Developing biosecurity strategies for an invasive reptile,

the plague skink *(Lampropholis delicata)* on Great Barrier Island

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"May your trails be crooked, winding, lonesome, dangerous,

leading to the most amazing view.

May your mountains rise into and above the clouds"

Edward Abbey

Abstract

Human exploration has a long history of removing geographical barriers and facilitating species dispersal. In the last 100 years alone, human activities and international trade have further circumvented these biogeographic boundaries, allowing species to access, establish and impact novel locations at an unprecedented rate.

The biological invasion process is composed of three distinct phases: arrival, establishment and spread. Once established, pest species across plant, invertebrate and vertebrate taxa can cause substantial harm to ecosystems and have severe socioeconomic impacts. Management of pest invasion has historically been reactionary, however, as the consequences have become increasingly apparent, conservation managers and invasion biologists have redirected their focus towards arrival prevention where possible. While efforts have been largely focussed on terrestrial vertebrates, there has been a distinct lack of progression in the development of effective control and eradication techniques for invasive herpetofauna.

The Australian plague skink *(Lampropholis delicata)* arrived in New Zealand in the late 1960s and has rapidly dispersed throughout much of the North Island. Additionally, it has established thriving populations on several off-shore islands in Auckland's Hauraki Gulf. It is now considered as New Zealand's only established pest reptile species since it's classification as an Unwanted Organism under the Biosecurity Act (1993) in 2010. The discovery of *L. delicata* on Great Barrier Island (Aotea) in April 2013 and subsequent biosecurity incursion response have provided the opportunity to test and develop effective control and eradication techniques in the absence of any existing management strategy for this species.

To test and evaluate techniques of detection for low density populations of *L. delicata*, we designed a surveillance system to locate the dispersal pathways and range limits of the plague skink population at Tryphena Wharf, Great Barrier Island. Approximately 750 traps were installed and serviced according to a mixture of standard and removal sampling designs. No skinks were found beyond the estimated boundary line which was supported statistically with 95% confidence.

To test and evaluate eradication techniques we designed a trapping grid system to parameterize the requirements of reducing the plague skink population to extinction using a highly adaptive and experimental operational framework. Two grids were set up using three standard herpetological monitoring trap types at varying spatial intensities according to the practicalities of the heterogeneous and often inaccessible landscape. Intensive trapping was implemented for several months before the effort and tools were statistically evaluated for their efficiency. Logistic ANCOVA indicates the effort was not sufficient, suggesting that greater intensity, greater efficiency or alternative methodologies are required.

To support a multi-facetted management approach a controlled laboratory trial was implemented to test the oral toxicity of acetaminophen to plague skinks. An 'Up-down procedure' was used to calculate the lethal dose required to kill 50% of the population (LD50). Acetaminophen was found to be lethal at a 550 mg/kg dosage, indicating its potential as a commercially viable toxicant.

An urgent focus on the development of effective techniques is required to manage invasive herpetofauna, which are becoming globally problematic at an increasing rate. Our operational field effort and research will make a practical and meaningful contribution towards the development of this field of invasion biology.

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Introduction

1.1 Introduction to invasion biology

The study of invasion ecology has emerged from a variety of associated and underpinning disciplines including agriculture, forestry, entomology, zoology, botany and pathology (Lockwood, Hoopes, & Marchetti, 2007). Due to concern of reduced crop yields, diseased livestock and transforming plant communities, scientists all over the world began to document the presence and observed impact of exotic species. This increased awareness and recognition of the negative effects of exotic species, and led to the development of the Scientific Committee on Problems of the Environment (SCOPE), catalysing a substantial increase in published literature on the subject (Fig 1.1) (Lockwood et al., 2007). A 2010 study examined the recorded global number of alien invasive species and found 542 species demonstrating evidence of negative biodiversity impact across 57 countries. Of these, 316 vascular plant, 101 marine and 44 freshwater fish, 43 mammal, 23 bird and 15 amphibian species were accounted for (McGeoch et al., 2010).
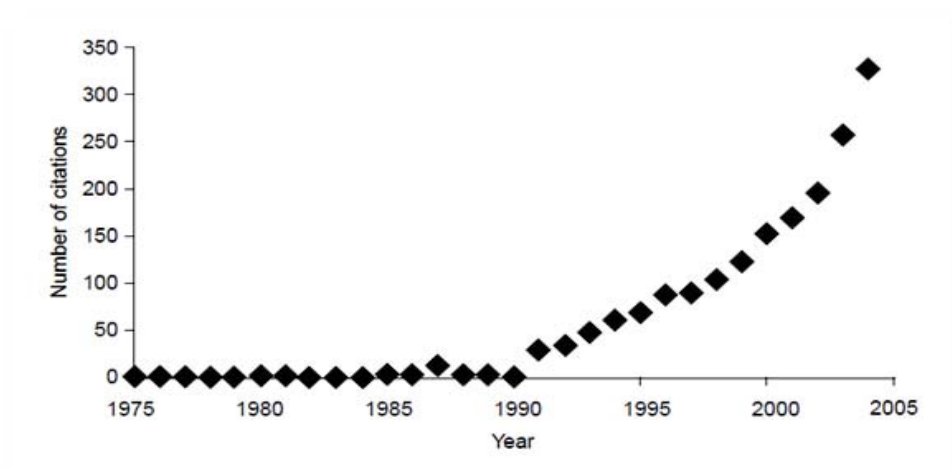
Figure 1.1: Number of citations returned from a search between the years 1975 and 2005 including the terms 'invas' and 'ecolog' (Lockwood et al., 2007)

Defining what characteristics or features constitute an invasive species appears to be somewhat problematic in the literature. While (Kraus, 2009) defines them as those causing harm that are transported and released outside of their native ranges via the activities of humans, either intentionally or otherwise, others argue that the word 'invasive' should only be ascribed to those species that have become abundant and expanded their geographic range (Lockwood et al., 2007). However for our purposes we shall combine the two and expand upon them to define an invasive species as one that has been transported to a novel geographical area, established a self-sustaining population, and causes either ecological, social or economic harm.

1.2 Introductions and pathways

In order for a species to be moved to a novel location, a transport vector and pathway must be made available to it. While species movement across geographical barriers is a natural occurrence (e.g. via wind and water currents), global human movements have facilitated the transfer of organisms across much broader geographic scales at much narrower temporal rates, enabling the establishment of species in areas that would have never naturally been available to them (Bergey et al., 2014).

A fundamental characteristic of invasion is the speed at which the geographical range of the population expands. In contrast with the natural dispersal process, human mediated dispersal often requires far less physiological resilience than that of natural dispersal processes due to extremely efficient transfer speeds (Neubert & Caswell, 2000).

R.N. Mack (2004) investigated the links between pathway strength and the likelihood of exotic species establishment. The number of species moving along a given pathway plus their respective establishment viabilities serve as indicators of pathway strength when combined with vector frequencies (Lockwood et al., 2007).

By undertaking a pathway analysis intervention can be better informed and managed in the case of future incursions. It is now known as a rule of thumb that that the unintentional transfer of taxonomic groups such as marine invertebrates and insects will generally occur as stowaway events or contaminated cargo. By contrast, mammal, fish,

bird and plant invasions are often the unintended result of an intentional introduction, with cargo movement resulting in the highest transfer of exotic frogs, snakes and lizards (Fig 1.2) (Kraus, 2009). Furthermore, historical pathway analysis of introduced herpetofauna reveals the importance of different pathways regionally, with North America and the Pacific receiving the greatest level of introduced species via the biocontrol and food pathways. The pet trade pathway poses the greatest risk to North America and Europe, while Australia's greatest invasion risk is via cargo (Kraus, 2009). Interestingly, the flow of invasion between areas is often uni-directional, with Carlton (1987) recording the inadvertent transportation of 14 marine species from the western Pacific to the Hawaiian Islands, only one of which was transferred bi-directionally.
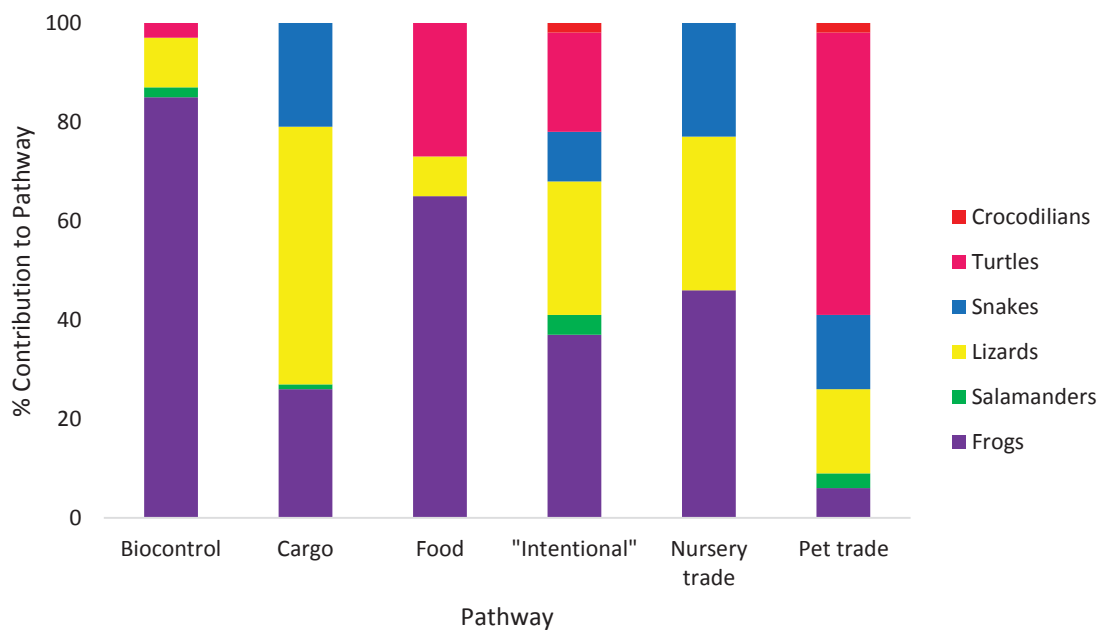
Figure 1.2: Frequency of taxonomic representation for each major pathway of herpetofaunal introduction. Chart replicated from Kraus (2009).

1.2.1 Unintentional vectors and pathways

Increases in the number and spread of exotic species have been strongly and positively associated with the increase in global trade and transport, particularly over the past 25 years (McGeoch et al., 2010). The nursery trade and ship ballast tanks are two vectors responsible for the inadvertent long-distance transfer of millions of organisms, and were estimated almost 20 years ago as transporting at least 10,000 species on any given trip (Carlton, 1987). However, since that time the rate of consumer globalization and free trade has increased the volume of worldwide shipping traffic by 400%, with the largest traffic increases occurring in the Indian and Pacific Oceans along with the Chinese seas (Fig 1.3) (Tournadre, 2014).
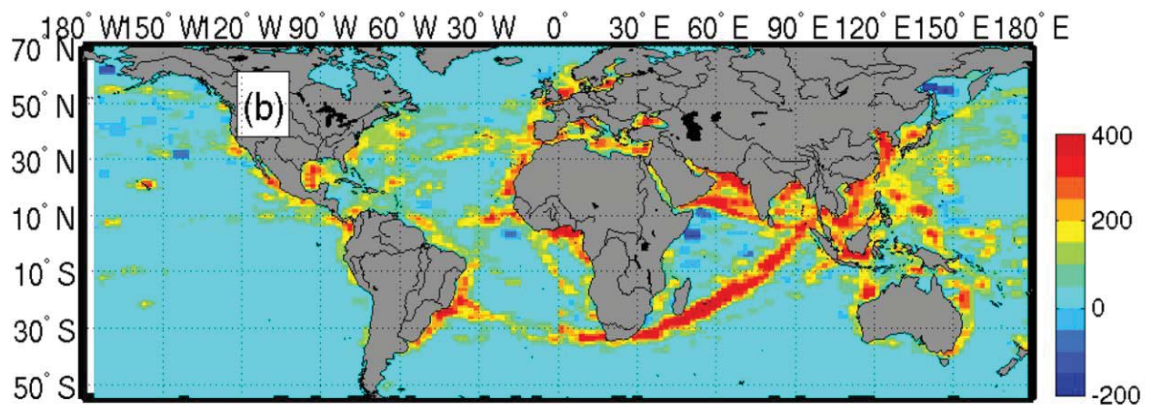
Figure 1.3: Evolution of ship traffic in percentage between 1992-2002 and 2003-2012 (Tournadre, 2014)

Similarly, the plant nursery trade relocates thousands of hitchhiking taxa to favourable habitats around the world (Bergey et al., 2014; Lockwood et al., 2007). This, combined with an often delicate transfer process due to the fragility of many nursery species, greatly increases the chances of survival of the contaminant or stowaway organism (Bergey et al., 2014; Kraus, 2009). A study investigating plant nurseries in the USA, Hawaii and Europe identified this pathway as a key mechanism for the long-distance dispersal of invasive land snails including the giant African snail (*Achatina Lissachatina fulica*). Despite efforts to remove them, the snail has now dispersed throughout 23

Brazil's 26 states, confirming that once established this species is very difficult to eradicate (Thiengo, Faraco, Salgado, Cowie, & Fernandez, 2007).

1.2.2 Intentional vectors and pathways

Intentional introductions are made when a species is perceived to be of use or value to humans (Kraus, 2009). Scientific research, international zoological breeding programmes, the pet trade, aquaria trade, agriculture, horticulture and biocontrol have all contributed to a substantial list of biological invasions. European snail species such as *Helix aspersa* have become a pest in many areas due to their international transfer as a food delicacy in the escargot trade. Similarly rainbow trout (*Oncorhynchus mykiss)* which by 1996 had been transported to every continent around the globe as a game fish species, has negatively impacted native trout species such as the Canadian westslope cutthroat trout via out-competition, hybridization and genetic introgression (Corsi, Eby, Barfoot, & Fleming, 2013; Fausch, Taniguchi, Nakano, Grossman, & Townsend, 2001).

1.3 Invasability – the major hypotheses

Invasion literature contains a number of hypotheses regarding the variance between communities to resist invasion events (Sol, 2000). In general, most studies have either approached ecosystem invasibility from one of two perspectives. From the perspective of the invader, trait differences between invasive and non-invasive species are examined across a variety of clades and ecosystems with the aim to understand and make predictions around the suite of traits that are likely to succeed in a given ecosystem (Moles, Gruber, & Bonser, 2008). From the perspective of the ecosystems ability to resist invasions, a variety of biotic and abiotic characteristics are examined to identify features and mechanisms which serve to invite or reject the advances of invading species. While controversy regarding the features that result in a successful invader persists, it appears that each situation is context-dependent, with a host of inter-related biotic and abiotic characteristics of both the exotic species and the recipient area determining invasion success. Understandably, these can be problematic to disentangle and as a result,

empirical evidence supporting many of the major hypotheses of the discipline is lacking (Moles et al., 2012).

1.3.1 The enemy release hypothesis

The enemy release hypothesis postulates that invaders can benefit from the lack of specialized natural enemies (e.g. predators, parasites and pathogens) in a novel environment (Colautti, Ricciardi, Grigorovich, & MacIsaac, 2004; Facon et al., 2006). This may play a key role in facilitating invasion success, particularly when an exotic species is found in a superior state (e.g. larger and more fecund) to both conspecifics of its native range and to native congeners in the new location. Numerous studies investigating this hypothesis in invasion biology theory have shown strong support for its validity across terrestrial, freshwater and marine ecosystems (Colautti et al., 2004).

1.3.2 Empty niche hypothesis

If no competitors or sympatric species exist in the area then a vacant niche can enhance the invasion process, sometimes resulting in dramatic changes to the novel ecosystem, as seen in New Zealand with the introduction of mammals (Moles et al., 2008). The empty niche hypothesis suggests that invaders are often able to make the most of the resources not favoured by natives (Kraus, 2009).

1.3.3 Environmental resistance hypothesis

Should a niche be occupied by a native species, the invader will only become successful if it is a superior competitor and can exceed the competition threshold set up by residents (Facon et al., 2006). These competition thresholds form part of the ecosystem resistance hypothesis (also known as the diversity-invasability hypothesis), which generally proposes that ecosystems with high biodiversity are more resistant to invasion than ecosystems with low biodiversity, via the mechanisms of competition, predation, parasitism and herbivory amongst others (D Antonio, Levine, & Thomsen, 2001; Jeschke et al., 2012).

D'Antonio et al. (2001) suggest that invasion resistance is a continuum which varies over time and depends on exogenous abiotic forces such as weather patterns and land management. The importance of abiotic factors in limiting the establishment of invaders in a novel area has been strongly emphasised by researchers over several decades, with harsh climatic conditions (e.g. toxic soil or prolonged drought periods) imposing simplistic, yet substantial constraints upon the invasion of less hardy species (D Antonio et al., 2001). Additional abiotic forces which can strongly determine invasion success include water provision, shelter and suitable climatic conditions. The physiological and behavioural capacity of the invader to tolerate these conditions will largely determine its persistence in the new location (Kraus, 2009; Lockwood et al., 2007).

1.3.4 Island invasibility hypothesis

The island susceptibility hypothesis states that invasive species are more likely to become established and have major ecological impacts on islands than continents (Jeschke et al., 2012; Sol, 2000). It is widely accepted and consistent with the environmental resistance theory due to islands naturally having fewer species than the mainland and therefore a greater vulnerability to invasion (Jeschke et al., 2012). Kraus (2009) describes the importance of landform size to herpetological invasion success by stating that small islands (<6000km²) have twice the rate of successful establishments than large islands (>8000km²), and quadruple that of continents. However, recent investigations demonstrate there to be very little empirical data to support this theory across a broader ecological spectrum, with only 11% confirming its validity due to the number of confounding factors that make this hypothesis difficult to test (Jeschke et al., 2012; Kolar & Lodge, 2001). Sol (2000) tested the hypothesis on introduced birds in two independent island-mainland comparisons; Australia versus New Zealand, and the USA versus Hawaii and found it was not supported by his outcomes. Similarly, Yiming, Zhengjun, and Duncan (2006) found human activity to be a strong determinant of invasion success for bullfrogs *Rana catesbeiana*, identifying that variance in hunting pressures between the island and mainland farms contributed to the ease with which the bullfrogs were able to invade the island farms (Yiming et al., 2006).

1.3.5 Disturbance hypothesis

The disturbance hypothesis is defined as "any relatively discrete event in time which disrupt an ecosystem, community or population structure and changes resources, substrate, availability or the physical environment" (Brokaw, Pickett, & White, 1985). It can include anything from stochastic weather events to regular herbivory, forest clearing or even alterations to soil ecology. Disturbance determinants of invasion include the intensity, frequency, duration, predictability, distribution and synergism of the event or regime (Lockwood et al., 2007; Sousa, 1984). Disturbance is thought to enhance the invasion process by opening up new ground for colonization (Moles et al., 2012). The majority of successful plant invasions in New Zealand correspond with areas subject to a regular disturbance regime or where resource availability has recently changed, for example pasture land and roadsides which are often dominated by a introduced species such as gorse (*Ulex europaeus*) and common broom (*Cytisus scoparius*) (Facon et al., 2006).

While this hypothesis has been widely accepted due to the apparent dominance of many introduced species at highly modified sites, it has also been dubbed as 'one of the most commonly accepted truisms in the field of invasion ecology' (Lockwood et al., 2007). While numerous studies have found disturbance to be positively correlated with the invasion of terrestrial plants, animals and marine organisms, the positive relationship between disturbance and invasion cannot be said for all taxa (Moles et al., 2012) In some instances, disturbance has been known to prevent invasion and in other examples the exotic species itself can be the driver of change, thus creating a disturbance or altering a disturbance regime. For example, the invasive earthworm *Lumbricus terrestris,* which has been known to dramatically affect soil structure, soil food webs and forest floor dynamics in northern North America (Ransom & Billak, 2015).

1.3.6 Propagule pressure hypothesis

Propagule pressure describes the number of individuals in an invading population and the frequency with which they are introduced to a novel location (Britton & Gozlan, 2013). It has become a central paradigm throughout invasion biology literature and proposes that the more individuals released into a location, the higher the likelihood of both establishment success and population persistence in the face of demographic and environmental stochasticity (Blackburn, Prowse, Lockwood, & Cassey, 2013; Britton & Gozlan, 2013; Lockwood et al., 2007; Simberloff, 2009a). However, the validity of this hypothesis appears to be somewhat contradictory throughout the literature. Where some report it as one of the best predictors of establishment success for a range of invading taxa (Blackburn et al., 2013), others feel that there isn't enough evidence to support it, stating that few studies have empirically tested effect of propagule size and number on establishment success, with none having addressed freshwater habitats or having been carried out over an extended period of time (Britton & Gozlan, 2013).

Propagule pressure interacts with environmental resistance to create spatial heterogeneity across various habitat types. When resistance is controlled largely by biotic factors, D'Antonio et al. (2001) theorized that high rates of propagule supply are more likely to be able to overcome it, due to the temporal and spatial variability of biological resistance. The rate of propagule supply may assist introduced populations to overcome challenges of Allee effects which reduce genetic fitness and population growth rates (Kolbe et al., 2004). High rates of propagule supply can serve to continually boost the genetic diversity of an exotic population, and in some cases lead to a higher level of genetic fitness in the invading population than its conspecifics still residing in its native habitat. For example, the brown anole lizard *(Anolis sagrei)* is a very well established and invasive species in Florida, first appearing in the late 1800's from its native home in the Caribbean islands. Genetic analyses indicate that at least 8 separate introduction events from independent source populations have occurred, resulting in a substantially greater genetic fitness level in the Florida population than any of the individual populations from which they came (Kolbe et al., 2004). This type of genetic 'reshuffling' can greatly enhance the invasiveness and impact of an exotic species in a variety of ways.

1.3.7 Invasion meltdown hypothesis

The invasional meltdown hypothesis states that the successful establishment of one or more invaders to a novel area can reduce ecosystem resistance, and make it easier for other arriving invaders to successfully establish (Kraus, 2009; Simberloff & Von Holle, 1999).

An example of this can be seen in New Zealand with banana passionfruit *(Passiflora tripartite* var. *mollissima)*, a noxious vine that is considered invasive in coastal forest patches throughout the country. A recent study demonstrated that the vine has formed invasive mutualisms with introduced bees for pollination purposes and with introduced mammals for seed dispersal. These relationships have exacerbated its spread and support the invasional meltdown hypothesis (Beavon & Kelly, 2015). However, many invaders may require a specific partner to carry out essential lifecycle functions, and an absence of the required species may mean that while it is able to survive the conditions of the novel environment, it is unable to reproduce and create the self-sustaining population required to progress to the next level of invasion (Lockwood et al., 2007).

1.4 The invasion process

Multiple ecological barriers must be overcome before a species can reach the final stages of invasion. The ultimate success of this process is hinged upon complex interactions between the localized ecology of the area, the life history traits of the species, the number of animals to arrive at that location and the extent to which human movement facilitates or inhibits it (Lockwood et al., 2007). Those that do overcome each of the barriers pose a substantial threat to local biodiversity via mechanisms of predation, competition, habitat alteration, hybridisation and as vectors of disease (Fig 1.4) (Mack et al., 2000).

The majority of exotic species will not have the ability to overcome these barriers to qualify as invasive. The 'tens rule' stipulates that 10% of exotic imported species will become naturalized, and 10% of naturalized species will become invasive (Kraus, 2009). While this postulate is based on statistical averages, the proportion of species that

transition from one stage to the next can vary between 5% - 20% (Lockwood et al., 2007), with substantial variation amongst herpetofaunal taxa (Kraus, 2009; Lockwood et al., 2007). Individual population dynamics may determine overall species invasiveness. For example a species that is classified as invasive in one location due to a negative impact on the local ecology, may be suppressed by local ecological constraints and consequently only qualify as exotic in another (Colautti & MacIsaac, 2004). Likewise characteristics or traits of that species may help it progress through one or more of the barriers, but act as a hindrance to the progress through other barriers. For example an adaptation to feeding in darkness may help fish in ballast tanks survive the transport phase of a journey, yet may also hinder their establishment in a novel environment with high water clarity (Kolar & Lodge, 2001). In this respect, the importance of researching the independent factors which affect the different stages of the invasion pathway should not be underestimated (García-Díaz, Ross, Ayres, & Cassey, 2014).
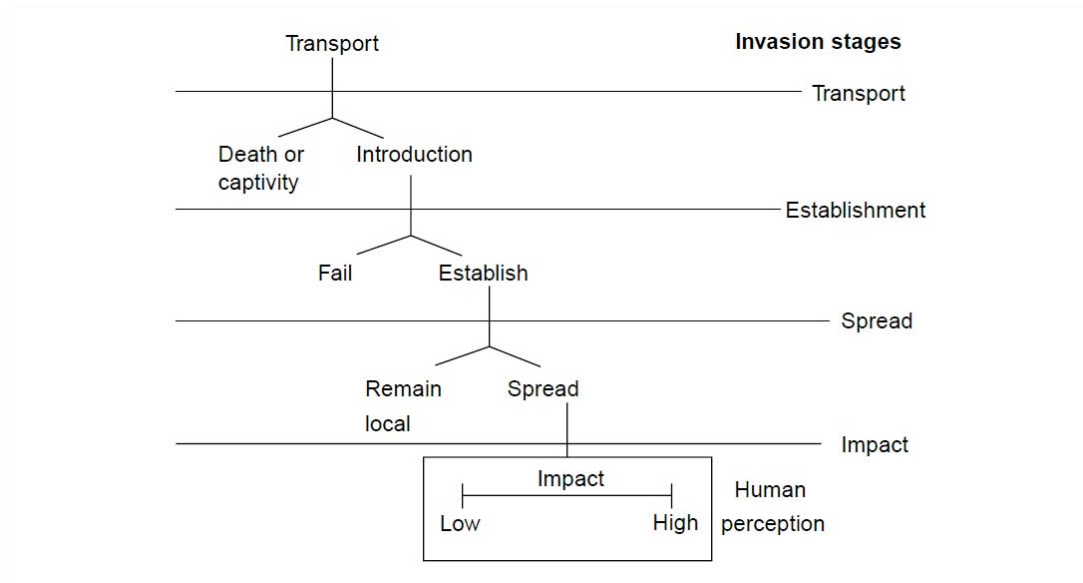
Figure 1.4: The invasion process from the initial transport phase through to impact (Lockwood et al., 2007)

1.4.1 Population growth & lag phase

The initial phase is to survive the process of being transported from the native range to a new location. If environmental conditions are favourable, a self-sustaining population must then be established, before growing in abundance and either expanding in geographic range or remaining localised. Species with high reproductive outputs and the ability to quickly populate a novel location will be less vulnerable to demographic stochasticity, however, a failure to adapt to novel environmental pressures may lead to negative growth or extinction (Sol et al., 2012).

A study examining 2760 introduction events from 428 introduced bird species found a high justification for an initial investment in adult survival over the value of rapid reproduction (Sol et al., 2012). This delay in reproductive effort is known as a lag phase and is characterised by a period of slow population growth followed by a rapid increase in growth rate (Fig 1.5) (Aagaard & Lockwood, 2014; Aikio, Duncan, & Hulme, 2010). Lag phases provide additional challenges to the discipline of invasion biology due to making it more difficult to predict, detect and manage invasive species (Aagaard & Lockwood, 2014). Life history traits and reproductive rates, along with numerous external ecological influences will generally determine the duration of this phase whereby the species itself is generally too low in numbers to be detected, or not deemed as a threat to either the local ecology or economy (Kraus, 2009). Very few authors have attempted to quantify lag phases, but those that have done so report that a duration of 50 years or more is not at all uncommon in plant species (Aagaard & Lockwood, 2014), with nearly all New Zealand weed species having at least a 20-30 year lag phase (Aikio et al., 2010). Aagaard and Lockwood (2014) examined lag phases for 17 species of exotic birds in Hawaii and found evidence of 14 species utilizing lag phase population growth patterns and concluded that while the duration is generally shorter than that of plants (averaging 11-15 years), the prevalence of lag phases are as common in birds as they are plants.

In some cases the rapid increase in population growth which follows the lag phase can result in an equally dramatic population crash, a concept known as 'boom and bust'. Although often associated with regular predator prey cycles, the mechanisms behind most collapses outside of obvious stochastic events (e.g. disease outbreaks) remain largely unqualified (Simberloff & Gibbons, 2004). This phenomenon has been seen to

occur in numerous invasive species which underwent rapid growth and were well established in the invasion process, before undergoing an apparent spontaneous decline, sometimes to the point of localized extinction (Simberloff & Gibbons, 2004). A well-documented example of this can be seen with the highly invasive cane toad *(Rhinella marina),* whose invasions in Australia, Puerto Rico, New Guinea, Bermuda, Barbados and the Philippines have all followed this pattern. While the rapid population crashes were generally attributed to food shortages or parasites and pathogens, Australia (the only country to empirically study the decline) ruled these out as causes of the poor body condition which was believed to have caused the decline of the long-established population quite literally overnight (Simberloff & Gibbons, 2004 Freeland, 1986 #57).
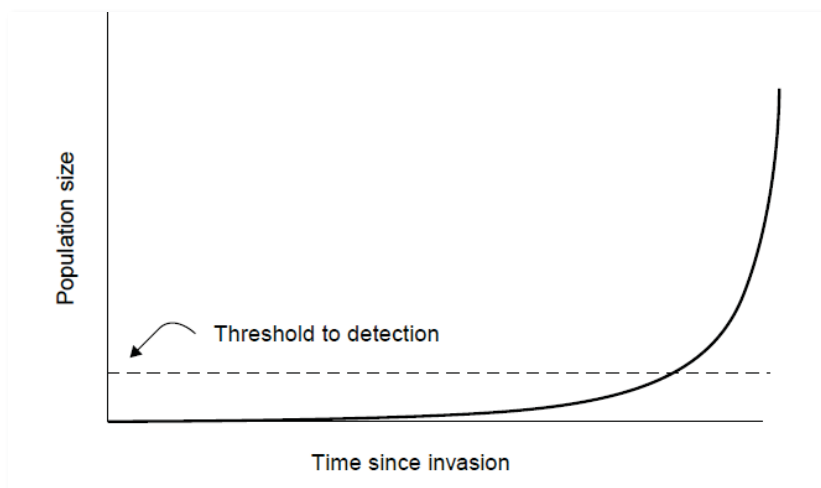
Figure 1.5 Illustration of how exponential population growth can lead to a lag phase in the invasion process (Lockwood et al., 2007)

1.4.2 Dispersal & range expansion

A growing population will naturally need to spread to acquire new resources either prior to or upon its habitat reaching carrying capacity. However, the drivers behind dispersal behaviours are complex and varied, and may be characterised by morphological, phenotypical, physiological or behavioural attributes at both species and individualistic scales (Clobert, Galliard, Cote, Meylan, & Massot, 2009). For example, the invading

species may be driven to disperse via population density thresholds, or conversely it may be genetically hardwired to disperse based on demographic factors such as sex or age class (Ryberg et al., 2004). Alternatively, dispersal patterns may be dominated by the pursuit of prey or the evasion of predators (Ramanantoanina, Hui, & Ouhinou, 2011). The combined effects of such internal and external drivers can generate complex species-specific movement patterns and strongly influence both colonization processes and population dynamics (Clobert et al., 2009). However, while a large number of studies have focused on the demographic contribution of dispersal behaviours, very few have addressed the morphological and behavioural traits that characterise dispersers (Clobert et al., 2009).

When undergoing range expansion a newly established population is often more dispersive than older populations (Duckworth, 2008). Western bluebirds (*Sialia Mexicana*) have been shown to strategize dispersal and range expansion by putting aggressive male dispersers with high fitness at the outer edges of their expanding range, and less aggressive males at the core of the established colony (Duckworth, 2008).

Phillips et al. (2008) identified an evolutionary component of dispersal dynamics when investigating dispersal rates in cane toads. They found an evolved shift in the individuals at the expanding frontline in the form of a steady increase in dispersal rate and identified it as a natural consequence of the dynamics of range expansion. The species is now known to achieve >55km/year in parts of their northern Australian range, compared with their initial rates of 10km/year (Phillips, Brown, Travis, & Shine, 2008).

1.5 Management

Over the past 20 years very little practical headway has been made in developing effective control and eradication methodologies of invasive species, despite an increasing awareness of their substantial environmental and economic impacts. The few attempts at control have been largely reactionary and are often skewed towards pests posing harm to agricultural interests, as is regularly noted in countries such as New Zealand who depend upon the primary production sector for a large proportion of its national income.

