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# An Australian Abroad: The secret life of the brushtail possum (*Trichosurus vulpecula*)

A thesis presented in fulfilment of the requirements for the degree of

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New Zealand

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**Dedication**

For Apple Monster

**Abstract**

The “superspreader” hypothesis relates disease transmission to social contacts and assumes transmission is driven by the frequency, type and distribution of contacts among infected and susceptible individuals. I investigated characteristics of brushtail possum *(Trichosurus vulpecula)* home ranges for six wild free-living subpopulations, (four grids were studied; all of them before possum depopulation and two of them after possum depopulation) constructing social networks relevant to bovine tuberculosis (TB) transmission before and after depopulation. I also experimentally infected possums with a novel strain of TB to monitor secondary case infections in relation to these contact and other factors, including population density and sex ratio.

Before depopulation home range estimates showed adult males had larger home ranges than female and younger possums. Home range overlap and area of overlap differed between subpopulations, and possum sex and age; with adult males having more and larger overlaps with other possums. Possums were fitted with proximity-logging collars and contacts registered between April and October, 2012. The number of connections an individual has with others and the probability of the distribution of contacts it has within the population, or node degree and betweenness, also known as the shortest distance between individuals, were associated with sex, with males having higher values for each. Males also contacted more possums than females. Post-depopulation results showed an influx of male possums, higher population density, and smaller home range sizes than before depopulation. Possums post-depopulation also lacked an apparent ‘routine’ in contact networks, interacting with other possums haphazardly. The greater level of contact among adult males, than before depopulation, and their effects

on recovering populations post-depopulation, was likely the cause of more TB infection in adults and males.

This thesis provides empirical evidence that adult male possums have home range and contact network characteristics that are likely to enhance their involvement in the transmission and persistence of TB, relative to female and younger possums. Observations of experimentally infected individuals showed that infected males survived longer than females and that, as a consequence, those males potentially acted as a “supershedding” subgroup. I therefore provide evidence that adult male possums are the most important drivers of TB transmission and persistence of infection in populations, and could be targeted for control measures.

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## Thesis structure and format

This thesis is presented as a series of seven chapters. Encompassed by a general introduction and discussion, five research chapters have been prepared and are presented for future publication in peer reviewed journals.

### Chapter one

**General introduction and literature review** introduces the concepts behind the research contained in this thesis by discussion and reviewing current literature of basic ecology of brushtail possums and disease ecology associated with the aspects of bovine tuberculosis.

### Chapter two

**Interacting determinants of brushtail possum (*Trichosurus vulpecula*) home range size and implication for possum population management** will be submitted to Australian Journal of Ecology

### Chapter three

**Home range overlaps in the brushtail possum (*Trichosurus vulpecula*): investigating potential intrinsic and extrinsic determinants** will be submitted to Australian Journal of Ecology

### Chapter four

**Construction of brushtail possum (*Trichosurus vulpecula*) contact networks to inform on bovine tuberculosis transmission between individuals, and its persistence in wild populations**

**Chapter five**

**Changes in population structure following depopulation; implications for TB transmission and persistence**

**Chapter six**

**Relating variation in tuberculosis (TB) transmission in brushtail possums (*Trichosurus vulpecula*) to potential drivers found at the environmental, population and individual level**

**Chapter seven**

**General discussion** summarises the significant findings of this study. The relevance and implications are discussed and future research directions are suggested.

**Chapter eight**

**Literature cited** has been collated at the end of the thesis to reduce repetition. Literature is referred to in the format consistent with the format used for the journal Ecology.

**Chapter nine**

**Appendix:** Improving animal welfare standards while reducing disease exposure risk during euthanasia of trapped brushtail possums (*Trichosurus vulpecula*), C. Rouco, K.S. Richardson, D.M. Tompkins. Published Animal Welfare 2015, Vol 24; pg 235-239.

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# Chapter 1
# General introduction and literature review

## 1.1 Study aim

Although disease treatments in the form of vaccinations and population control methods have helped limit disease spread in the 20th century (Cohen 2000, Gubler 2009), the world still suffers over 13 million deaths annually due to infectious disease. While emerging infectious diseases (EIDs) related to disease spill-over between domestic and wild animals threatens domestic animal, human health and the conservation of global biodiversity, changes in the disease causing microorganisms themselves are contributing to the re-emergence of previously controlled diseases (Cohen 2000, Daszak et al. 2000).

The ability of a disease to transmit is dependent on many complex factors, one of which is the degree of contact an infected individual has with a possible host. Thus managing species populations, often by reducing population density through euthanasia is used in wild animals (Wobeser 1994). However, other methods of control targeting both population abundance and disease transmission have been suggested by way of fertility control (see Cowan 1996, 2000) and vaccination in species such as badgers (*Meles meles*) in the U.K. (Swinton et al. 1997, Tuyttens and Macdonald 1998, White and Harris 1995) and possums in New Zealand (Barlow 1991b, 1996, 1997, Caley et al. 1999, Cowan 1996, Roberts 1996). Hypotheses that controlling populations may have an effect on disease transmission from changing the social behaviour within a population, has been a topic of growing interest (Woolhouse et al. 1997), but these hypotheses have yet to be investigated in detail.

Aside from density dependent transmission, frequency dependent transmission allows for disease spread where transmission is not proportional to the number of susceptible or infectious hosts in a given area but rather dependent on the frequency of contacts infected individuals have with healthy individuals (Thrall et al. 1993, Antonovics et al. 1995).

The factors associated in estimating disease transmission coefficients is a difficult parameter estimation problem (Anderson and May 1991) because transmission of infectious agents within host populations is influenced by many different sources of heterogeneity ranging from behavioural to spatial factors (Anderson and May 1991, Grenfell et al. 1995, Isham and Medley 1996, Manning et al 1995, Morand et al. 1996, Scott and Smith 1994,). Trying to determine these factors in the field presents an enormous challenge (McCallum, Barlow and Hone 2001) and as such, studies directed at investigating disease transmission coefficients in both the laboratory and the field are uncommon (Bouma et al. 1995, Hone et al. 1992, Swinton et al. 1997, Begon et al. 1999).

Another hypothesis that exists for controlling both population and disease transmission is to attempt to target specific risk groups or individuals within a population (Anderson and May 1991, Bundy 1990, Jamison et al. 1993, WHO 1990). This method of control is not deemed the most cost efficient and the extent to which targeted control should be used rather than mass control methods would depend on the heterogeneities in transmission rates and cost of the two methods (Woolhouse et al. 1997). This method of targeting select individuals or groups of individuals is largely dependent on the general notion that few individuals are responsible for the infection of many.

An estimated 20% of host individuals typically contribute around 80% of the net transmission potential of infectious agents; hence the effects of heterogeneity on disease spread and persistence are key considerations in modern epidemiology and infectious disease management (Barlow 1991a, Tompkins et al. 2015). For example, analyses of severe acute respiratory syndrome (SARS) cases indicated that the great majority of infected individuals were unlikely to transmit the virus and infect others, but a small proportion were highly infectious (Lloyd-Smith et al. 2005). Similar patterns are seen in livestock and wildlife-associated infectious diseases: for example the high variability in transmissibility of foot and mouth disease (FMD) virus makes epidemics difficult to predict (Duffield and Young 1985) and a single relatively uncommon bird species, the American robin, appeared to be responsible for the majority of West Nile virus transmission in North America (Jackson and Morris 1995). Further, host heterogeneity can play a key role in the emergence of new infectious agents (Tanner and Michel 1999).

These two concepts form the basis of the high-profile 'superspreader' and 'supershedder' hypotheses (Lloyd-Smith et al. 2005, Matthews et al. 2006, Chase-Topping et al. 2008). The superspreader hypothesis relates disease transmission to social contacts and assumes transmission is driven by the frequency, type and distribution of contacts among infected and susceptible individuals (Biet et al. 2005, Natoli et al. 2005). In contrast, the supershedder hypothesis relates disease transmission to the production and dissemination of infectious stages; high shedding individuals are more likely to transmit infection than low shedding ones (Cobbold et al. 2007, Whitford et al. 2014).

Numerous studies have presented compelling evidence to support both the superspreader and supershedder hypotheses in a variety of infectious diseases such as Sin Nombre virus (Buddle and Young 2000) and *Escherichia coli* O157 (Cobbold et al. 2007, Matthews et al. 2009, Nugent et al. 2015, Spencer et al. 2015). For example, Clay et al. (2009) found that a large majority (80%) of the contacts by individual deer mice transmitting Sin Nombra virus are made by a minority (20%) of the population, and the combination of the number and duration of contacts per individual is positively associated with the probability of that individual becoming infected. In comparison, Stephens et al. (2009) showed that *Escherichia coli* O157 prevalence in cattle is increased by the presence of supershedders. Although they represent less than 10% of the population, supershedding cattle are estimated to be responsible for > 99% of bacteria shed and > 90% of new infections (Omisakin et al. 2003, Matthews et al. 2006, Chase-Topping et al. 2007). Böhm et al. (2009) suggested that individual dominant cattle in herds act as 'hubs' for interspecific contact with wild badgers in the UK, and heard management for bovine tuberculosis (TB) will likely be more effective and efficient if targeted against these individuals.

In many epidemiological models used to inform policy for the management of different diseases, either the superspreader or supershedder hypothesis are assumed. However, real-world scenarios are likely to involve both mechanisms to a greater or lesser degree (Jewell et al. 2009, McCallum 2009). For example, many consider the main mode of TB transmission from badgers in cattle to be indirect contact via excretory products on pasture (Muirhead et al 1974). Thus, disease management based on direct contact patterns alone may not have the desired effect.

The aim of this thesis is to investigate the social and contact behaviour of possums in wild free-living populations, with reference to the potential transmission of TB. This study combines considerations of social behaviour and experimental infections of a novel stain of TB and employs existing possum contact networks as a possible, traceable route to track the spread of disease through an uncontrolled environment. I use this as a model system to investigate the generic hypotheses noted above, and then discuss implications of my findings for infectious disease epidemiology and management in general.

Brushtail possums in New Zealand are a suitable study species as the presence of superspreaders has been clearly demonstrated in a social network analysis of captive possums (Ramsey and Efford 2010a), and the scheduled 2016 extermination of possums in the study area allowed for the use of the novel TB strain. Research of the superspreader and supershedder hypothesis has yet to be conducted in a wild free-living population.

## 1.2 Study species – the brushtail possum

The brushtail possum (*Trichosurus vulpecula*) is a small nocturnal marsupial, about the size of a house cat with adults weights ranging from c. 1.5 to 4.5 kg (Clout and Gaze 1984, Buddle et al. 1994, King 2005). They were introduced into New Zealand from Australia around 1840. At first they were listed as a protected species in New Zealand, but soon flourished; exacting a heavy toll on native biodiversity and becoming the primary wildlife reservoir hosts for TB (Buddle et al. 1994, Paterson et al. 1995, Rodger 1996, Biet et al. 2005, Ramsey et al. 2009). By the end of the 20$^{th}$ century possums had

colonized up to 98% of the land area of the country and their nationwide population was estimated at 60 to 70 million individuals (Buddle et al. 1994).

Domestic livestock are highly susceptible to infection with *Mycobacterium bovis*, the causative agent of TB in cattle (Gortazar and Cowan 2013b). Cattle (*Bos taurus*) were introduced into New Zealand around 1840, and as in most countries *M. bovis* was introduced along with them (Cooke 2000). TB was first identified in possums in New Zealand in the late 1960s, Possums have been acknowledged as the key wildlife reservoir, acting as the greatest barrier to the eradication of TB from livestock in this country (Matthews et al. 2006). Possum management has been undertaken since 1971 in one form or another in an attempt to prevent, or at least reduce, the transmission of TB to farmed livestock such as cattle and red deer (*Cerus* spp.) (Nugent et al. 2000, Stephens et al. 2009).

### 1.3 *M. bovis* and its presence in possums

TB is a zoonotic disease that thrives in complex systems at the wildlife-livestock interface and is a global challenge in animal health. TB control in cattle, complicated by reservoirs of infection in the wild (Gortazar and Cowan 2013a), costs millions to taxpayers globally (Zinsstag et al. 2006).
Possums are highly susceptible to TB infection and the development of both lymphatic and pulmonary TB (Jackson et al. 1995).

A range of experimental 'challenge models' have been developed, including infecting possums with *M. bovis* via the lungs (Buddle et al. 1994, Corner et al. 2002b, Buddle et al. 2006, Ramsey et al. 2009) or via intra-conjunctival administration (Corner et al.

2003). However, these models are invariably non-representative of natural infection due to their faster rates of infection in challenged subjects, and different clinical patterns of disease progression (Buddle and Young 2000). Natural TB in wild possums is frequently identified by one or a small number of gross tuberculous lesions, or broad disseminated lesions most commonly affecting the lungs and the peripheral lymph nodes associated with the limbs (Jackson et al. 1995, Cooke 2000), and individuals occasionally survive for over a year with clinical disease (Ramsey and Cowan 2003).

### 1.4 Home range, distribution and behaviour

For infectious diseases in animal populations, transmission is generally linked to both animal density and the behaviours that bring individuals into close proximity or contact (Crawley 1973, Ramsey et al. 2002b). Possum population ecology has been studied in both Australia (Dunnet 1956, Porphyre et al. 2008, Jewell et al. 2009, Gortazar and Cowan 2013b) and New Zealand (Tyndale-Biscoe et al. 1987, Brockie 1992, Jackson and Morris 1995, Cobbold et al. 2007). In New Zealand, possums are now found from the coast to high mountains and occupy virtually all vegetated habitats in the North and South Islands, as a result of both human relocations and their natural spread (Jackson and Morris 1995). Due to this nationwide infestation, and the importance of possums with regards to both biodiversity protection and TB management, studies on them have been undertaken throughout the country in most of the habitat types that they occupy (Cowan and Clout 2000). These include podocarp broadleaved forest (Crawley 1970, Blackie et al. 2011), farmland/woodland/ native forest (Paterson et al. 1995, Efford et al. 2000, Yockney et al. 2013b, Whyte et al. 2014) and grassland/ shrub-land (Glen et al. 2012a, Rouco et al. 2013). It's been shown that home range size can vary depending

on habitat and population density and although once established, possums tend to remain within their home ranges, boundaries can vary from day to day (Efford et al. 2000). In continuously forested habitats, resident possums have discrete, stable home ranges usually less than 500 m in length (Clout 1977, Ward 1978), whereas possums occupying the grasslands of Central Otago have been shown to occur naturally at densities of less than one per hectare (Nugent et al. 2015).

Home range is considered to be "that area traversed by the individual in its normal activities of food gathering, mating, and caring for young" (Burt 1943). However, in reality an individual home range can vary substantially both across years and seasonally within years (Ward 1978). Possums do not maintain fixed home range borders; behavioural plasticity can occur with respect to territorial behaviour due to both breeding and changing resource requirements (Whyte et al. 2014). Although adult possums tend to occupy their home ranges for life, boundary shifts occur regularly, though never more than a few hundred meters (Cowan and Clout 2000, Nugent et al. 2000).

Males appear to have larger home ranges than females although this relationship is generally not found to be statistically significant (Whyte et al. 2014), and females also display smaller nightly areas of activity than their male counterparts (Ward 1978, Brockie 1992, Cowan and Clout 2000, Ramsey et al. 2002b). Also, home range sizes seem to be determined by the location of resources rather than by behaviour intrinsic to the possum itself (Brockie 1992). The majority of possum nightly activity is concentrated around a small portion of the individuals larger home range (Ward 1978, 1984, Brockie and New Zealand. Ecology Division. 1987, Brockie 1992, Paterson et al. 1995).

Although possums are not considered territorial creatures (Ramsey and Efford 2010a), individuals (mainly female) do exhibit some forms of territoriality by way of defending a core area within their home range (Efford et al. 2000). Offspring stop foraging with their mothers at approximately eight months of age but remain within the maternal home range (Winter 1976). Young possum dispersal occurs around 9 to 12 months of age (Dunnet 1956, Cown et al. 1996, Efford 1998) particularly young males (Jewell et al. 2009), and most possums in New Zealand disperse between the months of February and June (Paterson et al. 1995). Of these juveniles 20-25% undertake long range dispersal (Clout and Efford 1984, Cowan and Rhodes 1993, Cown et al. 1996, Efford 1998, Omisakin et al. 2003) with a sex bias of roughly 4:1 towards male dispersers (Clout and Efford 1984, Cowan et al. 1997, Cowan and Clout 2000). As the males tend to move further from their mothers, the young females are more likely to be recruited into local populations (Clout and Efford 1984); in the study of Clout and Efford 1984, 89% of home range centres of native females lay within 60 m of the centre of their maternal range, and 64% of native females were at sites within their mothers' known home range. This occurred in only 19% of young males.

Variation in the home range characteristics of pest populations can substantially affect the ability of managers to control populations and assess models of epidemiology and biodiversity impact (Arthur et al. 2002, Corner et al. 2003, Matthews et al. 2006, Tompkins and Ramsey 2007, Ramsey and Efford 2010b).

### 1.5 Contact networks

Studies on wildlife contact networks have previously established their relevance to the transmission of infectious diseases (Craft and Caillaud 2011, Tompkins et al. 2011). Such studies include infectious cancer in the form of facial tumours found in Tasmanian devils (Hamede et al. 2009) TB infection in badgers in the U.K. (Böhm et al. 2009) and canine distemper virus found among Serengeti lions (Craft et al. 2011). Possum networks have previously been analysed based on den sharing between individuals (Ramsey and Efford 2010a) and activity range overlap (Porphyre et al. 2008). However, while both of these approaches identified 'highly connected' individuals, they are not wholly congruent to considerations of superspreading, being based on proximity and not actual contacts. This highlights the importance of identifying and quantifying only those contacts relevant to the transmission paradigms being investigated (Perkins et al. 2009), and even with this knowledge, little is currently known about possum contacts in the wild. It is thought, however, that possums are essentially solitary and spend less than 1% of their time directly interacting, except during the breeding season (Ramsey and Efford 2010a).

Interactions between possums tend to be infrequent, and physical conflict seldom occurs; with most interactions ending with threat displays usually followed by short chases (Paterson et al. 1995). However, many of these contacts are likely to be close enough to transmit TB between animals via the respiratory route. A study of contacts between male and female possums in the wild revealed that contacts were primarily sexual in nature and occurred more frequently during breeding season where one female would interact with up to four males (Ji et al. 2005). However, because possum home range sizes are not fixed, and behavioural plasticity can occur as needs for resources arise (Whyte et al. 2014), brief and infrequent contacts among possums outside of breeding season do occur (Brockie 1992, Ji et al. 2005). This raises an interesting

question about how TB persists in possums outside their higher contact seasons. Possums are known to come into close contact through communal nesting. They are known to frequent more than one den site within their home range (Dunnet 1956), and den sharing is not uncommon with recorded observations of both female-female and male-female pairs (Winter 1976, Caley et al. 1998, Ramsey and Efford 2010a). In contrast, Paterson et al (1995) observed den sharing between mother and pairs of offspring only, yet there is general agreement that social interactions occur.

### 1.6 TB transmission in the brushtail possum

The mechanisms of TB transmission among possums are not well understood, transmission during mating, fighting, sequential den sharing and mother to pouch young transmission are all possible methods of infection (Jackson et al. 1995, Ramsey et al. 2009). Paterson (1995) states that evidence suggests transmission of tuberculosis is associated with denning areas where transmission to livestock is most likely to occur, however, this is speculative and further investigation of transmission mechanisms is necessary. Evidence has been found that TB infection in possums is not age-dependent (Jackson 1995a), and tends to be more prevalent in males than females (Buddle et al. 1994, Jackson et al. 1995, Zinsstag et al. 2006), and Brockie et al (1981) has stated that once infected, adult females have a higher survival rate than males, though all infected individuals are thought to die within four to six months of infection (Norton et al. 2005, Otterstatter and Thomson 2007).

While numerous studies have focused on possible drivers of disease such as population density (Cowan and Clout 2000, McCallum 2009, Whyte et al. 2014, Nugent et al.

2015) and home range size (Burt 1943, Ward 1978, Paterson et al. 1995, Arthur et al. 2002, Whyte et al. 2014, Nugent et al. 2015), it remains unclear which if any of these factors have an effect on possum interactions, and thus the potential for intra-specific transmission.

### 1.7 Thesis chapter contents

This thesis focuses on an analysis of possum social and contact behaviour, with the aim of identifying potential drivers of infectious disease transmission. I use a uniquely large data set from a highly amenable disease/wildlife system, to further our understanding of how disease transmits and persists in wildlife populations. Analyses for the following chapters is comprised of two seasons of mark and recapture data coupled with two seasons of contact data provided by proximity-logging radio collars, to investigate possum behaviour, the dynamics of contact networks and disease transmission.

This thesis comprises five analytical chapters investigating possum home range size, possum home range overlaps, possum contact networks and possible disease transmission among possums, with a chapter comparing differences between the first and second seasons. In Chapter 2, I estimate the home range size and investigate the drivers of home range size of possums (adult, juvenile and male, female) by minimum convex polygon (MCP) estimates on all four grids during the first season.

After estimating home range size, the data are used in Chapter 3 to look deeper into the determinants of possible home range overlaps among possums. Here I investigate the determinants of home range overlap, examining the role of possum age, sex and trapping grid.

The contact network chapter (chapter 4) explores high and low connectivity of individuals in the study subpopulations, with reference to the superspreader hypothesis, by constructing and analysing networks using data from contact-loggers with detection distances set at extreme close range (Ji et al. 2005). The collars record the close proximity of any other collared possum (detecting pulse controlled transmitters in the collars). Contact networks are also constructed for each subpopulation.

Chapter 5 employs analysis used in the previous three chapters to investigate any differences in home range sizes, home range overlaps and the contact networks following the first season depopulation and re-establishment.

Finally, in chapter 6, I apply the findings from Chapters 2-5 to investigate determinants of TB transmission in possums resulting from experimental challenges in wild free-living individuals. This involves using a novel strain of TB different to the local background strain to track possible secondary case infections through the population using the aforementioned concepts.

This thesis will conclude with a general discussion reporting all findings and discussing implications and recommended future work

# Chapter 2

# Interacting determinants of brushtail possum (*Trichosurus vulpecula*) home range size, and implications for possum population management

## 2.1 Introduction

The brushtail possum (*Trichosurus vulpecula*) is a serious conservation pest impacting native biodiversity and the main wildlife reservoir host of bovine tuberculosis (TB) in New Zealand. An understanding of home range (and 'activity range') behaviour is recognized as critical to many aspects of its management, informing models of disease epidemiology (Ramsey and Efford 2010a) and biodiversity impact (Holland et al. 2013), and guiding strategies for its population control (Matthews et al. 2006, Tompkins and Ramsey 2007, Monks and Tompkins 2012). Many studies have investigated the determinants of possum home range size. However, the picture obtained to date is inconsistent (Table 2-1).

While several determinants have been identified, only a negative relationship between home range size and density appears to be consistent, both within and across studies (Whyte et al. 2014, Nugent et al. 2015). Although age and sex are identified as other natural determinants, tests for their effects have not always shown significance (Table 2-1). However, an apparent association between failure to demonstrate such effects and

smaller sample size indicates this may be due to statistical power issues rather than inconsistency of effect. Such issues are common to ecological studies for two frequently unavoidable reasons (Jennions and Møller 2003, O'Brien et al. 2009). First, both the time and effort generally required to conduct such work restricts its scale. Second, establishing independent relationships can be difficult due to high levels of variation and multiple interacting and/or confounding factors in uncontrolled circumstances. For possum home range determinants, variation among studies in the number of individuals monitored and/or the amount of spatial data obtained per individual are thus both potential explanations for the lack of consistency observed (Table 2-1).

Here I take advantage of a uniquely large data-set to investigate whether or not effects of sex and age on possum home range size can be discerned. This opportunity was offered by large-scale possum population monitoring as part of TB transmission trials. If sex and age are significant determinants of home range size in these trials, this could support the hypothesis that their inconsistent effects across previous studies were indeed driven by statistical power issues. In contrast, failure to detect such effects would strongly suggest that they are not generic to all settings but rather are contingent on other factors. Although seasonality effects are also inconsistent across studies (Table 2-1), our data set has insufficient seasonal replication to provide a similar test for this determinant. Such effects are not explicitly investigated here, although possible implications of our findings for seasonality patterns are discussed.

**Table 2-1** Past studies investigating determinants of possum home range (or 'activity range') size, in relation to contextual factors. Under 'determinants', bold text indicates those reported as statistically significant. Sample size indicates number of individuals included in analyses, and is not necessarily a reflection of the data per individual. * Denotes an experimental manipulation.

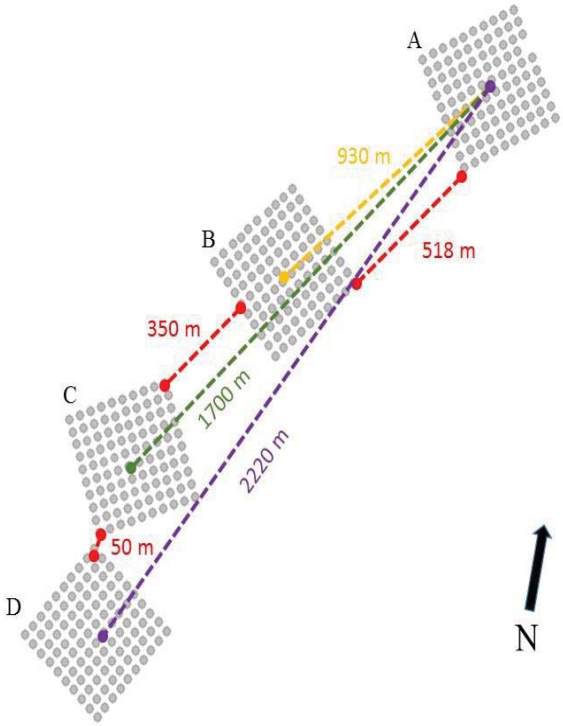
| Habitat | Date range | Site | Determinants investigated | Sample size | Density (ha-1) | Mean home range (ha) | Methods | Reference |
|---|---|---|---|---|---|---|---|---|
| Podocarp broadleaved forest | Feb 1966 Oct 1968 | Orongorongo Valley | **sex, age** | 301 | 6.4 - 10.6 | 0.46 - 0.81 | Live-trapping | Crawley et al. 1972 |
| Farmland woodland | Apr 1990 Apr 1992 | Wairarapa Region | **sex**, **season,** age | 114 | not reported | 4.4 | Live-trapping; radio-tracking | Paterson et al. 1995 |
| Farmland native forest | Jul 1993 Oct 1996 | Dunedin | **sex,** population perturbation* | 337 | 0.033 - 0.022 | 33.6 - 45.3 | Live-trapping; radio-tracking | Efford et al. 2000 |
| Farmland native forest | Sep 1996 Aug 2001 | Manawatu | **sex, sterilization*** | 82 | not reported | 0.5 | Live-trapping | Ramsey 2000 |
| Nothofagus spp. Forest | Oct 2007 Oct 2008 | Kaimanawa Range | season, **population perturbation*** | 50 | 0.8 – 5.6 | 3.1 | GPS tracking | Pech et al. 2010 |
| Podocarp broadleaved forest | Nov 2007 May 2009 | Waikato Region | sex (juvenile), **mother removal*** | 28 | 14 | 0.5 | GPS tracking | Blackie et al. 2011 |
| Grassland shrubland | Jan-05 | Molesworth Station | Sex | 29 | 1.7 | 5.1 | Radio-tracking | Glen et al. 2012 |
| Grassland shrubland | Mar-10 | Central Otago | **Density** | 370 | 0.4 - 0.7 | 36.2 - 54.1 | Live-trapping | Rouco et al. 2012 |
| Farmland | Sep 2009 Feb 2010 | Molesworth Station | **Sex** | 63 | <1 | 22.3 | GPS tracking | Yockney et al. 2013 |
| Farmland pine forest | Jan 2011 Feb 2012 | Canterbury Region | **density,** season, sex | 17 | 7-Jan | 1.2 - 12 | GPS tracking | Whyte et al. 2013 |

## 2.2 Methods and Materials

### *2.2.1 Study site*

The study site was a designated research area in the Orongorongo Valley on the North Island of New Zealand (41°21'S, 174°58'E). The valley is orientated north to south, runs between steep ridges rising to 670 m above sea level, and contains mixed beech/podocarp forest that supports high possum densities (6-12/ha; (Efford and Cowan 2004, Rouco et al. 2015)). Home range data were obtained from four square trapping grids (A, B, C, and D) situated horizontally between the ridge and the river on the east ridge of the valley, each made up of 100 traps at 40 m spacing covering approximately 13 ha. The grids were created specifically for this project and no previous studies had ever been conducted in the immediate vicinity. Grids A to D run north to south respectively with a distance of 518 metres between grids A and B and 350 metres between grids B and C. Due to the terrain of the valley and the borders of the Landcare Research study area, grids C and D we situated only 50 metres apart with a hillside ridge separating them (Figure 2-1). Possums were captured in Grieve wire cage traps (60 × 26 × 28 cm) with spring-assisted folding doors triggered by a pendulum bait hook (Buddle et al. 1994, Warburton et al. 2000).

