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Ship rat density in urban Dunedin and the development of a non-invasive estimation method

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ABSTRACT

In New Zealand ship rats (*Rattus rattus*) are one of the major threats to endemic fauna and flora. Rural ship rat populations have been implicated in the ongoing decline and extinction of many species of endemic wildlife. The role ship rats have in structuring urban ecosystems, directly through predation, and indirectly through food and habitat competition is poorly understood in New Zealand. Understanding the role of ship rats in the urban environment is impeded by a lack of information on their distribution and robust estimates of their density.

Rat presence and distribution across different urban habitats was determined by the identification of genus-specific bite marks on wax blocks. The results from the wax block survey suggest that rats are either absent from, or at very low densities within the housed residential sites sampled in this study. In urban bush fragments rats were detected infrequently using wax blocks. High rates of non-target species interference may obscure the rate of rat detection in urban areas.

Density is a fundamental biological parameter, however unbiased density estimation can be extremely difficult for certain species. Ship rats are nocturnal and highly dispersed, which makes them particularly difficult to sample using conventional techniques. Currently the most accurate and reliable estimates of absolute ship rat density are obtained through cage-trapping and spatially explicit capture-recapture analysis. This sampling method is both laborious and intrusive. Invasive sampling methods are also not always suitable for use in urban areas.

This study describes the application of a non-invasive genetic technique for the estimation of urban ship rat density. Individual genotyping of ship rats was facilitated by analysing nine microsatellite loci amplified from the tissue of ship rat hair follicles. Hair samples were collected using hair-snag tubes (220 mm lengths of 65 mm PVC down pipe). Hair samples were retained on adhesive coated rubber bands that partly occluded the opening at both ends of the hair tubes. Hair tubes were baited with peanut butter and set in a known array. Ship rat density was estimated using spatially explicit capture-recapture analysis (DENSITY 4.1). Maximum likelihood was used to fit a range of candidate models to the spatial dimensions of hair tube re-visitation data.

The efficacy of the hair tube methodology was initially verified in the Orongorongo Valley on a well studied population of ship rats. In the Orongorongo Valley the density estimate of 1.17 ± 0.42 (SE) rats/ha was in accordance with recent cage-trapping estimates from the same sampling grid.

Very low densities (0.26 ± 0.10 (SE) ha) of ship rats were found in Dunedin urban bush fragments. The overall effect of ship rats as predators on urban birdlife is inferred to be much less than in rural areas, where higher ship rat densities exist. If rats exist in high densities within urban Dunedin it seems likely they do so within small pockets of favourable habitat i.e. areas that are not frequently controlled, where food is abundant or where domestic cat densities are low.

Systematic sampling and genetic profiling of ship rat hair for spatially explicit density estimation requires fewer human resources than cage-trapping and provides robust estimates of absolute density, but involves increased costs in laboratory analysis.

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DEDICATION

For ZL3IC and my Lisa

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1 INTRODUCTION

1.1 Urban ecology

In the year 2007 the Population Reference Bureau estimated that 75% of people in the developed world live in urban areas; in New Zealand this figure is 86% (Haub *et al.* 2007). Movement of the human population into cities is a global phenomenon, with subsequent loss of surrounding rural land to urban sprawl. In Great Britain 6,000 ha of rural land is converted to urban use each year (Department of Environment Food and Rural Affairs 2003). When averaged New Zealand's rate of rural to urban expansion has been in the order of 4–5% per year over the last 25 years (Ward *et al.* 1996). With the ongoing transition of rural to urban habitats there is an increasing need to understand urban ecosystems better. Despite the fact that most of us live in the urban environment surprisingly little is known about the ecological interactions that take place within it.

The aggregation of people in urban areas is generally considered to be detrimental to global biodiversity (Harrison & Davies 2002), although some rare and threatened species can exist in urban areas, after being reduced in numbers or extirpated from other parts of their former range (Bland *et al.* 2004). Whether a species survives in the urban environment is dependent on its ability to live and reproduce in highly modified and regularly disturbed habitat that is continually being manipulated by humans. Modified habitats have been viewed as having little value for wildlife, but this attitude is changing. For example, Miller and Hobbs (2002) showed that a large proportion of the world's 'biodiversity hotspots' (Myers *et al.* 2000) currently harbour higher than average human population densities.

1.2 Valuing urban ecosystems

More often than not, conservation programmes focus on saving larger blocks of non-urban habitat as opposed to smaller urban habitat fragments. Prioritising conservation effort solely towards larger reserves using the 'bigger is better' approach is, however, not always justified. Some smaller reserves are functionally very important for certain species that are not present, or protected, within larger non-urban reserves (Shafer 1995). Most cities contain remnant

patches of semi-natural habitat and often substantial areas of residential gardens. These areas provide habitat for a diverse array of species as well as providing connectivity for animals travelling from one area of habitat to another (Rudd *et al.* 2002). Small patches of remnant habitat can contain genetically diverse sub-populations that are important for the long term genetic viability and persistence of surrounding populations (Trewick 1999). For example in Dunedin city a genetically distinct population of peripatus (*Peripatoides novaezealandiae*) persists within a 3.4 ha bush fragment in Caversham Valley (Trewick 1999). Likewise a small population of the declining jewelled gecko (*Naultinus gemmeus*) exists in tiny patches of remnant bush on the Otago Peninsula (Jewell & McQueen 2007). These populations are small but have high conservation value nationally (Trewick 1999; Whitaker *et al.* 2002).

The intrinsic values of urban wildlife should also not be underestimated. With the exception of commensal rodents, most urban residents highly value the presence of wildlife in their gardens (Baker & Harris 2007). For many city dwellers an experience with urban wildlife may be as close to 'wild nature' as they ever get. As cities continue to grow in both human population size and area, children will become increasingly cut-off from wild nature. If conservation programmes are to be sufficiently supported in the future then it is important that children nowadays are brought up with a basic understanding of conservation and the environment. Remnant patches within urban habitat and the species that live within them have the potential to be the primary focus of environmental education programmes for future generations.

1.3 Urban aliens

Biogeographic dispersal barriers no longer prevent the spread of alien species from one country to the next (Kowarik 2008). The spread of alien species into novel habitat has become one of the major focuses of urban ecology (Wittenberg & Cock 2001). Falinski (1998) states that the invasion and establishment of alien plant species is favoured by disturbance of community structure, human changes to the environment, fragmentation and enhanced migration. These features are pervasive in the urban environment.

As well as invasive plant species, the New Zealand urban environment is home to a suite of alien predators. Exotic mammalian predators have been implicated both directly and

indirectly in the decimation of New Zealand's endemic flora and fauna (Bell 1978). Species such as hedgehogs (*Erinaceus europaeus*), domestic cats (*Felis catus*), possums (*Trichosurus vulpecula*), rats (*Rattus* spp.), and mice (*Mus musculus*) are common in many urban areas. The majority of these species have been extensively studied in the non-urban environment (e.g. Fitzgerald & Karl 1979; Alterio 1996; King *et al.* 1996; Ruscoe *et al.* 2001; Sanders & Maloney 2002; Wilson *et al.* 2003a), but the influence these species exert as predators on urban wildlife has mostly been neglected. If urban ecosystems are to be enhanced or preserved it is essential to understand the impact that these predators have on other urban wildlife.

1.4 Commensal rats: a worldwide pest

Throughout the world rats are found living in close association with human settlements (Traweger *et al.* 2006). This is probably due to their ability to adapt to a generalist diet and occupy a variety of habitats (Innes 1990b). Commensal rats act as a vector for a number of virulent pathogens, making them a significant public health risk (Battersby *et al.* 2002). Rats are also a common agricultural and horticultural pest (Traweger *et al.* 2006). Despite these factors surprisingly little is known about the population ecology of commensal rats. Scientific studies investigating the ecology of rat populations in cities are uncommon, and reliable estimates of rat densities in urban areas are almost non-existent (Traweger *et al.* 2006).

1.5 Rats in New Zealand forests

In New Zealand rats have decimated endemic fauna and in some areas are subject to ongoing control programmes (Innes *et al.* 1995). Three species of rat exist in New Zealand, Pacific rats (*Rattus exulans*), ship rats (*R. rattus*) and Norway rats (*R. norvegicus*). Pacific rats came to New Zealand with the first Polynesians. They are the smallest of the three rat species with adults generally weighing between 60–80 grams (Atkinson & Moller 1990). Norway rats arrived in New Zealand with the first Europeans between 1790–1800 (Atkinson 1973) and are the largest of New Zealand's rat species, with most individuals weighing between 200–300 grams (King 2005). Ship rats spread throughout New Zealand after *c.* 1880 but may have arrived much earlier (Atkinson 1973); most adult ship rats weigh between 120–160 grams

(Innes 2005). Competitive exclusion has been observed as each successive species of rat has established in New Zealand (Harper 2002); with ship rats considered the superior competitor (Russell & Clout 2004). The once widespread Pacific rat is now rare on the mainland and found almost exclusively on offshore islands free of the ship and Norway rats (Atkinson & Moller 1990). Likewise, Norway rats were displaced from areas of previously suitable habitat following the introduction of ship rats (Atkinson 1973). Norway rats are now found most frequently in small local colonies near water, or on offshore island (Innes *et al.* 2001).

Rats are omnivorous and are relentless predators of native invertebrates, amphibians, reptiles and birds (Atkinson 1985; Newman & McFadden 1990; Thurley & Bell 1994; Cree *et al.* 1995; Towns *et al.* 2006). Rats may also affect non-prey species indirectly through food and habitat competition The detrimental effect of rats on plant communities is also often overlooked (Wilson *et al.* 2003a). Rats forage heavily on seeds, growth stems, leaves and fruits (Campbell & Atkinson 2002; Wilson *et al.* 2003a).

Ship rats are highly arboreal, whereas both Norway and Pacific rats are almost always found on the ground (Daniel 1972; Dowding & Murphy 1994; Hooker & Innes 1995). Ship rats are therefore considered a much greater threat to tree-nesting avifauna than other rat species present in New Zealand (Innes *et al.* 1999). The efficacy of ship rats as predators was cruelly demonstrated in 1964 when ship rats invaded Taukihepa (Big South Cape Island) (Bell 1978). Direct predation by ship rats on the fauna present on Big South Cape led to the extinction of the endemic Stead's bush wren (*Xenicus longipes*), Stewart Island snipe (*Coenocorypha aucklandica iredalei*) and the greater short-tailed bat (*Mystacina robusta*) (Bell 1978). The local extinction of the South Island saddleback (*Philesturnus carunculatus carunculatus*), the Stewart Island robin (*Petroica australis rakiura*), the South Island fern bird (*Bowdleria punctata*), the brown creeper (*Mohoua novaseelandiae*) and a large flightless weevil (*Hadramphus stilbocarpae*) also resulted (Bell 1978).

Ship rat densities recorded on mainland New Zealand and its associated offshore islands range substantially; from 1.7 rats/ha in the Orongorongo Valley (Daniel 1972), to 37.6–62.7 rats/ha on Taukihepa (Rutherford 2005).

1.6 Ship rats in the New Zealand urban environment

In New Zealand ship rats are the most common rat species found in urban areas (prey of household cats: Gillies 1998 and Y. van Heezik, unpubl. data; cage-trapping data: Morgan *et al.* 2009). The specific role ship rats have in structuring urban ecosystems, directly through predation and indirectly through food and habitat competition is poorly understood in New Zealand. In Canberra, Barratt (1997) showed commensal rats were a significant proportion of prey killed by household cats. Remarkably few estimates of urban rat density exist, considering the degree to which rats influence human populations (e.g. Emlen *et al.* 1949; Traweger *et al.* 2006). Ship rats are both nocturnal and highly dispersed making them particularly difficult to sample using conventional methods. Robust density estimates may provide an indication of the degree to which ship rats influence urban bird populations. Currently there is no low cost method readily available to estimate accurately absolute ship rat density.

The exact extent to which ship rats influence populations of urban wildlife is complicated by the presence of the domestic cat. Domestic cats are a 'super predator' in the urban environment due to the fact that they exist in unnaturally high densities (Woods *et al.* 2003; Baker *et al.* 2008; Sims *et al.* 2008). Few domestic cats rely on hunting prey for sustenance, but high cat densities ensure a substantial effect of secondary predation on certain prey species (Baker *et al.* 2005). Churcher and Lawton (1987) estimated that 30% of house sparrow (*Passer domesticus*) deaths in Bedfordshire England were due to cats. The flow-on effects caused by such levels of predation must influence the structure and functioning of urban ecosystems. However, eradication of cats from the urban environment could potentially have a paradoxical effect if meso-predators, such as ship rats exist. Uncontrolled populations of these predators could threaten wildlife to a far greater extent than the presence of cats (Fitzgerald 1988; Courchamp *et al.* 1999). The process whereby a reduction in superpredator abundance leads to a population explosion of mesopredators has been termed 'mesopredator release' (Fitzgerald 1990; Courchamp *et al.* 1999). In the urban environment the presence of a superpredator 'domestic cats' may in fact be indirectly beneficial to prey species (Baker *et al.* 2005). Fitzgerald and Karl (1979) suggest this is the case in the Orongorongo Valley forest, New Zealand, where a more dense population of native birds exist than would be expected if feral cats, that suppress the rat population, were not present.

The findings of Efford *et al.* (2006) also suggest an inverse relationship of cat and rat density in the Orongorongo Valley. Reducing the abundance and distribution of domestic cats in urban areas may therefore not be the best solution to protect endemic wildlife when mesopredators such as ship rats are present.

1.7 Abundance estimation

One of the fundamental issues involved in the research, conservation and management of rare or invasive species is determining precisely 'how many there are'. A range of techniques have been used for estimating the abundance of rare or highly dispersed species. The most accurate system is a complete enumeration where all individuals within the population are recorded. Counts like these are, however, seldom possible (Thompson *et al.* 1998; Stanley & Royle 2005), and in rats a complete population census would almost certainly be impossible. In most instances complete counts are only possible when a population is confined to a manageable area (island or fenced site), consists of a large conspicuous species, and exists in low numbers. For example mule deer (*Odocoileus hemionus*) in north western Colorado (Bartmann *et al.* 1986) or nesting northern royal albatross (*Diomedea sanfordi*) on the Otago Peninsula.

Indices of relative population density are almost always easier and quicker to obtain, making them cheaper than conventional methods for estimating absolute population density (Caughley 1977; Ruscoe *et al.* 2001). To estimate relative rat densities in New Zealand, snap trapping and ink tunnels are two commonly used methods (e.g. Taylor & Thomas 1993; Innes *et al.* 1995; King & Moller 1997; Innes *et al.* 1999; Harper 2005; Efford *et al.* 2006). However, there are several limitations to using these methods: (1) density indices vary significantly with sampling protocol and index type (Blackwell *et al.* 2002); (2) the assumption that an index value is correlated to the true population density is almost always unknown and rarely tested (Wilson *et al.* 2007); (3) indices make the possibly unwarranted assumption of equal catchability (or detectability) over time, and between different habitat types (Brown *et al.* 1996); (4) density indices cannot be compared between trapping sessions if factors such as trapping effort vary (McDonald & Harris 1999); and (5) seasonal variation in captures (or detections) may reflect changes in animal activity (Dowding & Murphy 1994), rather than changes in population density.

Measures of relative density appear most valuable in long-term studies that target a single study site where sampling methodology remains constant over the study period. For example, snap trap indices of Efford *et al.* (2006) show how a population of ship rats fluctuated over a 27-year period in the Orongorongo Valley. However, the inherent biases associated with index values reduce the wide-scale applicability of most conclusions (Blackwell *et al.* 2002).

Repeated live trapping and marking is a common technique for estimating the absolute abundance of small mammals (Otis *et al.* 1978). The profound cost associated with measuring absolute abundance currently limits its wide scale applicability.

Absolute abundance can be calculated by dividing a count by an estimate of the detection probability, obtained by fitting a statistical model to the captures and recaptures (Wilson & Delahay 2001). A range of alternative models have been developed to deal specifically with data collected from incomplete counts (see Pollock 1991).

1.8 Density estimation

In the past, converting estimates of abundance to estimates of absolute density has been complicated by the issue of defining an effective trapping area (ETA). Funding rarely allows for complete areas of habitat (e.g. national parks) to be sampled as a whole. In most instances the population being sampled is part of a much greater population in the surrounding region. Estimating density in this manner is clearly biased by an unknown 'edge effect' (Efford *et al.* 2005). In an undefined sample area animals are free to move in and out of the study site at any time. The population susceptible to sampling is therefore never constant. Attempts have been made to overcome this issue by estimating an ETA. Dice (1938) calculated an ETA by adding a boundary strip to the actual trapped area. Dice (1938) defined the boundary strip as the area equal to the average home-range radius of the animals within the trapped area. A severe limitation of this approach is that the size of an animal's home range can vary over time and between habitat types. Estimates of density that have been derived from an initial abundance estimate and an arbitrary approximation of ETA should therefore be treated with caution (Efford *et al.* 2004; Borchers & Efford 2008; Efford *et al.* 2009). Edge effect can be minimised by increasing the ratio of the size of the study area to the home range size of the

study species. However, increasing the area of a field site can make sampling unmanageable. This is particularly apparent in smaller studies that sample species with comparatively large home ranges.

1.9 Spatially explicit estimation of absolute density

Efford (2004) and Efford *et al.* (2004) demonstrated that absolute density could be estimated directly using spatially explicit capture-recapture (SECR). This eliminated the bias associated with relying on estimates of absolute abundance and the existence of an ETA to calculate density. In conventional capture-recapture (CR) the spatial dimensions of the trapping process are almost always ignored (Efford *et al.* 2009). Closed population capture histories are generally recorded in binary format with (1) representing a capture, and (0) no capture (Otis *et al.* 1978). A spatial capture history also records the location at which each capture occurred (Efford *et al.* 2009). Efford (2004) estimates density (D) directly by fitting a statistical model to the spatial dimensions of the trapping data (software: DENSITY).

Estimating absolute density is technically challenging, time consuming and expensive to perform (Innes 2005). However, an unbiased estimate of absolute population density is almost always a more useful and versatile parameter than an index (Efford 2004). Estimates of absolute density allow for direct comparison between sites, over time, and are free from many of the untested assumptions associated with indexing methods.

Currently in New Zealand the most reliable method for obtaining estimates of absolute ship rat density is thought to be live trapping and SECR analysis (e.g. Wilson *et al.* 2007). CR and live trapping of ship rats requires a grid of cage traps to be baited and set over a number of regular occasions. On each occasion rats are caught, possibly anaesthetised, marked in some manner (commonly ear tagged e.g. Latham (2006)), and released. The software DENSITY (Efford 2008) can then be used to estimate density directly from the spatial dimensions of the CR data. This approach relies heavily on recaptures of marked individuals on several subsequent occasions. For logistical reasons intense sampling effort like this is rarely possible on a large scale, which means most researchers resort to less desirable estimates of relative density.

1.10 Non-invasive sampling

Conventional CR using body tags requires direct capture and handling of animals at least once (Foran *et al.* 1997). Non-invasive sampling does not require direct human-target species contact at any stage during sampling. Sampling in this manner is particularly beneficial if the species in question is cryptic, secretive, elusive, vulnerable to human contact, highly dispersed, nocturnal or rare (Foran *et al.* 1997). Rats fit into nearly all of these categories; this makes them an ideal candidate for non-invasive sampling.

Injuries or death of target species resulting from hands-on capture is counterproductive when attempting to estimate population size, as well as being unethical in small or endangered populations. Direct handling of large carnivores, such as brown bear (*Ursus arctos*), can also be hazardous for researchers (Solberg *et al.* 2006).

Advances in modern photography and molecular techniques have allowed species that were previously difficult to sample, to be sampled more readily, and at a substantially reduced cost (Wilson & Delahay 2001). Remote camera trapping has been used where target species are individually distinguished by their external appearance. Karanth *et al.* (2004) estimated the density of tigers (*Panthera tigris*) in this manner using stripe patterns to recognise unique individuals. Advances in molecular genetic techniques have also lead to a proliferation of ecological studies investigating the abundance and distribution of species previously too difficult and costly to sample using invasive methods (Fernando *et al.* 2003).

1.11 Genetic sampling

In the last decade there has been a realisation that sloughed skin, saliva, feathers, regurgitates, faecal material, hair and eggshells are ready sources of template DNA (Taberlet & Luikart 1999). At present hair and faecal samples are used most frequently in non-invasive genetic studies (Waits 2004). Currently the most popular molecular markers in CR studies are microsatellites. By combining a number of independent polymorphic microsatellites a 'multilocus genotype' can be ascertained that is unique to each individual within a population (Palsboll 1999; McKelvey & Schwartz 2004). In the past macroscopic or microscopic examination of hair samples was used primarily to gain an indication of species or genera

present (Foran *et al.* 1997; Lindenmayer *et al.* 1999). Now a single hair follicle can be distinguished to the level of the individual by the analysis of polymorphic microsatellite loci (Goossens *et al.* 1998).

Non-invasive genetic techniques have been used to obtain population parameters of previously difficult-to-sample species without the need to capture, observe, or directly disturb the animal (Table 1.0). In addition to population parameters, collection of DNA can be utilised for auxiliary purposes such as information on sex ratio, home ranges, geographic distribution of genotypes, paternity and genetic relatedness (Kohn *et al.* 1999; Solberg *et al.* 2006). A further benefit of non-invasive genetic sampling is that it does not suffer the same issues of tag loss that traditional population studies have to deal with (Mills *et al.* 2000).

Nocturnal species like *Rattus* spp. can be extremely difficult to trap, and have been known to display trap avoidance behaviour. Cowan (1977) recorded neophobia or 'new object reaction' of ship rats to food baskets. Likewise Howard *et al.* (1987) suggests it may take up to several weeks for ship rats to enter foreign bait stations. Rats may also exhibit trap avoidance behaviour if the stress of the initial capture was substantial. In a cage-trapping study of ship rats, Innes (1977) failed to recapture 59% of rats after initial captures. Daniel (1972) also failed to recapture 53% of individual ship rats after initial captures. Innes (1977) proposed this could be due to death of individuals, migration, or trap shyness. Both migration and loss of individuals seem unlikely given the sample sessions of each of the aforementioned studies was run over a brief period of time i.e. days rather than months or years. Ship rats may therefore exhibit a strong behavioural response to cage-trapping in the form of trap avoidance. A non-invasive approach to sampling ship rats may decrease the likelihood of a 'trap avoidance response'. If rats are not constrained at any time then it is likely that they will not suffer the same capture related sampling stress of conventional CR using cage-trapping.

