Estimating population density and habitat selection of rats (*Rattus* spp.) and abundance of birds on the Otago Peninsula

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ABSTRACT

The introduction of rats (*Rattus* spp.) and conversion of native forest and scrub have had a devastating effect on the biodiversity of New Zealand's fauna and flora, threatening many native bird species with extinction. The Otago Peninsula is an area of land characterised by its fragmented landscape and presence of introduced mammalian predators, including rats.

This study aimed to estimate the abundance of rats at three sites of fragmented vegetation on the Otago Peninsula using spatially explicit capture-recapture (SECR) and non-invasive genetic sampling. Hair-snag tube grids and microsatellite genotyping were used in an attempt to identify individual rats and estimate population density. The results remain inconclusive in terms of estimating rat population density due to the high degree of genotyping error and inability to identify recaptures. As such, a simple estimate of rat population density at each site was obtained through the inclusion of a boundary strip around the hair-snag tube grid and using a minimum number alive of rats at each site. The boundary strip was calculated to establish an effective trap area to reduce the edge effect in the density calculation. The highest density of rats was found to be at Okia, with approximately 0.9 rats/ha. Leith Walk had a density of 0.7 rats ha^{-1}, while Hooper's Inlet had an estimated density of 0.3 rats ha^{-1}.

In addition to this, a habitat selection analysis was also undertaken. Number of tiers was used as an index of vegetation complexity and was found to be positively associated with probability of rat presence ($p=0.020$), as was percentage groundcover ($p=0.006$). While rat species could not be discerned from genotyping, these results suggest the species detected was likely ship rat (*Rattus rattus*). Distance to coast, presence or absence of fruiting trees, distance to freshwater, maximum canopy height, and site were all found to be nonsignificant variables in predicting rat presence.

A tracking tunnel line at each site also gave a rough index of rat abundance. Very low tracking rates of 10% at Hooper's Inlet and Okia confirmed rat presence in the area. No rat tracks were recorded at Leith Walk. There was no correlation between rat tracking rates and the number of rats detected from the hair-snag tube grids. Mice (*Mus musculus*) were frequently detected using both sampling methods, with tracking rates of 100% at all three sites.

Using 5-minute bird counts 24 bird species were counted across the same three sites in which the rat sampling was conducted. The most abundant bird species were bellbird (*Anthornis melanura*) at Leith Walk, and goldfinch (*Carduelis carduelis*) at Hooper's Inlet and Okia. Distance sampling allowed for analysis of ten of the most abundant species in DISTANCE software to estimate population density of these species. Fantails (*Rhipidura fuliginosa*) had the highest population density among bird species at Leith Walk (1.043 ha^{-1}), whereas silvereyes (*Zosterops lateralis lateralis*) had the highest estimated densities of species at both Leith Walk (0.175 ha^{-1}) and Okia (0.163 ha^{-1}).

These results provide a foundation for further study of rat density estimations and bird abundance on the Otago Peninsula, and can be used as a baseline when evaluating the impact of future rat eradication, which has been proposed to meet the goal of a Predator Free Peninsula by 2050.

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1 INTRODUCTION

1.1 New Zealand's changing environment

New Zealand's landscape has undergone drastic changes since the arrival of humans. The arrival of Māori marked the first sudden change, as large areas of land were cleared through the use of fire for settlements and agriculture (Cumberland 1971). Prior to human arrival, an estimated 85-90% of the country was covered by thick forest, with low scrub and herbaceous communities above the tree line (McGlone 1983; McWethy et al. 2010). By the mid 19th century, when New Zealand was being settled by Europeans, over 40% of the South Island's forests had been cleared through the use of fire and replaced by tussock grassland and bracken-dominated shrubland (Cumberland 1971; McGlone 1983; McGlone 2001; Mark and McLennan 2005; McGlone et al. 2005). Further forest clearance by Europeans transformed most of the remaining bracken-dominated shrubland into pastureland comprising mostly introduced plant species (McWethy et al. 2010). These large-scale landscape changes have resulted in a massive decrease in native habitat, which, coupled with the unintended consequences of deliberate and accidental introductions of non-native predatory mammal species, has resulted in the extinction of many of New Zealand's native fauna and the decline of most others (Sullivan et al. 2010).

1.2 The introduction of mammalian predators into New Zealand

In addition to five species of native bat, two of which are extant (the New Zealand long-tailed bat, *Chalinolobus tuberculatus*, and the lesser New Zealand short-tailed bat, *Mystacina tuberculata*), there are 31 species of naturalised terrestrial mammals currently found in New Zealand (Tennyson 2010). Most of these mammals were introduced for sport (e.g. red deer, *Cervus elaphus scoticus*; Bennett's wallaby, *Macropus rufogriseus rufogriseus*), many for utility (e.g. cattle, *Bos taurus*), and some for keeping as pets (e.g. cat, *Felis catus*; King 2005a). When some species, such as the European rabbit (*Oryctolagus cuniculus cuniculus*), became over-abundant throughout New Zealand,

other mammalian species were introduced as a form of pest control. In the case of the rabbit several mustelid species were introduced: the stoat (*Mustela ermina*), the common weasel (*M. nivalis vulgaris*), and the ferret (*M. furo*; Clapperton and Byrom 2005; King 2005b; King and Murphy 2005). While most of these introductions were deliberate, the unintended consequences on New Zealand's native plant and animal species were not realised until years later. Bird species that had evolved without mammalian predators now faced a new critical threat, as did native plant species from herbivorous mammals (King 2005a; Sullivan et al. 2010). However, mammals that arrived in New Zealand as stowaways posed a more concealed threat. All of the species that arrived in New Zealand as stowaways are from the same family of rodents – Muridae – and were able to establish themselves in New Zealand due to their commensal relationship with humans (Innes 2005a; Innes 2005b; King 2005b). These are three rat species in the genus *Rattus* and the house mouse (*Mus musculus*; King 2005a).

1.3 Rats in New Zealand

New Zealand has three introduced species of commensal rat; the kiore (*Rattus exulans*), the ship rat (*R. rattus*), and the Norway rat (*R. norvegicus*; King 2005a). While the kiore was brought in with the first Māori settlers, the ship and Norway rats were brought over by early Europeans, the Norway rat being the first of the two (Atkinson 1973; King 2005a). The kiore is the smallest of the three species, with an adult normally weighing around 60-80 g and a maximum head-body length (HBL) of 180 mm (Atkinson and Towns 2005). The ship rat is the next smallest with a weight of 120-160 g and maximum HBL of 225 mm. The largest, the Norway rat, weighs in at 200-300 g with a maximum HBL of 250 mm (King 2005a).

While all three species can climb trees and shrubs, the ship rat is far more agile and is the only one of the three considered to be highly arboreal. The kiore and Norway rat are almost always found on the ground (Atkinson and Towns 2005; Innes 2005a; Innes 2005b). For example, a study by Foster et al. (2011) found that while Norway rats can climb to similar heights as ship rats to access food, they did so much more slowly and

with less agility and were more easily defeated by obstacles. Foster et al. (2011) concluded that Norway rats seldom forage above ground, not because they cannot climb, but because the risk of doing so is deemed greater than the reward. Similarly, the kiore is considered to be ground dwelling (Atkinson and Towns 2005). This difference is likely one of the reasons why ship rats have been so successful in forested habitats and are virtually the sole rat species present in podocarp-broadleaved forests (Harper 2002). Habitat choice is influenced by competition and predation, something which is seen in the relative distributions of the three rat species, as there are few places on the mainland where all three species live together in the same area, suggesting competitive exclusion (Taylor 1975). Kiore for the most part have been displaced across much of the country, first by Norway rats, then even further by ship rats, which arrived later (King 2005a; Bramley 2014). In the nineteenth century the Norway rat was very widespread across New Zealand, occupying all types of habitat to 1200 m above sea level (Innes 2005a). Nowadays, the Norway rat is still widely found across the North and South Islands; however, this distribution is much patchier (Innes 2005a). The ship rat on the other hand has largely displaced the Norway rat to become by far the most common rat species distributed across New Zealand's three large islands (Innes 2005b).

A significant reason for the successful spread of these rat species, particularly Norway and ship rats, is that they are both omnivorous and opportunistic (King 2005a), although ship rats are selective in some instances (Clark 1981; Ruffino et al. 2011). In a recent study by Clapperton et al. (2019), ship rats in the Fiordland National Park in New Zealand's South Island consumed lepidopteran larvae, wētā (*Hemideina*/*Hemiandrus* spp.), vegetative plant matter, and beech seeds. Most importantly however, Clapperton et al. (2019) reported for the first time in New Zealand the presence of a lizard species as a prey item for ship rats: although the ship rat had been linked to the decline of some lizard species both in New Zealand and elsewhere, this had never been confirmed in New Zealand (e.g. Towns et al. 2006; Banks and Hughes 2012; Smith et al. 2012; Harper and Bunbury 2015). Norway rats are also known to consume lizards, as well as invertebrates, tree bark and other vegetation, and carcasses of dead animals (Allen et al. 1994; Innes 2005a). Both species have also been found to kill mice, particularly after mouse irruptions as a result of mast seed-fall in beech forests (Davis 1979; Innes 2005a; Clapperton et al. 2019). Additionally, all three species of rats have been found to prey on native birds at

various life stages (e.g. ship rats predating on eggs, chicks, and breeding adults of North Island robin, *Petroica longipes* and tomtit, *Petroica macrocephala*: Brown 1997). For ship rats in established mainland populations, birds are a minor diet item and eggshells or feathers in the stomachs of rats occur at a low frequency. However, due to the abundance of rats and their population densities being higher than those of other predators, together with being an arboreal species, they are the most frequent cause of losses of birds, eggs, and chicks in non-beech forests on the New Zealand mainland (Innes 2005b).

1.4 Rat habitats in New Zealand

Being highly adaptable, New Zealand's three rat species are found in a wide range of habitats (King 2005a). Typical habitats of Norway rats include wetland areas such as those near rivers, streams, lakes, estuaries and lagoons, although the largest rat populations live commensally in almost every town and city, as well as around farms and cropland (Innes 2005a). Isolated populations survive away from habitation but there are few comprehensive data on their distribution (Innes 2005a). Wild ship rats inhabit both native and exotic forests of all sizes, urban areas, farms, and hedgerows, but are most abundant in mature lowland podocarp-broadleaved forest of high diversity, although being uncommon and hence hard to detect in pure beech forest (*Nothofagus* spp.), except after mast seed-fall events (King and Moller 1997; Alterio et al. 1999; Studholme 2000; Blackwell et al. 2001).

Ship rats are known to have a relatively large home range, with a typical length of 100-300 m, although these range sizes can differ greatly according to habitat and population density (Innes et al. 2010). Norway rats have smaller ranges, with mean lengths of 49 m for females and 113 m for males (with a maximum of 330 m) on Motuhoropapa Island (Moors 1985).

1.5 Rats in New Zealand forest and scrub fragments

Currently, a large proportion of New Zealand's mainland is covered in agricultural and urban developed land, with remnants of native lowland forest. Much of the native forest in New Zealand's lowland rural landscapes is in the form of smaller fragments surrounded by pastureland and hedgerows. These small fragments are remnants of original forested ecosystems and are not well represented within public conservation land (Department of Conservation and Ministry for the Environment 2000; Green 2005; Innes et al. 2010). Native forest and scrub fragments therefore hold a disproportionately large percentage of the threatened species and ecosystems of lowland areas and play important roles in restoration strategies on both a regional and national scale (Walker et al. 2006; Ministry for the Environment New Zealand and Department of Conservation 2007; Innes et al. 2010).

Far fewer studies have been done on rat ecology in forest fragments than in larger forests. Some studies (e.g. Boulton 2006; King et al. 2011) have shown ship rats may be less common in unfenced forest fragments that are open to grazing by stock than in fenced forest fragments where livestock grazing is prevented. A study by Innes (2010) revealed that fencing forest fragments as protection from stock grazing increases the density of seedlings and saplings of understorey and subcanopy trees with time, creating more litter biomass and thus allowing for an increase in invertebrate abundance, in turn increasing ship rat food (Innes 2005b; Didham et al. 2009). Higher rat abundance could in part or fully be explained by the ability of rats to escape predators such as cats and stoats more effectively in denser vegetation (Innes 2005b; Innes et al. 2010). Given the patchy nature of fragments in agricultural landscapes, the survival of populations of native species in patches of suitable habitat, as well as the rate at which invasive species such as rats colonise those patches, is dependent on species-specific capacities for dispersal and inter-patch movements (Bowne and Bowers 2004; King et al. 2011).

Conservation of forest and scrub fragments poses challenges because it is difficult to prevent rats from rapidly reinvading an area after it has been cleared of the species, if the

surrounding area has not had any form of predator control. In these circumstances reinvasion is considered inevitable due to the sink effect, which is induced through the eradication of local populations that are connected to a wider meta-population (Russell et al. 2009; King et al. 2011). Reinvasions of areas that have undergone predator control provide good examples of source-sink population dynamics and are an important reason why some eradications of rats from protected areas are unsuccessful (Pulliam 1988; King et al. 2011). On islands eradication programmes are effective at clearing areas of increasingly larger size due to isolation from potential source populations and a much lower likelihood of individuals reinvading naturally compared to mainland sites (Brooke et al. 2007; Towns 2009). Some of the techniques used in the conservation management of islands could potentially be used in managing mainland forest fragments, as they share certain aspects, though the foremost constraint for managers of forest fragments is that isolation from possible reinvasion is the key condition that managers of forest fragments would need to meet to replicate the successes of pest eradication programmes on islands (Watling and Donnelly 2006; King et al. 2011).

Unless confined to larger areas that encompass nearby forest fragments and are isolated from reinvasion (‘eradication units’; King et al. 2011) eradication efforts are likely to fail. Innes et al. (2010) confirmed that ship rats readily reached forest fragments from nearby source populations at least 250 m away by dispersing across grazed pasture, despite there being a greater risk of predation by stoats and cats. In addition to making eradication efforts more difficult, dispersal also further adds to the already challenging task of estimating the abundance of animal populations (Thompson et al. 1998). Abundance estimates (number of individuals), together with density estimates (number of individuals per unit area; Thompson et al. 1998), are useful in evaluating the impacts of a species on an ecosystem and offer insight into where to target pest control efforts, estimating minimum control efforts, and to what scale these efforts are needed to successfully eradicate a species from an area (Norbury et al. 2015).

1.6 Abundance estimation

Two terms most useful in population estimates of animals are abundance and density. A way to get a reliable estimate of abundance of individuals in an area would be to count all individuals within a confined area (i.e. a census), however, this is both time costly and likely impossible for small, evasive species such as rats. Instead, indices of relative abundance are used, as they are easier, quicker, and cheaper than most conventional methods (Ruscoe et al. 2001).

1.7 Density estimation

In most cases, especially in the case of live trapping, sampling the entire habitat of a species' population is far too labour-intensive and costly and as a result only a section of the habitat is sampled. As an example, live trapping, which is a standard field method often used for cryptic and mobile species, utilises the construct of a grid of traps. Because an enormous number of traps would be required to encompass an entire habitat (e.g. protected areas or islands), a grid is established that samples a representative smaller section of the area. In this instance Efford et al. (2005) highlight the problem that arises with this method; the unknown "edge effect", which occurs due to animals moving on and off the trapping grid. The edge effect describes the occurrence of disproportionately high frequencies of animals caught in traps along the border of the grid (Stenseth and Hansson 1979). This leads to an overestimation of population size if the grid is used to calculate density (Tioli et al. 2009). In an attempt to account for edge effects and obtain estimates of density that are unbiased, the effective trapping area (ETA) was established (Dice 1938; Bondrup-Nielsen 1983), which is estimated by adding a boundary strip around the trapping grid, increasing the total area in the density equation (Tioli et al. 2009). Many different methods for estimating the size of this boundary strip have been used, for example using the mean maximum distance moved of small mammals by Monte Carlo methods (Wilson and Anderson 1985), or using a distance of half the average home range length (Dice 1938); however, most of these are still less than ideal and often over- or under-estimate population sizes (Parmenter et al. 2003).

Burge et al. (2017) found edge effects for rats and possums within a New Zealand wetland habitat, with rats almost exclusively caught at traps on the grid border. However, a study by Ruffell et al. (2014) reported contradictory results whereby it was found that ship rat capture rates actually decreased towards the edges of the forest fragments studied: this was subsequently linked to changes in vegetation structure. Therefore, edge effect bias can range greatly depending on trap layout and home range size, as well as habitat structure and the matrix in which the traps are situated (Efford et al. 2004; Ruffell et al. 2014).

1.8 Spatially explicit capture-recapture

Due in part to the advancements of modern computer technology, major improvements in statistical modelling have occurred, allowing for finer details of landscapes and spatially dependent biological processes to be simulated with precision (Deangelis and Yurek 2017).

One such advancement is the development of spatially implicit and spatially explicit models (SIMs and SEMs, respectively). SIMs are often used in ecology but are limited mainly to ecological theories. SEMs have "unique advantages for addressing pragmatic questions concerning species populations or communities in specific places, because local conditions, such as spatial heterogeneities, organism behaviors, and other contingencies, produce dynamics and patterns that usually cannot be incorporated into simpler SIMs" (Deangelis and Yurek 2017). To assess elusive or sparsely distributed populations of animals without the bias associated with ETAs, spatially explicit capture-recapture (SECR) was developed to estimate absolute population density with data from an array of passive detectors (e.g. live-capture traps, cameras; Efford et al. 2004; Efford et al. 2009). In most cases when trapping has been used to produce capture-recapture data, the spatial aspect has been ignored and is only represented in the binary format of captured/not captured (Otis et al. 1978; Efford et al. 2009). SECR estimates "spatial detection parameters on the basis of recapture locations of individual animals" and is therefore more informative than conventional capture-release (Wilson et al. 2017). The

emphasis of SECR is on maximising precision, as opposed to avoiding the biases that can arise with other methods (Efford et al. 2005). With the use of the R-package 'secr', data collected from passive detector arrays can be analysed (Efford 2019).

As its name suggests, SECR requires a certain number of recaptures of the same individuals in different points on the trap array to be able to run the analysis with any effectiveness (Efford et al. 2004; Wilson et al. 2017). With some animal species (e.g. European hedgehogs, *Erinaceus europaeus*: Kristiansson 1990), capture-recapture can be done in the form of live trapping. However, some species are potentially neophobic, such as rats, and may display trap-avoidance behaviour (Innes 2005a). Therefore, live trapping does not present a viable option for the capture-recapture of these species as they will not enter a trap during the first days or weeks of trap setup, which, depending on the study time frame, might encompass the entire length of the study (Amstrup et al. 2010). Additionally, if they were to enter a trap and be captured, it is likely they would not do so again, which would preclude an analysis that relies on recapture, such as in SECR (Efford et al. 2004; Amstrup et al. 2010).

1.9 Non-invasive passive sampling methods

"Passive" forms of detection methods are those that record individuals at one point in an array, and in which animals are only counted when they interact with a detector such as a live trap, camera, or hair-snag tube. Combined with capture-recapture this is a common source of data in experiments of closed populations (Otis et al. 1978; Efford et al. 2004). In some instances, it is difficult to live trap animals for capture-recapture or population monitoring, such as when an animal is elusive, dangerous, too large, displays trap-avoidance behaviour, or if it is simply too time consuming or costly to set up the appropriate equipment. As a result, alternative sampling methods have been developed which require no handling of the animals (Efford et al. 2004; Amstrup et al. 2010).

1.9.1 Tracking tunnels

First described in New Zealand by King & Edgar (1977), tracking tunnels have become one of the most common methods for monitoring small mammal abundance in New Zealand. This is due to their cost-effectiveness and relative ease of use in the field. The process by which data are collected from animals is simple; an individual is attracted by bait in the tunnel and runs through it over an ink pad, leaving its footprints behind for researchers to interpret (Gillies 2013; Gillies and Williams 2013). It is informative of a wide range of animals, from large invertebrates to small birds and mammals. It does not impact the population of any species present as it is non-invasive (Gillies and Williams 2013). At low population densities, tracking tunnels are considered more sensitive to detecting rodents than another commonly used but invasive method, snap traps. Elliott et al. (2018) showed that data gathered from tracking tunnels can also be used to estimate population growth rates in small mammals. Additionally, the method is less labour intensive as the tunnels can be left in the field over long periods of time. However, tracking tunnels only provide a rough index of relative abundance and cannot provide density estimates (Gillies and Williams 2013). Another issue with tracking tunnels is that the ink cards can become saturated with footprints in areas of high rodent densities, making interpretation of the cards difficult (Gillies and Williams 2013).

1.10 Genetic sampling

Technological advances in genetics in recent years have allowed for increasingly more sensitive DNA extraction methods. Nowadays, only a very small amount of DNA needs to be present in a sample to allow for a detailed analysis of the DNA. DNA from a single hair follicle can be amplified, and through the use of genetic markers such as microsatellites, genetic information can be determined right down to the individual of a species (Navidi et al. 1992; Foran et al. 1997; Goossens et al. 1998; Fernando 2003). Non-invasive genetic sampling through the collection of biological material (e.g. hair, skin, faeces) allows for the collection of population data from species that are evasive and hard to sample otherwise (Goossens et al. 1998). To distinguish individuals in a population

from DNA collected through various methods in a study site, the polymorphic microsatellites of one sample's collected DNA is analysed and compared to those of DNA collected from other samples in software such as GENEMAPPER to ascertain whether or not they are the same individual. Alleles found at different lengths to others indicate that the samples are likely the DNA of different individuals (Navidi et al. 1992; Kraus et al. 2015).

1.10.1 Hair-snag tubes

With the advancements in molecular testing, only small amounts of DNA need to be collected (although more is better, see: Kubasiewicz et al. 2016). This means that a passive, non-invasive sampling method that allows for the collection of biological material can be used effectively to provide informative data on a species' population. At present one of the most commonly used sources of DNA for non-invasive studies of fur-bearing mammals is from hair, which can be collected by barbed wire for larger mammals (e.g. for badgers, *Meles meles*: Alessandro et al. 2011; Latham et al. 2012; bears, *Ursus* spp.: Fisher et al. 2016) or hair-snag tubes and funnels with glue or sticky tape for small and medium-sized mammals (e.g. for wild hamsters, *Cricetus cricetus*: Reiners et al. 2011; O'Mahony et al. 2015; European pine martens, *Martes martes*: Kubasiewicz et al. 2016). DNA analysis can then be conducted on any hairs that have all or part of the hair follicle still attached.

1.11 Landscape scale pest eradications

Eradication of mammalian predators in New Zealand has progressed through offshore islands of increasing size towards mainland 'ecosanctuaries' that exclude predators from an area that has been encompassed by large, especially designed fences (e.g. Dunedin's Orokonui Ecosanctuary, Zealandia Ecosanctuary in Wellington). In recent years, eradication initiatives have developed further to encompass much larger unfenced areas of New Zealand's mainland. Some examples of these initiatives include Cape to City in

Hawke's Bay, which covers an area of 26,000 ha (Cape to City 2019), and Predator Free Peninsula, run by the Otago Peninsula Biodiversity Group, and within the umbrella group Predator Free Dunedin (Otago Peninsula Biodiversity Group 2019). All of these local groups, together with the government and other non-governmental organisations, are working in unison to achieve the 'Predator Free 2050' goal, an ambitious attempt to remove brushtail possums (*Trichosurus vulpecula*), stoats, and rats from the entirety of New Zealand's three main islands through various eradication programmes on both a local and national scale (Predator Free NZ 2019).

1.11.1 The Otago Peninsula Biodiversity Group

The Otago Peninsula Biodiversity group (OPBG) was established in 2008 as a non-profit organisation by residents of the Otago Peninsula, Dunedin. The main goal of this organisation has been to eradicate mammalian predators to enhance the biodiversity and economic values of the Peninsula. Presently the OPBG's main focus is on the eradication of possums (of which 16,000 have been removed from the Peninsula since 2011); however, this focus may shift towards rats and stoats in the future to align with the goals of Predator Free 2050. As such, an understanding of rat presence and abundance in the different habitats on the Peninsula is crucial to provide baseline information for future eradication efforts, and can be used as supplementary information for eradication plans such as the multispecies eradication plan drafted by the OPBG which is set to be completed soon (Otago Peninsula Biodiversity Group 2019).

In addition to possum eradication, the OPBG also organises local volunteers to conduct five-minute bird counts to collate data on bird abundance in the various habitat types on the Peninsula throughout years of possum control. This is an important part of the eradication programme as it is vital to monitor the outcomes (responses of native species' populations to the eradication of a species) as well as the outputs (number of predators removed) of predator eradication to gauge the success of the programme (Towns et al. 2013; Otago Peninsula Biodiversity Group 2019). While the monitoring of birds on the Peninsula gives a general idea of the responses of bird communities to possum control,

this study aims to evaluate those bird communities that are directly associated with the areas where rat abundance is also evaluated, before any eradication efforts begin.

