.4).

Little Barrier, Maud and Motuopao Islands had a zero prevalence of *Salmonella.* The maximum possible prevalence of *Salmonella* on these islands was 6.3%, 3.0% and 22.0% respectively. The remaining five islands were the source of between one and

three *Salmonella* serovars which were not found on any other island in this study (Table 2.3). *Salmonella* Bousso and *Salmonella* Mana were the only two serovars that were found on more than one island. *Salmonella* Bousso was found in shore skinks (*O. smithi*) on both Cuvier and Korapuki islands. *Salmonella* Mana was found on Mana Island and Matiu/Somes Island. The test prevalence of serovars on islands ranged from 0.7% - 6.9%. The true prevalence of serovars on islands ranged from 0 – 20.5%. *Salmonella* Bousso was found in the highest prevalence. 6.9% of lizards tested on Cuvier Island were positive for *Salmonella* Bousso.

Previous lizard translocations to the island did not have an effect on either the prevalence of *Salmonella* on the island nor the number of serovars found on the island (Table 2.5). The test prevalence of *Salmonella* on islands with a history of lizard translocations was found to be 4.8% (n=456) and the true prevalence is 5.9%. The test prevalence of *Salmonella* on islands without a history of lizard translocations was 4.5% (n=247) and the true prevalence is 5.1%. The difference was not statistically significant (χ^2 = 0.0493, DF = 1, p=0.824). The number of serovars found on the island was not influenced by the number of translocations that have occurred to the island (Table 2.5).

Table 2.3: Prevalence of *Salmonella* serovars found on New Zealand offshore islands and the true prevalence range at the 95% confidence interval

Island	**Salmonella test prevalence (%)**	**95%CI true prevalence**	**Serovars**	**No. lizards sampled**
Cuvier	6.9	0 – 20.6%	Bousso[a]	101
Korapuki	9.9	5.8% - 27.2%	IV 43:z4, z23:-[c] Warragul Bousso Mississippi	131
Little Barrier	0.0	0 – 6.3%		46
Mana	5.8	0 – 17.4%	Mana[b] 6,7:z:- 48:k:-	103
Matiu/Somes	2.7	0 – 7.7%	Mana Infantis	111
Maud	0.0	0 – 3.0%		99
Motuopao	0.0	0 – 22.0%		12
Stephens	4.0	0 – 12.2%	Saintpaul Typhimurium phage type 135 4,12:-:1,2	100

a, first isolates from New Zealand; b, first isolated from a takahe on Mana island in 1998; c, formally serovar Houten.

No difference was detected in the prevalence of *Salmonella* between males (P^T=5.5%, n=274) and females (P^T=5.4%, n=278) (χ^2 = 0.002, DF = 1, p=0.967). Similarly, juveniles (P^T=2.0%, n=151) were not found to differ in their *Salmonella* carriage rates compared with adults (P^T=5.4%, n=552) (χ^2 = 3.15, DF = 1, p=0.076).

Ten different lizard species were found to be host to *Salmonella* serovars (Table 2.4), eight species of skinks and two species of gecko. Sample sizes were too low to determine whether there is a difference in *Salmonella* distribution between species. Common skinks (*O. nigriplantare*, n=332) and common geckos (*H.maculatus*, n=29) were host to the highest diversity (n = 4) of *Salmonella* serovars (Table 2.4). Shore skinks (*O. smithi*) and egg laying skinks (*O. suteri*) were host to two and three serovars respectively. All others species were host to a single *Salmonella* serovar.

Salmonella Saintpaul and *Salmonella* 4,12:-:1,2 were found concurrently in two lizards on Stephens Island. All other individuals were host to a single serovar of *Salmonella*.

Those individuals caught on the coast within five metres of the high tide mark were much more likely to be carriers of *Salmonella* (P^T=11.2%, n=487) than those that were caught more than five metres from the high tide mark (P^T=1.8%, n=216) (χ^2 = 29.0, DF = 1, p=0.001).

A multiple regression was performed on weight (g) and snout – vent length (mm) and I found that *Salmonella* had no effect on the body condition of skinks (t=-0.977, n.s.) or geckos (t=-0.805, n.s.) (Appendix 1)

Table 2.4: Prevalence of *Salmonella* serovars found in New Zealand endemic lizards and the true prevalence range at the 95% confidence interval

Species	**Test prevalence %**	**95% CI range of true prevalence**	**Serovars**	**No. lizards sampled**
C. aenea	2.6	0 – 11.5%	Mana	39
C. alani	0.0	0 – 12.6%		22
C. macgregori	10.0	0 – 44.1%	6,7:z:-	20
C. oliveri	18.2	0 – 81.2%	43: z4, z23:-	11
C. whitakeri	0.0	0 – 23.8%		11
O. infrapunctatum	0.0	0 – 95.2%		1
O. lineoocellatum	2.0	0 – 7.8%	Infantis	51
O. moco	0.0	0 – 34.8%		7
O. nigriplantare	17.2	3.1% - 60.4%	Mana Saintpaul Typhimurium 4,12:-:1,2	29
O. smithi	16.4	12.4% – 47.8%	Bousso Mississippi	73
O. suteri	7.7	0 – 29.3%	Bousso IV 43:z4,z23:- Warragul	39
O. zelandicum	7.7	0 – 42.0%	Typhimurium	13
H. chrysosireticus	0.0	0 – 20.4%		13
H. duvauceli	9.5	0 – 41.8%	IV 43:z4,z23:-	21
H. maculatus	1.2	0 – 1.8%	IV 43:z4,z23:- Mana 6,7:z:- 48:k:-	332
H. pacificus	0.0	0 – 39.2%		6
H. stephensi	0.0	0 – 95.2%		1
N. manukanus	0.0	0 – 19.2%		14

Table 2.5: Number of lizard translocations to study sites and the corresponding number of *Salmonella* serovars found on the island

Island	**Number of lizard translocations**	**Number of *Salmonella* serovars found**	**Number of lizards caught**
Korapuki Island	4	4	131
Mana Island	4	3	103
Motuopao Island	2	0	12
Matiu/Somes Island	2	2	111
Maud Island	1	0	99
Little Barrier Island	0	0	46
Stephens Island	0	3	100
Cuvier Island	0	1	101

2.4 Discussion

This study found prevalence rates of *Salmonella* on offshore islands to be between 0 and 9.9% with a mean true prevalence of 4.7%. This is much lower than the 95% prevalence found amongst free-living reptiles in Indiana County, Pennsylvania, but similar to the 0% prevalence found in other studies (Richards et al 2004; Chambers and Hulse 2006).

The prevalence of *Salmonella* on islands ranged from 0 – 9.9%. Not all islands in this study showed faecal excretion of *Salmonella* amongst the lizard inhabitants. Little Barrier, Motuopao and Maud Islands were all found to be free of *Salmonella* in the inhabitant lizards, although it is likely that they were not free of *Salmonella* entirely and that the prevalence was at a lower level than detected by the sample sizes. This is indicated by the maximum possible prevalences of 6.3%, 3.0% and 22.0%. Why these islands should have lower prevalences of *Salmonella* than other islands is unclear. One possible explanation is that Little Barrier Island is New Zealand's oldest offshore nature reserve, hence strict quarantine procedures have been in place for this island for some time. Maud Island's quarantine procedures are also very strict in order to protect the

Maud Island frog (*Leiopelma pakeka*). These procedures may help to reduce the spread of *Salmonella* to the islands also. A very small sample size was obtained on Motuopao Island. Had a larger sample size been obtained it is possible we would have isolated some *Salmonella*. The maximum prevalence of *Salmonella* on Motuopao Island was 22.0% indicating this.

The remaining five islands surveyed were host to between one and four different serovars of *Salmonella* that are not found on any of the other islands. This suggests that translocation of lizards between islands could also introduce *Salmonella* serovars that have not previously existed on the island. At this stage the pathogenicity of these serovars to lizards is unknown so I am unsure as to whether this would have any adverse effects on the existing populations of lizard.

Two *Salmonella* serovars, *Salmonella* Bousso and *Salmonella* Mana, were found on more than one island. *Salmonella* Bousso was found amongst lizards on Cuvier Island (36°26′S, 175°46′E) and Korapuki Island (36°38′S, 175°52′E) in the Coromandel. *Salmonella* Mana was found on Mana Island (41°6′S, 174°48′E) and Matiu/Somes Island (41°16′S, 174°52′E) in the Wellington region. It is very difficult to isolate a spatial distribution from a species distribution as they are likely to be inter-related. Shore skinks (*O. smithi*) were captured on three islands in this study, Cuvier, Korapuki and Little Barrier (36°12′S, 175°8′E). No *Salmonella* was found on Little Barrier Island. *Salmonella* Bousso, however, was found on both Cuvier and Korapuki and only in *O. smithi*. To the best of my knowledge no lizard translocations have been conducted between these islands which could have spread *Salmonella* Bousso from one island to another.

If the distribution of *Salmonella* Mana is also due to a species influence, rather than geographical one, it is less clear from this study. *Salmonella* Mana was found in only two individuals on both Mana and Matiu/Somes. A single *O. nigriplantare* was found to be positive on both islands and *Salmonella* Mana was also isolated from a common gecko (*H. maculatus*) on Mana Island and a copper skink (*C. aenea*) on Matiu/Somes Island. *Salmonella* Mana was first isolated from a takahe in 1998. These isolates found amongst lizards on Mana Island and Matiu/Somes Island were the first isolations of this serovar since 1998. Fifty spotted skinks were translocated from Matiu/Somes Island to

Mana Island in 1998, shortly before *Salmonella* Mana was isolated from takahe on Mana Island. So, it is possible that Matiu/Somes Island was in fact the source of *Salmonella* Mana.

The prevalence of *Salmonella* and the number of serovars isolated varied between islands. It is unclear what would cause such a variation in prevalence from 0 – 9.9% as I did not find that increased *Salmonella* prevalence or serovar diversity was associated with a history of lizard translocations to the island.

Skinks (*Scincidae*) were far more likely to be carriers of *Salmonella* than geckos (*Gekkonidae*) in this study. Skinks are predominantly ground dwellers and feed on a range of food including fruits, insects and carrion (Gill and Whitaker 1996). Geckos are more arboreal species (Gill and Whitaker 1996). The green geckos (*Naultinus*) are diurnal and almost entirely arboreal, feeding predominantly on fruits and insects (Gill and Whitaker 1996; Department of Conservation 2002). Members of the genus *Hoplodactylus* are also inclined to an arboreal lifestyle but will forage both on the ground and in trees and plants and are nocturnal (Gill and Whitaker 1996; Department of Conservation 2002). *Salmonella* was only found within the *Hoplodactylus* species. Skinks and the two species of *Hoplodactylus* found to be shedders of *Salmonella* are known to eat carrion and regurgitated seabird fluid (personal observation). It is possible that this is the source of *Salmonella* infection in these animals. New Zealand seabirds have been found to be hosts of *Salmonella* by other authors (Clark et al 2002; Clark et al 2004; Tizard 2004). Arboreal lizards are less likely to come into contact with faecal infected food than those that live on the ground. This may also contribute to the difference in *Salmonella* rates found within these species.

Diet and lifestyle may also be the cause of lizards living within five metres of the high tide mark having higher prevalences of *Salmonella* than those living more than five metres from the shore. Many of the lizards that inhabit the coastal environments will readily eat carrion, indeed a fish head placed in a pitfall trap overnight will see some thirty or more lizards caught in the trap by morning on some island beaches (Figure 2.3), with little sign of the fish head remaining. More studies are required to determine whether diet is a likely source of infection in these animals.

Figure 2.3: *O. smithi* and *O. suteri* caught in a pitfall trap using dead fish as bait

Previous studies of both captive and wild caught tuatara have found an absence of detectable gastrointestinal shedding of *Salmonella* (Gartrell et al 2006; Gartrell et al 2007). It has been suggested that this may be due to a deficiency in the sampling technique, or that tuatara may not be carriers of *Salmonella*; either due to an acquired immunity or a lack of exposure (Gartrell et al 2006; Gartrell et al 2007). This study found that lizards co-existing with tuatara were excreting *Salmonella* which suggests that tuatara are most likely exposed. On both Stephens and Cuvier Islands, skinks live in very close proximity to the tuatara species *Sphenodon punctatus*. The tuatara and skink populations on Stephens Island are so dense that skinks and tuatara frequently inhabit the same burrows, with resident skinks apparently relying on their speed to avoid falling prey to the local tuatara (Walls 1981). Whilst skinks are abundant where tuatara live, they only form a small part of the diet of a tuatara, primarily because skinks are too swift for them (Walls 1981). Skinks, however, are on occasion caught and eaten by local tuatara (Walls 1981). It is therefore unlikely that tuatara on these islands would have been able to avoid exposure to the *Salmonella* serovars, for which these skinks are frequently hosts. Inadvertent introduction of *Salmonella* serovars unique to these islands through lizard translocations could therefore be detrimental to the survival of the

tuatara populations if their immunity is serovar specific. Matiu/Somes Island is also home to a dense population of skinks and the rarer species of tuatara *Sphenodon guntheri*. To the best of my knowledge no research has been conducted to determine whether this species also shows an absence in detectable faecal shedding of *Salmonella*.

Salmonella subspecies I (*enterica*) has been found to be the most commonly isolated subspecies from reptiles by other authors (Geue and Loschner 2002; Pasmans et al 2005; Bauwens et al 2006). This study provides evidence that this may also be the case in New Zealand. The vast majority of *Salmonella* serovars isolated in this study belonged to subspecies I, which is also the subspecies most commonly associated with human salmonellosis infections (Pasmans et al 2005). The only other subspecies isolated from New Zealand lizards in this study was subspecies IV (*houtenae*). Poikilothermic animals are generally regarded as usual carriers of *Salmonella* subspecies IV (Pfleger et al 2003). I found no *Salmonella* subspecies IIIa or IIIb in this study.

Eleven serotypes of *Salmonella* were identified in this study. Some are more common than others. *Salmonella* Mississippi is a relatively uncommon cause of human disease throughout the world (Ashbolt and Kirk 2006). In Europe and the United States, it accounts for less than 1% of all human salmonellosis infections (Ashbolt and Kirk 2006). However, it is a relatively common disease in Tasmania, Australia. Approximately 80% of all *Salmonella* Mississippi infections in Australia are reported among Tasmanian residents (Ashbolt and Kirk 2006). Individuals who had direct or indirect contact with wildlife were more likely to contract *Salmonella* Mississippi, suggesting Tasmanian wildlife may be acting as a reservoir for this serovar (Ashbolt and Kirk 2006). Previous studies have found *Salmonella* Mississippi from faecal samples of many native Australian species in Tasmania including; skinks, snakes, quolls, Tasmanian devils and kangaroos (Ashbolt and Kirk 2006). In New Zealand, *Salmonella* Mississippi is also an important serovar for human health, from 2003-2007 it was responsible for 71 reported cases of salmonellosis infection in humans (Environmental Science and Research 2008a). It has also been identified in a number of non-human isolates and is frequently isolated from bovine, ovine and equine sources (Environmental Science and Research 2008b).

Salmonella enterica serovars Typhimurium, Infantis and Saintpaul have also been isolated from reptiles overseas in relatively high densities (Baumler et al 1998; Chambers and Hulse 2006). Typhimurium was also the most common serovar isolated from Indiana County, Pennsylvania herpetofauna and was found amongst a number of snakes at Emperor Valley Zoo in Trinidad (Gopee et al 2000; Chambers and Hulse 2006), suggesting that this is a common serovar amongst reptiles throughout the world. *Salmonella* Infantis and Saintpaul have also been isolated from herpetofauna throughout the world, including in America and Sweden (de Jong et al 2005; Chambers and Hulse 2006). *Salmonella* Infantis, Mississippi, Saintpaul and Typhimurium are all commonly isolated from cattle, sheep and pigs in New Zealand.

Four of the *Salmonella* serovars isolated from New Zealand lizards during this study are also significant pathogens of humans. For example, *Salmonella* Typhimurium is responsible for more than 50% of reported cases of human salmonellosis in America. *Salmonella* Saintpaul, Infantis and Mississippi are also common causes of human salmonellosis. *Salmonella* Infantis is more commonly isolated from non-human sources. However, in Canada from 1983 – 1992, it was the fifth most common serovar isolated from humans (Khakhria et al 1997). *Salmonella* Saintpaul was the ninth most common isolate in Canada for the same period of time (Khakhria et al 1997).

Other serovars such as *Salmonella* Bousso and Warragul are less often associated with human disease but can still result in significant salmonellosis in certain circumstances. *Salmonella* Bousso was isolated from 14 lizards on the offshore islands of Cuvier and Korapuki. This is the first isolation of *Salmonella* Bousso in New Zealand, although it has been reported as the cause of salmonellosis amongst humans in North America (Centers for Disease Control and Prevention 2007).

The mean prevalence of *Salmonella* within the lizards on these eight offshore islands was 4.7%, this was higher than the rates reported for free-living reptiles overseas. A number of authors in North America have found that wild reptiles do not excrete *Salmonella* (Richards et al 2004). I have found no evidence that *Salmonella* excretion has an adverse effect on the health of lizards, as their body weights are not significantly different from non-*Salmonella* excreting individuals of the same length. Nor have I found any effect of a previous history of lizard translocations to the island on

Salmonella prevalence amongst the inhabiting lizards. However, members of the genus *Scincidae* (skinks) were far more likely to be shedders of *Salmonella* than geckos (*Gekkonidae*) and lizards that live within 5 metres of the high tide mark were more frequently found to be excreting *Salmonella* than those further than 5 metres. This suggests that *Salmonella* excretion may be related to diet and lifestyle. Future studies should examine the prevalence of *Salmonella* serovars amongst seabirds which regularly inhabit these islands as a potential source of the bacteria.

References:

Alley MR, Connolly JH, Fenwick SG, Mackereth GF, Leyland MJ, Rogers LE, Haycock M, Nicol C, Reed CEM. An epidemic of salmonellosis caused by *Salmonella* Typhimurium DT160 in wild birds and humans in New Zealand. *New Zealand Veterinary Journal* 50, 170-6, 2002

Ashbolt R, Kirk MD. *Salmonella* Mississippi infections in Tasmania: the role of native Australian animals and untreated drinking water. *Epidemiology and Infection* 134, 1257-65, 2006

Bager F, Petersen J. Sensitivity and specificity of different methods for the isolation of *Salmonella* from pigs. *Acta Veterinaria Scandinavica* 32, 473-81, 1991

Baumler AJ, Tsolis RM, Ficht TA, Adams LG. Evolution of host adaptation in *Salmonella enterica*. *Infection and Immunity* 66, 4579-87, 1998

Bauwens L, Vercammen F, Bertrand S, Collard JM, De Ceuster S. Isolation of *Salmonella* from environmental samples collected in the reptile department of Antwerp Zoo using different selective methods. *Journal of Applied Bacteriology* 101, 284-9, 2006

Cameron AR, Baldock FC. A new probability formula for surveys to substantiate freedom from disease. *Preventative Veterinary Medicine* 34, 1-17, 1998

Centers for Disease Control and Prevention. *Salmonella* surveillance: Annual summary, 2005. US Department of Health and Human Services, CDC, Atlanta, Georgia, 2007

Chambers DL, Hulse AC. *Salmonella* serovars in the Herpetofauna of Indiana County, Pennsylvania. *Applied and Environmental Microbiology* 72, 3771-3, 2006

Clark RG, Robinson RA, Alley MR, Nicol CM, Hathaway SC, Marchant RM. *Salmonella* in animals in New Zealand: the past to the future. *New Zealand Veterinary Journal* 50, 57-60, 2002

Clark RG, Fenwick SG, Nicol CM, Marchant RM, Swanney S, Gill JM, Holmes JD, Leyland M, Davies PR. *Salmonella* Brandenburg - emergence of a new strain affecting stock and humans in the South Island of New Zealand. *New Zealand Veterinary Journal* 52, 26-36, 2004

Daugherty CH, Patterson GB, Hitchmough RA. Taxonomic and conservation review of the New Zealand herpetofauna. *New Zealand Journal of Zoology* 21, 317-23, 1994

de Hamel FA, McInnes HM. Lizards as vectors of human salmonellosis. *Journal of Hygiene* 69, 247-53, 1971

de Jong BC, Andersson Y, Ekdahl K. Effect of regulation and education on reptile-associated salmonellosis. *Emerging Infectious Diseases* 11, 398-403, 2005

Department of Conservation. The Penguin guide to New Zealand wildlife: native and introduced birds, mammals, reptiles and amphibians, Penguin Books (NZ) Ltd, Auckland, 2002

Environmental Science and Research. Human *Salmonella* isolates 2007. www.surv.esr.cri.nz/enteric_reference/human_salmonella, Wellington, New Zealand, 2008a

Environmental Science and Research. Non-human *Salmonella* isolates 2007. www.surv.esr.crinz/enteric_reference/nonhuman_salmonella, Wellington, New Zealand,12 Jan. 2008b

Gartrell BD, Jillings E, Adlington BA, Mack H, Nelson NJ. Health screening for a translocation of captive-reared tuatara (Sphenodon punctatus) to an island refuge. *New Zealand Veterinary Journal* 54, 344-9, 2006

Gartrell BD, Youl JM, King CM, Bolotovski I, McDonald WL, Nelson NJ. Failure to detect *Salmonella* species in a population of wild tuatara (*Sphenodon punctatus*). *New Zealand Veterinary Journal* 55, 134-6, 2007