Table 1.0: Recent studies which have utilised non-invasive genetic sampling

Species	DNA source	Source
Grizzly bear (*Ursus arctos*)	Hair	(Mowat & Strobeck 2000)
Common wombat (*Vombatus ursinus*)	Faeces	(Banks *et al.* 2002)
American marten (*Martes americana*)	Hair	(Mowat & Paetkau 2002)
Black bear (*Ursus americanus luteolus*)	Hair	(Triant *et al.* 2004)
Humpback whale (*Megaptera novaeangliae*)	Skin biopsy and sloughed skin	(Palsboll *et al.* 1997)
European badgers (*Meles meles*)	Faeces	(Wilson *et al.* 2003b)
	Faeces	(Frantz *et al.* 2003)
Hairy-nosed wombat (*Lasiorhinus krefftii*)	Hair	(Sloane *et al.* 2000)
Brown bear (*Ursus arctos*)	Hair and faeces	(Taberlet *et al.* 1997)
	Faeces	(Solberg *et al.* 2006)
Mountain lion (*Puma concolor*)	Faeces	(Ernest *et al.* 2000)
Wolves (*Canis lupus*)	Faeces	(Creel *et al.* 2003)
Stoat (*Mustela erminea*)	Hair	(Efford *et al.* 2009)
Coyote (*Canis latrans*)	Faeces	(Kohn *et al.* 1999)
African elephant (*Loxodonta cyclotis*)	Faeces	(Eggert *et al.* 2003)

Ship rats are a typical nocturnal predator for which direct density estimation is often difficult. Ship rats are sensitive to human disturbance and can be difficult to recapture using conventional techniques such as cage-trapping, limiting the application of these methods to high density populations. A widely applicable technique to measure ship rat density, non-invasively and at low cost, stands to benefit conservation programmes throughout New Zealand.

1.12 Sampling in an urban area

People are a major component of the urban environment and it is important that their presence is taken into careful consideration before planning an urban ecology study. The presence of people and their pets constrains the methods that can be used in studies of urban ecology. For

example, lethal sampling of rats, using snap traps or poison, may pose an unacceptable risk to household cats or young children in urban areas. Urban residents may also interfere with field equipment either deliberately or accidently. This can complicate and compromise the results of urban ecology studies. Solving these methodological problems is a prerequisite for studying urban ecology.

1.13 Aims of this study

The overarching aim of this research was to gain an increased understanding of the distribution and density of ship rats within urban Dunedin: This constitutes an initial step towards ascertaining the role of ship rats as predators on urban bird populations. Knowledge regarding the distribution and density of ship rat populations may be useful for the planning and implementation of effective rat control measures.

This study focuses primarily on partly arboreal ship rats as opposed to ground-based Norway rats. Ship rats are of greater ecological interest in this study due to their potential ability to prey on, and suppress tree-nesting urban bird populations. Unpublished data on prey of household cats suggests that ship rats are the primary species present in urban Dunedin (Y. van Heezik, unpubl. data); assuming that household cats prey on either species of rat equally. In the following chapters I will refer to populations of ship rats that live in close association with humans as 'urban' or 'commensal' rats to distinguish them from 'non-urban' populations.

1.13.1 Specific objectives

The specific objectives of the project were to:

1. Determine the presence and distribution of rats (*R. Rattus* and *R. norvegicus*) within four distinct areas of the Dunedin urban environment: inner-urban bush fragments, peri-urban bush fragments, housed residential areas and an urban island.
2. Develop a non-invasive method to estimate ship rat density precisely and with minimum bias.

3. Test the density estimation technique on a well studied population of ship rats in the Orongorongo Valley.
4. Measure ship rat densities within urban Dunedin with a particular emphasis on areas where native bird populations breed most frequently i.e. urban bush fragments.
5. Compare the density of ship rats in urban forest fragments with that on an urban island free of other mammalian predators (possums, mustelids and cats).

2 METHODS

2.1 Field sites

The main field component of this research took place in Dunedin, New Zealand (45°52'S 170°30'E) (Figure 2.0). Sampling effort was focused in and around bush fragments, where native birds are most abundant and breed most frequently (van Heezik *et al.* 2008a). Four zones were initially defined: inner-urban bush fragment, peri-urban bush fragment, housed residential and urban island. Urban island was classified in this study as a body of land isolated from the mainland but within close proximity (<1km) to an urban area. The urban island was also one of the only sites in the Dunedin area that was free of household cats. Specific site selection within each study zone was driven by factors such as accessibility for monitoring purposes, suitability of habitat for rats (Morgan *et al.* 2009), habitat continuity and accessibility to the general public. Sites were only sampled if mammalian control operations had not taken place within them in the last 12 months.

2.1.1 Inner-urban: Dunedin town belt

The inner-urban bush fragment studied was the Dunedin town belt, a narrow stretch of inner city bush, ~200 m in width and over 5 km long, consisting of multi-tiered tree stands forming a closed canopy. The town belt is a patchy mosaic of native and introduced plant species with natives confined more to the northern half and exotics to the south. Of the native forest Scott (2001) describes two main types: kanuka-dominated and mixed broadleaf forest. This study focused sampling effort within areas of mixed broadleaf, which occurs most frequently in gullies and shaded sites. The canopy is predominantly mahoe (*Melicytus ramiflorus*), tree fuschia (*Fucshia excorticata*), lemonwood (*Pittosporum eugenioides*), kohuhu (*Pittosporum tenufolium*) and three finger (*Pseudopanax colensoi*). *Coprosma areolata* is present in an often sparse understory, while hounds tongue (*Microsorum pustulatum*) and hen and chicken fern (*Asplenium bulbiferum*) make up a patchy herb layer (Scott 2001). Two sample sites were selected within the town belt: 'Woodhaugh Gardens' (Figure 2.1) and 'Wallace block' (Figure 2.2).

2.1.2 Peri-urban: McGouns Creek bush

The peri-urban bush fragment was McGoun's Creek bush, a forested area of approximately 20 ha (Figure 2.3). McGouns Creek bush consists predominantly of native trees. Mahoe (*Melicytus ramiflorus*), broadleaf (*Griselinia littoralis*), rimu (*Dacrydium cupressinum*) and fuschia (*Fuschia excorticata*) form a mixed canopy. Supple-jack (*Ripogonum scandens*), wheki (*Dicksonia squarrosa*), and crown fern (*Blechnum discolor*) compose an occasionally dense understory.

2.1.3 Urban Island: Quarantine Island

Quarantine Island was used as the urban island study site. Quarantine Island is situated in Dunedin harbour ~200 m from Portobello marine laboratory and ~600 m from the township of Port Chambers on the opposite side of the harbour. Quarantine Island is 15 ha in size with a small stand (~6 ha) of dense native bush (Figure 2.4). Extensive re-planting has been carried out in recent years by the St. Martins Island Community trust to extend the remnant patch of forest. The bush consists largely of Halls totara (*Podocarpus totara*), lancewood (*Pseudopanax crassifolium*), flax (*Phormium tenax*), miro (*Podocarpus ferrugineus*) and matai (*Podocarpus spicatus*). The island is free of possums, mustelids and cats, but is believed to harbour rats (K. Mason, *pers. comm.*).

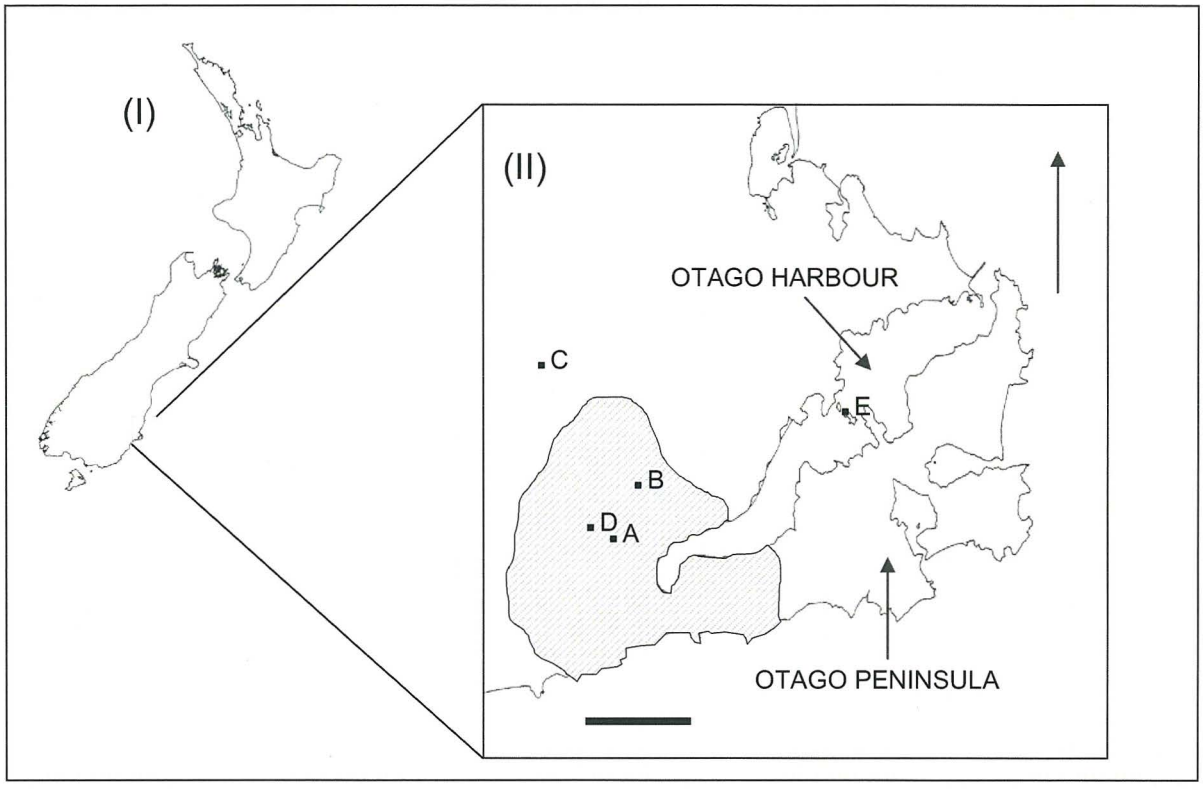

Figure 2.0: (I) Location of Dunedin and the Otago Peninsula in relation to the South Island of New Zealand. (II) Sample locations in and around urban Dunedin. A) Wallace block (inner-urban bush fragment); B) Woodhaugh Gardens (inner-urban bush fragment); C) McGouns Creek bush (Peri-urban bush fragment); D) Residential 1 (as defined by Freeman and Buck 2003) (housed residential zone) and E) Quarantine Island (urban island). The shaded region represents the central area of Dunedin. The scale bar equals 5 km.

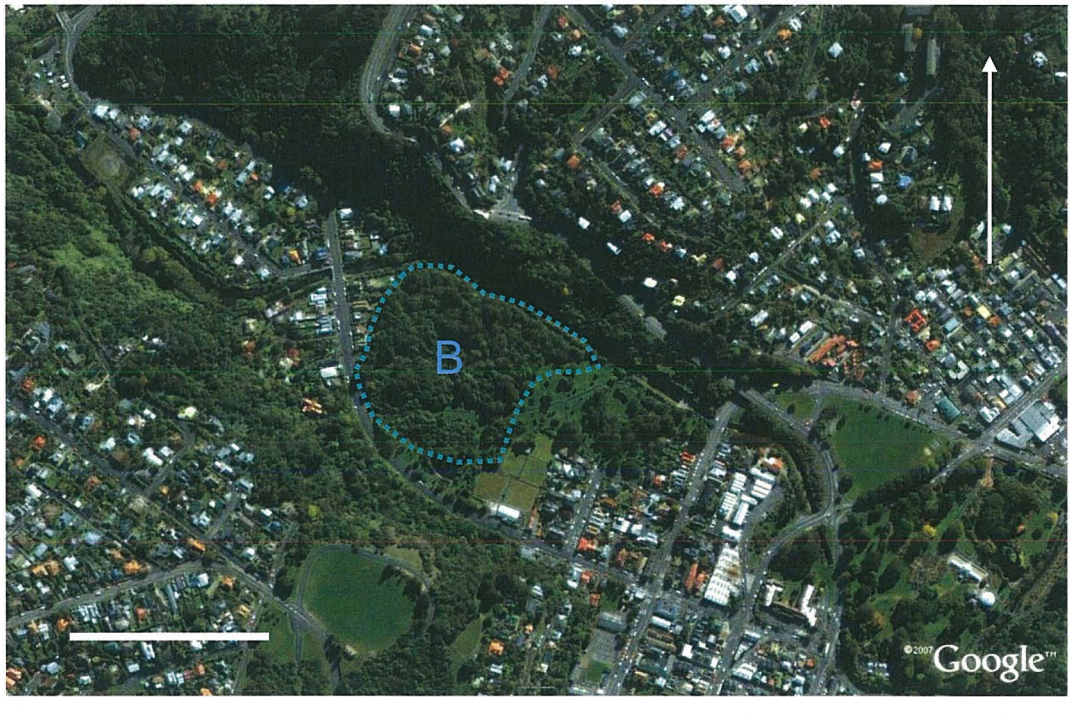

Figure 2.1: Composite aerial photography of (B) Woodhaugh Gardens study site (inner-urban bush fragment). The scale bar equals 300 m.

Figure 2.2: Composite aerial photography of (A) the Wallace block study site (inner-urban bush fragment). The scale bar equals 500 m.

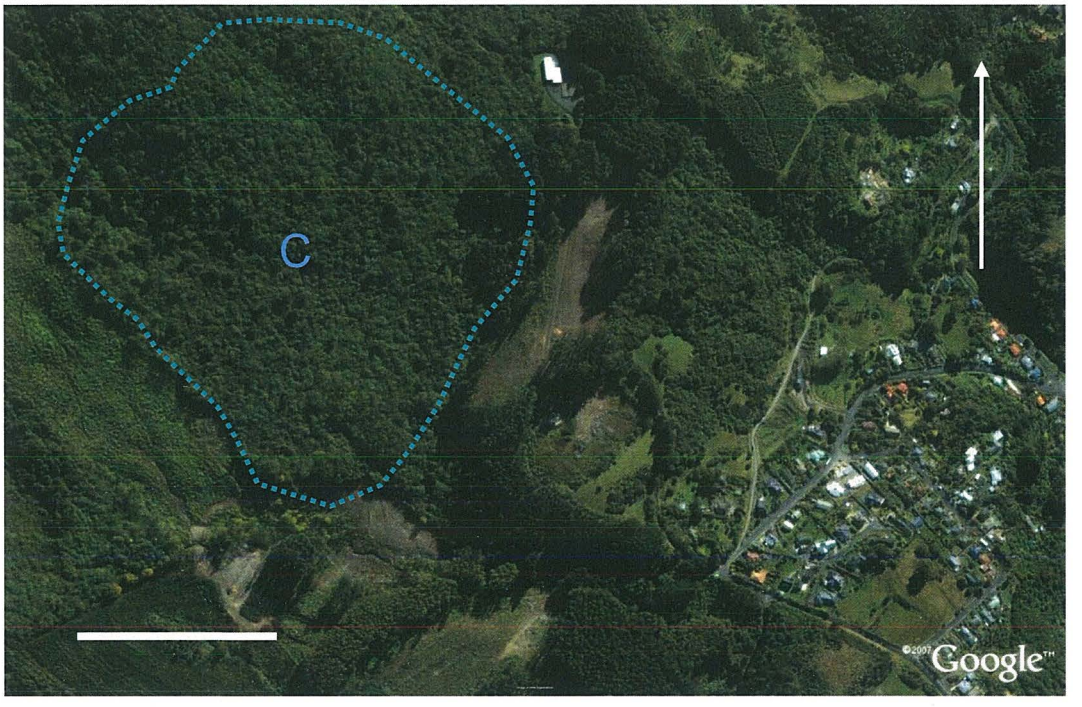

Figure 2.3: Composite aerial photography of (C) McGouns Creek bush study site (peri-urban bush fragment). The scale bar equals 300 m.

Figure 2.4: Composite aerial photography of (E) Quarantine Island bush fragment (urban island). The scale bar equals 500 m.

2.1.4 Housed residential

A 24 ha block of housed residential area was sampled with wax blocks only (Residential 1, as defined by Freeman & Buck 2003). This area contained garden-rich housing (1/3 of lot size as garden), with structured tree and shrub vegetation (Figure 2.5). The area of Residential 1 sampled was directly adjacent to the north-western extent of the town belt. Freeman and Buck (2003) distinguish two further housed residential areas (Residential 2 and 3). These contain smaller and less complex gardens and therefore most probably provide less habitat for rodents to live. These areas support lower densities of native birds, probably due to a reduction in suitable foraging and nesting habitat (van Heezik *et al.* 2008a). Residential sites 2 and 3 (Freeman & Buck 2003), were therefore considered of lower priority in this study.

Figure 2.5: Composite aerial photography of (D) Residential 1 study site (housed residential area). The scale bar equals 300 m.

2.1.5 Verification of methods: Orongorongo Valley

Non-invasive hair tube methodology has only recently been developed (results from stoat (*Mustela erminea*) hair tube data published in (Efford *et al.* 2009)) and hasn't previously been used to estimate rat density. Validation of the hair tube technique in an area known to support ship rats was therefore considered necessary.

Hair tube methodology was implemented in the Orongorongo Valley, New Zealand (41°21'S 174°58'E) (Figure 2.6 and 2.7). The Orongorongo field site harbours a population of ship rats which have been extensively studied since 1966 (Efford *et al.* 2006). The Orongorongo Valley consists of a mosaic of mixed podocarp-broadleaf forest and beech forest. By using existing trapping lines, the initial time consuming step of establishing a new survey grid could be avoided. Density estimates obtained in the Orongorongo Valley were compared to recent cage-trapping estimates (D.J. Wilson, A.E. Byrom, R. Pech, M. Perry, D.P. Anderson, Landcare Research, unpubl. data).

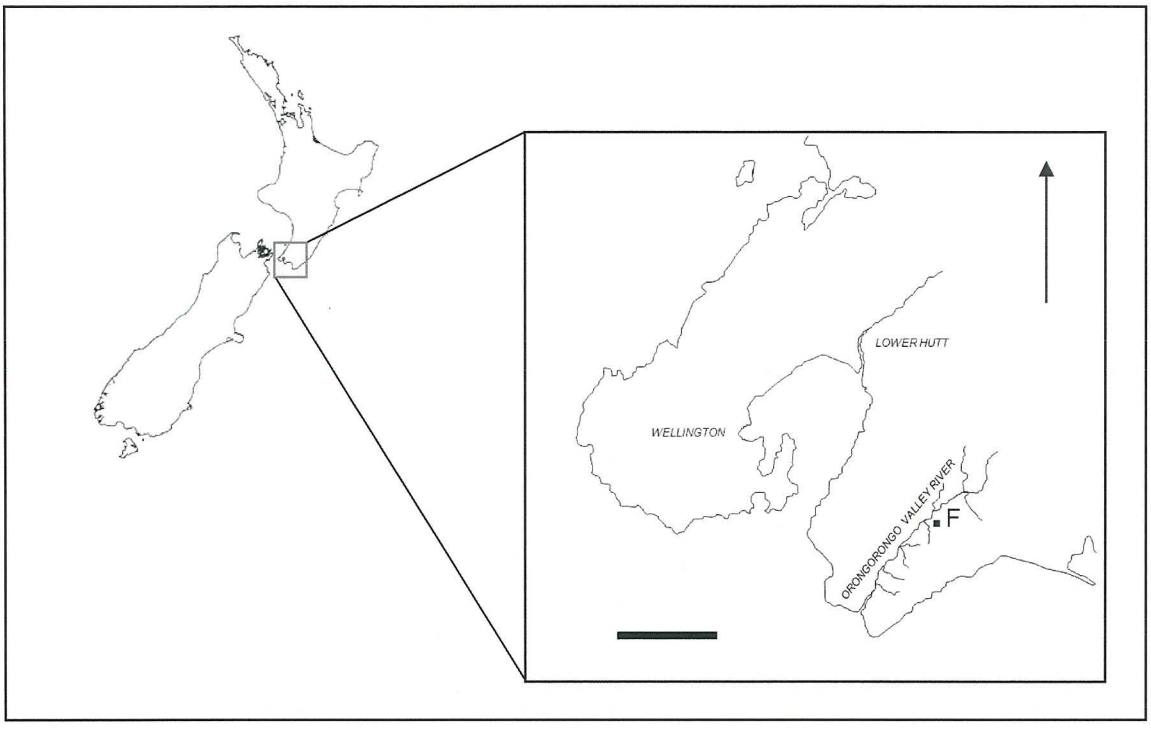

Figure 2.6: Location of (F) 'Woottons grid' at the Orongorongo Valley field site, in relation to the North Island of New Zealand. The scale bar equals 10 km.

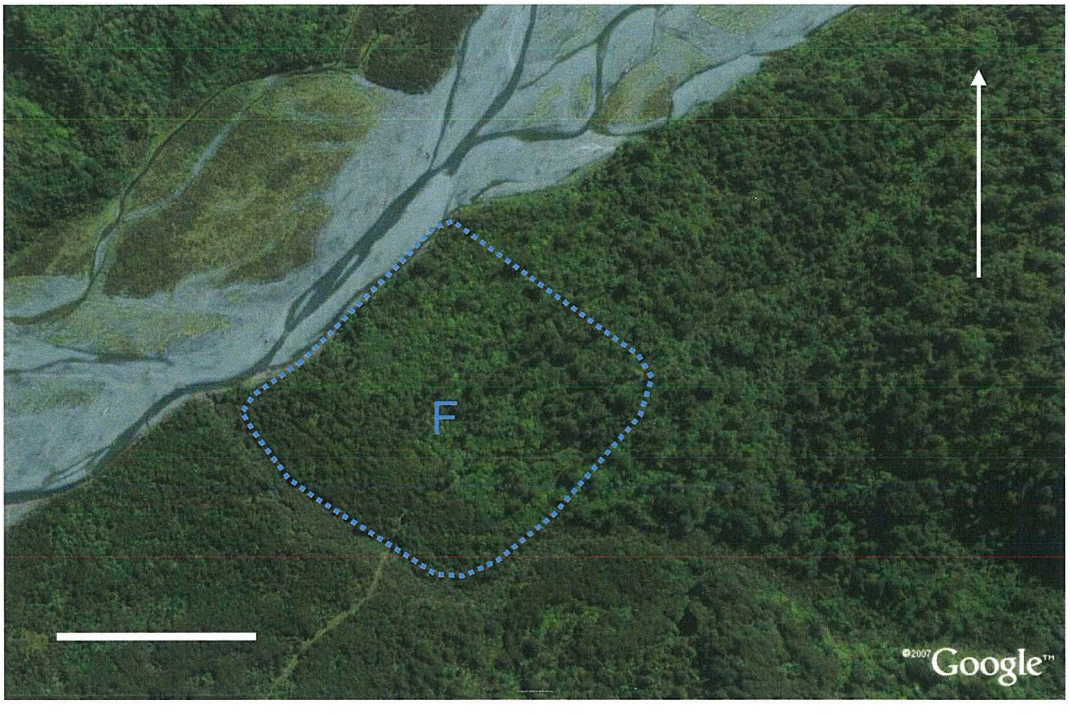

Figure 2.7: Composite aerial photography of (F) Woottons grid study site (Orongorongo Valley). The scale bar equals 200 m.

2.2 Field protocol

2.2.1 ***Phase 1:*** *Using wax blocks for the detection of rat presence and assessment of rat distribution in the Dunedin urban environment*

Wax blocks are a bait interference method of sampling species presence (Thomas *et al.* 2003). In this study rat presence and distribution across various urban habitats was determined by the identification of genus-specific bite marks on wax blocks. Wax blocks were used in this study because they are non-toxic, small, lightweight, easy to use in the field and cheap (NZ $0.85). These features of wax blocks are all highly desirable when sampling in urban localities. The nature of wax blocks also meant a wider sampling area could be covered compared with alternative techniques such as rodent tracking tunnels.

