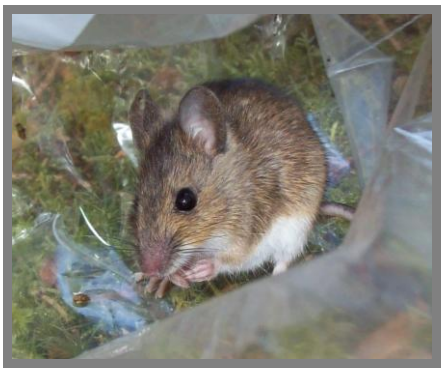
Population dynamics of rodents and their parasite communities in a naturally fragmented landscape

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Abstract

An island system with corresponding mainland sites, was used to study woodland rodent dynamics and their parasite communities within a naturally fragmented landscape. The study site, hosts and parasite species investigated within this thesis allowed the investigation of how natural fragmentation affects demographic and population dynamics of rodents (chapter 3). Reduced habitat connectivity is known to affect nearly every process in biology. Low degrees of fragmentation and high connectivity between habitats have been shown to provide the most stable conditions for populations to persist, as movement of organisms is less restricted. It is shown that in contrast to previous studies on fragmented populations, the fragmented landscape of the islands had little effect on the demographic characteristics of rodent populations in comparison to those on the mainland. There were few difference found in the demographics of wood mice and bank voles when compared to mainland sites. The results from this study then allowed the broader question of how parasites dynamics are affected by the spatial structure of a host population to be addressed.

Theory predicts that parasites are unable to persist in small, isolated host populations, due to small host population size as well as potential genetic factors increasing the risk of extinction. However parasites may become more prevalent in isolated populations as hosts may have a reduced ability to deal with infection. It is shown (chapter 4) that within this study system that despite some island populations being extremely small, there is no overall reduction in parasite species found within fragmented habitats. Furthermore, extinction of the parasites investigated within wood mice and bank voles is unlikely due to the direct life cycle of these parasites. Variation was seen in the prevalence of infection, however the majority of the parasite species on islands did not show a reduced prevalence of infection compared to mainland sites.

Finally parasite co-infection and co-aggregation and their dependency on host characteristics in woodland rodents (chapter 5) were investigated. Parasite species infecting hosts are normally studied individually, however this is not what is seen within natural populations. Co-infection is an important concept within natural systems as there is a vast diversity of parasite species that create

ample opportunity for concurrent infections. Therefore, it is proposed that studies should be focused on parasite interactions, as within host interactions can in turn affect the abundance and distribution at the level of the host population. This study focused on seven parasite taxa, and it was found that the maximum number of parasite species any individual was found to be infected with was five, with the mean number for both host species at around two. Parasites associations were also more common than expected within the same functional groups with co-occurrence being more common between parasite species associated with ectoparasites. Within this study, host aggregation was positively correlated with differing parasite taxa. Furthermore, looking at patterns of co-aggregation could aid in our understanding of parasite interactions within hosts. The nature of these interactions will determine whether aggregation is positively or negatively correlated across different parasite taxa. A small number of hosts maybe responsible for transmitting the majority of infections (20/80 rule). Identifying these individuals would be informative in helping to control disease spread. Host characteristics have been found to be informative in terms of single parasite species infections. Within this study it was found that juvenile bank voles were more likely to be co-infected than those within other age classes. No host characteristic explained patterns of co-infection in wood mice.

In conclusion I found that natural fragmentation does not have an overall negative effect on rodent host dynamics nor does it reduce the number or prevalence of infection of parasite species able to infect hosts. This thesis has highlighted the importance for using natural wildlife systems in empirical studies, and the need to further address multiple parasite interactions within a host community.

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Author's declaration

I declare that the work recorded in this thesis is entirely my own, expect where otherwise stated. Louise Mair, Alan Law, Mary Ryan and Matt Sullivan helped collect field data and carry out laboratory analysis. Plasma samples were tested by Dr Trevor Jones (The National Centre for Zoonosis Research University of Liverpool) by an immunofluorescence antibody assay. Elizabeth Kilbride (University of Glasgow) tested the red blood cells for *Bartonella* infection. Katya Mamonova (University of Glasgow) carried out a range of laboratory analysis. No part of this thesis has been submitted as part of any other degree.

