Minimising non-target impacts of anticoagulant rodenticide use for a highly susceptible species, the New Zealand lesser short-tailed bat (*Mystacina tuberculata*)

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Abstract

Anticoagulant rodenticides may cause mortality in non-target wildlife. In New Zealand, broad-scale anticoagulant use is essential for wildlife conservation, but also poses risks to threatened species. In 2009, >100 endemic lesser short-tailed bats (*Mystacina tuberculata*) died during a rodent control operation. In this thesis I confirm that these bats were intoxicated with the first-generation anticoagulant rodenticide diphacinone, and present several lines of research investigating the route of exposure, the effectiveness of management changes at minimising exposure and mortalities and the potential consequences of such non-target impacts on bat population viability.

I used infra-red video cameras at non-toxic baits in wild and captive settings to determine that the route of exposure of the bats to diphacinone was most likely through ingestion of contaminated arthropods. In a field trial, analysis of communal guano deposits revealed that an alternative baiting method reduced but did not prevent exposure of bats to diphacinone. However, this exposure was subclinical, as prolongation of mean blood prothrombin time was not evident. Furthermore, mark-recapture analysis of passive integrated transponder (PIT) tagged bats indicated zero to negligible effect of exposure on population survival.

Despite this result, sublethal exposure to anticoagulants is of concern because of the unknown effects on bat fitness and reproduction. An abundance estimate using closed-population mark-recapture analysis revealed that the study population was small (c.780 adults) relative to other lesser short-tailed bat populations, and thus particularly vulnerable to reductions. I developed a model describing the population dynamics of the bats to explore the potential effects of chronic reduction of survival and productivity on population viability. While model projections highlighted the need to suppress rodents in bat habitat, they also demonstrated that small annual reductions in survival could threaten population persistence.

This study has contributed to bat conservation management in New Zealand and highlights the delicate balance that needs to be achieved between managing invasive vertebrates and protecting native species that are highly susceptible to vertebrate

pesticides. Investigating the effects of sublethal exposure of bats to anticoagulant rodenticides should be a conservation priority as there are global implications for health and viability of other insectivorous bat species.

For BM and PB.

Thanks for taking me on all those bush walks to waterfalls when I was a kid.

Here again we are reminded that in nature nothing exists alone.

Rachel Carson
Silent Spring, 1962

Acknowledgements

In 2007, while working for the Department of Conservation (DOC) as a biodiversity ranger monitoring threatened species at Pureora Forest Park, I was contemplating returning to university to undertake post-graduate study. I asked Colin O'Donnell (DOC's bat scientist) for his thoughts on my proposal to embark on a research journey with conservation of the New Zealand lesser short-tailed bat at its heart. Without hesitation he said he thought it was a bl**dy good idea. And so it was.

I was determined that my research should contribute to some aspect of applied conservation management of the bats. I had not yet settled on a topic when an unforeseen event during seasonal bat monitoring work at Pureora led me down an unexpected path. Developing my research topic around the non-target mortality of the bats that occurred during a rodent control operation at Pureora that summer has proven to be varied, challenging and interesting. My research commenced as a Master's degree, but somewhere along the way Brett Gartrell's gentle persuasion convinced me not only that the topic had potential for the more in-depth research that a PhD allowed, but also that I was capable of accomplishing it. And so I did.

First and foremost my thanks go to that league of extraordinary gentlemen, my supervisors; Brett Gartrell, Doug Armstrong and Colin O'Donnell. Brett, thank you for welcoming me in to the Wildbase family and for your gentle encouragement and calm and steady guidance. I always came away from our meetings with renewed focus and feeling that any concerns had been allayed. I was in awe of your ability to instantly engage deeply and with clarity with whatever aspect of my research we were discussing. Doug, thank you for coming on board part way through when my studies evolved into a PhD and required complementary expertise and additional brain-power. Thank you for constantly challenging me and believing me capable of rising to the challenges. And thanks for guiding my entrée into the world of population modelling and making it fun. Colin, thanks for that enthusiastic response that got me started. I have valued your advice, support and guidance since you introduced me to my first bat in Fiordland in 1999. I greatly appreciate your generosity with your time and with sharing your in-depth knowledge and experience of all things bat.

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Chapter 1

General introduction

The New Zealand lesser short-tailed bat (*Mystacina tuberculata*), a threatened species susceptible to non-target poisoning. Photo courtesy of David Mudge, Nga Manu images.

1.1 Introduction

Anticoagulant rodenticides are commonly used in many parts of the world to alleviate the impacts of rodents and other vertebrate pests on agriculture and health (Jacob & Buckle, 2018) and to eradicate invasive rodents from islands for restoration of native ecosystems (Donlan *et al.*, 2003; Towns & Broome, 2003; Howald *et al.*, 2007). In New Zealand their use extends to broad-scale, sustained application in mainland conservation areas to manage populations of invasive rodents and other introduced mammalian predators (Innes & Barker, 1999; Russell *et al.*, 2015). Unfortunately, the broad-spectrum toxicity of anticoagulant rodenticides has undesirable consequences for non-target species.

The non-target impacts on wildlife resulting from anticoagulant rodenticide use are well documented for a wide range of species and in many countries, including New Zealand (Eason *et al.*, 2002; Stone *et al.*, 2003; Laakso *et al.*, 2010; Sánchez-Barbudo *et al.*, 2012; Lohr & Davis, 2018). Lethal or sublethal exposure of wildlife can result from animals feeding directly on toxic baits or on contaminated prey (Brakes & Smith, 2005; Dowding *et al.*, 2006; Riley *et al.*, 2007). Threatened wildlife can benefit from suppression of rodent populations in their habitat (O'Donnell & Hoare, 2012; Le Corre *et al.*, 2015; O'Donnell *et al.*, 2017) but species that are susceptible to anticoagulant rodenticide poisoning may be vulnerable to population reductions that threaten viability. Minimising the risk of non-target mortality in such species is therefore an important component of conservation management.

In this chapter I introduce the topic of anticoagulant rodenticides and the impacts of their use on non-target wildlife. I outline some of the key (chemical, operational and ecological) factors that affect the risk of wild animals being lethally exposed to anticoagulant poisons during pest control operations and the potential for population-level consequences. I describe the use of anticoagulant rodenticides for broad-scale conservation management in New Zealand, in particular at mainland sites, and how the history of non-target impacts on local wildlife has determined contemporary patterns of use. Finally, I introduce the lesser short-tailed bat (*Mystacina tuberculata*), one of only two endemic land-breeding mammals in New Zealand. I provide an overview of the characteristics that affect the susceptibility of this species to poisoning with anticoagulant rodenticides and explain why there is a need to provide effective rodent control in bat

habitat using methods that minimise non-target impacts. The lesser short-tailed bat will be used as the study species for the various lines of investigation undertaken in this thesis, which are outlined at the conclusion of this chapter.

1.2 Anticoagulant rodenticides and exposure of non-target wildlife

1.2.1 Classification of anticoagulant rodenticides

The rodenticidal properties of anticoagulant chemicals were first realised during the 1940s. Since then a series of compounds known as anticoagulant rodenticides have been developed and marketed for the management of rodent pests. They are broadly categorised as either first- or second-generation compounds according to when they were developed. They can also be classified as hydroxycoumarins or indandiones based on their chemical structure (Table 1.1). First-generation anticoagulant rodenticides initially dominated management of rodent pests worldwide, but with prolonged and intensive use came the discovery of evolving resistance in some rodent populations in the 1950s. This led to the development of a second generation of more potent compounds (Buckle, 1994).

Table 1.1 Classification of anticoagulant rodenticides according to 'generation' (period of development) and chemical class (Buckle, 1994; Hadler & Buckle, 1992).

Type	Chemical class	Compound	Developed
First-generation	Indanediones	Diphacinone†*	1940s-1960s
		Pindone†*	
		Chlorophacinone	
	Hydroxycoumarins	Warfarin	
		Coumatetralyl†*	
		Coumafuryl	
		Coumachlor	
Second-generation		Brodifacoum†*	1970s-1980s
		Bromadiolone†	
		Flocoumafen†	
		Difethialone†	
		Difenacoum	

†registered for use as vertebrate toxic agents in New Zealand (MPI, 2018)
*registered for use on New Zealand public conservation land(DOC, 2019)

1.2.2 Mode of action of anticoagulant rodenticides

Both first- and second-generation anticoagulant rodenticides share a common mode of action; they interfere with the synthesis of vitamin K-dependent blood clotting factors (II, VII, IX and X). This interaction occurs primarily in the liver cells of vertebrates and to a lesser degree in the kidney and spleen. During the normal production of clotting factors the active form of vitamin K is converted to an inactive form. Most of this compound is reactivated by enzymes in a two-step process (Figure 1.1) (Buckle, 1994). The reactivated compound represents the main source of vitamin K involved in the production of clotting factors, but small amounts can be supplied from gut bacteria and dietary sources. Anticoagulant rodenticides inhibit the enzymes and prevent the reactivation of vitamin K. The active form of the vitamin is gradually depleted and cannot be replaced in sufficient quantities from other sources. Consequently, the production of clotting factors eventually ceases (Hadler & Buckle, 1992).

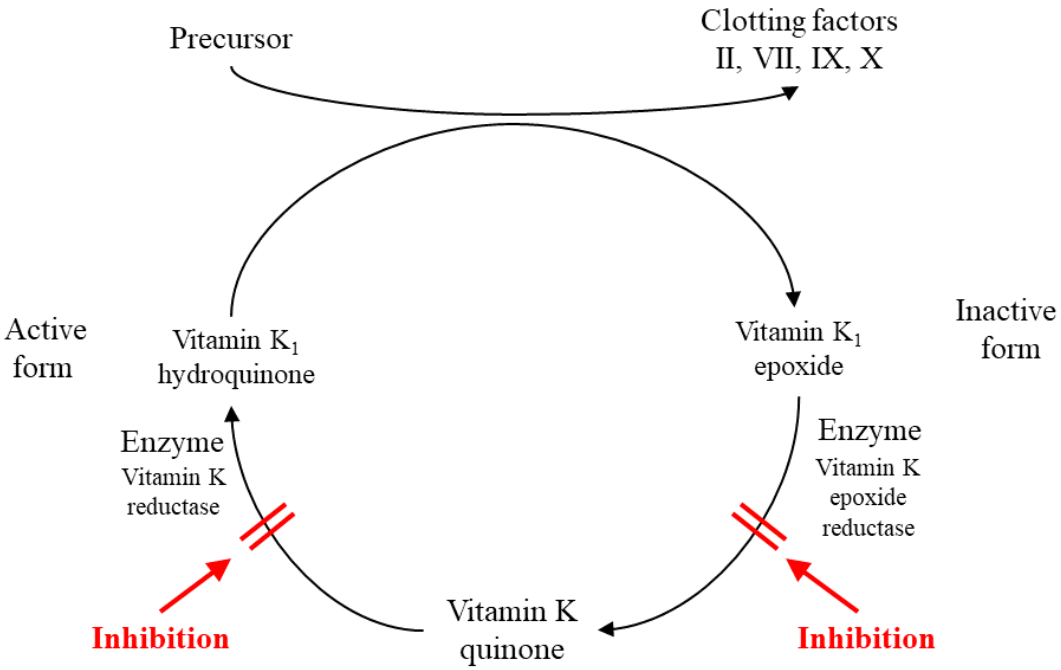

Figure 1.1 Sites of inhibition of vitamin K recycling by anticoagulant rodenticides in the liver of vertebrates (adapted from Buckle, 1994).

Following ingestion of an effective dose of an anticoagulant compound haemostasis is initially maintained by clotting factors already circulating in the blood, but once these have been depleted animals are vulnerable to fatal haemorrhaging. Death occurs several days to several weeks after ingestion of a lethal dose. The delay between ingestion of the anticoagulant and the onset of clinical effects prevents animals from associating the symptoms of poisoning with consumption of bait, and so bait avoidance ("bait shyness") does not occur. This is one of the key factors in the success of these compounds as rodenticides but is also of consequence for non-target poisoning risk (Hadler & Buckle, 1992; Buckle, 1994).

Second-generation anticoagulant rodenticides are more potent, more acutely toxic and more persistent in animal tissues than first-generation compounds and therefore present greater primary and secondary poisoning risks (Section 1.3.1). The greater potency and acuity of second-generation anticoagulants is due to their greater affinity for specific binding sites in the liver (Thijssen, 1995) and their ability to disrupt vitamin K_1 recycling at more than one point (Watt *et al.*, 2005). In contrast, repeated doses are required to maintain an effective concentration of first-generation compounds in the liver. However, once the enzymes are blocked, time until death does not differ significantly between first- and second-generation compounds (Hadler & Buckle, 1992). The greater accumulation and persistence of second-generation compounds in animal tissue is due to enterohepatic recirculation and the high lipid solubility of these compounds (Watt *et al.*, 2005).

1.2.3 Routes of exposure to anticoagulant rodenticides

Non-target wildlife can be exposed to anticoagulant poisons directly, by eating toxic bait (primary exposure), or indirectly, by consuming prey or feeding on the carcasses of animals that have eaten toxic bait or contaminated prey (secondary or tertiary exposure) (Eason & Spurr, 1995). Primary exposure occurs when toxic baits are accessible and attractive to non-target animals (Brakes & Smith, 2005; Pryde *et al.*, 2012). Indirect exposure is most commonly reported for predatory and scavenging birds and mammals (Howald *et al.*, 1999; Shore *et al.*, 1999; Stone *et al.*, 1999; Riley *et al.*, 2007; Albert *et al.*, 2010; Elmeros *et al.*, 2011). Invertebrates such as arthropods can also serve as a pathway of indirect exposure of non-target wildlife to anticoagulant poisons, in particular for ground-feeding insectivorous and omnivorous mammals and birds (Dowding *et al.*, 2006; Dowding *et al.*, 2010; Hernandez-

Moreno *et al.*, 2013). Invertebrates have been documented feeding on anticoagulant-laced baits (Craddock, 2003) and may also be exposed indirectly by ingesting contaminated soil (Booth *et al.*, 2003), carcasses (Howald, 1997) or faeces (Craddock, 2003; Fisher, 2009). Invertebrates have a different blood clotting system to vertebrates (Theopold *et al.*, 2004) and in general are thought to be unaffected by exposure to anticoagulant rodenticides, however there could be some exceptions (Booth *et al.*, 2001; Booth *et al.*, 2003).

1.2.4 Extent of impacts of anticoagulant rodenticides on non-target wildlife

The full extent of the impacts of anticoagulant rodenticide on non-target wildlife populations are most likely underestimated. Acute toxicity resulting in death is most commonly described, but surveillance effort and reporting systems vary between countries (Vyas, 1999; Erickson & Urban, 2004; Berny, 2007; Vyas, 2017). Even if surveillance is undertaken, detection of mortalities can be problematic; poisoned animals may be preyed on or move out of the area before they die, and carcasses may be difficult to detect, or be scavenged or decompose before detection (Vyas, 1999; Huso, 2011). Furthermore, the sublethal effects of exposure are often only known from experimental studies and may be difficult to detect or to attribute to exposure in wild animals. Thus, chronic poisoning of wildlife is seldom described (Mineau, 2005; Berny, 2007).

Analytical tests to confirm exposure are costly and the relationship between tissue residue levels and clinical signs of poisoning is highly variable with both interspecific and individual variation in metabolism and excretion, creating difficulties in correctly attributing the cause of death (Berny, 2007; Connell *et al.*, 2009; Murray, 2011; Sánchez-Barbudo *et al.*, 2012). Exposure to anticoagulants has been strongly inferred as the cause of death or a contributing factor by the detection of tissue residues in carcasses recovered through active surveillance following poisoning operations (e.g. Empson & Miskelly, 1999; Riley *et al.*, 2007). Sublethal exposure has been inferred by sampling or sacrificing live animals to test for tissue residues (e.g. Murphy *et al.*, 1998; Howald *et al.*, 1999). Passive surveillance, where mortalities are encountered incidentally (e.g. roadkill, presentation to wildlife carers), can involve less certainty about the relationship between detection of tissue residues and exposure as the cause of death (Murray, 2011). Fisher (2009) summarises liver brodifacoum concentrations measured in a range of non-target species recovered under these different scenarios, thus demonstrating the challenges of

interpreting the results of such monitoring. In general, despite these limitations, species-specific thresholds for exposure versus toxicity based on tissue residues have been suggested for a range of animals (e.g. Mineau *et al.*, 2001; Rattner *et al.*, 2014a).

1.2.5 Effects of anticoagulant rodenticide exposure

Clinical effects

Exposure to anticoagulant rodenticides may be lethal, sublethal or subclinical. Death may ultimately result from severe haemorrhage caused by impairment of coagulation or from some other less obvious or unknown effect of intoxication (Rattner & Mastrota, 2018). Subclinical exposure involves tissue concentrations that do not produce any observable harmful effects. Sublethal effects occur at doses below those causing death and are usually defined as non-lethal adverse effects that reduce an individual's fitness (Newman, 2010). Sublethal effects may be acute (short-term) or chronic (long-term), reversible or irreversible (Connell *et al.*, 2009).

Sublethal exposure to anticoagulant rodenticides may have physiological effects (e.g. reduced mobility) that may affect an individual's fitness and increase the likelihood of mortality from other causes (Brakes & Smith, 2005). Effects may manifest as weakness, slower reaction times, and susceptibility to accidents, infection or predation (Cox & Smith, 1992; Fournier-Chambrillon *et al.*, 2004; Riley *et al.*, 2007; Vidal *et al.*, 2009). There may also be more subtle behavioural or pathological effects which are as yet poorly understood and may be difficult to recognise (Berny, 2007; Fraser *et al.*, 2018). Anticoagulants can be passed to offspring (e.g. via the yolk, placenta or milk), and may cause death or debility of the developing embryo, foetus or juvenile individual (Hall *et al.*, 1980; Robinson *et al.*, 2005).

Population level response to anticoagulant rodenticide poisoning

Responses to anticoagulant exposure that occur at or below the cellular level (e.g. increased clotting time) may have consequences for individuals (e.g. reduced fitness, death). The adverse effects of exposure on individuals may combine to produce impacts at population level (Rattner *et al.*, 2014b). The consequences of impacts at population level will largely be determined by the proportion of individuals in the effective breeding population that are affected and the capacity of the population to recover from reductions.

(Spurr, 1979; Innes & Barker, 1999). Whether such impacts have long-term ecological significant may depend on the conservation status of the affected species.

1.3 Factors affecting the risk of non-target poisoning

The risk that a non-target animal will be fatally poisoned in the field is a function of hazard (the potential of a poison to cause harm to an animal) and exposure (the likelihood of an animal encountering and consuming a sufficient quantity of toxic bait or contaminated tissue to induce death) (Eason & Wickstrom, 2001; Crowell & Broome, 2004; Erickson & Urban, 2004) (Figure 1.2). Broadly grouped, risk factors relate to the properties of the poison used, the methods used in the pest control operation, the ecology of the non-target species and its sensitivity to the toxin. Aspects of these factors as they pertain to anticoagulant rodenticides and their use are considered in more detail below.

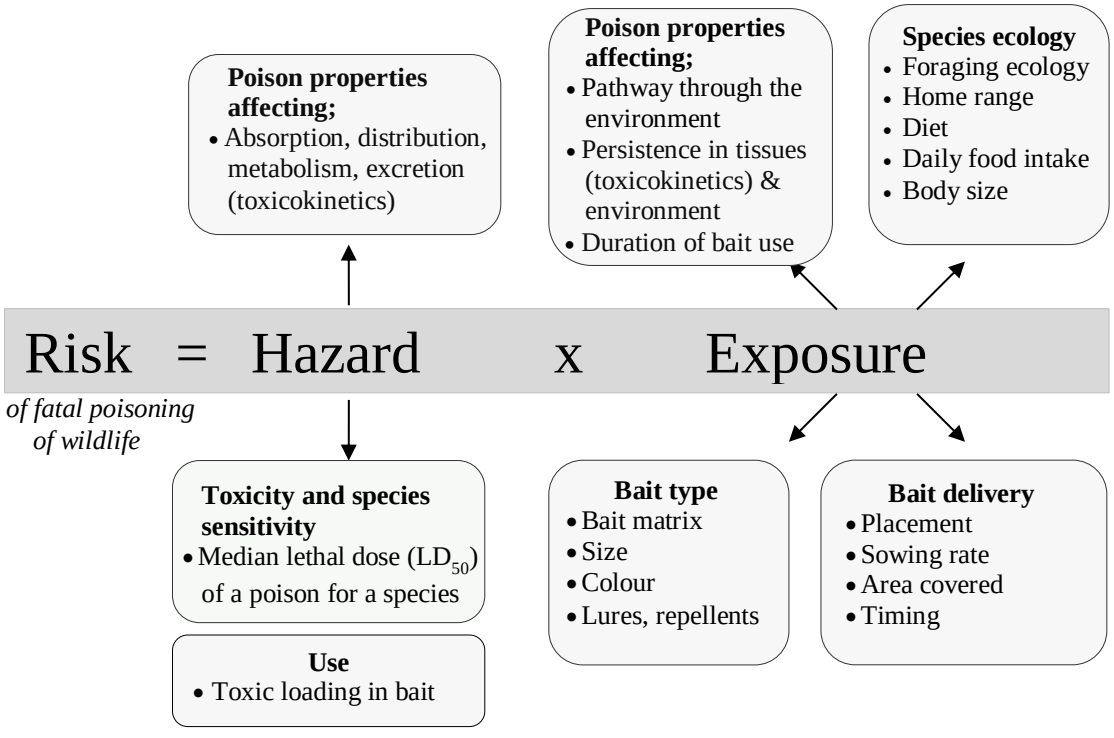

Figure 1.2 Factors affecting the risk of fatal poisoning of wildlife with anticoagulant rodenticides and other vertebrate pesticides (Developed from Eason & Wickstrom, 2001; Crowell & Broome, 2004; Erickson & Urban, 2004).

1.3.1 Properties of anticoagulant rodenticides

Toxicity

The potential of a toxic agent to cause harm (hazard) is related to its toxicity. Toxicity is measured under laboratory conditions and can be characterised by a variety of standardised tests. These tests can be used to determine the dose or concentration of a compound required to reach a specified lethal or non-lethal endpoint in a population of test organisms. Mortality is often used as the endpoint because it can be easily defined and interpreted, and is statistically repeatable (Connell *et al.*, 2009). The most commonly used measure of a substance's acute oral toxicity is LD_{50}, or median lethal dose. Acute oral LD_{50} is a statistically estimated single oral dose expected to be lethal to 50% of exposed individuals and is expressed as the weight of active ingredient (mg) per unit of organism body weight (kg) (Newman, 2010). Extrapolation of laboratory data to the real world involves considerable uncertainty, so LD_{50} values can only serve as a guideline for comparing toxicity among toxic agents and organisms (Erickson & Urban, 2004; Rattner & Mastrota, 2018). LD_{50} has been criticised as a crude measure of biological effect that ignores sublethal effects. Another system that is used is the 'no observable adverse effects' level (NOAEL), the maximum dose that does not produce a statistically significant harmful effect (Connell *et al.*, 2009). However, this metric has also come under criticism (Rattner *et al.*, 2014a). Probabilistic methods that take in to account the variability in response of species and individuals have been proposed as an alternative approach to risk assessment (Thomas *et al.*, 2011; Rattner & Mastrota, 2018). Nevertheless, LD_{50} is currently still widely used.

A smaller LD_{50} indicates greater toxicity relative to other compounds or other species. Second-generation anticoagulant rodenticides have lower LD_{50} values than first-generation compounds (Table 1.2) and generally death is induced by a single dose. In contrast, the potency of first-generation compounds increases when they are administered over several consecutive days, although chlorophacinone and diphacinone may kill some animals after a single feed (Erickson & Urban, 2004). Acute oral LD_{50} doses for first-generation compounds therefore underestimate their potential toxicity (Vyas & Rattner, 2012). While higher toxicity increases the efficacy of second-generation compounds as pest control agents, it also increases the risk of lethal poisoning of non-target animals (Eason & Spurr, 1995).

The differences in toxicity of first- and second-generation anticoagulant rodenticides affects their patterns of use in the field. First-generation compounds, having low acute toxicity, must be used in large quantities for prolonged periods to achieve the desired level of rodent control. Reduction of rodent populations using first-generation poisons requires surplus baiting; baits must be replenished periodically to ensure rodents have continuous access over several weeks to months, increasing the duration that non-target animals are at risk of exposure (Buckle, 1994; Witmer & Eisemann, 2005). Second-generation anticoagulant rodenticides are more acutely toxic and therefore typically require a single application to suppress or eradicate rodent populations (Buckle, 1994). However, the brief duration of use required is somewhat offset by their prolonged persistence in the environment and animal tissue (see below).

Persistence in the environment and in animal tissues

The persistence of poisons in the environment affects the duration of their availability for uptake by living organisms. Second-generation anticoagulants persist for longer in the environment than first generation compounds. Brodifacoum, for example, binds strongly to soil and is insoluble in water. Following disintegration of baits, brodifacoum is likely to remain absorbed in the soil and undergo slow degradation by soil microorganisms (aerobic soil metabolism half-life of 157 days) (EPA, 1998). These laboratory-based estimates are supported by the results of environmental monitoring on Palmyra Island, USA, where residues of brodifacoum were detected in soil samples collected seven months after an aerial bait application (Pitt *et al.*, 2015). Due to the high immobility of brodifacoum in soil, contamination is usually localised relative to bait placement. In contrast, the first-generation compound diphacinone (also immobile in soil and having low water solubility) breaks down in soil relatively quickly (aerobic soil metabolism half-life of c. 30 days) (EPA, 1998). In laboratory-based studies, traces of brodifacoum were found in earthworms (*Apporectodea calignosa)* and common garden snails (*Cantareus aspersus*) exposed to brodifacoum-spiked soil (Booth *et al.*, 2003). Uptake of anticoagulant residues from soil by invertebrates could present a pathway for secondary exposure of insectivores.

Table 1.2 Comparison of acute and multiple dose oral LD_{50} for Norway rats (*Rattus norvegicus*) determined under laboratory conditions for selected first- and second-generation anticoagulant rodenticides.

Class	Anticoagulant	Test animal *(Rattus norvegicus)* sex and strain where specified	Acute oral LD_{50} (mg/kg)	Multiple oral exposures LD_{50} (mg/kg)	Reference
Second-generation	Brodifacoum	male Lab strain	0.418		(EPA, 1998)
		female Lab strain	0.561		
	Bromadiolone	female Lab strain	1.1		
First-generation	Diphacinone	Wild	1.75	0.35/day for 5 days	(Erickson & Urban, 2004)
		Male	6.8		(EPA, 1998)
		Female	8		
	Chlorophacinone	Wild	0.8	0.16/day for 5 days	(Erickson & Urban, 2004)
		Male	3.15		(EPA, 1998)
		Female	10.95		
	Coumatetralyl	'Rat'	16.5	0.3/day for 5 days	(Eason & Wickstrom, 2001)
	Warfarin	Wild	2.20	0.44/day for 5 days	(Erickson & Urban, 2004)
		Norway rat	10-20		
	Pindone	Norway rat	75-100		(Eason & Wickstrom, 2001)

The persistence of anticoagulant poisons in animal tissue affects the potential for accumulation of a lethal dose with repeated exposure, as well as the duration of the period of risk of secondary exposure of non-target predators. Second-generation anticoagulants like brodifacoum are lipophilic, poorly metabolised, and bind strongly to receptors in the liver, pancreas and kidneys of vertebrates after absorption (Eason & Wickstrom, 2001; Watt *et al.*, 2005). Therefore, they are eliminated from the body slowly (Table 1.3). Brodifacoum can persist in the in the liver and kidneys of sublethally poisoned vertebrates for more than a year (Eason & Wickstrom, 2001), and repeated sublethal exposure to second-generation compounds can result in bioaccumulation (build-up) of toxic residues in tissues over time (Huckle *et al.*, 1988). In contrast, first-generation compounds are metabolised and excreted more rapidly so are likely to pose less primary or secondary non-target risk (Erickson & Urban, 2004; Table 1.3). With the delayed onset of the symptoms of anticoagulant poisoning, animals may continue to consume toxic bait for

several days after a receiving a lethal dose, further elevating tissue residues and the level of risk. Following death, residues of second-generation compounds can persist in carcasses for many months (Eason & Wickstrom, 2001), increasing the risk of secondary poisoning of scavengers (Howald *et al.*, 1999).

Table 1.3 Comparative elimination half-lives and likely persistence times of first- and second-generation anticoagulant rodenticides in liver tissue of female rats (*Rattus norvegicus* Wistar) administered acute sublethal (c. LD_{15}) doses (Fisher *et al.*, 2003).

Class	Compound	Elimination half-life (days)	Likely persistence time (weeks)
Second-generation	Brodifacoum	113.5	> 24
First-generation	Diphacinone	3	1-2
	Pindone	2.1	1-2

Persistence time in tissues for a given anticoagulant varies among species. For example, mean hepatic elimination half-life of diphacinone in sublethally dosed red deer (*Cervus elaphus scotius*) was 6 days and in pigs (*Sus scrofa*) 12.4 days (Crowell *et al.*, 2013). Further examples of persistence times of first- and second-generation anticoagulants in the tissues of a range of species (predominantly domestic) are presented by Eason *et al.* (2002) with the caveat that comparisons are crude due to differences in testing methodologies between researchers, as well as the inherent differences between species. Estimates of persistence times in wildlife are not readily available because of uncertainty about exposure histories. Persistence times of first- and second- generation anticoagulants in invertebrate tissue appear to be much shorter than in vertebrate tissue (Booth *et al.*, 2001; Brooke *et al.*, 2013). However, after bait removal invertebrates may be exposed to residues in soil (as mentioned above) or in contaminated carcasses, prolonging the period of risk to insectivores (Howald, 1997; Booth *et al.*, 2003).

1.3.2 Operational factors

Bait matrix formulation

Anticoagulant rodenticide baits are created by incorporating the active ingredient into an edible matrix (Buckle, 1994). Baits are used for ease of handling, storage and application,

and to make them attractive as a food source to target species. A wide variety of bait types are used to present anticoagulant compounds, including pellets, pastes, gels, liquids and powders (O'Connor & Eason, 2000). Relatively high palatability and acceptance of toxic bait to a high proportion of target pest animals is necessary to achieve effective control (Morriss *et al.*, 2008). Flavours can be added to bait formulations to attract target animals or mask the smell or taste of toxins (Ecroyd *et al.*, 1995; Spurr, 1999; Clapperton *et al.*, 2006) and colours or flavours can be added to make baits less attractive to non-target species (e.g. Hartley *et al.*, 2000). The attractiveness of different formulations to target and non-target species may be evaluated in laboratory or captive settings (Spurr, 1993; Clapperton *et al.*, 2006) or in the field (Morgan, 1982; Styche & Speed, 2002) but there is a general lack of field studies that assess the attractiveness of baits to non-target species.

Method of bait application

Anticoagulant baits can be applied by broadcasting baits or by placing baits in receptacles (bait stations) distributed throughout the site. Broadcast baits are scattered over the ground by aircraft or by hand permitting unrestricted access by non-target species throughout the treatment area (Howald *et al.*, 2007; Broome *et al.*, 2014). Although aerially broadcast baits can be placed with reasonable precision relative to site features, baits occasionally land off-target e.g. below the tideline or in lakes, introducing exposure risk to aquatic organisms (e.g. Masuda *et al.*, 2015; Pitt *et al.*, 2015).

Bait stations are permanent containers designed to contain baits in a way that allows access by target pests while excluding non-target species and minimising spillage (Spurr *et al.*, 2007). Baits are delivered in bait stations when required by regulation and when there is concern about access to bait by non-target animals (Pitt *et al.*, 2011). The use of bait stations concentrates bait availability at discrete locations, protects baits from the elements and reduces the amount of bait entering environment (Howald *et al.*, 2007). A large variety of designs are available and in some cases further modifications have been made to exclude specific non-target species (e.g. Erickson *et al.*, 1990; Whisson, 1999; Phillips *et al.*, 2007). Pitt *et al*. (2011) caution that the effect of design changes on accessibility should always be field tested on target as well as non-target species, as some rodent pests are neophobic. Observations or evidence of non-target species accessing bait contained in bait stations include arthropods (Craddock, 2003; Bowie & Ross, 2006),

reptiles (Hoare, 2006), land crabs (Griffiths *et al.*, 2011) and birds (Taylor, 1984). Furthermore, baits that are not anchored within bait stations can be spilled and dispersed, increasing accessibility by non-target species (Pryde *et al.*, 2012).

1.3.3 Non-target species factors

Sensitivity of non-target species

Further to differences in the inherent toxicity of anticoagulant compounds, species differ in their sensitivity to different poisons. Male Norway rats (*Rattus norvegicus*) for example, are more sensitive to a single oral dose of brodifacoum (LD_{50} 0.418 mg/kg) than of diphacinone (LD_{50} 6.8 mg/kg) (Table 1.2), but they are more sensitive to diphacinone than are mallard ducks (*Anas platyrhynchos*) (LD_{50} 3158 mg/kg). (Table 1.4) (EPA, 1998; Erickson & Urban, 2004). Furthermore, the sensitivity to a poison shown by individuals of the same species may vary with factors such as age, reproductive status and genetic variation (Connell *et al.*, 2009).

Table 1.4 Acute oral toxicity (mg/kg body weight) of selected species to the anticoagulant diphacinone, showing taxonomic level of relatedness between species.

Species	Class	Order	Acute oral LD_{50} (mg/kg body wt)	Source
Indian mongoose (*Herpestes auropunctatus)*	Mammalia	Carnivora	0.2	(Erickson & Urban, 2004)
Cat (*Felis catus*)			14.7	
Dog (*Canis familiaris*)			3 - 7.5	
Coyote (*Canis latrans*)			0.6	
Vampire bat (*Desmodus rotundus*)		Chiroptera	0.91	(Thompson *et al.*, 1972)
Wild Norway rat (*Rattus norvegicus)*		Rodentia	1.75	(Erickson & Urban, 2004)
Mouse (*Mus sp.)*			141 - 340	
Mallard duck (*Anas platyrhynchos*)	Aves	Anseriformes	3158	
Northern bobwhite quail (*Colinus virginianus)*		Galliformes	2014	(Rattner *et al.*, 2011)
American kestrel (*Falco sparverius*)		Falconiformes	97	

Sensitivity data are not available for all species, nor for wild populations, and the uniformity of test animals, environmental conditions and exposure patterns required for toxicity testing has been criticised for being ecologically unrealistic (Connell *et al.*, 2009; Rattner & Mastrota, 2018). However, it would be impractical (and in the case of threatened species, unethical) to submit all species of concern to laboratory toxicology tests. Instead, test organisms that exemplify a broader range of species are selected to be representative of the types of animals that are likely to be exposed to a poison (Connell *et al.*, 2009). In most cases toxicity data for one species cannot be used to accurately predict the sensitivity of another closely related species to a particular poison. However, there may be general trends within and between animal groups which allow evaluation of the level of risk (e.g. McIlroy, 1986; Rattner & Mastrota, 2018). Mammals, for example, tend to be more sensitive to anticoagulant rodenticides than birds, and among bird species raptors appear to be more sensitive to anticoagulants than other birds (Table 1.4).

Species ecology and susceptibility to non-target poisoning

A variety of ecological traits affect the risk of exposure of non-target species to toxic baits and whether such exposure will be harmful. An animal must live and feed in the area where and when baits are laid. Its foraging behaviour must lead to encounters with bait that is attractive and palatable as a food source in the case of primary exposure, or with contaminated prey in the case of secondary exposure. Their daily food requirements, sensitivity to the poison used and body size will have bearing on whether dietary exposure is likely to be lethal.

Species that are likely to encounter and ingest toxic bait (or contaminated prey) and eat sufficient to receive a lethal dose are susceptible to fatal exposure (Glen *et al.*, 2007). A species can be sensitive to a toxin but have low susceptibility to fatal exposure e.g. if individuals are unlikely to encounter baits (e.g. an arboreal forager), unlikely to eat baits if encountered (e.g. the baits are unpalatable); or if consumed, unlikely to ingest a lethal dose (small volume daily food intake). For example, there is evidence that free-living New Zealand native lizards will eat anticoagulant-laced baits (Hoare & Hare, 2006a). Toxicity testing of a surrogate species, the Western fence lizard (*Sceloporus occidentalis*), indicates likely sensitivity of New Zealand native lizards to pindone, relative to other anticoagulant rodenticides. However, based on the daily food intake of

native lizards and the concentration of pindone in baits they would be unlikely to consume sufficient bait to receive an acute lethal dose (Weir *et al.*, 2016). Conversely, animals that are not sensitive to a particular toxin may regularly encounter and consume baits with little or no adverse effects. Ascension Island land crabs (*Gecarcinus lagostoma*), for example, are known to interfere with bait stations during rodent eradication programmes and consume considerable quantities of brodifacoum-laced baits (Griffiths *et al.*, 2011). However, laboratory studies indicate that the crabs are unaffected by exposure to brodifacoum (Pain *et al.*, 2000).

De Lange *et al.* (2010) describe a further component of risk as population vulnerability, which considers how a susceptible population's capacity to recover from reductions can affect the long-term impacts on the population. Thus, susceptible species that have low reproductive rates and/or low dispersal ability are more vulnerable to the population-level impacts of non-target mortality than those that can recover their numbers quickly (Spurr, 1979).

1.4 Anticoagulant rodenticide use for conservation management in New Zealand

1.4.1 Mammalian pests in New Zealand

Prior to human contact with New Zealand the only terrestrial mammals present were three species of bat (Worthy & Holdaway, 2002). Intentional and accidental introductions of exotic mammal species accompanied Polynesian settlement (c. 1300 A.D.; Wilmshurst *et al.*, 2008) and visiting European explorers, sealers, whalers and trading vessels, but the most numerous introductions occurred following European settlement in the 1830s (King, 2005). Settlers and introduced mammals wrought destructive changes on the indigenous fauna, the native vegetation and the landscape (Atkinson, 2001; McGlone, 2009). Since human settlement, fifty-three of New Zealand's mainland endemic vertebrate species have been driven to extinction by habitat loss and degradation, hunting pressures, and predation and competition by introduced mammals (Atkinson & Cameron, 1993; Wilson, 2004). Today, fourteen introduced mammal species are widespread, and several species are considered pests (King, 2005). Mammalian pests are managed by national and regional government authorities and private landowners for three broad reasons; to protect agricultural and forestry production values; reduction of the vectors of bovine

tuberculosis; and the conservation of indigenous species and ecosystems (Parkes & Murphy, 2003).

The impact of vertebrate pests on native fauna and habitats is the dominant conservation issue in New Zealand (Craig *et al.*, 2000; Innes *et al.*, 2010; Russell *et al.*, 2015). Consequently, vertebrate pest management is a significant component of protecting biodiversity values (DOC, 2018b). Management of mammalian pest populations on New Zealand's public conservation land is one of the main responsibilities of the Department of Conservation (DOC), a central government organisation tasked with conserving New Zealand's natural and historic heritage. Public conservation land comprises a fragmented network of natural areas covering 8.5 mill hectares, more than one third of New Zealand's land area (DOC, 2018b). Most areas of conservation land support at least one vertebrate pest species (King, 2005).

Ship rats (*Rattus rattus*), Norway rats and house mice (M*us musculus*) are present throughout New Zealand, while Pacific rats or kiore (*Rattus exulans*) are now limited to offshore islands (King, 2005). Rats and mice have caused the declines or extinctions of numerous New Zealand endemic species including birds, lizards, amphibians, insects and plants (Newman, 1994; Ecroyd, 1996; Towns *et al.*, 2006; Innes *et al.*, 2010; St Clair, 2011). The last known population of greater short-tailed bats (*Mystacina robusta*) was most likely extirpated as a result of predation by ship rats that invaded Big South Cape island in the 1960s (O'Donnell *et al.*, 2010; Bell *et al.*, 2016). Rats and mice also slow forest regeneration and compete with native species for food resources by eating seeds, seedlings and invertebrates (Wilson *et al.*, 2003; Clapperton *et al.*, 2019). Thus rodent species are among the main vertebrate pests targeted by DOC (DOC, 2017) and the measures used to control them are amongst the most intensive and large-scale in the world (Innes & Barker, 1999; Eason & Wickstrom, 2001; Towns & Broome, 2003; Elliott & Kemp, 2016).

1.4.2 Use of anticoagulant rodenticides and non-target risks in New Zealand

Anticoagulant-laced baits are widely used to manage rodents and Australian brushtail possums (*Trichosurus vulpecula*) in New Zealand, often over large land areas (Innes & Barker, 1999; Parkes & Murphy, 2003). Aerial application of baits containing the second-generation anticoagulant brodifacoum is favoured for rodent eradication operations on

offshore islands (Towns & Broome, 2003) and at peninsular mainland sites (Dowding *et al.*, 2006). Bait drops are usually timed to coincide with periods when rodents are low in number and stressed by resource scarcity (Howald *et al.*, 2005) and, if possible, when risks to non-target species are considered to be low (Broome *et al.*, 2014; Pitt *et al.*, 2015).

Rodent management at mainland sites typically involves placement of anticoagulant-laced baits in permanent ground-based bait stations (Brown *et al.*, 2015). Bait stations are usually arranged on a grid pattern throughout the treated site (Thomas & Taylor, 2002), spaced at intervals with regard to the density, movement patterns and social behaviours of the target pest (Clapperton *et al.*, 2006). Temporary bait stations in the form of biodegradable bait bags stapled to trees were also used to deliver first generation anticoagulant baits until non-target poisoning issues compelled restrictions on their use with these toxins on public conservation land (DOC, 2018a; DOC, 2019). Eradication of pests from mainland sites is not possible unless exclusion fences are used (Burns *et al.*, 2012). Bait application is therefore required annually to reduce pests to acceptable densities during the breeding season of threatened species (e.g. Moorhouse *et al.*, 2003). This approach of 'sustained control' is the most common vertebrate pest control strategy used in New Zealand. In the year ending 30 June 2017 DOC reported treating >1 million hectares for rodent species (DOC, 2017).

Four anticoagulant rodenticides (Table 1.5) and the acute toxic agent 1080 (sodium monofluoroacetate) are the main vertebrate pesticides used for broad-scale control of rodents and other vertebrate pests on public conservation land in New Zealand (DOC, 2019). The use of second-generation anticoagulants (brodifacoum and bromadiolone) became prominent in the 1980s, with the development of techniques to eradicate rodents from New Zealand offshore islands (Towns & Broome, 2003). During the 1990s, brodifacoum displaced 1080 as the most commonly used vertebrate pesticide throughout mainland and insular New Zealand as a consequence of increasing concerns about the impacts of 1080 on human and wildlife health (Eason *et al.*, 1999b; Innes & Barker, 1999).

After almost a decade of intensive use of brodifacoum on the mainland, considerable evidence had mounted concerning lethal and sublethal poisoning of non-target wildlife and

accumulation in the tissues of game animals (Eason *et al.*, 1999a). Comprehensive reviews of the non-target impacts of brodifacoum use on New Zealand native wildlife are provided by Eason *et al.* (2002) and Hoare and Hare (2006b). This led DOC to introduce a policy limiting the use of second-generation anticoagulants on mainland public conservation land (DOC, 2018c; Table 1.5). Similar concerns have led to restrictions on field use of second-generation compounds in other countries (Erickson & Urban, 2004). Currently, brodifacoum is used for rodent eradications and incursion detections on offshore islands and there are strict conditions around its use on mainland public conservation land, although less restricted use under other authorities continues at other mainland sites (Hoare & Hare, 2006b; MPI, 2018; DOC, 2019). Three first-generation anticoagulant rodenticides, diphacinone, pindone and coumatetralyl, are currently registered for rodent control operations using bait stations on mainland public conservation land (Table 1.5). These poisons are less potent and persistent than second generation compounds and so pose lower non-target poisoning risk (Fisher *et al.*, 2003). Diphacinone has been considered a promising alternative to brodifacoum because it offers a compromise between the high toxicity to rodents demonstrated by second-generation anticoagulants, and the shorter persistence of first-generation-compounds (Fisher *et al.*, 2003; Eason *et al.*, 2010).

Diphacinone is the main anticoagulant rodenticide of concern to this study. Diphacinone has been used in New Zealand to control rats in mainland conservation reserves (Gillies *et al.*, 2006). In the U.S.A., diphacinone has been used to eradicate rodents from small islands using aerial and ground-based delivery systems (Donlan *et al.*, 2003; Witmer *et al.*, 2007; Hess & Jacobi, 2011) and at mainland U.S. sites it is used to control rodents in commensal, agricultural and rangeland settings (Erickson & Urban, 2004; Salmon *et al.*, 2007). Despite the lower potency and shorter persistence of diphacinone compared with second generation anticoagulants there is evidence of risk to non-target wildlife (Eisemann & Swift, 2006; Rattner *et al.*, 2012). Secondary poisoning of raptors has been demonstrated in laboratory trials using mice that were killed with diphacinone (Mendenhall & Pank, 1980), and residues of diphacinone have been detected in wild populations of non-target mammals (e.g. Riley *et al.*, 2007), birds (e.g. Stone *et al.*, 2003) and invertebrates (e.g. Johnston *et al.*, 2005).

Table 1.5 Accepted anticoagulant rodenticides registered for broad-scale use to control rodent pests on public conservation land in New Zealand. The status category "Accepted' means that the product can be used as long as all compulsory requirements on the performance standards sheets are adhered to. Adapted from Abridged Pesticides Status List (DOC, 2019).

Poison	Target pest	Trade name	Toxic loading	Type of bait	Method	Policies or compulsory restrictions that apply to this pesticide use
Brodifacoum	Rats & mice	Pestoff Rodent Bait 20R	0.02 g/kg	Cereal pellet	Aerial, bait stations, hand-laying	Restricted to the control of rodents on non-stocked off-shore islands or for rodent control carried out in accordance with the Code of Practice approved for this product.
		Pestoff Rodent Bait	0.02 g/kg	Cereal pellet	Bait stations	At mainland sites this pesticide use is restricted to one or two operations per lifespan for the longest lived native animal species likely to be exposed. (An operation is defined as the application of the pesticide for long enough to achieve the operational target.) This pesticide use can only be used for operations that: i) use captive baits in bait stations designed to exclude other animal pests present (especially possums); and ii) where pigs cannot be exposed to the toxin.
		Pestoff Rodent Blocks	0.02 g/kg	Block	Bait stations	
Coumatetralyl	Rats	Racumin Paste	0.375 g/kg	Block	Bait stations	
Diphacinone	Rats	Ditrac All-Weather Rodent Block	0.05 g/kg	Block	Bait stations	
		RatAbate Paste	0.05 g/kg	Hard paste		
		Pestoff Rat Bait 50D	0.05 g/kg	Cereal pellet		
		D-Block for the Control of Rats (RatAbate Blocks)	0.05 g/kg	Block		
		D-Block Extreme	0.05 g/kg	Block		
Pindone	Rats	Pindone Pellets	0.5 g/kg	Cereal pellet	Bait stations, aerial & hand-laying	Aerial & handlaying: Do not use in kea habitat as defined in the DOC Code of practice for aerial 1080 in kea habitat DOC-2612859.

1.5 Risk of non-target poisoning to New Zealand's endemic land mammals

New Zealand's only terrestrial native mammals are two endemic bat species; the New Zealand lesser short-tailed bat (*Mystacina tuberculata* Gray 1843, Mystacinidae) and the New Zealand long-tailed bat (*Chalinolobus tuberculatus* Forster 1844, Vespertilionidae) (King, 2005). Both are echolocating bats classified under suborder Yangochiroptera (Vespertilioniformes) (Teeling *et al.*, 2005; Hutcheon & Kirsch, 2006), previously Microchiroptera. These two bat species occur in habitats where poisons are applied for control of rodents and other vertebrate pests, and both have been considered at risk of non-target poisoning (Daniel & Williams, 1984; Eason & Spurr, 1995). While both species are insectivorous and could be vulnerable to secondary exposure, the risk of long-tailed bats encountering contaminated prey is thought to be low as they forage entirely on the wing, primarily along forest margins (O'Donnell, 2000; O'Donnell, 2001). The lesser short-tailed bat is thought to be at greater risk of primary or secondary exposure because it has an unusually broad diet and also spends time foraging on the ground in the forest interior (Daniel, 1976; Jones *et al.*, 2003; McCartney *et al.*, 2007). For this reason I will focus on the lesser short-tailed bat and aspects of its ecology that affect its susceptibility to non-target poisoning.

1.5.1 General ecology of the lesser short-tailed bat

Distribution

The lesser short-tailed bat is the only extant representative of the Mystacinid family, with no close living relatives (Hand *et al.*, 1998). The species was once widespread throughout New Zealand but significant populations are currently only known from 14 locations; two on small offshore islands (<3000 ha) and the remainder at mainland sites, all on public conservation land (Lloyd, 2005). This includes a previously unknown population detected in the Murchison mountains, Fiordland, in 2019 (R. Jackson, DOC, pers. comm., 2019) (Figure 1.3). Occasional records of low activity indicate that remnant populations may persist in other locations in low numbers (Lloyd, 2005). Populations are limited to extensive areas of old-growth native forest containing a diversity of food resources and large trees with cavities suitable for roosting, and all populations (O'Donnell *et al.*, 1999; Sedgeley, 2003; Lloyd, 2005).

Threats

Both of New Zealand's endemic bat species have declined in distribution and abundance since European settlement (O'Donnell, 2000; O'Donnell *et al.*, 2010). Lesser short-tailed bats are currently categorised as 'Vulnerable' by the IUCN, due to their extremely fragmented distribution and continuing population declines (O'Donnell, 2008). Within New Zealand, three recognised sub-species are currently ranked separately as either 'At Risk' or 'Threatened' under the National Threat Classification System (Hill & Daniel, 1985; Hitchmough *et al.*, 2007; O'Donnell *et al.*, 2010; O'Donnell *et al.*, 2018) (Table 1.6). Primary threats to lesser short-tailed bats include predation by and competition with introduced mammalian pests, habitat degradation, disturbance at roosts, potential exposure to vertebrate poisons and possibly disease (O'Donnell *et al.*, 2010). Declines may result from a combination of threats, but predation is of particular concern. Rats, possums, cats and stoats (*Mustela erminea*) are present in all mainland bat habitats and are known or suspected predators of New Zealand bats (Daniel & Williams, 1984; O'Donnell, 2000; Scrimgeour *et al.*, 2012). In Fiordland long-tailed bat population declines have been correlated with high densities of rats and stoats (O'Donnell *et al.*, 2017) and the disappearance of the greater short-tailed bat from mainland New Zealand coincides with the spread of kiore prior to European settlement (Worthy, 1997). The role of ship rats in the ultimate extinction of this species has been described above (Section 1.4.1). This highlights the need for effective predator control in bat habitats.

Reproduction

During late spring (October/November) lesser short-tailed bat colonies begin to congregate at maternity roosts. During summer these roosts are dominated by breeding females who give birth to a single pup between mid-December and mid-January (Sedgeley, 2003; Lloyd, 2005). Pups are crèched in maternity roosts until they begin flying at four to six weeks of age and are weaned several weeks later. The same maternity roost may be occupied for several days to weeks, with non-volant pups carried by their mothers when changing roosts (Lloyd, 2005). Lesser short-tailed bats employ a lek breeding system, with aggregations of males displaying from singing roosts clustered near maternity roosts to attract receptive females for mating (Toth *et al.*, 2015b).

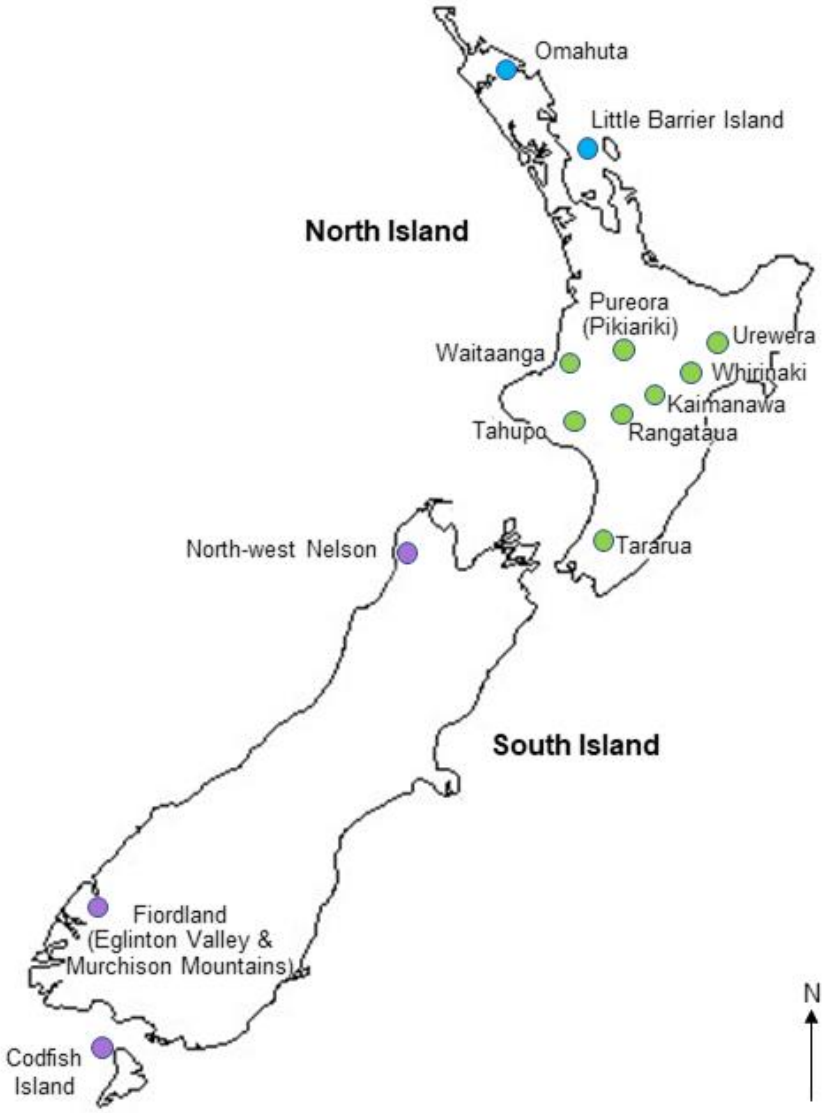

Figure 1.3 Locations of known lesser short-tailed bat (*Mystacina tuberculata*) populations in New Zealand, including a new population detected in the Murchison Mountains in 2019. The three recognised subspecies are indicated by dots of different colours. Blue dots: northern subspecies (*M. t. apourica*); Green dots: central subspecies (*M. t. rhyacobia*); Purple dots: southern subspecies (*M. t. tuberculata*). Modified from (Lloyd, 2005; O'Donnell *et al.*, 2010; R. Jackson, pers. comm. 2019).

Table 1.6 Current national and international threat rankings of the three sub-species of New Zealand lesser short-tailed bats (*Mystacina tuberculata*) (O'Donnell, 2008; O'Donnell *et al.*, 2018).

Subspecies	**New Zealand National Threat Ranking 2017**	**IUCN threat ranking** (assessed 2008)
Northern (*M. t. apourica*)	Threatened: Nationally vulnerable	Threatened: Vulnerable
Central (*M. t. rhyacobia*)	At risk: Declining	
Southern (*M. t. tuberculata*)	At risk: Recovering	

Foraging behaviour and diet

Lesser short-tailed bats are capable fliers and forage in the forest interior where they use echolocation to locate prey on the wing (Jones *et al.*, 2003; O'Donnell *et al.*, 2006). Unlike most other bat species they are also agile crawlers, allowing them to forage on the ground and on tree trunks and branches (Daniel, 1976; Jones *et al.*, 2003) (Figure 1.4). Around two thirds of their diet consists of forest arthropods, and the remainder comprises fruit, nectar and pollen (Daniel, 1979; Arkins *et al.*, 1999). Jones *et al.* (2003) suggest that these bats may use smell to locate fruit and nectar, and also to locate arthropods when foraging on the ground.

Figure 1.4 A New Zealand lesser short-tailed bat (*Mystacina tuberculata*) crawling on a tree trunk (left) and feeding on nectar from *Dactylanthus* flowers that grow on the forest floor (right). Photographs courtesy of David Mudge, Nga Manu Images.

Faecal analysis studies from north, central and southern populations have revealed that a wide variety of both volant and non-volant arthropod taxa are included in the diet in both winter and summer (Arkins, 1997; Arkins *et al.*, 1999; Czenze *et al.*, 2018). Early accounts of these bats feeding on carrion have not subsequently been substantiated (Stead, 1936; Blanchard, 1992; McCartney *et al.*, 2007). The daily food requirements of lesser short-tailed bats are substantial relative to their small body mass (mean adult mass excluding breeding females ± SD; 14.39 ± 1.84 g, n=954) (Lloyd, 2005). Daniel (1979) observed that captive bats consumed 36-50% of their pre-feeding body mass each night, and the

daily food intake of free-living bats has been estimated as 40% of body mass (c. 5.6 g, Lloyd & McQueen, 2000). Foraging activity of individuals studied in two populations was concentrated within one or more small core areas within much larger home ranges of several hundred to more than 1000 ha (Christie & O'Donnell, 2014; Toth *et al.*, 2015a). During winter short-tailed bats use torpor to conserve energy, but periodically rouse to feed when conditions are suitable (Christie & Simpson, 2006; Czenze *et al.*, 2017).

1.5.2 Risk of anticoagulant poisoning of lesser short-tailed bats

Anticoagulant rodenticide use in bat habitat

Invasive rodents and other vertebrate pests have been eradicated from the two offshore islands where lesser short-tailed bats occur, but these pests are still present in the habitats of all mainland bat populations. Control of rodents and other predatory mammals such as mustelids is likely to benefit bats by reducing predator impacts (O'Donnell *et al.*, 2011; Edmonds *et al.*, 2017; O'Donnell *et al.*, 2017) and improving food resources (Innes & Barker, 1999). Vertebrate pesticides, including anticoagulant rodenticides, are regularly or periodically used to suppress pest populations at several of these sites. The frequency of rodent control operations at managed sites depends on how the productivity of different forest types drives mammalian pest population dynamics. Annual control is required in podocarp-broadleaf forests, where rodents, especially ship rats, are generally abundant throughout the year, every year (King *et al.*, 1996). In beech forests, however, ship rats are scarce in most years, and predator control is only required following mast (heavy to moderate seeding) years, which result in irruptions of mouse, ship rat and stoat populations (King, 1983; Elliott & Kemp, 2016). This cycle is climate driven and occurs at irregular intervals of once every five or six years on average (Tompkins *et al.*, 2013).

Potential sensitivity of lesser short-tailed bats to anticoagulant rodenticides

The sensitivity of the lesser short-tailed bat to anticoagulant poisons is not known. However, vampire bats (*Desmodus rotundus*) are known to be sensitive to several first-generation anticoagulant compounds. Diphacinone, warfarin and chlorophacinone have been used to supress vampire bat populations in Central and South America to reduce the incidence of bat-transmitted rabies in cattle. Bats are exposed by ingesting toxic paste applied directly to their fur, or by secondary exposure to poison in the blood of livestock treated with sublethal doses (Linhart *et al.*, 1972; Thompson *et al.*, 1972; Arellano-Sota,

1988). Chlorophacinone has also been used for rapid and effective control of nuisance colonies of big brown bats (*Eptesicus fuscus*) in the United States (Corrigan, 1984).

The acute oral LD_{50} of diphacinone in vampire bats is 0.91 mg/kg (Thompson *et al.*, 1972) and of chlorophacinone 3.06 mg/kg (Linhart *et al.*, 1972) indicating that vampire bats are more sensitive to these toxins than Norway rats under laboratory conditions (Table 1.4). As previously noted (Section 1.3.3), toxicity data for one species cannot generally be used to accurately predict the sensitivity of another closely related species to the same poison. However, general trends in sensitivity within and between animal groups (e.g. McIlroy, 1986) suggest there is a strong risk that lesser short-tailed bats may also be sensitive to diphacinone and other anticoagulant poisons.

Risk of exposure due to foraging ecology

The unusual foraging behaviour and broad diet of lesser short-tailed bats increases their risk of exposure to vertebrate poisons during baiting operations in their habitat. As they forage they may be at risk of directly encountering and feeding on baits that are broadcast on the ground or placed in bait stations on tree trunks (primary exposure). Baits that are formulated to be palatable to other mammal species may also be attractive to these bats that feed on a variety of foods. The bats may also be at risk of encountering and feeding on contaminated arthropod prey as they forage on or near the ground (secondary exposure). The substantial daily food requirements of lesser short-tailed bats relative to their body mass and their possible sensitivity to anticoagulant rodenticides (see above) means that if exposed, there is a considerable risk that they will ingest a lethal dose acutely or over several consecutive days.

Assessment of non-target mortality risk in lesser short-tailed bats

There is a paucity of consideration of bats relative to birds in risk assessments for pest control operations in New Zealand, even though mammals are generally more sensitive than birds to anticoagulants and other rodenticides commonly used here (e.g. Table 1.4). This may in part be due to the dominance of bird species and the lack of native mammal species in the terrestrial fauna, which may also contribute to decisions by regulatory authorities to allow such widespread field used of vertebrate pesticides in New Zealand. Furthermore, bats are difficult to observe and monitor. A few practical risk assessments

for lesser short-tailed bats were made in the late 1990s around the time of peak concern for the non-target impacts of widespread brodifacoum use. Bait acceptance trials with captive lesser short-tailed bats indicated that the bats were unlikely to directly consume the cereal pellet baits that were typically used in pest control operations in New Zealand but could be at risk of secondary exposure (Lloyd, 1994) but no mortalities were detected when wild bats were monitored through pest control operations (Sedgeley & Anderson, 2000; Lloyd & McQueen, 2002). In a later captive study, however, wild-caught captive lesser short-tailed bats showed interest in a range of baits (Beath *et al.*, 2004). Long-tailed bats have been considered at low risk theoretically and so have not been the subject of any practical risk assessments. Toxicity testing to assess the sensitivity of New Zealand bats to anticoagulants would be unethical so inferences have relied on limited studies on vampire bats (Thompson *et al.*, 1972).

Consequences of non-target impacts for lesser short-tailed bat populations

Species with low reproductive output and poor dispersal capacity are have restricted ability to recover from population reductions and are therefore at greater risk of non-target mortality events impacting population viability (Spurr, 1979; De Lange *et al.*, 2010). Bats in general are long-lived with low rates of reproduction (Barclay & Harder, 2003). Most bat species produce one pup per year, particularly in temperate zones where the breeding season is limited (Barclay & Harder, 2003). The proportion of females that reproduce in a breeding season varies widely for bats but is typically < 100% (range 32% - 100%, *n*=257 species), particularly in the temperate zone (Barclay *et al.*, 2004). There is limited data on the rates of reproduction in lesser short-tailed bats, but colonies are known to breed once a year during the Austral summer with <100% of breeding-aged females producing a single pup (Sedgeley, 2003; Lloyd, 2005). Furthermore, the remaining significant short-tailed bat populations are geographically isolated. Although short-tailed bats are capable of flying tens of kilometres in a night, long-distance dispersal has not been recorded in contemporary populations and genetic studies suggest that movement between populations is rare (Lloyd, 2003a; Lloyd, 2003b). Lesser short-tailed bat populations may therefore be vulnerable to reductions in size, particularly if populations are already small (Caughley, 1994).

Non-target mortality of lesser short-tailed bats

In January 2009, 115 lesser short-tailed bat deaths were observed in Pikiariki Ecological Area, Pureora Forest Park in the central North Island during a rodent control operation using diphacinone-laced baits in the bats' core roosting habitat. The dead bats were discovered incidentally near a maternity roost during preparations to estimate abundance of the population. Post-mortem examination confirmed diphacinone poisoning, and this investigation is documented in Chapter 2 of the thesis. This was the first recorded non-target mass mortality of this species (Dennis & Gartrell, 2015; Chapter 2). Prior to this event, only one poison-related lesser short-tailed bat death had ever been documented; in 1977 a single dead bat was found on a fruit-lured cyanide bait laid for possum control (Daniel & Williams, 1984). It is possible, however, that other non-target deaths of this species have gone undetected since the bats live in remote, forested locations and have cryptic roosting behaviours and few populations are monitored.