1.12 Five-minute bird counts

Developed in New Zealand in the early 1970s by the Ecology Division of the Department of Scientific and Industrial Research as a tool for monitoring birds, the five-minute bird count (5MBC) method has since become the principal method in the country for collecting data on multispecies forest bird populations and has led to a large database of counts as a result (Hartley 2012). Hartley (Hartley 2012) and Hartley and Greene (2012) outline a few aspects of this method that should be taken into account when designing a study: (1) it is not a census method, but rather an index; (2) multiple factors affecting the number of birds being detected need to be considered, including species behaviour, season, and the observer; and (3) data generated from a count are specific to the habitat, species, and probably year, and precludes any inter-species comparisons, as well as comparisons of different sites at different years. Although there are a few iterations of bird counts, the point count method of 5MBCs is now preferred in most instances (Hartley and Greene 2012). This method has the observer recording all birds heard or seen while standing in one spot for five minutes, then walk to another spot 200 m away and do the same, and so forth (Dawson and Bull 1975; Hartley 2012). The 5MBC has a number of assumptions, including that the relationship between the index and true abundance is linear, the population remains closed during the sampling period, and that the fraction of individuals counted in an area is constant (Hartley and Greene 2012).

1.12.1 Distance sampling

Without accounting for detectability, 5MBCs can only be used as an index, not as a robust estimate of bird abundance with confidence intervals. The latter is preferable when attempting to demonstrate changes over time or between sites. Therefore, when performing 5MBCs, distance data can be taken (whereby the distance from the observer

to the bird is measured and recorded) which can be used in DISTANCE software to obtain a robust estimate of bird abundance, provided all assumptions are met and aspects of detectability in different habitats has been considered (Buckland et al. 2005; Greene and Efford 2012). Distance sampling accounts for variable detectability by using the distances to sightings to estimate a detectability function that takes into consideration the fact that an increase in distance from the observer results in a decrease in detectability of a bird, and that detectability can vary between species and habitats (Gottschalk and Huettmann 2011).

1.13 Aims of this study

The overall aim of this study was to determine the presence and identity of rat species at three sites on the Otago Peninsula, and estimate the density of the population of rats at those locations. Furthermore, this study aims to analyse rat habitat selection to assess which factors of the habitat are important in predicting rat presence. This will provide useful knowledge for future pest control as the Predator Free Peninsula programme progresses from the current possum control to eradication of rats and other introduced pest predators on the Peninsula (Wilson 2017).

As the distribution of kiore is limited to only a few locations across New Zealand, with the result that they are unlikely to be present in the sample populations (Atkinson and Towns 2005), this study focuses on the two other rat species; the Norway and the ship rat.

In addition to this, this study aims to obtain abundance estimates of birds and bird community composition at these sites. These will provide baseline information to allow evaluation of impacts of rat removal on avian communities.

1.13.1 Specific objectives

1. Determine presence and population densities of two rat species (*R. rattus* and *R. norvegicus*) at three sites on the Otago Peninsula through the use of hair-snag tubes, a non-invasive sampling method, and DNA analysis.
2. Compare the density of the populations at each site based on the habitat type and vegetation composition.
3. Estimate an index of relative abundance of rats at these sites using tracking tunnels, another non-invasive sampling method.
4. Determine important aspects of habitat that account for a higher probability of rat presence
5. Analyse the relationship between the tracking tunnel data and the data from the hair-snag tube grids.
6. Estimate relative abundance of bird species at each site using the point-count variation of the 5MBC method.

2 METHODS

2.1 Field sites: the Otago Peninsula

All field sites in this study were located on the Otago Peninsula, New Zealand (45°50'S 170°41'E; Figure 2.0). The three sites on the Peninsula were chosen based on vegetative growth, ease of access, and size, as the area needed to be large enough to fit a large number of hair-snag tubes in a grid. All three sites were deemed to be fragments of vegetation due to the large amount of converted land surrounding them, in most cases pastureland. Sites chosen were areas where possum control had taken place, but where no further mammalian pest control had taken place.

2.1.1 Leith Walk

The Leith Walk study had the least amount of past grazing. Leith Walk is situated just north-west of Hooper's Inlet, one of the two large inlets found on the Otago Peninsula, and about 700 m from Portobello township, separated by a hill. The sampling site was approximately 5.1 ha in size and consisted of two main vegetation types: mixed mature native bush (Figure 2.1a) and tall kānuka-dominated (*Kunzea ericoides*) forest (Figure 2.1b), with a few small patches of cleared area overgrown with introduced grasses (e.g. kikuyu, *Pennisetum clandestinum*), wild blackberry (*Rubus fruticosus*) and some deliberately planted shrubs and trees. The mixed mature native bush parts consisted mainly of high- to mid-canopy vegetation with old tree fuchsia (*Fuchsia excorticata*), lemonwood (*Pittosporum eugenioides*), māhoe (*Melicytus ramiflorus*), and kānuka, mid- to low-canopy level growth of round-leaved coprosma (*Coprosma rotundifolia*) and red matipo (*Myrsine australis*), and understorey fern growth consisting mainly of hen and chicken fern (*Asplenium bulbiferum*) and crown fern (*Blechnum discolor*). The kānuka-dominated forest was for the most part much drier (except in areas near flowing streams) and had stands of native mid- to low-canopy level shrubs, mainly red matipo, lemonwood, māhoe, round-leaved coprosma, broadleaf (*Griselina littoralis*), kōhūhū (*Pittosporum*

tenuifolium), patē (*Schefflera digitata*) and shining karamū (*Coprosma lucida*). Streams flowing throughout the sampling area meant there were parts of the area that were consistently in a fairly wet environment. In the past there has been some private pest control in the vicinity by landowners with a few traps that have caught or shown signs of possums, rats, and hedgehogs in relatively low numbers outside of the sampling area.

2.1.2 Hooper's Inlet

The area which encompassed the Hooper's Inlet study site is a section of 3.7 ha of privately-owned, fenced land bordering Hooper's Inlet, with agricultural land north and south-east of the forested area. This area consists of two main vegetation types: kānuka-dominated forest (Figure 2.2a), and two clearings with large macrocarpa stands (*Cupressus macrocarpa*; Figure 2.2b), one of which has low-growing introduced grasses while the other is inundated with various native and introduced herbaceous plants, shrubs, and weeds (e.g. poroporo, *Solanum laciniatum*; poison hemlock, *Conium maculatum*; red bidibid, *Acaena novae-zelandiae*). Throughout the kānuka-dominated forest are intermittent growths of native trees and shrubs; mainly māhoe and totara (*Podocarpus totara*; possibly Hall's totara, *Podocarpus laetus*). There is also a small area in the north-west corner comprising tree fuchsia, hen and chicken fern, and bush lawyer (*Rubus cissoides*). The site has obvious signs of extensive rabbit activity, especially in the clearings. The land is regenerating from a period of stock grazing which ceased in 1993 (M. Parker, *pers. comm.*), with subsequent growth of kānuka forest, a common primary successional species that can indicate regeneration from pastureland (Allen et al. 2009). The site is approximately 1.2 km east of the Leith Walk site. Other plant species commonly found throughout the area include pohuehue (*Muehlenbeckia australis*), ngaio (*Myoporum laetum*), ongaonga (*Urtica ferox*), and mānuka (*Leptospermum scoparium*).

2.1.3 Okia Flat

The Okia Reserve is a large area of approximately 231 ha of coastal land and is jointly owned by the Dunedin City Council and the Yellow-eyed Penguin Trust. It was purchased in 1991 as a reserve to aid in the conservation of yellow-eyed penguins (*Megadyptes antipodes*) which nest in the area, and stock grazing ceased in the area shortly after purchase (Johnson 1993). The sampling site was located in the northern end of the reserve, bordered in the north by large cliffs. The area consists of relict dune fields and the soil is mainly sandy in composition. There are some wet areas with pools of water, most of which dry out in the summer, and a small stream near the cliffs. The most dominant vegetation is bracken (*Pteridium esculentum*), which covers the majority of the area, together with a small area composed of sedges (*Carex* spp.), and a few intermittent native shrubs and shrub stands amongst the bracken, comprising mainly lemonwood, flax (*Phormium* sp.), broadleaf, māhoe, narrow-leaved lacebark (*Hoheria angustifolia*), and mikimiki (*Coprosma crassifolia*), commonly with kaihua (*Parsonsia heterophylla*) vines (Figure 2.3). Along the northern end of the site at the base of the cliffs is a thick stand of mixed native forest with ongaonga and broadleaf, among others. Around this area are also rocky outcrops and pillar formations with large boulders encircling them. On these rocky outcrops vegetation includes broadleaf, patotara (*Leucopogon fraseri*), kokihi (*Tetragonia implexicoma*), and korokio (*Corokia cotoneaster*). While not a forest fragment itself (though there are some scattered forest fragments), Okia Flat is a fragment of sorts in that it is predominantly native vegetation surrounded by agriculturally developed land and is henceforth referred to as a scrub or vegetation fragment. Possum control is ongoing, and there are also a number of stoat, ferret, (DOC 200, Fenn traps), weasel (Fenn traps), and cat traps (Steve Allen traps).

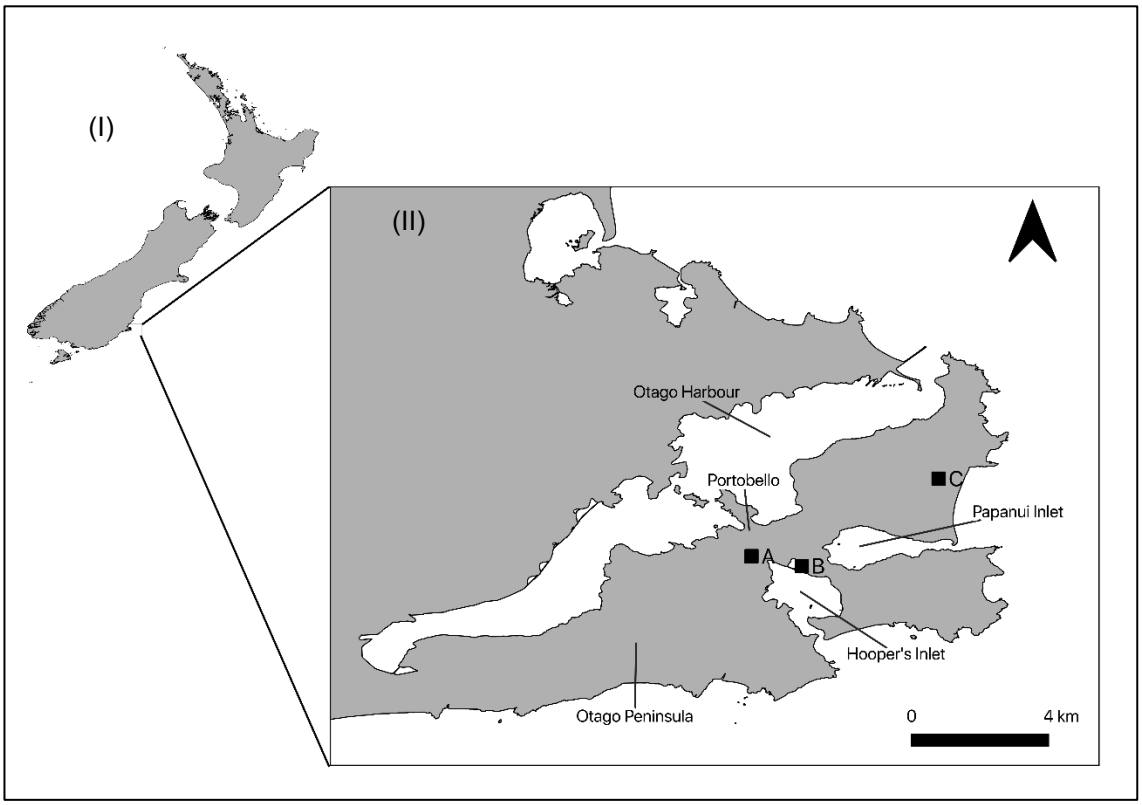

Figure 2.0: (I) Location of the Otago Peninsula on the South Island of New Zealand. (II) Sampling locations on the Otago Peninsula. A) Leith Walk site; B) Hooper's Inlet site; C) Okia flat site. The scale bar is equal to 4km.

Figure 2.1: The two typical vegetation types found at Leith Walk; (a) mature native bush and (b) kānuka-dominated mixed forest.

Figure 2.2: The two typical vegetation types found at Hooper's Inlet; (a) kānuka-dominated regenerating forest and (b) clearing with macrocarpa stands.

Figure 2.3: View of the bracken-dominated hollow dune landscape of Okia Flat, with cliffs towards the left-hand side of the image.

2.2 Field protocol

2.2.1 Hair-snag tubes

In order to collect tissue samples in a non-invasive manner, hair-snag tubes were used to collect hair from rodents so that the follicle DNA could be used to identify individuals. The tubes were set up in an array and checked daily for presence of hair over eight days. The hair-snag tubes consisted of 220 mm lengths of white 65 mm PVC pipe with two slits cut halfway through the tube about 40 mm in from each end. A 229 mm diameter, 16 mm wide rubber band was stretched between the slits so that the rubber band was strung across the inside centre of the tube. The entire sections of elastic band inside the tube were coated in diluted TRAPPER® glue, from Pest Management Services, Paraparaumu, which was used in a similar study (McCulloch 2009). Hair would adhere to the glue-coated rubber band as the rodent brushes past underneath it to reach the bait. The slits were covered using duct tape to prevent moisture from interfering with the glue (Figure 2.4). The glue was diluted by gently heating the container holding the glue in a hot water bath and mixing in toluene until the glue was the consistency of thick honey. The dilution process was carried out in a fume cupboard.

To bait the hair-snag tubes and entice rats into the tube a small amount (approximately 5 g) of peanut butter was added into the middle of the tube, between the two sections of rubber band. The tubes were pegged into the ground using a loop of number 8 wire to prevent movement. Each tube had a unique number written on it to identify the location of any collected samples. Each day, if hairs were found clinging to the rubber band, the entire rubber band was carefully extracted from the tube and placed into a small zip-lock back containing filter paper to absorb moisture and minimise DNA deterioration. Each bag containing a sample was labelled with the sampling location (tube number) and date. The tube was then reset with a fresh rubber band, glue, and peanut butter. Any tubes where an animal had consumed the peanut butter but failed to leave hair had fresh peanut butter added.

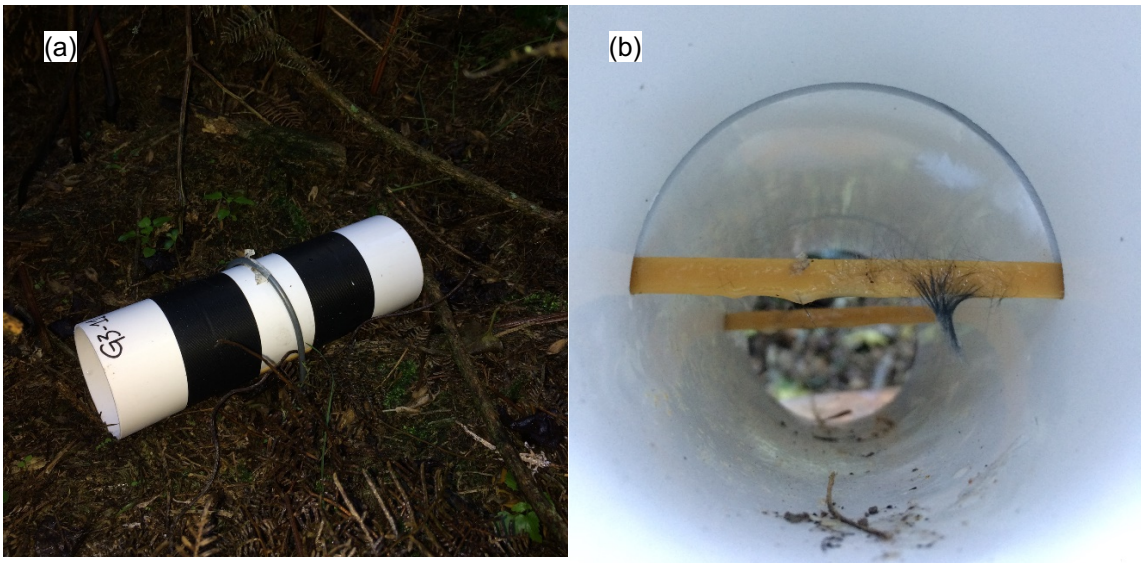

Figure 2.4: (a) Hair-snag tube in the field, showing duct tape covering the slits and wire loop for stabilisation. (b) Rubber band strung between slits as seen from inside of tube with glue and attached hair sample. Width of rubber band when stretched equals 10 mm.

The arrays of hair-snag tubes at each site differed in size and shape as each site had unique characteristics that constrained tube placement, such as dense vegetation and terrain. However, in all instances the tubes were spaced at intervals of 25 m. Sample points were created in Garmin® BaseCamp© version 4.6.3 and were located in the field using handheld GPSs. The Leith Walk site had ten lines spaced 25 m apart, with each tube along the line spaced 25 m apart (n = 87; Figure 2.5). The site at Hooper's Inlet consisted of six lines with the same spacing as the grid at Leith Walk (n = 70; Figure 2.6). The Okia site had six lines with the same spacing as the other two sites (n = 44; Figure 2.7).

Sampling was carried out within the same month (May 2018) to minimise potential impacts of variables associated with seasonal fluctuations in rat abundance and to maintain site comparability. The Leith Walk site monitoring ran from 2nd May 2018 – 9th May 2018, Hooper's Inlet from 3rd May 2018 – 10th May 2018, and Okia from 22nd May 2018 – 29th May 2018. Weather and logistical issues prevented the Okia site from being monitored during the same week as the Leith Walk and Hooper's Inlet sites. The tubes were laid on the first day, with the first samples being collected daily from the following day onwards. The tubes were collected again on the last day after the last samples had been collected. All samples were frozen at -20°C and kept in the freezer until they were

needed for identification and DNA extraction (extraction occurred between approximately 7 months 25 days and 14 months 26 days after collection).

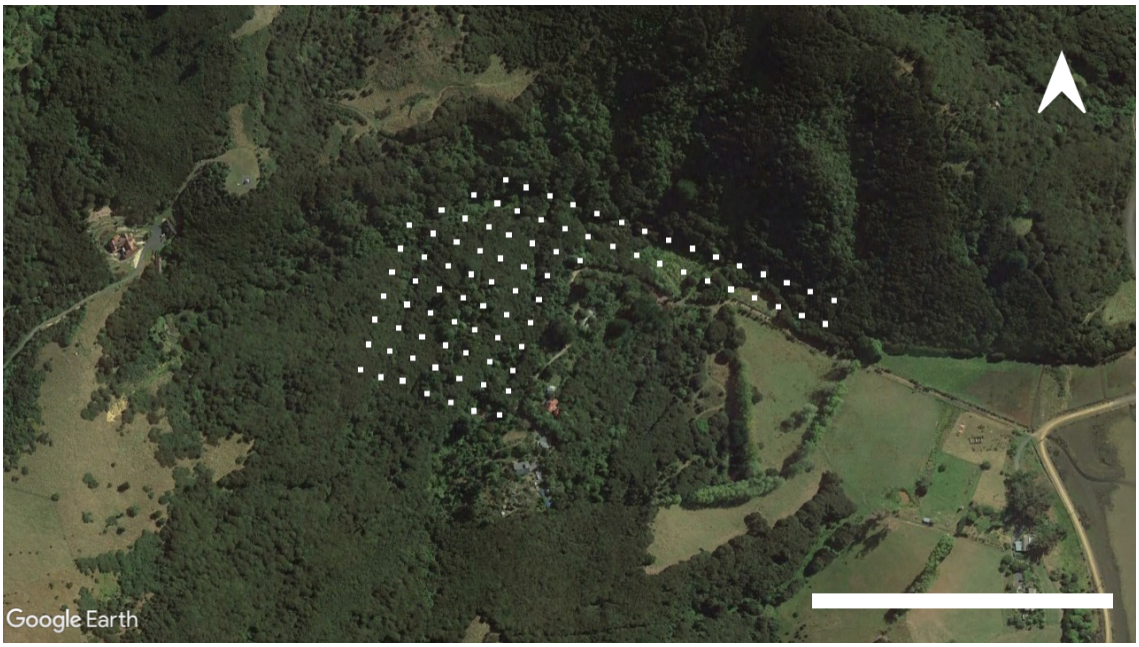

Figure 2.5: Composite aerial photograph of Leith Walk study site with hair-snag tube sampling grid overlaid (white squares; *n*=87). Tubes spaced 25 m along lines and 25 m between lines. Scale bar is equal to 300 m.

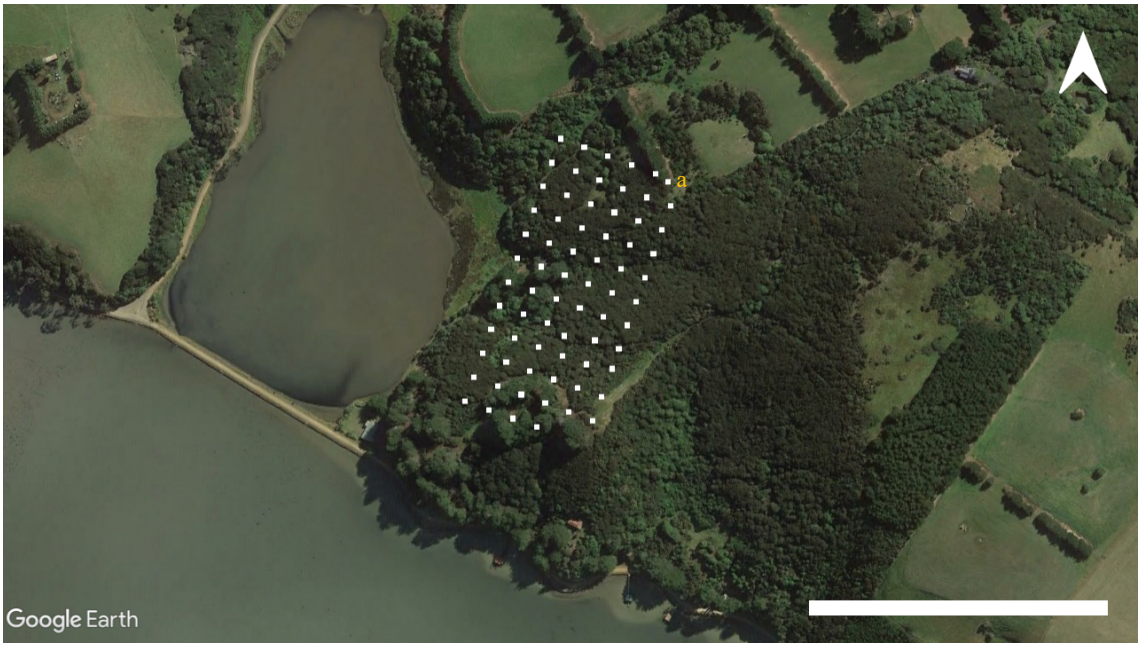

Figure 2.6: Composite aerial photograph of Hooper's Inlet study site with hair-snag tube sampling grid overlaid (white square; *n*=71). Tubes spaced 25 m along lines and 25 m between lines, except at (a) where tube location had to be altered due to site access restrictions. Scale bar is equal to 300 m.

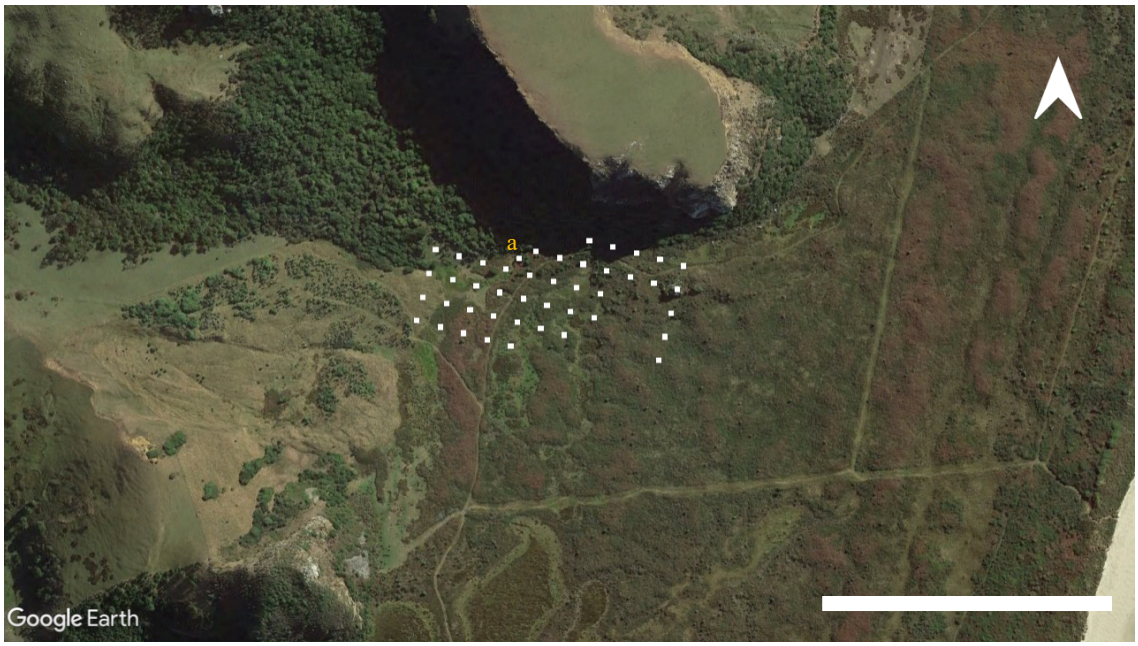

Figure 2.7: Composite aerial photograph of Okia study site with hair-snag tube sampling grid overlaid (white squares; *n*=44). Tubes spaced 25 m along lines and 25 m between lines, except at (a) where tube location had to be altered due to site access restrictions. Irregularity of the otherwise rectangular grid was due to restrictive terrain. Scale bar is equal to 300 m.