This study used wax blocks sold under the trademark 'Waxtag®' (Pest Control Research Ltd, Christchurch); each unit consisted of a small triangular piece of fluorescent plastic with a lump of non-toxic wax at the apex (Figure 2.8). Using a compass and handheld GPS, a single sampling grid was established at each field site. The locations of the sample points on each grid were recorded to within 10 m and uploaded to MapSource Version 6.13.7. Sample points were then overlaid on a topographic image of each sample site.

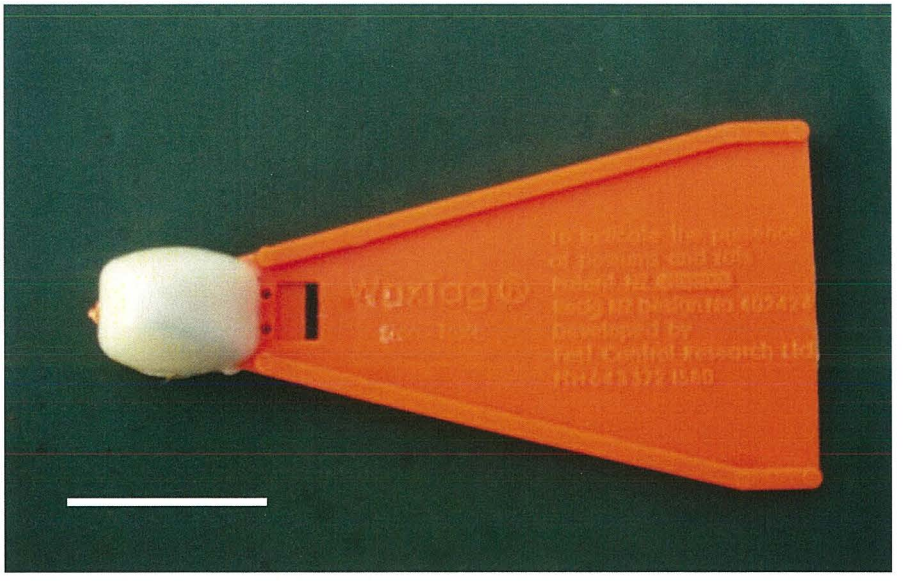

Figure 2.8: Wax block. The scale bar equals 30 mm.

A single wax block was placed at each sampling point. The number of sampling points (n) and their arrangement, varied between field sites, depending on the size and shape of each site. At McGouns Creek six lines of 17 wax blocks (n_{total} = 101) were spaced at 50 m intervals between lines and 25 m intervals along lines (Figure 2.9). To install a similar number of wax blocks in each study site it was necessary to restrict the distance between sampling points in inner-urban study sites. In the town belt Wallace block five lines were established, with wax blocks spaced at 25 m between lines and 25 m along lines (n = 91 wax blocks, Figure 2.10). In the housed residential study site a regular grid was less feasible due to access restrictions in certain areas. Random allocation of sampling points across the housed residential study site was therefore implemented (Figure 2.11). A simple random sample of points was overlaid on a satellite image of the field site. In each case, the nearest household to the random sample point was chosen for sampling. If one household did not wish to take part or a sample point was inaccessible, then the next sample point was chosen.

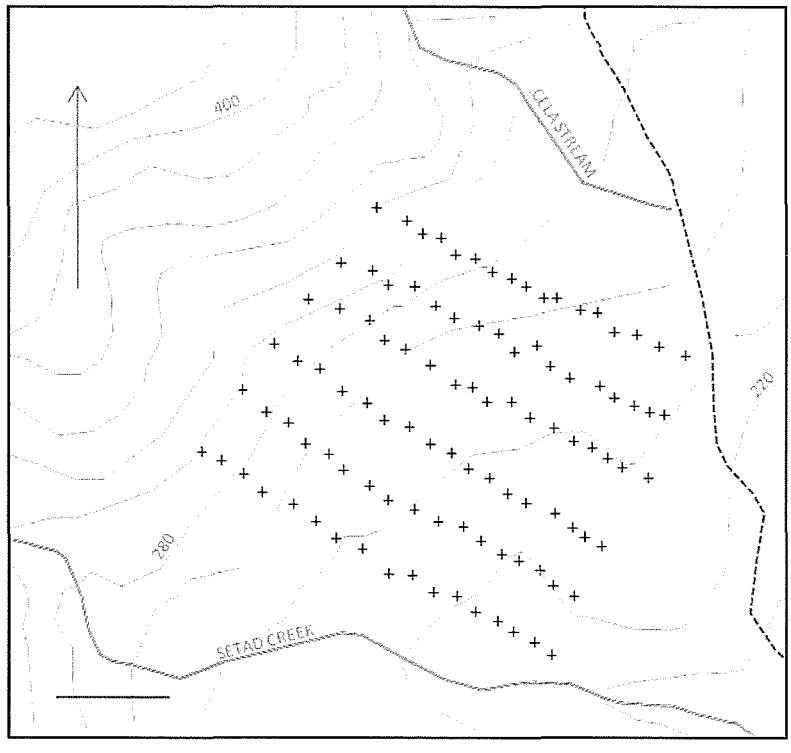

Figure 2.9: McGouns Creek bush sampling grid (n = 101) with wax blocks spaced at 25m along lines and 50m between lines. The scale bar equals 100 m.

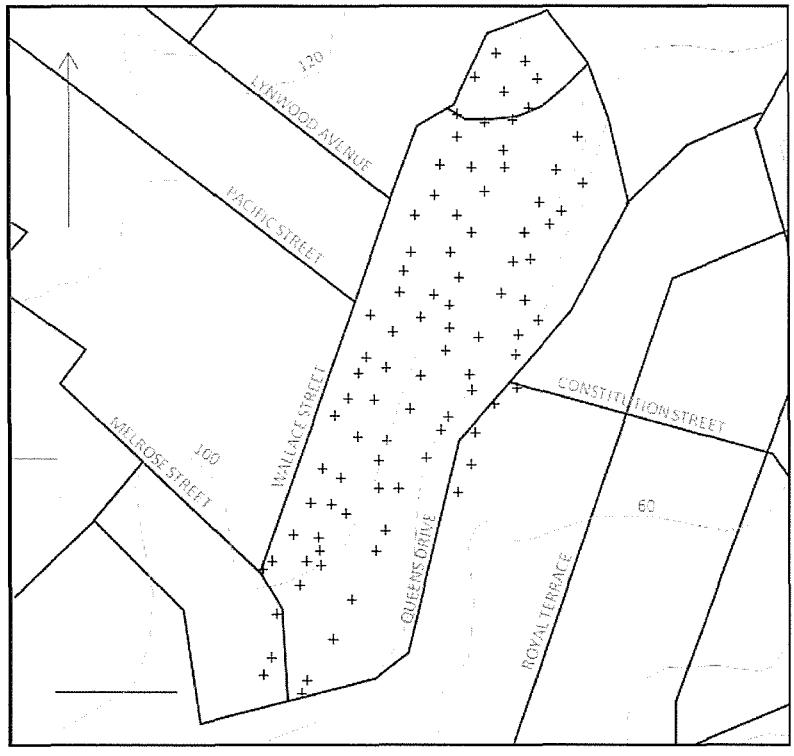

Figure 2.10: Wallace block sampling grid (n = 91) with wax blocks spaced at 25m along lines and 25m between lines. The scale bar equals 100 m.

Figure 2.11: Housed residential sampling grid (n = 56) with wax blocks spaced at varying intervals due to random allocation of sampling points. The scale bar equals 100 m.

Within the housed residential area wax blocks were placed after 5pm, when occupants were most likely to be home. Each household where a randomly allocated sample point was positioned was visited and asked whether they would mind a wax block being placed in their garden. Each participating household was given an information sheet briefly outlining the study and reassuring household occupants of the non-invasive and non-toxic nature of wax blocks.

Wax blocks were placed in the town belt from the 6th August 2008 – 10th August 2008, in McGouns Creek from the 21st July 2008 – 25th July 2008; and in the housed residential study site over three nights 17th – 19th March 2008 (collected 21st – 23rd March 2008). Peanut butter was smeared on wax blocks as a lure. Peanut butter is widely used in studies to increase the rate of ship rat detection or capture (e.g. Brown *et al.* 1996; Blackwell *et al.* 2002; McKay & Russell 2005). Unpublished data on the foraging patterns of ship rats suggests that ship rats forage most frequently under or close to dense vegetation, compared with more open habitat types (D. Wilson, unpubl. data). Wax blocks were therefore fixed as close to dense cover as possible and just above ground level (to exclude non-target interference from hedgehog and Norway rats).

2.2.2 ***Phase 2:*** *Using non-invasive hair-snag tubes to estimate rat density*

Tissue samples in the form of hair follicles were collected using hair-snag tubes. An array of hair tubes was placed in each study site and checked daily for the presence of hair, over seven days. Hair tubes consisted of 220 mm lengths of 65 mm PVC down pipe. Two slits were cut half way through the tube, 40 mm in from either end. A 16 mm wide rubber band with 229 mm diameter was stretched between the two slits so that the rubber band partly occluded the opening at both ends (Figure 2.12). The rubber bands were coated with diluted TRAPPER® glue, sourced from Pest Management Services, Paraparaumu. To dilute, the glue was heated gently in a 100 degrees oven for approximately 20 minutes (or until fluid). In a fume cupboard toluene was added until the glue formed a thick honey consistency.

Hair tubes were baited with about 5 g of peanut butter in the middle of the tube. To prevent movement tubes were pegged to the ground using a loop of number eight wire. During

monitoring, if hairs were found, the hair tube containing the hair was replaced. Each sample was removed with forceps and bagged individually and labelled according to sample location and sampling occasion. If hair tubes were disturbed by non-target species then the disturbance was noted and the tube reset. The population of ship rats at each study site was assumed to be closed i.e. no births, deaths or migrations during each sampling session.

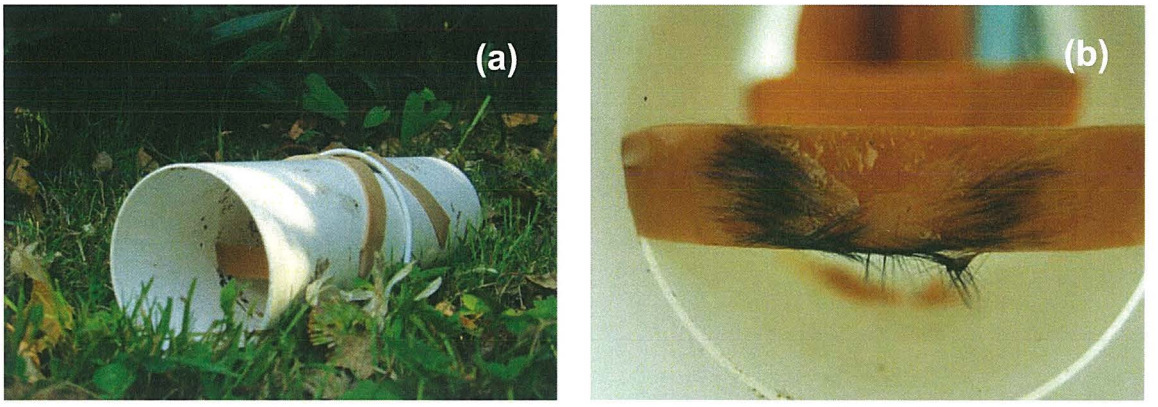

Figure 2.12: (a) Hair tube with rubber band occluding the opening of the tube at either end (b) hair sample retained on an adhesive coated rubber band, viewed inside a hair tube. The stretched width of the rubber band equals 10 mm.

2.3 Verification of hair tubes in the Orongorongo Valley

To verify the efficacy of the hair tube methodology a field trial was carried out in the Orongorongo Valley, Wellington. This field trial ran for one week from the 10th June 2008 – 17th June 2008. 100 hair tubes were placed in a 10 x 10 array on Woottons grid (a pre-existing sampling grid). Tubes were spaced at 25 m intervals; the polygon obtained by joining the outermost sampling points had an area of just over 5 ha (Figure 2.13). Tubes were set and checked daily following the same protocol as in the urban study sites. Rain on the final monitoring occasion meant a large number of samples were damp when collected. These samples remained damp until they were processed in the lab several days later.

2.4 Using hair tubes to estimate rat density in urban Dunedin

Hair tubes were placed on the existing wax block grids of the Wallace block and McGouns Creek, but not the housed residential site (after failing to detect a single rat using wax blocks).

After initial hair-tubing resulted in low rat numbers being detected, two additional sites were surveyed: Woodhaugh Gardens and Quarantine Island.

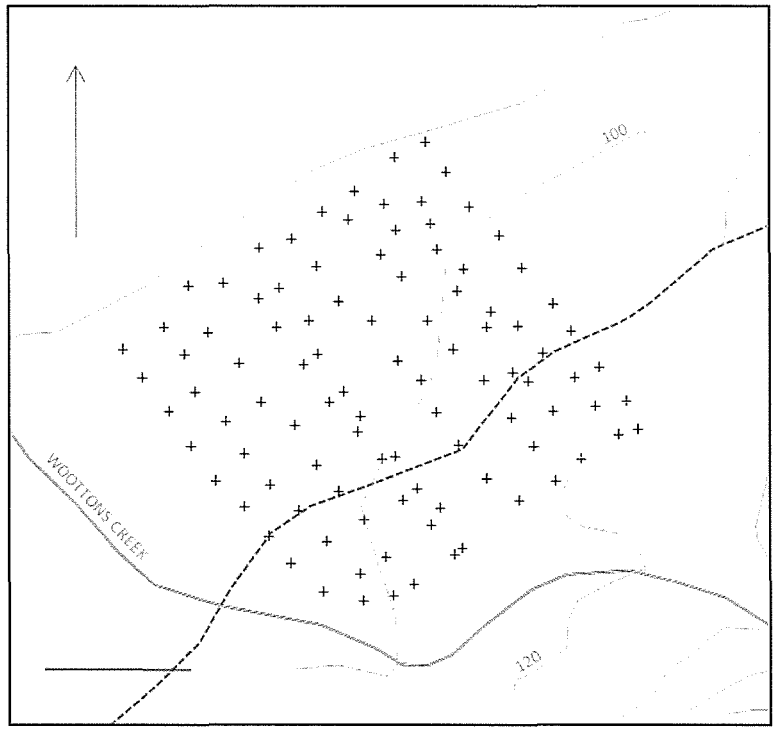

Figure 2.13: Woottons sampling grid (n = 100) (Orongorongo Valley) with detectors spaced at 25m along lines and 25m between lines. The scale bar equals 100 m.

Hair tube grids were established at Woodhaugh Gardens (Figure 2.14) and Quarantine Island (Figure 2.15) following the same procedure as in earlier field sites. Quarantine Island has areas of steep and densely vegetated terrain that were inaccessible to sample. This made establishing a regular grid on the island difficult and the spacing of sampling points was variable (Figure 2.15). The time period between sampling of different study sites was kept to a minimum to prevent complications associated with seasonal fluctuations in rat abundance and ensure comparability between sites. Within the Wallace block hair tubes were laid on the 18th July 2008 and collected on the 25th July 2008. Within McGouns Creek hair tubes were placed on the 7th July 2008 and collected on the 14th July 2008. In Woodhaugh Gardens hair tubes were placed on the 14th September 2008 and collected on the 21st September 2008. Lastly on Quarantine Island hair tubes were placed on the 13th September 2008 and collected on the 23rd October 2008. After failing to detect a single rat after a week of sampling on Quarantine Island, tubes were left in the field for an additional five weeks before collection; hair tubes were not monitored daily over this period.

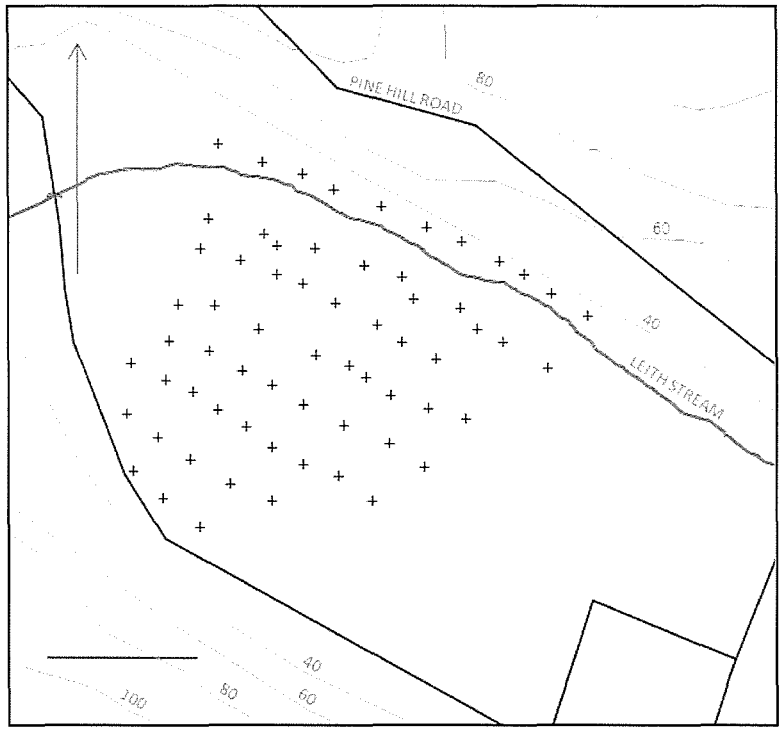

Figure 2.14: Woodhaugh Gardens sampling grid (n = 65) with detectors spaced at 25m along lines and 25m between lines. The scale bar equals 100 m.

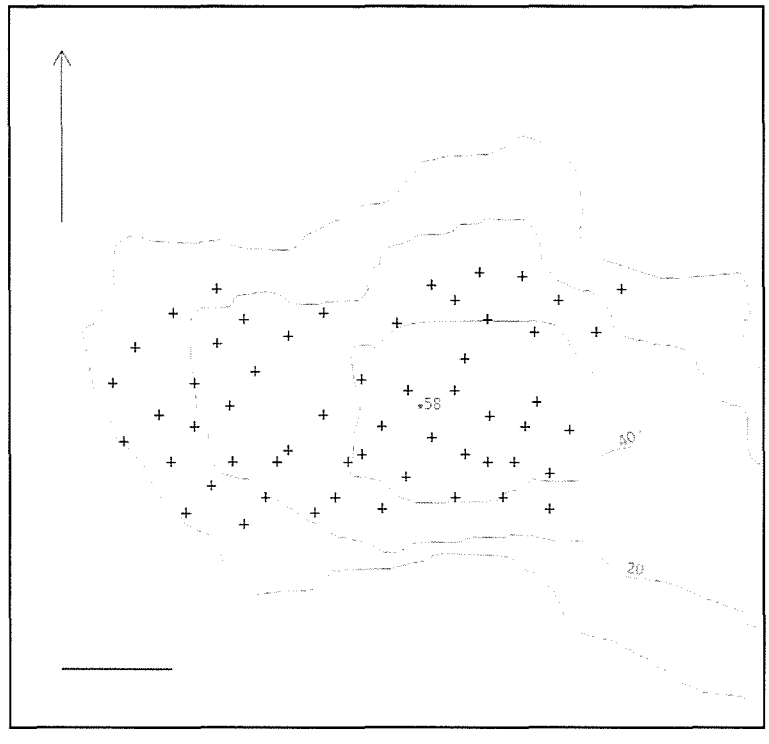

Figure 2.15: Quarantine Island sampling grid (n = 56) with detectors spaced at approximately 25m along lines and 25m between lines. The scale bar equals 100 m.

Samples collected from Woodhaugh Gardens were dried and stored for a month before being sent for microsatellite analysis. Unfortunately these samples got lost in the post, but eventually arrived in Auckland five weeks later. The time between collection and processing of Woodhaugh Gardens samples was therefore over eight weeks. This was much longer than the period between collection and analysis of other samples.

2.5 Analysis

2.5.1 ***Phase 1:*** *Examination of wax blocks*

Reference tags (provided by M. Thomas, Pest Control Research Ltd, Christchurch) were used to assess whether sign was due to rats, or other non-target species. Size and shape were the main characters used to differentiate the bite marks of different species. Rats left, upper- and lower-incisor bite marks (Figure 2.16 (a) & (b)) were much smaller (0.4–1.4 mm), than those from possums for example. Typically, possum interference was distinguished by wide (2–4 mm) incisor marks (Figure 2.16 (c)) and often also by deep canine and pre-molar grooves. Mouse bite marks were similar to those of rats but considerably narrower and shorter (Figure 2.16 (e)). Cat bite marks were distinguished by a series of overlapping grooves formed by the canine teeth (Figure 2.16 (f)). Likewise dog (*Canis familiaris*) bite marks were easily distinguished by the large carnassial grooves (Figure 2.16 (d)).

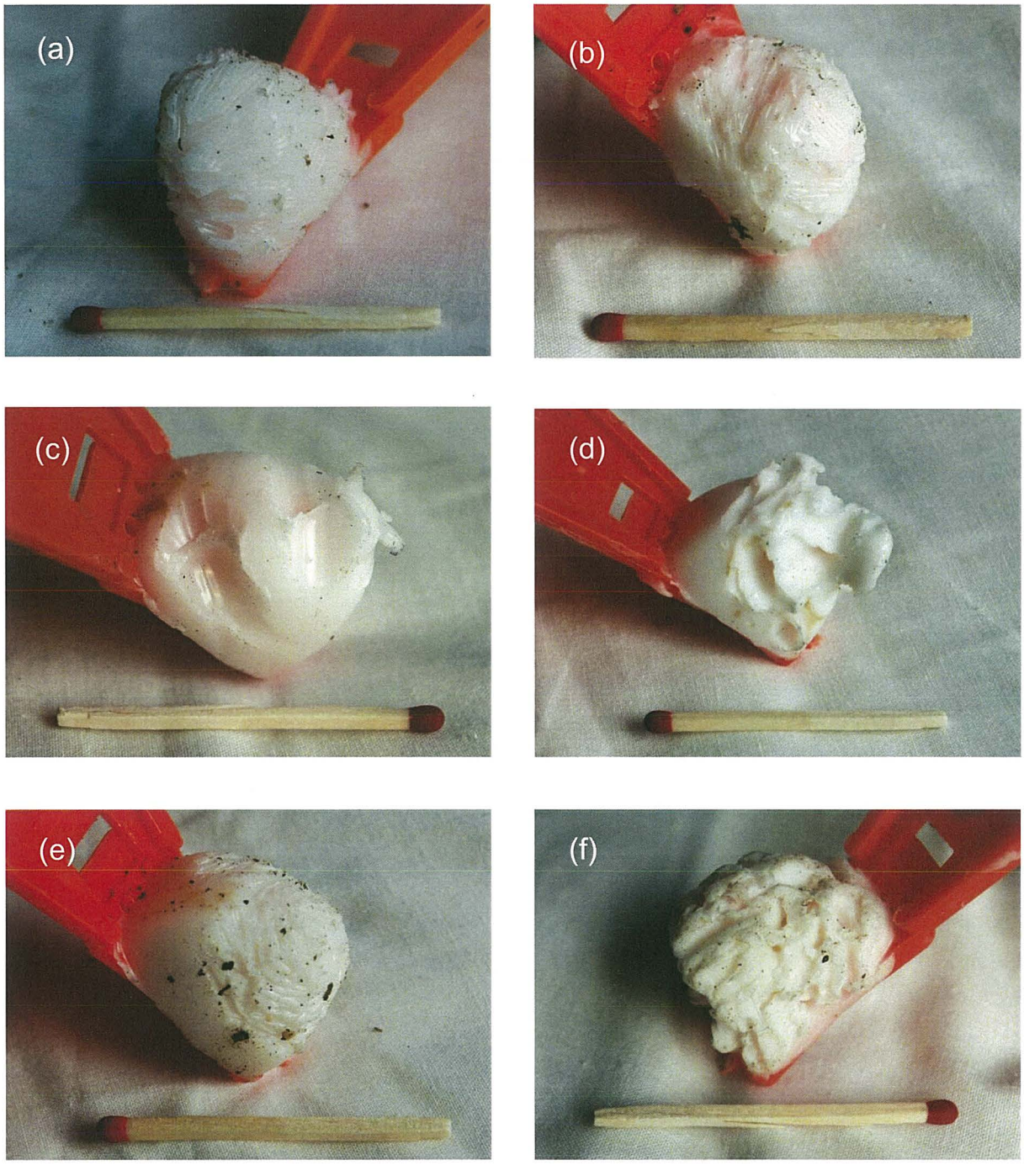

Figure 2.16: Reference wax blocks (a) & (b) ship rats incisors; (c) possum incisors; (d) dog carnassials; (e) mouse incisors; (f) cat carnassials. The match sticks provide scale.