1.6 Research aims and thesis structure

The studies in this thesis were initiated as a result of the non-target mortality of New Zealand lesser short-tailed bats caused by exposure to the first-generation anticoagulant diphacinone during a rodent control operation on public conservation land, as described above. The mortality event raised the obvious questions of 'how did it happen?' and 'how can it be prevented from happening again?' The field research undertaken in two chapters aims to address these questions, but the overall aim of the studies undertaken in this thesis is to ensure that the threatened lesser short-tailed bat and other species susceptible to non-target poisoning obtain long-term population benefits from the careful use of anticoagulant poisons to control rodent pests in their habitat.

The thesis is organised in to seven chapters centred around five research chapters, each written as stand-alone papers. While this format leads to some inevitable repetition, I have tried to minimise this where possible.

In **Chapter 2** I document the lesser short-tailed bat mortality event through first-hand involvement and report on the associated post-mortem investigation undertaken by Wildbase Pathology to confirm the cause of death of the bats.

In **Chapter 3** I investigate the likely route of exposure of lesser short-tailed bats to diphacinone by conducting bait trials with captive and free-living bats and arthropods. As lesser short-tailed bats have an unusually broad diet that includes both plant matter and arthropods either primary or secondary exposure (or both) could have occurred during the mortality event. The findings of the study have helped to guide management decisions about the choice of bait type and the bait delivery methods used in bat habitat.

In **Chapter 4** I conducted a 6-month field trial to assess the effectiveness of an alternative baiting method at reducing the risk of exposure and non-target mortalities of lesser short-tailed bats. I monitored the bat population at the site of the mortalities before, during and after a rodent baiting programme run by the Department of Conservation. I sought evidence of anticoagulant exposure and associated clinical effects by sampling individuals while measuring population survival using mark-recapture methods, with the purpose of demonstrating a causal relationship between exposure and population-level impacts.

In **Chapter 5** I used closed mark-recapture analysis to estimate the abundance of the lesser short-tailed bat population at the site where the mortalities occurred. At the time of the mortality event the only current estimate of adult population size was from roost emergence counts. This only provides a minimum estimate of population size because it is not possible to identify all of the roost trees used by a population on a single night. Furthermore, during the mortality event it is likely that only a proportion of the bats killed by diphacinone exposure were detected. I was therefore unable to make an accurate assessment of the true extent of the mortalities and the possible impact of the incident on population viability. The abundance estimate made during the current study will provide a reference point for assessing population trends in future assessments and improve evaluation of population-level impacts in the case of further mortalities.

In **Chapter 6** I used population modelling to consider the viability of the lesser short-tailed bat population over a 10-year timeframe under various management and non-target mortality scenarios. To achieve this I developed a model describing the population dynamics of the bat and included estimates of survival and abundance measured in the field study in my population projections. The model was used to demonstrate where the balance lies between the need to provide effective rodent management using toxins in bat habitat and the level of by-kill that can be tolerated without impacting population viability. The model was also used to explore the potential impacts on the viability of the bat population of chronic sublethal exposure of bats to anticoagulant rodenticides.

Chapter 7 concludes the thesis with a summary of the research findings and a general discussion of the implications of anticoagulant rodenticide exposure for the health and population viability of the study species, for New Zealand's only other bat species, the long-tailed bat, and for bat populations worldwide. I also provide recommendations for management and monitoring and identify important research needs in this field of study.

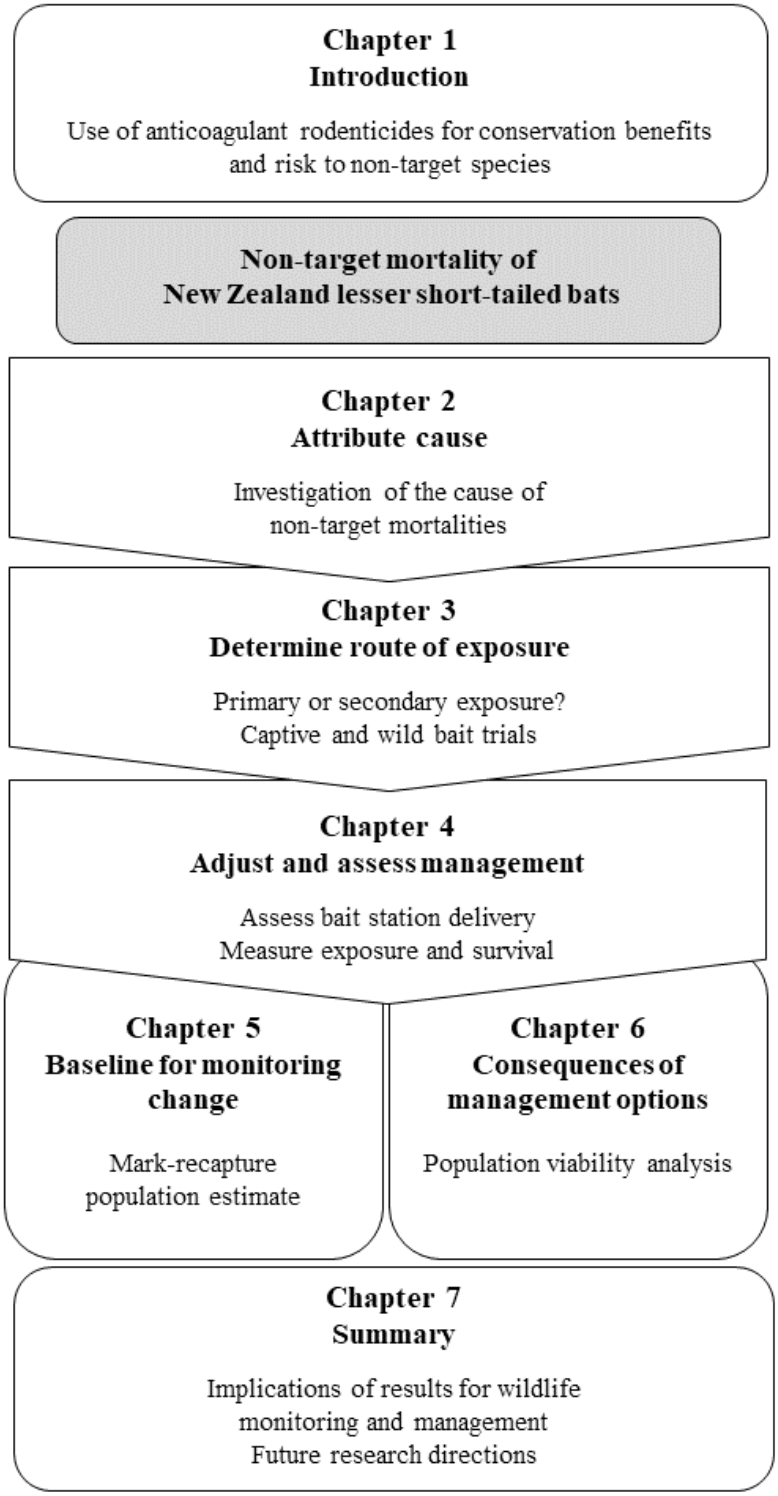

Figure 1.5 Outline of topics addressed in this thesis, in the context of minimising the risk of non-target impacts and ensuring long-term benefits of anticoagulant rodenticide use for a highly susceptible species, the New Zealand lesser short-tailed bat (*Mystacina tuberculata*).

Chapter 2

Non-target mortality of New Zealand lesser short-tailed bats (*Mystacina tuberculata*) caused by diphacinone

One of many lesser short-tailed bat pups found dead at the base of a maternity roost tree with signs of anticoagulant poisoning.

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2.1 Abstract

Primary and secondary poisoning of non-target wildlife with second-generation anticoagulant rodenticides has led to restrictions on their use, and increased use of first-generation anticoagulants, including diphacinone. Although first-generation anticoagulants are less potent and less persistent than second-generation compounds, their use is not without risks to non-target species. We report the first known mortalities of threatened New Zealand lesser short-tailed bats (*Mystacina tuberculata*) caused by diphacinone intoxication. The mortalities occurred during a rodent control operation in Pureora Forest Park, New Zealand, during the 2008-2009 Austral summer. We observed 115 lesser short-tailed bat deaths between 9 January and 6 February 2009, and it is likely that many deaths were undetected. At post-mortem, adult bats showed gross and histologic haemorrhages consistent with coagulopathy, and diphacinone residues were confirmed in 10 of 12 liver samples tested. The cause of mortality of pups was diagnosed as a combination of the effects of diphacinone toxicity, exposure, and starvation. Diphacinone was also detected in two of 11 milk samples extracted from the stomachs of dead pups. Eight adults and 20 pups were moribund when found. Two adults and five pups survived to admission to a veterinary hospital. Three pups responded to treatment and were released at the roost site on 17 March, 2009. The route of diphacinone ingestion by adult bats is uncertain. Direct consumption of toxic bait or consumption of poisoned arthropod prey could have occurred. We suggest that the omnivorous diet and terrestrial feeding habits of lesser short-tailed bats make them susceptible to poisoning with the bait matrix and the method of bait delivery used. We recommend the use of alternative vertebrate pesticides, bait matrices, and delivery methods in bat habitat.

Key words: Anticoagulant rodenticides, conservation, microchiroptera, Mystacinidae, pathology, pest control, toxicants, wildlife.

2.2 Introduction

Anticoagulant rodenticides are commonly used to control rodent pests in urban and agricultural settings worldwide. They are also widely used to eradicate introduced rodents from mammal-free island ecosystems for conservation purposes (Towns & Broome, 2003; Howald *et al.*, 2007). In New Zealand, where the only native land mammals are two species of bats (King, 2005), these pesticide compounds are also used to control rats (*Rattus* spp.) in mainland conservation reserves using broad-scale, sustained, ground-based applications (Innes & Barker, 1999).

Anticoagulant rodenticides are categorised as either first- or second-generation compounds. Both types act by interfering with the synthesis of vitamin K-dependent blood clotting factors in the liver of vertebrates, causing fatal haemorrhaging (Buckle, 1994). Second-generation compounds are more potent and more persistent in animal tissue than first-generation compounds (Fisher *et al.*, 2003; Erickson & Urban, 2004), increasing the risk of poisoning of non-target animals by direct consumption of poisoned bait (primary exposure) or by consumption of poisoned prey (secondary exposure) (Eason & Spurr, 1995). Mortalities and sublethal poisonings, caused through both primary and secondary routes of exposure to second-generation compounds, have been recorded for a wide range of non-target bird (e.g. Eason *et al.*, 2002) and mammal species (e.g. Fournier-Chambrillon *et al.*, 2004). Intoxication of non-target wildlife with first-generation compounds also occurs (e.g. Stone *et al.*, 1999), but much less frequently (Erickson & Urban, 2004).

Widespread concern about the continuing impacts of second-generation anticoagulant rodenticides on non-target wildlife has led to new restrictions on their use (DOC, 2006; EPA, 2008), and investigation of more suitable first-generation compounds, including diphacinone. Diphacinone has been considered a promising alternative to second-generation compounds because it has medium potency to rodents but relatively short persistence in animal tissue (Fisher *et al.*, 2003). In New Zealand diphacinone has proven effective for controlling rats in mainland conservation reserves (Gillies *et al.*, 2006). In the USA it is used to control rodents in commensal (Erickson & Urban, 2004), agricultural, and rangeland settings (Salmon *et al.*, 2007), and to eradicate rodents from small islands for conservation purposes (Witmer *et al.*, 2007b).

Although less potent and less persistent than second-generation anticoagulants, the use of diphacinone is not without ecologic risk (Eisemann & Swift, 2006; Rattner *et al.*, 2012). Secondary poisoning of raptors, fed mice killed with diphacinone, has been demonstrated in laboratory trials (Mendenhall & Pank, 1980), and residues of diphacinone have been detected in wild populations of non-target birds (e.g. Stone *et al.*, 2003), invertebrates (e.g. Johnston *et al.*, 2005) and mammals (e.g. Riley *et al.*, 2007).

We report non-target mortalities of New Zealand lesser short-tailed bats (*Mystacina tuberculata*) due to diphacinone intoxication. These threatened, endemic bats have a terrestrial foraging habit and a broad diet, comprising terrestrial, arboreal and aerial arthropods, nectar, pollen and fruit (Daniel, 1976; Arkins *et al.*, 1999). They have therefore been considered at risk of primary poisoning through consumption of toxic baits encountered while foraging (Eason & Spurr, 1995) or to secondary poisoning by consumption of arthropods which have fed on toxic bait (Lloyd & McQueen, 2000; Craddock, 2003).

This is the first reported case of lesser short-tailed bat deaths due to anticoagulant poisoning. The incident occurred during a rodent control operation in native forest on public conservation land in New Zealand, in January 2009. The mortalities were detected at two roost trees during the bats' breeding season (November–February), when pups are born and crèched in maternity roosts. We documented the mortality event and subsequent investigation to provide records for future reference.

2.3 Methods

2.3.1 Study site

The bat mortalities occurred in Pikiariki Ecological Area, Pureora Forest Park, in the North Island, New Zealand (38°31'S, 175°34'E). Pikiariki Ecological Area (hereafter "Pikiariki"), is a remnant of old-growth native podocarp-hardwood forest (457 ha) within Pureora Forest Park (78,000 ha). Pikiariki has been designated an Ecological Area in recognition of its high conservation values, and provides the core breeding and colonial roost tree habitat for a population of lesser short-tailed bats.

2.3.2 Bat mortalities

On January 9, 2009, we discovered dead and moribund lesser short-tailed bats at the bases of a maternity roost tree and a nearby colonial roost tree. At the time, a rodent control operation using diphacinone (0.005%) was taking place in Pikiariki to limit the ship rat (*Rattus rattus*) population. The active ingredient was presented in a cereal paste matrix (RatAbate®, Connovation Ltd, Auckland, New Zealand) delivered in biodegradable plastic bait bags, each containing 300 g of paste. Bait bags were stapled to tree trunks 0.2–1 m above the ground on a 150 x 50 m grid throughout Pikiariki. Baiting commenced on 21 October 2008, with subsequent bait deployments in November and December to maintain availability to rodents.

Daily checks for further mortalities at the two roost trees continued for 34 days. An additional eight known colonial and maternity roost trees were also monitored for signs of occupancy and for mortalities. We attempted to locate any unknown roost trees which might have been occupied. Eight adult bats in apparent good health were captured in mist-nets (38 mm, Avinet, USA) and fitted with radio-transmitters (BD2A, Holohil Systems, Carp, Ontario, Canada), attached between the scapulae on an area of partially trimmed fur, using a latex-based contact adhesive (Ados F2®, CRC Industries New Zealand, Auckland, New Zealand). Transmitters weighed ≤ 0.7g and represented <5% of bat body mass. Bats were radio-tracked to roosts during the day using a hand-held TR4 receiver (Telonics, Mesa, Arizona, USA) and a hand-held, 3-element Yagi aerial (Lotek, Havelock North, New Zealand). Intensive monitoring of the affected roosts continued for 5 days after the last casualty was found. The diphacinone-laced baits were removed from the field within 4 days of finding the first dead bats, following the preliminary post-mortem findings.

2.3.3 Pathology

Dead bats were chilled and transported to Massey University, Palmerston North. Those in suitable condition underwent post-mortem examination. Tissue samples were fixed in 10% neutral buffered formalin. Fixed tissues were embedded in paraffin, sectioned at 4 μm, and stained with haematoxylin and eosin for histologic examination. Fresh tissue samples of lung, liver, and kidney were taken from adults and pups for aerobic bacterial

culture. Stomach contents of freshly dead adults were examined. Pup stomachs containing milk were excised and frozen.

2.3.4 Toxicology

Liver samples were frozen separately for toxicologic analysis. The diphacinone content of selected liver samples and maternal milk extracted from the stomachs of pups was determined by high-performance liquid chromatography. The method detection limit was 0.05µg/g for liver, and is undetermined for milk. The uncertainty (95% CI) was ±20%. Assays were performed by CENTOX (Centre for Environmental Toxicology), Landcare Research, Lincoln, New Zealand.

2.3.5 Treatment of live bats

Moribund bats were given supportive care and transported to Massey University for treatment. Admitted bats were initially hydrated twice daily with warmed subcutaneous fluids (0.9% NaCl/ 2.5% glucose), and dosed with subcutaneous vitamin K (Konakion, Roche, Auckland, New Zealand) at 10 mg/kg. Bats then received 10 mg/kg oral vitamin K solution twice a day, prepared by a compounding pharmacist. Treatment with vitamin K continued for 34 days. General rehabilitation continued for a further 3 wk.

2.4 Results

2.4.1 Mortality, morbidity, and response to treatment

We collected 118 affected bats from two roost sites over 29 days (Figure 2.1). Recovery of dead and moribund adults (*n*=47 and 8, respectively) and nonvolant pups (*n*=43 dead and 20 moribund) included both sexes (*n*=39 males, 29 females and 50 unknown). Most affected bats were located on the ground within approximately 3 m of the base of the maternity roost tree. One moribund and two dead adult bats were located at the base of a colonial roost tree 65 m east of the maternity roost. The maternity roost remained active throughout the monitoring period. No mortalities or injured bats were detected at an additional eight known maternity and colonial roosts, and no new colonial or maternity roosts were identified through radio-tracking of eight tagged adult bats.

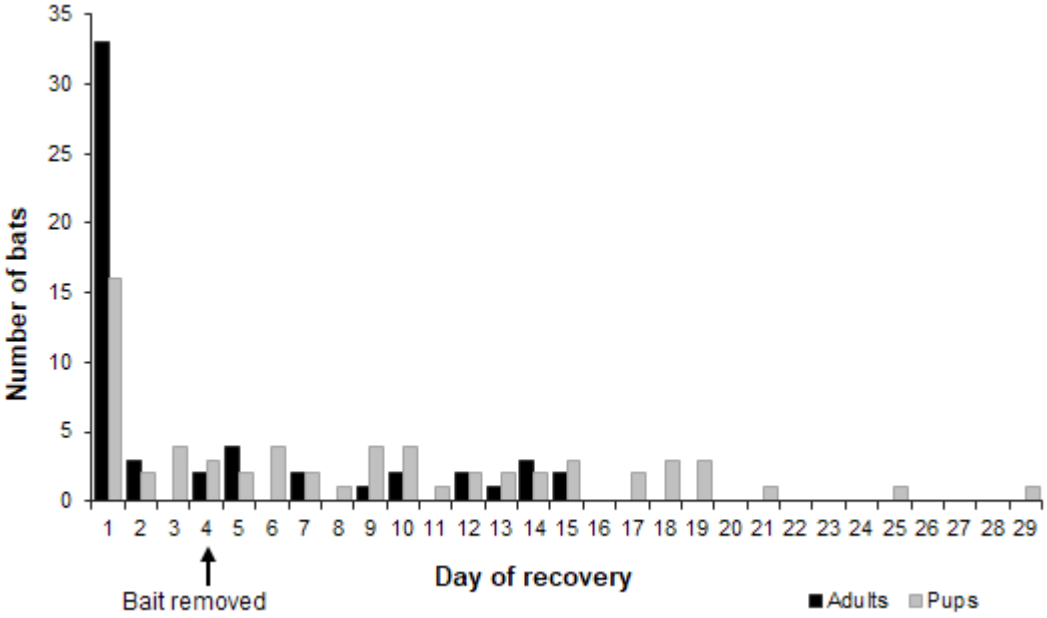

Figure 2.1 Numbers of dead and moribund lesser short-tailed bat (*Mystacina tuberculata*) adults and pups recovered each day over a 29-day period from beneath an active maternity roost tree and a nearby colonial roost tree, in Pureora Forest Park, New Zealand, during a large mortality event in 2009. The large number of bats recovered on day one reflects an accumulation of bat bodies over an unknown period of time prior to discovery. Intensive monitoring of the affected roost trees continued for five days beyond the last bat recovery on day 29.

Affected bats found alive were lethargic and did not resist handling. Four moribund adults and nine moribund pups died shortly after collection. The remaining four adults and 11 pups found alive were transported to Massey University. Two adults and five pups survived to admission. Three pups were successfully treated and were released at the roost site on 17 March, 2009.

2.4.2 Pathology findings

Dead bats were in various stages of decomposition, ranging from intact to desiccated specimens, indicating that the mortalities had occurred over several days to weeks. Subcutaneous bruising and haemorrhage on the abdomen, neck, thorax, legs and around the bones of the wings was noted on external examination of some of the fresher specimens, particularly on unfurred pups. There was dried blood in the mouth of one dead adult bat and around the anus of another, but no moribund bats exhibited signs of external bleeding.

Eleven adults and sixteen pups were in suitable condition for post-mortem. At post-mortem, one adult and four pups that died shortly after collection were in poor body condition. Two of these pups were severely dehydrated, as evidenced by skin turgor and tacky serosal surfaces. The most frequently observed post-mortem findings were subcutaneous haemorrhages, haemoperitoneum and pulmonary haemorrhage (Table 2.1). Subcutaneous haemorrhages were observed in a variety of locations on both adults and pups. In two adults internal haemorrhaging was severe. There were no skeletal fractures that might indicate trauma as a cause of haemorrhage. One adult and nine pups showed no evidence of internal haemorrhaging. Only one pup showed no evidence of any haemorrhaging. Pallor of the liver was observed in one adult and one pup, and pallor of the lungs in one pup.

Table 2.1 Location and incidence of haemorrhagic lesions in lesser short-tailed bat (*Mystacina tuberculata*) adults and pups with diphacinone toxicity, determined at post-mortem by gross and histological examination, New Zealand, 2009.

Site of haemorrhage	No. of bats	
	Adults (*n*=11)	Pups (*n*=16)
Subcutaneous	4	15
Peritoneal cavity	5	5
Lungs	6	3
Heart	3	0
Skeletal muscle	2	0
Meninges	1	0
Liver	1	0
Perineal region	1	0

The stomachs of six dead adult bats contained pollen and arthropod fragments. None contained evidence of cereal bait consumption. Forty dead pups were in suitable condition for examination of the abdominal organs. Twenty-two pups had an empty stomach, 17 stomachs contained milk, and one contained pollen and arthropod fragments. Six of the milk samples may have been bat milk formula that pups received while in care, and these were excluded from toxicologic analysis. The remaining 11 samples were maternal milk.

2.4.3 Histopathologic examination

Histopathologic findings in five of eight adults and three of seven pups examined were indicative of coagulopathy. Significant lesions in adults included recent haemorrhage in the myocardium and pericardium (*n*=2), in the pleura or alveoli of the lung (*n*=4), in the peritoneum and the serosal surfaces of abdominal viscera (*n*=2), and in the intestinal muscular wall (*n*=1). Significant lesions in pups included atelectasis in some areas of the lung (*n*=1), diffuse moderate accumulations of haemosiderin in the cytoplasm of hepatocytes (*n*=2), free erythrocytes in the stomach lumen (*n*=1), haemorrhage within the lumen of the alveoli (*n*=1), and large recent haemorrhages on the serosa of the kidney (*n*=1).

2.4.4 Microbiology

Ten species of bacteria were cultured from samples of liver, lung, and kidney from short-tailed bat adults (*n*=3) and pups (*n*=1); *Corynebacterium* sp. (*n*=3 bats), *Proteus* sp. (*n*=2), alpha haemolytic *Streptococcus* sp. (*n*=2), non-haemolytic *Streptococcus* sp. (*n*=2), *Micrococcus* sp. (*n*=1), *Staphylococcus aureus* (*n*=1), *Escherichia coli* (*n*=1), *Proteus mirabilis* (*n*=1), *Hafnia alvei* (*n*=1) and *Serratia* sp. (*n*=1). None of the bacteria isolated were considered to be primary pathogens.

2.4.5 Toxicology

Diphacinone was confirmed in liver samples from four of five adults (mean concentration $\pm$ SE; 0.29 ± 0.06 µg/g, *n*=4) and six of seven pups tested (0.32 ± 0.07 µg/g, *n*=6). Concentrations ranged from 0.19 to 0.68 µg/g of liver tissue. Diphacinone was also confirmed in two of the 11 maternal milk samples tested, at concentrations of 0.23 and 0.09 µg/g.

2.5 Discussion

Cause of death

Our investigation confirms a diagnosis of diphacinone-induced coagulopathy of lesser short-tailed bats. Diagnosis was supported by a history of exposure, clinical signs, gross and histologic lesions at post-mortem, toxicologic analysis and response to treatment. For 80 days leading up to the observed mortalities diphacinone-laced baits were distributed

throughout the bats' core roosting habitat. Diphacinone residues were confirmed in liver samples from both adults and pups and in two samples of maternal milk. Severe diffuse haemorrhage, as observed in the bats at the time of post mortem, is characteristic of anticoagulant poisoning (Berny, 2007).

Few of the affected bats which were recovered alive during the mortality event survived for more than a few days after collection. Following ingestion of a lethal dose of an anticoagulant there is a delay of several days until death (five to eight days in rats dosed with 3 mg/kg diphacinone) (Eason & Wickstrom, 2001). It is likely, therefore, that live bats that allowed us to approach and handle them were already severely affected by diphacinone. Furthermore stress, caused by handling and transport, could have exacerbated susceptibility to anticoagulant toxicosis (Robinson *et al.*, 2005).

The cause of mortality of bat pups was diagnosed as a combination of the effects of diphacinone toxicity, exposure, and starvation. The presence of diphacinone in the maternal milk collected from the stomachs of dead pups provided evidence that the poison was passed from lactating adult females to their pups. This route of intoxication with pesticides has been reported for bat pups in the US (e.g. Clark *et al.*, 1978; Clark *et al.*, 1988). The pathway of diphacinone intoxication of the adult bats at Pikiariki, however, is uncertain.

Route of exposure

Two likely routes of exposure of adult bats to diphacinone at Pikiariki are direct consumption of toxic bait or secondary poisoning after eating arthropods that had consumed toxic bait. The absence of bait in the stomachs of examined adult bats does not exclude the possibility of primary poisoning, due to the delayed mode of action of anticoagulants and the rapid gut transit time of microbats (Klite, 1965).

The method of presentation of the diphacinone-laced baits during the mortality event may have increased the potential for bait encounters and consumption by foraging adult bats. Diphacinone was formulated in cereal paste baits which were placed in biodegradable bags stapled to tree trunks, rather than in cereal pellet baits secured in bait stations. Bait acceptance trials indicate that captive lesser short-tailed bats are unlikely to consume the

cereal pellet baits typically used in vertebrate pest control operations (Lloyd, 1994). However, in a separate captive trial lesser short-tailed bats sampled cereal paste baits, similar to the RatAbate paste used at Pikiariki, although it was uncertain whether the quantities consumed were sufficient to put bats at risk of poisoning (Beath *et al.*, 2004).

Consumption of toxic bait by arthropods may have served as a pathway for secondary exposure of lesser short-tailed bats to diphacinone (Lloyd & McQueen, 2000). Pesticide-contaminated prey have been implicated in mortalities of insectivorous bats in the US (Clark *et al.*, 1988; O'Shea & Clark, 2002) and a wide variety of arthropod species have been observed on cereal pellet baits in New Zealand forests (e.g. Sherley *et al.*, 1999; Spurr & Drew, 1999). Residue analysis of arthropods exposed to anticoagulant baits in laboratory and field trials confirm that they may act as vectors of these compounds (Craddock, 2003; Fisher *et al.*, 2007).

Despite evidence to support a secondary route of poisoning, no mortalities have previously been detected in wild lesser short-tailed bat populations monitored through pest control operations using cereal pellet baits laced with anticoagulants, either when aerially broadcasted (Sedgeley & Anderson, 2000) or concealed in bait stations (O'Donnell *et al.*, 2011). Further investigation into the palatability and acceptance of a non-toxic RatAbate paste matrix to lesser short-tailed bats and forest arthropods is required.

Sensitivity to diphacinone

Species differ in their sensitivities to a particular toxicant (Erickson & Urban, 2004), although there may be general trends within animal groups (McIlroy, 1986). The microchiroptera may be relatively more sensitive to diphacinone than most other mammal groups. Vampire bats (*Desmodus rotundus*) are sensitive to diphacinone, and populations in Central and South America are controlled using this toxicant in systemic or dermal applications (Arellano-Sota, 1988). The acute oral median lethal dose (LD_{50}) of diphacinone determined for caged (unexercised) vampire bats is 0.91mg/kg (Thompson *et al.*, 1972) and in active bats may be closer to 0.3 mg/kg (Bullard & Thompson, 1977). Furthermore, LD_{50} figures for acute doses of first-generation anticoagulants are typically higher than multiple doses administered over several consecutive days, suggesting that

the risks associated with the use of these compounds are underestimated (Vyas & Rattner, 2012). The acute oral LD_{50} of diphacinone for lesser short-tailed bats is not known (Fisher & Broome, 2010) and caution must be observed when using data from similar species to predict sensitivity (McIlroy, 1986).

Extent of mortalities

The number of carcasses recovered in Pikiariki is likely to be an underestimate of the total mortality of lesser short-tailed bats resulting from diphacinone intoxication. Carcass counts are unreliable estimators of mortality (Vyas, 1999). The large number of decomposed bodies found on the first day of recovery suggests that deaths had been occurring for several days to weeks. Many adult deaths may have gone undetected between initial deployment of baits in October and the start of our surveillance 80 days later, on January 9. Pups are born in late December and so would have been susceptible to poisoning for a much shorter period, but maternal exposure may have caused prenatal losses through abortion (Robinson *et al.*, 2005). During the interval between death and our searches, carcasses may have decomposed or been removed by scavengers, and sick animals may have been taken by predators. Furthermore, dead and moribund lesser short-tailed bats encountered in our searches were difficult to see due to their colour and small size, and some bats may have been obscured from view by vegetation. An unknown number of bats may have died inside the affected roost trees, at other unidentified roost trees or away from roost sites.

Adult bat deaths were detected for several days following removal of toxic baits from Pikiariki, most likely due to the delayed onset of symptoms in lethally dosed animals. Sublethal intoxication of lactating females, or their eventual death, may account for the extended period of pup deaths observed. The hepatic elimination half-life of diphacinone is three days in female laboratory rats (Fisher *et al.*, 2003). If it is similar in lesser short-tailed bats the clotting mechanism would begin to recover three days after exposure and we would not expect mortalities to continue long after bait removal. However, sub-lethally exposed individuals may have been affected in ways which compromised their survival beyond this period (e.g. Riley *et al.*, 2007; Lemus *et al.*, 2011).

The overall impact of the bat mortalities on the viability of the Pikiariki population is not known. Bats are long-lived species with low reproductive output (Barclay & Harder, 2003). We lack information on population trends and the size of the Pikiariki lesser short-tailed bat population before and after the mortality event. There is a need for baseline data on population size and long-term studies on population dynamics to monitor the population's recovery and viability.

Management implications

These findings illustrate the hazards of diphacinone use to the lesser short-tailed bat. However, the risks to non-target species of anticoagulant rodenticide use should be weighed against the benefits of control of rodent pests (e.g. Pascal *et al.*, 2005). The lesser short-tailed bat, classified as vulnerable by the International Union for the Conservation of Nature (O'Donnell, 2008), is likely to benefit from rodent control (Pryde *et al.*, 2005). Furthermore, the consequences of failing to reduce rodent pest impacts can be extreme, as demonstrated by the probable extinction of the New Zealand greater short-tailed bat (*Mystacina robusta*) (Daniel, 1990; Worthy, 1997; O'Donnell *et al.*, 2010).

While the use of vertebrate pesticides remains a necessity, measures which reduce exposure to, and the adverse effects on non-target species are important (Witmer *et al.*, 2007a; Eason *et al.*, 2010). Determination of the route of exposure of the Pikiariki population of lesser short-tailed bats to diphacinone will help inform bait design and delivery to reduce the risk of further mortalities during future rodent control operations in bat habitat. The effectiveness of any operational changes in minimising risks to bats should be evaluated by appropriate monitoring of bat populations. Until more information is available, we recommend the use of less potent toxicants presented in cereal pellet baits delivered in secure bait stations, to control vertebrate pests in bat habitat.

2.6 Acknowledgements

We thank Department of Conservation staff at Pureora Field Centre, Maniapoto Area Office and Hamilton Conservancy Office for assistance during and following the mortality event. Special thanks to Jo O'Boyle, volunteers Tanja Straka and Kahori Nakagawa, Auckland University staff member Stuart Parsons and students Eru Nathan and Victor Obolonkin for assistance in the field, and to Stuart Parsons and Department of Conservation scientist Colin O'Donnell for advice during the event. Thanks also to Dawne Morton of Manawatu-Wanganui Bird Rescue and volunteers from Tongariro Natural History Society for transport of moribund bats to Massey University. Microbial cultures were performed by New Zealand Veterinary Pathology. Eighty-two lesser short-tailed bat specimens recovered during this study were deposited at the Museum of New Zealand Te Papa Tongerewa, Wellington, and are stored under Accession Lot 3599. GCD was employed by the Department of Conservation during the mortality event and undertook subsequent investigation in partial fulfilment of a Doctoral degree at Massey University, Palmerston North, New Zealand. Funding support was received from the Department of Conservation for diphacinone residue analysis of liver and milk samples. GCD received personal financial support during this study from the Julie Alley Bursary and JP Skipworth (Ecology) Scholarship through Massey University. This manuscript benefited from comments by Doug Armstrong of Massey University, and Colin O'Donnell.

2.7 Appendix 2.1

Statement of Contribution

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

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

Name of Candidate: Gillian Dennis

Name/Title of Principal Supervisor: Brett Gartrell

Name of Published Research Output and full reference:

Dennis, G.C. and Gartrell B. D. (2015) Non-target mortality of New Zealand lesser short-tailed bats (Mystacina tuberculata) caused by diphacinone. Journal of Wildlife Diseases, 51(1):177-186

In which Chapter is the Published Work: Chapter 2

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  GCD managed the field response to the mortality event for the Department of Conservation, extracted gut material from pups for toxicological analysis at post-mortem and researched and wrote the paper with editorial input from BG.

Gillian Dennis Digitally signed by Gillian Dennis Date: 2019.07.31 09:15:06 +12'00'
Candidate's Signature

31.7.19
Date

Brett Gartrell Digitally signed by Brett Gartrell Date: 2019.07.31 09:33:01 +12'00'
Principal Supervisor's signature

31/7/2019
Date

GRS Version 3– 16 September 2011

Chapter 3

Non-target anticoagulant poisoning of New Zealand lesser short-tailed bats (*Mystacina tuberculata*) is most likely due to secondary exposure rather than direct consumption of baits

A lesser short-tailed bat eating a wētā (Orthoptera) in the entrance to a singing roost. Photo courtesy of David Mudge, Nga Manu Images.

3.1 Abstract

Toxic baits containing anticoagulant rodenticides are widely used in New Zealand to control introduced mammalian pests on conservation land. Non-target wildlife may be at risk of primary or secondary poisoning, particularly species that are likely to consume toxic bait or to prey on or scavenge other species that have consumed bait. The endemic New Zealand lesser short-tailed bat (*Mystacina tuberculata*) often forages on the ground and has a broad diet, unusual traits for a bat that may place it at risk of primary or secondary poisoning when toxic baits are laid on or near the ground in its habitat. In 2009, at least 115 lesser short-tailed bats died from diphacinone exposure during a rodent control operation using baits containing this first-generation anticoagulant rodenticide in Pikiariki Ecological Area, Pureora Forest Park, New Zealand. Reducing the risk of further such mortalities occurring requires determining the route of exposure of the bats to diphacinone. I therefore used infrared cameras to record whether the bats consumed similar non-toxic bait in captive and wild settings, and to record whether the bait was consumed in the wild by arthropods known to be prey items for the bats. Ten captive bats were observed at two feed stations containing the non-toxic bait and their normal captive diet during 264 10-minute periods sampled over 12 nights, and no bat consumed the non-toxic bait. No free-living bat visited non-toxic baits during 252 h recorded at 12 sites observed for three nights each in Pikiariki Ecological Area. Arthropods visited non-toxic baits at all 12 wild sites and were observed on baits in 45% of 263 10-minute periods sampled. I conclude that lesser short-tailed bats are more at risk of poisoning by secondary ingestion of anticoagulant rodenticides in contaminated arthropod prey, than through direct ingestion of toxic bait.

Keywords: Arthropods, bait station, bykill, Chiroptera, diphacinone, insectivore, Mystacinidae, non-target mortality, Orthoptera, pest control, wētā.

3.2 Introduction

An unintended consequence of the widespread use of anticoagulant rodenticides is the exposure of non-target wildlife to these poisons. Wildlife surveillance has established the presence of anticoagulant residues in a wide range of non-target species in many countries, including New Zealand (Stone *et al.*, 1999; Eason *et al.*, 2002; Berny, 2007; López-Perea *et al.*, 2015). Anticoagulant rodenticide use in New Zealand includes broad-scale field applications to eradicate or suppress populations of introduced mammalian pests that threaten native ecosystems (Eason *et al.*, 2002). Consequently, exposure to anticoagulants has been reported for many of New Zealand's native and endemic species, including some that are rare and threatened (Hoare & Hare, 2006).

Anticoagulants are toxic to all vertebrates and exposure to these compounds can be lethal for non-target wildlife (e.g. Sánchez-Barbudo *et al.*, 2012). Even sublethal exposure can ultimately be fatal by predisposing animals to death from other causes such as disease (Lemus *et al.*, 2011) or predation (Cox & Smith, 1992; Vyas *et al.*, 2012). Non-target deaths are undesirable, particularly when effects on individuals combine to produce population-level impacts that outweigh the benefits of pest management (Innes & Barker, 1999; Eason *et al.*, 2002). The refinement of pest control operations to minimise the exposure of non-target wildlife to anticoagulant poisons is therefore essential (Witmer *et al.*, 2007), and can be informed by determining the route of exposure of affected species.

Exposure, the likelihood of an animal encountering and ingesting poison in some form, is an important component of poisoning risk (Eason & Wickstrom, 2001). Non-target wildlife can be exposed to poisons directly, by eating toxic bait (primary exposure), or indirectly, by preying on or scavenging the carcasses of animals that have themselves eaten toxic bait or contaminated prey (secondary or tertiary exposure). Primary and secondary non-target poisoning is most commonly reported for second-generation anticoagulant rodenticides (e.g. brodifacoum, bromadiolone), because they are highly toxic to a wide range of vertebrates, and persist and accumulate in animal tissue (Erickson & Urban, 2004). However, non-target mortalities also occur with less potent and less persistent first-generation compounds (e.g. diphacinone, chlorophacinone) (Riley *et al.*, 2007; Rattner *et al.*, 2012).

Primary exposure occurs when toxic baits are accessible to and attractive to non-target animals (Brakes & Smith, 2005; Pryde *et al.*, 2012). Bait matrices are typically formulated to be attractive to target pest species (Morriss *et al.*, 2008), with colours and flavours added to deter non-target species. In New Zealand, such modifications are generally directed at discouraging birds (e.g. Hartley *et al.*, 2000), because the only native terrestrial mammals are two species of bats (King, 2005). Indirect exposure is most commonly reported for predatory and scavenging birds and mammals (Howald *et al.*, 1999; Albert *et al.*, 2010; Elmeros *et al.*, 2011), and is more difficult to prevent (Buckle & Prescott, 2018).

Invertebrates can serve as a pathway of indirect exposure of non-target wildlife to anticoagulant poisons, in particular for ground-feeding insectivorous and omnivorous mammals and birds. A variety of invertebrate species, particularly arthropods, have been observed feeding on cereal baits that are used as matrices for anticoagulants or other vertebrate pesticides that are used to manage conservation pests in New Zealand (Sherley *et al.*, 1999; Spurr & Drew, 1999; Lloyd & McQueen, 2000; Wakelin, 2000; Spurr & Berben, 2004; Bowie & Ross, 2006). Arthropods may also be exposed to anticoagulants by ingesting contaminated rodent faeces (Craddock, 2003; Fisher, 2009), soil (Booth *et al.*, 2003), or tissue from carcasses (Howald, 1997) (Figure 3.1). Arthropod-mediated exposure to anticoagulants has been suspected in the mortality of non-target bird and mammal species in New Zealand and internationally (Dowding *et al.*, 1999; Dowding *et al.*, 2010), and dietary exposure to other pesticides has been implicated in mortalities in several species of insectivorous bats in the United States (O'Shea & Clark, 2002).

The lesser short-tailed bat (*Mystacina tuberculata*), one of New Zealand's two extant endemic bat species, has been considered at risk of primary or secondary exposure to anticoagulant rodenticides and other vertebrate pesticides because of its unusual diet and foraging behaviour (Eason & Spurr, 1995). These forest-dwelling bats are opportunistic foragers that have a broad diet that includes arthropods (c. 60%), fruit, nectar and pollen (Daniel, 1979; Arkins *et al.*, 1999). They are adapted to terrestrial locomotion and are adept at foraging on the ground and on the branches and trunks of trees (Daniel, 1976; Jones *et al.*, 2003; McCartney *et al.*, 2007), where poison baits are typically laid in aerial broadcast or bait station operations. Lesser short-tailed bats have therefore been

considered susceptible to primary exposure to toxic baits or to secondary exposure to poisons through consumption of contaminated arthropods (Figure 3.1). Furthermore, some bat species may be sensitive to anticoagulant poisons (Thompson *et al.*, 1972a).

These concerns were realised in January 2009 with the deaths of at least 115 lesser short-tailed bats in Pikiariki Ecological Area (hereafter Pikiariki) in Pureora Forest Park, New Zealand, during a rodent control operation using cereal paste baits laced with the first-generation anticoagulant diphacinone. Post-mortem examination confirmed diphacinone intoxication of adults and indirect exposure of nursing pups through contaminated maternal milk (Dennis & Gartrell, 2015; Chapter 2). The route of exposure of the adult bats to diphacinone is uncertain; baits were placed in single-use biodegradable bags that were attached to tree trunks, with potential for spillage and access by both bats and forest arthropods. Early reports that New Zealand short-tailed bats consume bird flesh have never been substantiated (Stead, 1936; Blanchard, 1992; McCartney *et al.*, 2007), so it is unlikely that the bats were exposed directly through scavenging poisoned carcasses.

Previous studies with captive and wild lesser short-tailed bats have indicated that these bats are unlikely to directly consume the cereal pellet baits that are typically used in pest control operations in New Zealand (Lloyd, 1994; Sedgeley & Anderson, 2000). In contrast, in a separate trial, wild-caught captive lesser short-tailed bats showed interest in a range of baits, including one similar to the toxic paste in use when the bat mortalities occurred at Pikiariki. However, there was not conclusive evidence that the bats in the trial ate quantities that would put them at risk of poisoning. The same study also found that the bats, given a choice of 10 scented bait lures, showed a preference for a peanut-scented lure, although considerably more visits were made to the honey-water control (Beath *et al.*, 2004). Given the subsequent fatal exposure of wild lesser short-tailed bats to anticoagulant rodenticides in Pikiariki, it is important to assess their attraction to the peanut-lured bait matrix used at the time of the mortalities.

In this study, I investigated two potential routes of exposure of lesser short-tailed bats to anticoagulant rodenticides by observing bats and arthropods interacting with a non-toxic version of the cereal paste baits that were used to deliver diphacinone during the 2009 bat mortalities. I conducted field observations at non-toxic baits laid in bat habitat in Pikiariki

to determine whether the baits were visited by free-living lesser short-tailed bats and/or forest-dwelling arthropods. I also conducted two-choice trials to assess whether captive lesser short-tailed bats would directly eat the non-toxic bait in the presence of their normal captive diet. Information about the route of exposure of bats to anticoagulant rodenticides will be valuable in guiding further research and in aiding conservation managers to make appropriate adjustments to pest control operations in bat habitat to reduce the risk of further bat mortalities.

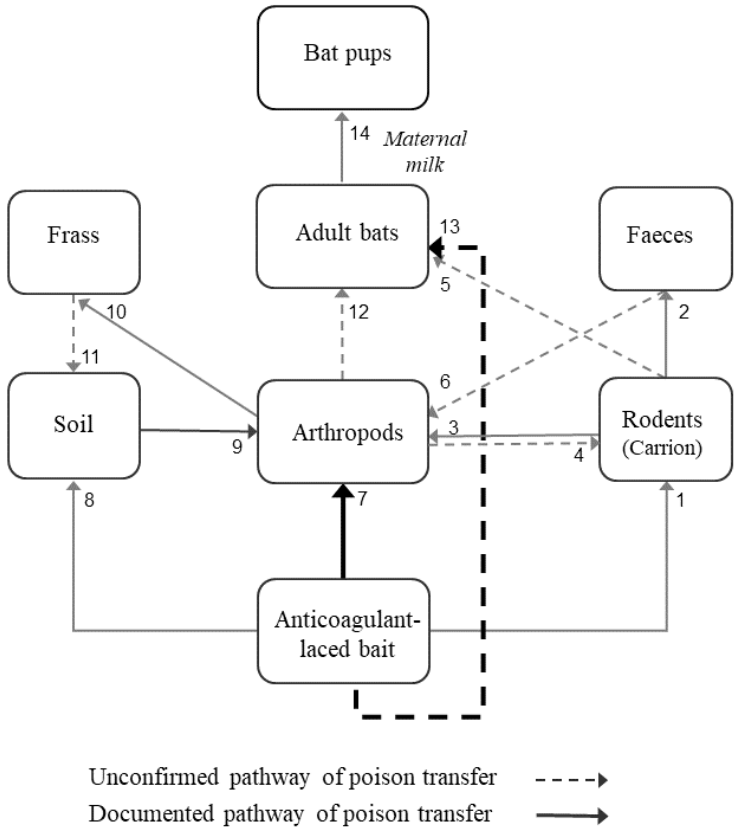

Figure 3.1 Potential routes of exposure of New Zealand lesser short-tailed bats (*Mystacina tuberculata*) to anticoagulant rodenticides presented in baits laid for mammalian pest control in bat habitat. Unconfirmed pathways are those that have been proposed, based on known food web relationships, but have not been tested. Dark lines indicate the two routes of exposure investigated in this study using a non-toxic cereal paste bait matrix. 1.(Buckle, 1994); 2. (Fisher, 2009); 3. (Howald, 1997); 4. (Innes, 2001); 5. (Stead, 1936); 6.& 7. (Craddock, 2003); 8. (Pitt *et al.*, 2015) 9. (Booth *et al.*, 2003); 10. (Hernandez-Moreno *et al.*, 2013); 11. (Fisher *et al.*, 2007); 12. & 13. (Eason & Spurr, 1995); 14. (Dennis & Gartrell, 2015).

3.3 Methods

3.3.1 Field trial

Study site

The field trial was conducted in Pikiariki Ecological Area (Pikiariki), a native forest remnant (457 ha), and contiguous areas of mature exotic conifer plantation forest within 78,000 ha Pureora Forest Park, North Island, New Zealand (38°31'S, 175°34'E) (Figure 3.2). Pikiariki is the only known location within Pureora Forest Park providing maternity and colonial roost tree habitat for a population of lesser short-tailed bats. The old-growth native podocarp-hardwood forest in Pikiariki is dominated by tawa (*Belschmeidia tawa*), matai (*Prumnopitys taxifolia*) and rimu (*Dacrydium cupressinum*). Unsealed roads border the forest and fragment the remnant in to three smaller blocks. The neighbouring land, once forested, now supports dairy pasture and mature stands and clear-felled compartments of commercial timber species including Douglas fir (*Pseudotsuga menziesii*) and radiata pine (*Pinus radiata*). Pikiariki was selectively logged until 1978 (King & Gaukrodger, 2015) but is now designated as an Ecological Area in recognition of its high conservation values (Norton & Overmars, 2012). The Department of Conservation manages the site and regularly undertakes pest control operations using anticoagulant poisons to target rodents (*Rattus* spp.) to enhance native wildlife populations and forest health.

Data collection

I used infrared cameras to record night-time activity of arthropods and bats at non-toxic baits at 12 sites in Pikiariki and contiguous areas of mature exotic conifer plantation forest (Figure 3.3). Three sites were surveyed simultaneously over three nights in one of four survey periods between 23 December 2009 and 16 January 2010. This corresponded broadly to the period when lesser short-tailed bat mortalities were observed in Pikiariki in January 2009 (Dennis & Gartrell, 2015). The non-toxic baits (Ferafeed, Connovation, Auckland, New Zealand) were green-dyed cereal paste baits with a peanut flavouring. This matrix was a non-toxic version of the diphacinone-laced bait matrix that was used for the rodent control operation at the time of the 2009 bat mortalities. Filming was conducted using custom-made CCTV digital recording systems designed for field use (Department of Conservation, Wellington, New Zealand). Each recording system employed a black and

white security camera in a waterproof housing, with 940 nm infrared LEDs for lighting. Each camera was connected by a cable to a SecuMate mini portable security recorder (SecuMate, Middleburg, The Netherlands) inside a waterproof case. The equipment was powered by 12 V 12 Ah rechargeable lead acid batteries. Recorded information was stored on secure digital (SD) cards.

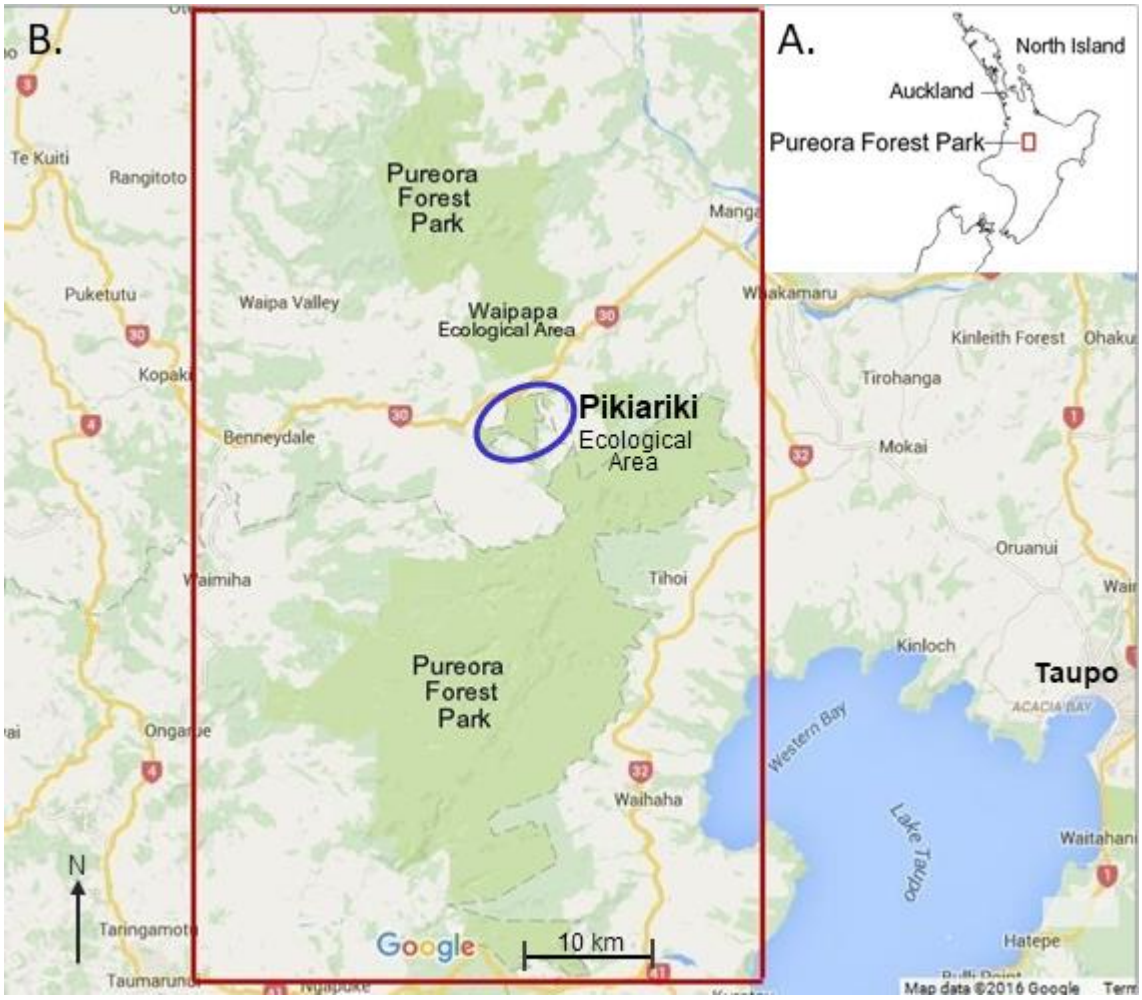

Figure 3.2 A. (Inset map) Location of Pureora Forest Park in the central North Island, New Zealand, and **B.** Location of Pikiariki Ecological Area within Pureora Forest Park.

I selected the 12 survey sites using systematic random sampling, based on a series of established bait lines with numbered permanent bait stations on a 150 m x 150 m grid throughout Pikiariki's native forest and contiguous areas of mature conifer plantation. Bait lines at the north east and south west extremes of the managed block were excluded from consideration to concentrate observations in the core of the remnant where bat activity or roosts had previously been detected (Wallace, 2006; Dennis, 2008). A single

bait line was randomly selected, then a further 11 bait lines were selected systematically from this starting point, resulting in four evenly spaced sets of three alternate bait lines. I allocated one site to each of the 12 chosen bait lines by randomly selecting a numbered bait station on each line. The four sets of lines corresponded to the four survey periods (S1-S4). The three points selected in each set of alternate lines corresponded to the sites surveyed simultaneously (Figure 3.3).

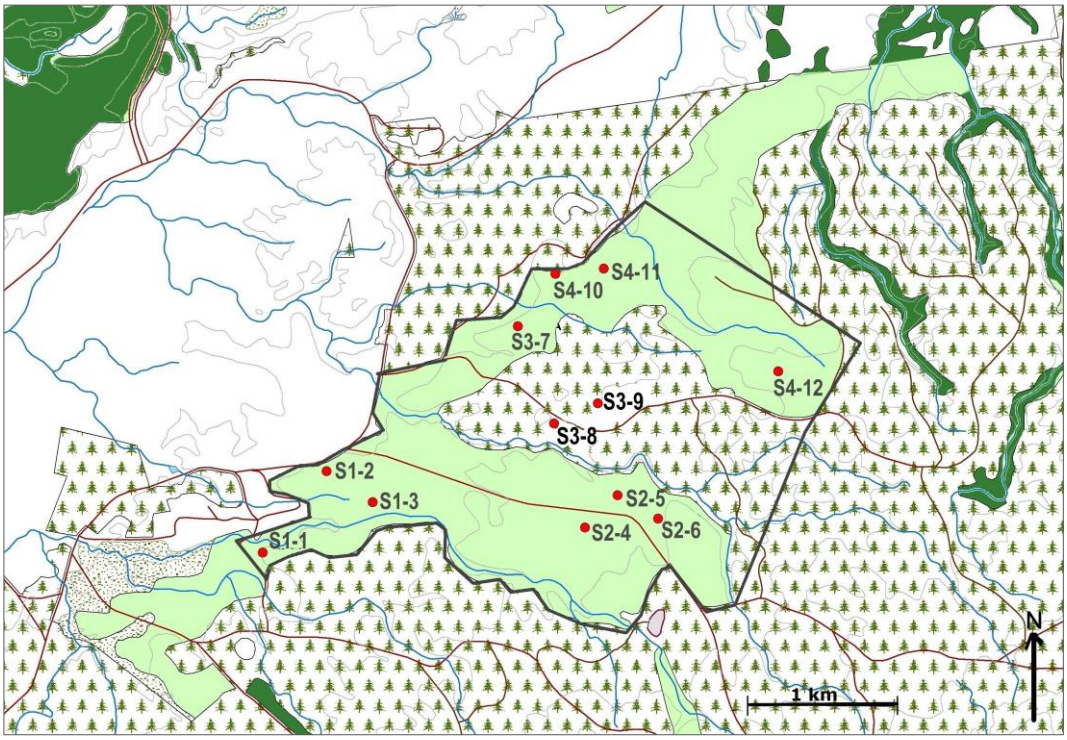

Figure 3.3 Pikiariki Ecological Area (light green), showing the area considered for the field trial (black boundary), and the 12 sites (1-12) where night-time activity at non-toxic baits was filmed during four survey periods (S1-S4). (White = dairy pasture; Δ = mature or clear-cut exotic conifer plantation; dark green = other native vegetation; brown lines = unsealed roads).

At each of the 12 sites, I placed 100 g of non-toxic bait at a point between 50–100 m from the selected bait station in surroundings that could be categorised as either ‘good’ bat habitat or ‘poor’ bat habitat. ‘Good’ habitats were sites within mature native forest where lesser short-tailed bats might be expected to forage. These sites were at least 200 m from the forest edge, with a closed canopy, dense leaf litter and a relatively uncluttered understorey, and. in some cases were near streams. ‘Poor’ habitats were sites where it was expected the bats might be less likely to forage. These were sites within mature native forest with a densely cluttered understorey and ground cover, in large tree-fall gaps, or

within exotic plantation forest (Grindal *et al.*, 1999; O'Donnell *et al.*, 1999; O'Donnell *et al.*, 2006; Racey, 2007). Sampling 'poor' habitat allowed for the possibility that bats might encounter and opportunistically investigate baits while engaged in non-foraging activities, such as commuting or night roosting. This approach compensated for the lack of information available at the time of the study about preferred foraging habitats in this area. At each site, I positioned a non-toxic bait on either an elevated surface (such as the trunk of a standing or fallen tree) or on the ground. This was to simulate patterns of operational use and bait dispersal at the time of the 2009 bat mortalities (Figure 3.4A–D). Non-toxic bait was placed on the ground at four 'good' and three 'poor' habitat sites, and in an elevated position at four 'good' and one 'poor' habitat site (Table 3.1).

Figure 3.4 A. A biodegradable bag containing diphacinone-laced cereal paste bait for rodent control nailed to a tree trunk in Pikiariki Ecological Area, Pureora Forest Park, New Zealand, January 2009. **B.** An empty bait bag. Bait has spilled on to the ground below. **C.** An infrared camera at a non-toxic paste bait on the ground, and **D**. on a tree trunk in Pikiariki, to record night-time visits by lesser short-tailed bats (*Mystacina tuberculata*) and forest arthropods, 23 December 2009 - 16 January 2010.

Table 3.1 Habitat type and placement of non-toxic baits at 12 survey sites in native forest in Pikiariki Ecological Area and adjacent exotic conifer plantation forest, Pureora Forest Park, New Zealand, 23 December 2009 – 16 January 2010.

Habitat Type	Bait on Ground	Description	Bait Elevated	Description
GOOD	Site 1	Mature native forest Open understorey Scattered ground cover 50 m from stream	Site 2	Mature native forest Open understorey Bait in tree fork at c. 1 m
	Site 4	Mature native forest Open understorey Scattered ground cover 15 m from stream	Site 5	Mature native forest Open understorey 20 m from stream Bait on tree trunk at c. 1 m
	Site 7	Mature native forest Open understorey Light ground cover of ferns	Site 10	Native transition (edge) forest Bat-pollinated *Dactylanthus taylorii* nearby but not in flower Bait on tree stump at c. 0.5 m
	Site 11	Mature native forest Open understorey Sparse ground cover	Site 12	Mature native forest Open understorey Bait on trunk of fallen tree at c. 1 m
POOR	Site 3	Large tree fall gap with cluttered boundary within mature native forest	Site 9	Exotic conifer plantation Densely cluttered regenerating native understorey c. 2 m high Bait on trunk of conifer at c. 1 m
	Site 6	Mature native forest Densely cluttered understorey and ground vegetation		
	Site 8	Exotic conifer plantation Open sub-canopy Small clearing in c. 2 m high regenerating native understorey Bait at base of conifer		

Cameras placed at non-toxic baits were programmed to record between 21:15 and 05:45 to coincide with the nocturnal activity patterns of lesser short-tailed bats (Christie, 2006). I placed a custom-made automatic bat detector (ABD) with two channels of heterodyned recording (28 kHz and 40 kHz) (Department of Conservation, Wellington, New Zealand) within 10 m of each camera to digitally record lesser short-tailed bat echolocation calls up to a maximum distance of approximately 30 m (S. Cockburn, pers. comm.). ABDs were deployed for one night prior to filming and for the subsequent three nights on which filming occurred at each site and were programmed to operate on the same schedule as the cameras. Although lesser short-tailed bat activity was not limited by rain in a Fiordland study (Christie & Simpson, 2006), filming was restricted to nights without rain to avoid compromising image clarity. At the end of each night of filming I downloaded SD cards, recharged and replaced batteries, and replenished bait if necessary.

Field trial data review

Camera recordings from the 12 survey sites were reviewed to determine activity at non-toxic baits. I viewed recordings in entirety at accelerated playback speeds to ascertain the number of lesser short-tailed bats that visited baits. I also reviewed one 10-minute period from each hour that bait was present on screen to record visits by wētā (Order Orthoptera), other forest arthropods and introduced mammals. A visit was defined as an animal making any contact with the bait. A pilot night of filming indicated that it was not going to be possible to accurately record the number of small arthropods visiting baits because numerous individuals visited simultaneously and moved in and out of view behind or beneath the bait. Nor was it going to be possible to determine whether small arthropods on the bait were eating it. I therefore reported the number of hourly 10-minute periods when at least one individual in a particular category (wētā, other arthropod or pest mammal) visited the bait. For each wētā recorded in contact with the bait during one of the sampled 10-minute periods, I also noted the start and end times of the entire visit. The starting time of the hourly 10-minute periods was selected at random.

I analysed recordings from ABDs using BatSearch 1.02 Software (Department of Conservation, Wellington, New Zealand) to determine the number of lesser short-tailed bat passes that were detected at each survey site per night. This provided a relative measure of bat activity in the vicinity of each bait, as there is no way to correlate the number of passes recorded with the number of individual bats present (Zabel & Seidman, 2001). A bat pass was defined as a sequence of two or more echolocation calls (recorded as audible clicks) with a pause of at least one second before the next sequence (Fenton, 1970; Zabel & Seidman, 2001).

3.3.2 Captive trial

Trial set up

A trial to determine the palatability of non-toxic bait to captive lesser short-tailed bats was conducted with 10 adults (seven males, three females) in an off-display enclosure at Auckland Zoo, where the bats had been held communally since September 2007. In a ‘two-choice’ experiment, I offered the bats a choice between their normal captive diet and non-toxic bait (hereafter ‘test bait’) identical to the bait used in the Pikiariki field trial. The choices were offered at two existing feed stations in the bats’ enclosure (Figures

3.5 and 3.6A) for 12 consecutive nights; six nights (period 1: 7-12 October, 2009) using the test bait without modification, and six nights (period 2: 13-18 October, 2009) using the test bait dyed with rhodamine B (0.3% w/v, Sigma-Aldrich N.Z. Ltd, Auckland). The dyed bait was trialled to assess the potential of using marked non-toxic baits to detect bait take by bats in the field. The low concentration of rhodamine B was not expected to alter palatability of the bait, as this has only been reported for concentrations of rhodamine B greater than 1% (Fisher, 1999). The bats' normal captive diet (hereafter 'food') consisted of mealworms (*Tenebrio molitor*) pre-fed on a substrate of bran and Wombaroo Insectivore Mix ™ (Wombaroo Food Products, Adelaide, Australia). Food and water were provided *ad libitum*. The total weight of mealworms offered to the bats collectively each night was determined by zoo staff according to standard procedures.

I placed one portion of test bait on each of the bats' usual feeding trays, in a separate compartment to the bats' food (Figure 3.6B). The test bait and food were presented on the same tray to eliminate any variation due to environmental factors (Prince *et al.*, 2004). The position of the test bait and the food items (i.e. left or right side of the feed station) was alternated on successive nights of the trial to minimise positional preferences (Thompson *et al.*, 1972b). I followed the zoo staff's usual feeding routine for the bats, placing a prepared feeding tray at each of the two feed stations at 16:30, before the bats emerged from their day roosts, and removing the trays the following morning at 08:30, after bats had returned to their day roosts. Two additional control baits on identical feeding trays were kept in rodent-proof conditions in an enclosed access area immediately outside the bats' enclosure to enable correction of test bait weights for natural moisture loss or gain (Fisher *et al.*, 2007). Each test and control bait weighed 300 g ± 1 g (UWGM digital bench scale, Wedderburn, Sydney, Australia) at the start of the first trial period. Each bait was replaced with 300 g of dyed bait at the start of the second trial period.

During the day, all baits were stored together in a rodent-proof location. Individual bat weights were monitored weekly during the trial to fulfil Massey University Animal Ethics Committee requirements to safeguard their well-being. No weight changes of concern were detected in the bats during the trial.