Geue L, Loschner U. *Salmonella enterica* in reptiles of German and Austrian origin. *Veterinary Microbiology* 84, 79-91, 2002

Gill B, Whitaker T. New Zealand Frogs and Reptiles, David Bateman Limited, Auckland, 1996

Gopee NV, Adesiyun AA, Caesar K. Retrospective and longitudinal study of salmonellosis in captive wildlife in Trinidad. *Journal of Wildlife Diseases* 36, 284-93, 2000

Haraga A, Ohlson MB, Miller SI. Salmonellae interplay with host cells. *Nature Reviews Microbiology* 6, 53-66, 2008

Johnson-Delaney CA. Reptile zoonoses and threats to public health. In: Mader DR (ed) *Reptile Medicine and Surgery*. Pp 20-32. WB Saunders, Philadelphia, USA, 1996

Khakhria R, Woodward D, Johnson WM, Poppe C. *Salmonella* isolated from humans, animals and other sources in Canada, 1983-92. *Epidemiology and Infection* 119, 15-23, 1997

Mermin J, Hutwagner L, Vugia D, Shallow S, Daily P, Bender J, Koehler J, Marcus R, Angulo FJ. Reptiles, amphibians, and human *Salmonella* infection: A population-based, case-control study. *Clinical Infectious Diseases* 38, S253-S61, 2004

Otokunefor TV, Kindzeka BI, Ibiteye IO, Osuji GU, Obi FO, Jack AWK. *Salmonella* in gut and droppings of three pest lizards in Nigeria. *World Journal of Microbiology & Biotechnology* 19, 545-8, 2003

Pasmans F, Martel A, Boyen F, Vandekerchove D, Wybo I, Van Immerseel F, Heyndrickx M, Collard JM, Ducatelle R, Haesebrouck F. Characterization of *Salmonella* isolates from captive lizards. *Veterinary Microbiology* 110, 285-91, 2005

Pfleger S, Benyr G, Sommer R, Hassl A. Pattern of *Salmonella* excretion in amphibians and reptiles in a vivarium. *International Journal of Hygiene and Environmental Health* 206, 53-9, 2003

Quinn PJ, Carter ME, Markey B, Carter GR. Clinical Veterinary Microbiology, Wolfe Publishing, London, 1994

Richards JM, Brown JD, Kelly TR, Fountain AL, Sleeman JM. Absence of detectable *Salmonella* cloacal shedding in free-living reptiles on admission to the Wildlife Center of Virginia. *Journal of Zoo and Wildlife Medicine* 35, 562-3, 2004

Robinson RA, Daniel MJ. The significance of *Salmonella* isolates from wild birds and rats in New Zealand. *New Zealand Veterinary Journal* 16, 53-5, 1968

Thrusfield M. Veterinary Epidemiology, 3rd edn. Blackwell Scientific, Oxford, 2005

Tizard I. Salmonellosis in wild birds. *Seminars in Avian and Exotic Pet Medicine* 13, 11-3, 2004

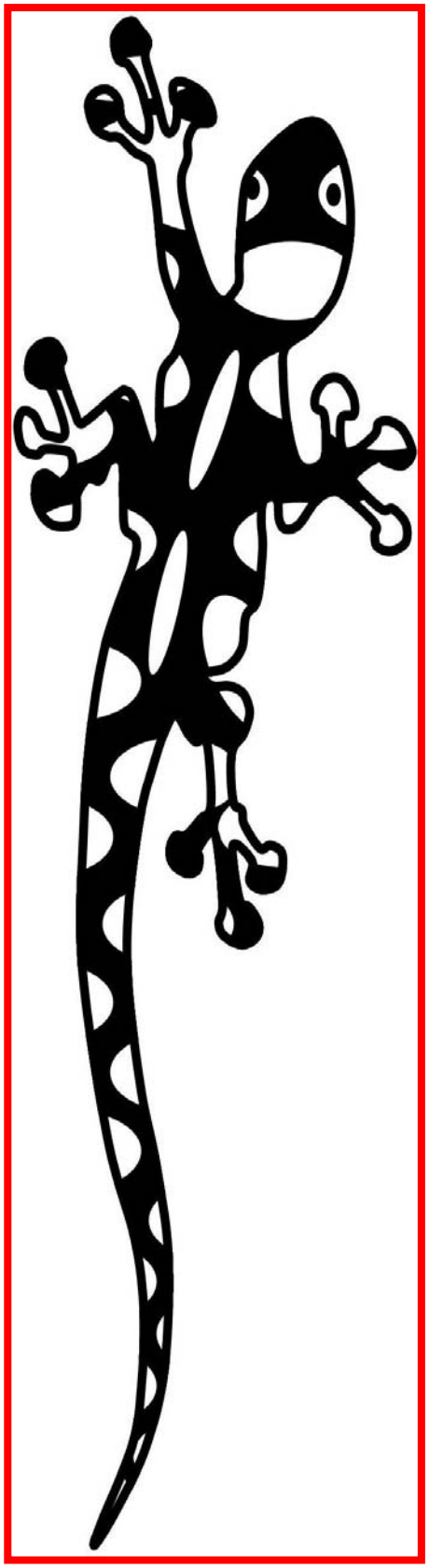
Towns DR. *Cyclodina* spp. recovery plan. Department of Conservation, Wellington, New Zealand, 1999

Towns DR, Daugherty CH, Cree A. Raising the prospects for a forgotten fauna: a review of 10 years of conservation effort for New Zealand reptiles. *Biological Conservation* 99, 3-16, 2001

Twentyman C. Diseases in New Zealand reptiles. *Surveillance* 26, 3-5, 1999

Walls GY. Feeding ecology of the tuatara (*Sphenodon punctatus*) on Stephens Island, Cook Strait. *New Zealand Journal of Ecology* 4, 89-97, 1981

Woodford MH, Rossiter PB. Disease risks associated with wildlife translocation projects. In: Olney PJS, Mace GM, Feistner ATC (eds). *Creative Conservation: Interactive management of wild and captive animals*. Pp 179-99. Chapman and Hall, London, 1994



Chapter Three:

A survey of potential pathogens in New Zealand native lizards from Mana Island, New Zealand

A survey of potential pathogens in New Zealand native lizards from Mana Island, New Zealand

Abstract

One hundred and three cloacal swabs were obtained from lizards on Mana Island, New Zealand in order to assess the prevalence of faecal excretion of *Salmonella, Aeromonas and Hafnia alvei* by New Zealand native lizards on Mana Island. These cloacal swabs were cultured specifically for *Salmonella, H. alvei* and *Aeromonas* using aerobic enrichment and culture methods. The prevalence of *Salmonella* on Mana Island was found to be 5.8%. Three different *Salmonella* serotypes were identified from the lizards sampled, they were *Salmonella* Mana, *Salmonella* 6,7 :z:- and *Salmonella* 48:k:-. Six sites were sampled across Mana Island and it was found that each site was host to *Salmonella* serovars that were not found on any other site in this study. No *Aeromonas* species were cultured from any of the cloacal swabs which suggests that, at the 95% confidence interval the maximum prevalence of *Aeromonas* amongst this population is less than 3%. *Hafnia alvei* was found at a prevalence of 1.9%. None of the organisms were found to have an effect on body condition of the reptiles. The spatial distribution of *Salmonella* serovars suggest that serovars tend to localise in populations. Translocated reptiles should continue to be screened for *Salmonella* in order to prevent infecting wild populations. The pathogenic and zoonotic potential of *H. alvei* remains unknown although its prevalence in this population was very low.

Keywords: *Aeromonas*, bacteria, disease prevalence, *Hafnia alvei*, lizard, reptile, *Salmonella*, serotypes, Squamata, translocation, wildlife, zoonosis

3.1 Introduction

New Zealand is home to approximately 60 species of lizard (Towns et al 2001; Department of Conservation 2002). Of these lizards, approximately half are registered as rare, threatened or endangered (Daugherty et al 1994). The Department of Conservation (DoC) has implemented management strategies for the recovery of these native lizards, which frequently involve the translocation of threatened lizards to offshore islands where introduced pests have been eradicated (Towns et al 2001). The presence and possible spread of disease is an important consideration when translocating reptiles, and indeed all animals, to new areas (Woodford and Rossiter 1994). Reptiles born in one area may have acquired immunity to diseases found in that area and may become symptomless carriers of the pathogens. This poses a threat to animals at the release site which may have been previously naïve to the pathogen. In much the same way, reptiles from one area may not have developed immunity to pathogens found at the release site and may therefore be at risk after release (Woodford and Rossiter 1994).

Salmonella and *Aeromonas* are important diseases amongst reptiles overseas and can also cause disease in humans, mammals and birds (Cooper and Jackson 1981; Quinn et al 1994; Mader 1996; Twentyman 1999; Mermin et al 2004; Richards et al 2004; Chambers and Hulse 2006). We know very little about the prevalence of these organisms in New Zealand. A recent study by Gartrell et al (2007) found *H. alvei* amongst tuatara (*Sphenodon punctatus*). The present study aims to assess the rate of intestinal carriage of *Salmonella, H. alvei and Aeromonas* by wild endemic lizards on Mana Island, New Zealand.

3.1.1 Mana (Te Mana o Kupe ki Aotearoa) Island

Mana Island (41°6′S,174°48′E) is a 217-ha scientific reserve that lies 2.5km from the mainland of the North Island at its closest point (Timmins et al 1987; Newman 1994; Miskelly 1999; Wotton 2002). Mana Island's classification as a scientific reserve allows for public access, and visitors are very common on the island during summer months (Miskelly 1999).

There is a long history of human occupation of Mana Island, both by Maori and Europeans (Timmins et al 1987; Miskelly 1999). From 1832 to 1986 the island was farmed, resulting in extensive modification of the landscape (Timmins et al 1987; Wotton 2002). By 1986, the indigenous vegetation was confined almost entirely to the cliffs and a small catchment on the flats (Miskelly 1999). An extensive replanting programme has been undertaken as part of the island restoration plan (Wotton 2002).

Figure 3.1: View across the valley on Mana Island

In the mid 1980s farming ceased on the island and all stock were removed (Timmins et al 1987). Despite the abundance of human activity on the island, house mice (*Mus musculus*) were the only non-stock mammal that inhabited the island and these too were removed through poisoning when an eradication programme began in August 1989 (Timmins et al 1987; Newman 1994; Wotton 2002). No mice or their signs have been seen on Mana Island since February 1990 (Newman 1994).

Ten species of reptile currently inhabit the island. They are the McGregor's skink (*Cyclodina macgregori*), copper skink (*Cyclodina aenea*), brown skink (*Oligosoma zelandicum*), common skink (*Oligosoma nigriplantare*), speckled skink (*Oligosoma infrapunctatum*), spotted skink (*Oligosoma lineocellatum*), goldstripe gecko (*Hoplodactylus chrysosireticus*), common gecko (*Hoplodactylus maculates*), Duvaucel's gecko (*Hoplodactylus duvauceli*) and Wellington green gecko (*Naultinus elegans elegans*). Copper skinks, common skinks and common geckos are common and widespread on the island (Miskelly 1999). There are two very important populations of lizard on Mana Island; the McGregor's skink is considered vulnerable (Vu) by the IUCN red list (IUCN 2007) and range restricted by the Department of Conservation's (DoC) threatened species classification (Hitchmough et al 2005). McGregor's skinks are currently found on only four islands, including Mana Island (Miskelly 1999). The goldstripe gecko is considered lower risk/least concern by the IUCN red list (IUCN 2007) and sparse to gradual decline by DoC (Hitchmough et al 2005). The Mana Island population is the only island population of this species. Thus, it is the only population free of predation by introduced mammals and is possibly the largest surviving population of goldstripe geckos (Miskelly 1999).

Archeological excavation of Mana Island found the remains of seven different species of reptile. Four of these species still inhabit the island; McGregor's skink (*C. macgregori*), copper skinks (*C. aenea*), common skinks (*O. nigriplantare*) and common gecko (*H. maculatus*). The remains of robust skinks (*C. alani*), Duvaucel's gecko (*H. duvauceli*) and tuatara (*Sphenodon* sp) were also found, although these species are no longer extant on the island (Miskelly 1999). The list of reptile species recorded from archaeological excavation is unlikely to be complete, highlighted by the absence of goldstripe geckos (*H. chrysosireticus*) and brown skink (*O. zelandicum*) remains (Miskelly 1999). It is therefore possible that any or all of the 17 species of reptile

recorded in the lower North Island may have been present on Mana Island at some time (Miskelly 1999).

Four of the ten species that currently inhabit Mana Island have been reintroduced to the island from various locations throughout New Zealand (Armstong 2008) (Table 3.1). A number of other reintroductions are planned for the future, including tuatara (*Sphenodon punctatus*), robust skink (*Cyclodina alani*) and Whitaker's skink (*Cyclodina whitakeri*) (Miskelly 1999). It is therefore important that we have a basic knowledge of the diseases found on Mana Island to ensure that we do not introduce new disease to the island with the reptiles, and endanger the survival of species already inhabiting the island or threaten the survival of the transferred species through infection with disease found on Mana Island.

Table 3.1: Source of lizard translocations to Mana Island. Data from Armstrong (2008)

Species	Source	Number released	Date of release
Hoplodactylus duvaucelii	North Brother Island	21	1997
Naultinus elegans elegans	Wellington region	12	1998/1999
Oligosoma infrapunctatum	Stephens Island	49	Unknown
Oligosoma lineoocellatum	Matiu/Somes Island	50	1998

3.1.2 *Salmonella*

Very little research has been conducted in New Zealand on the prevalence of *Salmonella* within wild populations of reptiles, although overseas studies have found *Salmonella* to be highly prevalent amongst wild and captive herpetofauna (Mader 1996; Chambers and Hulse 2006). Of the more than 2,300 *Salmonella* serovars more than 40% are primarily cultured from reptiles.

Within New Zealand it has been suggested that there is a spatial distribution of *Salmonella* (Twentyman 1999). If this is the case, then it is likely that lizards will have acquired immunity to serovars found at their natal site, but would not have this immunity to serovars introduced from other geographical areas. Moreover, translocated reptiles might serve as vectors of *Salmonella* serovars not present at the release site. This is therefore an important consideration when translocating reptiles to new areas.

Little is known about the prevalence of *Salmonella* serovars within New Zealand endemic lizard populations. A recent study found no *Salmonella* within free-living tuatara (*Sphenodon punctatus*) on Stephens Island. This could be due to a lack of exposure, resistance to infection or failure of methodology (Gartrell et al 2007). Geckos and particularly skinks regularly coexist with tuatara. Therefore, some knowledge of the prevalence of *Salmonella* within New Zealand native lizards would aid in assessing the risk of the spread of novel *Salmonella* serovars to geckos, skinks and tuatara.

3.1.3 *Hafnia alvei*

Hafnia alvei is a zoonotic organism found in mammals, birds, fish, soil, sewage, food and reptiles (Janda and Abbott 2006). However, very little is known about *H. alvei* and the role it plays in both human and veterinary medicine (Janda and Abbott 2006). *H. alvei* is rarely pathogenic (Quinn et al 1994), but has a possible association with gastroenteritis in humans (Rodriguez et al 1999; Janda and Abbott 2006). It has been found in tuatara in New Zealand (Gartrell et al 2007).

3.1.4 *Aeromonas*

Aeromonas is commonly associated with septicaemic disease and mouth infections of reptiles (Cooper and Jackson 1981; Mader 1996; Twentyman 1999), although the organism is also frequently isolated from clinically healthy lizards (Mader 1996). *Aeromonas* is only very rarely associated with disease in humans (Quinn et al 1994). There has been no comprehensive survey on the prevalence of this organism within New Zealand lizards.

A better base knowledge of these diseases amongst the geckos and skinks on Mana Island will help us to better manage disease risks. The potential risks are to humans involved in the translocation of these reptiles, to translocated lizards, and to other wildlife that may be present at the release site.

The aims of this study were to:

1. Assess the prevalence of *Salmonella*, *H. alvei* and *Aeromonas* on Mana Island, New Zealand
2. Identify the serovars of *Salmonella* present on Mana Island, New Zealand
3. Determine whether there is a spatial distribution of *Salmonella*, *H. alvei* or *Aeromonas* within herpetofauna on Mana Island
4. Determine the effect of age, sex and species on the carriage of *Salmonella*, *Aeromonas* and *H. alvei* amongst endemic lizards on Mana Island.

3.2 Materials and Methods

All procedures involving the use of animals were approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand (MUAEC 07/31). Mana Island is owned by the Crown. Access to the island and procedures involving the use of lizards were approved by the New Zealand Department of Conservation (DoC) and Ngati Toa who are kaitiaki of Mana Island.

3.2.1 Field methods

One hundred and three (103) lizards in the scientific reserve of Mana Island were sampled during March (southern hemisphere summer) 2006. Lizards from the families *Scincidae* and *Gekkonidae* were captured by hand or in pitfall traps and manually restrained for cloacal sampling (Figure 3.2). The Minitip cloacal swabs were stored in individual polypropylene tubes containing Aimes agar gel with charcoal (Copan Diagnostics Inc, 2175 Sampson Ave, Suite 124, Corona, CA 92879, USA) and stored on ice for up to five hours, followed by refrigeration at 4-6°C on the island for up to four days until transport to the laboratory where they were stored at 4°C until culturing.

For each individual, a measurement of weight and snout-vent length (SVL) was made. Lizards were measured along their abdomen from the tip of their jaw to their cloacal opening. This measurement was made to an accuracy of ±0.5mm. Weight was determined using Pesola scales with an accuracy of ±0.2g. This information was used to determine the body condition of each lizard. The species and sex of each individual was also recorded.

Figure 3.2: Cloacal sampling a common gecko (*H. maculatus*)

Six sites across the scientific reserve of Mana Island and in a range of different habitats were sampled in this study (Figure 3.3).

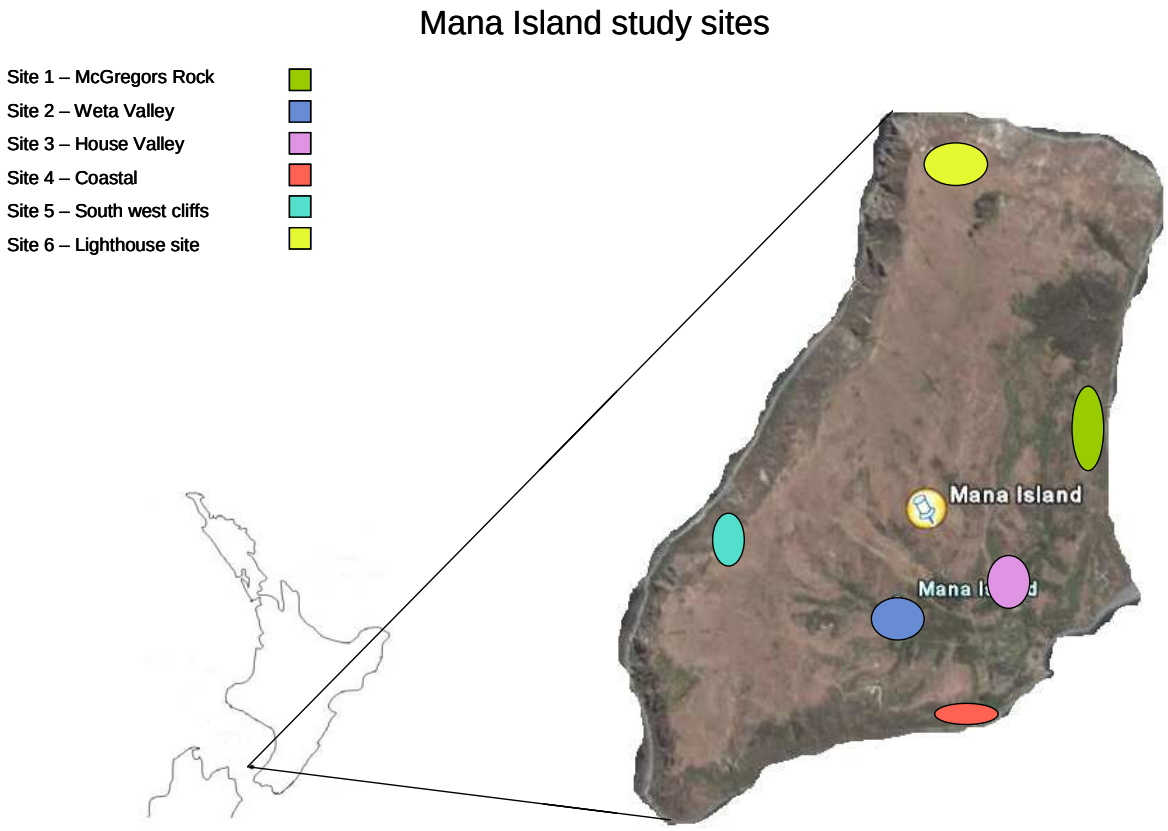


Figure 3.3: Aerial map of Mana Island showing location of sites surveyed (adapted from Google Earth)

3.2.2 Laboratory methods

The protocol for microbiological isolation of *Salmonella* has been described in Chapter Two of this thesis. No changes were made to this protocol in the present study.