2.5.2 ***Phase 2:*** *Identification of hair samples*

Each section of rubber band with adhering strands of hair was carefully removed and placed in a small zip-lock bag with a piece of filter paper to absorb any excess moisture. Hair samples were distinguished as rat or non-target species by careful visual examination. In cases where samples were not easily distinguished as rat by macroscopic examination, the cuticle scale pattern and medulla structure of hair strands were observed using a compound microscope, following Day (1966) .

Polyvinyl acetate (PVA) was used as a medium to produce cuticle scale casts. On each glass slide a thin and even film of dilute (50% solution of PVA and distilled water) PVA was applied. Using forceps, hair strands were placed on the PVA, gently pressed into the medium to ensure adequate surface contact, and left to set. Once the glue had set (about 20 minutes at room temperature), hair was carefully removed, leaving behind an impression of the surface structure of each hair strand. For viewing casts, a second slide was placed on top of the first and the sandwich was inverted. This arrangement ensured scale patterns were viewed as they actually appeared on each strand of hair. A photographic reference collection of scale casts from known ship rat and house mouse hair samples was initially made to compare hair tube samples against (Figures 2.17 & 2.18).

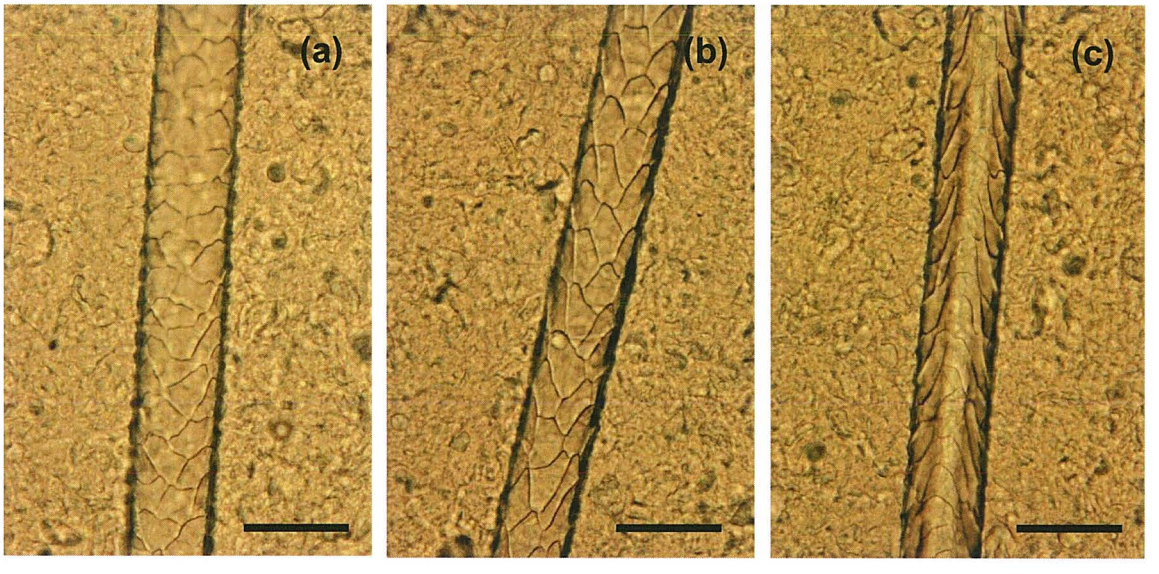

Figure 2.17: Cuticle scale casts of hair samples from a house mouse: (a) convex side of a primary guard hair with regular wave-like scales; (b) primary guard hair with broad petal-like cuticle scales; and (c) concave side of a primary guard hair, with regular wave-like scales. The scale bar equals 50 microns.

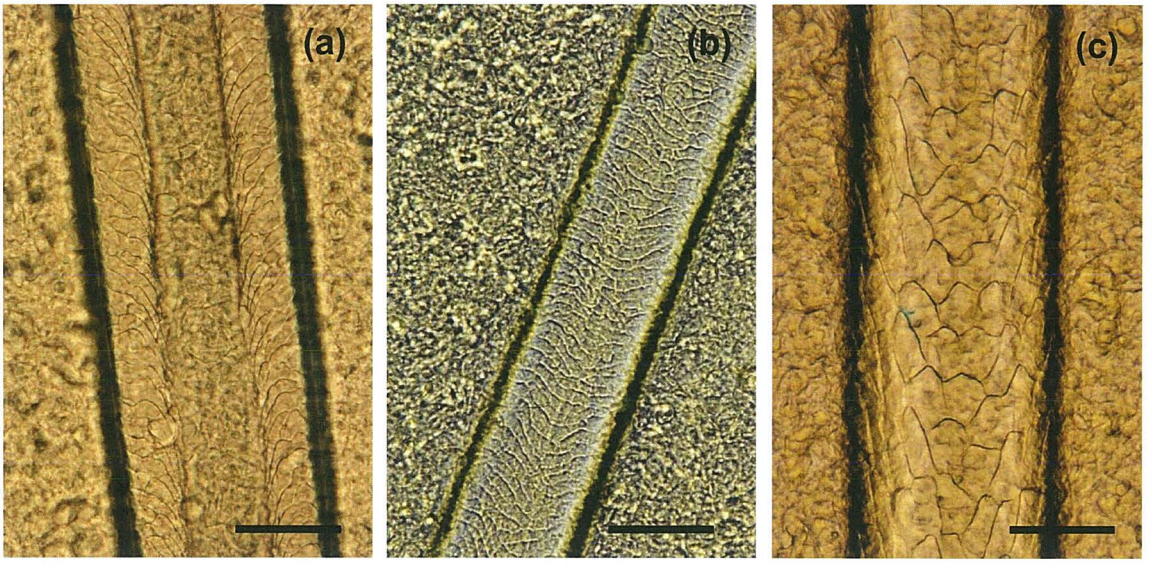

Figure 2.18: Cuticle scale casts of hair samples from a ship rat: (a) concave side of a primary guard hair with broad petal scale pattern; (b) irregular wave scale pattern, typically found near the tips of ship rat primary guard hairs; and (c) concave side of a primary guard hair with broad petal scale pattern and uneven scale edges. The scale bar equals 100 microns.

To view the structure of the medulla, strands of hair were mounted whole in paraffin oil. This allowed the appearance and pigmentation of the medulla to be easily viewed under a compound microscope. Reference photos (Figure 2.19) were taken of mounted hair strands of known origin. These were then compared to unidentified hair samples. If there was still uncertainty as to whether a hair sample was from a rat then the sample was sent for microsatellite analysis along with definite rat samples. Non-rat samples were readily distinguished from their microsatellite DNA and rejected from further analysis.

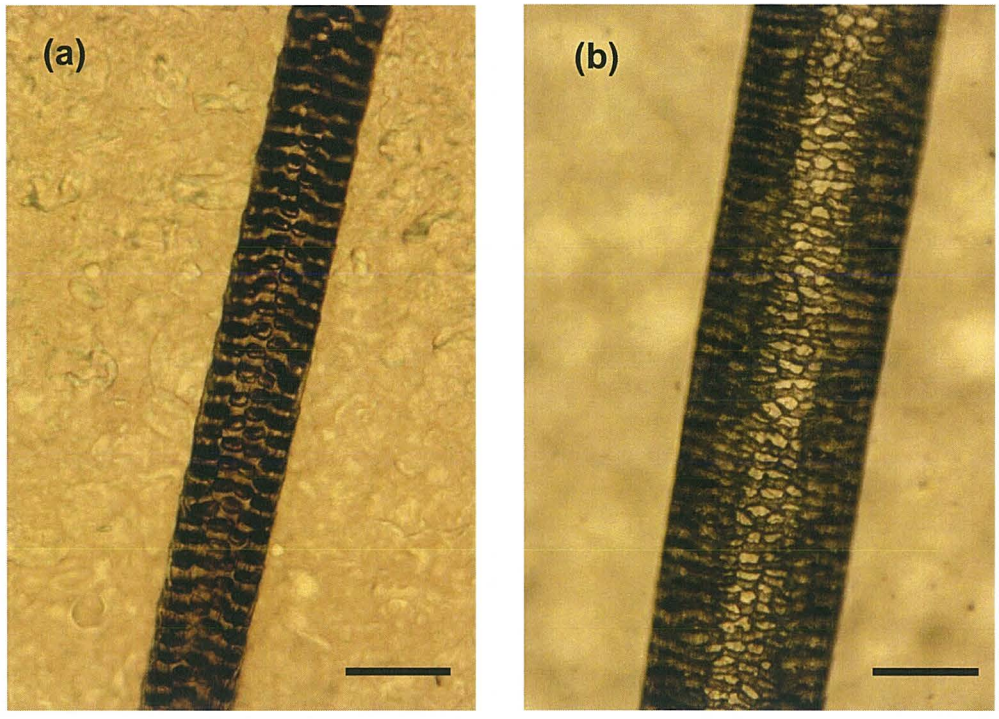

Figure 2.19: Whole mount of primary guard hairs showing the wide aeriform lattice medulla structure of (a) house mouse (scale bar equals 50 microns); and (b) ship rat (scale bar equals 100 microns).

2.5.2.1 Microsatellite analysis

Samples were sent to the Ecological Genetics Laboratory, Landcare Research, Auckland for microsatellite profiling and gender analysis. Nine polymorphic microsatellite loci were amplified (D2Rat234, D5Rat83, D7Rat13, D11Mgh5, D19Mit2, D10Rat20, D15Rat77, D16Rat81, D18Rat96); and one sex-specific marker. Individuals within a population are distinguished by their genotypes i.e. the base-pair lengths of the two alleles at each locus.

Samples of hair from ship rats and Norway rats were not morphologically distinguishable, but both may have been present. The primers used in microsatellite analysis are suitable for the analysis of both species. Norway rats exhibit short base-pair lengths at the D11Mgh5 and D19Mit2 loci (R. Howitt, *pers comm.*). Multilocus genotypes could therefore be easily screened for the presence of samples collected from Norway rats.

Genotyping errors, due to allelic dropout or false alleles (see Taberlet *et al.* 1996), were identified by comparison of similar genotypes. An individual's spatial detection history and

gender was also used to further confirm the presence of genotyping errors. Genotyping errors rates were reported as the average number of errors per locus (or per locus error rate).

2.5.2.2 Estimating the probability of individual identification among genotypes

When using microsatellite loci to construct multilocus genotypes there is a possibility that two or more individuals may have identical genetic profiles. Mills *et al.* (2000) proposed that in genetic profiling enough polymorphic microsatellite loci should be used to distinguish individuals with 99% confidence. Probability of identity (PI) is the probability of observing identical multilocus genotypes in two individuals drawn at random from the same population (Ayres & Overall 2004). PI should be small to avoid misidentification. In this study PI was estimated as an indication of whether the number of microsatellite loci was sufficient to consistently distinguish individuals. PI was computed using formula (1) below, which assumes Hardy-Weinberg equilibrium (Mills *et al.* 2000). PI at each locus (with multiple alleles) is calculated as the sum of squares of the expected frequencies of all possible genotypes (Paetkau *et al.* 1998; Mills *et al.* 2000). It is usual to calculate PI for one locus at a time, and to multiply these values together, assuming independence among loci. The following formula was used:

$$PI_{singlelocus} = \sum_i p_i^4 + \sum_i \sum_{j>i} (2p_i p_j)^2 \qquad (1)$$

where p_i and p_j are the frequencies of the ith and jth alleles (Mills *et al.* 2000).

In populations where close relatives are likely to be sampled Taberlet *et al.* (1999) recommend using the following formula to estimate the probability of identity between siblings (PI_{sibs}):

$$PI_{sib} = 0.25 + \left(0.5 \sum_{pi} 2\right) + \left[0.5 \left(\sum_{pi} 2\right)^2\right] - \left(0.25 \sum_{pi} 4\right) \qquad (2)$$

where p_i is the frequency of the i_{th} allele (Waits *et al.* 2001). PI_{sibs} is a conservative upper limit of the probability of observing two individuals with identical multilocus genotypes (Waits *et al.* 2001; Ayres & Overall 2004).

PI and PI_{sibs} values were calculated for each locus. The product of values for all loci was used to obtain an overall probability for both PI and PI_{sibs}:

$$PI_{overall} = \prod(PI_{single\ locus}) \qquad (3)$$

2.5.3 ***Phase 3:*** *Spatially explicit capture-recapture*

Once individual genetic profiles had been obtained, spatially explicit capture–recapture (SECR) models (e.g. Borchers & Efford 2008; Efford *et al.* 2009) were applied using DENSITY 4.1 (Efford 2008). For each data set the specific location of each hair tube (x, y coordinates) and the capture history of each individual was imported into DENSITY. Hair tubes were considered a proximity detector in DENSITY because they do not detain individuals at any stage during the sampling process.

In SECR the spatial location of each animal within the trapping grid is summarised as a single point, termed the 'home range centre'. The home range centres of all individuals are assumed to follow a 2-D Poisson distribution with density D. The probability that an individual will be detected at a particular hair tube is assumed to be a function of the distance from the individuals' home range centre to the given hair tube.

Three parameters were estimated concurrently from the trapping data: density (D), g_0 and σ. D is the key parameter of ecological interest, g_0 is the probability of detection when the distance r from a given detector equals 0, and σ represents the spatial scale of an individual's movements. The parameters σ and g_0 combine to make the 2-parameter spatial detection function $g(r)$. Maximum likelihood was applied numerically to estimate D and the parameters of the detection function g_0 and σ. At each study site populations were assumed 'closed' (population closure (demographic and geographic); equal probability of capture; and mark retention) over the seven day sampling period.

In DENSITY a set of *a priori* models was developed to incorporate more or less model complexity. By allowing for variation in capture probability due to time or behaviour for example, a better fitting model may result. Models can also become over complicated in an

effort to reduce bias, and therefore lose model precision. The best fitting model can be selected by comparing a set of candidate models that vary in structure and complexity. In this study, selection between candidate models was guided by lowest AICc (Akaike's Information Criterion corrected for small sample size), a measure of both fit to data and parsimony of model. AICc trades off model bias with model precision to select the so-called model of 'best fit'.

AICc values were calculated for each of the candidate models. Burnham *et al.* (2002) recommend three rules-of-thumb for model selection: (1) models are approximately equal if the difference in AIC value is less than 2; (2) if AIC values vary between 2 and 7 then there is some support for a real difference between alternate models; and (3) if the difference in AIC is greater than 7 then there is strong evidence to support an actual difference in the fit of alternate models. In this study normalised Akaike weights (w_i) were calculated in instances where AICc values differed by less than 7 (Burnham & Anderson 2002). Akaike weights are the probability that a given model is the 'best model' in the set. Weighted values give an indication of the degree to which one model is more or less supported than other alternative models.

3 RESULTS

3.1 Wax blocks

Interference was recorded on 91% of all wax blocks after four nights. The presence of rats was confirmed in McGouns Creek bush and the Wallace block, but not in the housed residential study site (Figure 3.0, 3.1 and 3.2). The 11 detections of rat in the Wallace block were localised to the north-eastern extent of the sampling grid. Only three wax blocks recorded rat interference in McGouns Creek, two of which were adjacent to one another.

The rate of interference on wax blocks by non-target species was high in all three study sites: 77% at the housed residential site; 79% in the Wallace block and 96% at McGouns Creek bush (Figure 3.3). Of the 232 wax blocks from which interference was recorded, approximately 5% were classified as unidentified. A single wax block was also removed by a person that lived in the residential site being sampled. On rare occasions (~3%) interference from two different species was observed on a single wax block.

The composition of species present, determined by comparing bite marks, varied substantially between study sites (Figure 3.3). Within the housed residential area, 63% of wax blocks were chewed by cats. In the Wallace block and McGouns Creek 50% and 52% of wax blocks respectively were chewed by possums. Interference by mice was common in both the McGouns Creek (42%) and the Wallace block (20%) and was also frequently detected in the housed residential area (9%).

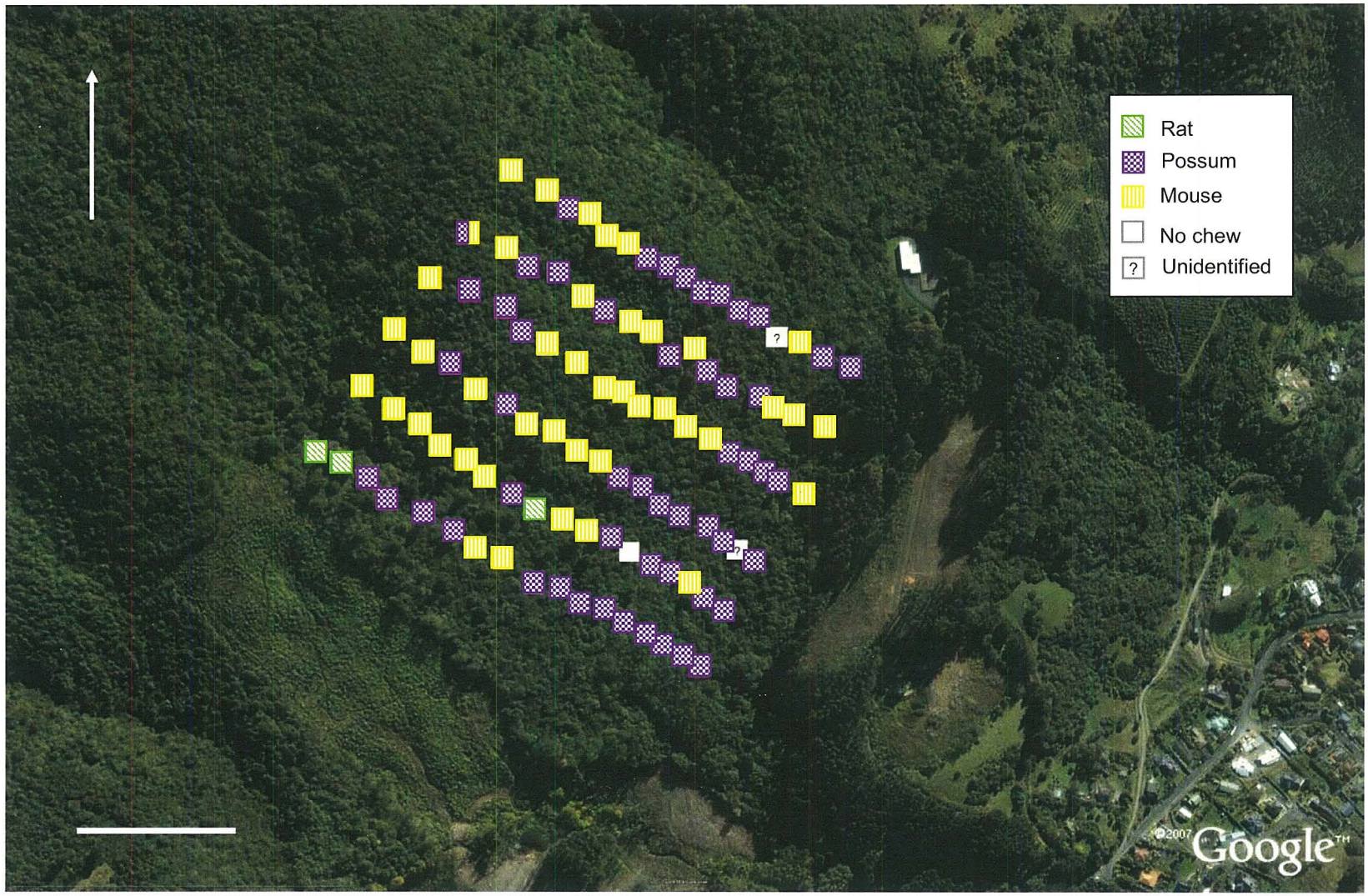

Figure 3.0: Composition of mammalian species detected in McGouns Creek, by observation of genus-specific bite marks on wax blocks. The marker composed of two colours represents a wax blocks in which interference was recorded by two different species. The scale bar equals 100 m.

Figure 3.1: Composition of mammalian species detected in the Wallace block, by observation of genus-specific bite marks on wax blocks. Markers composed of two colours represent wax blocks where interference was recorded by two different species. The scale bar equals 100 m.

Figure 3.2: Composition of mammalian species detected in the housed residential site, by observation of genus-specific bite marks on wax blocks. Scale bar equals 250 m. The marker composed of two colours represents a wax block in which interference by two different species was recorded. *Wax block taken.