Preventing the arrival of novel invaders requires a clear understanding of how they may be transported and what parameters determine pathway success. Once a species is established firmly in a new location, it is exceptionally difficult to eradicate without significant investment in both time and money. A well designed risk assessment which breaks the problem of invasion down into stages may prevent the arrival, spread and establishment of an invader in the first place, and can therefore be easily justified both economically and ecologically (Kolar & Lodge, 2001; Kraus, 2009).

A lot of research upon species traits as predictors of invasive success has been implemented, and while there is a reasonable amount of supporting evidence for this approach, the complexity of the invasion process requires it to be broken down into constituent stages in order for management to be adaptive and therefore tackled effectively (Cassey, Blackburn, Duncan, & Lockwood, 2005).

The failure to prevent an invasion leaves management options limited to either eradication or long-term control. In order to be effective, an eradication should be implemented as quickly as possible. According to Simberloff (2003) a simple 'brute force' approach using any and every tool available (chemicals and manual trapping) can be highly effective at stopping an invasion before it starts. However, it is too often the case that a lack in biological data has provided an excuse for inaction, resulting in a considerable number of established invasions that could have been prevented with a 'quick and dirty' operation (Simberloff, 2003).

1.5.1 Adaptive management

Conservation biology has been described as a crisis discipline as it typically require decisions to be made in the face of considerable uncertainty (McCarthy & Possingham, 2007). Despite the growing volume of research dedicated towards the discipline of invasion biology, it remains a frustration of conservation managers to not have sufficient guidance on how and when to undertake an eradication operation for a new invader. Simberloff (2009b) describes this as being simply the "nature of the beast" due to the complexity of and variance between invasion events, adding that for any general rule there will likely be many exceptions.

Adaptive management incorporates knowledge from research and practice into conservation decision-making and action to enhance project success and learning outcomes (Salafsky, Margoluis, & Redford, 2001). An adaptive management technique can be undertaken in two ways; active and passive. While the former places explicit value on learning about the effectiveness of management by monitoring its outcomes, the latter approach incorporates serendipitous learning into the management plan as it evolves (McCarthy & Possingham, 2007).

The holistic integration of design, management and monitoring of a system both tests assumptions and generates the flexibility that allows managers to react when conditions change, supporting the needs of many conservation projects and leading to a deeper understanding of complexities to account for. Furthermore, by managing for a range of acceptable outcomes, catastrophes and irreversible negative effects may be avoided and the likelihood of success enhanced (Johnson, 1999; Salafsky et al., 2001).

Although this approach is so important to the development of conservation management, it has not been widely incorporated into the discipline, even though the benefits may be far reaching and ensure other conservation managers around the world with the same objectives avoid similar pitfalls in their own projects (Keith, Martin, McDonald-Madden, & Walters, 2011).

1.6 Invasive reptiles

1.6.1 General

Invasive reptiles are a sub-set of alien invaders whose impacts until recently have been largely speculative and highly underestimated (Kraus, 2009). The global invasion rate of exotic herpetofauna has been estimated as rising from 1 species per 35,000 years to 20-30 per year, equating to a million fold increase and attributable primarily to economic globalisation. Recent attempts to quantify the number of global herpetological invaders resulted in the documentation of 5745 introductions from 675 taxa, resulting in 1060 successfully established populations involving 322 species (Kraus, 2010). The rate at which herpetofaunal species spread throughout their novel environment is underpinned

by human activity, species traits and characteristics of the invasive range and is an important consideration of risk assessment and management strategies (Fig 1.6) (Liu et al., 2014).

However, in spite of this number, very few studies have been done to a sufficient standard to bring herpetological invaders and their extensive ecological impacts into the global limelight. This lack of research has largely been attributed to the fact that most reptile and amphibian species are highly cryptic, rendering them difficult to detect and their impacts challenging to quantify (Kraus, 2009).

1.6.2 Ecological Impacts

Of the 322 invasive herpetofaunal species that have naturalized in novel locations, only 14 have had impacts either demonstrated or inferred, and only three have been studied in moderate detail (*Boiga irregularis, R. marina and R. catesbeiana)* (Kraus, 2009). However, with two thirds of all herpetofaunal introductions having only occurred within the last 60 years, it's likely that over time a much larger pool of impact evidence will emerge as lag phases end and invasions become increasingly problematic around the world (Kraus, 2009). Those impacts which have been associated with invasive herpetofauna are consistent with most other invasive taxa, and include: species extinctions, food web disruptions, community alteration, ecosystem conversion and homogenization, changes to nutrient cycling and primary production, agricultural loss, building damage, disease epidemics, genetic contamination (via hybridization), changes in morphological, physiological and behavioural traits (Kraus, 2009; Lockwood et al., 2007).

Whilst invasive species impacts are most commonly studied at the population level, these impacts may inevitably lead to substantial changes in community composition, which if left to their own devices can lead to impacts at landscape, regional and global levels (Lockwood et al., 2007). This has been demonstrated by the brown tree snake, a species listed as one of the world's top 100 most invasive alien species due to its devastating impact upon the flora and fauna of Guam (Invasive Species Specialist Group

(ISSG), n.d.). Native to Australia, Indonesia, Papua New Guinea and the Solomon Islands, *B. irregularis* is believed to have hitchhiked to Guam on military aircraft in the 1940's or 50's. Within 40 years it had established and dispersed throughout much of the island due to a lack of natural predators, ample prey and a severe underestimation of its damage potential (Lowe, Browne, Boudjelas, & De Poorter, 2000; Wiles, Bart, Beck, & Aguon, 2003). In addition to decimating most of the island's native bird and lizard species, it caused cascading ecological effects by removing native pollinators (including flying foxes (*Pteropus mariannus))*, thereby resulting in the decline of many native plant species as well (Invasive Species Specialist Group (ISSG), n.d.). Unsurprisingly, the removal of the islands insectivores released the local arachnid population, which is now 40 times higher than adjacent islands, with a 'cobweb stick' now a necessary addition to any self-respecting local ecologist's toolkit (Hance, 2012).

The two other equally well researched herpetological invaders are the *R. marina* and *R. catesbeiana.* Brought in for the purpose of bio-control to tackle the sugar cane pests in northern Queensland, *R. marina* led to the widespread decline of native frog-eating predators (via its secretion of bufotoxin) along with significant declines in the rainbow bee-eater (*Merops ornatus)*, numerous gecko and small snake species and a variety of invertebrates via predatory and competitive mechanisms (Taylor & Edwards, 2005). By contrast, *R. catesbeiana* (a species native to America), has been introduced to more than 40 other countries primarily as a human food resource (Pitt, Vice, & Pitzler, 2005). Poorly managed populations have caused escapees to spread and cause major ecological damage to native herpetofauna, primarily via competition and predation, but also through the transmission of *Batrachochytrium dendrobatidis,* the chytrid fungus responsible for the deadly chytridiomycosis disease (Ficetola, Thuiller, & Miaud, 2007).

While the bulk of herpetofaunal invaders cause direct disruptions to food-web ecology via predation, competition and disease vectoring, many secondary impacts can be equally severe or even offer inadvertent benefits. Several studies have demonstrated the top-down effects of coqui frogs (*Eleutherodactylus coqui)* on forest nutrient dynamics via the reduction of endemic invertebrate populations (Beard, Vogt, & Kulmatiski, 2002; Sin, Beard, & Pitt, 2008). It is theorized that they, along with greenhouse frogs (*E. planirostris*) may act as nutrient sinks and ecological pathway

disrupters due to their ravenous consumption of native invertebrates which are converted into readily available nutrients for plants and microbes (Beard et al., 2002). While this could be considered as beneficial to the local flora, it may also offer a direct competitive advantage to invasive plants in a system where native species are better adapted to the nutrient-poor conditions with which they have evolved (Sin et al., 2008).

1.6.3 Economic & Social impacts

The cost of biological invasion to national economies and human health can be immense, and herpetological invaders are no exception to this rule. Between 1978 and 1997, more than 1600 power outages were caused by the brown tree snake in Guam due to its propensity to nest in electrical infrastructure. The resulting power surges cost the United States approximately between US$1 million and $4 million per year in expenses relating to the repair and maintenance of electrical products as well as substantial investment in backup generators (Rodda & Savidge, 2007). Furthermore, in spite of already having spent $300,000 to install a permanent snake barrier at the airport, in 2006 alone $5.76 million was spent in screening Guam's exports and an additional $210,000 at the other end in Hawaii for inspections (Kraus, 2009; Rodda & Savidge, 2007; Vice & Pitzler, 2000). The additional 'hidden costs' budget equates to approximately $400,000 per year and is set aside to cover the costs of detection dogs, running a shop and staff associated with the Guam Wildlife Services program (Vice & Pitzler, 2000).

As with the brown tree snake, the cane toad has placed considerable strain on the Australian economy thanks to their heavy predation upon honey bees. The resulting loss for bee keepers and reduced crop-pollination services has necessitated the design and construction of specially made beehive stands that are inaccessible to the toads. The AU$1 million for construction along with labour, transport and 5 yearly replacement coupled with the additional $1 million spent on researching various associated ecological parameters signifies substantial investment has gone into the understanding of and control of this invasive species (Kraus, 2009; Taylor & Edwards, 2005).

Other economic impacts incurred by alien reptiles and amphibians are stemmed from social issues including healthcare, for example a study on annual salmonella infections in the United States attributed 6% of cases to reptile and amphibian contact (Mermin et al., 2004), and children under four years old were reported to make up 50% of all emergency room snakebite victims (Fritts, McCoid, & Haddock, 1990).

Herpetological invasions have also led to substantial reductions in real estate values in areas where they are problematic. In Hawaii the coqui frog is responsible for an estimated US $8 million per year in lost in revenue for houses within 500 meters of an infestation, with individual properties estimated at losing 0.16% in value (Kraus, 2009; Taylor & Edwards, 2005).

1.6.4 Management of invasive herpetofauna

Herpetological invaders have been largely ignored from a management perspective, despite invasion events having become common and widespread throughout much of the developed world (Kraus, 2009). The few preventative approaches that have been taken have generally arisen from lessons learned the hard way. Under the Lacey Act (1900) the USA Fish and Wildlife Service now prohibit the import, export or transport of *B. irregularis* between States as a result of their impact in Guam (U.S. Fish & Wildlife Service, 2015). Additionally the importation of the African tortoises infected livestock and the white-tailed deer (*Odocoileus virginianus*) with harmful ticks vectoring heart water disease, which led the US Department of Agriculture (USDA) to prohibit them due to the risk they posed to domestic ruminants (Jenkins, 2007).

As a result of the bold proactive approach not often having been taken in herpetological invasions an unacceptable number of opportunities to develop and parameterize much needed detection, control and eradication techniques have been missed. As a result, the limited number of attempts to either control or eradicate alien reptiles and amphibians have failed to achieve their goals due to both a lack of reliable and efficient tools, as well as an inherent failure by ecologists and conservation management to recognize the potential impacts of novel 'herpeto-invaders' (Kraus, 2009).

However, in accordance with its reputation as a world leader in invasive species management, New Zealand has taken a preventative approach to the threat of harmful herpetofauna and prohibits the importation of all snake species under its Biosecurity (1993) and Hazardous Substances and New Organisms (1996) Acts. The unfortunate arrival, localized establishment and recent discovery of the European alpine newt (*Ichthyosaura alpestris*) to the Waikato region has resulted in a multi-agency led eradication attempt by the Ministry for Primary Industries, Department of Conservation and Waikato Regional Council. As there are no existing or proven eradication strategies for this species, the approach is underpinned by a highly adaptive, experimental and research based methodology (D. van Winkel, personal communication, February 2015).

1.6.5 Plague skinks

The plague skink (also known in New Zealand as the rainbow skink) *Lampropholis delicata* is a native reptile of Australia, where it thrives in human-disturbed habitats and is one of the most commonly found skink species in urban regions of Australia's east coast (Chapple, Whitaker, Chapple, Miller, & Thompson, 2013). Its range spans the country's east coast from Cairns to Melbourne, as well as being found throughout areas of Tasmania and coastal regions of South Australia (Fig 1.6) (Peace, 2004). Within these areas *L. delicata* occupies a wide range of habitats including supra-littoral zones, forest margins, woodland, coastal zones and is commonly found in urban gardens and human occupied areas (Chapple et al., 2015; Howard, Williamson, & Mather, 2003). Microhabitat refuges can take the form of wood piles, leaf litter and stones, where positive correlations between areas of dense leaf litter connected to patches of bare ground below a higher canopy and skink abundance have been noted (Peace, 2004). This clear preference for both discrete and exposed areas is most likely connected with the requirement to forage and bask with quick retreat options to avoid predation and aggressive competition. In its native range, *L. delicata* has proven to be a robust species, maintaining viable populations in spite of high level subjection to both predation and competition.
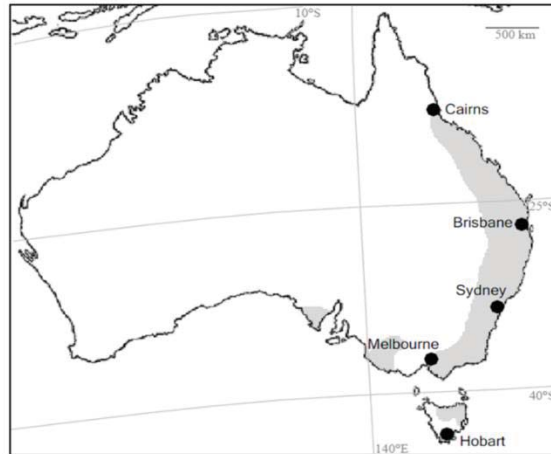
Figure 1.6: Distribution range of *L. delicata* in native Australia (Peace, 2004).

The plague skink is a small scincid lizard with adults measuring approximately 40-50mm from snout to vent (SVL), and a lifespan of 2-4 years. It is a heliothermic, diurnal forager with both active and passive foraging techniques (Howard et al., 2003). *L. delicata* is an opportunistic, generalist feeder, subsisting on a wide variety of invertebrates with a preference for insects and spiders (Chapple et al., 2015; Lunney, Ashby, Grigg, & Oconnell, 1989).

Like many of its native congeners *L. delicata* is an oviparous lizard, reaching sexual maturity in one year with eggs taking between 40-60 days to hatch from oviposition (Peace, 2004). Laboratory experiments have confirmed that embryos are capable of hatching up to 3 days early in response to predator cues such as vibration, with neonates dispersing quickly and immediately (Doody & Paull, 2013). As a gregarious species plague skinks often nest communally, with clutches having been anecdotally reported as containing up to several thousand eggs. Clutch size range can be markedly different between Australian and invasive populations (Australia 1-8, New Zealand 3-7, Hawaii 1-7 and Lord Howe Island 1-7) , and is significantly correlated with female body size, with mean individual clutch sizes varying between locations at 3.0-4.4, 4.75, 3.5-4.7 and 3.4 eggs respectively (Baker, 1979; Chapple et al., 2015; Peace, 2004). While hatchlings are typically observed in New Zealand over the summer months, an increasing number of hatchlings have been observed to emerge during the winter months, suggesting a high level of reproductive plasticity consistent with their native range.

1.6.5.1 Invasion history of the plague skink

An adaptive nature and resilience to human disturbance along with a flexible foraging ecology have enabled *L. delicata* to successfully invade locations outside of its native range (New Zealand, Hawaii and Lord Howe Island), with favourable microclimatic conditions containing an abundance of suitable refuge and food rapidly facilitating plague-like population densities. In all cases their introductions are thought to have been as inadvertent stowaways in freight, cargo or personal effects (Baker, 1979; Chapple, Miller, Kraus, & Thompson, 2013; Gill, Bejakovtch, & Whitaker, 2001).

In both Hawaii and Lord Howe Island, this species competes with native congeners and is suspected to have contributed towards the decline in both the vulnerable Lord Howe Island skink (*Oligosoma lichenigerum)* and Hawaii's native moth skink *(Lipinia noctua)* (Baker, 1979; Chapple et al., 2015; McKeown, 1978). It is assumed that the plague skink will have an impact on the native invertebrate community on Lord Howe Island along with the potential to introduce novel parasites and pathogens to native lizard species (Chapple et al., 2015).

L. delicata was first detected in New Zealand in the mid-1960s at the Otahuhu railyards of South Auckland (Chapple, Miller, et al., 2013). While it was suggested to have originated from a shipment of wooden railway sleepers, mitochondrial DNA analysis identified Tenterfield, New South Wales as the source population for one or more introductions of this species (Chapple, Miller, et al., 2013; Lever, 2003). While it remained localized to the Auckland region for almost 15 years, thereafter plague skink populations rapidly expanded across the North Island, establishing in the Waikato, Bay of Plenty, Coromandel, Wanganui, Palmerston North, the Manuwatu, Whangarei, Dargaville and Kaitaia regions (Chapple, Miller, et al., 2013). The last decade alone has seen an expansion of *L. delicata* across much of the northern half of the North Island (including several offshore islands) in an almost continuous range, via human mediated dispersal and high propagule pressure at North Island ports (Chapple, Miller, et al., 2013). Genetic analysis of 79 plague skinks intercepted between 2001 and 2008 were analysed, identifying 13 of them to be novel introductions from Australia (Chapple, Simmonds, & Wong, 2012). Within these areas of inhabitation, *L. delicata* is commonly found in highly urbanized and human modified areas such as industrial zones and

nurseries, as well as forested areas, open grasslands, farmland, scrublands and in coastal zones including sand dune sites and wetlands (Peace, 2004). The recent development of a species specific surveillance programme by the Auckland Council has identified plague skink populations in prolific densities at numerous nurseries and building supply yards throughout the region. These have been identified as high risk vector sites due to their nationwide product distribution including off-shore islands, via revegetation and building projects.

In spite of their presence in New Zealand for more than 40 years, a thorough understanding of the ecology and biology of plague skinks as an invasive species within indigenous ecosystems is still lacking, with little hypothesis based research having been undertaken on current and potential future impacts on native herpetofauna. However, it is assumed to be via their high reproductive capacity and subsequent competition for resources that *L. delicata* is expected to have the greatest impact within New Zealand ecosystems. Peace (2004) identified the copper skink (*Oligosoma aeneum*) as sharing the most ecologically similar niche with plague skinks due to their common microhabitat use and diurnal foraging behaviours as invertebrate generalists. However, given their substantial range expansion in the past decade alone along with their propensity to thrive in almost all habitat types, it could be hypothesized that *L. delicata* will adapt to whatever resources are available, potentially resulting in complete niche overlaps with all native species with which they come into contact. Furthermore, the degree to which plague skink populations are contributing towards elevated predator levels in all of their invaded regions remains poorly understood. Similarly, the invasive curious skink (*Carlia ailanpalai*) has reached extremely high population densities in Guam since its arrival in the 1960s, exacerbating the risk to native skink species by serving as a primary food resource to the brown tree snake and supporting its abundant population (Pitt et al., 2005). The same potential exists in New Zealand for rodent, cat, mustelid and hedgehog populations to grow in abundance in response to the high availability of *L. delicata*, and poses an increased threat to native lizard and invertebrate taxa (Reardon, 2014).

1.6.5.2 Plague skinks on Great Barrier Island

Great Barrier Island (Aotea) lies 90 kilometres north-east of downtown Auckland in New Zealand's Hauraki Gulf. Although it was given its English name by Captain Cook in 1769 (for the protection it provided to both the Gulf and mainland from the Pacific Ocean), the island has been continuously inhabited by local Maori since the 13th century when it was settled by people of Tainui and Arawa descent (Armitage, 2004; Department of Conservation, 2015). Great Barrier includes 23 islands and numerous islets and rocks (Armitage, 2004). Encompassing 285 square kilometres, 60% of the island's largely forested landscape is managed by the Department of Conservation (DOC) in order to protect its rich assemblage of biodiversity (Department of Conservation, 2015; Ogden & Gilbert, 2009). The rest of the island's ownership is private, with a permanent resident population of approximately 1000 people and visitor numbers swelling to in excess of 40,000 each year (Ogden & Gilbert, 2009). Two airlines service the island several times daily. Additionally, the Sealink ferry company run a barge service 5 days per week (increasing to 7 over the summer months) to both Tryphena and Port Fitzroy Wharves, which includes 2 freight transport days. There are no current biosecurity inspection regimes for any of the transport operators (Plate 1.1), although planes and ferries have signage advising passengers to watch out for pest stowaways. These transport vectors and pathways provide regular opportunities for invasive species to arrive on the island, the frequency of which facilitates sufficient propagule pressure for self-sustaining population establishments.

Under DOC's management the island is now free of many introduced pests including possums (*Trichosurus Vulpecula)*, mustelids *(Mustelidae* spp*.)*, hedgehogs (*Erinaceus europaeus*), deer *(Cervidae* spp*.)*, goats *(Capra hircus)* and Norway rats (*Rattus norvegicus*). Feral cats *(Felis catus)*, European rabbits *(Oryctolagus cuniculus),* ship rats *(R. rattus)*, kiore *(R. exulans)* and mice *(Mus musculus)* are still present in problematic proportions, placing the island's vulnerable taxa which includes the nationally threatened chevron skink (*O. homolonotum*), under increasing risk. An additional 12 species of lizards (3 skink and 2 gecko species) and 2 species of frogs (1 introduced and 1 native) inhabit the island making Great Barrier home to one of the richest assemblages

of herpetofauna per hectare, several of which are rare or extinct on the mainland (Armitage, 2004).

With the exception of Waiheke and Motutapu-Rangitoto islands, the presence of plague skinks on islands within the Hauraki Gulf was until recently largely unknown. A pilot survey to detect their presence or absence on Great Barrier Island was initiated in April 2013 by the Auckland Council, supported by the Great Barrier Island Local Board and implemented by the author (Wairepo, 2013). A breeding plague skink population was detected at Tryphena Wharf, Shoal Bay. Throughout the remainder of the year monitoring and a conservative level of intermittent trapping was implemented by the local Auckland Council Biosecurity Advisor while a plan to address the issue was formulated.

Plate 1.1: Sealink barge transporting cars, trailers and freight (H. Jamieson, 2013)

1.6.6 Management gaps

In recognition of the potential risk posed to our endemic herpetofauna, on July 7th 2010 *L. delicata* was removed from the Wildlife Act (1953) and listed as an Unwanted Organism by the Ministry for Primary Industries (MPI) under the Biosecurity Act (1993) (Ministry for Primary Industries, 2011).

Unfortunately, outside of the conservation community and Hauraki gulf boating-marine industry (see www.treasureislands.co.nz), there has been little success in communicating to the New Zealand public that this species is now considered a pest, with people commonly believing it to be a native species that is thriving in response to improved rodent control (Wairepo, 2013). Furthermore, due to its morphological similarities with the native copper skink, those that are aware of the plague skink's pest status are not generally equipped with the knowledge to distinguish between them. Species confirmation requires capture and identification via their single fronto-parietal head-scale which is located directly above the eye sockets (Fig 1.7), a challenging task for even experienced herpetologists. These factors combined with *L. delicata*'s propensity to travel and the regular opportunity to do so render numerous other ecologically sensitive off-shore islands increasingly vulnerable to invasion, particularly with Great Barrier Island now serving as a potential source population and gateway to the rest of the Hauraki Gulf (Reardon & Wairepo, 2014).
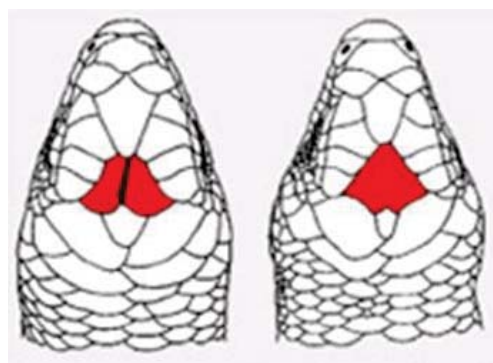
Figure 1.7: Identification of L. delicata (right) via single head scale compared with native New Zealand skink double scale (left) (Department of Conservation, 2011)

It is a reasonable to assume that the hypothesized future ecological impacts of plague skinks in New Zealand have until this point been largely underestimated, poorly understood, and therefore have not warranted the critical attention they require. This, along with the global lack of developed and tested management protocols, continues to provide the excuse to prolong the inaction, with very little progress having been made since the 1990s in identifying effective control methods in response to alien reptile invasions (Kraus, 2009). However, as plague skinks continue to increase in numbers and range throughout the mainland and its off-shore islands, it is of great importance that we begin to parameterize theoretical management protocols and develop novel detection and control techniques before this species finds its way to locations considered even more ecologically sensitive to the effects of exotic herpetofaunal invasion.

Furthermore, with conservation funds in New Zealand becoming increasingly limited it is crucial that biosecurity funding be channelled into projects that yield measureable beneficial outcomes within budget constraints. As no attempts to control plague skinks have yet been made on either mainland or island ecosystems it is important to determine if eradication and control efforts are even feasible in order to justify further conservation funding.

1.7 Research objectives

This study evaluates the methodologies employed to test and qualify the outcomes of a plague skink Biosecurity incursion response operation on Great Barrier Island. By combining field work with a laboratory toxicant trial, we attempt to parameterize the requirements of detecting and controlling plague skink populations in New Zealand by answering 3 key questions:

1. What is required to accurately delimit a plague skink population range?
2. Is it possible to eradicate a plague skink population through trapping alone?
3. Can plague skinks be controlled with a toxicant?

1.8 Thesis structure

This thesis has been divided into 5 chapters.

Chapter 1 introduces the discipline and driving principals of invasion ecology and describes the increasingly problematic issue of globally invasive herpetofauna.

Chapter 2 outlines the adaptive methodology utilized in an attempt to understand how far into the incursion area the plague skink population has dispersed. It then implement a power analysis to statistically support the success of our efforts.

Chapter 3 discusses the methodology used over a three month period to attempt to reduce the population and evaluate whether or not eradication is feasible using the tools that are currently available to us. The importance of adaptive management is highlighted and results are outlined.

Chapter 4 introduces the background, methodology and justification for an experimental laboratory trial to test the oral toxicity of acetaminophen to plague skinks. Baseline data for the lethal dose of 50% of the population (LD50) is provided along with

histology results performed and provided by Dr Cathy Harvey of the NZVP (New Zealand Veterinary Pathology).

Chapter 5 provides an overall synopsis which binds the previous chapters together and attempts to make sense of them within the context of practical management for invasion ecologists. It concludes with recommendations to progress the management of plague skinks on Great Barrier Island along with final concluding remarks of the study's operational and research outcomes.

Chapter 2

The development of an effective detection technique: Delimiting a plague skink *(Lampropholis delicata)* population range

2.1 Abstract

Context. It is now recognized that invasive alien species are one of the largest threats to indigenous ecosystems and global economies. Despite this there appears to be a widespread under-provision of biosecurity surveillance to prevent the arrival and establishment of exotic species. When border security systems fail to prevent the arrival of a novel invader, effective early detection systems may prevent its establishment. Species lag phases and unknown detection probabilities make early detection a challenging task and account for tremendous uncertainty faced by biosecurity managers with limited resources. The collection of presence/ absence data can be useful in biosecurity surveillance, however, imperfect detection can render such data unreliable for making inference, sound management decisions and scientific conclusions.

The arrival of the invasive plague skink (*Lampropholis delicata*) on New Zealand's Great Barrier Island was detected in April 2013 at Tryphena Wharf, however, due to a lack of effective rapid response reptile detection tools, the true population range remained unclear for the following 18 months.

Method. Between November 2014 and February 2015 approximately 750 invertebrate sticky traps were installed in an area surrounding Tryphena Wharf, across various transects and grids over an area encompassing 51,500m² to determine whether or not the population had dispersed beyond an estimated boundary line. All traps were installed in accordance with targeted biosecurity response protocols, with locations selected according to preferred skink habitat.

In order to make inferences about the power of the surveillance system, detection probabilities were quantified along with a retrospective power analysis to assess the optimum number of surveys, survey sites and likelihood of falsely declaring species absence.

Key results. No plague skinks were detected at or beyond the estimated boundary line. A retrospective power analysis indicated that effort was sufficient to have detected plague skinks for detection probabilities of ≥0.2 with 95% confidence and ≥0.3 with 99% confidence, with the likelihood of declaring a false absence calculated at $p = 0.05$.

Conclusion. Invertebrate sticky traps are an effective tool for the detection of plague skinks in low densities, when installed in a systematic yet targeted system. The effort implemented was deemed sufficient to have detected plague skinks with 95-99% confidence in all locations within the assessment area.

2.2 Introduction

2.2.1 Biosecurity surveillance

Biosecurity surveillance has been defined as the "collection, collation, analysis, interpretation and timely dissemination of information on the presence, distribution or prevalence of risk organisms and the plants or animals they affect" (Kean, Phillips, & McNeill, 2008). Traditionally, surveillance has focussed on species that have already arrived and is largely about monitoring (Hellström, 2008), however, the early detection of exotic pests (while it is still cost-effective to manage them), is critical to the success of any control or eradication effort. Despite being one of the most important surveillance outcomes, it remains a major challenge of the biosecurity industry (Hellström, 2008; Jarrad, Barrett, Murray, Parkes, et al., 2011).

Post-border biosecurity surveillance of pests and diseases that are already established in New Zealand aim to provide information to assist containment and/or eradication efforts, particularly for those subject to regional or national pest management strategies (Hellström, 2008). The ability to rapidly delimit the geographical range of a new population is fundamental to the success of pest containment or eradication efforts (Fox, Buckley, Panetta, Bourgoin, & Pullar, 2009). As tools and techniques may vary in their ability to detect populations at various stages of establishment or dispersal, the development of effective detection tools is crucial (Marshall, Storer, Fraser, & Mastro, 2010). For new tools and techniques to be utilized, they must first be evaluated against cost in terms of relative efficiency. As surveillance efforts can be expensive and resource limited, techniques that maximise the chance of detection are prioritized (Fox et al., 2009).

To be effective at detecting and monitoring pest species, surveillance can either be passive or active. While passive surveillance can be the fortuitous detection of an exotic species, enhanced passive surveillance may involve the use of specialist equipment including camera trap recording devices, tracking tunnels or chew tags, all of which provide information about species presence without interfering with species behaviour or activity (Froud, Oliver, Bingham, Flynn, & Rowswell, 2008). Conversely, targeted

active surveillance is a commonly used approach within New Zealand's biosecurity sector, referring to the process of selectively surveying an area vulnerable to invasion (or already invaded) by setting up monitoring or trap equipment in habitats known to be favoured by the target species (Fox et al., 2009; Hauser & McCarthy, 2009; Jarrad, Low-Choy, & Mengersen, 2015). Habitat suitability models may assist with the process by identifying environmental characteristics that predict invasive species occurrence (Hauser & McCarthy, 2009). High quality targeted surveillance is essential for all stages of an incursion and has been used as the common approach to support numerous pest management operations nationally and globally to minimize management costs and maximise detection (Hauser & McCarthy, 2009).

2.2.2 Survey design

A robust survey design is integral to the success of achieving study objectives for any wildlife survey (Garden, McAlpine, Possingham, & Jones, 2007; Larossi, 2006; Wintle, 2013). While a 'standard' approach is most commonly used; involving a specified number of repeat surveys at a specified number of sampling units, others may need to adopt a more adaptive approach to suit both the assessment area and any logistical constraints. A 'removal design' is one such option, and can be adapted from its common purpose which involves trapping and removing animals from a population, and using the subsequent decline in numbers to estimate population size (Farnsworth et al., 2002). In occupancy studies this can be modified to involve the surveillance of a site to a maximum of 'K' times until the target species is detected. Upon initial detection no further surveys of this site need to be conducted, effectively removing it from the pool of sites being actively surveyed and thus allowing for greater spatial replication and resource re-direction for a fixed level of effort (Azuma, Baldwin, & Noon, 1990; MacKenzie et al., 2006). Where detection probabilities are relatively constant, a removal design is likely to be more efficient than a standard design, however, can be somewhat restrictive to data analysis options. Conversely a standard design may provide greater modelling flexibility, but may not be the best use of resources. In such cases, recommend a hybrid approach of standard and removal designs to support both robustness and efficient use of limited resources.