Isolation of *Aeromonas* and *Hafnia alvei* was conducted by inoculating sheep blood agar (SBA) prepared from dehydrated media (Merck and Co., Inc, P.O. Box 100, Whitehouse Station, NJ 08889-0100, USA) and incubating for 24 hours at 35-37°C. Blood agar plates were examined for evidence of haemolysis and the characteristic pungent smell of *Aeromonas*. Colonies that were consistent with the appearance of *Aeromonas* and *H. alvei* on blood agar were then picked and stained using the Gram stain procedure. Gram negative colonies were submitted to oxidase and catalase tests and grown in lysine decarboxylase broth. Colonies that were oxidase negative, catalase positive and gave a positive lysine decarboxylase test were futher tested. Each suspect organism was tested for; fermentation of glucose, mannitol and xylose, hydrolysis of O-nitrophenyl-β-d-galactopyranoside (ONPG), indole production, acetoin production, citrate utilization, production of indolepyruvate and ornithine decarboxylase.

Positive *H. alvei* samples were sent to Ministry of Agriculture and Forestry (MAF) for confirmation of identification by PCR (polymerase chain reaction). *Salmonella* species were sent to ESR for serotyping by identification of their flagella (H) and somatic (O) antigens.

3.2.3 Statistical analysis

The test prevalence and true prevalence of *Salmonella* and *Hafnia alvei* on Mana Island was calculated using the methods described in Chapter Two of this thesis, making the assumptions that the *Salmonella* culture was 50% sensitive and 98% specific (Bager and Petersen 1991) and using confidence intervals of 95%. Due to the rarity of *H. alvei* as a cause of disease in both humans and animals there have been no studies into the sensitivity and specificity of biological tests for this organism. I have therefore predicted the sensitivity to be 50% and the specificity to be 95% based on results found by Rodriguez et al (1999) and the similarity of this organism to *Salmonella*.

Statistical analysis was applied to data collected to detect the 95% confidence range for the maximum possible prevalence of *Aeromonas* (Cameron and Baldock 1998) in the population, given our level of sampling. The software used was the Detection of Disease component of WinEpiScope 2.0 (Facultad de Veterinaria Zaragoza, Spain; Wageningen University, The Netherlands; and University of Edinburgh, UK). The population size of lizards on Mana Island was estimated at 10, 000 individuals.

Chi square analyses were performed on prevalence results between: sites, genus, species and sex to determine whether significant differences in prevalence existed between these groups.

Body weights and lengths were analysed using a multiple regression to determine whether infection with any of the potential pathogens had an adverse effect on body weight.

3.3 Results

3.3.1 *Salmonella*

The test prevalence of *Salmonella* excretion on Mana Island was found to be 5.8%. The true prevalence was calculated at 8.0% and 95% confidence interval for the true prevalence was 0 – 17.4%.

Salmonella excretion was found predominantly in skinks (*Scincidae*) (P^T=10.0%, n=30) and less often in geckos (*Gekkonidae*) (P^T=4.1%, n=73). This difference in prevalence was not found to be significant (χ^2=1.34, DF =1, p=0.246). Only three species of lizard were found to be shedders of *Salmonella* in this study, two species of skink (*C. macgregori* and *O. nigriplantare*) and one species of gecko (*H. maculatus*).

Lizards were caught and sampled across six sites (Figure 3.1). *Salmonella* excretion was found in lizards at only three of these sites. No statistically significant difference in the prevalence of *Salmonella* was found across these six sites (χ^2 = 4.83, DF = 5, p= 0.436) (Table 3.2).

Three serovars of *Salmonella* were isolated on Mana Island. The serovars were Mana, and two serovars that were unable to be fully typed but had the designations 6,7:z:- and 48:k:- based on their flagella (H) and somatic (O) antigens. Each of these serovars belongs to *Salmonella* subspecies I. *Salmonella* was found in only three of the sites sampled and each of these sites was host to a different *Salmonella* serovar (Table 3.2).

The prevalence of *Salmonella* excretion between females (P^T=5.3%, n=38) and males (6.9%, n=58) was not significantly different (χ^2 = 0.105, DF= 1, p=0.746). Adults (6.3%, n-=96) did not have an increased likelihood of *Salmonella* excretion than juveniles (0.0, n=7) (χ^2 = 0.465, DF = 1, p =0.495).

Those lizards that tested positive for *Salmonella* were not in poorer body condition, based on their snout-to-vent length and weight measurements, than lizards that were negative for *Salmonella* excretion (t = -0.11, n.s.) (Appendix 2).

Table 3.2: Distribution of *Salmonella* serovars amongst native lizards on Mana Island, New Zealand

Site	**Prevalence of *Salmonella***	**Lizard species**	***Salmonella* serovar**	**No. of lizards sampled**
McGregor's Rock	11.1%	*C. macgregori* (2) *H. maculatus*	6,7 : z:-	27
Weta Valley	0		Nil	13
House Valley	0		Nil	19
Coastal site	9.1%	*H. maculatus*	48 : k:-	11
South west cliffs	9.5%	*H. maculatus* *O. nigriplantare*	Mana	21
Lighthouse site	0		Nil	12

3.3.2 *Hafnia alvei*

Hafnia alvei was found in only two of the 103 samples giving a test prevalence of 1.9% amongst lizards on Mana Island and the true prevalence was within the range 0 – 2.1% at the 95% confidence interval. .

H. alvei infection was not found to have any effect on body weights of lizards when compared with uninfected lizards using a multiple regression (t = 0.83, n.s.) (Appendix 2).

3.3.3 *Aeromonas*

No *Aeromonas* species were cultured from any of the cloacal swabs, which suggests that, at the 95% confidence interval, the maximum prevalence of *Aeromonas* amongst this population is less than 3%.

3.4 Discussion

This study highlights the complex dynamics of *Salmonella* carriage among wild lizard populations. Herpetofauna have often been implicated as transmitters of *Salmonella*, *Aeromonas* and *H. alvei*. The literature pertaining to the prevalence of these organisms among reptiles is scant, and the vast majority of reptile species have never been sampled.

This study found the test prevalence of intestinal carriage of *Salmonella* in wild lizards on Mana Island, New Zealand to be 5.8%. The true prevalence was calculated as 8.0%. It might seem counterintuitive that the true prevalence is higher than the test prevalence but *Salmonella* cultures have a low sensitivity which we are estimating as 50% (Bager and Petersen 1991), therefore the calculation allows for the fact that our results will under-estimate the true prevalence. Some overseas studies have reported considerably higher rates of *Salmonella* carriage amongst wild reptiles. Chambers and Hulse (2006), showed the frequency of isolation from essentially healthy reptiles in Indiana County, Pennsylvania to be 95.3%. Other studies have found significantly lower prevalence

rates than this, for example Richards et al (2004) found a zero percent prevalence of *Salmonella* amongst free living reptiles (Richards et al 2004). The prevalence (5.8%) of *Salmonella* found in this study was not significantly higher than the mean test prevalence (4.7%) of *Salmonella* found in 703 wild New Zealand lizards in Chapter Two of this thesis

There has been little research into the prevalence of *Salmonella* among geckos and skinks in New Zealand. Serotypes *S.* Hindmarsh, *S.* Typhimurium, *S.* Victoria, *S.* Saintpaul, *S.* Mississippi and *S.* Wohlen have been found in New Zealand reptiles, particularly green geckos (Twentyman 1999), although no extensive surveys have been conducted. In this study, two of the sampled reptiles, a common skink and a common gecko, were found to have *Salmonella* Mana. *Salmonella* Mana is a serovar that was first isolated from a takahe (*Porphyrio mantelli hochstetteri*) on Mana Island in 1998. This serovar has since been identified in a lizard on Matiu/Somes Island (Chapter Two) and a human case was reported at Foxton Beach, New Zealand (unpublished). This demonstrates the ability of this serovar to cross species barriers and raises the possibility of a spatial distribution of serovars within New Zealand (Chapter 2). If such a distribution does exist, this would raise important considerations for the translocation process. Lizards from Mana Island may have developed immunity to *Salmonella* Mana but as demonstrated by the common gecko and skink in this survey, they are still capable of being asymptomatic carriers of the disease. Translocating lizards to or from Mana Island or outside of the greater Wellington region may introduce *Salmonella* Mana to reptiles previously naïve to this serovar. The consequences of this are unknown. The remaining two serovars identified are rarely cultured from humans and are more commonly identified from cold-blooded animals overseas. They have not previously been identified in New Zealand.

The three *Salmonella* serovars found in this study all belong to subspecies I. Previous studies have found that *Salmonella* subspecies IIIa and IIIb are most commonly isolated from reptilian sources (Mitchell 2006). In Chapter Two of this thesis I found that most *Salmonella* serovars sourced from New Zealand reptiles belonged to subspecies I. *Salmonella* subspecies I is also more likely to be associated with human salmonellosis infection than subspecies IIIa and IIIb.

In Chapter Two of this thesis I found that each of the eight islands surveyed were host to between one and three *Salmonella* serovars which were not found on any other island in the study. Within Mana Island, I found different *Salmonella* serovars at different sites. *Salmonella* Mana was found in lizards caught on the south-west cliffs; *Salmonella* 6, 7: z: - was found in a common gecko and two McGregor's skinks captured at McGregor's Rock and *Salmonella* 48: k: - was found in a common gecko caught at the coastal site. This study suggests that *Salmonella* serotypes may become established in an area, with the local reptiles developing immunity to endemic serovars and become asymptomatic carriers.

The hypothesis, that in most cases *Salmonella* serotypes are only carried by the lizards and only rarely cause significant disease, is further supported by my findings that *Salmonella* carriage was not correlated with a lower body weight compared with uninfected individuals in this study. Had the *Salmonella* resulted in disease in its host, we would have expected this to have shown as a reduction in body weight to length. Previous studies have also found that most herpetofauna are frequently asymptomatic carriers of *Salmonella* (Geue and Loschner 2002).

It is important that we gain a basic knowledge of the *Salmonella* serovars present on islands such as Mana. Like many New Zealand islands Mana Island is a sanctuary for native bird species as well as reptiles. Introductions of novel *Salmonella* serovars to the island through lizard translocations could have adverse effects on both the bird and lizard populations. Whilst some serovars of *Salmonella* appear to be highly species specific, others seem to be more generalist. This has been demonstrated on Mana Island as both lizards and takahe (*Porphyrio mantelli hochstetteri*) have been found to be carriers of *Salmonella* Mana. Reptiles infected with *Salmonella* are often non-symptomatic carriers of the disease. So prospective lizards for translocation may appear to be physically well and yet be carrying a serovar of *Salmonella* previously unrepresented on Mana Island and to which the island population does not have immunity, resulting in disease within the Mana Island natal population.

Hafnia alvei was found at a prevalence of 1.9% in this study. This is the first large scale study of *H. alvei* prevalence in New Zealand native lizards. Although, it has been found amongst free-living tuatara (*Sphenodon punctatus*) on Stephens Island, New Zealand

(Gartrell et al 2007). I suspect that *H. alvei* is not of importance in New Zealand lizard populations due to its low prevalence and a lack of evidence of any adverse effects. H. alvei is, however, likely to be of limited but not negligible risk to humans and other wildlife. The gastrointestinal tracts of mammals appear to be a common ecological niche of Hafniae (Janda and Abbott 2006). On rare occasions *H. alvei* has been known to result in gastroenteritis, septicaemia and urinary infections among humans (Proietti et al 2004; Janda and Abbott 2006). Immuno-compromised and debilitated hosts are most at risk (Janda and Abbott 2006).

Hafnia alvei has been isolated from a number of avian species at prevalences of between 3 – 16% (Janda and Abbott 2006). In most cases *H. alvei* exists as a commensal organism within the gastrointestinal tract of the birds, however in certain conditions it can cause significant disease within the birds (Janda and Abbott 2006). In 2004 *H. alvei* was the cause of multifocal necrotising hepatitis and splenitis of pullets in Italy (Proietti et al 2004). The risk of this occurring with a wild population of birds appears to be low since to date their have been no documented cases of wild bird fatalities due to *H. alvei*.

Hafnia alvei appears to be commensal within New Zealand native lizards on Mana Island as no reduction in body weight was found when compared with uninfected individuals. It should be noted however, that the sample size of *H. alvei* carriers in this study was very small (n=2).

The present study found lower rates of *Aeromonas* amongst reptiles than previous studies (Madsen 1996; Oros et al 2005; Turutoglu et al 2005). *Aeromonas* species are common bacteria in aquatic environments (Mader 1996) and the majority of studies in which *Aeromonas* has been identified have looked at essentially aquatic animals, such as Nile crocodiles (*Crocodylus niloticus*) (Turutoglu et al 2005), green anacondas (*Euncectes murinus*) (Miller et al 2004) and sea turtles (*Caretta caretta*, *Chelonia myads* and *Dermochelys coriacea*) (Oros et al 2005). The prevalence of *Aeromonas* among Nile crocodiles has been shown to be as high as 90% (Madsen 1996), dramatically higher than the 0% prevalence found in the current study. It is possible that, given the high rates at which *Aeromonas* can be found in rivers, lakes and ponds, aquatic reptiles are more likely to be exposed to *Aeromonas* and hence are more

susceptible to becoming carriers of the disease. Future studies should compare the prevalence of *Aeromonas* amongst reptiles that complete most of their life cycle in water versus those that spend most of their lives on land. *Aeromonas* is capable of causing significant disease in terrestrial reptiles, with symptoms ranging from stomatitis (mouth infections) to septicemia and bacteraemia, and occasionally resulting in death (Mader 1996). The absence of *Aeromonas* in this survey of 103 healthy reptiles suggests that *Aeromonas* may not be a commensal organism amongst New Zealand native terrestrial reptiles.

Before future translocations are undertaken to Mana Island a number of factors should be considered. These include the source of the individuals to be translocated and the serovars of *Salmonella* that have been previously identified on the island, the serovars of *Salmonella* found within the translocated animals, the serovars of *Salmonella* found within the source population and whether any of these serovars are novel to Mana Island.

References:

Armstong DP. 3 Jan. 2008. Reintroduction projects in New Zealand. http://www.massey.ac.nz/~darmstro/nz_projects.htm. 12 Feb 2008

Bager F, Petersen J. Sensitivity and specificity of different methods for the isolation of *Salmonella* from pigs. *Acta Veterinaria Scandinavica* 32, 473-81, 1991

Cameron AR, Baldock FC. A new probability formula for surveys to substantiate freedom from disease. *Preventative Veterinary Medicine* 34, 1-17, 1998

Chambers DL, Hulse AC. *Salmonella* serovars in the Herpetofauna of Indiana County, Pennsylvania. *Applied and Environmental Microbiology* 72, 3771-3, 2006

Cooper JE, Jackson OF. Diseases of the Reptilia, Academic Press Inc, London, 1981

Daugherty CH, Patterson GB, Hitchmough RA. Taxonomic and conservation review of the New Zealand herpetofauna. *New Zealand Journal of Zoology* 21, 317-23, 1994

Department of Conservation. The Penguin guide to New Zealand wildlife: native and introduced birds, mammals, reptiles and amphibians, Penguin Books (NZ) Ltd, Auckland, 2002

Gartrell BD, Youl JM, King CM, Bolotovski I, McDonald WL, Nelson NJ. Failure to detect *Salmonella* species in a population of wild tuatara (*Sphenodon punctatus*). *New Zealand Veterinary Journal* 55, 134-6, 2007

Geue L, Loschner U. *Salmonella enterica* in reptiles of German and Austrian origin. *Veterinary Microbiology* 84, 79-91, 2002

Hitchmough RA, Bull L, Cromarty PL. New Zealand Threat Classification Systems Lists. Science and Technical Publishings, Department of Conservation, Wellington, 2005

IUCN. 2007 IUCN red list of threatened species. www.iucnredlist.org, 2007

Janda JM, Abbott SL. The Genus *Hafnia*: from Soup to Nuts. *Clinical Microbiological Reviews* 19, 12-8, 2006

Mader DR. Reptile Medicine and Surgery, W.B. Saunders Company, Philadelphia, 1996

Madsen M. Prevalence and serovar distribution of *Salmonella* in fresh and frozen meat from captive Nile crocodiles (*Crocodylus niloticus*). *International Journal of Food Microbiology* 29, 111-8, 1996

Mermin J, Hutwagner L, Vugia D, Shallow S, Daily P, Bender J, Koehler J, Marcus R, Angulo FJ. Reptiles, amphibians, and human *Salmonella* infection: A population-based, case-control study. *Clinical Infectious Diseases* 38, S253-S61, 2004

Miller DL, Radi ZA, Stiver SL, Thornhill TD. Cutaneous and pulmonary mycosis in green anacondas (*Euncectes murinus*). *Journal of Zoo and Wildlife Medicine* 35, 557-61, 2004

Miskelly C. Mana Island Ecological Restoration Plan. Department of Conservation, Wellington, 1999

Mitchell MA. *Salmonella*: Diagnostic methods for reptiles. In: Mader DR (ed) *Reptile Medicine and Surgery*. Pp 900-1. Saunders Elsevier, Missouri, USA, 2006

Newman DG. Effects of a mouse, *Mus musculus*, eradication program and habitat change on lizard populations of Mana Island, New Zealand, with special reference to McGregor skink, *Cyclodina macgregori*. *New Zealand Journal of Zoology* 21, 443-56, 1994

Oros J, Torrent A, Calabuig P, Deniz S. Diseases and causes of mortality among sea turtles stranded in the Canary Islands, Spain (1998-2001). *Diseases of Aquatic Organisms* 63, 13-24, 2005

Proietti PC, Passamonti F, Franciosini MP, Asdrubali G. *Hafnia alvei* infection in pullets in Italy. *Avian Pathology* 33, 200-4, 2004

Quinn PJ, Carter ME, Markey B, Carter GR. Clinical Veterinary Microbiology, Wolfe Publishing, London, 1994

Richards JM, Brown JD, Kelly TR, Fountain AL, Sleeman JM. Absence of detectable *Salmonella* cloacal shedding in free-living reptiles on admission to the Wildlife Center of Virginia. *Journal of Zoo and Wildlife Medicine* 35, 562-3, 2004

Rodriguez LA, Vivas J, Gallardo CS, Acosta F, Barbeyto L, Real F. Identification of *Hafnia alvei* with the Microscan WalkAway System. *Journal of Clinical Microbiology* 37, 4186-8, 1999

Timmins SM, Atkinson IAE, Ogle CC. Conservation opportunities on a highly modified island: Mana Island, Wellington, New Zealand. *New Zealand Journal of Ecology* 10, 57-65, 1987

Towns DR, Daugherty CH, Cree A. Raising the prospects for a forgotten fauna: a review of 10 years of conservation effort for New Zealand reptiles. *Biological Conservation* 99, 3-16, 2001

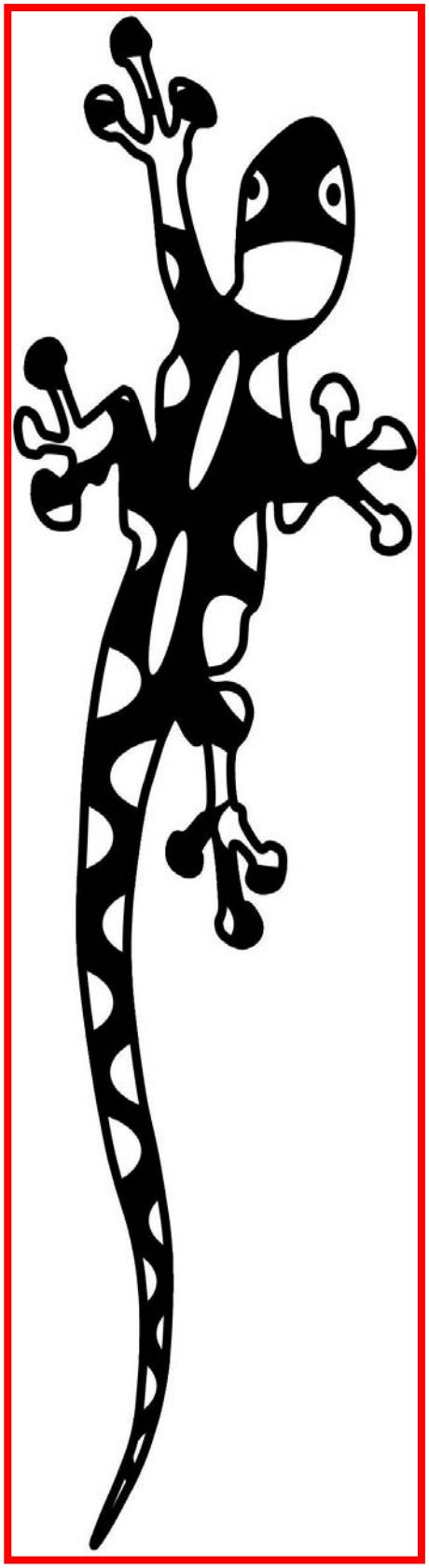
Turutoglu H, Ercelik S, Corlu M. *Aeromonas* hydrophila-associated skin lesions and septicaemia in a Nile crocodile (*Crocodylus niloticus*). *Journal of the South*

African Veterinary Association-Tydskrif Van Die Suid-Afrikaanse Veterinere Vereniging 76, 40-2, 2005

Twentyman C. Diseases in New Zealand reptiles. *Surveillance* 26, 3-5, 1999

Woodford MH, Rossiter PB. Disease risks associated with wildlife translocation projects. In: Olney PJS, Mace GM, Feistner ATC (eds). *Creative Conservation: Interactive management of wild and captive animals*. Pp 179-99. Chapman and Hall, London, 1994

Wotton DM. Effectiveness of the common gecko (*Hoplodactylus maculatus*) as a seed disperser on Mana Island, New Zealand. *New Zealand Journal of Botany* 40, 639-47, 2002



Chapter Four:

Disease prevalence in endemic New Zealand lizards at captive breeding facilities

Disease prevalence in endemic New Zealand lizards at captive breeding facilities

Abstract

This aim of this study was to assess the prevalence of faecal excretion of *Salmonella*, *Aeromonas* and *Hafnia alvei* by New Zealand native lizards from two captive bred populations in the North Island of New Zealand. Ninety-six cloacal swabs were obtained from lizards at two captive breeding populations. Population A was tested in its entirety whilst a representative sample of 56 individuals was made from population B. These samples were cultured specifically for *Salmonella, H. alvei* and *Aeromonas* using aerobic enrichment and culture methods. The mean test prevalence of faecal excretion of *Salmonella* in captive lizards sampled was 11.5%. There was a higher prevalence of *Salmonella* within captive population A (22.0%) than in population B (3.6%). Two different *Salmonella* serovars were found within the captive lizards, they were *Salmonella* Saintpaul and *Salmonella* Mississippi. No *Aeromonas* was cultured from any of the lizards which suggests that, at the 95% confidence interval the maximum prevalence of *Aeromonas* is less than 2.0%. *H.alvei* was found at a prevalence of 5.2%. The prevalence of *H. alvei* excretion within population A was found to be 9.8%, whilst within population B a prevalence of 1.8% was found. This difference was not statistically significant. This study supports other findings which have found a difference in the prevalence of *Salmonella* between captive breeding facilities. Captive populations of lizards should only be used to form or supplement wild populations if there are no other viable options. Where captive populations are used as the source of new wild populations wildlife managers should select the source of these lizards with care.