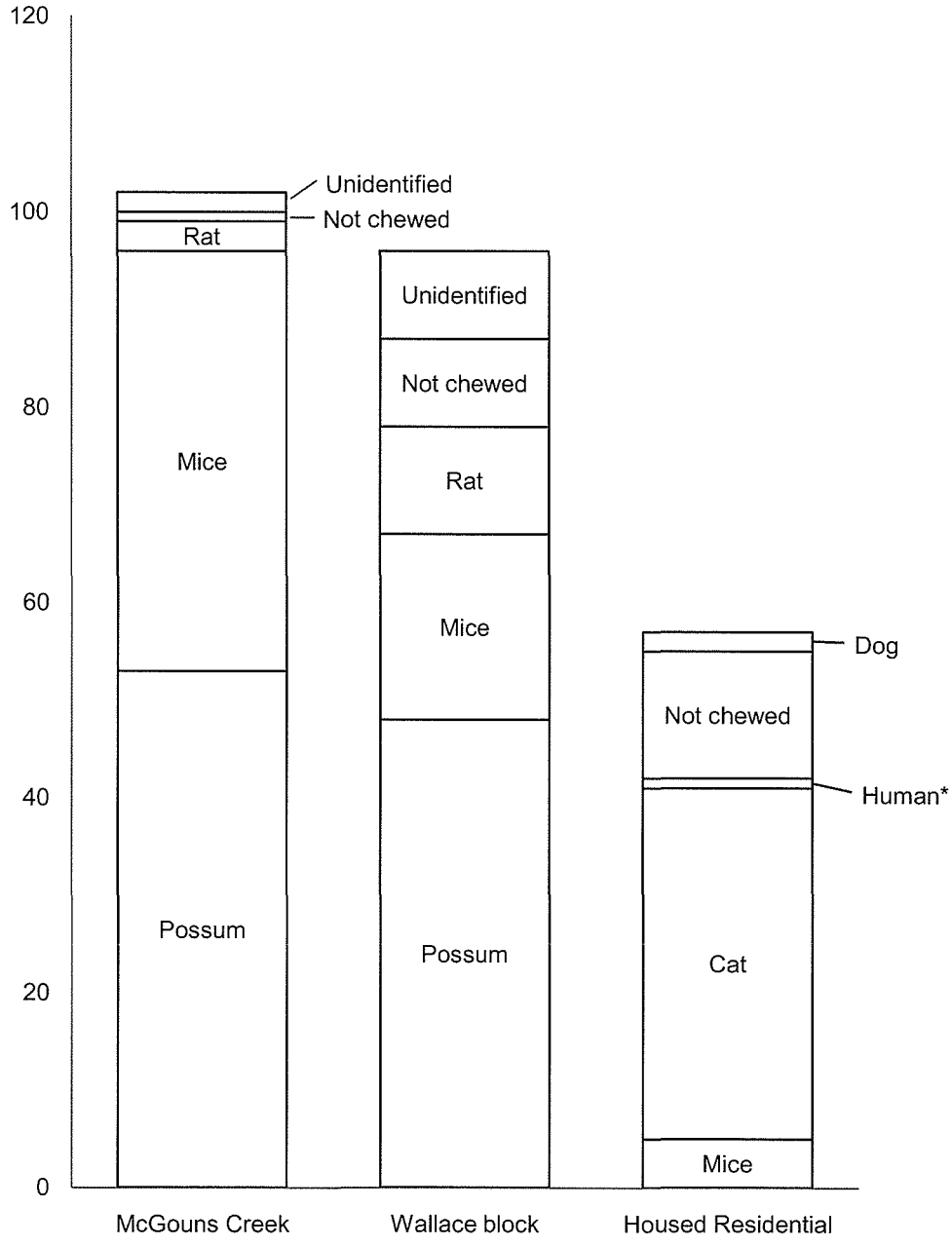

Figure 3.3: Presence of species in the three study sites, determined by genus-specific bite marks on wax blocks (McGouns Creek n = 102; Wallace block n = 97; and housed residential n = 57). *Wax block was removed by a member of the public.

3.2 Hair tubes: Orongorongo Valley

3.2.1 Data collection

One hundred and three samples were collected over seven days. Using microscopical techniques 69 of these samples (collected from 33 different hair tubes) were confirmed as ship rat hair. Non-target disturbance by possums and mice was recorded in approximately 4% of all hair tubes on the sample grid. The quantity of hair adhering to each section of rubber band ranged from two single strands to hundreds of strands (mean = 29.3 ± 3.0 SE, $n = 69$). Eighty-eight percent of hair samples collected were blonde or lemony-white in colour. These samples were all confirmed as rat hair using a combination of macroscopic and microscopic examination. Hair strands from nine further rubber bands were grey-brown in colour and could not be confidently identified using macroscopic and microscopic techniques. Ambiguous samples such as these were sent for microsatellite analysis to determine their origin. A single rat dropping was also found inside a hair tube; this was also sent for microsatellite analysis.

The number of hair samples collected daily increased from five after the first sampling occasion to 24 on the seventh sampling occasion (Figure 3.4). Hair tubes were revisited regularly, with rat hair being collected from one particular hair tube on all seven sampling occasions.

3.2.2 Genetic analysis: Amplification and individual identification

Of all the tissue samples collected, 56 (72%) were successfully amplified at all nine loci and confirmed as ship rat. Of the ambiguous hair samples collected, seven out of nine failed to successfully amplify. The genotyping success was 100% of samples identified as rat using macroscopic and microscopic identification, until the final day when it dropped to 27% (Figure 3.5). A genotype was obtained for the rat dropping.

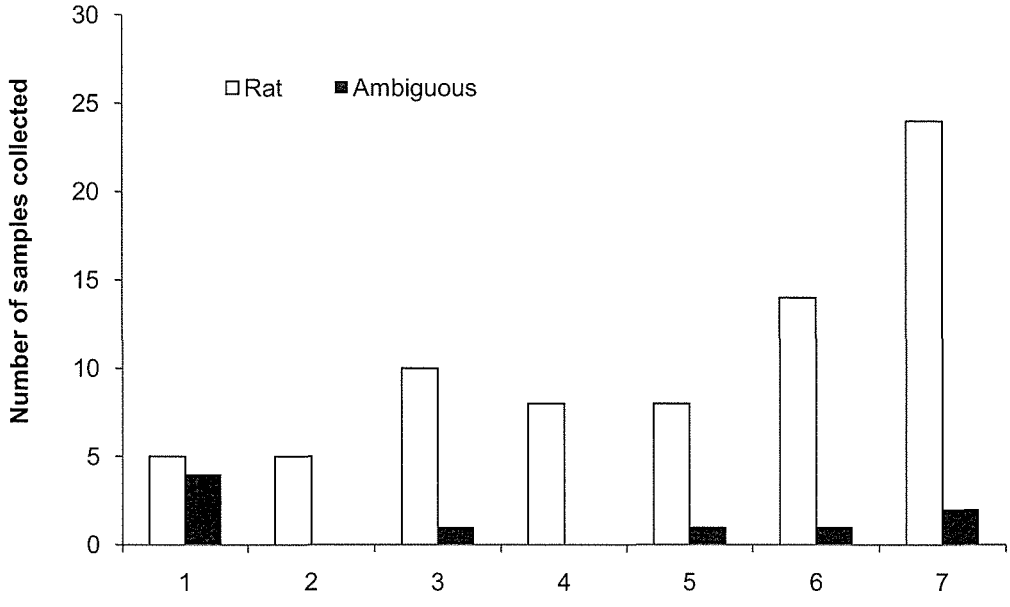

Figure 3.4: Samples collected for microsatellite analysis. Samples were distinguished as either rat or ambiguous using standard macroscopic and microscopic techniques. Samples that were deemed to be non-rat in origin were not genotyped.

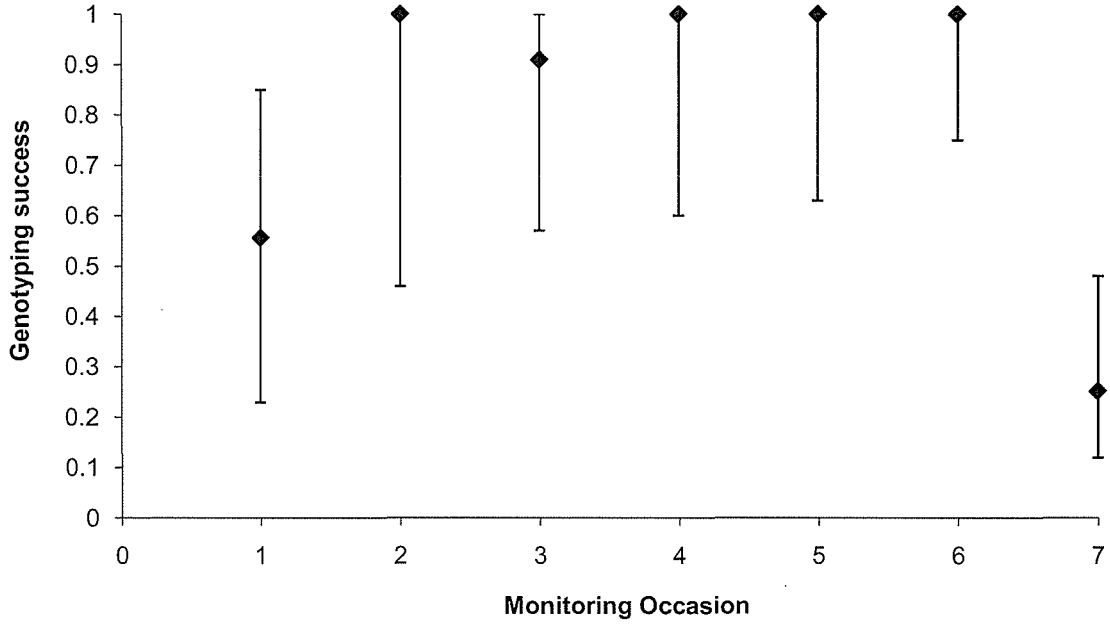

Figure 3.5: Mean genotyping success for rat and ambiguous samples at each monitoring occasion. Points encompassed by 95% binomial confidence limits.

From the 56 hair samples successfully amplified nine distinct genotypes were identified (6 males and 3 females) (Table 3.0). Each multilocus genotype was detected from 1 to 27 times, with a mean number of detections of 6.22 ± 2.75 (mean ± SE, n = 56). The greatest distance between recaptures of the same individual was 85 m (Rat 1; Figure 3.6).

Recaptures were recorded on all seven monitoring occasions (Figure 3.7). Individuals were regularly detected multiple times, at different hair tubes over a single occasion. A high rate of amplification failure on the final day caused the number of successfully genotyped captures to drop on the final sampling period. There was also a general increase in the number of samples collected over the sampling period (Figure 3.7).

3.2.3 Detection of genotyping errors

False alleles were observed at the locus D11Mgh5, where a single allele amplified to 286 instead of 264 (Rat 1). All other alleles amplified from different loci matched that of Rat (1). The sample in question was also collected in a location where Rat (1) was regularly detected over the sampling period. Overall the per-locus error rate recorded from microsatellite analysis was 0.4% (2/504).

A single instance of allelic dropout was also inferred. This error made a heterozygote (156, 176) at the D7Rat13 locus, appear to be homozygous (156, 156). The interpretation was supported by both samples being collected at the same hair tube, and both samples being female.

Table 3.0: Multilocus genotypes obtained of Orongotongo Valley ship rats at nine microsatellite loci. Nine distinct individuals were identified. 'Captures' refers to the total number of times a single multilocus genotype was detected over all seven monitoring occasions. M = male, F= female.

	Microsatellite loci																			
	D2Rat234		D5Rat83		D7Rat13		D11Mgh5		D19Mit2		D10Rat20		D15Rat77		D16Rat81		D18Rat96		Captures	Sex
Rat 1	100	118	174	176	152	156	264	264	223	233	98	98	233	233	158	160	235	235	25	M
Rat 2	100	118	176	176	156	156	278	286	223	223	98	127	247	251	158	158	244	244	7	F
Rat 3	89	122	168	176	156	156	276	278	221	233	98	98	233	237	158	160	235	242	14	M
Rat 4	118	122	172	176	156	156	284	286	221	223	98	98	233	233	158	158	235	235	4	M
Rat 5	89	118	176	176	156	166	278	286	223	227	98	118	233	241	158	158	232	240	1	M
Rat 6	100	122	174	176	156	156	264	264	223	227	98	98	233	235	158	158	235	235	1	M
Rat 7	100	100	172	174	156	176	276	278	223	223	98	98	233	241	158	160	235	235	2	F
Rat 8	100	118	172	176	156	176	248	278	223	233	98	98	233	233	158	160	240	240	1	F
Rat 9	100	100	170	172	152	156	264	278	225	225	98	98	233	239	158	160	231	240	1	M

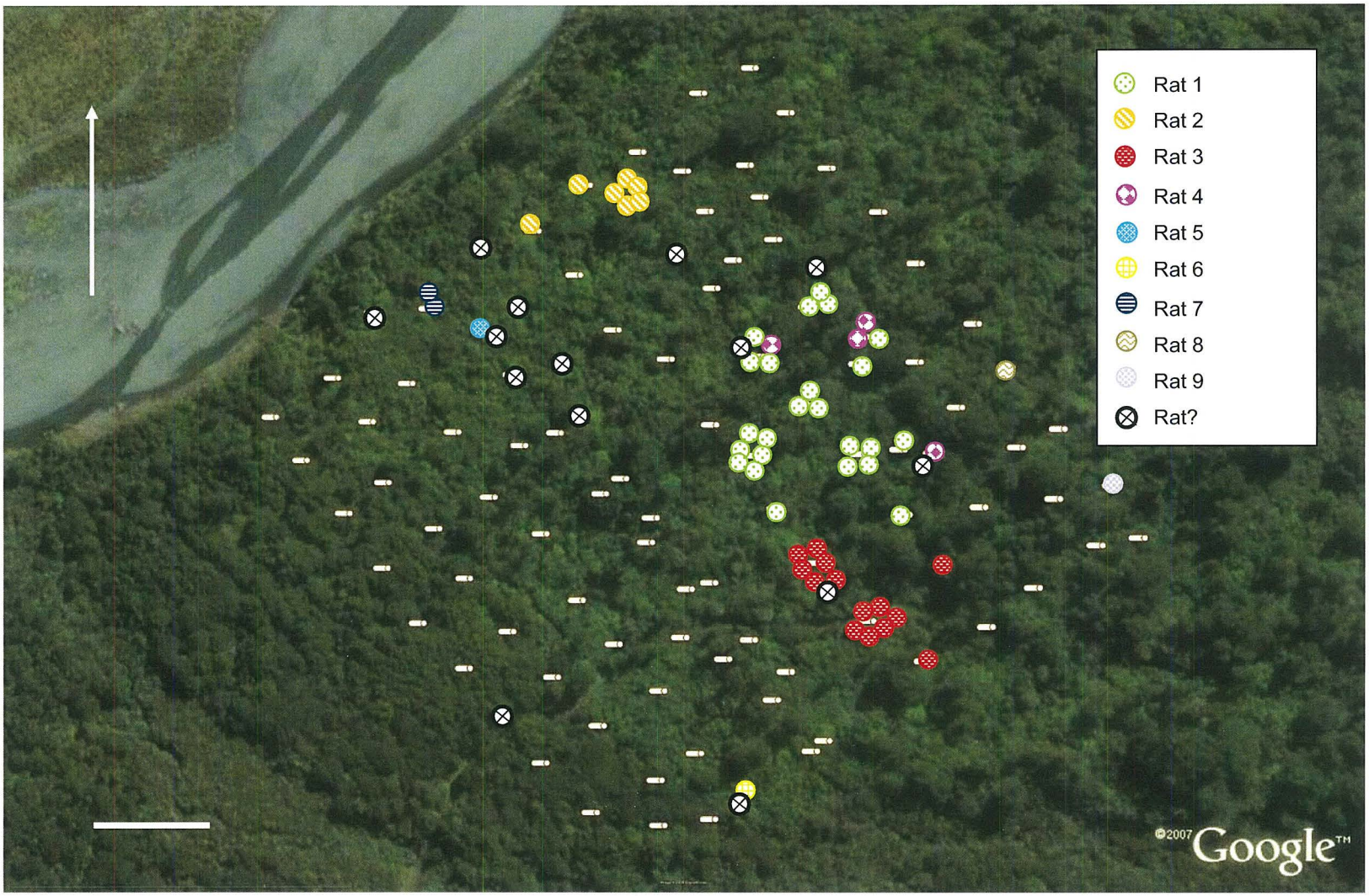

Figure 3.6: Composite aerial photography of the Orongorongo Valley field site with the hair tube grid and rat captures overlaid. Circles with crosses represent instances in which rat hair samples could not be successfully genotyped. Male rats = (1); (3); (4); (6); (8); and (9). Female rats = (2); (5); and (7). The scale bar equals 50 m.

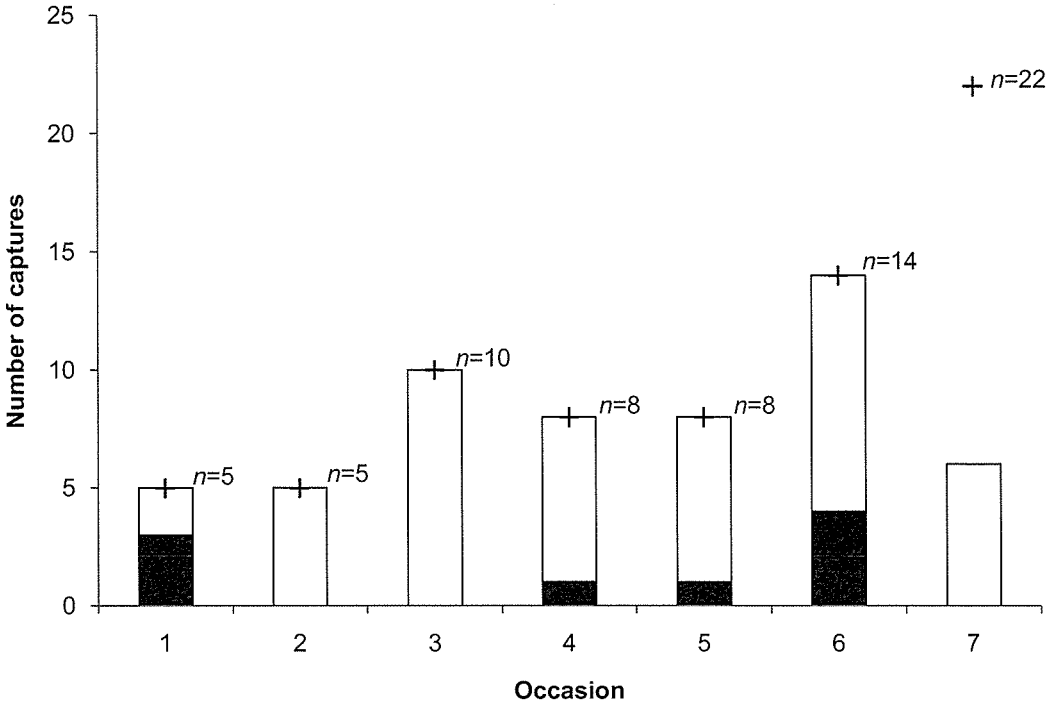

Figure 3.7: Distribution of first-time captures (filled bars) and recaptures (open bars), over the seven monitoring occasions. The crosses represent the total number of rat hair samples collected (ambiguous samples excluded) on each monitoring occasion. The gap between the bar and the cross (+) on occasion seven represents the number of genotyping failures recorded.

All nine loci were polymorphic, with a mean of 4.7 ± 0.53 (mean ± SE, $n = 42$) alleles per locus. After correcting for genotyping errors, all genotypes in the dataset differed at a minimum of four loci. Individuals could therefore be distinguished with a high degree of confidence.

3.2.4 Genetic matches: probability of rat identity

The combined probability of identity of the nine microsatellite loci was low ($PI = 2.1 \times 10^{-9}$; $PI_{sibs} = 0.0023$). Subsets of loci also yielded low PI. For example, not allowing for close relationship, the two most informative loci yielded a PI near zero. Even allowing for the possibility of close relationship, the probability of identity PI_{sib} was less than 1% when the six most informative loci were used (Figure 3.8).

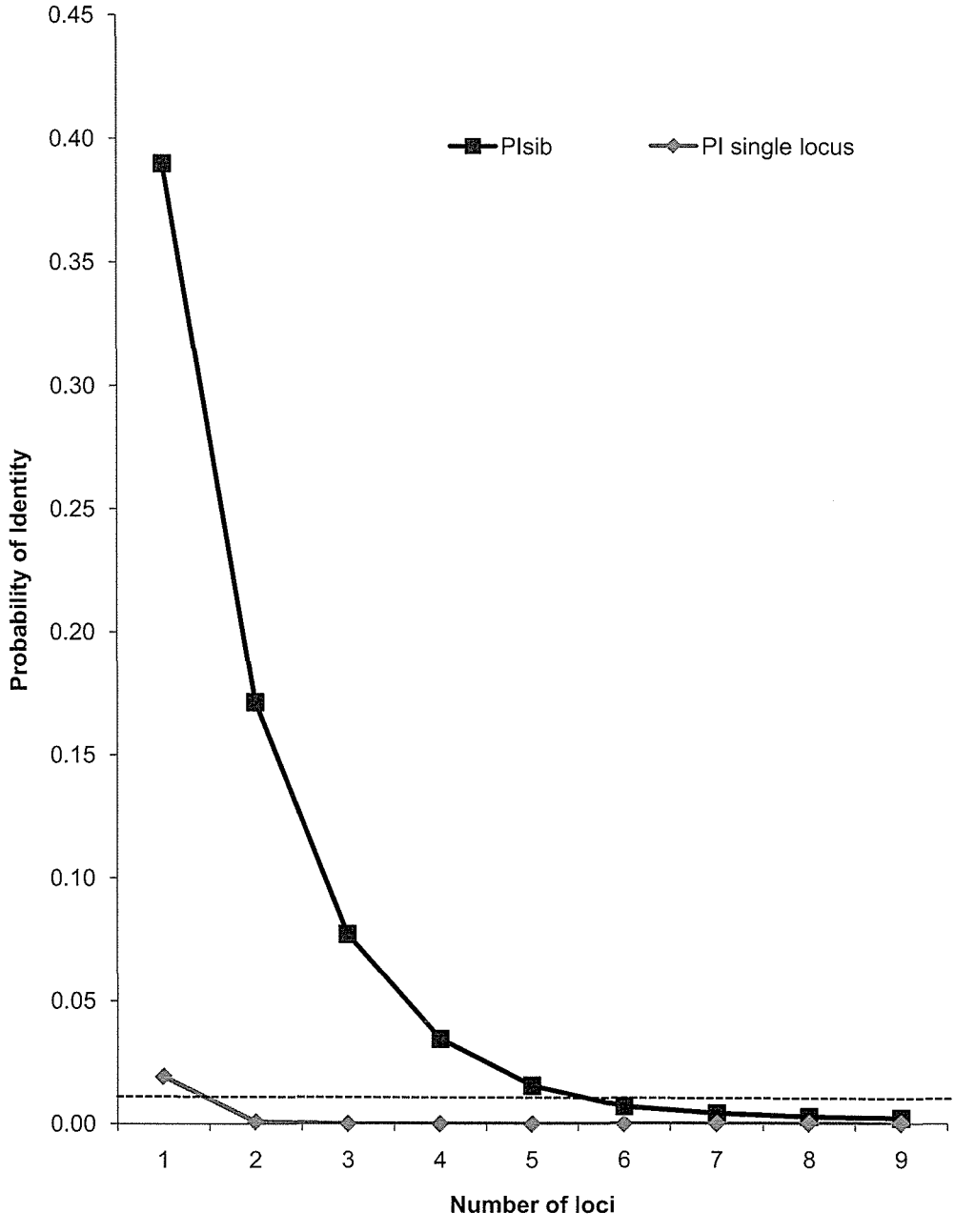

Figure 3.8: Decrease in probability of identity (PI) (grey line) for ship rat genotypes as additional microsatellite markers are added, in order of decreasing heterozygosity. The dotted line represents an arbitrary 1% threshold below which enough loci are typed to distinguish between individuals with 99% certainty. PI_{sibs} (black line) gives the upper bound.

3.2.5 Analysis of microsatellite capture-recapture data

Six models were compared using DENSITY 4.1 (Table 3.1). Models were fitted with additional parameters such as a behavioural ($D_{behaviour}$) response to capture (allowing for trap happy or trap shy behaviour) and change in capture probability over time. The assumption of equal detection probability over time may not be true, given the high rate of genotyping errors of samples collected on the last day. To incorporate this potential variation a model was constructed with a different detection probability (g_0) for the samples collected on the final monitoring occasion ($D_{time*behaviour}$). Density estimates ranged in the set of candidate models from 0.98–1.41 rats/ha (Table 3.1). AICc values suggest the best model was one that fitted a halfnormal detection function and incorporated a behavioural response to capture, with the probability of detection increasing after first-time capture. Akaike weights were calculated, as four of the best models (as determined by lowest AICc values), varied by less than 7. The weighted values show 81% support for the $D_{behaviour}$ halfnormal model relative to the candidate set. This model corresponds to a maximum likelihood density estimate of 1.17 ± 0.42 (SE) ship rats/ha.