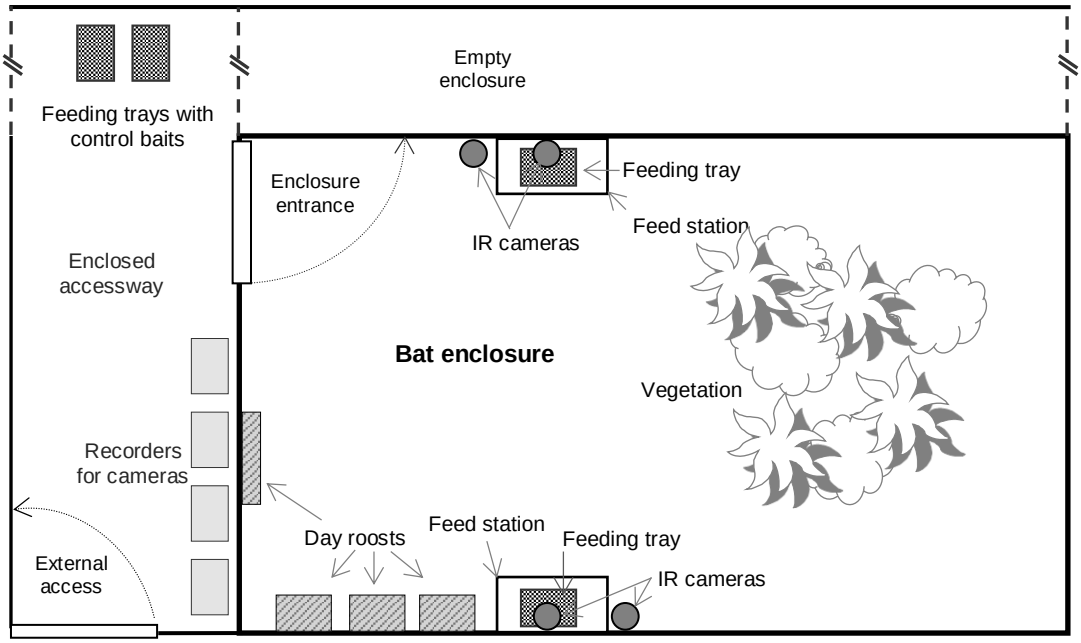

Figure 3.5 A representation of the set-up used in the off-display bat enclosure at Auckland Zoo, New Zealand, where a 'two-choice' experiment was conducted with 10 captive lesser short-tailed bats (*Mystacina tuberculata*) in October 2009 to test whether they would eat non-toxic bait in the presence of their normal captive diet.

Figure 3.6 A. One of the two usual feed stations (indicated by red arrow) within the lesser short-tailed bat (*Mystacina tuberculata*) off-display enclosure at Auckland Zoo, New Zealand, October 2009. **B**. Presentation of non-toxic test bait (right), food (mealworms, left front) and water (with rock, left rear) in a compartmentalised tray placed on a feed station. **C.** Two infrared cameras installed at a feed station. **D.** Cavities in a test bait where slugs (Order Pulmonata) were seen feeding the previous night.

Captive trial data collection, review, and analysis

I installed two infrared cameras (specifications as for field trial) at each feed station to record the feeding behaviour of the bats. One camera was placed above and the other to the side of each station (Figure 3.6C). Cables connected cameras to four recording devices (specifications as for field trial), placed outside the bat enclosure, that were programmed to record between 19:45 and 07:00 on each night of the trial.

I monitored changes in the weights of test and control baits after each night of the trial by subtracting the weight of a bait at 09:00 from its weight at 16:00 the previous afternoon. Corrected weights for test baits were calculated by adding the mean percentage overnight weight change of the two control baits to the starting weight of each test bait on the corresponding night (Fisher *et al.*, 2007). Test bait palatability was expressed as the total weight (grams) of test bait eaten divided by the total weight (grams) of normal food eaten by the bats collectively (Johnson & Prescott, 1994). Test baits were visually examined each morning for signs of feeding by bats.

For each night of the trial, I reviewed one 10-minute period per recorded hour from the overhead camera at each feed station. The starting time for the hourly 10-minute periods was selected at random. Bats could not be marked for individual identification, so for each of the sampled 10-minute periods I noted the total number of visits made by the bats collectively to the test bait and did the same for visits made to their food. All visits that started within a sampled 10-minute period were included in the count. A visit was defined as an animal entering the feeding tray compartment (partially or wholly) where the test bait or food was located. To assess whether my sampling regime captured bat feeding behaviour that was typical of the whole night, I also reviewed the overhead recordings for the entire first night, and again separately noted the total number of visits made by the bats collectively to either the test bait, or to food.

I recorded the duration, rounded up to the nearest second, of each visit made by a bat to the test bait or food during the sampled 10-minute periods. The mean duration (s ± SE) of visits to each food type was then calculated for the 10 bats collectively for the 12 nights of the combined trial periods. I also noted whether bats that entered the bait compartment appeared to consume any test bait, or whether a part of their body definitely, or possibly

came in to contact with the test bait. Reduced playback speeds and footage from side cameras were used to provide further clarity when necessary. Visits by free-living arthropods from the bat enclosure to the test bait compartment were also noted during each sampled 10-minute period.

I performed a chi-square goodness of fit test in Programme R (Version 3.1.1) to assess the suitability of the sampling regime. This tested whether the proportion of visits to test bait made during the 10-minute periods sampled on the first night differed significantly from the proportion of visits to bait made during the entire first night. Data from both feed stations was combined for this analysis to meet the conditions of the statistical test. I then used a chi-square test to determine whether the total proportion of visits by bats to test bait made during the sampled 10-minute periods for the 12 nights of the trial were independent of feed station (station 1 and station 2) and trial period (period 1: nights 1-6 and period 2: nights 7-12). Finally, I used a chi-square test to determine whether there was a statistical difference between the proportion of total visits to the test bait and the proportion of total visits to the food made during the sampled 10-minute periods for the 12 nights of the trial.

3.4 Results

3.4.1 Field trial

No lesser short-tailed bats were observed visiting non-toxic bait at any of the 12 surveyed sites during the 263 10-minute periods reviewed, nor during any of the remaining time while baits were present on screen. Arthropods, however, were frequent visitors to the baits (Figure 3.7). Recordings to assess visits were obtained on three nights at 11 sites, and on two nights at one site, providing the equivalent of 35 nights of recordings. The total time recorded across all sites was 279 h, out of a scheduled 306 h. This was because on several nights recording ceased before the programmed time due to equipment failure or flat batteries, and at one site the data recorded on one night was corrupted. Furthermore, on some nights the bait was completely eaten or removed by introduced mammals before recording ended. Therefore, bat visits to bait were reviewed for the 252 h when bait was present on screen. This included 263 hourly 10-minute periods, when visits to bait by arthropods and pest mammals were also noted.

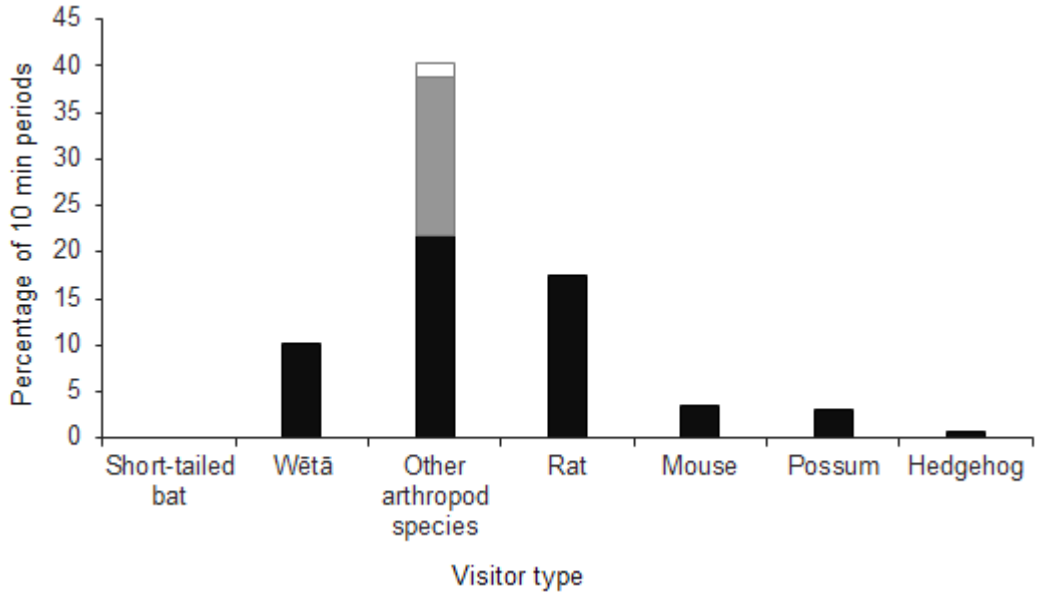

Figure 3.7 Percentage of 10-minute periods (*n*=263) when visits to non-toxic baits by lesser short-tailed bats (*Mystacina tuberculata*), wētā (Order Orthoptera), other arthropod species, rats (*Rattus* spp.), possums (*Trichosurus vulpecula*), mice (*Mus musculus*) and hedgehogs (*Erinaceus europaeus*) were observed at 12 sites surveyed on the equivalent of 35 nights using infrared cameras in Pikiariki Ecological Area, Pureora Forest Park, New Zealand, 23 December 2009 - 16 January 2010. 'Other arthropod species' present on bait were further classified according to their predominant mode of locomotion as either crawling (black shading) or flying (grey shading). White shading indicates that both crawling and flying arthropods were present during the same 10-minute period.

Although no bats were observed visiting non-toxic baits, automatic bat detectors (ABDs) confirmed bat activity in the vicinity at 10 of the 12 survey sites (Figure 3.8). There was a high level of activity detected at two sites (sites 7 and 8), but the mean number of bat passes per night was fewer than 20 at each of the other sites. No bat passes were detected at one 'good' habitat site (site 2: mature native forest with open understorey) where rat vocalisations dominated ABD recordings, and at one 'poor' habitat site (site 9: exotic conifer plantation with dense regenerating native understorey) (Table 3.1). Surveys with ABDs were limited to 40 of the 48 night-equivalents of planned deployment, due to occasional equipment failure. This included seven of the 12 nights before baits and cameras were placed at sites, and 33 of the 36 nights during filming at baits. Bat passes were recorded at 10 sites on 31 of the 40 nights of successful ABD deployment, including six nights before and 25 nights during filming at baits (Tables 3.2A and B).

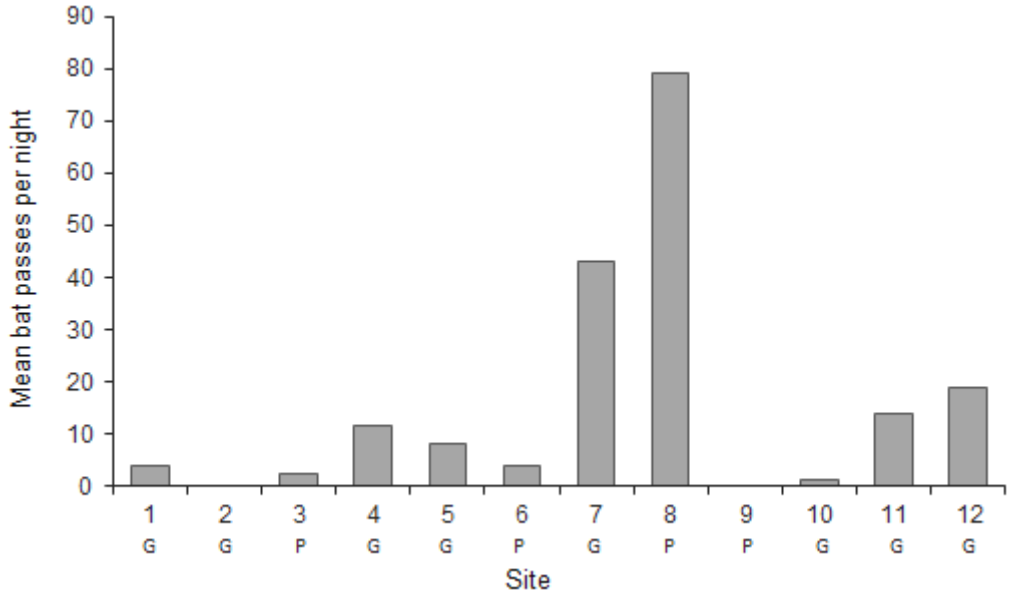

Figure 3.8 Mean lesser short-tailed bat (*Mystacina tuberculata*) passes per night detected using Automatic Bat Detectors (ABDs) at 12 sites in Pikiariki Ecological Area and adjacent exotic conifer plantation forest in Pureora Forest Park, New Zealand, one night prior to filming and three nights during filming at non-toxic baits, 23 December 2009 - 16 January 2010. (Number of nights equipment functioned at sites 1 & 4-9 *n*=4; sites 2, 3 & 12 *n*=3; site 10 *n*=2; site 11 *n*=1). Sites were expected to be good (G) or poor (P) bat foraging habitat.

Arthropods were observed visiting non-toxic baits at all 12 surveyed sites (Figure 3.9). Visits occurred on 32 nights during 118 (45%) of the hourly 10-minute periods sampled from recordings (*n*=263). This included visits by flying and crawling arthropods other than wētā at all 12 sites on 30 nights during 106 (40.3%) of the 10-minute periods sampled (Figures 3.7). This is most likely a conservative figure as some 10-minute periods may have included visits by small arthropods that were not visible on camera (e.g. underneath the bait). I was unable to consistently identify arthropod taxa that visited the baits (other than wētā), due to their small size (typically less than c. 1 cm long) and the poor resolution of recorded images. Glare from the infrared lights on the surface of the bait also reduced image clarity. Occasionally it was possible to recognise ants (Hymenoptera), cockroaches (Blattodea), harvestmen (Opiliones), beetles (Coleoptera), flies (Diptera) and spiders (Araneae) among the arthropods that visited baits.

Large- and small-bodied wētā were observed visiting baits at nine of the 12 sites (Figure 3.9). Visits occurred on 15 nights during 27 (10.3%) of the 10-minute periods sampled (*n*=263, Figures 3.7 and 3.10). At site 11, more than one wētā was observed in contact

with the bait simultaneously on three occasions. The mean bait contact time per individual wētā visit, within the sampled periods, was 4.9 min (± SE, range: ± 0.56 min, 0.1 – 10 min, *n*=31). When the boundaries of the sampled 10-minute periods were ignored, the mean bait contact time of these wētā, based on the entire length of each visit, was 16.8 min (± 2.3 min, 0.17 – 53.75 min, *n*=31).

Table 3.2 A. Number of night-equivalents of attempted surveys, successful surveys, and bat detections, and **B.** sites where lesser short-tailed bat (*Mystacina tuberculata*) passes were detected using Automatic Bat Detectors (ABDs) in Pikiariki Ecological Area and adjacent exotic conifer plantation forest in Pureora Forest Park, New Zealand, one night prior to filming (-1) and three nights during filming (1, 2, 3) at non-toxic baits placed in 'good' and 'poor' bat foraging habitats, 23 December 2009 - 16 January 2010. ✓ = Bats detected; × = Bats not detected; o = ABD faulty, not deployed; c = Recorded files corrupted.

A.

	Night-equivalents		
	Total	Prior to filming	During filming
Survey attempted	48	12	36
Survey successful	40	7	33
Bats detected	31	6	25

B.

	Habitat Type							
	'Good' habitat				**'Poor' habitat**			
Survey night	**-1**	**1**	**2**	**3**	**-1**	**1**	**2**	**3**
Site 1	✓	×	×	✓				
Site 2	o	×	×	×				
Site 3					o	✓	✓	✓
Site 4	✓	✓	✓	✓				
Site 5	✓	✓	✓	✓				
Site 6					✓	✓	✓	✓
Site 7	✓	✓	✓	✓				
Site 8					✓	✓	✓	✓
Site 9					×	×	×	×
Site 10	o	✓	✓	o				
Site 11	o	✓	o	c				
Site 12	o	✓	✓	✓				

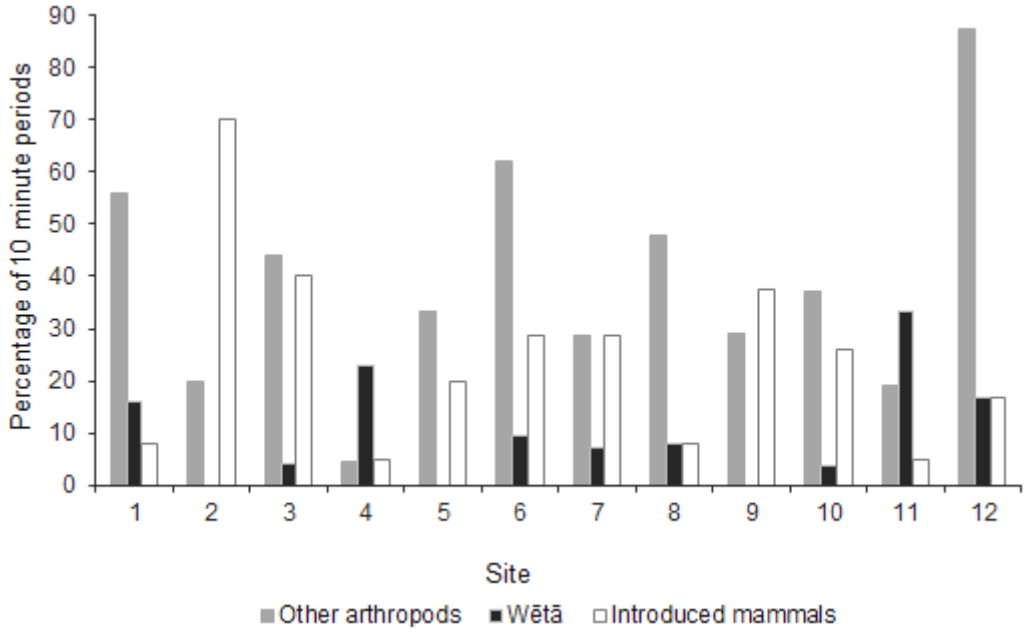

Figure 3.9 Percentage of 10-minute periods (*n*=263) when visits to non-toxic bait by wētā (Order Orthoptera), other forest arthropod species and one or more species of introduced mammal (rats, mice, possums and hedgehogs) were observed at 12 sites surveyed using infrared cameras in Pikiariki Ecological Area, Pureora Forest Park, New Zealand, 23 December 2009 - 16 January 2010. Surveys occurred for three nights at each site except at site 7, where recording equipment failed on one night.

Rats, possums (*Trichosurus vulpecula*), mice (*Mus musculus*) and hedgehogs (*Erinaceus europaeus*) were also observed visiting non-toxic baits. At least one or more of these pest species visited baits at all 12 sites during the sampled periods (Figure 3.9). Rats were the most frequent introduced mammalian pests to visit (Figure 3.7). There was a moderate negative correlation between the percentage of hourly 10-minute periods at each site that included visits to bait by rats, possums or mice, and the percentage that included wētā visits (r=–0.68, *n*=12 sites). On several occasions wētā were observed to leave the bait rapidly several seconds before the arrival of a pest mammal.

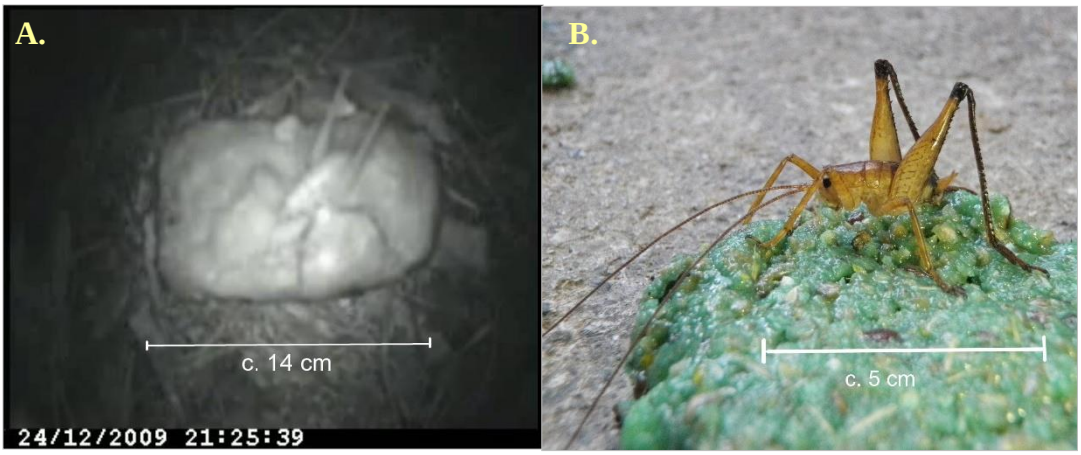

Figure 3.10 A. Infrared image of a large-bodied cave wētā (Family Rhaphidophoridae) feeding on non-toxic bait in the forest in Pikiariki Ecological Area, Pureora Forest Park, New Zealand, December 2009. **B.** A small-bodied cave wētā observed eating non-toxic bait in the field equipment storage area adjacent to the study site.

3.4.2 Captive trial

The test bait appeared to be unpalatable to lesser short-tailed bats in the captive trial, as none were observed consuming bait in reviewed footage. The mean duration of visits by bats to the test bait was short (mean s ± SE, 2.10 ± 0.44, max. 27 s, *n*=71), with 82% of visits lasting one second or less. The duration of visits by bats to food was on average much longer (19.41 ± 1.84, max. 335 s, *n*=609), with only 58% of visits lasting one second or less. I reviewed bat activity at the two feed stations during 264 x 10-minute periods (11 x 10-minute periods per station per night) from a total of 282 h recorded on the two overhead cameras over the 12 nights of the trial. Bats visited either test bait, food, or both during 49% of the 10-minute periods sampled across the 12 nights (*n*=264). Visits to food were more frequent than visits to test bait and this difference was highly significant (χ^2 [1, *n*=680] = 252.31, p< 0.01) and independent of station (χ^2 [1, *n*=680] = 0.07, p=0.79) and trial period (χ^2 [1, *n*=680] = 0.81, p=0.37). Sub-sampling of recordings was considered appropriate because there was no significant difference between the proportion of visits made by bats to test bait or food during 11 hourly 10-minute periods on the first night, and the proportion of visits made to test bait or food during the entire night (χ^2 [1, *n*=342) = 0.02, p=0.88).

Bats visited the bait compartments of the feed trays on 71 occasions. On 58 occasions contact clearly occurred between the bait and some part of a bat's body (Figure 3.11). On

14 of these occasions, definite contact was made between the bait and some part of a bat's head (Figure 3.12). On a further 13 occasions, there was definite contact with some other body part and possible contact between the bait and some part of the bat's head. On the remaining 31 occasions, contact involved the bat's hind legs, feet, ventral surface, wing membranes or thumbs, but no part of the bat's head, so during these visits there was no possibility that the bat could have directly sampled the bait. There were also six occasions when it was not clear whether a bat visiting the bait compartment made contact with the bait, and in three of these cases the possible contact involved the bat's head. However, on no occasions was it evident that any bat consumed bait during a visit to the bait compartment.

Even though no bats were observed consuming bait, a decrease in weight of 16 g was observed for the four tests baits collectively over the 12 trial nights. This weight loss was adjusted to 27 g after test bait weights were corrected for changes in moisture content using changes in the weights of control baits (Table 3.3), a mean loss of 1.13 g of test bait per station per night of the trial.

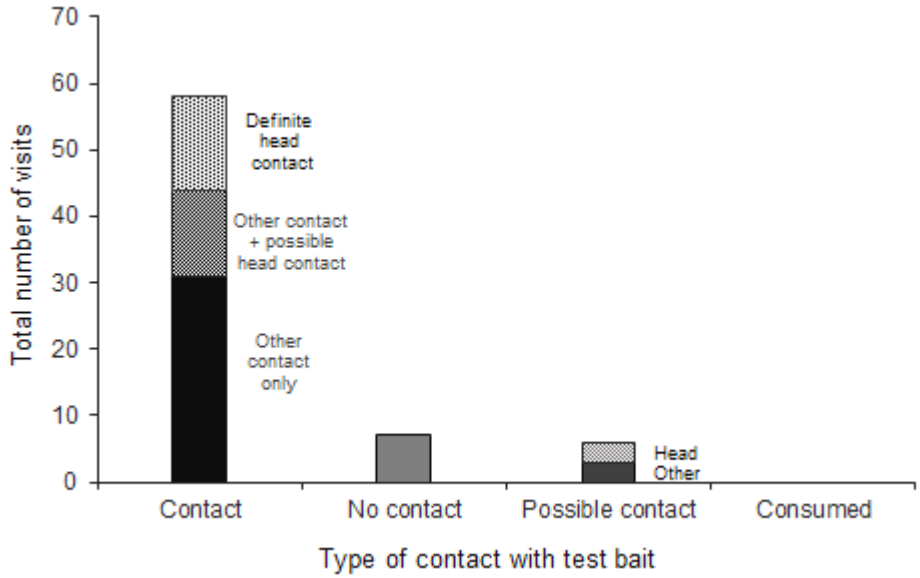

Figure 3.11 Frequency of different types of interaction of lesser short-tailed bats (*Mystacina tuberculata*) with test baits during visits (*n*=71) to the test bait compartment of feeding trays at stations 1 and 2 (combined data) during 264 sampled 10-minute periods over 12 nights at Auckland Zoo, New Zealand, October 2009.

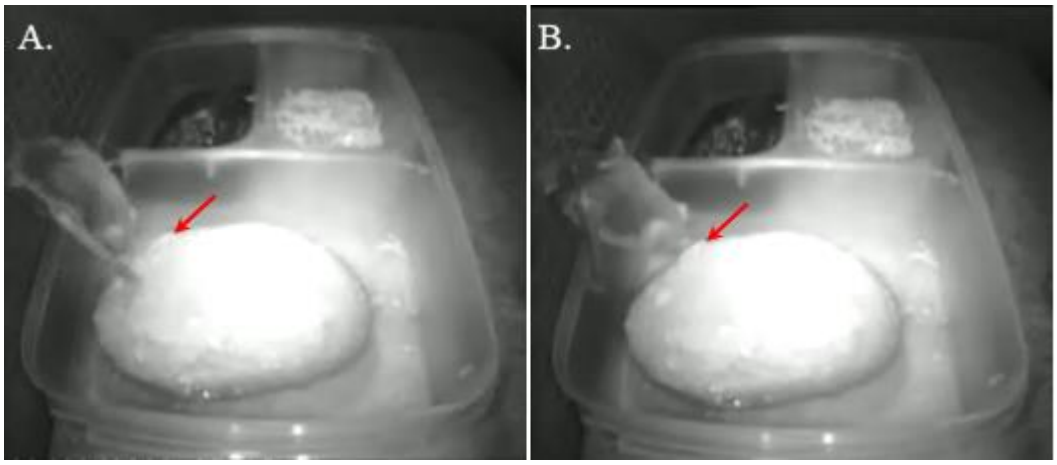

Figure 3.12 Video screen captures of a lesser short-tailed bat (*Mystacina tuberculata*) making contact with the non-toxic bait surface to retrieve a mealworm during a trial to assess the palatability of the bait to the bats at Auckland Zoo, New Zealand, October 2009.

Table 3.3 Total and individual weights (grams) lost from four 300 g test baits, before and after weight adjustment for moisture gain or loss based on the weights of control baits. Each test bait was used for six nights at one of two feed stations during one of two trial periods at Auckland Zoo, New Zealand, October 2009.

		Station 1	Station 2	Total
Unadjusted weight lost from test baits (g)	Period 1	-4	-4	-8
	Period 2	-2	-6	-8
	Total	-6	-10	**-16**
Corrected weight lost from test baits (g)	Period 1	-6	-6	-12
	Period 2	-5.5	-9.5	-15
	Total	-11.5	-15.5	**-27**

Slugs, snails (Order Pulmonata) and cockroaches living in the bat enclosure were also observed visiting the non-toxic test baits during the hourly 10-minute periods sampled. Arthropods were present on test baits during 115 of the hourly 10-minute periods reviewed (43.6%, *n*=264), and on 106 (92%) of these occasions the visitors were slugs. There was often more than one slug in a bait compartment at a time, and on one occasion there were more than 10. Individual slugs were frequently observed remaining in contact with the test baits at a single location for several hours. Inspection of test baits the morning after each trial night for signs of feeding by bats revealed cavities in the surface of the baits that could be matched to the sites of slug activity recorded during the preceding night (Figure 3.6D). Further evidence that slugs had consumed test bait was provided during the second trial period, when the red colour of the rhodamine B-dyed test bait was

visible in the gut of slugs found inside the bat enclosure during the day (Figure 3.13). As the bats were not observed consuming any of the dyed bait, there appeared to be no potential value in using marked non-toxic baits to detect bait take by bats in the field.

Figure 3.13 A slug with red internal colouring due to consumption of non-toxic test bait dyed with rhodamine B. The slug was found in the lesser short-tailed bat (*Mystacina tuberculata*) enclosure at Auckland Zoo, New Zealand during the October 2009 bait acceptance trial.

3.5 Discussion

New Zealand lesser short-tailed bats appear to be at greater risk of exposure to anticoagulant rodenticides by feeding on arthropods that have consumed toxic bait, than by directly feeding on bait themselves. This study demonstrated that nocturnal forest-dwelling arthropods in the bats' habitat at Pikiariki were attracted to a non-toxic cereal bait matrix similar to that used to deliver diphacinone for rodent control at the site during January 2009, when at least 115 bats died from diphacinone poisoning (Dennis & Gartrell, 2015). Although my ability to consistently identify arthropod taxa was limited by the low resolution of the recording equipment, I confirmed that both crawling and flying arthropods visited the non-toxic baits, and that both small- and large-bodied wētā spent considerable time grazing the baits. No wild bats, however, were observed visiting the non-toxic baits during the field trial. My observations in the wild appeared to be corroborated in captivity, where I saw no evidence that captive lesser short-tailed bats at Auckland Zoo consumed the same non-toxic bait, despite frequently coming in to contact with baits placed in the vicinity of their regular food. Arthropods living within the bat enclosure, however, visited and consumed the bait.

Wētā and many other types of arthropods commonly occur in the diet of lesser short-tailed bats (Daniel, 1979; Arkins *et al.*, 1999; Lloyd, 2005; Appendix 3.1). Arkins *et al.* (1999) determined that Orthoptera (wētā) were among the four arthropod orders, along with Coleoptera (beetles), Lepidoptera (moths) and Diptera (flies), that always made up at least 50% of the diet of lesser short-tailed bats on Little Barrier Island/Hauturu (based on percentage frequency in faecal pellets). The diet of the island bats was most diverse during summer, and the importance of different invertebrate groups varied with season. Wētā were also an important component of the diet of lesser short-tailed bats on Codfish Island/Whenua Hou, along with cockroaches, beetles, flies and spiders (Lloyd, 2005). Based on the results of dietary studies from other sites wētā were also expected to be important in the diet of the bats in Pikiariki. In contrast, using molecular techniques to examine the frequency of different prey orders in guano pellets, Czenze *et al.* (2018) detected a low frequency of wētā in the summer diet of lesser short-tailed bats in Pikiariki but found that moths and flies were important. Despite this, wētā could still play an important role in toxin transfer to the bats. I will discuss below how a single large-bodied wētā could potentially contain a lethal dose of toxin for bats.

Previous studies have recorded wētā, beetles, cockroaches and harvestmen among a wide variety of arthropod taxa on a range of cereal bait types used for vertebrate pest control in New Zealand (Sherley *et al.*, 1999; Spurr & Drew, 1999; Lloyd & McQueen, 2000; Wakelin, 2000; Craddock, 2003; Spurr & Berben, 2004; Bowie & Ross, 2006; Appendix 3.1). In comparative studies, significantly more arthropods were observed on baits at night than during the day (Spurr & Drew, 1999; Lloyd & McQueen, 2000). While the species and number of individuals visiting baits in these studies varied depending on the bait type tested, wētā were common visitors to a variety of bait types. Ground, tree and cave wētā (Families Anostostomatidae and Rhapidophoridae) were among the most frequently observed taxa on broadcast cereal baits of the types commonly used with 1080 (sodium monofluoroacetate) (Sherley *et al.*, 1999; Spurr & Drew, 1999; Lloyd & McQueen, 2000; Spurr & Berben, 2004). Craddock (2003) observed that wētā (as well as cockroaches) spent considerable time in permanent bait stations in contact with brodifacoum-laced cereal baits.

Although I was not able to confirm in my field study whether all arthropods observed visiting the non-toxic baits consumed it, numerous residue studies provide evidence that many wild arthropod species feed on the toxic baits they visit. In New Zealand, residues of brodifacoum have been detected in wētā, beetles, cockroaches and other arthropods found on broadcast baits or in and around bait stations (Booth *et al.*, 2001; Craddock, 2003). In Hawaii, residues of diphacinone have been detected in invertebrates collected from pellet baits following aerial application (Spurr *et al.*, 2015). Anticoagulant residues have also been detected in arthropods indirectly exposed to sources of toxins; fly larvae collected from a bat carcass in Pikiariki contained residues of diphacinone (Chapter 4), and brodifacoum residues were detected in necrophagous invertebrates that fed on rodent carcasses in an experimental field trial in Canada (Howald, 1997). Regardless of the route of exposure, it is generally accepted that most invertebrates are not affected by anticoagulant poisoning as their blood clotting system differs to that of vertebrates (Theopold *et al.*, 2004). Laboratory trials indicate that neither acute nor chronic exposure to brodifacoum (60 days) or diphacinone (64 days) is likely to cause mortality of wētā (Booth *et al.*, 2001; Bowie & Ross, 2006; Fisher *et al.*, 2007). However, in a recent captive trial Parli (2018) found that Wellington tree wētā exposed to brodifacoum showed changes in behaviour including aggression, boldness, and increased emergence but decreased activity. This could have implications for predator avoidance and the probability of bats catching a contaminated wētā rather than a healthy one.

The lack of susceptibility to anticoagulants observed in most invertebrates to date has raised concerns that they may accumulate large residue loads following repeat exposures. However, persistence (and thereby the potential for accumulation) of anticoagulant residues in invertebrate tissues appears to be much shorter than in vertebrate tissues. Fisher *et al*. (2007) determined that Wellington tree wētā (*Hemideina crassidens*) exposed to diphacinone-laced baits in the laboratory for 64 days did not accumulate toxic residues. Peak diphacinone concentration (mean μg/g ± SE, 4.85 ± 0.73, n=4) in wētā occurred after four days of exposure, suggesting fairly rapid excretion. In a separate laboratory trial exposing *H. crassidens* to a single dose (10 μg/g) of brodifacoum, no toxic residues were detectable after four days (Booth *et al.*, 2001). Residues persisted for 42 days in captive Central American giant cockroaches (*Blaberus giganteus*) exposed to brodifacoum baits

ad lib for 10 days, but the bulk of elimination occurred in the first two weeks following bait removal (Brooke *et al.*, 2013).

In the wild, residues of diphacinone were detected in invertebrates collected in pitfall traps up to one week, one month, and three months after an aerial application of diphacinone-laced pellet baits in Hawaii (Spurr *et al.*, 2015). In New Zealand native forest, Craddock's (2003) field study showed that brodifacoum residues in beetles, wētā, cockroaches and other arthropods took 3-4 weeks to return to background levels after bait was removed from bait stations. Traces of toxin were still detectable in some of the wild arthropods after 10 weeks. It is possible, though, that the wild arthropods at the site had access to other sources of bait or toxin in the environment after bait was removed from the bait stations, or that some invertebrates had the ability to store food in a crop for extended periods prior to digestion (e.g. Guthrie & Tindall, 1968).

The risk of secondary poisoning of lesser short-tailed bats from consumption of contaminated invertebrate prey has previously been acknowledged (Eason & Spurr, 1995). However, to my knowledge only bird species have been considered in published theoretical risk assessments for secondary poisoning of New Zealand native insectivores with anticoagulants (Craddock, 2003; Bowie & Ross, 2006; Fisher *et al.*, 2007). Mammals are generally more sensitive to anticoagulants than birds (EPA, 1998), so lesser short-tailed bats are likely to be at higher risk of mortality from exposure compared to insectivorous birds. Toxicological sensitivity data for anticoagulant poisons is not available for lesser short-tailed bats, but bats in general may be particularly sensitive to these compounds. This supposition is based on an acute oral LD_{50} (median lethal dose) of 0.91 mg/kg diphacinone for the vampire bat (*Desmodus rotundus*) (Thompson *et al.*, 1972a). Lesser short-tailed bats may also be more likely to encounter contaminated prey than insectivorous birds because the bats forage throughout the night (Christie, 2006), when invertebrate activity at baits is higher (Spurr & Drew, 1999). Furthermore, microbats in general have substantial daily food requirements relative to their small size (e.g. Encarnação & Dietz, 2006; Kalka & Kalko, 2006), adding to the risk of lethal exposure to anticoagulants through dietary sources.

Adult lesser short-tailed bats at held captive at Wellington Zoo regularly consumed up to 50% of their pre-feeding body mass in arthropods each night, and the daily food intake of free-living lesser short-tailed bats has been estimated as 40% of body mass (Lloyd & McQueen, 2000). Based on peak mean diphacinone residue concentration in Wellington tree wētā (Fisher *et al.*, 2007), a lesser short-tailed bat of average body mass (c. 14 g, Lloyd, 2005) could receive a lethal dose of diphacinone from 2.7g of contaminated wētā (around 50% of the bat's estimated daily food intake), assuming that these bats are as sensitive to diphacinone as vampire bats. A single Wellington tree wētā on average weighs more than 2.7 g (Mean ± SE; females 3.40 ± 0.34 g, n=11, males 2.82 ± 0.23 g, n=9) (Fisher *et al.*, 2007). An even smaller quantity of contaminated wētā could ultimately be lethal if eaten daily for several consecutive days, because, like other first-generation anticoagulants, diphacinone is more potent when administered in consecutive daily doses (Buckle, 1994). Fisher *et al*. (2007) suggested that most of the diphacinone residues in wētā exposed to toxic baits in laboratory trials were probably concentrated in their gut contents, so the fact the bats tend to clip off and discard legs of large prey items (Lloyd, 1994) would not reduce the amount of poison they ingested per wētā.

Direct consumption of bait would present a much greater hazard to bats than consumption of contaminated arthropods because the toxin would be more concentrated in the bait. For example, one gram of bait containing 0.005% diphacinone would deliver at least 10 times as much poison as the same weight of contaminated wētā (based on peak mean residue diphacinone concentration in wētā; Fisher *et al.*, 2007). Although captive lesser short-tailed bats were not observed consuming cereal bait in my study, nor in a captive trial by Lloyd (1994), it is possible that my sub-sampling approach failed to capture a very low incidence of bats consuming small amounts of bait, or that the recording set-up did not allow me to detect bats sampling bait in less obvious ways, such as licking. In a study by Beath *et al.* (2004) observers reported that wild-caught lesser short-tailed bats held temporarily in captivity appeared to sample small amounts of cereal bait, but were unable to estimate the amount of bait consumed. Based on the time the wild-caught bats spent apparently feeding on baits (on average less than 10 seconds per bait type per 3 h trial) it was uncertain whether sufficient bait was ingested to be of concern. The mean duration of visits by bats to baits in my study was also very brief, and many visits did not involve a bat placing its head near the bait.

Although consumption of bait by bats wasn't evident in my captive trial, I frequently observed bats landing on the baits or coming in to contact with baits with some body part. Contact with anticoagulant-laced baits could potentially be hazardous to bats. Topical application of anticoagulants is one of the methods used for the control of vampire bats; treated bats return to their roost where they contaminate roost mates and ingest the poison while grooming (Arellano-Sota, 1988). Beath *et al*. (2004) also noted that wild-caught captive lesser short-tailed bats walked through the soft baits offered in their trial, dispersing them and inadvertently spreading them on themselves. The amount of bait transferred to bats' fur by contact most likely depended on the consistency of the bait matrix, with pastes more likely to result in contamination than cereal pellet baits. It is uncertain whether any bait material was transferred to bats as a result of contact in my captive trial, and my ability to measure changes in bait weight attributable to contact by bats was confounded by the consumption of bait by slugs living in the bat enclosure.

The feeding behaviours that I observed in bats in the captive trial may have been an artefact of captive conditions (e.g. Willson & Comet, 1993). The bats used in my captive study may have been habituated to certain foods and feeding routines. Auckland Zoo staff reported that these bats showed a strong preference for mealworms over all other food items offered. Caution should therefore be exercised before extrapolating the results from the captive trial to represent the feeding preferences of wild bats. However, the field trial at Pikiariki also demonstrated no visits to baits by wild bats, despite evidence of bat activity in the vicinity of baits at 10 of the 12 sites surveyed, provided by automatic bat detectors. Furthermore, on many occasions during the captive trial, contact with baits may have been a result of the large size of the baits and their placement in the largest compartment of each feed tray, where mealworms were usually offered.

While the results of this study support an arthropod-mediated route of exposure of lesser short-tailed bats to anticoagulant rodenticides, not all populations may be equally at risk; different forest types inhabited by the bats in different parts of their range are likely to vary naturally in their arthropod fauna (Moeed & Meads, 1986; Moeed & Meads, 1987b; Lloyd, 2005). This may partly explain why no mortalities were detected in wild southern lesser short-tailed bat populations monitored through pest control operations using cereal pellet baits containing pindone (O'Donnell *et al.*, 2011) or brodifacoum (Sedgeley &

Anderson, 2000). Furthermore, natural variation in the abundance of some arthropod taxa between years (Moeed & Meads, 1987a) may correspond to different levels of risks at the same site from year to year. Further to this, Craddock (1997) reported that more arthropods visited baits at sites where pest mammal populations were suppressed, most likely due to reduced predation pressure and habitat recovery (e.g. Watts *et al.*, 2011). Even in the short-term, suppression of pest populations could alter bait availability to arthropods and indirectly influence poisoning risk to non-target insectivores. For example, low bait-take by rodents in Pikiariki following the December 2008 baiting round (Fisher & Broome, 2010) may have been of consequence regarding the bat mortalities detected there the following month.

Arthropod-mediated secondary poisoning of lesser short-tailed bats could potentially be a risk during baiting operations done at any time of year. New Zealand forest arthropods are active throughout the year (Moeed & Meads, 1984; Moeed & Meads, 1986; Moeed & Meads, 1987a), and during winter lesser short-tailed bats will rouse from torpor to feed when weather conditions are suitable (Sedgeley, 2001; Christie & Simpson, 2006). Many of the observations of arthropods on cereal baits in New Zealand forests occurred during winter (Sherley *et al.*, 1999; Spurr & Drew, 1999; Lloyd & McQueen, 2000; Wakelin, 2000; Spurr & Berben, 2004), when baiting operations with 1080 typically occur. Craddock (2003), however, noted that more arthropods entered bait stations during summer compared to other seasons.

The attractiveness of different bait formulations to arthropods could also affect the potential for secondary poisoning of lesser short-tailed bats to occur. In a comparative field study, Spurr and Drew (1999) established that more arthropods were attracted to plain baits (as used with brodifacoum) than to baits flavoured with cinnamon oil (as used with 1080). The colour of the baits had no effect. In a captive study, however, the number of cave wētā and cockroaches feeding on baits was not reduced by cinnamon oil (McGregor *et al.*, 2004). The relative preference of arthropods for the peanut-flavoured bait used in the current study has not been tested.

Further Research and Management Recommendations

Modifications to baiting practices in bat habitat that reduce the uptake of toxic bait by arthropods are required to minimise the risk of further non-target poisoning of lesser short-tailed bats. Such measures would presumably also benefit insectivorous forest bird species. No lesser short-tailed bat mortalities were detected in Fiordland when cereal pellet baits containing pindone were delivered enclosed in Philproof-Mini bait stations (Philproof Pest Control Products, Hamilton, New Zealand) (O'Donnell *et al.*, 2011), and this method of bait delivery should be trialled in Pikiariki. However, bait stations do not exclude arthropods, and studies at other sites provide evidence that arthropods contain toxic residues after feeding on pellet baits contained within the stations (Craddock, 2003). It seems likely, therefore, that bait station delivery would not prevent exposure of bats to anticoagulant rodenticides, but there was no assessment of whether bats were sublethally exposed to pindone in the Fiordland trial.

The result of the Fiordland trial may also have been affected by the species of arthropods available to bats in their beech forest habitat. Furthermore, pindone is one of the least potent and persistent anticoagulant rodenticides (Eason & Wickstrom, 2001). It is also reported to have insecticidal properties, although this has not been tested using the pindone-laced cereal baits used in New Zealand (Fisher, 2013). The success of the Fiordland trial, therefore, may have been toxin- and/or site-specific. Monitoring survival and exposure of the lesser short-tailed bat population at Pikiariki will therefore be essential to assess the effectiveness of using permanent bait stations and pellet baits to minimise any potential negative impacts of anticoagulant rodenticide use.

If the recommended adjustments to baiting practice prove inadequate to safeguard bats in Pikiariki, alternative methods to manage pest such as kill trapping (Carter *et al.*, 2016), or chemical methods to repel invertebrates from bait may be necessary. Invertebrate antifeedants have proven effective in laboratory trials and these should be further tested to assess their effectiveness in the field (McGregor *et al.*, 2004). To be acceptable to managers, this approach would need to incur minimal additional cost and guarantee that the additives would not compromise pest control operations by reducing bait palatability to target species. Research to determine whether important arthropod species in the diet of the Pikiariki lesser short-tailed bat population are likely to be involved in toxin transfer

could potentially guide the choice of antifeedant. Seasonal variations in the importance of these prey items in the bats' diet could help to limit the time when antifeedant-laced bait was required. This may help to address issues concerning the longevity of the effect of antifeedant compounds (Spurr & McGregor, 2003). Long-term dietary studies of the bat population could potentially establish whether the risk of arthropod-mediated secondary poisoning was likely to vary annually. Finally, given that New Zealand's other endemic bat species, the long-tailed bat (*Chalinolobus tuberculatus*) is entirely insectivorous, investigation should be made into the risk of dietary exposure for this species.

3.6 Acknowledgements

Volunteers Irene Weinberger and Laura Bergner assisted with field work. Andrew Nelson, Nicole Kunzman, Natalie Clarke, Amy Symes and other Auckland Zoo staff provided assistance with the captive trial. Auckland Zoo Animal Ethics Committee gave approval for the zoo trial. Massey University Animal Ethics Committee gave approval for both zoo and field trials (Protocols 09/83 and 09/91 respectively). Laurence Barea, Craig Gillies and Colin O'Donnell from the Department of Conservation (DOC) provided advice on trial designs. Alastair Robertson from Massey University and Alisdair Noble, formerly of Massey University, provided advice on trial design and statistical analysis. This study was supported by grants from the JS Watson Trust and the DOC Science Advisory Fund. DOC also provided in-kind support during the field trials. GCD received personal financial support during this study from the Julie Alley Bursary and JP Skipworth (Ecology) Scholarship through Massey University.

3.7 Appendix 3.1

Table 3.4 Arthropod taxa identified in bat guano in four studies done on three lesser short-tailed bat (*Mystacina tuberculata*) populations in New Zealand, (adapted from Lloyd, 2005), and relevant arthropod taxa observed on toxic and non-toxic cereal baits used in New Zealand vertebrate pest control operations. Frequency in bat diet: ✓**Occasional** ✓✓**Common** ✓✓✓**Abundant.**

	Lesser short-tailed bat diet [a]	Sherley et al., 1999	Spurr & Drew, 1999	Lloyd & McQueen, 2000	Wakelin, 2000	Craddock, 2003[b]	Berben & Spurr, 2004	Bowie & Ross, 2006
Cereal bait type (+toxin)		Wanganui No. 7 (Non-toxic; +1080)	Wanganui No.7, RS5, AgTech, (Non-toxic)	Wanganui No. 7 (+1080)	Wanganui No.7 (Non-toxic)	Pest-off (+Brodifacoum)	Wanganui No. 7 (+1080)	Talon 50WB (+Brodifacoum)
Region and forest type		Central NI: mixed podocarp/broadleaf	West Coast SI: 2 rata/kamahi forests	Central NI: Beech, podocarp/broadleaf	Lower NI; podocarp/ broadleaf	Upper NI: 1 coastal, 2 podocarp/ broadleaf	Lower NI; podocarp/ broadleaf	East SI: scrub, coastal forest, pines, grassland
Time of year surveyed		Jun - Oct	July & Sep	August	July	Jan - Dec	August 23-25	7 February
Time of day surveyed		Night	Night & day	Night	Night	Dusk till dawn	Within 3 h of sunset	10pm-11pm
ACARINA (mites)	✓	•	•		•		•	
ARANEA (spiders)	✓ - ✓✓	•		•	•	•	•	• [c]
OPILIONES (harvestmen)	✓	•	•	•	•	•	•	
AMPHIPODA (crustaceans e.g. isopods/woodlice)	✓	•	•	•	•		•	• [d]
MYRIAPODA (millipedes and centipedes)	✓	•		•	•		•	
BLATTODEA (cockroaches)	✓✓	•	• [e]	•		•	•	• [c]
ORTHOPTERA (wētā) tree and ground wētā	✓✓ - ✓✓✓	•	•	•		•	•	• [d]
cave wētā	✓✓ - ✓✓✓	•	•	•	•	•	•	• [d]
HEMIPTERA (bugs)	✓	•			•			• [c]
THYSANOPTERA (thrips)	✓							
NEUROPTERA (lacewings)	✓							
COLEOPTERA (Beetles, weevils)	✓ - ✓✓✓	•	•	•	•		•	• [d]
DIPTERA (Flies, midges)	✓ - ✓✓✓	•			•		•	
LEPIDOPTERA (Moths)	✓ - ✓✓✓	•			•		• [f]	
HYMENOPTERA (Ants, wasps, bees)	✓	•	•		•		•	

a. Lesser short-tailed bat dietary studies: (Arkins *et al*., 1999), Little Barrier Island; McQueen & Lloyd unpublished data, Rangataua Forest, Central North Island; (Arkins, 1997), Codfish Island; Lloyd & McCartney, unpublished data, Codfish Island. **b**. Craddock (2003) also observed arthropods <5 mm (including small wētā, harvestmen, bristletails and others) on baits but treated these as a single category without identifying individual species. **c.** Observed inside loaded bait station in large numbers, not specified whether on bait **d.** Observed foraging on bait inside bait station **e.** Not specified which of the tested bait type/s this arthropod taxon was observed on. **f**. Caterpillars

Chapter 4

Changes to rodent baiting practices reduces non-target mortality of New Zealand lesser short-tailed bats (*Mystacina tuberculata*) but does not prevent exposure to diphacinone

Sampling blood from the brachial vein of a wild lesser short-tailed bat to measure blood prothrombin time. Photo courtesy of Raewyn Empson.

4.1 Abstract

The use of toxic baits to control introduced mammals is essential for wildlife conservation in New Zealand, but also poses risks to non-target species, including the lesser short-tailed bat (*Mystacina tuberculata*). In 2009, 115 lesser short-tailed bats were found dead during a rodent control operation in Pikiariki Ecological Area, Pureora Forest Park, New Zealand. Post-mortem examination of bats confirmed poisoning with the anticoagulant rodenticide diphacinone. This most likely occurred due to secondary exposure through contaminated arthropod prey. The type of bait used and the method of delivery were thought to be precipitating factors in the mortalities. Bats are long-lived, slow breeders and may therefore be vulnerable to population impacts from non-target mortalities, but they are also threatened by invasive predators. Successful conservation of bats requires effective pest control using baiting methods that minimise the risk of exposure of bats. The aim of this study was to assess whether an alternative baiting method prevented extensive lethal exposure of the bats in Pikiariki to diphacinone, as had occurred in 2009.

I conducted a 6-month field trial and measured bat population survival before, during and after the rodent baiting operation. I sought to demonstrate a causal relationship between exposure and survival by providing evidence of exposure and clinical effects. The bat population's exposure to diphacinone was confirmed by detecting residues in communal guano during 5 months of the study. No overt clinical signs of anticoagulant poisoning were observed in live bats sampled monthly (n=256) and dried blood spot (DBS) analysis failed to detect residues of diphacinone in blood samples (n=20). Prolongation of mean blood prothrombin time was not evident during the period of exposure, nor when compared to mean blood prothrombin time of bats from an unexposed population (n=33) indicating that exposure was subclinical. Mark-recapture survival analysis indicated that there was zero to negligible effect of the exposure on survival of a sample of PIT-tagged bats (n=580).

My results suggest that in this season the alternative baiting method considerably reduced the risk of extensive mortalities of bats resulting from diphacinone exposure. However, while the pathway of exposure remains intact I suggest that the risk of adverse effects on bats could vary annually with variation in the abundance of arthropod prey involved in toxin transfer to the bats. Furthermore, sublethal exposure of bats to anticoagulant

rodenticides could have harmful effects on reproduction and health. I recommend that surveillance and survival monitoring continue at exposed bat populations to assess annual variations in survival and long-term population trends.

4.2 Introduction

The impact of vertebrate pests on native fauna is one of the dominant conservation issues in New Zealand (Innes *et al.*, 2010; Russell *et al.*, 2015). Consequently, vertebrate pest management is a significant component of protecting biodiversity values (DOC, 2018). Native wildlife populations can benefit from reductions in the densities of introduced mammals such as ship rats (*Rattus rattus*) (Pryde *et al.*, 2005; O'Donnell & Hoare, 2012), but they are also potentially at risk of lethal or sublethal exposure to the toxins used to manage pests in their habitats. Non-target mortalities due to primary or secondary poisoning with broad-spectrum vertebrate pesticides such as anticoagulant rodenticides have been documented for a wide range of species in New Zealand (Eason *et al.*, 2002) and overseas (Laakso *et al.*, 2010) during pest control operations for conservation purposes. Despite this, a low incidence of non-target deaths may be considered acceptable relative to the population-level impacts of predators likely to occur in the absence of management and the potential levels of recovery achievable (e.g. Empson & Miskelly, 1999).

Monitoring wildlife populations that are vulnerable to non-target impacts of toxin use is a key requirement for establishing that pest management results in overall conservation gains. Carcass counts following poison operations tend to underestimate the extent of mortalities, primarily due to lack of detection by observers, rapid decomposition or removal by predators (Huso, 2011). Alternative methods for estimating impacts are available, and the most appropriate should be selected for the species and the situation (Spurr & Powlesland, 2000). If animals can be captured and individually marked or otherwise identified, mark-recapture (or mark-resight) methods offer a rigorous technique for estimating the impacts of poison operations on native wildlife populations. This method is particularly useful for cryptic species that are difficult to observe because it overcomes the problem of imperfect detection (Kunz *et al.*, 2009a).

Mark-recapture studies can be designed to measure population survival rates before, during and after poison operations so that changes in mortality rates associated with the period of poison exposure can be estimated (Williams *et al.*, 2002). Model selection procedures are used to understand the relationship between any observed changes in survival and the timing of the poison operation, while also considering any other factors thought to influence survival (Burnham & Anderson, 2002). This method of monitoring has been used in New Zealand to

measure the impacts of poison operations on a variety of native species, including birds (Armstrong & Ewen, 2001; Armstrong *et al.*, 2001; Davidson & Armstrong, 2002), bats (Edmonds *et al.*, 2017) and insects (Spurr & Berben, 2004). Inferences from this method of monitoring can be strengthened by showing evidence of exposure by testing for residues in the tissues of recovered carcasses (Edmonds *et al.*, 2017). Causal relationships can be inferred when tissue residues are considered with evidence of clinical or pathological effects that are consistent with poisoning (Gabriel *et al.*, 2012; Dennis & Gartrell, 2015; Chapter 2).

New Zealand's two endemic microbat species, the lesser short-tailed bat (*Mystacina tuberculata)* and the long-tailed bat (*Chalinolobus tuberculatus*) are likely to benefit from reductions in rodent densities in their habitats (O'Donnell *et al.*, 2011; Edmonds *et al.*, 2017; O'Donnell *et al.*, 2017). However, lesser short-tailed bats are vulnerable to non-target poisoning as they often forage on or near the ground where baits are laid (Daniel, 1976). In 2009, 118 lesser short-tailed bats of the central North Island subspecies (*M. t. rhyacobia*) were found dead or dying during a Department of Conservation rodent control operation in Pikiariki Ecological Area (hereafter Pikiariki), Pureora Forest Park, as a result of exposure to the anticoagulant rodenticide diphacinone (Dennis & Gartrell, 2015: Chapter 2). During the poison operation, diphacinone was presented in cereal-based paste baits that were delivered in biodegradable plastic bags stapled to tree trunks, with potential for spillage. This bait delivery method was considered a contributing factor to the mortalities. Furthermore, in some countries anticoagulant toxins are used for the control of bats (Johnson *et al.*, 2014), and captive studies with vampire bats (*Desmodus rotundus*) suggest that bats may be sensitive to diphacinone (Thompson *et al.*, 1972). Long-tailed bats are considered less at risk of non-target poisoning as they are aerial insectivores that forage predominantly along forest edges and so are therefore supposed less likely to encounter contaminated arthropods from the forest floor (Daniel & Williams, 1984; O'Donnell, 2000b; O'Donnell, 2001a).

The central subspecies of lesser short-tailed bat is listed as 'At Risk' under the New Zealand threat classification system (Hitchmough *et al.*, 2007). Without effective control of introduced predators bat populations are predicted to decline (O'Donnell *et al.*, 2010). Therefore, with the aim of reducing the risk of further non-target poisoning of bats, the Department of Conservation banned the use of anticoagulant-laced paste baits in short-

tailed bat habitats (O'Donnell *et al.*, 2011), and cereal pellet baits contained in bait stations were subsequently used to deliver diphacinone for rodent control in Pikiariki and a neighbouring forest block. This decision was guided by the results of a trial in the Eglinton Valley, Fiordland, South Island, where survivorship of a sample of southern lesser short-tailed bats (*M. t. tuberculata*) was high before, during and after a pest control operation using bait stations containing cereal pellet baits laced with the first-generation anticoagulant rodenticide pindone (O'Donnell *et al.*, 2011). The Fiordland trial, however, did not assess whether the bats had been sublethally exposed to pindone.

Bait trials in captive and wild settings indicate that lesser short-tailed bats are unlikely to directly consume cereal-based pellet baits (Lloyd, 1994; Sedgeley & Anderson, 2000) but are more at risk of secondary exposure to poisons by consumption of contaminated arthropod prey (Lloyd & McQueen, 2000; Chapter 3). Forest arthropods containing residues of the anticoagulant rodenticide brodifacoum have been detected during pest control operations using cereal-based pellet baits in bait stations (Craddock, 2003), suggesting that there is still a risk of secondary exposure of bats when this baiting practice is used. Furthermore, potential differences in the composition of arthropod fauna between Fiordland beech and Pikiariki podocarp forests (Moeed & Meads, 1987b) and the higher potency and longer persistence of diphacinone in mammal tissue compared to pindone (Eason & Wickstrom, 2001; Fisher *et al.*, 2003) suggest that caution should be exercised in generalising the results of the Fiordland trial to the Pikiariki site. Therefore, as well as monitoring survival of bats through the pest control operation at Pikiariki, assessment of exposure and evidence of associated clinical effects was critical to ensure that the revised baiting practice also prevented sublethal exposure of bats.

Sublethal exposure of bats to diphacinone and other anticoagulant rodenticides is of concern because of the potential adverse effects of these chemicals on mammalian health and reproduction. Exposure to sublethal doses of anticoagulants may cause weakness, slower reaction times, and increased susceptibility to accidents or predation (Fournier-Chambrillon *et al.*, 2004), increased pathogen or parasite burdens (Riley *et al.*, 2007; Lemus *et al.*, 2011), liver damage (Eason & Jolly, 1993) and loss of appetite (Oliver & Wheeler, 1978). Anticoagulants may be passed to the offspring of mammals via the placenta or milk and may cause death or debility of the developing embryo, foetus or

young (Hall *et al.*, 1980; Robinson *et al.*, 2005; Dennis & Gartrell, 2015: Chapter 2). Furthermore, sublethal exposure to anticoagulants may interact with other stressors to increase sensitivity to the toxin or to induce mortality from other causes (Robinson *et al.*, 2005; Vidal *et al.*, 2009). Recent evaluation of genome-wide expression patterns in bobcats (*Lynx rufus*) in California, U.S.A., has revealed that sublethal anticoagulant exposure disrupts regulation of genes involved in immune function and other systemic defence processes, with potentially serious consequences for individual fitness and persistence of populations (Fraser *et al.*, 2018).

Sublethal exposure of living animals to anticoagulant toxins can be confirmed by detection of residues in blood samples (Fisher, 2009; Schaff & Montgomery, 2013) or faeces (Sage *et al.*, 2010; Seljetun *et al.*, 2018). It is also important to determine whether exposure is above some unknown dose threshold that produces harmful effects (Connell *et al.*, 2009). The primary action of the anticoagulant poisons is their effect on haemostasis. Prothrombin time (one measure of blood clotting time) is a useful measure of sublethal exposure as it is normally prolonged before the onset of clinical signs of anticoagulant poisoning (Murphy & Talcott, 2006), so provides an early and sensitive indicator of exposure (Woody *et al.*, 1992). Prolongation of prothrombin time following oral administration of an effective dose of anticoagulant compounds is obvious and rapid. In a study on four dogs (*Canis familiaris*) dosed with brodifacoum for three consecutive days, mean prothrombin time on day four was twice as long normal (Woody *et al.*, 1992). A seven-fold increase in prothrombin time was observed in 30 California ground squirrels (*Spermophilus beecheyi*) 72 h after dosing with diphacinone (Whisson & Salmon, 2002). Shlosberg and Booth (2006) suggest that an increase in prothrombin time ≥ 25% above baseline values can be indicative of coagulopathy.

The aim of this study was to assess whether modifying baiting practices for rodent control prevented extensive lethal non-target exposure of lesser short-tailed bats in Pikiariki, as had occurred in 2009 (Dennis & Gartrell, 2015; Chapter 2), and resulted in net benefit to the population. I therefore monitored the survival of the bat population over a six-month period, before, during and after the Department of Conservation's rodent control operation using diphacinone-laced pellet baits in bait stations. Mark-recapture methods that account for the probability of detection were used to estimate survival because these

bats are small, nocturnal, and roost in tree cavities, and so are difficult to detect. Population exposure was monitored by monthly testing of guano for diphacinone residues. I sought evidence of clinical effects associated with anticoagulant poisoning by measuring prothrombin times of a sample of bats each month, and by visually examining bats for overt signs of intoxication. This would support a causal relationship if survival appeared to be reduced in association with the rodent control operation, or otherwise provide evidence of sublethal exposure. Prothrombin time was also measured in an unexposed population of lesser short-tailed bats to provide reference values. The opportunity was also taken to assess the risk of non-target exposure of long-tailed bats by measuring diphacinone residues in guano collected from the Pikiariki population during the rodent control operation.

4.3 Methods

4.3.1 Study sites

Research was conducted at two sites. The primary study site was Pikiariki Ecological Area ('Pikiariki') in the central North Island, New Zealand (38º31'S, 175 º34'E) (Figure 4.1). Pikiariki is a small remnant (457 ha) of old-growth native podocarp-hardwood forest, dominated by emergent matai (*Prumnopitys taxifolia*) and rimu (*Dacrydium cupressinum*), with a predominantly tawa (*Beilschmiedia tawa*) canopy. The remnant is situated within Pureora Forest Park (78,000 ha) but is isolated from more extensive tracts of native forest by pasture, shrubland, unsealed roads and standing or cutover exotic conifer plantation forest. Pikiariki was designated as an Ecological Area in 1979 in recognition of its high conservation values (Norton & Overmars, 2012). The site contains the only known roost tree habitat in the park for a population of lesser short-tailed bats.

Pureora Forest Park is managed by the New Zealand Department of Conservation (DOC). Both Pikiariki and nearby Waipapa Ecological Area (hereafter referred to as Waipapa; 5112 ha, Figure 4.1) are the focus of ecosystem-style restoration efforts, with annual toxic baiting programmes aimed at enhancing native wildlife populations and forest health. This is achieved by supressing populations of introduced rodents (*Rattus* spp. and *Mus musculus*) and other mammalian pests to low levels during vulnerable periods in the

breeding cycles of threatened species. My research was conducted at Pikiariki over two bat breeding seasons; November 2012-February 2013 and October 2013-April 2014.