2.2.2 Tracking Tunnels

Tracking tunnels were used to provide additional data on rat presence or absence as they are relatively light and the ink cards used are cheap (NZ$1.78), although for this study they were kindly donated by the OPBG.

A line of tracking tunnels was laid at each site, with each line comprising ten tunnels set 50 m apart within the same area encompassed by the hair-snag tube grid. Due to the relatively small sizes of the study sites, only one line of tunnels was established at each site (Figure 2.8). The tunnels themselves were constructed from a 2 cm thick plank of wood, approximately 15 cm wide and 55 cm long, which served as the base, and a sheet of black corflute attached to either side of the wood and folded over the wood to form a tunnel with a height of approximately 11 cm. A 49 cm length of card sold as The Black Trakka™ (Gotcha Traps Ltd, Auckland) with ink in the middle and white card on either

end was secured to the wood with pushpins (Figure 2.9; Figure 2.10). A small amount of peanut butter (approximately 5 g) was smeared onto the black corflute at either end of the tunnel following Department of Conservation procedure (Gillies and Williams 2013). Tunnels were set out at all three sites on 3rd June 2018. The following day on 4th June 2018 ink cards were collected and labelled with sampling location (tunnel number) and date. Fresh cards were placed and left for four days until 8th June 2018 when the tunnels were collected again.

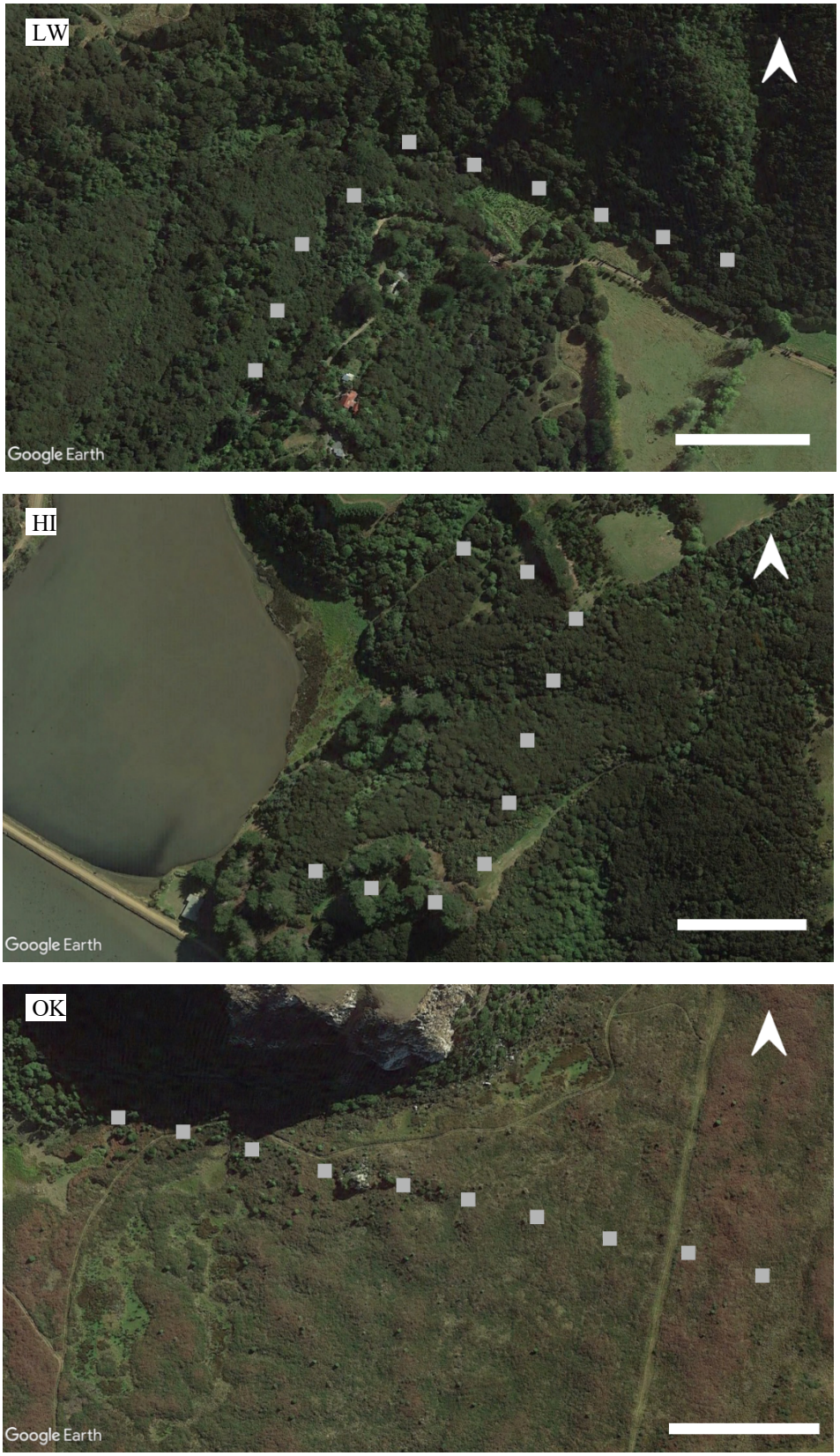

Figure 2.8: Composite aerial photography of the three tracking tunnel lines, one at each of the three study sites; Leith Walk (LW), Hooper's Inlet (HI), and Okia (OK). Tunnels are spaced at 50 m apart. Scale bars represent 100 m.

Figure 2.9: Tracking tunnel supplied by the OPBG, with ink tracking card attached to wooden base. Peanut butter used as bait was smeared on both ends of the tunnel.

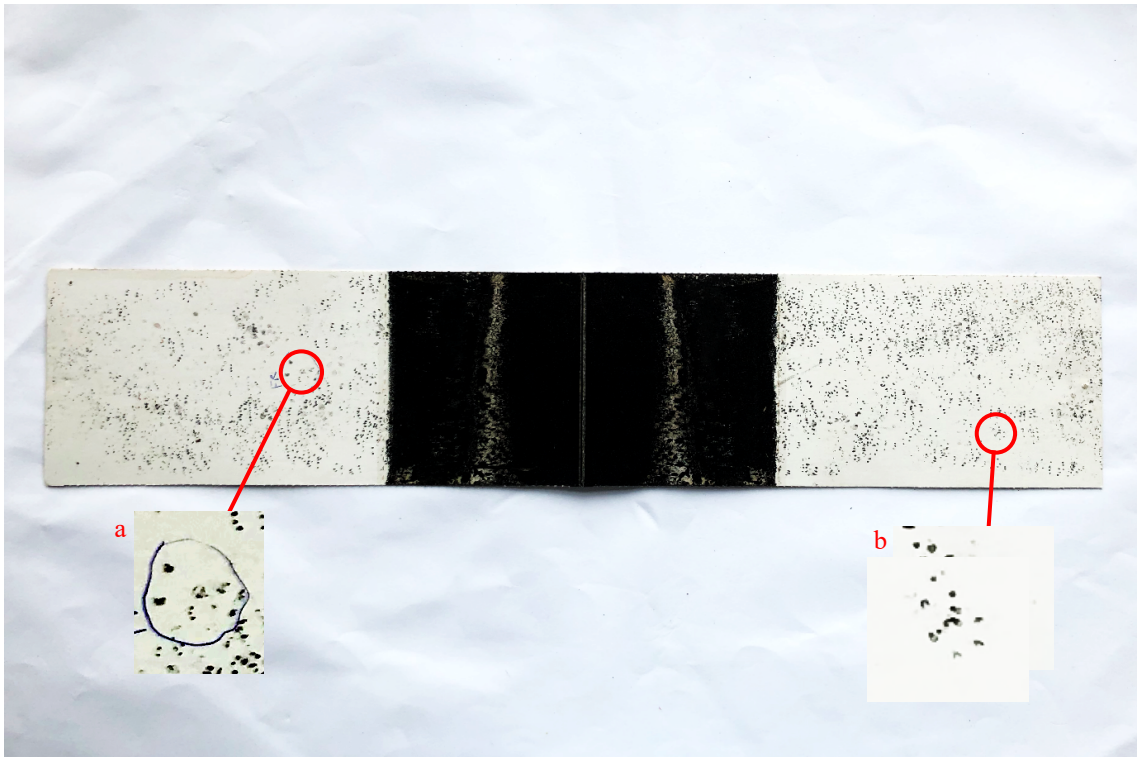

Figure 2.10: Ink tracking card showing ink in middle and a strip of white card on either side. Footprints were identified as (a) rat and (b) mouse. End to end the ink card measures 49 cm.

2.2.3 Bird counts

To gain an understanding of bird abundance in the areas where sampling took place, 5MBC were conducted at each site following DOC procedure (Hartley and Greene 2012). The 5MBC consisted of a line of 'stations' within the same area used for the hair-snag tubes. These stations were set at 200 m walking intervals, and each site had three stations (Figure 2.11). At these stations 5 minutes was timed and all birds seen or heard within that timeframe were recorded. Distance from observer to the bird was estimated using a Nikon ProStaff Laser 440 rangefinder. Birds seen were recorded separately from birds heard so that it could be used as a covariate when detectability was modelled in DISTANCE later on. The bird counts were conducted in early-summer, as birds were deemed to be most active in spring-early summer (U. Ellenberg, *pers. comm.*). Bird counts were conducted over four days from 19th October 2018 to 22nd October 2018. All counts were done in the mornings after 7 am and before 12 pm when birds would be most active (U. Ellenberg, *pers. comm.*).

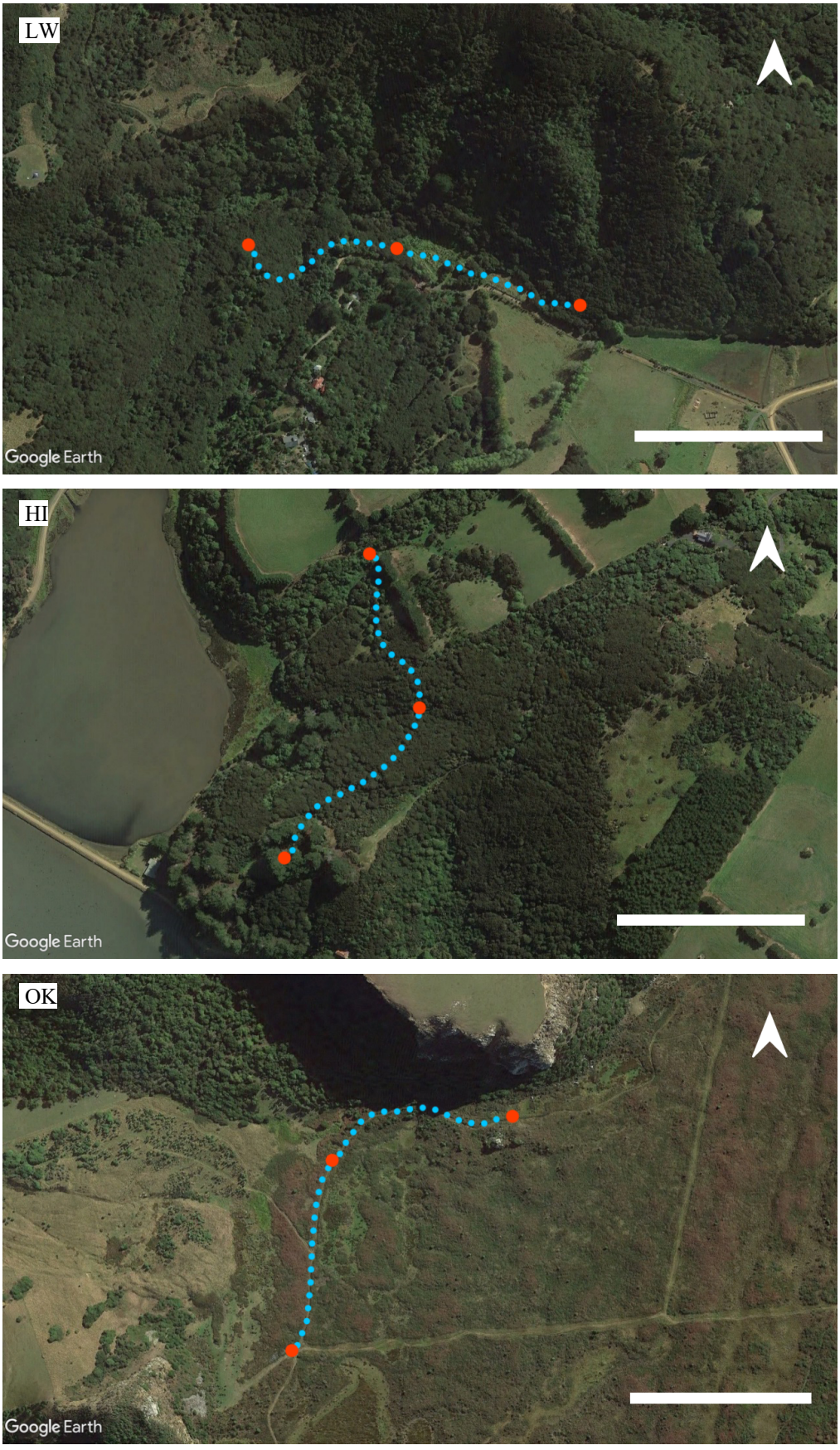

Figure 2.11: Composite aerial photograph showing the 5MBC stations (red) and the paths walked (blue dotted) for the three study sites; Leith Walk (LW), Hooper's Inlet (HI), and Okia (OK). Scale bar is equal to 200 m.

2.3 Analysis

2.3.1 Identification of hair samples

Samples collected were first examined under a microscope to distinguish rat hair from hairs from non-target species, through comparison of the hair's medulla structure (Figure 2.12). Hair attached to the rubber band was extracted with forceps and placed on a glass slide. Due to the hair being covered in glue, no additional medium was needed to keep the hair secured on the slide and the glue did not interfere with the appearance or visibility of the hair. Several plates were created as reference slides from known ship rat hair samples, taken from a dead specimen donated to the study, from known mouse hair samples, also taken from dead specimens donated to the study, and some reference slides from a past study were used for mustelid hair references. While mouse hair was in most cases readily distinguishable, it was more difficult to definitively identify rat hair, and the hair could not be distinguished between rat species. Therefore, these identifications were not treated as absolute and microsatellite analysis was needed to confirm species as well as identify individuals.

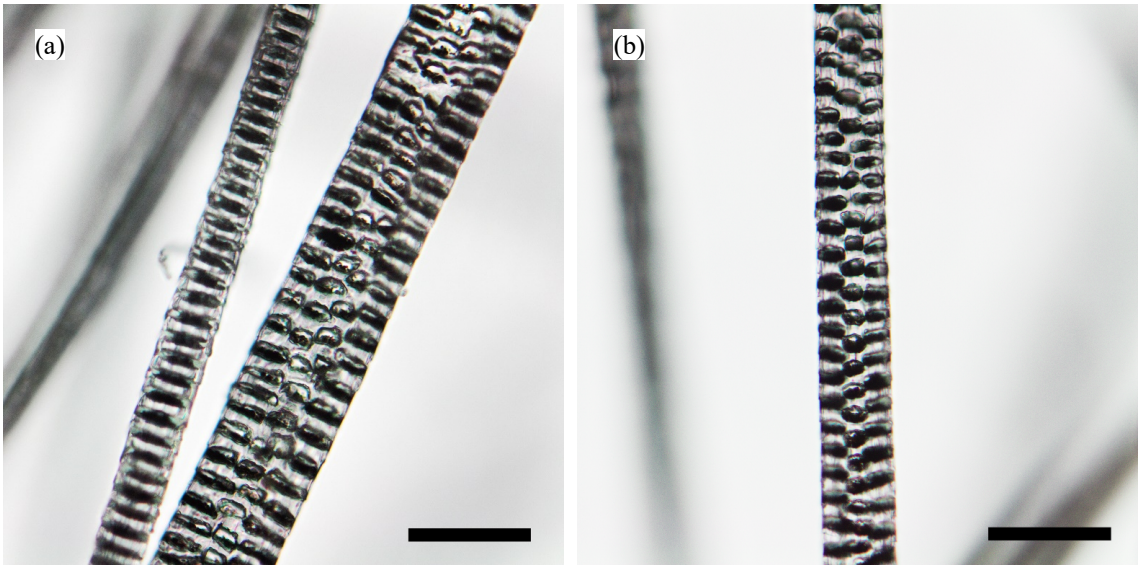

Figure 2.12: Aeroform lattice medulla structure of (a) an underfur hair (left) and a guard hair (right) taken from a ship rat specimen, and (b) a guard hair taken from a mouse specimen. Scale bars are 50 micrometres.

2.3.1.1 Microsatellite analysis

To distinguish rat species and possibly identify individual rats, a microsatellite analysis was employed. Hair samples were removed from the collected rubber bands using forceps and placed into a 1.5 ml microcentrifuge tube. The DNA in the follicles of the hair were extracted using the Quick-DNA™ Miniprep Plus Kit (Zymo Reseach Corp.) according to the manufacturer's instruction manual with the addition of dithiolthreitol (DTT) for hair samples (with the exception of six samples where no DTT was added). A small amount of the extracted DNA was analysed using a Nanodrop ND-1000 spectrophotometer to assess concentration of DNA, and samples with high concentrations were diluted to ~10-15ng/µl with water, although this was discontinued in later samples as the spectrophotometer gave inconsistent results and few samples actually had enough hair collected to produce a significantly high concentration of DNA. Extracted DNA was stored at 4°C for a period of 1-2 days, or 37-50 days.

Another method of extraction was also briefly used in the form of cetrimonium bromide (CTAB; following modified process of Doyle and Doyle 1987). However, this was only used for the extraction of 12 samples as the consequent results were not satisfactory. The DNA of some samples were precipitated by eluting the DNA in a mixture of sodium acetate (NaOCa), ethanol, and linear polyacrylamide (LPA; from GenElute™ Sigma-Aldrich), in an attempt to increase purity of the DNA. This process was found to have no effect on PCR success and was subsequently not used in further samples.

Extracted DNA was added to PCR wells and dried in an Eppendorf Concentrator Plus vacuum concentrator. Water, both of the forward and reverse primers (Sigma-Aldrich, Missouri), the M13 primers, and Type-It were added to the wells, and the PCR was run (following Townsend et al. 2012). The dyes used were 6-FAM (blue), VIC (green), NED (yellow), and PET (red). The PCR products were sent to Otago Genetic Analysis Service, Department of Anatomy, University of Otago, Dunedin and run on an ABI 3730xl DNA Analyser. The resulting information could be uploaded to the computer software GeneMapper© version 4.1 (Thermo Fisher, Massachusetts) to visualise the genotypic

information and allow comparisons of polymorphic microsatellite loci. Conducting a PCR allowed for small amounts of DNA expected from hair-snag sampling to be amplified to give higher probabilities of success in genotyping.

Specific primers were chosen based on their ability to amplify specific loci according to previous studies (Love et al. 1990; Hearne et al. 1991; Kondo et al. 1993; McCulloch 2009; Russell et al. 2010). Some primers amplified either rat or mouse loci but not both and allowed the genera to be distinguished from one another (Table 2.0). This was done to allow a sample to be assessed as coming from a mouse if not from a rat, as opposed to providing a false positive genotyping error where the result could either indicate error or coming from a mouse. Hair and tissue samples taken from two donated wild rat and two wild mouse specimens, along with hair from a laboratory mouse from the Microbiology Department, University of Otago, were used at the beginning to test primers and develop a multiplex panel using MultiplexManager 1.0 (Holleley and Geerts 2009), to aid in identification of genotypes from DNA taken from the samples from the hair-snag tubes.

In total, 23 polymorphic microsatellite loci and one sex-linked marker were amplified. Nine of these were specific for rat genotyping (D15Rat77, D18Rat96, D20Rat46, D16Rat81, DXRat2, D10Rat20, D11Mgh5, D7Rat13, D2Rat234, D5Rat83, D19Mit2). As Norway rats have been shown to have shorter base-pair lengths at the D11Mgh5 and D19Mit2 loci than ship rats (McCulloch 2009), these two species should be separated based on the base-pair lengths of alleles at these loci.

Comparing similar genotypes also allowed for the identification of any genotyping errors by allelic dropout or false alleles and were classified as the average number of errors per locus (following Taberlet 1996; McCulloch 2009).

2.3.1.2 Locus failure rate

To determine the rate of failed amplifications for a locus, the number of samples in which no alleles were amplified was divided by the number of samples in which that locus was used.

Table 2.0: The 23 microsatellite loci used, with chromosome in which locus is found with map location in centimorgans for rats and mice, the primer sequence for forward (f) and reverse (r) primers (both in 5' to 3' orientation), the expected size of the polymerase chain reaction (PCR) product as size in base pairs of alleles (via Genbank and Love et al. 1990), the repeat unit of the microsatellites, and the colour label used for the 23 microsatellite loci used in this study. Source of locus information given. N/A denotes data not available Table adapted from Love (1990).

Locus	Chromosome	Primer sequences 5' to 3'	PCR product size (bp)	Repeat unit	Colour label	Multiplex	Source
Acrg	Mouse: 1	F: ACCGTTCACAGCTGACCTAGT	112	$(CA)_{12}$	PET	2	[1, 2]
		R: GGGACACAGATGTACTAAGCT					
Bcl-2	Mouse: 1	F: CATTATCAATGATGTACCATG	132	$(CA)_{23}$	NED	1	[1, 3]
		R: GCAGTAAATAGCTGATTCGAC					
D0Nds2	Mouse: N/A	F: CTCTTATTCCTGTTCTACTCA	88	$(CT)_{15}$	6-FAM	2	[1]
		R: ATTCTTTAGCATTTGTGGATC					
D10Rat20	Rat: 10	F: GATTGCCATACCTGCCT	123	$(TG)_{32}$	VIC	1	[4]
		R: GAAATGGCCAGGATAAACCA					
D11Mgh5	Rat: 11	F: CATCTAATTCCAGAAAGGTTT	242	$(GT)_{23}$	VIC	1	[4]
		R: GAATCGATTGACAGATGTCTGTG					
D15Rat77	Rat: 15	F: CATGTGGGGAAAGCATTACC	233	$(AC)_{25}$	PET	1	[4]

		R: ACAGAGGGAACCCATCACAG					
D16Rat81	Rat: 16	F: GAGCCTTAGCACAGTGGCTT	153	$(GT)_{28}$	6-FAM	2	[4]
		R: GGCCCACATGTGCATGTATA					
D18Rat96	Rat: 18	F: TGGACATCCTCAATGGACCT	247	$(TG)_{24}(AG)_{25}$	6-FAM	1	[4]
		R: GCAGATCTCTCCTCCACAGC					
D19Mit2	Rat: 19	F: AAGGTTGGCAGTTTCCCAG	193	$(CT)_{18}(GT)_{19}$	NED	1	[4]
		R: ACCATTTATGTGCCCAGATG					
D20Rat46	Rat: 20	F: AAGTACTGAGTGGGCTGCGT	168	$(TG)_{23}$	PET	1	[4]
		R: GGCAAAACACCAATGCCTAT					
D2Rat234	Rat: 2	F: GTAGAGCAAGATGGGGTGGA	120	$(GT)_{27}$	PET	1	[4]
		R: ATATTCAAGCTGGCTTCCCC					
D5Rat83	Rat: 5	F: GGTCTTCAGGATGGCAATGT	198	$(AC)_{29}$	VIC	1	[4]
		R: ACTTGGAAACAGGGAGATGG					
D7Rat13	Rat: 7	F: GACTTCTGCTACACGCCACA	168	$(TG)_{22}$	6-FAM	1	[4]
		R: CAGCCCTAGAAGGAAATGCA					
DXRat2	Rat: X	F: GCACAAGGTGTCACAGG	160	$(GT)_{32}$	VIC	2	[4]

		R: CCAGCCTGAACCCTATCTCA					
Gfap	Mouse: 11	F: TGAATTCTAGGACCAGCCAAGGCT	277	$(GA)_{21}A(GAA)_8$	PET	2	[1, 2, 3]
		R: ACCTCTAAGATCCTGTGCGAGGCT		$(GA)_2GAAA(GT)_{15}$			
Hsp68	Mouse: 17	F: GTAATTGCGTTGACTGTTAAAT	96	$(TA)_{14}$	VIC	2	[1]
		R: AGTGCTGCTCCCAACATTACT					
Il-1b	Mouse: 2	F: CCAAGCTTCCTTGTGCAAGTA	257	complex $(TC)_n$	VIC	2	[1, 3]
		R: AAGCCCAAAGTCCATCAGTGG					
Il-5	Mouse: 11	F: CCTTTCTGAAAGTATTAAGAGT	288	$(AT)_{17}(GT)_{13}(AT)_5$	6-FAM	2	[1, 2, 3]
		R: ACAACCATCTGCATATCCAGC		$(GTAT)_8AT(GT)_{16}$			
Myc	Mouse: 15	F: CGTCACTGATAGTAGGGAGTA	107	$(CA)_{20}$	NED	2	[1, 2, 3]
		R: TCAGCGTGCTGTACTTCCAAG					
Myla	Mouse: 11	F: ACTAGTCCTACCGGTCTTCCA	205	$(GT)_{16}$	NED	2	[1, 2]
		R: TGTCTGTTGCTTACTATGTGC					
Qa-4	Mouse: 17	F: CCTGGAGGAATATCAATAGTG	214	$(TTC)_{31}(CT)_{32}$	PET	2	[1]
		R: ATACAGAGAAACCCTATCTCAA					
	Rat: Y	F: TGCAATGGGACAACAACCTA	N/A	N/A	6-FAM	2	[5]

SRYf-Peakall		R: TAGTGGAACTGGTGCTGCTG					
Tnfa	Mouse: 17	F: GTTTCAGTTCTCAGGGTCCTA R: CAGGATTCTGTGGCAATCTGG	102	$(CA)_{20}$	6-FAM	1	[1, 2]

[1] (Love et al. 1990)
[2] (Hearne et al. 1991)
[3] (Kondo et al. 1993)
[4] (Russell et al. 2010)
[5] H. Roberts, *pers. comm.*

2.3.2 Spatially explicit capture-recapture

Original plans to implement a SECR model to estimate rat density were abandoned as recaptures of rats could not be identified. A simple estimation of rat density was undertaken in its place.