Table 3.1: Maximum likelihood (ML) density estimates (rats/ha) from six closed capture-recapture (CR) models incorporating different constraints on detection probability. Halfnormal or hazard refers to the shape of the detection curve. Models were compared using Akaikes Information Criterion for small sample sizes (AICc) and AIC weights indicate the relative support for each model. SE, LCL and UCL represent the standard error, lower confidence limit and upper confidence limit respectively. Model notation is given in the text. Estimates were generated using DENSITY 4.1.

Model	AICc	Akaike weight	ML Density	SE	LCL	UCL
D *behaviour* Halfnormal	360.9	0.812	1.17	0.42	0.59	2.33
D *null* - Hazard	365.5	0.082	0.80	0.29	0.41	1.58
D *time*behaviour* - Halfnormal	366.2	0.056	1.38	0.58	0.63	3.03
D *null* - Halfnormal	367.4	0.032	0.98	0.34	0.51	1.90
D *behaviour* - Hazard	368.6	0.017	1.17	0.43	0.59	2.34
D *time*behaviour* - Hazard	384.7	0.000	1.41	0.58	0.65	3.08

3.3 Hair tubes: Urban Dunedin

3.3.1 Data collection

One hundred and forty-six hair samples were collected from the four Dunedin study sites and 32% of these were confirmed as rat hair using standard microscopy techniques. Fifty-five percent were identified as hair samples belonging to non-target species, such as mice. The species of 19 further samples could not be determined. Ambiguous samples were genotyped along with definite rat samples. Three samples were collected from Quarantine Island after hair tubes were left set for one month. Rat droppings and burrows typical of Norway rats, were also observed on Quarantine Island. Sixteen of the nineteen ambiguous samples were collected in McGouns Creek. McGouns Creek also had a high incidence of non-target disturbance by mice (44/75). Over all four sampling sites the number of rat hair samples collected appears to increase across each session (Table 3.2).

Table 3.2: Samples collected over seven sampling occasions from four Dunedin study sites. Samples from Quarantine Island are not included in this table as they were collected after the seven day sampling session. Samples of non-rat origin were not genotyped. R = Rat; A = Ambiguous.

	Monitoring Occasion													
	1		2		3		4		5		6		7	
	R	A	R	A	R	A	R	A	R	A	R	A	R	A
Wallace	0	0	1	0	2	0	2	1	2	0	3	2	3	0
McGouns	0	0	0	1	0	4	2	4	4	3	6	3	3	1
Woodhaugh	3	0	1	0	2	0	3	0	2	0	2	0	3	0
Quarantine	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pooled total	3	0	2	1	4	4	7	5	8	3	11	5	9	1

The amount of hair retained on each section of rubber band varied from four individual strands to hundreds of strands. Ninety percent of hair samples that were determined as rat were blonde or lemony-white in colour. All ambiguous samples analysed were grey-brown in colour.

3.3.2 Genetic analysis: Amplification and individual identification

Of the 66 tissue samples collected, 42 (64%) were confirmed as rat and successfully amplified at all nine loci. Of the ambiguous samples collected, 19 out of 19 failed to successfully amplify.

When the 19 ambiguous samples were excluded, the genotyping success of confirmed rat hair was 89%. Genotyping failure occurred in 2/15 samples from McGouns Creek and 3/10 samples from the Wallace block. All samples collected from Woodhaugh Gardens and Quarantine Island contained DNA and were successfully genotyped.

Of the 42 samples from all four sites, 10 distinct multilocus genotypes were identified (4 female, 6 male) (Figures 3.9, 3.10, 3.11, 3.12 & Table 3.3). Each multilocus genotype was detected 1–7 times, with a mean number of detections of 4.2 ± 0.57 (mean ± SE, $n = 42$). Polymorphism was observed at all nine loci with a range of eight to eleven alleles per locus (9.33 ± 0.37, mean ± SE, $n = 84$). The greatest distance between detections of the same individual was 134 m by Rat (4) in McGouns Creek bush (Figure 3.10).

Rats (6) and (10) (Table 3.3) have substantially lower base-pair lengths at the D11Mgh5 and D19Mit2 loci – in the range of Norway rats (R. Howitt, *pers. comm.*). This is illustrated in Figure 3.13, which shows the base-pair lengths at each locus, of all the rats genotyped in this study (including samples from the Orongorongo Valley). Rat (10) was detected on Quarantine Island while Rat (6) was detected on the banks of the Leith Stream.

Combined results from Woodhaugh Gardens, the Wallace block and McGouns Creek bush show that the number of daily recaptures increased across the sampling session while the number of new captures declined (Figure 3.14).

Table 3.3: Consensus multilocus genotypes obtained from nine microsatellite loci. Ten distinct individuals were identified from all four study sites. 'Captures' refers to the total number of times a single multilocus genotype was detected over all seven monitoring occasions. M = male, F= female.

		Microsatellite loci																			
		D2Rat234		D5Rat83		D7Rat13		D11Mgh5		D19Mit2		D10Rat20		D15Rat77		D16Rat81		D18Rat96		Captures	Sex
Wallace	Rat 1	100	122	166	186	181	183	280	282	225	231	129	131	233	233	166	166	231	246	5	F
	Rat 2	108	118	166	191	156	166	282	284	223	233	125	129	241	243	168	168	242	242	5	M
McGouns	Rat 3	100	102	172	186	158	181	266	280	225	225	129	131	233	249	160	160	239	239	5	F
	Rat 4	118	122	170	174	166	185	282	284	225	225	98	98	247	249	160	172	231	240	6	M
	Rat 5	100	100	174	186	158	183	282	284	225	225	98	100	233	241	166	168	240	240	2	F
Woodhaugh	Rat 6	108	108	168	174	160	160	248	248	195	195	109	109	229	259	163	163	245	247	4	M
	Rat 7	130	130	192	198	164	164	290	292	233	237	105	142	241	241	167	179	236	247	7	M
	Rat 8	106	108	184	198	180	180	286	292	233	241	137	139	241	257	173	173	245	247	1	M
	Rat 9	108	118	192	192	180	190	286	290	229	245	105	105	245	249	173	173	245	247	4	F
Quarantine	Rat 10	120	120	168	174	160	160	243	243	195	197	109	109	253	255	167	167	236	242	3	M

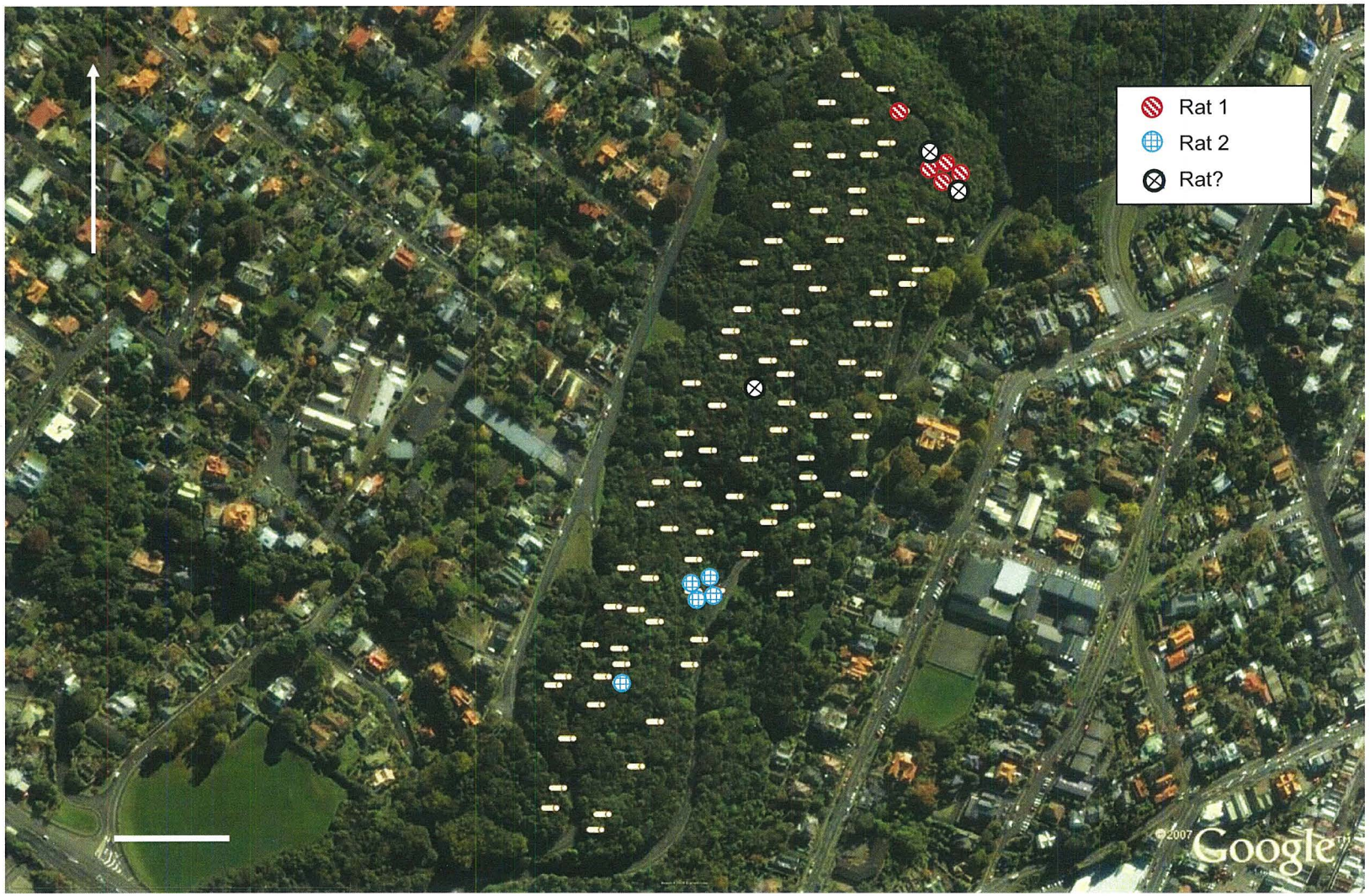

Figure 3.9: Composite aerial photography of the Wallace block (inner-urban bush fragment) with the hair tube grid and rat captures overlaid. Circles with crosses represent instances in which rat hair samples could not be successfully genotyped. Male rat = (1). Female rat = (2). The scale bar equals 75 m.

Figure 3.10: Composite aerial photography of McGouns Creek bush (peri-urban bush fragment) with the hair tube grid and rat captures overlaid. Circles with crosses represent instances in which rat hair samples collected could not be successfully genotyped. Male rat = (4). Female rats = (3); and (5). The scale bar equals 50 m.

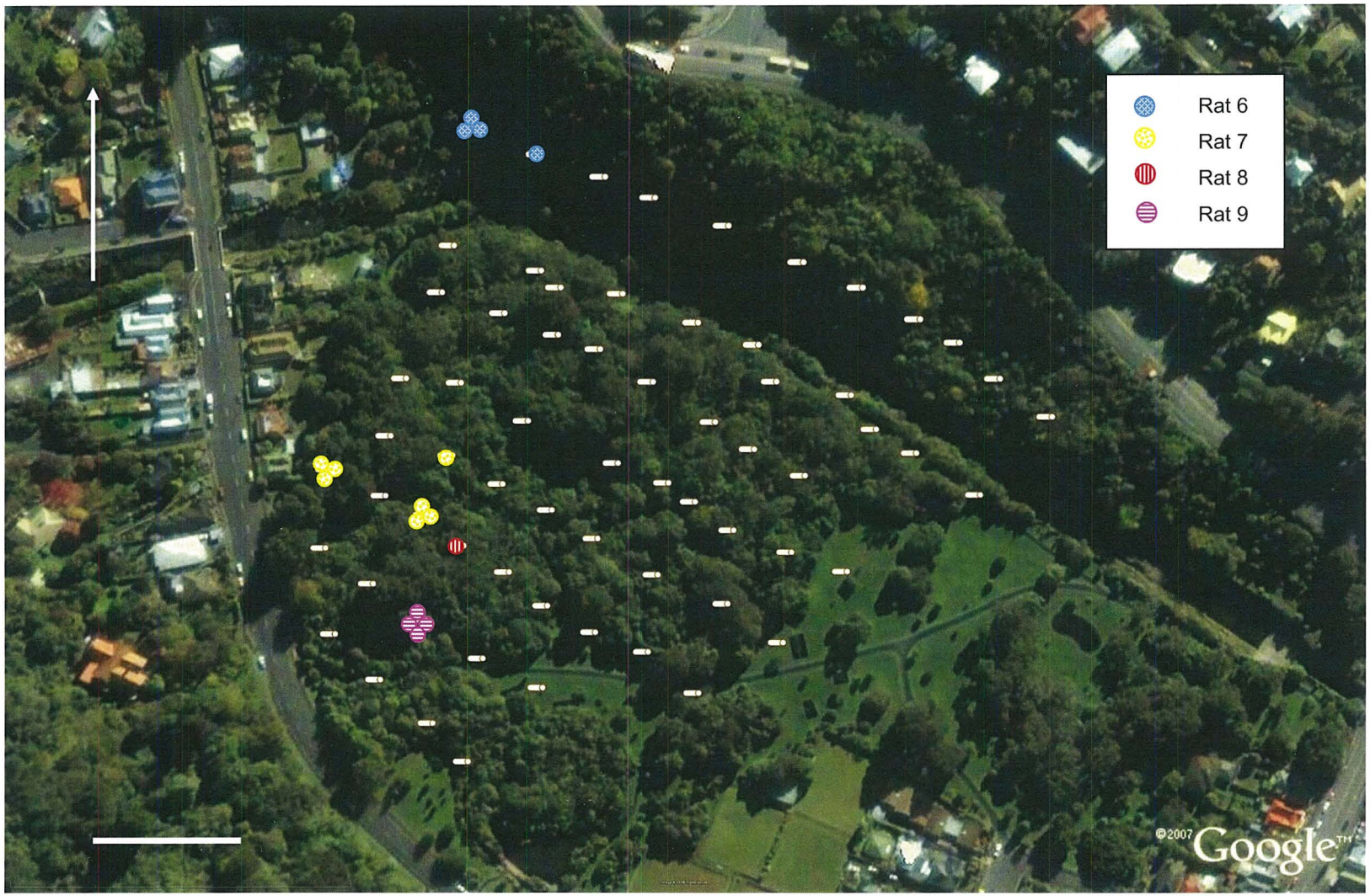

Figure 3.11: Composite aerial photography of Woodhaugh Gardens (inner-urban bush fragment) with the hair tube grid and rat captures overlaid. Male rats = (6); (7); and (8). Female rat = (9). The scale bar equals 50 m.

Figure 3.12: Composite aerial photography of Quarantine Island (urban island) with the hair tube grid and rat captures overlaid. Male rat = (10). The scale bar equals 50 m.

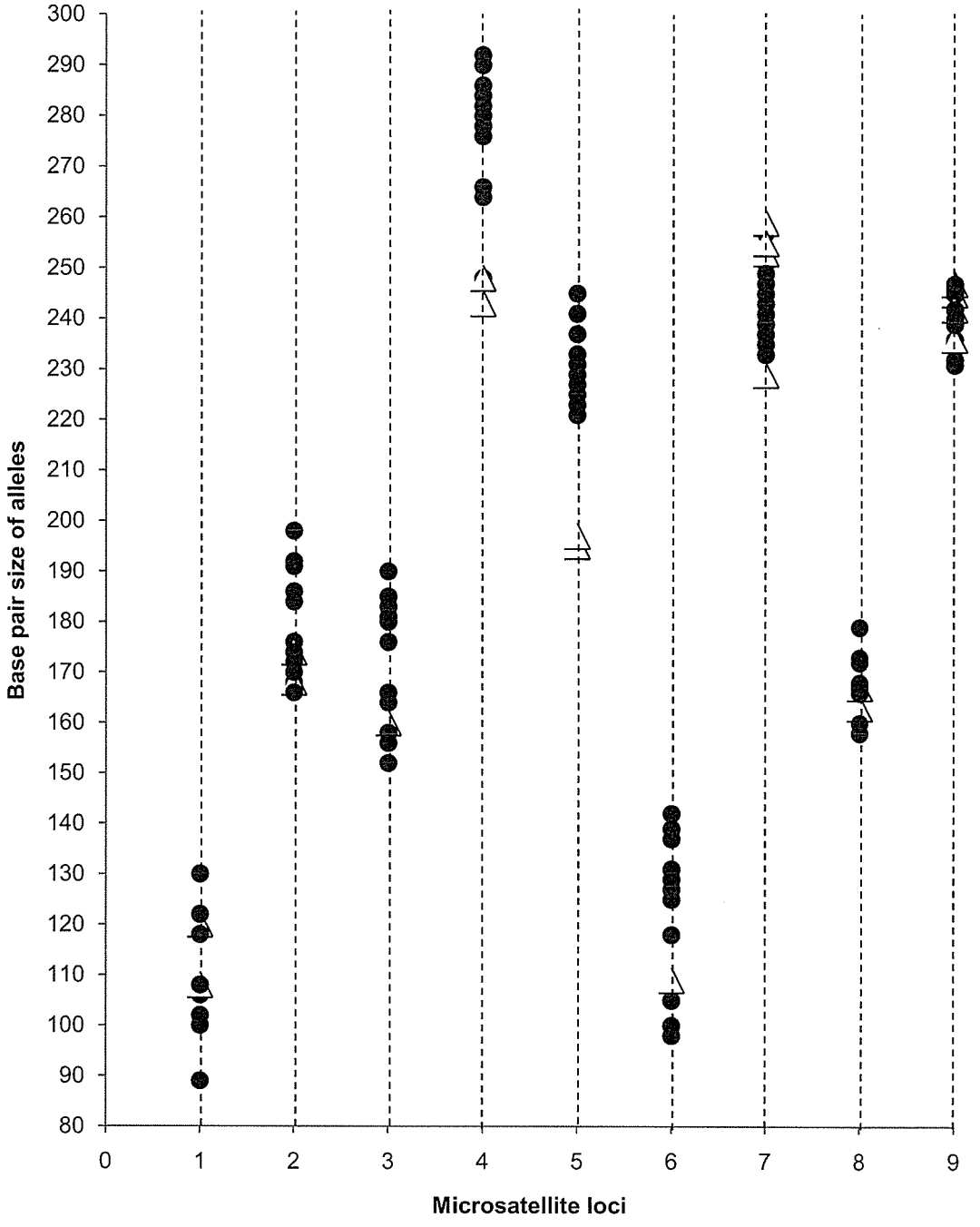

Figure 3.13: Allele base pair lengths for nine microsatellite loci examined in rats sampled in this study (n = 19). Filled black circles represent probable ship rats (n = 17). Open triangles represent individuals (6) and (10) which are probably Norway rats (n = 2). Norway rats were identified from lower base pair lengths at both the D11Mgh5 and D19Mit2 loci (R. Howitt, *pers. comm.*). The numbers on the x-axis refer to microsatellite loci: (1) D2Rat234; (2) D5Rat83; (3) D7Rat13; (4) D11Mgh5; (5) D19Mit2; (6) D10Rat20; (7) D15Rat77; (8) D16Rat81; and (9) D18Rat96.

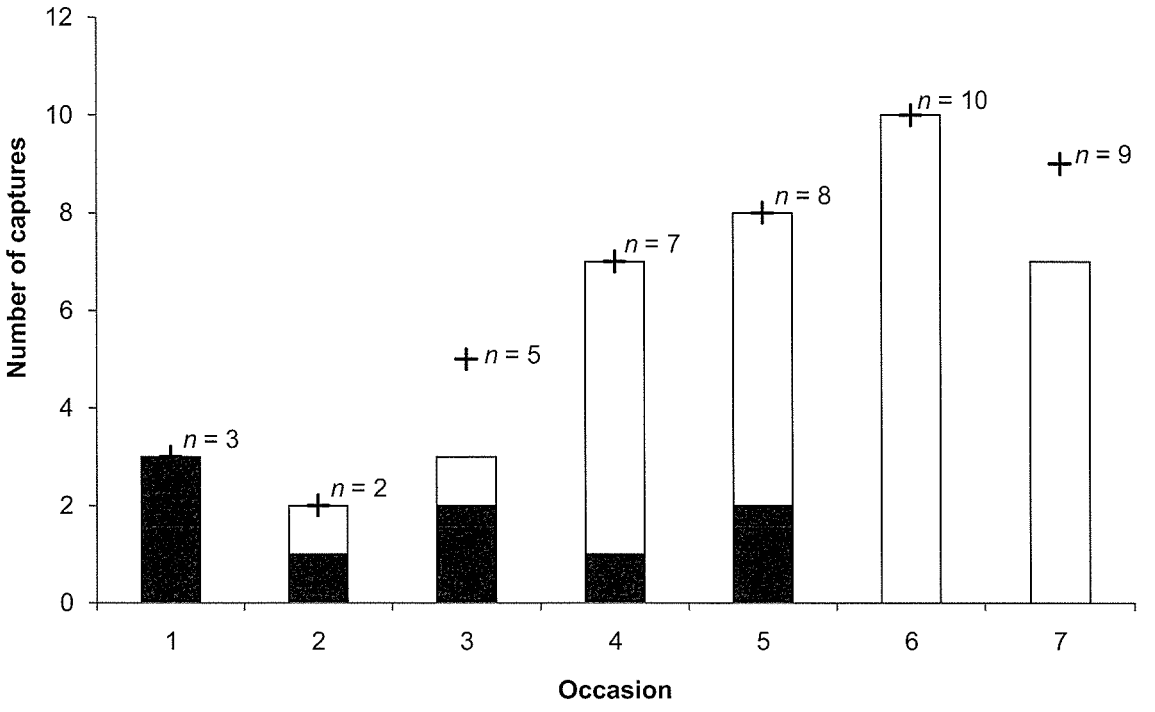

Figure 3.14: Distribution of first time captures (filled bars) to recaptures (open bars), for study sites in which rats were detected combined from each monitoring session (McGouns Creek, Wallace block and Woodhaugh Gardens). Crosses represent the total number of rat hair samples collected (ambiguous samples excluded) on each monitoring occasion. The gap between the bars and the crosses, at occasions three and seven, represent the number of genotyping failures

3.3.3 Detection of genotyping errors

The per-locus error rate from samples collected in urban Dunedin was 0.26% (1/378). Of the 42 successfully amplified samples, a single instance of allelic dropout was recognised. Allelic dropout occurred at the D19Mit2 locus where the animal in question appeared to be homozygous (225, 225). Three additional captures, at the same hair tube, of an animal that is heterozygous (225, 231) at the D19Mit2 locus (with an otherwise identical multilocus genotype), indicates the likely occurrence of allelic dropout. Allelic dropout was supported further when both the suspect sample and the confirmed sample were found to be female. The probability of errors at two or more loci in the same genotype is negligible given how rarely genotyping errors occurred at a single locus. It is therefore highly probable that each of the 10 multilocus genotypes in this data set corresponds to a unique individual. Too few individuals were detected at each Dunedin study site to allow a probability of identity statistic to be calculated from locally determined allele frequencies.

3.3.4 Analysis of urban Dunedin capture-recapture data

Very low rat numbers were detected within all urban sites sampled. With such a low number of recaptures, DENSITY could not calculate individual density estimates for each site. CR data was therefore pooled between all three urban study sites and a combined density estimate was calculated. Quarantine Island data was excluding from pooling after failing to detect the presence of ship rats following the first seven monitoring occasions.