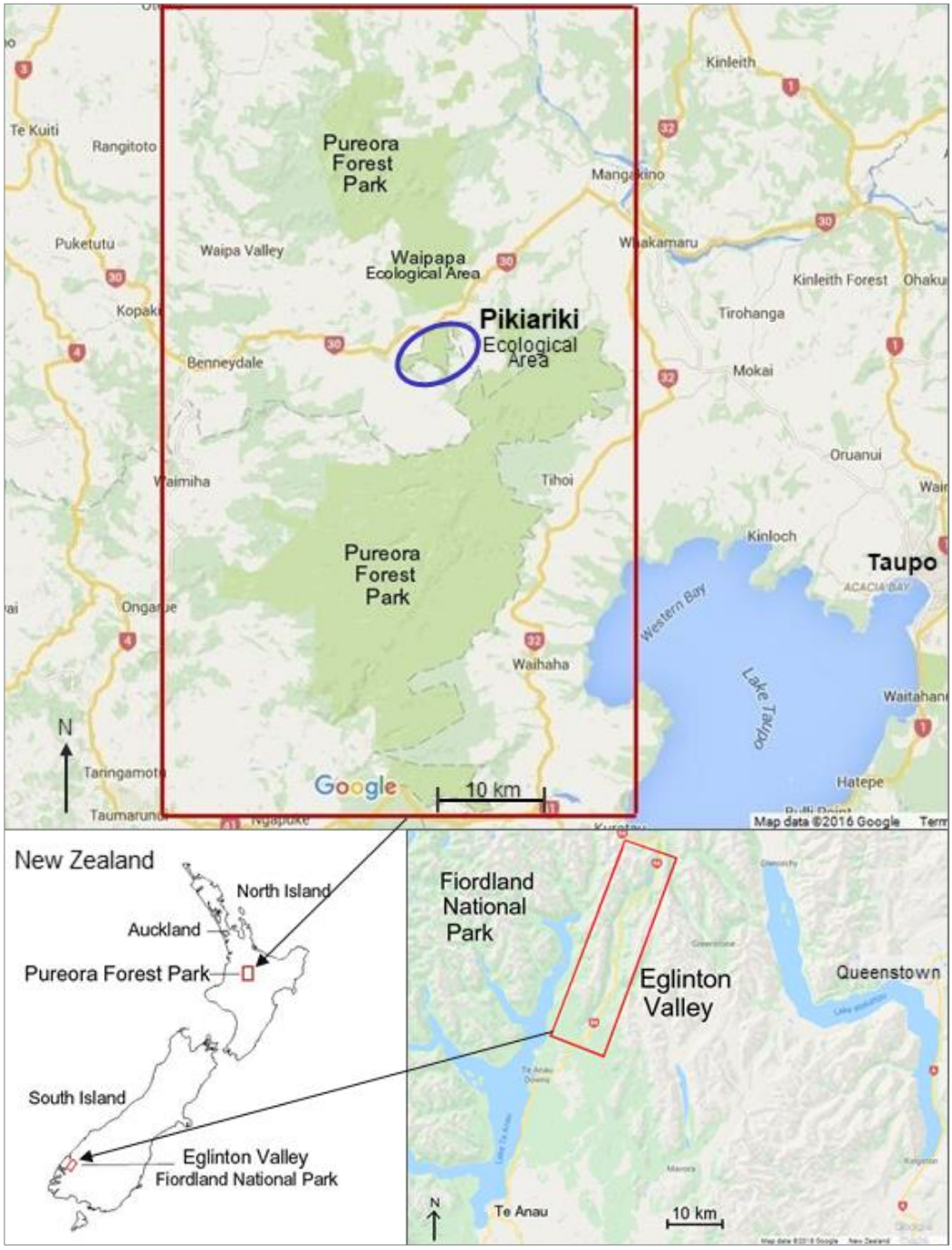

Figure 4.1 General location of study sites at Pureora Forest Park in the central North Island, and Eglinton Valley in Fiordland National Park in the South Island, New Zealand (bottom left); Locations of Pikiariki Ecological Area (primary study site) and Waipapa Ecological Area in Pureora Forest Park (top); and location of Eglinton Valley in Fiordland National Park (secondary study site, bottom right).

The secondary study site was in the Eglinton Valley, Fiordland National Park, South Island, New Zealand (44°58'S, 168°00' E) (Figure 4.1). The Eglinton is a U-shaped valley of glacial origin, with steep sides and a flat floor up to 2 km wide. A main road runs along the length of the valley floor, parallel to the Eglinton River. Mature mixed southern beech forest *(Lophozonia menziesii* and *Fuscospora* spp.) covers the valley floor and lower hill slopes. On the valley floor the forest is interspersed with shrubland and large pockets of modified tussock grassland. A population of the southern subspecies of lesser short-tailed bats (*M. t. tuberculatus*) inhabits the forest of the mid- to upper valley (O'Donnell *et al.*, 1999). Toxic baiting is only necessary during mast years (every two to six years) in beech forest, when synchronised mass production of beech seeds results in local irruptions of mammalian pests (Elliott & Kemp, 2016). The Fiordland bat population therefore served as a control during the second season of my study (2013/14) as no baiting was required during the period when bats were sampled there.

4.3.2 Rodent baiting operations

Rodent baiting operations in Pikiariki were managed by DOC staff using an existing network of Philproof bait stations (Philproof Pest Control Products, Hamilton, New Zealand) distributed on a 50 m x 150 m grid throughout the treatment area (Figure 4.2). DOC staff commenced baiting in Pikiariki on 2 August 2012, prior to the first field season of my study, filling bait stations with 400 g of diphacinone-laced cereal pellet baits (Pestoff Rat Bait 50D, 0.005%, Animal Control Products Ltd, Whanganui, New Zealand). Bait stations were refilled (if required) with 400 g of pellets on 28 August and 8 November, and with 300 g of pellets on 31 January 2013. Any remaining bait was removed from bait stations on 2 May 2013. Between 20 August 2012 and 18 April 2013 diphacinone-laced pellet baits were also present in bait stations in the south block of Waipapa (Figure 4.1).

During the second field season of my study, the duration of bait deployment in Pikiariki was shortened so that bats could be monitored before, during and after the poison operation within a six-month period. Bait stations in Pikiariki were filled with 400 g of diphacinone-laced pellet baits between 25 and 29 November 2013, and refilled (if required) with 300 g of pellets between 16 and 20 December. Any bait remaining in bait stations in Pikiariki was removed between 17 and 21 February 2014. Diphacinone-laced

pellet baits were also present in bait stations in the south block of Waipapa between 13 September 2013 and 14 April 2014. Rat tracking indices were measured in Pikiariki in November, January and April by Department of Conservation staff following best practice methods to assess rodent densities before, during and after the baiting operation (Brown *et al.*, 1996; Gillies & Williams, 2013).

Figure 4.2 Methods used to deliver diphacinone-laced baits for rodent control in Pikiariki Ecological Area, Pureora Forest Park, New Zealand; **A.** A biodegradable bag containing cereal-based paste bait nailed to a tree trunk in January 2009, and **B.** A bait station containing cereal pellet baits used for rodent control operations in 2012/13 and 2013/14.

4.3.3 Population-level study

Roost location

During the 2012/13 field season I limited my study to known maternity roosts in Pikiariki. During the 2013/14 field season I located additional maternity roosts by radio-tracking bats. During October 2013 and March 2014 I used mist-nets (38mm, Avinet, Dryden, USA) at suitable sites in Pikiariki to capture bats away from their roosts (Kunz *et al.*, 2009b). Adult females weighing no less than 14 g were fitted with radio-transmitters (BD2, Holohil Systems, Carp, Ontario, Canada), attached between the scapulae on an area of partially trimmed fur, using a latex-based contact adhesive (Ados F2®,CRC industries, East Tamaki, New Zealand). Transmitters weighed ≤ 0.7 g and were therefore

< 5% of bat body mass, in accordance with recommended guidelines (Aldridge & Brigham, 1988). I tracked radio-tagged females during the day to locate active maternity roosts using a hand-held TR4 receiver (Telonics, Arizona, USA) and a hand-held, 3-element Yagi aerial (Sirtrack, Havelock North, New Zealand). Entrances of newly located roosts were identified by hauling a TR4 receiver up the tree trunk while the roost was occupied by a bat carrying a transmitter. Three known maternity roosts were also inspected daily during October 2013 for evidence of occupation in the form of fresh guano deposits beneath the roost entrance, and by using a hand-held Batbox III bat detector (Stag electronics, Steyning, U.K.) to detect ultrasonic bat calls coming from the roost.

Guano collection and analysis and surveillance for mortalities

Communal samples of bat guano were collected from bat roosts in Pikiariki during the 2012/13 rodent baiting period and tested for diphacinone residues. Guano was collected once a week for 12 weeks between 23 November 2012 and 20 February 2013, from tarpaulins placed beneath the entrances of active maternity roosts. Tarpaulins were cleared once a week, and two to four days later accumulated guano was collected, stored in plastic bags and frozen until analysis. DOC staff carried out sample collection on my behalf during weeks when I was unable to visit the site.

I also monitored bat population exposure to diphacinone during the 2013/14 study period, one month before rodent bait deployment in Pikiariki, three months while baits were present, and two months after surplus baits were removed from bait stations. I collected guano from tarpaulins beneath active maternity roosts during one week each month between 4 November 2013 and 9 April 2014. Guano collection coincided with weeks when individual bats were sampled (Section 4.3.4). Tarpaulins were cleared at the start of the week, and samples were collected daily up to four times during the week, frozen separately, and later pooled for analysis.

Opportunistic surveillance for lesser short-tailed bat mortalities occurred during visits to active roosts. Dead bats were collected and sent to Wildbase Pathology, Massey University, Palmerston North for post-mortem examination by a veterinary pathologist. Long-tailed bat guano was collected by Department of Conservation staff on my behalf during January 2014 to assess the risk of poison exposure to this aerial insectivore.

Deposits from bats captured in harp-traps during routine monitoring were pooled to provide a single sample that was tested for diphacinone residues.

The diphacinone content of guano, liver tissue from dead bats and necrophagous invertebrates collected from decomposing bats was determined by high performance liquid chromatography. The method detection limit (MDL) is 0.02 μg/g for liver, 0.2μg/g for invertebrate tissue and is undetermined for guano. The uncertainty (95% C.I.) is ±20%. Assays were performed by CENTOX (Centre for Environmental Toxicology), Landcare Research/Manaaki Whenua, Lincoln, New Zealand.

Bat marking and data collection

Permanent marking of bats with passive integrated transponder (PIT) tags was required to generate data for population survival estimates, and also to uniquely identify bats that were captured and individually sampled each month. PIT-tagging was undertaken with assistance from DOC staff and other trained personnel between 29 October and 1 November 2013. Bats were captured bats at evening emergence using a 4.2 m^2 harp trap (Austbat Research Equipment, Melbourne, Australia) suspended across the entrance of a roost. Previously, 188 bats in the population had been permanently marked with PIT tags (between January 2012 and February 2013). Therefore, captured bats were sexed and checked for an existing PIT tag using a hand-held Scanflex AFX-100 scanner (ISO Compatible RF/ID Pocket Reader, Allflex Australia Pty Ltd, Capalaba, Australia). If no tag was detected an Allflex PIT tag (FDX-B 11 mm x 2.1 mm glass transponder implant) was inserted subcutaneously on the back between the scapulae using a sterile single-use 12 gauge needle (Allflex, Capalaba, Australia) on a Henke-ject insertion gun (Henke-Sass Wolf, Tuttlingen, Germany), following Department of Conservation best practice (Sedgeley *et al.*, 2012). Juveniles and additional adults were PIT-tagged between 4 and 8 February 2014, once juveniles had become volant.

PIT-tagged bats were detected remotely using custom-made RFID (radio-frequency identification) data loggers (Department of Conservation, Wellington, New Zealand) at roosts. DOC staff and I custom-made antennae and installed these around one or more entrances at seven maternity roost trees (Cockburn, 2013). Antennae were connected to loggers that were powered by an external 12-volt battery. Each time a PIT-tagged bat passed

through an antenna loop, the date, time and unique PIT tag code were recorded on the logger. Loggers were programmed to operate each night for the hours that bats were active. Loggers were visited once or twice a week to change batteries and to download data to an Archer Field PC (Juniper Systems, Logan, U.S.A) for transfer to a database.

I monitored PIT-tagged bats over 186 days between 29 October 2013 and 2 May 2014. Monitored bats included individuals PIT-tagged during the study, and any of the bats PIT-tagged prior to the study that were 'encountered' during the survival period. 'Encountered' means that the unique code of a PIT tag was recorded as a bat passed through an RFID aerial and/or was manually scanned and recorded by handlers following capture in a harp-trap.

Mark-recapture survival analysis

I selected eight encounter occasions from the data logged at roosts after the initial physical capture and PIT-tagging session in October 2013. The encounter occasions divided the survival period into eight intervals (Table 4.1); interval one (22 days) in November before poison baits were laid in Pikiariki, intervals two through five (28, 27, 20 and 11 days respectively) between late November and mid-February while poison baits were present in Pikiariki, and intervals six through eight (29, 29 and 17 days respectively) between mid-February and early May after poison baits were removed from Pikiariki. Bat encounters for each occasion comprised roost data logger records pooled from two consecutive nights. The end point of a survival interval was taken as the mid-point of the two pooled nights that followed that interval**.** The selection of dates for encounter occasions was constrained by the delineation between poison and non-poison periods, and by the timing of the PIT-tagging session in February 2014.

I measured the apparent monthly survival (ϕ) of PIT-tagged bats in Pikiariki over 183 days (1 November 2013 to 2 May 2014, referred to hereafter as the survival period) to assess the effect of the poison operation on the population. Apparent survival combines the probability that a marked individual has survived and the probability that the individual has not permanently emigrated out of the study area between encounter occasions (Williams *et al.*, 2002). I constructed encounter histories of individual bats for the initial PIT-tagging session and eight subsequent encounter occasions and used these

to estimate monthly survival and encounter probabilities. Encounter probability (*P*) is the probability that a bat is detected on an encounter occasion, conditional on the bat being alive and in the study area (Williams *et al.*, 2002). Bats with existing PIT tags were considered to enter the study on the first occasion that they were encountered, while unmarked bats entered the study when they were PIT-tagged. Data were modelled in Program MARK (Version 7.2) (White & Burnham, 1999) using the Cormack-Jolly-Seber open population model for live recaptures (Lebreton *et al.*, 1992).

I constructed a global model that included all the parameters that I considered important in affecting survival (ϕ) and encounter (*P*) probabilities. I considered that age (adult or juvenile) and sex (male or female) were important factors, based on previous studies on New Zealand bat species (Pryde *et al.*, 2005). I also wanted to determine whether different age- or sex- classes were more susceptible to poisoning. The global model therefore included the parameters age (*a*), sex (*s*), and time (*t*), and was defined as ϕ (*a*s*t*), *P*(*a*s*t*). This model estimates survival and encounter probabilities separately for each interval for each sex- and age-class. I also considered that PIT-tagging could have an effect on survival of newly-tagged bats in the interval immediately following tagging, based on preliminary examination of data. I therefore constructed an alternative global model ϕ(*a*s*t*tag*), *P*(*a*s*t*). This model included separate estimates of survival probabilities for bats with new PIT tags in the interval after they were tagged.

To assess the effect of tagging on the fit of the global models, I structured encounter history data to recognise six groups; adult males and females with existing PIT tags (two groups), adult males and females with new PIT tags (two groups), and juvenile males and females with new PIT tags (two groups). Adult bats with existing PIT tags were those that had been tagged prior to the current study. Unmarked adult bats that entered the study during either of the two PIT-tagging occasions were modelled as newly-tagged bats for the survival interval following tagging, and thereafter were treated as bats with existing PIT tags. All juveniles entered the study during the February PIT-tagging session, and therefore all were treated as newly PIT-tagged juveniles in the first interval after tagging, and as juveniles with existing PIT tags thereafter. I compared the two global models using AIC_c (Akaike's Information Criterion) adjusted for small sample size (Burnham & Anderson, 2002). The best global model (that with the lowest AIC_c) was then tested for

goodness of fit using the median c-hat approach in Program MARK and adjusted for over-dispersion.

I assessed the effect of the poison operation on survival by comparing models that included differences in survival probability based on whether or not there was a risk of the bats being exposed to poison. Poison exposure risk (PER) was modelled as a constraint of the time parameter, where time relates to whether survival intervals occurred before, during or after the poison operation. Two PER models were based on the *a priori* hypothesis that the presence of baits in Pikiariki would negatively affect bat survival. In the model (PER-121), survival rates were estimated separately for intervals when poison baits were present and when they were absent, with survival in the intervals before and after the baiting operation being the same. In the model (PER-123), separate survival rates were estimated for intervals before, during and after the operation, based on the assumption that survival before and after the operation would differ (Table 4.1). This could occur due to a lag between ingestion and the effects of exposure to poison (anticoagulants have a delayed onset) and exposure to residual poisons in the environment after removal of bait from bait stations. It was also possible that baiting would have a positive effect on bat survival through reduction in rodent densities. Rat tracking indices were measured before, during and after the baiting operation, so changes in bat survival rates associated with changes in rodent densities could be described using a model with the same structure as PER-121 or PER-123. Under either of these models, changes in rodent tracking rates could aid interpretation of results if an increase in bat survival was observed during the baiting period.

A third poison exposure model, (PER-guano), was compared after I received the results of residue testing of guano samples. In this model, separate survival rates were estimated for intervals when diphacinone residues were detected in bat guano and intervals when there was no evidence of exposure (Table 4.1). I also compared two models without a poison exposure effect, one with full time variation in estimates of survival for each interval, and the other with constant survival across all intervals. I considered additive and interactive effects between the poison exposure risk parameter and age and sex of bats. Support for any of the models including poison exposure risk would suggest unusual

survival in the intervals when poison baits were present or when diphacinone was detected in guano.

Selection of the best approximating model from the set of candidate models was based on the quasi Akaike's information criterion ($QAIC_c$), corrected for small sample size and adjusted for overdispersion. I initially reduced the encounter model using the best global survival model, then used the most parsimonious encounter model to compare the candidate survival models (Lebreton *et al.*, 1992) The best model is the one with the lowest $QAIC_c$, indicating the best compromise between model fit (low deviance) and simplicity (few parameters) (Burnham & Anderson, 2002).

Table 4.1 Treatment of survival intervals in Program MARK (Version 7.2) under different poison exposure risk (PER) models to consider the effects of a poison operation on lesser short-tailed bat (*Mystacina tuberculata*) survival in Pikiariki Ecological Area, Pureora Forest Park, New Zealand, 1 November 2013 – 2 May 2014. For each model, cells with the same number indicate intervals constrained to have the same survival probability. The same patterns of poison exposure risk were used to model the effects of the poison operation on bat prothrombin time measured in samples of blood collected from individual bats once a month for the six months spanning the eight survival intervals (Section 4.3.4.). PIT-tagging of bats occurred at the start of survival intervals 1 and 5.

	November	December	January	February	March	April		
Poison in Pikiariki								
Poison in Waipapa								
Poison in guano	✓		✓	✓		✓	✖	
Blood sampling								
Survival interval	1	2	3	4	5	6	7	8
Survival models								
PER-121	1	2	2	2	2	1	1	1
PER-123	1	2	2	2	2	3	3	3
PER-GUANO	1	1	1	1	1	1	2	2

4.3.4 Individual-level study

I captured bats in harp traps at maternity roosts in Pikiariki once a month between 4 November 2013 and 11 April 2014 (six occasions) to assess individuals for clinical evidence of exposure to diphacinone. This involved collection of blood to measure prothrombin time and diphacinone residues, and visual examination of individuals for

any overt signs of anticoagulant toxicosis. I also examined individuals and measured prothrombin time in a sample of lesser short-tailed bats from the poison-free Eglinton Valley, Fiordland site during January 2014 to serve as a control.

Prothrombin time

I measured prothrombin time of lesser short-tailed bat blood at point of collection using a CoaguChek$^{(R)}$ XS Plus coagulation monitor (Roche Diagnostics GmbH, Mannheim, Germany). Wildlife vets from Wildbase, Massey University, Palmerston North assisted with collection of a small volume of whole blood (c. 10 μl) from a sample of bats in Pikiariki each month by pricking the brachial vein with a 25-gauge needle (Smith *et al.*, 2010). Individual sampling occurred during the same weeks as guano was collected for residue analysis.

In Pikiariki blood was sampled from up to 15 individuals in each of four demographic groups each month; adult males, non-breeding adult females, breeding adult females and juveniles (from February to April only). In general, only bats with PIT tags were sampled to ensure assessment of different individuals each month. The exception was in February, when blood sampling and PIT-tagging of adults and newly volant juveniles were performed during the same week to minimise disturbance at roosts. PIT-tagging by DOC staff was allowed to proceed only after I had measured prothrombin time in a sample of untagged juveniles to assess the risk of haemorrhaging. Thereafter, to minimise stress to individuals, only untagged juveniles and adult bats with existing PIT tags had blood samples taken. Sampled juveniles were marked by fur-clipping so that they would be excluded from the PIT-tagging procedure, and to prevent resampling if they were recaptured during the February sampling week. Only juveniles that had been PIT-tagged during February were targeted for sampling in the following months.

I followed the manufacturer's instructions for performing the tests with the coagulation monitor. A two-level, quality control test is automatically performed within the test chamber of the monitor as part of every blood test. Duplicate determinations with the Coaguchek XS Plus system reported by the manufacturer showed high precision (C.V.= 2.0%) for INR (Internationalised Normalised Ratio) results of whole venous blood (n = 399 human patients). Regression statistics yielded a correlation coefficient of 0.974 for

comparison of venous whole blood INR with a standard laboratory reference (n = 811 samples from 412 human patients). The precision and accuracy of the Coaguchek X Plus system has not been tested for bat blood, but in this study measures of prothrombin time were only used for relative comparisons among individuals of the same species.

I expected that the prothrombin time of bats would be prolonged if they were exposed to diphacinone. The effect of poison exposure on prothrombin time was assessed by constructing models with the same poison exposure risk (PER) scenarios that were used to model survival probability; PER-121, PER-123 and PER-guano (Table 4.1). Under model (PER-121), I expected to observe a difference in prothrombin time among blood samples collected during the three months when baits were present in Pikiariki (December-February) and those collected in months when baits were absent (November, March and April). Under model (PER-123) I expected to observe a difference in prothrombin time based on whether samples were collected before (November), during (December-February) or after poison baits were present (March and April). I expected prothrombin time in the post-exposure months to differ from prothrombin time in the pre-exposure month under this model because bats exposed to poison in the final days of the baiting operation could show signs of toxicosis more than a week later, due to the delayed onset of the symptoms of anticoagulant poisoning. There could also be on-going exposure of bats to residual poisons in the environment after bait was removed from bait stations. Under model PER-GUANO, prothrombin time in the months when diphacinone residues were detected in bat guano samples was expected to differ from prothrombin time in months in which there was no evidence of exposure.

I considered that both the likelihood of exposure and the effects of exposure to diphacinone could vary among age, sex and reproductive classes of bats based on differences in foraging ecology (Christie & O'Donnell, 2014), energy demands (Racey & Speakman, 1987) and potential differences in susceptibility to toxins among these groups (Connell *et al.*, 2009). I also considered that differences in prothrombin time could be attributed to time of year (month), as circannual variations in the percentages of blood coagulation factors have been described in some species with seasonal activity cycles (De Wit *et al.*, 1984). Therefore, demographic group (age and sex, and reproductive status of adult females only) and time (month) were included as factors in the models.

Prothrombin time can be inversely affected by body temperature (Rohrer & Natale, 1992). In the absence of a suitable method to measure the internal body temperature of the bats, I regularly recorded air temperature in the vicinity of the sampled bats using a Kestrel (R) 3000 Weather Meter (Kestrel, Boothwyn, Pennsylvania, USA). A linear regression was performed to determine whether air temperature should be modelled as a covariate of prothrombin time. Time of day can also affect prothrombin time (Labrecque & Soulban, 1991) but as all measurements were taken at a similar time of day diurnal variations in prothrombin time were not considered in the analysis.

I developed a set of *a priori* General Linear Models in Program R (Version 3.1.1) to address hypotheses about how the factors described above could affect prothrombin time. An information-theoretic approach was used to select the best approximating model from the set of candidate models. Model selection was based on the Akaike's information criterion, corrected for small sample size (AIC_c) (Burnham & Anderson, 2002). The data were checked for normality using a one-sample Kolmogorov-Smirnov test in Program R (Version 3.1.1) which did not indicate significant departure from normality ($D_{256} = 0.078$, $P > 0.05$).

Comparison with unexposed population

The normal prothrombin time of lesser short-tailed bats is not known. I therefore measured prothrombin time in a sample of lesser short-tailed bats from the Eglinton Valley population in Fiordland (Figure 4.1) to provide reference values. The Fiordland population served as a control as there was no poison bait laid in the bats' habitat at the time of sampling due to naturally low rat densities in Fiordland during summer 2013/14. Blood samples collected from bats in Fiordland between 20-23 January were compared to samples collected from bats in Pikiariki between 3-8 February. At this time poison baits were present in Pikiariki and the bats at each site were at approximately the same stage of the breeding cycle.

I assessed whether there was a difference between the prothrombin times of bats from potentially exposed (Pikiariki) and unexposed (Fiordland) populations by conducting a one-way ANOVA. I also used ANOVA to test for differences among the four demographic groups (described above) within and between sites and used Tukey's post-

hoc pairwise comparison of means to identify specific differences. The combined prothrombin time data from the two populations were checked for normality using the Kolmogorov-Smirnov test in Program R (Version 3.1.1) which did not indicate significant departure from normality (D_{77}=0.096, P >0.05). There was homogeneity of variances of prothrombin times between sites (Bartlett's test, $X^2_{77,1}$=0.12, P=0.73), and among demographic groups within and between sites (Bartlett's test, $X^2_{77,7}$ =11.3, P=0.13).

The time that each bat was sampled was not recorded during the Fiordland sampling sessions, so the relationship between temperature and prothrombin time could not be modelled in this analysis. Instead a two-tailed t-test was used to compare mean temperature at each site. Temperature data did not differ significantly from normal (Shapiro-Wilk test, W_{35}=0.97, P=0.45) and there was equality of variance in temperatures between sites (Bartlett's test, $X^2_{35,1}$=3.74, P=0.053). Reported statistics are expressed as means ± SE.

Clinical signs of toxicosis

I assessed individual bats from Pikiariki and Fiordland populations for clinical signs of anticoagulant poisoning prior to sampling their blood. In particular, bats were visually assessed for any evidence of dried blood on or bleeding from any orifice (respiratory, oral, gastrointestinal, genitourinary tract) or PIT tag entry site; signs of bruising or subcutaneous haemorrhage on bare skin and in wing membranes, particularly around wing bones and joints; or pallor of mucous membranes (Herring & McMichael, 2012). Any abnormalities in behaviour or movement (e.g. shaking, lethargy, stiffness of movements) were also noted.

Blood diphacinone concentration

I collected an additional sample of 50-100 µl of blood from a subset of bats sampled from the Pikiariki and Fiordland populations. Each blood sample was placed on a dried blood spot (DBS) card (Whatman 903 Protein Saver Card, Sigma-Aldrich, Auckland, New Zealand) for analysis of diphacinone concentration. I tested DBS cards from the bats that had the longest prothrombin times during each month that poison was present in Pikiariki (December, January and February) and during the two months of the post-baiting period

(March and April). Four DBS samples from bats from the unexposed Fiordland population served as controls to validate the method. Analysis of diphacinone concentration in dried blood spots was determined by high performance liquid chromatography. The method detection limit was 0.05 ppm. Assays were performed by CENTOX (Centre for Environmental Toxicology) Manaaki Whenua/Landcare Research, Lincoln, New Zealand.

4.4 Results

4.4.1 Population-level study

Roost location

I found three active maternity roosts in Pikiariki during the 2012/13 field season (November-February) by inspecting 11 known roost trees for signs of occupation. The following field season, between October 2013 and April 2014 maternity colonies were found occupying five known and two previously unknown roost trees for part or all of the study period. I identified six of these roosts through radio-tracking four adult female bats and by inspection of known roost trees. DOC staff located the sixth active roost in March by radio-tracking a breeding female to the tree.

Diphacinone residue analysis

Diphacinone residues were detected in eight of the 14 bat guano samples collected from occupied maternity roosts between 26 November 2012 and 20 February 2013, while poison baits were present in Pikiariki and Waipapa (Table 4.2). Twelve of the samples were collected on a weekly basis from a single maternity roost that was occupied for the entire period. During December a second maternity roost was occupied for several weeks, and on two occasions samples were collected from both roosts on the same date. Guano could not be accessed at a third occupied roost. Four pups were found dead at the base of one maternity roost in January 2013. Diphacinone (0.029 μg/g) was detected in the liver of one of the two pups tested. The remaining two pups were too decomposed for post-mortem analysis.

Table 4.2 Diphacinone concentration (μg/g) in lesser short-tailed bat (*Mystacina tuberculata*) guano samples collected from beneath active maternity roost trees between 26 November 2012 and 20 February 2013, and 4 November 2013 and 9 April 2014, in Pikiariki Ecological Area, Pureora Forest Park. The concentration of diphacinone in tissue and invertebrate samples taken from dead bats recovered from beneath roosts is also shown. The presence or absence of diphacinone-laced baits in bait stations in Pikiariki and nearby Waipapa Ecological Areas at the time of guano sampling is indicated. Intervals pertaining to a bat survival analysis conducted in 2013/14 are shown for the corresponding months.

Guano sample collection dates	**No. days accumulated**	**Poison baits in Pikiariki**	**Poison baits in Waipapa**	**Diphacinone in guano (μg/g)**	**Diphacinone in bat liver (L) or maggots (M) (μg/g)**
2012/13 field season					
November 2012	4	✓	✓	0.51	
	3	✓	✓	1.18	
December 2012	2	✓	✓	<MDL	
	2	✓	✓	0.37 <MDL*	
	2	✓	✓	0.16 <MDL*	
January 2013	2	✓	✓	0.19	0.029 (L)
	2	✓	✓	0.33	
	2	✓	✓	<MDL	
	1	✓	✓	0.1	
February 2013	2	✓	✓	0.17	
	2	✓	✓	<MDL	
	2	✓	✓	<MDL	
2013/14 field season	**No. days pooled**				
November 2013 Survival interval #1	3	×	✓	0.32	
December 2013 Survival interval #2	3	✓	✓	0.67	
January 2014 Survival interval #3	2	✓	✓	0.52	
February 2014 Survival intervals #4 & 5	2	✓	✓	0.85	
March 2014 Survival interval #6	1	×	✓	0.04	
April 2014 Survival intervals #7 & 8	1	×	✓	<MDL†	0.21 (M)†

MDL=method detection limit

* Sample was collected from a second maternity roost on the same date as the sample above

†Guano sample was collected on 9 April, maggots on 2 April. Bait was removed from Waipapa between 7-14 April.

During the second season of guano collection (2013/14), diphacinone residues were detected in five of the six monthly samples collected from beneath occupied maternity roosts from November to April (Table 4.2). Positive detections occurred in samples collected from three different roosts during the three months when poison baits were present in Pikiariki (December-February), and also in samples collected during November (pre-baiting in Pikiariki) and March (after bait was removed from Pikiariki). Baits were present in Waipapa during all of the months when diphacinone was detected in guano. Poison was not detected in the guano sample collected in Pikiariki on 9 April 2014, but residues (0.21 μg/g) were detected in maggots collected from the carcass of a juvenile bat found at the base of a roost tree in Pikiariki one week earlier. This was approximately six weeks after bait had been removed from Pikiariki (21 February) but before baits had been removed from Waipapa (14 April). Another pup found dead at the base of a maternity roost tree in Pikiariki on 7 January 2014 was too decomposed for residue analysis. Diphacinone residues (0.16 μg /g) were also detected in the long-tailed bat guano sample pooled from bats captured in Pikiariki in January 2014.

PIT-tagging and RFID detection of bats

During the 2013/14 study period 580 individual PIT-tagged bats (206 adult males, 234 adult females, 140 juveniles) were detected by RFID aerials at roosts or by physical capture. This included 392 bats that were PIT-tagged during the study; 202 adults (61 males, 141 females) tagged between 29 October and 1 November 2013, and 50 adults (20 males, 30 females) and 140 juveniles (76 males, 64 females) tagged between 4-8 February 2014. The remaining 188 bats (125 adult males, 63 adult females) detected had been PIT-tagged prior to the study. A total of 62,419 PIT tag detections were registered on roost loggers. PIT-tagged adults were detected by RFID aerials and logged at seven monitored roosts on 172 of a possible 186 days between 29 October 2013 and 2 May 2014. PIT-tagged juveniles were logged at three roosts on 66 of a possible 88 days between 4 February and 2 May 2014. No PIT tags were registered on days when logger batteries were flat, when bats first moved to a previously unknown roost without a logger installed, and when bats switched from logged to unlogged roost exits in known roost trees.

Survival analysis

The survival analysis initially included the 580 PIT-tagged bats logged at roosts during the survival period. However, nine months after the completion of the study, DOC staff captured a bat with a protruding PIT tag, indicating that PIT tag loss could be an issue. DOC staff and I searched around the bases of seven maternity and 20 solitary roost trees using PIT tag scanners and located 52 PIT tags. A search of database records from roost data loggers revealed that 17 of these PIT tag losses occurred from the monitored sub-population of tagged bats during the survival period. Sixteen of these tags were lost from newly-tagged bats, including six during the two post-tagging intervals.

I suspected that other bats that 'disappeared' during the study (meaning that at some point they ceased to be detected on the data loggers), particularly the comparatively large number of newly-tagged bats that were last detected during the two post-tagging intervals, may also have lost PIT tags that were not recovered. However, I was unable to estimate a rate of PIT tag loss for the study population as the bats were not double-banded. To aid with reducing some of the uncertainty around patterns of PIT tag loss I searched database records for the year following the study and determined that 54 bats had only 'disappeared' (ceased to be detected during the study period) temporarily. For the remainder of the bats that had 'disappeared' (n = 82) I could not distinguish between bats that had died and bats that had lost tags that were not recovered. As 57% of the bats with unknown fates were newly-tagged bats that were last detected in the interval post-tagging, I retained the POST-TAG variable in the survival models, which allowed separate estimation of mortality (and/or PIT tag loss) rates for newly-tagged bats in the two post-tagging intervals. Tag-loss could also be viewed in terms of survival, where PIT tags either 'survived' (stayed in the bat) or 'died' (were lost). I adjusted encounter histories to reflect removal of the 17 recovered tag identities from the data set following their last recorded encounter and reran the survival analysis.

The fit of the global model was clearly improved by including a post-tagging effect on survival probability in the interval following tagging (Table 4.3) and so ϕ (a*s*t* POST-TAG), P(a*s*t) was accepted as the global model. In the process of reducing the global survival model I determined that there was strong support for retaining an interaction term to describe the effect of tagging on survival of newly-tagged adult bats as a result of sex,

so all of the reduced models included this term. The reduced encounter model with the most support included the effects of age, sex and time variation. In this model $P(a + s * t_{1=5})$, encounter probabilities were allowed to vary on each encounter occasion, except on the first and fifth occasions. On these two occasions, some bats temporarily used unlogged roost exits, resulting in fewer encounters relative to other occasions. Therefore, encounter probabilities of bats were constrained to be equal on these two occasions. This only applied to adult bats as juveniles did not enter the study until the fourth occasion. Estimated encounter probabilities varied for each age and sex class; 0.32–0.99 (mean 0.70) for adult males; 0.44-0.91 (mean 0.70) for adult females; 0.67-0.99 (mean 0.90) for juvenile males; and 0.77-0.97 (mean 0.90) for juvenile females.

Table 4.3 Comparison of global models describing factors affecting survival (ϕ) and encounter (*P*) probabilities of lesser short-tailed bats (*Mystacina tuberculata*) in Pikiariki Ecological Area, New Zealand, 1 November 2013 – 2 May 2014. Models were compared using Program MARK (Version 7.2).

Model	**Model Structure**[a,b]	**AIC$_c$**[c]	**ΔAIC$_c$**[d]	w_i[e]	**K**[f]	**Dev**[g]
Global with post-tag	ϕ (a*s*t* POST-TAG), *P*(a*s*t)	3828.73	0	0.9999	48	660.26
Global	ϕ (a*s*t), *P*(a*s*t)	3847.07	18.34	0.0001	44	686.90

[a] Model notation: a = age (adult or juvenile); s = sex (male or female); t = time (encounter occasion); Post-tag = survival of newly-tagged bats different in the interval following PIT-tagging; * interactions between factors as well as additive effects; [c] Akaike's Information Criterion corrected for small sample size and overdispersion ($\hat{c}$=1.27); [d] differences in AIC$_c$ value from the best model in the candidate set; [e] Akaike's weights, indicating relative support for the models; [f] number of parameters in model; [g] deviance

The top two survival models accounted for >70% of cumulative QAIC$_c$ weights (w_i) (Table 4.4). The top-ranked model (POST-TAG) included the effects of sex and age, and a post-tagging effect for newly-tagged bats, but no effect for poison exposure risk. The second, third and fourth ranked models differed from the top-ranked model only by the inclusion of poison exposure risk. The second ranked model (POST-TAG + PER-121) had competing support for the data (ΔQAIC$_c$ = 0.65) and the third and fourth ranked models, (POST-TAG + PER-GUANO and POST-TAG + PER-123 respectively) also had some support (ΔQAIC$_c$ < 3). However, the inclusion of PER has not improved the fit of the models to the data. The best model without PER has 20 parameters, whereas the top PER model has 21

Table 4.4 The top four models, as well as the global survival model, describing factors affecting survival (ϕ) and encounter (*P*) probabilities of PIT-tagged adult and juvenile lesser short-tailed bats (*Mystacina tuberculata*) in Pikiariki Ecological Area, New Zealand, before, during and after a rodent control operation using diphacinone-laced cereal pellet baits in bait stations,1 November 2013 – 2 May 2014. Models were compared using Program MARK (Version 7.2).

Model name	**Model Structure**[a,b]	**QAIC$_c$**[c]	**ΔQAIC$_c$**[d]	***w$_i$***[e]	**K**[f]	**QDev**[g]
POST-TAG	ϕ (a + s + post-tag) + (s_{ad} * post-tag), *P*	2917.49	0	0.41	20	524.72
POST-TAG + PER-121	ϕ (a + s + post-tag + PER-121) + (s_{ad} * post-tag), *P*	2918.14	0.65	0.30	21	523.33
POST-TAG + PER-GUANO	ϕ (a + s + post- tag + PER-GUANO) + (s_{ad} * post-tag), *P*	2919.52	2.03	0.15	21	524.71
POST-TAG + PER-123	ϕ (a + s + post-tag + PER-123) + (s_{ad} * post-tag), *P*	2919.65	2.16	0.14	22	522.81
GLOBAL ϕ, REDUCED *P*	ϕ (a * s * t * post- tag), *P*	2945.07	27.57	0	39	513.39

[a] Model notation: a = age (adult or juvenile); s = sex (male or female); t = time (encounter occasion); Post-tag = survival in the following interval affected by PIT-tagging; PER-*i* = survival affected by different patterns of poison exposure risk (PER-121: Survival is the same in pre- and post-poison intervals, and different in intervals when poison is laid in Pikiariki; PER-123: survival differs in intervals before, during and after bait is laid; P-GUANO, survival is affected in intervals when poison is detected in bat guano; * interactions between factors as well as additive effects; +, additive effects only, ad = effects acting on adults only. [b] $P = P\ (a + s * t_{1=5})$ Detection model; $t_{1=5}$: detection probabilities constrained to be the same on the first and fifth encounter occasions; [c] Quasi Akaike's Information Criterion corrected for small sample size and overdispersion ($\hat{c}$=1.27); [d] differences in QAIC$_c$ value from the best model in the candidate set; [e] Akaike's weights, indicating relative support for the models; [f] number of parameters in model; [g] deviance.

parameters. The difference in the number of parameters explains most of the difference in the AIC*c* values between the top-ranked model and PER models, indicating negligible effect of PER (Burnham & Anderson, 2002).

In the top-ranked model (POST-TAG) monthly survival of bats with existing tags was constant over time within each demographic group (Figure 4.3). In the two models where the risk of poison exposure related to the presence of baits in Pikiariki (POST-TAG + PER-121 and POST-TAG + PER-123) survival for each demographic group was lower in the risk period than in the non-risk periods. In the model where poison exposure risk was defined by the presence of diphacinone in guano (POST-TAG + PER-GUANO) survival was higher in the risk period than in the non-risk period. Under all four models, survival was higher for adults than juveniles, and higher for females than males. Survival in the interval following tagging was lower for newly-tagged adult bats compared to those with existing tags in the same demographic group. This comparison was not possible for juveniles.

Where the model selection process indicates ambiguity in model choice, $QAIC_c$ weights can be used to compute weighted averages of parameter estimates across all the models considered so that model selection uncertainty can be accounted for. However, averaging models is not recommended when competing models have differing interpretations with regards to explanatory variables of primary interest (Burnham & Anderson, 2002). As the top-ranked and competing models in this analysis differed only by the inclusion or omission of the effect of poison exposure, I did not consider it informative to derive parameters that averaged contrasting effects. Instead, for each of the three models that included poison exposure risk I calculated the percentage additional mortality for adult bats with existing tags during the period of poison exposure risk, relative to survival probabilities for these groups during non-risk periods (Table 4.5). This was calculated using $1 - \hat{S}_P/\hat{S}_E$, where $\hat{S}_P$ is survival probability during poison exposure risk intervals and $\hat{S}_E$ is the expected survival probability, based on intervals without poison exposure risk. Standard errors for the estimates of additional mortality were calculated using the delta method which is used to find the variance of a function of two or more variables (Seber, 1982). Similar estimates of additional mortality could not be made for juveniles

under these models, as the effects of both poison exposure risk and PIT-tagging occurred to all juveniles in the same interval, so these effects could not be separated.

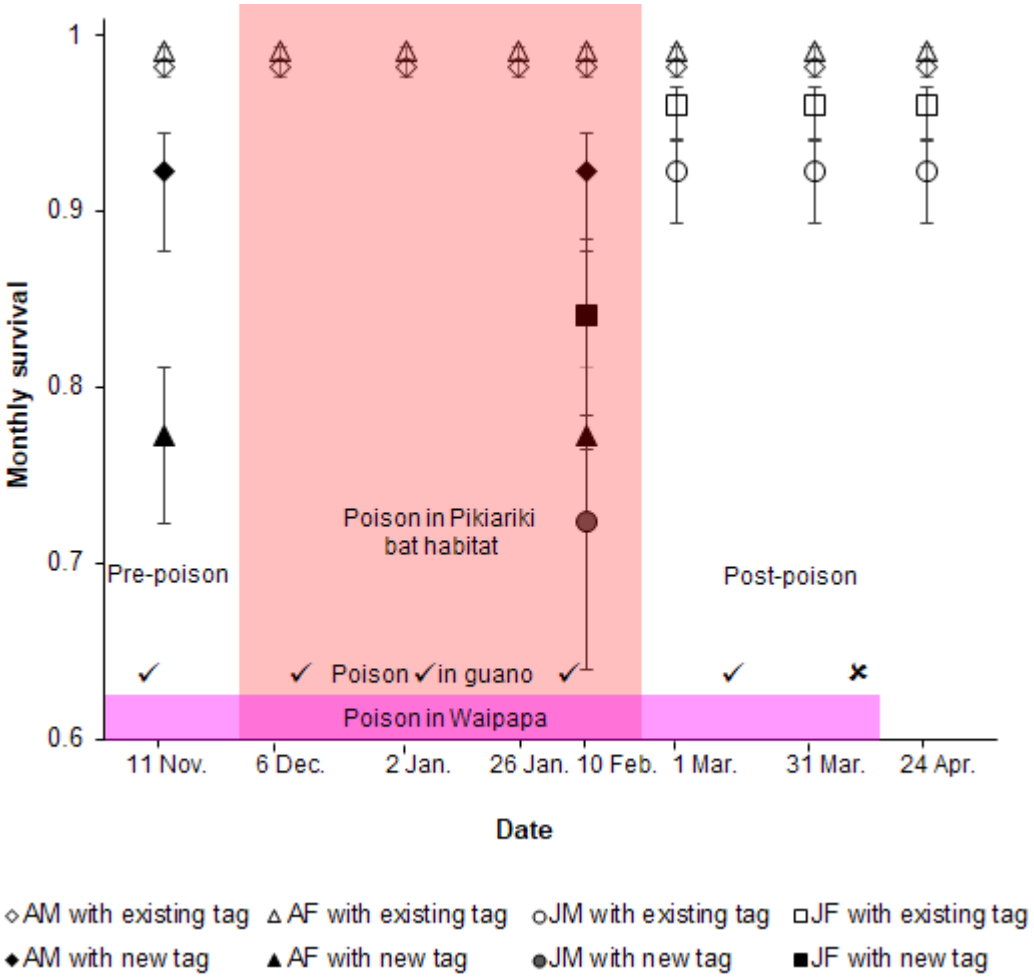

Figure 4.3 Monthly survival rates (± SE) of lesser short-tailed bats (*Mystacina tuberculata*) (*n*=580) before, during and after a rodent control operation using diphacinone-laced baits in bait stations in Pikiariki Ecological Area, Pureora Forest Park, New Zealand, 1 November 2013 - 2 May 2014. AM = adult males; AF = adult females; JM = juvenile males; JF = juvenile females. Markers are shown at the mid-point of each of eight time-intervals. Juveniles entered the study in the fifth interval. Adults and juveniles PIT-tagged during the study (new tags) became animals with existing tags in the second interval following their tagging. Survival rates are from the top ranked model in Table 4.4 and were estimated using Program Mark (Version 7.2). Periods when poison baits were present in Pikiariki where bats roost and nearby Waipapa Ecological Area are indicated by shaded areas as labelled. Approximate dates that bat guano tested positive (✓) or negative (×) for diphacinone residues are indicated.

Survival of adult males with existing tags was reduced by <3% during the 86-day poison exposure period under the second ranked model PER-121 + POST-TAG. For adult females with existing tags survival under the same model survival was reduced by <2% for this period. Similar reductions in survival were estimated for adult males (< 4%) and adult females (< 2 %) with existing tags for the same period under the fourth-ranked model PER 123 + POST-TAG. In the third ranked model, PER-GUANO + POST-TAG, estimated additional mortality was negative, indicating that there was a small (< 1%) increase in survival probability of both adult males and females with existing tags during the 137-day period of poison exposure risk defined by detection of residues in guano. However, 95% confidence intervals for all of the estimates spanned zero, indicating that none of these results were significant (Table 4.5). Rat tracking rates in Pikiariki were 22% in November, 10% in January and 22% in April. Although the Department of Conservation's target of ≤5% rat tracking rate was not achieved during the peak of the breeding season, some improvement in survival rates might have been expected during this period.

Table 4.5 Estimated percentage additional mortality of adult lesser short-tailed bats (*Mystacina tuberculata*) in Pikiariki Ecological Area, Pureora Forest Park, New Zealand, during the period of risk of exposure to diphacinone-laced baits laid in their habitat, 25 November 2013 - 21 February 2014. Estimates are based on survival rates for bats with existing PIT tags estimated under three poison exposure risk (PER) models in Program MARK (Version 7.2). Model abbreviations and structures as described in Table 4.4.

Model	Duration of PER period in model (Days)	% additional mortality (95% C.L.) of bats with existing tags during PER period	
		Adult males	Adult females
PER-121+POST-TAG	86	2.6 (-1.9, 7.2)	1.2 (-0.9, 3.4)
PER-GUANO +POST-TAG	137	-0.3 (-8.2, 7.6)	-0.2 (-4.1, 3.8)
PER-123 + POST-TAG	86	3.5 (-0.9, 7.9)	1.7 (-0.5, 3.8)

The additional apparent mortality of newly PIT-tagged individuals in the post-tagging intervals could also have been due to the loss of tags, but I was unable to distinguish between the mortality and tag-loss. Estimating survival of newly-tagged bats separately

in these intervals reduced the influence of this unanticipated problem on survival estimates for unaffected bats. The additional 'mortality' (i.e. death and/or PIT tag loss) of newly-tagged adult bats in the two post-tagging intervals was calculated using $1 - \hat{S}_T/\hat{S}_E$, where $\hat{S}_T$ is 'survival' probability of newly-tagged bats during the intervals post-tagging and $\hat{S}_E$ is the survival probability of bats with existing tags during the same intervals. Under the top-ranked model and each of the three poison exposure risk models, the additional 'mortality' of newly-tagged adult females during the 33-days of the two combined post-tagging intervals was >20% (Table 4.6). The additional tagging-related 'mortality' rates for adult males for the same period under each model were much lower (<7%) and 95% confidence intervals for estimates spanned zero, indicating that results for males were not significant (Table 4.6). For juveniles, I was unable to estimate additional 'mortality' of newly-tagged bats due to the tagging effect or additional mortality due to poison exposure risk, as these effects could not be separated for juveniles.

Table 4.6 Estimated percentage additional mortality of newly PIT-tagged adult lesser short-tailed bats (*Mystacina tuberculata*) in Pikiariki Ecological Area, Pureora Forest Park, New Zealand, during the post-tagging intervals in November 2013 and February 2014. Estimates are based on survival rates estimated under the top-ranked (POST-TAG) model and three poison exposure risk (PER) models in Program MARK (Version 7.2). Model abbreviations and structures as described in Table 4.4.

Model	Total duration of two post-tagging intervals (Days)	% additional mortality (95% C.L.) of newly-tagged bats in post-tagging intervals	
		Adult males	Adult females
POST-TAG	33	6.6 (-0.3, 13.1)	23.9 (13.8, 32.8)
PER-121+POST-TAG	33	3.8 (-2.9, 10)	23 (13.1, 31.8)
PER-GUANO +POST-TAG	33	6.8 (-0.9, 13.8)	23.9 (13.9, 32.8)
PER-123 + POST-TAG	33	3.7 (-2.9, 9.9)	22.4 (12.4, 31.2)

The annual survival rate of adult bats from the Pikiariki population was extrapolated from estimated monthly survival rates under the top-ranked model, with standard errors calculated using the delta method (Seber, 1982). The annual survival rate calculated for adult females from Pikiariki was within the range of annual survival rates measured for adult females in the Fiordland population in a non-mast year when predators were naturally low and in a mast-year when predator irruptions were supressed to low levels

using poison baits (M. Pryde, DOC unpub. data, 2015; Thakur *et al.*, 2017). The annual survival rate calculated for adult males from Pikiariki was lower than annual survival rates measured for adult males in the Fiordland population in these years (M. Pryde, DOC unpub. data, 2015), but each rate was contained within the 95% confidence interval of the other two rates for males, suggesting that there was no difference between these estimates (Figure 4.4).

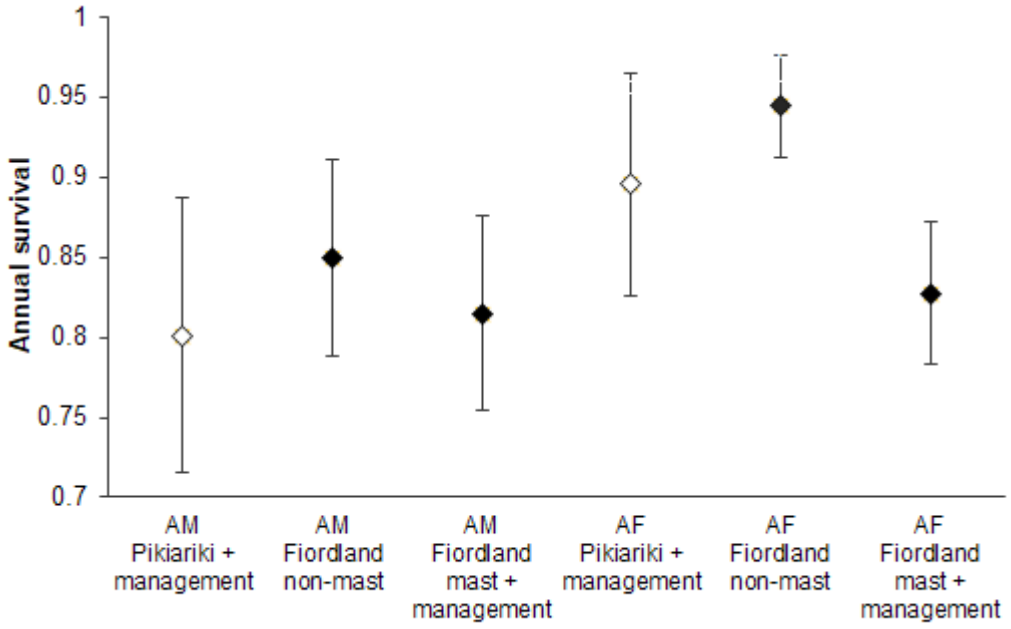

Figure 4.4 Comparison of extrapolated annual survival rates (± 95% confidence intervals) of adult male and female lesser short-tailed bats (*Mystacina tuberculata*) from the Pikiariki Ecological Area, North Island population and the Eglinton Valley, Fiordland, South Island population. Pikiariki rates were extrapolated from monthly survival rates estimated using Program Mark (Version 7.2) to model mark-recapture data collected between 1 November 2013 and 2 May 2014. Fiordland rates are from annual estimates made between 2008-2015 using mark-recapture methods (M. Pryde, DOC unpub. data; Thakur et *al.*, 2017). AM = adult males; AF = adult females. Mast = year with heavy synchronised seeding of southern beech (*Lophozonia menziesii* and *Fuscospora* spp.) leading to high predator numbers; Non-mast = year when predator numbers are naturally low; Management = predator control using pesticides.

4.4.2 Individual-level study

Prothrombin time

I measured the prothrombin time of blood samples from 256 bats (81 breeding adult females, 44 non-breeding adult females, 83 adult males and 48 juveniles) from the Pikiariki population. An estimated 28% of the total adult population was sampled, based on a pre-breeding population size estimate done in November 2013 (Chapter 5). Samples were collected monthly over the 6-month sampling period (November 2013 to April 2014). Preliminary analysis showed that there was a small but significant negative correlation ($R = -0.191$; $P < 0.05$) between air temperature and prothrombin time. Temperature was therefore included as a covariate in all of the models that were compared to assess factors affecting variation in prothrombin time.

The model that best described variation in prothrombin time included the effects of temperature and demographic group (TEMP. + DEM. GROUP) but no effect of poison exposure risk (Table 4.7; Figure 4.5). The second-, third- and fourth-ranked models differed from the top-ranked model only by the inclusion of the effect of poison exposure risk. In the second-ranked model (TEMP. + DEM. GROUP + PER-GUANO) the effect of poison exposure risk was based on detection of diphacinone residues in guano. In the third-ranked model (TEMP. + DEM. GROUP + PER-121) the effect of poison exposure risk was based on the presence of poison baits in the bats' habitat. Both of these models had competing support for the data ($\Delta AIC_c < 2$). However, the inclusion of PER has not improved the fit of the models to the data. The best model without PER has 6 parameters, whereas the top PER model has 7 parameters. The difference in the number of parameters explains most of the difference in the AIC*c* values between the top-ranked model and PER models, indicating negligible effect of PER (Table 4.7, Figures 4.6 & 4.7). The fourth-ranked model ($\Delta AIC_c > 3$) differed from the third ranked model only by distinction of the pre- and post-poison periods (TEMP. + DEM. GROUP + PER-123). In the fifth ranked model, time (month), not poison exposure risk was included as an explanatory variable, as well as demographic group. There was virtually no support for month as a factor explaining variation in prothrombin time (Table 4.7, Figure 4.8). Together, the top five models accounted for 99% of cumulative AIC_c weights (w_i). Models that did not include the effect of demographic group were not supported (Table 4.7). (Burnham & Anderson, 2002).

Table 4.7 Comparison of general linear models describing factors affecting prothrombin time of blood samples collected from 256 lesser short-tailed bats (*Mystacina tuberculata*) in Pikiariki Ecological Area, Pureora Forest Park, New Zealand. Samples were collected monthly between November 2013 and April 2014, before, during and after a rodent control operation using diphacinone-laced cereal pellet baits in bait stations. Models were fitted using Program R (Version 3.1.1).

Model[a]	**AIC$_c$**[b]	**ΔAIC$_c$**[c]	**w_i**[d]	**K**[e]	**LL**[f]
TEMP.+DEM. GROUP	1074.85	0.00	0.45	6	-531.25
TEMP.+DEM. GROUP +PER GUANO	1076.45	1.60	0.20	7	-531.00
TEMP.+DEM. GROUP +PER-121	1076.64	1.80	0.18	7	-531.10
TEMP.+ DEM. GROUP +PER-123	1078.11	3.26	0.09	8	-530.76
TEMP.+DEM. GROUP + MONTH	1078.62	3.77	0.07	11	-527.77
TEMP.	1088.72	13.87	0.00	3	-541.31
TEMP.+PER-121	1090.4	15.55	0.00	4	-541.12
TEMP.+MONTH	1090.86	16.01	0.00	8	-537.14
TEMP.+PER-123	1092.34	17.49	0.00	5	-541.05

[a] Model notation; Temp. = air temperature at sampling station during sample collection; Dem. Group, demographic group (breeding adult females; non-breeding adult females; adult males; juveniles); Month = consecutive months of blood testing (Nov.2013 to Apr. 2014); PER-*i* = survival affected by different patterns of poison exposure risk (PER-121: Survival is the same in intervals pre- and post- the poison operation, and different in intervals when poison bait is laid in Pikiariki; PER-123: survival differs in intervals before, during and after the poison operation; PER-GUANO, survival is different in intervals when poison is detected in bat guano); [b] AIC$_c$, Akaike's Information Criterion corrected for small sample size; [c]ΔAIC$_c$, differences in AIC$_c$ value from the best model in the candidate set; [d] w_i, Akaike's weights indicating relative support for the models; [e]K = number of parameters in model; [f] LL = log likelihood.

Post-hoc analysis of the top-ranked model revealed that mean prothrombin time of adult males from the Pikiariki population was longer than mean prothrombin time of each of the other demographic groups. The differences were small but significant; ANCOVA with Bonferonni-corrected between-pairs comparisons of adjusted mean prothrombin times of adult males (18.85 ± 0.21, n=83) and breeding adult females (17.89 ± 0.22, n=81, P <0.05); adult males and non-breeding adult females (17.59 ± 0.30, n=44, P <0.01); and adult males and juveniles (17.5 ± 0.30, n=48, P <0.01) (Figure 4.5).

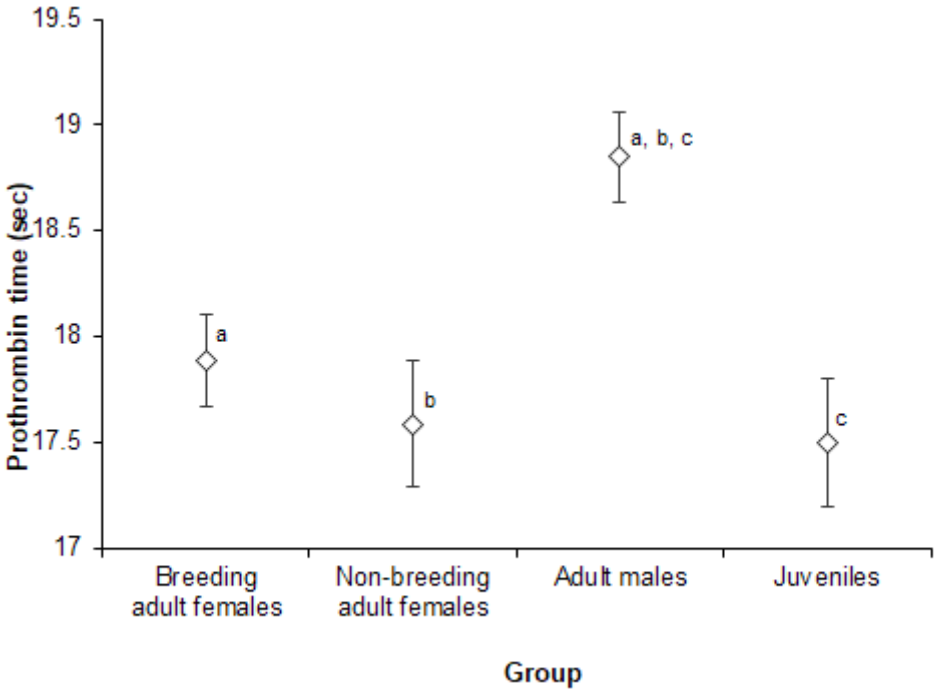

Figure 4.5 Prothrombin time (mean sec. ± SE) of lesser short-tailed bats (*Mystacina tuberculata*) (*n*=256) from four demographic groups in Pikiariki Ecological Area, Pureora Forest Park, New Zealand under the top-ranked model TEMP + DEM GP in Table 4.7. The means were calculated from monthly measurements taken for six months for adults (November 2013 - April 2014) and for three months for juveniles (February - April 2014), before, during and after a rodent control operation using diphacinone-laced cereal pellet baits in bait stations. Prothrombin times have been adjusted for the effects of temperature. Differences between means that share a letter are statistically significant based on post-hoc tests.

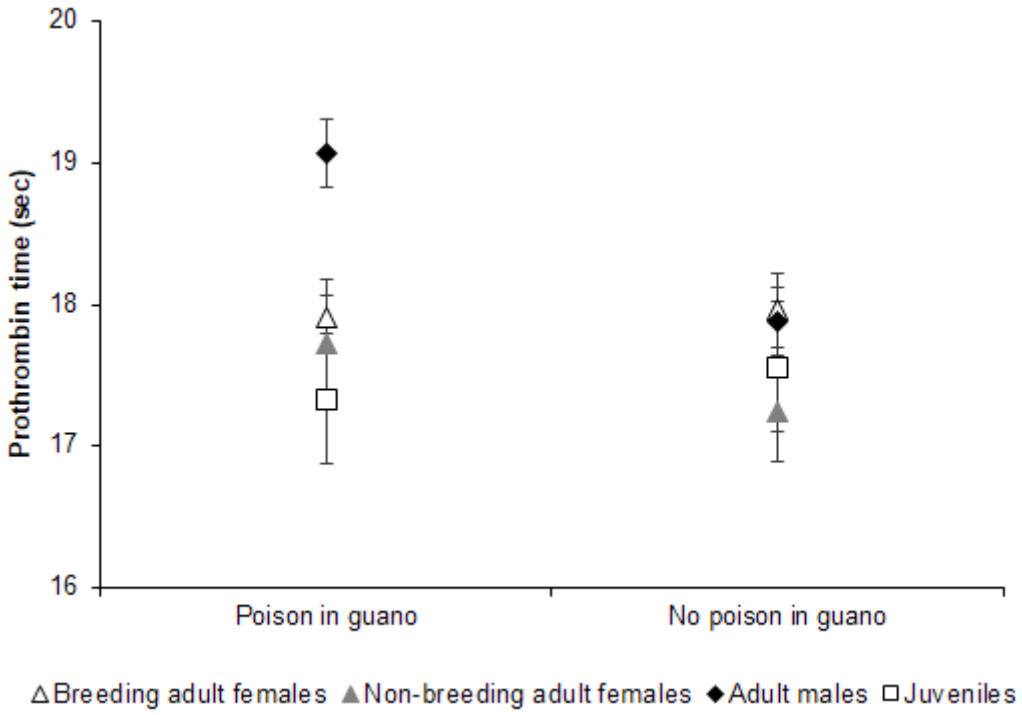

Figure 4.6 Prothrombin time (mean sec. ± SE) of lesser short-tailed bats (*Mystacina tuberculata*) (*n*=256) from four demographic groups in Pikiariki Ecological Area, Pureora Forest Park, New Zealand, under the model PER-GUANO in Table 4.7. Means were calculated from monthly measurements for the period when poison residues were detected in guano (November 2013 - March 2014 for adults, and February - March 2014 for juveniles) and for the period when no residues were detected (April 2014). A rodent control operation using diphacinone-laced cereal pellet baits in bait stations took place in Pikiariki from December 2013 to February 2014, and in nearby Waipapa Ecological Area from August 2013 to early April 2014. Prothrombin times have been adjusted for the effects of temperature.