The effectiveness of different survey types not only depends on selecting the most appropriate method, but also the level of survey effort (Wintle, 2013). The detection power of a wildlife survey or surveillance system is generally defined by its ability to detect both the presence of a given target species and/or reduce the probability of a Type II error (false declaration of absence) (Barrett, Whittle, Mengersen, & Stoklosa, 2010). While trap efficiency is crucial to successfully detecting low density populations of cryptic species and invaders, it is equally essential to understand and define the optimum survey effort allocation required, in terms of trap numbers (trap intensity) and trapping duration. Until recently, little attention has been given to the statistical foundation of risk, power and system optimisation, upon which the design of biosecurity surveillance systems should ideally be based (Jarrad, Barrett, Murray, Parkes, et al., 2011). To detect incursions of exotic species across national borders or to offshore island systems, sampling designs often need to incorporate a heterogeneous landscape which stratifies for differential risk, management of labour costs and provision of sufficient power of detection (Barrett et al., 2010). Sound statistical designs can provide a guide to optimising effort in the face of limiting resource constraints, which generally determine the number of detection devices implemented and how they are allocated across a landscape (Barrett et al., 2010). Multiple site visits may be laborious on time and budgets, however, the expenditure of extra effort to obtain unbiased estimates should be preferable to obtaining flawed data (MacKenzie et al., 2002). Furthermore, the incorporation of both risk and power into a survey design can be tailored to meet any given precision requirement.

2.2.3 Detectability

Occupancy-based surveillance and monitoring programs rely on survey data to infer the probability that a sampling unit in an area of interest is occupied by a target species (Canessa, Heard, Parris, & McCarthy, 2012). However, in most practical situations, it is likely that animals or even entire species will go undetected when present at a site, leading to erroneous inferences of absence, particularly when rare or cryptic (Canessa et al., 2012; MacKenzie et al., 2006; Wintle, 2013). Detection probability (or

'detectability') is the estimated likelihood that at least one individual of a species will be detected given that the species inhabits the area of interest (McCarthy et al., 2013). However, the issue of detection probabilities less than 1 (or 100%) have often been neglected based on the default assumption that if a species is present, it will be detected (Canessa et al., 2012). Numerous predictors may influence detection probability, such as weather effects, time of year, habitat, abundance, predatory and competitive pressures. As a result, detection probabilities of a single population of any given species can be variable (Canessa et al., 2012; Nichols et al., 2008).

The problems associated with false negatives have been repeatedly addressed, with several statistically based solutions having been developed to overcome this issue (Canessa et al., 2012; Mackenzie, 2005a, 2005b; MacKenzie et al., 2002; MacKenzie et al., 2006; MacKenzie & Royle, 2005; McArdle, 1990; Nichols et al., 2008; Rota, Fletcher Jr, Dorazio, & Betts, 2009; Wintle, 2013). If detection probabilities can be included in the design stage of a survey, then programs can be targeted and maximise the probability of detecting a species where present. However, once detection probabilities and statistical estimates have been incorporated into a survey design, it is not uncommon for the optimal effort (e.g. number of visits to a survey site) to be unfeasible or impossible to achieve under resource constraints. In such cases MacKenzie (2005) recommends a minimum of 3 trap nights per sampling unit where detection probabilities are estimated as >0.5. For anything below this, the number of trap nights should be increased. Moseby & Read (2001) investigated the influence of trapping period and trap intensity on capture rates of small terrestrial reptiles and found that over a 10 trap night period, the first 3 nights yielded significantly higher trap success than nights 6-10, with a general decline from night 4 onwards. They specified that where survey time is limited fewer trap nights should be balanced by a greater number of traps, and quantified 10 traps and 3 trap nights as sufficient to detect 70% of a local reptile inventory. However, a trade-off between the number of survey sites and the number of surveys may lead to an under-estimate of effort and reduce the precision of any occupancy estimates (MacKenzie et al., 2006).

Consideration of inherent biological characteristics which may affect the detectability of the target species should also underpin survey design. For example, Carter, Heinsohn, Goldizen, and Biro (2012) found that bold male Namibian rock agamas *(Agama planiceps)* were detected and trapped more frequently and quickly than shy individuals, while Duckworth (2008) found that bolder male western bluebirds (*Sialia Mexicana*) had greater exploratory tendencies and lead population dispersal at the outer edges of an expanding range. Both boldness and dispersal are traits possessed by many successful invaders, often increasing their detectability and acting as distinguishing characteristics which set them apart from other less successful invaders (Rehage & Sih, 2004).

Exploratory differences in behaviour between congeneric species have also been noted, with the contrast distinguishing between invasion success and failure. Plague skinks *(Lampropholis delicata)* are found to be more exploratory than their non-invasive congener the garden skink (*L. guichenoti)*, exhibiting both cautious and cryptic behaviours at low densities, and gregarious, curious and bold tendencies when 'en masse.' This is suspected to enable them to more effectively assess risk and therefore locate, explore and habituate to new environments (Chapple et al., 2012; Rodríguez-Prieto, Martín, & Fernández-Juricic, 2011).

2.2.4 Detecting invasive reptiles

Detecting and monitoring rare or cryptic reptile species are challenging tasks which are well documented throughout the literature as crucial to successful herpetological conservation (Garden et al., 2007; Guimarães, Doherty Jr, & Munguía-Steyer, 2014; Mazerolle et al., 2007). Lag phase phenomena further hinders the likelihood of detecting a new species, and while the percentage of alien herpetofauna that exhibit this is unknown, observation suggests that they may frequently exhibit long lag phases prior to exceeding detection threshold barriers (Lockwood, Hoopes, & Marchetti, 2013). Additionally, lack of data may be confounded by lags in detection, as illustrated by the presence of the coqui frog (*Eleutherodactylus coqui*) in Hawaii for a decade before it exploded in number and was subsequently re-labelled as invasive (Crooks, Soulé, Sandlund, Schei, & Viken, 1999); (Kraus, 2009).

Conventional methods for detecting lizard populations are often time consuming, ineffective or uneconomical due to financial costs of both labour and equipment (Bell, 2009). Furthermore, these techniques are often limited in New Zealand with many surveys consisting of informal search efforts undertaken by people with minimal expertise in lizard behaviour and ecology (O'Donnell & Hoare, 2012). Such inadequacies in sampling may result in non-detection of a species that is present. These sampling flaws combined with the cryptic behaviour and spatially clumped distributions of reptiles, may render many rapid detection and monitoring efforts inappropriate and unproductive, and result in the premature abandonment of management efforts (McCarthy et al., 2013).

Due to the increasing number of exotic herpetofaunal introductions globally, the need for efficient and proven detection and monitoring techniques of rare and cryptic species has become increasingly important (Kraus, 2009). Differences in relative effectiveness of trap types can bias herpetofaunal sampling, and can be best evaluated by their ability to detect elusive species upon arrival or shortly thereafter with as much speed and accuracy as possible (Greenberg, Neary, & Harris, 1994).

Both active and passive detection techniques are employed in the monitoring of New Zealand reptiles. These typically include live traps such as pitfalls or G-minnow traps, artificial cover objects (ACOs), tracking tunnels with inked cards, visual encounter surveys and active hand searching (Anderson, Bell, Chapman, & Corbett, 2012). Each technique comes with its own unique combination of advantages and disadvantages, with a range of factors affecting success such as animal body size, home range, microhabitat fidelity, seasonal activity and weather patterns (Greenberg et al., 1994; Hoare, O'Donnell, Westbrooke, Hodapp, & Lettink, 2009).

The need for a lizard trap which supports biosecurity priorities by minimising identification errors and maximising capture rates across all age classes is novel in New Zealand. Invertebrate sticky traps (described in Chapter 3 in greater detail) are the only tool of those currently available which satisfy these requirements while simultaneously allowing for the minimization of native by-catch.

2.3 Study rationale

The imperfect detection of an invader may seriously impede the ability to make reliable, informed management decisions (Barclay & Hargrove, 2005; Barrett et al., 2010; Jarrad, Barrett, Murray, Stoklosa, et al., 2011; MacKenzie, 2005b). While New Zealand is recognized for its comprehensive strategy on introduced species, monitoring for rapid detection of invaders and an adequate structure for a rapid response are two acknowledged weaknesses in our approach (Simberloff, 2009b). The 'Early Detection Rapid Response' (EDRR) is considered as the second line of defence against introduced species when prevention has failed, and is the preferred management strategy for preventing the establishment and spread of an invasive species (Simberloff & Rejmánek, 2010). The benefits from early detection and subsequent rapid response are abundant, and include minimal and short-term impacts on the environment, followed by the ability to restore the invaded habitat to a natural state, potentially improving it for resident native species (Kraus, 2009; Lockwood et al., 2007; Simberloff & Rejmánek, 2010).

New Zealand's current biosecurity gaps in early pest detection are illustrated by the establishment of *L. delicata* on several of New Zealand's off-shore islands, including Great Barrier. While it is unknown how long they were present on the island prior to their detection in April 2013, given their dense populations and the extent to which they appear to have dispersed throughout the local environment, the assumption of a decade or more is not unreasonable.

Understanding the dispersal pathways of plague skinks and delineating the population boundary is crucial to the development of a future management strategy for the incursion on Great Barrier Island. To make reliable, informed management decisions, surveillance programs must be designed to include information about detection probability (MacKenzie, 2005b). Statistical power analyses and likelihood models can be used to estimate parameters of detection and habitat occupancy, which can then be used to: 1) evaluate the power of the system, 2) identify bias, and 3) form meaningful conclusions (Guimarães et al., 2014). Furthermore, parameterising what is required to delineate an established population accurately via a likelihood-based analysis of

presence/absence data, is a critical component in the development of an overarching National Management Strategy for this species.

2.4 Aims

This objectives of this chapter are twofold:

1) To design a detection survey to delimit the range of the plague skink operation on Great Barrier Island, using an adaptive, targeted and replicable methodology. We test the null hypothesis that the population has not spread beyond the easement track which encircles the core infestation.

2) To test the power of the designed surveillance system to verify operational outcomes. We identify the effort required to achieve power of ≥0.95 to detect outlying individual dispersers.

These aims support the priorities of the Auckland Council, Department of Conservation and the Plague Skink Tactical Group, with the overarching objective of developing a containment barrier and future-proofing management options.

2.5 Methodology

2.5.1 Background

An intensive plague skink trapping operation was implemented between November 2013 and April 2014 at Tryphena Wharf. Two trapping grids encompassing an area of 4,400m² were set up to run alongside passive detection tracking tunnel transects, which were installed around the perimeter of the trapping grids to try and delineate the population's boundary line. Additional transects were set up along Shoal Bay Road and across all properties at Gibb's Landing. By March 2014 it was established that plague skinks had already spread beyond the estimated boundary and existing trapping grid area, resulting in the detection transects being moved further afield to encompass a much larger area of approximately 63,500m² (Fig 2.1).

The inadequacies of using tracking tunnels for this purpose had become apparent and included card ink drying up quickly, snail damage destroying data, heavy rodent 'foot-traffic' and indiscernible skink prints. Furthermore, the time taken for the tracking tunnels to confirm that plague skinks had passed the estimated boundary line was perceived as too long.

Due to the increased level of urgency required to delimit the incursion zone, it was decided to discontinue using tracking tunnels and replace them with invertebrate sticky traps to improve species identification efficiency, speed and reduce doubt. Trapper® Monitor and Insect traps were selected. Additionally, the Auckland Council along with the Department of Conservation and Plague Skink Tactical Group required a significantly higher confidence threshold (≥95%) for any conclusions or inferences made about the true population range, as these would ultimately determine future management outcomes and resource allocations for the following financial year(s).

Figure 2.1: Map displaying original delimitation line (estimated as the out limit of the population core highlighted in yellow frame) and relocated delimitation boundary for this study (highlighted by the red frame).

2.5.2 General

The general methods employed to achieve the objectives of this chapter are as follows:

1. Installation of 10 transects and grids upon and surrounding the easement track to detect the presence or absence of plague skinks at this estimated boundary line.

2. Installation of 14 transects within the assessment area in order to identify dispersal pathways used by plague skinks and thus identify which areas of the boundary line are most vulnerable to being breached.

   a. To calculate detection and occupancy probabilities for traps and transects that capture plague skinks and statistically assess the overall power of the surveillance system.

3. Application of the resulting power analysis data to Bayes' Theorem of probability in order to estimate the likelihood of area occupancy where non-detections have occurred.

2.5.3 Sticky trap shelter construction & deployment

Invertebrate sticky traps (hereafter referred to as ISTs) are vulnerable to deterioration from heat and rain so needed to be protected in a shelter. Purpose built trap shelters were constructed from white corflute to minimize heat absorption, allow for flexibility in site selection and negate any possible risk averseness skinks may have had to the large open-ended tracking tunnels, while maintaining adequate airflow. Trap shelters were secured to the ground with a garden peg and inserted with invertebrate sticky traps which were secured to the shelter with a paperclip (Plate 2.1).

Plate 2.1: Custom made corflute trap shelters (Wairepo, 2015)

2.5.4 Predation mitigation

In addition to rodents and ground dwelling avian predators, ants pose a significant threat to lizards trapped on ISTs. Dense populations of carnivorous ants (not formally identified but most likely *Monomorium antarcticum* and possibly *M. antipodum)* are abundant on Great Barrier Island and are extremely prevalent in the Tryphena area (J. Warden, personal communication, August 2015). To mitigate against the risk of predation in areas with high density ant populations (particularly around sunny clay bank sites), ant bait was applied near trap shelters in close proximity to ant nests and covered over with rocks and vegetation. Alternatively where appropriate, bait was

inserted directly underneath the trap shelter which was then re-secured tightly to the ground ensuring no accessibility to bees which are vulnerable to the toxicant.

2.5.5 Weather conditions

Optimal conditions were considered as warm and sunny with no or little wind. Conversely sub-optimal weather conditions consisted of rain, cool temperatures and high wind. Due to the variability of island weather within a 24 hour timeframe, weather conditions of the morning during peak skink activity (roughly between the hours of 9am – 12pm) were generally those which determined overall optimality. Trap nights were discounted if either the morning or entire day had experienced sub-optimal weather conditions. Temperature, wind and light were scored against a numbered scale, with 1-3 qualifying as optimal trap conditions and acceptable to be included in the trap night count. Weather scores of 4-5/6 qualified conditions as sub-optimal and were therefore considered unacceptable to be included in the trap night count (Table 2.1).

Table 2.1: Weather scale on datasheets to identify optimal vs sub-optimal trapping conditions

Temperature Average during survey/trap service period		*Wind* Average during survey/trap service period		*Light* General weather conditions during survey/trap service period	
6 freezing	< 0°C	**1**	Calm (Beaufort 1)	**1**	Fine/sunny
5 cold	0-5 °C	**2**	Light breeze - Leaves rustle (Beaufort 2)	**2**	Part cloudy (4/8 cloud cover)
4 cool	6-10 °C	**3**	Mod breeze - Leaves and branches in constant motion (Beaufort 3 and 4)	**3**	Overcast (8/8 cloud cover)
3 moderate	11-15 °C	**4**	Gusty - Branches or trees sway (Beaufort 5)	**4**	Showers
2 warm	16-22 °C	**5**	Strong winds – (Beaufort 6 and 7)	**5**	Rain
1 hot	> 22 °C				

2.5.6 Capture and dispatch

All captured skinks were species identified using the single fronto-parietal head scale. This is an easily recognisable and definitive characteristic that distinguishes plague skinks from all native New Zealand skinks (refer to Chapter 1, Plate 1.2). Skinks captured on ISTs were carefully released by soaking the direct area with Canola vegetable oil and gently prying the animal away with the aid of a toothpick (Plate 3.5). Animals were released from tail to snout as this reduced the likelihood of stressed animals dropping their tails once body and limbs were free from the adhesive. All sinks were wrapped in tissue to remove excess oil. Native skinks were then placed in a bucket with vegetation before being collectively released outside of the immediate vicinity of the transect and into the known plague skink incursion area. This was done to both reduce the likelihood of individual re-capture as well as to maintain intraspecific competitive pressures upon the plague skink population. All captured plague skinks were euthanized using blunt force trauma to the head, the approved field technique for small lizards weighing <1kg (Gartrell & Kirk, 2005).

2.5.7 Dispersal pathway identification

Between October 2014 and February 2015, four separate survey trips were made to Great Barrier Island, each lasting between 1 and 5 weeks. A total of 15 transects and a single grid were installed and serviced within the estimated plague skink population range, using a total of 238 trap stations installed at 2-3m intervals (Fig 2.2). Global Positioning System (GPS) coordinates were taken for all traps using a Garmin Oregon 550. The range boundary line was estimated as the location of an existing track and the purpose of these were to identify dispersal pathways within this boundary, and locate the true moving front of the dispersing population in all directions spanning from the population core at Tryphena Wharf.

Due to a pre-existing understanding of habitat types preferred by this species, all transects and grids were installed in manner consistent with biosecurity targeted surveillance techniques. Sunny areas, path edges and vegetation edges were selected in favour of damp gullies or areas which were heavily shaded or overly exposed. All traps were tucked into vegetation where patch proximities and land gradients were considered. Lettink and Seddon (2007) tested the importance of trap proximity to microhabitat and found it to be a significant factor in trapping success. As such, habitat suitability was prioritized over rigid spacing intervals meaning some traps varied between 1 – 3m intervals according to practicalities.

As the purpose of these traps was to identify dispersal pathways, this section of the survey was set up according to a removal design methodology. Once plague skinks were detected, all traps in the entire transect or grid were decommissioned and relocated to another untested area. This meant the number of trap nights varied between sites with those yielding presence running a minimum of a single night, and those not showing presence being serviced a maximum of 20 nights. The number of surveys taken to detect presence provided the relevant information for calculating detection probabilities and making statistically grounded inferences upon the rest of the system which lay at the boundary line and beyond (MacKenzie et al., 2006; Steidl, Hayes, & Schauber, 1997).

2.5.8 Delimitation

In addition to identifying dispersal pathways within the perceived area of infestation, a single staggered transect was set up to encompass an area of 63,500m². This boundary line was named the 'new delimitation line' (NDL) and was selected based on an existing track which ran from the northern side of the wharf area to the southern end of Gibbs Landing; a residential bay where plague skinks had been identified the previous summer in low densities. The track facilitated ease of access (with landowner permission) and also provided a wide berth to the perceived plague skink population boundary. In early January 2015, 320 trap shelters were installed at 2-3m intervals. Traps were installed to alternate between each side of the track depending on site suitability, which was assessed according to vegetation and coverage, substrate and morning sun exposure. This transect was set up to run as a standard survey design, whereby irrespective of detecting presence or absence, the line was to be actively serviced for a minimum of 12 optimal trap nights by 4 people to ensure sufficient survey effort had been applied to the entire perimeter. In addition to this 3 trap grids and 4 transects were installed on the outside of the NDL at high risk sites in the event that dispersing individuals evaded capture on the NDL. These sites were deemed as high risk due to habitat suitability, gaps in the canopy and the resulting sunlight exposure. All grid traps were set up to the same standard survey design as the NDL.

The minimum timeframe of 12 optimal trap nights was selected according to previously calculated species detection probabilities ranging between 0.25-1.0, confirming the sufficiency of 4 trap nights (5 days and a 'standard working week'). This was consistent with the recommendations of Moseby & Read (2001) and estimated to be sufficient to detect plague skinks within various areas of their existing population range. This, coupled with the recommendations of MacKenzie and Royle (2005) (the optimum number of surveys to conduct at each site (*K*)), resulted in a total of 16 total nights, or 4 working weeks for contractors. Allowances for up to 4 nights of sub-optimal conditions were factored into time and monetary budgets.
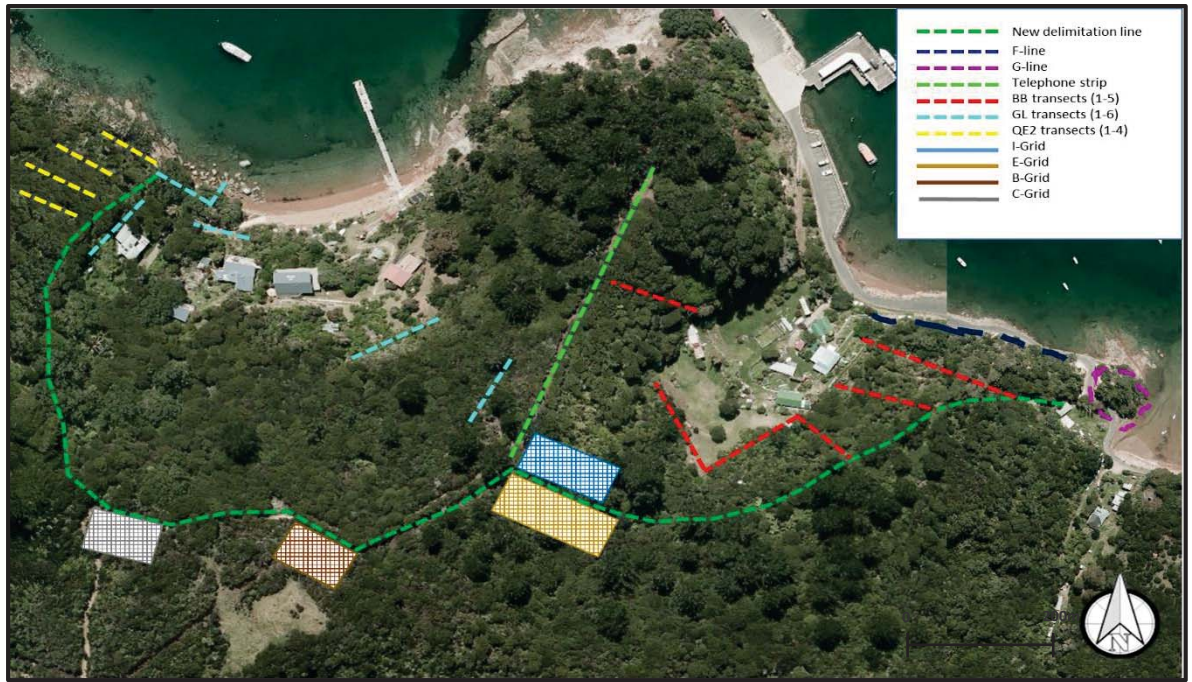
Figure 2.2: Transects and grids installed to detect dispersal pathways and range boundaries at plague skink incursion site at Tryphena Wharf, Great Barrier Island (36⁰19'13.04"S 175⁰29'09.26 E).

2.5.9 The Model

A power analysis was performed using an inference based probability model (Kery, 2002; McArdle, 1990), which was designed to calculate cumulative detection probabilities for multi-survey plans, the number of sampling visits (N) required to detect a species given a probability of detection (p), and a pre-defined confidence limit (%) or 'power level' (α). This type of retrospective analysis of power uses Bayesian theory and the acquired dataset to estimate the effort required to infer the genuine absence of the target species and thus reduce the probability of incurring a false negative in accepting the null hypothesis.

The probability of detecting plague skinks *(p)* was calculated for each transect and grid yielding their presence.

Detection probabilities for each trap that captured a plague skink were calculated according to the equation:

$$p = 1/n$$

where (n) represents the number of trap nights taken to detect presence. The resulting detection probability was assumed constant for each of the traps within its corresponding detection transect throughout its respective trapping duration. Occupancy estimates (Ψ) at each of these transects were then calculated as:

$$\Psi = \text{no. of detecting traps / total no. of traps}$$

The calculated p and Ψ values were then used as proxies to estimate the probability of detection and occupancy of plague skinks at transects and grids where no detections had occurred, in order to conclude that recorded absence on the delimitation line and beyond were genuine. The model assumes that all visits are comparable and independent and is calculated as follows:

Equation 1:   Probability of non-detection of plague skinks in N sample visits =

$$\alpha = (1-p)^N$$

From this we can calculate:

Equation 2:   Probability of detecting plague skinks in N visits =

$$\alpha = 1-(1-p)^N$$

From this we can then solve for N and get:

$$\log(\alpha) = N \times \log(1-p)$$

Equation 3: $N = \log(\alpha) / \log(1-p)$

To calculate the minimum number of visits ($N\ min$) necessary to declare a species absent from a site, two power levels (acceptable minimum and maximum standard errors) were set (α_{min} = 95%, α_{max} = 99%) to get:

$$\text{Equation 4:} \qquad N\ min = \frac{\log\ (1\text{-}\ \alpha)}{\log\ (1\text{-}p)}$$

Additional analyses were performed upon the dataset to quantify the optimum number of traps required at the boundary line and beyond in order to further investigate whether sufficient effort was made to detect plague skink presence to the required precision levels.

$$\text{Equation 5:} \qquad s = \frac{\varphi}{\text{Var}(\varphi)}\left((1-\varphi) + \frac{1-p*}{p*-Np(1-p)^{N-1}}\right)$$

where $p*$ is the probability of detecting the species at least once in N trap nights according to the model:

$$p* = 1-(1-p)^N.$$

Finally, the overall likelihood of the species having been present in areas of non-detection in accordance with Bayes theorem was calculated:

$$\text{PR(species present}\mid \text{species not detected)} = \frac{\text{PR(species present } and \text{ not detected)}}{\text{PR(species not detected)}}$$

$$\text{Equation 6:} \qquad = \frac{\Psi_{i,t}\prod_{j=1}\left(1-p_{i,j,t}\right)}{\left(1-\psi_{i,t}\right)+\psi_{i,t}\prod_{j=1}\left(1-p_{i,j,t}\right)}$$

2.6 Results

2.6.1 General

Between November 2014 and February 2015, a total of 753 traps divided amongst 23 transects and grids were installed and serviced to detect the presence, absence and dispersal of plague skinks beyond the known population boundary. Four contractors serviced each transect and grid for a trap night duration of 1-14 nights, equating to a total survey count of 9415 trap nights.

No plague skinks were trapped in any unit either on or beyond the NDL, however, they were detected in several new sites within the assessment area, with two captures revealing their dispersal to within 15m of the NDL (Table 2.2, Fig 2.3).

Three native skink species were regularly trapped throughout the duration at 83% of the detection transects: copper skink *(Oligosoma aeneum)*, moko skink *(O. moco)* and ornate skink *(O. ornatum)*, supporting habitat suitability of targeted surveillance areas.

Table 2.2: Transects and grids with their respective number of traps, the number of nights which they were actively trapping, vegetation type at surrounding location and skink detection information.

Transect / Grid ID	# traps	# trap nights	Vegetation description	Plague skink detection	Native skink detection
New delimitation line (NDL)	320	14	Various: Grass, clay, manuka/kanuka scrub, pine litter	X	*O. aeneum* *O. moco* *O. ornatum*
F-line	70	14	Grass, clay	X	*O. aeneum* *O. moco*
G-line	25	14	Coastal scrub	X	*O. aeneum* *O. moco* *O. ornatum*
Gibbs Landing transect 1 (GLT1)	5	9	Grass	X	X
Gibbs Landing transect 2 (GLT2)	10	14	Coastal leaf litter	X	*O. aeneum* *O. moco* *O. ornatum*
Gibbs Landing transect 3 (GLT3)	5	14	Grass / pavement edge	✓	*O. aeneum* *O. moco*
Gibbs Landing transect 4 (GLT4)	10	14	Grass	X	*O. aeneum*
Gibbs Landing transect 5 (GLT5)	15	3	Coastal leaf litter	✓	*O. aeneum* *O. ornatum*
Gibbs Landing transect 6 (GLT6)	15	14	Pine litter	X	*O. aeneum* *O. ornatum*
QE2 transect 1 (QE2T1)	5	14	Coastal grass / rockface	X	*O. aeneum* *O. moco*
QE2 transect 2 (QE2T2)	5	14	Coastal leaf litter	X	*O. ornatum*
QE2 transect 3 (QE2T3)	5	14	Coastal leaf litter	X	*O. aeneum*
QE2 transect 4 (QE2T3)	5	14	Coastal leaf litter	X	*O. aeneum* *O. moco* *O. ornatum*
Telephone strip (TS)	20	2	Grass & shrub – open track	✓	*O. aeneum* *O. moco*
Burrell property transect 1 (BB1)	12	5	Grassy track edge, coastal scrub	X	*O. moco*
Burrell property transect 2 (BB2)	12	5	Coastal leaf litter	X	X
Burrell property transect 3 (BB3)	10	5	Coastal leaf litter	X	X
Burrell property transect 4 (BB4)	16	1	Grass and fern edge	✓	X
Burrell property transect 5 (BB5)	10	5	Coastal leaf litter	X	X
Exterior grid (E-grid)	60	14	Grass, pine litter	X	*O. aeneum*
Interior grid (I-grid)	40	4	Coastal leaf litter, coastal scrub	✓	*O. aeneum*
Corner grid (C-grid)	60	14	Grass, coastal leaf litter	X	*O. aeneum* *O. moco* *O. ornatum*
Back grid (B-grid)	20	14	Grass, coastal scrub	X	*O. aeneum* *O. ornatum*

2.6.2 Dispersal pathways

A total of 4 detection transects and 1 detection grid identified the presence of plague skinks within the NDL boundary line, ranging in duration between 1-9 nights and taking an average of 3 nights (±2.5 SD). The resulting detection probabilities ranged between p = 0.1-1.0 (median 0.33 ±0.36 SD) with area use (occupancy) estimates ranging between Ψ = 0.05-0.2 (median 0.19 ±0.06 SD). The distances from the wharf (arrival location and original core breeding area) were measured, ranging between 120 – 224m (median 163 ±29.65 SD) (Table 2.3).

Figure 2.3: Map displaying location of plague skinks detected (*) in new areas relative to the estimated boundary and previously known dispersal range.

Table 2.3: Detection probability and estimated occupancy of plague skinks detected at various transects/grids

Transect ID	Trap ID	# trap nights to detect	Distance from core (wharf) (m)	Detection probability (p)	Estimated transect occupancy (Ψ)
TS	7	2	160	0.5	0.05
BB4	11	1	133	1	0.19
BB4	15	1	120	1	0.19
BB4	16	1	126	1	0.19
Igrid	D10	4	182	0.25	0.05
GLT3	3.1	9	224	0.1	0.20
GLT5	5.2	3	163	0.33	0.20
GLT5	5.4	3	173	0.33	0.20
GLT5	5.11	3	185	0.33	0.20
Median ($\bar{X}$±SD)		**3 ±2.5**	**163 ±31**	**0.33 ±0.36**	**0.19 ±0.06**

2.6.3 Delimitation

The NDL was serviced for a total of 14 trap nights, of which 13 were considered to have optimal weather conditions. While this was 2 trap nights short of the originally planned 16 night duration (due to set up delays), it was still within the level of acceptability.

No plague skinks were captured at any of the 480 traps situated at or beyond the assumed boundary line. Native skinks of all 3 species were trapped at 26% of traps on the NDL (with 32% of those ($n = 27$) capturing multiple animals throughout the duration) and 30% of traps in the outer surrounding grids. Based on no captures at the NDL the plague skink population range was estimated and mapped (Fig 2.4).

Figure 2.4: Map displaying identified plague skink distribution within the assessment area.

2.6.4 Data Analysis

To make conservative inferences about the power of the surveillance system at various areas within the assessment zone, calculations of detection probability and occupancy estimates were made for all transects where plague skinks were captured. MacKenzie et al. (2006) state that a rare species may have a detection probability of ≤0.05, therefore this has been included as calculations of 0.1 as a precautionary measure to account for individual outliers.