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1 General Introduction

1.1 Disease ecology

Disease ecology investigates how spatio-temporal patterns of disease are influenced by the interactions between pathogens and their hosts. These interactions may depend on environmental changes, as well as changes within host, vector and pathogen populations. Research into disease ecology began by looking at the life cycle of parasites; later researchers realised that parasitology had important links to many other disciplines in biology (Hudson, Dobson et al. 2002). The combination of parasitology and quantitative population biology has shown that host-parasite interactions are complex and involve dynamic processes, including the flow of parasites from one host to another, and this in turn is determined by host abundance and contact rates (Anderson and May 1978). Building on this, disease ecologists then focused on applying this knowledge to not only investigate human infections but also natural diseases in wildlife systems.

Infections within wildlife populations can be epidemic, where outbreaks are sudden with rapid spread, or endemic, where the prevalence of infection does not fluctuate widely temporally and is found in a defined spatial area (Hudson, Rizzoli et al. 2001). The basic reproductive number (R_0) is the expected average number of secondary cases caused by a typical infectious individual in a susceptible population (Heesterbeek 2002). The value of R_0 is dependent on the factors that affect transmission. This includes contact rate between hosts, mixing patterns, infectiousness and susceptibility, and the duration of the infectious period (Heesterbeek 2002). Therefore R_0 can vary between different infectious diseases but also within the same disease between differing populations. If $R_0 > 1$ then each infectious individual will produce more than one new infection; this can lead to an epidemic outbreak. If $R_0 < 1$ then an infectious individual will not be able to produce enough new infections and the disease will fadeout. If $R_0 = 1$ then there is no spread amongst the population, with stochastic extinction happening in a short space of time. Since R_0 determines whether a parasite can spread within a host population, it is a highly important concept in disease ecology.

Heterogeneities of infections are generated by variation between individuals and their environment, the condition of the host, and the amount of exposure hosts have to a particular parasite. Heterogeneity in transmission is important at multiple levels within disease ecology: (1) the level of different host and parasites species, (2) the level of populations and how they are spatially distributed, and (3) at the individual host level. The consideration of these three levels forms an overarching theme for this thesis. Two of them, spatial population structure and individual variation, will be discussed in the following in more detail.

1.2 Spatial epidemiology

The term spatial epidemiology describes research that investigates the causes and consequences of spatial variation in disease risk or incidence (Ostfeld *et al*. 2005). Important questions when considering the distribution of diseases are: which landscape-level variables affect local persistence of diseases, how the disease is affected by the structure of the community, and how disease risk is spatially distributed. A fundamentally important area of spatial epidemiology is how factors affect the spatial positions of parasites, hosts or vectors, and in addition their chances of close encounter.

Naturally, there is spatial structure and patchiness in the distribution of hosts, and it has been proposed that metapopulation theory is applicable to host-parasite dynamics. A metapopulation can be described as a group of spatially separated populations or patches that are connected by dispersal and thus are able to interact (Hanski and Gilpin 1997). Persistence of such a system relies on a balance between population extinction and colonisation events. The persistence of a patchily distributed species is affected by the existence of an equilibrium between extinction and colonisation and this idea is the common thread between epidemiological and metapopulation theory (Grenfell and Harwood 1997), as persistence in large communities and the fadeout in small communities is similar to that of a mainland-island metapopulation. Here, each host can represent a patch containing a population of parasites, where infection

is equivalent to a colonisation event and either the death or recovery of an individual is equivalent to local extinction (Lawton, Nee et al. 1994). Local extinction could occur when a host dies and the parasites are unable to migrate to another nearby susceptible host. The availability of such hosts will be influenced by the local population density as well as the behaviour of hosts (such as propensity to form aggregations). It will also be strongly influenced by chance in small and sparsely spread populations. In small host populations there is a risk of simultaneous local extinctions leading to complete global extinction. Therefore spatial structure of populations may affect whether parasites are prone to extinction or not (Hudson, Rizzoli et al. 2001).