Figure 2-1 Study grids layout with distances measured in metres.




*2.2.2 Data collection*

Possums were captured during monthly trapping sessions from March–December 2012, from the start of the Austral autumn through to early summer. April–June is the peak possum breeding season at the study site (Fletcher and Selwood 2000). Each trapping session consisted of four consecutive trap nights, with traps being checked and re-baited each day. Upon initial capture, individuals were anaesthetized by intra-muscular injection of Zoletil 100® (Virbac New Zealand Ltd, Auckland, New Zealand; (Morgan et al. 2012), tagged in both ears with unique numbered tags (National Band & Tag Co. size 3, Kentucky, USA), and had their sex and juvenile/adult status recorded before being released at the trap site where they were caught. Possums were classified as belonging to either a 'juvenile cohort' or an 'adult cohort' based on the developmental stage of the pouch/testes at initial capture (Corner et al. 2003).

*2.2.3 Population-scale density and mean home range estimates*

A maximum likelihood approach in version 5.0 of the program DENSITY (Efford 2004, Borchers and Efford 2008); http://www.otago.ac.nz/density/) was used to estimate possum density and mean home range parameters from the trapping data, employing the spatially explicit capture–recapture (SECR) module. The probability of catching an animal was modelled based on the distance between the trap and the home range centre, with the spatial position of home-range centres assumed to follow a Poisson distribution. The simplest spatial detection function available in DENSITY (half-normal) was utilized (Efford 2004). This function has two parameters, the first ($\sigma$) corresponding to a measure of home range size ($2.45\sigma$ = 95% home range radius assuming a circular shape; (Efford et al. 2005), and the second ($g_0$) being the one-night probability of capture at the home range centre. The default settings were used for all computations, except for 'buffer width' which was set to 200 m. This was checked retrospectively using the spatially explicit capture–recapture log likelihood tool to ensure these settings were appropriate (i.e. density estimates did not change as buffer width was increased).

Population-scale density and mean home-range parameter estimates were obtained for each trap grid independently, utilizing the full 10 months of data on the basis of 'between-sessions' models of variation in $g_0$ and $\sigma$. Corrected Akaike's information criterion (AICc) values were used to choose between a null model ($M_0$) with constant $g_0$ and $\sigma$, and models in which both parameters varied according to temporal variation ($M_t$) in detection parameters over the trapping period, behavioural response to capture (either permanent ($M_b$), or temporary ($M_{b1}$) affecting only the next capture) and considering

either sex differences ($M_{sex}$) or age differences ($M_{age}$) in $g_0$ and σ (Borchers and Efford 2008, Nugent et al. 2015).

*2.2.4 Determinants of individual home range size*

All trap locations were recorded in the field as Universal Transverse Mercator coordinates (UTM) using a Garmin-12 Global Positioning System (GPS) portable receiver. The 100% Minimum Convex Polygon method (MCP; (Mohr 1947) was used to estimate home range sizes on an individual basis across the full 10 months of data. This was implemented in the program ArcView 3.2 (Environmental Systems Research Institute), with a buffer area set as half the distance between traps (i.e. 20 m). MCP estimates were obtained for all animals that were trapped at least five times during the ten months, and log-transformed to satisfy assumptions of normality. They were then analysed with respect to sex (female versus male), age (juvenile versus adult) and grid by analysis of variance (including all interaction terms) in Statistica v9.0 (Statsoft, Tulsa), with the number of occasions that a possum was trapped as a covariate.

## 2.3 Results

*2.3.1 Population-scale density and mean home range estimates*

There were 7697 captures of 608 possums during the 10 months of the study (Table 2-2). The population across all four trapping grids comprised 42% adult female, 36% adult male, 12% juvenile male and 10% juvenile female. In the program DENSITY, the model in which the home range parameters were allowed to vary to reflect a temporary behavioural response to capture ($M_{b1}$) was the most parsimonious for grids A, B and D,

while the model with the permanent behavioural response to capture ($M_b$) was the best fit for grid C. There was very strong support for the most parsimonious models, being more than two AICc units from the second best model for each grid (Table 2-3). Based on these models, mean (SE) possum density was estimated as 4.87 (0.19), 6.92 (0.29), 4.08 (0.21) and 4.20 (0.19) $ha^{-1}$ for grids A–D respectively. There was no significant support for sex or age effects on DENSITY home range parameters.

**Table 2-2** Brushtail possum sex ratio, age structure and individual trapping frequency (across the ten months study period), for the marked sub-populations on trapping grids A-D. Mean trapping frequencies were similar across grids, sexes and age cohorts.

| | | | Grid A | Grid B | Grid C | Grid D |
|---|---|---|---|---|---|---|
| All individuals | | Number | 146 | 178 | 144 | 140 |
| | | Mean times trapped (range) | 15 (3,41) | 11 (1,36) | 10 (3,37) | 12 (1,33) |
| Adult individuals only | All adults | Number (percentage of trapped population) | 126 (86%) | 130 (73%) | 102 (71%) | 114 (81%) |
| | | Mean times trapped (range) | 15 (3,41) | 11 (3,36) | 10 (3,37) | 12 (1,33) |
| | Male only | Number (percentage of trapped population) | 63 (43%) | 52 (29%) | 51 (35%) | 51 (36%) |
| | | Mean times trapped (range) | 17 (3,41) | 14 (3,36) | 11 (3,37) | 14 (3,32) |
| | Female only | Number (percentage of trapped population) | 63 (43%) | 78 (44%) | 51 (35%) | 63 (45%) |
| | | Mean times trapped (range) | 12 (3,40) | 9 (3,26) | 9 (3,25) | 10 (3,33) |
| Juvenile individuals only | All juveniles | Number (percentage of trapped population) | 20 (14%) | 48 (27%) | 42 (29%) | 26 (19%) |
| | | Mean times trapped (range) | 14 (3,39) | 11 (1,27) | 11 (3,24) | 14 (3,29) |
| | Male only | Number (percentage of trapped population) | 8 (6%) | 30 (17%) | 24 (17%) | 16 (11%) |
| | | Mean times trapped (range) | 15 (3,39) | 11 (1,27) | 12 (3,23) | 17 (3,29) |
| | Female only | Number (percentage of trapped population) | 12 (8%) | 18 (10%) | 18 (13%) | 10 (8%) |
| | | Mean times trapped (range) | 15 (3,34) | 11 (3,22) | 9 (3,24) | 11 (4,24) |

**Table 2-3** Estimates of possum density $\widehat{D}$ (ha$^{-1}$) and the parameters $\hat{g}_0$ and $\hat{\sigma}$ of a half-normal detection function for each trapping grid. Estimated standard errors are shown in brackets. ΔAICc is the difference between the corrected AIC of the model in question and the best model (with lowest AICc). See Methods for an explanation of the different models explored.

| | Model | $\widehat{D}$(ha-1) | $\hat{g}$ 0 | $\widehat{\sigma}$(m) | AICc | ΔAICc |
|---|---|---|---|---|---|---|
| Grid A | Mb1 | 4.87 (0.19) | 0.088 (0.006) | 45.82 (1.41) | 8183 | 0 |
| | Mb | 4.68 (0.20) | 0.084 (0.007) | 48.55 (1.63) | 8216 | 32.7 |
| | M0 | 5.03 (0.17) | 0.138 (0.006) | 37.34 (0.70) | 8282 | 98.8 |
| | Msex | 5.03 (0.17) | 0.138 (0.006) | 37.33 (0.70) | 8282 | 99 |
| | Mt | 5.03 (0.17) | 0.138 (0.006) | 37.35 (0.70) | 8285 | 101.7 |
| | Mage | 5.20 (0.17) | 0.163 (0.006) | 36.28 (0.63) | 8471 | 288.5 |
| Grid B | Mb1 | 6.92 (0.29) | 0.105 (0.008) | 31.68 (0.98) | 6193 | 0 |
| | Mb | 6.39 (0.30) | 0.104 (0.010) | 34.15 (1.24) | 6212 | 18.9 |
| | Mt | 6.64 (0.23) | 0.168 (0.008) | 26.53 (0.55) | 6228 | 35.1 |
| | Msex | 6.67 (0.21) | 0.162 (0.008) | 26.92 (0.55) | 6234 | 41.3 |
| | M0 | 6.66 (0.23) | 0.161 (0.008) | 27.00 (0.55) | 6255 | 61.8 |
| | Mage | 7.23 (0.88) | 0.129 (0.008) | 29.56 (2.21) | 6323 | 130.1 |
| Grid C | Mb | 4.08 (0.21) | 0.054 (0.005) | 60.03 (2.53) | 6247 | 0 |
| | Mt | 5.18 (0.21) | 0.074 (0.004) | 42.08 (1.10) | 6261 | 13.8 |
| | Mb1 | 4.81 (0.23) | 0.049 (0.004) | 54.07 (1.97) | 6266 | 19.5 |
| | M0 | 5.20 (0.21) | 0.065 (0.004) | 44.73 (1.11) | 6324 | 76.6 |
| | Msex | 5.21 (0.21) | 0.065 (0.004) | 44.67 (1.11) | 6324 | 77.2 |
| | Mage | 5.88 (0.79) | 0.071 (0.013) | 41.85 (3.55) | 6423 | 176.3 |
| Grid D | Mb1 | 4.20 (0.19) | 0.059 (0.004) | 59.29 (2.08) | 7849 | 0 |
| | Mb | 3.63 (0.17) | 0.071 (0.006) | 63.70 (2.68) | 7851 | 2.3 |
| | Mt | 4.63 (0.17) | 0.081 (0.004) | 47.63 (1.05) | 7902 | 53 |
| | M0 | 4.67 (0.17) | 0.081 (0.004) | 47.64 (1.04) | 7929 | 80.2 |
| | Msex | 4.68 (0.17) | 0.081 (0.004) | 47.53 (1.04) | 7931 | 82.6 |
| | Mage | 4.56 (0.52) | 0.086 (0.007) | 50.42 (2.98) | 8130 | 281.7 |

*2.3.2 Determinants of individual home range size*

Of the 608 possums caught across the four trapping grids, 530 were trapped at least five times and had their MCP home ranges estimated. Estimated mean (SD) home range size across all grids varied from 1.08 (0.05) ha for adult females to 1.55 (0.07) ha for adult males, and were 1.17 (0.13) ha and 1.09 (0.14) ha for female and male juveniles, respectively. Mean MCP home range size was significantly negatively correlated to mean possum density across the four trapping grids ($r_{spearman} = –0.98$, $p = 0.02$).

In the analysis of MCP home range area with respect to sex, age and grid, a statistically significant effect of grid was observed (Table 2-4). This was expected from the observed differences in possum density among grids, and the negative relationship between mean home range size and density demonstrated at the population scale (Table 2-3). Also reflecting the population-scale analyses, there were no significant independent effects of either sex or age on MCP home range size. However, there was a significant interaction between the two (Table 2-4). While home range size was similar for female and male juveniles, it was smaller on average for female than male adults. This interaction appears to reflect male adults have significantly larger home ranges than all other age/sex combinations (Figure 2-1).

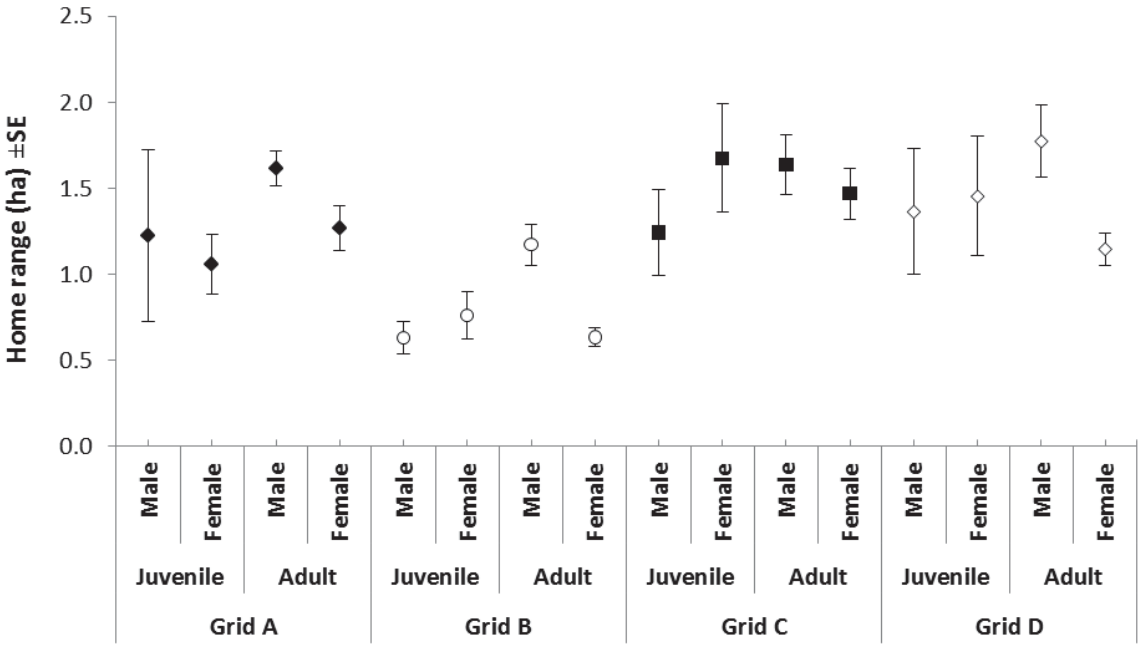
**Table 2-4**

F values from the factorial analysis of variance of the effect of grid (A, B, C, D), age (juvenile versus adult) and sex (male versus female) on possum home range size, including the number of times each possum was trapped as a covariate.

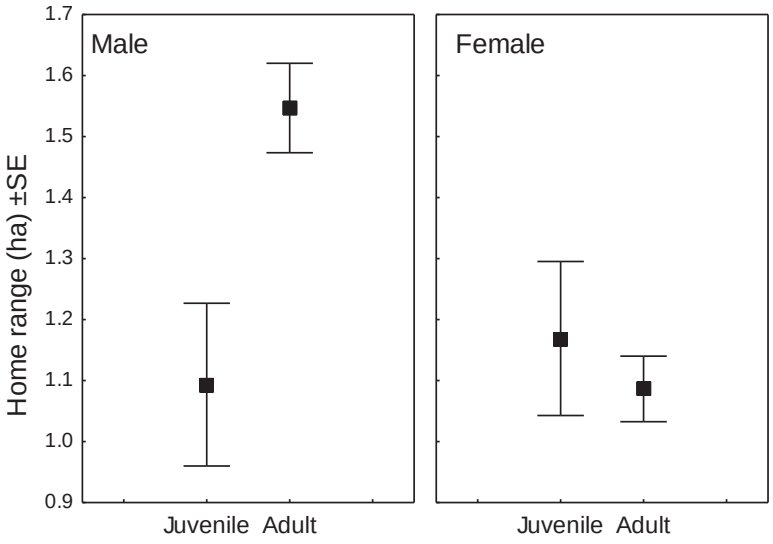
| | DF | ***F*** | P |
|---|---|---|---|
| **Intercept** | 1 | 27.68 | **<0.0001** |
| **Covariate** | 1 | 36.83 | **<0.0001** |
| **Grid** | 3 | 8.84 | **<0.0001** |
| **Age** | 1 | 1.02 | 0.312 |
| **Sex** | 1 | 1.51 | 0.219 |
| **Grid x Age** | 3 | 0.12 | 0.947 |
| **Grid x Sex** | 3 | 0.91 | 0.171 |
| **Age x Sex** | 1 | 7.35 | **0.006** |
| **Grid x Age x Sex** | 3 | 0.11 | 0.951 |

**Figure 2-1** Brushtail possum home range estimates (minimum convex polygon, calculated from trapping data) by age and sex for (A) trapping grids A–D separately, and (B) for the four grids combined.

A)




(B)



## 2.4 Discussion

Our results suggest that while differences in statistical power are partly responsible for previous inconsistencies across studies in sex and age effects on possum home range size (Table 2-1), this is not the whole story. Despite our data set being uniquely large (Table 2-2), we found no statistically significant independent effects of sex or age (Table 2-3; Figure 2-1). Rather, there was a significant interaction between the two (Figure 2-1). Such interactions among determinants not only raise the data requirements of studies to demonstrate them, but also may obscure effects when not accounted for. Hence this interaction is likely to be at least partly responsible for the previous inconsistencies. For example, in (Paterson et al. 1995) where an age effect was not demonstrated, only a small sample of juveniles was available for analysis. In contrast, the positive effect of age in Crawley (1973) was likely to be driven by adults being trapped over three times more frequently than younger possums. Since home range estimates initially increase with the number of times trapped (Crawley 1973), this is possibly an example of low sample size, or an inappropriate statistical test, leading to a flawed conclusion.

In studies where sex was identified as a significant determinant of possum home range size, the sample of animals studied consisted either wholly or mainly of adults (Efford et al. 2000, Yockney et al. 2013a). With such samples, there would be few juvenile similarities (as observed here; Table 2-1) to potentially mask the sex effect. In contrast, in some cases where a sex effect was not demonstrated, the samples analysed were all juvenile (Blackie et al. 2011, Glen et al. 2012b). In other such cases age was not considered, and thus the unaccounted variance may have been a cause of sex effects not being significant (Whyte et al. 2014). Considering the potential for such interactions in

both study design and analysis appears essential when investigating determinants of possum home range size. Their omission may also be responsible for season being indicated as a determinant by Paterson et al. (1995), but not by the studies of Nugent et al. (2000) and Whyte et al. (2014). Age effects not being properly partitioned in the Paterson et al. (1995) study (see above) may have caused the incorrect observation of a seasonal effect (Table 2-1).

The additional insight into determinants of brushtail possum home range size offered by the present study indicates that sex and age effects, by which adult males have markedly larger home ranges than all other individuals, are likely consistent. Such consistency has implications for the planning and implementation of possum population management for TB eradication and biodiversity benefit. The age effect indicates that populations with younger age structures may be relatively harder to control than established populations due to animals with smaller home ranges being less likely to encounter control devices or baits. Such populations can occur as a result of re-establishment following a prior control event (e.g. Clout and Efford (1984)). With respect to TB epidemiology, the sex effect indicates that infected adult male possums may play a relatively greater role in disease transmission, persistence and spread, if their larger home ranges bring them into contact with more individuals. Finally, the interacting effects of age and sex driving adult female home range sizes below the average size of males and females of both cohorts may result in predicted optimal strategies for possum eradication (for example by trapping or bait-station delivery of control agents; Ball et al. 2005; Tompkins & Ramsey 2007) under-estimating the effort required to sufficiently control the reproductive output of the population. In conclusion, our findings indicate that population structure needs to be considered along with population density in the design and application of possum management approaches.

# Chapter 3

# Home range overlaps in the brushtail possum (*Trichosurus vulpecula*): investigating potential intrinsic and extrinsic determinants

## 3.1 Introduction

Studies of wildlife habitats and home range characteristics including overlaps between individuals, are common (Lugton 1997, Nugent et al. 2013a). Numerous studies attribute infectious disease transmission to contact among individuals (Brockie 1992, Day and O'Connor 2000, Whyte et al. 2014). In solitary species, such as the brushtail possum, that don't generally undergo large spatial movement, (Crawley 1973, Nugent et al. 2000), home range overlaps with infected individuals occupying the same space are the most likely determinants of disease transmission. Such thinking is the basis of density-dependent transmission used in epidemiological models, where the force of infection increases linearly with population density (Morris et al. 1994, Begon et al. 2002). In recent years, however, studies have shown that the assumptions of density dependent transmission (on which management is based) rarely hold completely due to species behaviour (Morris et al. 1994). As a result, efforts to manage disease can fail due to a lack of understanding of species behavioural components and how they influence disease transmission, particularly as host population density decreases.

In Chapters 1 and 2 I detail the general lack of information (with some notable exceptions; for example see Ji et al. 2005) regarding social interactions in possums.

With TB being an infectious disease, such a lack leads to critical knowledge gaps with regards to how TB is transmitted and persists within possum populations. Experimental evidence has shown that *M*. bovis can survive for long periods of time in the environment (Biet et al. 2005). This survival varies drastically over time depending on its environmental situation as it can be deposited in grass, soil, water or carcass tissue (see Little et al. 1982, Duffield et al. 1985, Tanner et al. 1999, Young et al. 2005). In New Zealand *M*. bovis can survive for up to two weeks in a suitable environment, however, when exposed to ultra violet light the survival time of TB drops to a matter of days (Jackson et al. 1995). This, along with the natural breakdown of TB exposed in the environment makes it less likely for a possum to come into contact with a dose suitable for infection. Because *M. bovis* is fragile in the environment, possums must come within proximity of one another for horizontal transmission to occur, either through individual contacts or through co-occupation of the same habitat areas close enough in time for *M. bovis* shed from one individual to be picked up by another from the shared contaminated environment. Such transmission may occur through the overlap of foraging ranges or sequential use of the same den sites (see Chapter 2). If there are identifiable drivers of proximity (i.e. intrinsic behavioural or extrinsic environmental factors), particularly those that introduce non-linear dynamics with respect to density, then understanding these drivers will result in a better understanding of TB transmission dynamics in possums, with implications for TB management.

Possums adhere to a number of basic behavioural characteristics that often require them or lead them to come into proximity with other individuals. Breeding (Bell 1981, Cowan 1993, Ramsey et al. 2002a) and communal nesting (Cowan 1989, Caley et al. 1998) are both well documented mechanisms by which possums come into proximity. Possums are also known to undergo home range plasticity as need arises ((Whyte et al.

2014) see Chapter 2); such as when foraging for food (Paterson et al. 1995). Previous studies have shown a strong correlation between food sources and possum density (Ramsey et al. 2002a, Harper et al. 2008, Rouco et al. 2013) and as a result, increasing population densities could lead to overlapping home ranges.

In Chapter 2 I investigated the determinants of possum home range size. Here, I investigate the determinants of possum home range overlap by addressing two questions: Are there determinants of who overlaps with whom? And when home ranges overlap, are there determinants of the extent of overlap that occurs? The importance of understanding how animals utilize a given habitat and co-exist with one another has key implications for disease transmission, spread and persistence within populations.

## 3.2 Methods and materials

### *3.2.1 Study site*

As for the home range size analyses presented in chapter 2, data for the home range overlap analyses presented here were collected from possum populations in the Orongorongo Valley Study Area (41°21'S, 174°58’E). The sampling was based on those possums living in the area covered by four square trapping grids (A, B, C, and D), each grid being made up of 100 traps at 40 m spacing. Grieve wire cage traps (60 × 26 × 28 cm) were used with spring-assisted folding doors triggered by a pendulum bait hook (Crawley 1973, Donnelly et al. 2006). See chapter 2 for more details.

### *3.2.2 Data collection*

Possums were captured during monthly trapping sessions from March to December 2012. All methods were conducted as detailed in chapter 2.

### *3.2.3 Analysis*

To assess home range overlaps, all trap locations were recorded in the field as Universal Transverse Mercator coordinates (UTM) using a Garmin-12 Global Positioning System (GPS) portable receiver. As for home range size estimation in chapter 2, home range locations of study animals were determined on an individual basis across the full 10 months of data using the 100% Minimum Convex Polygon method (MCP; (Mohr 1947)). This was implemented in the program ArcView 3.2 (Environmental Systems Research Institute), with a buffer area set as half the distance between traps (i.e. 20 m). MCP estimates were obtained for all animals that were trapped at least five times during the ten months and log-transformed to satisfy assumptions of normality.

Whether the home range of each possum on each grid overlapped with that of each other possum, and the extent of such overlaps when they occurred, were then calculated based on the geo-located MCPs, also using ArcView 3.2. This resulted in two types of outcome variable: one at the possum level (i.e. the number of home ranges overlapped for each possum) and one at the level of the overlap (i.e. the area of overlap for each pair of possums or dyad).

The 'number of home ranges overlapped' comprised four separate outcome variables stratified by age and gender; namely the number of overlaps each possum had with adult males, adult females, juvenile males and juvenile females. The explanatory variables considered for this outcome were the possum's age, sex, grid and area of home range.

The ‘area of overlap’ comprised a single outcome; namely the area of overlap for each pair of overlapping home ranges. The explanatory variables considered were the sex and age combinations of the dyad and the grid in which the overlaps occurred. For sex this was the combination of sexes for each pair; namely male/male, male/female or female/female dyads. For age this was the combination of ages for each pair; namely adult/adult, juvenile/juvenile or adult/juvenile dyads).

Outcome variables were log transformed prior to analysis and their relationships with each explanatory variable was assessed using a Poisson regression model and an analysis of variance in Statistica v9.0 (Statsoft, Tulsa). Since the analysis of home range overlap size included a degree of pseudoreplication, as individuals had multiple overlaps, a more stringent critical probability was used when assessing statistical significance (P= 0.01).