Four models were compared using DENSITY 4.1 (Table 3.4). Density estimates ranged in the set of candidate models from 0.25–0.53 rats/ha (Table 3.4). AICc values suggest the best model was the null model with a hazard detection curve. A behavioural ($D_{behaviour}$) response to capture (allowing for trap happy or trap shy behaviour) was incorporated in two density models but these models had higher AICc values than the comparative null models. Akaike weights were calculated, as the AICc values for each model differed by less than 7. AICc weights suggest that the D_{null} hazard model had 92% support for being the best model in the candidate set. This model corresponds to a pooled maximum likelihood density estimate of 0.26 ± 0.10 (SE) ship rats/ha.

Table 3.4: Maximum likelihood (ML) density estimates (rats/ha) from four closed capture-recapture (CR) models incorporating different constraints on detection probability. Halfnormal or hazard refers to the shape of the detection curve. Models were compared using Akaikes Information Criterion for small sample sizes (AICc) and AIC weights indicate the relative support for each model. SE, LCL and UCL represent the standard error, lower confidence limit and upper confidence limit respectively. Model notation is given in the text. Estimates were generated using DENSITY 4.1.

Model	AICc	Akaike weight	ML Density	SE	LCL	UCL
D *null* - Hazard	337.0	0.921	0.26	0.10	0.13	0.53
D *behaviour* - Hazard	342.0	0.076	0.53	0.15	0.16	0.80
D *null* - Halfnormal	349.0	0.002	0.25	0.09	0.13	0.49
D *behaviour* Halfnormal	351.0	0.001	0.29	0.11	0.14	0.59

3.3.5 Overall success of hair tubes and microsatellite analysis

The hair tube methodology, almost always, was successful at obtaining sufficient rat hair for microsatellite analysis. Of the 249 samples collected from in Dunedin and the Orongorongo Valley 41% were verified as rat, 11% as ambiguous and 48% as belonging to non-target species - nearly always mice.

A total of 19 distinct individuals were recognised, including 7 females and 12 males. Females were captured on average 3.7 ± 0.8 times (± SE, n = 26), while male rats were captured on average 6 ± 2 times (± SE, n = 72). The mean number of captures for male rats was inflated by Rat 1 in the Orongorongo Valley, which was recaptured 25 times. When Rat (1) was excluded from analysis, the mean number of captures for male ship rats was 4.3 ± 1.7 (± SE, n = 71). Genotyping success was high when ambiguous samples were excluded from analysis, 82% (n = 119). The per-locus genotyping error rate was low in all samples, 0.34% (3/882). This included a single instance of false alleles and two instances of allelic dropout.

4 DISCUSSION

4.1 Value of wax blocks to assess distribution and abundance

Wax blocks were extremely quick to set up and analyse once collected. Field equipment in cities has a tendency to get stolen or manipulated by members of the public. Therefore, one of the biggest advantages of using wax blocks in this study was that they were cheap to purchase. Residents weren't concerned about having a wax block in their garden, particularly because they are non-invasive and non-toxic. The single household that did not wish to take part in the wax block survey had a young boy who was allergic to peanuts. Understandably these residents did not want to risk having a peanut-coated wax block in their garden.

Thomas *et al.* (1999) suggest that the use of wax blocks may be a feasible method to observe changes in rat abundance over time. The findings of this study suggest that in urban Dunedin it would be very difficult to determine changes in rat abundance using wax blocks. Wax blocks cannot be used to distinguish between different individuals of the same species (or between Norway and ship rats). Of the wax blocks chewed in the Wallace block all were close together, and could easily have been chewed by a single rat. Wax blocks therefore present many of the traditional pitfalls of index-methods. For example, variation in home range size may influence the number of wax blocks chewed on any given sampling occasion. Rats that exist in low densities may become more active and obtain larger home ranges than rats that exist at higher densities (Blackwell *et al.* 2002). Dowding and Murphy (1994) found that the home ranges of two male ship rats increased in spring. Wax block indices collected from different seasons would therefore not be comparable.

When total interference by non-target species is high the effective level of sampling effort drops to near zero and the sampling method becomes completely unreliable. Interference from non-target species potentially obscures the detection of rat presence. High rates of non-target interference in the residential study site may have meant rats did not get the opportunity to chew wax blocks. Alternatively since the interference was largely caused by cats, rats may have

actively avoided wax blocks. Mice were, however, detected regularly in the residential area. If rats were actively avoiding wax blocks due to the presence of cats then it seems likely that mice may also display this behaviour.

Peanut butter appeared to be an effective lure for rats, mice, possums and surprisingly cats. In McGouns Creek and the Wallace block a large proportion of wax blocks were chewed by possums. It is likely that one possum could have chewed a substantial number of wax blocks over a single night. Interference from other species may have severely diminished the chance rats had to chew the wax blocks, or obscured rat bite marks completely. The use of tracking tunnels would have decreased the level of non-target interference observed in this study and perhaps increased the rate of rat detection. The use of tracking tunnels is initially more time consuming than using wax blocks. However, a decrease in non-target interference would mean the effective sampling effort would increase, and allow for more rigorous conclusions to be drawn.

4.2 Efficacy of density estimation using hair tubes

Hair tubes proved extremely effective at plucking sufficient hair for microsatellite analysis. Hair tubes were often re-visited by the same rat, which suggests rats did not perceive the sampling process as unpleasant. The ship rats did not appear to exhibit neophobia (sometimes called new 'object reaction') (Cowan 1977), with some individuals being detected from the very first sampling occasion.

Microsatellite analysis showed that samples whose species identification was uncertain from microscopical examination almost never belonged to rats. Non-target interference by mice was common and determining the difference between rat and mouse hair was often difficult, particularly if very few guard hairs were retained. Ambiguous samples in which genotypes were not successfully obtained most probably belonged to mice. Distinguishing the difference between rat and mouse hair was complicated by a flaw in the hair tube design. The arrangement of the rubber bands in the hair tubes meant that it was common for animals to go over the top of the adhesive coated rubber band, rather than beneath the rubber band. Belly hair was therefore

often retained more frequently than the diagnostically useful guard hairs, which are found on the back of most species (Triggs & Brunner 2002). Under-hairs of small mammal species are generally of little diagnostic value (Triggs & Brunner 2002). A conservative approach was therefore taken with marginal samples that could have belonged to rats but probably belonged to mice. In hindsight, microscopic techniques were very accurate. Subjectively removing ambiguous samples before genotyping would save considerable cost and only marginally reduce the number of rats captured.

Once ambiguous samples were omitted from analysis, the overall amplification success at all nine loci was 77%. This is approximately average in comparison to recent studies that also utilised non-invasive genetic sampling (Table 4.0). The failure rate of 75% of samples on the final monitoring occasion in the Orongorongo Valley was unacceptably high. Samples collected on the final monitoring occasion were wet, and remained damp until they were processed over one week later and so the genotyping failure was probably due to DNA degradation between the time of collection and microsatellite analysis. The method in which samples are stored between collection and DNA extraction can substantially influence the rate of genotype failure (Banks *et al.* 2002). Studies that have recorded the highest rates of genotyping success have generally collected fresh samples and carried out DNA extractions only hours after collection (Banks *et al.* 2002). Clearly this is not always possible and a greater emphasis on correct sample storage is desirable.

The estimated 'probability of identity' (PI) statistics suggest that the nine loci used in this study would be sufficient to distinguish between individual rats, including siblings, with 99% certainty. Targeting a sufficient number of polymorphic microsatellite loci increases the chance of distinguishing between individuals within the same population. This is particularly important when the population in question has lower than average genetic variability, such as one from an isolated island. The analysis of nine microsatellite loci is approximately average relative to previous studies, which also utilised non-invasive genetic sampling (Table 4.0).

Table 4.0: Amplification success of microsatellite DNA in studies that utilised non-invasive genetic sampling.

Tissue type	Species	Loci	Genotyping success
Hair follicles	*R. rattus* + *R. norvegicus*	9	77%[1]
Faecal and hair follicles	*Ursus arctos*	24	~16%[2]
Faecal	*Canis latrans*	3	48%[3]
Faecal	*Puma concolor*	12	>75%[4]
Faecal	*Ovis aries* + *Rangifer tarandus*	6	93%[5]
Faecal	*Ursus arctos*	6–7	~70%[6]
Faecal	*Vombatus ursinus*	6	83%[7]
Hair follicles	*Ursus arctos*	6	77%[8]
Faecal	*Ursus arctos*	6	73%[9]
Hair follicles	*Martes americana*	6	80%[10]
Hair follicles	*Ursus americanus* +*Ursus arctos*	6	~88%[11]
Hair follicles	*Ursus americanus*	8	~62%[12]
Faecal	*Meles meles*	7	74%[13]
Urine and faecal	*Gulo gulo*	10	40%–65%[14]
Hair follicles	*Mustela erminea*	6	73%[15]
Hair follicles	*Pan troglodytes*	11	50%[16]
Hair follicles	*Lasiorhinus krefftii*	10	96%[17]
Hair follicles	*Lasiorhinus krefftii*	10	93%[18]
Faecal	*Loxodonta cyclotis*	6	72%[19]
Faecal	*Lutra lutra*	9	20%[20]
Faecal	*Canis lupus*	5	53%[21]

Sources: [1]This study; [2](Taberlet *et al.* 1997); [3](Kohn *et al.* 1999); [4](Ernest *et al.* 2000); [5](Flagstad *et al.* 1999); [6](Bellemain *et al.* 2005); [7](Banks *et al.* 2002); [8](Mowat & Strobeck 2000); [9](Solberg *et al.* 2006); [10](Mowat & Paetkau 2002); [11](Woods *et al.* 1999); [12](Triant *et al.* 2004); [13](Wilson *et al.* 2003b); [14](Hedmark *et al.* 2004); [15](Efford *et al.* 2009); [16](Gagneux *et al.* 1997); [17](Banks *et al.* 2003b); [18](Banks *et al.* 2003a); [19](Eggert *et al.* 2003); [20](Dallas *et al.* 2003); [21](Lucchini *et al.* 2002).

4.3 Validation of the hair tube technique in the Orongorongo Valley

To test the efficacy of the hair tube methodology a well studied population of ship rats in the Orongorongo Valley was initially sampled. Identification failures on the final sampling occasion greatly reduced the number of captures that could be used for CR modelling in the Orongorongo Valley dataset. However, the density estimate of 1.17 rats/ha was in accordance with recent estimates obtained from the same sampling grid using cage-trapping (Figure 4.0). Cage-trapping estimates from Figure 4.0 were also calculated using SECR. In the May 2008 session of Figure 4.0 nine rats were removed from the Orongorongo Valley sampling grid (two months prior to the June 2008 hair tube estimate), the model used for the May 2008 estimate allowed for these removals (D. Wilson, *pers. comm.*). To be consistent between all other sampling sessions, the null model was selected for each of the cage-trapping estimates in Figure 4.0 (D. Wilson, *pers. comm.*). The June hair tube density estimate is low in comparison with previous published estimates of ship rat density in forests (Table 4.1). The removal of rats helps explain the lower cage-trapping density estimates for May and June, followed by re-colonisation and a higher estimate for October, 2008. There appeared to be a slight decline in rat density from May to June. However the large confidence limits make it difficult to draw significant conclusions. Improving precision of the data would enable more robust conclusions to be drawn as the confidence intervals would be reduced and the likelihood of observing any trends present would thus increase.

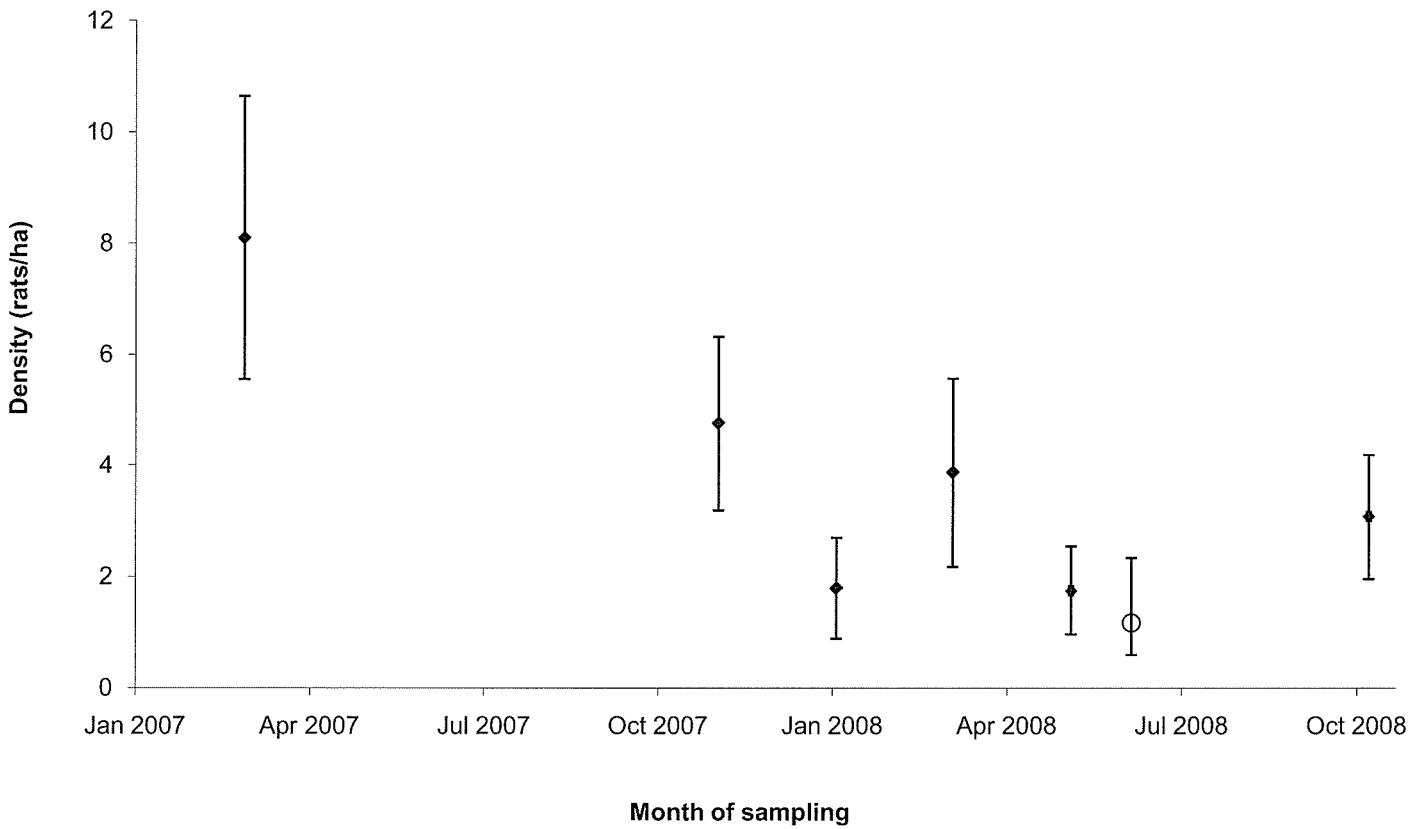

Figure 4.0: Estimates of absolute ship rat density on the 'Woottons grid', Orongorongo Valley (January 2007 – October 2008). The open circle represents the June 2008 hair tube estimate. Black squares represent cage-trapping estimates (D.J. Wilson, A.E. Byrom, R. Pech, M. Perry, D.P. Anderson, Landcare Research, unpubl. data). DENSITY has two methods of fitting SECR models: conditional likelihood produces symmetrical confidence limits (cage-trapping estimates); full likelihood produces asymmetrical confidence limits (hair tube estimate).

Table 4.1: Estimates of absolute ship rat densities from mainland New Zealand and associated offshore islands'. Adapted from Latham (2006) .

Location	Season	Year	Density (rats/ha)
Orongorongo Valley, Rimutaka Range	September-November	1966	2.5–3.7[1]
	December-February	1966–1967	3.2–2.5[1]
	April-May	2003	5[2]
	April	2004	9[2]
Puketi Forest, Northland	September-October	1993	2.9[3]
Rotoehu Forest, Bay of Plenty	January	1992	6.2[4]
Kaharoa Forest, Bay of Plenty	January	1994	6.5–7.8[5]
Lake Waikaremoana, Urewera	June	1998	8.2[6]
Halfmoon Bay, Stewart Island	August-September	1984	2.0–2.5[7]
Goat Island, Warkworth	April-June	2005	3.3[8]
Ponui Island, Hauraki Gulf	July	2005	22.4[9]
	August	2005	16.2[9]
	September	2005	6.7[9]
	October	2005	11.2[9]
	December	2004	6[10]
	February	2005	10[10]
Taukihepa (Big South Cape Island)	December	2004	8.1–10.7[11]
	February	2005	37.6–62.7[11]
Keeble's Bush, Manawatu	March-March	1976–1977	2.8[12]

Sources: [1]Daniel (1972) , [2]Wilson *et al.* (2007) , [3]Dowding and Murphy (1994) , [4]Hooker and Innes (1995) , [5]Brown *et al.* (1996) , [6]Blackwell (2000) , [7]Hickson *et al.* (1986) , [8]MacKay and Russell (2005) , [9]Latham (2006) , [10]Shapiro (2005) , [11]Rutherford (2005) , [12]Innes (1977) .

The results from the Orongorongo Valley suggest that hair tubes and SECR was an effective technique to estimate ship rat density accurately and with greater precision for given effort. Density estimation in this study relies on three main assumptions: (1) population closure (demographic and geographic); (2) equal probability of capture; and (3) mark retention. It is unlikely that births, deaths or migration affected the outcome of this study as each session was over a very short time period (7-days). Because hair tubes do not harm the individuals sampled, the likelihood of animals showing a negative behavioural response to capture is decreased. This can result in an increased number of recaptures. Ship rats in the Orongorongo Valley appeared to display a 'trap-happy' response following initial capture. To allow for this variation in capture probability, a behavioural function was incorporated into the Orongorongo Valley DENSITY models ($D_{\text{behaviour}}$). Model selection is an important part of CR studies. Unless there is an *a priori* biological reason to choose one model over another, then model selection should be consistent in estimates being compared between different sampling sessions. The null model is frequently selected in CR studies when the power of a dataset is low (Mowat & Strobeck 2000). The null model is a naive estimator which assumes equal detectability of individuals, within and between sampling sessions. Equal detectability is unlikely to ever be true in wild populations (Pollock 1991). Mark retention can severely compromise conventional CR studies. In this study the 'mark' is the genetic profile of each individual sampled. Genetic profiles remain unchanged throughout the life of the individual although genotyping errors can occur (pg 69–72).

4.3.1 Spatial distribution of male and female ship rats

Of the nine rats detected at the Orongorongo Valley study site there was no evidence that hair tubes sampled one sex more effectively than the other. Further hair tube sampling is, however, necessary to verify this. Females sampled were spatially separate from the males. Whether this is an actual ecological pattern or just a coincidence is unclear from the data available. In radio-tracking studies by Hooker and Innes (1995) and Dowding and Murphy (1994) male and female ship rats did not appear to have spatially separate distributions. Factors such as season, rat density and the presence of other predators may influence how male and female rats are distributed. Further sampling at this site may clarify how male and female rats are spatially distributed within an area of habitat.

4.4 Using spatially explicit capture-recapture to estimate rat density

This current study indicates, as in Efford *et al.* (2009), that SECR can be applied to relatively small datasets. Efford *et al.* (2009) suggests as a rule-of-thumb that a minimum of 20 recaptures is necessary to obtain acceptable levels of precision (using DENSITY); with precision generally increasing with the number of recaptures obtained (see Efford *et al.* (2004)). A substantial advantage of using hair tubes over an invasive sampling method such as cage-trapping is the potential to gain a greater number of recaptures over the same number of sampling occasions. Hair tubes allow for multiple captures at more than one tube, over a single sampling occasion. In this study a comparatively low number of distinct individuals were sampled, however a high number of recaptures were recorded (Table 4.2). In some instances rats became extremely trap-happy, for example Rat (1) from the Orongorongo Valley, was recaptured on average 3.6 times per sampling occasion. The high recapture rate of Rat (1) and not other rats implies a difference in detectability between individuals, otherwise called 'individual heterogeneity'. However, sample size was probably too low overall to incorporate individual heterogeneity in DENSITY models (M. Efford, *pers. comm.*). In a larger dataset, individual heterogeneity may be worth incorporating as a model variable in DENSITY.

The precision of CR estimates can be improved by increasing the number of recaptures obtained. Low recapture rates of ship rats using cage-trapping have been recorded in several studies. For example, Innes (1977); Daniel (1972); and Latham (2006) failed to recapture (using cage-trapping) 59%, 53% and 39% of individual ship rats respectively following initial capture. Of all the ship rats sampled in this study only 26% were not recaptured over each sampling session. Wilson *et al.* (2007) recorded very high initial capture rates, followed by very low recapture rates, of ship rats in the Orongorongo Valley using cage-traps (Table 4.2). To increase the number of recaptures, for the purpose of density estimation, Wilson *et al.* (2007) pooled samples between sites. Pooling meant the total number of first-time captures was over ten times greater than that of first time captures recorded in the Orongorongo Valley in this study. However, the number of recaptures in the pooled data of Wilson *et al.* (2007) remained under half the number of recaptures recorded in this study. Furthermore, Wilson *et al.* (2007) recorded a number of capture-related mortalities (Table 4.2). It seems likely that the ship rats studied in Wilson *et al.*

(2007) exhibited some degree of trap shyness following initial capture. It is difficult to establish whether the ship rats in Wilson *et al.* (2007) exhibited trap shyness, or whether it was just that ship rats in this study became trap happy, or both.

Table 4.2: Numbers of first-time captures and recaptures using cage trapping in three sites (April 2004) and hair tubes in one site (June 2008), in the Orongorongo Valley. Capture-related mortalities are also recorded.

Dataset	Sites	First Captures	Recaptures	Mortalities
Wilson *et al.* (2007) (Cage-trapping)	1	21	7	0
	2	55	15	1
	3	32	5	2
Pooled total	3 sites	108	27	3
This study (Hair tubes)	1 site	9	56	0*

*No known mortalities recorded during the sampling session using hair tubes.

4.5 Evaluation of genetic sampling

An initial proliferation of studies advocating non-invasive genetic sampling in CR studies has been tempered by the realisation that these techniques have some serious pitfalls (Taberlet & Luikart 1999). Genetic errors associated with non-invasive CR could potentially nullify the apparent advantages of such studies. Identification and removal of genotyping errors is a crucial step in producing reliable estimates of population parameters from non-invasive CR data (McKelvey & Schwartz 2004). Error proofing allows managers to use DNA-based CR data much more widely and with a higher degree of confidence. Extraction and amplification success of microsatellite loci depends on the quality and quantity of starting 'template' DNA. Non-invasive sampling often means dealing with minute quantities of low quality DNA (Frantzen *et al.* 1998), and this can lead to a high rate of genotyping errors (Taberlet & Luikart 1999). Two main forms of error were recorded in this study: 'false alleles' and 'allelic dropout' (Taberlet *et al.* 1996; Taberlet & Luikart 1999). If genotyping errors remain unidentified then the false genotypes are misinterpreted as new individuals.