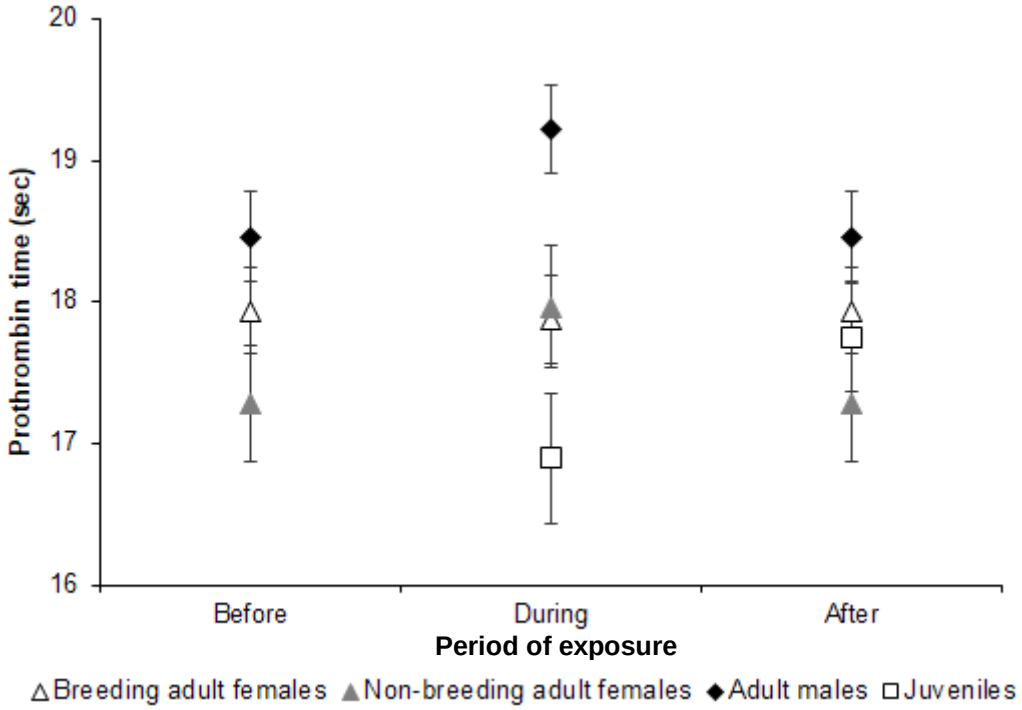

Figure 4.7 Prothrombin time (mean sec. ± SE) of lesser short-tailed bats (*Mystacina tuberculata*) (*n*=256) from four demographic groups in Pikiariki Ecological Area, Pureora Forest Park, New Zealand under the model PER-121 in Table 4.7. Means are calculated from monthly measurements for the periods before (November 2013), during (December 2013 - February 2014) and after (March - April 2014) a rodent control operation in Pikiariki using diphacinone-laced baits in bait stations. Juveniles entered the study in February. Prothrombin times have been adjusted for the effects of temperature.

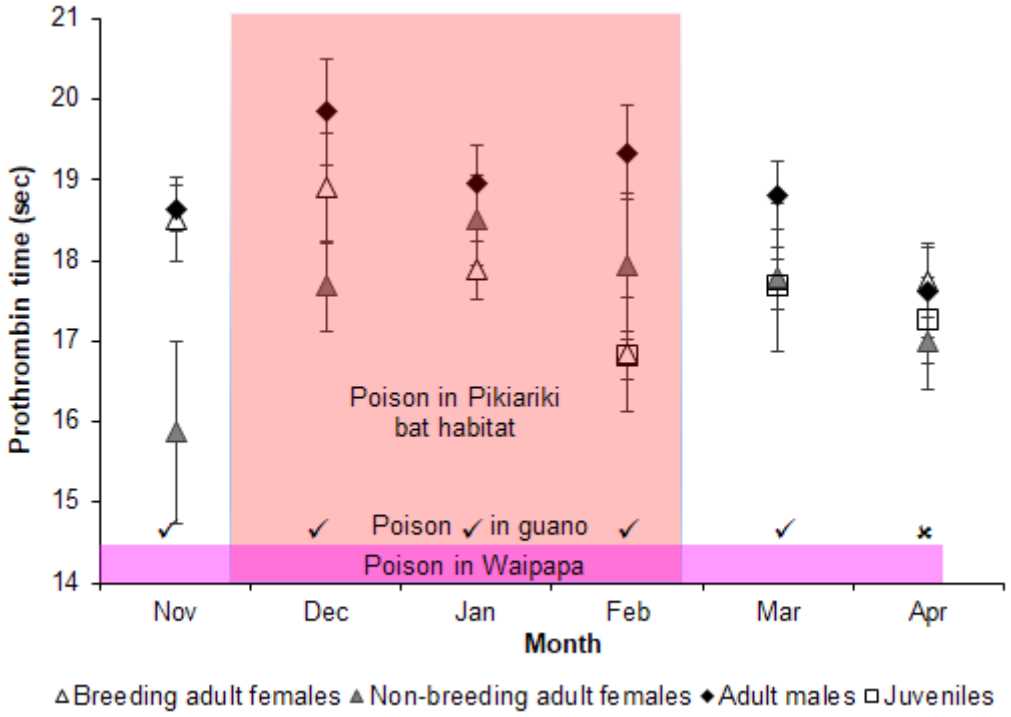

Figure 4.8 Prothrombin time (mean sec. ± SE) of lesser short-tailed bats (*Mystacina tuberculata*) (*n*=256) from four demographic groups in Pikiariki Ecological Area, Pureora Forest Park, New Zealand under the model PER-MONTH in Table 4.7. The means were calculated from monthly measurements taken for six months for adults (November 2013 - April 2014) and for three months for juveniles (February - April 2014), before, during and after a rodent control operation using diphacinone-laced cereal pellet baits in bait stations. Periods when poison baits were present in Pikiariki where bats roost and nearby Waipapa Ecological Area are indicated by shaded areas as labelled. Approximate dates that guano tested positive (✓) or negative (×) for diphacinone residues are shown above the x-axis.

Comparison of prothrombin times of bats from the population exposed to diphacinone (Pikiariki) with bats from the unexposed population (Fiordland) gave no support to exposure having any clinical effects (Figure 4.9). The mean prothrombin time of bats from Pikiariki (17.65 ± 0.32 sec., *n*=44) assessed in early February 2014 when baits were deployed in their habitat, was significantly shorter than the mean prothrombin time of bats from the non-treatment site in Fiordland (19.49 ± 0.39 sec., *n*=33) assessed during late January 2014 (ANOVA: $F_{1,75}$=13.94, P <0.001). This difference can be attributed to differences in mean prothrombin times between particular demographic groups at each site (ANOVA: $F_{7,69}$ =5.37, P <0.001). Mean prothrombin times of breeding adult females (16.84 ± 0.71, *n*=10) and juveniles (16.82 ± 0.30, *n*=18) from Pikiariki were significantly shorter than mean prothrombin times of breeding adult females from Fiordland (19.84 ± 0.49, *n*=15 Tukey's test; Q=5.3, P <0.01 and Q=6.24, P < 0.001 respectively) and of mean

prothrombin times of adult males from Fiordland (20.68 ± 1.42, *n*=6, Tukey's test: Q=5.38, P <0.01 and Q=5.92, P <0.005 respectively). The mean prothrombin time of juveniles from Pikiariki was also significantly shorter than that of adult males from Pikiariki (19.35 ± 0.59, *n*=13 Tukey's test: Q=5.01, P <0.05) in February. There was no significant difference between the mean prothrombin times of adult males from Pikiariki and adult males from Fiordland during the months compared (Tukey's test: Q=0.85, P=0.86).

As I was unable to assess the relationship between temperature and prothrombin time for the Fiordland data, I instead compared mean temperature (°C ±SE) at each site, based on hourly temperature readings taken each night during the sampling sessions. Mean temperature was significantly lower during the sampling sessions in Fiordland in January (11.87 ±0.39 *n*=10) than during the sampling sessions in Pikiariki in February (14.36 ±0.45, *n*=25, *t*(33)=3.27, P < 0.01).

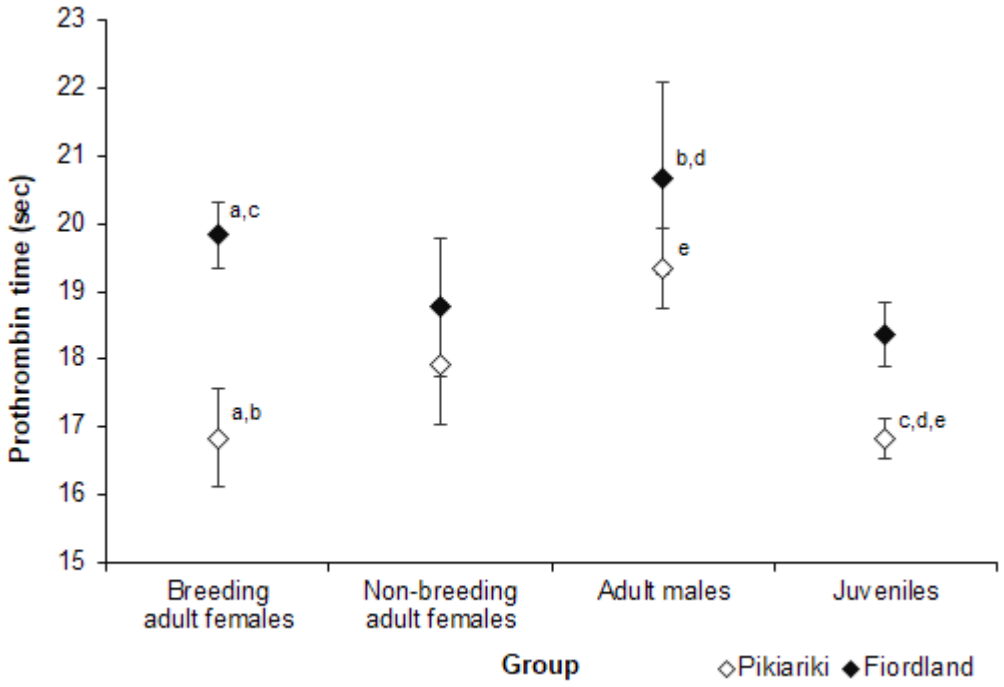

Figure 4.9 Prothrombin time (mean sec. ± SE) of lesser short-tailed bats (*Mystacina tuberculata*) from the Eglinton Valley, Fiordland (20 - 23 January 2014; *n*=35) and Pikiariki Ecological Area, Pureora Forest Park, New Zealand (3 - 8 February 2014; *n*=46). Diphacinone-laced cereal pellet baits for rodent control were present in bait stations in Pikiariki at the time of measurement but not in Fiordland. Differences between means that share a letter are statistically significant.

Clinical signs of toxicosis

All but two of the 256 bats in the Pikiariki population that were visually assessed were free of clinical signs of anticoagulant toxicosis. The exceptions were one non-breeding adult female assessed in December and one adult male assessed in April that both had pale mucous membranes. The prothrombin times of these bats (17.3 and 17.2 seconds respectively) were similar to the averages for their respective demographic groups (Figure 4.5) and there were no other clinical signs that would indicate a coagulopathy. Both of the bats were detected by loggers at roosts on multiple occasions after sampling, with the latest detection of each on 2 May 2014, the final day of the study.

Blood diphacinone concentration

Twenty DBS cards selected for analysis represented samples from bats in the Pikiariki population with the longest prothrombin times (mean 20.24 $\pm$ 0.61, *n*=20; range 14.8 - 26.9 seconds) in each month that poison was laid in the bats' habitat (December, January and February) and for the two months of the post-poison period (March and April). The concentration of diphacinone in the blood was below the method detection limit (0.05 ppm) for the 20 samples tested.

4.5 Discussion

Outcome of baiting trial

The use of cereal pellet baits enclosed in bait stations did not prevent exposure of lesser short-tailed bats to diphacinone during two rodent control operations in their roosting habitat during 2012/13 and 2013/14 as evidenced by residues of diphacinone detected in communal guano deposits. These residues were detected during the two periods of bait deployment, providing evidence of population-level exposure. Detection of residues in a dead pup in 2013 and in a dead juvenile in 2014 provided further confirmation of exposure. The risk of exposure for the bats extended over a larger landscape scale than considered when planning the bait trial, as residues were detected in guano during periods when toxic baits were absent from the forest remnant where the bats roosted (Pikiariki) but present in a nearby forest remnant (Waipapa) within the wider Pureora Forest Park. However, despite evidence of exposure, mark-recapture survival analysis and monthly measurements of prothrombin time carried out during the second year of the study

suggested that exposure of the bats to diphacinone was subclinical. The tested baiting method, therefore, considerably reduced the risk of extensive lesser short-tailed bat mortalities resulting from diphacinone exposure, such as those observed in 2009 (Dennis & Gartrell, 2015; Chapter 2) but did not prevent the sublethal exposure of bats.

Population-level exposure

At the time that my study was designed there was very limited evidence that bats from Pikiariki foraged in Waipapa. Bats had been identified foraging around the boundaries of Waipapa on only two occasions and acoustic surveys in the forest interior had detected low pass rates (Wallace, 2006; Toth *et al.*, 2015a: T. Thurley, pers. comm., 2015) so I did not expect to find evidence of exposure in the population in the periods when baits were absent from Pikiariki but present in Waipapa. Waipapa is easily within foraging range of Pikiariki, based on home-range size and commuting distances of lesser short-tailed bats studied in the Eglinton Valley, Fiordland (O'Donnell *et al.*, 1999; Christie, 2006; Christie & O'Donnell, 2014). Individual bats in the Eglinton Valley commuted large distances (mean 18.9 km) between roosting and one or more foraging areas in contiguous native forest and occasionally crossed areas of grassland up to 2 km wide (O'Donnell *et al.*, 1999; Christie & O'Donnell, 2014). In contrast, Toth *et al*. (2015a) found that bats in the Pikiariki population commuted shorter distances and had smaller foraging ranges. More recently, Bennett (2019) found that some lesser short-tailed bats commuted through forest patches and farmland between Pikiariki and Waipapa, but she did not determine their activity within Waipapa.

Testing guano for diphacinone residues has proved an effective, non-invasive method for confirming population-level exposure that does not require bat capture. However, the variation in diphacinone concentrations detected during the different sampling periods cannot be used to infer corresponding levels of individual exposure in the population or the number of bats affected. To illustrate, a sample accumulated over four days during November 2012 had less than half the diphacinone concentration of a sample accumulated over three days at the same roost later the same week. Factors such as the number of bats using a roost, retention time of guano inside roosts and weather conditions leading up to guano collection may have affected the concentration of residues in the guano deposits outside of roosts. Nevertheless, there may be plausible explanations for the results of the March and April tests, when the concentration of diphacinone in guano was low (March)

or below the detection limit (April), even though baits were still present in Waipapa. These results could reflect depleted bait supplies and reduced bait toxicity late in the season, seasonal changes in dietary preferences of bats (Arkins *et al.*, 1999; Czenze *et al.*, 2018), or changes in bat foraging ranges associated with different stages of the breeding cycle (O'Donnell, 2001b; Christie & O'Donnell, 2014).

Clinical effects of exposure

Monthly assessments of prothrombin time provided no evidence of a clinical effect of diphacinone in bats, despite the detection of residues in guano indicating that individuals in the Pikiariki population had been exposed. There was no significant difference in the mean prothrombin times of bats in each age- and sex-class in the months before (November), during (December-February) or after (March-April) toxic baits were present in Pikiariki, nor between the periods when diphacinone was detected in guano (November-March) and when it was not (April). Comparison of monthly mean prothrombin times for each demographic group did not reveal any significant patterns that might be associated with seasonal variation as has been described in other mammals with seasonal cycles of activity (De Wit *et al.*, 1984). The longer mean prothrombin time of adult males compared to other demographic groups was not pronounced enough to be consistent with a clinical coagulopathy. In rodents, clinically effective poisoning with anticoagulants is indicated when prothrombin times increase by 3.6x or more (Garg & Singla, 2015). I suggest the most likely interpretation of these results is that there was a sex difference in prothrombin time between the adult bats. Similar sex-related differences in prothrombin times have been observed in other mammal species (e.g. Siroka *et al.*, 2011).

The presence of diphacinone residues in guano in the months outside of the baiting period in Pikiariki (November and March), presumably due to bats foraging in Waipapa, confounded comparison of prothrombin times for discrete periods before, during and after poison exposure. Nevertheless, if exposure had produced a clinical effect on prothrombin time, some differences in mean clotting times among individuals might have been expected within these two months, as not all bats sampled would have foraged in Waipapa (Toth *et al.*, 2015a). In the Eglinton Valley, Fiordland, adult male long-tailed bats, juveniles older than two weeks and post-lactating females had larger foraging ranges than

lactating and non-breeding females and younger juveniles (O'Donnell, 2001b). In contrast, Christie & O'Donnell (2014) determined that in a sympatric population of lesser short-tailed bats adult males had the smallest foraging ranges, but bats were only monitored during late summer/early autumn when breeding males frequent roosts close to maternity colonies for mating opportunities (Toth *et al.*, 2015b). Further radio-tracking studies are needed to determine whether particular demographic groups from the Pikiariki lesser short-tailed bat population are more likely to forage in Waipapa.

As reference values for lesser short-tailed bat prothrombin times were not available, it was important to compare values measured in the Pikiariki population with clotting times of healthy bats from an unexposed population. This comparison provided further confirmation that there was no measurable effect of diphacinone exposure on prothrombin times in the Pikiariki population. In fact, the mean prothrombin times of adult males and breeding adult females in Fiordland during late January were statistically significantly longer than mean prothrombin times of juveniles and breeding adult females in Pikiariki during early February. This difference in coagulation times is most likely due to thermal effects on coagulation physiology. Based on the negative correlation between temperature and prothrombin time observed in the Pikiariki population this effect was likely to be small. To confirm this theory, we would have needed to more accurately record and model the cooler Fiordland temperatures as a covariate of prothrombin time for the Fiordland population. As a matter of interest, there was no significant difference between mean prothrombin times of adult males in Pikiariki and Fiordland.

Only two of the bats assessed in Pikiariki showed any of the clinical signs that might be associated with anticoagulant poisoning. In both cases, this finding was a subjective assessment of pallor of mucous membranes, used here as a gross indicator of anaemia. One of these cases was in April, during the only sampling period when diphacinone residues were not detected in guano. The prothrombin times of each of these bats was similar to the average for their respective demographic groups; neither was indicative of a coagulopathy, and logging data showed that both bats survived the entire study period. Mucous membrane pallor is not specific to anaemia and can also be due to a number of other factors including illness, dehydration, shock and stress (Ohad, 2017).

Diphacinone could not be detected in individual lesser short-tailed bat blood samples, despite guano analysis confirming evidence of population exposure during the same week that blood was taken in all months except April 2014. Diphacinone was also detected in a pup's liver in 2013 and in maggots from a pup in 2014 confirming that guano residues were not a result of contamination from other species. Individual bats may have had blood concentrations of diphacinone below the minimum detectable limit of the DBS test, or the volume of blood collected for each sample may have been inadequate. Alternatively, the exposure of bats within the population could have been uneven and DBS sampling may have been too insensitive to detect a low prevalence of exposure. It is also possible that the method requires further validation. DBS analysis has previously been used to measure the concentration of the anticoagulant brodifacoum in domestic chicken (*Gallus gallus*) blood (Fisher, 2009) but it has not previously been used in bats. Fisher (2009) was only able to detect residues within four days of dosing chickens, but this is unlikely to have been a limitation in my study, given the coincident detection of diphacinone residues in guano at the time of blood sampling in all but one month.

Effect of exposure on survival

Analysis of survival of bats through the 2013/14 baiting operation in Pikiariki indicated that there was zero to negligible effect of exposure to diphacinone on survival of bats with existing PIT tags over the 6-month monitoring period. The lack of a strong negative effect on survival during the exposure risk period was corroborated by the results of the monthly assessments of individual health. Two competing models that were equally plausible gave different interpretations of the effect of the poison operation on monthly survival of bats with existing PIT tags over the 6-month monitoring period. Under these two models the alternatives were (1) constant survival before, during and after the Pikiariki poison operation (November - April); or (2) a small decrease in survival during the period when baits were laid in Pikiariki (December - February). There was weaker support for two other models that included poison exposure risk. One of these models indicated a small increase in survival during the period when poison was detected in guano (November - March). The other indicated different survival rates before, during and after baiting in Pikiariki, with the lowest rates during the baiting period (December - February). Under the models that included poison exposure risk, the change (increase or decrease) in mortality rate was negligible. The lack of a strong negative effect during the exposure

risk period was corroborated by the results of the monthly assessments of individual health.

The reduced survival rates estimated for the period when baits were laid in Pikiariki, under two of the poison exposure risk models, could have been the result of normal seasonal stresses associated with reproduction and development (Tuttle & Stevenson, 1982; Welbergen, 2011). It is also possible that some of the apparent mortalities were due to PIT tag losses that weren't accounted for in the analysis. Reduced rodent densities could have contributed to the small increase in survival observed under the other poison exposure risk model. However, the period when survival was higher under this model (November-March, when poison residues were detected in guano) does not align with the timing of the reduction in rodent density. Pryde *et al*. (2005) reported reduced annual adult survival (<0.6) of long-tailed bats in Fiordland during a year when rat tracking rates of 30% were measured in November and February. Although tracking rates are indicative and not directly comparable between sites or years (Blackwell *et al.*, 2002) this suggests that lower survival rates might have been expected when tracking rates in Pikiariki were at 22% in November.

Annual survival rates calculated for adult males and females from the Pikiariki population were high compared to rates for microbat species generally (Lentini *et al.*, 2015), and were consistent with estimates of annual survival of lesser short-tailed bats in Fiordland during years when predator numbers were either naturally low or suppressed to low densities using vertebrate pesticides (Thakur *et al.*, 2017; M. Pryde, DOC, unpub. data). The Pikiariki rates also compared favourably with estimates of annual survival for New Zealand long-tailed bats in years with low predator densities (Pryde *et al.*, 2005; Pryde *et al.*, 2006; O'Donnell *et al.*, 2017) and for other tree-dwelling insectivorous microbat species internationally (Schorcht *et al.*, 2009; Papadatou *et al.*, 2012), all estimated using mark-recapture methods.

Annual survival rates for adults from Pikiariki were extrapolated for bats with existing PIT tags from monthly survival rates estimated under the top-ranked (constant survival) model over a 6-month period, as no information about seasonal variation in survival rates of lesser short-tailed bats was available at the time of analysis. Temperate microbat

species, particularly those that hibernate, are expected to have higher mortality rates over winter due to depleted fat reserves. Sendor & Simon (2003) found no evidence of this in pipistrelle bats (*Pipistrellus pipistrellus*) and suggested that temperate bats should have high winter survival rates to offset low reproductive rates. Preliminary analysis of PIT tag logging data from the Pikiariki population since completion of my analysis suggests that adult survival rates remained high between May and October 2014 (Thurley, 2017), thus my extrapolated survival rates provide a reasonable comparison.

Loss of PIT tags

Loss of marks, here PIT tags, violates one of the key assumptions of open population mark recapture models (Pollock *et al.*, 1990). Violation of this assumption would result in underestimation of survival and loss of precision of estimates (Arnason & Mills, 1981). I addressed this problem in part by removing recovered PIT tag identities from encounter histories following their last detection. It is likely that other PIT tags were lost during the study that weren't recovered, and uncertainty around the extent and timing of additional losses could not be fully resolved by examining the pattern of loss of the recovered tags. However, the pattern of 'disappearance' of bats (i.e. failure to be detected) suggested that tag losses were more likely in the post-tagging intervals. By estimating survival rates separately for newly-tagged bats in the two post-tagging intervals I reduced the influence of possible unaccounted for PIT tag loss on survival estimates for bats with existing tags that were less prone to tag loss. Feldhiem *et al*. (2002) found that PIT tags were more likely to be lost within a few days of insertion in lemon sharks (*Negaprion brevirostris*), due to the tag exiting through the insertion hole before it had healed. It is possible, though, that PIT tag losses also occurred in later intervals in my study, because some of the recovered PIT tags were last detected in intervals other than the two post-tagging intervals, and one of the recovered PIT tags came from a bat that had been tagged prior to the study.

It is possible that some of the newly-tagged bats that were last detected in the post-tagging intervals died rather than lost PIT tags. Deaths could have occurred unnaturally as a result of increased stress or injury from the PIT-tagging procedure, which could have been exacerbated by sublethal exposure to diphacinone (Vidal *et al.*, 2009). Breeding females and juveniles were predominant among the losses in the post-tagging intervals in October

and February respectively, and these groups could have been more sensitive to the effects of the rodenticide (Connell *et al.*, 2009). Alternatively, newly-tagged bats could have been preyed on by morepork (*Ninox novaezeelandiae*) on their release. Several morepork were observed on perches above tagging sites, no doubt attracted by the vocalisations of the captured bats. Some loss of bats during the post-tag intervals could also have been due to natural mortality. PIT-tagging of juveniles occurred during the early flight stage, when higher natural mortality is expected (Tuttle & Stevenson, 1982). Transience of individuals is an unlikely explanation for the 'disappearance' of bats following tagging. Low rates of transient behaviour have been observed among long-tailed bat social groups with overlapping home ranges in the Eglinton Valley, Fiordland (O'Donnell, 2000a). However, the nearest known short-tailed bat populations are c. 80 km from Pikiariki, and fidelity to roost sites is typically high among breeding females and newly volant juveniles (Kunz *et al.*, 2009a).

PIT tag loss has been reported for a variety of species, and rates of loss appear to vary widely (Gibbons & Andrews, 2004). Low *et al.* (2005) propose that higher rates of PIT tag loss may occur in flighted species because of movement of skin and flight muscles around the insertion site. Loss of PIT tags has been observed in other species of bats, although not in other populations of lesser short-tailed bats (O'Donnell *et al.*, 2011). A three-year double-banding study of Daubenton's bats (*Myotis daubentonii*) in the U.K. concluded that behaviour, health and reproductive success of individuals was not significantly affected by PIT-tagging (Rigby *et al.*, 2012). However, tags were lost or had ceased to function in 16% of recaptured bats that had previously been tagged under anaesthesia. In a study on the population dynamics of the critically endangered southern bent-winged bat (*Miniopterus orianae bassanii*) in Naracoorte, Australia, the estimated rate of PIT tag loss was < 5% within the first 10 days following tagging (E. Van Harten, pers. comm., 2019). In a multi-year study on big brown bats (*Eptesicus fuscus*) in Fort Collins, U.S.A the estimated a rate of PIT tag loss was 1.6% (O'Shea *et al*., 2004). In my study, tag loss was confirmed by recovery of shed tags for c.3% of the tagged the bats that were monitored. The maximum rate of tag loss, in the unlikely event that all of the missing bats in the study were due to tag loss, would have been c. 17%.

Lebl & Ruf (2010) successfully reduced the rate of PIT tag loss in edible dormice (*Glis glis*) by sealing the puncture site with a topical tissue adhesive, and this could be trialled with lesser short-tailed bats in Pikiariki. To assess the effectiveness of this technique, small freeze branding marks could be applied to the pelage of a sample of bats. Freeze branding has been used safely and effectively as a permanent marking method on several species of bats (Sherwin *et al.*, 2002).

Risk of exposure for long-tailed bats

Detection of diphacinone residues in long-tailed bat guano collected in Pikiariki confirms that non-target exposure of these bats to anticoagulant rodenticides does occur. Secondary exposure is the more plausible explanation, as these bats would be unlikely to encounter baits directly. Long-tailed bats are entirely insectivorous and are known to feed on moths and occasionally their larvae (Gurau, 2014). Moth larvae have been observed on cereal pellet baits in New Zealand (Spurr & Berben, 2004), and Hawaii (Dunlevy *et al.*, 2000). Furthermore, flies are an important dietary item for long-tailed bats (Gurau, 2014), and diphacinone residues were detected in maggots collected from a contaminated lesser short-tailed bat carcass in Pikiariki in 2014. If toxic residues are retained through metamorphosis, larvae of flying invertebrates could be a potential route of toxin transfer.

No mortalities or overt signs indicative of anticoagulant poisoning were observed in long-tailed bats by DOC staff during routine monitoring sessions for annual survival analysis that occurred over a 6-week period when toxic baits were present in Pikiariki (T. Thurley, pers. comm., 2014). Further investigation is required to understand the implications of exposure of long-tailed bats in Pikiariki and elsewhere to diphacinone and other anticoagulant rodenticides. The importance of this has been highlighted by the subsequent detection of residues of brodifacoum in the liver of a dead long-tailed bat from a separate population that was recovered by Department of Conservation staff from private farmland near Grand Canyon cave, c.50 km west of Pikiariki (T. Thurley, pers. comm. 2015).

Further research and management recommendations

Although the use of bait stations to deliver cereal pellet baits did not prevent exposure of lesser short-tailed bats to diphacinone, I was unable to detect any lethal or sublethal effects of that exposure. Monthly survival was high throughout the monitoring period,

and it is likely that the poison operation had no or negligible impact on survival rates. However, while the pathway of secondary exposure remains intact I suggest that the bats are potentially still at risk of non-target impacts from anticoagulant use in their habitat. Exposure levels in bats could vary from year to year with natural variations in the composition and abundance of dietary invertebrate species involved in toxin transfer (Moeed & Meads, 1987a). Furthermore, the effects of the level of exposure observed in the current study on bat reproduction and pup survival are unknown. In light of their findings that sublethal exposure to anticoagulants can have disruptive systemic effects, Fraser *et al*. (2018) caution that attention may be misplaced by focussing only on the direct lethal impacts of anticoagulant exposure and suggest that the sublethal effects of these pesticides should be a priority for future research.

Management of rodents to low densities is vital for persistence of both species of New Zealand bats. With the widely adopted national vision of a predator-free New Zealand by 2050, considerable effort is currently being directed towards refining existing pest control techniques, developing toxins with higher species specificity and developing efficient mechanical control methods (Murphy *et al.*). Until such tools become available, the use of cereal pellet baits in bait stations should be standard practice for delivering broad spectrum toxins like anticoagulant rodenticides in bat habitat. Such practices must be applied on a scale that includes both roosting and foraging ranges. As a precaution, I recommend the use of less potent and less persistent rodenticides such as pindone in bat habitat to further minimise risks.

Given that the recommended method of bait delivery does not completely prevent exposure, and that natural variation in annual survival rates is expected (Thakur *et al.*, 2017), long-term monitoring of annual survival of the Pikiariki lesser short-tailed bat population has been implemented since completion of this study to confirm that the benefits of pest control continue to outweigh potential risks. Identification of important prey species involved in toxins transfer through the food chain may help to inform whether timing and duration of bait delivery in bat habitat can be manipulated to further minimise risks to bat populations.

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Chapter 5

Estimating abundance of a cryptic species, the New Zealand lesser short-tailed bat (*Mystacina tuberculata*), using closed mark-recapture analysis

Lesser short-tailed bats are difficult to detect because of their cryptic roosting behaviour. Photo courtesy of David Mudge, Nga Manu Images.

5.1 Abstract

Estimates of abundance provide vital information for assessing population status of threatened species. This can be challenging for cryptic species like the endemic New Zealand lesser short-tailed bat (*Mystacina tuberculata*). These bats are difficult to detect because they are small, nocturnal and roost in tree cavities, with a single population dispersed among multiple roosts. In such cases, abundance estimation methods that account for the probability of detection are useful. I therefore used mark-recapture analysis to estimate the pre-breeding abundance of lesser short-tailed bats in Pikiariki Ecological Area, Pureora Forest Park, New Zealand in November 2013. Bats were captured at three maternity roost trees on six occasions over a 14-day period, and a combination of PIT-tagging and fur-clipping was used to mark individuals. Closed population models were used to estimate the number of adult males and females and model averaging was employed to account for uncertainty in model selection. The analysis produced a population estimate of 777 adults (95% CI 618-1021), of which 395 were males (95% CI 258-655), and 382 were females (95% CI 350-457). Low detection probabilities for males (0.02-0.08) resulted in low precision of the abundance estimate for this group. However, previous studies show that this method provides more accurate estimates of population size than roost emergence counts (which just give a minimum number) and this is the first estimate of abundance for a lesser short-tailed bat population obtained using mark-recapture closed population analysis based on more than two capture occasions. The abundance estimate for the bat population will provide a baseline for monitoring future trends. Monitoring abundance is also of importance for other lesser short-tailed bat populations, as those remaining on the New Zealand mainland are under threat from introduced mammalian predators, as well as being vulnerable to non-target poisoning from the vertebrate pesticides laid in their habitat to control these pests.

Keywords: detection probability, Chiroptera, Mystacinidae, PIT tag loss, population trends, threat status, Vespertilioniformes

5.2 Introduction

Measuring abundance is central to the management of wildlife populations (Williams *et al.*, 2002). For threatened species, assessment of population size is often the first step towards prioritising and implementing management (Mace *et al.*, 2008), and thereafter provides a reference point for assessing population trends and appraisal of management practices (Dutton *et al.*, 2005). Abundance is also a vital parameter in models used to predict the response of threatened wildlife populations to alternative management scenarios (e.g. Pryde *et al.*, 2005).

Almost a quarter of all Vespertilioniformid bat species are globally threatened (Mickleburgh *et al.*, 2002), but estimating abundance of bat populations is often challenging because of their cryptic nature. Many species are difficult to observe and capture because they are nocturnal, relatively small, volant, highly mobile, patchily distributed and roost where they are inconspicuous and difficult to access (O'Shea *et al.*, 2004; Kunz *et al.*, 2009a). This includes the New Zealand lesser short-tailed bat (*Mystacina tuberculata*), an endemic species with only 14 significant populations known (See Chapter 1, Figure 1.3). These populations are restricted to extensive stands of old-growth native forest, containing large trees with cavities suitable for colonial roosting (Lloyd, 2005).

There are three recognised subspecies of lesser short-tailed bats that are ranked separately under the New Zealand National Threat Classification System (Hill & Daniel, 1985; Hitchmough *et al.*, 2007; O'Donnell *et al.*, 2010) (See Chapter 1, Table 1.6). In 2017, the threat status of the southern subspecies (*M. t. tuberculata*) was changed from 'Threatened-Nationally Endangered' to 'At Risk-Recovering' (O'Donnell *et al.*, 2018). This reassessment was partly based on a documented increase in the size of Eglinton Valley, Fiordland population of this sub-species, determined by long-term annual monitoring (Thakur *et al.*, 2017). Up-to-date information on population size is lacking for most other populations, including a population of the central North Island subspecies (*M. t. rhyacobia*) at Pikiariki Ecological Area in Pureora Forest Park (hereafter "Pikiariki"). The central subspecies is currently classified as 'At Risk-Declining', while the northern subspecies (*M. t. apourica*) is classified as 'Threatened-Endangered' (O'Donnell *et al.*, 2018).

Two basic approaches are available to determine population size in colonial bat species; complete counts (or census), and estimates based on sampling. A census can be achieved by counting bats inside accessible roosts or during evening emergence, but only if the whole population is aggregated at a single location and if all bats roosting or emerging can be observed (Kunz *et al.*, 2009a). If only a proportion of the individuals in a population can be observed or captured, then sampling methods that account for the probability of detection are required (Pollock *et al.*, 1990). If individual bats in the population can be captured and marked, mark-recapture methods can be used to overcome the problem of imperfect detection (Kunz *et al.*, 2009a).

Mark-recapture methods are suitable for abundance estimation of lesser short-tailed bat populations because their cryptic roosting behaviour makes them difficult to detect. During the colder months, individuals frequently roost solitarily and use torpor to conserve energy, but during the breeding season large numbers roost colonially (Sedgeley, 2001; Lloyd, 2005). Their breeding season is highly synchronised, and during spring adult males and females begin to congregate at maternity roosts (Daniel, 1979; Lloyd, 2005). Breeding females dominate the maternity roosts, whereas males and non-breeding females move between the maternity colony and other roosting sites (Sedgeley, 2003; Lloyd, 2005; Dennis, 2008). On any one day, members of a population will be dispersed among one or more maternity roosts and numerous smaller colonial and solitary roosts, so simple counts of bats emerging from a single maternity roost will detect only a proportion of the population (Sedgeley, 2012b). The same maternity roost may be occupied for several days to weeks, but the composition of individuals occupying the roost is likely to vary from day to day (O'Donnell *et al.*, 1999; Sedgeley, 2003; Lloyd, 2005). Lloyd (2005) suggests that a large proportion (c. 80-90%) of reproductively mature adult females breed each year. Females give birth to a single pup between mid-December and mid-January and pups are crèched in the maternity roost until they begin flying at four to six weeks of age (Daniel, 1979; Lloyd, 2005).

The most suitable time to estimate adult population size is during the weeks leading up to parturition. During this window of time, large numbers of adult bats aggregated at maternity roosts can be captured and marked, and the risk of stressing nursing mothers and pups is avoided. The population is effectively demographically and geographically

'closed' during this period, with no births occurring and negligible changes in population size expected due to deaths, immigration or emigration (see Methods for further explanation).

I used mark-recapture for closed populations to estimate the abundance of the adult lesser short-tailed bat population in Pikiariki during October-November 2013. The primary incentive for obtaining an abundance estimate for this population was the death of a large number of lesser short-tailed bats at the site in January 2009. This occurred as a result of non-target poisoning with diphacinone used in baits laid for rodent control in the bats' habitat (Dennis & Gartrell, 2015; Chapter 2). One hundred and fifteen bat deaths were observed, but it is likely that many mortalities were undetected. The size of the adult population in Pikiariki had not previously been assessed, other than a minimum pre-breeding estimate of c. 500 adult bats, based on roost emergence counts (Dennis, 2008). The true size of the adult population before the poisoning event was unknown, so the actual extent of the mortalities and the recovery trajectory of the population could not be assessed.

The current abundance estimate will provide a baseline reference for monitoring future population trends in Pikiariki and adds to knowledge about regional and national lesser short-tailed bat abundance that will assist with future threat-ranking assessments and prioritising management. The abundance estimate will also provide a vital parameter in a population model used to assess the viability of the local population under alternative pest management scenarios and potential non-target mortality scenarios associated with the use of vertebrate pesticides (Chapter 6).

5.3 Methods

5.3.1 Study site

The study was conducted in Pikiariki Ecological Area (457 ha) within Pureora Forest Park (78,000 ha), in the North Island of New Zealand (38°31'S, 175°34'E). Pikiariki is a remnant of old-growth native forest which provides roosting habitat for lesser short-tailed bats. The forest canopy is dominated by tawa (*Beilschmiedia tawa*), with abundant emergent podocarps such as rimu (*Dacrydium cupressinum*), matai (*Prunopitys taxifolia*)

and totara (*Podocarpus totara*). The understorey is dense and cluttered in places, due to regeneration following cessation of selective native logging in 1978. Pikiariki is isolated from other tracts of native forest by pasture, regenerating scrub and a patchwork of mature and clear-felled stands of exotic timber tree species. The remnant itself is fragmented into three blocks by forestry roads. The site is approximately 549 m a.s.l. and has a cool temperate climate. Mean monthly minimum temperatures range from 1.3 ºC in July to 10.3 ºC in February, with 142.5 mm rainfall per month on average (NIWA, 2016). Pikiariki is inhabited by populations of several protected species of plants and animals and has been designated as an Ecological Area in recognition of its high conservation values (Norton & Overmars, 2012).

5.3.2 Data collection

I conducted a mark-recapture study between 29 October and 11 November 2013 by trapping bats at three roosts on six occasions. During this period adults were congregated at maternity roosts, but females had not yet given birth. During early October, I used 9 m. mist-net rigs (38mm, Avinet, Dryden, USA) at suitable sites in Pikiariki to capture bats away from their roosts (Kunz *et al.*, 2009b). Mist netting took place 2-4 h after sunset to coincide with high levels of bat activity. Activity was detected using custom-made automated heterodyne bat detectors (Department of Conservation, Wellington, New Zealand) set to 28 kHz to detect echolocation calls of lesser short-tailed bats (Sedgeley, 2012a). Captured bats weighing no less than 14 g were fitted with radio-transmitters (BD2, Holohil Systems, Carp, Ontario, Canada), attached between the scapulae on an area of partially trimmed fur, using a latex-based contact adhesive (Ados F2®, CRC industries, East Tamaki, New Zealand). Transmitters weighed ≤ 0.7 g and were therefore <5% of bat body mass, in accordance with recommended guidelines (Aldridge & Brigham, 1988). During the day, I tracked radio-tagged bats on foot to locate their roosts, using a hand-held TR4 receiver (Telonics, Arizona, USA) and a hand-held, 3-element Yagi aerial (Lotek, Havelock North, New Zealand). Three known colonial roosts were also inspected daily for evidence of occupation in the form of fresh guano deposits beneath the roost entrance.

Once an active roost had been identified, I assessed its suitability for trapping by counting the number of bats emerging from the roost and identifying the number and nature of

roost exits used. Counts involved observing bats at evening emergence using an infra-red video camera (Sony Handycam DCR-HC90, Sony Corporation, Tokyo, Japan) and a spotlight with an infra-red filter (Lightforce SL 170mm Striker Handheld Spotlight and Lightforce IR Infrared Filter Lens Cover) (Sedgeley, 2012b). Observations and filming commenced approximately 30 minutes after dusk, shortly before bats began emerging (Lloyd, 2005), and ceased 90-120 minutes later, once no bats were seen leaving the roost for at least 10 minutes, or there were more bats entering the roost than leaving it. Emergence counts were later confirmed by reviewing recordings.

When an occupied roost suitable for trapping had been identified, I captured bats on the first calm dry night that followed. Bats were captured during evening emergence using a 4.2 m^2 harp trap (Austbat Research Equipment, Melbourne, Australia) suspended across the roost entrance. Different roosts were targeted on consecutive trapping occasions, so that a minimum of two days was left between trapping the same roost. This was to minimise disturbance and to allow time for natural mixing of bats in the wider population using different roost trees. During each trapping session, the harp-trap was periodically lowered and raised again to allow transfer of captured bats to cloth bags for processing and release. This was to comply with suggested guidelines on time limits for holding individual bats (Sedgeley *et al.*, 2012).

On the first four capture occasions I collaborated with Department of Conservation (DOC) staff and other trained personnel to permanently mark approximately 200 bats with passive integrated transponder (PIT) tags. There were already 188 bats in the population that had previously been marked with PIT tags, between January 2012 and February 2013. Therefore, on each capture occasion bats were sexed and checked for an existing PIT tag using a hand-held Scanflex AFX-100 scanner (ISO Compatible RF/ID Pocket Reader, Allflex Australia Pty Ltd, Capalaba, Australia). On the first four capture occasions, if no PIT tag was detected an Allflex PIT tag (FDX-B 11 mm x 2.1 mm glass transponder implant) was inserted subcutaneously on the back between the scapulae using a sterile single-use 12 gauge needle (Allflex Australia Pty Ltd, Capalaba, Australia) mounted on a Henke-ject insertion gun (Henke-Sass Wolf, Tuttlingen, Germany), following the current Department of Conservation (DOC) best practice protocol (Sedgeley *et al.*, 2012).

If the PIT-tagging procedure failed, the bat was given a temporary mark by trimming a small patch of fur in a specific location on its back. The location of a fur-clip was specific to the capture occasion (e.g. top left back on the first occasion, top right back on the second occasion etc.). Each time a bat with one or more existing fur-clips was captured on a subsequent occasion it was given a new fur-clip in a new location, and PIT-tagging was not attempted. On the fifth capture occasion no PIT-tagging was done, and any unmarked bats captured were marked only using fur-clipping. On the final capture occasion no PIT-tagging was done and no new fur-clip marks were given to any marked or unmarked bats captured.

5.3.3 Data analysis

I used closed-population mark-recapture analysis (Otis *et al.*, 1978) to estimate the abundance of male and female adult lesser-short-tailed bats in the Pikiariki population. Capture and recapture data were converted to encounter history format for analysis. Encounter histories were constructed by recording a '1' for each occasion when an individual was captured, and '0' for each occasion when an individual was not captured. Bats captured only on the third and fifth trapping occasions, for example, would have the encounter history '001010'. Individual encounter histories were coded for sex so that two attribute groups, "male' and 'female' could be considered in the analysis.

Encounter histories were entered into Program MARK (Version 6.0) and the 'closed captures' procedure was then used to model factors which may have influenced capture and recapture probabilities of bats on each trapping occasion. The programme used the method of maximum likelihood estimation to estimate the parameters p_j (the probability that an animal in the population will be captured for the first time on occasion j), c_j (the probability that a previously marked animal will be captured on occasion j), and N_g (the abundance of individuals in attribute group g) (Otis *et al.*, 1978; White & Burnham, 1999).

5.3.4 Assumptions of closed population models

Estimation of abundance using closed mark-recapture models requires that four underlying assumptions are met; (1) the population is closed during the study period; (2) animals do not lose their marks during the study period; (3) marks are correctly identified

and recorded on each trapping occasion; and (4) all animals (marked and unmarked) have equal capture probability on each trapping occasion (Otis *et al.*, 1978).

Population closure (assumption 1) implies that there is no recruitment (births or immigration), or losses (deaths or emigration), of bats during the study, and the population therefore remains constant in size and composition across all sampling occasions. Statistical tests for closure have been developed, but these generally fail to reject closure unless the departure from closure is strong and the sample is large, and they cannot distinguish animals with low capture rates from those that have emigrated or died (Otis *et al.*, 1978). Furthermore, none of these tests is comprehensive (e.g. Stanley & Burnham, 1999). Otis *et al*. (1978) recommend instead that the assumption of population closure is assessed on a biological basis.

Therefore, I designed the mark-recapture study so that the assumption of closure could be met approximately by conducting the study over a relatively short time frame, and by timing the trapping period to occur before the birth pulse. The study ended on 11 November, more than one month before any births were expected (Lloyd, 2005). The probability of immigration or emigration occurring during the study period was likely to be very low; genetic analysis indicates that movement between populations in the central North Island is rare (Lloyd, 2003c).

Loss of marks during the study period (assumption 2) would result in the overestimation of abundance because fewer recaptures would be recorded than actually occurred (Pollock *et al.*, 1990). Loss of PIT tags was expected to be negligible, based on several years of monitoring of PIT-tagged lesser short-tailed bats in the Eglinton Valley, South Island population (H. Edmonds, Department of Conservation, pers. comm., 2015). However, there is potential for PIT tags to be lost, either by exiting the needle hole soon after insertion (Feldheim *et al.*, 2002), or if recognised as a foreign body and rejected by the tagged individual (Roark & Dorcas, 2000). The loss of one tag during this study was later confirmed (see Results). Marks made by fur-clipping, although temporary, cannot 'fall off'. Observations of the rate of hair re-growth in patches of trimmed hair on captive lesser short-tailed bats indicate that the time required for hair re-growth at this time of year far exceeds the period of the mark-recapture study (G. Dennis unpublished data).

If marks were overlooked (assumption 3), this would also result in the overestimation of abundance (Pollock *et al.*, 1990). Failure of a scanner to read PIT tags (e.g. Low *et al.*, 2005), or migration of PIT tags away from the point of insertion could cause violation of this assumption. Detection of migrated tags was unlikely to be a problem as the bats are small. Variation in bat-handling skills and consistency in the size and placement of fur-clips could potentially result in marks occasionally being overlooked. Scanned PIT tag numbers were directly downloaded to a database, thus avoiding transcription errors, but there was potential to introduce errors during manual recording of fur-clip marks. Despite there being several potential sources of error, the rate of overlooking fur-clip marks or PIT tags was expected to be very low.

A fourth assumption of closed population mark-recapture models, that all animals (marked and unmarked) have equal capture probability, is unrealistic for most wild animal populations. The assumption can generally be relaxed in mark-recapture studies with more than two capture occasions by the use of appropriate models to address heterogeneity in capture probability among sampling occasions and attribute groups (e.g. age, sex) (Otis *et al.*, 1978). I took this approach as I was unable to model individual heterogeneity because bats that were marked with fur-clips instead of PIT tags could not be individually identified. This can result in underestimation of abundance (Otis *et al.*, 1978).

5.3.5 Model construction

A set of candidate models to estimate the probability of bat capture was defined *a priori*. I based identification of plausible models on information in the published literature about the ecology of the lesser short-tailed bat and other bat species, several years of personal experience working with the study species at the study site, and observations specific to the period of data collection (Burnham & Anderson, 2002). I considered that the following four factors could affect the probability of bat capture; (1) time (i.e. variation among capture sessions) (*t*), (2) roost (i.e. variation among capture sites) (*r*), (3) sex (i.e. male or female) (*s*), and (4) behaviour (i.e. more or less prone to recapture after initial capture) (*b*).

In models where capture probabilities vary with time (M_t), all individuals are at equal risk of capture on the j^{th} trapping occasion, but the probability of capture varies among trapping occasions (Otis *et al.*, 1978). This could occur in response to changes in environmental, ecological or anthropogenic factors on each occasion. Nightly differences in ambient temperature, precipitation or cloud cover, the presence of aerial predators, and changes in colony size can affect the time and rate of emergence of bats from roost sites (Erkert, 1982; Kunz & Anthony, 1996), and this in turn could affect capture probability. Trap placement and tension, which can vary between trapping occasions, can also affect the probability of bat capture (Kunz & Anthony, 1977).

I considered a model where recapture probability varied with roost (M_r) as a constrained variation of the time model. Under this model, all individuals trapped at the same roost are at equal risk of capture. The probability of capture varies among occasions when different roosts are trapped. Size, number and configuration of roost exits vary between roost trees at Pikiariki, and this could affect the probability of catching bats. Bats leaving roosts through large exits or crevices tend to rely on spatial memory and may thus be at risk of being trapped, while those flying through small spaces tend to be more 'acoustically' aware and therefore more likely to detect and avoid a trap (Kunz & Anthony, 1977). In roosts with multiple access points, bats may leave via untrapped exits. Variation in size and configuration of roost exits can also affect rates of emergence (Kunz & Anthony, 1996), as well as optimal trap placement. Capture is more likely when there is little room to manoeuvre between the roost exit and the trap, and bats encounter traps with reduced flight momentum (Kunz & Anthony, 1977).

Heterogeneity in capture probabilities can occur due to characteristics such as age, sex or size of individuals, or some unrecognised attribute (Otis *et al.*, 1978). For example, age- and sex-related differences best described the recapture probabilities of New Zealand long-tailed bats (*Chalinolobus tuberculatus*) in a mark-recapture study in Fiordland, New Zealand (Pryde *et al.*, 2005). A difference in the time of emergence has been noted between sexes for some overseas bat species, (Kunz, 1974; Lee & McCracken, 2001) so catches at roosts could potentially be biased towards one sex, depending the timing of trap placement. Bats may have other sex-related characteristics that make them more or less prone to capture. Breeding females, for example, may have less manoeuvrability due

to increased wing loading during pregnancy (Kunz & Anthony, 1996). In lesser short-tailed bat colonies, differences in roosting behaviour between sexes could affect recapture probabilities (Sedgeley, 2003; Lloyd, 2005). Models incorporating sex (M_s) were therefore used to model potential differences in capture probabilities between male and female lesser short-tailed bats in my study.

Animals that have previously been captured may be more prone to recapture, or 'trap-happy'($p_j<c_j$), or less prone to recapture, or 'trap-shy' ($p_j>c_j$) (Otis *et al.*, 1978). In models with a behaviour effect (M_b), on the j^{th} trapping occasion all marked animals have one probability of capture and all unmarked animals have a different probability of capture. Pryde *et al.* (2005) considered trap-response was unlikely when harp-trapping New Zealand long-tailed bats at tree roosts, due to placement of traps. However, optimal trap placement is not always possible and, unlike long-tailed bats, lesser short-tailed bats may occupy a roost for several weeks during the breeding season (O'Donnell, 2000; Lloyd, 2005), and so may associate capture with a specific site. It is possible that previously captured bats could be more acoustically aware of their environment when exiting a roost and could potentially avoid recapture if they are able to detect and manoeuvre around a trap or use an alternative roost exit. They may also choose to remain inside the roost or use an alternative roost site.

The global model (i.e. the model containing all of the important factors) was defined as *p*(sex*time*behaviour), *c*(sex*time*behaviour). 'Roost' is a constrained variation of 'time', so these factors cannot appear in the same model. The global model and a set of alternative models were run in Program MARK using the logit link for additive models and the sin link for models with interaction terms. Additive effects on capture and recapture probabilities express a relationship that varies in parallel between two or more of the modelled factors, while interaction effects indicate that the relationship between two or more factors is not constant (Cooch & White, 2018).

5.3.6 Model selection

I used an information-theoretic approach to select the best approximating model from the set of candidate models. Models were compared using Akaike's information criterion (AIC) corrected for small sample size (AIC_c). AIC_c provides an objective means of selecting the most parsimonious model in the candidate set, that is, the model that gives the best compromise between explaining variation in the data while using few parameters. The model with the lowest AIC_c value therefore provides the optimal balance between precision and bias of parameter estimates and is ranked highest in the set of candidate models. AIC_c differences (Δ_i) between each competing model ($AIC_{c,i}$) and the best approximating model ($AIC_{c,\,min}$) provide information about the relative plausibility of each model, given the data. For the highest ranked model Δ_i=0. Δ AIC_c values are used to calculate Akaike weights (w_i). Akaike weights are proportional to the relative likelihood of each model and weigh the evidence in favour of a particular model being the best model relative to all others in a given set of models. They are calculated by normalising model likelihoods for a set of candidate models to generate a set of values which adds to one, using

$$w_i = \frac{\exp[-\frac{1}{2}\Delta_i]}{\sum \exp[-\frac{1}{2}\Delta_i]}$$

The larger the w_i, the more plausible the model. As Δ_i increases, model weight decreases indicating that the relative plausibility of a model decreases (Burnham & Anderson, 2002).

5.3.7 Model averaging

When more than one model in the candidate set has substantial support, more robust inferences can be obtained by model averaging. Model averaging accounts for both model-specific variation and model-selection uncertainty in estimates of parameters and variance. I therefore undertook model averaging in Program MARK. This provides separate model-averaged estimates of abundance for males ($\widehat{\bar{N}}_m$) and females ($\widehat{\bar{N}}_f$) calculated as

$$\widehat{\bar{N}}_g = \sum w_i \widehat{N}_{g,i}$$

where w_i is the Akaike weight and $\widehat{N}_{g,i}$ is the sex-specific abundance estimate for model *i* (Burnham & Anderson, 2002).

I then calculated 95% confidence intervals around each of the model-averaged estimates. Estimation of confidence limits for $\bar{\widehat{N}}_g$ uses the number of animals not captured, $\bar{\widehat{U}}_g$, under the assumption that this measure follows a log-normal distribution, thus avoiding the bias inherent in interval estimation under the assumption of normality. I obtained the number of male animals not captured using $\bar{\widehat{U}}_m = \bar{\widehat{N}}_m - (M_{t+1})_m$ where M_{t+1} is the number of marked individuals in the study. The calculation was repeated to obtain $\bar{\widehat{U}}_f$ for females. I then obtained the unconditional variance (i.e. variance that is not conditional on any one model) by squaring the unconditional standard error given in the output for model averaging provided by Program MARK. Finally, I calculated the upper and lower bounds of the 95% confidence interval around $\bar{\widehat{N}}_g$ for each sex using

$$\left[M_{t+1}\left(\bar{\widehat{U}}_g / C\right), M_{t+1} \times \left(\bar{\widehat{U}}_g \times C\right)\right]$$

where

$$C = \exp\left(1.96\left[\ln\left(1 + \frac{\mathrm{var}\left(\bar{\widehat{N}}_g\right)}{\bar{\widehat{U}}_g^2}\right)\right]^{1/2}\right)$$

(Williams *et al.*, 2002).

5.3.8 Estimation of total population size and 95% confidence interval

To obtain an abundance estimate for the entire bat population ($\bar{\widehat{N}}_T$), I added together the model-averaged abundance estimates for males and females. To estimate confidence limits for $\bar{\widehat{N}}_T$ I first calculated the number of uncaptured males ($\bar{\widehat{U}}_m$) and females ($\bar{\widehat{U}}_f$), as done above in section 5.2.7, but in this case using the model-averaged estimates of abundance for each sex. The total number of animals not captured is then given by $\bar{\widehat{U}}_T = \bar{\widehat{U}}_m + \bar{\widehat{U}}_f$. I then obtained confidence limits for $\hat{\beta}_T$, the natural logarithm of $\bar{\widehat{U}}_T$, using the delta method. The delta method is used to approximate the standard error (SE) for a

function of two or more estimates, each with its own estimate of variance (Seber, 1982). The SE of $\hat{\beta}_T$ is approximated by

$$\mathrm{SE}(\hat{\beta}_T) = \frac{1}{\widehat{U}_T}\sqrt{\{[\widehat{U}_m^2\, SE(\hat{B}_m)^2] + [\widehat{U}_f^2\, SE(\hat{B}_f)^2] + [2\,\widehat{U}_m\widehat{U}_f\, Cov(\hat{B}_m, \hat{B}_f)]\}}$$

where $\hat{\beta}_m$ and $\hat{\beta}_f$ are the beta (natural log-transformed) estimates of $\widehat{\widehat{U}}_m$ and $\widehat{\widehat{U}}_f$ respectively, and $\mathrm{Cov}(\hat{\beta}_m, \hat{\beta}_f)$ is the covariance among these estimates (available in Program MARK). I then calculated the approximate 95% confidence limits around $\hat{\beta}_T$ using $\hat{\beta}_T \pm 1.96SE(\hat{\beta}_T)$. The results were back-transformed to obtain real estimates of the confidence limits of $\widehat{\widehat{U}}_T$. Finally, the total number of male and female bats captured $(M_{t+1})_{m,f}$ was added to the upper and lower confidence limits for $\widehat{\widehat{U}}_T$ to obtain upper and lower 95% confidence limits for $\widehat{\widehat{N}}_T$, the estimated total lesser short-tailed bat population size (Williams *et al.*, 2002; McCartney *et al.*, 2006).

5.4 Results

5.4.1 Radio-tracking and roost emergence counts

Four adult female bats were captured and fitted with radio-transmitters between 19 and 29 October. I confirmed occupation of one previously unknown and three known maternity roosts by tracking the radio-tagged bats during the day. Emergence counts of 95 bats at one roost on 22 October, and 330 bats at different roost on 24 October indicated that bats were congregating at the roosts in numbers suitable for commencing the mark-recapture study.

5.4.2 Number of captures

During the six trapping occasions I captured 407 adult bats, comprising 318 individuals (104 males, 214 females) and 89 recaptures (13 males, 76 females) (Table 5.1). Only 9 males and 26 females were fur-clipped, as the remainder had new or existing PIT tags or were captured for the first time on the final occasion, when marking was not necessary. The number of bats captured on each occasion varied, and the proportion of females in each sample varied but was always > 0.5. I trapped bats at the same roost tree (r_1) on the first, third and fifth capture occasions, at a second roost tree (r_2), on the second and fourth

capture occasions, and at a third roost tree (r_3) on the final capture occasion. The three roosts trees differed in characteristics that may have affected trapping success; r_1 had a single, large exit, r_2 had a single, small exit but was adjacent to a tree that interfered with trap placement, and r_3 had multiple small or narrow exits that were widely spaced. Recapture of PIT-tagged individuals at different sites confirmed that there was exchange of bats between the three roosts trapped; 35% of uniquely marked individuals were captured at two different roosts and a further 10% were captured at all three roosts.

Table 5.1 Number of newly captured and recaptured adult male and female lesser short-tailed bats (*Mystacina tuberculata*) caught in harp-traps at three maternity roosts on six occasions between 29 October and 11 November 2013 in Pikiariki Ecological Area in Pureora Forest Park, New Zealand.

Trapping occasion	Roost	No. captured (new, recaptures)		
		Males	Females	Total
1	r_1	10 (10, 0)	51 (51, 0)	61 (61, 0)
2	r_2	22 (22, 0)	49 (32, 17)	71 (54, 17)
3	r_1	33 (30, 3)	76 (56, 20)	109 (86, 23)
4	r_2	20 (16, 4)	34 (22, 12)	54 (38, 16)
5	r_1	8 (7, 1)	21 (14, 7)	29 (21, 8)
6	r_3	24 (19, 5)	59 (39, 20)	83 (58, 25)
	Total	117 (104, 13)	290 (214, 76)	407 (318, 89)

5.4.3 Loss of marks

The potential shedding of PIT tags by lesser short-tailed bats was first observed by DOC staff during a capture session more than a year after the completion of this study. Consequently, between February and April 2015 DOC staff and I used hand-held PIT tag scanners to search for lost tags around the bases of seven maternity and 20 solitary roost trees. Fifty-two PIT tags were recovered. I searched database records and determined that only one of the recovered PIT tags had been inserted or last detected during the 2013 closed mark-recapture study. Database records were compiled from automated PIT tag readers that had been operating at six maternity roosts since DOC staff and I installed

them for survival monitoring during the 2013-14 field season (Chapter 4), and from physical captures during PIT-tagging sessions in February 2014 and 2015. The PIT tag that was lost during the M-R study came from a female that was detected only once, on the occasion that she was tagged in October 2013. Her encounter history was therefore adjusted to reflect removal from the study on the occasion of her final (and only) capture.

Although only one of the recovered PIT tags affected the M-R study, it is possible that during the study other bats lost tags that were not recovered. To determine whether this may have been the case, I also searched the database to ascertain whether any of the tagged bats in the study that were not seen on the final capture occasion had been detected after that. This revealed a further 19 bats (three males and 16 females) that were detected only once during the study, at the time of tagging. It is possible that these tags had also been lost soon after insertion, or that the bats had died following tag insertion due to the procedure or as a result of predation by moreporks at the release site. I removed these tag identities from the data set. Models were re-run after these adjustments had been made. The effect of this assumption on the abundance estimate is discussed.

5.4.4 Population size estimate

Two models explained the data better than all other models considered, accounting for 100% of the cumulative Akaike weights (*w*) (Table 5.2). The two top-ranked models both included temporal variation (*t*) and sex-specific differences (*s*) in capture probabilities. In the best model, M_{s+t}, the relative difference between capture probabilities for males and females remained constant among trapping occasions, while in the second-ranked model, M_{s*t}, this relationship differed on each occasion. There was no support for the third ranked model (w=0) in which time-varying capture probabilities did not differ between the sexes. Nor was there support for the model in which capture probabilities differed between the sexes but remained constant over time (w=0). Models that included a roost effect or a behavioural response to capture received no support. Models with an interaction term that included a behavioural effect could not be fitted and were excluded from consideration.

Table 5.2 Comparison of candidate models used in program MARK (Version 6.0) to estimate abundance of adult lesser short-tailed bats (*Mystacina tuberculata*) in Pikiariki Ecological Area, Pureora Forest Park, New Zealand, 29 October – 11 November 2013. Models describe factors affecting capture and recapture probabilities of bats.

	Model [a]	**AIC$_c$** [b]	Δ_i [c]	w_i [d]	**K** [e]	**Deviance**
1.	M_{s+t}	-979.18	0	0.79	9	95.47
2.	M_{s*t}	-976.48	2.69	0.21	14	88.04
3.	M_t	-966.06	13.11	0	8	110.60
4.	M_{s+r}	-927.08	52.09	0	6	153.61
5.	M_s	-926.49	52.69	0	4	158.23
6.	M_{s*r}	-925.65	53.53	0	8	151.02
7	M_{s+b}	-922.54	56.64	0	6	158.16
8.	M_r	-914.07	65.11	0	5	168.64
9.	M_b	-911.60	67.58	0	4	173.12

[a] Factors assumed to affect capture (*p*) and recapture (*c*) probabilities under different candidate models (*M*) include: *s* = sex (male or female); *t* = time (trapping occasion); *r* = roost (trapping site); *b* = behaviour; * = interactions between factors as well as additive effects; + = additive effects only.

[b] Akaike's Information Criterion corrected for small sample size.

[c] Differences in AIC_c value from the best model in the candidate set.

[d] Akaike's weights, indicate relative support of each model.

[e] Number of parameters in model.

While the top-ranked model received most of the support (*w*=0.79) the second-ranked model also had some plausibility (*w*=0.21). Model averaging was therefore undertaken in Program MARK to account for model-selection uncertainty. Based on model averaging, the lesser short-tailed bat colony at Pikiariki is estimated to have 777 (CI 95% 618-1021) adult bats, with 49% of these being females (Table 5.3). Model-averaged estimates of capture probabilities ranged from 0.06-0.2 (mean 0.13) for females and 0.02-0.08 (mean 0.05) for males.