## 3.3 Results

### *3.3.1 Number of overlaps*

Among the 816 individuals monitored across the four trapping grids, 24,064 home range overlaps occurred. Of the total recorded home range overlaps, 74% occurred between adult possums, while only 22% of overlaps were between adults and juveniles, and only 4% were between juveniles (Table 3-1). Juvenile possums had more home range overlaps with adults than they did with other juveniles, and this is partly attributable to the majority of the population being adult. When analysing the total number of home range overlaps against the expected proportion of home range overlaps (Table 3-2), or the proportion of overlaps for each dyad based on the number of possible

overlaps for each grid (assuming all animals on each grid can overlap with one another), adult males were found to have more overlap with other groups than expected. They were observed to have >13 times more home range overlaps than juvenile males and 28 times more overlaps than juvenile females, when rates based solely on population composition were expected to be nine times and 14 times greater respectively. Also, the number of home range overlaps between adult males and adult females was close to parity even though the expected proportion of overlaps showed adult females having twice as many overlaps as adult males.

**Table 3-1** Total number of home range overlaps segregated by group sex and age. Percentages are calculated from the total number of home range overlaps.

| | N | Adult male | Adult female | Juvenile male | Juvenile female |
|---|---|---|---|---|---|
| Adult male | 280 | 5708 (24%) | 6094 (25%) | 1604 (7%) | 1044 (4%) |
| Adult female | 368 | | 5952 (25%) | 1641 (7%) | 1085 (4%) |
| Juvenile male | 93 | | | 446 (2%) | 287 (1%) |
| Juvenile female | 75 | | | | 204 (1%) |

**Table 3-2** Expected numbers of overlaps for each dyad based on the number of possible overlaps among grids and across grids. Percentages are calculated from the total number of home range overlaps.

| | | N | Adult male | Adult female | Juvenile male | Juvenile female |
|---|---|---|---|---|---|---|
| All grids | Adult male | 280 | 9823 (18%) | 13107 (24%) | 3199 (5%) | 2644 (4%) |
| | Adult female | 368 | | 17275 (32%) | 4293 (7%) | 3522 (6%) |
| | Juvenile male | 93 | | | 1096 (2%) | 891 (1%) |
| | Juvenile female | 75 | | | | 691 (1%) |
| Grid A | Adult male | 82 | 3321 (21%) | 4018 (26%) | 615 (4%) | 779 (5%) |
| | Adult female | 98 | | 4753 (30%) | 735 (5%) | 931 (6%) |
| | Juvenile male | 15 | | | 105 (1%) | 143 (1%) |
| | Juvenile female | 19 | | | | 171 (1%) |
| Grid B | Adult male | 73 | 2628 (14%) | 4125 (22%) | 1095 (6%) | 876 (4%) |
| | Adult female | 113 | | 6328 (33%) | 1695 (9%) | 1356 (7%) |
| | Juvenile male | 30 | | | 435 (2%) | 360 (2%) |
| | Juvenile female | 24 | | | | 276 (1%) |
| Grid C | Adult male | 57 | 1596 (17%) | 1938 (21%) | 741 (8%) | 513 (6%) |
| | Adult female | 68 | | 2278 (24%) | 884 (9%) | 612 (7%) |
| | Juvenile male | 26 | | | 325 (4%) | 234 (2%) |
| | Juvenile female | 18 | | | | 153 (2%) |
| Grid D | Adult male | 68 | 2278 (18%) | 3026 (24%) | 748 (6%) | 476 (4%) |
| | Adult female | 89 | | 3916 (31%) | 979 (8%) | 623 (5%) |
| | Juvenile male | 22 | | | 231 (2%) | 154 (1%) |
| | Juvenile female | 14 | | | | 91 (1%) |

There were significant relationships between grid, sex, and age and the number of home range overlaps (grid, F= 43.6, df= 3, P< 0.00001, sex, F= 17.3, df= 1, P< 0.0001, age, F= 9.97, df= 1, P< 0.002). With regards to grid, there were fewer home range overlaps on grid B than on other grids, while adults had more overlaps than juveniles, and males had more overlaps than females (Figure 3-1 and Table 3-3).

**Figure 3-1** Mean number of home range overlaps across all four grids broken down by sex and age of possums with overlapping boundaries. Error bars represent standard errors.

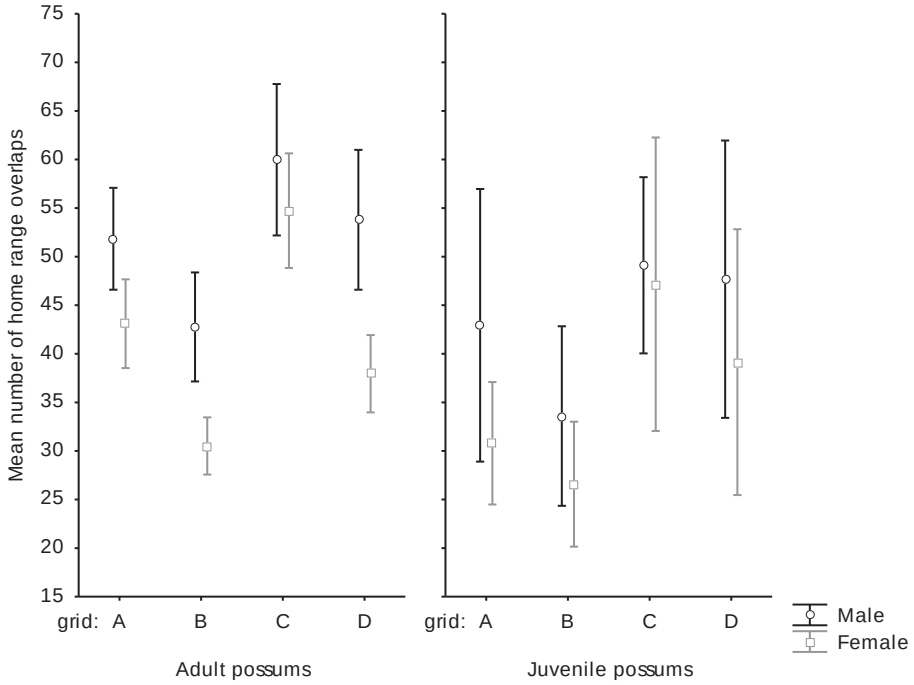


**Table 3-3** Regression model estimates for the model with the number of overlaps with adult males, adult females, juvenile males and juvenile females as the outcome variables.

| | Adult male | | | Adult female | | | Juvenile male | | | Juvenile female | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Coefficient | SE | P-value | Coefficient | SE | P-value | Coefficient | SE | P-value | Coefficient | SE | P-value |
| Intercept* | 2.83 | 0.03 | | 2.86 | 0.03 | | 1.48 | 0.05 | | 1.11 | 0.04 | |
| Grid A | 0.08 | 0.04 | 0.07 | 0.03 | 0.05 | 0.53 | -0.53 | 0.12 | *0.00001* | -0.24 | 0.09 | *0.006* |
| Grid B | -0.31 | 0.05 | 0 | -0.23 | 0.05 | *0.00002* | -0.11 | 0.07 | 0.09 | -0.16 | 0.07 | *0.02* |
| Grid C | 0.15 | 0.04 | *0.0001* | 0.16 | 0.04 | *0.0003* | 0.56 | 0.05 | 0 | 0.42 | 0.05 | 0 |
| Sex (Female vs. Male) | -0.1 | 0.03 | *0.00005* | -0.12 | 0.03 | *0.00002* | -0.13 | 0.05 | *0.004* | -0.08 | 0.04 | 0.06 |
| Age (Adult vs. Juvenile) | 0.08 | 0.03 | *0.002* | 0.08 | 0.03 | *0.006* | 0.12 | 0.05 | *0.008* | 0.1 | 0.04 | *0.02* |
| Sex & Age | -0.01 | 0.03 | 0.81 | 0.02 | 0.03 | 0.46 | -0.005 | 0.05 | 0.91 | 0.04 | 0.04 | 0.33 |
| Grid & Sex | 0.07 | 0.04 | 0.07 | 0.08 | 0.04 | 0.07 | 0.11 | 0.05 | *0.05* | 0.07 | 0.05 | 0.22 |
| Grid & Age | 0.01 | 0.03 | 0.81 | -0.01 | 0.04 | 0.85 | 0 | 0.05 | 0.94 | -0.01 | 0.05 | 0.83 |
| Grid & Sex & Age | -0.01 | 0.04 | 0.88 | 0 | 0.04 | 0.94 | -0.02 | 0.05 | 0.68 | 0.03 | 0.05 | 0.57 |

*Male juvenile on Grid D

*3.3.2 Area of overlaps*

Analysis showed that when possums home ranges overlapped, the area shared between individuals was lower on grid B than all other grids ($F=368.4$, sd= 3, $P< 0.0001$). As for numbers of overlaps, the area of home overlap was also dependent on both sex and age ($F= 92.7$, sd= 1, $P< 0.00001$ and $F=50.1$, sd= 1, $P< 0.00001$, respectively). In addition to male possums having more home range overlaps with other possums, than did female possums (see above), they also had larger overlap areas when they occurred. Likewise, in addition to juvenile possums having fewer home range overlaps with other possums, than did adult possums (see above), they also had smaller overlap areas when they occurred.

Significant interactions (using the threshold of $P = 0.01$) were also observed between grid and sex, and between grid, sex and age (Figure 3-2 and Table 3-4). Both interactions were driven by only possums on grid A showing a large difference in overlap size between males and females for juvenile possums, an effect most likely driven by a small sample size of juvenile females on that grid with smaller home ranges by chance.

**Figure 3-2** Mean (SE) of the area of home range overlap shared among possums separated by sex and age cohorts found across all four grids.

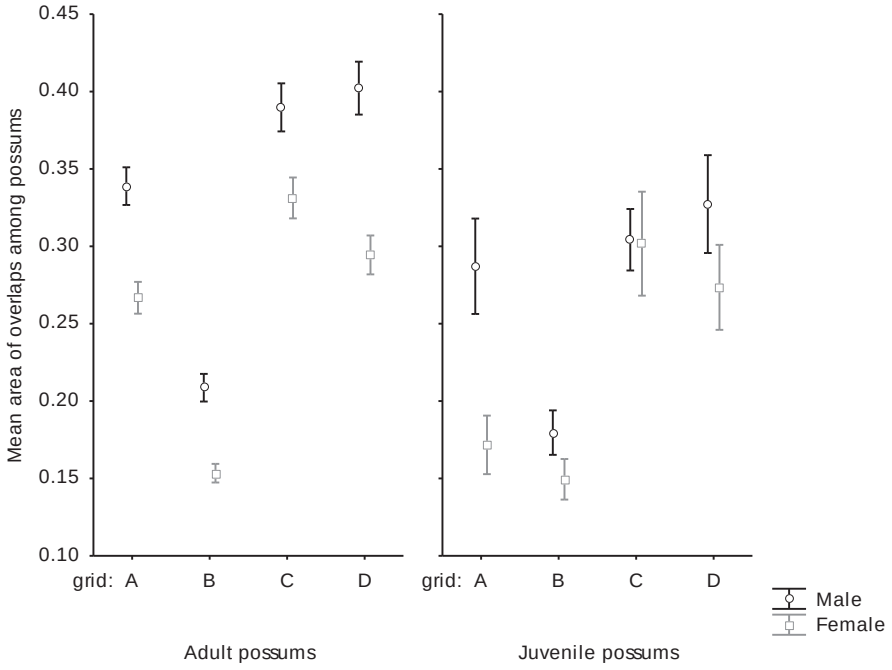


**Table 3-4** P value model estimates for the area of home range overlap shared between possums with possum sex, age and grids as outcome variables.

| | df | F value | P value |
|---|---|---|---|
| Intercept | 1 | 11721.2 | 0.00000 |
| grid | 3 | 368.4 | 0.00000 |
| sex | 1 | 92.66 | 0.00000 |
| age | 1 | 50.14 | 0.00000 |
| grid x sex | 3 | 26.91 | 0.00001 |
| grid x age | 3 | 8.02 | 0.04557 |
| sex x age | 1 | 0.47 | 0.49432 |
| grid x sex x age | 3 | 12.65 | 0.00547 |

Analysis of home range overlaps broken down into sex pairs or dyads showed the greatest area of home range overlap is shared between males ($F= 393.1$, $df= 2$, $P< 0.0001$), while female/female pairs had the smallest amount of shared area (Figure 3-3).

**Figure 3-3** Mean (SE) of the area of home range overlaps shared among pairs of male/male (M-M), male/female (M-F) and female/female (F-F) across all four grids

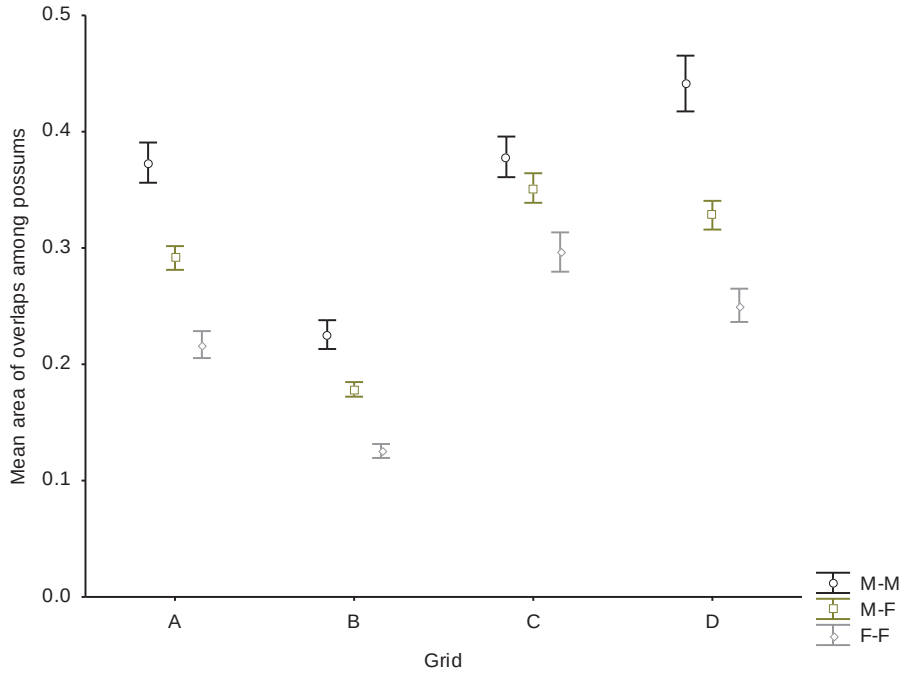
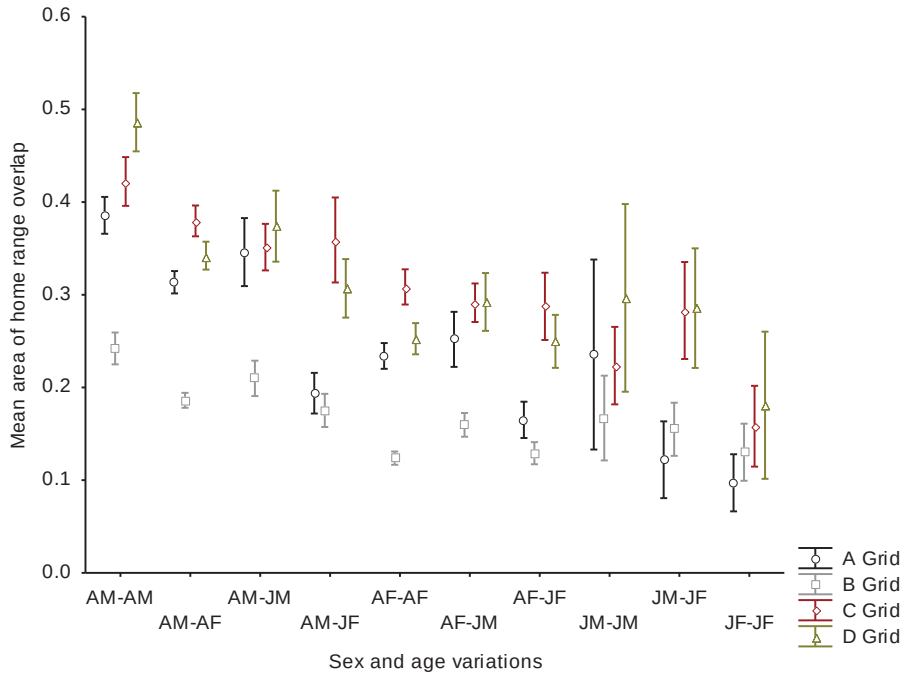



Figure 3-4 shows when the area of home range overlap is separated by age and sex, the pattern of adult males sharing the largest home range overlaps across all four grids is clear (F= 541.6, sd= 9, P< 0.0001). The grid effect is also clearly evident, with home range overlaps being smaller on grid B (F= 104.1, sd= 3, P< 0.0001). There was also a clear interaction of grid and dyad observed (F= 104.9, sd= 27, P< 0.0001).

**Figure 3-4** Mean (SE) of the area of home range overlap expressed by each possible sex and age paring across all four grids. Adult male/adult male (AM-AM), adult male/adult female (AM-AF), adult male/juvenile male (AM-JM), adult male/juvenile female (AM-JF), adult female/adult female (AF-AF), adult female/juvenile male (AF-JM), adult female/juvenile female (AF-JF), juvenile male/juvenile male (JM-JM), juvenile male/juvenile female (JM-JF) and juvenile female/juvenile female (JF-JF)




### 3.4 Discussion

In general, the results are mainly as one would expect from the analyses of home range size presented in Chapter 2. In Chapter 2, I provided strong evidence in agreement with previous studies (e.g Ramsey et al. 2002a, and Whyte et al. 2014), that adult males have larger home ranges than adult females. Here this translates into males generally having

more, and larger, home range overlaps with other possums than do females. When looking at the duel identity of home range overlapping possums (i.e. the ‘dyads’), this logically translates into overlaps between males being largest and overlaps between females being smallest, with male/female home range overlaps being of intermediate size (Figure 3-4).

However, there was a key departure in the results of the current home range overlap analyses, from what one may predict from the considerations of home range size differences in Chapter 2. While Chapter 2 showed that juvenile possums did not necessarily have smaller home ranges than adult possums, the findings of the current chapter are that they do still have fewer and smaller home range overlaps with other possums. This reinforces the findings of Blackie (2011) and is likely due to behavioural differences, with juvenile possums perhaps showing a greater level of avoidance behaviour with respect to other individual possums than adult possums have, or adult possums being less tolerant of juveniles in their home ranges than they are of other adults.

In addition to these intrinsic drivers of possum home range overlap, extrinsic effects were also apparent through the significant grid effect. Here, home range overlaps were fewer (per possum) and smaller when they occurred on Grid B than on the other trapping grids. This is the subpopulation that was at significantly higher density during the study (Chapter 2), resulting in smaller home ranges sizes on this grid than on the other grids (Chapter 2), with this effect linking through in the current chapter to each possum having fewer and smaller home range overlaps with other individuals in the high density grid.

### *3.4.1 Implications*

With regards to the transmission of TB among possums, and the persistence and spread of the disease in possum subpopulations, my findings have several key implications regarding our understanding of the dynamics of the disease and potentially also for management. First, the smaller number and size of juvenile than adult possum home range overlaps (irrespective of population density), means that they likely find themselves less frequently in proximity with TB infected individuals. Regardless of the actual mechanism of transmission, be it through environmental contamination or actual contact, this strongly suggests that they are at a much lower risk than adults of contracting the disease. This hypothesis is supported by the generally much lower rate of TB infection observed in juvenile than adult possums (Crawley 1973, Buddle et al. 1994, Zinsstag et al. 2006, Gortazar and Cowan 2013a). Also, even if juvenile individuals do in some way become infected (e.g. through contact with an infected mother) they will play a relatively small role with them more likely to act as 'dead-end' host individuals that do not pass the infection on.

Second, the larger number and size of home range overlaps (again, irrespective of population density) for adult male possums than for adult female possums (apparently driven simply by them having larger home range sizes), means that this population subgroup may play a disproportionally larger role in TB disease dynamics. Infected adult males will come into close proximity with more susceptible individuals than infected adult females. Vice-versa, males are more likely than females to come into proximity with any infected individual in any neighbourhood. In such a way, this population subgroup may be

acting as a disease ‘superspreading’ group (Lloyd-Smith et al. 2005, Matthews et al. 2006, Chase-Topping et al. 2008).

Finally, the differences in possum home range parameters noted for the subpopulation on Grid B, at markedly higher population density than the other three subpopulations, imply issues for TB management through population reduction. If high density is indeed the driver of the observed overall smaller individual home ranges on Grid B (Chapter 2) having fewer and smaller overlaps with other individuals (this chapter), the flip-side is that home range overlaps per individual will be both more and larger at lower density increasing the likely hood of disease transmission encounters with TB infected individuals. This hypothesis complicates the findings of Barlow (1991a, b) who states that populations held under 40% carrying capacity would allow for the eradication of TB, and Caley (1999) who states the same depopulation at 22% carrying capacity would eradicate TB. These phenomena could lead to non-linearity in the relationship between possum density and disease transmission rates, with infected individuals potentially being more likely to pass the infection on at lower density. This is the inverse effect to the logic that the use of population reductions to control disease is based on. Of course, possums with smaller home ranges at higher densities may still encounter (or come into proximity with) those individuals who they do share space with at a higher frequency rate (leading to greater opportunities for disease transmission).

The balance of these effects, and the potential for adult males to also drive TB transmission through their larger home ranges, will be further explored through the analysis of data from contact logging collars placed on the individuals in the four subpopulations studied. Of critical importance to TB dynamics, will be ascertaining how the home range patterns

documented in both this and the previous chapter translate into actual contact/proximity rates between individuals.

For the study of infectious disease dynamics in vertebrate wildlife in general, the evidence here for certain population groups potentially having larger roles in transmission than others, and the suggestion that per-capita opportunities for transmission may increase at low density rather than decrease due to home range behaviour effects, provides avenues for exploration for other systems where failures of population control to manage disease occur.

# Chapter 4

# Construction of brushtail possum (*Trichosurus vulpecula*) contact networks to inform on bovine tuberculosis transmission between individuals, and its persistence in wild populations

## 4.1 Introduction

As outlined in Chapter 1, brushtail possums (*Trichosurus* vulpecula) are New Zealand's main wildlife reservoir for tuberculosis (TB), and disease transmission from infected possums to cattle is the major contributor to infected cattle herds (Nugent et al. 2015). However, as mentioned in Chapter 3, *M. bovis* is extremely fragile in the environment (Morris et al. 1994); this rapid deterioration reduces the probability of another animal becoming infected from free-living bacteria in the environment. This means that transmission of TB most likely occurs between individuals in proximity of each other. However, the main wildlife reservoir, the possum, is considered to be a relatively solitary species (Ji et al. 2005, Ramsey and Efford 2010a). Therefore, the debate among scientists is not focussed on whether possums are primarily responsible for transmitting TB to cattle, but rather what are the mechanisms of intra-species TB transmission that allow for the persistence of TB in possum populations?

Although possums do not tend to be territorial, individuals do occupy a home range or area of activity (Burt 1943, Ward 1978, Buddle and Young 2000, Efford et al. 2000) where behavioural plasticity can occur as needs for mating and resources arise (Whyte et al. 2014), meaning social contacts among possums do occur (Brockie 1992). Ji (2005) states that interactions between individuals are primarily associated with mating, with contacts outside of the breeding season being brief and infrequent. With contemporary wisdom being that possums generally die from clinical disease within four to six months of infection with TB (Norton et al. 2005, Otterstatter and Thomson 2007), the critical question is how is disease transmission sufficient (most notably outside of the breeding season) for the disease to persist year round in possum populations.

Outside of the breeding season, once possums come into close quarter most encounters are a result of foraging or happenstance, and primarily consist of threat displays and hissing (Crawley 1973). Communal nesting is also an accepted method of possum social contacts. Possums are known to frequent more than one den site within their home range (Dunnet 1956), and den sharing is not uncommon with recorded observations of both female-female and male-female dyads (Winter 1976, Caley et al. 1998, Ramsey and Efford 2010a).

If TB transmission between possums is associated with behaviours such as fighting and den sharing (Barlow 1991b), then understanding possum social networks is a vital element towards understanding TB transmission. Such an approach has proven invaluable in understanding infectious disease dynamics (Buddle and Young 2000, Matthews et al. 2009, Nugent et al. 2015), including for TB in wild vertebrate populations (Donnelly et al. 2006). For TB in possums, little is known about the interactions and contact networks of possums or how they may be determining disease transmission and thus persistence. While

numerous studies have focused on possible drivers of disease such as population density (Buddle and Young 2000, McCallum 2009, Whyte et al. 2014, Nugent et al. 2015) and home range size (Burt 1943, Ward 1978, Paterson et al. 1995, Arthur et al. 2002, Whyte et al. 2014, Nugent et al. 2015), it remains unclear which if any of these factors have an effect on possum interaction/proximity probability and the potential for intra-specific transmission. Furthering our knowledge of social interactions among possums will give us a better understanding of species behaviour, informing on how TB persists in this species. Such knowledge may allow more targeted TB control actions aimed at reducing population and area transmission, and crossover infection into other species (such as domestic cattle).

Although possum contacts always occur at an individual level, to identify interactions between possum sex and interactions among grids, I investigate the social interactions of possums at three levels. I have used the terms: population level, individual level and contact level. The population level analysis offers a large scale view of the basic structure of possum social order, informing our understanding of general social network characteristics. The individual level analysis provides further detail by allowing investigation of any differences between male and female adult possums. Results from previous work on home range size (Chapter 2) and overlap (Chapter 3) indicates that such differences are likely. Finally the contact analysis delves deeper into the possum social order by investigating the behaviour of specific possums, their interactions with other possums and possible drivers of these interactions. This study uses a six month subset of contact data from the larger bovine tuberculosis study in which possums were fitted with proximity tracking devices (a six month subset of the time window covered in Chapter 2-3).

I hypothesise that even though possums are solitary animals, social networks do exist within wild free-living possum populations, and these possum to possum interactions are of a manner that may help to support year round transmission of diseases such as tuberculosis. If the spread of TB through possums is associated with possum to possum interactions, then the examination of contact network measures of node degree, edge strength and betweenness (see Table 1 for definitions) would provide crucial data to investigate a possible link between contacts and transmission. Based on the findings from Chapters 2 and 3, I predict that male possums will have greater contact rates than females, since males have more and larger home range overlaps with other individuals. I also expect to find differences among the four grid subpopulations studied, linked to the differences in home range size and home range overlap observed among grids. Note that I cannot explore here how the adult versus juvenile differences in home range size and overlap identified in Chapters 2 and 3 translate into actual contact/proximity differences, since I was only able to fit collars to adult possums for animal welfare reasons.