The optimal number of surveys were calculated against all detection probabilities using precisions of both 0.05 and 0.01 in order to assess the variance in effort required between these sensitivity levels (Fig 2.5). For detection probabilities ≥0.2, the 14 night trap effort was sufficient to have detected plague skinks with 95% confidence (Nmin = 13), and for detection probabilities ≥0.3 with 99% confidence (Nmin = 13). However, the likelihood of having detected outlying individuals dispersing ahead of the invading population frontline within 14 trap nights was calculated at 51-77%, falling short of the required detection power of ≥95% (Table 2.4).
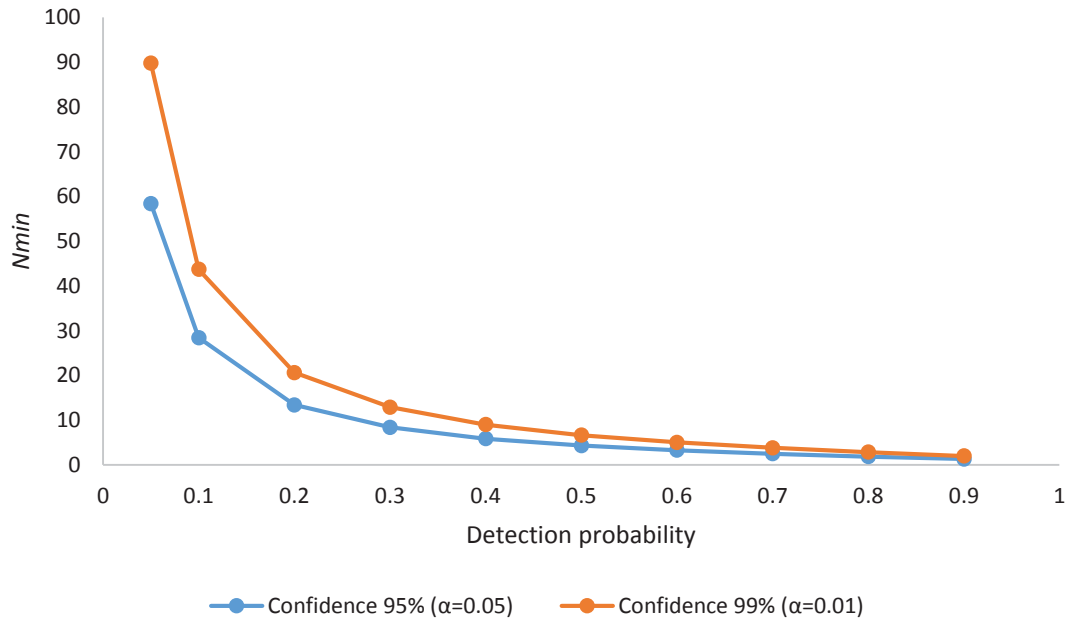
Figure 2.5: Detectability curve illustrating the relationship between detection probabilities and the corresponding minimum number of trap nights (Nmin) required for confidences (detection power) of 95% and 99%. Nmin decreases with increasing detectability.

The optimal number of traps to survey for the fixed survey effort of 14 nights were calculated for all detection and occupancy probability estimates using precisions of 0.05, 0.03 and 0.01 (See Appendix 2.1). In almost all cases our effort of 480 traps exceeded the minimum power threshold, achieving 0.97. For values of $p = 0.1$, $\Psi = 0.4\text{-}0.9$, our effort exceeded the minimum threshold requirement for a power of 0.95. For detection probabilities ≥0.4, the optimum number of traps required remains the same due to the 100% likelihood of detection within the trapping timeframe.

The probability that plague skinks had already dispersed beyond the assumed boundary line was calculated conservatively using Bayes' theorem of inference using the lowest likely detection probability ($p = 0.1$) and the median value of estimated occupancy likelihood ($\Psi = 0.19 \pm 0.06$ SD). The resulting value PR = 0.05 (± 0.2 SD) indicates that the null hypothesis that plague skinks had not dispersed beyond the boundary line, should not be rejected.

2.7 Discussion

2.7.1 Survey design

Most ecological surveys associated with species occurrence (particularly for rare or cryptic species), are subject to sampling error and underestimations due to factors including method efficiency, effort level and species population density (Fielding & Bell, 1997). Those with a conservation focus are often restricted to rapid assessment methods which are often not sufficient to detect such species (MacKenzie, 2005b).

This survey did not detect any plague skinks either at or beyond the NDL, which is a successful operational outcome. Furthermore, 26% of traps at the NDL did capture native skinks. This indicates that the placement of traps in terms of location and micro-habitat were sufficient to detect presence of 3 native species that are generally considered to be more cryptic than the gregarious invader, which in turn supports the required design power (Bell, 2009).

To design an effective study for the collection of presence/absence or count data, 'efficiency' must be defined. This may require a specific level of precision for the minimal total survey effort, or alternatively a maximum precision for a set level of survey effort. Deciding how to maximise survey efficiency is problematic regardless of the species being targeted, and may require testing both precision approaches before an evaluation can be made. Lewis and Gould (2000) investigated the effect of survey duration on the detection power of 20 raptor species to monitor migration trends. They found that changing survey length affected power, but results varied and depended on the migration pattern of each species at specific sites.

As no efficient detection methods had been developed for *L. delicata*, there was uncertainty regarding trap night duration and intensity requirements. Therefore design decisions were made according to estimated detection probabilities and recommendations in the literature regarding optimal survey effort for lizard inventories (Mackenzie, 2005a, 2005b; Moseby & Read, 2001). While Moseby & Read (2001) recommend 25 pitfall trap nights to efficiently compile an accurate inventory accounting

for 90% or more of all reptile species in an area, they clearly specify that 'more traps' is better than 'more trap nights' where time and resources are limited.

Conversely, MacKenzie et al. (2006) theorize that "increasing the number of sites surveyed at the expense of decreasing the number of repeat surveys may not result in a better design", as imperfect detection of a target species will cause parameter estimators to be biased (MacKenzie, 2005b). MacKenzie and Royle (2005) demonstrated evidence of this in a study which identified the standard error for occupancy at 200 sites surveyed twice (total 400 surveys) was 0.11 compared with 0.07 for 80 sites surveyed five times. A 36% reduction of the standard error was achieved by reallocating the same number of surveys in a more efficient manner (MacKenzie & Royle, 2005).

The conflicting literary advice provided the rationale to split the overall effort between standard and removal designs, with effort allocated in favour of duration at the boundary line while simultaneously testing detectability and its associated timeframes within it. Within the NDL, in all but one case plague skinks were intercepted within the first four trap nights which was consistent with both species behaviour and design expectations based on Moseby & Read's (2001) recommendations.

The transition from the use of tracking tunnels to invertebrate sticky traps in detection transects and grids can be considered as critical to its success. By eliminating the possibility of a Type I error as well as being self-baiting and discretely sized, this tool addressed and corrected for the failings of the previous seasons effort at achieving the same goal. In the face of time and resource constraints, they have proven to be extremely efficient at capturing skinks quickly, rendering them a useful tool for rapid assessment surveys for invasive reptiles.

While ISTs are not expensive to purchase, they did require a trade off in time, which ultimately equated to labour cost. In areas of high herpetofaunal richness, these traps are capable of capturing several animals (Plate 2.2) and species (Plate 2.3) in a single trap. However, the time taken to release native by-catch should not be underestimated

and may substantially increase labour costs, hence should be included into any future plague skink surveillance design plans going forwards.

2.7.1.1 Design bias

Unlike conventional hypothesis testing which sets out to reject the null hypothesis, this survey employed two different systems designed to achieve opposing goals. The NDL which was set up according to a standard design was located to detect absence at an estimated boundary line for the purpose of eventual containment. Conversely, the habitat-targeted locations of transects and grids installed within the NDL boundary line (grassy track and road edges, and sunny patches of low level scrub) were located to detect presence and identify the proximity of the population range to this boundary line. The combination of these opposing approaches resulted in an over-dispersed, zero-inflated dataset which did not reflect a standard population distribution, and as such restricted the types of analyses that could be performed. Despite these analytical limitations it was an important technique to employ, as habitat is known to directly influence the detectability of a species by way of population density, individual behaviour and the efficiency of the sampling method (Gu & Swihart, 2004; MacKenzie et al., 2002). Structural differences in vegetation patches can cause variation between meta-populations in home range size, leading to more or less extensive movement and fluctuations in detectability (Gu & Swihart, 2004; Moore & Swihart, 2005). By understanding how patch variability may influence species occupancy and detectability, the success of studies such as this that seek to identify species dispersal pathways across a landscape stratified by distance from the population's core, may be greatly enhanced by habitat targeted surveillance. This was found to be the case for an Australian study incorporating a heterogeneous landscape into a survey design for invasive rats *(Rattus norvegicus and R. exulans).* Although the survey had originally been designed to detect incursions of the big-headed ant *(Pheidole megacephala),* the system was robust to adaptation when due consideration was given to ecological differences. By spatially stratifying levels of invasion risk against various habitat types, the increased surveillance power was capable of detecting a novel rat incursion on Barrow Island, Australia (Jarrad, Barrett, Murray, Parkes, et al., 2011).

While this type of selective sampling may be considered inappropriate for other types of species distribution surveys (e.g. those whose primary objectives are to identify the relationship between habitat covariates and occupancy or abundance), it is an important tool in the adaptive management methods commonly employed by conservation managers faced with limited resources and tremendous uncertainty (Cook, Liu, Murphy, & Lonsdale, 2010; Jarrad et al., 2015).

Plate 2.2: 6 plague skinks captured on a single IST (Wairepo, 2014)

Plate 2.3: Copper skink (small) and ornate skink (large) captured on a single IST (Wairepo, 2014)

2.7.2 Dispersal pathway

Dispersal is widely recognised to be a key process in ecology and has been described in general terms as the spreading if individuals away from each other (Van Dyck & Baguette, 2005). The pathways and movement patterns utilized by a given species is the result of several different mechanistic scenarios. Developing an observational understanding of dispersal tendencies can improve the ability of conservation managers to both target for species presence and manipulate its movement patterns (Van Dyck & Baguette, 2005).

Dispersal pathways from the core breeding site by Tryphena wharf are now apparent, with skinks dispersing in all directions following both the edges (road and coast in both an easterly and westerly direction), as well as tracking up the ridge via open or sunshine

pathways towards the telephone pylon strip and coming within 15 meters of the NDL. It is via this pathway that the greatest level of risk was expected in relation to the NDL. In response a 60 meter long drift fence was installed along this section to reduce the potential for further dispersal (Plate 2.4). Additionally, the E-grid was installed on the outer side of the drift-fence to determine whether or not the population had already crossed over the NDL and continued to disperse further up the ridge. No plague skinks were detected in this grid.

Plate 2.4: 60m of drift fencing installed at the top of the Telephone strip

Interestingly, there are large areas within the detection zone where plague skinks had not dispersed, despite the suitability of the habitat. Neither the F-line nor G-line had detections of plague skinks, despite high population densities within approximately 15 meters of the inner end of the F-line (Fig 2.2). It has been suggested that where spatial homogeneity selects for dispersal, heterogeneity selects against it (Van Dyck & Baguette, 2005). The Burrell property provides an abundance of optimal and diverse habitat choices for plague skinks and is the likely reason for the abrupt discontinuation of roadside dispersal (Fig 2.6). The undulating terrain on the property transitions between dry, sunlit coastal broadleaf vegetation and damp rain-channelling gullies, and therefore provides a natural dispersal pathway towards the ridge. Interestingly, other invasive species have been observed following similar dispersal patterns, including the highly invasive Argentine ant *(Linepithema humile)* and various rodent species, often displaying a clear preference for following straight edges such as fence lines or roadsides and sunny pathways (J. Cook, personal communication, 2015).

Dispersal along the north-westerly bankside appears to follow a similar trend, with plague skinks well established coastally up to a point (approximately half way along the Gibbs Landing residential properties) before ascending via a sunshine pathway towards the ridge. The vegetation on this side of the ridge is markedly different, being almost entirely composed of manuka scrub with a dense, dry ground layer, which may explain the possible slower dispersal rate. *L. delicata* has been found to thrive in numerous, diverse habitat types and while it shows a clear preference for dry, sunny, well vegetated areas (Peace, 2004), it has shown that it is not averse to travelling through less hospitable terrain to reach more favourable areas in its travers through approximately 30-50m of shaded pine forest to reach the sunny ridge.

Figure 2.6: Dispersal pathways from the core, highlighting change of direction at reaching the Burrell property.

2.7.3 The model

Early detection is critical to the prevention of invasive species establishment and there is increasing emphasis on the incorporation of statistical power and differential spatial risk in survey designs prior to their undertaking (Barrett et al., 2010; Burgman, 2005). In the absence of detailed information about species specific population dynamics, presence/absence data can be used to create models to better understand the relationships between occurrence and habitat variables (MacKenzie, 2005b; Yackulic et

al., 2013). This type of dataset, which is often much easier and less costly to collect than abundance data, facilitates inferences to be made around a wide variety of ecological processes such as extinction and colonization probabilities (Gu & Swihart, 2004).

Doherty Jr, Boulinier, and Nichols (2003) state that invasion fronts, range edges and areas of range overlaps with competing species are where the population is most dynamic, and therefore subject to rapid changes in occupancy and low-detection probabilities. Although no plague skinks were detected beyond the NDL, the presence of absence is not confirmation of absence of presence, and the proximity of plague skinks to the boundary line at the telephone strip ridgeline places a great level of reliance upon the strength of the surveillance system with respect to power and risk.

When a null hypothesis is not rejected, it is important to assess the statistical power of the test (Steidl et al., 1997). If the null hypothesis is accepted and the estimated power of the system and tests are considered as high (>0.8) (Jarrad, Barrett, Murray, Parkes, et al., 2011), then it can be considered as reasonable to contend it to be true. However, if power is low then the test is inconclusive (Steidl et al., 1997). For this reason, a retrospective power analysis was selected as the most appropriate way to statistically test our outcomes.

It was found that the effort in terms of trap night duration (*N = 14)* and trap intensity (753) were sufficient to yield a power of 0.95-0.99 in most cases. This means that our efforts were sufficient to have detected the population if it had crossed the NDL in low to medium densities. However, the power was not sufficient to detect the individual dispersers at the forefront of the population range with detectabilities ≤0.1. In this scenario the power of the system was calculated at 0.77, meaning it was 18% below the pre-determined power threshold required by Auckland Council management. Barclay & Humble (2009) discuss the challenges of meeting statistical requirements to ensure a reasonable probability of detecting invasive species when they vastly outstrip resources. In order to achieve the 0.95 power requirement, a minimum number of 28 trap nights should have been run at the both the NDL and outer trapping grids to ensure that even individual dispersers would have been detected (Fig 2.5).

While estimates of power are subject to bias and reduced precision and ideally should be undertaken *a priori*, in some circumstances calculations of power after a study are appropriate when being used for decision making (Gerard, Smith, & Weerakkody, 1998). This highlights one of the key limitations of this study, whereby the power estimates should have ideally been calculated a priori using existing available data from the previous season and used as a 'gold standard' approach to be worked towards within the logistical constraints. This may have yielded a reduction in bias and enhanced the biological meaning of the study by allowing the specificity of true differences that are compatible with the data (Gerard et al., 1998).

Bayesian probability calculations indicated a 5% likelihood of individual plague skinks having dispersed beyond the line unnoticed ($p = 0.05 \pm 0.02$ SD). This means that in spite of the system not meeting power requirements to detect these solitary dispersers, there is a significant likelihood that skinks were absent. The application of Bayesian inference to ecological questions has been extensively applied to population and community ecology and forms the basis for adaptive management and environmental decision making (Ellison, 2004). Furthermore, this type of inference is particularly useful in modelling population dynamics of single species, and estimating species occurrence from geographically or logistically constrained samples (Dorazio & Johnson, 2003; Ellison, 2004). For example, a study examining demographic data from a colony of the endangered greater horseshoe bat *(Rhinolophus ferrumequinum)* was analytically restricted by its limited dataset. Through the application of Bayesian integrated population modelling, they were able to estimate fecundity, survivorship and population growth to a precision level that wouldn't have been available to them if using alternative methods (Schaub, Gimenez, Sierro, & Arlettaz, 2007).

2.8 Conclusions

The operational goals of this study were twofold. By identifying dispersal pathways we were able to locate the directional movements and range limits of the population with respect to the NDL, which was the estimated range boundary. It was theorized that this line would be suitable for the installation of a containment barrier fence to prevent plague skinks from dispersing further into the environment while management plans were being developed. The research aims of this study were to assess the success of the operational goals via an analysis of statistical power.

Both operational and research aims were successfully fulfilled. However, our retrospective power analysis revealed an insufficient effort had been implemented to detect independently dispersing individuals at the NDL. Despite there being a significant likelihood of skinks not having dispersed beyond this point, an additional 14 trap nights (outside of the timeframe of this study) were run to meet the 95% confidence level required to detect individual dispersers ($Nmin$ = 28). During this time a single plague skink intercept was subsequently made at the telephone line area where the section of drift fencing had been installed. This supports both the validity of installing a containment structure around the length of the boundary line, as well as highlighting the urgency with which it should be done.

Furthermore, the field work and post-hoc data analysis have provided a framework against which similar field operations can be implemented in the future for plague skinks and other invasive herpetofauna. With due consideration given to ecological differences, the surveillance design methodology which spatially stratifies risk across a heterogeneous sampling frame, could be applicable across a wide variety of invertebrate and vertebrate species (Jarrad, Barrett, Murray, Parkes, et al., 2011).

Chapter 3

Can plague skinks *(Lampropholis delicata)* be eradicated by trapping alone?

3.1 Abstract

Context. Plague skinks (*Lampropholis delicata)* are the only exotic reptile to have successfully established in New Zealand. Since their arrival in the late 1960's they have dispersed throughout much of the North Island and in recent years have established populations on several off-shore islands in the Hauraki Gulf. Their discovery on Great Barrier Island in April 2013 prompted a renewed momentum to develop effective eradication and control techniques, given its important endemic herpetofaunal assemblage and proximity to other ecologically sensitive off-shore islands. Few studies have addressed the capture efficiencies of standard reptile monitoring tools and to date there are no proven eradication strategies or monitoring programmes for invasive lizards, which is an issue that is growing in significance globally.

Method. We installed two trapping grids at Tryphena Wharf, the incursion site on Great Barrier Island using a selection of reptile monitoring tools at varying spatial intensities. An intensive trapping programme ran from November 2013 to June 2014, however, efforts were the highest and most consistent over the months of January, February and March. Areas of high skink activity were intensified with trap clusters as part of an adaptive management approach. Statistical evaluation using a Logistic ANCOVA was used to test the validity of field observations by assessing capture rates and comparing the efficiencies of the two main trap types used.

Key results. Approximately 507 plague skinks were captured across all age classes. Capture rates increased throughout the trapping period due to a growing neonatal population, and in spite of the increased trap effort applied, the system was not sufficient to make a significant impact population reduction ($p = 0.250$). The two trap types were found to be statistically equal in their efficiency ($p = 0.488$).

Conclusion. Our trap efforts were insufficient to eradicate the plague skink population which was found to be more widely dispersed and dense than estimated. Pitfall traps were found to be impractical as a biosecurity response tool, however, invertebrate sticky traps (ISTs) G-minnow traps were found to be effective in varying situations. Careful experimental design to address the required spatial intensity of traps and optimal capture probabilities should be prioritized to progress our abilities to effectively control plague skinks in New Zealand.

3.2 Introduction

The rapid rate at which exotic species are increasingly breaching biogeographic boundaries is adding increasing pressure to limited global conservation funds (Capinha, Essl, Seebens, Moser, & Pereira, 2015). One of the single most challenging aspects of eradicating exotic species has been identified as the absence of rigorous and comprehensive early warning systems that are so critical success (Simberloff, 2009b). Paradoxically, detection is often unlikely to happen until the species reaches numbers that bring it above the detection threshold, by which time it may already be established and above the eradication threshold (Lockwood, M. F. Hoopes, & M. P. Marchetti, 2013). Statistical modelling, increased efficiency in survey design and techniques, along with improvements in targeted surveillance efforts are ways in which the invasion biology community has attempted to address this conundrum (Lockwood et al., 2013).

When traditional preventative methods fail to stop the establishment of an invasive species then three responses can occur. 1. Do nothing, as in some cases they may be productive or provide habitat (Rodriguez, 2006). 2. Control the population by reducing numbers to an acceptable threshold and mitigate its impact upon the invaded ecosystem. 3. Attempt an eradiation operation which involves the complete and permanent removal of all individuals from a wild population to prevent impacts to native species, their ecosystems or other values such as primary production (Genovesi, 2010).

Despite having been described as the 'step-child' of invasion ecology, the study of eradication and biological control is subject to criticism thanks to several well publicised failures and the small window of opportunity for success (Lockwood et al., 2013; Simberloff, 2009b). Those that have succeeded have done so with rapid response "low-tech, scorched-earth" approaches, often at the expense of time consuming research or the development of sophisticated scientific methodologies (Simberloff, 2009b).

3.2.1 History of eradication

Eradication management arose for the primary purpose of preventing health risks to humans from livestock, particularly due to invertebrates vectoring diseases such as sleeping sickness (a widespread tropical disease that can be fatal and is spread by the tsetse fly (*Glossina* spp.)) and malaria (Genovesi, 2010). Invasive invertebrates have become increasingly problematic, posing risks to food security and agriculturally driven economies, resulting in many politically motivated and often unsuccessful eradication attempts (Myers, Simberloff, Kuris, & Carey, 2000). For example, the failed attempt to eradicate the fire ant (*Solenopsis Invicta*) (an invader whose impacts affected both humans and native ant fauna) from the southern USA cost more than $200 million between the late 1930's – 1950's. More recently, annual ant management costs were estimated at $500 million, accounting for $200 million in management costs and an additional $300 million worth of damage to livestock, wildlife and public health (Lofgren, Banks, & Glancey, 1975; Myers et al., 2000; Pimentel, Zuniga, & Morrison, 2005).

Conversely, one of the earliest and most impressive eradication successes was the removal of the African *Anopholes gambiae* (a malaria vector) from a 54,000km² area of habitat in northeast Brazil during the 1930's and early 1940's (Coggeshall, 1944). After more than 14,000 human deaths, an integrated programme based upon larval control successfully eradicated the species from the area, which lead to the methodology being successfully implemented in Egypt and Zambia in subsequent years (Killeen, Fillinger, Kiche, Gouagna, & Knols, 2002; Myers et al., 2000).

It wasn't until the 1980's that eradication began to be viewed as a tool to protect biological diversity, and was formally listed as a priority conservation tool at the highest policy levels in the 1990's. At the Convention on Biological Diversity in 1992, all member state parties were called upon to adopt a hierarchical approach to prevent unwanted biological introductions (Genovesi, 2010). With more than 86% of the 11,000+ eradication attempts carried out globally considered as successful, it has now become an important management tool for conservation initiatives, particularly for island ecosystems in need of removing exotic vertebrates as part of restoration efforts (Genovesi, 2010; Lockwood et al., 2013; Parkes & Panetta, 2009) (Fig 3.1).
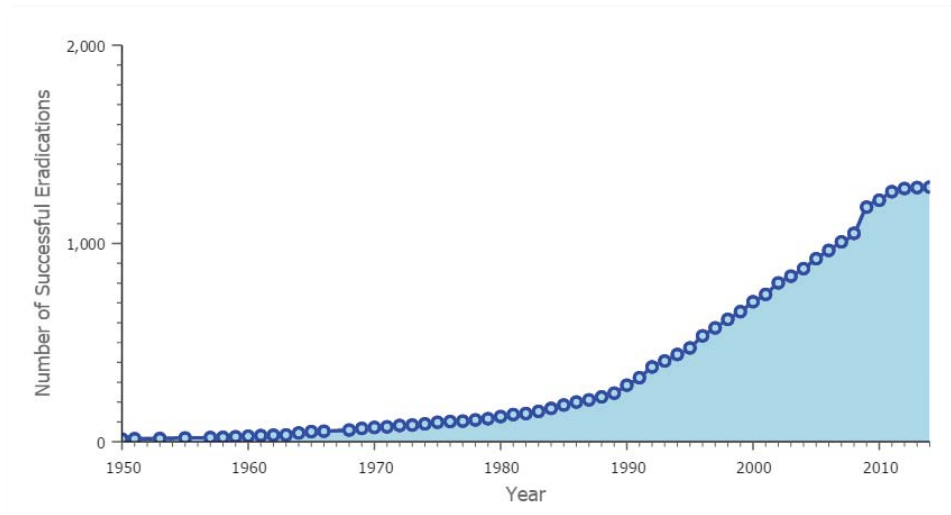
Figure 3.1: Cumulative number of successful island vertebrate eradications globally by year since 1950 (DIISE, 2015)

3.2.2 Requirements of a successful eradication

Feasibility studies are an important pre-cursor to eradication proposals. Their primary function serves to inform policy and convince funding agencies of the importance of eradication to eliminate adverse impacts upon native ecology. They should include a peer reviewed project design and rigorous cost benefit analyses (Cromarty, Broome, Cox, Empson, & Hutchinson, 2002; Genovesi, 2010; Parkes & Panetta, 2009).

While the implicit assumption of most feasibility studies is that the benefits of eradication outweigh the costs, minimizing harm to non-target species is a major component of eradication planning (Cowan & Warburton, 2011). An advanced ecological understanding of how the proposed eradication protocol may impact non-target species and the wider ecosystem is critical, and must allow for potentially unpredictable results (Cowan & Warburton, 2011). Failure to understand and quantify the often complex interactions between the target organism and other species through a comprehensive feasibility plan may not only lead to eradication failure, but also risk the loss of both credibility and public support (Myers et al., 2000; Parkes & Panetta, 2009).

Under certain circumstances successful eradications are possible, however, timing is critical. If discovered early, a newly introduced species which has not yet established

may be successfully eradicated. Both Genovesi (2010) and Simberloff (2003, 2005, 2009) highlight the importance of rapidly responding to incursions, suggesting that when the incipient population remains in its infancy, the application of rapid, effective force can often be critical to eradication efforts. For example the removal of the black-striped mussel (*Mytilopsis sallei*) from Cullen Bay in Darwin Harbour, Australia when it was discovered within 6 months of arrival. Via the enactment of emergency legislation, within 9 days a team of over 280 people had quarantined and treated the 600 mega-litre manmade marina with 260 tonnes of liquid sodium hypochlorite and 9 tonnes of copper sulphate at a cost of $1.6 million (Coles & Eldredge, 2002).

The standard requirements for a successful eradication operation are the result of numerous attempts at setting specific criteria for assessing the feasibility of an eradication proposal. The identification of six factors mixing both essential rules and desirable attributes are now considered as the critical elements of a successful eradication (Table 3.1) (Bomford & O'Brien, 1995; Parkes & Panetta, 2009).

Table 3.1: Essential requirements and desirable attributes of an eradication (Cromarty et al., 2002)

Criteria No.	Requirement Description
1	Funding and resources should be sufficient to fund the program to its conclusion.
2	All necessary actions must be permitted, with the lines of authority having been made clear.
3	The biology of the target species must make it susceptible to the control technique(s) being applied. The target species must essentially be removed at a rate which exceeds that of its own rate of increase.
4	Immigration / reinvasion must be accounted for and prevented, otherwise it will be only temporary.
5	The target species must be detectable at low densities.
6	Should the target species play a keystone role in the ecosystem it is being removed from, restoration efforts must be made to negate any adverse impacts upon native species (Myers et al., 2000).

3.2.3 Eradication, control or containment?

The four broad strategic goals for managing biological invasions have been identified as prevention, eradication, containment and control (Fig 3.2) (Grice, 2009). In cases where prevention has failed and eradication has been deemed infeasible, containment may be the most appropriate strategy for a species during the early expansion stage, whereas a control strategy is likely to be most suitable for an advanced stage with a large and extensive population (Fraser, Cook, Mumford, Wilby, & Waage, 2006; Grice, 2009). Both management strategies serve to reduce a population's density and range to a level which does not compromise the integrity of the ecosystem, while simultaneously striking a balance between the economic and resource constraints of ongoing management (Heikkilä & Kettunen, 2014).

In some cases control may aim to completely remove the invading population, however, efforts may not result in eradication. In these cases approaches such as 'management to zero density', may be used (N. Waipara, personal communication, November 2013), which enables the operation to forge ahead providing the benefits of eradication but inbuilt with a type of 'safeguard' (Genovesi, 2010). While this safeguard may be initially beneficial, it does require an indefinite investment of time, tools and money to keep the invasive population sufficiently suppressed (Zavaleta, Hobbs, & Mooney, 2001).
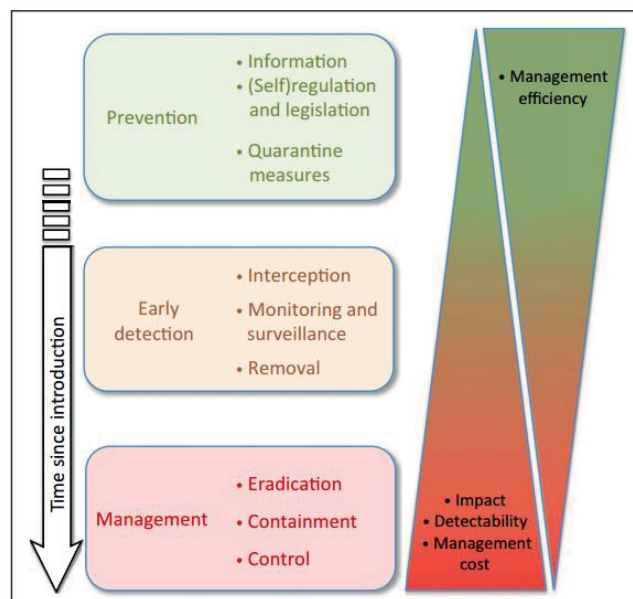
Fig 3.2: Strategic process involved with managing biological invasions
(Simberloff et al., 2013)

3.2.4 Development of techniques

Significant advances in species removal techniques led to both a greater number of operations resulting in success and a greater variety of species being targeted (Genovesi, 2010; Griffiths, 2011; Pluess et al., 2012). Two general strategies employed in eradication. The first involves a single operation that assumes all pests will be killed. The second is where repeated control efforts are applied until no individuals of the target species remain. While methods typically involve trapping, shooting, poisoning or manual hand removal, many can require an integrated approach combining several methods throughout the stages of the eradication (Parkes & Panetta, 2009). For example feral cat populations may be initially reduced by poisoning an area, with residual animals being hunted or trapped (Nogales et al., 2004). It can be assumed that the latter approach will be subject to both recruitment and immigration between the pulsed control efforts, which can be problematic and require creative and adaptive management regimes to monitor dispersal (Parkes & Panetta, 2009; Ramsey, Parkes, Will, Hanson, & Campbell, 2011). As such, a hybrid strategy may be useful where it is possible to remove all target species, but where reinvasion is highly likely (Parkes & Panetta, 2009).

Other alternatives including biocontrol have been widely used. However, the potential for these to result in unknown ecological impacts or even failure has historically been underestimated, therefore caution is needed when introducing further exotic species (Follett & Duan, 2012). More sophisticated methods may also be used in conjunction with traditional techniques to enhance the operation. For example, the removal of disturbance regimes or alteration of habitat to inhibit the establishment of invasive species or even provide native species with a competitive edge (Kraus, 2009).

3.2.4.1 Mammals

Systematic eradication campaigns did not begin in New Zealand until the mid-1980s. The country is now considered a world leader in this field, particularly for the removal of mammals from off-shore islands where more than 250 vertebrate eradications have been attempted to clear them of species including but not limited to rodents (*Muridae*

spp.), rabbits (*Oryctolagus cuniculus*), possums *(Trichosurus vulpecula)* mustelids (*Mustelidae* spp*.)*, feral cats (*Felis catus*), pigs (*Sus scrofa*) and goats (*Capra hircus*) (Towns, West, & Broome, 2013). Invasive rodents are responsible for a large number of native species extinctions on island ecosystems, and as such have been the focus of conservation efforts seeking to restore these ecologically sensitive areas (Lockwood et al., 2013).

Management protocols and techniques developed in New Zealand are largely the result of two key technical developments which have set a unique precedent for addressing complex, multi-tiered eradications across trophic groups, and have since been adopted and utilized globally (Towns & Broome, 2003). The first arose from the availability of second-generation anticoagulant toxins while the second involved the transition from manual bait delivery methods to aerial distribution. Not only did these totally revolutionize rodent eradication efforts but also countered the previously held idea that invaders occupying areas of greater than 1000 hectares were not reasonable targets for eradication (Simberloff, 2003; Towns et al., 2013).

3.2.4.2 Invertebrates

Pest invertebrate management has different challenges and as a result requires creative and novel techniques to be effective. One successful technique includes mating disruption which involves the release of large quantities of sex attractant in an area can confuse males and prevent them from finding females. Alternatively, the sterile insect release (SIR) involves rearing, sterilizing and releasing large numbers of males to mate with wild females who will then produce inviable eggs (Suckling et al., 2014). These can work well in conjunction with the male annihilation technique, which uses insecticide permeated traps to rapidly reduce the number of wild males in the environment (Myers et al., 2000).

There are several successful examples of pest invertebrate eradications, including the repeated and recent reinvasion of the Queensland fruit fly (*Bactrocera tryoni*) to New Zealand which are successfully managed by putting local area movement restrictions in

place and using lures and insecticidal baits to detect, trap and kill all individuals (Ministry for Primary Industries, 2015).