Keywords: *Aeromonas*, bacteria, captive, disease prevalence, *Hafnia alvei*, lizard, New Zealand, reptile, *Salmonella*.

4.1 Introduction

Reptiles have been recognised as a source of *Salmonella* and *Aeromonas* for many years. *Salmonella* and *Aeromonas* are often found as commensal organisms within herpetofauna and only rarely result in disease within the host (Cooper and Jackson 1981; Bradley et al 2001; Pfleger et al 2003). When these reptiles are kept as pets or in captivity however, there is increased opportunity for the transmission of *Salmonella* to humans, domestic animals and other reptiles (Bradley et al 2001). *Hafnia alvei* has been isolated from a wide range of hosts including mammals, birds, fish and reptiles however little is known about the pathogenicity of this organism in reptiles (Janda and Abbott 2006).

Numerous studies have been conducted overseas into the prevalence of *Salmonella* excretion amongst captive reptiles. This is primarily due to the increasing interest in maintaining exotic reptiles as pets. In the USA, reptiles constitute the fastest growing sector of the pet market (Mitchell and Shane 2000). The infection rates found in these studies have varied from 0 to 84.1% (Geue and Loschner 2002; Pfleger et al 2003). The prevalence of *Salmonella* excretion has also been shown to vary significantly between captive reptile populations. Geue and Loschner (2002) showed that the prevalence of *Salmonella* between captive populations varied from 0 - 80%. Many authors have found that reptiles kept in captivity have much higher prevalence rates of *Salmonella* excretion when compared with free-living reptiles (Otokunefor et al 2003; Awad-Masalmeh et al 2005; Ebani et al 2005; Nakadai et al 2005; Pasmans et al 2005).

There is some discrepancy between authors as to whether there is an increased risk of horizontal transmission of *Salmonella* between all members of the population once a single individual is infected. Geue and Loschner (2002) found that identical serotypes, plasmid profiles and genotypes were isolated from different reptiles belonging to the same owner. In contrast, reptiles living together in one terrarium have been found to excrete *Salmonella* of different serotypes (Pfleger et al 2003). In both studies, some animals failed to show any *Salmonella* excretion although they were living together with *Salmonella* excreting animals (Pfleger et al 2003).

Free-living and captive reptiles are often symptomless carriers of *Salmonella* species. This may indicate that the bacterium exists as a commensal flora in the animal's gut (Sanyal et al 1997). A single reptile may be host to several different *Salmonella* serovars and an intermittent shedder of the bacterium (Sanyal et al 1997; Gopee et al 2000; Bradley et al 2001). Pfleger et al (2003) observed continuous excretion of *Salmonella* was never found in their study of captive reptiles and amphibians. Stress, which may result from overcrowding, water deprivation and changes in diet or excessive heat exposure, can result in increased faecal shedding. This is thought to be a direct result of changes in the normal intestinal flora of the gut (Quinn et al 1994; Richards et al 2004). It is for this reason that captive reptiles are thought to excrete *Salmonella* more often than free-living reptiles.

Salmonella enterica subspecies II, IIIa, IIIb, IV and VI are known to be mainly associated with cold-blooded animals (Sanyal et al 1997; Baumler et al 1998; Mitchell 2006), whilst members of subspecies I are most frequently isolated from avian and mammalian hosts (Baumler et al 1998). Despite this, serovars belonging to subspecies I are often the most commonly isolated serovars from many captive collections (Geue and Loschner 2002; Pfleger et al 2003).

Aeromonas has been isolated from clinically healthy reptiles overseas but is also associated with septicaemic disease and mouth infections (Cooper and Jackson 1981; Mader 1996; Twentyman 1999). Very rarely this organism is also associated with human infections (Quinn et al 1994).

Hafnia alvei has been isolated from a wide range of hosts including mammals, birds, fish and reptiles as well as in environments such as sewage, food and soil (Janda and Abbott 2006). Little is known about *H. alvei* and the role it plays in human and veterinary medicine (Rodriguez et al 1999; Janda and Abbott 2006). *H. alvei* has been found amongst tuatara (*Sphenodon punctatus*) in New Zealand, however no comprehensive study of its prevalence in endemic lizards has been conducted (Gartrell et al 2007).

All New Zealand native lizard species have 'absolute protection' under the Wildlife Act 1953. This means that no native lizard may be collected from the wild, or handled or disturbed in any way without written authority from the Department of Conservation (DoC) and local iwi. All native lizard keepers in New Zealand must therefore hold a permit from DoC and their only legitimate source of lizards is to obtain them from another captive breeder. There are strict protocols in place for native lizard breeders in New Zealand. In general, only the less threatened species may be kept in captivity, however rarer species may be held if the breeding contributes to an approved conservation programme.

This study aims to assess the risk involved in translocating captive bred lizards back into the wild, by assessing the prevalence of *Salmonella*, *H. alvei* and *Aeromonas* amongst two populations of captive reptiles in the North Island of New Zealand.

4.2 Materials and Methods

All procedures involving the use of animals were approved by the Massey University Animal Ethics Committee (MUAEC 07/31), Palmerston North, New Zealand.

Two populations of lizards were sourced from two captive breeding facilities in the lower North Island of New Zealand. In order to preserve the privacy of the reptile collection owners these two collections are referred to simply as population A and population B. All 41 individuals were sampled from captive population A and a representative sample of 55 individuals from approximately 200 individuals was taken from captive population B. Information gathered and recorded for each individual included: species, weight, snout-vent length (SVL) and gender. An estimate of age was also recorded.

Electronic scales with an accuracy of ±0.01g were used to weigh the captive reptiles (Figure 4.1). Snout-to-vent length measurements were obtained to an accuracy of ±0.5mm.

Small cotton Minitip swabs were used to obtain a cloacal sample (Figure 4.2). These were inserted into the cloaca and twisted several times before being placed into polypropylene tubes containing Aimes agar gel with charcoal (Copan Diagnostics Inc,

2175 Sampson Ave, Suite 124, Corona, CA 92879, USA) and stored on ice for up to five hours until returning to the lab in the evening. At the lab, swabs were stored at 4°C for up to 48 hours before inoculating an enrichment broth.

Figure 4.1: Weighing a common gecko (*Hoplodactylus maculatus*)

Figure 4.2: Minitip swabs used for obtaining cloacal samples

The swabs were used to inoculate sheep blood agar plates (SBA) prepared from dehydrated media (Merck and Co. Inc, PO Box 100, Whitehouse Station, NJ-08889-0100, USA) and selenite broths (Fort Richard Laboratories, 12 Huia Rd, Otahuhu, Auckland, NZ) which were then incubated overnight in normal atmosphere at 35-37°C. The selenite broths were used to inoculate xylose lysine deoxycholate (XLD) plates (Fort Richard Laboratories) which were incubated in normal atmosphere at 35-37ºC for a further 24 hours. Colonies that were suspected to be *Salmonella* on XLD were used to inoculate triple sugar iron (TSI) agar slopes, urea slopes and lysine decarboxylase tubes (Fort Richard Laboratories). The biochemical tests used to identify *Salmonella* species are explained in detail in Chapter Two of this thesis. This protocol was used unchanged in this study.

Blood agar plates were examined for evidence of haemolysis and the characteristic pungent smell of *Aeromonas* colonies. Colonies that were consistent with the appearance of *Aeromonas* and *H. alvei* on blood agar were picked and stained using the Gram stain procedure (Quinn et al 1994). Gram negative colonies were submitted to oxidase and catalase tests and grown in lysine decarboxylase broth. Colonies that were oxidase negative, catalase positive and gave a positive lysine decarboxylase test were further tested. Each suspected organism was tested for; fermentation of glucose, mannitol and xylose, hydrolysis of O-nitrophenyl-β-d-galactopyranoside (ONPG), indole production, acetoin production, citrate utilization, production of indolepyruvate and ornithine decarboxylase. *Aeromonas*, *H. alvei* and *Salmonella* species were identified based on the results of these tests.

Positive *H. alvei* samples were sent to the Ministry of Agriculture and Forestry (MAF) for confirmation of identification by polymerase chain reaction (PCR). *Salmonella* species were sent to ESR for serotyping through identification of the O (somatic) and flagella (H) antigens.

4.2.1 Statistical analyses

The test prevalence and true prevalence of *Salmonella* and *H. alvei* in each captive population was calculated using the methods described in Chapter Two of this thesis, and making the assumptions that the *Salmonella* culture was 50% sensitive and 98% specific (Bager and Petersen 1991) and using confidence intervals of 95%. Due to the rarity of *H. alvei* as a cause of disease in both humans and animals, there have been no studies into the sensitivity and specificity of biological tests for this organism. We have therefore predicted the sensitivity to be 50% and the specificity to be 95% based on the results found by Rodriguez et al (1999) and the similarity of this organism to *Salmonella*.

Statistical analysis was applied to the data collected to detect the 95% confidence range for the maximum possible prevalence of *Aeromonas* (Cameron and Baldock 1998) in the population, given our level of sampling. The software used was the Detection of Disease component of WinEpiScope 2.0 (Facultad de Veterinaria Zaragoza, Spain, Wageningen University, The Netherlands, and University of Edinburgh, UK).

A chi-square test was applied to the data to determine statistical significance in the *Salmonella* prevalence between populations and between families. A power analysis of length versus weight was conducted in order to determine if there was any effect of *Salmonella* infection on body weight. Only members of the genus *Naultinus* were included in the regression as this was the only genus in which I found any *Salmonella* or *H. alvei* excretion.

4.3 Results

4.3.1 Salmonella

The mean prevalence of *Salmonella* excretion between the two captive populations (n=96) was found to be 11.5%. The true prevalence was calculated at 19.79% with a 95% confidence interval of 6.5-33.0%.

Nine of the 41 individuals (P^T=22%) in captive population A were positive for *Salmonella* excretion. The true prevalence of *Salmonella* within this population was

determined to be 41.7% and between 15.2 – 68.0% at the 95% confidence interval. In contrast only two of the 55 lizards sampled at population B were positive for *Salmonella* excretion. The true prevalence was calculated to be between 0 - 13.4% at the 95% confidence interval. The difference in *Salmonella* excretion between lizards in these two populations was found to be significant (χ^2=7.77, DF = 1, p=0.005).

Table 4.1: Lizard species sampled in population A and the percentage prevalence of *H. alvei* and *Salmonella* within species

Reptile Taxonomy	Percentage prevalence of *H. alvei*	Percentage prevalence of *Salmonella*	No of reptiles sampled
Population A			
Gekkonidae	**%**	**%**	
Hoplodactylus chrysosireticus	0.0	0.0	2
Hoplodactylus granulatus	0.0	0.0	2
Naultinus elegans elegans	0.0	44.4	9
Naultinus grayii	20.0	20.0	5
Naultinus rudis	14.3	28.6	14
Naultinus stellatus	0.0	0.0	9
Total	9.80%	22.00%	41

Table 4.2: Lizard species sampled in population B and the percentage prevalence of *H. alvei* and *Salmonella* within species

Reptile Taxonomy	Percentage prevalence of *H. alvei*	Percentage prevalence of *Salmonella*	Number of reptiles sampled
Population B			
Gekkonidae	**%**	**%**	
Hoplodactylus duvaucelii	0	0	3
Hoplodactylus granulatus	0	0	3
Hoplodactylus maculatus	0	0	16
Hoplodactylus pacificus	0	0	2
Naultinus elegans elegans	0	11.1	9
Naultinus grayii	11.1	0	9
Naultinus rudis	0	0	3
Naultinus stellatus	0	16.7	6
Scincidae			
Cyclodina macgregori	0	0	2
Oligosoma microlepis	0	0	1
Oligosoma waimatense	0	0	1
Total	1.80%	3.60%	55

All the samples found to be positive for *Salmonella* originated from individuals belonging to the genus *Naultinus*, the diurnal green geckos (Figure 4.3). Inclusive of both populations I sampled 92 geckos (*Gekkonidae*) and only four skinks (*Scincidae*) (Tables 4.1 and 4.2). There was insufficient data to make any conclusions about the likely prevalence of *Salmonella* between these families. However, within the family *Gekkonidae* 64 members of the genus *Naultinus* were sampled and 28 members of the genus *Hoplodactylus*. Eleven members of the *Naultinus* genus tested positive (P^T=15.6%) in contrast to none of the *Hoplodactylus* species. Within these two captive populations *Naultinus* geckos were found to have significantly higher prevalences of *Salmonella* excretion than *Hoplodactylus* geckos at the 95% confidence interval (χ^2 = 5.47, DF = 1, p=0.019).

Figure 4.3: *Naultinus elegans* one of the only species found positive for *Salmonella* in this study

Infection with *Salmonella* did not result in any significant weight loss, as there was no correlation between weight and *Salmonella* infection (t= 0.44, n.s) (appendix 3). If significant, persistent illness was present in the individual we would expect this to be evident as a reduced body condition in comparison with unaffected healthy individuals.

Between both populations a total of 62 females, 32 males and two three day old juveniles (which were not sexed were tested). Of these, five females (P^T=8.1%), four males (P^T=12.5%) and two juveniles (P^T=100.0%) were found to be positive for *Salmonella* excretion. Males did not have a significantly higher prevalence than females of *Salmonella* excretion when tested with a chi-square analysis at the 95% confidence interval (χ^2 = 0.480, DF = 1, p=0.489). The sample size of juveniles was too small to determine whether juvenile lizards were more likely to be shedders of *Salmonella* than adults.

Only two *Salmonella* serovars were isolated between these populations. Both individuals found to have *Salmonella* in population B were carrying *Salmonella* Saintpaul, a serovar belonging to *Salmonella enterica* subspecies I. *S.* Saintpaul was also isolated from a single individual in population A, a female *N. grayii*. All other individuals shedding *Salmonella* at population A were found to have *S.* Mississippi, which is also a member of *Salmonella enterica* subspecies I.

4.3.2 Aeromonas

No *Aeromonas* was detected in any of the 96 samples taken. Given that all members of captive population A were sampled the prevalence of *Aeromonas* is zero. All of the 55 individuals sampled in population B tested negative. Given this level of sampling, at the 95% level of confidence the prevalence of *Aeromonas* in population B is less than 2.0%.

4.3.3 Hafnia alvei

The mean test prevalence of faecal excretion of *H. alvei* within the 96 reptiles sampled was 5.2% and the true prevalence was in the range 0 – 15.9% at the 95% confidence interval (Tables 4.1 and 4.2).

Hafnia alvei was isolated from four out of the 41 (P^T=9.8%) animals sampled at population A which we tested in its entirety. The true prevalence of *H. alvei* infection in this population was 10.6% and the 95% confidence interval is between 0 – 30.8%. A single lizard (P^T=1.8%) was found to be shedding *H. alvei* at population B and the true prevalence was within the range 0 – 14.9% at the 95% confidence level. Population A

did not have significantly higher prevalence of *Salmonella* excretion than population B at the 95% confidence interval (χ^2 = 3.00, DF = 1, $p \leq 0.083$).

The difference in *H. alvei* excretion between *Naultinus* geckos (P^T=7.8%, n=64) and *Hoplodactylus* geckos (P^T=0.0, n=28) was not found to be significant when tested with a chi square analysis (χ^2=2.31, DF = 1, p=0.128).

Infection with *H. alvei* does not appear to be correlated with a decrease in body condition (t = 0.67, n.s) (appendix 3).

4.4 Discussion

Previous studies of reptile collections overseas have found that the prevalence of *Salmonella* can vary greatly between collections, with some facilities having prevalence as high as 80% and others without any *Salmonella* excretion at all (Geue and Loschner 2002). In the present study we found a 22% prevalence amongst lizards at population A and only 3.6% prevalence at population B. There are a number of possible explanations for the variance seen in *Salmonella* prevalence between reptile collections. Firstly, we must remember that *Salmonella* is only intermittently shed from the gastrointestinal tract, often as a result of stress (Richards et al 2004). Future studies should consider repeated sampling to check whether the prevalence of *Salmonella* within population B remains at this low level. The present study was conducted during the relatively warm months of March–April; sampling during winter when the weather is colder and the lizards are likely to be more stressed may give a different result. The difference in prevalence between these breeders is significant and they were both sampled within a fortnight of each other, so we would expect that they would both be experiencing similar levels of seasonal stress.

Another possible explanation for the differences in prevalence between these two populations is husbandry. Transmission between captive reptiles could occur via a faecal-oral route due to poor hygiene, direct transmission from humans or consumption of contaminated food (Richards et al 2004). Captive reptiles are often kept in very close contact in cages where they share food and water bowls, and where there is frequently more than one species that may not naturally come into direct contact with each other.

Owners often handle many individuals in succession without washing their hands, and in general little separates cages other than wire mesh. It is therefore likely, given the environmental stability of *Salmonella* that once it has entered a captive population it will spread rapidly throughout it. Whilst this appears to have been the case for captive population A, the same cannot be said for population B. Individuals in population B were also more physically separated, with space between each cage rather than just wire mesh separating cages. Perhaps more significantly the individuals in population B were handled less by the owner, therefore reducing the likelihood of nosocomial *Salmonella* transfer between individuals.

An outbreak of *S.* Mississippi within captive population A approximately three months prior to this survey had resulted in the death of a number of geckos. Interestingly, we have found *S.* Mississippi in two six week old *N. elegans elegans* born after the outbreak had apparently ceased to result in fatality. Reptiles may become infected with *Salmonella* before birth while in the ovary, oviduct or cloaca (Mermin et al 2004; Richards et al 2004), alternatively the lizard may have become infected after birth via contact with infected faeces. This highlights the potential for *Salmonella* serovars to cause significant disease in reptiles which have not had an opportunity to develop immunity. *S.* Mississippi resulted in significant illness amongst a number of individuals in this population. The period of illness and fatality occurred over a very short space of time. This suggests that *S.* Mississippi was introduced to this population which had previously been naïve to it. Those individuals that survived the initial infection developed immunity to this serovar and some were non-symptomatic carriers of the bacteria. The juveniles may have been exposed to a very low number of this bacterium, either prior to birth or after enabling it to develop immunity. Research by Connolly et al (2006) showed that the clinical signs demonstrated by sparrows experimentally inoculated with *Salmonella* were dose dependent.

Within these captive populations only two *Salmonella* serovars were found, *S.* Saintpaul and *S.* Mississippi. The two individuals shedding *Salmonella* in population B were shedders of *S.* Saintpaul. *S.* Mississippi was a much more common isolate within population A whereas *S.* Saintpaul was isolated from a single female *N. grayii*. Reptile breeders in New Zealand frequently sell and swap lizards in order to maintain their populations at a manageable size and in order to help prevent inbreeding depression.

These exchanges are often made without any prior health screening of the individual which could result in a spread of *Salmonella* serovars between populations. This may explain the presence of *S.* Saintpaul amongst both populations. A comprehensive survey of all the reptile breeders in New Zealand is advisable in order to determine whether this constant exchange has resulted in a pool of *Salmonella* serovars that are found commonly amongst all breeders.

Salmonella serovars Saintpaul and Mississippi belong to *Salmonella* subspecies I (Centers for Disease Control and Prevention 2007). They are also common pathogens of humans. During 2007, *Salmonella* Mississippi composed approximately 1% of the reported *Salmonella* isolates from human sources in New Zealand (Environmental Science and Research 2008). During the same period, *Salmonella* Saintpaul was responsible for approximately 2% of reported cases (Environmental Science and Research 2008). *Salmonella* Mississippi is an uncommon serotype in many parts of the world. It accounts for 1% of reported salmonellosis infections in the USA (Ashbolt and Kirk 2006). However, amongst residence of Tasmania, Australia the incidence of *S.* Mississippi infection is considerably higher than this. A recent study by Ashbolt and Kirk (2006) found that wildlife were acting as a reservoir of *S.* Mississippi in Tasmania and direct contact with wildlife was not required in order to contract the disease. This highlights the potential importance of lizards as a source of *Salmonella* in New Zealand, both to humans and other wildlife.