2.3.3 Rat density estimation

Following Dice (Dice 1938), a rat density estimate (minimum number of rats alive per hectare) was obtained using the number of rats identified at each grid through genetic analysis divided by the area of the corresponding grid plus the area of a boundary strip surrounding the grid. The boundary strip was calculated as half of the average taken from the mean home-range length from two studies: Hickson (1986), from a rat population in regenerating coastal forest on Stewart Island during spring-summer (142 m); and Wilson (2007a), from a rat population in mixed podocarp-broadleaf forest at Orongorongo Valley in the southern North Island during autumn (144-184 m). These estimates were chosen based on the vegetation type and season in which sampling took place. These estimates were calculated for ship rats; it was assumed the rats detected in this study were ship rats based on anecdotal evidence (D. Wilson, *pers. comm.*) and inferences made from the results of this study in the habitat selection analysis. The boundary strip area at Okia had to be reduced due to the presence of a steep cliff on the northern side of the grid, and at Hooper's Inlet due to the shoreline on the eastern side of the grid. At Leith Walk the boundary strip also encloses residential properties. It was assumed that the population of rats at each study site was closed and that there were no deaths, births or migrations during the time in which sampling was conducted.

2.3.4 Habitat selection analysis

To analyse the possible habitat selection of rats, tube locations at each of the three sites were revisited and vegetation surveys were conducted. Seven variables were chosen based on habitat features that were thought to be possibly important in predicting rat presence (Innes 2005a; Innes 2005b; D. Wilson, *pers. comm*.); percentage groundcover, number of height tiers with vegetation cover over 20% representing the complexity of the vegetation (loosely following the Recce method; Hurst and Allen 2007), presence or absence of large (>3 mm length) fruited or seeded tree species, maximum canopy height, distance from the tube to a source of freshwater, distance from the tube to the coastline, and the three separate sites.

Tube locations were chosen based on whether hair was collected from the tube over the sampling period or if no sample was collected from there. At two sites (Leith Walk and Okia) all tubes where no samples were collected were included, however at the Hooper's Inlet site the tubes with no samples collected were randomly chosen to bring the overall number of tube locations to be revisited to 70 (ten lines of data per variable to allow statistical viability). This resulted in a ratio of 22 positive locations to 48 negative locations.

At each tube location a circle with a radius of five metres from the tube was estimated and all plants within that area were photographed for later identification. The percentage vegetation cover within the 5 m radius was also estimated at four tiers; less than 30 cm (groundcover), 30 cm to 2 m, 2 m to 12 m, and 12 m and above. Citizen science was implemented in the identification of the plant species using iNaturalist (www.inaturalist.org) where multiple users would reach a consensus on the identification of the plant pictured. These identifications were then additionally cross-referenced manually for accuracy. Presence or absence of large fruited or seeded species was based on the plants identified that fruited during the same season as when hair-tube sampling took place (autumn). Plant fruiting season was taken primarily from the New Zealand Plant Conservation Network database (www.NZPCN.org.nz) and, where records were

lacking, from the Landcare Research Ecotraits database (ecotraits.landcareresearch.co.nz). The main criterion as to whether or not a large fruited or seeded plant species should be considered was whether any research suggests the fruits of a certain species had been observed as being ingested by rats (e.g. Daniel 1973; Wilson et al. 2007b; Grant-Hoffman and Barboza 2010).

Distance to freshwater was recorded where possible in the field and otherwise measured using QGIS 3.8 software (QGIS Development Team 2019), as was distance to coastline.

2.3.4.1 Model selection

All seven variables were used as predictors of the response variable 'rat presence', which was a binary variable indicating whether a rat had been detected (1), at a tube over any one of the sampling occasions or not (0), in the original full generalised linear model to be used in diagnostic plots and exploratory data analysis. Rat presence was recorded by tube, not sampling occasion; i.e. if multiple rats were detected over multiple sampling occasions at the same tube, then it was recorded as one capture at that tube location. The function *glm* from the package *stats* was used to create the generalised linear model in R version 3.6.1, with family = *binomial*, link = *logit* (R Core Team 2019).

Following exploratory data analysis, the predictor variable 'canopy' (the maximum canopy height recorded at a hair-snag tube location) was removed as it showed a high degree of collinearity with another predictor variable 'tiers' (number of tiers at a tube location). The variable 'tiers' was chosen over 'canopy' due to the higher importance of 'tiers' as indicated through the exploratory data analysis phase. The predictor variables were all scaled and centred using the function *rescale* from the package *arm* in R to reduce collinearity and put the coefficients on a comparable scale to allow better insight into effect sizes (Gelman and Su 2018). Variance inflation factor checks were conducted before and after variable rescaling to assess collinearity of the parameters using the *vif* function from the package *car* in R (Fox and Weisberg 2019).

Candidate models with more parameters can explain more variation in the data, however, this can result in increased model complexity and inflated variance. As a means to balance bias and variance, AIC (Akaike's Information Criterion) can be used to compare models to determine the model with the best fit for the data while minimising the number of parameters (known as the principle of parsimony; (Buckland et al. 2001). As this study had a low sample size, a version of AIC corrected for small sample size, AICc, was used. The *dredge* function from the package *MuMIn* in R was used to generate AICc values for all possible candidate models, ranked by ΔAICc and Akaike weights, which indicated the relative importance of a particular model (Nakagawa and Freckleton 2011; Symonds and Moussalli 2011; Bartoń 2019). All models with ΔAICc values <8 were considered, with those with <2 denoting the models as approximately equal (Burnham and Anderson 2002; Nakagawa and Freckleton 2011; Symonds and Moussalli 2011).

Confidence intervals were calculated for each of the variables, those with confidence intervals excluding zero were considered important. The 'importance' of each parameter, as the sum of the Akaike weights of all of the models in which that parameter is present, was also calculated, using the *importance* function in the *MuMIn* package (Bartoń 2019). One model was selected with all the important variables, and together with the full model an analysis of variance (ANOVA) was run on each to obtain a p-value for each parameter using the *Anova* function in the *car* package in R. A McFadden's pseudo R^2 value was obtained to show how much variation in the data is being explained by each model, and was calculated for each model using the equation:

$$1 - \left(\frac{residual\ deviance}{null\ deviance}\right) \quad (1)$$

Where residual deviance is deviance of the fitted model and null deviance is the deviance of the null model (McFadden 1973).

The dispersion of both the full model and the reduced model was calculated to assess whether the assumption of a generalised linear model that the mean and variance are equal was met. The dependent variable was then plotted against each dependent variable in the reduced model using the *ggplot* function from the *ggplot2* package (Wickham 2016).

The interactions between predictor variables were originally included in the full model to see which interactions showed significance, however, these were then discarded as the significantly increased the collinearity of the variables.

2.3.5 Identification of ink cards from rat tracking tunnels

Tracks left on the card by animals were compared to reference tracks from card distributor (gotchatraps.co.nz), and cards with suspected rat tracks were double-checked by a Landcare Research scientist with extensive knowledge of rat tracking (J. Innes, *pers. comm.*). Tunnels were recorded as either having rat tracks (1) or not (0). The same was done separately for mouse tracks.

2.3.6 Estimating relative abundance of rats from tracking tunnels

Relative abundance of rats from the tracking tunnel data was calculated as the tracking index and specified as the mean percentage of tunnels tracked by rats per line set in each site. This allows for some basic comparisons between sites and can be used in conjunction with the hair-snag tube data to confirm presence or absence of rats in that area. To calculate the tracking index, the mean percentage-tracking rate per line was calculated by dividing the number of tunnels with either rat or mouse tracks (species calculated separately) on each line by the number of tunnels in that line (ten) and multiplied by 100.

A Pearson's correlation test was conducted to assess any correlation between tracking rate from tracking tunnels and number of rats detected on the hair-snag tube grid at the corresponding site using the *cor.test* function from the package *stats* in R (R Core Team 2019).

2.3.6 Estimating abundance of birds through distance analysis

The data collected through the 5MBC were used to estimate abundance of birds at each site using the measurements of distance from the observer to an identified bird. Distance sampling is useful as the distribution of distances of an observed bird from an observer can be used to estimate a detection probability function that accounts for birds that may be present in the area but were not detected when calculating density. To obtain a reliable abundance estimate using the 5MBC method, four assumptions must be met: (1) birds directly above the observer are always detected, (2) birds are detected before any movement, (3) distances are measured accurately, and (4) sightings are independent (Buckland 1993).

The data were uploaded into DISTANCE 6.0 software (Thomas et al. 2009). The type of survey was entered as a point transect by a single observer, with clusters of objects (birds) observed. For each of the bird species with a high enough number of detections, multiple models were analysed in DISTANCE. Models were tested with various levels of truncation, with or without stratification, different key functions (hazard-rate or half-normal) and series expansions (cosine, simple polynomial, or hermite polynomial), with or without birds detected by sound as a covariate (in which case the analysis engine for the model was changed from conventional distance sampling to multiple covariates distance sampling), and differing numbers of intervals were tested to find the most parsimonious model to estimate density of the bird species. A global multiplier was also added to the model. Plausible model candidates were compared using AICc to find the model with the best fit, with models with a lower ΔAICc considered best. Lower percentage coefficient of variances, the size of the confidence limits, and the chi-squared

goodness of fit test value were also kept in consideration when choosing the model with the best fit.

3 RESULTS

3.1 Abundance estimation

3.1.1 Data collection

In total, 452 hair samples were collected from hair-snag tubes at the three study sites on the Otago Peninsula, 253 of which came from Leith Walk study site, 82 from Hooper's Inlet, and 117 from Okia. There were more samples collected on the last sampling occasion than on the first at each site (Table 3.1).

Each site varied in the number of tubes from which hair samples were collected; Leith Walk had the highest proportion with hair samples collected from 92% of the tubes in the grid, Okia had samples collected from 77% of tubes, and Hooper's Inlet had the lowest incidence rate with samples collected from 43% of tubes.

Of the 452 samples only 65 (14%) were identified through examination of the hair's medulla using microscopic techniques as possibly being rat hair. These samples were collected from 48 different hair-snag tubes across the three grids; 26 from Leith Walk, nine from Hooper's Inlet, and 13 from Okia. The number of hairs extracted from each collected rubber band ranged from one hair to several hundreds. The hairs were all a similar colour, and of varying lengths; characteristics that did not contribute to conclusive identification.

Table 3.1: Number of hair samples collected from hair-snag tubes at three sites on the Otago Peninsula (n= 452). Sampling dates vary by site, but all were conducted over seven days.

Site	**Sampling Occasion**							**Total samples collected**
	1	2	3	4	5	6	7	
Leith Walk	11	26	32	45	36	56	47	253
Hooper's Inlet	7	6	11	7	18	14	19	82
Okia	8	12	16	17	21	25	18	117

3.1.2 Microsatellite genotyping

3.1.2.1 Amplification of specimens

DNA was collected from two dead ship rat specimens and used to compare the allele base-pair sizes to the DNA of collected hair samples. Both tissue and hair samples of rat1 and rat2 had alleles successfully amplified at 18 and 19 of the 23 loci, respectively. Tissue samples produced much more strongly amplified alleles that were easier to identify compared to hair samples collected from these two rats. The same was also true in the samples collected from the mouse specimens. The tissue sample from mouse1 amplified 17 out of the 23 loci, whereas the hair sample from mouse2 amplified 15 loci. Six loci (D10Rat20, D5Rat83, D11Mgh5, D19Mit2, D2Rat234, D20Rat46) were amplified only in the rat DNA, and four (Il-5, Hsp68, Il-1b, Myla) were identified only in the mouse DNA. As there were multiple specimens of each species (ship rat, n=2; mouse, n=3), the amplification of some loci and the lack of amplification of others was deemed to be a relatively reliable way to distinguish between the two genera using the DNA collected from the hair-snag tubes (for example, if the DNA had alleles amplified at D10Rat20, it was deemed probable that this sample contained rat DNA). The use of the specimens

allowed a visual to be created with the base-pair sizes of all the alleles amplified and could be used to compare the collected hair samples to in order to aid in the identification of the DNA origin of the samples (Figure 3.1).

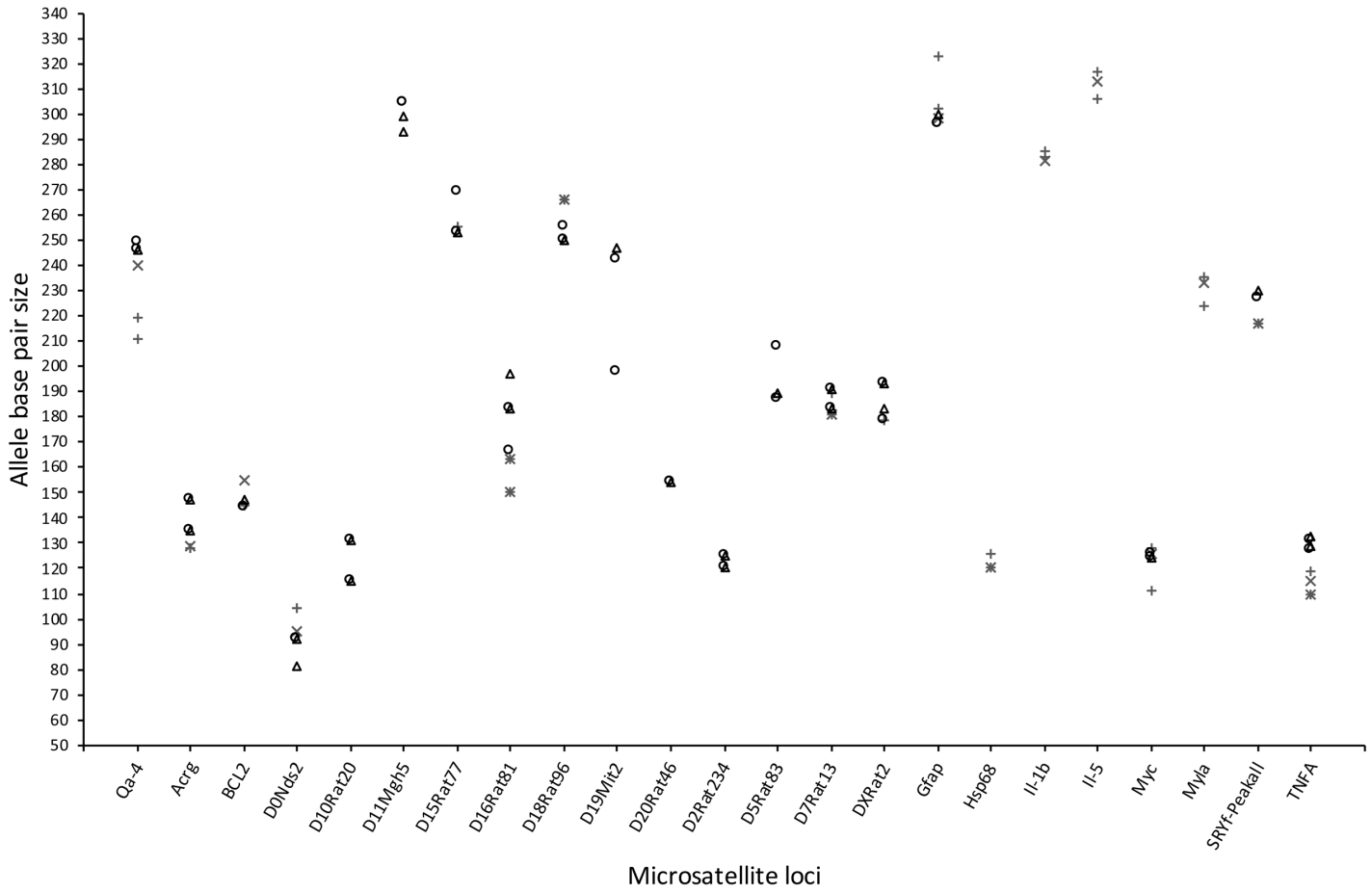

Figure 3.1: Base pair lengths for alleles from hair and tissue samples taken from dead mouse (excluding donated lab hair sample; n=2) and rat (n=2) specimens for the 23 microsatellite loci used, for comparison to samples collected in this study. Open triangles (△) represent rat 1, open circles (○) represent rat 2. Both individuals were identified as ship rats. Vertical grey-outlined crosses (+) represent mouse 1, diagonal grey-outlined crosses (×) represent mouse 2.

3.1.2.2 Amplification of collected samples

One hundred and eleven samples collected from the hair-snag tubes were further analysed through microsatellite genotyping. Of the 65 samples identified by microscope as possible rat hair, only 31% (20 samples) could be confirmed as actually containing rat DNA, while 40% did not result in any identification due to lack of allelic amplification. Twenty-six of the 65 samples (40%) were identified as containing mouse DNA (seven samples contained both rat and mouse DNA). The remaining 46 samples were identified by microscope as mouse hair and were chosen from tubes where rats were recorded on a different sampling occasion. Of these, two were identified as containing rat DNA after microsatellite genotyping (Figure 3.2; Figure 3.3; Figure 3.4).

The number of samples with rats varied greatly between sampling occasions and between sites, with no rats identified from the first and the last sampling occasion at all sites. No samples from the fourth sampling occasion at Hooper's Inlet were genotyped as no samples from this site or sampling occasion had been identified by microscope as possible rat hair (Figure 3.5).

Very few alleles were successfully amplified, with eight loci not amplifying in any of the samples (D5Rat83, D11Mgh5, D19Mit2, SRYf-Peakall, Il-5, Hsp68, DXRat2, Il-1b). As a result, confirmation of rodent species was difficult in most samples, and impossible in others. The average percentage failure rate of the loci used was 89.3% (Table 3.2).

Six samples had alleles at some loci amplified, but the alleles were not at base-pair lengths that could easily be differentiated among species and thus were uninformative in the identification of the sample. A further 37 samples failed to amplify at any loci whatsoever (approximately 33% of all the samples that were genotyped). Only ten samples had six or more loci amplified, with four of those samples having nine loci successfully amplified, the maximum number of loci successfully amplified in this study. Of those four samples, three were samples that had a moderate amount of hair attached to the

rubber band (roughly 5-15 hairs). In samples with very few hairs (<5 hairs) collected on the rubber band, two or less loci were successfully amplified in 65% of those samples, with only 9% of those samples with few hairs having five or more loci amplified successfully. In samples with a large number of hairs (>15 hairs), 71% had five to nine loci successfully amplified and 29% had two or less. Approximately 20% of samples with a moderate amount of hair per sample had five to nine loci successfully amplify, with 54% amplifying two or less. Overall, 37 of the samples that were genotyped had no loci amplified whatsoever. Of these samples, 54% were samples with few hairs, and 43% with a moderate amount of hair (Table 3.3).

Because so few alleles were successfully amplified, identification of individual rat identity was impossible as the allelic combinations of one sample could not be systematically compared to that of another. However, 22 samples could be identified as containing the DNA of rats based on base-pair sizes of alleles amplified or alleles amplified at rat specific loci, with two rats detected on two different sampling occasions at one trap at Leith Walk (Figure 3.2) and two traps each with two rats detected on two different sampling occasions at Okia (Figure 3.4). Two of the loci that failed to have any alleles amplified in any of the samples (D11Mgh5 and D19Mit2) would have been used to indicate the species of rat (Norway or ship rat). Consequently, the genetic material collected from rats in this study could only be identified to genus level and not down to species level. The sex of each identified rat could also not be determined as the sex-linked locus SRYf-Peakall failed to have alleles successfully amplify in the samples.

Table 3.2: Percentage failure rate in the genotyping of collected samples per locus, together with the test specimen DNA in which alleles were amplified at that locus during initial loci testing. Also shown is the allele base pair length recorded from the specimen DNA, and the multiplex that contained that locus for the 23 microsatellite loci used in this study.

Locus	% Failure	Multiplex	Specimen	Allele base pair length
Acrg	52.2	2	Mouse	128-129
			Rat	135-147
Bcl-2	72.1	1	Mouse	144-155
			Rat	144
D0Nds2	70.3	2	Mouse	95-104
			Rat	81-92
D10Rat20	90.1	1	Rat	114-131
D11Mgh5	100	1	Rat	293-304
D15Rat77	99.1	1	Mouse	255
			Rat	253-269
D16Rat81	91.0	2	Mouse	150-163
			Rat	166-197
D18Rat96	98.2	1	Mouse	266
			Rat	250-255
D19Mit2	100	1	Rat	198-247
D20Rat46	99.1	1	Rat	154
D2Rat234	98.2	1	Rat	120-125
D5Rat83	100	1	Rat	187-208
D7Rat13	94.6	1	Mouse	181-189
			Rat	183-191

DXRat2	100	2	Mouse	179
			Rat	179-193
Gfap	95.5	2	Mouse	298-323
			Rat	296-300
Hsp68	100	2	Mouse	120-126
Il-1b	100	2	Mouse	281-285
Il-5	100	2	Mouse	281-285
Myc	62.2	2	Mouse	111-128
			Rat	124-126
Myla	66.7	2	Mouse	224-235
Qa-4	91.0	2	Mouse	211-240
			Rat	246-249
SRYf-Peakall	100	2	Mouse	217
			Rat	227-230
Tnfa	73.9	1	Mouse	110-119
			Rat	129-133

Table 3.3: Number of samples processed through microsatellite analysis from collected hair samples in relation to the number of loci that were successfully amplified in those samples, in three categories of approximately estimated number of hairs per sample.

Number of hairs per sample	**Number of loci**										**Total**
	0	1	2	3	4	5	6	7	8	9	
<5 hairs	20	3	5	7	4	3	0	1	0	0	43
5-15	16	5	8	9	5	6	2	0	0	3	54
>15 hairs	1	2	1	0	0	6	2	1	0	1	14
Total	37	10	14	16	9	15	4	2	0	4	111

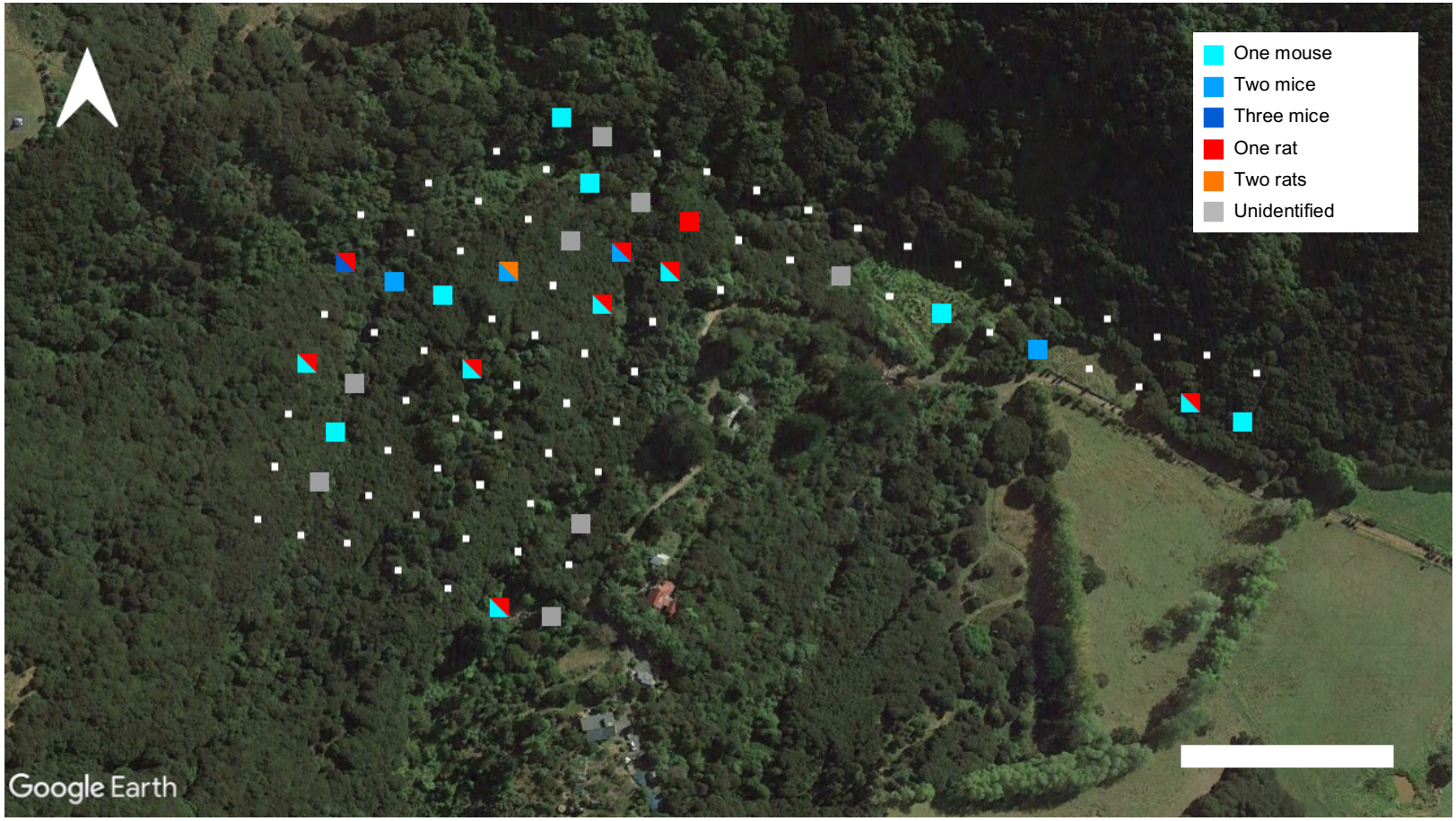

Figure 3.2: Detection of rats and mice at the hair-snag tube grid at Leith Walk, Otago Peninsula, using genetic analysis techniques. The colours of the squares differ based on whether a rat or mouse was detected and whether multiple individuals were identified at that tube. Squares with two colours represent that both rats and mice were identified at the tube from either one sample or multiple samples collected on different days. Grey squares show tubes from which samples were analysed but could not be successfully genotyped. Scale bar shows 100 m.