## 4.2 Methods and materials

### *4.2.1 Data collection*

This study uses the four possum subpopulations described in the previous chapters, in the Orongorongo Valley New Zealand from April 2012 to November 2012, examining contact network information collected both during the breeding season (April- June) and up to five months after. During initial capture months (April and May 2012) when grid populations were being established, 40 adult possums (20 male and 20 female) on each trapping grid

were fitted with Sirtrack™ radio-collars with proximity-logging capability. Collars were set-up to record contacts between collared possums when they came within one meter of each other. During a contact, each collar usually recorded the ID of the opposing collar as well as the date, time and duration of the contact (two way contacts). In some cases only one-way contacts occurred (i.e. only recorded by one collar). Such records would have been caused by possums around the extreme of the 1m contact zone at which minor differences in possum orientation and positioning, or remaining collar battery life, can lead to one collar recording contact without reciprocation.

### *4.2.2 Analyses*

As noted in the introduction, possum social networks were analysed at three scales: a population-level contact network analysis, an individual-level analysis (examining gender and age effect on possum contacts) and a contact-level analysis (examining nocturnal and diurnal contact patterns, with the intention that higher scale findings would inform the analysis of lower scale data. Using this data obtained from the deployed collars, I characterize the population network structure on each of the four trapping grids (population level analysis) independently. I then analyse the total number and the duration of contacts that individual possums exhibited within each grid for the individual level analysis, while controlling for grid effects. I then examine the potential drivers of social interactions between individuals in the contact level analysis, again controlling for grid effects. It is important to note that the proximity collars fitted to each possum had a battery life of around six months, which determined our analytical time frame. Thus there were insufficient contacts per month to allow a meaningful analysis of how contacts may have varied through the study.

*4.2.2.1 Population-level analysis*

Population summaries of node degree, edge strength and betweenness were compared between grids, as were the transitivity values for each grid (see Table 1 for definitions and references for these measures). To standardize data for across grid comparisons, relative values for node degree and betweenness were estimated using the following equations (see Table 1 for notation definitions): Relative degree centrality ($RDc_i$) where $RDc_i = \frac{Dc_i}{N-1}$ and relative betweenness centrality for undirected networks ($RBc_i$) where $RBc_i = \frac{Bc_i}{(N-1)(N-2)/2}$ (Lugton 1997). Due to the lack of data regarding which individuals were the initiating contactors, and due to the occurrence of one-way contacts, all interactions between possums were considered as single contacts regardless of whether they were identified as one or two way contacts, meaning the initiating possum was deemed irrelevant as long as a contact was made.

Data were analysed using R 3.1.2 studio packages dplyr, ggplot2 and igraph. Dplyr was used to set up a working database. Ggplot2 was used for grouping the possums into contactors (possums making contacts) and contactees (possums being contacted). Igraph was used to construct network graphs/webs for viewing the distribution of contacts between individuals.

**Table 4-1** Definitions of the contact network values and the equation notations used in the population-level analysis (Lugton 1997).

| **Notations for population level analysis** | **Notation Definitions** |
|---|---|
| Node degree | The number of other possums an individual possum comes into contact with. |
| Edge strength | The strength of the ties between two nodes or the number of contacts between each pair of possums. |
| Betweenness | An indicator of a nodes centrality in a given network, the shortest path to all vertices that pass through a given node or how connected a possum is with neighbouring possums. |
| Transitivity/Clustering coefficient | The measure of the degree in which nodes cluster together or the measure by grid of how many possums are contacting each other. |
| **Equation notations for population level analysis** | **Notation Definitions** |
| I= Node | An individual with a collar. |
| N= Number of nodes in the network | The number of total collared possums on the individuals trapping grid. |
| $RDc_i$= Relative degree centrality of node i | The relative number of total collared possums on the individuals trapping grid used for grid comparison. |
| $Dc_i$= Degree centrality of node i | The measure of the number of contacts node i has. |
| $RBc_i$= Relative betweenness centrality of node i | The relative measure of the number of contacts node i has used for grid comparison. |
| $Bc_i$= Betweenness centrality of node i | An estimate of the probability that the shortest path between any pair of nodes of the network passes through node i. |

#### 4.2.2.2 *Individual-level analysis*

In this analysis I considered the determinants of network variables at the level of the individual possum. These included: the number of other collared possums each individual possum interacted with (node degree); the total number of contact events that each individual had, and the summed length of time each individual spent in contact with other possums. These were analysed with respect to grid and sex of the contactor using a Generalised Linear Model (Poisson regression). Due to the setup of trapping grids there was a structural contact bias meaning that possums located near to the centroid of each grid had a greater chance of contacting other collared possums than possums located on the perimeter of the grids. As a ways of controlling for this bias in the analyses, both the number of possums with overlapping home ranges to each contactor, or the summed area of overlap with all other possums for each contactor, were explored as covariates in the fitted GLM models. Home ranges were estimated using the 100% Minimum Convex Polygon method (MCP; (Tompkins et al. 2007)) detailed in Chapter 2.

Poisson regression was used to investigate the interaction summaries of contactors, with male and female contactees separately, with regards to the contactor sex and grid and with the home range measures noted above as covariates. A binomial distribution was used to analyse the total number of contacts, and the summed contact times were log10 transformed for analysis to conform to assumptions of normality.

#### *4.2.2.3 Contact-level analysis*

Variables at the level of the individual contact (i.e. an interaction between a dyad) were examined to determine their relationship with grid and gender. The number of contacts and

summed time in contact for each possum dyad, where contact occurred, were analysed with respect to both trapping grid and dyad type (male/male, male/female or female/female) using Poisson regression. Both the number of contacts made and the summed time in contact per 100 days were log-transformed prior to analysis to conform to assumptions of normality.

Due to the staggered introductions of collars into the field (April and May 2012), and inconsistency of collar battery life, the numbers of individual contacts and the contact times were standardized into rates per 100 days (with three months generally being the infectious period of possums with clinical TB; (Buddle et al. 1994)). This was accomplished by dividing the number of collar contacts and contact times by the total days the collar was in the field, and multiplying by 100 days.

As for the individual level analyses, I explored the number of possums with overlapping home ranges and the summed area of overlap with all other possums for each contactor as covariates in the fitted GLM models. Due to differing day time and night time behaviour expressed among possums (Paterson et al. 1995), data for each time frame (hours of dawn/day time and dusk/night time) were analysed separately. Time scales were adopted from the Royal Astronomical Society of New Zealand (http://rasnz.org.nz/in-the-sky/sun-rise-and-set), and dawn and dusk times were deemed to be one hour pre and post documented sun rise and sun set. Recorded day time contacts with durations lasting fewer than three seconds were dropped from the study as these contacts were likely human influenced due to post-handled possums stumbling into already occupied den-sites.

Backward elimination processes were employed to resolve the most suitable model fit using two-way ANOVA. Since applying this analytical approach to dyadic data, while providing valid model fits, consists of pseudoreplication that can inflate p-values (each individual can occur in multiple dyads), I use a more stringent probability level than generally accepted to infer significance ($p < 0.01$) for these analyses.

**Figure 4-1** Diagrams of social networks for each grid, generated by Igraph in R studio. Numbers located at the nodes denote individual possum IDs. Line thickness is proportional to edge strength. Yellow arrows indicate challenged individuals

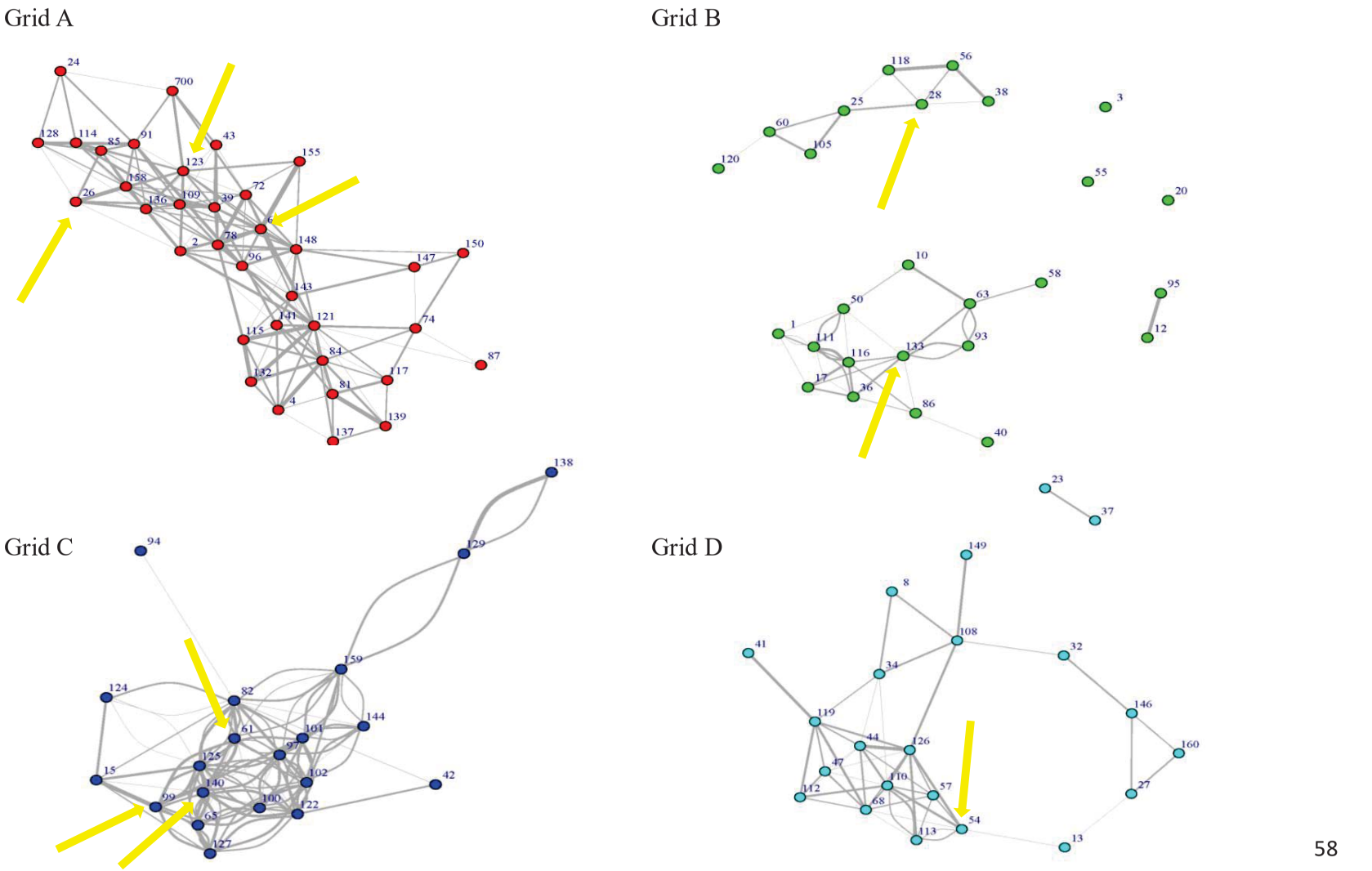


**Figure 4-2** Left: Network structure across all grids. Dark blue (C grid) and light blue (D grid) have various recorded overlaps due to two corners of the grids lying one fifty meters apart. Below: Layout of the Orongorongo Valley trapping grids. A, B, C and D grid are shown from left to right. Notice the corners of C and D grids, explaining the contact overlaps between these two grids.



## 4.3 Results

### *4.3.1 Population-level summaries of network characteristics*

Of the 160 proximity-logging collars put on possums, 110 (49 male and 61 female) were retrieved for analysis registering 37,238 contacts between the months of April and October of 2012. Grids A, B, C and D are represented by 35, 27, 26 and 22 collars, respectively. Relative node degree ($F= 40.1$, $df= 3$, $P< 0.00001$), edge strength ($F= 13.5$, $df= 3$, $P< 0.0001$) and relative betweenness ($F= 4.0$, $df= 3$, $P= 0.01$) differed significantly between grids. Analysis of pairwise comparisons revealed that grid C was significantly different from all other grids for relative node degree, grids A and C were significantly different from B and D for edge strength, and grid B was significantly different from grids A and D for relative betweenness (maximum $P= 0.02$). The network characteristics for each grid are summarised in table 4-2.

Analysis of these different measures clearly reveals a difference between grids in contact network and possum sociality (mostly among males) showing grids A and C to be similar containing possums with greater network values in both node degree and edge strength, and grids B and D as being similar containing possums with lower network values. These sociality differences were independent of density and number of collars retrieved.

**Table 4-2** Mean (standard error) of network characteristics of each grid, stratified by sex of possums. Adjusted values are the relative values adjusted for the number of radio-collared individuals in each grid.

| | | | Network parameter estimate (Standard error) | | | |
|---|---|---|---|---|---|---|
| Grid | Sex | No. possums | Node degree/ *adjusted* | Edge strength | Betweenness/ *adjusted* | Clustering coeff. |
| | Males | 15 | 10.5(1.05)<br>*0.31(0.03)* | 344 (76.4) | 86.5 (16.3)<br>*0.15(0.03)* | |
| A | Females | 20 | 7.0 (0.54)<br>*0.21(0.02)* | 282 (51.3) | 24.0 (7.16)<br>0.04(0.01) | |
| | **All** | **35** | **8.5 (0.62)**<br>***0.25 (0.02)*** | **309 (43.6)** | **50.7 (9.5)**<br>***0.09(0.02)*** | **0.49** |
| | Males | 13 | 3.8(0.62)<br>*0.14(0.02)* | 64.3 (21.1) | 10.2 (2.88)<br>*0.03(0.01)* | |
| B | Females | 14 | 2.9 (0.72)<br>*0.11(0.03)* | 36.7 (8.04) | 3.14 (2.63)<br>*0.01(0.01)* | |
| | **All** | **27** | **3.3 (0.48)**<br>***0.13 (02)*** | **48.4 (11.2)** | **6.5 (2.0)**<br>***0.02(0.01)*** | **0.48** |
| | Males | 10 | 21.1(2.22)<br>*0.84(0.09)* | 325 (43.8) | 35.7 (13.3)<br>*0.12(0.04)* | |
| C | Females | 16 | 12.4 (1.53)<br>*0.50(0.06)* | 383 (109.7) | 16.0 (5.53)<br>*0.05(0.02)* | |
| | **All** | **26** | **15.8 (1.51)**<br>***0.63 (0.06)*** | **361 (68.9)** | **23.6 (6.3)**<br>***0.08 (0.02)*** | **0.54** |
| | Males | 11 | 4.4 (0.97)<br>*0.21(0.05)* | 44.1 (11.1) | 18.2 (6.14)<br>*0.09(0.03)* | |
| D | Females | 11 | 4.9 (0.84)<br>*0.23(0.04)* | 79.0 (31.2) | 21.7 (6.43)<br>*0.10(0.03)* | |
| | **All** | **22** | **4.6 (0.63)**<br>***0.22 (0.03)*** | **61.6 (16.6)** | **19.9 (4.4)**<br>***0.09 (0.02)*** | **0.60** |

Relative node degree and betweenness were both associated with sex, with males tending to have higher values for both compared to females (F= 14.2, df= 1, P= 0.0003 for relative node degree, and F=10.5, df= 1, P= 0.001 for relative betweenness). There was a significant interaction between sex and grid for these variables (F= 6.5, df= 3 P= 0.0004 for relative node degree and F=3.0, df= 3 P= 0.03 for relative betweenness). The interaction could be explained by the different trend in grid D compared to the other three grids (Table 4-2). This is probably due to three females on grid D having extremely high numbers of contacts and time spent together, skewing the result for this grid. It is important to note that these analyses are comprised of only contacts between possums for which collars were retrieved and downloaded n=110.

### *4.3.2 Individual-level analysis*

Though the collars were active and in circulation on the grids for their six month battery life, the vast majority of contacts occurred between the months of April and June showing a strong correlation of higher possum contacts during the breeding season. 'Contactors' are defined as collared possums that were re-captured and the collars retrieved, and 'contactees' as all possums contacted by contactors, including possums for which collars were not retrieved (N.B. this is in contrast to the section above where only contacts between possums for which collars were retrieved and downloaded were included in the analysis).

The mean (s.e.) number of possums contacted during season one is shown stratified by gender in table 4-3. Males contacted more possums on average than females, as described above. Contacts between males, and between male and female possums, were similar. Female possums, however, tended to contact relatively fewer other female possums (mean = 1.85) compared to males (mean = 2.62) (Table 4-3).

**Table 4-3** The mean (se) number of contacts between ‘contactors’ and ‘contactees’ stratified by sex.

| | Number of contacts (standard error) | |
|---|---|---|
| | Downloaded collared possum gender | |
| Contactee gender | Male | Female |
| Male | 3.00 (0.45) | 2.62 (0.35) |
| Female | 3.22 (0.36) | 1.85 (0.19) |
| All | 6.22 (0.76) | 4.48 (0.50) |

Poisson regression modelling was used to test for significant differences in contacts between male and female possums while adjusting for age, structural contact bias and the effect of grid. The number of contacts with male and female ‘contactees’ were used as outcome variables, and the covariates were: sex of ‘contactor’; grid; age of ‘contactor’; and the number of overlapping home ranges. Only the difference between the number of males and females (contactors) contacting females was significant, with females again less likely to contact other females compared to males (Deviance = 17.8, 1 df, P=0.00003, Table 4-4), confirming the univariate summary in table 4-3.

**Figure 4-3** Mean (SE) total number of standardized contacts during season one across all four grids.

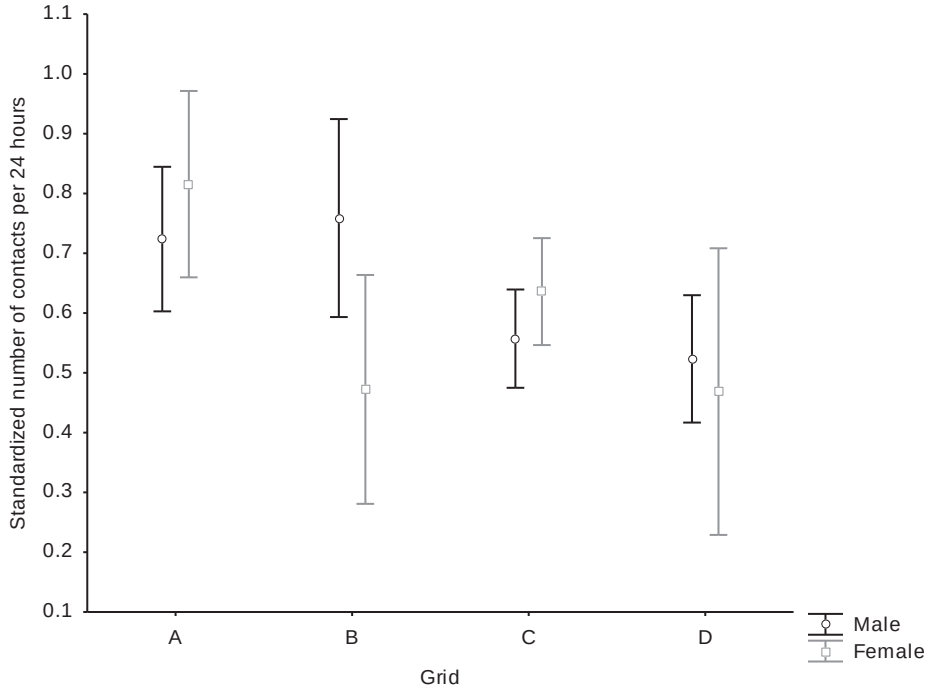



**Table 4-4** Poisson regression model estimates for the model with the number of contacts with female contactees as the outcome variable.

| Variable | Coefficient | Standard error | P value |
|---|---|---|---|
| Intercept* | 0.58 | 0.28 | |
| gridB | -0.07 | 0.21 | 0.75 |
| gridC | 0.58 | 0.16 | 0.0002 |
| gridD | 0.07 | 0.20 | 0.72 |
| Age (adult vs juvenile) | 0.18 | 0.18 | 0.33 |
| Number of overlapping home ranges | 0.01 | 0.01 | 0.11 |
| Female vs Male | -0.54 | 0.13 | 0.00003 |

*Juvenile male in grid A

Table 4-5 shows the distribution of the summed duration of contacts between contactors and contactees per 100 days stratified by sex (duration of contacts was log-normally distributed). Although there was a very wide range in the summed duration of contacts,

the median summed duration was similar for all pairs of males and females, with the exception of lower female/female contact durations (median duration 35 seconds and 67 seconds conditional on contact). Hence females were less likely to contact other females, and if they did they spent less time in contact.

**Table 4-5** Duration in seconds of contacts between contactors and contactees stratified by sex. The top half of the table is for all potential contacts, the bottom is conditional on at least one contact occurring.

| | | Centiles of distribution of length of contacts (seconds) | | | | |
|---|---|---|---|---|---|---|
| | Contactor | 0% | 25% | 50% | 75% | 100% |
| Contact with male contactees | Male | 0 | 10 | 129 | 566 | 422715 |
| | Female | 0 | 0 | 170 | 961 | 713700 |
| Contact with female contactees | Male | 0 | 64 | 339 | 1473 | 212412 |
| | Female | 0 | 2 | 35 | 329 | 1105536 |
| All contacts | Male | 4 | 140 | 662 | 1852 | 424188 |
| | Female | 1 | 48 | 418 | 1640 | 1106383 |
| | | Conditional on contact | | | | |
| Contact with male contactees | Male | 1 | 45.25 | 266.5 | 705 | 422715 |
| | Female | 1 | 73 | 640 | 1334 | 713700 |
| Contact with female contactees | Male | 1 | 67.75 | 340 | 1493.75 | 212412 |
| | Female | 1 | 15.5 | 67 | 679.5 | 1105536 |

Regression modelling was used to test for significant differences in duration of contacts between male and female possums while adjusting for age, structural contact bias and the effect of grid. The log duration of contacts (in seconds + 1) with male and female 'contactees' were used as outcome variables, and the covariates were: sex of 'contactor'; grid; age of 'contactor'; and the number of overlapping home ranges. Only the duration of contacts between females was significantly different (lower) from other combinations (F value = 4.1, df=1, P= 0.046 for all possums), however when only possums that had

had contact with females were considered (i.e. conditional on contact) this was not significant (F value =1.1, df=1, P= 0.3).

*4.3.3 Contact-level analysis*

A trend was observed among contacting dyads showing male to female pairings being greater in quantity than both same sex pairings (Table 4-6).

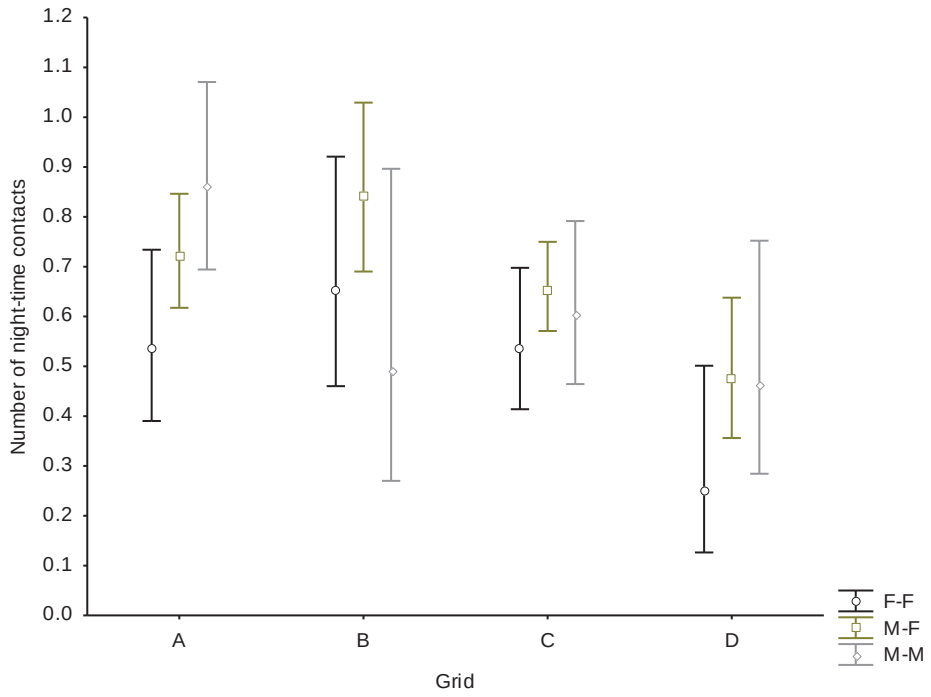
**Table 4-6** Recorded male to male (M-M), male to female (M-F) and female to female (F-F) dyads across all grids separated by daytime and night-time contacts.

| | Number of dyads | | |
|---|---|---|---|
| | M-M | M-F | F-F |
| Grid A | 40 | 94 | 36 |
| Grid B | 19 | 38 | 15 |
| Grid C | 47 | 125 | 45 |
| Grid D | 13 | 38 | 17 |
| **Total** | **119** | **295** | **113** |

| | Daytime Dyads | | | Night-time Dyads | | |
|---|---|---|---|---|---|---|
| | M-M | M-F | F-F | M-M | M-F | F-F |
| Grid A | 1 | 10 | 3 | 39 | 95 | 36 |
| Grid B | 1 | 4 | 0 | 19 | 38 | 15 |
| Grid C | 1 | 9 | 4 | 47 | 125 | 45 |
| Grid D | 1 | 0 | 1 | 13 | 38 | 17 |
| **Total** | **4** | **23** | **8** | **118** | **296** | **113** |

Analysis of the total number of night-time dyad showed a grid effect (F=13.8, sd=3, P< 0.004) with dyads on grid A making more contacts on average than the other grids, and male/female parings occurred more frequently at night than male/male or female/female pairs though this was not significant F=8.4, sd= 2, P= 0.15 (Figure 4-4).

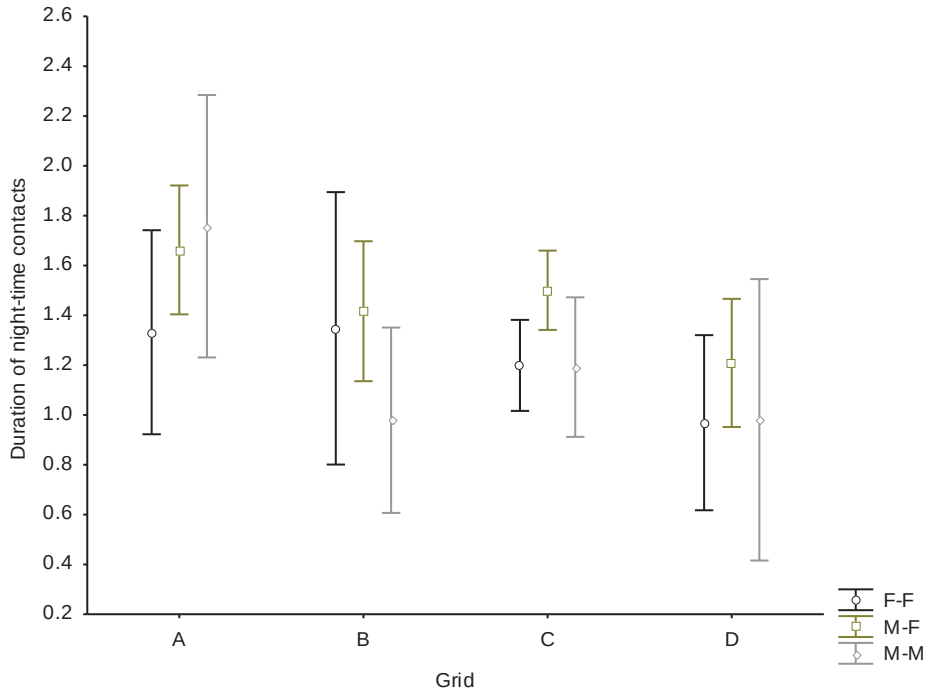
**Figure 4-4** Mean, SE (±) standardized number of night-time contacts shared among female/female (F-F), male/female (M-F), and male/male (M-M) dyads.