An area of interest within spatial epidemiology is the concept of host population size threshold or critical community size, below which an infectious organism cannot persist (Black 1966). Seminal work on critical community size was carried out by Bartlett (1957) on the analyses of measles. This was an important concept as it allowed tractable models to be created that showed the probability of measles persisting in different host populations. The majority of the research into host population size thresholds done to date has tended towards measles and other notifiable human diseases. Very few empirical examples of critical community size exist for wildlife systems (Lloyd-Smith *et al*. 2005). The lack of empirical examples for the host population size threshold is due in part to the difficultly of obtaining long-term spatio-temporal data in wildlife populations. Therefore there is a need to carry out field studies in natural systems in order to better understand how relevant and applicable these concepts are to parasite dynamics in non-human parasites.

Investigations into critical community size to date tended to be based on single parasites, as apposed to entire parasite communities. Begon *et al*. (2003) provides one of the best empirical examples of population thresholds in wildlife to date. Their study investigated cowpox dynamics in wood mice and bank voles on 14 woodland islands that varied in size from 0.02×10^4 m^2 to 1.14×10^4 m^2. Islands were located within a lake and were monitored over a 2-year period, along with an adjacent mainland population (8 hectares in size). The dynamics of bank voles on the islands and mainland sites where grouped into large islands, small islands and the mainland site. The pattern of their dynamics where found

to be indistinguishable when comparing across these habitat types, thus suggesting that there was no intrinsic variation in host dynamic patterns of bank voles or wood mice with island size. Documented movement of individuals among islands or between islands and mainland was rare but most common in male wood mice. The authors therefore suggested that the dynamics seen on most of the islands were mostly determined by the demographic processes within populations. As movement did occur within and between the islands, and the amount of movement was high enough so that no islands remained isolated for long periods of time, the network of islands appeared to function as a metapopulation. The study found no evidence for the existence of a critical community size for cowpox in terms of host density, but instead found that the total number of hosts influenced cowpox persistence and this was not shown to be density dependent. There was also a suggestion of invasion and persistence thresholds on the islands but their distinction was not clear-cut. There seemed to be a progression from no presence of cowpox to ecological invasion (hosts are occasionally infected but with no apparent transmission), to epidemiological invasion (succession of infected hosts) to persistence on the mainland. The authors also highlighted the importance for a separation between invasion and persistence thresholds that cannot occur when using deterministic models. Therefore using stochastic rather than deterministic models will show a distinction between invasion and persistence thresholds and therefore show any potential fade out of the disease. This study provided an excellent example of disease dynamics within a fragmented host community, which allowed for a study on the limits to parasites persistence and CCS. However this study only applies to a single parasite species and not entire parasite communities that are typically found within natural populations. The spatial scale was also rather small allowing for disease dynamics to be closely coupled across the entire metapopulation. So far, the concept has also only been applied to acute, immunising infections whereas its wider applicability to other diseases remains largely untested. Thus, more research into CCS is needed, especially considering the relative lack of empirical examples in wildlife populations (Lloyd-Smith *et al*. 2005).

Habitat fragmentation is a process where a large continuous area of habitat is reduced and divided into smaller patches. The remaining patches not

only differ in size from the original area by being smaller, they also have a greater proportion of edge in relation to the total area, and that the edges are closer to another edge than the previous habitat. Furthermore the patches are more isolated for other patches than the original continuous habitat (Pullin 2002). The most stable environments are said to be those that are highly connected and show a low degree of fragmentation (Christensen, Ecke et al. 2008; Mortelliti, Amori et al. 2009), as it is well documented that fragmentation has negative conservation implications, and tends to result in species extinctions (Ekross, Heliola et al. 2010; Krauss, Bommarco et al. 2010) as small patches tend to be more vulnerable to due the edge effect. However, fragmentation does not always have to be thought of in terms of a negative impact on a population. Species are known to adapt and respond to changes in their environment as species responses are highly variable. Changes within the environment have the potential to change the demography of species, which in turn can likely affect ecological interactions with other species. This includes not only changes between species such as within the food web, but also host-parasite interactions. This area of research has received relatively little attention in fragmented landscape research. Fragmented habitats provide an extreme example of spatial heterogeneity and can therefore provide opportunities for empirical wildlife studies to investigate the limits of parasites persistence and critical community size.