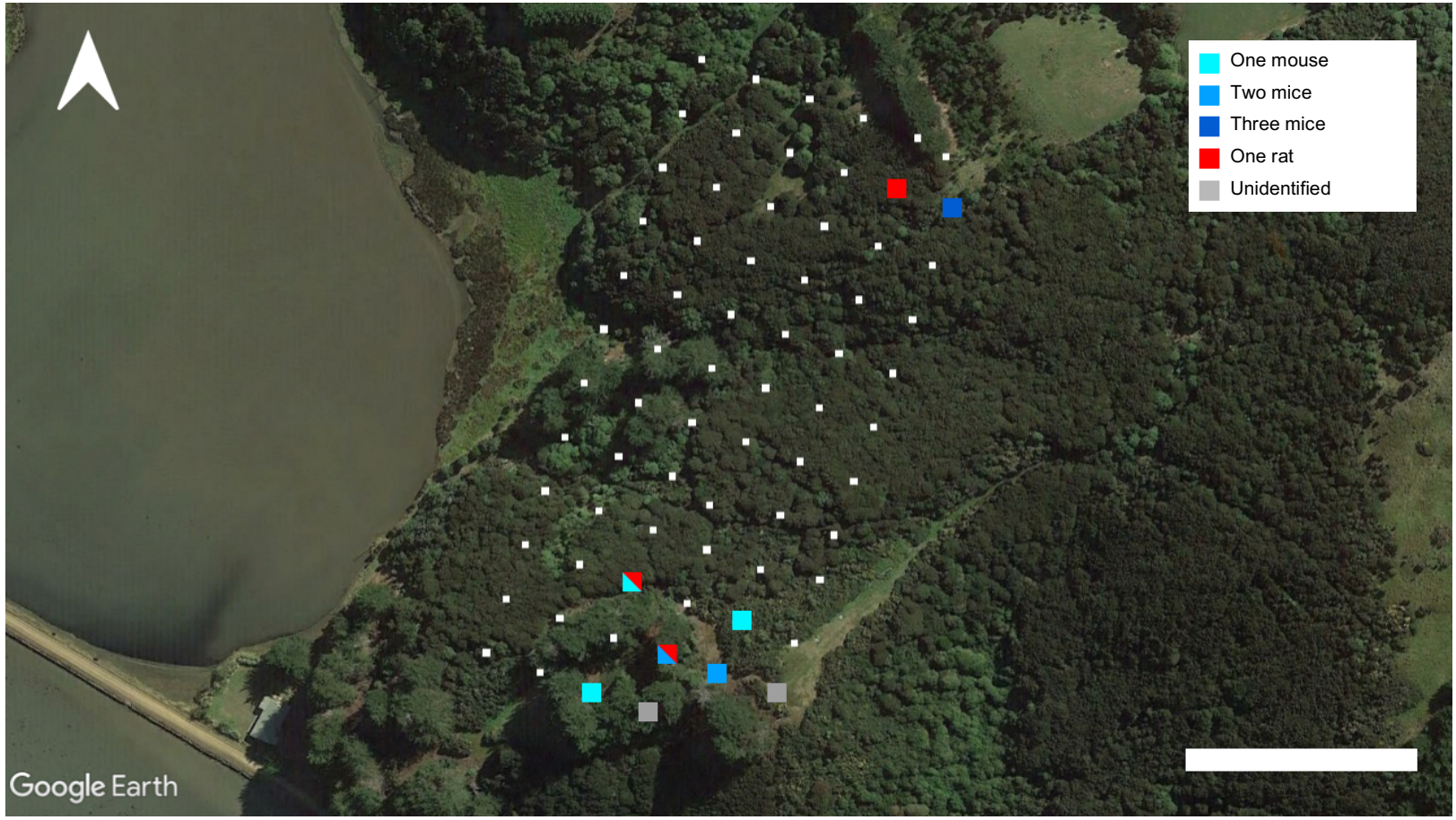

Figure 3.3: Detection of rats and mice at the hair-snag tube grid near Hooper's Inlet, Otago Peninsula, using genetic analysis techniques. The colours of the squares differ based on whether a rat or mouse was detected and whether multiple individuals were identified at that tube. Squares with two colours represent that both rats and mice were identified at the tube from either one sample or multiple samples collected on different days. Grey squares show tubes from which samples were analysed but could not be successfully genotyped. Scale bar shows 100 m.

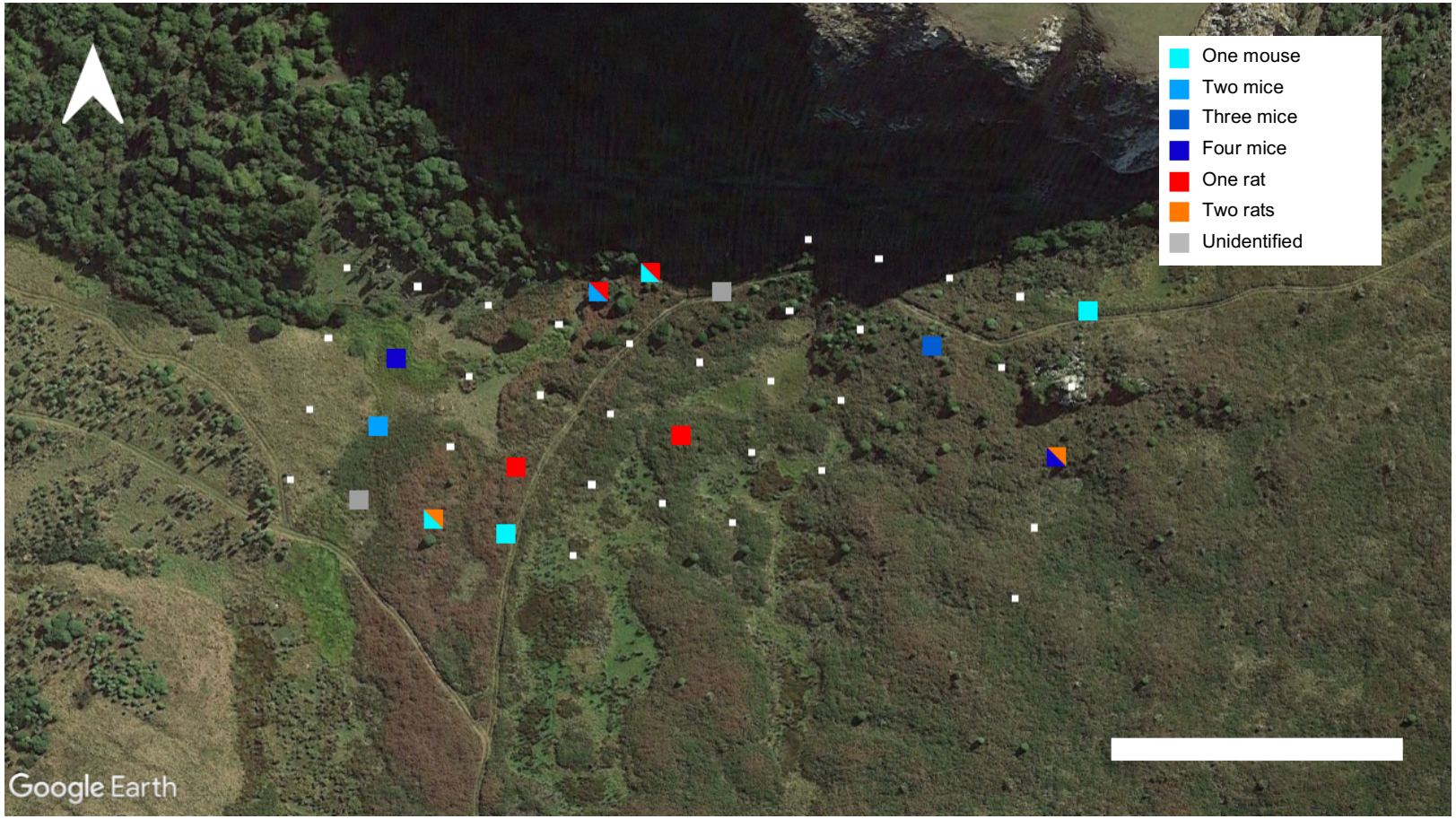

Figure 3.4: Detection of rats and mice at the hair-snag tube grid at Okia, Otago Peninsula, using genetic analysis techniques. The colours of the squares differ based on whether a rat or mouse was detected and whether multiple individuals were identified at that tube. Squares with two colours represent that both rats and mice were identified at the tube from either one sample or multiple samples collected on different days. Grey squares show tubes from which samples were analysed but could not be successfully genotyped. Scale bar shows 100 m.

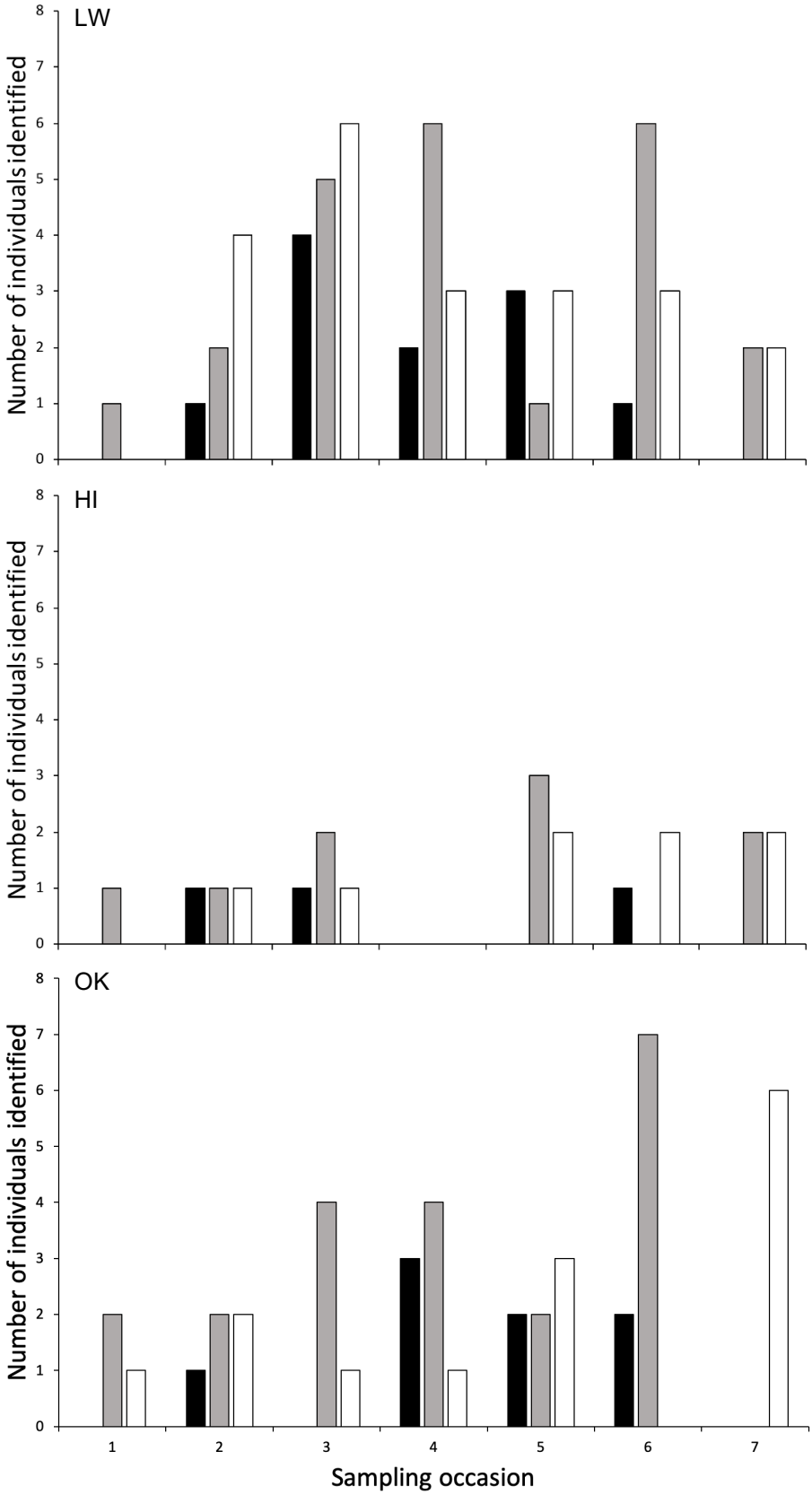

Figure 3.5: Number of individuals identified through microsatellite genotyping from samples collected over seven days at three sites on the Otago Peninsula (LW: Leith Walk, HI: Hooper's Inlet; OK: Okia). Black bars represent rats (n=22), grey bars represent mice (n=53), and white bars signify samples from which not enough information was produced to allow identification (n=43).

3.1.2.2.1 CTAB and samples without DTT

Of the six samples that were done without DTT, two had no loci amplified, one had three loci amplified (all three had few hairs in the sample), and three had five loci amplified (one had few hairs, the other two had a large number of hairs). All 12 samples where the CTAB technique was used failed to have any loci successfully amplify.

3.1.3 Rat density estimation

As a result of poor genotyping success, recaptures could not be distinguished and the planned SECR analysis could not be conducted. As a result, only a simple density estimate (minimum number alive per hectare) could be calculated using the grid area plus a boundary strip of half of the average of two estimated mean home-lengths of 142 m from Hickson (1986) and 144-184 m from Wilson (2007a). The total area plus boundary strip (effective trap area, ETA) for each site was calculated at: 10.7 ha at Hooper's Inlet; 14.9 ha at Leith Walk; and 8.93 ha at Okia. The ETA at Hooper's Inlet was reduced due to encroachment onto the inlet and at Okia due to a steep cliff near the grid. From this simplistic calculation, Okia was found to have the highest rat density at 0.90 rats ha^{-1}, followed by Leith Walk with 0.74 ha^{-1}. Hooper's Inlet had the lowest density of rats at an estimated 0.28 ha^{-1}.

3.1.4 Habitat selection analysis

Seventy hair-tube points across the three grids were revisited and vegetation surveys conducted. Data was collected for six variables: percentage groundcover of vegetation below 30 cm, number of vegetation tiers with vegetation cover over 20%, distance to the coast, maximum canopy height, distance to freshwater, presence or absence of large-fruited or -seeded plants. Site was also included as the seventh variable in the glm model. The seven selected variables were analysed through an exploration of the data, and the

variable ‘maximum canopy height’ was removed due to evidence of indication of collinearity with the variable ‘number of vegetation tiers’ as deduced from early analytical correlation plots. “Number of tiers” was kept over “canopy height” due to the greater importance of the former presented throughout the exploratory data analysis. Originally interactions were also kept in the model, though only one interaction was significant. Including any interaction in the model greatly inflated the variance terms, so no interactions were included. The final, full model was chosen with rat presence as the response variable, and number of tiers, distance to coast, distance to freshwater, percentage groundcover, presence/absence of large fruited/seeded plant species, and site were chosen as the predictor variables. The McFadden’s pseudo-R^2 value for the full model was low at 0.168. Only two predictors showed a statistically significant p-value: “percentage groundcover” (ANOVA; p=0.047) and “number of vegetation tiers” (ANOVA; p=0.009), indicating a significant relationship between each of these two predictors and the probability of rat presence. These two variables were then incorporated into a reduced model to simplify the model as, while adding more variables increases the R^2 value, this further complicates the model and the same two predictors remain as the only significant variables.

This reduced model contained only “percentage groundcover” and “number of vegetation tiers” as predictor variables of the response variable “rat presence”. The reduced model explains only a slightly smaller amount of the variation in the data than the full model did, with a relatively low McFadden’s pseudo-R^2 value of 0.115. The p-values for both variables remained significant for both percentage groundcover (ANOVA; p=0.006) and number of vegetation tiers (ANOVA; p=0.020). The assumption that the mean and variance of a generalised linear model (glm) are equal was met as the dispersion of the model is not far from 1 at 1.094.

3.1.4.1 Percentage ground cover

There is evidence that the percentage of ground cover is a significant predictor of the probability of rat presence (GLM; p=0.006). The fitted reduced model shows a weak

positive relationship between percentage groundcover and probability of rat presence (Figure 3.6).

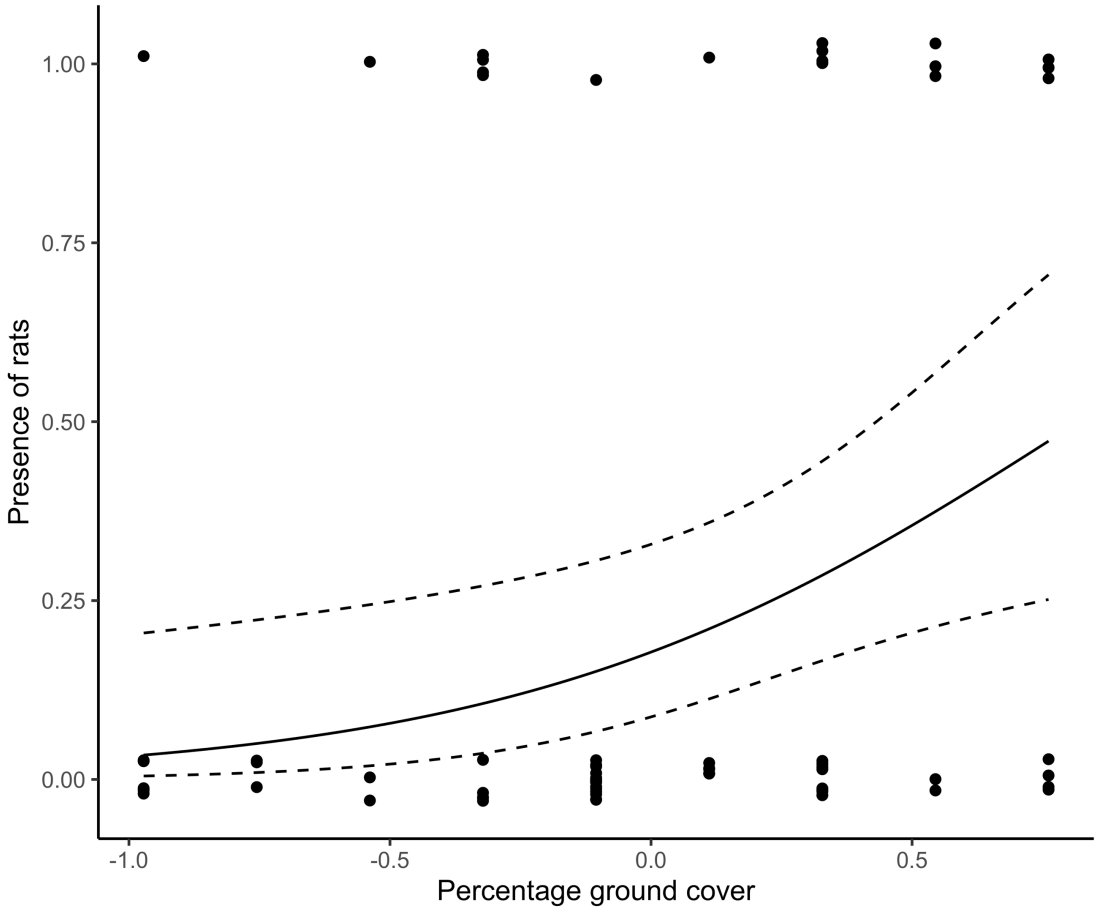

Figure 3.6: Probability of rat presence in relationship to percentage of ground cover predicted by a fitted model and plotted together with the observed data (centred and scaled; circular points). A jitter has been applied to the observed data for clarity. Dashed lines are the 95% confidence intervals.

3.1.4.2 Number of vegetation tiers

There is evidence that the number of vegetation tiers with vegetation cover of 20% or more is a significant predictor of the probability of rat presence (GLM; p=0.020). A positive relationship between number of tiers and probability of rat presence is shown in the fitted reduced model, though the large 95% confidence intervals are due to the small sample size (Figure 3.7).

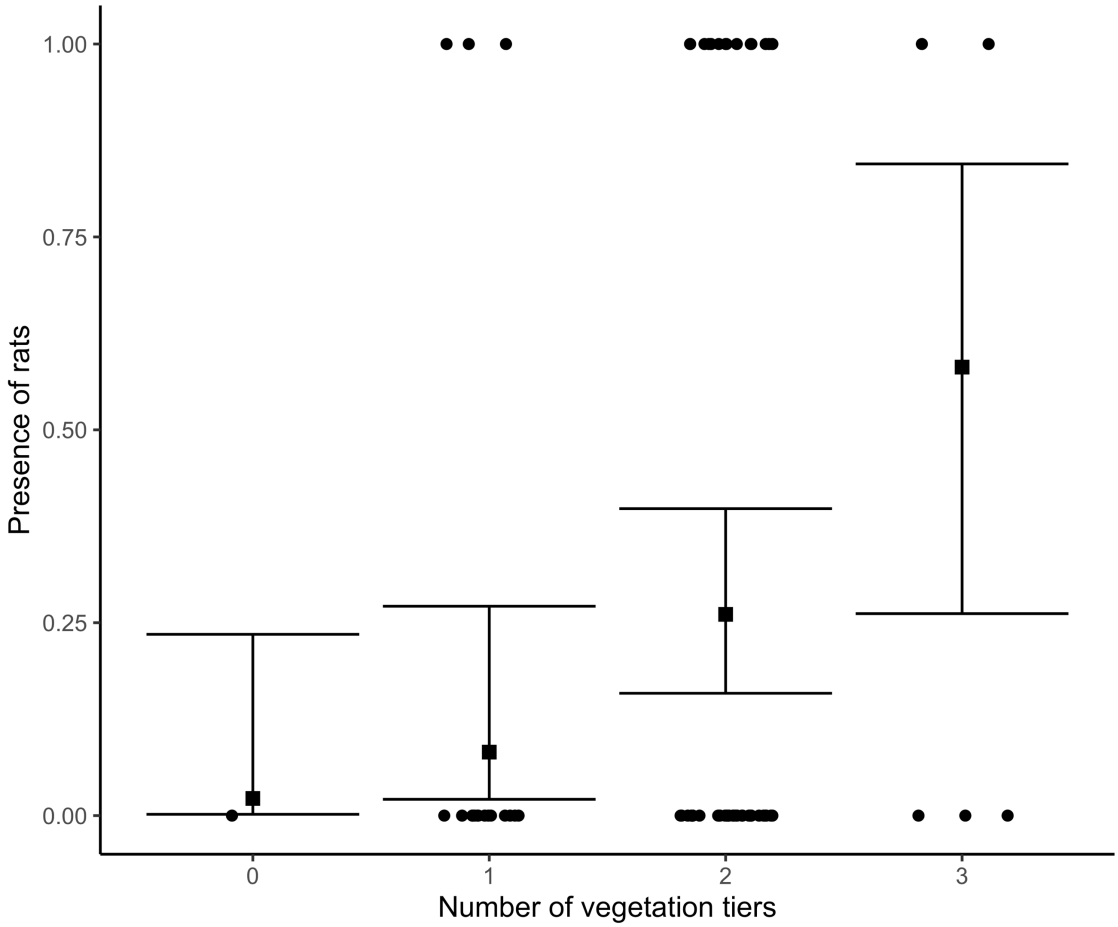

Figure 3.7: Probability of rat presence in relationship to number of vegetation tiers (each tier counted with 20% or more of vegetation cover) predicted by a fitted model (square points) and plotted together with the observed data (circular points). A jitter has been applied to the observed data for clarity. Error bars either side of the plotted model estimate points represent the 95% confidence intervals.