3.2.5 Methods for lizard and amphibian eradications

Reptiles can make excellent stowaways due to their cryptic behaviours assisting with undetected movement in transportation networks. Their eggs which require no parental care, are also highly transportable. Additionally, a widespread perception that alien herpetofauna are environmentally benign along with their often high reproductive outputs has meant that the necessary rapid response required to eradicate developing incipient populations is delayed beyond the point whereby the operation may have been successful (Kraus, 2009, 2010; Pitt et al., 2005). As such, adequate management of alien herpetofauna lags significantly behind that of most other vertebrates, with only a few successful amphibian eradications and only a single successful reptile operation (*Varanus gouldii* from Reevesby Island*)* having been documented for island ecosystems (DIISE, 2015), despite of the fact islands are reported to have a higher overall eradication success. The few successes are recorded as the result of breeding requirements limiting the populations initial dispersal and allowing them to be targeted for control (Kraus, 2010). For example the Australian frog *Limnodynastes dumerilii* which was privately reared in captivity from an egg clutch found in the North Island. Authorities were alerted to the matter and a rapid response eradication operation was initiated. Within 2 months all animals and eggs had been destroyed (Seymour et al., 2005).

When even one of the essential requirements of an eradication are not met, the risk of failure increases. The coqui frog (*Eleutherodactylus coqui*) has quickly proliferated across the islands of Hawaii since its arrival in the 1980's via shipping and commercial plant transport (Beachy et al., 2011). While they remain firmly established on the main island of Hawai`i and are subject to ongoing control efforts on the islands of Kaua`i, Maui and Moloka`i, they were temporarily eradicated from the island of O`ahu, where 4 sparsely distributed populations were removed due to a quick multiagency response (Beachy et al., 2011). Unfortunately 2 new captures were subsequently reported in 2014,

illustrating the temporary nature of eradication success (DIISE, 2015; Star Advertiser, 2014, May 2). Similarly, despite numerous attempts to remove Nile monitor lizards *(Varanus niloticus)* from Florida due to the threat posed to native ground-dwelling bird species and various endangered turtle species, the species remains firmly entrenched (Mauldin & Savarie, 2010).

3.2.6 Traditional reptile capture techniques

Reptile sampling in New Zealand has traditionally been for the purpose of detecting and monitoring native herpetofauna. While techniques range from passive surveillance tools to active traps, few studies have confirmed the accuracy and sensitivity of each technique to detect population trends and response to management (Lettink, O'Donnell, & Hoare, 2011). Furthermore, their utility in trapping large numbers of lizards as possible eradication tools do not yet appear to have been evaluated.

The choice of capture methods require due consideration of the ecology and behaviour of the target species. As it is unlikely that any single sampling technique will be suitable for capturing lizards across a heterogeneous landscape, the use of multiple complementary sampling techniques is likely to vastly improve the detection, monitoring, or eradication outcomes (Ribeiro-Júnior, Gardner, & Ávila-Pires, 2008). Furthermore, understanding the sensitivity of techniques in terms of its effectiveness across habitat types is fundamental to the implementation of robust studies and suitable management strategies (Ribeiro-Júnior et al., 2008).

Live trapping can be an effective method of accurately identifying and monitoring species composition and distribution patterns both spatially and over set timeframes, with the ability to accurately extrapolate data to make relative species richness and abundance predictions (Greenberg et al., 1994). Live trapping techniques may be highly advantageous via the reduction of certain biases such as observer bias, and diurnal/nocturnal species bias by remaining activated over 24 hours. However, they also come with their own set of disadvantages. For example, in accordance with the Wildlife Act (1953), all live traps must be serviced within a 24 hour timeframe to minimize harm

to both target and non-target species, making them laborious, restrictive and potentially unviable for long-term projects (Anderson et al., 2012). Efforts should be made to minimize predator accessibility and when not in use must be fully decommissioned to avoid mortality events, as lizards have been known to drown in pitfall traps where there is a shallow water table and known to trap species along with their predators (Padley, 1997). Some trapping methods are weather dependent, and once captured, animals may become 'trap shy' which may affect subsequent re-capture efforts in a monitoring situation (Clark, 2006).

Trap placement plays an important role in capture rate, with its relationship to proximity of microhabitat having been demonstrated for two New Zealand endemic skink species (*Oligosoma maccani and O. nigriplantare polychrome),* whose capture rates were statistically correlated with distance from microhabitat cover (Lettink & Seddon, 2007). Furthermore, there is a substantial body of evidence to show the effectiveness of trapping may be enhanced by the use of drift fences, and should be used where habitats and budgets allow (Davis, Fleming, Craig, Grigg, & Hardy, 2008; Ellis & Bedward, 2014; Garden et al., 2007; Greenberg et al., 1994; Moseby & Read, 2001). Bait is commonly used to improve trap catch, with food such as banana, pear and cat food proving palatable to some New Zealand lizard species (Hare, 2012a; Jamieson & Neilson, 2007).

3.2.6.1 Hand capture

This technique involves diurnal and nocturnal surveying by searching through appropriate substrates and habitat for the target species at the appropriate time of day (van Winkel, 2008). Considered as the simplest effective technique for the surveillance of terrestrial (and arboreal) lizard species, the success of this methodology not only depends on the elusivity and abundance of the target species, but also on the skill level of the observer to sight, identify and capture (Garden et al., 2007; van Winkel, 2008). While Garden et al. (2007) found this to be the best technique for the detection of large reptiles, they do recommend it be used in conjunction with other techniques such as pitfall trapping due to the strong seasonal and weather dependence which is disadvantageous to this technique.

3.2.6.2 Pitfall traps

Pitfall traps are containers buried into the soil whose surface sits flush with the soil surface (Plate 3.1). Containers have drainage holes punched into them to prevent animals from downing, and covers are placed over the trap to protect any captured creatures from predation and desiccation (Padley, 1997). Pitfall traps are traditionally baited with fruit and should also contain a piece of damp sponge to further protect trapped animals from desiccation. Traps must be checked within 24 hours in accordance with the requirements of the Wildlife Act (1953) (Hare, 2012c).

3.2.6.3 G-minnow traps

Funnel traps have been widely used around the world for herpetofaunal monitoring for many years (Hare, 2012a). They generally consist of a cylinder with inverted an inverted funnel at either one (single-ended) or both ends (double-ended) and are often home-made using semi-rigid or rigid mesh, resembling constructions made for fish trapping. The G-minnow fish trap is simply an adaptation of the funnel design and has been adopted in New Zealand herpetofaunal sampling in recent years (Plate 3.1) (Anderson et al., 2012; Hare, 2012a). This type of trap has proven to be an effective live trapping technique for some terrestrial lizards including geckos and the nationally vulnerable chevron skink (*Oligosoma homalonotum)* (Anderson et al., 2012; Bell, 2009; Davis et al., 2008).

As with pitfall traps, damp sponge and bait should be used to provide food and water resources and improve trap catch, but traps must be checked daily and decommissioned when not in use to avoid mortality events.

3.2.6.3 Invertebrate sticky traps (ISTs)

Rodent glueboards or 'sticky traps' have been used in international herpetological monitoring for several decades (Bauer & Sadlier, 1992). Traps can be secured discretely at the base of habitat patches or along pathway edges to target terrestrial lizards, or alternatively on tree trunks or branches to capture arboreal species. A lizard becomes entrapped from the moment they come into contact with the glue surface, with a single foot often proving sufficient. Vegetable cooking oil is all that is required to remove the adhesive, with excess wiped off with a tissue leaving no harm to the lizard (Bauer & Sadlier, 1992).

Effective of January 1st 2015, the New Zealand Animal Welfare Act (2009) now strictly prohibits the sale and use of rodent glueboards due to concerns over the pain and distress caused to captured rodents, however, in certain biosecurity related circumstances (e.g. for pest free islands) exemptions may be granted (Ministry for Primary Industries, 2014). An alternative to these has been identified for the purpose of invasive herpetological surveillance, with invertebrate sticky cards offering the same benefits of a glueboard without the restrictions.

The difference between traditional rodent glueboards and invertebrate cards are in their shape and glue viscosity. While the glueboards have a much thicker layer of glue (approximately 1cm), sticky cards have an extremely fine film of glue (>1mm), rendering lizard removal much easier and allowing rodent bycatch to self-extract (Plate 3.1).

Plate 3.1: Left: Pitfall trap (Dunson, 2010), Centre: G-minnow trap, Right: Trapper Monitor & Insect glue trap (Wairepo, 2015)

3.2.7 Measuring success

When attempting to remove all individuals of a population, knowing when to declare the invader status as officially eradicated hinges on detectability of the last few remaining individuals. As the number of individuals within a population decrease, the cost per effort is known to increase substantially as the last remaining individuals become increasingly difficult to detect and therefore require greater resources to do so (Lockwood et al., 2013). With conservation and biosecurity budgets becoming increasingly scarce, it is not unreasonable to be conservative of spending limited funds on bio-statistical consultations when conservation outcomes can be achieved using simple counts, population indices and situational experience to assess how the target population responds to an applied management technique (Griffiths, Foster, Wilkinson, & Sewell, 2015).

It is rarely possible to count all the individuals of a population either before or after the treatment has been applied, and as such the comparison of spatial or temporal count statistics can assist with making inferences from the captured population to the entire population (Hare, 2012b).

Measures of a population such as catch-per-unit-effort (CPUE) and density (number of individuals per unit area) can be helpful with assessing population trends among sites within a particular habitat type or identifying population trends (Bjorndal et al., 2010; G.H. Rodda et al., 2012). CPUE indices are generated by dividing the number of animals captured by the expended effort which can be quantified by n/100 trap nights or n/person/hour and can be adjusted to include variables which may influence capture data (e.g. temperature) (Hare, 2012b). Estimates of density are generated by dividing the number of captured individuals by the area searched (n/hectare) and may be bias due to assumptions associated with capture probabilities. In spite of these, abundance indices are often sufficient to identify basic biological patterns and are generally much easier and cheaper to perform, and may in some circumstances alleviate the requirement for intensive statistical analysis (Hare, 2012b). Where a deeper understanding is required, user friendly software such as MARK and Removal Sampling 2 are increasingly available. Not only does this make statistical inference less complex

to conservation practitioners, but also may assist with the difficult decision of an eradication being successful. Failure to determine this accurately may result in what is known as the 'Lazarus effect'; the release of control pressures on the remaining few which enables the population to rebound (Morrison, Macdonald, Walker, Lozier, & Shaw, 2007).

3.2.8 Advantages and disadvantages of eradication

3.2.8.1 Ecological

It is essential to critically evaluate whether an eradication is not only biologically feasible, but also environmentally, politically and socially acceptable as it can be a subject of considerable controversy due to the potential for ecological damage, their risk to any non-target species and the wider ecosystem (Liebhold, MacDonald, Bergdahl, & Mastro, 1995; Myers et al., 2000).

While some ecological impacts will be unavoidable, it becomes all the more crucial to predict any potentially adverse scenario (Genovesi, 2010). For example, impacts resulting from environmental toxins or poisoned baits yield direct impacts, while indirect impacts may arise via secondary ingestion within predator prey systems (Eason, Ross, & Miller, 2013).

Zavaleta et. al (2001) emphasise the importance of understanding the change to food webs, tropic cascades and predator-prey dynamics in invaded systems so as to avoid unexpected system reactions to species removal efforts. As the ecological context of eradications can become increasingly complex over time, they suggest that by quantitatively evaluating functional roles and trophic interactions, any post-eradication monitoring can be anticipated and built into an adaptive management framework (Lockwood et al., 2013). Additionally, by understanding the influence of the invader upon native species, the effects of the eradication upon the entire ecosystem can be better managed.

In spite of the numerous challenges posed by eradication operations, the corresponding ecological, social and financial benefits of doing so can quickly become apparent. New

Zealand's offshore islands have seen rapid recoveries in native ecosystems within very short timeframes upon the removal of various mammalian species. Tiritiri Matangi Island, an open sanctuary in Auckland's Hauraki Gulf, is a fitting example of what can be achieved once exotic invaders are removed and native vegetation is restored. After several centuries of human occupation and environmental degradation (primarily due to farming), only 6% of the island's forest cover remained (Galbraith & Hayson, 1995). It wasn't until the island became a Scientific Reserve under the Reserves Act (1977) that farming ceased and paved the way for more than 300,000 native trees to be planted (Graham & Veitch, 2002). The removal of wild populations of cats, rabbits, kiore and goats resulted in increased populations of 5.5 - 178.6% of various native bird species that had managed to persist on the island with the number of bellbirds (*Athornis melanura*) doubling between 1990 - 1998 (Graham & Veitch, 2002). Furthermore, two populations of extant species not known to be present on the island re-appeared several years later, the common gecko (*Hoplodactylus maculatus*) (2004) and the giant centipede (*Cormocephalus rubriceps*) (2007). Approximately 15 native fauna species were translocated between 1973 and 2011, demonstrating what can be achieved with upon removal of pest species (Galbraith & Cooper, 2013).

3.2.8.2 Economic

Invasion economics is a sub-discipline of invasion ecology that specifically deals with the allocation of resources to either reduce the risk of repeat introductions, or to control the existing damages caused by the invader (Perrings, 2010). While almost all eradications will require a substantial financial investment, the few studies which have compared the operational cost of eradication against the ongoing costs associated with inaction, have confirmed the financial utility of taking such measure (Heikkilä & Kettunen, 2014). Prior to the successful eradication of the painted apple moth (*Orgyia anartoides*) in New Zealand, the estimated ecological and economic cost of action ($63.8 million) versus inaction ($50-350 million over 20 years) were carefully evaluated prior to undertaking the eradication operation (Ministry for Primary Industries (formerly MAF), 1999; Suckling et al., 2007).

However, failure to carefully evaluate costs can prove to be an expensive error, as illustrated by Italy, a country that suffers economic losses in excess of €4 million each year by not removing the invasive copyu (*Myocastor coypus*) (also known as a river rat or nutrias), compared with Britain where they were eradicated in 1989 for approximately €5 million (Baker, 2006; Genovesi, 2010).

Several statistical models have been created to help parameterize variables such as the costs associated with rates of invader dispersal, to evaluate optimal funding allocation such as eradication versus containment, control or abandonment (Chalak, Pannell, & Polyakov, 2011). Chalak et al. (2011) found that as the probability of dispersal decreases for an invader, so do the economic damages caused by it. By accounting for border control costs, they identified that it is optimal to eradicate or contain an invasive species when the invasion is in the corner or on the edge of a landscape (as opposed to the middle) *and* when controlling boundaries can be effective.

3.2.8.3 Social

An understanding of the role played by local communities and the general public is critical to effectively tackle the problems associated with invasive species (García-Llorente, Martín-López, González, Alcorlo, & Montes, 2008). To foster a positive and informed public perception of invasive species management, local stakeholder groups should be involved in the decision making process wherever possible. Large-scale projects pose a much greater potential threat to non-target species, and consequently tend to be more socially controversial and costly (Myers et al., 2000). Public awareness and support are necessary components of an eradication, without which can act as major restraint. Operations involving chemical controls are often opposed by the public out of concern for human and environmental health. In an analysis of social factors that influence the willingness to support and fund invasive alien species management, Garcia-Llorente et al. (2011) found that people were more willing to pay for eradication than prevention; and public support was influenced by an individual's knowledge and perception of invasive species, an active interest in nature and socio-demographic attributes.

To create the awareness and understanding required to gain public support, various communication and engagement strategies such as educational pamphlets or brochures can be distributed or meetings can be held, which ultimately may be costly (Myers et al., 2000). This has been the case in New Zealand concerning the use of mono sodium fluoro-acetate (commonly known as 1080), where there is fierce opposition against its use amongst environmental lobbyists, in spite of the proven protection it offers indigenous wildlife and reduction in bovine tuberculosis (*Mycobacterium bovis*). In a study on public opposition to 1080 based upon risk perception and agency response, Green and Rohan (2012) identified 525 opposing public submissions against the application to reassess the use of the toxin in 2007 with grounds largely consisting of the perception that poisons are unsafe and indiscriminatory to non-target species due to the reported secondary poisoning of native species including kiwi (*Apteryx*), kea (*Nestor notabilis*), kaka (*N. meridionalis*), keruru (*Hemiphaga novaeseelandiae)* and morepork (*Ninox novaeseelandiae*). Additionally ethical concerns continue to be expressed due to the risk imposed to domestic farm and hunting dogs (*Canis lupus familiaris*) and game species such as deer (*Cervus* spp.), along with environmental concerns for freshwater ecosystems and human health (Brown & Sherley, 2002; Environmental Protection Authority, 2013; Green & Rohan, 2012). Many reported the practice was inconsistent with the clean '100% Pure', green image of the country and that this posed both economic and social risks to New Zealand's international reputation which could further impinge upon national export and employment opportunities (Green & Rohan, 2012).

3.3 Study rationale

Plague skinks were officially declared as an Unwanted Organism in 2010 under the Biosecurity Act (1993), however, since then there has been little research or progress made in containing their spread and impacts on native ecosystems.

As plague skinks continue to increase in numbers and range throughout the warmer regions of New Zealand's North island, it is important that control and eradication protocols be developed and tested to inform the overarching National Management Strategy and ensure the South Island and off-shore conservation sanctuaries remain free of this pest via the implementation of an effective response plan.

3.4 Aims

In the absence of alternative methodologies, the identification in April 2013 of a breeding plague skink population at Tryphena Wharf, Great Barrier Island has provided the opportunity to parameterize the operational methods of an eradication or control operation. This chapter seeks to:

- Assess the impact of intensive and sustained trapping upon the plague skink population throughout their active breeding season

- Evaluate the 'rapid response' efficiency of 3 herpetofaunal monitoring tools in a biosecurity incursion operation.

The outcomes of this operation are expected to provide a clearer understanding of the effort required to control plague skinks, with the overarching objective of developing a strategic incursion response framework which may serve to eradicate or control a novel infestation in the future. These objectives have been prioritised by the Auckland Council and Department of Conservation.

3.5 Methodology

3.5.1 General

Between November 2013 and January 2014 two trapping grids were installed across the known area of infestation adjacent to Shoal Bay at Tryphena Wharf (Fig 3.3). The methodology behind trap selection and placement was practical, operational and adaptive in nature.

Figure 3.3: Trapping grid boundaries (red outline) at Tryphena Wharf (36°19'13.04"S 175°29'09.26E) (Auckland Council GIS viewer)

Each trapping grid consisted of horizontal transects 2-5 meters apart on the vegetated banks adjacent to the wharf. Three trap types were installed (ISTs, G-minnow traps and pitfall traps) at varying spatial intensities along each transect (Fig 3.4).

Between January and April 2014 the trapping grids were set up to run four nights per week with up to 329 traps set per night. Traps were activated on a Monday and deactivated on a Friday, subject to weather conditions. Thereafter this effort was reduced to four nights per fortnight until June 2014, at which time it was decided that the effort and costs were not justified by the diminishing capture returns, and that trapping throughout winter was to be most effective when 3 to 4 consecutive fine days of weather were forecast.

3.5.2 Grid 1

Grid 1 consisted of 53 traps laid out across five transects (A – H). Transects A and B were set two metres apart with ISTs placed at two metre intervals along each of the transects. Transects C, D and E were considered inaccessible and were therefore each set with six g-minnow traps at five meter intervals on the lizard gondola pulley system. Two additional g-minnow traps were placed along the top of the grid and accessed from a walkable track leading around to Gibbs Landing.

3.5.3 Grid 2

Grid 2 consisted of 187 traps across seven transects (A-G). Transects A and B consisted solely of ISTs placed at two meter intervals along each transect to reflect the accessibility of the area, the optimality of the habitat and the proximity to higher densities of skinks at the core of the infestation. Transects C, D, E & F consisted of G-minnow traps and ISTs and set at 5m intervals.

3.5.4 Trap clusters

Where 'hotspots' of plague skinks were identified (either visually or through repeated trapping at neighbouring stations) 'cluster sites' were installed. These consisted of a cluster of between five and ten additional ISTs installed between the transects. These clusters were designed to be adaptive and follow the movement of plague skinks across the grids, throughout the course of the trap season. An additional 6 ISTs in a single cluster were installed in Grid 1, while an additional 83 ISTs were set up across 8 clusters in Grid 2.

3.5.5 Lizard gondolas

Due to the steep and inaccessible terrain across the majority of Grid 1 and numerous sections in Grid 2, a pulley system ('Lizard Gondolas') was designed to maintain the spatial integrity of the transects. The Grid 1 system consisted of a sequence of G-minnow traps secured to droplines which were then rotated through a support line that had been installed at the upper end of the grid (Plate 3.2).

Plate 3.2: Installation of Grid 1 lizard gondola system

Due to the heterogeneous terrain of Grid 2, a mixture of both individual and multiple G-minnow traps were either set up on gondola systems or secured to trees on droplines and lowered into inaccessible areas (Plates 3.3).

Plate 3.3: Grid 2 lizard gondolas being set in place and activated

3.5.6 Trap types

3.5.6.1 Invertebrate sticky traps (ISTs)

Individual invertebrate Trapper® Monitor and Insect traps were folded to shape, affixed into customised shelters (made from folded corflute or plastic tracking tunnel covers) and secured with a paperclip. The shelters were then secured to the ground with either a garden peg or hammered in with a nail. Traps were checked and/or serviced multiple times throughout the day to minimize the chance of captured lizards suffering mortality via heat stress or predation.

3.5.6.2 G-minnow traps

G-minnow traps were partially filled with leaf litter and baited with pear. A damp sponge was added to reduce the chance of dehydration of any captured lizards prior to the trap being serviced. Traps were secured and covered in vegetation to integrate them into the

habitat. Traps on drop-down lines and the lizard gondola system were covered with white muslin cloth to provide shade in sun-baked areas. Traps were serviced once daily.

3.5.6.3 Pitfall traps

Pitfall traps were partially filled with leaf litter and a damp sponge and baited with pear. Lids were set in place leaving a 1-2cm gap using small sticks on the rim and secured with a heavy rock. Traps were serviced once daily.

Key

○ (yellow)	IST
● (red cluster)	IST cluster site
◄ (blue)	G-minnow trap
✚ (purple)	Pitfall trap

Grid 2

Grid 1

25m

0

Fig 3.4: Grid 2 layout by trap type per transect (A-G). White lines connecting G-minnow traps indicate installation and servicing via the Lizard Gondola system. Red lines indicate trap grid boundary lines.

3.5.7 Identification

All captured skinks were identified to species using the single fronto-parietal head scale (Fig 2.12). This is an easily recognisable and definitive characteristic that distinguishes plague skinks from all native New Zealand skinks (Plate 3.4).

Plate 3.4: Single fronto-parietal head scale of plague skink, indicated by red frame (Wairepo, 2013)

3.5.8 Release

Skinks captured on ISTs were released by soaking the direct area with Canola vegetable oil and gently prying the animal away with the aid of a toothpick (Plate 3.5).

Plate 3.5: Native copper skink being released (Wairepo, 2013)

Native skinks were initially released outside the trapping grids (within 500m of trapping site) to reduce unnecessary repeated captures of non-target species. However, this practice was stopped after the first three weeks due to concerns about; i) destabilising native lizard guilds, ii) the potential for it to facilitate an increased spread of plague skinks by removing the competitive pressures imposed by native skinks, and iii) evidence from other studies showing reduced survival of lizards when relocated into unimproved

habitat already at carrying capacity. Thereafter, all native skinks were released back within the grid as close as possible to their original point of capture.

3.5.9 Morphometric data collection

Until mid-March, all captured plague skinks had the morphometric data collected to identify important demographic information about the population. This included sex, snout-vent length (SVL), vent-tail length (VTL), age class (neonate 15-19mm, juvenile 20-24mm, sub-adult 25-33mm, adult 34-55mm), weight and tail regeneration length if applicable. Thereafter, only age class and sex were recorded to speed up processing times.

3.5.10 Euthanasia

Plague skinks were euthanised using rapid blunt force, the accepted field practise for lizards with a bodyweight of 5g or less (Gartrell & Kirk, 2005). Female lizards were dissected to inspect for eggs, which were removed and stored in ethyl-alcohol for advocacy purposes (Plate 3.6). A small number of animals also had tails clipped and stored for potential future use (i.e., genetic screening).
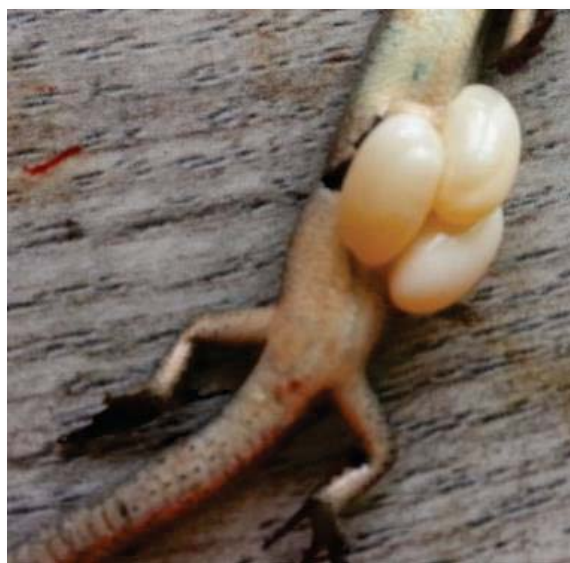
Plate 3.6: Euthanized female rainbow skink with exposed egg clutch (Wairepo, 2014)

3.5.11 Data analysis and statistics

As trapping between November and December was inconsistent due to incomplete trap grids, data analysis was restricted to the months of January, February and March. As no pre or post operation population abundance estimates were made, a binomial Generalised Linear Model (GLM) was used to assess whether time was a predictor of capture rate in an effort to interpret the impact of trapping on the capture data. The relative efficiencies of G-minnow traps and ISTs were simultaneously compared using the same model.

All descriptive statistics were calculated using Microsoft Excel. Statistical analyses were run using R 'stats' package (R Studio). Significance set at $p=<0.05$.

3.6 Results

3.6.1 Capture results

   3.6.1.1 Trap type

Eighty two percent of plague skinks were captured by ISTs (*n* = 417) across all age classes, capturing 84% of all neonates/juveniles (*n* = 216) and 79% of sub-adults/adults (*n* = 185). G-minnow traps captured 8% of total catch (*n* = 40), capturing 17% of all sub-adults/adults. No juveniles or sub-adults were caught in these traps.

Pitfall traps captured a single adult accounting for 0.4% of the age class (*n* = 1) and the remaining 9.6% of captures (*n* = 49) were done so opportunistically by hand with 16.3% of juveniles (*n* = 16) and 3% of adults (*n* = 7) (Table 3.3).

   3.6.1.2 Demographics

Between 20th November 2013 and 28th March 2014 a total of 507 plague skinks and 619 native skinks across 3 species (*O. aeneum* = 166*, O. moco* = 355*, O. ornatum* = 98*)* were captured over 62 nights in Grids 1 and 2, equating to an approximate total of 16,000 trap nights.

233 plague skinks were of breeding age, classified as either sub-adult or adult (98 male, 114 female, 44 unspecified) and 258 were juveniles or neonates. 16 skinks did not have sex or age class recorded.

A total of 115 eggs were removed from 31 euthanized females with an average SVL measurement of 41.48mm ± 3.9SD and 3.58 ± 1.1SD eggs per clutch which were not found to be significantly correlated (*r* = 0.197, *p* = 0.179). A large communal nest was unearthed, revealing a total of 120 hatched shells and 25 unhatched egg. Upon removal eggs were stimulated to prematurely hatch, resulting in the capture of an additional 5 neonates.

Table 3.3: Plague skink capture results with descriptive statistics by age class and trap type for January, February and March. Not recorded indicates number of skinks captured with missing age class data.

# Plague skink captures	JANUARY (12 trap nights)					FEBRUARY (17 trap nights)					MARCH (18 trap nights)				
	IST	G-Minnow	Pitfall	Hand	TOTAL	IST	G-Minnow	Pitfall	Hand	TOTAL	IST	G-Minnow	Pitfall	Hand	TOTAL
Juvenile/neonate	46	7	0	1	54	60	0	0	13	73	110	0	0	24	134
Sub-adult/adult	45	0	0	3	48	51	19	0	2	72	43	14	1	4	62
Not recorded	1	0	0	0	1	2	0	0	0	2	0	0	0	0	0
Total	92	7	0	4	103	113	19	0	15	147	153	14	1	28	196
Mean	30.67	2.33	0.00	1.33	34.33	37.67	6.33	0.00	5.00	49.00	39.33	4.67	0.33	9.33	65.33
Std. deviation	25.70	4.04	0.00	1.53	29.02	31.21	10.97	0.00	7.00	40.71	55.43	8.08	0.58	12.86	67.06
Std. error	14.84	2.33	0.00	0.88	16.76	18.02	6.33	0.00	4.04	23.50	32.01	4.67	0.33	7.42	38.72

3.6.1.3 Demographic variation over time

The representation of each age class and sex varied across months. While the adult to juvenile ratio was fairly even in January and February, juveniles made up a higher proportion of the captures in March (Figure 3.5). Similarly, while the capture ratio between sexes was fairly balanced in January and February, the proportion of males appears to be reduced by March but cannot be confirmed due to the high proportion of captured animals with sex not recorded (Figure 3.6).
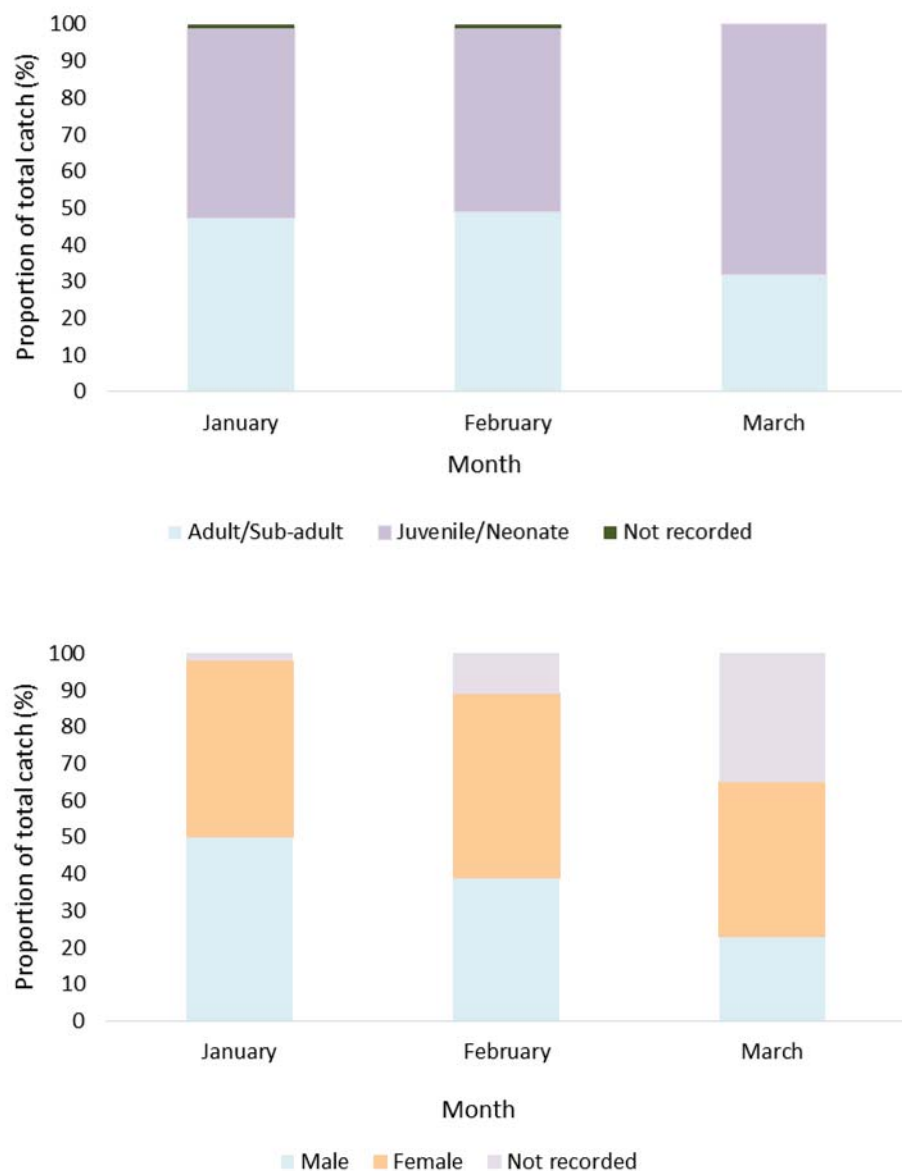
Figure 3.5, 3.6: Total captures across January, February and March divided by proportion of age class (Fig 3.5: top) and sex (Fig 3.6: bottom). Where a skink was captured and did not have age class or sex recorded, they were still included in each chart.