1.3 Individual heterogeneity

Nested within spatially structured populations is heterogeneity at the individual level. Heterogeneities in parasites infections can arise from individual differences in the exposure and susceptibility to parasite infection (Hudson and Dobson 1995, Shaw and Dobson 1995). The variation seen between hosts in the likelihood of acquiring and/or transmitting infections is a feature of how infectious diseases are spread (Cattadori, Albert et al. 2007). The magnitude of the heterogeneity seen can determine the probability of the invasion and persistence of a disease within a host population (Lloyd- Smith, Schreiber et al. 2005). The aggregated distribution of parasites is well documented, in the extreme case resulting in super spreading. Super spreaders are defined as those

individuals that cause unusually high levels of transmission, therefore forming a group with a higher prevalence of infection that those in the general population (Garske and Rhodes 2008). This phenomenon may be relatively common and its impact on disease dynamics is therefore of interest (Garske and Rhodes 2008). However the majority of examples of super spreaders (Lipsitch, Cohen et al. 2003; Galvani and May 2005; James, Pitchford et al. 2007) have considered hosts that are infected with a single parasite species rather than hosts infected with multiple parasite species.

There is a potential interaction of individual heterogeneity with spatial heterogeneity of disease dynamics. The majority of studies that have used host characteristics to explain patterns of parasite infections have again been of single parasite infections. The sex of the host plays an empirical role in parasite intensities, with well-documented evidence of males exhibiting higher parasite loads than females (Perkins, Cattadori et al. 2003; Ferrari, Cattadori et al. 2004; Ferrari, Rosa et al. 2007). Other work has argued that it is not the sex of the individual that is the driver but its size or body mass. In mammals, males tend to be larger than females and it has been suggested that greater size of an individual presents a larger target for parasites. For example, adult chimpanzees had a higher prevalence of nematode infections that sub-adults, with no difference between sexes (Gillespie, Lonsdorf et al. 2010; Harrison, Scantlebury et al. 2010). The mass of an animal in some cases can be used as a proxy for the age of the individual, as seen in rodents. Thus, individuals that are older may have a higher prevalence of infection due to longer exposure to parasite infection. This therefore highlights an important mechanism when concerning a multitude of parasites infections in hosts. If a host is older (and larger) they are more likely to become infected with a range of parasite species across a variety of functional groups, and this host characteristic could explain a pattern of co-infection. Those studies that give evidence for sex playing an empirical role investigated endoparasites whilst the evidence for age used ectoparasites in rodents. However multiple factors explaining infection heterogeneity have been identified but these are likely to differ according to both host and parasite species. Further to this, being in breeding condition has been linked to increased susceptibility to infection in some species. For example, male yellow-necked mice (*Apodemus flavicollis*) in breeding condition had double the number of

Heligmosomiodes polygyrus fecund eggs *in utero* when compared to their non-breeding counterparts (Luong, Perkins et al. 2010). This showed an interaction between sex and breeding status. An interaction between sex and breeding status could potentially allow co-aggregation of parasites species within certain host individuals. The chances of infection with a range of parasite species are increased when individuals are interacting and moving around more during the breeding season. One may further expect that individuals in poor body condition are more susceptible to infection, and this can result in a vicious cycle, if parasites further negatively impact their host's health (Beldomenico, Telfer et al. 2008). Again, the effect of poor body condition may depend on host sex. For cowpox in field voles, males in poor body condition were twice as likely to become infected compared to those in good condition (Beldomenico, Telfer et al. 2009). It could be hypothesised that this effect of poor body condition could extend to multiple parasite species, making such individuals likely candidates for high parasite loads across multiple species and thus co-aggregation. At the same time, one can suspect that the vicious cycle between infection susceptibility and deteriorating body condition (Beldomenico, Telfer et al. 2009) would apply even more strongly in this case.