Allelic dropout occurs when only a single allele of the two alleles present in a heterozygous paired individual is amplified (Taberlet *et al.* 1996). This produces a 'false homozygote'. 'False alleles' may result when contaminated DNA is amplified instead of the targeted template DNA (Morin *et al.* 2001). DNA degradation through exposure to moisture, heat, and ultraviolet radiation can increase the rate at which genetic errors of either kind occur (McKelvey & Schwartz 2004).

Underestimation of population size may occur if multiple individuals appear to have the same genotype. This has been termed the 'shadow effect' (Mills *et al.* 2000) and this occurs most often when too few microsatellite markers are used or alternatively, when the markers lack heterozygosity (McKelvey & Schwartz 2004). Samples from populations with a high degree of genetic relatedness may be harder to differentiate; such populations tend to lack genetic variation. Therefore, use of an increased number of polymorphic loci is advised when studying island populations that may display very low levels of genetic variation (Paetkau *et al.* 1998). By using a larger number of polymorphic microsatellite loci, the shadow effect should rarely be an issue. The low PI_{sibs} value obtained from the Orongorongo Valley data confirmed that the microsatellite markers used in this study were sufficiently polymorphic to distinguish between siblings with similar genotypes.

Lastly, laboratory errors can occur. These can result from human mistakes such as incorrect labelling, switching or mixing of samples and errors in running the polymerase chain reaction (PCR) (Fernando *et al.* 2003). Laboratory error is rarely tested and almost never reported in non-invasive genetic studies (Gagneux *et al.* 1997). In most professional laboratories it seems likely that laboratory error rates are low.

The observed rate of allelic dropout was low in this study. Allelic dropout is one of the biggest shortcomings of non-invasive genetic CR studies (McKelvey & Schwartz 2004). The effect of failing to identify a false homozygote is two-fold; the presence of a 'new' individual elevates the first time capture rate and also decreases the recapture rate. This kind of error can have a severe affect on population estimation. Creel *et al.* (2003) found that genotyping errors caused up to a 5.5 fold increase in the estimated population size of wolves (*Canis lupus*) in Yellowstone

National Park. The problem of allelic dropout affects more than just population estimates. The rate of spurious 'new' individuals increases if more microsatellite loci are analysed and if the number of samples collected increases. This is because genotyping errors occur at the level of the individual locus (McKelvey & Schwartz 2004). Even if the single locus error rate is low, the probability of an error occurring in a multilocus genotype can be high. This also means that in a CR study every recapture could potentially equate to a 'new' capture. Thus a genetically sampled CR study that has a high number of recaptures may suffer high rates of genotyping errors and ironically produce a less reliable estimate of animal abundance than a concurrent invasive study that acquires less recaptures.

Nearly all organic tissue contains enzymes that degrade DNA and compromise genetic analysis (Foran *et al.* 1997). Enzymes can be inhibited by storing samples in an appropriate manner, such as freezing. Often the most practical field method is moisture removal, i.e. using silica beads or filter paper in a sealed container.

The hair tubes used in this study almost always plucked sufficient quantities of hair for microsatellite analysis. Initially several different hair tube and rubber band setups were trialled to establish which was the most consistent at obtaining sufficient hair samples. Genetic errors occur most frequently when low quantities of template DNA are amplified (e.g. Morin *et al.* 2001; McKelvey & Schwartz 2004). Taberlet *et al.* (1996) found that ≈56 picograms of template DNA was a critical threshold below which genetic errors increased substantially. Likewise Goossens *et al.* (1998) found that rates of genetic error dropped (14% to 4.9% to 0.3%) by increasing the number of alpine marmot (*Marmota marmota*) hairs analysed (1 to 3 to 10). Non-invasive sampling methodology should be designed to ensure sufficient quantities of template DNA are obtained. In general, fresh samples will provide more ready sources of template DNA than older samples (Banks *et al.* 2002). In this study successful genotypes were obtained from all Quarantine Island and Woodhaugh Gardens samples, even though they were analysed at least six weeks after collection. These samples were dried thoroughly and the results suggest that drying is the key to achieving high rates of genotyping success.

To further prevent genotyping errors Taberlet *et al.* (1996) proposed a 'multiple tubes' approach for obtaining reliable genotypes from non-invasively collected DNA. This involves repeating analyses several times for each locus and for each extract, which greatly increases the time and expense of laboratory analysis (Fernando *et al.* 2003). Sloane *et al.* (2000) concluded that the use of the multiple tubes approach is unnecessary because genotyping errors occur only rarely and they will nearly always be detected if a careful and conservative approach is taken to sampling and laboratory analysis. Genotyping errors should be reduced to negligible levels by using strongly polymorphic microsatellite markers. Potential genotyping errors can be dealt with by reanalysing genotype pairs that are very similar e.g. differ at only one locus (Woods *et al.* 1999). Likewise sexing or comparing the geographic location of similar 'suspect' genotypes may help determine whether an error exists. Care must be taken to ensure extreme movements are not removed from the dataset 'cleaned up'. If the screening process used to identify genotyping errors was insufficient in this study then the identification of false individuals may have resulted. However, Sloane *et al.* (2000) points out that genotyping errors mostly occur at a single allele. In this study all multilocus genotypes in the dataset varied at a minimum of four loci. It is therefore unlikely that genotyping errors occurred and were not identified.

More recent studies have emphasised that the rates of genetic error can be reduced to inconsequential levels in comparison with errors and bias associated with traditional CR methods (reviewed in Mills *et al.* 2000; Sloane *et al.* 2000; Creel *et al.* 2003; Paetkau 2003). Misidentifications occur in all tagging systems (Woods *et al.* 1999). Physical tags may be lost or misread, transmitters can malfunction and tattoos can fade or become distorted over time (Woods *et al.* 1999).

4.6 Distribution of rats in urban Dunedin

In New Zealand published estimates of urban ship rat population density and distribution are currently not available. Worldwide, studies which investigate the ecology of rats in towns are uncommon (e.g. Langton *et al.* 2001; Traweger *et al.* 2006; Morgan *et al.* 2009). Traweger *et al.* (2006) detected very low densities of Norway rats in the city of Salzburg and found that populations were distributed in a mosaic of 'hot spots' throughout the city. Likewise, Dickman

and Doncaster (1987) found that small mammals in Oxford, U.K., had widespread and patchy distributions, with some habitat patches supporting very dense populations and others none.

In this study rats were either present in residential gardens at very low densities (i.e. too low to be detected using wax blocks) or there detection obscured by other non target species such as cats. In Salzburg, using a combination of hair tubes (for the detection of rat presence) and cage traps, Traweger *et al.* (2006) recorded low detection rates of Norway rats in well maintained urban habitats such as household gardens. In Dunedin city, forested habitat appears to be commonly associated with the presence of ship rats. Similar results to these were recorded in Hamilton city where Morgan *et al.* (2009) failed to detect the presence of rats using wax blocks in a housed residential site, but confirmed the presence of rats in urban gullies, which contained fewer buildings and more vegetation. In Auckland, Gillies and Clout (2003) found that ship rats were a common prey item of household cats in homes located in urban/forest fringe habitat. In a fully urban site, isolated from forest, ship rats did not figure in Gillies and Clout (2003) that recorded prey of household cats. In Great Britain the utilisation of gardens by small mammals significantly decreases with increased urbanisation (Baker & Harris 2007). A number of factors may account for this, such as a reduction in the amount of traffic and the number of roads, reduced human disturbances, larger areas of residential gardens, and larger areas of natural and semi-natural habitat (Baker & Harris 2007).

4.6.1 Factors that may limit the abundance of rats

Previous studies have found small mammals reluctant to cross roadways, which leads to fragmented populations (e.g. Dickman & Doncaster 1987; Gerlach & Musolf 2000). The high density of roads surrounding urban forest fragments may act as a significant dispersal barrier for ship rats. Roads may restrict and fragment urban ship rat populations. This factor should be taken into careful consideration when planning a pest control operation (Traweger & Slotta-Bachmayr 2005; Traweger *et al.* 2006). The Dunedin City council owns and manages most of the major bush fragments in Dunedin City. These areas are weeded regularly and are also subjected to intermittent pest control (S. McLean, *pers. comm.*). Pest control consists of cage trapping for possums, and laying of poisonous bait for rats and mice. In the sites sampled in this study no pest control had taken place since January 2007. More precise and effective control

operations may be implemented if effort is targeted at habitat fragments with increased risk of rat infestation (Traweger & Slotta-Bachmayr 2005). Habitat suitability modelling using GIS has successfully been used to predict Norway rat infestation in Salzburg (Traweger & Slotta-Bachmayr 2005). Investigating the level of genetic relatedness of ship rat populations from different urban forest fragments may elucidate the degree to which ship rats disperse between discontinuous urban habitats. (Abdelkrim *et al.* 2009) found very little genetic structure in a ship rat population in Puketi Forest Conservation Reserve. However, ship rat populations in fragmented urban landscapes may contain high levels of genetic structure. Recognition of migration corridors, by the observation of genetic structure, would provide valuable information for the management of urban ship rat populations.

4.6.2 Habitat preference

Ship rats in urban areas may have a preference for living in buildings as opposed to in residential gardens. Of the properties infested with Norway rats in an English House Condition Survey, Langton *et al.* (2001) found a greater prevalence of Norway rats living in the outdoors (87.6%) compared with living indoors (12.4%) (n = 202). However, food, shelter and protection from predators may be more readily obtained for rats that live indoors. In both Morgan *et al.*'s (2009) and this study wax blocks were placed in residential gardens and not in or under houses. Additional research is necessary to determine whether rats are commonly found in housed residential areas and whether they prefer to live indoors or in residential gardens (Morgan *et al.* 2009). Although no rats were detected in the residential site sampled in this study, a number of residents mentioned that their cats often brought home rats. GPS-tracking of household cats may help determine exactly where rats are being caught i.e. indoors, in residential gardens or within urban forest fragments.

4.6.3 High detection rates of non-target species

Extremely high levels of non-target species interference on the wax blocks in the residential site may have masked the presence of rats. House mice and possum were detected regularly in all three study sites. High detection rates of mice may be predictive of low ship rat densities and vice versa. Innes *et al.* (1995) and Miller and Miller (1995) found that in non-urban areas

measured mouse abundance increased rapidly following large-scale poison operations targeted at ship rats. Likewise, Brown *et al.* (1996) recorded a significant increase in tracking tunnel use by mice after reducing rat abundance using snap traps. Brown *et al.* (1996) suggests that mice were deterred from entering tracking tunnels while rats existed in high densities, and a reduction in rat density meant the rate of mice detection increased. In urban Dunedin, high detection rates of mice may therefore be the result of low rat densities. Alternatively the neophilic nature of mice (Wolff & Sherman 2007), may have meant rats had less of an opportunity to chew wax blocks when mice were present. Rats were, however, detected in both McGouns Creek and the Wallace block where similar levels of non-target interference were recorded by possum.

4.7 Density of rats in urban Dunedin

Extremely low rat densities were recorded in urban Dunedin bush fragments. The pooled estimate of ship rat density, 0.26 rats/ha, is lower than any published estimate from New Zealand mainland forests, except those areas that have been actively controlled (Table 4.1). One of the initial aims of this study was to determine whether ship rat density varied between different areas within the urban environment. However, with such low all round detection rates, no conclusions could be drawn with regard to whether ship rat densities were higher or lower in inner-urban bush fragments versus peri-urban bush fragments. The density estimates reported in this study were recorded during different seasons. Using snap-trap indices Efford *et al.* (2006) found that seasonal variation in ship rat density less than two-fold on average, perhaps because rats unlike mice often live for more than one year. It is therefore unlikely that substantially different results would have been obtained by sampling in other seasons.

In most non-urban environments food availability is likely to be the limiting factor that determines ship rat carrying capacity (Harper 2005; Latham 2006). However, in urban areas this assertion may not be true. In residential areas household cats can reach extremely high densities, for example in urban Britain Sims *et al.* (2008) recorded between 132 and 1580 cats per square kilometre. Human provisioning of food means cat population densities are independent of prey availability. Household cats have huge potential to influence the density and distribution of rats in Dunedin city. In Dunedin an estimated 35% of all households own a cat (Y. van Heezik,

unpubl. data). Individual cats vary widely in the number of prey they catch, with most taking very few prey, or none at all (Churcher & Lawton 1987). However, collectively household cats are likely to be responsible for killing huge numbers of prey each year in urban areas (Baker *et al.* 2005). In Dunedin it is estimated that household cats may kill approximately 28,366 rats per annum (Y. van Heezik, unpubl. data). Even if the rate of secondary predation on rats was low, such high cat densities may result in an overall reduction in rat numbers. In Bristol, Baker *et al.* (2003) suggests that decreased mouse abundance in areas of increasing urbanisation are probably due to higher household cat densities. If rats exist in housed residential areas, it seems likely they do so in locations where cat density is low, or where there is sufficient refuge or cover from cats.

The extremely low rat densities within urban bush fragments indicated by both hair tubes and wax blocks may be due to a combination of high cat density, intermittent pest control or food and habitat limitations. Non-target interference by mice on wax blocks and hair tubes was frequently recorded. In the sites sampled mice appear to be present in higher densities than rats. Teeth marks of mice were found on imitation eggs placed in artificial nests in Dunedin gardens and bush fragments (van Heezik *et al.* 2008b). Further research could aim to quantify mouse densities in urban areas and the rate of mice predation on urban tree-nesting bird species.

4.7.1 The detection of Norway rats

The results from this study suggest that Quarantine Island is free of ship rats but appears to harbour Norway rats. The presence of Norway rats was initially suspected after finding active burrows, typical of Norway rats (Innes 1990b). Microsatellite results also show that the rat sampled on Quarantine Island was unlikely to be a ship rat because there were lower allele base-pair lengths at the D11Mgh5 and D19Mit2 loci (R. Howitt, *pers. comm.*). The microsatellite results also show that a single rat from Woodhaugh Gardens was potentially a Norway rat. Figure 4.1 illustrates this by plotting the D19Mit2 base-pair lengths of all the samples collected in this study, against D19Mit2 base-pair lengths from known Norway and ship rats (S. Miller, unpubl. data). The two rats recorded in this study (Rat 10, Quarantine Island; and Rat 6, Woodhaugh Gardens) had base-pair lengths which lay within the range for Norway rats. All other rats sampled in this study had distinct base-pair lengths from the known Norway rats, at the D19Mit2 locus. It is therefore inferred that all other rats sampled in this study were ship rats.

In this study Quarantine Island was sampled because it was a peri-urban site in which cats were absent. It was hypothesised that household cats suppressed the density of ship rats in urban Dunedin. Therefore if household cats were absent it was inferred that ship rat densities may have been high. However, after a month of sampling no ship rats were detected on Quarantine Island. A single Norway rat was detected; Norway rats are therefore either present in very low densities, or exhibited extreme neophobia. Norway rats exhibit neophobia most often when an unfamiliar object is placed in a stable, familiar environment (Innes 2001). Taylor and Thomas (1989) detected no evidence of neophobia in an isolated island population of non-commensal Norway rats. However, after hair tubes were left on Quarantine Island for one month, only three samples from a single rat were collected. Burrows 60–90 mm in diameter were commonly observed under rocks and the base of tree roots and no other burrowing animal is present on the island. The number of burrows indicates Norway rat density may be a lot higher than hair tube results suggest, unless the burrows are old and vacant. Norway rats may not have visited hair tubes for other reasons, such as a lack of attraction to peanut butter, or reluctance to run through the 65 mm diameter hair tubes.

Norway rats are a threat to ground-based fauna such as lizards, invertebrates and seabirds; they also may affect the regeneration of trees and shrubs (e.g. Allen *et al.* 1994; Wilson *et al.* 2003a; Clayton *et al.* 2008). The presence of Norway rats should be taken into careful consideration in future management of Quarantine Island. Predation pressure on tree-nesting bird species may be substantially reduced on Quarantine Island in comparison with nearby mainland urban areas, where ship rats and household cats are present. Further work could aim to quantify the breeding success of tree-nesting birds on Quarantine Island versus the breeding success of birds in urban areas in which ship rats and household cats are present. Wild populations of Norway rat in New Zealand are almost always found in close association with water (Innes 1990a). In the city of Salzburg, Traweger *et al.* (2006) found that the occurrence of Norway rats was positively associated with habitats that were within close proximity to water. It is therefore no surprise that the Norway rat from Woodhaugh Gardens (Rat 6) was detected on the banks of the Leith Stream, which, consisted of natural soils and rocks, surrounded by vegetation. These characteristics provide ideal habitat for Norway rats (Traweger *et al.* 2006).

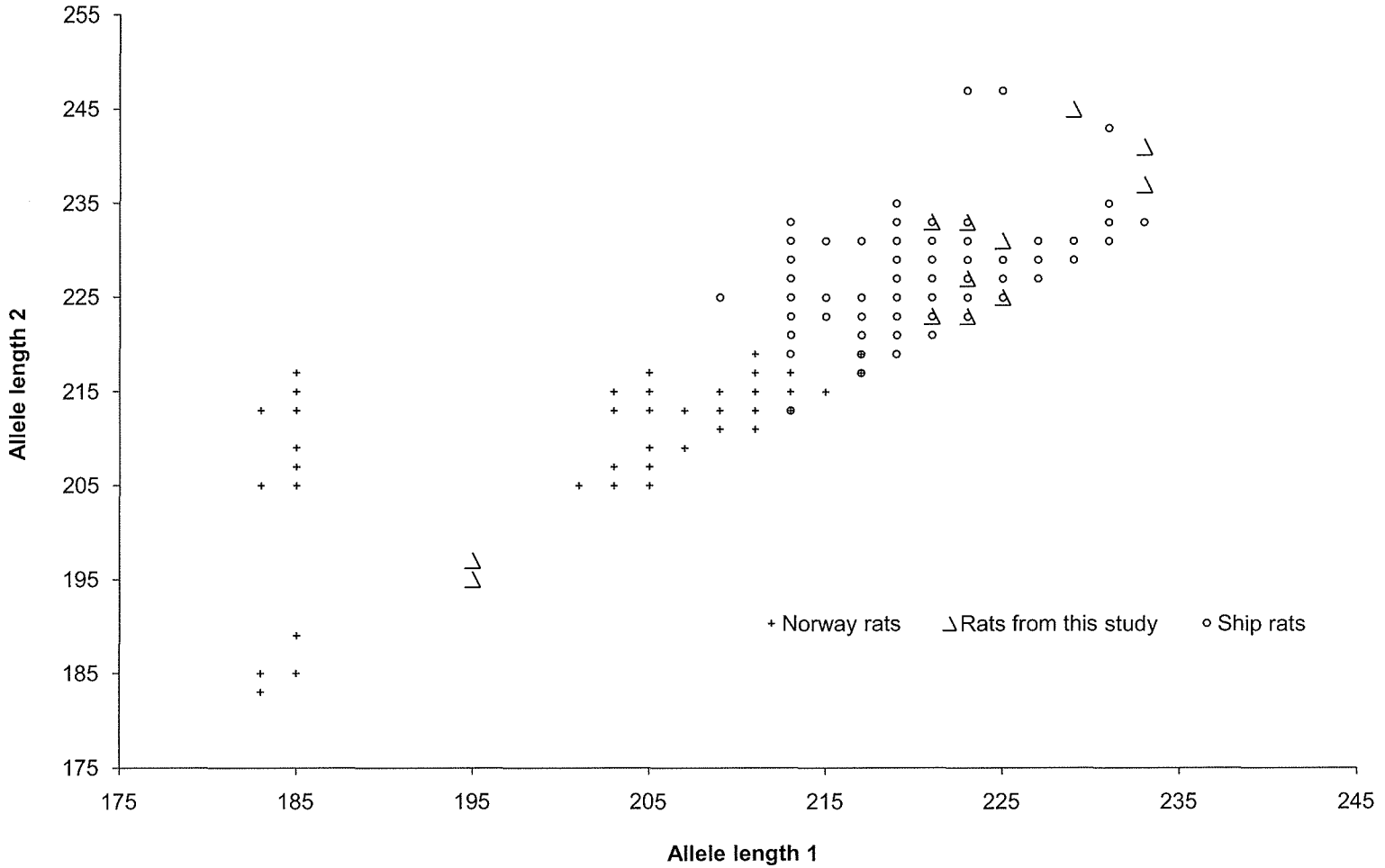

Figure 4.1: Comparison of allele base pair lengths (at the D19Mit2 locus) between rats sampled in this study (open triangles) (n = 19) versus samples from confirmed Norway rats (crosses) (n = 378) and ship rats (open circles) (n = 575) (S. Miller, unpubl. data). Two individuals in this study have base pair lengths, at the D19Mit2 locus, which fall within the range of Norway rats: Rat (6) Woodhaugh Gardens (195, 195); and Rat (10) Quarantine Island (195, 197).

4.8 Conclusions

This study has described a reliable non-invasive protocol for estimating ship rat density without the use of cage trapping. The validity of the hair tube methodology was confirmed in the Orongorongo Valley. The density estimate obtained from the Orongorongo Valley was low but comparable to recent cage-trapping estimates from the same sampling grid.

Wax block results suggest that ship rats are either absent from, or at very low densities within the housed residential sites sampled in this study. In urban bush fragments ship rats were detected infrequently using either wax blocks or hair tubes, and density was also inferred to be low. Ship rats were not detected on Quarantine Island, but after hair tubes were left on the island for a month a single Norway rat was detected. The efficacy of the hair tube approach to estimate Norway rat density was not tested. Neophobia in Norway rats, or inherent reluctance to enter the hair tubes, could compromise the efficacy of hair tubes.

The density of ship rats in Dunedin bush fragments appears to be much lower than the density of ship rats in non-urban forests. Low ship rat densities may be due to the combined effects of household cat predation and intermittent council pest control. Predation pressure by ship rats on native birds is likely to be minimal in Dunedin city in comparison to non-urban areas.

This study has demonstrated the feasibility and utility of applying polymorphic microsatellite loci for genetic profiling of ship rats. Given the difficulties associated with sampling elusive animals, genetic data from DNA extracted from hair follicles allows previously difficult-to-sample species, like ship rats, to be studied more easily. The collection of hair samples is less laborious and intrusive than trapping, anaesthetising, ear-tagging, releasing and recapturing ship rats. While immediate field identification of individuals is not possible using non-invasive genetic sampling, genetic sampling does enable researchers to carry out an array of population genetic analyses.

Non-invasive genetic sampling can be reliable and worthwhile in studies investigating population structure, providing strict guidelines are followed to reduce potential sources of error. Non-invasive genetic techniques will not be suitable for all situations given the relatively expensive laboratory work required. However, as the cost of genotyping continues

to drop, non-invasive genetic sampling will become more readily available and will undoubtedly become increasingly applied in conservation management. The techniques described in this study should be beneficial to managers who wish to deal with robust estimates of absolute density, rather than rely on index-based estimates of relative density. The hair tube approach to sampling may also be useful to managers who wish to validate index-based measures of relative density.

Further work is necessary to determine how hair tubes perform in sampling dense populations of ship rats. Trap saturation and mixed samples have the potential to compromise the results of genetic studies. Likewise, further research is necessary over a greater time scale to determine the degree to which ship rat density fluctuates in Dunedin bush fragments. If rats exist in high densities within urban Dunedin, it seems likely they do so within small pockets of favourable habitat. Additional sampling in Dunedin may confirm the presence of high density populations, or alternatively add support for the assertion of overall low ship rat density. Sampling within different New Zealand cities may also help confirm whether low ship rat density is a phenomenon common to all urban areas.

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