3.6.2 Spatial distribution

Spatial plots of cumulative captures by month show areas of higher plague skink densities. These underpinned the targeted and adaptive placement of traps, and identified the locations to intensify trap effort with hotspot clusters (Fig 3.7).
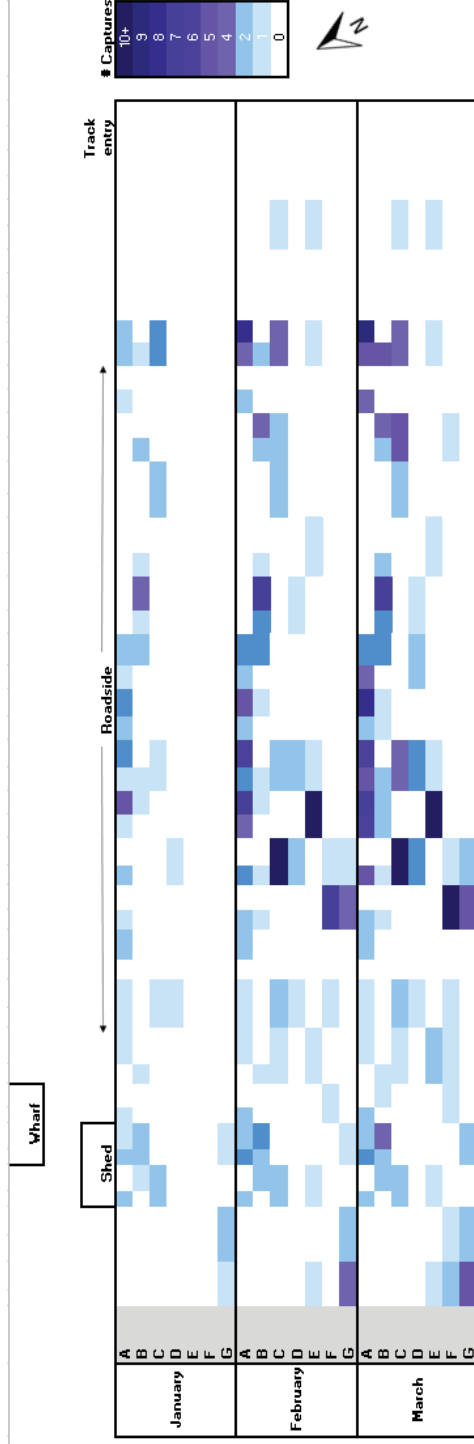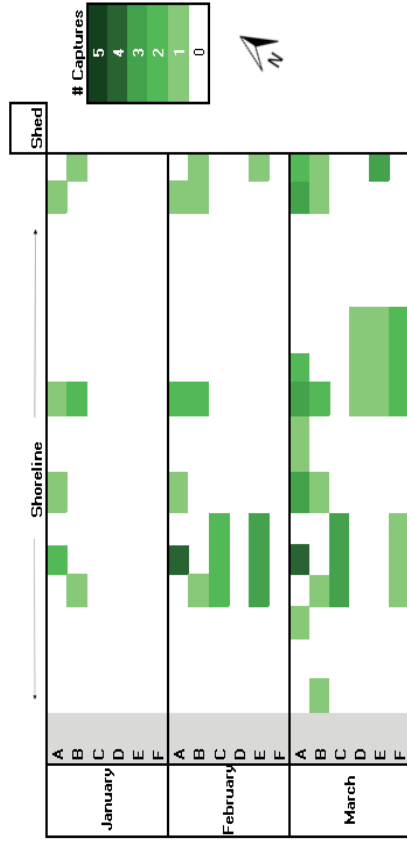
Figure 3.7: Grid 1 (top) and Grid 2 (bottom) spatial distribution diagram by month, of cumulative plague skink captures of all age classes per individual trap across all transects between January – March 2014. Each square in transects A and B represent a spatial trap density of of 2 meters, and each square in transects C,D,E,F and G represents a trap density of 5 meters. Cell colour represents the total number of captures an individual trap has made across the 3 month timeframe.

3.6.3 Capture trend

The total number of plague skink captures increased each month (January, n = 103, February, n = 147, March, n = 196), resulting in a cumulative number of captures for both grids, each displaying a steady increase over time. Both grids showed a decreasing rate in cumulative adult captures, however, the overall increase continued to be driven by the growing neonatal population (Figure 3.8).
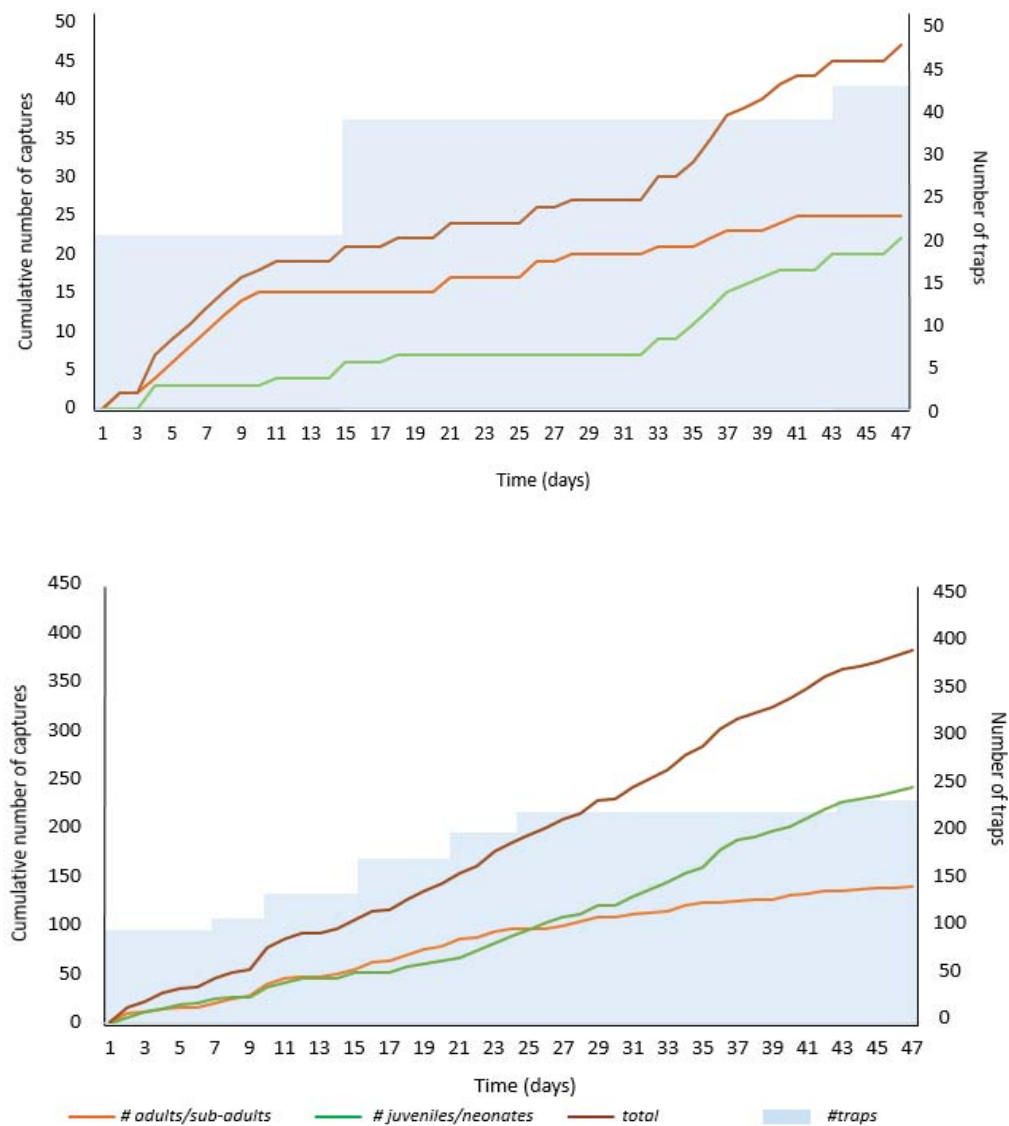
Figure 3.8: Cumulative number of plague skinks captured (left Y axis) in Grid 1 (top) and Grid 2 (bottom). Age class is separated by juveniles/neonates and sub-adults/adults and measured against the number of traps running each night (right Y axis) increasing over time.

3.6.4 Statistical analysis

3.6.4.1 Capture reduction and trap comparison

The relationship between the proportion of traps for each transect capturing skinks over time was examined for a sub-set of the Grid 2 data, where trap effort was consistent and balanced between trap types, using logistic ANCOVA with a logit link function (R 'stats' package). The model failed to reveal a significant reduction in the number of plague skinks captured by each trap over time (z = -1.150, p = 0.250, df = 236). Additionally, a comparison of relative efficiency between the trap types within revealed there was no significant difference between G-minnow traps or ISTs (z = 0.693, p = 0.488, df = 236) (Fig 3.9).
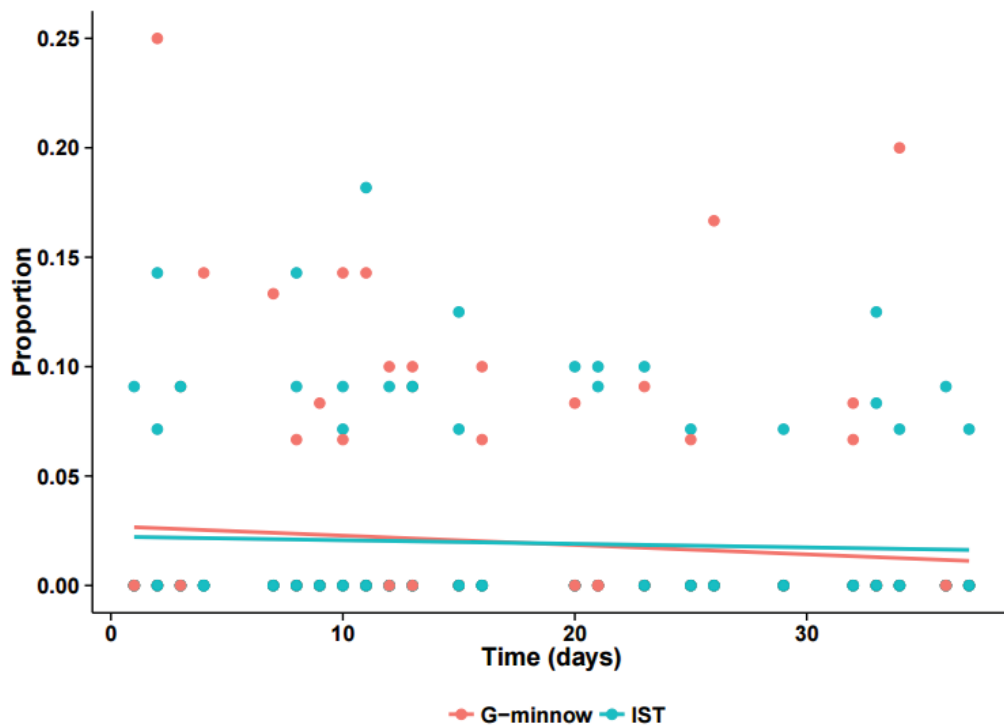
Fig 3.9: Logistic ANCOVA displaying capture data over time for a balanced number of G-minnow traps and ISTs within Grid 2. Points represent the proportion of traps capturing a skink per night per transect. Lines represent the estimated regression that best fits the data for each of the trap types (blue = IST, pink = G-minnow). There was no significant change in the proportion of traps capturing skinks over time, neither was there a significant difference between the capture returns of each trap type.

3.7 Discussion

As with numerous other countries which have become increasingly susceptible to invasion by alien herpetofauna, New Zealand was slow to recognize the potential ecological threat imposed by plague skinks. Due to its conservation values and proximity to other more ecologically sensitive off-shore islands inhabited by rare endemic herpetofauna, the incursion on Great Barrier Island provided a much needed impetus to re-address the risk of this species to native lizard fauna. As such, ours was the first attempt to parameterize the requirements of plague skink control in New Zealand and possibly internationally.

The research aim of this chapter was to assess the efficacy of the operational management response to the plague skink incursion on Great Barrier Island, the priority of which was to remove as many individuals from the population as possible. While the application of ongoing adaptive management throughout the course of the operation was critical to improving capture success, it introduced several biases into the evaluation process, making robust and statistically grounded conclusions challenging.

3.7.1 System evaluation

Our data shows a steady increase in the number of plague skink captures between January and March. This, combined with the discovery of a large communal nest along with the wide spatial distribution of skinks of all age classes enabled two conclusions to be reached. Firstly, the plague skink population was substantially more abundant and widespread than previously thought, and secondly, the trapping effort was insufficient to trap the population to extinction.

Interestingly however, the data suggests that while the overall cumulative number of captures increased, they appear to have been primarily driven by an abundance of neonates recruiting into the population. While the cumulative number of adult captures also continued to rise over time, the capture plots suggest a slow down towards the end of the capture period. Although it is tempting to suggest that this is evidence of the demographic having been impacted by the effort, when the adult and sub-adult capture

data was statistically evaluated, the model did not provide any support for this observation. This suggests that the perceived reduction in capture rates illustrated by the decreasing trend (Fig 3.9) may have been driven by other biological and ecological factors that the model did not account for.

Behaviours such as trap avoidance and anti-predator responses to the regular and sustained anthropomorphic disturbance are well documented in the literature and are likely to have contributed to some degree towards the demographic variability in the capture data. Frid & Dill (2002) report that the same economic principles employed by prey encountering predators may also be used when faced with generalized threatening stimuli such as a loud noise or rapidly approaching object.

In spite of the increase in the number of traps targeting the growing neonatal population, the capture data suggests that the effort was insufficient to curb this trend, as illustrated in Figure 3.8. However, no robust quantification of true demographic distribution and variation can be made with our data, as it was constrained by the high number of skinks that did not have age class or sex recorded. Additionally, our research did not seek to explore the external variables which influenced capture rates, and without these key pieces of information it is difficult to form meaningful conclusions.

Kraus (2009) describes the key issues that result in the failure of herpetofaunal eradication attempts as insufficient funding, poor planning, an initial misconception of the size of the target population and a lack of involvement by trained management. A number of examples illustrate this stance, including the US$51,000 1 year eradication programme of the Nile monitor (*Varanus niloticus*) in 2002 which was forced to be down-scaled to a research program when it quickly became apparent that the density and range of the population exceeded that which was feasible to eradicate with limited time and funds (Kraus, 2009).

Our operational failure mechanisms are not only consistent with the above description, but were further confounded by a high likelihood of sustained propagule pressure of

invading skinks from the mainland, and a lack of efficient tools for detecting invaders and dispersers at low densities.

3.7.2 Tool evaluation

The GLM model did not report any statistical difference in capture rates between two of the tools utilized in this operation. However, from a practical standpoint, their benefits and limitations quickly became apparent.

3.7.2.1 Pitfall traps

Garden et al. (2007) and Ribeiro- Júnior (2008) found pitfall traps to be the most effective and cost efficient of several herpetofaunal traps, yielding the greatest level of detection success of small, terrestrial lizards. Conversely anecdotal evidence suggests this may not be the case for plague skinks, as reported by a Ranger on Motutapu Island who has watched plague skinks actively avoid pitfall traps on several occasions (A. Polkanov, personal communication, April 2013).

The pitfall traps installed at the outer periphery of Grid 2 captured a total of 15 skinks, of which 14 were native, providing some support for the findings reported by Polkanov. They were not included in the analysis due the low number installed (determined by only a limited number of locations having level ground) and poor capture result, which may be explained by their location and distance from the core infestation. However, these practicalities are both a realistic and common scenario on any of New Zealand's off-shore conservation islands, many of which possess steep, heterogeneous landscapes. Should the eradication of any invasive reptile be required in a future scenario, pitfall traps should only be used as a supplementary aid to other trapping techniques with a proven superior efficiency, or as part of a post-operative monitoring regime.

3.7.2.2 G-minnow traps

While the G-minnow traps only contributed 8% of the total skink reduction, they did account for 17% of all adult and sub-adult captures. This combined with their quick

installation and low environmental disturbance makes them a promising tool in a biosecurity context. Unfortunately we found this particular model to have an inappropriate mesh size which allowed neonates and juveniles to evade capture. Their quick set up time was also offset by the time it took to service them properly, which involved sifting through vegetation for skinks while ensuring anything captured didn't escape. By creating this microhabitat with food and water, it elevated the risk to trapped skinks through the increased potential to attract predators. While structural adaptations such as finer mesh and excluder modifications at entry points could mitigate against this risk by preventing rodent sized predators or larger, it wouldn't protect against invertebrate predators such as swarms of carnivorous ants or giant centipedes which have been known to opportunistically predate upon trapped lizards (H. Jamieson, personal communication, November 2013).

3.7.2.3 Invertebrate sticky traps

Unlike G-minnow traps, ISTs did capture high numbers of plague skinks across all age classes, capturing 82% of all skinks, 84% of all juveniles and neonates and 79% of all adults and sub-adults. These figures along with observational evidence suggest ISTs are superior to other trap types, which may be explained by the ease with which an animal becomes captured, requiring nothing more than a single foot to make contact with the glue. This contrasts with pitfall and G-minnow traps which require an element of behavioural boldness to function efficiently. While the use of them in herpetofaunal monitoring is extremely limited and somewhat novel in New Zealand, our findings are consistent with those reported almost twenty years ago by (Downes & Borges, 1998) and (Whiting, 1998), who found them to be extremely effective in capturing large numbers of small, fast and aggregative terrestrial lizards. Interestingly, Whiting (1998) observed an increased rate of capture for males of an unspecified *Lampropholis* species when lured by trapped females.

ISTs can be discretely camouflaged along dispersal pathways, capturing the more cryptic and neophobic lizard species that would normally be considered as hard to sample (Bauer & Sadlier, 1992). Their compact size enables placement in low-level vegetation,

rock banks and other areas that are too small and impractical for the bulkier traps (Downes & Borges, 1998). Additionally, the self-baiting mechanism of the attractant-infused Trapper and Insect Monitor® gluecards further enhances trap efficiency.

Two important disadvantages of ISTs were quickly identified. The immobilization of captured skinks renders them particularly vulnerable to predation by ants, rodents and birds, as well as increasing mortality risk via stress and heat. This was also observed and noted by Vargas, Krakauer, Egremy-Hernandez, and Carlton (2000) who reported high fatality rates when 47.6% of sticky-trap captured *Anolis carolinensis* expired. Additionally, Rodda, McCoid & Fritts (1993) reported the fatality of all *Emoia atrocostata* specimens within 6 hours and suggested that mortality may have a species-related component due to the survivorship of others. While our efforts to actively prevent these unacceptably high mortality rates were successful, our results do indicate that native copper and ornate skinks were almost 1.5 times as vulnerable to mortality when trapped on ISTs (15% and 14.8% mortality respectively) than moko and plague skinks at 8% and 9% mortality, thereby supporting the suggestions of Rodda et al. (1993).

Bauer & Sadlier (1992) and Vargas et al. (2000) noted the limitations of ISTs with reduced glue adhesion after rainfall and frequently becoming fouled with leaf litter, soil or debris rendering them ineffective. However, we observed the phenomenon to be an act of 'biological sabotage' by resident ant communities that were able to completely cover the sticky surface with leaf litter in a matter of hours, disabling it and discounting it for an entire trap night (Plate 3.7).

Plate 3.7: ISTs 'deactivated' due to native ants covering them in leaf litter (Wairepo, 2015)

3.7.3 Study limitations - balancing operational and research goals

Despite the resurgence of interest in reptile conservation over the past twenty years, obtaining population data for them remains a significant challenge due to their cryptic and often highly seasonal patterns of behaviour which are intrinsically tied to prevailing weather conditions (Griffiths et al., 2015). While numerous statistical tools are now available to provide a detailed analysis and robust estimates of a given population, herpetological data collection methods have changed very little over the last quarter century and may therefore be riddled with violations of assumptions that are critical to the accuracy of statistical assessments (Sewell, Griffiths, Beebee, Foster, & Wilkinson, 2013). Furthermore, the technological advancement of such tools often requires specialist training that falls outside of the expertise of many conservation practitioners who question the contribution of statistically rigorous population assessments to conservation decision making (Griffiths et al., 2015).

While it is extremely important to be able to statistically evaluate descriptive data and observational evidence, the primary objective of the field effort was to capture as many plague skinks as possible. The nature of biosecurity responses such as these mean robust experimental designs are not prioritized. However, the opportunity to examine

the data collected and assess whether the system, effort and tools applied were effective at achieving the operational goals was an important measure to have taken. While our model and its outputs do answer the research questions, they must be interpreted with caution, as they were limited by an unbalanced design and practical constraints.

As this was the first attempt of its kind, very little information was available to assist with decisions surrounding grid design and spatial intensity. As such, a rationale grounded in resource availability determined these decisions.

It would have been desirable to apply equal 2 meter spatial intensities across all transects, however was not possible due to the time associated with processing skinks, particularly given the high number of native captures which was greater than the number of plague skink captures. While it is acknowledged that the presence of native species will influence the outcomes and impacts of any eradication effort (Zavaleta et al., 2001), these unexpected densities meant a great deal of limited conservation resources were re-directed towards the processing and release of native bycatch. While this was far from ideal, it was critical to mitigate against unacceptable levels of native bycatch mortality, and as such, flexibility needed to be built into the working timeframes of each day.

An important quality of the adaptive approach is that it deals with uncertainty through a structured improvement of relevant knowledge while simultaneously minimising risks associated with ongoing management (Keith et al., 2011). The balance between minimizing harm to native lizards and capturing as many plague skinks as possible further justified the adaptive integration of targeted clusters of traps in known skink hotspots. Additional biases of the system included high intensity effort applied in a stratified manner by distance from the core population, and use of a single trap type areas with higher skink numbers due to an un-tested yet perceived superior efficiency.

Our spatial distribution diagrams (Fig 3.7) confirms the clustered distribution of skinks throughout the area analysed by the model. This further complicates the system

evaluation due to model assumptions that the sample represents a constant proportion of the population (Hare, 2012b). Temporal, spatial and individual heterogeneity in detection and capture probabilities are typical of many animal populations (White, 1982), with variance between habitat, population density and interspecific interactions all identified as influential over capture rates (Lettink & Armstrong, 2003; Wiedemer, Wilson, Mulvey, & Clark, 2007).

To reconcile some of the modelling issues, juveniles were excluded (due to not being captured in G-minnow traps) and the analysis was restricted to a small sub-set of Grid 2 capture data where the trap effort was balanced and constant. This adjustment satisfied closure assumptions which are an important requirement when modelling population data. Closure designates that animals neither leave nor enter a population during the course of a study. This can be either geographic (immigration and emigration) or demographic (births and deaths), and given its rarity in biological populations (Otis, Burnham, White, & Anderson, 1978; Stanley & Burnham, 1999), must be considered when interpreting modelling outputs.

3.7.4 Learning and innovation

Challenges encountered in invasion biology and all other areas of ecological management must be viewed as opportunities for important learning outcomes if these disciplines are to continue advancing in knowledge, skill and expertise. The steep, often inaccessible and heterogeneous terrain of the trapping grids is typical of many New Zealand landscapes. Our lizard gondola pulley system was an innovation designed to maintain the spatial integrity of the trapping grids in inaccessible areas. While various similar systems had previously been designed to erect traps or survey equipment into canopy systems for the purpose of collecting seed, sampling epiphytes, insects and arboreal vertebrates (Faulds & Crabtree, 1995; Tucker & Powell, 1992), ours appears to be the first to have integrated arboricultural canopy access and herpetological monitoring techniques across a landscape to access steep terrain.

While the lizard gondola system did not capture a high number of plague skinks, it did successfully trap them in both grids, confirming species presence and distribution where it would otherwise be unknown. The system provides a foundation to build upon as a novel tool to be used across a diverse range of disciplines in wildlife management and conservation biology, particularly for native herpetological species. Not only does it alleviate the requirement of specialist skillsets in order to survey or monitor a range of species in inaccessible terrains, but it also minimizes the high level of environmental disturbance which is so often associated with such efforts (Towns, 1991). By substituting G-minnow traps for custom made, weighted IST shelters at an increased density, the lizard gondolas may have the potential to yield a much greater capture rate and resulting impact upon the plague skink sub-population of Grid 1.

3.8 Conclusion

In spite of the challenging complexities surrounding the eradiation and control of invasive herpetofauna, the rapidly increasing momentum to develop and test novel management techniques warrants a sense of optimism in this emerging field of invasion biology. Our data suggests that both our trap type combination and spatial intervals between them were not sufficient to eradicate plague skinks from a heavily invaded area. While this can be considered a key limitation to the operational success of this study, carefully designed experiments testing trap density and effort requirements should considered as an important next step to take. The implications of this may be far reaching in New Zealand biosecurity as the frequency with which herpetological invaders arrive upon our shores increases.

Chapter 4

Lessons from Guam – Testing the toxicity of acetaminophen to plague skinks (Lampropholis delicata)

4.1 Abstract

Context*.* Plague skinks (*Lampropholis delicata)* are the only exotic reptile to have successfully established in New Zealand. As plague skinks continue to increase in numbers and range throughout the warmer regions of New Zealand's North and South Islands, it is of great importance that the incidence of incursion to off-shore islands be minimized. Surveys of potential vector sites across the Auckland region between 2011 and 2013 have resulted in the identification of nurseries, marinas and building supply yards as providers of regular off-shore transport pathways to plague skinks. The development of a toxin to target this species in these mainland areas may provide an effective solution to this problem with little to no impact on native congeners.

Acetaminophen is a lethal oral toxicant to reptiles and has been effectively used to control invasive populations of brown tree snakes (*Boiga irregularis)* in Guam. In controlled lab conditions it was also shown to be an effective toxicant to juvenile Burmese pythons *(Python molurus bivittatus*) and Nile monitor lizards (*Varanus niloticus*), both of which are invasive in Florida.

Method. We determined the effectiveness and required toxicity of acetaminophen (in the form of paracetamol) as a reptile toxin using a controlled laboratory trial. An up-down procedure was used to estimate the median lethal dose (LD50), with each animal treated sequentially in a staircase design. A total of 9 plague skinks were tested at 3 different dosages of 0.175, 0.55 and 1.75mg/g.

Key results. Acetaminophen was found to be acutely toxic to plague skinks resulting in mortality for 4/9 individuals. Statistical software AOT425Pgm, version: 1.0 calculated the LD50 at 0.55mg/g (0.385-1.530 SD, 95% CI).

Conclusion. The results of this trial are expected to provide valuable insight into whether or not acetaminophen based baits could be developed commercially for the eradication of plague skink infestations at mainland vector sites in New Zealand.

4.2 Introduction

4.2.1 Toxicant use in invasion biology and conservation

New Zealand employs a wide use of chemical toxicants including pesticides, fungicides, insecticides, fumigants and broad spectrum anti-coagulants for the control of various exotic flora and fauna as part of integrated management regimes (Goldson et al., 2015). While the use of toxicants has been successful, the general public, along with various environmental and animal welfare groups have expressed concern for the impact of such chemicals upon forest, soil and freshwater ecosystems, along with non-target indigenous species (Goldson et al., 2015). While a great deal of progress has been made to address the concern and develop less environmentally harmful products, the use of synthetic toxicants is still necessary to suppress exotic invaders (Goldson et al., 2015).

Unlike other countries such as Australia, New Zealand's forests are extremely vulnerable to mammalian browsers due to not having evolved in their presence and therefore not having developed natural anti-herbivory toxins (Eason & Wickstrom, 2001). New Zealand has achieved a number of successful pest eradications due to the use of various poisons targeting mammalian predators, some of which use synthetically manufactured equivalents of these natural toxins. Sodium-flouroacetate (commonly referred to as 1080) is a manufactured version of flouroacetate, a naturally occurring toxic component of numerous plant species that grow on high fluoride soils in countries including Australia, Sri Lanka, India, Africa and South America (Eason, Miller, Ogilvie, & Fairweather, 2011). It has been used extensively in New Zealand pest control efforts since the 1950's, and along with several other chemical toxicants, has contributed substantially towards positive conservation outcomes. It is the only toxicant registered in New Zealand for aerial control of possums (*Trichosurus Vulpecula*), however, it has been tested on a number of diverse species including birds, mammals and reptiles, with highly variable LD50 values according to the mode of delivery (Eason et al., 2011; Eisler, 1995). While the primary administration mechanism is via the ingestion of baited pellets, 1080 can be inhaled or absorbed through dermal cuts and abrasions (Saunders & Stacey, 1949).

4.2.2 Ecotoxicology of reptiles

The complexity of herpetofaunal life cycles leads to diverse ways in which they are exposed to environmental contaminants, with both skin and food uptake routes playing an important role (Sparling, Linder, Bishop, & Krest, 2010). However, until recently very few laboratory test procedures have been implemented to evaluate the effects of chemicals on reptiles, in spite of the knowledge that they are sensitive to environmental chemicals and may serve as surrogate indicators of environmental health (Hopkins, 2000; Sparling et al., 2010). This distinct gap in the literature can in part be attributed towards the slow growth rates, long life cycles and complexity of sexual maturity that make it challenging to establish captive breeding populations of many reptile species for developing standard test methods (Sparling et al., 2010).

New Zealand's indigenous herpetofaunal assemblage is vulnerable to a wide range of pest management strategies which incorporate the use of numerous pesticides, herbicides, fumigants and bait pellets. This has resulted in a number of key studies aimed at understanding the toxicological impact of these specific chemicals upon native skink and gecko species, which is a key focus of applied toxicology (Fisher, 2011; Hopkins, 2000; Sparling et al., 2010). An increasing body of evidence has now demonstrated that indigenous herpetofaunal species will routinely consume bait pellets along with toxin-loaded invertebrates, with the effect of brodifacoum being noted in 3 reptile species: Duvaucel's gecko (*Hoplodactylus duvaucelii),* the common gecko (*H. maculatus)* and shore skinks *(Oligosoma smithi)* (Fisher, 2011; Hoare & Hare, 2006; Wedding, Ji, & Brunton, 2010)*.* The results of these combined with similar investigations into the effects of pindone (a first generation anti-coagulant used for rabbit control) and 1080 on New Zealand's McCann's skink (*O. maccanni*), suggest that while direct mortality due to anticoagulant toxicosis does not appear to be a risk due to the vast quantities that would need to be consumed, the chronic and sub-lethal impact remains to be thoroughly evaluated (Freeman, Hickling, & Bannock, 1996; Wedding et al., 2010). Interestingly, in trials evaluating risk to native herpetofauna by way of direct or secondary poisoning in 1080 pest-operations, Australian amphibian and reptile species were found to be more tolerant to 1080 poison than most other animals (McIlroy, King, & Oliver, 1985).

4.2.3 Invasive reptiles

The phenomenon of globalization has resulted in herpetofaunal species crossing biogeographic boundaries at unprecedented rates, and in spite of their overall global declines are increasingly becoming invaders capable of reaching high density populations in novel locations and imposing devastating effects on native and endemic species (Fisher, 2011). Examples include the Asian house gecko (*Hemidactylus frenatus*), which has invaded many islands throughout the Pacific, the gold-dust day gecko (*Phelsuma laticauda*), a species of increasing concern as it disperses south of Hawai'i, and various skink species including the curious skink (*Carlia ailanpalia*) that is rapidly displacing native Micronesian skink species as it spreads throughout the region (Fisher, 2011). This escalating level of invasion has prompted an increased awareness of alien amphibians and reptiles, which has historically lagged behind that of other vertebrate species (Reed & Kraus, 2010).

Managing populations of invasive herpetofauna through trapping or shooting has been successful at reducing numbers, but will not achieve effective control or eradication outcomes without an integrated approach incorporating the use of toxicants (Avery, Eisemann, Keacher, & Savarie, 2011). As such, a number of toxicants have been trialled in recent years for their potential as management tools. For example, toxicant testing of zinc phosphide and acetaminophen on invasive black spiny-tailed iguanas (*Ctenosaura similis*) has shown zinc phosphide's potential to lethally control populations where they are a problem in Florida, resulting in 100% mortality at low doses of only 25mg per lizard in clinical trials (Avery et al., 2011).