Finally, movement of an individual is likely to affect its probability of coming into contact with a parasite. The aforementioned higher parasite loads in males could thus also be a function of males tending to have larger home ranges and moving around more (Brown, Macdonald et al. 1994). However it is often not known whether this increase in movement is the cause or consequence of parasite infection. Increased host movement should also increase the potential for spreading and transmission and some parasites are able to modify host behaviour in that respect. Recent work has further pointed to individual variation in movement behaviour (personality) affecting infection. In Siberian chipmunks, *Tamias sibiricus,* individual differences in movement had an indirect effect on tick load, in that 'bolder' individuals were move likely to become parasitized (Boyer, Reale et al. 2010). Further to this, in terms of co-aggregation, individuals that are moving more widely in space are more likely to encounter not only more parasites but also a greater diversity of parasite species. Therefore you might expect these individuals to be more heavily parasitized and show signs of co-aggregation.

1.4 Wild rodents as model systems in population and disease ecology

Rodents are often used as model species, not only in the lab but also in ecological field studies. They often have high abundance, are relatively easy to handle, have a high reproductive rate, and the cost of trapping/sampling are relatively low. Metapopulations studies often used rodents as a model species. Rodents have been used to investigate population demographics (Merriam, Kozakiewicz et al. 1989; Telfer, Dallas et al. 2003; Zhigalsky and Belan 2004), predation (Telfer, Holt et al. 2001; Banks, Norrdahl et al. 2004) and habitat quality (Schooley and Branch 2007) in the context of metapopulations. As well as studies on the effect of metapopulation structure on disease dynamics (Grenfell and Harwood 1997; Harding, Begon et al. 2011; Jesse, Mazzucco et al. 2011).

Laboratory mice have long been used to study disease and infection dynamics, providing a useful background and experimental counterpart for epidemiological studies of wild rodents. Rodents are well documented as reservoirs of disease and especially zoonotic diseases, including plague (Gross 1995), leptospirosis (Holt, Davis et al. 2006) and hantavirus (Deter, Chaval et al. 2008). Aside from rodents being of interest as sources of human infections, they also serve as good model systems for understanding disease ecology in wildlife populations. Within Europe, mice (*Apodemus* spp.) and voles (*Myodes glareolus, Microtus* spp.) have been particularly well studied with respect to their disease ecology. These rodents carry a number of parasite species and pathogens that are transferable within and between species. Many studies have investigated rodent parasites empirically within naturally settings and for a range of parasite functional groups. This includes (1) ectoparasites such as fleas (Smith, Telfer et al. 2005) and ticks (Devevey and Brisson 2012) (2) gastrointestinal parasites (Abu-Madi, Behnke et al. 2000; Behnke, Bajer et al. 2008; Gerbert 2008) and (3) microparasites, such as cowpox (Hazel, Bennett et al. 2000; Begon, Hazel et al. 2003; Carslake, Bennett et al. 2005), *Bartonella* (Telfer, Begon et al. 2007) and murid gamma herpes virus (Telfer, Bennett et al. 2007).

The importance of studying parasites communities as a whole and investigating patterns of co-infection is becoming more widely recognised (Pedersen & Fenton 2007; Telfer, Lambin et a. 2010). Most host individuals are infected simultaneously with a variety of parasite species. Again, rodents provide an ideal model hosts to study this as they naturally are infected with a wide range of parasite species across a range of functional groups. Moreover the majority of the parasites that they harbour tend to be non-lethal in the majority of hosts. Microparasites communities (cowpox, *Babesia mictoti, Anaplasma phagocytophilium* and *Bartonella* species) have been studied in long-term data-sets of field voles, *Microtus agrestis* (Telfer, Lambin et al. 2010). Further work on macroparasite co-infections within rodent hosts has included studies of yellow-necked mice (*Apodemus flavicollis*) and white-footed mice (*Peromyscus leucopus*) and two nematodes, *Syphacia* and *Heligmosomoides polygyrus* (Grear and Hudson 2011). Despite an increasing number of studies on co-infection, the direction and strength of parasite interactions within hosts and their underlying mechanisms remain largely open questions.