Members of *Salmonella enterica* subspecies II, IIIa, IIIb and IV are frequently isolated from reptilian sources (Sanyal et al 1997; Baumler et al 1998; Mitchell and Shane 2000). Serovars belonging to subspecies I are more commonly isolated from homeothermic species (Baumler et al 1998). In the two populations surveyed in this study only *Salmonella* serotypes belonging to subspecies I were isolated. Subspecies I serovars are often isolated more frequently than other subspecies in captive populations (Geue and Loschner 2002; Pfleger et al 2003). Captive reptiles may be more exposed to subspecies I serovars due to their increased contact with humans and their mammalian pets. However, in Chapter Two of this thesis we found *Salmonella enterica* subspecies I were most commonly isolated from native wild reptiles in NZ also.

In this study, only geckos belonging to the genus *Naultinus* were shedders of *Salmonella*. This is quite different to the result I obtained when sampling wild New Zealand lizards (Chapter 2). When studying wild lizards from offshore islands of New Zealand, we found that only geckos belonging to the genus *Hoplodactylus* were shedders of *Salmonella*. However, we were unable to obtain a large enough sample of wild *Naultinus* geckos to determine whether this was statistically significant (Chapter 2). In the present study, *Naultinus* geckos were far more abundant within the captive populations than *Hoplodactylus* species. Thus, it is possible, that infection with *Salmonella* is correlated with density. More research is required in order to verify this.

No previous systematic studies of *Salmonella* amongst captive or free-living geckos and skinks have been conducted in New Zealand. However, there have been reported cases of *Salmonella* amongst New Zealand native lizards and these have often occurred in the green geckos (*Naultinus*) (Twentyman 1999). In Chapter Two of this thesis I found that skinks were far more likely to be carriers of *Salmonella* than geckos. Only four skinks were available in this study so I am unable to make any conclusions about whether this would also be the case in captivity.

Infection with *Salmonella* did not appear to be having an adverse effect on health of lizards in this study. If the *Salmonella* infection had resulted in disease the lizards should have suffered some weight loss, thereby giving a smaller weight: length ratio than uninfected individuals, this was not found to be the case (appendix 1). This suggests that *Salmonella* infection within these lizards is non-symptomatic. *Salmonella* is commonly isolated from both reptiles and amphibians that show no symptoms of illness (Pfleger et al 2003).

In Chapter Two of this thesis the prevalence of *Salmonella* was tested amongst 703 wild, free-living individuals. It was found that 4.5% of all wild individuals tested were *Salmonella* shedders. This is significantly lower than the 11.3% of captive reptiles found to be positive for *Salmonella* excretion (χ^2=6.7, $p \leq 0.0123$, DF=1). I would therefore advise that captive reptiles may not form an appropriate source of individuals for release into the wild. Where it is essential to use these reptiles to form wild populations the captive facility should be chosen carefully and extensive and repeated disease screening should be conducted prior to release into the wild.

There is also a difference in the prevalence of *H. alvei* between breeders. 9.8 percent of population A was positive for *H. alvei* in contrast to 1.8% of population B. The causes for the differences in prevalence between breeders are likely to be the same as those for *Salmonella*. The mean prevalence of *H. alvei* in the captive reptiles sampled in this study was 5.2%. In Chapter Three of this thesis we sampled 103 wild lizards on Mana Island for *H. alvei* excretion. The mean prevalence of *H. alvei* amongst wild reptiles on Mana Island was 1.9%. The prevalence of *H. alvei* amongst captive New Zealand lizards is not significantly higher than that amongst wild endemic lizards ($\chi^2 = 0.474$, DF = 1, $p \leq 0.491$).

Little is known about the importance of *H. alvei* in veterinary or human medicine. We do not know if infection with this disease has any effect on reptiles. *H. alvei* infection of birds, however, is known to result in loss of appetite, decreased egg productivity, catarrhal enteritis, and fulminant septicemia (Janda and Abbott 2006). These results of this study and those of Chapter Three indicate that *H. alvei* is most likely a commensal organism of reptiles with no known significant to reptile populations. However, it is of limited but not negligible risk to humans and other wildlife.

The results from this study are consistent with the results we obtained in Chapter Three of this thesis which found a 0% prevalence of *Aeromonas* amongst wild New Zealand lizards. Previous studies of reptiles overseas have found prevalences of *Aeromonas* as high as 90% (Madsen 1996; Oros et al 2005; Turutoglu et al 2005). As was discussed in Chapter 3 of this thesis, *Aeromonas* species are common bacteria in aquatic environments (Mader 1996) and the majority of studies in which *Aeromonas* has been identified have looked at essentially aquatic animals such as Nile crocodiles (*Crocodylus niloticus*) (Turutoglu et al 2005), green anacondas (*Euncectes murinus*) (Miller et al 2004) and sea turtles (*Caretta caretta*, *Chelonia myads*, *Dermochelys coriaceae*) (Oros et al 2005). Captive bred lizards are unlikely to have contact with such infected water since their drinking supply is generally chlorinated water from the household tap (personal observation). Since I also found no *Aeromonas* amongst the 103 wild lizards caught on Mana Island (Chapter Three) nor in this study of 96 captive lizards, it would be fair to assume that New Zealand lizards are less at risk of infection with *Aeromonas* due to their terrestrial and often arboreal lifestyle. Given the high rates at which *Aeromonas* can be found in rivers, lakes and ponds, aquatic reptiles are more

likely to be exposed to *Aeromonas* and hence are more susceptible to becoming carriers of the disease.

Hafnia alvei and *Salmonella* species are known to cause significant disease in immuno-compromised human individuals. Disease symptoms include gastroenteritis, urinary infections and septicaemia. It would therefore be advisable that children, pregnant women and immuno-compromised individuals limit their contact with reptiles in order to prevent transmission of the disease. Lizard handlers and keepers should also be made aware of the risks and follow appropriate hygiene practices in order to limit the spread of disease.

Both *Salmonella* and *H. alvei* were found at prevalence rates significant enough to cause concern within captive populations. *Salmonella* was found at prevalence rates significantly higher within captive populations than in wild populations. For this reason, captive populations of lizards should only be used to form or supplement wild populations if there are no other viable options. Where captive populations are used as the source of new wild populations, wildlife managers should select the source of these lizards with care, as the prevalence of disease appears to vary between breeders. Disease screening should also be an essential part of any such translocation.

In both of these captive collections *Salmonella* and *H. alvei* were only found in *Naultinus* geckos. Reptile owners should therefore be careful about mixing species in their collections. Where possible, species should be separated into their own cages or at the very least cages should only contain individuals of the same genus. Handling of lizards should also be limited in order to prevent nosocomial transmission of *Salmonella* and *H. alvei* between lizards in a collection. Since *Salmonella* is highly environmentally stable I would suggest that lizard cages should be physically separated rather than being separated only by wire mesh. This would help to prevent the spread of *Salmonella* between reptiles in different cages.

More research is required in order to determine whether the constant movement of reptiles between captive collections has resulted in a common pool of *Salmonella* serovars which are found in all collections. Our research suggests that this may not be the case however, since *S.* Mississippi was found in lizards in captive population A but

not population B. In order to prevent the spread of *Salmonella* serovars between populations I would advise that all lizards which are moved between populations should be screened for *Salmonella* and *H. alvei* prior to movement.

References

Ashbolt R, Kirk MD. *Salmonella* Mississippi infections in Tasmania: the role of native Australian animals and untreated drinking water. *Epidemiology and Infection* 134, 1257-65, 2006

Awad-Masalmeh M, Romanik G, Herkal C. The occurrence of salmonellae in reptiles kept privately or in zoos in Austria. *Tierarztliche Umschau* 60, 70-4, 2005

Bager F, Petersen J. Sensitivity and specificity of different methods for the isolation of *Salmonella* from pigs. *Acta Veterinaria Scandinavica* 32, 473-81, 1991

Baumler AJ, Tsolis RM, Ficht TA, Adams LG. Evolution of host adaptation in *Salmonella enterica*. *Infection and Immunity* 66, 4579-87, 1998

Bradley T, Angulo F, Mitchell M. Public health education on *Salmonella* spp and reptiles. *Journal of the American Veterinary Medical Association* 219, 754-5, 2001

Cameron AR, Baldock FC. A new probability formula for surveys to substantiate freedom from disease. *Preventative Veterinary Medicine* 34, 1-17, 1998

Centers for Disease Control and Prevention. *Salmonella* surveillance: Annual summary, 2005. US Department of Health and Human Services, CDC, Atlanta, Georgia, 2007

Connolly JH, Alley MR, Dutton GJ, Rogers LE. Infectivity and persistence of an outbreak strain of *Salmonella enterica* serotype Typhimurium DT160 for house sparrows (*Passer domesticus*) in New Zealand. *New Zealand Veterinary Journal* 54, 329-32, 2006

Cooper JE, Jackson OF. Diseases of the Reptilia, Academic Press Inc, London, 1981

Ebani VV, Cerri D, Fratini F, Meille N, Valentini P, Andreani E. *Salmonella* enterica isolates from faeces of domestic reptiles and a study of their antimicrobial in vitro sensitivity. *Research in Veterinary Science* 78, 117-21, 2005

Environmental Science and Research. Human *Salmonella* isolates 2007. www.surv.esr.cri.nz/enteric_reference/human_salmonella, Wellington, New Zealand, 2008

Gartrell BD, Youl JM, King CM, Bolotovski I, McDonald WL, Nelson NJ. Failure to detect *Salmonella* species in a population of wild tuatara (*Sphenodon punctatus*). *New Zealand Veterinary Journal* 55, 134-6, 2007

Geue L, Loschner U. *Salmonella enterica* in reptiles of German and Austrian origin. *Veterinary Microbiology* 84, 79-91, 2002

Gopee NV, Adesiyun AA, Caesar K. Retrospective and longitudinal study of salmonellosis in captive wildlife in Trinidad. *Journal of Wildlife Diseases* 36, 284-93, 2000

Janda JM, Abbott SL. The Genus *Hafnia*: from Soup to Nuts. *Clinical Microbiological Reviews* 19, 12-8, 2006

Mader DR. Reptile Medicine and Surgery, W.B. Saunders Company, Philadelphia, 1996

Madsen M. Prevalence and serovar distribution of Salmonella in fresh and frozen meat from captive Nile crocodiles (Crocodylus niloticus). *International Journal of Food Microbiology* 29, 111-8, 1996

Mermin J, Hutwagner L, Vugia D, Shallow S, Daily P, Bender J, Koehler J, Marcus R, Angulo FJ. Reptiles, amphibians, and human *Salmonella* infection: A population-based, case-control study. *Clinical Infectious Diseases* 38, S253-S61, 2004

Miller DL, Radi ZA, Stiver SL, Thornhill TD. Cutaneous and pulmonary mycosis in green anacondas (*Euncectes murinus*). *Journal of Zoo and Wildlife Medicine* 35, 557-61, 2004

Mitchell MA, Shane SM. Preliminary findings of *Salmonella* spp. in captive green iguanas (*Iguana iguana*) and their environment. *Preventive Veterinary Medicine* 45, 297-304, 2000

Mitchell MA. *Salmonella*: Diagnostic methods for reptiles. In: Mader DR (ed) *Reptile Medicine and Surgery*. Pp 900-1. Saunders Elsevier, Missouri, USA, 2006

Nakadai A, Kuroki T, Kato Y, Suzuki R, Yamai S, Yaginuma C, Shiotani R, Yamanouchi A, Hayashidani H. Prevalence of *Salmonella* spp. in pet reptiles in Japan. *Journal of Veterinary Medical Science* 67, 97-101, 2005

Oros J, Torrent A, Calabuig P, Deniz S. Diseases and causes of mortality among sea turtles stranded in the Canary Islands, Spain (1998-2001). *Diseases of Aquatic Organisms* 63, 13-24, 2005

Otokunefor TV, Kindzeka BI, Ibiteye IO, Osuji GU, Obi FO, Jack AWK. *Salmonella* in gut and droppings of three pest lizards in Nigeria. *World Journal of Microbiology & Biotechnology* 19, 545-8, 2003

Pasmans F, Martel A, Boyen F, Vandekerchove D, Wybo I, Van Immerseel F, Heyndrickx M, Collard JM, Ducatelle R, Haesebrouck F. Characterization of *Salmonella* isolates from captive lizards. *Veterinary Microbiology* 110, 285-91, 2005

Pfleger S, Benyr G, Sommer R, Hassl A. Pattern of *Salmonella* excretion in amphibians and reptiles in a vivarium. *International Journal of Hygiene and Environmental Health* 206, 53-9, 2003

Quinn PJ, Carter ME, Markey B, Carter GR. Clinical Veterinary Microbiology, Wolfe Publishing, London, 1994

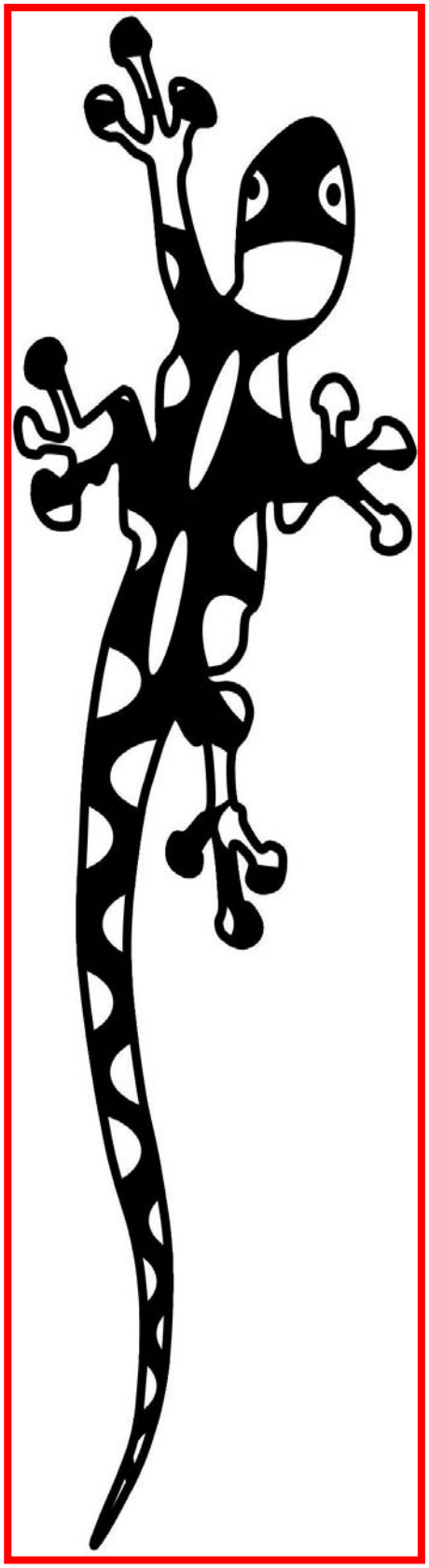
Richards JM, Brown JD, Kelly TR, Fountain AL, Sleeman JM. Absence of detectable *Salmonella* cloacal shedding in free-living reptiles on admission to the Wildlife Center of Virginia. *Journal of Zoo and Wildlife Medicine* 35, 562-3, 2004

Rodriguez LA, Vivas J, Gallardo CS, Acosta F, Barbeyto L, Real F. Identification of *Hafnia alvei* with the Microscan WalkAway System. *Journal of Clinical Microbiology* 37, 4186-8, 1999

Sanyal D, Douglas T, Roberts R. *Salmonella* infection acquired from reptilian pets. *Archives of Disease in Childhood* 77, 345-6, 1997

Turutoglu H, Ercelik S, Corlu M. *Aeromonas* hydrophila-associated skin lesions and septicaemia in a Nile crocodile (*Crocodylus niloticus*). *Journal of the South African Veterinary Association-Tydskrif Van Die Suid-Afrikaanse Veterinere Vereniging* 76, 40-2, 2005

Twentyman C. Diseases in New Zealand reptiles. *Surveillance* 26, 3-5, 1999



Chapter Five:

Microbiological isolation of *Salmonella* from New Zealand Herpetofauna

Microbiological isolation of *Salmonella* from New Zealand Herpetofauna

Abstract

This study assesses the efficacy of different isolation procedures used to detect six *Salmonella* serovars commonly found in New Zealand endemic reptiles. The aim of this study was to assess the efficiencies of two media and two temperatures for the isolation of *Salmonella* serovars obtained from New Zealand endemic reptiles. Seven hundred and three cloacal swabs obtained from lizards on New Zealand's offshore islands were tested on XLD and MacConkey agar and incubated at 27°C and 37°C. For 10 of the 11 serovars detected, 37°C was the more reliable incubation temperature. The exception was *Salmonella* Saintpaul, which was isolated better at 27°C than 37°C. XLD was a more effective media than MacConkey for all serovars except Mississippi and Saintpaul. Mississippi was isolated most effectively from MacConkey agar incubated at 37°C whilst *Salmonella* Saintpaul showed no preference for MacConkey or XLD agar. For all serovars isolated in this survey the choice of media appeared to be more important than temperature.

Six of the *Salmonella* serovars obtained in the survey of native lizards were further tested by inoculating both XLD and MacConkey agar with a 10µL suspension of known concentration. Each serovar was tested ten times with each method over a period of five days. Incubation temperature had no effect on the growth of these serovars, however XLD agar was found to be the more effective media for isolation of all six serovars. The colony counts for five out of the six serovars tested decreased gradually from days 1 to 5 of the experiment indicating that time from sampling may be a factor in the isolation of *Salmonella* serovars from reptilian sources.

Keywords: Incubation, isolation, lizard, MacConkey, New Zealand, reptile, *Salmonella*, Squamate, temperature, XLD,

5.1 Introduction

The process of isolating *Salmonella* from reptiles is prone to failure. Because *Salmonella* are shed only intermittently from most reptiles, it is often impossible to detect the presence of the disease in a carrier (Richards et al 2004). Even if one does sample a *Salmonella* carrier that is shedding at the time of sampling, *Salmonella* may be lost during culture (Busse 1995). If *Salmonella* is present as a major component of the sample then *Salmonella* may dominate the flora of the enrichment broth. When this is the case, then the chances of isolating and finding *Salmonella* are improved greatly (Fricker 1987; Busse 1995). If *Salmonella* organism numbers are low, they may be out competed in the enrichment broth and the probability of successful detection is greatly reduced (Fricker 1987; Busse 1995). This is when the choice of enrichment broth and plating media becomes important (Busse 1995). The aim of this study is to test different methods of identifying *Salmonella* in New Zealand reptiles to determine which method/s are most effective.

Bacteria are seldom uniformly dispersed within an individual, and gastro-intestinal shedding of *Salmonella* is often intermittent (Fricker 1987). For this reason, repeat samplings and larger sample sizes are frequently beneficial when seeking *Salmonella* in faeces. The presence of *Salmonella* in a sample does not necessarily guarantee that one will find *Salmonella* following microbiological isolation. Some healthy bacterial cells are often killed when added to selective culture media. Hence, if the bacteria are present in low numbers within the sample, this may result in the death of all *Salmonella* organisms present. Furthermore, bacterial cells which have been damaged due to environmental stress are more sensitive to selective agents than undamaged cells, and hence the likelihood of culturing these organisms is further reduced (Fricker 1987).

A number of steps are usually required for the detection of bacteria from reptile, bird, mammalian or environmental sources:

- Enrichment culture in a liquid medium
- Isolation as individual colonies on a selective solid medium
- Confirmation of the identity of the organism using morphological, biochemical and physiological tests

There are a range of microbiological isolation methods which can be used for the identification of *Salmonella* from a reptilian source. Following an enrichment step, the inoculated enrichment broth is plated onto a selective agar. The most commonly used selective agars for isolation of *Salmonella* from reptiles are MacConkey agar and XLD agar (Quinn et al 1994). Selective agars can affect the recovery of certain *Salmonella* serotypes (Chau and Leung 1978). XLD agar has been used successfully in the isolation of *Salmonella* from chelonians and squamates (Mitchell 2006). Chau and Leung (1978) found that the range of human pathogenic serotypes tested in their study grew better on MacConkey agar than XLD agar, although XLD agar was better at inhibiting the growth of many other Gram-negative intestinal bacteria.

During the microbiological isolation of *Salmonella*, the sample is typically incubated twice, once after the enrichment step and again after plating onto selective agars. Each of these incubation steps generally last 24 hours (Quinn et al 1994). Incubation of *Salmonella* is normally conducted at 37°C. This is in order to mimic as closely as possible the body temperature of a typical homeotherm. The body temperatures of New Zealand lizards are generally much lower because they are poikilotherms and the climate is cool. The preferred body temperatures of common geckos (*H. maculatus*) and McCann's skinks (*C. maccanni*) are 23.9±1.2°C and 24.5±1.8°C respectively (Besson and Cree 2007).