Table 5.3 Model-averaged estimates of abundance ($\widehat{\widehat{N}}$) of adult male and female lesser short-tailed bats (*Mystacina tuberculata*) and total population size, in Pikiariki Ecological Area, Pureora Forest Park, New Zealand, 29 October – 11 November 2013. Estimates were obtained using closed mark-recapture analysis in Program MARK (Version 6.0).

Group	$\widehat{\widehat{N}}$	95% CI
Males	395	258–655
Females	382	330–457
Total	777	618-1021

5.5 Discussion

Population size estimate

An estimated 777 adult lesser short-tailed bats in Pikiariki suggests that this population is small relative to other populations of this species that have been assessed. Population size estimates reported for other sites since the mid-1990s include Little Barrier Island/Hauturu (c. 5000); Eglinton Valley (c. 3000); Codfish Island/Whenua Hou (c. 2000); Waitaanga (c. 2700); and Rangataua (c. 7,000) (Sedgeley & Anderson, 2000; Lloyd, 2005; O'Donnell *et al.*, 2010; C. O'Donnell, pers. comm. 2018). Estimates made at these sites were based on roost emergence counts and are therefore likely to be minimum population sizes. Roost emergence counts tend to underestimate population size for lesser short-tailed bats because it is unlikely that all roosts used by a colony on a single day will be located (O'Donnell *et al.*, 1999; O'Donnell & Sedgeley, 2012). The population estimate for lesser short-tailed bats in Pikiariki is, to my knowledge, the first estimate of abundance for this species that was obtained using mark-recapture closed population analysis based on more than two capture occasions.

Study limitations

The accuracy and precision of the abundance estimates in this study may have been affected by the roosting behaviour of the bats. Captures were biased towards females, as was expected, because breeding females dominate the composition of maternity roosts (Sedgeley, 2003) where trapping effort was focussed by necessity. Males and non-

breeding females may not have had the same availability for capture as breeding females because they switch between the maternity roost and other roosting sites, including singing roosts for breeding males (Sedgeley, 2003; Lloyd, 2005; G. Dennis unpub. data; Toth *et al.*, 2015). As a consequence, the average capture probability for males was low, and the average capture probability for females may have been negatively biased because I did not distinguish between breeding and non-breeding individuals, the latter thought to comprise around 10-20% of the reproductively mature adult female population in any year (Lloyd, 2005).

Low capture probabilities can result in overestimation of abundance. Using simulation data that satisfied the assumptions of time dependent models, Otis *et al.* (1978) demonstrated that bias in abundance estimates became significant (e.g. >10%) if average capture probabilities were less than 0.1. Low average capture probabilities in the simulation also resulted in large variance estimates and hence wide confidence intervals around the abundance estimate. The low average capture probability of male lesser short-tailed bats in my study is reflected in the greater uncertainty around the estimate of male abundance, which is likely to be less reliable than the abundance estimate obtained for females. Conversely, unmodeled individual heterogeneity could have resulted in underestimation of abundance for both sexes. The greater the amount of heterogeneity in the population, the greater the bias (Otis *et al.*, 1978). Even so, mark-recapture methods have proven to be more useful in estimating abundance of the cryptic components of wildlife populations than other methods (Pryde *et al.*, 2006; Katzner *et al.*, 2011).

More capture sessions may be required to increase the sample size of males to improve estimates of their abundance (Otis *et al.*, 1978), though care must be taken not to extend the capture period in to the late stages of pregnancy to avoid stress or injury to breeding females and unborn pups. An alternative approach to increasing detection probabilities for cryptic components of the population is to passively detect bats using remote PIT tag reader technology. In a mark-recapture survival study comparing conventional (i.e. physical) capture methods of big brown bats (*Eptesicus fuscus*) with remote detection using PIT tag readers installed at roost entrances, Ellison *et al.* (2007) found that remote detection provided more encounters, resulting in higher, more precise estimates of capture probability. Furthermore, remote readers detected around half of the bats that were never

physically recaptured following initial capture for insertion of PIT tags. Remote detection of PIT-tagged animals, used in combination with video-ed roost emergence counts, could potentially be used to estimate bat abundance using multi-occasion mark-resight methods (M. Pryde, Department of Conservation, pers. comm., 2017). Such a method would have the advantage of reduced disturbance at roosts, as well as providing improved precision of estimates because data from marked, unmarked, and marked but not identifiable individuals are used in the analysis (Rich *et al.*, 2014). However, this approach is only useful if PIT tag loss is not an issue, or if the rate of tag loss can be estimated.

The loss of PIT tags, if unaccounted for, would result in underestimation of capture probabilities and overestimation of abundance. The loss of PIT tags from bats in the Pikiariki population was unexpected. In a Fiordland study 97% of PIT-tagged lesser short-tailed bats monitored were detected after a five-month period, indicating that tag loss was unlikely to be a concern in this species (O'Donnell *et al.*, 2011). PIT tag loss has been reported in some overseas bat species, however (Freeland & Fry, 1995; Rigby *et al.*, 2012). A three-year double-banding study of Daubenton's bats (*Myotis daubentonii*) in the UK found that PIT tags were lost or had ceased to function in 16% of recaptured bats (Rigby *et al.*, 2012). Estimation of the rate of PIT tag loss in double-banding studies can be used to adjust estimates of abundance (Williams *et al.*, 2002). Unfortunately, double-banding of lesser short-tailed bats is not possible. Banding trials with captive and free-living lesser short-tailed bats concluded that forearm bands cause an unacceptable rate of injury, due to the unusual wing morphology and terrestrial foraging habit of these bats. Currently, PIT tags are the only approved method of permanent marking for lesser short-tailed bats (Sedgeley *et al.*, 2012). If the loss of PIT tags had been anticipated as a potential issue in the current study, a fur-clip mark made in a specific location could have been used as an alternative to double-banding.

PIT tag loss was confirmed through tag recovery for only one bat in the study. The assumption of PIT tag loss for an additional 19 bats that were seen only once during the study, at the time of tagging, was reliant on a time-consuming search to confirm their absence from a large database of detections made remotely by roost loggers for 15 months after the completion of the study. Another plausible explanation is that the capture and tagging process either directly or indirectly caused the unnatural mortality of these bats

through stress or injury, or through predation by moreporks (*Ninox novaeseelandiae*) that were occasionally observed pursuing released bats at the capture and processing sites. Alternatively, some of the bats could have suffered natural mortality. Permanent or temporary emigration seems an unlikely explanation for the lack of detection of these bats immediately following tagging, given the geographic isolation of this population from other known populations and evidence from genetic studies that indicates movement between populations is rare (Lloyd, 2003c; Lloyd, 2003b).

Conservation implications

The lesser short-tailed bat population in Pikiariki and other populations throughout New Zealand are currently smaller and more isolated than they would have been prior to human settlement c. 1280 A.D (Lloyd, 2003a; Wilmshurst *et al.*, 2008). Bats were once abundant and widespread throughout the vast tracts of old-growth native forests that once dominated much of New Zealand. Genetic studies suggest that at least 12.5 million bats inhabited the pre-human forests of the central and southern North Island (Lloyd, 2003a). Extensive deforestation and other anthropogenic impacts that followed human settlement have contributed to a significant reduction in the species' range and population size. In 2001, only c. 50,000 bats were estimated to remain nationally. This includes c. 40,000 bats of the central subspecies, distributed primarily among eight known relict populations that are geographically isolated by the fragmented landscape (Lloyd, 2003a; Lloyd, 2003b; O'Donnell *et al.*, 2010).

With a larger number of bats spread over several sites, the central subspecies is considered more secure than the northern and southern subspecies (O'Donnell *et al.*, 2010). However, none of the central subspecies populations on its own is considered secure (O'Donnell, 2010). As with all mainland populations, on-going declines are predicted due to existing threats, including introduced mammalian predators and competitors, disturbance of roosts, on-going habitat loss and degradation, and potential secondary poisoning from vertebrate pesticides applied in their habitat (O'Donnell *et al.*, 2010; Dennis & Gartrell, 2015; Chapter 2). The only populations that are considered secure are one from each of the northern and southern subspecies that occur on offshore predator-free islands (O'Donnell, 2010). Attempts to establish new populations in secure locations have thus far failed (Ruffell & Parsons, 2009), so mainland populations must be managed *in situ*.

Previously, in times when lesser short-tailed bats were widespread, they may have lived in discrete, socially isolated colonies with overlapping home ranges, much as sympatric long-tailed bat colonies still do in the Eglinton Valley, Fiordland (O'Donnell, 2000; Lloyd, 2003b; Lloyd, 2003a). Information is lacking on the potential size of the remaining, now isolated lesser short-tailed bat colonies (now synonymous with populations) in the absence of anthropogenic impacts. Evidently, lesser short-tailed bat colonies can comprise several thousand individuals, even in today's less-than-pristine habitats, and it is likely that the Pikiariki population is smaller than it should be because of the likely effects of habitat fragmentation and loss, predators, and possibly other nontarget mortality events that could have occurred since predator management using vertebrate pesticides began at the site in the mid-1990s (Dave Smith, DOC, pers. comm, 2014).

This may be of consequence because Allee affects can be important in populations that are small relative to their natural state. Allee effects describe a positive relationship between population size and individual fitness (a component Allee effect) that may manifest as negative population growth (a demographic Allee effect) if the effect is strong (Courchamp *et al.*, 2008). The susceptibility of bats to Allee effects has been suggested because many species are highly social, and individuals may derive benefits from cooperative behaviours for activities such as thermoregulation and information transfer about roosting or foraging sites (Kerth, 2008; Gregory & Jones, 2010). Furthermore, small populations are more vulnerable to extinction through inbreeding, genetic drift, and stochastic events (Caughley, 1994). Monks and O'Donnell (2017) reported collapse of a colony of 88 long-tailed bats over a two-year period when consecutive beech mast (mass seeding) events led to high rodent densities in the Eglinton Valley, Fiordland.

Although small relative to other populations, the Pikiariki population is of high importance. It is one of the few known locations where bats still provide pollination services for *Dactylanthus taylorii*, an endangered root-parasite and the only bat-pollinated ground-flowering plant in the world (Cummings *et al.*, 2014). Ecroyd (1996) suggested that declines in bat distribution and abundance throughout New Zealand may have contributed to declines in *Dactylanthus.* Although remote from large urban centres, Pikiariki has one of the most easily accessible lesser short-tailed bat populations in New

Zealand, providing valuable opportunities for research and advocacy. During the past 10 years six post-graduate research projects have based their investigations on this population, providing important advancements in various aspects of the ecology and conservation of the species.

Recommendations

An abundance estimate is useful for establishing the size of a population at a given point in time, but its value is limited without further assessments to determine the direction of population trends. Only four lesser short-tailed bat populations have had recent assessments of abundance as of 2018 (including that reported here). While population growth on predator-free islands is expected to be positive, periodic reassessment to confirm trends would be prudent, given the importance of these populations as secure sites. The status of populations at the remaining mainland sites needs to be updated urgently to prioritise and gain support for management, and to provide more up-to-date information for future assessments of threat status. If a negative trend is detected, investigation may be required to determine the processes driving the decline, followed by monitoring to assess the effectiveness of any subsequent management actions. These actions may come too late for at least three populations (North-west Nelson, Omahuta and Tararuas) where O'Donnell *et al*. (2010) warn that bat sightings have been rare in recent years.

5.6 Acknowledgements

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Chapter 6

Population viability analysis shows the benefits of rodent control for the conservation of New Zealand lesser short-tailed bats (*Mystacina tuberculata*) and the importance of minimising non-target mortalities

A lesser short-tailed bat emerging from a roost. Photo courtesy of David Mudge, Nga Manu Images.

6.1 Abstract

Lethal or sublethal exposure of non-target wildlife to anticoagulant rodenticides is a widespread and undesirable consequence of vertebrate pest management. Broadscale field application of anticoagulant rodenticides to manage pests that threaten conservation values can result in non-target mortality of threatened species. Low rates of by-kill may be tolerated because non-target wildlife populations are expected to thrive following suppression or eradication of pests. However, species with low reproductive rates and low dispersal ability, like the lesser short-tailed bat (*Mystacina tuberculata*), may be less tolerant to population reductions. The lesser short-tailed bat is susceptible to poisoning from anticoagulant rodenticides used to control rodent pests in its habitat but is also at risk from predation and competition by these invasive mammals. Therefore, a balance is needed between effective rodent control and minimising the impacts from anticoagulant rodenticides used to ensure bat population viability.

I developed a model describing the population dynamics of the lesser short-tailed bat to explore the viability of a small, isolated population over a 10-year timeframe under a series of rodent management and non-target mortality scenarios. My projections aimed to demonstrate how bat population growth was affected by rodent management or lack thereof, and to quantify the magnitude and frequency of poison exposure-related non-target mortalities that could be tolerated under different rodent control regimes. The model was also used to explore the potential impacts on bat population viability of reduced productivity that could result from chronic sublethal exposure of bats to anticoagulant rodenticides.

Model projections indicated that without effective rodent control the population will decline and could be close to extinction within 10 years if predator densities are high. However, effective rodent control involving annual non-target mortality of adults of c.5-12% could also potentially impact viability over this timeframe even if annual survival rates of adult females were as high as 0.8-0.9. Population growth was most sensitive to impacts on adult survival, but I also demonstrated that chronic reductions in productivity have the potential to impact viability over longer timeframes than considered in my analysis. The modelling has demonstrated the need for careful use of anticoagulant rodenticides in bat habitat to ensure the viability of such vulnerable isolated remnant populations.

6.2 Introduction

Invasive rodents (*Rattus* spp.) threaten many wildlife species through predation and competition for resources (Innes *et al.*, 2010; St Clair, 2011; Doherty *et al.*, 2016). Field studies provide evidence that suppression or eradication of rodent populations enhances survival of many threatened species including bats, reptiles and birds (Reardon *et al.*, 2012; Le Corre *et al.*, 2015; O'Donnell *et al.*, 2017). Anticoagulants rodenticides are used to eradicate populations of introduced rodents for conservation gains on offshore and oceanic islands globally (Duron *et al.*, 2016). In New Zealand, their use extends to mainland public conservation land where they are used extensively to suppress rodent populations (Eason *et al.*, 2002; Brown *et al.*, 2015). However, non-target wildlife is at risk through primary (direct) or secondary (indirect) exposure to anticoagulant poisons. Lethal and sublethal impacts of first- and second-generation anticoagulant rodenticide exposure have been documented for many wildlife species, including bats (Eason *et al.*, 2002; Dennis & Gartrell, 2015; Chapter 2).

While prevention of all non-target deaths would be ideal, low levels of non-target mortality associated with toxin use are generally tolerated because the long-term population-level benefits of suppressing rodent populations are expected to more than compensate for individual losses. This has clearly been demonstrated on islands, where rodent eradication operations typically involve one or two aerial applications of anticoagulant rodenticide baits or bait station campaigns of limited duration (Howald *et al.*, 2007). Exposure-related mortalities have been documented in many studies that followed the fates of selected non-target species during rodent eradication operations on islands. However, in most cases post-operation monitoring demonstrated that populations of the affected species quickly recovered to pre-eradication levels or higher in the rodent-free environment (Empson & Miskelly, 1999; Davidson & Armstrong, 2002; Croll *et al.*, 2016; Newton *et al.*, 2016). In rare exceptions non-target losses have outweighed long-term population benefits because the entire local population of a non-target species was extirpated (Taylor, 1984).

Special measures are sometimes taken during island rodent eradication campaigns to minimise risks to non-target species. For example, bait stations were modified to prevent access by non-target mammal species during a rodent eradication operation on Barrow Island, Australia (Morris, 2002) and to prevent skinks accessing baits on Bird Island in the

Seychelles (Merton *et al.*, 2002). In rare cases, security populations of vulnerable species of high conservation value are temporarily held in captivity during the baiting period and reintroduced to the rodent-free site post-eradication (McClelland, 2002; Howald *et al.*, 2009; Oppel *et al.*, 2016). Such extreme measures are not likely to be practical with all species or at all sites.

At unfenced mainland conservation sites eradication of rodents is not currently feasible. Following broadscale pest control operations, rat densities in some New Zealand native forest types has been reported to reach pre-operation levels within two to five months due to reinvasion from surrounding unmanaged areas (Innes *et al.*, 1995). Therefore, annual application of toxins is necessary to reduce rodent pests to low densities during vulnerable periods for native wildlife, usually commencing before the onset of the breeding season each year (Austral summer).

In New Zealand, broad-scale field use of potent and persistent second-generation anticoagulant rodenticides (e.g. brodifacoum) has been restricted on mainland public conservation land since the year 2000 because of documented levels of mortality of non-target wildlife and contamination of game meat (Eason *et al.*, 1999; DOC, 2000; Eason *et al.*, 2002; DOC, 2018). Field use of first-generation compounds (e.g. pindone, diphacinone and coumatetralyl) has subsequently increased, and although less potent and persistent than their second-generation counterparts their use is not without risk (Eisemann & Swift, 2006; Rattner *et al.*, 2012). Populations of non-target species may be exposed to these poisons repeatedly as a result of annual pest management programmes. The cumulative effects of any non-target mortalities occurring on an annual basis and the potential long-term effects of sublethal exposure could affect population viability of vulnerable species.

Species that have low fecundity and low dispersal ability (or are isolated from other populations) may be particularly vulnerable to non-target impacts as they have low capacity to recover their numbers between mortality events. Even small reductions in survival on a regular basis could affect population viability of such species (Spurr, 1979). Temporary captivity would not be a practical solution to safeguard such species at sites where rodent control operations are required annually. Modification of bait stations may reduce direct access to baits but may be ineffective at preventing poisoning if a secondary or tertiary

route of exposure is involved e.g. if toxins moved through the food chain via invertebrates or poisoned rodent carcasses (Howald, 1997; Howald *et al.*, 1999; Dowding *et al.*, 2006; Chapter 3).

The New Zealand lesser short-tailed bat (*Mystacina tuberculata*) is one such vulnerable species. This bat is one of only two native terrestrial mammals in New Zealand and is threatened by rodents and other introduced mammalian predators (O'Donnell *et al.*, 2010). Mainland populations of the bats occur in large tracts of native forest and anticoagulant baits are regularly applied to suppress rodents at several of these sites. Rodent control is required annually in mainland podocarp/broadleaf forests while in mainland southern beech forest (*Lophozonia menziesii* and *Fuscospora* spp.) management is only necessary during mast (mass seeding) years that result in irruption of rodent populations (King, 1983; Elliott & Kemp, 2016). These events occurred on average every 2-6 years at the end of last century but are reported to be increasing in frequency with changes in climate (Wardle, 1984; Tompkins *et al.*, 2013).

Lesser short-tailed bats have a broad diet that includes terrestrial arthropods, putting the bats at risk of secondary exposure to rodenticides through eating contaminated prey (Daniel, 1976; Eason & Spurr, 1995; Chapter 3). Bats may also be sensitive to anticoagulant poisons (Thompson *et al.*, 1972). At least 115 lesser short-tailed bat mortalities caused by anticoagulant rodenticide poisoning occurred during a rodent control operation using diphacinone in Pikiariki Ecological Area, Pureora Forest Park in the North Island, New Zealand in 2009 (Dennis & Gartrell, 2015; Chapter 2). Unprotected delivery of cereal paste baits was considered a precipitating factor in the mortalities (Chapter 4).

The recovery potential of lesser short-tailed bats following population reduction is poor. These bats have low fecundity, producing one pup per breeding female each year (Lloyd, 2005) and evidence from studies on overseas bat species suggests they are likely to be long-lived (>15 years) with low extrinsic rates of mortality (Wilkinson & South, 2002; Barclay & Harder, 2003; Podlutsky *et al.*, 2005). Lesser short-tailed bats are capable fliers but known populations are geographically isolated and genetic studies suggest that long-distance dispersal between populations is rare (Lloyd, 2003).

Following the 2009 mortality event at Pikiariki, populations of lesser short-tailed bats at two locations were monitored through rodent control operations using anticoagulant-laced cereal pellet baits delivered in bait stations. Short-term survival was high in both studies. In the Fiordland population in the South Island, at least 97% of passive integrated transponder (PIT) tagged bats detected prior to baiting with pindone pellets in bait stations were still alive five months later (O'Donnell *et al.*, 2011). In the Pikiariki population, the six-month survival rates of adult males and females monitored through a bait station operation using diphacinone pellets were 0.9 and 0.95 respectively (Chapter 4). Support for a 'poisoning effect' on survival of the bats in the Pikiariki study was equivocal, but if any additional mortality did result from the poison operation it was low (1.2% for adult females, 2.6% for adult males). However, sublethal exposure of bats in the Pikiariki population was confirmed by testing communal guano deposits for residues of diphacinone. Repeated sublethal exposure of bats to anticoagulant poisons during annual pest control operations is of concern because of the potential effects of these chemicals on mammalian reproduction (Hall *et al.*, 1980; Robinson *et al.*, 2005) and other aspects of fitness (Fraser *et al.*, 2018). The effects of sublethal exposure of bats to anticoagulant poisons and the potential consequences of these effects at a population level is unknown.

Although short-term survival of bats was high in the two studies described above, there are three issues that deserve further consideration; (1) the occurrence of sublethal exposure signals that there is potential for further mortalities to occur e.g. natural variation in the abundance of some arthropod taxa between years (Moeed & Meads, 1987) could affect the level of secondary exposure of the bats; (2) sublethal exposure could reduce population viability through cryptic effects on survival and reproduction that manifest over longer time frames (Robinson *et al.*, 2005; Riley *et al.*, 2007; Fraser *et al.*, 2018); and (3) a low incidence of annual non-target mortality in a species with slow recovery potential may accrue over several years to affect population viability.

I conducted a population viability analysis to explore the viability of the lesser short-tailed bat population at Pikiariki over a 10-year period under a series of alternative rodent management and non-target poisoning scenarios. Population viability analysis (PVA) is a useful tool for assessing population viability under different conditions and comparing a range of management alternatives and different levels of threat (e.g. Basse *et al.*, 2003;

Pryde *et al.*, 2005; Armstrong *et al.*, 2007; Fessl *et al.*, 2010; Maggs *et al.*, 2015; O'Donnell *et al.*, 2017). My analysis aimed assess how bat population growth was affected by rodent management and to quantify the magnitude and frequency of poison exposure-related non-target mortalities of lesser short-tailed bats that could be tolerated under different rodent control regimes. I also used the model to explore the potential effects on population viability of reduced productivity that could potentially result from sublethal exposure of bats to anticoagulants. Results of such modelling can help managers to design appropriate rodent control regimes for bat habitat and to identify suitable monitoring requirements for bat populations.

There are few mathematical models of bat population dynamics in the published literature due to the logistical challenges of studying this group of mammals (Frick *et al.*, 2010; O'Shea *et al.*, 2011; Lentini *et al.*, 2015). Furthermore, the great diversity of species and life history traits among bats means that no single model among those developed fits all species (Hallam & Frederico, 2009). Currently, there is no specific model representing the population dynamics of the lesser short-tailed bat. Therefore, I created a simple model of the population dynamics of the species that allowed me to explore the potential effects on bat population viability of using anticoagulant-laced baits to control rodents in their habitat.

6.3 Methods

6.3.1 Simulation model

I created a female-only, discrete-time, age-structured model of lesser short-tailed bat population growth. The structure of the population model was based on a female-only conceptual life-cycle model (Figure 6.1). I developed the conceptual model using information from the published literature on lesser short-tailed bats (O'Donnell *et al.*, 1999; Lloyd, 2005) and other yangochiropteran (formerly microchiropteran) bat species (Tuttle, 1976; Tuttle & Stevenson, 1982; Racey & Entwistle, 2000; Altringham, 2011) and through discussions with a New Zealand bat ecologist (C. O'Donnell, pers. comm., 2015). A female-only model was used for simplicity and because lesser short-tailed bats have a lek-mating system, where a male may fertilise many females, and females are therefore the limiting sex (Toth *et al.*, 2015).

I created the population model in spreadsheet format in Microsoft Excel® which provides an easy to use, transparent and readily available platform for simple population modelling, with a level of accuracy sufficient for the intended purpose (White, 2000a). The model tracked the number of adult females alive at the start of each breeding season. Each column in the spreadsheet represented a discrete year, and each row contained a specific calculation pertaining to reproduction and survival of individuals in the population during that year.

I incorporated age- and sex-specific survival and reproduction rates into the model. These rates were not affected by changes in population size. Density-dependent population regulation mechanisms in bat species are not well understood, and no general models of density dependence have been developed for bats (Hallam & Frederico, 2009). However, density independent population growth is unrealistic for most species (Lebreton & Gimenez, 2013). I therefore limited the potential of the lesser short-tailed bat population to grow indefinitely under the model by using a relatively short time-horizon of 10 years. This also had the benefit of serving to minimise the propagation of any errors associated with uncertainties in model structure or parameterisation (Beissinger & Westphal, 1998; Hallam & Frederico, 2009).

Population growth at Pikiariki is unlikely to be limited by habitat availability within the time frame modelled; the population modelled is small relative to other known lesser short-tailed bat populations (Chapter 5) and currently the core roosting area occupies <1% of the surrounding 78,000 ha Pureora Forest Park (Wallace, 2006; Dennis, 2008). Changes in population size due to immigration and emigration were not considered as long-distance movement between currently known populations has not been recorded and genetic studies suggests that movement between populations in the central North Island is rare (Lloyd, 2003).

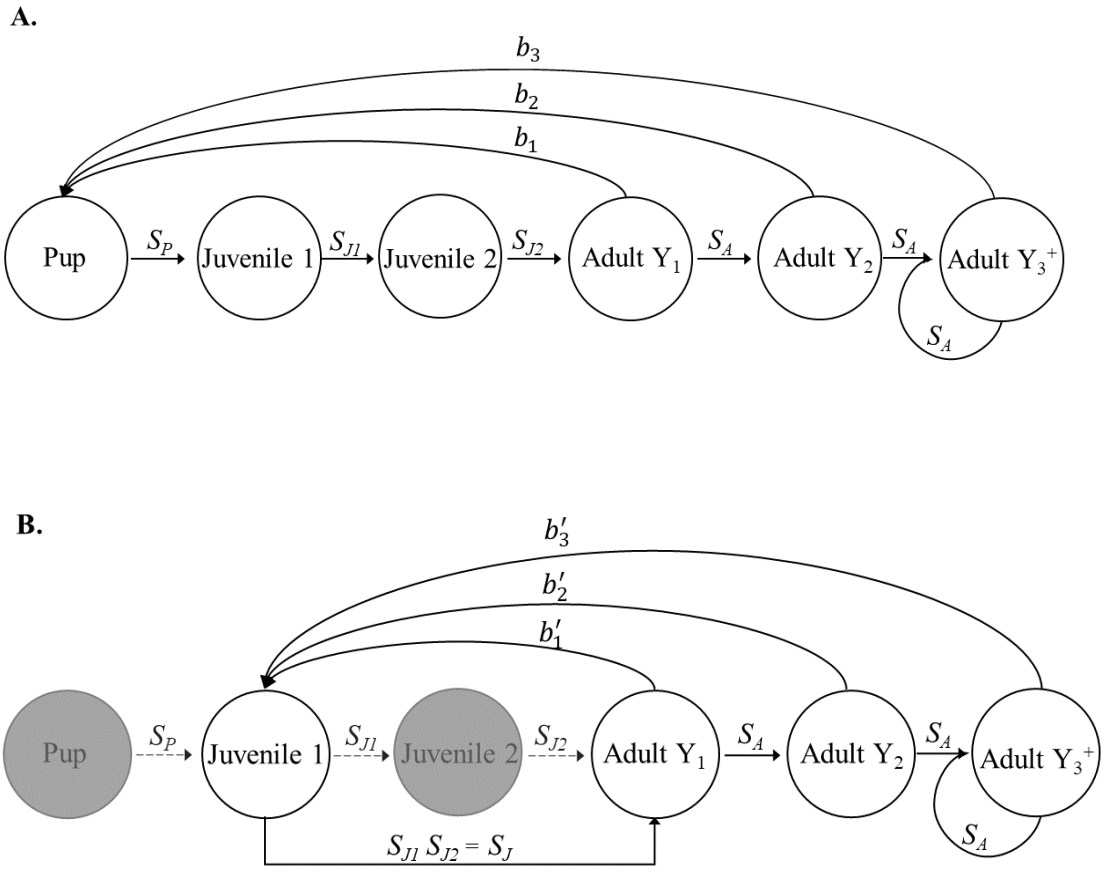

Figure 6.1 A. Conceptual female-only life-cycle model proposed for the lesser short-tailed bat (*Mystacina tuberculata*), and **B**. Model adapted to accommodate known vital rates. Pup (P)=pre-flight (0–c. 4 weeks); Juvenile 1 (J_1)=stage-1 juvenile (early flight, c.4-10 weeks); Juvenile 2 (J_2)=stage-2 juvenile (c. 10 weeks-1 year); Adult Y_1=adults aged between 1-2 years; Adult Y_2=adults aged between 2-3 years; Adult Y_3^+=adults aged $\geq$ 3 years; S_i=probability of young surviving to the next age-class (S_P, S_{J1} ,S_{J2}), or adults surviving to the next time interval (S_A); b_i=No. pups produced by adult age-class *i*, calculated as $N_{Yi}a_{Yi}R_P sr_P$ (No. Y_i adults x proportion of Y_i adults breeding x mean no. pups produced per breeding female x sex ratio for female pups); b'_i=No. stage-1 juveniles produced by age-class *i*, calculated as $N_{Yi}a_{Yi}R_{J1}sr_{J1}$ (No. Y_i adults x proportion of Y_i adults breeding x mean no. stage-1 juveniles produced per breeding female x sex ratio for female stage-1 juveniles).

6.3.2 Sources of Parameter Estimates

I used the conceptual model (Figure 6.1A) to identify the parameters that were required to model the dynamics of the lesser short-tailed bat population then adapted the conceptual model to accommodate the parameter estimates available (Figure 6.1B). Where possible I sourced parameter estimates and associated measures of uncertainty from published and unpublished studies on lesser short-tailed bats (Table 6.1). Where information was lacking

for lesser short-tailed bats I used the New Zealand long-tailed bat (*Chalinolobus tuberculatus*) as a surrogate (Caro *et al.*, 2005). The lesser short-tailed bat is the only extant representative of the Mystacinid family, with no close living relatives (Kirsch *et al.*, 1998). However, the two New Zealand bat species share many characteristics. The long-tailed bat is a long-lived, forest-dwelling, tree-roosting, insectivorous bat, similar in size to the lesser short-tailed bat. Long-tailed bats are known to occur at many of the old-growth forest sites where lesser short-tailed bat populations persist. Both species breed once a year, with breeding females producing a single pup during December (the Austral summer). The timing of births is highly synchronised within colonies (O'Donnell, 2002; Lloyd, 2005). This 'pulsed' birthing pattern is well suited to a discrete-time population model with an annual pre-breeding census.

I sourced estimates of the proportion of females in each age-class that reproduced each year ($\hat{a}_i$) from published studies on long-tailed bats, as this information is not currently available for lesser short-tailed bats (O'Donnell, 2002; Pryde *et al.*, 2005) (Table 6.1). The survival rate of pups from birth to stage-1 juvenile (pre-flight to early flight stage) has not been measured for either species and is typically difficult to measure for bat species in general (Tuttle & Stevenson, 1982). I therefore adjusted the conceptual model to utilise an estimate of the mean number of stage-1 juveniles produced per breeding adult female long-tailed bat each year ($\hat{R}_{J1}$), a rate that incorporates both production rate and survival of pups (Figure 6.1B). The sex ratio of stage-1 juvenile lesser short-tailed bats was assumed to be equal, as for long-tailed bats (O'Donnell, 2002).

Initial adult female population size ($\widehat{N}_0$) was sourced from the lesser short-tailed bat population at Pikiariki (Table 6.1). The estimate was obtained using closed mark-recapture analysis of data collected from PIT-tagged individuals during November 2013 (Chapter 5). Age-specific annual survival rates ($\hat{S}_i$) for the model were sourced from the Pikiariki and Fiordland lesser short-tailed bat populations (Table 6.1). Survival rates from the Pikiariki population were measured by monitoring PIT-tagged individuals over a six-month period (29 October 2013 – 2 May 2014) for adults and a three-month period (6 February – 2 May 2014) for juveniles (Chapter 4). I extrapolated the survival rates for these periods to provide estimates of annual survival for adults ($\hat{S}_A$), and for juveniles from stage-1 (first flight) to year-1 of adulthood ($\hat{S}_J$). This produced estimates of annual adult and juvenile survival that

Table 6.1 Estimates and standard errors (where available) of population parameters used to model the dynamics of a population of lesser short-tailed bats (*Mystacina tuberculata*) in Pikiariki Ecological Area, Pureora Forest Park, New Zealand.

Model parameter	Population parameter	Estimate	SE	Species[a]	Geographic location[b]	Source
$\widehat{N}_0$	No. adult females in starting population	382	31.74	*M. t. rhyacobia*	Pikiariki, N. I., N. Z.	Chapter 5
$\hat{a}_{Y1,}\hat{a}_{Y2,}\hat{a}_{Y3}$	Proportion of Y_1 adult females breeding	0.04	NA	*C. tuberculatus*	Fiordland, South Id., N. Z.	O'Donnell, 2002 Pryde *el* al., 2005
	Proportion of Y_2 adult females breeding	0.60	NA			
	Proportion of Y_3^+ adult females breeding	1	NA			
$\hat{R}_{J1}$	No. of stage 1 juveniles produced per breeding adult female	0.91	0.07	*C. tuberculatus*	Fiordland, South Id., N. Z.	O'Donnell, 2002
$\widehat{sr}_{J1}$	Sex ratio for female stage-1 juveniles	0.5	NA	*C. tuberculatus*	Fiordland, South Id., N. Z.	O'Donnell, 2002
$\hat{S}_J$	Extrapolated annual survival probability for juvenile females (low rat density)	0.740	0.067	*M. t. rhyacobia*	Pikiariki, North Id., N. Z.	Chapter 4
	Annual survival probability for juvenile females (low rat density)	0.689	0.065	*M. t. tuberculata*	Fiordland, South Id., N. Z.	Unpub. analysis on *M. t. tuberculata*, DOC, N. Z.
	Annual survival probability for juvenile females (high rat density)	0.353	0.074			
$\hat{S}_A$	Extrapolated annual survival probability for Y_1^+ adult females (low rat density)	0.896	0.036	*M. t. rhyacobia*	Pikiariki, North Id., N. Z.	Chapter 4
	Annual survival probability for Y_1^+ adult females (low rat density)	0.828	0.023	*M. t. tuberculata*	Fiordland, South Id., N. Z.	Unpub. analysis on *M. t. tuberculata*, DOC, N. Z.
	Annual survival probability for Y_1^+ adult females (high rat density)	0.526	0.043			

[a] *M. t. rhyacobia* = *Mystacina tuberculata rhyacobia*, New Zealand lesser short-tailed bat, central subspecies; *M.t. tuberculata*, New Zealand lesser short-tailed bat, southern subspecies; *C. tuberculatus* = *Chalinolobus tuberculatus*, New Zealand long-tailed bat; [b] Pikiariki, N. I., N. Z.= Pikiariki Ecological Area, Pureora Forest Park, North Island, New Zealand; Fiordland, S. I., N. Z. = Eglinton valley, Fiordland National Park, South Island, New Zealand; Rangataua, N. I., N. Z. = Rangataua Conservation Area, North Island, New Zealand. N. Z. = New Zealand; NA = not available; DOC = Department of Conservation.

were consistent with estimates of annual survival of lesser short-tailed bats in Fiordland during years when predator numbers were either naturally low or suppressed to low densities using vertebrate pesticides (O'Donnell *et al.*, 2017; Thakur *et al.*, 2018; M. Pryde, DOC, unpub. data). I considered this acceptable as the intention was to demonstrate a range of possible outcomes in the PVA rather than a definitive result. As only one season of survival data was available for the Pikiariki population, I also modelled annual survival rates from two years of the Fiordland study to demonstrate a range of possible outcomes (Edmonds *et al.*, 2017; M. Pryde, DOC, unpub. data). Estimates of survival in the Fiordland population were measured by DOC (Department of Conservation) staff using mark-recapture analysis of data collected from PIT-tagged individuals.

The survival estimates used in the model were taken from years in which rodent populations in bat habitat were managed using seasonal application of first-generation anticoagulant rodenticides. Survival estimates for the Pikiariki population were measured during a period when the density of rodents was reduced, based on a comparison of rodent tracking tunnel indices measured pre-baiting (22%) with indices measured during the peak of the bats' breeding season (10%) (Brown *et al.*, 1996; Gillies & Williams, 2013; H. Matthews, DOC, pers. comm. 2014). From the Fiordland study, I selected survival rates from two years that included mast seeding by southern beech trees that led to high rodent abundance. Rodents were controlled effectively during one of the selected years (≤ 1% tracking) (Hill, 2012), and annual survival of adult female bats for that year approximated mean annual survival over the eight-year study (2008-2015) (Edmonds *et al.*, 2017). In the other year selected from the Fiordland study, pest management only occurred over a limited area of the known bat habitat (Edmonds *et al.*, 2017). Rodents remained abundant (c. 60% tracking) during the peak of the bats' breeding season (Elliott & Suggate, 2007), and annual survival of adult female bats was the lowest measured for the population to date (Thakur *et al.*, 2018). Note that rodent tracking indices should not be used to compare abundance between sites with different forest types (Blackwell *et al.*, 2002), and are only intended to be broadly indicative of high or low rat abundance in the context of this chapter.

Estimates of annual survival measured during high rodent abundance were not available for lesser short-tailed bat populations in podocarp forest habitat. Therefore, I used the predicted rate of decline for central North Island populations in the absence of rodent management

(1% per annum) to simulate population growth under these conditions (O'Donnell *et al.*, 2010). Mean population growth (λ) of 0.99 over 10 years was simulated in the model by adding appropriate calculations to cells that contained survival parameters.

6.3.3 Sources of uncertainty

I initially constructed a deterministic model with fixed parameter estimates. I then incorporated three types of uncertainty in to the model; parameter estimate uncertainty, demographic stochasticity and model-selection uncertainty (Armstrong & Reynolds, 2012). Environmental stochasticity was not incorporated in to the model as long-term data sets are required to estimate random annual variation (White, 2000a). Parameter estimate uncertainty was incorporated into the model by randomly sampling values from distributions on each iteration of the model. This was done for adult and juvenile survival rates ($\hat{S}_A$ and $\hat{S}_J$ respectively), the number of stage-1 juveniles produced per breeding female ($\hat{R}_{J1}$), and the number of adult females in the starting population ($\widehat{N}_0$).

For survival parameters, the NORMINV function in Excel® was used to generate a distribution defined by the logit-transformed estimate and its standard error. Real estimates were obtained by back-transforming randomly sampled values. For production of stage-1 juveniles, the same method was followed using log-transformed values to generate the distribution (Armstrong & Reynolds, 2012). The number of adult females in the starting population ($\widehat{N}_0$) was sampled from a log-normal distribution defined by the number of adult females in the population that were not captured during the process of abundance estimation, and the associated standard error (Chapter 5). The number of females in the starting population was obtained on each run of the model by back-transforming a value randomly sampled from the distribution and adding it to the number of adult females that were known to be in the population (Armstrong & Reynolds, 2012). Model-selection uncertainty was also incorporated into this parameter by using a model-averaged estimate of abundance (Chapter 5).

Finally, I incorporated demographic stochasticity into the model for adult and juvenile survival probabilities and for the production of stage-1 juveniles. This was achieved by sampling values from appropriate distributions in each of the model cells that used these parameters in calculations to model population dynamics (section 6.3.4).

6.3.4 Modelling population dynamics

Annual production of stage-1 juveniles (J_1) was calculated in three steps. First, the number of females in each adult age-class breeding each year was sampled from the binomial distribution using the Excel® function CRITBINOM (trials, probability_s, alpha), where trials is the number of individuals in an adult age-class (N_{Yi}), probability is the proportion of individuals in that age-class breeding (a_{Yi}) and alpha is a uniform random number from 0 to 1. Next, the number of stage-1 juveniles produced by each adult age-class was sampled from the binomial distribution using the CRITBINOM function. In this step, trials is the number of females in each adult age-class breeding (from the previous step), and probability is the mean number of stage-1 juveniles produced per breeding female ($\hat{R}_{J1}$). This was constrained to be ≤ 1 as twins are expected to be rare in New Zealand bats, as with many other temperate climate bat species (Barclay & Harder, 2003; Lloyd, 2005). Finally, the number of stage-1 juveniles produced by each adult age-class was summed and multiplied by the sex ratio (sr_{J1}) to provide the total number of stage-1 juvenile females produced in the year.

The number of females in each age-class surviving to the next time step was also sampled from the binomial distribution using the CRITBINOM function (trials, probability_s, alpha), where trials is the maximum number of individuals that can make the transition to the next age-class, probability is age-class specific survival rate ($\hat{S}_i$), and alpha is a uniform random number between 0 and 1. The number of females surviving in each age-class was then summed to provide the total number of adult females alive at the end of the time-step.

6.3.5 Population projections

I used the population model to assess the viability of the adult female lesser short-tailed bat population over a 10-year period. Viability was explored in three scenarios that simulated population growth at different rodent densities with different magnitudes and frequencies of non-target mortalities imposed in years when management involved poison use. The three scenarios were (1) no non-target impacts of annual poison use on survival, (2) consistent non-target impacts of annual poison use on survival and/or reproduction, and (3) periodic non-target impacts of annual poison use on survival. Environmental conditions were assumed to remain constant over the projected period. Each projection within the four scenarios was produced by running the model for 1000 iterations to obtain a distribution of

outcomes (White, 2000b). In all projections where non-target mortalities were imposed the rate was assumed to be additive to the normal mortality rate; for example, under a 20% reduction the annual survival of adult females in Pikiariki would be lowered from 0.90 to 0.70.

Scenario 1

In the first scenario (no poison impacts), I compared projected growth of the bat population using survival parameters estimated or predicted at high or low rodent densities (see section 6.3.2). In this scenario, no non-target mortalities were imposed in association with the use of poisons to manage rodents. Population growth at low rodent densities was modelled using (a) high adult and juvenile survival rates measured at low rodent densities in Pikiariki (low rats A), and (b) moderately high adult and juvenile survival rates measured at low rodent densities in Fiordland (low rats B). In both cases the survival rates used were estimated when rodent populations were suppressed using anticoagulant rodenticides (Table 6.2). To simulate population growth when rodents were at high densities I modelled (a) the predicted rate of decline (1% per annum) of the central North Island lesser short-tailed bat subspecies in the absence of pest management (high rats A, slow decline) and (b) low adult and juvenile survival rates measured in Fiordland during a mast year when pest control using anticoagulant rodenticides was ineffective and rats remained abundant (high rats B, rapid decline) (Table 6.2).

Table 6.2 Abbreviations and estimates used for the sets of survival rates used in the model to simulate lesser short-tailed bat (*Mystacina tuberculata*) population growth at different rodent densities.

Abbreviation	Description and survival estimates
Low rats A	High adult (0.9) and juvenile (0.74) survival rates measured in Pikiariki when rodents were suppressed to low abundance using anticoagulant rodenticides.
Low rats B	Moderately high adult (0.83) and juvenile (0.69) survival rates measured in Fiordland during a beech-mast year when rodents were suppressed to low abundance using anticoagulant rodenticides.
High rats A (slow decline)	Predicted rate of decline (1% per annum) of the central North Island lesser short-tailed bat subspecies in the absence of pest management.
High rats B (rapid decline)	Low adult (0.53) and juvenile (0.35) survival rates measured in Fiordland during a mast year when pest control using anticoagulant rodenticides was ineffective and rats remained abundant.

Scenario 2

In the second scenario (consistent, annual poison impacts), I modelled population growth using the survival rates estimated during low rat abundance (low rats A and low rats B, Table 6.2) and imposed reductions of different magnitudes (0-40%) on breeding output and survival of different age-classes. This was to simulate the potential effects of lethal and sublethal exposure of the bats to anticoagulant rodenticides during annual rodent control operations. For simplicity I assumed that mortalities would affect each age-class at the same rate but in reality, sex, age and other individual variations can affect sensitivity to a poison among individuals in the same population (Connell *et al.*, 2009). For example, juvenile little brown bats (*Myotis lucifugus*) were found to be 1.5 times more sensitive to DDT (dichlorodiphenyltrichloroethane) than adults (Clark *et al.*, 1978).

In the first set of projections, adult and juvenile survival were reduced to simulate potential exposure-related non-target mortalities in these age-classes. In this simulation the production of pups was also reduced as a consequence of the death of breeding females. In the second set of projections, production and survival of pups only were reduced. This was to simulate the possible effects of sublethal exposure of adults on breeding success. In the third set of projections, production of pups and survival of pups, juveniles and adults were reduced to simulate potential impacts caused by both lethal and sublethal exposure (meaning that productivity would be impacted directly by the death of breeding females and indirectly by sublethal exposure of breeding females). Mean population growth rate over 10 years was compared for each projection.

Scenario 3

In the third scenario (periodic poison impacts), additional mortalities of up to 60% were imposed periodically across adult and juvenile age-classes on a frequency of one-, two-, three-, four-, five- or six-yearly intervals in a rodent control regime with 10 consecutive years of rodent management using poisons. This was to simulate potential non-target mortalities that could vary in frequency in association with changes in some unknown environmental factor that affected the probability of the bats being lethally exposed. One hypothesis underlying this approach is that annual fluctuations in the abundance of arthropod species that feed on toxic baits could affect the amount of secondary exposure of bats and therefore the risk of non-target mortalities occurring (Chapter 3). Periodic reductions in survival of adults and juveniles could also represent exposure-related non-

target impacts during mast-years when toxic baiting was required for predator control in beech forest environments such as the Eglinton Valley, Fiordland. In reality the frequency of such events would be random, not evenly spaced as demonstrated here for the purpose of making comparisons. The effect of periodic mortalities on population growth was modelled using the survival rates estimated during low rat abundance (low rats A and low rats B, Table 6.2). Mean population growth rate over 10 years was compared for each projection.

6.4 Results

6.4.1 Population viability analysis

Scenario 1

In the first scenario (no poison impacts), projected population growth over 10 years was positive when female lesser short-tailed bat population dynamics were modelled using survival rates measured during years when rodents were suppressed to low densities. In the projection using high survival rates (low rats A, Table 6.2) the population grew quickly (mean λ=1.13, 95% prediction interval 1.02-1.21). The number of adult females doubled by year six and increased >3-fold in 10 years (Figure 6.2A). Moderately high survival rates (low rats B, Table 6.2) produced a mean population growth rate of 1.05 (0.99-1.11). Under this projection the number of adult females almost doubled in 10 years (Figure 6.2B).

The decrease in the number of adult females over the 10-year period was barely perceptible (mean N_0=382 (326-457); mean N_{10}=360 (136-667)) when the population declined slowly in the absence of rodent management (high rats A Table 6.2, mean λ=0.99 (0.9-1.06)) (Figure 6.2A). However, when low survival rates measured during high rat abundance were used to model growth (high rats B, Table 6.2), the population declined on average 40% per year (mean λ=0.6, 0.48-0.75). In this projection, the number of adult females had more than halved in two years. Fewer than 100 (30-124) adult females remained after four years, and fewer than 10 (0-22) by year nine (Figure 6.2B).

Scenario 2

In the second scenario (consistent annual poison impacts), population growth was more sensitive to annual reductions in survival of adult and juvenile females than to reductions

in the production and survival of pups. Additional exposure-related mortality of adults and juveniles >11.6% (5-17.5%) annually resulted in population decline ($\lambda<1$) when high survival rates (low rats A, Table 6.2) were used to model population growth over 10 years. When moderately high survival rates (low rats B, Table 6.2) were modelled, the population declined if > 4.9% (0-10%) additional mortality of these two age-classes occurred annually for 10 years (Figure 6.3A).

If poison exposure only affected production and survival of pups, reductions in these parameters >40% (30-65%) annually for 10 years resulted in population decline when high survival rates (low rats A, Table 6.2) were modelled. When moderately high survival rates (low rats B, Table 6.2) were modelled, the population declined if pup production and survival was reduced >15% (0-30%) annually for 10 years (Figure 6.3B). If reductions in pup production and survival occurred as a result of both lethal and sublethal exposure of breeding females this resulted in only a small decrease in the level of impact that could be tolerated annually compared to if there were no sublethal effects on reproduction. Population decline resulted from >9% (5-14%) annual reduction in pup production and survival of all three age-classes when high survival rates (low rats A, Table 6.2) were used, and from >4% (0-8%) annual reduction in these parameters when moderately high survival rates (low rats B, Table 6.2) were used in the model (Figure 6.3C).

Scenario 3

In the third scenario (periodic poison impacts), quite large reductions in survival of adult and juvenile females could be tolerated without net population decline if survival rates were initially high and additional exposure-related mortalities occurred less frequently than annually in a pest control regime with 10 consecutive years of management. When high survival rates (low rats A, Table 6.2) were used to model population growth, population decline occurred with additional mortalities >22% every second year (or five times in 10 years), >34% every third year (or three times in 10 years), >46% every fourth or fifth year (or twice in 10 years), or >60% every sixth year (or once in 10 years) (Figure 6.4A). When moderately high survival rates (low rats B, Table 6.2) were modelled, the population declined if >9.5% reduction in adult and juvenile survival occurred every second year (or in five years out of 10), >15.5% every third year (or three times in 10 years); >22.5% every fourth or fifth year (or twice in 10 years); or >39% every sixth year (or once in 10 years) (Figure 6.4B).

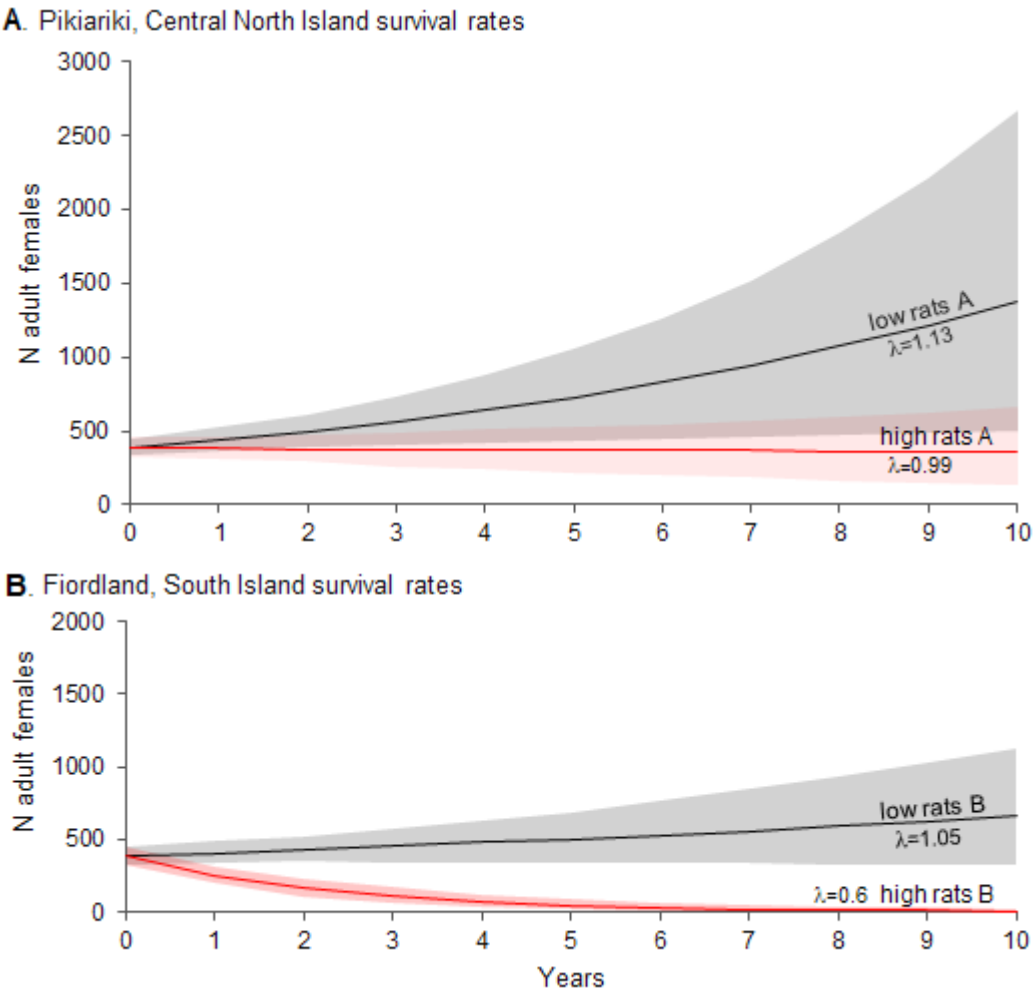

Figure 6.2 Projected changes in the median number of adult female lesser short-tailed bats (*Mystacina tuberculata*) at Pikiariki, central North Island, over 10 years. Population growth was modelled using **A.** high survival rates measured in Pikiariki during low rat abundance (low rats A), and the predicted rate of decline (1% per annum) of central North Island lesser short-tailed bat populations in the absence of rodent management (high rats A), and **B.** moderately high survival rates measured in Fiordland, South Island, during low rat abundance (low rats B), and low survival rates measured in Fiordland during high rat abundance (high rats B). Shaded areas indicate 95% prediction intervals. Mean population growth rate (λ) over 10 years is given for each projection. Each projection was based on 1000 simulations of a female-only population model created in Microsoft Excel®. The initial population size (N_0) for each simulation was randomly sampled from a distribution based on a 2013 pre-breeding abundance estimate and the associated standard error ($\widehat{N}$=382, SE=31.74) determined for the Pikiariki population using closed population mark-recapture analysis (Chapter 5). The predicted decline rate for the central North Island subspecies was sourced from O'Donnell *el al.*, (2010). See Table 6.1 for sources of other parameter estimates used in the model and Table 6.2 for explanation of rodent densities.

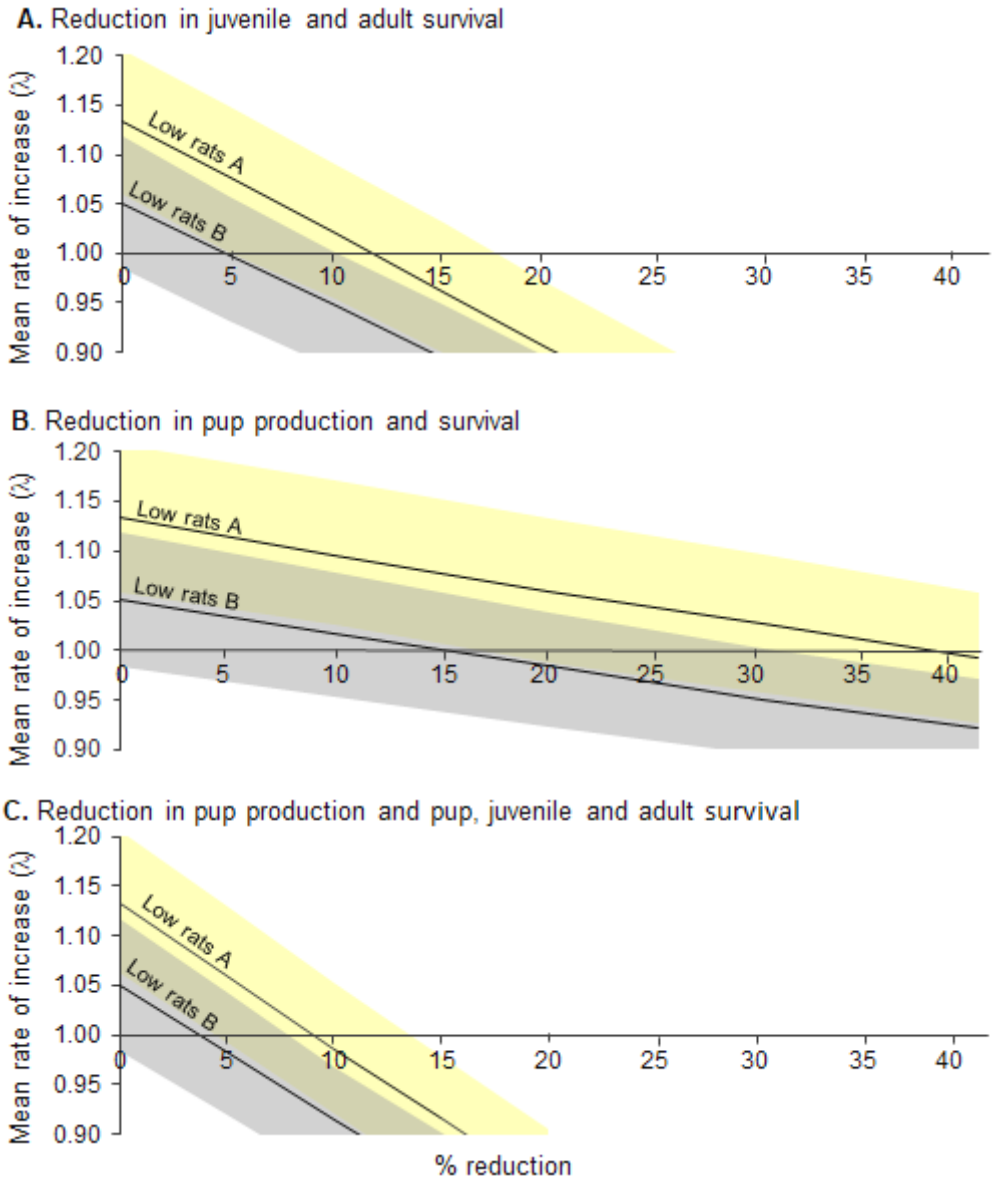

Figure 6.3 Projected mean population growth rates (λ) of adult female lesser short-tailed bats (*Mystacina tuberculata*) at Pikiariki, central North Island subject to 10 years of annual non-target reductions of a range of magnitudes in **A**. survival of female juveniles and adults, **B.** production and survival of female pups, and **C.** production of female pups and survival of female pups, juveniles and adults. Population growth was modelled using high survival rates measured in Pikiariki during low rat abundance (low rats A), and moderately high survival rates measured in Fiordland during low rat abundance (low rats B) to simulate 10 consecutive years of rodent control. Shaded areas indicate 95% prediction intervals. Each projection was based on 1000 simulations of a female-only population model created in Microsoft Excel®. Selection of the initial population size for each simulation, the source of parameter estimates and explanations of rodent densities are as described for Figure 6.2.

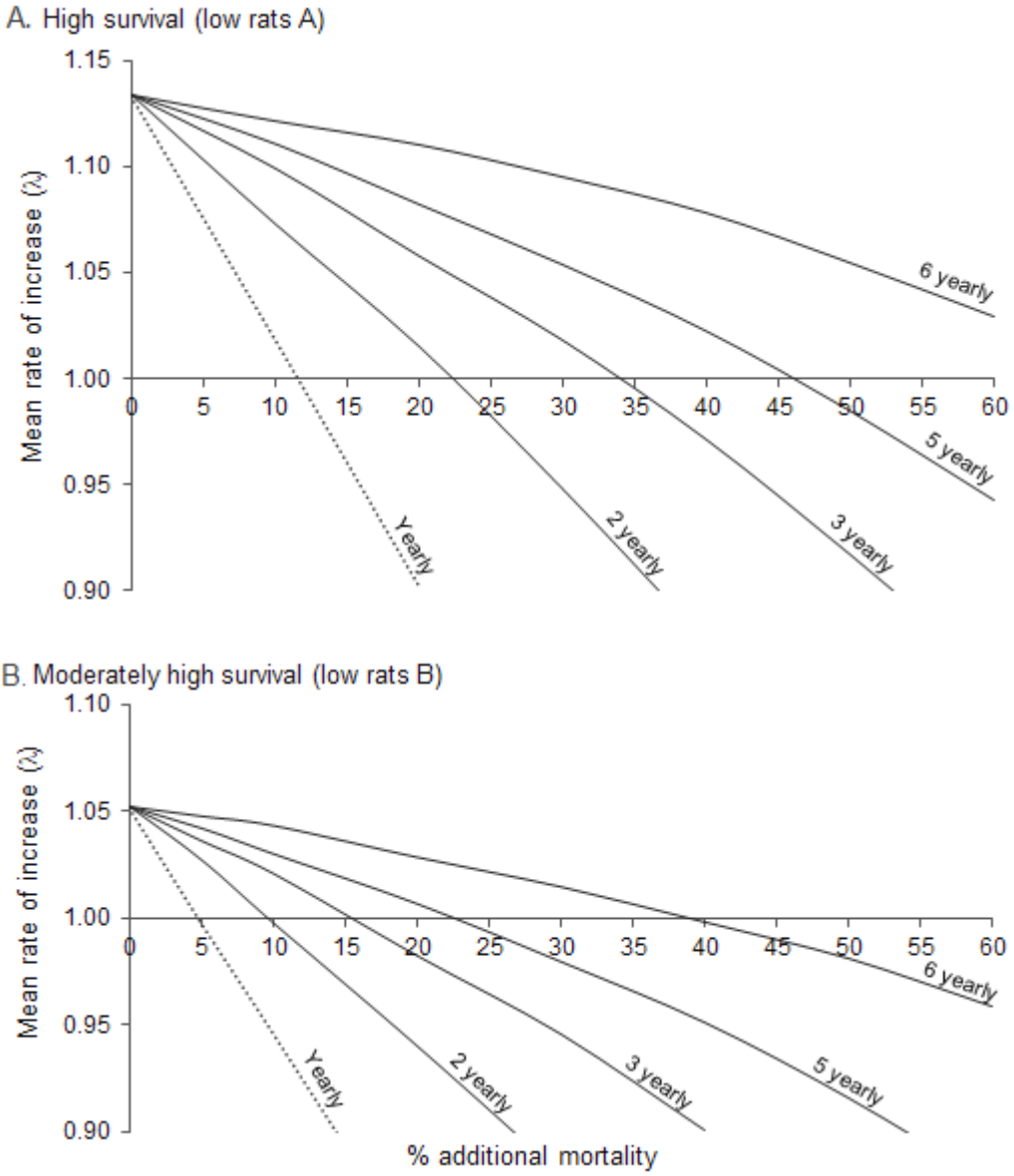

Figure 6.4 Projected mean population growth rates (λ) of adult female lesser short-tailed bats (*Mystacina tuberculata*) at Pikiariki, central North Island subject to periodic non-target reductions in adult and juvenile survival at a variety of frequencies and magnitudes over 10 years. Population growth was modelled using **A.** high survival rates measured in Pikiariki during low rat abundance (low rats A), and **B.** moderately high survival rates measured in Fiordland during low rat abundance (low rats B). Annual reductions in adult and juvenile survival of different magnitudes from Figure 6.3A are shown as dotted lines for comparison. 95% prediction intervals have been omitted for simplicity. Each projection was based on 1000 simulations of a female-only population model created in Microsoft Excel®. Selection of the initial population size for each simulation, the source of parameter estimates and explanations of rodent densities are as described for Figure 6.2.

6.5 Discussion

The effect of rodent density on bat population viability

My simulation modelling indicates that if rodents are not effectively managed in lesser short-tailed bat habitat at Pikiariki the bat population will decline. At high rat densities decline would occur rapidly and the Pikiariki population would be close to extinction within 10 years. The population is already small relative to other known populations (Chapter 5) and if bats are subject to Allee effects (inverse density-dependence at low population densities (Courchamp *et al.*, 2008) as suggested by (Gregory & Jones, 2010), the rate of decline could accelerate as the population size decreases. In contrast, evidence of the slow rate of decline predicted for central North Island lesser short-tailed bat populations in the absence of pest control would be difficult to substantiate within a 10-year period without intensive monitoring of survival parameters using statistically robust methods. The reality would more likely be a mean rate of decline somewhere between these two extremes, with annual variation in survival rates associated with rodent densities and environmental conditions (O'Donnell *et al.*, 2017).