3.2 Tracking tunnels

After the first of five nights no rat prints were detected, whereas mice were detected at tracking rates of 0.9, 0.7, and 0.8 at Leith Walk, Hooper's Inlet, and Okia, respectively. After another four nights mice were tracked at all three sites. Only two ink cards detected rat prints after the additional four nights. Wētā and other invertebrate prints were also detected, though only at Hooper's Inlet (Table 3.4). Hooper's Inlet and Okia both had rat tracking rates of 0.1, while at Leith Walk no rats were ever detected (Figure 9). The location of the tracking tunnel which detected a rat was not consistent with the locations of rats identified on the hair-snag tube grid at Okia; however, at Hooper's Inlet the tunnel with the rat detection was in an area near where a rat had been identified from a hair-snag tube (Figure 3.2; Figure 3.3; Figure 3.4; Figure 3.8).

A Pearson's product-moment correlation test was conducted to assess any correlation between the detection rates of rats from each tracking tunnel line and the number of rats detected at each hair-snag tube grid (estimate= -0.786), together with a t-test, which was found not to be significant (p=0.425).

Table 3.4: Tracking tunnel rates for rat, mouse, and invertebrate detection from three lines of ten tracking tunnels (n=30), one line at each of three sites on the Otago Peninsula; Leith Walk (LW), Hooper's Inlet (HI), and Okia (OK). Ink tracking cards were collected after the first night and new cards placed, which were collected after a further 4 nights.

Tracks	**Nights out**	**Tracking rates (positive ID per trap)**		
		LW	HI	OK
Rat	1	0	0	0
	4	0	0.1	0.1
Mouse	1	0.9	0.7	0.8
	4	1	1	1
Invertebrate	1	0	0.4	0
	4	0	0.2	0

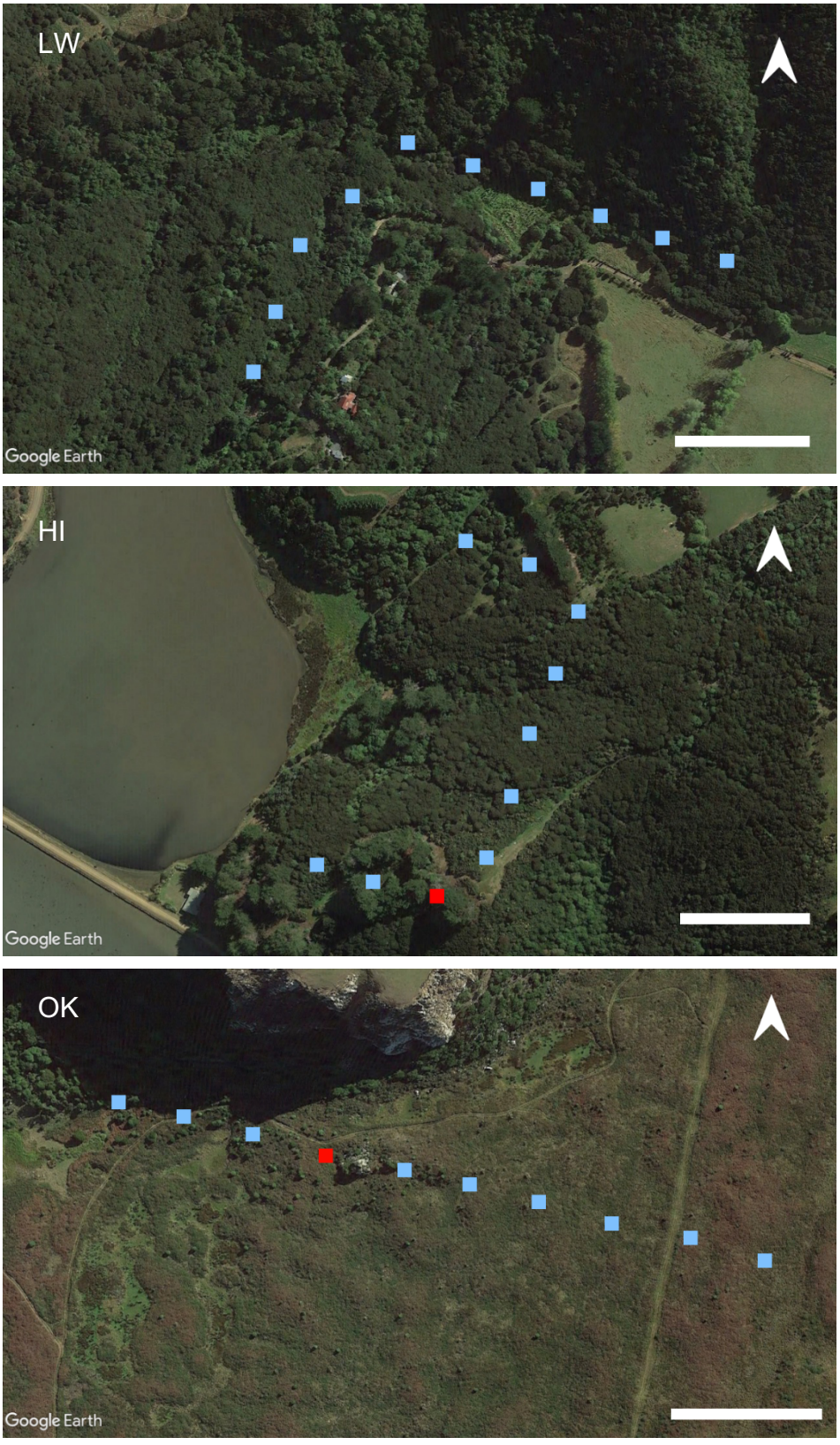

Figure 3.8: Detection of rats on the tracking tunnel lines at each of the three study sites on the Otago Peninsula (LW: Leith Walk; HI: Hooper's Inlet; OK: Okia). Rats detected are presented as red squares (n=2), blue squares denote no rats detected at that particular tunnel. All tunnels detected mice. Scale bars at 100 m.

3.3 Bird counts

In total, 24 bird species were counted during the 5MBCs at the three sites, three species (brown creeper *Mohoua novaeseelandiae*; kereru *Hemiphaga novaeseelandiae*; rifleman *Acanthisitta chloris*) only at Leith Walk, and six (black-backed gull *Larus dominicanus*; mallard *Anas platyrhynchos*; paradise shelduck *Tadorna variegata*; pukeko *Porphyrio melanotus*; skylark *Alauda arvensis*; welcome swallow *Hirundo neoxena*) were counted only at Okia (Table 3.5). A total of 148 observations were recorded, the most at Hooper's Inlet with 43% of the total observations.

The most abundant species at Leith Walk was bellbird (*Anthornis melanura*), followed by chaffinch (*Fringilla coelebs*) and dunnock (*Prunella modularis*). The goldfinch (*Carduelis carduelis*) was the most abundant species at both Hooper's Inlet and Okia, followed by chaffinch and dunnock at Hooper's Inlet, and by dunnock and paradise shelduck at Okia (Figure 3.9). The bellbird was the most abundant species overall, despite being recorded only at two sites (Leith Walk and Hooper's Inlet; Table 3.5).

The counts of ten of the bird species detected could be used in DISTANCE analysis. These species had enough detections to allow DISTANCE to compute density estimates, percentage coefficient of variance, and upper and lower confidence limits. Species with a low number of detections, as well as some species with large number of detections (e.g. bellbird), had high variance and hence large confidence intervals. The densities calculated from the 5MBCs were all low. Fantails had the highest density at Leith Walk (1.043 ha^{-1}, LCL=0.587, UCL=1.851), whereas silvereyes had the highest estimated densities at both Hooper's Inlet (0.969 ha^{-1}, LCL=0.075, UCL=12.53) and Okia (0.761 ha^{-1}, LCL=0.177, UCL=3.273), although the confidence limits and percentage coefficient of variance are large for this species, indicating this estimate lacks precision. The lowest estimated densities were for blackbirds at Leith Walk (0.175 ha^{-1}, LCL=0.128, UCL=0.239) and Okia (0.163 ha^{-1}, LCL=0.113, UCL=0.236) (Figure 3.10; Table 3.6). Despite being the most abundant species recorded, the bellbird data could not be used in the DISTANCE analysis, as the calculated percentage coefficient of variance was too

large, yielding an imprecise density estimate since the confidence intervals were wide. Species with a low number of counts (<14) could also not be used.

Table 3.5: Number of birds counted at the three stations visited during the seven bird counting occasions at each of the three sites on the Otago Peninsula, together with the total count of each species per site. Birds that were counted but could not be identified are presented as 'Unidentified'.

Species	**Point count locations**											
	Leith Walk				**Hooper's Inlet**				**Okia**			
	1	**2**	**3**	**Total**	**1**	**2**	**3**	**Total**	**1**	**2**	**3**	**Total**
Bellbird	12	39	28	79	13	3	15	31	0	0	0	0
Black-backed gull	0	0	0	0	0	0	0	0	1	1	0	2
Blackbird	8	6	7	21	6	13	12	31	2	13	4	19
Brown creeper	0	0	1	1	0	0	0	0	0	0	0	0
Chaffinch	18	3	9	30	13	12	10	35	0	4	0	4
Dunnock	5	13	8	26	7	16	10	33	15	11	2	28
Fantail	5	8	2	15	1	1	1	3	0	0	0	0
Goldfinch	5	0	2	7	33	10	20	63	8	7	17	32
Greenfinch	1	0	0	1	0	0	1	1	0	0	0	0
Grey warbler	3	1	9	13	4	8	0	12	0	1	4	5
Harrier	0	0	1	1	0	1	0	1	0	1	0	1

Kereru	1	2	0	3	0	0	0	0	0	0	0	0
Mallard	0	0	0	0	0	0	0	0	2	0	0	2
Paradise shelduck	0	0	0	0	0	0	0	0	6	9	8	23
Pukeko	0	0	0	0	0	0	0	0	0	3	0	3
Redpoll	0	0	0	0	0	1	0	1	1	3	2	6
Rifleman	4	0	5	9	0	0	0	0	0	0	0	0
Silvereye	3	1	6	10	0	4	1	5	0	0	3	3
Skylark	0	0	0	0	0	0	0	0	4	2	3	9
Song thrush	6	9	10	25	5	4	11	20	2	9	0	11
Starling	0	0	0	0	0	0	2	2	3	0	0	3
Tui	14	4	2	20	1	1	9	11	0	0	0	0
Welcome swallow	0	0	0	0	0	0	0	0	2	0	0	2
Yellowhammer	1	0	0	1	0	0	2	2	3	2	6	11
Unidentified	18	8	17	43	30	14	25	69	8	6	31	45

Table 3.6: Ten bird species analysed in DISTANCE, with a density estimate (ha^{-1}), percentage coefficient of variance (% CV), and upper and lower confidence limits generated for each species at each site. The amount of truncation is based on what produced the most parsimonious model for each species. The most parsimonious model for each species was with a half-normal key function and a cosine series expansion.

Species	**Truncation**	**Number of observations before/after fitted**	**Site**	**Density estimate (individuals ha^{-1})**	**% CV**	**Confidence limit**	
						Lower	**Upper**
Blackbird	5% of largest distances	69/67	LW	0.175	15.73	0.128	0.239
			HI	0.251	18.50	0.174	0.362
			OK	0.163	18.55	0.113	0.236
Chaffinch	100 m	69/69	LW	0.338	26.68	0.200	0.570
			HI	0.311	26.65	0.184	0.524
			OK	0.675	26.41	0.402	1.134

Dunnock	93 m	87/81	LW	0.278	18.58	0.193	0.401
			HI	0.360	18.85	0.248	0.522
			OK	0.342	18.95	0.236	0.497
Goldfinch	110 m	94/94	LW	0.321	38.05	0.134	0.768
			HI	0.481	12.15	0.379	0.612
			OK	0.300	11.97	0.237	0.380
Grey warbler	5% of	30/30	LW	0.576	59.99	0.183	1.812
	largest		HI	0.620	47.02	0.249	1.542
	distances		OK	0.517	45.58	0.213	1.257
Song thrush	5% of	56/54	LW	0.463	42.63	0.204	1.052
	largest		HI	0.445	42.78	0.196	1.013
	distances		OK	0.579	47.06	0.235	1.423
Silvereye	100 m	16/16	LW	0.508	39.37	0.219	1.774
			HI	0.969	58.20	0.075	12.53
			OK	0.761	41.97	0.177	3.273
Tui	100 m	30/30	LW	0.256	27.44	0.148	0.443

			HI	0.387	62.94	0.084	1.788
Fantail	90 m	18/18	LW	1.043	27.59	0.587	1.851
			HI	0.695	27.53	0.392	1.233
Paradise shelduck	5% of largest distances	14/13	OK	0.313	36.65	0.148	0.661

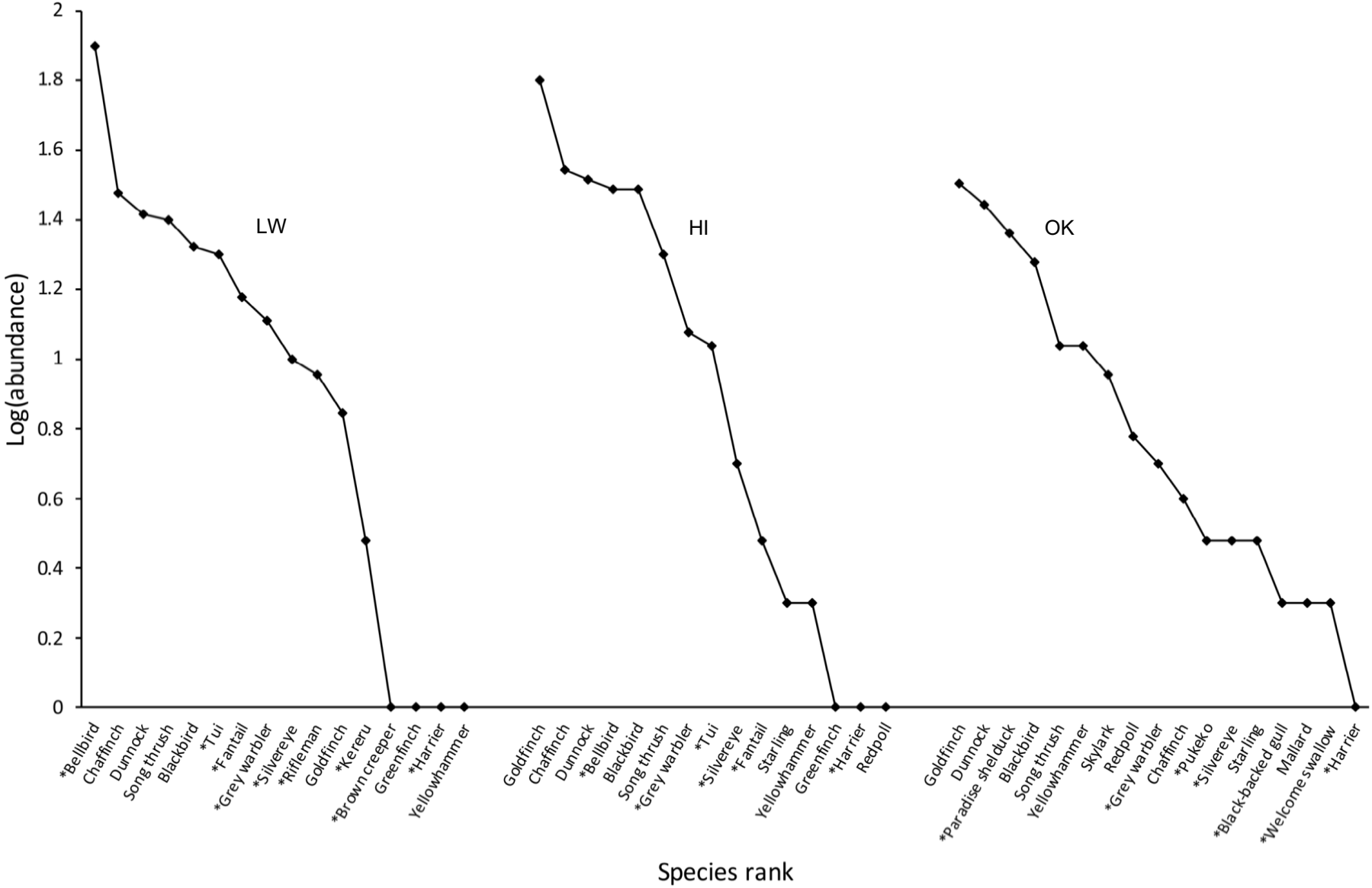

Figure 3.9: Rank abundance plot of log transformed abundance estimates for bird species counted at the three sites on the Otago Peninsula (LW: Leith Walk; HI: Hooper's Inlet; OK: Okia). Birds that were counted but could not be identified are presented as 'Unidentified'. Asterisks (*) denote native species.

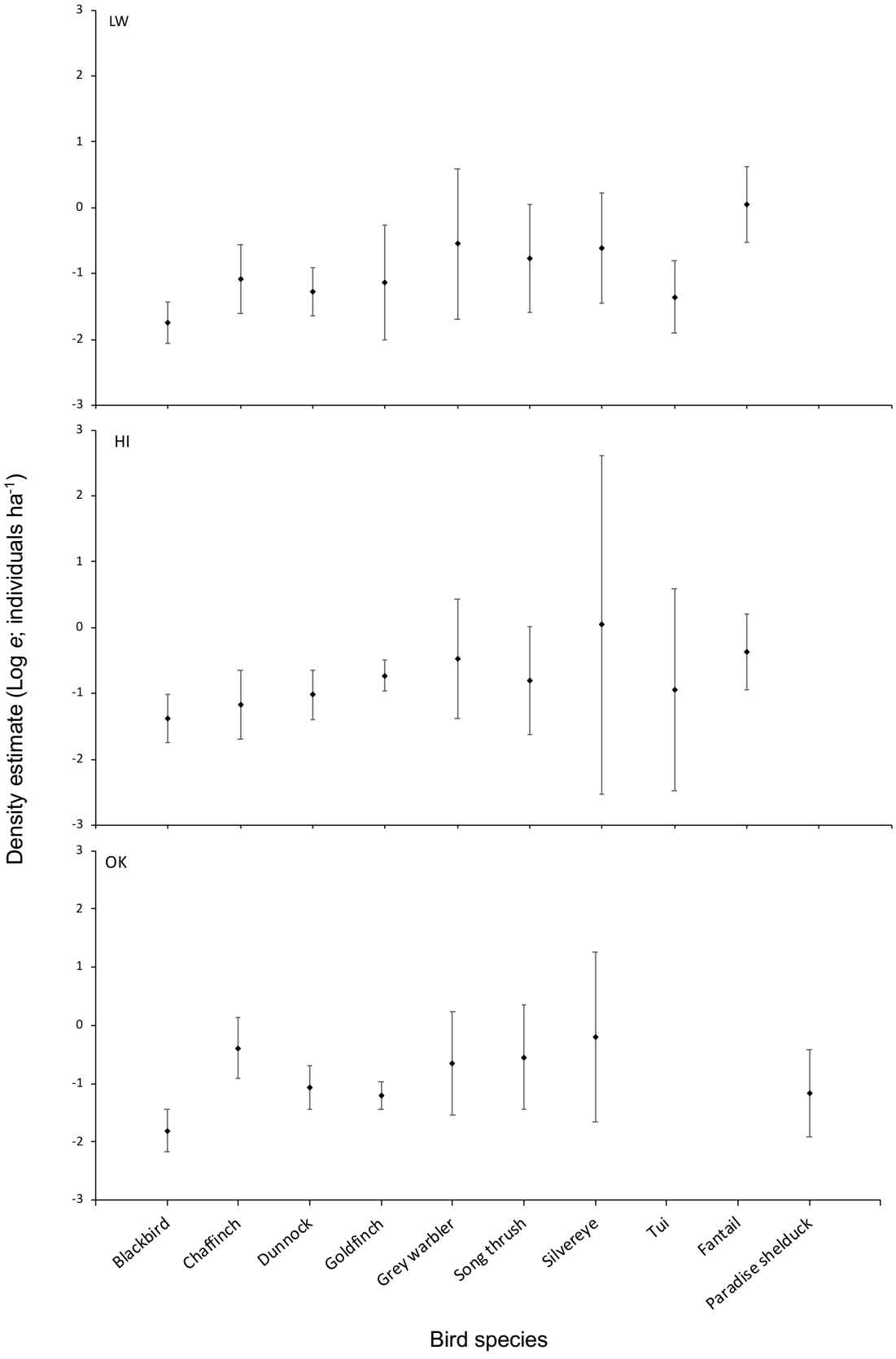

Figure 3.10: Log base *e* transformed density estimates as number of individuals per hectare for the ten most counted bird species separated by site (LW: Leith Walk; HI: Hooper's Inlet; OK: Okia). The gaps for some birds represent the lack of observations of those species at a given site. Error bars are 95% confidence intervals.

4 DISCUSSION

4.1 Rat density estimation

Due to the lack of recapture data and the inability to distinguish individual rats, the spatially explicit capture-recapture model could not be used to achieve an accurate estimate of rat population density. However, a simple density estimate of 0.74, 0.28, and 0.90 rats ha^{-1} was calculated for Leith Walk, Hooper's Inlet, and Okia, respectively, using the number of rats identified (minimum number alive) per the effective trap area of the hair-snag tube grid, which included a boundary strip added around the grid area (Dice 1938). The estimates themselves are very much at the lower end of reported density estimates for rats in mixed forests and scrub in New Zealand (1-12 ha-1; Blackwell et al. 2002; Wilson et al. 2007a). Estimating density using MNA is less than ideal as no confidence intervals can be calculated. Additionally, MNA will always produce lower density estimates than SECR density calculations as it does not account for undetected individuals (Efford et al. 2009; van Heezik, *pers. comm*.).

A study estimating rat population density in urban and peri-urban forest fragments around Dunedin found a similarly low density (0.26 rats ha^{-1}), which was hypothesised to be due to the presence of domestic cats in the area or intermittent pest control (McCulloch 2009). It is unknown if the houses near the study sites owned cats or not, but at Okia feral cats were spotted during sampling occasions. This could be one reason for the low densities of rats reported in this study, though cat density is unlikely to be as high in these rural sites compared to in urban areas and therefore has less of an impact on rat populations. Along the same lines, there may be a high density of other rat predators (e.g. ferrets, stoats) that are suppressing rat populations (King 2005a). Additionally, while infrequent trapping had taken place in areas near the Leith Walk site, the last rat to have been killed had been from well over a year earlier; therefore, this trapping effort is unlikely to have influenced the density of rats at this site (S. Cutler, *pers. comm.*). No trapping of rats had been untaken at Hooper's Inlet or Okia (D. McFarlane, *pers. comm*.; M. Parker, *pers. comm*.).

The boundary strip was incorporated into the density calculation as a means of minimising edge-effect bias (Stenseth and Hansson 1979), and was calculated using the average home range lengths for ship rats from Hickson et al. (1986) and Wilson et al. (2007a), as the home range length of rats could not be calculated from the data in this study. As home range size will affect the width of the boundary strip, which will in turn influence the density estimate, the papers were chosen based on time of year when sampling was conducted and forest type in order to be as similar to sampling conditions in this study as possible, with Hickson et al. (1986) being one of the few studies conducted in southern New Zealand and not in beech forest. Using the home-range size from other studies introduces potential inaccuracy since home-ranges of rats are known to be extremely variable between populations, over time and between habitats (Wilson et al. 2007a). This variability means that assuming a constant boundary strip will lead to unreliable density estimates (Efford et al. 2004). The home ranges of male rats tend to be larger than those of females (possibly due to reproductive behaviour with males searching larger areas for mates; Dowding and Murphy 1994) and may even double during the breeding season (Hickson et al. 1986; Dowding and Murphy 1994; Whisson et al. 2007).

Home-range sizes can also depend on the energy requirements of the individual, which are determined by food availability (Harestad and Bunnell 1979). It can therefore be expected that home range size may contract in more productive habitats, due to there being a larger amount of resources available in a smaller area (McNab 1963). Home-ranges at the sites sampled in this study would likely differ between sites, with home-ranges perhaps smallest at Leith Walk, a site with a possibly greater resource availability than at Hooper's Inlet, which was predominantly kānuka. Indeed, King et al. (1996) reported fewer ship rats caught in early successional habitats compared to complex native forests. As the kānuka forest at Hooper's Inlet is successional growth on land previously grazed by stock, the trend of lower density of rats here may be similar as described by King et al. (1996).