In recent years, there has been a substantial amount of research into the effect of temperature for the isolation of *Salmonella*. The majority of this research has considered the elevation of temperature from 37°C to 43°C (Carlson et al 1967; Van Schothorst et al 1977). Many of these studies have found that there is an increase in the number of *Salmonella* isolations from foods and faeces when incubation temperature is increased (Harvey and Price 1968; Carlson and Snoeyenbos 1972; Van Schothorst et al 1977). Other studies have found that some *Salmonella* serotypes are not able to reproduce at 43°C (Carlson and Snoeyenbos 1974).

No previous studies have considered the effects of lowering the incubation temperature of *Salmonella* samples from reptilian sources to mimic more closely the average body temperature of lizards, which is said to range from 18°C to 32°C (Mader 1996).

This study aims to:

- determine if XLD agar or MacConkey agar differ in the efficacy with which they isolate *Salmonella* serotypes from New Zealand lizards.
- assess whether identification of *Salmonella* serovars isolated from New Zealand endemic lizards is more easily achieved at 27°C or 37°C.

5.2 Methods

5.2.1 Survey

Each of the 703 cloacal swabs reported on in Chapter Two of this thesis were tested using each of the two media xylose lysine deoxycholate (XLD, Fort Richard Laboratories, Otahuhu, Auckland, NZ) and MacConkey agar (MC, Fort Richard Laboratories) and incubated at 27°C and 37°C. Positive samples obtained using each of these four methods were recorded.

5.2.2 Experiment

Six of the *Salmonella* serovars obtained from native lizards reported in Chapter Two of this thesis were further tested. All serovars were isolated from cloacal samples and stored in glycerol broths at -80°C after their isolation. The serovars used were *Salmonella* Bousso, Infantis, Mississippi, Warragul, and Mana from *Salmonella* subspecies I, and 43:z4,z23:- from *Salmonella* subspecies IV.

The isolation of each of the six serovars was tested using MacConkey agar (MC) and xylose lysine deoxycholate agar (XLD).

Overnight broth cultures of six of the *Salmonella* serovars were diluted in sterile saline. Serial dilutions were then made and 10µL of each was plated onto sheep blood agar (SBA) prepared from dehydrated media (Merck and Co., Inc, P.O. Box 100, Whitehouse Station, NJ 08889-0100, USA). Five replicates of each dilution were plated for all serovars. The plates were then incubated for 24 hours at 37°C. The colonies on these plates were counted and for each serovar the dilution that resulted in between 80 and 120 colony forming units (CFUs) per 10µL was selected for use in the following experiment.

Ten microlitres of the diluted bacterial suspension was used to inoculate the surface of XLD or MC agar and distributed homogenously around the surface of the plate using a sterilised glass spreader. Each serovar was tested on both XLD and MacConkey agar and at 37°C and 27°C. Ten replicates of each combination were made over five days with two experimental sessions run each day. All possible replicates were tested in each session; however the order that each replicate was tested was allocated randomly.

After incubation for 24 hours, the number of colony forming units on each plate was counted and recorded.

5.2.3 Statistical analysis

An analysis of variance was performed comparing colony counts with media, temperature and day, to determine whether the numbers of colonies formed were affected by any of the above variables. Results were analysed using one- and two-way ANOVAs on SAS 9.1.3 (SAS Institute Inc, 100 SAS Campus Drive, Cary, NC 27513-2414, USA).

5.3 Results

5.3.1 Survey

Each of the 703 cloacal samples obtained from lizards as described in Chapter Two of this thesis were tested using the four methods described. A total of 11 serovars were found.

Table 5.1: Number of samples found to be positive when tested using each of the four microbiological methods

	Percentage of positive samples found using the given methods				
Serovar	**37°C XLD**	**27°C XLD**	**37°C MacConkey**	**27°C MacConkey**	**Number of individuals host to this serovar**
Bousso	90.9	18.2	54.5	36.4	11
Mississippi	50.0	50.0	100.0	50.0	2
IV 43:z4,z23:-	60.0	60.0	40.0	20.0	5
Warragul	100.0	66.7	33.3	33.3	3
Infantis	100.0	100.0	100.0	100.0	1
Mana	100.0	0.0	50.0	0.0	2
6,7:z:-	100.0	100.0	100.0	50.0	2
48:k:-	100.0	100.0	100.0	100.0	2
Saintpaul	50.0	100.0	50.0	100.0	2
Typhimurium	100.0	100.0	100.0	50.0	2
4,12:-:1,2	100.0	0.0	100.0	100.0	1

For serovars Bousso and subspecies IV 43:z4,z23:- no technique detected 100% of *Salmonella* present. These serovars also had the largest sample sizes. For all other serovars at least one of the four techniques tested resulted in 100% *Salmonella* isolation. Table 5.1 shows that for most serovars isolated in this study 37°C was the more reliable incubation temperature. This was not the case for *Salmonella* Saintpaul which was isolated better at 27°C than 37°C, although this was not a significant result. XLD was a more effective media than MacConkey for all serovars except Mississippi and Saintpaul and 4,12:-:1,2. Mississippi was isolated most effectively from MacConkey agar incubated at 37°C whilst *Salmonella* Saintpaul showed no preference for MacConkey or XLD agar.

For most serovars detection of *Salmonella* was more affected by the choice of media than by the choice of temperature The exceptions were serovars Mana, Saintpaul and 4,12:-:1,2.

5.3.2 Experiment

I began this experiment with cell cultures containing between 80 and 120 colonies per 10µL. The general linear model detected a significant serovar effect. Despite having started with fairly uniform concentrations, the mean CFUs obtained for each serovar were markedly different (Table 5.2). The highest mean CFU count was obtained by serovar Bousso (148.8) and the lowest was obtained from serovar 43:z4,z23:-. Serovar Bousso and Mississippi had mean CFU counts higher than the concentrations prepared during the methods. Serovars Mana and IV 43:z4,z23:- had mean CFU counts lower than the concentrations prepared in the methods.

Table 5.2: Mean CFUs for six reptile-associated *Salmonella* serovars

Serovar	**Mean CFUs**
Bousso	148.8
Infantis	94.5
Mana	63.8
Mississipp	122.0
Warragul	118.2
IV 43:z4,z23:-	41.4

Temperature was not found to affect the growth of any of the serovars in this experiment ($F_{1,89}$=0.457, p=0.501). The overall mean CFUs at 27°C was 99.45 and 96.86 at 37°C. Different serovars were, however, found to behave differently at different temperatures ($F_{5,89}$ = 3.692, p= 0.004). Most serovars had a slight drop in CFUs from 27°C to 37°C. In contrast the mean CFUs for Mississippi increased slightly from 27°C to 37°C. The mean CFUs for Mana and Warragul remained similar at both 27°C and 37°C (Table 5.3).

Table 5.3: Mean CFUs growth at 27°C and 37°C

Serovar	Mean CFUs 27°C	Mean CFUs 37°C
Bousso	157.6	140.1
Infantis	108.4	80.7
Mana	64.1	63.6
Mississippi	117.9	126.2
Warragul	118.7	117.8
IV 43:z4,z23:-	30.0	52.9

The medium used had a very significant effect on the number of colony forming units produced ($F_{1,89}$=400.31, p=0.000). XLD (mean = 136.52) was found to be the more effective for the isolation of these serovars when compared with MacConkey agar (mean = 59.79). A serovar medium interaction was detected indicating that different serovars behaved differently at different temperatures ($F_{5,89}$ = 55.18, p=0.000). Figure 5.1 shows that for most serovars there is a marked increase in the number of colony forming units grown on XLD agar when compared with MacConkey agar. However, serovar Mana only had a mean increase of six colonies. No medium – temperature interaction was observed.

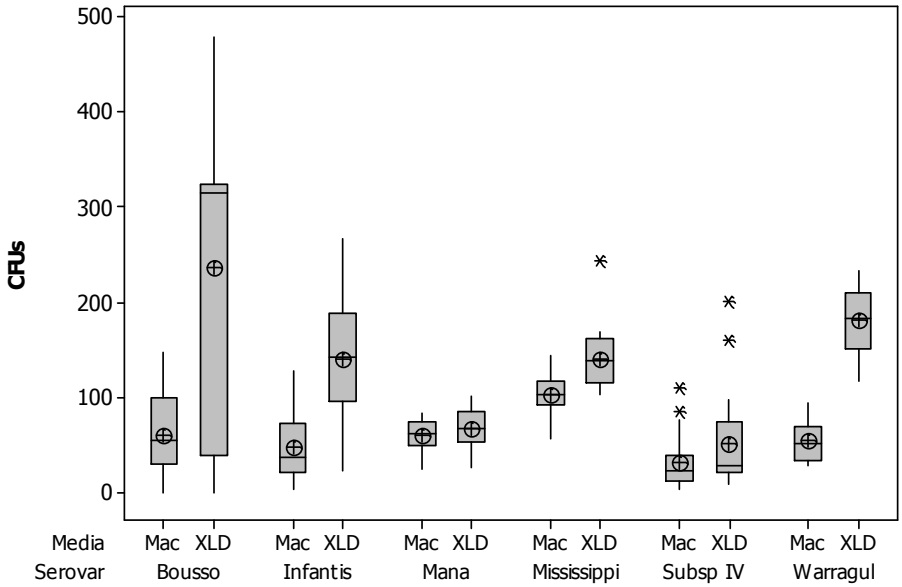


Figure 5.1: Boxplot of CFUs for reptile-associated *Salmonella* serovars grown on XLD and MacConkey agar (Mac).

There was a significant day effect suggesting that the number of colony forming units counted each day was variable ($F_{4,240}$=19.412, p=0.000). Figure 5.2 shows that for most *Salmonella* serovars there is a gradual decline in colony counts from days one to five. This was not the case for serovar Bousso which was highly variable in the number of colony counts obtained across the five days.

No order effect was found in this experiment ($F_{23,204}$ =1.205, p=0.243). It was therefore removed as a factor from the model. Time was found to be a factor in the number of colony counts obtained. This was entirely random.

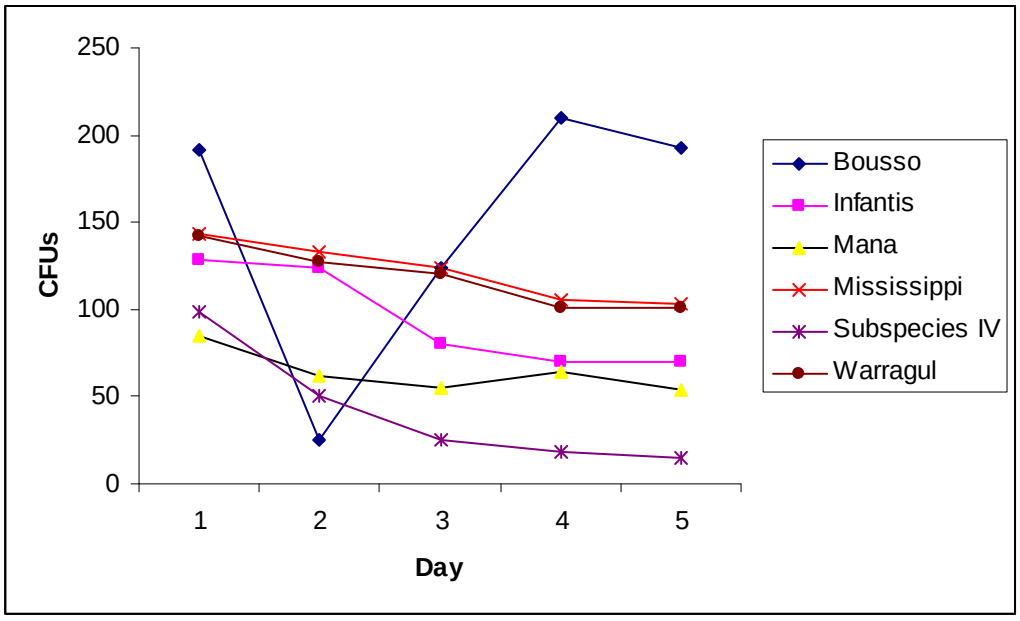


Figure 5.2: Mean CFUs grown each day for six reptile-associated *Salmonella* serovars

5.4 Discussion

There was marked variation in success of different culture media for *Salmonella* serovars isolated from reptiles in New Zealand. In my survey of wild reptiles, for some *Salmonella* serovars isolated, no culture method was completely effective. For most serovars, incubation at 37°C was more reliable, but for *Salmonella* Saintpaul, 27°C was more reliable although our sample size of this serovar was very low. For most serovars medium was more important than incubation temperature, with the exception of *Salmonella* Mana, Saintpaul and 4,12:-:1,2.

The type of medium used was important and XLD was more effective than MacConkey agar. These findings were confirmed by my experimental trial which showed that *Salmonella* serovar and culture media had significant and interactive effects on the success of microbial isolation. This experimental trial has found that XLD agar is far more effective at the isolation of five out of the six reptile-associated *Salmonella* serovars. Serovar Mana grew equally well on both MacConkey and XLD agar. Despite being isolated from reptiles in New Zealand, many of the serovars tested are also pathogens of humans. The results are at variance with those reported by Chau and Leung (1978) who found that *Salmonella* serovars from a human source were more easily isolated on MacConkey agar than XLD agar. Other studies on isolation of reptile-associated serovars have found that XLD agar is more effective than MacConkey agar (Mitchell 2006).

Most of the *Salmonella* serovars isolated from New Zealand reptiles, as outlined in Chapter Two of this thesis, belong to subspecies I. Members of *Salmonella* subspecies I are also common isolates of mammals, whilst other subspecies such as IIIa, IIIb and IV are often associated with reptiles (Pfleger et al 2003; Pasmans et al 2005; Mitchell 2006).

New Zealand geckos and skinks have considerably lower body temperatures than mammals. *H. maculatus* and *O. maccanni* have preferred body temperatures between 22.7°C and 26.3°C (Besson and Cree 2007). It might therefore be expected that *Salmonella* species which are specific pathogens of these hosts would have evolved an optimum growth temperature closer to their host's normal body temperature. Based on the results of this study, however, this is not the case. Five of the six isolates tested showed no difference in growth patterns at 27°C or 37°C. The isolates tested in this study are also common pathogens of mammals. It is perhaps unsurprising, therefore, that we found incubation temperature had no effect on the growth of any of the serovars tested in this study. The majority of *Salmonella* serovars I isolated from New Zealand reptiles belong to subspecies I. This group of Salmonella is generally thought to infect mammals. These findings suggest that *Salmonella* bacteria have evolved with sufficient adaptability to environmental temperature to survive in both poikilothermic and homoeothermic hosts.

Salmonella subspecies IV are isolated from cold-blooded reptiles overseas, however they are less often isolated from warm-blooded hosts (Pasmans et al 2005). Thus, it might have been expected that members belonging to this subspecies would grow better at 27°C than 37°C. This was not found to be the case in this study, as *Salmonella* subspecies IV 43:z4,z23:- grew equally well at both 27°C and 37°C. This suggests that this organism could be capable of infecting warm-blooded hosts.

Five of the six serovars tested showed a decrease in *Salmonella* colony counts from days 1 to 5. The concentration of serovars Mana and subspecies IV had decreased the most, dropping significantly in the 36 hours between preparation of the broth and the beginning of the experiment. All other serovars, except Bousso, decreased gradually in their production of CFUs from days 1 to 5. This highlights the importance of prompt investigation of *Salmonella* samples following collection, particularly if they are from a source in which the levels of *Salmonella* may be low. If *Salmonella* samples are left in storage for more than a week and the bacterial numbers in the original sample are low, all *Salmonella* may die before testing. It is advisable that, when isolating *Salmonella* serovars from New Zealand endemic reptiles, samples should be inoculated into an enrichment broth and incubated within 48 hours of collection in order to detect the likely low numbers of bacteria in these species.

Isolation of *Salmonella* serovars from New Zealand endemic reptiles appears to be best achieved using XLD agar. MacConkey agar will also detect all serovars. However, if the numbers of bacteria within the sample are low, the chances of isolating them seem to be enhanced using XLD agar. Incubation temperature had no effect on the isolation of *Salmonella* from New Zealand lizards. In order to increase the likelihood of isolating *Salmonella* from cultures where the numbers of bacteria may be low, it appears that samples should be tested as soon as possible after collection and preferably within 48 hours as the numbers of bacteria decline steadily over time. Based on the results of the survey together with the experiment I would advise that in order to achieve the highest likelihood of detecting all *Salmonella* present in a sample a number of different media should be used and samples should be tested as promptly as possible following collection.

References

Besson AA, Cree A. Preferred temperatures, activity responses and tolerance of cool temperature: a comparison between two sympatric lizards from southern New Zealand (*Hoplodactylus maculatus* and *Oligosoma maccanni*). In: '12th Biennial Conference of the Society for Research on Amphibians and Reptiles in New Zealand'. University of Otago, Dunedin, New Zealand p 261. (New Zealand Journal of Zoology) 2007

Busse M. Media for *Salmonella*. *International Journal of Food Microbiology* 26, 117-31, 1995

Carlson VL, Snoeyenbos GH, McKie BA, Smyser CF. A comparison of incubation time and temperature for the isolation of *Salmonella*. *Avian diseases* 11, 217-25, 1967

Carlson VL, Snoeyenbos GH. Relationship of population kinetics of *Salmonella* Typhimurium and cultural methodology. *American Journal of Veterinary Research* 33, 177-84, 1972

Carlson VL, Snoeyenbos GH. Comparitive efficacies of selenite and tetrathionate enrichment broths for isolation of *Salmonella* serotypes. *American Journal of Veterinary Research* 35, 711-8, 1974

Chau PY, Leung YK. Inhibitory action of various plating media on the growth of certain *Salmonella* serotypes. *Journal of Applied Bacteriology* 45, 341-5, 1978

Fricker CR. The isolation of *Salmonellas* and *Campylobacters*. *Journal of Applied Bacteriology* 63, 99-116, 1987

Harvey RWS, Price TH. Elevated temperature incubation of enrichment media for isolation of *Salmonellas* from heavily contaminated materials. *Journal of Hygiene Cambridge* 66, 377, 1968

Harvey RWS, Price TH. *Salmonella* isolation from reptilian faeces: A discussion of appropriate cultural techniques. *Journal of Hygiene Cambridge* 91, 25-32, 1983

Mader DR. Reptile Medicine and Surgery, W.B. Saunders Company, Philadelphia, 1996

Mitchell MA. *Salmonella*: Diagnostic methods for reptiles. In: Mader DR (ed) *Reptile Medicine and Surgery*. Pp 900-1. Saunders Elsevier, Missouri, USA, 2006

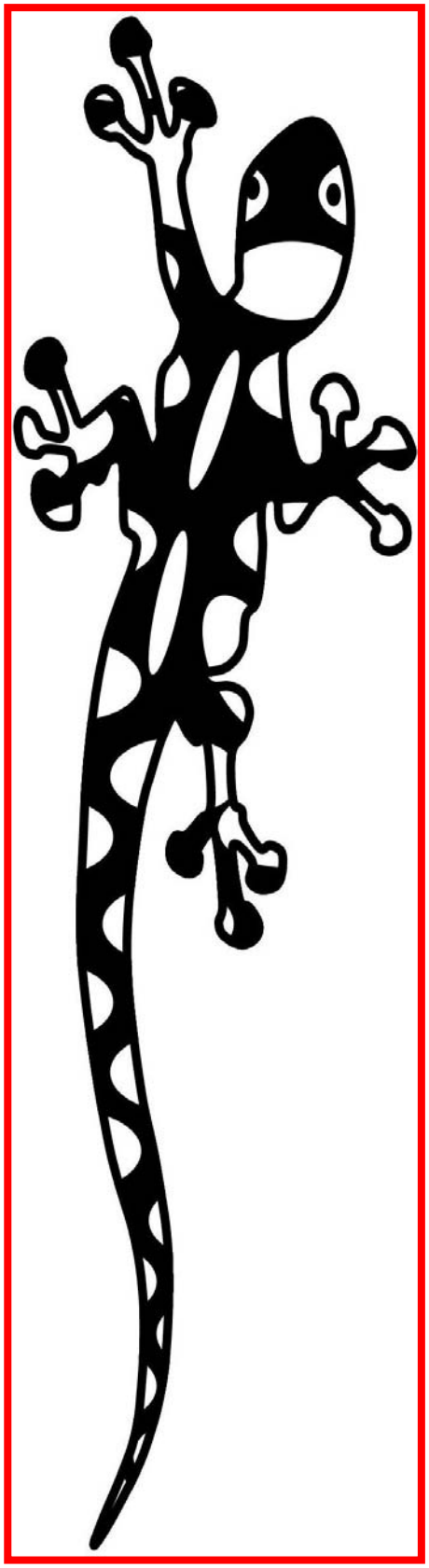
Pasmans F, Martel A, Boyen F, Vandekerchove D, Wybo I, Van Immerseel F, Heyndrickx M, Collard JM, Ducatelle R, Haesebrouck F. Characterization of *Salmonella* isolates from captive lizards. *Veterinary Microbiology* 110, 285-91, 2005

Pfleger S, Benyr G, Sommer R, Hassl A. Pattern of *Salmonella* excretion in amphibians and reptiles in a vivarium. *International Journal of Hygiene and Environmental Health* 206, 53-9, 2003

Quinn PJ, Carter ME, Markey B, Carter GR. Clinical Veterinary Microbiology, Wolfe Publishing, London, 1994

Richards JM, Brown JD, Kelly TR, Fountain AL, Sleeman JM. Absence of detectable *Salmonella* cloacal shedding in free-living reptiles on admission to the Wildlife Center of Virginia. *Journal of Zoo and Wildlife Medicine* 35, 562-3, 2004

Van Schothorst M, Van Leusden FM, Jeunink J, deDreu J. Studies on the multiplication of *Salmonella* in various enrichment media at different incubation temperatures. *Journal of Applied Bacteriology* 42, 157-63, 1977



Chapter Six:

Summary and Recommendations

Potential Pathogens in New Zealand native lizards: Summary and Recommendations

Since the arrival of humans there have been dramatic declines in the distribution and abundance of New Zealand lizards (Daugherty et al 1994; Towns and Daugherty 1994; Towns et al 2001). This is primarily due to human-induced habitat loss and degradation as well as predation by introduced mammals (Towns and Daugherty 1994). The conservation of New Zealand lizards has therefore become reliant on the formation of appropriate management strategies which frequently involve the translocation of our rarest endemic lizards to offshore islands or mainland "island" sites where introduced predators have been eradicated (Towns 1999; Towns et al 2001).