Acetaminophen has received wide attention for its potential use as an effective toxicant to control invasive reptiles, after having been used to manage the brown tree snake (*Boiga irregularis)*, known perhaps as the most notorious of all invasive reptiles due to having decimated native bird and reptile populations in Guam since its accidental introduction 60 years ago (Brooks, Savarie, & Johnston, 1998; Savarie, Shivik, White, Hurley, & Clark, 2001). Researchers spent several years assessing the oral and dermal toxicity of more than 20 chemicals including rotenone, propoxur, natural pyrethrins, allethrin, resmethrin, diphacinone, warfarin and asparin (Brooks et al., 1998; Johnston, Mauldin, Savarie, Brooks, & Primus, 2001). While many of these toxicants demonstrated

potential, acetaminophen was selected after rigorous testing due to its benign environmental impact, minimal risk to non-target organisms, widespread availability, low cost and potential for registration under the Federal Insecticide, Fungicide and Rodenticide Act (1988) (Savarie et al., 2001; U.S. Department of Agriculture, 2011).

4.2.4 Classes of toxicants

A toxicant is defined as any chemical of natural or synthetic origin, which is capable of causing harm to a living organism. It is distinguishable from a toxin, which is the metabolic product of a living organism, evolved as a defence mechanism for deterring or killing predators or pathogens (Cope, Leidy, & Hodgson, 2004).

Toxicants can be categorized according to their form, which rarely remains static upon release into the environment causing harm as air pollutants, or degrading into water and soil pollutants capable of entering nutrient cycles and food webs (Cope et al., 2004). Exposure to ubiquitous metals (including lead and mercury), food additives, solvents and therapeutic drugs may be through air, water or food sources, while agricultural chemicals such as pesticides, herbicides and fumigants, are transported through the soil matrix resulting in nitrate and organophosphate accumulation and leaching into drinking water (Melamed, Cao, Chen, & Ma, 2003). This level of industrial advancement has led to the increasing occurrence of chronic disease as a result of unregulated exposure of carcinogens via inhalation, physical contact or ingestion (Hodgson, 2004).

4.2.5 Metabolism of toxicants

Several defence mechanisms and barriers prevent and regulate the entry, absorption and distribution of toxicants into the body (Fig 4.1) (Baynes & Hodgson, 2004). While the skin acts as a physical barrier to absorption, alternative routes of entry include the alimentary tract, skin, lungs, eye or via injection (Ensley, 2013). The gastrointestinal track's microbes and enzymes offer poor resistance to chemicals which will be absorbed and distributed via the circulatory system to various tissues across the body which will attempt to detoxify itself via the metabolic system. The extent to which it will succeed

is largely determined by the rate at which the toxicant is being simultaneously eliminated via metabolic and excretory mechanisms of the liver (Baynes & Hodgson, 2004; Ensley, 2013).

The ability of a given species to efficiently remove toxic substances is critical to its survival and is interlinked with the complexity of its structure and form, as well as the concentration of the compound (Rose & Hodgson, 2004). While passive diffusion may be sufficient for a unicellular organism to remove a toxicant, more complex species eliminate toxicants and their metabolites via the renal and hepatic systems and as such, the liver and kidneys are important sites to examine for signs of chemical toxicity (Ensley, 2013).
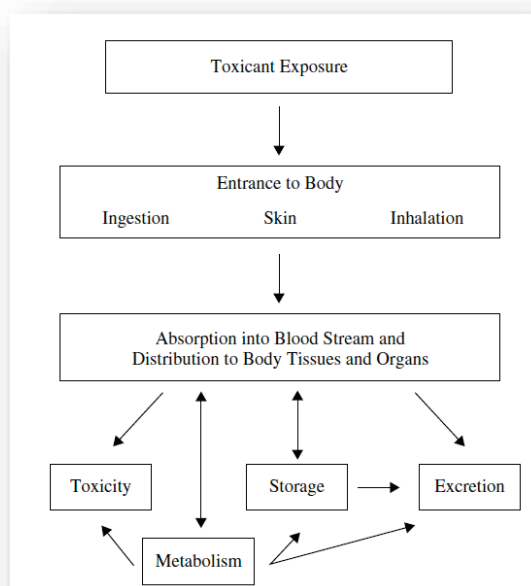
Figure 4.1: Fate and effect of toxicants in the mammalian body (Hodgson, 2004).

4.2.6 Toxicity Testing

Toxicity testing is defined as the determination of potential hazards that may be produced by a test substance, and typically employs a wide range of tests in different animal species via the long term administration of a drug, regular monitoring of physiological and biochemical responses along with detailed post mortem examinations to detect histological abnormalities (Arome & Chinedu, 2013). An experimental toxicant can be administered orally, dermally, as an inhalant or injected. Where gastrointestinal absorption is required, the chemical can be mixed with food, water or inserted directly via the use of a catheter. Before running any toxicity based experiment, information on the chemical structure and physiochemical properties of the test substance should be obtained along with the results of any previous experiments using it (Cunny & Hodgson, 2004).

While the benefits of toxicity testing should always be assessed as outweighing the harm, it is often controversial in nature due to its use of experimental animals, their associated welfare issues and extrapolation of such data to human physiology (Cunny & Hodgson, 2004). For this reason, all toxicity tests should ideally be conducted using animals which are likely to elicit responses similar to the species which they are trying extrapolate the data to (Hayes & Kruger, 2014).

4.2.6.1 Acute toxicity

There are two primary perspectives for considering acute toxicity. It may act as a qualitative indicator of an incident of poisoning, implying acute exposure. Alternatively, subject mortality occurrence within 2 days may represent an acute effect (LeBlanc, 2004). Both perspectives can provide useful information on doses associated with target-organ toxicity and lethality, the effects of which are generally measured through testing the lethal dose of 50% of the test population, otherwise known as 'LD50' testing (Hayes & Kruger, 2014).

While it is at this 50% response point that the greatest level of confidence can be achieved, placing too much emphasis on the outcome of an LD50 may be erroneous as

lethality is only one of many critical indices in assessing acute toxicity, which also includes dose-response curve, time to death, clinical signs of pain and histopathological findings (Hayes & Kruger, 2014).

The optimality of the experimental environment is critical to attaining accurate results, with major deviations from ideal temperature, humidity and photoperiod influencing stress and associated metabolic and chemical responses (Hodgson, 2004). Dose-response curves indicate the relationship between the change of dose and the lethal response and may provide insight into the intrinsic toxic characteristics of a compound or even the mechanism of toxicity (Fig 4.2) (Hayes & Kruger, 2014). They can be quantified by dosing a minimum of three concentrations at defined time periods, which can then be used to calculate the LD50 along with an estimated threshold dose, which is the lowest dose of the chemical eliciting a response (Hodgson, 2004). Calculations are based on the assumption that dosage is directly related to the percentage that responds, and is presented as mg/kg with confidence limits.
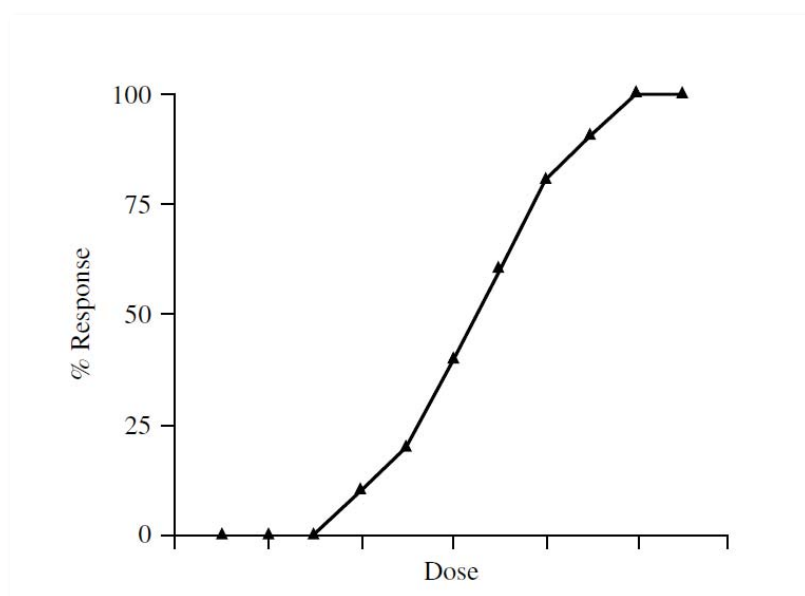
Figure 4.2: Typical dose-response curve (Hodgson, 2004)

4.2.6.2 Alternative methods

Traditional LD50 testing has received a high level of criticism due to the number of experimental animals used (normally consisting of 5 of each sex for each dose), resulting in the development of alternative methodologies to determine acute toxicity including the 'up-and-down procedure' (UDP), 'fixed-dose procedure' (FDP) and the 'acute-toxic-class method' (Stallard & Whitehead, 1995). Compared with classical procedures, each of these alternatives offer a major reduction in the number of animals used (Bruce, 1985).

The UDP uses a sequential 'staircase' design in combination with sophisticated computational methods and binary experimental endpoints (yes/no outcomes) to determine the LD50 dose of a toxicant (Rispin et al., 2002). The UDP uses a ½ log dose progression factor (3.2) to a maximum of 5000mg/kg, with a starting dose based on characteristics of the chemical being evaluated and a flexible stopping point allowing for a wide range of slopes of the dose-response curve (Rispin et al., 2002). The test involves the administration of a single test dose to a healthy, fasted young adult, followed by the observation of the specimen for clinical signs of toxicosis or mortality over the subsequent 14 days. These include the initial phase prior to the onset of clinical signs, a period of sickness behaviour when animals are most likely to feel pain and the final phase preceding death when animals may be unconscious (Eason et al., 2011).

Depending on the outcome of each test the dosage is progressively increased or decreased and the outcome is recorded. Providing the initial estimate of the LD50 is within a factor of 2 of the true LD50, this procedure should only require 6-10 animals (Bruce, 1985). To further reduce numbers, tests are only undertaken on animals of a single sex to reduce variability in the test population (Rispin et al., 2002). (Bruce, 1985) recommends the use of females alone due to their increased sensitivity in many species, however, the use of males may be preferable to avoid changes associated with female reproductive cycles (Hodgson, 2004).

A full necropsy should be implemented in order to confirm the toxicant's role in mortality, and rule out alternative underlying biological factors. This process typically

involves the removal of tissues to be examined for gross lesions and masses before being buffered in formalin for histologic examination (Hodgson, 2004).

4.2.7 Acetaminophen

Acetaminophen is a widely used analgesic and antipyretic, commonly referred to in New Zealand as paracetamol. It comes in the form of a white, odourless, crystalline powder and is taken to treat a wide variety of conditions involving musculoskeletal, headache, arthritic and rheumatic pain, as well to ease symptoms of the common cold and other viral infections (See table 4.1 for chemical properties) (Bessems & Vermeulen, 2001; International Agency for Research on Cancer, n.d.). It is available without prescription in many countries, and is an alternative to aspirin based products for patients with coagulation disorders (International Agency for Research on Cancer, n.d.).

The molecular formula for acetaminophen is $C_8H_9NO_2$ (Fig 4.3), however, standard 500mg tablets may include additional ingredients including but not limited to calcium stearate, cellulose, docusate sodium, sodium lauryl sulphate, hydroxypropyl methylcellulose and polyethylene glycol (International Agency for Research on Cancer, n.d.).
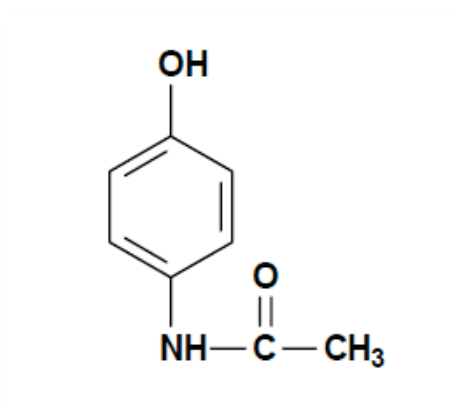
Figure 4.3: Molecular formula for acetaminophen
(International Agency for Research on Cancer, n.d.).

Table 4.1: Chemical properties of acetaminophen (International Agency for Research on Cancer, n.d.)

Description	Melting point	Density	Solubility	Stability
White, odourless, crystalline powder; large monoclinic prisms from water	169-170.5^{o}C	1.293g/cm³ at 21°C	Water: Soluble (1:70 at 100°C) Ethanol: Soluble (1:17) Acetone: Soluble (1:13) Chloroform (1:50) Methanol (1:10)	Stable to 45°C

4.2.7.1 Environmental risk

Water pollution by pharmaceuticals has been acknowledged as an environmental problem, entering waterways via the consumption of personal hygiene products, industry waste, hospital waste and therapeutic drugs, and often not effectively removed by conventional treatments (Rivera-Utrilla, Sánchez-Polo, Ferro-García, Prados-Joya, & Ocampo-Pérez, 2013). Acetaminophen is one of the most frequently found anthropogenic compounds due to its continuous release into watersheds, primarily through wastewater, although it does appear to have limited persistence in the environment (Kolpin et al., 2002). While investigating environmental and non-target organism effects for its potential application in Guam, it was found that typical chlorination processes were capable of degrading acetaminophen into other compounds, of which the risk to human health via drinking water was unclear yet deemed negligible (Kolpin et al., 2002; U.S. Department of Agriculture, 2011).

4.2.7.2 Animal testing

As with all chemicals manufactured for the medical industry, acetaminophen has been rigorously tested for human safety on numerous non-human species. Hepatic necrosis following administration of acetaminophen was first reported in rats (Boyd & Bereczky, 1966), however, has also been documented extensively in mice. While the main signs of this have been described as hydropic vacuolation, centrilobular necrosis, macrophage infiltration and regenerative activity, there is considerable variance between species

where hamsters and mice have demonstrated higher sensitivity than rats, rabbits and guinea-pigs (International Agency for Research on Cancer, n.d.).

In laboratory trials, Savarie et. al (2001) evaluated the efficiency of acetaminophen on brown tree snakes by treating 29 animals weighing between 47 – 300g with doses of 0, 10, 20, 40 or 80mg/ snake. While no mortality was observed at the 10mg dose, 67% mortality was observed at the 20mg does and 100% morality was achieved for both the 40 and 80mg treatments within 24 hours (Mauldin & Savarie, 2010). The mechanism of toxicity remains unidentified, however, treated snakes displayed severe methemoglobinemia, a condition also observed in cats which disrupts the ability of haemoglobin to transport oxygen within red blood cells and may result in tissue hypoxia (Mauldin & Savarie, 2010). Alternative suggested mechanisms include the possibility of glutathione depletion and hepatic necrosis, as has been recorded with some mammals (Savarie et al., 2001). As a rigorous assessment of its potential threat to the critically endangered, endemic Mariana crow (*Corvus kubaryi)* of Guam, fish crows (*Corvus ossifragus)* were tested as surrogates and showed some resistance towards the toxicant, with 0/5 and 1/5 birds dying at 80mg and 160mg doses respectively (Avery, Tillman, & Savarie, 2004)*.*

4.3 Study Rationale

The Australian plague skink (*Lampropholis delicata)* is the first and only invasive reptile to establish in New Zealand. As it continues to increase in number and range throughout the warmer regions of the North Island it is of great importance that we develop effective means for its control, particularly in locations of high dispersal risk. It is likely that island incursions may be reduced by targeting mainland vector sites, identified as high risk due to the regular transport pathway they provide to mainland pests. Numerous building supply yards, nurseries and marinas throughout the Auckland region have been identified as potential source sites for plague skinks with many harbouring high densities of plague skink populations and few, if any, native skinks. The development of a toxin to target these areas may provide an effective solution to this problem and thereby substantially reduce the risk of further transport of plague skinks

to human inhabited islands or those undergoing restoration, whereby plants and their media are sourced from mainland nurseries and therefore pose a level or risk.

Acetaminophen is widely and cheaply available in New Zealand as a non-prescription pharmaceutical, rendering it an easy and convenient option to assess. A trial to test its toxicity to *L. delicata* could provide valuable insight into whether or not acetaminophen based baits could be developed commercially for the eradication of plague skink infestations at mainland vector sites in New Zealand.

4.4 Aims

The two key aims of this chapter are outlined as follows:

1. To test the toxicity of acetaminophen to *L. delicata* through a controlled laboratory experiment
2. If found to be acutely toxic, to find the lethal median dose (LD50) in accordance with the guidelines set out by Rispin et al. (2002), the 'Up-Down-Procedure for Acute Oral Toxicity'.

4.5 Methods

This experiment was undertaken between late March and early May 2015, in accordance with Massey University's Animal Ethics Committee stipulations for research procedures using live animals, approval 14/23 (16-5-14).

4.5.1 Pre treatment

Male plague skinks were captured individually by hand from the Auckland region and immediately transferred into a sterile container for transport to Massey University's environmentally controlled reptile laboratory. Each skink was transferred into a sterilized cage with access to water, cover and basking under UV lights which were

maintained at a 12 h light/12 h dark photo-cycle, with temperature consistently ranging between 23.5-26°C at 92-92.5% relative humidity. Each skink was held and fasted for a 24 hour acclimatization period prior to receiving treatment.

4.5.2 UDP dosage methodology

The UDP recommends that the first animal dosed receive a step below the best preliminary estimate of the LD50, however, where no previous estimates have been made, the recommended starting dose commences at 175mg/kg (0.175mg/g). In most cases this dose is sub-lethal and therefore serves to reduce the level of pain and suffering (OECD). All dose progression factors were based on a half log progression unit of 3.2. Using this default progression factor, doses get selected from the sequence 1.75, 5.5, 17.5, 55, 175, 550, 1750, 5000 (mg/kg). If the skink died or survived, the following one would receive the subsequent dosage reduction or increase respectively.

4.5.3 Toxicant preparation

The acetaminophen dosage was prepared using standard 500mg Paracetamol tablets. For each dosage a single tablet was ground to a fine powder using a mortar and pestle for exactly 60 seconds (Plate 4.1) before being transferred to a microbalance (A&D GR-202) precision scales and measured into a sterile petri dish. To calculate the required administration dosage for each skink, its bodyweight was multiplied by the specified UDP progression dose to be tested, which had been converted into milligrams per gram. For example, a skink weighing 1g testing a UDP test dose of 175mg/kg would require 0.175mg of acetaminophen to be administered.

Plate 4.1: 500mg Acetaminophen (paracetamol) tablet
ground up before prior to being measured (Wairepo, 2015)

Once measured, the acetaminophen was added to a pre-measured volume of pure distilled carrier water to be administered orally. Due to the small size of the skinks it was not practical to administer any more or less than a single 0.01ml droplet at a time. For this reason all dosages were prepared to be administered in 2 separate 0.01 droplets of carrier water.

Due to the volume required it was impractical to prepare such a tiny dosage. For this reason all dosages (both acetaminophen and carrier fluid amounts) were multiplied by a minimum of 20 and a maximum of 40 in order to make up a sufficient volume at the correct concentration (Table 4.2). This meant that batches of either 0.4ml or 0.8ml of carrier water were pipetted into the petri dish to be thoroughly mixed with the corresponding amount of acetaminophen to achieve the correct concentration.

Table 4.2: Preparation dosage calculations to plague skinks (based on a skink weighing 1g). Calculated dosages are for amounts of either 0.4ml or 0.8ml of carrier water. Bold indicates starting dosage.

UDP Progression Dosage (1/2 log dose scale) (mg/g)	Acetaminophen Administration Dosage for skink of 1g (mg)	Acetaminophen Preparation amount / 400microlitres (0.4ml) carrier water (mg) (X 20)	Acetaminophen Preparation amount / 800microlitres (0.8ml) carrier water (mg) (X 40)
5	5	100	200
1.75	1.75	35	70
0.55	0.55	11	22
0.175	**0.175**	**3.5**	**7**
0.055	0.055	1.1	2.2
0.0175	0.0175	0.35	0.7
0.0055	0.0055	0.11	0.22

4.5.4 Administration

Prior to administration, each skink was allocated a randomized computer generated ID number, weighed and examined for clinical signs of disease, including body condition, ambulation and any external visible abnormalities. In order to administer the toxicant two people were required; one to restrain the skink and open its mouth, and the other to deliver the dosage via a pipette into the back of its mouth in 2 separate 0.01ml drops at 5 minute intervals.

4.5.5 Post treatment

After the complete dosage was administered, each skink was returned to its cage and given a variety of insects (mealworms, juvenile crickets and locusts) and water (Plates 4.2, 4.3). Skink behaviour was observed for the following 15 minutes for signs of discomfort or stress. During daytime hours skinks continued to be observed regularly for behavioural indicators of toxicity. A time lapse video camera was connected to a laptop

and set up above the cage to record skink behaviour over the total observational period of 40 hours, or until mortality occurrence. The recording schedule was set at 15 minute intervals every hour for the first 6 hours, then 2 hourly for hours 7 - 24, and 6 hourly from hours 25 - 40.

Plate 4.2: Skink returned to enclosure and provided with water and food (Wairepo, 2015)

Plate 4.3: Skink 'playing' with its prey (Wairepo, 2015)

All skinks receiving a fatal dose plus two that received a non-fatal dose were dissected to expose the coelomic cavity before being put into a solution of 10% buffered formalin to preserve tissues and prevent the onset of autolysis. From there they were taken to Auckland Zoo's New Zealand Veterinary Pathology clinic for blind, experimental histopathological analysis.

Skinks that survived the 40 hour observational period were subsequently euthanized using blunt force, in accordance with guidelines outlined by (Gartrell & Kirk, 2005). The use of this method is consistent with the field euthanasia practices outlined in chapters 2 and 3.

4.5.6 Analysis

The acute oral toxicity study to determine the LD50 of acetaminophen to plague skinks was conducted using UPD (Up-and-down Procedure) according to OECD/OCDE Test Guideline 425 on Acute Oral Toxicity under a computer-guided Statistical program - AOT425Pgm, version: 1.0, at a limit dose of 5000 mg/kg body weight / oral route and default of Sigma at 0.5. Where a chemical is expected to be toxic or when little or no information about its toxicity is available, the 'main test' should be used. The software calculates the outcome using the maximum likelihood method as follows, as set out by (Rispin et al., 2002):

$L = L_1 \, L_2 \, ...Ln$

where

L is the likelihood of the experimental outcome, given *mu* and *sigma,* and n the total number of animals tested.

$Li = 1 - F(Zi)$ if the ith animal survived, or

$Li = F(Zi)$ if the ith animal died,

where

F = cumulative standard normal distribution,

Zi = [log(di) – *mu*] / *sigma*

di = dose given to the ith animal, and

sigma = standard deviation in log units of dose (which is not the log standard deviation) – an estimate of 0.5 is used unless a better generic or case-specific value is available

The LD50 estimate is the value of the LD50 that maximises the likelihood when the slope is fixed at 1/assumed *sigma.* Confidence intervals are calculated using a profile-likelihood-based computational procedure that is expected to contain the true LD50 95% of the time.

4.5.6.1 Stopping point

The stopping rule is calculated by the software AOT425Pgm based on fulfilment of one of 3 criteria:

A. 3 consecutive animals survive at the upper bound;
B. 5 reversals occur in any 6 consecutive animals tested;
C. at least 4 animals have followed the first reversal and the specified likelihood-ratios exceed the critical value.

4.6 Results

4.6.1 LD50

Testing was concluded upon fulfilment of Criteria B in the OECD/OCDE Guidelines, whereby 5 reversals that occur in any 6 consecutive animals tested indicates a stopping point.

The results of acetaminophen dose-related mortality and approximate time-to-death are summarized in Appendix 3. Due to the on-set of toxicity related symptoms occurring throughout the night, time-to-death estimates are in some cases imprecise due to the inability to distinguish between sleep, paralysis or mortality.

No mortality occurred in either of the 2 skinks dosed at 0.175mg/g which was the start dose and step below the estimated LD50 of 0.55mg/g. Mortality occurred in 1 of the 4 skinks tested at the next progression dosage of 0.55mg/g, which was the one dose with a partial response. All of the 3 skinks tested at the subsequent progression dose of 1.75mg/g resulted in mortality (Table 4.3) (Fig 4.4).

Statistical calculations by the AOT425 software estimated the LD50 at 0.55mg/g (0.385 - 1.530 SD, 95% CI).

Table 4.3: Acetaminophen dose-related mortality, post dosage body weight and approximate time-to-death data (mean ± SD). Values in parentheses are body weight ranges of the tested skinks.

Acetaminophen dose (mg)	Body mass (g) ± SD	Dosage (mg acetaminophen per g body mass) ± SD	Mortality	Approximate time to death (hours)
0.175	1.16 ± 0.07 (1.11 - 1.21)	0.2 ± 0.01 (0.19 - 0.21)	0/2	No mortality
0.55	1.33 ± 0.14 (1.12 - 1.5)	0.75 ± 0.01 (0.62 - 0.83)	1/4	(12-15)
1.75	1.24 ± 0.04) (1.2 - 1.28)	2.17 ± 0.1 (2.1 - 2.24)	3/3	(5-20)
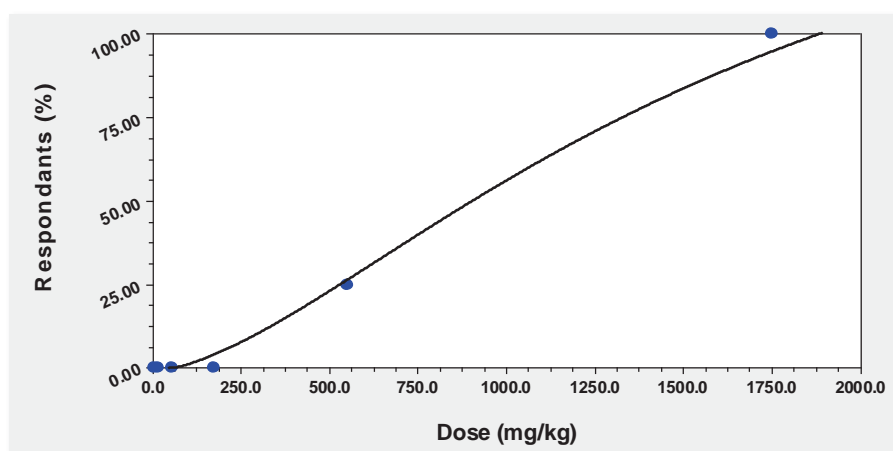
Figure 4.4: Dose response curve showing mortality response to acetaminophen at various dosages. Blue dots indicate an administered lethal dosage.

4.6.2 Signs of toxicity

All treated animals tolerated the administration of the acetaminophen suspension well, displaying active alert behaviours immediately upon being returned to their enclosure. Skinks that did not receive a lethal dose continued to behave normally throughout the 40 hour observational period, and were seen hunting invertebrates, drinking and sleeping throughout the night as would be expected.

All animals that did receive a lethal dose displayed behaviours similar to those receiving a non-lethal dose for the first 5-10 hours approximately, however, thereafter 3 skinks (ID 12, 17 and 38) displayed signs of toxicity with impaired ambulation (ID17), apparent joint stiffness in the rear legs (ID38) and immobilization (ID12) prior to death which occurred 12 – 20 hours post treatment in all cases of mortality.

4.6.3 Post mortem

There was an overall weight reduction in the mean weight of skinks before (1.26g ±0.04 SD, n=9) and after treatment (1.22g ±0.05 SD, n=9). Of the five skinks that received a non-lethal dosage, 2 gained weight and 3 lost weight over the 40 hour observational period. Conversely, all 4 skinks that received a fatal dose lost weight, however, a paired samples t test failed to reveal a statistically reliable difference between means of pre-treatment and post-treatment body weight (t = 1.301, df = 8, p = 0.229) (Table 4.3).

4.6.4 Histopathology

Histopathological tissue analysis identified mild to acute liver and kidney disease in all skinks receiving a fatal dosage. Of the two skinks that did not receive a fatal dose, the one that received the lowest dose (ID28) did not have any notable cellular damage to either hepatic or renal systems, while the one that received the LD50 dosage (ID16) suffered more severe cellular damage than skinks receiving higher dosages (Table 4.4). Skink ID12 was unable to be examined due to insufficient tissue preservation and resulting autolysis.

Renal necrosis was identified as the likely result of hypoxia (inadequate oxygen supply) which is consistent with the effects of toxicants or drugs. Similarly, hepatocellular steatosis (reversible change in fat content of liver cells) was also consistent with hypoxia and toxicants. All examined skinks (with the exception of ID28) had increased melanomacrophages (aggregates of pigmented cells located in kidney), which may be associated with inflammation or antigenic stimulation of the immune system.

Table 4.4: Skink histology outcomes (see Appendix 4 for further detail)

Skink ID	Dosage	Lived / died	Liver	Kidney	Notes
28	0.175	Lived	-	-	No significant findings
16	0.55	Lived	Moderate hepatocellular steatosis Elevated # melanomacrophages	Moderate/acute tubular necrosis	
49	0.55	Died	Slightly elevated # melanomacrophages	Minimal/acute Normal/tubular necrosis	
38	1.75	Died	Elevated # melanomacrophages	Minimal/acute Normal/tubular necrosis	May be within normal range
17	1.75	Died	Mild hepatocellular steatosis Multifocal Elevated # melanomacrophages	Moderate/acute tubular necrosis	
12	1.75	Died	-	-	Tissues unable to be examined due to autolysis

4.7 Discussion

During the past decades, reptilian toxicology has made up a disproportionately small percentage of toxicological studies of vertebrates (McClellan-Green, Celander, & Oberdorster, 2005). Those studies that have addressed the effects of contaminants have done so for conservation driven outcomes in recognition of the global decline in so many herpetofaunal species. Ironically, this research is motivated by the emerging sub-discipline of invasion biology that recognizes the threat of alien reptile species to vulnerable indigenous ecosystems, and seeks to develop an effective toxicant to contribute towards eradication and control efforts of invasive reptiles. As acetaminophen is a known lethal oral toxicant to some reptiles, having been effectively used to control *B. irregularis* in-situ, along with juvenile *V. niloticus* and *P. molurus bivittantus* in controlled laboratory trials, it makes sense to utilize the volume of existing research to test its toxicity on *L. delicata.* Furthermore, the outcomes may directly assist with conservation management decisions concerning plague skink incursions, the allocation of limited biosecurity funds and the use of toxins for reptile control.

4.7.1 Case study: Brown tree snake (B. irregularis)

The attempt to eradicate the brown tree snake from Guam has received global attention due to the novel and highly innovative technique with which the attempt was made. The species arrived on the island accidently after World War II, and rapidly proliferated across the island due to the abundant prey base and an absence of natural predators (Office of Insular Affairs, 2015). It took several decades for researchers to identify *B. irregularis* as the cause for the sharp and sudden decline in the islands bird populations, however, by 1994 the US government began to commit substantial annual resources towards combating the environmental and socioeconomic problems created by (Buckley, 2015). Efforts involved dispersal prevention and mitigating the daily impacts of the species using a variety of tools including barriers, custom designed traps, hand capture, snake-detector dogs and advocacy measures (U.S. Department of Agriculture, 2011). After 5 years no significant headway had been made and the decision was made to support the ongoing costly control programs with a full eradication operation. After

experimenting intensively with baits, floatation devices and various other techniques for 14 years, acetaminophen laced neonatal mice were selected to be aerially dropped over two targeted 55 hectare forest plots across the island using small double-ended cardboard streamer parachutes. The streamers were 100% biodegradable, and were designed to become entangled in the canopy, reducing the likelihood of mortality events of ground dwelling scavengers such as coconut crabs *(Birgus latro)*, hermit crabs (*Coenobita* spp.) and monitor lizards (*Varanus indicus)* (U.S. Department of Agriculture, 2011). While it remains unclear whether or not this novel technique will result in a total eradication of this invader, in the past 12 months the United States Department of Agriculture (USDA) Wildlife Services has experimentally demonstrated that populations of this invader can be suppressed over large forested areas via aerial broadcast of dead mouse baits, with preliminary results showing significant declines in snake activity in the two sites (Sablan, August 26, 2015).