Entire populations of Mystacinid bats have been rapidly extirpated by rodents in the past. Extinction of the last known population of greater short-tailed bats (*M. robusta*) took place on Big South Cape Island, New Zealand, over a just a few years following invasion by ship rats (*Rattus rattus*) c. 1963 (Bell *et al.*, 2016). The lesser short-tailed bat population on the island was also locally extirpated (Towns, 2009). Other bat species are also vulnerable to rapid declines. Monks & O'Donnell (2017) reported collapse of a colony of 88 long-tailed bats over a two-year period when consecutive beech mast (mass seeding) events led to high rodent densities in the Eglinton Valley, Fiordland. Predicative modelling by Pryde *et al*. (2005) indicated that without effective rodent control during beech mast years, the entire long-tailed bat population in the Eglinton Valley would decline to extinction within 50 years. This may happen even more rapidly with the predicted increase in frequency of mast years (O'Donnell *et al.*, 2017). Recent experience demonstrates that failure to act urgently on knowledge of declines can result in species extinctions, as was the case with the Christmas Island pipstrelle (*Pipistrellus murrayi*) (Martin *et al.*, 2012).

Effects of non-target mortalities on viability

A low incidence of by-kill associated with pest control operations is generally tolerated because the population-level benefits of reducing rodent densities are expected to more than compensate for individual losses (Brakes & Smith, 2005; Jones *et al.*, 2016). Here I have used predictive modelling to quantify the levels of additional non-target mortality that could be tolerated at specified survival rates in 10 consecutive years of pest control while maintaining positive population growth. The predictions indicated that even with annual survival rates between 0.8-0.9 for adult females and 0.69-0.74 for juvenile females, additional annual mortality of c.5-12% could result in net population decline. For a small population such as that at Pikiariki (777 adults, 95% C.I. 618-1021, Chapter 5) 5% of the population would be 39 (31-51) adults and 13 (10-17) juveniles (based on the proportion of juveniles in the population at a stable age distribution in the model). This assumes equal effects on all age-classes but in reality juveniles are likely to be more sensitive to poisons (Connell *et al.*, 2009). Furthermore, fewer adult and juvenile mortalities annually would result in decline if there were also reductions in breeding success and pup survival due to sublethal exposure of breeding females.

While changes in baiting practices at Pikiariki have reduced the risk of extensive non-target mortalities recurring on the scale observed in 2009, on-going exposure of bats to diphacinone (and later pindone) has been confirmed and poses an unknown level of risk (Dennis & Gartrell, 2015; Chapters 2 & 4; Thurley, 2017). Twelve dead bats (four adults, one juvenile, five pups and two bats of unknown age) have been found beneath active maternity roost trees during periods of anticoagulant rodenticide use between January 2013 and April 2015. While some natural mortality is expected, five of the six bats that were tested contained residues of diphacinone or pindone, although it is not known whether exposure caused these deaths (Thurley, 2017; Chapter 4). Given the small size of lesser short-tailed bats, their high mobility, cryptic roosting behaviour and the nature of their habitat it is likely that there were further deaths that were undetected.

To more accurately assess the extent of mortalities, appropriate long-term monitoring is necessary. This means having suitably large sample sizes and using methods such as mark-recapture survival analysis that account for detection probabilities (Williams *et al.*, 2002). Using simulation capture-recapture data DeSante *el al*. (2009) applied Cormack-

Jolly-Seber models to assess the sample the sizes needed for statistical power of 80% to detect trends in survival of bird species. In a population with an annual survival rate of 0.8, detection probabilities of 0.8, 0.5, 0.35, 0.2 or 0.05 required that c. 40, 80, 100, 200 or 1000 animals respectively be captured, marked and released each annual sampling period over 20 years for 80% power (α=0.1) to detect a linear trend in survival of 5% (i.e. within the same population over time). Lower survival rates, lower detection probabilities or lower statistical significance levels required larger sample sizes for the same level of power. Survival monitoring of adults and juveniles at Pikiariki using remote detection of PIT-tagged individuals with RFID (radio-frequency identification) aerials at roosts provided high mean detection probability (mean >0.7) of large numbers of bats (> 500) (Chapter 4), so detecting small trends in annual survival of these age-classes should be possible.

Effects of sublethal exposure on viability

Bats are generally long-lived species with high adult survival, low extrinsic mortality and low rates of reproduction (Barclay & Harder, 2003). Population growth rates of such species are particularly sensitive to changes in adult survival rates (Pryde *et al.*, 2005; Schorcht *et al.*, 2009; O'Shea *et al.*, 2011), and this was also indicated by the sensitivity analysis performed using my model. Despite this, I demonstrated that even when mean annual adult female survival rates were moderately high (c. 0.82) a reduction of c. 15% in mean annual production and survival of pups potentially resulting from sublethal exposure of breeding adults to anticoagulant rodenticides could affect the viability of the bat population within a 10-year period. Smaller reductions in mean rates of productivity could equally affect viability if considered over longer time frames.

Chronic reduction of productivity has been identified as the cause of population declines in other species. Decline of Galapagos blue-footed booby (*Sula nebouxii excisa*) populations in Ecuador has been attributed to chronic lack of breeding success since 1998, circumstantially linked to reduced availability of preferred prey (Anchundia *et al.*, 2014). In Australia, progressive regional loss of hooded robin (*Melanodryas cucullate)*, a ground-foraging woodland bird, was recorded over 30 years. The driving force in the decline was chronically low production of young due to high nest predation in fragmented landscapes, with insufficient recruits to replace adult mortality (Ford *et al.*, 2009).

Furthermore, chronic reduction in productivity would slow recovery of numbers should the bat population in Pikiariki be affected by a natural catastrophe or another extensive non-target mortality event impacting adult and juvenile survival. This problem has been demonstrated in in Antarctica, where reduced mean breeding success of the Emperor penguin (*Aptenodytes forsteri*) population has prevented recovery since abrupt changes in climate and ocean environment regimes reduced the population by 50% in the 1970s (Jenouvrier *et al.*, 2009). While numbers remain unnaturally low affected populations are at increased risk of extinction from demographic, catastrophic or genetic causes (Caughley, 1994).

Detecting changes in the rates of reproduction of lesser short-tailed bat populations presents some challenges. The proportion of adult females breeding annually and pup survival rates are unknown for lesser short-tailed bats. Population monitoring currently relies on measures of adult and juvenile survival, so changes in productivity could go undetected. Measurement of productivity parameters in the Eglinton Valley, Fiordland population during non-mast years, when rat densities are naturally low and toxic baiting is therefore not required, would provide reference values that could be used to update predictions from the population model. However, estimation and monitoring of these parameters in free-living bat populations would be challenging because roosting behaviour prevents observation, capture and marking of pups, and sampling adult females, by necessity at maternity roosts, is likely to be biased towards breeders (Sedgeley, 2003; Pers. obs. 2009-2016).

Periodic exposure risk

Although mark-recapture survival monitoring detected no or negligible mortality associated with poison exposure during the 2014/15 field season (Chapter 4) long-term survival monitoring should continue in order to establish whether exposure risk varies annually. Arkins (1999) found that there was variation in the frequency of some groups of arthropods in the diet of lesser short-tailed bats on Little Barrier Island among years. This has not been investigated in Pikiariki.

Reductions in survival >20% of adult and juvenile lesser short-tailed bats in the Pikiariki population could be tolerated if they occurred less frequently than annually when

underlying survival rates are high (>0.8). Mortality events of this scale would be easier to detect and measure due to the increased probability of finding carcasses and the magnitude of the change in annual survival rates. However, it is unlikely that managers would be willing to tolerate ongoing losses of this magnitude because the frequency of such events would be unpredictable, not at regular intervals as demonstrated here.

In beech forest habitats the increasing frequency of mast years will mean an increased frequency of pest control operations and therefore lower levels of tolerance for non-target mortalities. Furthermore, climate changes are also predicted to promote increased density and distribution of invasive predators (Christie, 2014) which may further increase requirements for toxin use in beech forest and the associated levels of exposure risk for bat populations in those habitats.

Model limitations

Parsimony in model building improves model predictive ability (Burnham & Anderson, 2002) but models must still be complex enough to capture the ecology of the studied species (Boyce, 1992). Furthermore, the value of models based on limited data has been widely debated (e.g. Morris *et al.*, 1999; Brook *et al.*, 2000; White, 2000b). My model is fairly simple and the survival parameters incorporated from the Pikiariki population are based on sparse data. The model also draws on data from a surrogate species to supplement the information currently available about lesser short-tailed bat population dynamics. However, I suggest that the model is sufficient to provide a tentative assessment of the potential impacts of non-target mortalities on bat population viability until more data and species-specific parameters are available.

The projections produced by my population model may be optimistic as other threats beside non-target mortality have not been included and my parameter estimates do not incorporate environmental stochasticity (Brook, 2000). Omission of random catastrophic events such as severe weather events (Jones *et al.*, 2001) or mass predation events (Scrimgeour *et al.*, 2012) can also result in overestimation of persistence (Morris *et al.*, 1999). As such events are generally rare, data are limited so it is difficult to estimate their frequency and magnitude. Furthermore, incorporating significant random events during the short time frame modelled here would confound comparison of projections.

In contrast, projections can be pessimistic if 'double dipping' occurs i.e. effects are modelled in the PVA that are already included in vital rates (Brook, 2000). All of the lesser short-tailed bat survival parameters used in my population model were estimated in years when anticoagulant rodenticides were used in pest control operations in bat habitat, so survival parameters could potentially incorporate non-target mortality. However, the model that best described survival of the Pikiariki population did not include a 'poison exposure' effect. If survival estimates borrowed from the Fiordland population included any mortality due to poison exposure the rate was likely to be small as there was no evidence of non-target deaths at the monitored roosts (M. Pryde, pers. comm, 2015).

Recommendations for management, monitoring and research

Building the population model has helped to identify knowledge gaps with respect to population parameters and life history traits of lesser short-tailed bats. As targeted data is collected the population model can be refined to improve its predictive ability for the Pikiariki population specifically and for lesser short-tailed bats in general. Long-term data sets will capture variation in vital rates and enable better estimation of uncertainty, and so improve model predictive ability. If sublethal exposure is found to have effects that threat population viability or if mortalities exceed predicted tolerance levels there will be a need to further reduce or eliminate exposure e.g. by formulating baits that are repellent to forest invertebrates or by switching to entirely non-chemical methods of pest control such as trapping.

Trapping networks could be used to replace toxic baiting or used periodically to offset exposure related mortalities and provide respite from chronic sublethal exposure. However, establishing a trap network and checking and maintaining traps is labour-intensive, increasing costs and potentially limiting the size of the trapped area (Smith & Meyer, 2015). Bat colonies have large collective foraging ranges (O'Donnell *et al.*, 1999; Christie & O'Donnell, 2014) so trapping may only be practical to cover core roosting areas. Automated gas traps are now available for broadscale rodent management in New Zealand. Networks of these traps have proven successful in reducing rats to very low densities in 200 ha of forest during a beech-mast/rat plague event in Fiordland (DOC, 2015). The safety of automated gas traps has not been tested with lesser short-tailed bats that may be attracted to lures designed to attract rodents (Beath *et al.*, 2004) and they could most likely access the traps where they are located

on tree trunks. Captive trials to assess the suitability of using these traps in lesser short-tailed bat habitats is recommended.

While the use of toxins in bat habitat continues, surveillance for mortalities should be accompanied by long-term monitoring of adult and juvenile survival using rigorous methods that can detect small changes in survival trends. This can be used to assess the effectiveness of applied management strategies., the determine patterns and correlates of non-target mortality risk and to ensure thresholds of acceptable levels of annual mortality are not exceeded. The effect of chronic sublethal exposure of lesser short-tailed bats to rodenticides needs to be investigated. Such exposure could have detrimental effects on reproduction and pup survival that are difficult to detect and that affect population viability over time frames longer than considered in this study. Abundance estimates should be done periodically to check population growth against model projections.

Chapter 7

General Discussion

A lesser short-tailed bat foraging on *Dactylanthus* flowers on the forest floor. Photo courtesy of David Mudge, Nga Manu Images.

7.1 Summary of results

The risk of primary or secondary non-target poisoning of the New Zealand lesser short-tailed bat (*Mystacina tuberculata*) with anticoagulant rodenticides had previously been considered a possibility (Eason & Spurr, 1995) but had not been confirmed. Risk assessments that measured survival of wild bats through pest control operations or evaluated the attractiveness of cereal pellet baits to captive and wild bats led to the conclusion that the risk of non-target poisoning was low (Lloyd, 1994; Lloyd & McQueen, 2000; Sedgeley & Anderson, 2000; Lloyd & McQueen, 2002). The incidental discovery in 2009 of 118 dead or dying lesser short-tailed bats during a broadscale rodent control operation using diphacinone on public conservation land established the susceptibility of this species to anticoagulant rodenticide poisoning.

The diagnosis of anticoagulant rodenticide poisoning was supported by a history of use of diphacinone-laced baits in the bats' habitat, clinical signs of anticoagulant toxicosis, gross and histologic lesions at post-mortem, analysis of liver tissue for toxic residues and response of moribund bats to treatment. Details of the mortality incident and the subsequent post-mortem examination have been documented in this thesis and in the published literature to raise awareness within New Zealand and internationally of the risk of broadscale anticoagulant rodenticide use to bats (Dennis & Gartrell, 2015; Chapter 2). In particular, the incident has brought to light the potential hazards associated with using the first-generation anticoagulant rodenticide diphacinone in bat habitat. Diphacinone has been used increasingly for broadscale application on public conservation land in New Zealand and internationally since being considered a suitable lower risk alternative to more potent and persistent second-generation compounds like brodifacoum (Donlan *et al.*, 2003; Gillies *et al.*, 2006; Eason *et al.*, 2010). The method of bait delivery and the bait formulation that were used during the 2009 mortality incident also came under scrutiny.

The 2009 mortality incident provoked the central question underlying my study; how can the risk of non-target poisoning of susceptible species be minimised to ensure that their populations benefit from the broadscale use of vertebrate pesticides to manage conservation pests in their habitat? Specifically, this question was investigated using lesser short-tailed bats and their exposure to diphacinone as the model system under

study, but the process involved could also be applied to resolving this problem for other susceptible species. The applied objective of my study was to provide information to guide New Zealand conservation managers in the design and delivery of future pest control operations in bat habitat that would minimise the risk of further non-target mortalities occurring.

This led me to investigate the route of exposure of lesser short-tailed bats to diphacinone (Chapter 3). Through captive and field trials I determined that the bats had most likely been exposed to diphacinone by eating contaminated arthropod prey rather than by feeding on baits directly; captive and free-living bats showed no interest in non-toxic cereal-based bait similar to that used during the mortality event, while forest arthropods in bat habitat were frequent visitors. My conclusion is supported by (1) previous assessments where captive lesser short-tailed bats showed no interest in cereal-based baits (Lloyd, 1994), (2) extensive observations of forest arthropods visiting and feeding on cereal-based baits in laboratory and field studies (Spurr & Drew, 1999; Craddock, 2003; Bowie & Ross, 2006), (3) detection of anticoagulant residues in the tissues of captive and wild forest arthropods exposed to cereal-based toxic baits (Craddock, 2003; Fisher *et al.*, 2007), and (4) knowledge of the diet and foraging behaviour of lesser short-tailed bats (Daniel, 1976; Daniel, 1979; Arkins *et al.*, 1999; Czenze *et al.*, 2018). The only evidence to date to suggest that primary exposure of bats could also occur is the observation by Beath *et al.* (2004) that wild lesser short-tailed bats held in temporary captivity appeared to sample small amounts of two cereal-based baits. However, the researchers were unable to estimate the amount of bait consumed during the very short time bats spent apparently feeding on these baits, and without this information the risk of primary exposure of bats during pest control operations remains equivocal.

Following the decision by the Department of Conservation (DOC) to use an alternative bait matrix and delivery method for rodent control at the site of the 2009 mortalities, I assessed the effectiveness of these changes at reducing the risk of exposure and non-target mortalities of lesser short-tailed bats during a field trial in 2013/14 (Chapter 4). I determined that replacing cereal-based paste baits with cereal pellet baits and enclosing them in bait stations instead of using biodegradable bags reduced but did not prevent exposure of lesser short-tailed bats to diphacinone. Despite evidence from previous

studies that forest arthropods feed on cereal pellet baits in bait stations (Craddock, 2003; Bowie & Ross, 2006), the change in bait type and delivery method presumably reduced access of arthropods to baits or reduced the amount of bait consumed relative to the amounts consumed during the 2009 mortality event. The question remains as to whether there were other factors that could have affected exposure risk between years, such as differences in abundance of key invertebrate species involved in trophic transfer.

Analysis of communal guano deposits during the field trial confirmed exposure of bats to diphacinone at a population level, and residues were also detected in the bodies of a small number of adults, juveniles and pups found dead during the study (Chapter 4). However, exposure appeared to be subclinical. No overt signs of anticoagulant poisoning were observed in live bats and dried blood spot (DBS) analysis failed to detect residues of diphacinone in blood samples. Prolongation of mean blood prothrombin time was not evident during the period of exposure, nor when compared to mean blood prothrombin time of bats from an unexposed population. Furthermore, mark-recapture survival analysis showed that there was zero to negligible effect of the baiting operation on lesser short-tailed bat survival in the exposed population. This study is noteworthy in that it has attempted to demonstrate a causal relationship between the effects of anticoagulant rodenticide exposure and population survival in wildlife by providing evidence of both exposure and an associated clinical effect.

Lack of a robust estimate of population size at the time of the 2009 mortality incident limited assessment of the potential population-level impact of the mortalities and precluded measurement of population recovery time. Therefore, I estimated population size using mark-recapture methods during the 2013/14 field trial to provide a baseline from which to measure population trends and to enable better assessment of population impacts in case of further mortality events (Chapter 5). The population is small relative to other lesser short-tailed bat populations of known size, so population-level impacts (whether caused by poisoning or predation) are more likely to have severe consequences.

Estimates of abundance and survival from this population were used as parameters in the model that I developed to describe the population dynamics of lesser short-tailed bats (Chapter 6). I used the model to explore the viability of the population over a 10-year

period at high or low rodent densities and when subjected to different magnitudes and frequencies of non-target mortality associated with use of anticoagulant rodenticides. Model projections demonstrated that without effective rodent control the population in Pikiariki will decline. In the worst-case scenario, at high rodent densities, the population could be close to extinction within 10 years. However, I also demonstrated that with annual rodent control using anticoagulant rodenticides, non-target mortality of adults of c.5-12% each year could result in net population decline in a 10-year period, even when annual survival rates of adults were as high as 0.8-0.9. Population growth was most sensitive to impacts on adult survival, but projections also suggested that chronic reductions in productivity had the potential to impact viability over longer timeframes than considered in my analysis. These results confirm the vulnerability of the lesser short-tailed bat population, where population persistence is balanced on the need for effective rodent control with minimal non-target mortality of the bats.

7.2 Management and monitoring recommendations

Based on the results of the survival and exposure study (Chapter 4), the use of cereal pellet baits enclosed in baits stations is recommended for the delivery of anticoagulant rodenticides to control pests in Pikiariki and other bat habitats in New Zealand. This method, where employed to minimise the risk of exposure of bat populations in an area, should be applied at an appropriate landscape scale that considers both bat roosting and foraging habitats. However, given that this method of delivery did not prevent sublethal exposure of bats in the field study, I recommend the use of less potent first-generation anticoagulant poisons in bat habitat until we have a better understanding of the consequences of the level of exposure that occurs, or can develop lower risk control agents (Murphy *et al.*, 2019) or products that deter arthropods from feeding on baits without compromising pest control targets (McGregor *et al.*, 2004).

Pindone is the only other first-generation anticoagulant rodenticide currently registered for broadscale use in New Zealand. Pindone and diphacinone have similar persistence times in mammal tissue (Fisher *et al.*, 2003) but pindone is generally less toxic to mammals than diphacinone (Eason & Wickstrom, 2001; Fisher, 2005). However, it's use is not without risk; lizards may be more sensitive to pindone than to other anticoagulant rodenticides (Weir *et*

al., 2016); some raptor species are sensitive to both pindone and diphacinone (Martin *et al.*, 1994; Twigg *et al.*, 1999; Rattner *et al.*, 2011); and pindone has been implicated in non-target poisoning of wildlife in Australia and New Zealand (Twigg *et al.*, 1999; Lohr & Davis, 2018). The toxicity of pindone to New Zealand native fauna has not been well studied and requires attention.

An alternative poison currently registered for broadscale use on public conservation land is 1080. The use of 1080 in bat habitat has been considered safe and beneficial for lesser short-tailed bats following field assessment in Fiordland. However, the potential consequences of sublethal exposure of lesser short-tailed bat to 1080 was not considered in the field assessment, despite detection of residues in the tissues of one bat found dead (Edmonds *et al.*, 2017). Defects in foetal development and impaired sperm production has been observed in laboratory rats exposed to sublethal doses of 1080 (Eason *et al.*, 1999; Eason & Turck, 2002) but the effect of sublethal exposure to 1080 has not been studied in bats.

Survival and exposure monitoring of the bat population at Pikiariki is recommended to assess the risk of nontarget impacts associated annual pindone application. Monitoring survival of adults and juveniles should continue for a minimum 10-year period using methods that can detect small trends in population growth (DeSante *et al.*, 2009; Chapter 6). Surveillance for mortalities at roosts, post-mortem examination and residue testing of any recovered bodies and testing communal guano for residues are also recommended so that poison exposure risk can be considered when modelling survival. An estimate of adult abundance should be repeated at 5 yearly intervals using methods more robust than roost exit counts so that population growth can be compared with projections from the population model. Slower than expected population growth could be indicative of reduced breeding success if adult and juvenile survival rates are not affected.

Use of a point of collection coagulation monitor to measure clotting time of lesser short-tailed bats in the field during my study was a novel application of this device (Chapter 4). Collection of this data was time consuming and invasive and the method is therefore of limited practical value for DOC field staff monitoring bat survival and exposure. However, prior to my study the normal prothrombin time of lesser short-tailed bats was not known, and no information on prothrombin times of other bat species could be found

in the published literature. The measures taken during my study provide a range of reference values in case of future mortality incidents or for studies examining sublethal exposure and are of value in the event of affected bats requiring veterinary treatment.

Since the mortality event in 2009 DOC has prohibited the use of biodegradable bags to deliver anticoagulant rodenticide baits (DOC, 2018). Furthermore, anticoagulant-laced paste baits paste baits cannot be used in areas where bats are present (O'Donnell *et al.*, 2011). Based on the recommendations provided to DOC at the completion of my field study pindone pellets in bait stations have been used for rodent control in Pikiariki since the 2013/14 field trial and survival monitoring and surveillance for mortalities has continued (Thurley, 2017). Annual survival rates of adults have been > 0.8 each year (2013-2017), but sublethal exposure of the population continues, with residues of pindone detected in communal guano deposits and in the livers of a small number of dead bats recovered.

7.3 Implications for research and conservation management

Implications of sublethal exposure of lesser short-tailed bats to anticoagulant rodenticides

I confirmed that sublethal exposure of adult and juvenile lesser short-tailed bats to diphacinone occurred during the 2013/14 field study (Chapter 4) and established that during the mortality event in 2009 diphacinone was passed to pups in the milk of exposed females (Dennis & Gartrell, 2015; Chapter 2). There is extensive evidence in the literature of widespread sublethal exposure of nontarget wildlife, particularly mammals and birds, to anticoagulant rodenticides. This includes detection of residues in live, dead or sacrificed animals as a result of active surveillance associated with pest control operations (e.g. Murphy *et al.*, 1998; Howald *et al.*, 1999; Hosea, 2000; DOC, 2007; Riley *et al.*, 2007; McMillin *et al.*, 2008; Sánchez-Barbudo *et al.*, 2012) and opportunistic residue testing in animals that died from other or unknown causes (e.g. Shore *et al.*, 1999; Stone *et al.*, 1999; Fournier-Chambrillon *et al.*, 2004; Albert *et al.*, 2010; Elmeros *et al.*, 2011; Murray, 2011).

Aside from coagulopathy, there are limited data concerning the effects of acute or chronic sublethal exposure of wildlife to anticoagulant rodenticides. Correlation between sublethal exposure of urban predators to anticoagulant rodenticides and disease (Riley *et al.*, 2007) and

immune function (Serieys *et al.*, 2018) suggests there could be cryptic and complex effects of these chemicals that reduce fitness. The mechanisms behind these associations is not yet understood. The effects of sublethal exposure to anticoagulants are better understood from human therapy and controlled exposure studies on laboratory or domestic animals. Effects in humans dosed sublethally with coumarin derivatives (e.g. warfarin) during pregnancy include abnormal foetal development, abortion and stillbirth (Hall *et al.*, 1980). In sheep (*Ovis aries*), an increased incidence of stillborn and nonviable lambs was observed in pregnant ewes sublethally dosed with pindone, and sperm motility was reduced in treated rams (Robinson *et al.*, 2005). Female lab rats sublethally dosed with flocumafen dispalyed transient infertility resulting in delayed conception (Sangha *et al.*, 1992). Other effects of sublethal exposure observed in humans include reduced bone density in children chronically dosed with warfarin (Barnes *et al.*, 2005).

The effects of chronic seasonal exposure of lesser short-tailed bats to anticoagulant rodenticides during annual pest control operations in podocarp-broadleaf forest are not known and should be investigated. In particular, research should be concerned with defining thresholds for clinical effects in lesser short-tailed bats, identifying any effects of sublethal exposure on production and survival of pups, and assessing the consequences of these potential effects on population viability. These questions will be challenging to answer as a surrogate species would be required for clinical trials, and the difficulty of accessing and monitoring pups will be problematic in establishing cause and effect for reproduction parameters in wild populations.

Risk to short-tailed bat populations in beech forest habitat

The risk of non-target exposure to anticoagulant rodenticides is not the same for all lesser short-tailed bat populations. Two populations are secure on predator-free offshore islands where they number in the thousands (O'Donnell *et al.*, 2010) (see Chapter 1, Figure 1.3). On the mainland, the risk of non-target mortalities and the potential for impacts from chronic sublethal exposure of bats is much lower for populations in beech forest habitats compared to podocarp-broadleaf forest ecosystems because toxin use is only required to suppress predator irruptions during mast years (on average once every five or six years). However, the frequency of mast-seeding events and associated seed-driven predator irruptions is predicted to increase with climate change (Tompkins *et al.*, 2013; Christie,

2014). Consequently toxin application will be required more frequently in beech forest habitats.

Implications of sublethal exposure of long-tailed bats to anticoagulant rodenticides

The risk of anticoagulant rodenticide exposure of long-tailed bats may have been underestimated in previous assessments (Daniel & Williams, 1984; Eason & Spurr, 1995). A communal guano sample collected in Pikiariki in January 2014 provided evidence of sublethal exposure of long-tailed bats to diphacinone during the period when pelletised baits were deployed in bait stations (Chapter 4). This raises questions about whether toxic residues in larval forms of volant arthropods that feed on bait are retained and possibly magnified through metamorphosis (Kraus *et al.*, 2014). Confirmation of this route of exposure would have implications for sublethal exposure a large number of bat species that are aerial hawkers. Access of long-tailed bats to contaminated prey also raises questions about the potential spread of contaminated arthropod prey beyond the forest interior and the foraging strategies of long-tailed bats in fragmented podocarp forest habitats.

Given that the changes to baiting practice in Pikiariki in 2013/14 reduced exposure of lesser short-tailed bats to diphacinone relative to the lethal exposure that occurred in 2009, it seems reasonable to contemplate whether lethal exposure of long-tailed bats might also have occurred in 2009 (assuming long-tailed bats are also sensitive to anticoagulant rodenticides). No long-tailed bat mortalities were observed at the time. However, mark-recapture population monitoring by DOC did not commence until January 2011 (Pers. obs.) and incidental detection of long-tailed bat mortalities would have been highly unlikely as they roost in small colonies and change roost trees almost every day (O'Donnell, 2000; Dennis, 2011).

Chronic sublethal exposure poses unknown risks to long-tailed bat populations at field sites where anticoagulant rodenticides are used. Furthermore, the risk of lethal exposure may be greater for populations in some locations. Long-tailed bats are 100% insectivorous and on average they are about 30% smaller than lesser short-tailed bats (O'Donnell, 2001a). They have large home ranges (O'Donnell, 2001b) and many populations persist in more modified landscapes outside of the conservation estate,

including at or near mainland sites where the second-generation anticoagulant rodenticide brodifacoum is used by private landowners and regional councils to manage pests. Long-tailed bats may also be more vulnerable to population reductions than lesser short-tailed bats: as well as having low reproductive rates their colony sizes are much smaller (O'Donnell, 2000). Furthermore, long-tailed bat populations that inhabit modified, fragmented landscapes near Geraldine in the South Island were found to have lower survival rates, poorer body condition and lower reproductive success than populations in extensive native forest habitats. This was possibly associated with poorer quality roost sites in the modified landscapes (Sedgeley & O'Donnell, 2004). Stress effects can increase susceptibility to toxins, and toxins can lower susceptibility to other stressors (Vidal *et al.*, 2009).

I recommend monitoring anticoagulant residues in long-tailed bat guano as part of the existing mark-recapture survival study at Pikiariki so that exposure can be modelled as a factor potentially affecting survival. Any dead bats recovered in the course of monitoring should be submitted for post-mortem examination to check for signs of anticoagulant toxicosis and liver residues. The diet of long-tailed bats in Pikiariki has previously been studied by (Gurau, 2014) who identified flies and moths (and occasionally moth larvae) as important items in the summer diet of the bats. Uptake of toxins from baits and poisoned carcasses by these arthropods in their adult and larval forms should be investigated, as should the fate of anticoagulant residues through metamorphosis. Investigation into the exposure of long-tailed bat populations at sites where second-generation anticoagulant rodenticides are routinely used should be a priority. If exposure is detected, monitoring to assess potential population impacts is recommended. Strong evidence of harm will be necessary to convince land management agencies and private landowners to use less potent toxins or alternative methods of pest control.

Implications of potential anticoagulant rodenticide exposure for other bat species

Bats have a global distribution, with more than 1300 species spread across every continent except Antarctica, and 60% of species with distributions on offshore or oceanic islands. They occur in a wide variety of terrestrial habitats, from natural areas at one end of the spectrum to production landscapes and highly modified urban environments at the other (Jones *et al.*, 2009; Fenton & Simmons, 2015). Many bats species are likely to live and

feed in locations where there is sustained or short-term use of first- or second-generation anticoagulant rodenticides to control or eradicate vertebrate pests, including agricultural and pastoral lands, conservation land, plantation forests, urban areas and islands where conservation restoration projects are undertaken (e. g. McDonald *et al.*, 1998; Parshad, 1999; Witmer & Eisemann, 2007; Guitart *et al.*, 2010; Duron *et al.*, 2016) review).

A large number of bat species are totally or partially insectivorous (Fenton & Simmons, 2015) and there are a variety of foraging strategies among them (Schnitzler & Kalko, 2001). Bats that feed entirely on invertebrates and forage near the ground or glean non-volant prey from the ground or from surfaces (Siemers & Ivanova, 2004; Denzinger & Schnitzler, 2013) may be at most risk of encountering contaminated prey in areas where anticoagulant rodenticides are used. Some bat species that are predominantly aerial hawkers but include non-volant invertebrates in their diet at certain times of year may also be at risk (Hope *et al.*, 2014).

Risk assessments during island eradication projects rarely consider bats nor include them in surveillance or monitoring programmes designed to assess non-target impacts (Duron *et al.*, 2016). In the few assessments that I located that did consider bats, the risk of exposure to anticoagulant poisons was considered low, primarily because the bats present were not expected feed directly on baits or to encounter sufficient contaminated prey to warrant concern (Cory *et al.*, 2011; Priddel *et al.*, 2011; U.S.Fish and Wildlife Service, 2011). Eisemann & Swift (2006) gave careful consideration to the risk of secondary exposure of the Hawaiian hoary bat (*Lasiurus cinereus semotus*) during aerial rodent baiting operations using diphacinone-laced baits in Hawaii. These bats feed primarily on Lepidoptera (moths) as well as other flying invertebrates (Jacobs, 1999). Lepidoptera larvae, flies and one adult moth had previously been observed on non-toxic cereal pellet baits of the type used with diphacinone (Dunlevy *et al.*, 2000). The authors acknowledged that the bats could theoretically ingest sufficient diphacinone-contaminated prey in one night to be lethal but concluded that the bats were only at risk of mortality if they were as sensitive as vampire bats, and if larval arthropods feeding on baits retained toxic residues through metamorphosis. No dead hoary bats were found following trial broadcasts of toxic baits. Surveillance methods were not described in the published literature and it is not evident that this potential route of exposure was investigated to assess the possibility

of sublethal exposure of the bats. Although a challenging undertaking, monitoring bat population survival through pest eradication operations on islands where they are present and collecting guano samples to assess sublethal exposure would improve understanding of the real level of risk for this group of animals.

The risks of pesticide exposure for bats in agricultural landscapes, however, may finally be gaining some attention. In June 2019, the European Food Safety Authority released a scientific statement on the coverage of bats by the current pesticide risk assessment for birds and mammals in European countries (Hernandez-Jerez *et al.*, 2019). The authors concluded that the current approach in standard risk assessment scenarios gave inadequate consideration to oral exposure of bats to pesticide-contaminated food. Potential dermal exposure through spraying of pesticides was also highlighted as an important exposure route. Knowledge gaps and research requirements were identified to inform recommendations for further actions.

There is a vast amount of literature reporting non-target mortalities associated with anticoagulant rodenticide exposure during field use of these agents (e.g. Eason *et al.*, 2002; Stone *et al.*, 2003; Riley *et al.*, 2007; Albert *et al.*, 2010; Elmeros *et al.*, 2011; Gabriel *et al.*, 2012; Sánchez-Barbudo *et al.*, 2012). Yet the non-target mortality of bats from anticoagulant exposure described in this thesis (Dennis & Gartrell, 2015; Chapter 2) appears to be the first reported. There could be several possible explanations for this; short-tailed bats may be more sensitive to anticoagulants than other bat species; the delayed onset of anticoagulant poisoning and the cryptic behaviour of bats makes the probability of detecting dead bats extremely low; or chronic sublethal exposure causes deaths indirectly that are seemingly unrelated to anticoagulant rodenticide use. Studies on bats show evidence of exposure to other toxic compounds (e.g. DDT, organochlorines, heavy metals) (O'Shea & Johnston, 2009; Hernout *et al.*, 2016) and similar investigations could be done to test for anticoagulant residues in guano deposits at communal roosting sites in areas where these poisons are used. Bat fatalities detected at windfarms (e.g. Valdez & Cryan, 2013) could potentially provide opportunities to test for liver residues.

Approximately 15% of bat species worldwide are threatened (Critically Endangered, Endangered or Vulnerable) and a further 21% are data deficient (IUCN, 2019).

Recognised threats include habitat loss and degradation, climate change, disease, invasive predators, disturbance, harvesting and exposure to environmental contaminants (O'Shea & Johnston, 2009; Weller *et al.*, 2009; Voigt & Kingston, 2016; O'Donnell *et al.*, 2017). The incidence of chronic sublethal exposure of bats to anticoagulant rodenticides is unknown, nor whether the effects of such exposure alone or combined with other stressors might be contributing to population declines (Vidal *et al.*, 2009). Bats in general are long-lived and have low reproductive rates (Barclay & Harder, 2003) and are therefore slow to recover from population level impacts. The extent of anticoagulant rodenticide exposure among bat species internationally should be investigated and research on the effects of sublethal exposure should be a priority. A better understanding of the threats to bats will help to guide development of appropriate management solutions.

7.4 Conclusion

The research undertaken in this thesis evolved out of a mortality event that confirmed previous theoretical assessments that lesser short-tailed bats could be at risk of secondary poisoning from anticoagulant rodenticides and other vertebrate pesticides during pest control operations. The results of this thesis have informed conservation management of bats in New Zealand, in particular the need to minimise the chances of arthropod-mediated transfer of anticoagulant poisons from baits to bats. This study has global implications for the use of anticoagulant rodenticides to manage vertebrate pests in bat habitats. In the New Zealand context, this study highlights the delicate balance that needs to be achieved between controlling invasive mammalian predators and protecting the highly susceptible bats. Questions remain around the effect of sublethal exposure on bat health, the potential population-level consequences of such exposure and the role that volant arthropods may play in the secondary poisoning of susceptible insectivores.

References

Albert, C.A., Wilson, L.K., Mineau, P., Trudeau, S. & Elliott, J.E. (2010) Anticoagulant rodenticides in three owl species from western Canada, 1988–2003. *Archives of Environmental Contamination and Toxicology,* **58,** 451-459.

Aldridge, H. & Brigham, R. (1988) Load carrying and maneuverability in an insectivorous bat: a test of the 5%"rule" of radio-telemetry. *Journal of Mammalogy,* **69,** 379-382.

Altringham, J.D. (2011) *Bats. From Evolution to Conservation,* 2 edn. Oxford University Press, Oxford.

Anchundia, D., Huyvaert, K. & Anderson, D. (2014) Chronic lack of breeding by Galápagos blue-footed boobies and associated population decline. *Avian Conservation and Ecology,* **9**.

Arellano-Sota, C. (1988) Biology, ecology, and control of the vampire bat. *Reviews of Infectious Diseases,* **10,** S615-S619.

Arkins, A.M. (1997) Report on a study of short-tailed bat winter diet and activity on Codfish Island June-July 1996. *Pekapeka,* **4**.

Arkins, A.M., Winnington, A.P., Anderson, S. & Clout, M.N. (1999) Diet and nectarivorous foraging behaviour of the short-tailed bat (*Mystacina tuberculata*). *Journal of the Zoological Society of London,* **247,** 183-187.

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

Armstrong, D.P. & Ewen, J.G. (2001) Estimating impacts of poison operations using mark-recapture analysis and population viability analysis: an example with New Zealand robins (Petroica australis). *New Zealand Journal of Ecology,* 29-38.

Armstrong, D.P., Perrott, J.K. & Castro, I. (2001) Estimating impacts of poison operations using mark-recapture analysis: hihi (Notiomystis cincta) on Mokoia Island. *New Zealand Journal of Ecology,* 49-54.

Armstrong, D.P. & Reynolds, M.H. (2012) Modelling reintroduced populations: the state of the art and future directions. Chapter 6. *Reintroduction biology: integrating science and management* (eds J.G. Ewen, D.P. Armstrong, K.A. Parker & P.J. Seddon), pp. 165-222. Wiley-Blackwell, Oxford, UK.

Arnason, A.N. & Mills, K.H. (1981) Bias and loss of precision due to tag loss in Jolly-Seber estimates for mark-recapture experiments. *Canadian Journal of Fisheries and Aquatic Sciences,* **38,** 1077 - 1095.

Atkinson, I.A.E. (2001) Introduced mammals and models for restoration. *Biological Conservation,* **99,** 81-96.

Atkinson, I.A.E. & Cameron, E.K. (1993) Human influence on the terrestrial biota and biotic communities of New Zealand. *Trends in Ecology and Evolution,* **8,** 447-451.

Barclay, R.M.R. & Harder, L.D. (2003) Life Histories of Bats: Life in the Slow Lane. *Bat ecology* (eds Kunz TH & Fenton MB), pp. 209-253. University of Chicago, Chicago, Illinois.

Barclay, R.M.R., Ulmer, J., MacKenzie, C.J.A., Thompson, M.S., Olson, L., McCool, J., Cropley, E. & Poll, G. (2004) Variation in the reproductive rate of bats. *Canadian Journal of Zoolgy,* **82,** 688-693.

Barnes, C., Newall, F., Ignjatovic, V., Wong, P., Cameron, F., Jones, G. & Monagle, P. (2005) Reduced bone density in children on long-term warfarin. *Pediatric Research,* **57,** 578.

Basse, B., Flux, I. & Innes, J. (2003) Recovery and maintenance of North Island kokako (*Callaeas cinerea wilsoni*) populations through pulsed pest control. *Biological Conservation,* **109,** 259-270.

Beath, A., Thorne, J. & Robertson, A.W. (2004) Evaluating the attractiveness of pestcControl baits and lures to captive short-tailed bats, *Mystacina tuberculata*. *DOC Science Internal Series 189*, pp. 19. Department of Conservation, Wellington.

Beissinger, S.R. & Westphal, M.I. (1998) On the use of demographic models of population viability in endangered species management. *The Journal of Wildlife Management,* 821-841.

Bell, E.A., Bell, B.D. & Merton, D.V. (2016) The legacy of Big South Cape: rat irruption to rat eradication. *New Zealand Journal of Ecology,* **40,** 212-218.

Bennett, R.S. (2019) Understanding Movement and Habitat Selection of the Lesser Short-tailed Bat to Infer Potential Encounters with Anticoagulant Bait. Unpublished MSc thesis, Massey University, Palmerston North.

Berny, P. (2007) Pesticides and the intoxication of wild animals. *Journal of Veterinary Pharmacology and Therapeutics,* **30,** 93-100.

Blackwell, G.L., Potter, M.A. & McLennan, J.A. (2002) Rodent density indices from tracking tunnels, snap-traps and Fenn traps: do they tell the same story? *New Zealand Journal of Ecology,* 43-51.

Blanchard, B. (1992) The New Zealand lesser short-tailed bat Mystacina tuberculata at the Wellington Zoological Gardens. *International Zoo Yearbook,* 92-96.

Booth, L.H., Eason, C.T. & Spurr, E.B. (2001) Literature review of the acute toxicity and persistence of brodifacoum to invertebrates. *Science for Conservation 177A*, pp. 9. Department of Conservation, Wellington.

Booth, L.H., Fisher, P., Heppelthwaite, V. & Eason, C.T. (2003) Toxicity and residues of brodifacoum in snails and earthworms. *DOC Science Internal Series 143*, pp. 14. Department of Conservation, Wellington.

Bowie, M.H. & Ross, J.G. (2006) Identification of wētā foraging on brodificoum bait and the risk of secondary poisoning for birds on Quail Island, Canterbury, New Zealand. *New Zealand Journal of Ecology,* **30,** 219-228.

Boyce, M.S. (1992) Population viability analysis. *Annual Review of Ecology and Systematics,* **23,** 481-497.

Brakes, C. & Smith, R.H. (2005) Exposure of non-target small mammals to rodenticides: short-term effects, recovery and implications for secondary poisoning. *Journal of Applied Ecology,* **42,** 118-128.

Brook, B.W. (2000) Pessimistic and optimistic bias in population viability analysis. *Conservation Biology,* **14,** 564-566.

Brook, B.W., O'Grady, J.J., Chapman, A.P., Burgman, M.A., Akcakaya, H.R. & Frankham, R. (2000) Predictive accuracy of population viability analysis in conservation biology. *Nature,* **404,** 385.

Brooke, M. de L., Cuthbert, R.J., Harrison, G., Gordon, C. & Taggart, M.A. (2013) Persistence of brodifacoum in cockroach and woodlice: Implications for secondary poisoning during rodent eradications. *Ecotoxicology and Environmental Safety,* **97,** 183–188.

Broome, K.G., Cox, A., Golding, C., Cromarty, P., Bell, P. & McClelland, P. (2014) Rat eradication using aerial baiting: Current agreed best practice used in New Zealand (Version 3.0). *New Zealand Department of Conservation internal document*, pp. 24. Wellington.

Brown, K., Elliott, G., Innes, J. & Kemp, J. (2015) *Ship rat, stoat and possum control on mainland New Zealand: an overview of techniques, successes and challenges*. pp. 36 Department of Conservation, Wellington.

Brown, K., Moller, H., Innes, J. & Alterio, N. (1996) Calibration of tunnel tracking rates to estimate relative abundances of ship rats (*Rattus rattus*) and mice (*Mus musculus*) in a New Zealand forest. *New Zealand Journal of Ecology,* **20,** 272-275.

Buckle, A. & Prescott, C. (2018) Anticoagulants and Risk Mitigation. *Anticoagulant Rodenticides and Wildlife (Vol. 5)* (eds N.W. van den Brink, J.E. Elliott, R.F. Shore & B.A. Rattner), pp. 319-355. Springer, Cham.

Buckle, A.P. (1994) Rodent Control Methods: Chemical. *Rodent pests and their control* (eds A.P. Buckle & R.H. Smith), pp. 127-160. CAB International, Wallingford, UK.

Bullard, R.W. & Thompson, R.D. (1977) Efficacy and safety of the systemic method of vampire bat control. *Interciencia,* **2,** 149-152.

Burnham, K.P. & Anderson, D.R. (2002) *Model Selection and Multimodal Inference. A Practical Information - Theoretic Approach, second ed.* Springer, New York.

Burns, B., Innes, J. & Day, T. (2012) The Use and Potential of Pest-Proof Fencing for Ecosystem Restoration and Fauna Conservation in New Zealand. *Fencing for Conservation* (eds M.J. Somers & M.W. Hayward), pp. 65-90. Springer, New York, NY.

Caro, T., Eadie, J. & Sih, A. (2005) Use of substitute species in conservation biology. *Conservation Biology,* **19,** 1821-1826.

Carter, A., Barr, S., Bond, C., Paske, G., Peters, D. & van Dam, R. (2016) Controlling sympatric pest mammal populations in New Zealand with self-resetting, toxicant-free traps: a promising tool for invasive species management. *Biological Invasions,* **18,** 1723–1736.

Caughley, G. (1994) Directions in Conservation Biology. *Journal of Animal Ecology*, **63,** 215-244.

Christie, J. (2014) *Adapting to a changing climate: a proposed framework for the conservation of terrestrial native biodiversity in New Zealand*. Department of Conservation.

Christie, J.E. (2006) Noctural activity patterns of the lesser short-tailed bat (*Mystacina tuberculata)* in temperate rainforest Fiordland New Zealand. *New Zealand Journal of Zoology*, **33,** 125-132.

Christie, J.E. & O'Donnell, C.F.J. (2014) Large home range size in the ground foraging bat, *Mystacina tuberculata*, in cold temperate rainforest, New Zealand. *Acta Chiropterologica*, **16,** 369-377.

Christie, J.E. & Simpson, W. (2006) Influence of winter weather conditions on lesser short-tailed bat (*Mystacina tuberculata*) activity in *Nothofagus* forest, Fiordland. *New Zealand Journal of Zoology*, **33** 133-140.

Clapperton, B.K., Maddigan, F., Chinn, W. & Murphy, E.C. (2019) Diet, population structure and breeding of Rattus rattus L. in South Island beech forest. *New Zealand Journal of Ecology*, **43,** 1-8.

Clapperton, B.K., Robbins, L., Porter, R.E.R. & Tallentire, K. (2006) Testing the attractiveness, palatability and longevity of stoat lure and bait formulations. *DOC Research and Development Series 241*. Department of Conservation, Wellington.

Clark, D.R., Jr., Bagley, F.M. & Waynon Johnson, W. (1988) Northern Alabama colonies of the endangered grey bat *Myotis grisescens*: organochlorine contamination and mortality. *Biological Conservation*, **43,** 213-225.

Clark, D.R., Jr., Kunz, T.H. & Kaiser, T.E. (1978a) Insecticides applied to a nursery colony of little brown bats (*Myotis lucifugus*): lethal concentrations in brain tissues. *Journal of Mammalogy***,** 84-91.

Clark, D.R., Jr., LaVal, R.K. & Swineford, D.M. (1978b) Dieldrin-induced mortality in an endangered species, the gray bat (*Myotis grisescens*). *Science*, **199,** 1357-1359.

Cockburn (2013) Instructions for setting up RFID readers, dataloggers and antennae. DOCDM379889. Unpublished manual. 25pp. Department of Conservation, Wellington.

Connell, D.W., Lam, P., Richardson, B. & Wu, R. (2009) *Introduction to ecotoxicology*. John Wiley & Sons, Hoboken.

Cooch, E.G. & White, G.C. (2018) Program MARK. A Gentle Introduction (18th edition), Available from: <http://www.phidot.org/software/mark/docs/book>.

Corrigan, R.M. (1984) Nuisance bats: Current technology in their management and control. *Proceedings of the Eleventh Vertebrate Pest Conference, University of Nebraska, Lincoln* (ed. D.O. Clark), pp. 174-179. University of California, Davis.

Cory, F., Wilson, A., Priddel, D., Carlile, N. & Klomp, N. (2011) Eradication of the House Mouse (*Mus musculus*) from Montague Island, New South Wales, Australia. *Ecological Management & Restoration*, **12,** 102-109.

Courchamp, F., Berec, J. & Gascoigne, J. (2008) *Allee Effects in Ecology and Conservation*. Oxford University Press, Oxford, New York, USA.

Cox, P. & Smith, R. (1992) Rodenticide ecotoxicology: pre-lethal effects of anticoagulants on rat behaviour. *15th Vertebrate Pest Conference.* (eds J.E. Borrecco & R.E. Marsh), pp. 165-170. University of Calif., Davis.

Craddock, P. (1997) The effect of rodent control on invertebrate communities in coastal forest near Auckland. Unpublished MSc thesis. University of Auckland.

Craddock, P. (2003) Aspects of the ecology of forest invertebrates and the use of brodifacoum. Unpublished PhD thesis, School of Biological Sciences, University of Auckland.

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

Croll, D.A., Newton, K.M., McKown, M., Holmes, N., Williams, J.C., Young, H.S., Buckelew, S., Wolf, C.A., Howald, G. & Bock, M.F. (2016) Passive recovery of an island bird community after rodent eradication. *Biological Invasions,* **18,** 703-715.

Crowell, M., Eason, C., Hix, S., Broome, K., Fairweather, A., Moltchanova, E., Ross, J. & Murphy, E. (2013) First generation anticoagulant rodenticide persistence in large mammals and implications for wildlife management. *New Zealand Journal of Zoology***,** 1-12.

Crowell, M.D. & Broome, K.G. (2004) Risk assessment system for pesticides used by Department of Conservation. *DOC Science Internal Series 184*, pp. 10. Department of Conservation, Wellington.

Cummings, G., Anderson, S., Dennis, T., Toth, C. & Parsons, S. (2014) Competition for pollination by the lesser short-tailed bat and its influence on the flowering phenology of some New Zealand endemics. *Journal of Zoology,* **293,** 281–288

Czenze, Z.J., Brigham, R.M., Hickey, A.J.R. & Parsons, S. (2017) Winter climate affects torpor patterns and roost choice in New Zealand lesser short-tailed bats. *Journal of Zoology,* **303** 236-243.

Czenze, Z.J., Tucker, J.L., Clare, E.L., Littlefair, J.E., Hemprich-Bennett, D., Oliveira, H.F., Brigham, R.M., Hickey, A.J. & Parsons, S. (2018) Spatiotemporal and demographic variation in the diet of New Zealand lesser short-tailed bats (*Mystacina tuberculata*). *Ecology and evolution,* **8,** 7599-7610.

Daniel, M.J. (1976) Feeding by the short-tailed bat (*Mystacina tuberculata*) on fruit and possibly nectar. *New Zealand Journal of Zoology,* **3,** 391-398.

Daniel, M.J. (1979) The New Zealand short-tailed bat, *Mystacina tuberculata*; a review of present knowledge. *New Zealand Journal of Zoology,* **6,** 357-370.

Daniel, M.J. (1990) Greater short-tailed bat. *The Handbook of New Zealand Mammals* (ed. C.M. King), pp. 131-136. Oxford University Press, Auckland.

Daniel, M.J. & Williams, G.R. (1984) A survey of the distribution, seasonal activity and roost sites of New Zealand bats. *New Zealand Journal of Ecology,* **7,** 9-25.

Davidson, R.S. & Armstrong, D.P. (2002) Estimating impacts of poison operations on non-target species using mark–recapture analysis and simulation modelling: an example with saddlebacks. *Biological Conservation,* **105,** 375-381.

De Lange, H., Sala, S., Vighi, M. & Faber, J. (2010) Ecological vulnerability in risk assessment—a review and perspectives. *Science of the Total Environment,* **408,** 3871-3879.

De Wit, C.A., Persson, G., Nilsson, I.M. & Johansson, B.W. (1984) Circannual changes in blood coagulation factors and the effect of warfarin on the hedgehog *Erinaceus europaeus*. *Comparative Biochemical Physiology,* **80A,** 43-47.

Dennis, G. (2008) A study of short-tailed bats (*Mystacina tuberculata rhyacobia*) in Pikiariki Ecological Area, Pureora Forest Park. November 2007 – January 2008. Internal Report DOCDM-315190. pp. 15. Department of Conservation.

Dennis, G. (2011) Pilot study for monitoring long-tailed bats (*Chalinolobus tuberculatus*) in Pikiariki Ecological Area, Pureora Forest Park, 10 January-18 February, 2011. DOCDM-714728, Department of Conservation Unpublished report. pp. 34.

Dennis, G.C. & Gartrell, B.D. (2015) Non-target mortality of New Zealand lesser short-tailed bats (*Mystacina tuberculata*) caused by diphacinone. *Journal of Wildlife Diseases,* **51,** 177-186.

Denzinger, A. & Schnitzler, H.-U. (2013) Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. *Frontiers in physiology,* **4,** 164.

DeSante, D.F., Kaschube, D.R., Saracco, J.F. & Hines, J.E. (2009) Power to detect differences and trends in apparent survival rates. *Bird Populations,* **9,** 29-41.

DOC (2000) New direction for DOC's use of Brodifacoum (Talon, Pestoff). Fact Sheet. Department of Conservation, Wellington, New Zealand.

DOC (2006) Use of second generation anticoagulants on public conservation lands. Department of Conservation, Northern Regional Office, Hamilton, New Zealand. (Unpublished).

DOC (2007) Trounson Kauri Park Mainland Island Annual Report (Combination of two years work) 2004/5 and 2005/6. *Unpublished report, No. docdm114819. Kauri Coast Area Office, Department of Conservation, Dargaville.*, pp. 63.

DOC (2015) Rat control (100m x 50m) Harts Hill - Fiordland Project Report DOC-2562031. Department of Conservation, Wellington.

DOC (2017) Department of Conservation Te Papa Atawhai Annual Report for the Year Ended 30 June 2017. pp. 113. Wellington.

DOC (2018a) Choose your control method for rats. DOCDM-799089. *Department of Conservation internal document*, pp. 11.

DOC (2018b) Department of Conservation Te Papa Atawhai Annual Report for the year ended 30 June 2018. pp. 143. Wellington.

DOC (2018c) Use of second-generation anticoagulants policy – docDM-97398. pp. 7. Department of Conservation, Wellington, New Zealand (Unpublished).

DOC (2019) Abridged pesticides status list. Unpublished report, DOCDM-22655, Department of Conservation, Wellington. Available at https://www.doc.govt.nz/get-involved/run-a-project/our-procedures-and-sops/managing-animal-pests/standard-operating-procedures/#abridged-status-list.

Doherty, T.S., Glen, A.S., Nimmo, D.G., Ritchie, E.G. & Dickman, C.R. (2016) Invasive predators and global biodiversity loss. *PNAS,* **113** 11261-11265.

Donlan, C.J., Howald, G.R., Tershy, B.R. & Croll, D.A. (2003) Evaluating alternative rodenticides for island conservation: roof rat eradication from the San Jorge Islands, Mexico. *Biological Conservation,* **114,** 29-34.

Dowding, C.V., Shore, R.F., Worgan, A., Baker, P.J. & Harris, S. (2010) Accumulation of anticoagulant rodenticides in a non-target insectivore, the European hedgehog (*Erinaceus europaeus*). *Environmental Pollution,* **158,** 161-166.

Dowding, J., Murphy, E. & Veitch, C. (1999) Brodifacoum residues in target and non-target species following an aerial poisoning operation on Motuihe Island, Hauraki Gulf, New Zealand. *New Zealand Journal of Ecology,* **23,** 207-214.

Dowding, J.E., Lovegrove, T.G., Ritchie, J., Kast, S.N. & Puckett, M. (2006) Mortality of northern New Zealand dotterels (*Charadrius obscurus aquilonius*) following an aerial poisoning operation. *Notornis,* **53,** 235.

Dunlevy, P.A., Campbell, E.W., III, & Lindsey, G.D. (2000) Broadcast application of a placebo rodenticide bait in a native Hawaiian forest. *International Biodeterioration & Biodegradation,* **45,** 199-208.

Duron, Q., Shiels, A.B. & Vidal, E. (2016) Control of invasive rats on islands and priorities for future action. *Conservation Biology,* **31,** 761-771.

Dutton, D.L., Dutton, P.H., Chaloupka, M. & Boulon, R.H. (2005) Increase of a Caribbean leatherback turtle *Dermochelys coriacea* nesting population linked to long-term nest protection. *Biological Conservation,* **126,** 186–194.

Eason, C., Henderson, R., Hix, S., MacMorran, D., Miller, A., Murphy, E., Ross, J. & Ogilvie, S. (2010) Alternatives to brodifacoum and 1080 for possum and rodent control—how and why? *New Zealand Journal of Zoology,* **37,** 175-183.

Eason, C. & Jolly, S. (1993) Anticoagulant Effects of Pindone in the Rabbit and Australian Bushtail Possum. *Wildlife Research,* **20,** 371-374.

Eason, C. & Spurr, E. (1995) Review of the toxicity and impacts of brodifacoum on non-target wildlife in New Zealand. *New Zealand Journal of Zoology,* **22,** 371-379.

Eason, C.T., Milne, L., Potts, M., Morriss, G., Wright, G.R.G. & Sutherland, O.R.W. (1999a) Secondary and tertiary poisoning risks associated with brodifacoum. *New Zealand Journal of Ecology,* **23,** 219-224.

Eason, C.T., Murphy, E.C., Wright, G.R.G. & Spurr, E.B. (2002) Assessment of risks of brodifacoum to non-target birds and mammals in New Zealand. *Ecotoxicology,* **11,** 35-48.

Eason, C.T. & Turck, P. (2002) A 90-day toxicological evaluation of compound 1080 (sodium monofluoroacetate) in Sprague-Dawley rats. *Toxicological Sciences,* **69,** 439-447.

Eason, C.T. & Wickstrom, M. (2001) Vertebrate pesticide toxicology manual (Poisons). Information on poisons used in New Zealand as vertebrate pesticides. *DOC Technical Series 23,* pp. 122. Department of Conservation, Wellington.

Eason, C.T., Wickstrom, M., Turck, P. & Wright, G.R.G. (1999b) A review of recent regulatory and environmental toxicology studies on 1080: results and implications. *New Zealand Journal of Ecology,* **23,** 129-137.

Ecroyd, C.E. (1996) The Ecology of *Dactylanthus taylorii* and threats to its survival. *New Zealand Journal of Ecology,* **20,** 81-100.

Ecroyd, C.E., Franich, R.A., Kroese, H.W. & Steward, D. (1995) The use of *Dactylanthus* nectar as a lure for possums and bats. *Science for Conservation 19,* pp. 31. Department of Conservation, Wellington.

Edmonds, H., Pryde, M. & O'Donnell, C.F. (2017) Survival of PIT-tagged lesser short-tailed bats (*Mystacina tuberculata*) through an aerial 1080 pest control operation. *New Zealand Journal of Ecology,* **41,** 186-192.

Eisemann, J.D. & Swift, C.E. (2006) Ecological and human health hazards from broadcast application of 0.005% diphacinone rodenticide baits in native Hawaiian ecosystems. *Procedings of the Vertebrate Pest Conference,* **22,** 413-433.

Elliott, G. & Kemp, J. (2016) Large-scale pest control in New Zeland beech forests. *Ecological Management and Restoration,* **17,** 200-209.

Elliott, G. & Suggate, R. (2007) Opertion Ark. Three year progress report. Department of Conservation, Christchurch. pp. 84.

Ellison, L.E., O'Shea, T.J., Neubaum, D.J., Neubaum, M.A., Pearce, R.D. & Bowen, R.A. (2007) A comparison of conventional capture versus PIT reader techniques for estimating survival and capture probabilities of big brown bats (*Eptesicus fuscus*). *Acta Chiropterologica,* **9,** 149-160.

Elmeros, M., Christensen, T.K. & Lassen, P. (2011) Concentrations of anticoagulant rodenticides in stoats *Mustela erminea* and weasels *Mustela nivalis* from Denmark. *Science of the Total Environment,* **409,** 2373-2378.

Empson, R.A. & Miskelly, C.M. (1999) The risks, costs and benefits of using brodifacoum to eradicate rats from Kapiti Island, New Zealand. *New Zealand Journal of Ecology,* **23,** 241-254.

Encarnação, J.A. & Dietz, M. (2006) Estimation of food intake and ingested energy in Daubenton's bats (*Myotis daubentonii*) during pregnancy and spermatogenesis. *European Journal of Wildlife Research,* **52,** 221–227.

EPA (1998) Reregistration Eligibility Decision (RED): Rodenticide Cluster. EPA738-R-98-007., pp. 307.

EPA (2008) Risk Mitigation Decision for Ten Rodenticides May 28, 2008 (revised June 24, 2008). pp. 37. US Environmental Protection Agency. Office of Prevention, Pesticides and Toxic Substances, Washington, D.C.

Erickson, W.A., Marsh, R.E. & Halvorson, W.L. (1990) A roof rat bait station that excludes deer mice. *Wildlife Society Bulletin (1973-2006),* **18,** 319-325.

Erickson, W.A. & Urban, D.J. (2004) Potential risks of nine rodenticides to birds and nontarget mammals: a comparative approach. pp. 225. US Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Washington, D.C., USA.

Erkert, H.G. (1982) Ecological aspects of bat activity rhythms. *Ecology of bats* (ed. T.H. Kunz), pp. 201-242. Plenum Press, New York.

Feldheim, K.A., Gruber, S.H., De Marignac, J.A.C. & Ashley, M.V. (2002) Genetic tagging to determine passive integrated transponder tag loss in lemon sharks. *Journal of Fish Biology,* **61,** 1309-1313.

Fenton, M.B. (1970) A technique for monitoring bat activity with results obtained from different environments in southern Ontario. *Canadian Journal of Zoology,* **48,** 847–851.

Fenton, M.B. & Simmons, N.B. (2015) *Bats: a world of science and mystery*. University of Chicago Press.

Fessl, B., Young, G.H., Young, R.P., Rodríguez-Matamoros, J., Dvorak, M., Tebbich, S. & Fa, J.E. (2010) How to save the rarest Darwin's finch from extinction: the mangrove finch on Isabela Island. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **365,** 1019-1030.

Fisher, P. (1999) Review of using rhodamine B as a marker for wildlife studies. *Wildlife Society Bulletin,* **27,** 318-329.

Fisher, P. (2005) *Review of house mouse (Mus musculus) susceptibility to anticoagulant poisons*. Department of Conservation Wellington.

Fisher, P. (2013) Non-target risks of using pindone and 1080 for rabbit control. pp. 26. Landcare Research New Zealand Limited.

Fisher, P. & Broome, K. (2010) Diphacinone. A review of current knowledge. *Pesticide Review Series,* pp. 38. Department of Conservation, Research and Development Group, Hamilton, New Zealand (unpublished).

Fisher, P., O'Connor, C., Wright, G. & Eason, C. (2003) Persistence of four anticoagulant rodenticides in the livers of laboratory rats. *DOC Science Internal Series 139,* pp. 19. Department of Conservation, Wellington.

Fisher, P., Spurr, E.B., Ogilvie, S.C. & Eason, C.T. (2007) Bait consumption and residual concentrations of diphacinone in the Wellington tree wētā (*Hemideina crassidens*) (Orthoptera: Anostomatidae). *New Zealand Journal of Ecology,* **31,** 00-00.

Fisher, P.M. (2009) Residual concentrations and persistence of the anticoagulant rodeniticdes brodifacoum and diphacinone in fauna. Unpublished PhD thesis. Lincoln University.

Ford, H.A., Walters, J.R., Cooper, C.B., Debus, S.J. & Doerr, V.A. (2009) Extinction debt or habitat change?–Ongoing losses of woodland birds in north-eastern New South Wales, Australia. *Biological Conservation,* **142,** 3182-3190.

Fournier-Chambrillon, C., Berny, P.J., Coiffier, O., Barbedienne, P., Dasse, B., Delas, G., Galineau, H., Mazet, A., Pouzenc, P. & Rosoux, R. (2004) Evidence of secondary poisoning of free-ranging riparian mustelids by anticoagulant rodenticides in France: implications for conservation of European mink (*Mustela lutreola*). *Journal of Wildlife Diseases,* **40,** 688.

Fraser, D., Mouton, A., Serieys, L.E.K., Cole, S., Carver, S., Vandewoude, S., Lappin, M., Riley, S.P.D. & Wayne, R. (2018) Genome-wide expression reveals multiple

systemic effects associated with detection of anticoagulant poisons in bobcats (*Lynx rufus*). *Molecular Ecology***,** 1-18.

Freeland, W. & Fry, K. (1995) Suitability of passive integrated transponder tags for marking live animals for trade. *Wildlife Research*, **22,** 767-773.