Similar to the number of night-time dyad contacts above, the analysis of the summed time spent together among dyads at night showed a grid effect (F= 14.9, sd= 3, P< 0.002) with possums on grid A spending more time together than possums on other grids. A non-significant trend in dyads was also found (F= 8.23, sd= 2, P= 0.016) showing males and females spending slightly more time together than male/male or female/female parings (Figure 4-5). However, no interaction between grid and dyad occurred (F=5.8, sd= 6, P= 0.44). Although analysis revealed significant values in both number of dyads and summed contact time, as outlined in the methods, I chose a critical

probability of 0.01 for this analysis hence only p values < 0.01 were considered significant.

**Figure 4-5** Mean, SE (±) duration of night time contact spent among dyad pairings of female/female (F-F), male/female (M-F), and male/male (M-M) dyads across all grids.




Analysis of possum dyads and summed times dyads spent together during the day were inconclusive as grids B and D were missing female/female and male/female dyads respectively. Male/male dyads were found on all four grids. These results show clear differences in possum contact networks and emphasises the nocturnal and diurnal difference of behaviour exhibited by possums.

## 4.4 Discussion

This study identified some significant differences in possum contact structure and behaviours between genders and grids. Here, I provide evidence that male possums have significantly more contacts than female possums shown in both node degree and betweenness. Although these results differed significantly between grids, contacts between female dyads were less likely than male-female or male dyads and their contact durations were much shorter than all other dyads. This is likely to be in part due to male possums having larger home ranges (Efford et al. 2000, Yockney et al. 2013a) as outlined in Chapter 2 and more and larger home range overlaps with other possums (see Chapters 3), although the relationship was still significant after adjusting for the number of home range overlaps. Most contacts recorded during this study occurred at night (this is supported by Paterson et al. (1995), see paper for documented nocturnal movements) with male-female dyad contacts being more frequent than single sex dyad contacts.

Previous research has provided evidence that classifies possums as highly solitary creatures (see Day et al. 2000) who exhibit minimal contact amongst each other even during peak breeding season (see Ji et al. 2005). Although we do not disagree with the hypothesis that possums are more anti-social outside of their breeding season (as evinced by the temporal pattern of contacts observed here; Figure 4-3), we challenge the notion that possums are "solitary". Although the effect of the breeding season was clear, contacts continued to occur outside of the breeding season, providing one possible explanation for how TB persists in possum populations all year round. Furthermore, with a limited number of proximity logging collars introduced into a population, social contacts among possums could easily be underestimated, as individuals within populations show varying levels of tolerance toward interactions with other possums.

This may have occurred in the study of Ji et al. (2005). In an ideal study, every adult possum would be fitted with a proximity logging collar. Therefore, I acknowledge that my findings concerning possum contacts are limited by the number of collars used during this study (40 proximity tracking collars per grid). However, with the larger resources expended allowing for greater effort on social contact monitoring here, I have provided evidence that although contact rates are decreased, possums are not "solitary" animals outside of their breeding season but rather exhibit dynamic social networks with more interactions among individuals than previously suggested.

The different possum sub-populations, population groups, and individuals at our study site display clear differences in their levels of sociality, as evinced in population, individual and contact level analyses of data conducted both during the breeding season and after. The amount of both contacts and contact times recorded far exceeded the study expectations; although reduced, the fact that there was still consistent contacts after the breeding season doesn't completely support the hypothesis that the majority of possum contacts are sexual in nature (see Ji et al. 2005). Rather, possums appear to contact each other in numerous social interactions that are not always sexual in nature. The study of Ji et al. (2005) reported a 24 hour contact rate of one contact every two days during breeding season and "clearly infrequent" contacts outside (before and after) breeding season suggesting that other means than direct contact may be significant in the transmission of disease in possums. This is a slightly lower per possum contact rate than in the current study and could partly be due to only interactions between male and female possums being reported by Ji et al. (2005). However, it is also very likely to be linked to population density with the current study being carried out in high density possum subpopulations.

My expectation that male possums would have more contact/proximity with other individuals than do female possums, based on the prior observation of males having larger home ranges, and both more and larger home range overlaps with other individuals than females, was upheld in the current study. That male possums in any population at any given time do have larger and more overlapping home ranges than female possums does indeed mean that they contact more individuals, have more contacts with other individuals, and have contacts of longer duration than do female possums. This resulted in there being far more contact activity for dyads involving males (either male/male or male/female dyads) than for female-only dyads. Although I could not examine the contact rates of juvenile possums in the current study, it is likely that the fewer and lower home range overlaps of juveniles compared to adults observed in Chapter 3 and the juvenile behaviour traits associated with home range found in (Blackie et al. 2011) likely translates into fewer contacts/less proximity for such individuals with other possums in any population at any given time.

Interestingly, the subpopulation differences in home range size and overlaps noted in Chapters 2 and 3 (with home range size, and the per capita number and size of home range overlaps, all being negatively correlated with subpopulation density), did not translate into differences in actual contacts among trapping grids in the current chapter. While trapping grid differences were apparent, it was not the case that the particularly high density subpopulation (on Grid B) was associated with different contact characteristics compared to the other three subpopulations. Rather, at the population level, it was Grids A and C that had more 'social' network characteristics, while Grids B and D had less 'social' network characteristics. Similarly, Grid B did not stand out as being distinct from the other possum subpopulations in either the individual-level or contact-level analyses. This shows that possum population-level metrics, such as

density, mean home range size, mean numbers of home range overlaps, and mean sizes of home range overlaps are not necessarily good predictors of the actual overall contacts/proximity occurring in any particular possum population at any particular time

The findings of this chapter have potential key implications for understanding the dynamics of TB in possum populations and its management. If the assumption of required proximity or contact for TB transmission among possums holds true (Lloyd-Smith et al. 2005) (an assumption that is very likely true given lack of evidence for long term *M. bovis* survival in the environment, and no indication of any role of insect vectors etc.) one can make two key predictions. First, possum density is unlikely to be a good predictor of per-capita TB transmission rates, as it was not a good predictor of the mean rates of contact or proximity (even though it did strongly influence mean home range characteristics). Second, irrespective of prevailing population characteristics, one could expect males to be playing more of a role in TB transmission than females with observed larger home ranges for males translating into larger and more home range overlaps. In turn these translate into greater contact/proximity with other individuals in a population. Furthermore, as noted above, a prediction of adults playing a greater role in TB transmission than juveniles can similarly be made, if the within population patterns in home range characteristics noted in Chapters 2 and 3 similarly translate into difference in proximity/contact rates.

Investigating these predictions with regards to TB transmission within possum populations is the focus of Chapter 6, in which I interpret the results of experimental TB challenge trials in wild possum populations in light of the considerations raised here.

# Chapter 5

# Changes in population structure following depopulation; implications for TB transmission and persistence

## 5.1 Introduction

Projects involving the live trapping of brushtail possums generally conclude with the termination of the study individuals. This is due to the status of this species as a pest that impacts native biodiversity and acts as New Zealand's primary wildlife reservoir of *Mycobacterium bovis* (the causative agent of Bovine Tuberculosis (TB); (Buddle et al. 1994)). Post-depopulation studies have been undertaken to evaluate the effectiveness of the control measures used (Jane 1979, Brockie et al. 1997) and to monitor the population response after treatment (Efford et al. 2000, Tompkins et al. 2011). However, little is known about the impact of control activities on possum behaviour and its potential effects on disease transmission. Such a knowledge gap is a concern particularly with regards to TB management in New Zealand; studies from Europe show how wildlife population perturbation can lead to increased TB transmission and a higher incidence in wildlife reservoir populations (Corner et al. 2008).

Research into contact networks has been used for understanding disease transmission in both humans (Buddle et al. 1994, Jolly et al. 2001) and livestock (Keeling et al. 2001, Webb 2005). However, research into contact networks of wildlife is still a relatively new avenue of study (Lloyd-Smith et al. 2005, Otterstatter and Thomson 2007, Grange

et al. 2014). Therefore, few wildlife studies have used this approach to document how the behaviour of wildlife populations may change post control and what this may mean for disease transmission.

Here, I continue the characterisation of possum home ranges and social networks for four subpopulations in the Orongorongo Valley (New Zealand) in 2012 (Chapter 2-4), by assessing the structure of two of the original four subpopulations at eight months post termination of the 2012 trial. Trapping grids were subsequently depopulated. This chapter uses data from the ongoing TB transmission trials in 2013, where only grids C and D were opened for trapping. The purpose of the study was to assess how the behaviour and social structure of the newly formed populations (rebuilt by dispersal in from the surrounding uncontrolled area) compare to those documented prior to control, and discuss changes and what they may mean for TB transmission, persistence and management in brushtail possum populations. If, for example, possum populations return to their previous structure, this would indicate possum home ranges are potentially dictated by environmental factors (e.g. topography). If this were the case, it may be possible to predict population characteristics from environmental factors. Alternatively, the disturbance associated with depopulation may result in a breakdown of previously identified population structure (see Chapter 2 for possum home range structure). Evidence for such breakdowns, which may potentially enhance TB transmission, has been observed in badgers (Tuyttens et al. 2000). It is important to note that depopulation events did not account for 100% of possums, therefore new populations were a mix of existing residents and dispersing individuals. Further, any inferences made from this analysis will be limited by the absence of a non-depopulated control group.

## 5.2 Methods and materials

### *5.2.1 Study site*

The study site was the Orongorongo Valley on the North Island of New Zealand (41°21'S, 174°58'E) during 2013-14. The grids of cage traps (delineating Orongorongo Valley possum subpopulations) used for the post-control characterisation are grids C and D (see Chapter 2 for full details). Prior to the first season depopulation, grids C and D had population sizes of 144 and 140 individuals respectively, with adults accounting for 71% (male and female = 35%) and 81% (male = 36%, female = 45%) of the grid populations respectively. Possums were euthanized by blunt force trauma. It is important to note that not all possums were removed from the grids at the conclusion of the first season. Grids A and B (see Chapter 2) were not part of the ongoing TB transmission trial at the site during the 2013/14 season, and were not available for the assessment conducted here.

### *5.2.2 Data collection*

Possums were captured in bi-monthly trapping sessions from August 2013 to April 2014; all other aspects of data collection are as reported in previous Chapters 2-4.

### *5.2.3 Analysis*

Analysis methods for this chapter are as detailed in Chapters 2 (home range analysis) and 4 (contact network analysis). Possum contact networks were analysed at the same three scales used in Chapter 4 with the only difference being the number of grids processed (two instead of four), and the months during which collars were active in the field (April - October 2012 for pre-depopulation; August- January 2013-14 for post

depopulation). A Chi-square test was used to investigate any sex differences between seasons.

#### *5.2.3.1 Population-level network analysis*

Data were analysed using R 3.1.2 studio packages and methods mentioned in Chapter 4. Differences in analysis are as mentioned above in the analysis section.

#### *5.2.3.2 Individual-level network analysis*

Data were analysed using GLM and a two-way ANOVA used in Statistica as detailed in Chapter 2.

#### *5.2.3.3 Contact-level network analysis*

Data were analysed using R 3.1.2 studio packages and methods mentioned in Chapter 4. Differences in analysis are as mentioned above in the analysis section.

## 5.3 Results

### *5.3.1 Home range*

Of the 362 individual possums captured on grids C (N=188) and D (N=174) during the post-depopulation assessment period (August 2013 to April 2014), 115 were trapped at least five times and had their MCP home ranges estimated. These grid population sizes are both approximately 30% higher than the pre-depopulation sizes reported in the previous year (see Chapter 2). Estimated mean home ranges on grids C and D were 0.34

(0.02 SD) in adult males, 0.27 (0.03 SD) in adult females, 0.33 (0.04 SD) in juvenile males and 0.41 (0.2) in juvenile females.

Unlike the pre-control assessment, where a significant grid effect (home range size negatively correlated with density) and a significant interaction between possum age and sex was observed, no variables were significantly associated with MCP home range size in the post-control assessment (possum age F= 2.40, df 1, P>0.1; possum sex F= 0.02, df 1, P>0.8; trapping grid F= 0.37, df 1, P>0.5). However, home ranges in the populations post-depopulation were all approximately three times smaller than those recorded pre-depopulation (see Chapter 2). In addition, although the population age structure (i.e. adult: juvenile ratio) was similar both pre and post-depopulation, there were relatively more males post-depopulation (approximately 60% male compared to 50% male pre-depopulation, chi-square = 4.5894 P= 0.032 Table5-1). It is important to note that not all possums were killed between the first and second seasons and all season two individuals were treated as new animals.

**Table 5-1** Brushtail possum sex ratio, age structure and individual trapping frequency (across the nine month study period) for the marked sub-populations on trapping grids C and D.

| | | | Grid C | Grid D |
|---|---|---|---|---|
| All individuals | | Number | 188 | 174 |
| | | Mean times trapped (range) | 3.99 (1,20) | 3.94 (1,20) |
| Adult individuals only | All adults | Number (percentage of grid population) | 153 (81%) | 145 (83%) |
| | | Mean times trapped (range) | 3.99 (1,20) | 3.94 (1,20) |
| | Male only | Number (percentage of grid population) | 96 (51%) | 77 (44%) |
| | | Mean times trapped (range) | 3.99 (1,20) | 3.94 (1,20) |
| | Female only | Number (percentage of grid population) | 57 (30%) | 68 (39%) |
| | | Mean times trapped (range) | 4.04 (1,14) | 3.88 (1,14) |
| Juvenile individuals only | All juveniles | Number (percentage of grid population) | 35 (19%) | 29 (17%) |
| | | Mean times trapped (range) | 3.77 (1,9) | 3.76 (1,15) |
| | Male only | Number (percentage of grid population) | 20 (11%) | 22 (13%) |
| | | Mean times trapped (range) | 3.8 (1,3) | 3.79 (1,15) |
| | Female only | Number (percentage of grid population) | 15 (8%) | 7 (4%) |
| | | Mean times trapped (range) | 3.77 (1,9) | 3.72 (1,8) |

### *5.3.2 Population-level analysis*

Of the 80 possums with proximity-logging collars, 53 (35 male and 18 female) were retrieved for analysis registering 554 contacts between the months of August 2013 and January 2014. Grids C and D are represented by 32 and 21 collars, respectively. Overall, node degree, edge strength and the clustering coefficient were all lower for these new populations than for the pre-depopulation populations, while betweenness was higher (Table 5-2).

There were no significant differences in relative node degree (F= 0.78, df= 1, P= 0.39), edge strength (F= <0.001, df= 1, P= 0.1) or relative betweenness (F= 3.2, df= 1, P= 0.08) between the two grids. There were also no differences in relative node degree or edge strength associated with sex (F= 0.1, df= 1, P=0.75 for relative node degree and F= 1, df= 1, P= 0.32 for edge strength). However, relative betweenness was associated with a grid/sex interaction in which males had higher values compared to females on grid C and the inverse was observed on grid D (F= 21.6, df= 1 P< 0.00007). It is important to note that these analyses are comprised of only contacts between possums for which collars were retrieved and downloaded n=53, and the power to detect differences was lower in the current post-depopulation study than in the pre-depopulation due to smaller sample size (see Table 5-2).

**Table 5-2** Post and pre depopulation mean (standard error) of network characteristics of trapping grids C and D, stratified by sex of possums. Adjusted values are the relative values adjusted for the number of radio-collared individuals in each grid. The post-depopulation data is from the fieldwork described in this chapter; pre-depopulation data is from Chapter 4.

Post-depopulation network parameter estimate (Standard error)

| Grid | Sex | No. possums | Node degree/ adjusted | Edge strength | Betweenness/ adjusted | Clustering coeff. |
|---|---|---|---|---|---|---|
| | | | 4.76 (0.56) | | 50.3 (10.9) | |
| | Males | 21 | 0.15 (0.02) | 26.0 (6.50) | 0.11 (0.02) | |
| | | | 3.82 (0.74) | | 10.2 (5.10) | |
| C | Females | 11 | 0.12 (0.02) | 30.1 (16.7) | 0.02 (0.01) | |
| | | | **4.44 (0.45)** | | **36.5 (8.06)** | |
| | **All** | **32** | **0.14 (0.01)** | **27.4 (7.00)** | **0.08 (0.02)** | **0.27** |
| | | | 2.29 (0.37) | | 13.6 (5.32) | |
| | Males | 14 | 0.11 (0.02) | 28.2 (11.3) | 0.07 (0.03) | |
| | | | 2.86 (0.59) | | 54.3 (12.8) | |
| D | Females | 7 | 0.14 (0.03) | 26.4 (7.86) | 0.29 (0.07) | |
| | | | **2.48 (0.31)** | | **27.2 (6.86)** | |
| | **All** | **21** | **0.12 (0.02)** | **27.6 (7.84)** | **0.14 (0.04)** | **0.33** |

Pre-depopulation network parameter estimate (Standard error)

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | 21.1 (2.22) | | 35.7 (13.3) | |
| | Males | 10 | 0.84 (0.09) | 325 (43.8) | 0.12 (0.04) | |
| | | | 12.4 (1.53) | | 16.0 (5.53) | |
| C | Females | 16 | 0.50 (0.06) | 383 (109.7) | 0.05 (0.02) | |
| | | | **15.8 (1.51)** | | **23.6 (6.30)** | |
| | **All** | **26** | **0.63 (0.06)** | **361 (68.9)** | **0.08 (0.02)** | **0.54** |
| | | | 4.40 (0.97) | | 18.2 (6.14) | |
| | Males | 11 | 0.21 (0.05) | 44.1 (11.1) | 0.09 (0.03) | |
| | | | 4.90 (0.84) | | 21.7 (6.43) | |
| D | Females | 11 | 0.23 (0.04) | 79.0 (31.2) | 0.10 (0.03) | |
| | | | **4.60 (0.63)** | | **19.9 (4.40)** | |
| | **All** | **22** | **0.22 (0.03)** | **61.6 (16.6)** | **0.09 (0.02)** | **0.6** |

*5.3.3 Individual-level analysis*

The mean (s.e.) number of possums contacted was stratified by gender and is shown in table 5-3; there were no significant differences between male and female contactors, F= 0.78, df= 51, P= 0.38. Interestingly, although the population sizes were larger on these recolonised grids, in addition to the home ranges being smaller the number of other possums that individuals contacted was approximately half that observed on the pre-depopulation grids (see Chapter 4).

**Table 5-3** Post and pre depopulation mean (se) number of individual contactees between 'contactors' and 'contactees' stratified by sex.

| Post-depopulation | Number of contacts (standard error) | |
|---|---|---|
| | Downloaded collared possum gender | |
| Contactee gender | Male | Female |
| Male | 1.49 (0.21) | 1.50 (0.28) |
| Female | 1.23 (0.17) | 1.44 (0.28) |
| **All** | **2.71 (0.24)** | **2.94 (0.49)** |

| Pre-depopulation | Number of contacts (standard error) | |
|---|---|---|
| | Downloaded collared possum gender | |
| Contactee gender | Male | Female |
| Male | 3.00 (0.45) | 2.62 (0.35) |
| Female | 3.22 (0.36) | 1.85 (0.19) |
| **All** | **6.22 (0.76)** | **4.48 (0.50)** |

As in Chapter 4, Poisson regression modelling was used to test for significant differences in contacts between male and female possums while adjusting for age, structural contact bias and the effect of grid (Table 5-4). The number of contacts with male and female 'contactees' were used as outcome variables, and the covariates were: sex of 'contactor'; grid; age of 'contactor'; and the number of overlapping home ranges. Pre-depopulation analysis revealed males contacted more possums on average than

females and that females contacted other females less than males did, however no such significant differences were found in the post-depopulation analyses F=1.648, df= 48 P = 0.18.

**Table 5-4** Poisson regression model estimates for the model with the number of contacts with female contactees as the outcome variable.

| Variable | Coefficient | Standard error | P value |
|---|---|---|---|
| Intercept* | 0.28 | 0.36 | 0.43 |
| Grid D | 0.22 | 0.24 | 0.37 |
| Age (adult vs juvenile) | -0.06 | 0.27 | 0.82 |
| Number of overlapping home ranges | -0.01 | 0.01 | 0.59 |
| Female vs Male | 0.12 | 0.26 | 0.64 |

* Juvenile male in C

Table 5-5 shows the distribution of the duration of contacts between contactors and contactees stratified by sex (duration of contacts was log-normally distributed). Although there was a very wide range in the duration of contacts, the median duration was similar for all pairs of males and females, F= 1.4, df= 1, P= 0.3.

The summed duration of contacts was an order of magnitude lower in the post-depopulation networks. This is most likely due to the contact loggers not being on the possums in the post-depopulation assessment during the breeding season, but may also be driven by the much smaller home ranges of possums in the post-depopulation

assessment leading to smaller home range overlaps between individuals (shown in Chapter 4 to be one driver of contact numbers and duration).

**Table 5-5** Pre and post depopulation duration of contacts between contactors and contactees stratified by sex. The top half of the table is for all potential contacts, the bottom is conditional on at least one contact occurring.

| | | Centiles of distribution of length of contacts (seconds) | | | | |
|---|---|---|---|---|---|---|
| **Post-depopulation** | **Contactor** | **0%** | **25%** | **50%** | **75%** | **100%** |
| Contact with male contactees | Male | 0 | 1 | 7 | 37.5 | 1910 |
| | Female | 0 | 1 | 12 | 54.25 | 570 |
| Contact with female contactees | Male | 0 | 1 | 3 | 21 | 309 |
| | Female | 0 | 1 | 4 | 16.5 | 114 |
| All Contacts | Male | 1 | 7.5 | 25 | 99 | 1930 |
| | Female | 1 | 2.75 | 27 | 86.25 | 574 |
| | | | | Conditional on contact | | |
| Contact with male contactees | Male | 0 | 1 | 7 | 37.5 | 1910 |
| | Female | 0 | 1 | 12 | 54.25 | 570 |
| Contact with female contactees | Male | 0 | 1 | 3 | 21 | 309 |
| | Female | 0 | 1 | 4 | 16.5 | 114 |
| **Pre-depopulation** | **Contactor** | **0%** | **25%** | **50%** | **75%** | **100%** |
| Contact with male contactees | Male | 0 | 10 | 129 | 566 | 422715 |
| | Female | 0 | 0 | 170 | 961 | 713700 |
| Contact with female contactees | Male | 0 | 64 | 339 | 1473 | 212412 |
| | Female | 0 | 2 | 35 | 329 | 1105536 |
| All Contacts | Male | 4 | 140 | 662 | 1852 | 424188 |
| | Female | 1 | 48 | 418 | 1640 | 1106383 |
| | | | | Conditional on contact | | |
| Contact with male contactees | Male | 1 | 45.25 | 266.5 | 705 | 422715 |
| | Female | 1 | 73 | 640 | 1334 | 713700 |
| Contact with female contactees | Male | 1 | 67.75 | 340 | 1493.75 | 212412 |
| | Female | 1 | 15.5 | 67 | 679.5 | 1105536 |

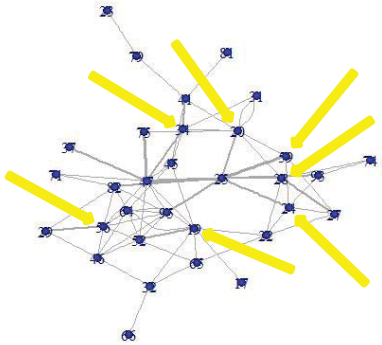
As in Chapter 4, regression modelling was used to test for significant differences in duration of contacts between sex while adjusting for age, structural contact bias and the effect of grid. The log duration of contacts (in seconds + 1) with male and female 'contactees' were used as outcome variables, and the covariates were: sex of 'contactor'; grid; age of 'contactor'; and the number of overlapping home ranges as seen in Chapter 4. Pre-depopulation analysis showed contacts between females to be significantly different from (lower than) other combinations, but again, no post-depopulation differences were found between either sex (males, F= 1.2, df= 1, P=0.33; females, F= 1.1 df= 1, P= 0.39). Covariate stats were non-significant for both males t= 1.36, P= 0.18 and females t= 0.93, P= 0.36.

### *5.3.4 Contact-level analysis*

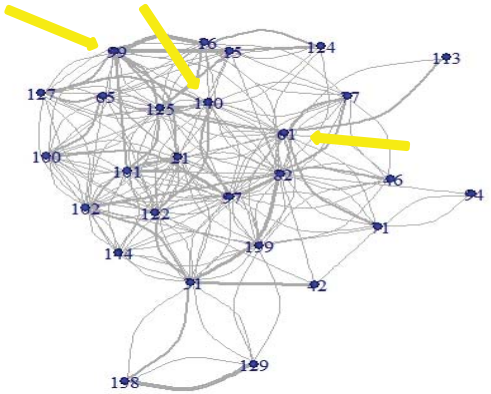
Analysis for night-time possum contacts found no significance among frequency of dyads contacts (at the P = 0.01 significance level), however, male to female dyads occurred more frequently (F=5.89, df= 2, P= 0.05) than male/male or female/female dyads across both grids. This result differs slightly from the pre-depopulation results where there was a trend found in dyad frequency but no clear differences (see Chapter 4). No differences were found in dyads recorded during the day (F= 0.49, df= 2, P= 0.78), however, this analysis was based on just 18 observations and would have had very low power.

**Figure 5-1** Pre and post depopulation diagrams of social networks for the two trapping grids re-examined, generated by Igraph in R studio. Post-depopulation grid C (1a), pre-depopulation grid C (1b), post-depopulation grid D (2a), pre-depopulation grid D (2b). Numbers located at the nodes denote individual possums IDs. Network line thickness is proportional to edge strength. Arrow indicates challenged possums.