4.7.2 Treatment on other reptiles

This Guam based research has paved the way for several other studies to also assess the utility of acetaminophen as a population control tool for other invasive reptiles, including Nile monitor lizards *(Varanus niloticus)* and Burmese pythons *(Python molurus bivittantus*) in Florida (Mauldin & Savarie, 2010). In laboratory trials of juvenile Nile monitors, acetaminophen doses of 10, 20 or 40mg resulted in 0, 50 and 100% mortality respectively, while in Burmese pythons doses of 20, 40 or 80mg resulted in 14.3, 85.7 and 100% mortality respectively. Negative correlation between dosage and time to death was observed in both species, neither of which exhibited signs of pain or discomfort following treatment, with basking, resting and sleeping the most commonly observed behaviours (Mauldin & Savarie, 2010). Extreme lethargy just prior to death was observed in half of the Nile monitors, while a few individual pythons became bloated due to fluid accumulation in the lungs and trachea (Mauldin & Savarie, 2010). It was concluded that acetaminophen was effective for juveniles and recommended investigation into its efficacy with adults of the two species.

4.7.3 LD50 results

The acetaminophen dose of 1.75mg/g (1750mg/kg) resulted in 100% mortality, however, due to the partial response of the subsequent lower dosage 0.55mg/g (550 mg/kg), the LD50 was identified at this point with 95% confidence intervals ranging between 0.39mg/g and 1.53mg/g. These results are consistent with the findings of Mauldin & Savarie (2010) who reported that acetaminophen doses of 522 - 2438mg/kg and 263 - 703mg/kg were uniformly lethal to juvenile Nile monitors and Burmese pythons respectively. Similarly, 100% mortality of brown tree snakes was observed when dosed with 40mg acetaminophen tablets for individuals ranging between 47-300g, equating to a lethal dosage of 66 mg/kg for the largest specimen. To manage for the fact that brown tree snakes are known to grow in excess of 300g in the field, the dosage was doubled to 80 mg, bringing it up to 267mg/kg for a 300g snake and ensuring mortality would occur for much larger animals (Mauldin & Savarie, 2010).

Interestingly, the results for plague skinks suggest they have a higher tolerance to acetaminophen than both the brown tree snake and Burmese Python, and a similar tolerance threshold to the Nile monitor lizards. Conversely, trials undertaking on black spiny tailed iguanas found them to be significantly more tolerant to acetaminophen, with lethal doses for 100% mortality exceeding practical limits for field applications (Avery et al., 2011). While it remains unclear why such variance in response to this toxicant occurs between species, reptiles possess unique sets of traits (enzymatic detoxification, dietary patterns and behavioural traits) which may influence contaminant uptake and tissue accumulation both within and between species. Furthermore, reptilian life history strategies are known to influence susceptibility to certain toxicants, particularly those that disrupt endocrine pathways (Gardner & Oberdorster, 2005).

4.7.4 Histopathology results

While toxicological research in various other taxa has provided the foundation for reptilian studies, (the majority of which investigate tissue residue research), the field of reptile toxicology remains in its infancy (McClellan-Green et al., 2005). Our histopathology results were consistent with those found in brown tree snakes, which were found to have methemoglobinemia, a condition resulting in hypoxia and a likely outcome of the treatment. Additionally, we found evidence of hepatic and renal deterioration. While it has no known anatomic significance, an important feature of reptile livers is the large amount of melanin pigment distributed throughout the hepatocellular parenchyma, and can sometimes constitute up to 20% of the liver cell volume (McClellan-Green et al., 2005). Four out of the five skinks analysed displayed elevated levels of these, which are often associated with inflammation. It is difficult to interpret this type of information despite a large body of literature reporting contaminant concentration in reptiles because of the lack of data specific to toxicity testing.

Not only are our findings consistent with previous similar experiments on reptiles, they have also been well documented in cats and dogs due to the high number of domestic pets that have inadvertently ingested the analgesic. (Schlesinger, 1995) reports a greater sensitivity to the drug in cats, who react with severe symptoms including cyanosis, methemoglobinemia, Heinz body anemia, facial swelling and death after a single 325mg tablet. Dogs, on the other hand are reported as suffering from vomiting, anorexia, tachycardia and acute liver necrosis at approximately 150-200mg/kg body weight. Interestingly, cyanosis (a clinical sign of hypoxia resulting in a bluish discolouration of the skin due to inadequate blood oxygenation) in reptiles and mammals (Schumacher, 2011), was also noted in all of the plague skinks that received a fatal dose.

4.7.5 Signs of toxicity

To observe signs of toxicity throughout the night an infra-red camera was set up to a recording schedule. (Mauldin & Savarie, 2010) and (Avery et al., 2011) reported that while there were no overt signs of pain or discomfort in any of their test subjects, all three reptile species (*C. similis, V. niloticus and P. molurus bivittatus)*, were observed with extreme lethargy and unresponsiveness prior to death. Similarly plague skinks were notably less active, however, due to the overnight timing of the experiments it was difficult to distinguish normal sleeping behaviour from lethargy induced by the drug. While half of the Nile monitor lizards were reported to have vomited just before death, plague skinks did not react to the treatment in this way.

A notable sign of toxicity was the impaired ambulation displayed by skinks ID17 and 38, who each suffered from reduced joint mobility, specifically in the knee joints of the hind legs. While unreported as visible effects of toxicity in Nile monitor lizards or Burmese pythons, diminished locomotor activity is an unsurprising outcome given that locomotion is made possible by the underlying biomechanics, physiology and biochemical processes which support it (Linder, Palmer, Little, Rowe, & Henry, 2010). These in turn are reliant upon integrated interactions between gastrointestinal physiology, neurophysiology and skeletomuscular and behavioural mechanisms (Linder et al., 2010).

Our observation period of 40 hours per skink was shorter than those undertaken by Avery et al. (2011) and Mauldin & Savarie (2010), who observed a post-treatment period of 14 and 7 days respectively. This, combined with their use of a different LD50 testing methodology which required treating many more animals meant that long term signs of toxicity such reduced appetite (and resulting loss in body mass) remain unexplored or inconclusive in plague skinks. Our small sample size combined with a failure to estimate time-to-death with precision (due to mortality occurring overnight), limited our ability to statistically evaluate the relationship between these variables. Simple ways that a repeat study could correct for this would be by treating skinks later on in the evening so

that any severe reduction in activity prior to the onset of death would occur diurnally and therefore be more visually obvious.

4.8 Conclusions

Acetaminophen is an effective toxicant in plague skinks when administered orally, however, the wide confidence interval of our LD50 result indicates further research into dose relationships is required to ascertain a true lethal dose for wild skinks. Before it can be considered for use in an urban setting, further investigation into minimising secondary hazards and developing an eventual method of delivery must also be implemented due to the extremely toxic effect on domestic animals. Should these challenges be resolved, it is possible that lessons from Guam may be employed and the use of acetaminophen as part of an integrated approach to plague skink management in New Zealand may be a viable option.

Chapter 5

Synopsis, management options and final remarks
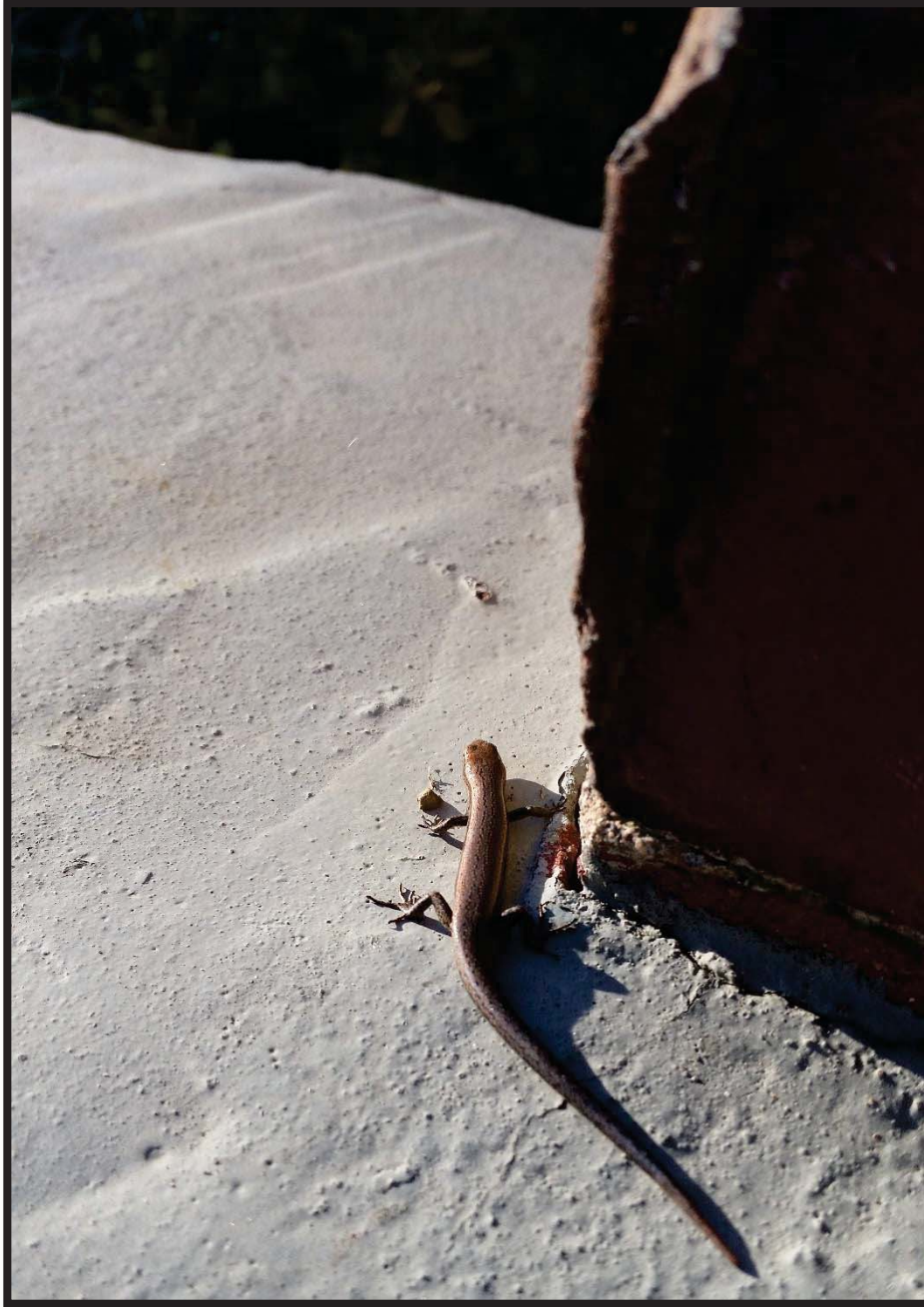
5.1 Synopsis

Interest and research in invasive herpetofauna has historically lagged behind other taxa such as mammals, plants and invertebrates, largely due to the ability of many of these invaders to cause economic harm and degrade ecosystems, often in a visually obvious and rapid manner (Reed & Kraus, 2010). As a result, in spite of the exponential growth in herpetofaunal introductions globally, effective management interventions have been limited and largely ineffective (Kraus, 2009).

The unhindered introduction, establishment and rapid expansion of plague skinks (*Lampropholis delicata)* throughout much of New Zealand's North Island provides a clear illustration of the poor understanding of the potential impact of invasive reptiles. This is of particular significance in a country renowned as world leaders in biosecurity strategy development. This may be attributed to plague skinks not yet having posed large scale ecological or economic harm to merit the effort of the extensive research or funding received by mammalian or agriculturally destructive invaders. Unfortunately, this naïve misconception of ecological benignity has allowed New Zealand's first and only invasive reptile to rapidly encroach upon ecologically sensitive indigenous habitats, leaving little time to be wasted if threatened native species are to be protected from potential harm.

My study addresses the research and management gaps of dealing with *L.delicata* as an invasive species and is the first of its kind. My comprehensive literature review details the issues and complexities faced by invasion biologists, particularly with respect to herpetofauna and the challenges associated with managing their introduction, expansion and impact on a global scale.

For a biosecurity incursion response to be efficient and effective, it must incorporate a range of management approaches and tools, which allow for detectability at low population densities. Chapter 2 addresses the need to accurately detect plague skink boundaries by locating dispersal pathways and the moving invasion front through a combination of intensive field effort followed by post-hoc power analyses utilizing detection probabilities and 95% confidence requirements. My results support they

hypothesis that if the population had dispersed beyond the tested boundary line I am 95% confident they would have been detected by my methodology.

I aimed to parameterize operational methods to eradicate a plague skink population and evaluate standard herpetological monitoring tools for their utility in a biosecurity response operation. An operational strategy underpinned by an adaptive framework was employed to maximise capture returns, however, despite an intensive trapping effort my grid system was insufficient to reduce the population significantly. Invertebrate sticky traps and G-minnow traps were effective tools in this context, each with their own set of advantages and disadvantages.

Chapter 4 aimed to test the acute toxicity of acetaminophen to plague skinks, with the rationale that mainland vector sites known to transfer skinks and their eggs may benefit from this type of management approach. I found the toxicant to be effective and estimated the LD50 at 550mg/kg, confirming its potential as an alternative control tool for future development.

5.2 Management options

5.2.1 Management

With the information and experience gained to date it is now important to re-evaluate the situation, identify clear operational goals, and develop a comprehensive strategy for the determined programme of work over the next several years on Great Barrier Island.

According to Lockwood et al. (2013), population spread can be considered as the penultimate stage before an invasive species inflicts economic or ecological harm. The capture history over my two field seasons demonstrates the area supports a healthy population of native copper *(Oligosoma aeneum)*, moko (*O. moco*) and ornate (*O. ornatum)* skinks. As such, the Shoal Bay area can be considered as highly representative of both the herpetofaunal and overarching conservation values of Aotea.

The key options for managing the population of plague skinks on Great Barrier Island have been identified as follows, with Option 2 having been previously identified as the recommended option due to its feasibility both operationally and financially.

1.   Total and rapid eradication of plague skinks from GBI through eradication by research
2.   Containment for the medium-term to be concluded with eradication by research
3.   Sustained containment of the incursion
4.   Do nothing and declare plague skinks established on Great Barrier Island (Reardon & Wairepo, 2014).

The main risk with Option 2 is that medium term containment efforts fail prior to the development of an effective eradication strategy and availability of sufficient funds. The choices for Option 2 are:

A) Construction of a physical barrier that runs the entire length of the boundary line

B) Regular sustained trapping of the delimitation trapping framework coupled with ongoing trapping at the core.

The first of these (A) is likely to be breached at some point in the future therefore to be effective it would require a careful design under the guidance of a professional Engineer to fulfil the following requirements (Wairepo, 2015):

•   Retention of track's structural stability and width
•   Must be unobtrusive and not hinder the landowner's access
•   Mitigate for water run-off and allow drainage to continue downhill without compromising the integrity of the barrier structure
•   Materials would need to be resilient for the duration required of approximately 2-3 years
•   Minimal maintenance required.

The sustained containment Option 3 is the strategy that represents the highest risk and cost, and would soon accumulate to be many more times as expensive as an Option 2.

The final Option 4, to declare plague skinks established on the island is obviously the least costly strategy but also the least desirable (Reardon & Wairepo, 2014). Potential ecological impacts to the island include direct competition with native herpetofauna for prey and habitat. It is also theoretically possible that plague skinks could operate in the same way as rodents and rabbits operate in many New Zealand ecosystems, acting as a primary prey source for predators that results in an elevated predator population. For example, rats, cats and kingfishers might grow in abundance due to the presence of plague skinks and together with competitive pressures, place native taxa at an elevated risk of predation and displacement (Reardon & Wairepo, 2014). Furthermore, the increased plague skink population has potential to act as an inoculum source, vectoring diseases and pathogens between regional endemic skink species that may have never previously had contact with each other (N. Waipara, personal communication, September 2015).

5.2.2 Research

A number of key research gaps have been identified throughout duration of this study. To progress plague skink management in New Zealand these gaps need to be urgently filled. I have identified 5 possible study areas which will contribute towards achieving this.

1. Trap density experiment: Five meter spatial intervals between traps were not sufficient to eradicate the population, however, trap density requirements remain unknown. A careful experimental design in a controlled field trial using a known number of skinks may be an effective study to achieve this (Reardon, personal communication, April 2014).

2. Quantification of the effort (time, labour, cost) required to achieve detection and capture probabilities of 1.0 in all densities.

3. Trap optimization experiments designed to mitigate against heat and predation by rodents and ants.

4. Acetaminophen trial progression to the next stage of toxicant development.

5. Dispersal dynamics, particularly for females and investigating behavioural cues for next site selection. This may assist with locating communal nests.

5.3 Final remarks

This research set out to achieve three clear goals which are hoped to feed into the development of a national management strategy for plague skinks. Firstly, to implement biosecurity incursion responses actions at Tryphena Wharf with the objectives of delimiting the population and removing as many individuals as possible to test the feasibility of eradication. Secondly, to utilize the acquired datasets to evaluate the success of the field efforts and form meaningful conclusions about what might be required to successfully eradicate the population. And finally, to address the need for an effective toxicant by undertaking a laboratory trial to test the toxicity of acetaminophen. While a thriving population of plague skinks remain on Great Barrier Island, this study successfully achieved its research goals. It is hoped that the findings of this research will make an important contribution towards the discipline of invasion biology, the ongoing development of effective management techniques for invasive lizards, and the protection of New Zealand's rare and precious endemic herpetofauna.

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Appendix 1: Optimal number of traps required over 14 trap nights to precision levels of 0.05, 0.03 and 0.01. Precision levels are synonymous with 'power' and are presented as a percentage of confidence (95%, 97%, 99%). Figures in bold and green denote the number of traps that were required for the average (mean & median) detection and occupancy probability estimates of plague skinks that were detected by our system. The number of traps in the system exceeded 97% confidence/power requirements.

Optimal number of traps (N =14)

Detection probability	Probability of occupancy (Ψ)																										
	0.1			**0.2**			**0.3**			**0.4**			**0.5**			**0.6**			**0.7**			**0.8**			**0.9**		
	95%	97%	99%	95%	97%	99%	95%	97%	99%	95%	97%	99%	95%	97%	99%	95%	97%	99%	95%	97%	99%	95%	97%	99%	95%	97%	99%
0.1	**58**	**162**	**1455**	108	301	2711	151	408	3766	185	513	4621	211	586	5277	229	637	5732	240	665	5132	242	671	6043	236	655	5898
0.2	38	106	950	68	189	1699	90	250	2249	104	289	2598	110	305	2748	108	300	2698	98	272	2098	80	222	1997	54	150	1347
0.3	36	101	911	**65**	**180**	**1621**	85	237	2132	98	271	2442	102	284	2553	99	274	2463	87	242	2174	67	187	1684	40	111	995
0.4	36	100	900	64	178	1600	84	233	2100	96	267	2400	100	278	2500	96	267	2400	84	233	2100	64	178	1600	36	100	900
0.5	36	100	900	64	178	1600	84	233	2100	96	267	2400	100	278	2500	96	267	2400	84	233	2100	64	178	1600	36	100	900
0.6	36	100	900	64	178	1600	84	233	2100	96	267	2400	100	278	2500	96	267	2400	84	233	2100	64	178	1600	36	100	900
0.7	36	100	900	64	178	1600	84	233	2100	96	267	2400	100	278	2500	96	267	2400	84	233	2100	64	178	1600	36	100	900
0.8	36	100	900	64	178	1600	84	233	2100	96	267	2400	100	278	2500	96	267	2400	84	233	2100	64	178	1600	36	100	900
0.9	36	100	900	64	178	1600	84	233	2100	96	267	2400	100	278	2500	96	267	2400	84	233	2100	64	178	1600	36	100	900

Appendix 2: High impact, research and collection permit (# 36317-FAU) for the Auckland Council to handle and release native lizard species as part of the Biosecurity incursion response on Great Barrier Island.

Department of Conservation
Te Papa Atawhai

High Impact, Research and Collection Permit

National Permit Number: 36317-FAU

Her Majesty the Queen, acting by and through the Minister of Conservation (the Grantor) GRANTS to the Peter Anderson of the Auckland Council (formerly Auckland Regional Council) (the Permit Holder) a Permit under the Wildlife Act 1953, subject to the details and conditions listed in Schedule One and Two.

Attach original application form to the approve permit.

Schedule One

(1) **Permit Holder and field assistants involved**

> Peter Anderson

(2) **Approved activity (including approved quantities) and reasons for undertaking the research**

Permits to collect, capture, handle release or kill:

General:

- Handling and identifying lizards and recording native frog presence during the normal course of the Permittee's work carrying out ecological assessments, surveys and lizard rescues if necessary.
- Collection of basic data such as species identification, weight, snout-vent length and tail length.
- Relocation within the same site **to a maximum distance of 500 metres** of absolutely protected lizard species within the Auckland Conservancy if the animals are considered to be threatened by development.

(Wider scale translocations of species away from the footprint of proposed developments requires coordination and consultation with the Department of Conservation (DOC) and may require a translocation application.)

This is NOT an authority to undertake such work on public conservation land apart from areas where site development is proposed. In this regard the permit holder is responsible for obtaining all additional consents and permits before undertaking any work on public conservation land.

Specific:

- Physical searching for skinks, geckos and Hochstetters frogs during the update of Tamaki and part of Rodney Ecological Districts. These include new or previously identified natural areas listed under the Protected Natural Areas Programme (PNAP), Sites of Special Wildlife Interest (SSWI) and Ecological Linkage Areas (LA) programmes. Initially sites located within North Shore City Council will be covered and this work may be extended to the new Auckland Super City from October 2010.
- Assistance towards survey, rescue and release of lizards during land use resource consent activities and other developments that are occurring within North Shore City and later the Auckland Super City. This includes private, local government and Crown land sited.

be sent to the relevant Area Manager, Department of Conservation, C/-Private Bag 68908, Newton, Auckland 1145 within one (1) month of any capture/release being completed.

26. The permit holder shall submit completed Amphibian and Reptile Distribution System (ARDS) cards (http://www.doc.govt.nz/conservation/native-animals/reptiles-and-frogs/species-information/herpetofauna-data-collection/ards-card/) to Benno Kappers, Department of Conservation, , Hawkes Bay Area Office, PO Box 644, Napier or herpetofauna@doc.govt.nz for all herpetofauna sightings and captures.

27. If any sick or dead frog is found, the attached DOC Fact Sheet "Handling Sick or Dead Frogs" must be followed.

SIGNED by		SIGNED by	
Dated	20/May/2013	Dated	
Jonathan Miles	*Jonathan Miles*	AS APPLICANT	
Auckland Area Manager			

ACTING UNDER DELEGATED AUTHORITY FROM THE DIRECTOR GENERAL OF CONSERVATION

("The Grantor")

In the presence of	Bruce Tubb	In the presence of	
Witness Signature		Witness Signature	
Occupation	Temporary Ranger	Occupation	
Address	Auckland Area Office	Address	

Appendix 3: Acetaminophen trial data for each skink dosed. Dosage volumes, pre and post dosage observations and measurements, mortality, estimated time-to-death and signs of toxicosis are included.

Specimen ID	UDP Progression Dosage (PD) (mg/g)	Pre-dosage bodyweight (BW) (g)	Dosage (PD X BW)	Pre-dosage Body condition	Pre-Dosage Ambulation	Mortality status (x=fatal, ✓= survival)	Estimated time till death (hrs)	Post-dosage weight at stopping point	Weight loss (-) or gain (+) (g)	Post-dosage Behavioural signs of toxicity	Post-mortem physical signs of toxicity
28	0.175	1.11	0.19	good	good	✓	-	1.25	+0.14	-	-
49	0.55	1.12	0.62	good	good	x	12 - 15	1.00	-0.12	Reduced activity, lethargy	Blue discolouration on lower jaw and belly
7	0.175	1.21	0.21	good	good	✓	-	1.31	+0.1	Reduced activity	-
22	0.55	1.5	0.83	good	good	✓	-	1.46	-0.04	-	-
12	1.75	1.25	2.19	good	good	x	5 - 18	1.13	-0.12	Reduced activity, lethargy	Blue jaw, dehydrated rear leg
16	0.55	1.36	0.75	Missing terminal segments from several toes on 3 feet	good	✓	-	1.32	-0.04	-	-
17	1.75	1.28	2.24	good	good	x	12 - 20	1.17	-0.11	Reduced activity and mobility - walking appears challenging	Blood in right ear enlarged, slight blue discolouration on snout
42	0.55	1.34	0.74	good	good	✓	-	1.29	-0.03	-	-
38	1.75	1.2	2.10	good	good	x	14 - 16	1.04	-0.16	Reduced activity and mobility in rear knee joints	-

Appendix 4: Toxicology report from Dr Cathy Harvey of the NZVP. The report discusses the histophathological findings of all skinks treated with a lethal dosage of acetaminophen, and two skinks that were treated with a sub-lethal dosage.

NEW ZEALAND VETERINARY PATHOLOGY LTD

HAMILTON: Cnr Thackeray & Anglesea Streets, PO Box 944, Hamilton 3240 Ph: 07 839 1470 Fax: 07 839 1471
PALMERSTON NORTH: Tennant Drive, PO Box 325, Palmerston North 4440 Ph: 06 353 3983 Fax: 06 353 3986
AUCKLAND: Motions Road, Western Springs, PO Box 44422, Point Chevalier 1246 Ph: 09 378 9006 Fax: 09 378 8773
FREEPHONE: 0800 VETLAB or 0800 838 522 WEBSITE: www.nzvp.co.nz

CASE NO : A15004061

Submitter:	JACQUI WAIREPO	**Species:**	Reptile **Age:** Unknown
	MASSEY UNIVERSITY	**Breed:**	RAINBOW SKINKS **Sex:** Unknown
	ALBANY CAMPUS		
	ALBANY HIGHWAY	**Date Sent:**	13 May 2015
		Date Received:	13 May 2015 10:19 am
Submitter Reference:		**Date Tested:**	18 May 2015
Animal ID:	16,17,28,38,49		
Owner:	JACQUI WAIREPO	**Notification:**	Fax, E-mail
	MASSEY UNIVERSITY, ALBANY CAMPUS	**<fax: EMAIL >**	

Test Requested: 2 x Fixed Tissue - Multiple Histology specimens,

HISTOLOGY

A1504061

Patient: RAINBOW SKINKS

Gross examination:
#16 CxA=3, CxB=1
#17 CxC=2, CxD=2
#28 CxE=1, CxF=1
#38 CxG=2, CxH=2
#49 CxI=2, CxJ=2
CH 13-05-2015

Histopathology:
All tissues have good preservation with minimal autolysis.
All contain an adequate amount of kidney (tubules and glomeruli) and liver
tissue to examine and evaluate.
All are males with active spermatogenesis.

Control #28 -
Kidney - normal - Tubules are lined by cuboidal epithelial cells with lightly
eosinophilic cytoplasm, or by cuboidal to columnar epithelial cells with deeply
eosinophilic granular cytoplasm (distal convoluted tubules "sexual granules /
segment" seen in male lizards especially when sexually active). There is mild
anisokaryosis of tubular epithelial cells. Small numbers of tubules are lined
by smaller cuboidal epithelial cells with lightly basophilic cytoplasm. There
are rare interstitial granulocytes and mononuclear cells. Glomeruli contain
small numbers of tightly packed cells in the tuft. Bowman's capsule parietal

Report Continued ....

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epithelial cells are flattened.

Liver – normal – All hepatocytes moderate amount of cleared cytoplasm. There
are small numbers of bile ducts and small numbers of melanomacrophages.

Testicle – Active spermatogenesis

Stomach – lumen full of insect material

Brain, Heart, Thyroid, Oesophagus, Skin, Dermal glandular tissue, Skeletal
muscle, Cartilage, Bone marrow, Spinal cord, Nerve roots, Lung, Intestines,
Spleen, Adipose tissue of fat body – no significant findings

#16 –
Kidney – Many of the tubules are dilated with epithelial cells sloughed into
the lumen and pyknotic (degeneration). Some have attenuated epithelial cells.
There are small numbers of interstitial infiltrates of granulocytes and
mononuclear cells.

Liver – Most hepatocytes contain cytoplasmic small clear vacuoles (steatosis).
There are small numbers of interstitial granulocytes and macrophages. There
are moderate numbers of melanomacrophages.

Lung – There are slightly increased numbers of interstitial cells, granulocytes
and macrophages with cytoplasmic black-brown granular and slightly refractile
pigment.

Stomach – lumen empty

Spleen – Small numbers of melanomacrophages.

Testicle – Active spermatogenesis

Skin, Skeletal muscle, Eye, Glandular tissue adjacent to Eye, Bone, Bone marrow,
 Cartilage, Oral cavity, Brain, Oesophagus, Adipose tissue of fat body – no
significant findings

#17 –
Kidney – Many of the tubules with epithelial cells with deeply eosinophilic
granular cytoplasm are dilated, epithelial cells sloughed into the lumen and
pyknotic (degeneration). The tubules with lightly eosinophilic cytoplasm
contain small amounts of debris in the lumen, occasional degenerate cells and
granulocytes.

Liver – Some hepatocytes contain cytoplasmic small clear vacuoles (steatosis).
There are moderate numbers of melanomacrophages.

Report Continued ....

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Stomach – lumen scant contents

Testicle – Active spermatogenesis

Skin, Skeletal muscle, Eye, Glandular tissue adjacent to Eye, Bone, Bone marrow, Cartilage, Oral cavity, Brain, Oesophagus, Thyroid, Lung, Heart, Intestines, Pancreas, Spinal cord, Nerve roots, Adipose tissue of fat body – no significant findings

#38 –
Kidney – Rare tubules contain small amounts of debris in the lumen and occasional degenerate cells.

Intestines – There are small to moderate numbers of mucosal protozoa (presumptive coccidia).

Liver – There are moderate numbers of melanomacrophages.

Lung – There is a small amount of basophilic granular material in airways.

Spleen – Small numbers of melanomacrophages.

Testicle – Active spermatogenesis

Stomach – lumen empty

Brain, Heart, Thyroid, Oesophagus, Skin, Dermal glandular tissue, Skeletal muscle, Bone, Cartilage, Bone marrow, Spinal cord, Nerve roots, Pancreas, Adipose tissue of fat body – no significant findings

#49 –
Kidney – Many of the tubules with epithelial cells with deeply eosinophilic granular cytoplasm are dilated, epithelial cells sloughed into the lumen and pyknotic (degeneration). The tubules with lightly eosinophilic cytoplasm contain small amounts of debris in the lumen, occasional degenerate pyknotic cells and rare granulocytes.

Liver – Small to moderate numbers of melanomacrophages.

Stomach – lumen empty

Testicle – Active spermatogenesis

Heart, Lung, Oesophagus, Skin, Dermal glandular tissue, Skeletal muscle, Bone, Cartilage, Bone marrow, Spinal cord, Nerve roots, Intestines, Adipose tissue of fat body – no significant findings

Case Number: *A15004061*   *HISTOLOGY*
Submitter: *JACQUI WAIREPO*
Owner: *JACQUI WAIREPO*

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Morphologic Dx:
#28  - Control - no significant findings

#16  -
Kidney - Tubular Nephrosis, moderate, acute
Liver - Hepatocellular Steatosis, moderate, diffuse; Increased numbers of
melanomacrophages.

#17  -
Kidney - Tubular Nephrosis, mild to moderate, acute
Liver - Hepatocellular Steatosis, mild, multifocal; Increased numbers of
melanomacrophages.

#38  -
Kidney - Normal vs Tubular Nephrosis, minimal, acute
Liver - Increased numbers of melanomacrophages.

#49  -
Kidney - Tubular Nephrosis, moderate, acute
Liver - Mildly increased numbers of melanomacrophages.

Comment:
Jacqui, I have charged at two x the multiple tissue rate.

Compared to #28
#16 and #49 appear to have moderate kidney disease, and #16 liver disease.
#17  may have mild kidney and liver disease.
#38 may be "within normal" or have minimal changes in the kidney and liver.

It can be difficult to interpret early changes in the kidney tubules due to
autolysis or necrosis (nephrosis).  Most of the tissues in the animals were
well preserved and tissues adjacent to the kidneys were not autolysed, so the
changes are likely early nephrosis - which can be due to hypoxia, toxins or
drugs.

Hepatocellular steatosis (fatty change, lipidosis) is the accumulation of
lipid-containing vacuoles in the cytoplasm of hepatocytes and is a non-specific
reversible form of cell injury.  It can occur secondary to starvation /
anorexia, hypoxia, toxins or drugs.

Increased melanomacrophages in reptiles are a nonspecific finding and can be
associated with inflammation or antigenic stimulation of the immune system.

Cathy Harvey
B.V.Sc, Diplomate ACVP, Registered Specialist in Veterinary Anatomic Pathology
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Signed   *(signature)*
               Cathy Harvey (Veterinarian)
Print Date: **18 May 2015**   **Final Report - HISTOLOGY**   **Report Fee: $228.10**