The likely home range for rats at Okia on the other hand is harder to predict/estimate as there is little information in the literature regarding rat habitat use in predominantly bracken growth on hollow dunes. Nevertheless, approximately 36% of the rats identified came from Okia, suggesting this area is suitable for rats. In a study assessing the tracking rates of tracking tunnel lines at sites on the Otago Peninsula, the line at Leith Walk recorded some of the highest tracking rates of the study (often exceeding 20%), while the line at Okia (although in a grazed area different to where the grid was located in this study) presented lower tracking rates (Wilson 2017). Okia offers a theoretically ideal habitat for rats as there is a lot of dense, low growth and it is populated by southern grass skinks (*Oligosoma* aff. *polychroma* Clade 5) and cryptic skinks (*Oligosoma inconspicuum*), which are likely prey items of rats (King 2005a). Invertebrate species such as wētā may also face predation by rats in the area (Ruscoe et al. 2013). As there are no studies on rat population dynamics in predominantly bracken habitats, the same averaged estimate used for the other two sites was applied to the home-range calculation here as well. This is less than ideal as home range sizes are known to differ between habitats (Innes 2005a; Innes 2005b), and the resulting boundary strip calculated from these home range sizes will greatly affect the density estimates by either over- or under-estimating density (Parmenter et al. 2003). As such, the use of predominantly bracken habitats by rat populations leaves potential for future study.

4.2 Rat habitat selection

More rats were identified at the grid at Leith Walk (50% of samples identified as containing rat hair were collected from tubes here) than either of the other two grids. Whisson et al. (2007) reported that active rats were most frequently located in dense blackberry thickets, and only 6% of locations with active rats were not associated with any kind of vine cover. Similar results were found in this study, as vegetation complexity (measured as number of tiers above 0.3 m with over 20% vegetation cover present in a 5 m radius at a hair-snag tube) and percentage groundcover (vegetation cover ≤0.3 m) were both found to be positively associated with the probability of a rat being present at the hair-snag tube location. This suggests rats prefer habitats with greater vegetation complexity and denser groundcover, which may allow for increased mobility within their

home-range as they are able to climb throughout complex vegetation and be protected from airborne predators in areas with dense groundcover (Innes 2005b; Innes et al. 2010). The importance of groundcover in predicting rat presence could explain the lower density of rats estimated at Hooper's Inlet, which comprises mainly regenerating kānuka forest with only a few mid-canopy height plants growing, such as māhoe and ongaonga. Indeed, some of the highest density estimates of rats on mainland New Zealand, outside of masting beech forests, have been reported in a mixed broadleaf/hardwood forest remnant (Brown et al. 1996), and a kohekohe (*Dysoxylum spectabile*)/tawa (*Beilschmiedia tawa*) dominated forest (Hooker and Innes 1995), forest types that provide relatively high vegetation complexity and are ideal for arboreal ship rats (Harper 2002).

Complex and denser vegetation growth may be favoured by rats due to the ability to escape predators more effectively (Innes 2005b; Innes et al. 2010). The presence of more complex vegetation could also imply that home-range lengths are shorter than in habitats with low growing vegetation, as in most studies this is measured on a horizontal, two dimensional plane, whereas the home-range is likely to be three dimensional and include vertical regions accessible by climbing trees as well (Innes 2005b). As ship rats are more arboreal than Norway rats (Innes 2005a; Innes 2005b), the significant relationship of complex vegetation to probability of rat presence suggests the rats identified could be ship rats. Conversely, the significant relationship of percentage groundcover to rat presence might suggest presence of Norway rats, however ship rats are not exclusively arboreal, and in fact frequently use the ground (Dowding and Murphy 1994; Hooker and Innes 1995). Unfortunately, the two loci used in this study (D11Mgh5 and D19Mit2) that could have distinguished hair collected between the two species failed to successfully amplify any alleles, so which rat species was sampled remains unknown, though the assumption for the density estimate was made that it was most likely ship rats based on anecdotal evidence from Peninsula home-owners.

Norway rats are often associated with urban areas and wetland habitats (Innes 2005b). Okia, with its sporadic freshwater ponds, may be close to the type of habitat commonly associated with Norway rats. However, the lack of any significant relationship found between distance from freshwater and probability of rat presence again suggests that the

rats identified were likely ship rats. In addition, as no significant relationship between site and rat presence was found, it is unlikely that the inclusion of two forested sites could have confounded these results; one would expect, for example, that if only Norway rats were sampled at Okia, and only ship rats at Leith Walk and Hooper's Inlet, that there would be a significant difference in the probability of rat presence between sites due to the differing habitats.

Distance to coast had been hypothesised as being an important factor in explaining the presence of rats, in that rats would be more likely to choose habitat closer to the coast (D. Wilson, *pers. comm.*). This behaviour may be found in Norway rats rather than ship rats, as they are more often associated with habitats near water than ship rats (Innes 2005a). However, there was no significant relationship between the distance from the coastline and the probability of a rat being present in the area. A reason for this nonsignificant relationship could be the fact that at Leith Walk and Hooper's Inlet a road separates the sites from the shore, and although the area does not have a high traffic volume, it may be enough to prevent an important association of rats with the shoreline. Dickman and Doncaster (1987) found a reluctance of small mammals in an urban environment to cross roads, and a similar effect may perhaps be observed in this study at those sites where a road separates the vegetation fragment from the shoreline.

Similarly, the presence of large fruited and seeded trees was thought to be a potentially important indicator of the presence of rats; however, no significant relationship was found. Ship rats eat a large variety of native fruits and seeds, such as those from tree fuchsia (*Fuchsia excorticata*), rimu (*Dacrydium cupressinum*), and totara (*Podocarpus totara*), as well as the fruits and seeds of introduced species such as hawthorn (*Crataegus monogyna*) and bramble (*Rubus fruticosus*; Wilson et al. 2003; D. Wilson, *pers. comm.*), all of which were present in at least one of the sites. The species considered in the habitat selection analysis as large fruited and seeded were species that would have had fruit or seeds towards the end of Autumn, when the hair-snag tube grids were set. The non-significant result suggests the presence of large fruited and seeded plant species is not as important in explaining rat presence as previously believed, though a more complex

interaction may be occurring. A larger sample size would ensure more comprehensive results.

Maximum canopy height and number of tiers were found to be correlated, which could be explained by higher maximum canopy heights being more likely to have a higher number of tiers, as the shorter the maximum canopy height, the fewer tiers can be attributed to the site (i.e. a tube location with a maximum canopy height of 2 m could only have a maximum of one tier attributed to it, whereas with a maximum canopy height of 15 m a theoretical maximum of three tiers). Therefore, maximum canopy height was removed from the model and could not be used to explain any variation in the data.

The reduced model, containing only the two significant variables (number of tiers and percentage groundcover) and no interactions, explained only a relatively small amount of the variation in the data (pseudo R^2=0.168). It is therefore likely that there are variables, or complex interactions of variables that were not considered that may be as, or even more, important in explaining the probability of rat presence.

4.3 Abundance and density of birds

At the same three sites where rat population density was estimated, bird species' densities and community composition were profiled in order to provide baseline values against which the impacts of various predator removal programmes on bird populations on the Otago Peninsula could be evaluated in the future.

Many of the most abundant birds counted in this study were introduced birds, particularly chaffinch, goldfinch, dunnock, blackbird, and song thrush; however, native birds such as bellbird, tui, and grey warbler were also well represented. The introduced bird species at high densities are species that have adapted to New Zealand's habitats and are even quite abundant in urban areas (Chace and Walsh 2006; van Heezik et al. 2008). Macleod et al.

(2009) reported that the density estimates in farmland of varying habitat types for 11 introduced European birds in New Zealand, including chaffinch, dunnock, song thrush, blackbird, goldfinch, and yellowhammer, were on average 22 times higher than in comparable habitat types in the UK. The mean densities in that study are in a similar range to those estimated in this study (Macleod et al. 2009). Other studies also reported much higher densities of introduced birds (e.g. yellowhammer; Macleod et al. 2005) in New Zealand compared to their native ranges. The success of these introduced birds in New Zealand may be due to enhanced niche opportunities compared to their native range, with possible higher quality resources available, a release resulting from lower predation pressure, environmental factors such as less extreme weather patterns, or a combination of some or all of these factors (Shea and Chesson 2002; Macleod et al. 2009). In an earlier study collating data from OPBG bird count transects, introduced birds were also found to be abundant, along with silvereyes, bellbirds, grey warblers, fantails, and tuis (Ewans 2017). The collated data show a general positive trend in relation to possum control in the abundance of many of the bird species counted; however, statistical significance was not tested therefore the data can only be used as a rough index (Ewans 2017). A study assessing the effects of predator eradication on the bird community at Zealandia, a fenced ecosanctuary in Wellington, found that of the native and introduced bird residents in the area, only tui had a dramatic increase in abundance, whereas other species, such as fantail, silvereye, and grey warbler, had a marked decline in counts 17 years after eradication and after the establishment of a diverse native bird community with reintroduced birds (Miskelly 2018). This indicates that the most commonly counted species at the three sites on the Otago Peninsula are unlikely to be greatly affected by predator control. However, species noted in low abundance in those areas, and that are perhaps more sensitive to predator presence, such as tomtits, may benefit greatly from predator eradication (Baber et al. 2009). In this way the avian community composition on the Otago Peninsula following predator eradication may change to favour a higher abundance of the currently less abundant native birds.

The Otago Peninsula is known to have an abundance of birdlife, particularly seabirds and waders (Ewans 2017). Ongoing efforts by volunteers of the OPBG have aimed at monitoring changes in bird relative abundance following the control of possums, and in future, other predator species (Otago Peninsula Biodiversity Group 2019). These efforts

consist of repeated slow walk transect bird counts at set transect routes in various areas and habitats along the Peninsula and have been regularly undertaken since 2012 (Ewans 2017). Unlike the bird counts conducted in this study, which were 5-minute bird counts and consisted of counts at stationary points ('stations'; following Hartley and Greene 2012), the OPBG is monitoring relative abundance of birds and relies on volunteers to conduct these counts. As such, discrepancies between volunteers can arise in terms of which bird species are correctly identified, as some volunteers may mis-identify a bellbird call as a tui, for example, despite training from the OPBG (U. Ellenberg, *pers. comm*.). Both 5MBC and the slow walk transect method are known as incomplete counts due to the fact that these methods make it hard to differentiate between a bird being absent and a bird being present but undetected (Ewans 2017). However, ideally, the OPBG volunteers should use distance sampling, which accounts for undetected individuals and allows for the calculation of confidence intervals and would therefore allow more robust analyses of trends to be made (Buckland et al. 2005). This method is recommended in combination with 5MBC at a number of permanent counting stations, instead of the slow walk method.

A few issues were encountered in using the 5MBC method. One issue was that bird song identification had to be learnt, an issue present in any kind of bird count. This proved particularly difficult to a newly trained observer when trying to distinguish bird songs by species, as some species (e.g. tui and bellbird, or blackbird and song thrush) have very similar calls and perhaps only seasoned bird counters might achieve accurate distinctions. Counting birds by hearing their calls is a fairly inaccurate method when applying distance sampling as the distance to the bird has to be estimated and depending on the habitat (forested Leith Walk vs. open low scrub Okia), differing weather conditions, or species, can lead to variations in distance estimates. For example, bellbird calls are clear and can be heard from a greater distance than those of grey warblers. Therefore, one might assume based on call volume that the two birds may be at a similar distance, whereas the bellbird is actually further away. This is perhaps part of the reason why bellbird density, despite their high rates of detection, could not be estimated accurately in DISTANCE software. Bellbirds counted were more often heard than seen and this may have resulted in the high percentage coefficient of variance that reduced the precision of the density estimate. A similar explanation may be applicable to the density estimates of song thrush, tui (at

Hooper's Inlet), silvereye (at Hooper's Inlet and Okia), and grey warbler. Interestingly, fantails were the 7th most detected bird species at Leith Walk, yet had the highest overall density estimate, with a comparatively low percentage coefficient of variance. Conversely, blackbirds were counted frequently but had low density estimates. In the case of fantails, the result may be due to their small size, which meant they were detected more often at distances nearer the counting point. With blackbirds the opposite may be true, as more calls were heard from further away. Additionally, blackbirds were heard more often than seen, as were fantails, so this could have an effect on the results.

Birds with lower densities estimated at Okia than at other sites included blackbirds, goldfinches, and grey warblers. Interestingly, no bird species were in their highest estimated density at Leith Walk apart from fantails. Whether this coincides with the higher rat densities at Okia and Leith Walk, or whether it is due to the different habitat types, is unknown, but opens up the possibility of further research regarding direct effects of rat population density on bird density at Okia. No fernbirds or New Zealand falcons were counted in this study, despite OPBG counts indicating the presence of these species (Ewans 2017).

4.4 Further research

4.4.1 Technical recommendations

4.4.1.1 Hair-snag tubes in estimating rat density

The hair-snag tube, comprising a PVC tube, rubber band, and glue, is a cost-effective method of collecting DNA from small mammals in the form of hair samples, which can be used in non-invasive genetic capture-recapture studies to provide estimates of a species' population density when coupled with especially designed data processing programmes, such as R-packages. Due to their light-weight construction and relatively small size, the tubes proved relatively easy to set out over a large area, with the assistance

of only a small number of volunteers, although translating the grid from the theoretical computer design into the field proved more difficult and grids had to be reshaped in order to conform to the landscape. In remote areas and at sites that are already difficult to traverse, such as the thick undergrowth of the forest in some parts of the Leith Walk site, the lightweight tube design can be valuable in keeping the study logistically feasible, and offers an advantage over more expensive methods and those that use larger DNA collection traps. In animal genetic population studies, the importance of using a cost-effective method for the collection of DNA becomes evident when the high cost of the genetic analysis that follows is considered. The use of hair-snag tubes for monitoring predator species populations in New Zealand is not widely used, however some studies (e.g. Gleeson et al. 2010; Pickerell et al. 2014) have successfully used this method within New Zealand.

Peanut butter was a good bait for the both the tubes and tracking tunnels, as the majority of the tubes had to be reset daily and tracking tunnel ink cards were all saturated with footprints by rodents (mainly mice). Peanut butter is a popular and widely used food bait for rodent traps due to its cost-effectiveness and particularly its easy accessibility (Takács et al. 2018). Whether it was the fault of the bait or low numbers of rats in the areas studied remains a question, as I did not have any devices that detected whether rats were attracted to the tubes but did not enter them (e.g. cameras). Another factor that may help explain the low number of rats detected could be trap-shyness, as rats are known to express new-object avoidance behaviour (neophobia; Cowan 1977; Innes 2005b; Amstrup et al. 2010) and may avoid baited traps if other food is abundant (Leung and Clark 2005). However, neophobia is more common in commensal than non-commensal rat populations, although this can differ with different genetic populations (Kiyokawa et al. 2017). No rats were detected on the first sampling occasion, which suggests neophobia on the first night at least, and some individuals might have avoided the new object for the entirety of the sampling period. Additionally, the assumption is made that the probability of a rat interacting with a tube remains constant throughout the sampling period. This assumption may be violated if encounter rates of rats are affected by variation in rat activity due to changes in the weather, for example (Griffiths and Barron 2016).

Some problems were encountered using the hair-snag tube method. The glue was difficult to mix to achieve an acceptable consistency. This may be due to the fact that the glue used was fairly old (leftover from a 2009 study; McCulloch 2009) but was used because access to this type of glue was restricted as it was no longer being produced in New Zealand. The glue further created problems later on during the microscopic analysis of the hairs, as hair clumps were often covered in the glue which made it difficult to extract hairs without breaking them. Another difficulty encountered was in the in-field removal of rubber bands from the tubes. The rubber band had to be carefully pulled up through the slits in the tube and this may have resulted in the loss of genetic material and damage of the hair, making it difficult to identify the medulla structure. One solution would be to find a way to non-destructively extract DNA from the glue itself, without the presence of glue harming the DNA extraction or amplification processes. Other methods of passive hair removal could be considered in place of the glue-covered rubber bands; however hair size would need to be considered as methods utilising Velcro or barbed wire tend to be used with larger mammals (e.g. Latham et al. 2012; Fischer et al. 2016) and thus might not be effective in collecting the smaller hair of rodents.

Due to the ongoing control of possums from the Otago Peninsula, possum interference with the tubes was not observed. Similarly, due to the relatively remote locations studied, other large species such as dogs did not interfere with the hair-snag tubes. A trial using hair snag tubes conducted in the Dunedin Town Belt (K. Miller, unpub.) highlighted that possum and dog (and possibly cat) interference could be a major problem in achieving viable results as these animals are large enough to completely displace tubes in an effort to obtain the peanut butter used as bait, and thus render the tube useless.

Assessing the hair's medulla microscopically also proved to be difficult as the structures of the medulla of rat and mouse hair were very similar, especially hairs from the rat's belly. However, the use of reference hairs from the rat and mouse specimens aided in the identification of the hairs. Of the samples identified as rats in the microsatellite genotyping analysis (n=22), only two had been identified as false positive identifications using the hair's medulla structure (91% successful identification). Conversely however, of the samples identified as rats in the microscopic analysis (n=65), only 20 were

positively confirmed as containing rat hair after genotyping (31% successful identification).

In a similar study in Dunedin, McCulloch (2009) looked at the hairs' outer cuticle scale pattern in addition to the medulla to aid identification and set the hair in PVC glue to create a relief of the cuticle structure. While this would indeed add another layer of confidence in the identification of a sample hair's origin, it also relies on the collection of multiple hairs from each rubber band, as this process may damage the hair follicle DNA, so the same hair could not be used in both the scale pattern identification and the genetic analysis. This was often not the case in this study. Additionally, it would have taken an even greater amount of time to incorporate this process given the 452 samples that were collected, and only the guard hair of an animal would have been informative.

DNA degradation could have occurred due to the amount of time between collection and DNA extraction, though the DNA was kept in a freezer at -20°C and should have remained stable, even for that amount of time. The use of filter paper would have ensured moisture did not affect DNA quality, but the effect of glue on the DNA quality remains an unknown factor.

4.4.1.2 The use of SECR in estimating rat density

Unfortunately, the failure to amplify a decent genotype in almost all samples that were analysed resulted in no rat identities being deciphered. At least 20 recaptures are desirable to produce an accurate estimation of an animal's population density, and accuracy increases with number of recaptures (Efford et al. 2004; Efford et al. 2009). Two of the basic requirements of a SECR model are that individuals are identifiable, and that there are individuals that are detected more than once and at two or more locations (Efford et al. 2004). As individual genotypes could not be deciphered, and thus no recaptures could be identified, neither of these two requirements were met and as a result the SECR model could not be applied to estimate rat density.

Achieving robust density estimates of rats and other invasive mammal species are vital in conservation efforts to assess the effects of the threats of these species on native fauna, and to identify where conservation efforts should be concentrated. However, as Després-Einspenner et al. (2017) point out, despite studies with empirical validations of conventional monitoring methods allowing researchers to assess the accuracy of density estimates and the usefulness of common survey methods, such studies are virtually non-existent, perhaps due to the added effort. Validating the monitoring methods to be used should nevertheless be considered in any future studies aiming to estimate rat population densities.

4.4.1.3 Assessment of the genetic sampling method

In any study that incorporates a genetic analysis component, errors can be introduced at any point from data collection in the field, to analysis of genetic material in the laboratory (Williams et al. 2009). The failing to achieve the desired result in this study can be attributed to several points in each of the methods used to collect rodent hair-follicle and analyse the DNA from wild populations on the Otago Peninsula.

DNA is known to degrade rapidly in the presence of moisture and warmth (Murphy et al. 2007; Al-Griw et al. 2017). As a precaution against this, samples collected from hair-snag tubes and placed in a zip-lock bag with filter paper were kept in the freezer at a temperature of -20°C until they were taken out to be examined microscopically, and again when the DNA was extracted from those samples deemed to possibly contain rat hair. The samples would have only been exposed to atmospheric temperatures for a period of maximum five hours from collection to freezing. Prior to that, any hair caught in the tube would have been in the field for a maximum of 26 hours before collection if a rodent had run through the tube immediately after it had been reset, although it would have been more likely to be between 15 and17 hours, from dusk until dawn. In any case, this is not enough time for the DNA to degrade at a very significant rate (Mowat and Paetkau 2002;

Al-Griw et al. 2017), also keeping in mind that field work was conducted in May (late Autumn), when average temperatures were fairly low.

Another factor in regard to possible DNA degradation as a result of time is that the samples that were to be extracted were kept in the freezer between seven (first batch processed) and 15 months (second batch processed). Pre-extracted DNA can remain in good condition for a long period of time when kept at temperatures around -20°C (Straube and Juen 2013). However, multiple freeze-thaw cycles can affect DNA integrity (Brunstein 2015). With the samples being kept at such a low temperature, together with the filter paper absorbing any excess moisture in the zip-locked bag, despite undergoing at least one freeze-thaw cycle, it is unknown if this could have resulted in the level of degradation of the DNA needed to explain the low number of successfully genotyped samples in this study.

After extraction, DNA remained in the fridge (at 4°C) between 1-2 days, or 37-50 days. Extracted DNA is even more stable than frozen DNA (Straube and Juen 2013) and could be kept at a higher (though still very low) temperature. There was no difference in the number of alleles amplified from samples that were in the fridge for a longer amount of time between extraction and being sent off for PCR compared to ones that had been in the fridge for a shorter amount of time. Nevertheless, the entire analysis from collection of samples to DNA processing should be done in the shortest amount of time possible to avoid any errors in genotyping that may occur from longer time frames between methods.

Another factor to consider that may have influenced the low success rate of genotyping samples is the probability that most samples had a very small amount of genetic material attached to the rubber band. It has been demonstrated that genotyping success in non-invasive capture-recapture studies increases with the number of hairs in a sample, and that the number of hair roots in a sample is positively correlated with the number of scorable loci (Mowat and Paetkau 2002; Belant et al. 2007). Many (~39%) of the samples collected in this study had fewer than five hairs attached to the rubber band, and of these samples the majority (65%) had only had two or fewer loci amplified. Only 9% of the

samples with fewer than five hairs had five or more loci amplified, whereas samples with 15 or more hairs had a 71% chance of having five or more loci amplified. This seems to be in line with findings of lower genotyping success with lower numbers of hairs (Mowat and Paetkau 2002). Additionally, the presence of glue, the type of glue, the age of the glue (~9 years), or a combination of the three, may have had an influence on DNA quality. Further study would need to be done to assess this. Presence of the glue in the extraction of DNA and subsequent PCR may have contributed to the unsuccessful genotyping of the samples as DNA could have been degraded by the glue or left behind when removing the hairs from the rubber band. Method of hair removal from the glue could also be the issue, as hair was removed from the glue with forceps, whereas in a study using non-invasive genetic methods on the American marten (*Martes americana*), Mowat and Paetkau (2002) used xylene to dissolve the glue and remove the hair, and found the use of xylene to have no effect on genotyping success. While it should be noted that they used commercial glue traps and the glue type may be different, this method could be considered for future efforts.

In a non-invasive genetic tagging study on white-tailed deer (*Odocoileus virginianus*), Belant et al. (2007) noted that four under-fur hairs contain approximately the same amount of DNA as is found in one guard hair. The follicle size of guard hairs is larger, and therefore contains a larger amount of DNA than the follicles of under-fur hair (Long et al. 2008). As rats have both guard hairs and under-fur hairs, the possibility that mainly under-fur hairs were collected exists. If a rat were to climb over the rubber band instead of under, it would leave behind the thinner, less diagnostically suitable belly hairs, as the guard hairs are mainly found on its back (Brunner and Triggs 2002).

The number of loci (19) used to decipher genotypes in rats, and the number of loci (17) used for mice was greater than the 10 and 11 used in a study by Williams et al. (2009), the numbers of which were deemed to be a balance between too few loci and too many. Too few loci could result in underestimates of population size due to an increase in the probability that two individuals share the same genotype, whereas with too many loci an overestimation of population size due to genotyping error can occur (Mills et al. 2000; Waits and Leberg 2000; Waits et al. 2001). However, the number of loci used in this study

was deemed to still be in accordance with the balance between too many and too few loci. With that said, the number of loci used could have been narrowed down to include fewer loci in each multiplex, and indeed some loci (D5Rat83, D11Mgh5, D19Mit2, SRYf-Peakall, Il-5, Hsp68, DXRat2, Il-1b) did not amplify at all, so the effective number of informative loci was 14 for rats and 12 for mice. Time and cost could have been saved if these loci had been omitted from the analysis; however, it could not have been known that they would not amplify as they had all amplified well in the DNA of the rats and mice specimens. Interestingly, of the loci that failed to amplify in any of the samples, three had been shown to amplify only in rats (D5Rat83, D11Mgh5, D19Mit2), and three only in mice (Il-5, Hsp68, Il-1b). The successful amplification of alleles in any of these loci would have been extremely helpful in deciding whether a sample contained DNA from a rat or a mouse and would have helped in the genotyping of an individual. As such, only three and one unique loci for rats and mice were able to produce any information, respectively. In future studies it may be useful to test a larger number of loci on the DNA of rats and mice, and use only those which amplify in one or the other species, or those that amplify alleles at such different base pair lengths that it limits confusion when scoring the alleles.

In many genetic studies, problems can occur at the PCR stage, with subsequent errors occurring during allele scoring (Pompanon et al. 2005). Two errors which can occur frequently are allelic drop out and the scoring of false alleles. Allelic drop out occurs when an allele fails to amplify at a locus. This can result in the scoring of the genotype as being homozygous at that locus when in actuality it may be heterozygous, and one of the two alleles has experienced allelic dropout (Murphy et al. 2007). False alleles occur when an allele-like artefact is generated by PCR (Pompanon et al. 2005). In genetic studies using an electropherogram to visualise alleles at base pair lengths, this would show as a peak at a given base-pair size within a locus, much like in normal alleles, so may be easily mistaken and falsely attributed to a genotype (Murphy et al. 2007). Both of these errors can lead to the misidentification of individuals as the same, or of one individual as multiple individuals, thus inflating the population size estimate as the two genotypes may be falsely identified as two different individuals (Kubasiewicz et al. 2017). For a certain locus, false alleles occur more frequently than allelic drop out (Kubasiewicz et al. 2017). The lack of amplification of alleles in this study may be

attributed to allelic drop out. Genotyping errors such as allelic dropout and false alleles are known to occur frequently when DNA quantity and/or quality is low (Pompanon et al. 2005).