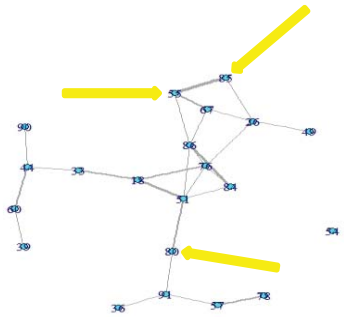
1a



1b



2a



2b

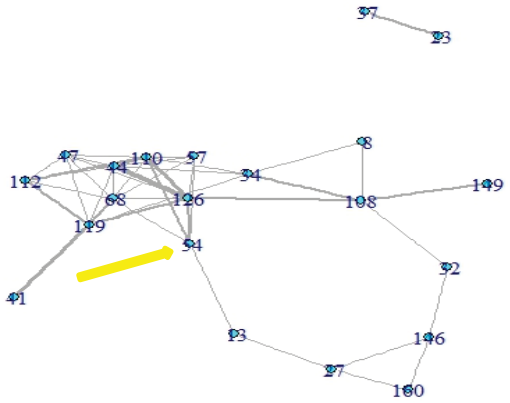
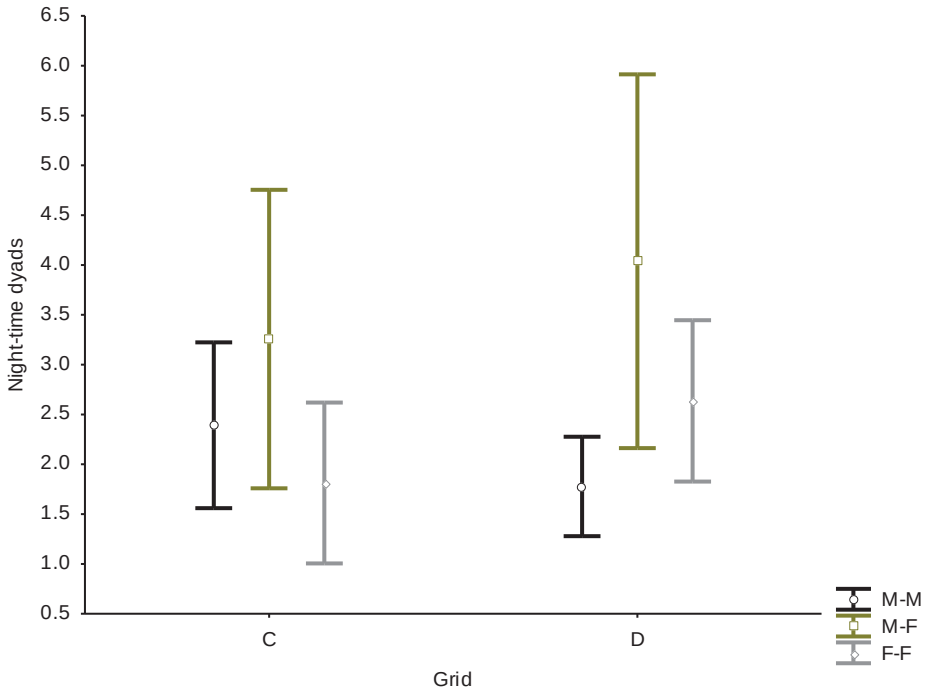


**Figure 5-2** Total number of night-time dyad pairs recorded on grids C and D separated into male/male (M-M), male/female (M-F), and female/female (F-F) dyads.




## 5.4 Discussion

Numerous studies have been undertaken to assess and better understand the social networks of populations of wild, free-living animals (Ramsey et al. 2002b, Ji et al. 2005, Lloyd-Smith et al. 2005, Godfrey et al. 2009, Matthews et al. 2009) as well as their home range characteristics (Buddle and Young 2000, Efford et al. 2000, McCallum 2009), yet little is known about these aspects of populations after a depopulation or their recovery dynamics. Efford (2000) addresses possum home range shifts in the wake of controlled buffer zones up to a year after depopulation, but subsequent data analysed was based on changes in location rather than the size of re-established possum home ranges or other elements of their behaviour.

In the present study, the depopulation conducted was on a much smaller scale than earlier examined depopulations, and the study populations rapidly recovered through possum immigration from the surrounding habitat. Indeed, at eight months post-depopulation the grid populations were approximately 30% greater than they had been previously, associated with a bias towards males. There are numerous reasons why the depopulated population may be larger than the pre-depopulated population. It may take time for the social structure to re-establish on the grids meaning possums may be vying to settle on the grids before the unsuccessful individuals are forced to move on. Also, the depopulation may have allowed a period of vegetation regrowth which could support higher numbers of individuals until the area is browsed back to normal levels of resources. The male bias is not surprising as they tend to be the more dispersing sex (Crawley 1973, Cowan and Clout 2000), and would have formed a larger part of the immigrating population. Interestingly, here I characterised the post-depopulation populations as having smaller home ranges than previously. This is in line with findings of higher densities and the generally observed negative relationship between possum density and home range size, but is in contrast to the findings of a previous study by Ramsey et al. (2002b), where larger home ranges post-depopulation were observed. However, the observations of Ramsey et al. (2002b) were soon after depopulation, before population density had recovered through immigration.

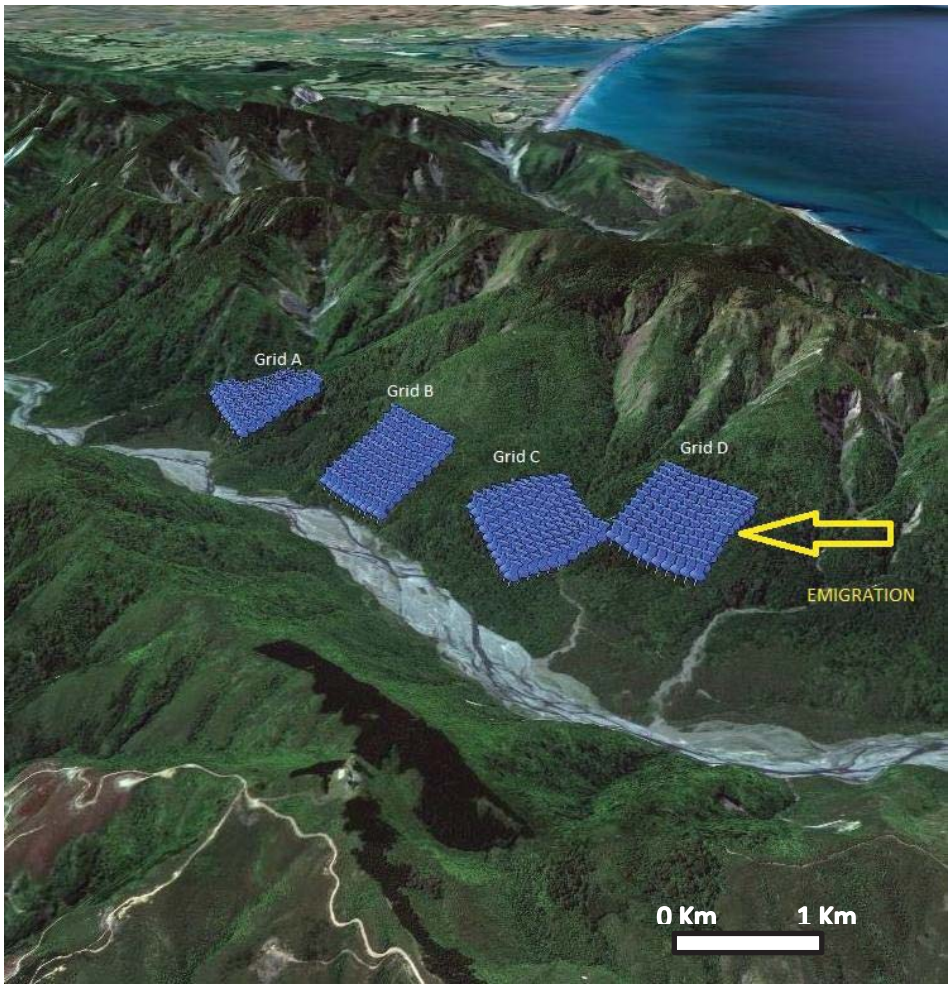
Associated with the higher population density (and male bias) observed here at eight months post-depopulation, compared to the pre-depopulated populations, were (1) smaller home range sizes, (2) networks with lower node degree, clustering and edge strengths, (3) fewer individuals in-contact per possum, and (4) a reduction in total contact duration (although this was more likely caused by the post-depopulation observations being outside the breeding season). Also there was much less structure to the home ranges and contacts/contact rates (i.e. fewer observable effects of possum age and sex). I acknowledge that the differences observed

between the pre and post depopulation studies may be due in part to variables such as the difference in the data collection season mentioned in the analysis, or low statistical power associated with the lack of temporal data. However, because no real trends for significant effects were observed the previous year (Chapter 2-4), the differences in observations between the two seasons are more likely due to the post-depopulation populations being in a stage of flux (with normal population structuring factors yet to re-establish, even after eight months of population recovering) than other factors. The observed relationships in the post-depopulation populations between higher density and smaller overall home ranges agrees with the home range/density relationships found previously for possums (including in Chapter 2), while the lower overall number of contacts recorded individuals agrees with the previously observed negative relationship between home range size and number of contacts (Chapter 4) that is driven by reductions in the home range overlap between individuals (Chapter 3). The second season did have a lower recapture frequency than that of the first season. This could be a result of newer, unsettled individuals with smaller home ranges moving on and off the grids or not coming into contact with as many traps. However, because individuals trapped fewer than five times were omitted from the home range analysis the lower recapture frequency likely has little effect on the home range results and no effect on the contact network results.

The hypothesis that the post-depopulation populations are in a state of flux is supported by the differences observed here between the two grids on which possum populations were monitored post-depopulation (Figure 5-3). These differences suggest that although possums filled in the empty space on the cleared out grids within months of depopulation, it may take longer for individuals to establish themselves in terms of home range establishment and possum dominance. This could explainthe differences in home range findings between seasons one and two. The findings outlined in the home range section above show evidence

that males are dispersing further and faster and interacting with other possums on grid C while females are establishing themselves on the better settled grid D. This adds to the idea that once an area is repopulated, it takes longer for individuals to establish a social structure. It is important to note that grids A and B underwent depopulation from the season one four months prior to the activation of grids C and D for season two. Due to this depopulation and referencing Efford (2000), by treating this section of the valley as one large buffer zone, a bottle neck effect of possums emigrating north up the valley should have occurred to fill the vacant area on grids C and D as shown in figure 5-3. Here, area above and below the grids is limited by the ridge and the river. Therefore the most likely place for possums to come from is south of grid D. This would mean that after the depopulation on grids A and B, possums would have moved south onto grid A while possums on Grid C would have moved north onto B grid. Possum immigration onto grids A and B and emigration from grid C could illustrate how grid D (the grid least likely to be affected by the depopulation on grids A and B) is more likely to be a better settled grid than C.

**Figure 5-3** Orongorongo Valley study grid placement with grid A as the northern most grid.




The post depopulation contact data analysed here confirms that possums are socialising, even though these data were collected completely outside of the breeding season. However, the types of interactions and the reasons for these interactions remain unclear. Even with a relatively large sample size of confirmed contacts (n=554), season two possums were lacking the social structure found in the first season as no significance in node degree or edge strength that would indicate a familiarity between two or more possums was found. Betweenness is as indicator of a nodes centrality in a given network (see Chapter 4 Table 4-1). The significance of relative betweenness found on both grids and the lack of structure for

the other two variables implies that possums are not contacting neighbouring possums out of habit or routine, but that contact is being made at random by many different individuals.

So, what are the implications of these observed pre and post depopulation differences in possum populations for TB transmission and dynamics? First, since it is apparent that the populations are still in a stage of flux at eight months post-depopulation, we cannot address the question of whether or not similar population structures are reformed (with such similarity indicating that possum home ranges and populations are, to at least some degree, dictated by environmental influences). The evidence presented here is that at eight months post-depopulation, the reformed populations have yet to re-stabilise. Assessment of populations over a longer period post-depopulation are needed to assess how long stabilisation and re-establishment of equilibrium social dynamics takes, and whether or not once an equilibrium is reached the home ranges and population structure bear any resemblance to that observed prior to depopulation.

Second, as is observed in many other wildlife disease systems including TB in badgers in the UK, the period of population disruption may have implications for TB transmission and dynamics in possums. However, what those implications are is not immediately intuitive. The higher population densities, greater network between-ness indicating more random contacts between individuals, and related lower apparent social structure, indicates potential for increased infectious disease transmission. However, in contrast, the smaller home range sizes and consequently overall lower rates of between possum contacts and contact durations (most likely due to lower home range overlaps – not assessed here, but such a relationship is demonstrated in Chapter 4), indicates potential for reduced infectious disease transmission. I investigate these potentially contrasting effects in Chapter 6, where I equate the outcomes of artificially induced TB transmission (resulting from the experimental challenge of

individuals) to the population differences observed in the pre and post depopulation possum populations, in addition to investigating population subgroup (e.g. male versus female, juvenile verses adult) and individual drivers of transmission (building on this Chapter, and Chapters 2-4).

Further studies on population recovery are needed to identify population settling dynamics of home range after depopulation.

# Chapter 6

# Relating variation in tuberculosis (TB) transmission in brushtail possums (*Trichosurus vulpecula*) to potential drivers found at the environmental, population and individual level

## 6.1 Introduction

Two processes by which few individuals generate a disproportionate or higher than average number of secondary cases of infection in a given population are supershedding, whereby infected individuals who shed disproportionately high levels of infectious stages lead to greater numbers of secondary cases of infection (Matthews et al. 2009), and superspreading, whereby infected individuals who come into disproportionately greater contact or proximity with other individuals lead to a greater number of secondary cases of infection (McCaig et al. 2011). (Matthews et al. 2009, McCaig et al. 2011). However, identifying individuals as supershedders or superspreaders is difficult as empirical data of sufficient resolution to distinguish their effects within a population is rare (Matthews et al. 2009, Spencer et al. 2015). Therefore, methods of identifying these individuals for purposes of disease control have yet to be established.

The largest barrier preventing the eradication of Tuberculosis (TB) in New Zealand and its consequential spillover to livestock is infection in the brushtail possum (Matthews et al.

2006). Pathways of TB transmission are not well understood (see Chapter 4), however, prior studies on old world badgers and foxes in the U.K. have presented evidence suggesting that, although disease transmission may not be limited to physical contacts, there is a positive correlation between close proximity interactions (Chase-Topping et al. 2008, Corner et al. 2008), and transmission via direct contact cannot be ruled out (Lloyd-Smith et al. 2005). Such findings are not surprising for an infectious disease such as TB that survives for a limited length of time in the environment and is not known to be vectored by insects or small mammals such as rodents. We now know possums exhibit variation in contact behaviour between individuals driven by factors such as sex (Chapter 4), variation in density and home range size (Chapter 2), and potentially disruption of populations (Chapter 5) and variation in the proximity (i.e. home range overlap) with which possums associated with others (Chapter 3). These findings all point to the potential for 'superspreading' to be playing a role in TB transmission among possums.

Likewise, given that TB is a bacterial disease, and other bacterial diseases clearly vary in their levels of shedding from different individuals, there is also potential for 'supershedding' to be playing a role in TB transmission among possums. Indeed, the variation in the progression and extent of bacterial lesions in infected possums is similar to that observed for E. coli in cattle, the system for which the 'supershedder' hypothesis was originally formulated. Understanding what role heterogeneities such as superspreading and supershedding play in TB dynamics in possum populations has important implications for the management of the disease (see Figure 6-1 for vector risk areas). For example, if predictable subsets of individuals are responsible for a disproportionately large amount of disease transmission (and thus are potentially responsible for driving its persistence and spread), identifying those subsets are priority for management could improve the efficacy of disease control (assuming such targeting was possible). Alternatively, blanket control may need to be

of a higher intensity than otherwise predicted, if successful disease eradication is reliant on suppressing a subset of a possum population rather than the overall population.

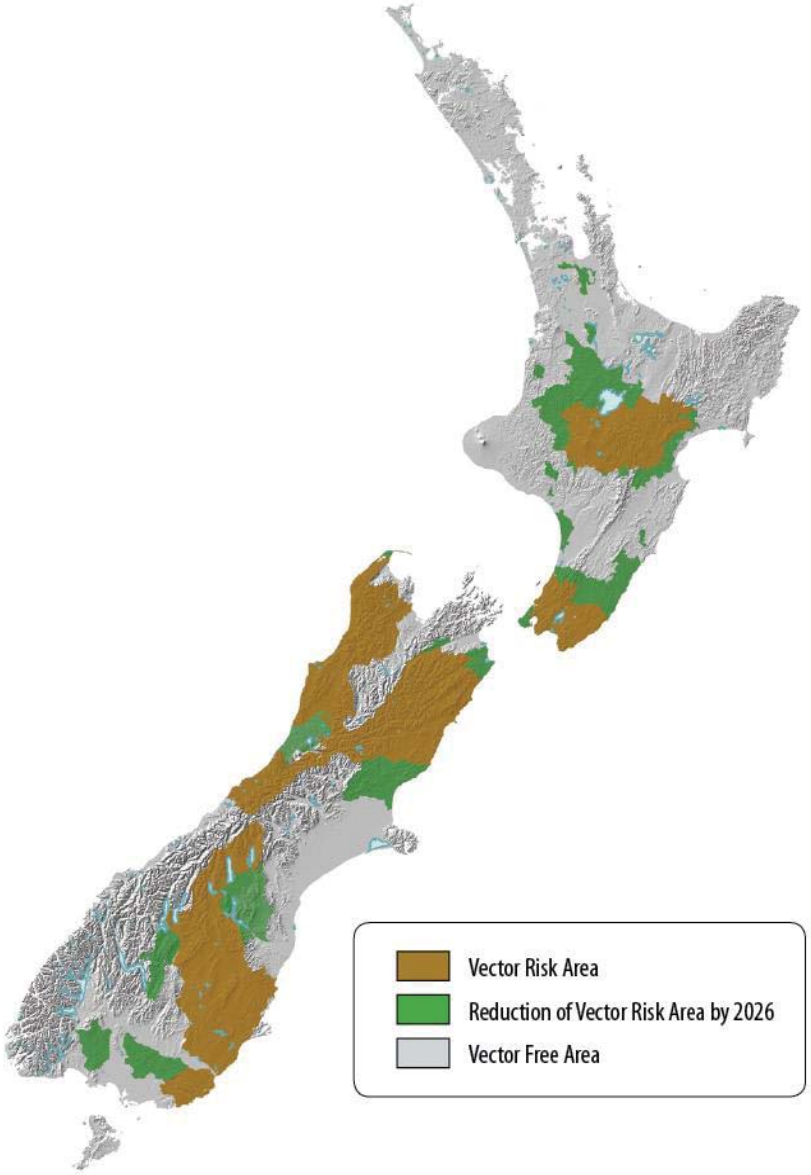



**Figure 6-1** Map of New Zealand with "vector risk areas" or areas with known cases of TB. TB infection identified in brown, vector free areas shown in grey, and the goal area of vector reduction by the year 2026 in green as described by TBfree New Zealand. www.tbfree.org.nz/risk-based-assessment-of-new-zealand

Two previous studies set out to establish infection pathways in wild free-living possums using experimental challenge methods (Corner et al. 2002a, Tompkins et al. 2007). These attempted to track subsequent natural infection rates, but failed to record any secondary cases infections due to a low incidence of the disease (Whitford et al. 2014).

My study aimed to identify the drivers of TB transmission among possums by conducting experimental challenges; infecting wild free-living possums with a strain of TB that was distinguishable from natural background infection and documenting ongoing transmission in secondary cases. I then related the patterns of transmission to potential environmental, population and individual-level drivers, drawing on the information and knowledge collated here in Chapters 2-5. Understanding the determinants of possum TB transmission is important for understanding the persistence and re-emergence of TB in possum populations.

## 6.2 Methods and materials

In season one and two (years one and two), I studied six subpopulations of possums (four in the first season and two in the second season), to investigate potential environmental, population and individual scale drivers of home range and contact network characteristics (Chapters 2-4-5). These subpopulations were employed in subsequent TB transmission trials, in which individuals were experimentally challenged with *M. bovis* and secondary transmission monitored. Observed patterns of transmission were related to the possum home range and social network characteristics explored in Chapters 2-5, in addition to other relevant factors such as population density and patterns of mortality and disease progression in primary and secondary case TB infections.

*6.2.1 Experimental design*

From the beginning of each field season, trapping grids were opened for four nights on a monthly basis, with every possum trapped evaluated for external evidence of the presence of TB infection by visual checks and physical palpation of cervical, axillary and inguinal lymph nodes. If swollen or enlarged lymph nodes were detected, the possum id and the size and location of the node were documented. Attempts to collect infection samples were made by using sterile swabs or aspirates (21 grade needle and 1ml syringe) and all collected samples were forwarded to AgResearch for bacteriological culture. Each season was concluded in April with a final possum kill-out where every trapped possum was euthanized. Possums were euthanized with an air rifle or blunt force trauma (Rouco et al. 2015), and taken to the on-site Landcare laboratory for subsequent necropsies. All animal manipulation for this research was undertaken with approval of the Landcare Research Animal Ethics Committee (approval no 12/02/01).

In season one; possums were experimentally challenged with TB on all four grids in July 2012. However, seven out of the eight challenged possums on grids A and B died unexpectedly in the first month following infection (during a period of very cold and wet weather in which many other non-challenged possums also died). The remaining possum was also lost to follow-up, but no body was recovered. Therefore, a new selection of possums on those grids was challenged in November 2012. Season two challenges were conducted during November 2013. These trials were only carried out on grids C and D, eight months after the previous season depopulation (see Chapter 5).

During the first season, four adult possums (two male and two female) per grid were challenged, on each of grids A, B, C and D, with the causative agent of TB, *M. bovis*. In this first season, highly social individuals (i.e. adult possums with node degree values within the

top 25% of collared possums on grids A and C) and low social individuals (i.e. adult possums with node degree values within the bottom 25% of collared possums) on grids B and D received the experimental infection challenge. Season two challenge possum selections differed from season one. Twice as many individuals per grid were selected to receive experimental infection (eight instead of four – four males and four females) due to fewer than expected secondary case infections being observed during the first season (see Results). As a result of this higher experimental infection intensity per grid, only two grids were included in the second season trial. In the second season, of the eight challenged individuals per grid, four received the same infection dose as in season one (see below), while the other four received a higher infection dose (with equal sex ratios in each group again maintained). In season two there was no a-priori selection of challenged individuals based on their social network characteristics.

During the second season, 16 adults (half male and half female) were also selected at random as control possums. These possums underwent the same treatment as the challenged individuals, but with challenge injections consisting of sterile water rather than *M. bovis* suspension (see below).

### *6.2.2 Experimental infection methodology*

The *M. bovis* challenge process consisted of possums being live-trapped under the same methods mentioned in Chapter 2, at which point possums were transported in a sack to a work area on the grid previously set up for infection administrations. Once all challenge candidates were collected, possums were anaesthetised (see Chapter 2 for details), inspected for the presence of existing clinical TB signs (challenged individuals showed no signs of prior TB infection), and their body weight, body length and tooth wear were recorded (see Chapter 2 for details). They were then, fitted with a new proximity collar (see Chapter 4 for

details), and infected with TB via sub-cutaneous injection, a method thought to more closely represent the natural route of TB infection in wild possums (McCaig et al. 2011). Possums were challenged with a strain of *M. bovis* (REA type 115, from the central North Island of New Zealand; (Buddle et al. 1994, Spencer et al. 2015)) that is different from that found naturally at the study site. This enable subsequent cases of infection in other individuals to be confirmed by strain typing as secondary cases resulting from transmission from the experimentally challenged individuals, as opposed to cases arising from background infection with the endemic strain, (Buddle et al. 1994, Spencer et al. 2015). Once infections were administered to a possum, it was placed back into the sack and returned to its original trap location.

In the first season, possums were each challenged with 20 cfu into the hind foot between the third and fourth toe. In the second season, as mentioned above, *M. bovis* inocula were administered at two dose levels: 20cfu (denoted “low dose”) and 200cfu (denoted “high dose”) using the same “hind foot” method as the first season. Personal protective equipment (PPE) was utilized during all challenge procedures including face mask, gloves, eye protection, and disposable full body gowns. During the first season, possums were trapped at monthly intervals after the experimental challenges to monitor both the progression of TB in challenged individuals, and new cases of secondary infection. During the second season possums were radio tracked between monthly trapping sessions to assess mortality status of challenged possums (each new collar fitted to challenged possums was fitted with mortality sensors) in order to better track the development of clinical disease.

## 6.3 Analyses

### *6.3.1 Survival rates of challenged possums*

In the second season on Grids C and D, mean and median survival times were compared between experimentally challenged and control possums, using the Kaplan–Meier product estimator in the SURVIVAL package of the R statistical computing environment (version 3.1.2, R Core Team 2013). Censored observations included possums that were still alive at the end of the study or otherwise had their radio-collar removed or experienced collar failure (i.e. right censoring). We used an exponential (constant hazard rate) model to test for differences in survival rates between challenge doses, sex, and trapping grids, from which instantaneous mortality rates were estimated (Ramsey and Cowan 2003, Ramsey and Efford 2010a, Nugent et al. 2013b)

### *6.3.2 Population-level drivers of transmission*

Numbers of observed secondary cases per challenged subpopulation (three from season one, and eight from season two) were compared across subpopulations with respect to (1) information on subpopulation density, mean home range size, sex ratio and age structure (data from Chapters 2 and 5), (2) information on challenge time of year, and challenge dose(s) used, and (3) information on subpopulation social network characteristics (data from Chapters 4 and 5) using GLM analysis in Stastistica version 7.0.

### *6.3.3 Subgroup-level drivers of transmission*

All secondary cases of infection were compared as a single group of individual transmission events with respect to the sex of the individual that had received infection (male versus female) and the age of the individual (adult versus juvenile).

*6.3.4 Individual-level drivers of transmission*

Home range estimates and home range overlaps for all possums analysed across grids and by sex were taken from Chapters 2, 3 and 5 and used to identify the home ranges of the challenged possums and any possible overlap occurring with possums showing secondary case TB infections during both seasons one and two. Node degree, edge strength and betweenness values from the population level, individual level and contact level were taken from Chapters 4 and 5 to evaluate any contact network information between experimentally challenged possums and possums with secondary case infections.

GLM analysis in Stastistica version 7.0 was employed to compare the mean home range overlap and area of overlaps possums shared with both challenged and un-challenged possums (with challenged possums split into males and females). This analysis was only carried out for one experimentally challenged subpopulation (Grid C in season two see Results), as it was the only occasion in which sufficient number of secondary cases occurred.