The presence and possible spread of disease is an important consideration when translocating reptiles to new areas and disease screening is required prior to the translocation of lizards to prevent the spread of disease to naïve wild populations (Woodford and Rossiter 1994). Unfortunately, many factors such as lack of funding, limitations of diagnostic testing and lack of a basic biomedical knowledge of the species involved, means that disease screening is not carried out as intensively or as accurately as required (Woodford and Rossiter 1994; Gartrell et al 2007). Current pathogenic threats to endemic New Zealand lizards are predominantly unknown and there is a need for focused research on the prevalence and distribution of potential pathogens amongst New Zealand native lizards in order to improve the management and long term survival of these species.

This research is the largest study of the prevalence of the potential pathogens, *Salmonella*, *H. alvei* and *Aeromonas* in endemic New Zealand lizards. The prevalence of *Salmonella* excretion by wild endemic New Zealand lizards was found at a mean prevalence of 4.7%. In this study *Salmonella* was not found on all islands, those islands on which it was found were host to between one and three *Salmonella* serovars which were not found on any other island in this study. This indicates that identification solely to the genus level is not sufficient in disease screening of lizards for translocation and all suspect *Salmonella* organisms should be sent to ESR for serotyping.

In this thesis *Salmonella* was found to have minimal impact on the health of lizards based on their snout-to-vent lengths. However, *Salmonella* has been demonstrated to cause significant disease in humans and naïve wildlife (Alley et al 2002; Tizard 2004; Wells et al 2004; Wybo et al 2004). At this stage the pathogenicity of these serovars in reptiles is unknown and no studies have yet considered the effect of introducing new serovars of *Salmonella* to naïve populations. Until further research has been conducted in this area we cannot be sure what effect introduction of new serovars of *Salmonella* to offshore islands would have. It therefore seems prudent that disease screening of *Salmonella* continues to be an integral part of the translocation process of any native lizard. Maintaining a database of *Salmonella* serovars found amongst populations would also help in identifying *Salmonella* serovars found within the source population and at the site of release, thereby reducing the risk of introducing novel *Salmonella* serovars to the population.

Salmonella serovars are known to behave differently in different species. No effect of *Salmonella* was identified in the lizards sampled in this study, however me must take care not to disregard any potential effects these serovars may have on other species. The clinical manifestations of salmonellosis depends upon the virulence of the serotype, nature of the lesion and innate immunity of the host (Gopee et al 2000). For example, serovar Typhi is known only to infect human hosts in contrast to *Salmonella* Typhimurium which can infect a range of hosts including humans, reptiles and mammals (Haraga et al 2008). In New Zealand *Salmonella* Typhimurium DT160 resulted in the mortality of humans and birds in urban areas between 1998 and 2001 (Alley et al 2002). The human associated *Salmonella* serovar Typhimurium DT195 resulted in the mortality of a number of hihi on Tiritiri Matangi Island following its introduction to the island by tourists (Ewen et al 2007). Further study is required in order to determine the pathogenicity of the serovars identified in this thesis in both reptiles and other wildlife.

Skinks were found to have significantly higher prevalences of *Salmonella* than geckos, which could be a reflection of their diet and more terrestrial lifestyle (Gill and Whitaker 1996). Diet may also be the cause of lizards living within 5 metres of the high tide mark being more prone to *Salmonella* excretion than those living more than five metres from the high tide mark. Lizards living on the coast have been observed eating carrion

and regurgitated sea-bird fluids which may form the source of infection. Alternatively, the increased *Salmonella* infection by some reptiles could be the result of different gastro-intestinal flora amongst species or different intestinal pH levels. More research is required in this area.

Salmonella serovars were also identified within captive populations of lizards. The prevalence of *Salmonella* within the two captive populations (11.5%) surveyed in this thesis was considerably higher than the prevalence found in wild populations (4.7%). There was also a difference in prevalence between the two populations of captive lizards which is consistent with results from overseas which have shown the prevalence of *Salmonella* excretion by captive reptiles to vary significantly between breeders (Geue and Loschner 2002). This suggests that some captive facilities may be more suitable as source populations than others and wildlife managers should research the source of captive lizards for translocation carefully.

Within these captive populations only two *Salmonella* serovars were isolated, *S.* Saintpaul and *S.* Mississippi. Reptile breeders in New Zealand frequently sell and swap lizards in order to maintain their populations at a manageable size and in order to help prevent inbreeding depression. These exchanges are often made without any prior health screening of the individual which could result in a spread of *Salmonella* serovars between populations. This may explain the presence of *S.* Saintpaul amongst both populations. A comprehensive survey of all the reptile breeders in New Zealand is advisable in order to determine whether this constant exchange has resulted in a pool of *Salmonella* serovars that are found commonly amongst all breeders.

Only *Naultinus* lizards were excreting *Salmonella* at the time of sampling in the captive populations, this is in contrast to the result I obtained from wild lizards on offshore islands. *Naultinus* geckos were far more abundant in the captive populations. This raises the possibility that *Salmonella* infection is correlated with density. It is known that stress which may be experienced from overcrowding often results in increased *Salmonella* excretion (Quinn et al 2002; Richards et al 2004). Reptile owners should therefore be careful about mixing species in their collections. Where possible, species should be separated into their own cages or at the very least cages should only contain individuals of the same genus and overcrowding should be avoided. Since *Salmonella*

is highly environmentally stable I would suggest that lizard cages should be physically separated rather than being separated only by wire mesh. This would help to prevent the spread of *Salmonella* between reptiles in different cages. Handling of lizards should also be limited in order to prevent nosocomial transmission of *Salmonella* between lizards in a collection.

All except one of the eleven *Salmonella* serovars isolated in this study belong to *Salmonella* subspecies I. Many *Salmonella* serovars belonging to subspecies I are common pathogens of humans. Overseas reptile-associated salmonellosis is more likely to involve infants than any other *Salmonella* infection, it is also more likely to be associated with invasive disease and lead to hospitalisation (Mermin et al 2004). Due to the stability of *Salmonella* in the environment, direct contact with the organism is not required for transmission of the disease. In New Zealand, wild geckos and skinks inhabiting the kitchens of homes in Otago resulted in an outbreak of salmonellosis caused by *Salmonella* Saintpaul. Appropriate hygiene measures should therefore be taken by wildlife managers involved in the translocation process and reptile owners in order to prevent infection with the bacteria.

A range of methodologies for isolation of *Salmonella* from a reptilian source was investigated in Chapter Five of this thesis. Two different media types (XLD and MacConkey agar) were incubated at 27°C and 37°C. All 703 cloacal swabs obtained from wild endemic lizards were tested using each of the four methods. For some of the serovars no single method detected 100% of *Salmonella* serovars obtained. For most serovars isolated in this thesis 37°C was the more reliable incubation temperature. This was not the case for *Salmonella* Saintpaul which was isolated better at 27°C than 37°C. XLD was a more effective media than MacConkey for all serovars except Mississippi and Saintpaul and 4,12:-:1,2. For most serovars media was more important than incubation temperature except for serovars Mana, Saintpaul and 4,12:-:1,2. Six of the *Salmonella* serovars obtained from native lizards reported in Chapter Two of this thesis were further tested using pure cultures at known concentrations. In this experiment XLD agar was found to more effective at isolating *Salmonella* serovars. Temperature did not have any effect on isolation of these six serovars. Isolation of *Salmonella* serovars from New Zealand endemic reptiles appears to be best achieved using XLD agar. MacConkey agar will also detect all serovars, however, if the numbers of bacteria

within the sample are low the chances of isolating them seem to be enhanced using XLD agar. Based on the results of the survey and the experiment I would suggest that a range of methodologies are required in order to identify all *Salmonella* serovars present in a sample.

Five of the six *Salmonella* serotypes showed a marked reduction in colony counts from days 1 to 5. This highlights the importance of prompt investigation of *Salmonella* samples following collection, particularly if they are from a source in which the levels of *Salmonella* may be low. If *Salmonella* samples are left in storage for more than a week and the bacterial numbers in the original sample were low, all *Salmonella* may have died before testing. It is advisable that, when isolating *Salmonella* serovars from New Zealand endemic reptiles, samples should be inoculated into an enrichment broth and incubated within 48 hours of collection in order to detect the likely low numbers of bacteria in these species.

No *Aeromonas* was found in any of the wild Mana Island samples or captive samples in this study. *Aeromonas* species are common bacteria in aquatic environments (Mader 1996) and the majority of studies in which *Aeromonas* has been identified have looked at essentially aquatic animals, such as Nile crocodiles (*Crocodylus niloticus*) (Turutoglu et al 2005), green anacondas (*Euncectes murinus*) (Miller et al 2004) and sea turtles (*Caretta caretta*, *Chelonia myads* and *Dermochelys coriacea*) (Oros et al 2005). The prevalence of *Aeromonas* among Nile crocodiles has been shown to be as high as 90% (Madsen 1996), dramatically higher than the 0% prevalence found in the current study. It would be fair to assume that given the high rates at which *Aeromonas* can be found in rivers, lakes and ponds, aquatic reptiles are more likely to be exposed to *Aeromonas* and hence are more susceptible to becoming carriers of the disease.

This was the first study into the prevalence of *H. alvei* in New Zealand endemic lizards. The present study found relatively low prevalences of *Salmonella* amongst wild (1.9%) and captive lizards (5.2%). At this stage little is known about the pathogenicity of *H. alvei* in reptiles, there have been no reported cases of reptile fatalities due to this bacteria and it has been found at low levels amongst tuatara and lizard populations. *Hafnia alvei* infection had minimal to no impact on the health of lizards in this study

and is not thought to be the cause of significant disease within endemic reptiles. Future screening of reptiles for *H. alvei* prior to translocation is probably not required.

Hafnia alvei is commonly found in the gastro-intestinal tract of mammals and is known to cause severe reproductive losses and disease in birds (Janda and Abbott 2006). In humans *H. alvei* is known to cause septicaemia and gastrointestinal infections (Proietti et al 2004; Janda and Abbott 2006). For this reason it is advisable that immuno-compromised people limit contact with lizards, especially captive bred reptiles which have been found to have prevalences as high as 10%.

A number of future research questions have been raised by this thesis. Recent research has failed to identify *Salmonella* in wild or captive populations of tuatara (Gartrell et al 2006; Gartrell et al 2007), however in this thesis I have identified *Salmonella* in geckos and skinks co-habiting with tuatara. I have also found that skinks are much more likely to be carriers of *Salmonella* than geckos in the wild. Future studies should consider why some reptiles are more prone to *Salmonella* infection than others. In doing this it would be prudent to consider comparing the gastro-intestinal flora of tuatara, *Salmonella* infected lizards and un-infected lizards in order to determine whether bacterial competition has any effect. The intestinal pH of lizard species and tuatara could also be considered as a potential source of this variation.

It was also observed in this thesis that lizards living within five metres of the high tide mark had much higher prevalences of *Salmonella* than those living more than five metres. This together with the different rates of *Salmonella* infection between geckos and skinks raises a possible dietary component to the infection. Future studies should consider sampling common food sources of the lizards in order to determine the potential source of the infection.

Salmonella was found in five of the eight islands surveyed in this thesis. Each of these islands was host to between one and three *Salmonella* serovars amongst the herpetofauna which were not found on any other island. Future studies should consider investigating *Salmonella* prevalence and the spatial distribution of its serovars amongst other species which also inhabit the island. Sea birds could be of particular interest due

to their often migratory behaviour and the fact that they are not confined to only one island.

References:

Alley MR, Connolly JH, Fenwick SG, Mackereth GF, Leyland MJ, Rogers LE, Haycock M, Nicol C, Reed CEM. An epidemic of salmonellosis caused by *Salmonella* Typhimurium DT160 in wild birds and humans in New Zealand. *New Zealand Veterinary Journal* 50, 170-6, 2002

Daugherty CH, Patterson GB, Hitchmough RA. Taxonomic and conservation review of the New Zealand herpetofauna. *New Zealand Journal of Zoology* 21, 317-23, 1994

Ewen JG, Thorogood R, Nicol C, Armstrong DP, Alley M. *Salmonella* Typhimurium in hihi, New Zealand. *Emerging Infectious Diseases* 13, 788-90, 2007

Gartrell BD, Jillings E, Adlington BA, Mack H, Nelson NJ. Health screening for a translocation of captive-reared tuatara (Sphenodon punctatus) to an island refuge. *New Zealand Veterinary Journal* 54, 344-9, 2006

Gartrell BD, Youl JM, King CM, Bolotovski I, McDonald WL, Nelson NJ. Failure to detect *Salmonella* species in a population of wild tuatara (*Sphenodon punctatus*). *New Zealand Veterinary Journal* 55, 134-6, 2007

Geue L, Loschner U. *Salmonella enterica* in reptiles of German and Austrian origin. *Veterinary Microbiology* 84, 79-91, 2002

Gill B, Whitaker T. New Zealand Frogs and Reptiles, David Bateman Limited, Auckland, 1996

Gopee NV, Adesiyun AA, Caesar K. Retrospective and longitudinal study of salmonellosis in captive wildlife in Trinidad. *Journal of Wildlife Diseases* 36, 284-93, 2000

Haraga A, Ohlson MB, Miller SI. Salmonellae interplay with host cells. *Nature Reviews Microbiology* 6, 53-66, 2008

Janda JM, Abbott SL. The Genus *Hafnia*: from Soup to Nuts. *Clinical Microbiological Reviews* 19, 12-8, 2006

Mader DR. Reptile Medicine and Surgery, W.B. Saunders Company, Philadelphia, 1996

Madsen M. Prevalence and serovar distribution of *Salmonella* in fresh and frozen meat from captive Nile crocodiles (*Crocodylus niloticus*). *International Journal of Food Microbiology* 29, 111-8, 1996

Mermin J, Hutwagner L, Vugia D, Shallow S, Daily P, Bender J, Koehler J, Marcus R, Angulo FJ. Reptiles, amphibians, and human *Salmonella* infection: A population-based, case-control study. *Clinical Infectious Diseases* 38, S253-S61, 2004

Miller DL, Radi ZA, Stiver SL, Thornhill TD. Cutaneous and pulmonary mycosis in green anacondas (*Euncectes murinus*). *Journal of Zoo and Wildlife Medicine* 35, 557-61, 2004

Oros J, Torrent A, Calabuig P, Deniz S. Diseases and causes of mortality among sea turtles stranded in the Canary Islands, Spain (1998-2001). *Diseases of Aquatic Organisms* 63, 13-24, 2005

Proietti PC, Passamonti F, Franciosini MP, Asdrubali G. *Hafnia alvei* infection in pullets in Italy. *Avian Pathology* 33, 200-4, 2004

Quinn PJ, Markey BK, Carter ME, Donnelly WJC, Leonard FC. Veterinary Microbiology and Microbial Disease, Blackwell Science Ltd, London, 2002

Richards JM, Brown JD, Kelly TR, Fountain AL, Sleeman JM. Absence of detectable *Salmonella* cloacal shedding in free-living reptiles on admission to the Wildlife Center of Virginia. *Journal of Zoo and Wildlife Medicine* 35, 562-3, 2004

Tizard I. Salmonellosis in wild birds. *Seminars in Avian and Exotic Pet Medicine* 13, 11-3, 2004

Towns DR, Daugherty CH. Patterns of range contractions and extinctions in the New Zealand herpetofauna following human colonisation. *New Zealand Journal of Zoology* 21, 325-39, 1994

Towns DR. *Cyclodina* spp. recovery plan. Department of Conservation, Wellington, New Zealand, 1999

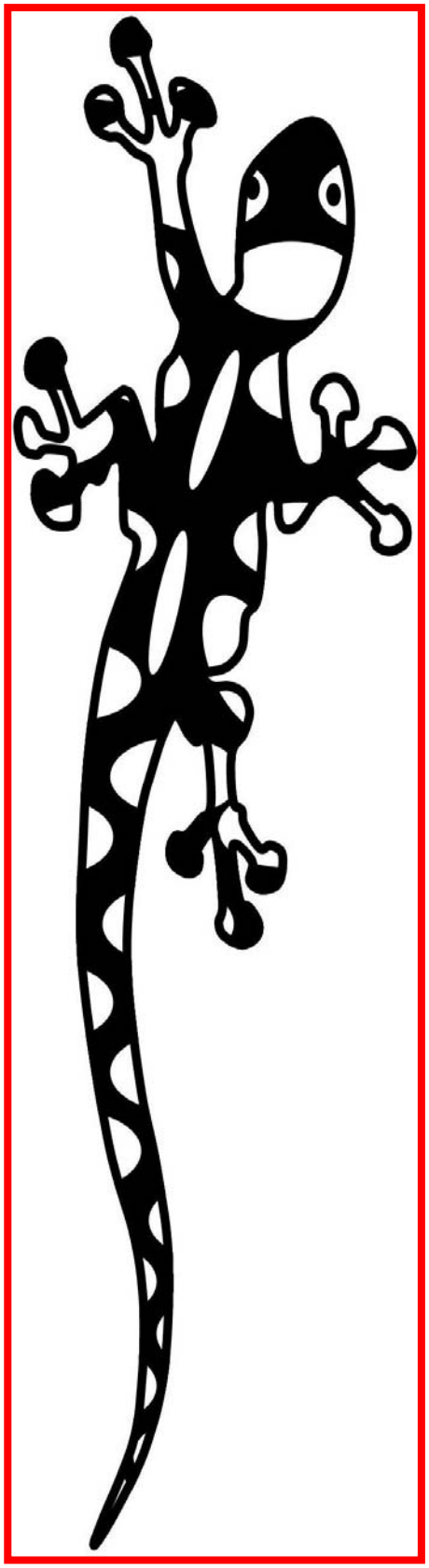
Towns DR, Daugherty CH, Cree A. Raising the prospects for a forgotten fauna: a review of 10 years of conservation effort for New Zealand reptiles. *Biological Conservation* 99, 3-16, 2001

Turutoglu H, Ercelik S, Corlu M. *Aeromonas* hydrophila-associated skin lesions and septicaemia in a Nile crocodile (*Crocodylus niloticus*). *Journal of the South African Veterinary Association-Tydskrif Van Die Suid-Afrikaanse Veterinere Vereniging* 76, 40-2, 2005

Wells EV, Boulton M, Hall W, Bidol SA. Reptile-associated salmonellosis in preschool-aged children in Michigan, January 2001 June 2003. *Clinical Infectious Diseases* 39, 687-91, 2004

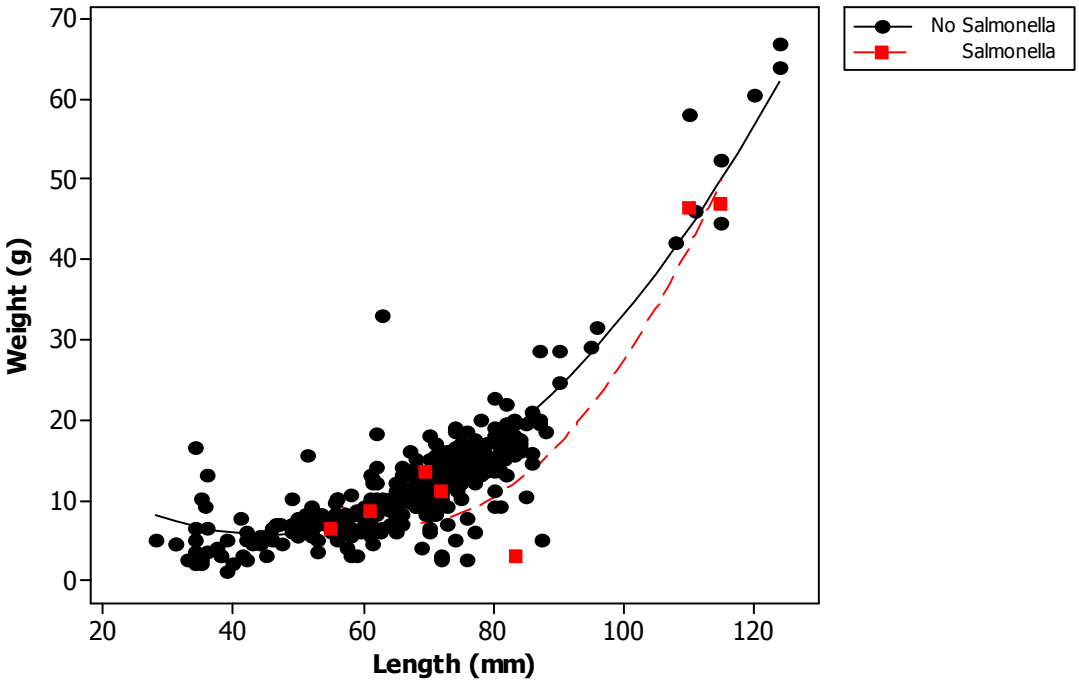
Woodford MH, Rossiter PB. Disease risks associated with wildlife translocation projects. In: Olney PJS, Mace GM, Feistner ATC (eds). *Creative Conservation: Interactive management of wild and captive animals*. Pp 179-99. Chapman and Hall, London, 1994