Some studies employ the multi-tubes approach, designed to obtain more accurate genotypes through the repetition of amplification per DNA sample (Taberlet 1996). Solberg et al. (2006) found that four replicates of the same DNA sample was the ideal number to yield reliable genotypes, whereas Taberlet (1996) found seven to be a good number of replicates per DNA sample. The multi-tubes approach is best used when the DNA is taken from a known source (e.g. Taberlet 1996). In a non-invasive genetic study, it may be difficult as DNA could be from multiple individuals or multiple species that could share overlapping allele base-pair lengths at some loci (e.g. rats and mice). This method would also increase the cost and effort of genetic study and may only be considered viable in studies with fewer samples, or in studies with a large amount of funding, but might increase the accuracy of the results of a genetic analysis.

Further errors that could have caused discrepancies include contamination and human error (Pompanon et al. 2005). Contamination from human error could have occurred at any point in the study from collection to genotyping, though most likely would have occurred in the DNA extraction stage or the PCR stage. Several precautions were undertaken at the DNA extraction and processing stages, including disinfecting benches and equipment (e.g. forceps) used, and strict lab regulations limited the possibility of contamination from others' work, therefore contamination from lab practices remains a fairly unlikely source of error. However, low DNA quantity and/or quality noticeably increase the risk of contamination, as low numbers of DNA template molecules increase the probability of contaminant molecules being amplified; (Taberlet 1996; Pompanon et al. 2005). With the low number of hairs in many of the samples collected in this study, the DNA quantity is likely to have been low in these samples, despite amplification, and would therefore have had a higher risk of contamination than samples with a larger number of hairs. Unfortunately, this was not tested, although early use of a control suggested no contamination occurred in those instances. Human error is always present in such studies as these. The amount can differ, with one study attributing 93% of the

errors to human error (Hoffman and Amos 2005). Scoring of alleles also represents an important form of human error. Bonin et al. (2004) reported that manual scoring represented the main source of discrepancy in the amplified fragment-length polymorphism data sets amongst independent scorers of electropherograms. While discrepancies among scorers of alleles using electropherograms were not a factor in this study, due to there only being one scorer, discrepancies still arose when scoring was undertaken on separate days. To combat this, alleles were scored again after the last samples had been processed, with all samples analysed on the same day.

The DNA of some of the samples in this study was extracted using the CTAB process when the extraction kit had run out. The CTAB process is often used in genetic studies as a method of DNA extraction and is a much cheaper alternative and has been shown in some studies to be just as effective (e.g. Mirimin and Roodt-Wilding 2015). In DNA extraction, CTAB is particularly useful in isolating DNA from tissues that contain large amounts of polysaccharides to promote cell lysis (Clarke 2009). CTAB can uphold the quality of DNA where other extraction methods may fail (Mirimin and Roodt-Wilding 2015). Unfortunately, in this study no DNA was amplified at any of the loci in those samples that underwent extraction through the CTAB process. This may be due to similar factors as with the original extraction method, for example the inherently low quantity of DNA present in many samples. If the issues can be identified and ironed out, this would be a much more cost-effective option in genetic studies than the use of pre-made extraction kits.

4.4.1.4 Tracking tunnels as rat detection devices

Interestingly, despite a greater number of rat detections from samples collected from the grid at Leith Walk, the tracking tunnel line placed there was the only line that did not detect any rats whatsoever. The line at Hooper's Inlet had a low tracking rate of 10%, with only one rat detected. Similarly, Okia had the same tracking rate (10%) and number of rats (1) detected. A reason for this low tracking rate could be the fact that tunnels were not left out for a long enough period of time before the tracking commenced, as rats have

been known to display neophobia, a fear of new objects placed in their habitat (Cowan 1977). Cooper et al. (2018) confirmed that rats were indeed very cautious when approaching an unfamiliar tracking tunnel. Due to time and volunteer constraints, the recommended period of time of three weeks to leave the tracking tunnel lines out before the first survey session (Gillies and Williams 2013) was not implemented. Leaving the tunnels unbaited for a period of time before sampling occurred could have resulted in larger tracking rates, as rats would have been more accustomed to the tunnels (Gillies and Williams 2013).

Knox et al. (2012) reported average rat-tracking rates of approximately 40% in ungrazed shrubland and regenerating kānuka forest sites. These higher tracking rates in comparison to this study could be due to bait placement in the middle of the tracking tunnel, as in this study bait was placed at tunnel ends following Gillies and Williams (2013) guidelines (Wilson 2017). There was found to be no correlation between tracking rates and number of rat detections from the hair-snag tube grid, though the sample size (number of grids) was fairly small so a nonsignificant result was to be expected.

The tracking tunnel that detected a rat at Hooper's Inlet was in the general vicinity of a hair-snag tube that also detected a rat. Given the average home-range size estimates reported for rats in various mainland forested habitats (0.17-1.80 ha excluding beech forest; Daniel 1972; Hooker and Innes 1995) it is possible the rats detected were the same individual. The tracking tunnel that detected a rat on the tunnel line at Okia was not as close to where a hair-snag tube had detected a rat from the tube grid as at Hooper's Inlet, but the distance of approximately 35 m could still be well in the range of the rat, or rats (two rats were identified from one hair snag tube but could not be distinguished as the same individual or as two), detected at that hair snag tube. This would imply a minimum range of 35 m moved by an individual but would not explain why hair-snag tubes in closer proximity to the location of the tracking tunnel failed to pick up rat presence. While rat home-range sizes may change with season (Innes 2005b), it is unlikely that the home-range of the rats would have changed much in the time between hair-snag tube and tracking tunnel sampling occasions (less than a week) and explain the difference in detection locations, though it is possible. Additionally, a search of the literature revealed

no indication that hair-snag tube grids, nor tracking tunnel lines, have been set in this exact location at Okia before, therefore it is unlikely that an individual rat showed "trap-happy" behaviour towards tracking tunnels that it may have become familiar with from previous tracking efforts (Cooper et al. 2018). It may be that the hair-snag tubes were more accessible to rats, which would explain the lower expected tracking rates from the tunnel lines at Leith Walk and Okia especially, or the fact that the hair-snag tubes had bait set in the middle of the tubes. Blackwell et al. (2002) found variation in the standard tracking tunnel density indices was only significantly related to rat density indices of Fenn traps when data were collected over a long period of time (27 months) and only in populations of higher density. Similar trends may be present in hair-snag tube methods, where detection rates of rats were higher than in tracking tunnels. As population density of rats was not estimated to be very high, leaving tracking tunnels out over a longer period may result in higher tracking rates of rats, and be more in line with the results from the hair-snag tube sampling.

Ideally, in a larger area, multiple tracking tunnel lines would be set up, which would allow for a more robust index of relative abundance of rats (Gillies and Williams 2013). As a consequence of doing so, however, a much larger amount of time and effort would be needed. Some drawbacks of tracking tunnels, similar to those of hair-snag tubes, is that the probability of detection of animals can be biased. The biases can arise depending on non-target species interference, the type of bait used, environmental food availability, and weather conditions (Pickerell et al. 2014; Carter et al. 2016; Anton et al. 2018). As tracking tunnels are usually placed on lines, index values also depend on rats encountering the device and interacting with it, neither of which behaviours are usually observed (Wilson et al. 2007a). As such, encounter rates could vary with habitat structure, home-range size, and activity of rats (Dowding and Murphy 1994), and probability of detection can vary with time and space (Thompson 2004). Additionally, in a study employing remote cameras, Anton et al. (2018) recorded instances when rats interacted with tracking tunnels but did not enter them.

4.5 Detection of mice

Both the hair-snag tube and tracking tunnel surveying methods picked up mice in extremely high numbers. Together with a large number of the samples identified as possibly originating from mice using microscopic and genotypic methods, tracking tunnels showed mouse tracking rates of 100% at all three sites, indicating a high density of mice. However, this study was aimed at estimating rat density, and a more accurate estimation of mouse population densities at these sites would require a study designed specifically to detect mice, for example, smaller spacing between detection devices or a narrower hair-snag tube used. This is recommended with respect to the aim of the OPBG to rid the Otago Peninsula of predators by 2050 (Otago Peninsula Biodiversity Group 2019). A problem common to many conservation efforts in New Zealand is that mice are last on the list of predator species to be eradicated or left out of eradication plans altogether. The removal of larger predator species such as rats, stoats, and feral cats, all of which are known to prey on mice, could trigger meso-predator release (Courchamp et al. 1999; McQueen and Lawrence 2008; Ritchie and Johnson 2009; Bridgman et al. 2018). Several studies have reported an increase in the frequency of mouse detections following the successful control of rats (e.g. Innes et al. 1995; Harper and Cabrera 2010; Bridgman et al. 2018). Bridgman et al. (2013) and Bridgman et al. (2018) also found that while direct predation of ship rats on mice did occur in their study, there was also evidence that presence of rats limited mouse foraging opportunities. As the two species share some diet overlap, it is also possible they are competitors for some of the same food resources (Bridgman et al. 2013). Therefore, as well as a potential meso-predator release, there may also be a competitive release of mice with the eradication of rats (Nelson et al. 2016). This predator/competitor relationship is described as an asymmetrical intraguild predator relationship (Polis et al. 1989). As such, activity of mice, and as a result their rates of detection, may be suppressed in the presence of rats (Brown et al. 1996; Harper and Cabrera 2010; Bridgman et al. 2018). Thus, the high rates of mouse detections observed in this study may suggest that rat density is low enough at these sites to allow high activity of mice.

House mice can be arboreal (Innes et al. 2018) and prey mainly on seeds and invertebrates, but are also important predators of lizards, and have been reported to prey on the eggs and chicks of birds (Ruscoe 2005; Norbury et al. 2014; O'Donnell et al. 2017). With the important biodiversity of fauna on the Otago Peninsula, including at-risk species of lizard such as jewelled geckos (*Naultinus gemmeus*), korero geckos (*Woodworthia* "Otago/Southland large"), southern grass skinks, and cryptic skinks, mice pose a large threat to the abundance of these species on the Peninsula, as even at densities considered moderately high, the impact of mice on the ecological aspects of a habitat may still be quite significant (Wilson et al. 2018). A study of jewelled geckos on the Otago Peninsula noted the significantly higher incidences of tail loss and lower density of the geckos in ungrazed *Coprosma* shrubland, which coincided with higher activity rates of rodents at these sites (Knox et al. 2012).

Removal of mice from an area as large as the Otago Peninsula, however, remains a difficult task. Even in fenced sanctuaries where cats, stoats, ferrets, weasels, and rats have all been removed, mice either survive the eradication attempts or subsequently reinvade (Innes et al. 2012), leaving mice to inhabit the area with much reduced predation and intraspecific competition (Wilson et al. 2018). Therefore, while potentially difficult, mouse eradication should still be considered in the predator management plans of the Otago Peninsula Predator Free 2050 and Predator Free New Zealand 2050 plans. Large-scale control may be difficult to achieve at first, so sites with important native species populations and sites of possible high mouse density, such as the sites on the Peninsula, need to be prioritised.

4.6 Implications for forest and scrub fragments

As the Otago Peninsula comprises only approximately 5% native forest or scrub, most of which is fragmented by developed agricultural and urban land (Ewans 2017), conservation of these fragments remains a challenge as rats can easily reinvade across grazed pasture despite the predation risks (Innes et al. 2010). Additionally, the Leith Walk site sampled had a nearby development of residential properties that could encourage and

sustain commensal rat populations, particularly Norway rats (Innes 2005a). The relatively high diversity of bird species recorded at each of the three study sites shows the importance of conserving these fragments and areas surrounded by pastureland. Fencing those fragments surrounded by active grazing could have a positive effect on bird fecundity (e.g. Armstrong et al. 2014), however, it could also have negative effects with respect to rat populations, as ship rats have been found to be more common in fenced forest fragments than in unfenced fragments (Boulton 2006; King et al. 2011). A combination of predator control in the fragment itself, as well as in the surrounding area, together with the fencing of the fragment could therefore be beneficial to bird communities.

4.7 Conclusion

This study has provided an insight into rat population densities at three sites on the Otago Peninsula. These results can be used as a foundation for future study of rat abundance on the Otago Peninsula, and inform the Otago Peninsula Predator Free 2050 plan. Areas with higher rat population density, such as at Okia and Leith Walk, together with areas of high vegetation complexity and dense groundcover, can be targeted first when control of rat species begins as part of the eradication plans. This would ensure that control would be most efficient at early stages.

The relatively large number of bird species counted at each site indicates the importance of conserving these vegetation fragments on the Peninsula. While the abundance of most native bird species on the Peninsula should increase with rat eradication, the effect may be more marked in native species due to the enormous impact rats have already had on native bird populations (Miskelly 2018). This could in turn perhaps influence the overall bird community composition by increasing the abundance of native birds relative to introduced birds. A similar trend of increasing native bird abundance following rat eradication as observed in North Island robins in central North Island broadleaf-podocarp forest fragments (Armstrong 2017) may be seen in the vegetation fragments of the Peninsula.

Bird counts at each of these areas allowed for an estimation of current bird densities and abundance at each of these sites and provide a baseline for future evaluation of the impact of rat eradication efforts on bird populations. Bird surveys are important for areas such as the Otago Peninsula as they provide robust estimates of bird abundance and community composition and enable the evaluation of outcomes of eradication efforts. The current regular bird counts undertaken by members of the OPBG provide simple estimates of bird abundance but can only be used as an index. The method used for the last ten years means it can be continued to be used to allow comparisons to earlier years. This, however, may not be a good enough rationale to continue with this less than ideal method of bird abundance monitoring. Such a large number of transects (21; Ewans 2017) to be completed on a regular basis requires considerable effort and dedicated volunteers and a large amount of data on bird abundance has been collected. However, seasonal, twice yearly, or even annual bird abundance estimates through distance sampling by one or a few contracted persons at permanent bird counting stations would allow more robust estimates of bird density and allow for more informative comparisons of avian community compositions and the effects of future predator species' eradication. These could be targeted in areas such as those suggested above; areas with high rat densities, high vegetation complexity, and dense groundcover, along with areas important to stoat, ferret, weasel, hedgehog, and mouse populations. Starting this before any eradication efforts of other species goes ahead ensures the ability to draw before and after comparisons of bird abundance. The bird counts could also be combined with regular invertebrate and herpetological monitoring to increase knowledge of species found on the Otago Peninsula and their response to predator control.

Similar to the OPBG bird counts, tracking tunnels are a fairly simple method of obtaining an index of population abundance (Gillies and Williams 2013). The index can be used to estimate population growth of small mammals (Elliott et al. 2018), but not population density. The combination of hair-snag tube grids and genetic analyses aims to estimate density, with confidence intervals. If procedures are perfected, hair-snag tubes as a non-invasive sampling technique offers the ability to achieve robust density estimates of small mammals and could be used in future studies to obtain density estimates at many more

sites across the Peninsula. Tracking tunnel lines could then be used in parallel to hair-snag tubes, which would allow the two methods and their detection rates to be compared. One current limitation in genetic sampling for non-invasive methods is the cost associated with genotyping the collected DNA samples and this remains a barrier for studies with a low budget. Large-scale non-invasive sampling efforts will only be viable once these costs are reduced and materials are made more readily available.

In terms of conservation work on the Peninsula, the fragmented nature of native forest and scrub on the Peninsula poses a challenge. Vegetation fragments must be dealt with as unique cases and not be subject to assumptions based on estimations and efforts from other unfragmented native habitats or islands around New Zealand. This is crucial in ensuring effective eradication of rats and the efficient use of relevant control methods. Additionally, the low densities reported in this study may be beneficial for the survival of current bird populations, but this itself will pose a challenge to eradication efforts as the rats will be harder to detect and may evade eradication. It was also shown that rats were detected in a range of habitats, and therefore eradication efforts should not be limited to what might be thought of as traditional rat habitat. Further complicating conservation efforts on the Peninsula is the presence of residential areas and public opinion on pest control. Nevertheless, control of multiple predator species simultaneously is the most effective option for pest control on the Peninsula, in order to avoid meso-predator and/or competitor releases. A large-scale short-term eradication effort of the full predator suite, followed by long-term ongoing monitoring and control to prevent reinvasion, is suggested to realistically meet the Predator Free Otago Peninsula 2050 deadline.

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6 APPENDIX

Table 6.1: Alleles base pair sizes for alleles amplified from hair samples collected from hair-snag tubes on the Otago Peninsula at eight of the 11 loci used in multiplex 1, alongside the identification of that sample. Present are all hair samples that had at least one allele successfully amplified in either multiplex 1 or multiplex 2 (see Table 6.2 in Appendix). Asterisks denote alleles amplified at the exact base-pair size as in the either of the rat specimens.

Sample	Identification	Microsatellite Loci														
		BCL2		D10Rat20		D15Rat77	D18Rat96		D20Rat46	D2Rat234		D7Rat13		TNFA		
A10-02.6	Mouse															
A10-02.8	Rat			118	118									105	126	
A2-01.7	Mouse															
A2-02.7	Mouse															
A2-02.8	Rat			112	129											
A2-05.7	Mouse (>1)	145	145				265	265						110	115	119
A2-05.9	Mouse	145	145													
A2-07.6	Mouse	145	145													
A2-12.6	Rat	135	135									190	190	105	126	
A2-13.9	Ambiguous															
A2-14.6	Mouse	147*	147*											104	110	
A2.07.6	Mouse													109	119	
A3-02.6	Rat/Mouse	143	147*	131*	131*					111	111			105	126	
A3-03.10	Mouse	147*	147*													
A3-03.6	Rat													105	126	
A3-03.7	Mouse	145	147*													
A4-02.6	Rat/Mouse	147*	147*							120	120					
A4-04.5	Rat	147*	147*									180	180	105	110	

A4-04.7	Rat/Mouse	121	147*					265	265			180	180	105	110
A4-04.9	Mouse														
A5-05.10	Mouse	143	147*											105	126
A5-06.4	Mouse	147*	147*											105	126
A5-06.9	Mouse														
A5-07.5	Mouse														
A5-07.8	Rat/Mouse	147*	147*	112	129										
A5-07.9	Mouse	147*	147*												
A6-04.7	Rat/Mouse					247	247					190	190	105	126
A7-07.9	Rat/Mouse	135	147*												
A8-06.9	Mouse														
E4-01.5	Mouse	138	145											113	120
G3-13.4	Rat			112	115*									105	126
G3-15.5	Mouse													109	113
G3-13.9	Ambiguous														
G4-14.5	Rat/Mouse	135	145	112	128									105	126
G4-14.7	Mouse														
G5-05.8	Rat											190	190	105	126
G5-13.7	Mouse	145	145											105	126
G5-14.4	Mouse														
G5-14.9	Mouse														
G6-05.?	Mouse	147*	147*											105	110
G6-05.3	Mouse														
G6-05.7	Mouse	147*	147*											105	110
X1-03.24	Rat			112	129										
X1-03.26	Rat			115*	118										
X1-03.28	Mouse														
X1-04.26	Ambiguous													105	126
X1-04.27	Mouse													109	109
X2-02.26	Mouse	121	147*												

X2-02.27	Ambiguous												
X2-02.28	Mouse	147*	147*										
X2-04.28	Rat												
X3-02.23	Ambiguous												
X3-02.25	Mouse												
X3-02.26	Mouse	147*	147*										
X3-02.27	Mouse	145	145										
X3-02.28	Mouse	145	145										
X3-06.28	Rat			112	129							105	126
X4-11.23	Mouse											109	109
X4-11.24	Mouse	121	145										
X4-11.25	Mouse												
X4-11.26	Rat												
X4-11.27	Rat	147*	147*					154*	154*				
X4-11.28	Mouse											109	109
X5-04.24	Mouse	145	147*										
X5-04.26	Rat			112	129							105	126
X5-04.28	Mouse	145	147*										
X5-05.25	Mouse												
X5-05.27	Rat			112	129					190	190	105	126
X5-06.26	Mouse												
X5-06.28	Mouse											105	110
X5-09.25	Mouse												
X5-09.26	Mouse												
X5-09.28	Mouse												
X6-11.23	Mouse											109	111

Table 6.2: Alleles base pair sizes for alleles amplified from hair samples collected from hair-snag tubes on the Otago Peninsula at seven of the 12 loci used in multiplex 2, alongside the identification of that sample. Present are all hair samples that had at least one allele successfully amplified in either multiplex 1 (see Table 6.1 in Appendix) or multiplex 2. Asterisks denote alleles amplified at the exact base-pair size as in the either of the rat specimens.

Sample	Identification	Microsatellite Loci															
		Qa-4				Acrg		D0Nds2		D16Rat81		Gfap		Myc		Myla	
A10-02.6	Mouse					128	128	95	95					110	110	224	224
A10-02.8	Rat							90	90								
A2-01.7	Mouse	186	186					95	95								
A2-02.7	Mouse							95	104					110	110	224	228
A2-02.8	Rat					126	128										
A2-05.7	Mouse (>1)	164	169	216		128	128	95	104	161	161			110	127	228	228
A2-05.9	Mouse	169	169			128	128							128	128	228	228
A2-07.6	Mouse											299	299				
A2-12.6	Rat																
A2-13.9	Ambiguous					127	127										
A2-14.6	Mouse															228	228
A2.07.6	Mouse					127	127							122	122	224	228
A3-02.6	Rat/Mouse					127	127	104	104			298	298	113	127	224	224
A3-03.10	Mouse					128	128	95	95					108	127	228	235
A3-03.6	Rat							90	90								
A3-03.7	Mouse					128	128	95	95					110	127	228	235
A4-02.6	Rat/Mouse	163	164	208	225	127	128	95	95	143	161	299	299	108	113	234	235
A4-04.5	Rat	164	169			128	128										
A4-04.7	Rat/Mouse					128	128	95	95	161	161						
A4-04.9	Mouse					128	134	95	95					121	127		
A5-05.10	Mouse					128	128	95	95					121	125	228	235
A5-06.4	Mouse	183	213			128	128	95	95					108	108		

A5-06.9	Mouse			128	128							108	127	228	228
A5-07.5	Mouse			128	128	95	95					110	110		
A5-07.8	Rat/Mouse											108	127	228	228
A5-07.9	Mouse			127	127							114	128	224	228
A6-04.7	Rat/Mouse			128	128	95	95								
A7-07.9	Rat/Mouse	163	186	128	128	90	92*					110	110		
A8-06.9	Mouse			128	128	95	95					110	110	235	235
E4-01.5	Mouse			127	127										
G3-13.4	Rat			126	126	95	103								
G3-15.5	Mouse	183	215	126	128							110	128	228	228
G3-13.9	Ambiguous			127	127							110	110		
G4-14.5	Rat/Mouse			127	127	104	104					108	110	228	228
G4-14.7	Mouse			127	127	104	104								
G5-05.8	Rat			127	127										
G5-13.7	Mouse	163	182	128	128	95	95	161	161	298	298	110	110	228	228
G5-14.4	Mouse											110	110		
G5-14.9	Mouse			128	128							110	127	228	228
G6-05.?	Mouse			126	126	95	104	161	161			110	127	224	235
G6-05.3	Mouse			126	126							110	110		
G6-05.7	Mouse					95	104	161	161			110	127	224	235
X1-03.24	Rat														
X1-03.26	Rat														
X1-03.28	Mouse			127	127							110	110	228	228
X1-04.26	Ambiguous			127	127										
X1-04.27	Mouse													228	228
X2-02.26	Mouse			128	128	104	104					113	125	235	235
X2-02.27	Ambiguous					90	90								
X2-02.28	Mouse			128	128	95	104					114	126**	228	228
X2-04.28	Rat					80	80	195	195						
X3-02.23	Ambiguous			127	127										

X3-02.25	Mouse				127	127									
X3-02.26	Mouse				128	128								228	228
X3-02.27	Mouse				127	127	95	95					113	127	228	228
X3-02.28	Mouse				127	135*	104	104					113	127	228	235
X3-06.28	Rat															
X4-11.23	Mouse	162	213		128	128							110	114	234	234
X4-11.24	Mouse				128	128	104	104					110	113	235	235
X4-11.25	Mouse				128	128							110	126**	235	235
X4-11.26	Rat	246*	246*		128	135*										
X4-11.27	Rat										299	299				
X4-11.28	Mouse				128	128										
X5-04.24	Mouse				128	128	104	104					111	114	228	228
X5-04.26	Rat															
X5-04.28	Mouse				127	127							127	127	224	228
X5-05.25	Mouse				128	128							128	128	224	224
X5-05.27	Rat															
X5-06.26	Mouse				128	128			195	195						
X5-06.28	Mouse				127	128			195	195			127	127	224	228
X5-09.25	Mouse				126	128	95	95					110	113	228	228
X5-09.26	Mouse								195	195						
X5-09.28	Mouse												111	111		
X6-11.23	Mouse				128	128							110	127	228	234