## 6.4 Results

*6.4.1 Survival rates of challenged possums in season 2*

Survival rates were only investigated during the second season when mortality tracking devices were fitted to the possum collars (no mortality tracking devices were used during the first season). Of the 16 radio-collared possums that were experimentally challenged with *M. bovis* in November 2013 (second season), mortality sensors monitored every two weeks showed 14 individuals died between 12 and 26 weeks post-challenge. From the 14 challenged possums, an overall survival probability was 94% 13 weeks post-challenge. After the 13 week period remaining individuals died rapidly. There was no significant difference in

survival durations of possums infected with low doses or high doses ($Z$= 0.63, $P$= 0.52), or between grids C and D ($Z$= 0.73, $P$= 0.46). Median survival times were 17 weeks for both infection doses and mean survival times (with 95% confidence intervals) were 18.3 (16.4- 24.0) and 18.5 (15.0- 21.0) weeks. Median survival times for male and female possums were 22.0 and 16.0 weeks respectfully. Mean survival times (with 95% confidence intervals) were 21.0 (18.3- 23.7) and 15.9 (14.2- 17.6) weeks for males and females which equates to mortality rates of 2.47 and 3.27 deaths per possum per year for challenged male and female possums respectively. This provided evidence that male possums had a significantly (P=0.03) greater survival rate than did challenged female possums (Table 6-1, Figure 6-2 and 6-3).

**Table 6-1** Results from a survival rate analysis using an exponential (constant hazard) model showing the effect of Tb Dose (High or Low), Grid (A and B) and Sex (male or female) from possums in the Orongorongo Valley. Estimate of regression coefficient (RC), hazard ratio (HR) and standard errors (SE (RC)), z-statistic and associated P-values are shown

| Treat | RC | HR | SE(RC) | z-statistic | P-value |
|---|---|---|---|---|---|
| Dose (low) | 0.4663 | 1.5941 | 0.6951 | 0.671 | 0.5023 |
| Grid (A) | 0.3903 | 1.4774 | 0.5959 | 0.655 | 0.5125 |
| Sex (male) | 2.1870 | 8.9082 | 1.0031 | 2.180 | **0.0292** |

**Figure 6-2** Kaplan–Meier survival probability plot for female versus male *M. bovis*-challenged possums (male gets *n* = 8, female gets n=8). Mean (± 95% CI) survival based on the raw data was 19.2 ± 2.2 weeks overall, and 17.4 ± 3.1 and 20.9 ± 3.5 weeks for females and males respectively. Kaplan–Meier calculations predicted a median survival time of 17.1 weeks overall and 16.2 and 22 weeks for females and males, respectively.

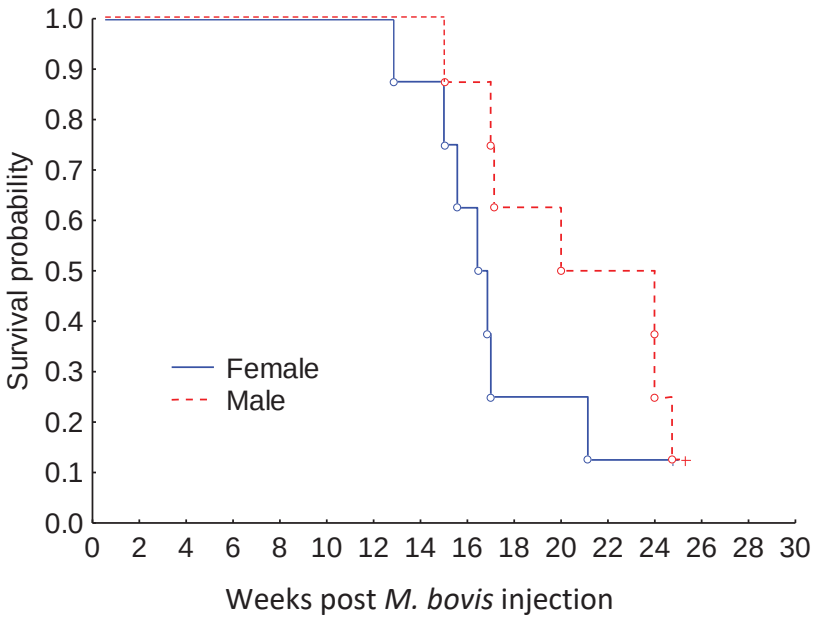
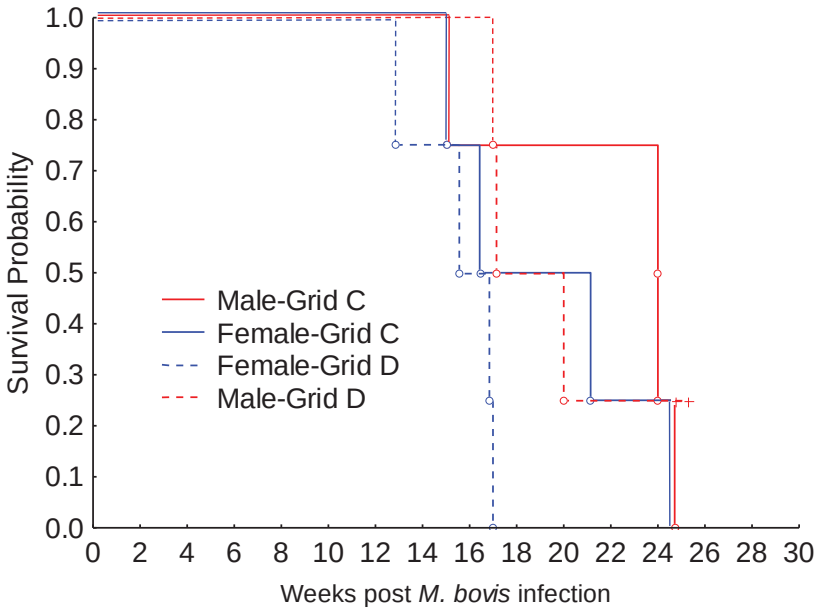


**Figure 6-3** Kaplan–Meier survival probability plot for female versus male *M. bovis*-challenged possums separated by grid ($n$ = 16). Mean (± 95% CI) survival based on the raw data was 19.3 ± 7.1 and 22.0 ± 7.2 weeks for females and males in Grid C, respectively, and 15.6 ± 3.1 and 20.0 ± 6 weeks for females and males for Grid D, respectively. Kaplan–Meier calculations predicted a median survival time of 18.2 and 24 weeks for females and males in Grid C respectively, and 16.2 and 18.0 weeks for females and males in Grid D, respectively.




*6.4.2 Population-level drivers of transmission*

During the first season the number of possums with home range overlaps with experimentally challenged possums varied from 80 to 142 across the four grids (Table 6-2; Figure 6-4) and three of these non-challenged individuals (two on Grid B and one on Grid C) were culture-confirmed as being infected with TB. No secondary case infections were found on grids A or D. Of the three secondary cases detected, two were typed as the novel strain of *M. bovis* used for the experimental challenge possums (REA type 115). The third case found on grid B was

not typed due to laboratory error. However, for testing purposes it was treated as a secondary case resulting from the experimental challenges as no other natural TB strain was detected in any of the 939 individuals handled over the full course of the first season.

During the second season the number of possums with home range overlaps with experimentally challenged possums was 166 and 182 on grids C and D respectively, where eight of these non-challenged individuals (seven and Grid C and one on Grid D) were culture-confirmed as being infected with the challenged strain of *M. bovis* (REA type 115, see Table 6-2, Figure 6-5).

**Table 6-2** Number of non-challenged possums whose MCP (100% minimum convex polygon) home ranges overlapped that of at least one of the experimentally *M. bovis*-challenged possums; total number of individual possums trapped on each grid during the same period; and the number of secondary infections detected for home range overlapping possums.

| | Grid | Experimental challenge date | Number of non-challenged possums with overlapping home range | Possums trapped within Grid | Secondary infected possums |
|---|---|---|---|---|---|
| 1st season | A | Nov. 2012 | 80 (53) | 120 (80) | 0 |
| | B | Nov. 2012 | 142 (75) | 211 (115) | 1[2]* |
| | C | July 20012 | 101 (61) | 125 (96) | 1 |
| | D | July 20012 | 128 (83) | 150 (111) | 0 |
| 2nd season | C | Nov. 2013 | 166 (100) | 194 (114) | 7 |
| | D | Nov. 2013 | 182 (81) | 184 (98) | 1 |

[2]* represents a sample lost in the laboratory prior to bacterial typing. Therefore only one sample was available for identification rather than the two originally found.

**Figure 6-4** Season One: Minimum convex polygon (MCP) home ranges of possums (*n* = 4 per grid) experimentally challenged with TB (in grey), and the non-challenged possums with culture-confirmed TB (N= in black) for each of the four study grids (dotted line represents secondary case of unknown TB strain). Graphs show the number of collared possums whose home ranges overlap the experimentally challenged possums on each grid (e.g. on gird A, 25 non-challenged possums had a home range overlap with at least one challenged possum)

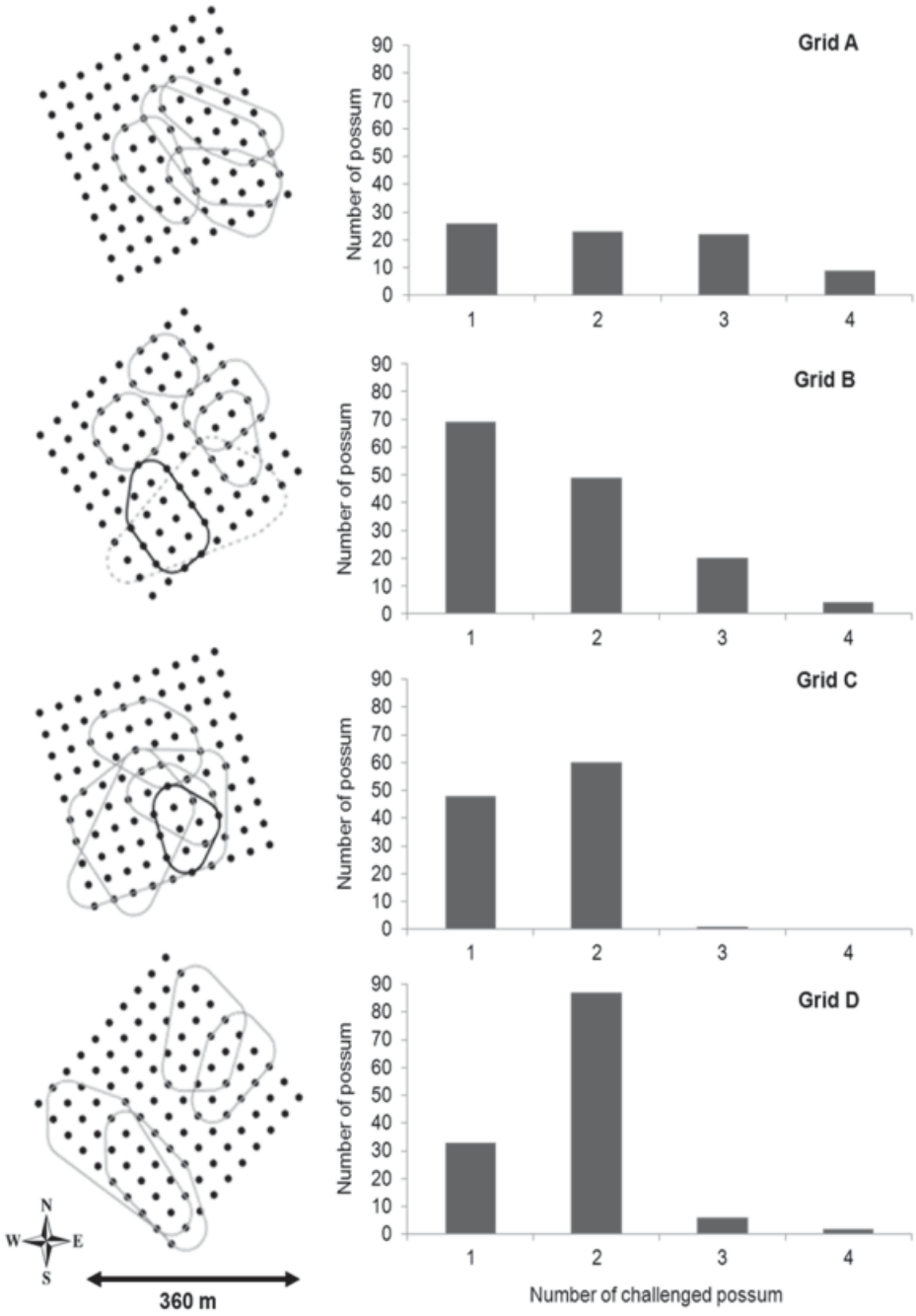
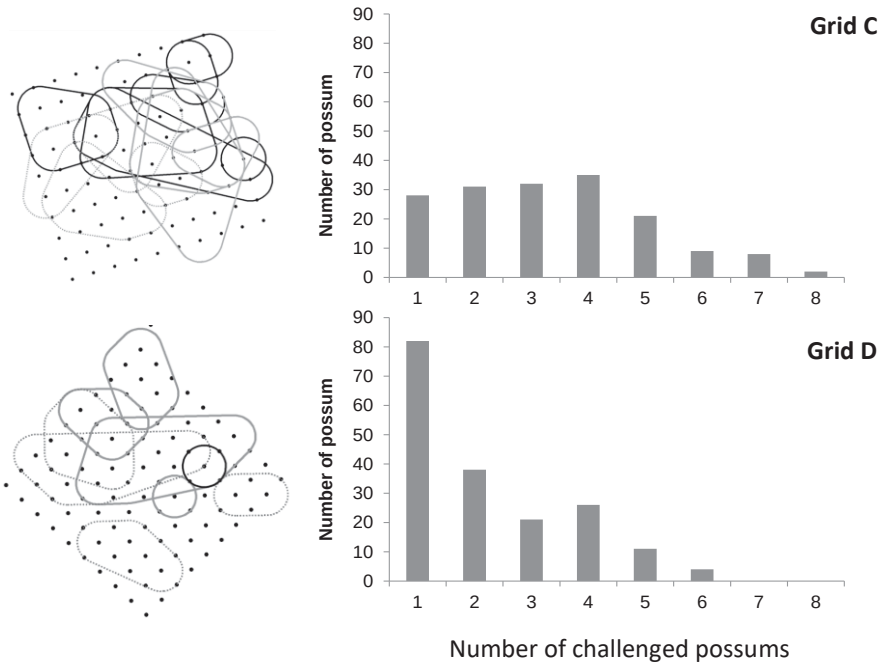


**Figure 6-5** Minimum convex polygon (MCP) home ranges of the season two possums (*n* = 8 per grid) experimentally challenged with TB (in grey), and the non-challenged possums with culture-confirmed TB (in black) for each study grids, seven in grid C and one in grid D. Graphs show the number of possums whose home ranges overlap those of the one to four experimentally challenged possums on each grid (e.g. on gird C, 30 non-challenged possums had a home range overlap with at least two challenged possums).




*6.4.3 Subgroup level considerations*

Of the 11 secondary case infections found in seasons one and two, nine secondary cases were males and only two were females. The novel strain infections were found primarily in adult possums (adults= 10, juveniles= 1), and on grid C (A= 0, B= 1, C= 8, D= 1). While the natural background strain was found only in adult possums with one strain on grids C and D (Table 6-3).

**Table 6-3** Number of secondary case infections found during seasons one and two across all four grids. Novel strain represents the secondary case infections derived from the introduced TB strain and the background strain represents the naturally occurring TB strain.

| | | Grid A | Grid B | Grid C | Grid D |
|---|---|---|---|---|---|
| 1st season | Novel strain | 0 | 1* | 1 | 0 |
| | Background strain | 0 | 0 | 0 | 0 |
| 2nd season | Novel strain | 0 | 0 | 7 | 1 |
| | Background strain | 0 | 0 | 1 | 1 |
| Total | Novel strain | 0 | 1 | 8 | 1 |
| | Background strain | 0 | 0 | 1 | 1 |

* represents a sample lost in the laboratory prior to bacterial typing. Therefore only one sample was available for identification rather than the two originally found.

*6.4.4 Individual-level drivers*

Analysis of home range found no sex effect for possums sharing home range overlaps with challenged individuals for either the number of overlaps F= 1.37, sd= 1, P= 0.24 or the shared area of the overlaps F= 1.21, sd=1, P= 0.27. Adult possums did share more home range overlaps with challenged individuals F= 4.65, sd= 1, P=0.03 but no significance was found for the area of home range shared between adult and juvenile possums with infected individuals. Note, individual-level drivers are only assessed from season two grid C.

## 6.5 Discussion

Although a relatively low number of secondary case TB infections were produced from season one and two (season three has yet to be analysed), variation in the population subgroups found secondary case infection of TB tend to be found mostly in adults and mostly in male possums. This finding is not surprising when considering the results from Chapter 2 and 3 where I predicted adult males could be the drivers of TB infection or "superspreaders" in wild free-living possum populations due to their larger home ranges (Chapter 2, Ramsey et al. 2002b, Whyte et al. 2014) and their greater number of home range overlaps accounting for more shared area with other possums than any other age or sex combination (Chapter 3). This finding is also supported by the results in Chapter 4 that showed adult male possums having more contacts with a greater number of individuals. Without knowing the type of interaction (fighting, breeding or den sharing) that occurs during possum contacts, it's difficult to predict if TB transmission is a result of intrinsic or extrinsic factors. However, more home range overlaps or greater chances of interactions with TB infected individuals likely results in greater risk of TB transmission occurring among possums. It's likely that the infected juvenile male captured on grid C was infected by an adult possum (possibly his mother) and was moving off the grid. This possum was only captured once on the edge of the grid during the final kill-out month, therefore the lack of individual data makes it impossible to investigate his trap or infection origins.

The possum mortality and survival rates found in this chapter also give weight to my prior prediction of male "superspreaders", as male possums experimentally challenged with TB had significantly higher survival rates than did females that underwent the same procedures of infection. Here, the mean survival rate of the challenged possums (~ 4.6 months) was similar to Ramsey and Cowan (2003) naturally acquired TB survival estimate in possums of

4.7 months. This survival rate is in contrast with the findings of Corner et al. (2002a) and Ramsey et al. (2009) who challenged possums via the lungs and estimated survival rates between 2-5 and 2-3 months respectively. Although I predicted male possums were the drivers of TB in possum populations, as a male sex-bias has been found in TB prevalence in possums (Lugton 1997, Ramsey and Cowan 2003), it was surprising to find a significant difference in the rate of survival between sexes since no differences were discovered in previous challenge trials (Ramsey et al. 2009). Reasons for the absence of significant survival rates may be due to the previous study survival rates being too short to discern differences (Nugent et al. 2013a), it's also possible that unnatural severity of infection produced in previous TB challenge models could have masked any sex differences. As noted in previous chapters, to differentiate between naturally occurring TB infection and secondary case TB infections transmitted from my experimentally challenged individuals, a novel strain of TB was introduced to the population which differed from the local background strain. It is unknown whether the different strains of TB affect the survival rates of individuals and it is important to note that this study did not investigate survival rates of possums infected with the natural background strain.

During the first season the sex ratio of possums on all grids was close to parity, however the perturbation on grids C and D from the first season depopulation led to a higher than normal adult male population on grid C during the second season (see Chapter 5). No significant correlates of MCP home range size were discovered in season two and a lack of difference in node degree, edge strength and relative betweenness between the grids indicated that contact networks among possums had not yet been established (see Chapter 5). However, relative betweenness was associated with sex where males had higher values than females on grid C meaning contact networks on grid C were random and driven by male possums. This transition may in part account for the lack of TB infection in juvenile possums, and would

also explain the significance of relative betweenness in male possums found on grid C in Chapter 5.

Evidence suggests an interaction of males with the environment, working in two distinct mechanisms that may act in synergy to create pulses of TB transmission which may allow disease persistence in a metapopulation context. According to the findings here of challenged possum survival rates, males may live longer once they had become infected with TB. This study does concede during the time of experimental infection, males were in better body condition than females possibly allowing for them to survive longer with TB (Rouco unpublished data), Ramsey et al. (2009) also found that seasonality may affect survival times following TB infection, therefore these two arguments may be linked to body condition. However, healthy body condition upon initial infection may lead to prolonged infection in individuals while periods of poor condition in later months may cause future relapses of infected possums.

Where this study cannot provide sufficient evidence that contact among individuals is the primary pathways of TB transmission in possums, the male/male interactions are likely to be key factors. This study does provide evidence that possum behaviours associated with contact frequency and contact duration vary among individuals. This makes it impossible to determine if sick individuals display different social behaviours than they would if they were healthy. There remains a possibility that other drivers of transmission cause infection from individuals simply inhabiting the same space (Corner et al. 2002a), in either case a home range overlap is necessary for horizontal transmission to occur. Here, the perturbation of grids C and D after the first depopulation left the two grids in a state of social flux for season two. Season one showed that possum populations, though not socially advanced, do possess very important individuals in social networks -ie. settled populations of possums seem to

interact out of routine rather than at random. Some possums spend much more time interacting with their neighbours than others, which could result in low TB prevalence in the sense if there is little TB present, interactions among possums don't present high pathways of infection to take hold. However, in disturbed populations where immigrating possums are still trying to settle into a new area, random contacts among unfamiliar individuals would lead to a greater chance of disease transmission. This could also help to explain cases of reoccurring disease in previously TB free zones in New Zealand.

# Chapter 7

# General discussion

During this thesis I investigate the social structure of possum populations in New Zealand and examine the possible implication it could have on the transmission of TB in brushtail possums. I hypothesise that TB is transmitted horizontally from possums to possum via physical/close quarter interactions and that contrary to popular belief (see Day et al. 2000 and Ji et al. 2005), possums partake in a year round complex social network which allows for the persistence of TB within populations.

In order to achieve a thorough understanding of the characteristics of disease spread through possum populations, knowledge of their behaviour with regards to interaction and potential infectious contact needs to be investigated. As discussed previously in this thesis, the pathways and mechanisms of TB transmission in possums remain the subject of much debate. Yet whether transmission occurs via intrinsic (possum contact) or extrinsic (sequential den sharing) factors, a home range overlap between infected and susceptible possums must exist. An understanding of possum home range behaviour is recognized as critical to many aspects of management and for informing models of disease epidemiology (Ramsey and Efford 2010a). Therefore, firstly I investigated the drivers of possum home range, (Chapter 2), and secondly the drivers of home range overlap between individuals; such overlap is considered necessary for disease transmission to occur (Chapter 3).

### 7.1 Possum home range

Possum densities vary greatly spatially and throughout New Zealand (Owen and Norton 1995, Nugent et al. 2000), and the Orongorongo Valley at the bottom of the North Island (in which I carried out my studies) historically has high densities of possums (Brockie 1992, Efford et al. 2000). During my first field season (2012), when analysing for drivers of home range size I provided evidence of differences between possum subpopulations, whereby animals on one of my four cage trapping grids (grid B) had significantly smaller home ranges than possums on the other three trapping grids (A, C and D). These smaller home ranges on grid B were likely to be driven by density dependence, as this subpopulation contained more possums than those on the other three grids.

I also investigated whether or not effects such as sex and age on possum home range size could be discerned. Here, possums trapped at least five times had their MCP home ranges estimated. This method is viewed as unorthodox by some, as home range measurement is typically plotted against the number of locations until it approaches stability. However, because home range estimates increase with the number of times trapped (Crawley 1973), a population estimate of possums with a minimum of five times trapped removes the home range under-estimation that would occur from leaving the individuals with low trap numbers in the analysis. There was no significant support for independent effects of possum age or sex on home range parameters; however, adult males did have significantly larger home ranges than all other age and sex combinations. This finding is supported by many previous studies (Burt 1943, Clout and Barlow 1982, Cowan 1993, Efford et al. 2000). A significant negative correlation between MCP home range and possum density across subpopulations was also found.

The chapter investigating determinants of possum home range size (Chapter 2) provides evidence that studies evaluating only sex or age of possums to determine home range characteristics may be misleading, since my data indicates that these two factors interact with each other, providing effect modification. Therefore, effects may be masked in analyses when only one or the other factor is considered in isolation.

### *7.1.1 Home range overlaps*

This thesis provides evidence that home range overlaps shared between possums may be required for disease transmission. I mention in Chapter 4 how previous studies have found TB to be relatively fragile in the environment (see Morris et al. 1994) and as such is likely to reduce the probability that another possum would contract TB from free-living bacteria contaminating the environment. Hence, it is likely that two individuals need to come into proximity for transmission to occur, which means their areas of operation must cross or overlap. When looking at home range overlaps between possums in Chapter 3, I found that 74% of all recorded overlaps in my study subpopulations were among adult possums while only 22% were between adult and juvenile possums, and only 4% between juvenile possums. If TB transmission does indeed require proximity of two individuals, this thesis presents evidence that adults are far more likely than juvenile possums to be involved in TB transmission. When surveys of infected wild populations are conducted, TB is found in significantly more frequently in adult possums than in juveniles (Coleman 1988, Jackson 1995b, Pfeiffer et al. 1995, Montague 2000). This, coupled with the findings of home range overlap here, make it unlikely that juvenile possums contribute to the persistence of TB in possum populations. It is possible that my home range overlap findings may be biased due by greater 'trapability' of adult possums compared to juveniles. However, analysis of the observed versus expected proportions of the number of home range overlaps based on the

structures of the subpopulations studies showed that the number of adult male overlaps with other possums were ~4x and ~14x greater than expected when compared to juvenile males and females respectively, and roughly equal to adult females when their expected number of overlaps should have been half as many.

Significant relationships between subpopulations and possum age and sex with home range overlap were identified. Here, the number of home range overlaps and the area of the overlap shared between two possums were significantly smaller in the possum subpopulation on cage-trap grid B (the grid with the higher density of possums). An explanation for this may be extrinsic influences of the topography in which this subpopulation occurred. Grid B topographically lacks the gullies that may attract possums for resources and shelter (Rouco unpublished data).

The sex and age effects on possum home range overlap identified in these studies show that adults have more and larger home range overlaps with other possums than do juveniles, and that male possums have more and larger home range overlaps with other possums than do females. The greatest area of home range overlaps occurred within male dyads (interacting pairs) and the smallest areas occurred within female dyads. This is not surprising, as male possums are known to have larger home ranges than females (Ramsey et al. 2002b, Whyte et al. 2014). What was surprising was that, although Chapter 2 found that juvenile possum home range sizes were not necessarily smaller than adults, both the number of home range overlaps and the shared area of the overlaps were significantly less. This lack of shared home range with other individuals lowers the likelihood that juvenile possums interact routinely with other possums until they become more mature, and again indicates that juvenile possums play little role in TB transmission and persistence in possum populations.

## 7.2 Possum contact networks

The proximity tracking collars used in Chapter 4 of this thesis allowed me to accurately record the date, time, duration of the contact and apposing collar identification of two collared possums once they came within one meter of each other. This method of tracking allows for data collection of contacting individuals to be recorded without being witnessed. However, one limitation to this method is, when trying to investigate disease transmission among individuals, it is impossible to discern the type of contact that occurred. Hence contact times fewer than three seconds were omitted from the contact data analysis presented in Chapter 4. This decision was made in an attempt to capture individual interactions that could result in TB transmission, and reduce the likelihood of analysing contacts where two possums simply crossed paths.