Wybo I, Potters D, Plaskie K, Covens L, Collard JM, Lauwers S. *Salmonella* enterica subspecies houtenae serotype 44 : Z(4), Z(23): - As a rare cause of meningitis. *Acta Clinica Belgica* 59, 232-4, 2004

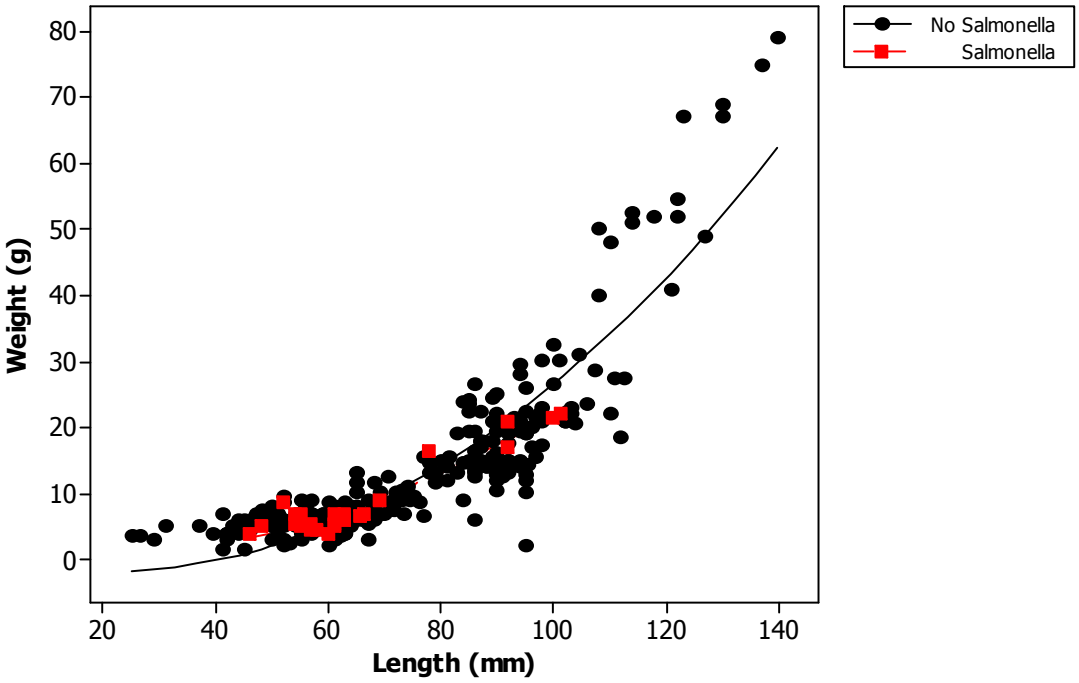


Appendices

Appendix 1

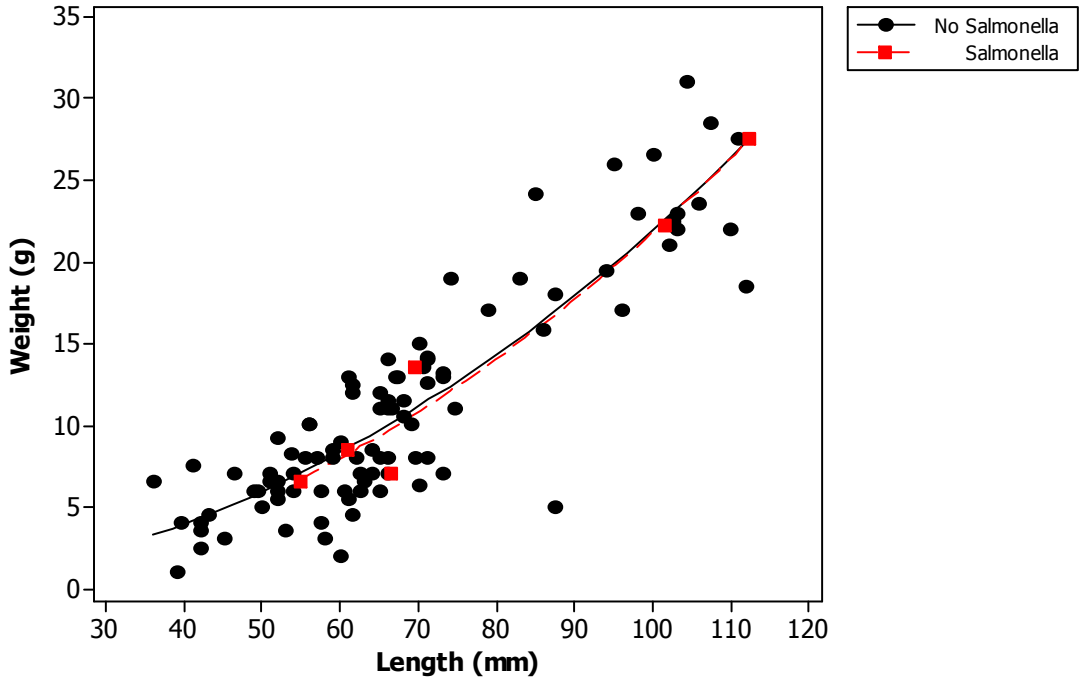


Appendix 1a: Body weights of *Salmonella* excreting and non-*Salmonella* excreting geckos on New Zealand offshore islands

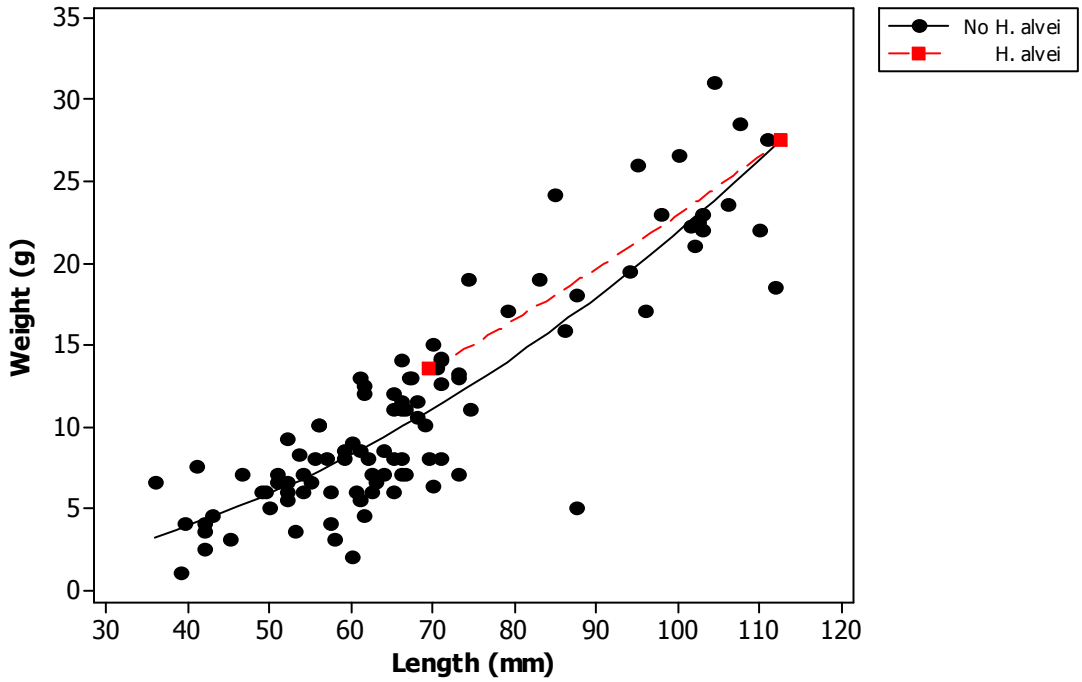


Appendix 1b: Body weights of *Salmonella* excreting and non-*Salmonella* excreting skinks on New Zealand offshore islands

Appendix 2

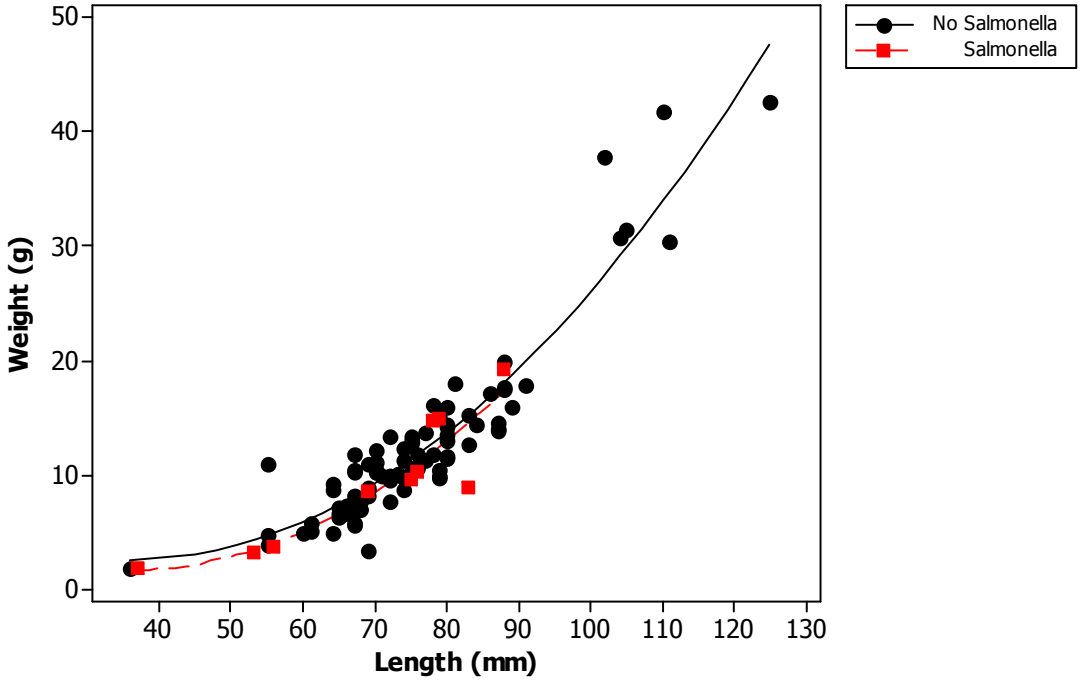


Appendix 2a*:* Body weights of *Salmonella* excreting and non-*Salmonella* excreting lizards on Mana Island, New Zealand

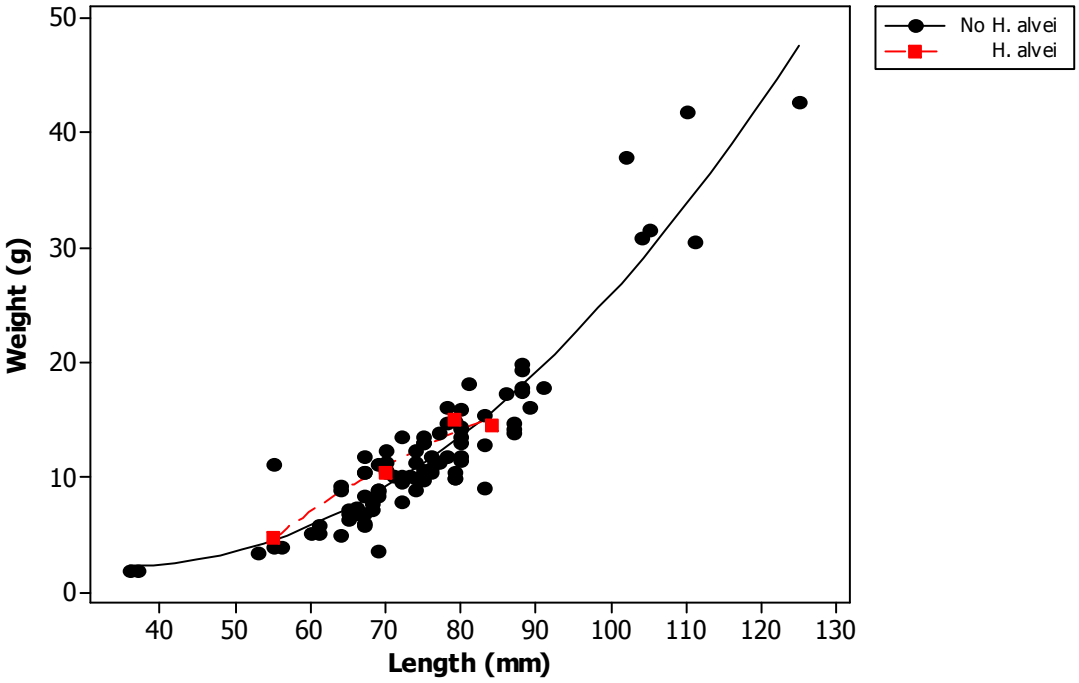


Appendix 2b: Body weights of *H. alvei* excreting and non-*H. alvei* excreting lizards on Mana Island, New Zealand

Appendix 3



Appendix 3a*:* Body weights of *Salmonella* excreting and non-*Salmonella* excreting lizards from two captive populations in New Zealand



Appendix 3b*:* Body weights of *H. alvei* excreting and non-*H. alvei* excreting lizards from two captive populations in New Zealand

Appendix 4

Appendix 4a: General linear model of main effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	853745.675(a)	35	24392.734	6.394	.000
Intercept	2312217.704	1	2312217.704	606.075	.000
Serovar	317705.907	5	63541.181	16.655	.000
Media	325746.490	1	325746.490	85.384	.000
Temperature	397.452	1	397.452	.104	.747
Order	105754.266	23	4598.012	1.205	.243
Day	68510.192	4	17127.548	4.489	.002
Time	8062.004	1	8062.004	2.113	.148
Error	778273.621	204	3815.067		
Total	3944237.000	240			
Corrected Total	1632019.296	239			

a R Squared = .523 (Adjusted R Squared = .441)

Appendix 4b: General linear model

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1553492.367(a)	150	10356.616	11.738	.000
Intercept	2312217.704	1	2312217.704	2620.596	.000
Serovar	317812.671	5	63562.534	72.040	.000
Media	353203.538	1	353203.538	400.310	.000
Temperature	403.004	1	403.004	.457	.501
Day	68510.192	4	17127.548	19.412	.000
Time	8062.004	1	8062.004	9.137	.003
Serovar * Media	224392.737	5	44878.547	50.864	.000
Serovar * Temperature	16286.171	5	3257.234	3.692	.004
Serovar * Day	209210.558	20	10460.528	11.856	.000
Serovar * Time	24402.171	5	4880.434	5.531	.000
Media * Temperature	1690.704	1	1690.704	1.916	.170
Media * Day	34383.942	4	8595.985	9.742	.000
Media * Time	145.704	1	145.704	.165	.685
Temperature * Day	2659.808	4	664.952	.754	.558
Temperature * Time	357.704	1	357.704	.405	.526
Day * Time	41362.308	4	10340.577	11.720	.000
Serovar * Media * Temperature	8735.771	5	1747.154	1.980	.089
Serovar * Media * Day	91479.908	20	4573.995	5.184	.000
Serovar * Media * Time	7149.571	5	1429.914	1.621	.163
Serovar * Temperature * Day	13796.642	20	689.832	.782	.728
Serovar * Temperature * Time	7910.271	5	1582.054	1.793	.122
Serovar * Day * Time	78050.642	20	3902.532	4.423	.000
Media * Temperature * Day	4408.692	4	1102.173	1.249	.296
Media * Temperature * Time	2.604	1	2.604	.003	.957
Media * Day * Time	35387.192	4	8846.798	10.027	.000
Temperature * Day * Time	3687.858	4	921.965	1.045	.389
Error	78526.929	89	882.325		
Total	3944237.000	240			
Corrected Total	1632019.296	239			

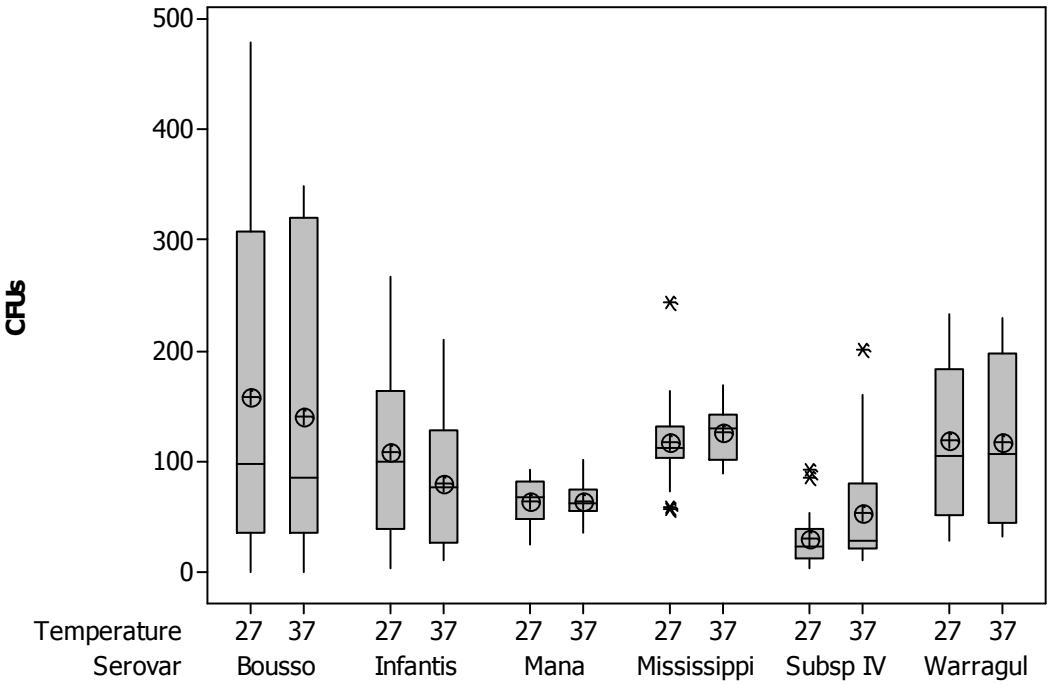
a R Squared = .952 (Adjusted R Squared = .871)

Appendix 4c: Mean *Salmonella* colony counts by time

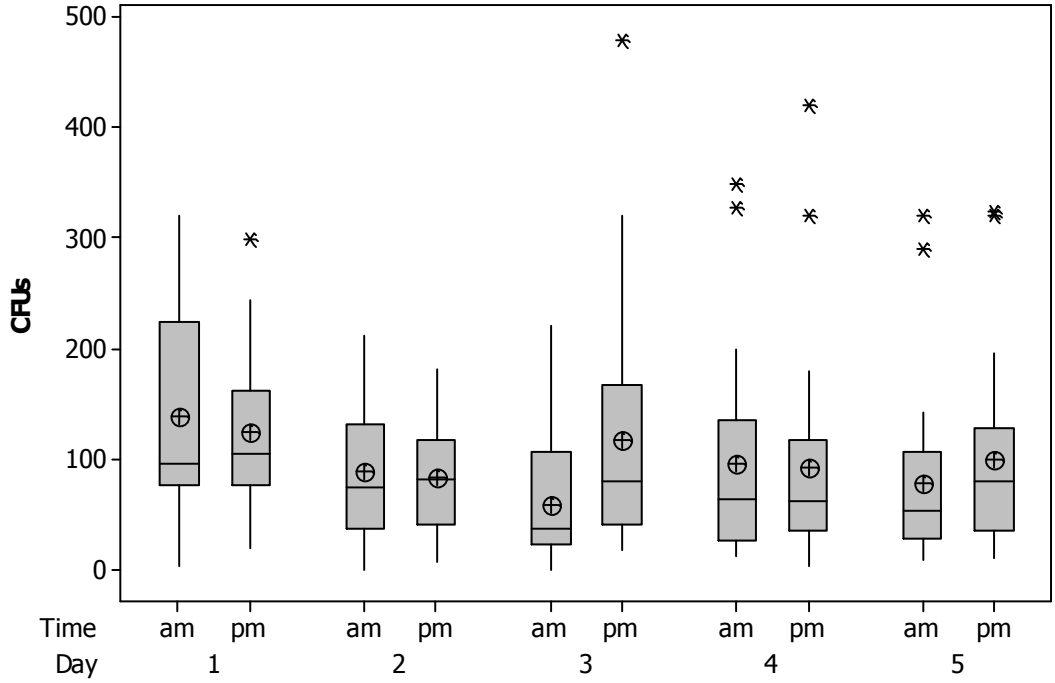
Time	Mean CFUs
Am	92.4
Pm	104.0

Appendix 4d: Mean *Salmonella* colony counts for each day of the experiment

Day	Mean CFUs
1	131.5
2	87.0
3	88.2
4	94.6
5	89.4



Appendix 4e: Colony counts of six *Salmonella* serovars incubated at two temperatures



Appendix 4f: Colony counts of *Salmonella* for each experimental session