Frick, W.F., Pollock, J.F., Hicks, A.C., Langwig, K.E., Reynolds, D.S., Turner, G.G., Butchkoski, C.M. & Kunz, T.H. (2010) An emerging disease causes regional population collapse of a common North American bat species. *Science*, **329,** 679-682.

Gabriel, M.W., Woods, L.W., Poppenga, R., Sweitzer, R.A., Thompson, C., Matthews, S.M., Higley, J.M., Keller, S.M., Purcell, K., Barrett, R.H., Wengert, G.M., Sacks, B.N. & Clifford, D.L. (2012) Anticoagulant rodenticides on our public and community lands: spatial distribution of exposure and poisoning of a rare forest carnivore. *PLoS ONE*, **7,** e40163.

Garg, N. & Singla, N. (2015) Blood clotting response test for detecting resistance to second generation anticoagulant bromadiolone in house rat (Rattus rattus). *Indian J. Anim. Res*, **49,** 607-611.

Gibbons, W.J. & Andrews, K.M. (2004) PIT tagging: simple technology at its best. *Bioscience*, **54,** 447-454.

Gillies, C., Styche, A., Bradfield, P., Chalmers, K., Leach, M., Murphy, E., Ward-Smith, T. & Warne, R. (2006) Diphacinone bait for ground control of rats on mainland conservation land. *Science for Conservation 270*, pp. 20. Department of Conservation, Wellington.

Gillies, C.A. & Williams, D. (2013) DOC tracking tunnel guide v2.5.2: Using tracking tunnels to monitor rodents and mustelids. Department of Conservation, Science & Capability Group, Hamilton, New Zealand. www.doc.govt.nz.

Glen, A., Gentle, M. & Dickman, C. (2007) Non-target impacts of poison baiting for predator control in Australia. *Mammal Review*, **37,** 191-205.

Gregory, S. & Jones, G. (2010) Bats and Allee effects: When social behaviours go batty. *The Biologist*, **57,** 198-205.

Griffiths, R., Miller, A. & Climo, G. (2011) Addressing the impact of land crabs on rodenteradications on islands. *Pacific Conservation Biology*, **17,** 347-353.

Grindal, S., Morissette, J. & Brigham, R. (1999) Concentration of bat activity in riparian habitats over an elevational gradient. *Canadian Journal of Zoology*, **77,** 972-977.

Guitart, R., Sachana, M., Caloni, F., Croubels, S., Vandenbroucke, V. & Berny, P. (2010) Animal poisoning in Europe, Part 3: Wildlife. *The Veterinary Journal*, **183,** 260-265.

Gurau, A. (2014) The diet of the New Zealand long-tailed bat, *Chalinolobus tuberculatus*. Master of Zoology, Massey University.

Guthrie, D.M. & Tindall, A.R. (1968) *The Biology of the Cockroach*. Edward Arnold Ltd., London.

Hadler, M.R. & Buckle, A.P. (1992) Forty five years of anticoagulant rodenticides - past, present and future trends. *Fifteenth Vertebrate Pest Conference*, pp. 149-155. University of Nebraska, Lincoln.

Hall, J.G., Pauli, R.M. & Wilson, K.M. (1980) Maternal and fetal sequelae of anticoagulation during pregnancy. *American Journal of Medicine,* **68,** 122-140.

Hallam, T.G. & Frederico, P. (2009) Application of dynamic population models to bats. *Ecological and Behavioural Methods for the Study of Bats* (eds T. Kunz & S. Parsons), pp. 177-194. The John Hopkins University Press, Baltimore.

Hand, S.J., Murray, P., Megirian, D., Archer, M. & Godthelp, H. (1998) Mystacinid Bats (Microchiroptera) from the Australian Tertiary. *Journal of Paleontology*, **72,** 538-545.

Hartley, L., Waas, J., O'Connor, C. & Matthews, L. (2000) Colour preferences and coloured bait consumption by weka *Galliralus australis*, an endemic New Zealand rail. *Biological Conservation*, **93,** 255-263.

Hernandez-Jerez, A., Adriaanse, P., Aldrich, A., Berny, P., Coja, T., Duquesne, S., Gimsing, A.L., Marina, M., Millet, M., Pelkonen, O., Pieper, S., Tiktak, A., Tzoulaki, I., Widenfalk, A., Wolterink, G., Russo, D., Streissl, F. & Topping, C. (2019) Scientific statement on the coverage of bats by the current pesticide risk assessment for birds and mammals. *European Food Safety Authority Journal*, **17,** 1-81

Hernandez-Moreno, D., I. de la Casa-Resino, Lopez-Beceiro, A., Fidalgo, L.E., Soler, F. & Perez-Lopez, M. (2013) Secondary poisoning of non-target animals in an Ornithological Zoo in Galicia (NW Spain) with anticoagulant rodenticides: a case report. *Veterinarni Medicina*, **58,** 553–559.

Hernout, B.V., Arnold, K.E., McClean, C.J., Walls, M., Baxter, M. & Boxall, A.B. (2016) A national level assessment of metal contamination in bats. *Environmental Pollution*, **214,** 847-858.

Herring, J. & McMichael, M. (2012) Diagnostic Approach to Small Animal Bleeding Disorders. *Topics in Companion Animal Medicine*.

Hess, S.C. & Jacobi, J.D. (2011) The history of mammal eradications in Hawai`i and the United States associated islands of the Central Pacific. *Island invasives: eradication and management* (eds C.R. Veitch, M.N. Clout & D.R. Towns), pp. 67-73 IUCN, Gland, Switzerland.

Hill, G. (2012) Threatened species protection in the Eglinton Valley. Annual report 2010/11. Internal report. Department of Conservation, Te Anau., pp. 25.

Hill, J.E. & Daniel, M.J. (1985) Systematics of the New Zealand short-tailed bat Mystacina Gray, 1843 (Chiroptera: Mystacinidae). *Bulletin of the British Museum of Natural History (Zoology)*, **48,** 279-300.

Hitchmough, R., Bull, L. & Cromarty, P. (2007) *New Zealand threat classification system lists: 2005*. pp. 194. Department of Conservation, Wellington.

Hoare, J.M. (2006) Novel predators and naïve prey: how introduced mammals shape behaviours and populations of New Zealand lizards. Unpublished PhD Thesis, Victoria University, Wellington, pp 207.

Hoare, J.M. & Hare, K.M. (2006a) Hoplodactylus maculatus (common gecko) toxin consumption. *Herpetological Review,* **37,** 86-87.

Hoare, J.M. & Hare, K.M. (2006b) The impact of brodifacoum on non-target wildlife: gaps in knowledge. *New Zealand Journal of Ecology,* **30,** 157-167.

Hope, P.R., Bohmann, K., Gilbert, M.T.P., Zepeda-Mendoza, M.L., Razgour, O. & Jones, G. (2014) Second generation sequencing and morphological faecal analysis reveal unexpected foraging behaviour by Myotis nattereri (Chiroptera, Vespertilionidae) in winter. *Frontiers in zoology*, **11,** 39.

Hosea, R.C. (2000) Exposure of non-target wildlife to anticoagulant rodenticides in California. *Nineteenth Vertebrate Pest Conference*, pp. 236-244. San Diego.

Howald, G., Donlan, C.J., Faulkner, K.R., Ortega, S., Gellerman, H., Croll, D.A. & Tershy, B.R. (2009) Eradication of black rats *Rattus rattus* from Anacapa Island. *Fauna & Flora International, Oryx,* **1,** 30-40.

Howald, G., Donlan, C.J., Galvan, J.P., Russell, J.C., Parkes, J., Samaniego, A., Wang, Y.W., Veitch, D., Genovesi, P., Pascal, M., Saunders, A. & Tershy, B. (2007) Invasive rodent eradication on islands. *Conservation Biology,* **21,** 1258-1268.

Howald, G., Mineau, P., Elliott, J. & Cheng, K. (1999) Brodifacoum poisoning of avian scavengers during rat control on a seabird colony. *Ecotoxicology*, **8,** 431-447.

Howald, G.R. (1997) *The risk of non-target species poisoning from brodifacoum used to eradicate rats from Langara Island, British Columbia, Canada*. Unpublished Master of Science thesis, Department of Animal Sciences, The University of British Columbia.

Howald, G.R., Faulkner, K.R., Tershy, B., Keitt, B., Gellerman, H., Creel, E.M., Grinnell, M., Ortega, S.T. & Croll, D.A. (2005) Eradication of black rats from Anacapa Island: biological and social considerations. *Proceedings of the sixth California Islands symposium*, pp. 299-312. Institute for Wildlife Studies, Arcata.

Huckle, K., Hutson, D. & Warburton, P. (1988) Elimination and accumulation of the rodenticide flocoumafen in rats following repeated oral administration. *Xenobiotica,* **18,** 1465-1479.

Huso, M.M.P. (2011) An estimator of wildlife fatality from observed carcasses. *Environmetrics,* **22,** 318-329.

Hutcheon, J.M. & Kirsch, J.A. (2006) A moveable face: deconstructing the Microchiroptera and a new classification of extant bats. *Acta Chiropterologica,* **8,** 1-10.

Innes, J. (2001) Advances in New Zealand mammalogy 1990–2000: European rats. *Journal of the Royal Society of New Zealand,* **31,** 111-125.

Innes, J. & Barker, G. (1999) Ecological consequences of toxin use for mammalian pest control in New Zealand - an overview. *New Zealand Journal of Ecology,* **23,** 111-127.

Innes, J., Kelly, D., Overton, J.M. & Gillies, C. (2010) Predation and other factors currently limiting New Zealand forest birds. *New Zealand Journal of Ecology* **34,** 86-114.

Innes, J., Warburton, B., Williams, D., Speed, H. & Bradfield, P. (1995) Large-scale poisoning of ship rats (*Rattus rattus*) in indigenous forests of the North Island, New Zealand. *New Zealand Journal of Ecology,* **19,** 5-17.

IUCN (2019). https://newredlist.iucnredlist.org/ retrieved 21.7.2019.

Jacob, J. & Buckle, A. (2018) Use of Anticoagulant Rodenticides in Different Applications Around the World. *Anticoagulant rodenticides and wildlife* (eds N.W. Van den Brink, J.E. Elliott, R.F. Shore & B.A. Rattner), pp. 11-44. Springer, Cham.

Jacobs, D.S. (1999) The diet of the insectivorous Hawaiian hoary bat (Lasiurus cinereus semotus) in an open and a cluttered habitat. *Canadian Journal of Zoology,* **77,** 1603-1608.

Jenouvrier, S., Barbraud, C., Weimerskirch, H. & Caswell, H. (2009) Limitation of population recovery: a stochastic approach to the case of the emperor penguin. *Oikos,* **118,** 1292-1298.

Johnson, N., Aréchiga-Ceballos, N. & Aguilar-Setien, A. (2014) Vampire bat rabies: ecology, epidemiology and control. *Viruses,* **6,** 1911-1928.

Johnson, R.A. & Prescott, C.V. (1994) The laboratory evaluation of rodenticides. *Rodent Pests and their Control* (eds A.P. Buckle & R.H. Smith), pp. 405. University Press, Cambridge.

Johnston, J.J., Pitt, W.C., Sugihara, R.T., Eisemann, J.D., Primus, T.M., Holmes, M.J., Crocker, J. & Hart, A. (2005) Probabilistic risk assessment for snails, slugs, and endangered honeycreepers in diphacinone rodenticide baited areas on Hawaii, USA. *Environmental Toxicology and Chemistry,* **24,** 1557-1567.

Jones, G., Webb, P.I., Sedgeley, J.A. & O'Donnell, C.F.J. (2003) Mysterious *Mystacina*; how the New Zealand short-tailed bat (*Mystacina tuberculata*) locates insect prey. *The Journal of Experimental Biology* **206,** 4209-4216.

Jones, H.P., Holmes, N.D., Butchart, S.H., Tershy, B.R., Kappes, P.J., Corkery, I., Aguirre-Muñoz, A., Armstrong, D.P., Bonnaud, E. & Burbidge, A.A. (2016) Invasive mammal eradication on islands results in substantial conservation gains. *Proceedings of the National Academy of Sciences,* **113,** 4033-4038.

Jones, K.E., Barlow, K.E., Vaughan, N., Rodríguez-Durán, A. & Gannon, M.R. (2001) Short-term impacts of extreme environmental disturbance on the bats of Puerto Rico. *Animal Conservation Forum*, pp. 59-66. Cambridge University Press.

Jones, K.E., Mickleburgh, S.P., Sechrest, W. & Walsh, A.L. (2009) Global overview of the conservation of island bats: importance, challenges and opportunities. *Island Bats: Evolution, Ecology, and Conservation.* (eds T.H. Fleming & P.A. Racey), pp. 496-530. University Press, Chicago.

Kalka, M. & Kalko, E.K.V. (2006) Gleaning bats as underestimated predators of herbivorous insects: diet of Micronycteris microtis (Phyllostomidae) in Panama. *Journal of Tropical Ecology,* **22,** 1-10.

Katzner, T.E., Ivy, J.A.R., Bragin, E.A., Milner-Gulland, E.J. & DeWoody, J.A. (2011) Conservation implications of inaccurate estimation of cryptic population size. *Animal Conservation,* **14,** 328-332.

Kerth, G. (2008) Causes and Consequences of Sociality in Bats. *Bioscience,* **58,** 737-746.

King, C. (1983) The relationships between beech (*Nothofagus* sp.) seedfall and populations of mice (*Mus musculus*), and the demographic and dietary responses

of stoats (*Mustela erminea*), in three New Zealand forests. *The Journal of Animal Ecology***,** 141-166.

King, C., Innes, J., Flux, M., Kimberley, M., Leathwick, J. & Williams, D. (1996) Distribution and abundance of small mammals in relation to habitat in Pureora Forest Park. *New Zealand Journal of Ecology,* **20,** 215-240.

King, C.M. (2005) *The Handbook of New Zealand Mammals,* 2nd ed. pp. 596. Oxford University Press, Melbourne.

King, C.M. & Gaukrodger, D.J. (2015) The Moratarium and Afterwards, 1978-87. pp203-224. *The Drama of Conservation: The History of Pureora Forest, New Zealand* (eds C.M. King, D.J. Gaukrodger & N.A. Ritchie), pp. 355. Springer, London.

Kirsch, J.A.W., Hutcheon, J.M., Byrnes, D.G.P. & Lloyd, B.D. (1998) Affinities and historical zoogeography of the New Zealand short-tailed bat, *Mystacina tuberculata* Gray 1843, inferred from DNA-hybridization comparisons. *Journal of Mammalian Evolution,* **5,** 33-64.

Klite, P. (1965) Intestinal bacterial flora and transit time of three neotropical bat species. *Journal of Bacteriology,* **90,** 375-379.

Kraus, J.M., Walters, D.M., Wesner, J.S., Stricker, C.A., Schmidt, T.S. & Zuellig, R.E. (2014) Metamorphosis alters contaminants and chemical tracers in insects: implications for food webs. *Environmental Science & Technology,* **48,** 10957-10965.

Kunz, T.H. (1974) Feeding ecology of a temperate insectivorous bat (*Myotis velifer*). *Ecology,* **55: ,** 693-711.

Kunz, T.H. & Anthony, E.L.P. (1977) On the efficiency of the Tuttle bat trap. *Journal of Mammalogy,* **58,** 309-315.

Kunz, T.H. & Anthony, E.L.P. (1996) Variation in the timing of nightly emergence behavior in the little brown bat *Myotis lucifugus* (Chiroptera: Verspertilionidae). *Contributions in Mammalogy: J. Knox Jones Memorial Volume***,** 225-235.

Kunz, T.H., Betke, M., Hristov, N.I. & Vonhof, M. (2009a) Methods for assessing colony size, population size, and relative abundance of bats. *Ecological and Behavioral Methods for the Study of Bats* (eds T. Kunz & S. Parsons), pp. 133-157. The Johns Hopkins University Press, Baltimore.

Kunz, T.H., Hodgkison, R. & Weise, C.D. (2009b) Methods of Capturing and Handling Bats. *Ecological and Behavioral Methods for the Study of Bats* (eds T.H. Kunz & S. Parsons), pp. 3-35. The John Hopkins University Press, Baltimore.

Laakso, S., Suomalainen, K. & Koivisto, S. (2010) *Literature review on residues of anticoagulant rodenticides in non-target animals*. Nordic Council of Ministers. TemaNord, Copenhagen.

Labrecque, G. & Soulban, G. (1991) Biological rhythms in the physiology and pharmacology of blood coagulation. *Chronobiology International,* **8,** 361-372.

Le Corre, M., Danckwerts, D., Ringler, D., Bastien, M., Orlowski, S., Rubio, C.M., Pinaud, D. & Micol, T. (2015) Seabird recovery and vegetation dynamics after Norway rat eradication at Tromelin Island, western Indian Ocean. *Biological Conservation,* **185,** 85-94.

Lebl, K. & Ruf, T. (2010) An easy way to reduce PIT-tag loss in rodents. *Ecological Research,* **25**.

Lebreton, J.-D., Burnham, K.P., Clobert, J. & Anderson, D.R. (1992) Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. *Ecological Monographs,* **62,** 67-118.

Lebreton, J.D. & Gimenez, O. (2013) Detecting and estimating density dependence in wildlife populations. *The Journal of Wildlife Management,* **77,** 12-23.

Lee, Y.F. & McCracken, G.F. (2001) Timing and variation in the emergence and return of Mexican free-tailed bats, *Tadarida brasiliensis mexicana*. *Zoological Studies,* **40,** 309-316.

Lemus, J.A., Bravo, C., García-Montijano, M., Palacín, C., Ponce, C., Magaña, M. & Alonso, J.C. (2011) Side effects of rodent control on non-target species: Rodenticides increase parasite and pathogen burden in great bustards. *Science of the Total Environment,* **409,** 4729-4734.

Lentini, P.E., Bird, T.J., Griffiths, S.R., Godinho, L.N. & Wintle, B.A. (2015) A global synthesis of survival estimates for microbats. *Biology Letters,* **11,** 20150371.

Linhart, S.B., Flores Crespo, R. & Mitchell, G.C. (1972) Control of vampire bats by topical application of an anticoagulant, chlorophacinone. *Boletin De La OSP-English Edition,* **6,** 31-38.

Lloyd, B. & McQueen, S. (2000) An assessment of the probability of secondary poisoning of forest insectivores following an aerial 1080 possum control operation. *New Zealand Journal of Ecology,* **24,** 47-56.

Lloyd, B.D. (1994a) A brief summary of work undertaken to assess the hazards of aerial baiting with talon to kakapo and short-tailed bats: Period October 1992-March 1994. A report to the Kakapo Recovery Group. Unpublished report. pp. 2. Department of Conservation, Invercargill.

Lloyd, B.D. (1994b) Evaluating the potential hazard of aerial 1080 poison operations to short-tailed bat populations. *Conservation Science Advisory Notes No. 108*. Department of Conservation, Wellington.

Lloyd, B.D. (2003a) The demographic history of the New Zealand short-tailed bat Mystacina tuberculata inferred from modified control region sequences. *Molecular Ecology,* **12,** 1895-1911.

Lloyd, B.D. (2003b) Intraspecific phylogeny of the New Zealand short-tailed bat Mystacina tuberculata inferred from multiple mitochondrial gene sequences. *Systematic Biology,* **52,** 460-476.

Lloyd, B.D. (2003c) Short-tailed bats in time and space: a phylogeographic investigation. *Conservation Science Newsletter No. 46,* pp. 12-15. Department of Conservation, Wellington.

Lloyd, B.D. (2005) The lesser short-tailed bat. *The handbook of New Zealand Mammals, second edition* (ed. C.M. King), pp. 110-126. Oxford University Press, Melbourne.

Lloyd, B.D. & McQueen, S.M. (2002) Measuring mortality in short-tailed bats *(Mystacina tuberculata)* as they return from foraging after an aerial 1080 possum control operation. *New Zealand Journal of Ecology,* **26,** 53-59.

Lohr, M.T. & Davis, R.A. (2018) Anticoagulant rodenticide use, non-target impacts and regulation: A case study from Australia. *Science of the Total Environment,* **634,** 1372-1384.

López-Perea, J.J., Camarero, P.R., Molina-López, R.A., Parpal, L., Obón, E., Solá, J. & Mateo, R. (2015) Interspecific and geographical differences in anticoagulant rodenticide residues of predatory wildlife from the Mediterranean region of Spain. *Science of the Total Environment,* **511,** 259-267.

Low, M., Eason, D. & McInnes, K. (2005) Evaluation of passive integrated transponders for identification of kakapo (*Strigops habroptilus*). *Emu,* **105,** 33-38.

Mace, G.M., Collar, N.J., Gaston, K.J., Hilton-Taylor, C., Akc¸akaya, H.R., Leader-Williams, N., Milner-Gulland, E.J. & Stuart, S.N. (2008) Quantification of extinction risk: IUCN's system for classifying threatened species. *Conservation Biology,* **22,** 1424–1442.

Maggs, G., Nicoll, M., Zuël, N., White, P.J., Winfield, E., Poongavanan, S., Tatayah, V., Jones, C.G. & Norris, K. (2015) *Rattus* management is essential for population persistence in a critically endangered passerine: Combining small-scale field experiments and population modelling. *Biological Conservation,* **191,** 274-281.

Martin, G., Kirkpatrick, W., King, D., Robertson, I., Hood, P. & Sutherland, J. (1994) Assessment of the Potential Toxicity of an Anticoagulant, Pindone (2-Pivalyl-1, 3-Indandione), to Some Australian Birds. *Wildlife Research,* **21,** 85-93.

Martin, T.G., Nally, S., Burbidge, A.A., Arnall, S., Garnett, S.T., Hayward, M.W., Lumsden, L.F., Menkhorst, P., McDonald-Madden, E. & Possingham, H.P. (2012) Acting fast helps avoid extinction. *Conservation Letters,* **5,** 274-280.

Masuda, B.M., Fisher, P. & Beaven, B. (2015) Residue profiles of brodifacoum in coastal marine species following an island rodent eradication. *Ecotoxicology and Environmental Safety,* **113,** 1-8.

McCartney, J., Armstrong, D.P., Gwynne, D.T., Kelly, C.D. & Barker, R.J. (2006) Estimating abundance, age structure and sex ratio of a recently discovered New Zealand tusked wētā *Motuweta riparia* (Orthoptera, Anostostomatidae), using mark-recapture analysis. *New Zealand Journal of Ecology,* **30,** 229-235.

McCartney, J., Stringer, I.A.N. & Potter, M.A. (2007) Feeding activity in captive New Zealand lesser short-tailed bats (*Mystacina tuberculata*). *New Zealand Journal of Ecology,* **34,** 227-238.

McClelland, P. (2002) Eradication of Pacific rats (*Rattus exulans*) from Whenua Hou Nature Reserve (Codfish Island), Putauhinu and Rarotoka Islands, New Zealand. *Turning the tide: the eradication of invasive species,* **27,** 173-181.

McDonald, R.A., Harris, S., Turnbull, G., Brown, P. & Fletcher, M. (1998) Anticoagulant rodenticides in stoats (*Mustela erminea*) and weasels (*Mustela nivalis*) in England. *Environmental Pollution,* **103,** 17-23.

McGlone, M.S. (2009) Postglacial history of New Zealand wetlands and implications for their conservation. *New Zealand Journal of Ecology,* **33,** 1-23.

McGregor, P.G., Peterson, P.G., Berben, P.H., Arnold, G.C. & Spurr, E.B. (2004) Antifeedant effects of diethyl toluamide and neem oil on native cave wētā, cockroaches, and amphipods. *DOC Science Internal Series 159*, pp. 14. Department of Conservation, Wellington.

McIlroy, J.C. (1986) The sensitivity of Australian animals to 1080 poison IX. Comparisons between the major groups of animals, and the potential danger non-target species face from 1080 poisoning campaigns. *Australian Wildlife Research,* **13,** 39-48.

McMillin, S.C., Hosea, R.C., Finlayson, B.F., Cypher, B.L. & Mekebri, A. (2008) Anticoagulant rodenticide exposure in an urban population of San Joaquin kit fox. pp. 163-163.

Mendenhall, V. & Pank, L. (1980) Secondary poisoning of owls by anticoagulant rodenticides. *Wildlife Society Bulletin***,** 311-315.

Merton, D., Climo, G., Laboudallon, V., Robert, S. & Mander, C. (2002) Alien mammal eradication and quarantine on inhabited islands. *Turning the tide: the eradication of invasive species***,** 226-232.

Mickleburgh, S.P., Hutson, A.M. & Racey, P.A. (2002) A review of the global conservation status of bats. *Oryx,* **36** 18-34.

Mineau, P. (2005) A review and analysis of study endpoints relevant to the assessment of "long term" pesticide toxicity in avian and mammalian wildlife. *Ecotoxicology,* **14,** 775-799.

Mineau, P., Baril, A., Collins, B.T., Duffe, J., Joerman, G. & Luttik, R. (2001) Pesticide acute toxicity reference values for birds. *Reviews of Environmental Contamination and Toxicology,* **170,** 13-74.

Moeed, A. & Meads, M.J. (1984) Vertical and seasonal distribution of airborne invertebrates in mixed lowland forest of the Orongorongo Valley, Wellington, New Zealand. *New Zealand Journal of Zoology,* **11** 49-58.

Moeed, A. & Meads, M.J. (1986) Seasonality of litter-inhabiting invertebrates in two native-forest communities of Orongorongo Valley, New Zealand. *New Zealand Journal of Zoology,* **13,** 45-63.

Moeed, A. & Meads, M.J. (1987a) Seasonality and density of emerging insects of a mixed lowland broadleaf podocarp forest floor, OrongorongoValley, New Zealand. *New Zealand Journal of Zoology,* **14,** 477-492.

Moeed, A. & Meads, M.J. (1987b) Seasonality and density of litter and humus invertebrates in broadleaf-podocarp and hard beech forests in Orongorongo Valley, New Zealand. *New Zealand Journal of Zoology. ,* **14,** 51-63.

Monks, J. & O'Donnell, C. (2017) Social implications of a colony collapse in a highly structured vertebrate species (long-tailed bat, *Chalinolobus tuberculatus*). *Animal Conservation,* **20,** 360-369.

Moorhouse, R., Greene, T., Dilks, P., Powlesland, R., Moran, L., Taylor, G., Jones, A., Knegtmans, J. & Wills, D. (2003) Control of introduced mammalian predators improves kaka Nestor meridionalis breeding success: reversing the decline of a threatened New Zealand parrot. *Biological Conservation,* **110,** 33–44.

Morgan, D.R. (1982) Field acceptance of non-toxic and toxic baits by populations of the brushtail possum (*Trichosurus vulpecula* Kerr). *New Zealand Journal of Ecology,* **5**.

Morris, K. (2002) The eradication of the black rat (*Rattus rattus*) on Barrow and adjacent islands off the north-west coast of Western Australia. *Turning the tide: the eradication of invasive species*, **27,** 219-225.

Morris, W., Doak, D., Groom, M., Kareiva, P., Fieberg, J., Gerber, L., Murphy, P. & Thomson, D. (1999) A practical handbook for population viability analysis. *The Nature Conservancy*.

Morriss, G.A., O'Connor, C.E., Airey, A.T. & Fisher, P. (2008) Factors influencing palatability and efficacy of toxic baits in ship rats, Norway rats and house mice. *Science for Conservation 282*, pp. 26. Department of Conservation, Wellington.

MPI (2018) ACVM register-veterinary medicines, agricultural chemicals and vertebrate toxic agents. Ministry for Primary Industries. https://eatsafe.nzfsa.govt.nz/web/public/acvm-register Accessed 11.9.2018.

Murphy, E.C., Clapperton, B.K., Bradfield, P.M.F. & Speed, H.J. (1998) Brodifacoum residues in target and non-target animals following large-scale poison operations in New Zealand podocarp-hardwood forests. *New Zealand Journal of Zoology,* **25,** 307-314.

Murphy, E.C., Russell, J.C., Broome, K.G., Ryan, G.J. & Dowding, J.E. (2019) Conserving New Zealand's native fauna: a review of tools being developed for the Predator Free 2050 programme. *Journal of Ornithology***,** 1-10.

Murphy, M.J. & Talcott, P.A. (2006) Chapter 30: Anticoagulant Rodenticides. *Small Animal Toxicology* (eds M.E. Peterson & P.A. Talcott), pp. 563-577. Elsevier Saunders, Missouri.

Murray, M. (2011) Anticoagulant Rodenticide Exposure and Toxicosis in Four Species of Birds of Prey Presented to a Wildlife Clinic in Massachusetts, 2006-2010. *Journal of Zoo and Wildlife Medicine,*, **42,** 88-97.

Newman, D.G. (1994) Effects of a mouse, *Mus musculus*, eradication programme and habitat change on lizard populations of Mana Island, New Zealand, with special reference to McGregor's skink, *Cyclodina macgregori*. *New Zealand Journal of Zoology,* **21,** 443-456.

Newman, M.C. (2010) *Fundamentals of Ecotoxicology, 3rd Ed*. CRC Press/ Taylor & Francis Group, Boca Raton, Florida.

Newton, K.M., McKown, M., Wolf, C., Gellerman, H., Coonan, T., Richards, D., Harvey, A.L., Holmes, N., Howald, G. & Faulkner, K. (2016) Response of native species 10 years after rat eradication on Anacapa Island, California. *Journal of Fish and Wildlife Management,* **7,** 72-85.

NIWA (2016) The National Climate Database. https://cliflo.niwa.co.nz/ Accessed 27.5.2016.

Norton, D.A. & Overmars, F.B. (2012) Ecological areas – premier protected natural areas. *New Zealand Journl of Ecology,* **36,** 108-120.

O'Connor, C.E. & Eason, C.T. (2000) Rodent baits and delivery systems for island protection. *Science for Conservation 150*, pp. 1173-2946 Department of Conservation, Wellington.

O'Donnell, C. (2008) *Mystacina tuberculata*. The IUCN Red List of Threatened Species 2008: e.T14261A4427784. Downloaded on 14 September 2012 and 01 June 2018. http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T14261A4427784.en.

O'Donnell, C. & Sedgeley, J. (2012) Inventory and monitoring toolbox: DOCDM-590819 Bats: Trapping at roosts: estimating population size.

O'Donnell, C., Christie, J., Hitchmough, R., Lloyd, B. & Parsons, S. (2010) The conservation status of New Zealand bats, 2009. *New Zealand Journal of Zoology,* **37,** 297-311.

O'Donnell, C.F. (2000) Conservation status and causes of decline of the threatened New Zealand Long-tailed Bat Chalinolobus tuberculatus (Chiroptera: Vespertilionidae). *Mammal Review,* **30,** 89-106.

O'Donnell, C.F. & Hoare, J.M. (2012) Quantifying the benefits of long-term integrated pest control for forest bird populations in a New Zealand temperate rainforest. *New Zealand Journal of Ecology,* **36,** 131

O'Donnell, C.F., Pryde, M.A., van Dam-Bates, P. & Elliott, G.P. (2017) Controlling invasive predators enhances the long-term survival of endangered New Zealand long-tailed bats (*Chalinolobus tuberculatus*): Implications for conservation of bats on oceanic islands. *Biological Conservation,* **214,** 156-167.

O'Donnell, C.F.J. (2000a) Cryptic local populations in a temperate rainforest bat *Chalinolobus tuberculatus* in New Zealand. *Animal Conservation,* **3,** 287-297.

O'Donnell, C.F.J. (2000b) Influence of season, habitat, temperature, and invertebrate availability on nocturnal activity of the New Zealand long-tailed bat (*Chalinolobus tuberculatus*). *New Zealand Journal of Zoology,* **27,** 207-221.

O'Donnell, C.F.J. (2001a) Advances in New Zealand mammalogy 1990–2000: Long-tailed bat. *Journal of the Royal Society of New Zealand,* **31,** 43-57.

O'Donnell, C.F.J. (2001b) Home range and use of space by *Chalinolobus tuberculatus*, a temperate rainforest bat from New Zealand. *Journal of Zoology,* **253,** 253-264.

O'Donnell, C.F.J. (2002) Timing of breeding, productivity and survival of long-tailed bats Chalinolobus tuberculatus (Chiroptera: Vespertilionidae) in cold-temperate rainforest in New Zealand. *Journal of Zoology,* **257,** 311-323.

O'Donnell, C.F.J. (2010) The ecology and conservation of New Zealand bats. *Island Bats: Evolution, Ecology and Conservation* (eds T.H. Fleming & P.A. Racey), pp. 460-495. Chicago University Press, Chicago.

O'Donnell, C.F.J. & Hoare, J.M. (2012) Quantifying the benefits of long-term integrated pest control for forest bird populations in a New Zealand temperate rainforest. *New Zealand Journal of Ecology,* **36,** 131-140.

O'Donnell, C.F.J., Borkin, K.M., Christie, J.E., Lloyd, B., Parsons, S. & Hitchmough, R.A. (2018) *Conservation status of New Zealand bats, 2017.* Department of Conservation, Wellington.

O'Donnell, C.F.J., Christie, J., Corben, C., Sedgeley, J.A. & Simpson, W. (1999) Rediscovery of short-tailed bats (*Mystacina* sp.) in Fiordland, New Zealand: preliminary observations of taxonomy, echolocation calls, population size, home range, and habitat use. *New Zealand Journal of Ecology,* **23,** 21-30.

O'Donnell, C.F.J., Christie, J.E. & Simpson, W. (2006) Habitat use and nocturnal activity of lesser short-tailed bats (*Mystacina tuberculata*) in comparison with long-tailed bats (*Chalinolobus tuberculatus*) in temperate rainforest. *New Zealand Journal of Zoology,* **33,** 113-124.

O'Donnell, C.F.J., Edmonds, H. & Hoare, J.M. (2011) Survival of PIT-tagged lesser short-tailed bats (*Mystacina tuberculata*) through a pest control operation using the toxin pindone in bait stations. *New Zealand Journal of Ecology,* **35,** 291.

O'Shea, T.J. & Clark, D.R., Jr. (2002) An overview of contaminants and bats, with special reference to insecticides and the Indiana bat. *The Indiana Bat: Biology and Management of an Endangered Species* (eds A. Kurta & J. Kennedy), pp. 237-253. Bat Conservation International, Austin, Texas.

O'Shea, T.J., Ellison, L.E. & Stanley, T.R. (2011) Adult survival and population growth rate in Colorado big brown bats (*Eptesicus fuscus*). *Journal of Mammalogy*, **92,** 433-443.

O'Shea, T.J. & Johnston, J.J. (2009) Environmental contaminants and bats; investigating exposure and effects. *Ecological and behavioral methods for the study of bats/edited by Thomas H. Kunz and Stuart Parsons.*

O'Shea, T.J., Ellison, L.E. & Stanley, T.R. (2004) Survival estimation in bats: historical overview, critical appraisal, and suggestions for new approaches. *Sampling rare or elusive species: concepts, designs, and techniques for estimating population parameters* (ed. M.L. Thompson), pp. 297-336. Island Press, Washington, DC.

Ohad, D.G. (2017) Pallor. *Textbook of Veterinary Internal Medicine. Eighth Edition* (eds S.J. Ettinger, E.C. Feldman & E. Cote), pp. 206-208. Elsevier-Health Sciences Division, Philadelphia, United States.

Oliver, A. & Wheeler, S. (1978) Toxicity of the Anticoagulant Pindone to the European Rabbit, Oryctolagus Cuniculus and the Sheep, Ovis Aries. *Wildlife Research*, **5,** 135-142.

Oppel, S., Bond, A.L., Brooke, M. de L., Harrison, G., Vickery, J.A. & Cuthbert, R.J. (2016) Temporary captive population and rapid population recovery of an endemic flightless rail after a rodent eradication operation using aerially distributed poison bait. *Biological Conservation Restoration and Sustainability,* **204,** 442–448.

Otis, D.L., Burnham, K.P., White, G.C. & Anderson, D.R. (1978) Statistical inference from capture data on closed animal populations. *Wildlife Monographs***,** 3-135.

Pain, D.J., Brooke, M.d.L., Finnie, J. & Jackson, A. (2000) Effects of brodifacoum on the land crab of Ascension Island. *The Journal of Wildlife Management***,** 380-387.

Papadatou, E., Pradel, R., Schaub, M., Dolch, D., Geiger, H., Ibañez, C., Kerth, G., Popa-Lisseanu, A., Schorcht, W. & Teubner, J. (2012) Comparing survival among species with imperfect detection using multilevel analysis of mark—recapture data: a case study on bats. *Ecography,* **35,** 153-161.

Parkes, J. & Murphy, E. (2003) Management of introduced mammals in New Zealand. *New Zealand Journal of Zoology*, **35,** 335-359.

Parli, A. (2018) Sub-lethal effects of brodifacoum pesticide exposure on Wellington tree wētā, Hemideina crassidens. Unpublished MSc Thesis, Department of Zoology, University of Otago, Dunedin.

Parshad, V. (1999) Rodent control in India. *Integrated Pest Management Reviews,* **4,** 97-126.

Pascal, M., Siorat, F., Lorvelec, O., Yésou, P. & Simberloff, D. (2005) A pleasing consequence of Norway rat eradication: two shrew species recover. *Diversity and Distributions,* **11,** 193-198.

Phillips, R.B., Harris, D.B. & Snell, H.L. (2007) Bait stations for detection and control of alien rats in Galapagos. *The Journal of Wildlife Management,* **71,** 2736-2742.

Pitt, W.C., Berentsen, A.R., Shiels, A.B., Volker, S.F., Eisemann, J.D., Wegmann, A.S. & Howald, G.R. (2015) Non-target species mortality and the measurement of brodifacoum rodenticide residues after a rat (*Rattus rattus*) eradication on Palmyra Atoll, tropical Pacific. *Biological Conservation,* **185,** 36-46.

Pitt, W.C., Sugihara, R.T., Driscoll, L.C. & Vice, D.S. (2011) Physical and behavioural abilities of commensal rodents related to the design of selective rodenticide bait stations. *International Journal of Pest Management,* **57,** 189-193.

Podlutsky, A.J., Khritankov, A.M., Ovodov, N.D. & Austad, S.N. (2005) A new field record for bat longevity. *Gerontology: Series A, Biology,* **60,** 1366–1368.

Pollock, K.H., Nichols, J.D., Brownie, C. & Hines, J.E. (1990) Statistical inference for capture-recapture experiments. *Wildlife Monographs***,** 3-97.

Priddel, D., Carlile, N., Wilkinson, I. & Wheeler, R. (2011) Eradication of exotic mammals from offshore islands in New South Wales, Australia. *Island invasives: eradication and management.* (eds C.R. Veitch, M.N. Clout & D.R. Towns), pp. 337-344. IUCN, Gland, Switzerland.

Prince, J.S., LeBlanc, W.G. & Maciá, S. (2004) Design and analysis of multiple choice feeding preference data. *Oecologia,* **138,** 1-4.

Pryde, M.A., Lettink, M. & O'Donnell, C.F.J. (2006) Survivorship in two populations of long-tailed bats (*Chalinolobus tuberculatus*) in New Zealand. *New Zealand Journal of Zoology*, **33,** 85-95.

Pryde, M.A., O'Donnell, C.F.J. & Barker, R.J. (2005) Factors influencing survival and long-term population viability of New Zealand long-tailed bats (*Chalinolobus tuberculatus*): Implications for conservation. *Biological Conservation,* **126,** 175-185.

Pryde, M.A., Pickerell, G., Coats, G., Hill, G.S., Greene, T.C. & Murphy, E.C. (2012) Observations of South Island Robins eating Racumin®, a toxic paste used for rodent control. *New Zealand Journal of Zoology,* **40,** 255-259.

Racey, P. (2007) The importance of the riparian environment as a habitat. *Behaviour and Ecology of Riparian Mammals* (eds N. Dunstone & M.L. Gorman), pp. 69-92. Cambridge University Press, New York.

Racey, P.A. & Entwistle, A.C. (2000) Life-history and reproductive strategies of bats. *Reproductive biology of bats***,** 363-414.

Racey, P.A. & Speakman, J.R. (1987) The energy costs of pregnancy and lactation in heterothermic bats. *Symposia of the Zoological Society of London*, **57,** 107-125.

Rattner, B.A., Horak, K.E., Lazarus, R.S., Eisenreich, K.M., Meteyer, C.U., Volker, S.F., Campton, C.M., Eisemann, J.D. & Johnston, J.J. (2012) Assessment of toxicity and potential risk of the anticoagulant rodenticide diphacinone using Eastern screech-owls (*Megascops asio*). *Ecotoxicology,* **21,** 832-846.

Rattner, B.A., Horak, K.E., Lazarus, R.S., Goldade, D.A. & Johnston, J.J. (2014a) Toxicokinetics and coagulopathy threshold of the rodenticide diphacinone in eastern screech-owls (*Megascops asio*). *Environmental Toxicology and Chemistry,* **33,** 74-81.

Rattner, B.A., Horak, K.E., Warner, S.E., Day, D.D., Meteyer, C.U., Volker, S.F., Eisemann, J.D. & Johnston, J.J. (2011) Acute toxicity, histopathology, and coagulopathy in American kestrels (*Falco sparverius*) following administration of the rodenticide diphacinone. *Environmental Toxicology and Chemistry,* **30,** 1213-1222.

Rattner, B.A., Lazarus, R.S., Elliott, J.E., Shore, R.F. & van den Brink, N. (2014b) Adverse outcome pathway and risks of anticoagulant rodenticides to predatory wildlife. *Environmental Science & Technology,* **48,** 8433-8445.

Rattner, B.A. & Mastrota, F.N. (2018) Chapter 3. Anticoagulant Rodenticide Toxicity to Non-target Wildlife Under Controlled Exposure Conditions. *Anticoagulant rodenticides and wildlife* (eds N.W. Van den Brink, J.E. Elliott, R.F. Shore & B.A. Rattner), pp. 45-86. Springer, Cham.

Reardon, J.T., Whitmore, N., Holmes, K.M., Judd, L.M., Hutcheon, A.D., Norbury, G. & Mackenzie, D.I. (2012) Predator control allows critically endangered lizards to recover on mainland New Zealand. *New Zealand Journal of Ecology,* **36,** 141-150.

Rich, L.N., Kelly, M.J., Sollmann, R., Noss, A.J., Maffei, L., Arispe, R.L., Paviolo, A., De Angelo, C.D., Di Blanco, Y.E. & Di Bitetti, M.S. (2014) Comparing capture-recapture, mark-resight, and spatial mark-resight models for estimating puma densities via camera traps. *Journal of Mammalogy,* **95,** 382-391.

Rigby, E., Aegerter, J., Brash, M. & Altringham, J. (2012) Impact of PIT tagging on recapture rates, body condition and reproductive success of wild Daubenton's bats (Myotis daubentonii). *Veterinary Record,* **170,** 101-101.

Riley, S.P.D., Bromley, C., Poppenga, R.H., Uzal, F.A., Whited, L. & Sauvajot, R.M. (2007) Anticoagulant exposure and notoedric mange in bobcats and mountain lions in urban southern California. *Journal of Wildlife Management,* **71,** 1874-1884.

Roark, A.W. & Dorcas, M.E. (2000) Regional body temperature variation in corn snakes measured using temperature-sensitive passive integrated transponders. *Journal of Herpetology,* **34,** 481-485.

Robinson, M.H., Twigg, L.E., Wheeler, S.H. & Martin, G.R. (2005) Effect of the anticoagulant, pindone, on the breeding performance and survival of merino

sheep, (*Ovis aries*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology,* **140,** 465-473.

Rohrer, M.J. & Natale, A.M. (1992) Effect of hypothermia on the coagulation cascade. *Critical care medicine,* **20,** 1402-1405.

Ruffell, J. & Parsons, S. (2009) Assessment of the short-term success of a translocation of lesser short-tailed bats Mystacina tuberculata. *Endangered Species Research,* **8,** 33-39.

Russell, J.C., Innes, J.G., Brown, P.H. & Byrom, A.E. (2015) Predator-Free New Zealand: Conservation Country. *Bioscience,* **65,** 520–525

Sage, M., Fourel, I., Coeurdassier, M., Barrat, J., Berny, P. & Giraudoux, P. (2010) Determination of bromadiolone residues in fox faeces by LC/ESI-MS in relationship with toxicological data and clinical signs after repeated exposure. *Environmental Research,* **110,** 664-674.

Salmon, T.P., Whisson, D.A., Berentsen, A.R. & Gorenzel, W.P. (2007) Comparison of 0.005% and 0.01% diphacinone and chlorophacinone baits for controlling California ground squirrels (*Spermophilus beecheyi*). *Wildlife Research,* **34,** 14-18.

Sánchez-Barbudo, I.S., Camarero, P.R. & Mateo, R. (2012) Primary and secondary poisoning by anticoagulant rodenticides of non-target animals in Spain. *Science of the Total Environment,* **420,** 280-288.

Sangha, G., Bilaspuri, G. & Guraya, S. (1992) Effects of oral administration of rodenticide flocumafen on the rat ovary. *Pesticide Biochemistry and Physiology,* **44,** 15-20.

Schaff, J.E. & Montgomery, M.A. (2013) An HPLC–HR-MS-MS Method for Identification of Anticoagulant Rodenticides in Blood. *Journal of Analytical Toxicology,* **37,** 321–325.

Schnitzler, H.-U. & Kalko, E.K. (2001) Echolocation by Insect-Eating Bats: We define four distinct functional groups of bats and find differences in signal structure that correlate with the typical echolocation tasks faced by each group. *Bioscience,* **51,** 557-569.

Schorcht, W., Bontadina, F. & Schaub, M. (2009) Variation of adult survival drives population dynamics in a migrating forest bat. *Journal of Animal Ecology,* **78,** 1182-1190.

Scrimgeour, J., Beath, A. & Swanney, M. (2012) Cat predation of short-tailed bats (Mystacina tuberculata rhyocobia) in rangataua forest, Mount Ruapehu, central North Island, New Zealand. *New Zealand Journal of Zoology,* **39,** 257-260.

Seber, G.A.F. (1982) *The Estimation of Animal Abundance: And Related Parameters*. Macmillan, New York.

Sedgeley, J. (2012a) Department of Conservation Inventory and Monitoring Toolbox: DOCDM-590733 Bats: counting away from roosts—automatic bat detectors v1.0. pp. 12.

Sedgeley, J. (2012b) Department of Conservation Inventory and Monitoring Toolbox: DOCDM-590789 Bats: exit counts at roosts—cameras and recorders v1.0. pp. 21.

Sedgeley, J. & Anderson, M. (2000) Capture and captive maintenance of short-tailed bats on Codfish Island and monitoring of wild bats during the Pacific rat eradication programme, winter 1998. pp. 64. Unpublished report, Southland Conservancy, Department of Conservation, Invercargill.

Sedgeley, J., O'Donnell, C.F.J., Lyall, J., Edmonds, H., Simpson, W., Carpenter, J., Hoare, J. & McInnes, K. (2012) Department of Conservation best practice manual of conservation techniques for bats. DOCDM-131465. pp. 169.

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

Sedgeley, J.A. (2003) Roost site selection and roosting behaviour in lesser short-tailed bats (*Mystacina tuberculata*) in comparison with long-tailed bats (*Chalinolobus tuberculatus*) in Nothofagus forest, Fiordland. *New Zealand Journal of Zoology*, **30,** 227-241.

Sedgeley, J.A. & O'Donnell, C.F. (2004) Roost use by long-tailed bats in South Canterbury: examining predictions of roost-site selection in a highly fragmented landscape. *New Zealand Journal of Ecology***,** 1-18.

Seljetun, K.O., Eliassen, E., Karinen, R., Moe, L. & Vindenes, V. (2018) Quantitative method for analysis of six anticoagulant rodenticides in faeces, applied in a case with repeated samples from a dog. *Acta Veterinaria Scandinavica*, **60,** 1-11.

Sendor, T. & Simon, M. (2003) Population Dynamics of the Pipistrelle Bat: Effects of Sex, Age and Winter Weather on Seasonal Survival. *Journal of Animal Ecology*, **72,** 308-320.

Serieys, L.E., Lea, A.J., Epeldegui, M., Armenta, T.C., Moriarty, J., VandeWoude, S., Carver, S., Foley, J., Wayne, R.K. & Riley, S.P. (2018) Urbanization and anticoagulant poisons promote immune dysfunction in bobcats. *Proceedings of the Royal Society B: Biological Sciences*, **285,** 20172533.

Sherley, G., Wakelin, M. & McCartney, J. (1999) Forest invertebrates found on baits used in pest mammal control and the impact of sodium monofluoroacetate ("1080") on their numbers at Ohakune, North Island, New Zealand. *New Zealand Journal of Zoology*, **26,** 279-302.

Sherwin, R.E., Haymond, S., Stricklan, D. & Olsen, R. (2002) Freeze-Branding to Permanently Mark Bats. *Wildlife Society Bulletin*, **30,** 97-100.

Shlosberg, A. & Booth, L. (2006) Veterinary and clinical treatment of vertebrate pesticide poisoning–a technical review. *Landcare Research, Lincoln, New Zealand*.

Shore, R.F., Birks, J.D.S. & Freestone, P. (1999) Exposure of non-target vertebrates to second-generation rodenticides in Britain, with particular reference to the polecat *Mustela putorius*. *New Zealand Journal of Ecology*, **23,** 199-206.

Siemers, B.M. & Ivanova, T. (2004) Ground gleaning in horseshoe bats: comparative evidence from Rhinolophus blasii, R. euryale and R. mehelyi. *Behavioral Ecology and Sociobiology*, **56,** 464-471.

Siroka, Z., Krocilova, B., Pikula, J., Bandouchova, H., Peckova, L. & Vitula, F. (2011) Blood coagulation parameters in fallow deer (Dama dama). *Veterinarni Medicina*, **56,** 119-122.

Smith, C.S., De Jong, C.E. & Field, H.E. (2010) Sampling small quantities of blood from microbats. *Acta Chiropterologica,* **12,** 255-258.

Smith, R.H. & Meyer, A.N. (2015) Rodent Control Methods: Non-chemical and Non-lethal Chemical, with Special Reference to Food Stores. Chapter 5 pp101-122. *Rodent Pests and their Control, 2nd Edition* (eds A.P. Buckle & R.H. Smith), pp. 432. CAB International, Boston.

Spurr, E. (1979) A theoretical assessment of the ability of bird species to recover from an imposed reduction in numbers, with particular reference to 1080 poisoning. *New Zealand Journal of Ecology***,** 46-63.

Spurr, E. & Drew, K. (1999) Invertebrates feeding on baits used for vertebrate pest control in New Zealand. *New Zealand Journal of Ecology,* **23,** 167-173.

Spurr, E. & Powlesland, R. (2000) Monitoring the impacts of vertebrate pest control operations on non-target wildlife species. *Department of Conservation technical series,* **24,** 51.

Spurr, E.B. (1993) Feeding by captive rare birds on baits used in poisoning operations for control of brushtail possums. *New Zealand Journal of Ecology*, ***17,*** 13-18.

Spurr, E.B. (1999) Developing a long-life toxic bait and lures for mustelids pp.1-24 in Department for Conservation 1999: Progress in mammal pest control on New Zealand conservation lands. *Science for Conservation 127,* pp. 74. Department of Conservation, Wellington.

Spurr, E.B. & Berben, P.H. (2004) Assessment of non-target impact of 1080-poisoning for vertebrate pest control on wētā (Orthoptera: Anostostomatidae and Rhaphidophoridae) and other invertebrates in artificial refuges. *New Zealand Journal of Ecology,* **28,** 63-72.

Spurr, E.B., Foote, D., Lindsey, G.D. & Perry, C.F. (2015) Aerial-broadcast application of diphacinone bait for rodent control in Hawai'i: efficacy and non-target species risk assessment. Hawai‘i Cooperative Studies Unit, Technical Report TR-HCSU-071. University of Hawai‘i at Hilo. pp24.

Spurr, E.B. & McGregor, P.G. (2003) Potential invertebrate antifeedants for toxic baits used for vertebrate pest control. A literature review. *Science for Conservation 232*, pp. 36. Department of Conservation, Wellington.

Spurr, E.B., Morriss, G.A., Turner, J., O'Connor, C.E. & Fisher, P. (2007) Bait station preferences of ship rats. *DOC Research & Development Series 271*, pp. 21. Department of Conservation, Wellington.

St Clair, J.H.H. (2011) The impacts of invasive rodents on island invertebrates *Biological Conservation,* **144,** 68-81.

Stanley, T.R. & Burnham, K.P. (1999) A goodness-of-fit test for capture-recapture model *Mt* under closure. *Biometrics,* **55,** 366-375.

Stead, E.F. (1936) Notes on the short-tailed bat (*Mystacops tuberculatus*). *Transactions of the Royal Society of New Zealand,* **66,** 188-191.

Stone, W., Okoniewski, J. & Stedelin, J. (2003) Anticoagulant rodenticides and raptors: recent findings from New York, 1998–2001. *Bulletin of Environmental Contamination and Toxicology,* **70,** 34-40.

Stone, W.B., Okoniewski, J.C. & Stedelin, J.R. (1999) Poisoning of wildlife with anticoagulant rodenticides in New York. *Journal of Wildlife Diseases,* **35,** 187.

Styche, A. & Speed, H. (2002) Rodent bait preferences in nontarget birds. *DOC Science Internal Series 40*, pp. 11. Department of Conservation, Wellington.

Taylor, D.P. (1984) The identification and detection of the rats in New Zealand and the eradication of ship rats on Tawhitinui Island. Unpublished Dissertation for Diploma in Parks and Recreation, Lincoln College, Canterbury, pp 73.

Teeling, E.C., Springer, M.S., Madsen, O., Bates, P., O'brien, S.J. & Murphy, W.J. (2005) A molecular phylogeny for bats illuminates biogeography and the fossil record. *Science,* **307,** 580-584.

Thakur, S., Edmonds, H. & Pryde, M. (2017) Eglinton valley lesser short-tailed bat monitoring programme 2016/2017. Internal report. Department of Conservation, Te Anau.

Thakur, S., Edmonds, H. & Pryde, M. (2018) Eglinton valley lesser short-tailed bat monitoring programme 2017/2018. Internal report. Department of Conservation, Te Anau., pp. 12.

Theopold, U., Schmidt, O., Soderhall, K. & Dushay, M.S. (2004) Coagulation in arthropods: defence, wound closure and healing. *Trends in Immunology,* **25**.

Thijssen, H.H. (1995) Warfarin-based rodenticides: Mode of action and mechanism of resistance. *Pesticide Science,* **43,** 73-78.

Thomas, B. & Taylor, R. (2002) A history of ground-based rodent eradication techniques developed in New Zealand, 1959–1993. *Turning the tide: the eradication of invasive species***,** 301-310.

Thomas, P.J., Mineau, P., Shore, R.F., Champoux, L., Martin, P.A., Wilson, L.K., Fitzgerald, G. & Elliott, J.E. (2011) Second generation anticoagulant rodenticides in predatory birds: probabilistic characterisation of toxic liver concentrations and implications for predatory bird populations in Canada. *Environment International,* **37,** 914-920.

Thompson, R.D., Mitchell, G.C. & Burns, R.J. (1972a) Vampire bat control by systemic treatment of livestock with an anticoagulant. *Science,* **177,** 806-808.

Thompson, R.D., Shumake, S.A. & Bullard, R.W. (1972b) Methodology for measuring taste and odor preference of rodents. *Proceedings of the 5th Vertebrate Pest Conference*. pp. 36-42. Lincoln, Nebraska.

Thurley (2017) Pikiariki short-tailed bat monitoring report 2013-2017. pp. 26. Department of Conservation unpublished report DOC-3026540.

Tompkins, D.M., Byrom, A.E. & Pech, R.P. (2013) Predicted responses of invasive mammal communities to climate-related changes in mast frequency in forest ecosystems. *Ecological Applications,* **23,** 1075-1085.

Toth, C.A., Cummings, G., Dennis, T.E. & Parsons, S. (2015a) Adoption of alternative habitats by a threatened, “obligate” forest-dwelling bat in a fragmented landscape. *Journal of Mammalogy,* **96,** 927-937.

Toth, C.A., Dennis, T.E., Pattemore, D.E. & Parsons, S. (2015b) Females as mobile resources: communal roosts promote the adoption of lek breeding in a temperate bat. *Behavioral Ecology*, **26,** 1156–1163.

Towns, D.R. (2009) Eradications as reverse invasions: lessons from Pacific rat (*Rattus exulans*) removals on New Zealand islands. *Biological Invasions,* **11,** 1719–1733.

Towns, D.R., Atkinson, I.A.E. & Daugherty, C.H. (2006) Have the harmful effects of introduced rats on islands been exaggerated? *Biological Invasions*, **8,** 863-891.

Towns, D.R. & Broome, K.G. (2003) From small Maria to massive Campbell: forty years of rat eradications from New Zealand islands. *New Zealand Journal of Zoology,* **30,** 377-398.

Tuttle, M. (1976) Population ecology of the gray bat (*Myotis grisescen*s): Factors influencing growth and survival of newly volant young. *Ecology,* **57,** 587-595.

Tuttle, M.D. & Stevenson, D. (1982) Growth and survival of bats. *Ecology of Bats* (ed. T.H. Kunz), pp. 105-150. Plenum Press, New York.

Twigg, L.E., Lowe, T.J., Martin, G.R. & S.Gray, G. (1999) A review of the anticoagulant pesticide Pindone. *Department of Agriculture and Food, Western Australia, Perth.*

U.S.Fish and Wildlife Service (2011) Desecheo Island National Wildlife Refuge. Rat Eradication to Promote Ecosystem Restoration: Final Environmental Assessment. pp. 239. Caribbean Islands National Wildlife Refuge Complex, Puerto Rico.

Valdez, E.W. & Cryan, P.M. (2013) Insect prey eaten by hoary bats (Lasiurus cinereus) prior to fatal collisions with wind turbines. *Western North American Naturalist,* **73,** 516-525.

Vidal, D., Alzaga, V., Luque-Larena, J.J., Mateo, R., Arroyo, L. & Viñuela, J. (2009) Possible interaction between a rodenticide treatment and a pathogen in common vole (*Microtus arvalis*) during a population peak. *Science of the Total Environment,* **408,** 267–271.

Voigt, C.C. & Kingston, T. (2016) Bats in the Anthropocene. *Bats in the Anthropocene: conservation of bats in a changing world*, pp. 1-9. Springer, Cham.

Vyas, N.B. (1999) Factors influencing estimation of pesticide-related wildlife mortality. *Toxicology and Industrial Health,* **15,** 187-192.

Vyas, N.B. (2017) Rodenticide incidents of exposure and adverse effects on non-raptor birds. *Science of the Total Environment*, **609,** 68-76.

Vyas, N.B., Hulse, C.S. & Rice, C.P. (2012) Chlorophacinone residues in mammalian prey at a black-tailed prairie dog colony. *Environmental Toxicology and Chemistry*, **31,** 2513-2516.

Vyas, N.B. & Rattner, B.A. (2012) Critique on the use of the standardized avian acute oral toxicity test for first generation anticoagulant rodenticides. *Human and Ecological Risk Assessment: An International Journal,* **18,** 1069-1077.

Wakelin, M.D. (2000) Invertebrates seen on cereal baits. A study of video and manual observation methods. *Science for Conservation 137*, pp. 36. Department of Conservation, Wellington.

Wallace, J. (2006) Short-tailed Bats in Pikiariki Ecological Area, Pureora Forest Park January to April 2006. Internal Report DOCDM-258163. pp. 24. Department of Conservation.

Wardle, J. (1984) *The New Zealand beeches: ecology, utilisation and management*. New Zealand Forest Service, Wellington.

Watt, B.E., Proudfoot, A.T., Bradberry, S.M. & Vale, J.A. (2005) Anticoagulant rodenticides. *Toxicological Reviews,* **24,** 259-269.

Watts, C.H., Armstrong, D.P., Innes, J. & Thornburrow, D. (2011) Dramatic increases in wētā (Orthoptera) following mammal eradication on Maungatautari – evidence from pitfalls and tracking tunnels. *New Zealand Journal of Ecology,* **35,** 261-272.

Weir, S.M., Yu, S., Knox, A., Talent, L.G., Monks, J.M. & Salice, C.J. (2016) Acute toxicity and risk to lizards of rodenticides and herbicides commonly used in New Zealand. *New Zealand Journal of Ecology,* **40,** 342-350.

Welbergen, J.A. (2011) Fit females and fat polygynous males: seasonal body mass changes in the grey-headed flying fox. *Oecologia,* **165,** 629-637.

Weller, T.J., Cryan, P.M. & O'Shea, T.J. (2009) Broadening the focus of bat conservation and research in the USA for the 21st century. *Endangered Species Research*, **8,** 129-145.

Whisson, D.A. (1999) Modified bait stations for California ground squirrel control in endangered kangaroo rat habitat. *Wildlife Society Bulletin,* **27,** 172-177.

Whisson, D.A. & Salmon, T.P. (2002) Effect of diphacinone on blood coagulation in *Spermophilus beecheyi* as a basis for determining optimal timing of field bait applications. *Pest Management Science*, **58,** 736-738.

White, G.C. (2000a) Modelling population dynamics *Ecology and Management of Large Mammals in North America* (eds S. Demarais & P.R. Krausman), pp. 85-107. Prentice Hall, Upper Saddle River, NJ.

White, G.C. (2000b) Population viability analysis: data requirements and essential analyses. *Research techniques in animal ecology: controversies and consequences. Columbia University Press, New York, New York, USA***,** 288-331.

White, G.C. & Burnham, K.P. (1999) Program MARK: survival estimation from populations of marked animals. *Bird Study,* **46,** 120-139.

Wilkinson, G.S. & South, J.M. (2002) Life history, ecology and longevity in bats. *Aging cell,* **1,** 124-131.

Williams, B.K., Nichols, J.D. & Conroy, M.J. (2002) *Analysis and Management of Animal Populations. Modeling, Estimation and Decision Making.* Academic Press, San Diego.

Willson, M.F. & Comet, T.A. (1993) Food Choices by Northwestern Crows: Experiments with Captive, Free-Ranging and Hand-Raised Birds. *The Condor,* **95,** 596-615.

Wilmshurst, J.M., Anderson, A.J., Higham, T.F.G. & Worthy, T.H. (2008) Dating the late prehistoric dispersal of Polynesians to New Zealand using the commensal Pacific rat. *PNAS,* **105,** 7676-7680.

Wilson, D.J., Lee, W.G., Webster, R.A. & Allen, R.B. (2003) Effects of possums and rats on seedling establishment at two forest sites in New Zealand. *New Zealand Journal of Ecology*, **27,** 147-155.

Wilson, K.-J. (2004) *Flight of the Huia. Ecology and conservation of New Zealand's*

Witmer, G. & Eisemann, J. (2005) An overview of the 2nd national invasive rodent summit. *Proceedings of the 11th Wildlife Damage Management Conference.* (D.L. Nolte, K.A. Fagerstone, Eds). pp. 102-111. University of Nebraska.

Witmer, G. & Eisemann, J. (2007) Rodenticide use in rodent management in the United States: an overview. *12th Wildlife Damage Management Conference* (eds D.L. Nolte, W.M. Arjo & D.H. Stalman), pp. 114-118. Nebraska.

Witmer, G., Eisemann, J.D. & Howald, G. (2007a) The use of rodenticides for conservation efforts. *USDA National Wildlife Research Center-Staff Publications***,** 780.

Witmer, G.W., Boyd, F. & Hillis-Starr, Z. (2007b) The successful eradication of introduced roof rats (*Rattus rattus*) from Buck Island using diphacinone, followed by an irruption of house mice (*Mus musculus*). *Wildlife Research*, **34,** 108-115.

Woody, B.J., Murphy, M.J., Ray, A.C. & Green, R.A. (1992) Coagulopathic effects and therapy of brodifacoum toxicosis in dogs. *Journal of Veterinary Internal Medicine*, **6,** 23-28.

Worthy, T. (1997) Quaternary fossil fauna of South Canterbury, South Island, New Zealand. *Journal of the Royal Society of New Zealand*, **27,** 67-162.

Worthy, T.H. & Holdaway, R.N. (2002) *The Lost World of the Moa. Prehistoric Life of New Zealand.* Indiana University Press, Bloomington.

Zabel, C.J. & Seidman, V.M. (2001) Bat activity along intermittent streams in northwestern California. *Journal of Mammalogy*, **82,** 738–747.