During my first field season, I found that the relative network parameters constructed from the contact data collected for the four studied possum subpopulations (node degree, edge strength and relative betweenness) differed significantly between those subpopulations. The analysis indicated that the possum subpopulations on cage-trap grids A and C had higher values for all network parameters than the subpopulations on grids B and D. The data collected showed clear differences in possum sociality at both the population and individual level, and that sociality in possums varies independently of density (contact events did not correlate directly to the home range and home range overlap results from Chapters 2 and 3, or the densities of the different grid subpopulations). However, sex effects were found (in agreement with influences on home range size and home range overlap), showing that adult male possums have significantly more contacts than all other age and sex groups (dyad findings of contact level analysis Chapter 4), and their contacts are also longer in duration when they occur. Adult male possums are likely to contact each other more frequently than

other subgroups of a possum population. Although juvenile possum contact rates could not be determined, as previously mentioned their lack of home range overlaps with other individuals makes it unlikely that they play a significant role in possum social networks or disease transmission. My findings also supported those of Ji et al. (2005) and Day et al. (2000), in that most contacts occur during the breeding season. However, I also found that possum social interactions carry on long after the breeding season providing a potential mechanism for year-round TB persistence in possum populations.

### 7.3 Post-depopulation possum dynamics

In Chapter 5 I investigated the possum subpopulations occurring on two of the cage-trap grids (grids C and D), eight months after they had been depopulated in my first field season. Here I explored the population dynamics of possum home range, home range overlap and re-established contact networks, in the newly recolonised subpopulations, to investigate whether or not similar possum social structures were being rebuilt.

The reformed populations (in my second field season, eight months after depopulation) were 30% larger than the pre-depopulation populations (first field season data reported in Chapter 2). Not every individual was removed in the first season depopulations; therefore, the reformed populations consisted of possums from the first season as well as possums that had immigrated into the grids from surrounding areas.

As detailed in Chapter 5, it is possible that the depopulation may have had an effect on both the home range and contact characteristics of the new possum subpopulations in comparison to their pre-depopulation characteristics, although it is important to acknowledge that there was no undisturbed control population for comparison and the time of year was different for

the pre and post-depopulation studies (the second study did not include the breeding season). The home range sizes post-depopulation were on average three times smaller than they were pre-depopulation sizes, and there was a significant sex bias towards males following the depopulation. This sex bias was not surprising as male possums are known to be the first to disperse to new areas (Montague 2000). Unlike the pre-depopulation populations, no variables tested (age, sex, grid) were significantly associated with MCP home range estimates for the post-depopulation populations. Also, the social network values for the subpopulations, constructed from the possum contact collar data collected (node degree, edge strength and betweenness), were all lower post-depopulation. Fewer determinants of these network parameters were identified in the post-depopulation study – there was only a significant grid and sex interaction with regards to relative betweenness, whereby adult males in one of two subpopulations (on grid C) had higher values than for those on grid D. Although this may be due to lower statistical power in the smaller post-depopulation study, this could indicate that the gender equilibrium had not been reached on grid C, and that the possums where further from re-establishing an equilibrium social order on this grid than on grid D.

The significant difference in betweenness found post-depopulation indicates that unlike the established populations (as found in the first season) possums did not appear to follow a 'routine' or have a regular pattern to their night-time behaviours. This means individuals probably wake and start to move around at the same time and probably navigate their way around the forests in familiar paths, leading them to encounter the same individuals. I provided evidence of this in my fieldwork, with possums continually being caught in the same traps. Although this observation is likely to be a response to the trap baiting, the fact that particular possums are contacting each other more frequently shows possums may indeed follow a routine. The possums in the post-depopulation did not appear to be making contacts with particular possums. Instead, they seemed to be making short duration contacts with

many different individuals. I acknowledge this difference in behaviour may be due to the difference in the season the contact-logging collars were active for (pre-depopulation collars were active during the breeding season, while post-depopulation ones were not). However, although the amount of possum contacts and contact durations during the first season dropped significantly after breeding season, possum behaviour associated with contacts was similar. It is therefore most likely that the difference in contacts found between the two seasons is driven by the disturbance caused by the depopulation effect.

### 7.4 TB transmission

Numerous studies attribute contact among individuals to infectious disease transmission (Koopman and Longini Jr 1994, May et al. 2001, Hamede et al. 2009). I attempted to identify the drivers of TB transmission among possums by conducting experimental challenges, infecting wild free-living possums with a novel strain of TB (REA type 115) distinguishable from the natural background strain found in the Orongorongo Valley. By using this novel strain of TB I was able to track the secondary case infections resulting from the challenge possums. The pre-depopulation first season trial yielded a lower than expected secondary infection rate as the grids that had secondary case infections had over 100 individuals sharing home range overlaps with infected possums. The post-depopulation second season trial, conducted on grids C and D had a higher number of secondary cases. As mentioned previously, the post-depopulation individuals seemed to contact with a variety of unique individuals, rather than numerous contacts with the same individual. This behaviour may be due to a large number of possums trying to re-establish an area, and these contacts with numerous, unfamiliar possums may be one reason for the difference in number of secondary cases observed between the pre and post-depopulation populations. Alternatively the

increased number of secondary cases in the second season may be due to the larger number of experimentally challenged index cases, or may just represent stochastic variation.

Although a relatively low number of secondary cases were identified from seasons one and two, resulting in low statistical power, I identified significant variation between population subgroups with regards to both natural background TB infections and secondary cases resulting from experimental challenged index cases. Infections were found mostly in adults and mostly in male possums. The study provided evidence of two distinct mechanisms involving male possums (survival rate once infected with TB and greater contact behaviour) that may act in synergy to enhance TB transmission and allow disease persistence in a metapopulation. Here, survival rates of the group of 16 experimentally challenged possums during the second season indicated that male possums were capable of surviving with TB longer than female possums.

My thesis provided evidence to support a hypothesis that adult males are the main drivers of TB transmission and persistence in brushtail possum populations. Their larger home range size, greater numbers of home range overlaps and larger shared area of those overlaps suggest that adult males have a greater opportunity for events that could result in TB transmission. The contact data presented in this thesis supports these findings by showing that males both have larger home range overlaps make contact/come into proximity with more other individual possums than females or juveniles of either sex. This means according to my contact network findings and secondary case infection and survival rates, male possums could act as both superspreaders (due to their enhanced contact behaviour) and supershedders (due to their longer survival once infected). With these two mechanisms acting in synergy, it may enable adult males to drive TB transmission and persistence in possum populations.

## 7.5 Implications and management

The largest barrier preventing the eradication of TB in New Zealand, and its consequential spillover to livestock, is the brushtail possum (Hutchings et al. 2011). The post-depopulation results presented here suggest that population extermination procedures may not be the best method for control of either possum populations or TB, as all it accomplished was an influx of more possums with higher disease prevalence. I acknowledge that the depopulation event that took place during this study was by no means an extermination, however studies have shown possum immigration over time after a mass kill-out ( see Efford et al. 2000). This shows that even if possums are eradicated from an area, more possums will re-populate the area without constant population control. This may help answer the question of how both possums and TB re-emerge in areas that have been previously "exterminated".

Both possums and TB do need to be controlled in New Zealand; however I propose control methods need to be better informed by an understanding of possum behaviour and infection dynamics. The results of this thesis provide evidence that the best way to control possums and TB may be to target male possums either by selective depopulation or recently hypothesised methods of fertility control (Tompkins and Ramsey 2007, Ji 2009, Ramsey and Efford 2010a) . Without a costly ten-year mass extermination, where possum populations would have to be reduced by 75% and maintained below 40% to eliminate TB (Barlow 1991b), it may be better to explore options aimed at targeting males. This could reduce TB in the populations by reducing the individuals most likely to be driving disease transmission and persistence, and would also reduce the number of possums nationwide. Furthermore, these findings may be valid beyond the control of TB and brushtail possums. The details presented here pertaining to superspreaders may apply to numerous studies and study species including foxes and old world badgers in the United Kingdom, by showing that not all individuals are

equal, and that subgroups may be driving disease within populations. If these subgroups can be identified for other disease systems, they can likewise be targeted for more effective and efficient management.

Further studies of the role of males as drivers of disease are needed, particularly studies of contact networks. As acknowledged earlier, the secondary case infection chapter (Chapter 6) of this thesis lacks statistical power. A recently completed third season will add more secondary cases to the study, but this may not provide enough data to make firm conclusions on the drivers of TB transmission.

I recommend a continuation of a similar study to this thesis to further our knowledge of the "supershedders and superspreaders" of TB in possum populations, along with a separate study of possum control based on a catch and release model where only male possums are euthanized throughout the duration of the study. Because wildlife culling is almost never an effective means to eradicate a wildlife-related disease (Donnelly et al. 2006), this method would reduce population numbers by removing individuals while maintaining an established population. In accordance with this study, this method of control may eliminate TB by removing possible superspreaders and avoiding a social collapse that would invoke an influx of new possums.

# Chapter 8

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# Chapter 9

# Appendix

*Animal Welfare*

*Running title: Air rifle vs. blunt force trauma*

## *Improving animal welfare standards while reducing researcher disease exposure risk: euthanasia of trapped possums*

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## *Abstract*

*In New Zealand there is a long history of research studies working with wild free-living possums where euthanasia of large numbers of animals is frequently required. The most commonly used method for euthanizing possums trapped alive is by blunt-force-trauma. If possums being euthanized are potentially infected with bovine tuberculosis (TB), however, the blunt-force-trauma approach is associated with risks of researcher exposure to TB infection (e.g. risk of being scratched, bitten or blood splattered). Here we trial the use of a point-blank shot to the head with an high velocity air rifle as an alternative approach for the euthanasia of wild free-living possums. Our trial revealed that the air rifle approach reduced the potential disease exposure risks to researchers by minimizing blood-spatter area, and by completely avoiding the likelihood of both accidents due to animal handling and animal escapes. While both methods induced immediate unconsciousness leading to death, the air rifle approach was quicker (i.e. shorter conscious handling and total time) and arguably had better animal welfare performance by significantly reducing the time to unconsciousness. Thus, we conclude that the air rifle approach to possum euthanasia is a suitably humane approach that should be considered when dealing with potentially TB infected possums.*

## *Keywords*

*Air rifle, blunt force trauma, euthanasia, shooting, Trichosurus vulpecula, tuberculosis, wildlife capture*

### *Introduction*

In New Zealand the brushtail possum *(Trichosurus vulpecula)* is a major pest impacting on a range of conservation values and acting as the main wildlife reservoir of bovine tuberculosis (TB; Cooke *et al* 1995; Nugent *et al* 2015). There is thus a long history of research studies working with wild free-living possums (e.g. Cowan 2001; Tompkins *et al* 2009; Gormley *et al* 2012; Nugent *et al* 2015) where euthanasia of large numbers of animals is frequently required to, for example, assess diet (e.g. Glen *et al* 2012; Sweetapple *et al* 2013), enumerate parasites (Cowan *et al* 2002, 2006), or detect subclinical cases of TB (e.g. Whitford *et al* 2014: Nugent *et al* 2015).

Animal welfare standards for trapping and/or killing animals should be a priority, and should minimise the pain and disruption for the species being handled (Iossa *et al* 2007). Regardless of the status of the species (e.g. as a recognised pest species such as the possum in New Zealand), they must be killed using the most humane method possible (Littin *et al* 2004). In New Zealand, the most commonly used method for euthanizing possums trapped alive is by blunt-force-trauma (NPCA 2009). This consists of removing the animal from the trap by its tail, placing its head over a solid object (e.g. a rock or a hard tree root), and delivering a hard 'stunning' blow to the back of the head (usually using a hammer), followed by additional blows until the skull is crushed. The use of the blunt-force-trauma method for any animal is less aesthetically acceptable than other alternatives (e.g. Inhaled anesthetics like carbon monoxide or halothane, and injectable anesthetics like pentobarbital combinations, AVMA 2013), however, when properly performed, it can be appropriate for some animals (e.g. lambs, Finnie *et al* 2000; kangaroo joeys, McLeod & Sharp 2014). Through decades of experience and refinement in New Zealand, it is considered an effective and humane

approach for euthanizing possums (NPCA 2009). If possums being euthanized are potentially infected with TB, however, the blunt-force-trauma approach is associated with risks of researcher exposure to TB infection. For example, the necessity of removing individuals from cages prior to euthanasia puts the handler at risk of being scratched and bitten (e.g. Goldstein & Abrahamian 2015). In addition, a blow to the head can cause blood splash which could be a potential source of TB contamination if it comes into contact with the skin (Twomey *et al* 2010).

Here we trial the use of a close-range shot to the head with a high velocity air rifle as an alternative approach for the euthanasia of wild free-living possums. We hypothesise that by both removing the need to handle individuals, and reducing the extent of blood-spatter produced compared to blunt-force-trauma, this approach will reduce potential disease exposure risks to researchers while maintaining humane animal ethics standards. Past assessments of air rifles (muzzle velocities less than 400 feet/sec) for this purpose concluded that they were not suitable for euthanizing possums, as they are less powerful than conventional fire-arms  and thus cause insufficient penetration of the skull and damage to the brain to reliably kill possums with a single shot (NPCA 2009). However, modern air rifles have muzzle velocities up to 1200 feet/sec, based on reports for other small animals (Whiting *et al* 2011); we considered that a close range head shot at these higher velocities is sufficiently powerful to reliably kill a medium-sized mammal such as the possum.

## *Material and methods*

### *Study site and possum trapping*

We took advantage of an ongoing research project investigating TB transmission among wild free-living possums (Whitford *et al* 2014), for which purpose the individuals studied here already required euthanasia. The study population inhabits a 1200 ha research area in the Orongorongo Valley (lower North Island, New Zealand [lat.-41˚21'S; long.-174˚58'E]. The study site is characterised by mixed native broadleaf-conifer forest interspersed with small areas of scrub, which has supported possum densities of about nine per hectare for the past 40-50 years (Efford 2000).

Possums were caught in Grieve wire cage traps (60 × 26 × 28 cm), with a spring-assisted folding door triggered by a pendulum hook (Montague & Warburton 2000), in four nights consecutive trapping in June 2014. Traps were set on the ground, baited each morning with apple sprinkled with powdered sugar, flour with anise oil applied as a lure, and checked every day.

### *Experimental procedure*

To compare the extent of blood-spatter produced by possum euthanasia by the high velocity air rifle with the established blunt-force-trauma approach, while assessing to ensure that welfare standards of performance are not compromised by this potentially new approach, several measures were compared between two groups of 30 adult possums (15 male and 15 female), one group for each treatment. For the air rifle method, possums received a single shot from a Gamo® Big Cat 1000 (4.5mm calibre, minimum velocity 1000 feet/second; Barcelona, Spain) through the bars of the trap at no greater than 5 cm distance from the

possum, aimed at the centre of the animals' forehead to ensure severe brain damage (Fig 1, but see Longair *et al.* 1991). For ammunition we used a super-heavy pointed pellet for high impact and maximum expansion (4.5mm calibre with 0.75 grams weight each; Stoeger Airguns® X-Magnum, Accokeek, U.S.A.). Air rifle used was single shot model and pellets were only chambered directly before dispatching possums in order to reduce hazards to the operator. Any rocks located underneath the cage should have been removed before setting up the trap for both; to reduce the risk of ricochet and in order to improve animal welfare by avoid moving the trap with a trapped possum inside. Although we predicted that animals would be immediately unconsciousness as a result of this method, to minimise any unnecessary suffering we planned to (1) give them a second shot within 10 seconds, and (2) euthanize them by blunt-force-trauma, is they failed to lose consciousness. The blunt-force-trauma approach followed the protocol outlined in the Introduction (NPCA 2009). For both approaches, the largest diameter of any blood-spatter area produced was recorded using a tape measure (to the nearest 5 cm for any spatter over 10 cm), in addition to any scratches received by handling staff, and any situation where there was a likelihood of the handled possum escaping. All animal manipulation for this research was undertaken with approval of the Landcare Research Animal Ethics Committee (approval no. 12/02/01).

We adapted welfare standards of performance from Iossa *et al* (2007). For each animal we recorded conscious handling time (CHT; i.e. from a one meter approach to the cage to unconsciousness), time to unconsciousness (TU; i.e. from shot/blunt force to loss of blink response), time to death (TD; i.e. from shot/blunt force to breath cessation), total time (TT; i.e. from a one meter approach to the cage to death), and the number of hit/shots used to kill each animal. During subsequent necropsy for TB, animals were also examined to assess air rifle pellet penetration and determine pellet location (i.e. still present in the cranium, or

having gone clean through). Comparisons of all measures between the two methods were made by two-tailed t-test using the package ‘stats’ in program R version 3.1.1 (R Core Team 2013). All time measures were log-transformed to satisfy assumptions of normality and homogeneity of variances.

### *Results*

For the thirty possums euthanized by high speed air rifle, did not cause any blood splatter, instead blood flowed in a small stream from the hole created by the pellet ending in a small puddle on the ground. In contrast, blood-spatter areas ranging in diameter from 5–100 cm (average diameter 17.2 cm) were produced from the thirty possums euthanized by blunt-force-trauma. Although the mean linear distance travelled by blood contamination from the air rifle approach was slightly larger than the mean diameter of blood-spatter area from the blunt-force-trauma approach, the area coverage (and variance of spread) was much greater for the latter. Two possums escaped, and one minor handler injury (a small scratch) occurred, both during euthanasia via blunt-force-trauma. In contrast, no such issues occurred with the air rifle approach.

For the welfare standards of performance, both conscious handling time (CHT) and time to unconsciousness (TU) were significantly shorter with the air rifle than with the blunt-force-trauma approach (*t-test* = 6.11 and $P < 0.00001$, and *t-test* = 3.11 and $P = 0.0041$, respectively; Fig. 2a,b). No differences were found in the time to death (TD) produced by the two methods (*t-test* = 1.1, $P = 0.27$, Fig. 2c). Seven possums out of 30 were shot twice to ensure unconsciousness; however, possums required significantly less shots than blunt-force-trauma possums required hits (means ± 95%CI of 1.23±0.16 and 3.1±0.54 respectively; *t-test*

= 6.7, $P < 0.00001$). No back-up blunt-force-trauma was needed for any possum euthanized by air rifle. Finally, the total time (TT) it took to euthanize a possum was significantly shorter with the air rifle approach than with blunt-force-trauma (*t-test* = 4.7, $P < 0.0001$, Fig. 2d). Necropsy examination showed that air rifle pellets caused obvious physical destruction of the majority of the brain to the brain in all cases; 90% of pellets were found inside the skull cavity with exits wounds noted for the other 10%.

### *Discussion*

This is the first published study to assess the effectiveness of shot by high velocity air rifle versus blunt-force-trauma for the humane killing of brushtail possums in the field. Our trial revealed that the high speed air rifle approach to euthanize possums reduced the potential disease exposure risks to researchers by minimizing blood-spatter area, and by completely avoiding the likelihood of accidents due to both animal handling and animal escapes. This is important when dealing with a disease that can be transmitted to an operator through a handling accident (Cooke *et al* 2002; Twomey *et al* 2010). While both methods induced immediate unconsciousness leading to death, the air rifle approach was quicker (i.e. shorter conscious handling (CHT) and total time (TT)) and had better welfare performance by significantly reducing the time to unconsciousness. In addition, restraint and removal in cage traps causes stress to possums (Warburton *et al* 1999); the air rifle approach would therefore likely decrease possum stress levels prior to euthanasia since there is no need to handle the animal to remove it from the cage.

Both methods are inexpensive to use (after the capital investment is done, i.e. NZ$ 20 *versus* NZ$ 320 for a hammer and an air rifle with 500 pellets, respectively). However, manually

applied blunt-force-trauma has some advantages compared to the use of air rifles for possum euthanasia; it is effective when performed correctly, easier to carry in the field than rifles and leaves no environmental residue. However, it does depend on the strength of the operator for consistency of application (Erasmus *et al* 2010), and may be difficult to apply humanely to large numbers of animals (AVMA 2013). In contrast, while air rifles are light and easy to carry in the field, and do not need licencing, their use for euthanasia would require appropriate and well-maintained equipment and well-trained personnel. The main concern in the use of this method would be the safety risks for personnel associated with the chance of ricochet. In the current trial, our results showed that air rifle pellets went through the skull of the animal on only three occasions (and the risk of injury via ricochet would be further reduced by the impact with the skull greatly reducing the speed of the pellet). We conclude that the air rifle method to possum euthanasia is a suitably humane approach that clearly minimize pain, distress and overall improve animal welfare to possums while reducing significantly disease exposure risk to the operator. Therefore it should be considered when dealing with potentially TB infected possums, and its potentially used with other similar-sized mammals elsewhere.

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## Figures captions

**Figure 1.** Illustration showing brain location in the brush-tailed possum, and the air rifle target for euthanasia used here. The air rifle pellet should enter the skull at the centre of the possums' head, slightly below a line drawn midway between the ears, to be centred on the brain.

**Figure 2.** Mean (± 95% confidence intervals, CI) of conscious handling time (CHT), time to unconsciousness (TU), time to death (TD) and total time (TT) in seconds, of possums euthanized using air rifle shot (N=30) and blunt force trauma (N=30).

Figure 1

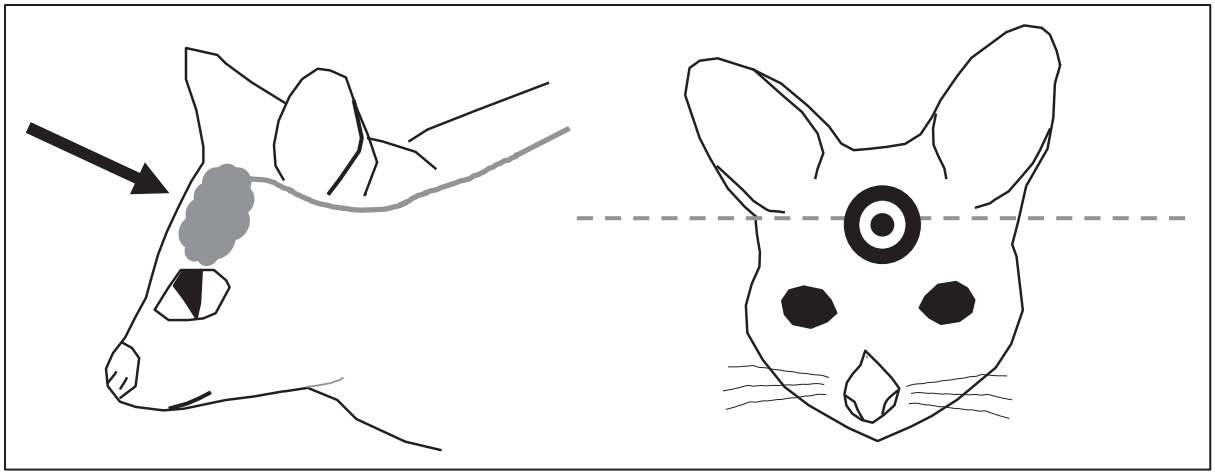
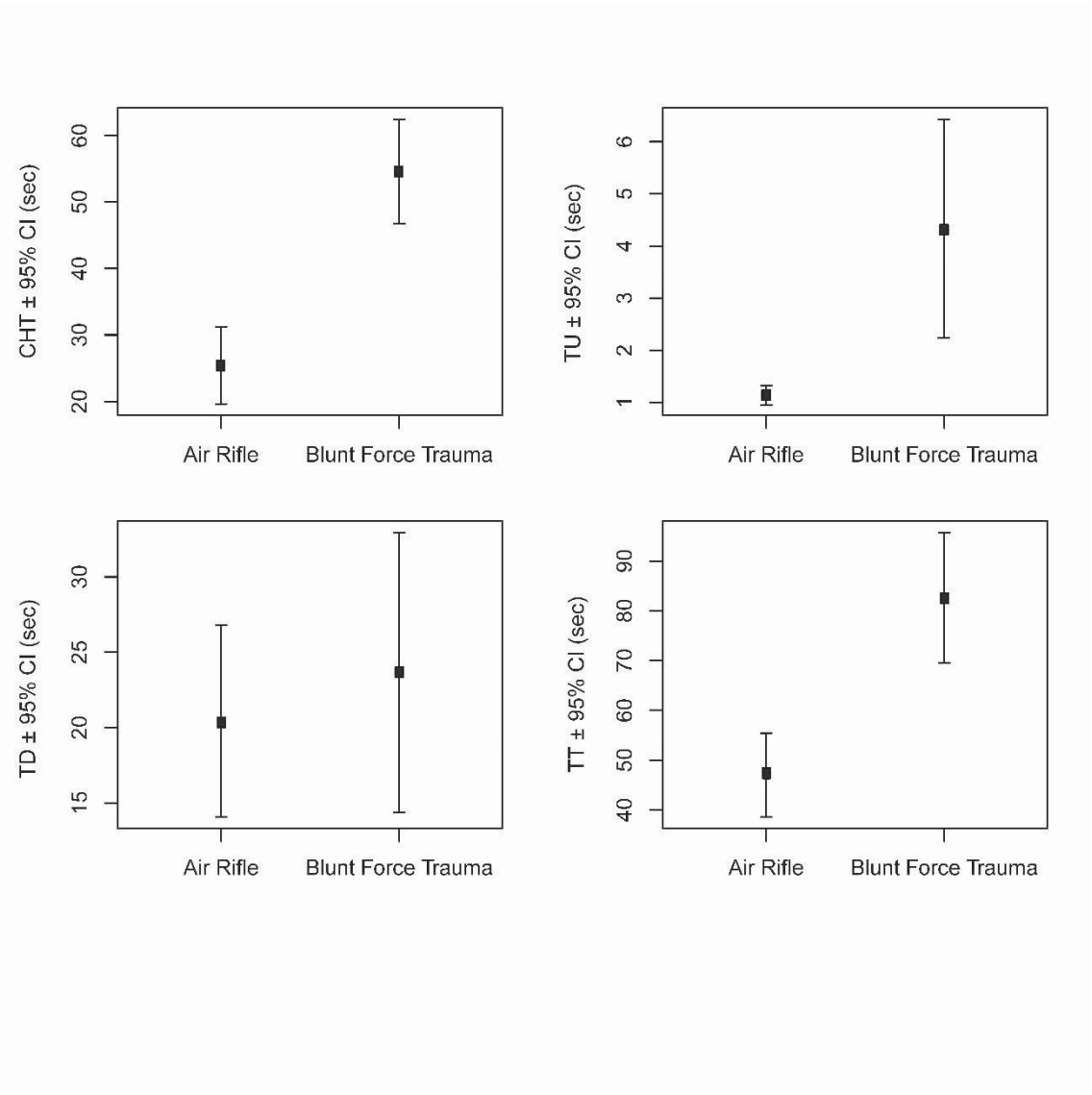


Figure 2


CHT ± 95% CI (sec)
20
30
40
50
60
Air Rifle
Blunt Force Trauma
TU ± 95% CI (sec)
1
2
3
4
5
6
Air Rifle
Blunt Force Trauma
TD ± 95% CI (sec)
15
20
25
30
Air Rifle
Blunt Force Trauma
TT ± 95% CI (sec)
40
50
60
70
80
90
Air Rifle
Blunt Force Trauma