1.5 Aims of thesis

The main aim of my thesis was to investigate how population dynamics of rodents and their parasite communities are affected by a naturally fragmented landscape. This was addressed for two woodland rodent species, wood mice, *Apodemus sylvaticus*, and bank voles, *Myodes glareolus*, and a variety of naturally occurring parasites, in the naturally fragmented island system of Loch Lomond. **Chapter 2** is a general methods chapter, giving an overview of the study system, species, parasites, and techniques used. **Chapter 3** deals with the question of how fragmentation affects population parameters of rodents inhabiting isolated patches. I investigated whether host populations on islands exhibit any fundamental differences in terms of their comparative demography and population dynamics compared to those on the mainland. This was investigated by (1) determining whether these population parameters were altered on islands, compared to continuous habitat on the nearby mainland, and (2) by examining whether these changes would have the potential to also alter parasite infection dynamics. **Chapter 4** investigates the consequences of habitat

fragmentation on rodent parasite distribution, prevalence and intensity of infection. It further assesses how parasite life history may affect the probability of persistence in fragmented host populations. **Chapter 5** examines patterns of co-infection and co-aggregation in host individuals and their dependency on host characteristics, by investigating (1) the patterns of co-infection among rodent parasites, in terms of species presence/absence, (2) evidence for host aggregation being positively or negatively correlated among different parasite species or functional groups, (3) what host characteristics best explain patterns of co-infection and co-aggregation within rodent hosts and finally (4) to what extent the identified relationships were consistent between the two rodent host species. Lastly, **chapter 6** gives a synthesis of the data chapters, implications of my work, and future research directions.

2 General methods: Study area, rodent trapping, and identification of parasite species

2.1 Introduction

This chapter outlines the methods used for the field and laboratory techniques, and is divided into two sections. The first section outlines the study area and the methods used to collect data in the field. The second section gives the techniques used for identifying ectoparasites, microparasites and gastro-intestinal parasites.

2.1.1 Study area

Fieldwork was conducted on the 12 southern islands of Loch Lomond as well as on three mainland sites located on the western, eastern and southern shores of Loch Lomond, respectively (Figure 2.1).

Loch Lomond is the largest freshwater area in Great Britain and Scotland's largest loch, sitting in a glacially carved valley (Mitchell 2001). Loch Lomondside has retained much of its semi-natural broad leave woodland, with a nearly complete surrounding of continuous tree cover (Mitchell 2001). An abundance of oak at Loch Lomond is due to the demand for tannins in the leather industry from the early eighteenth century. After the industrial revolution the demand decreased, and now the area is managed in order to preserve the woodlands (Dickinson 1994). The density of understorey on the islands and mainland sites varies. Dense areas of bracken; *Pteridium aquilnum*, blaeberry; *Vaccinium myrtillus* and bramble; *Rubus fruticosus*, are found across the mainland site LU, and four of the island sites (CA, CO, TA and TO). The other mainland sites at M1 and KN have a sparser understorey that mainly consists of a variety of grass species, blaeberry and a few brambles. The remaining islands (BU, CR, and MO) have a mixed understorey of blaeberry, bramble, rhododendrons, moss and grass species (Table 2.1).

The islands of Loch Lomond range in size from 3 hectares (ha) (BU), to 63 ha (CO) (Table 2.1). Distance among islands varies between 50metres and

2320m, whereas distance from the mainland ranges from 186m (island: TA) to 2720m (island: CO) (Figure 2.1). All islands are easily accessible by boat. Some of the islands are inhabited with permanent residents (FA, MU and TA) whilst others have holiday houses (CR and LO). Visitors to the area are numerous due to the close proximity to the city of Glasgow, and especially since Loch Lomond is within the Loch Lomond and Trossachs National Park, established in 2002. The area is managed by the Loch Lomond and Trossachs National Park Authority (LLTNPA), which aims to conserve the natural beauty as well as the natural and cultural heritage, enhance visitor experience and promote rural development (National Park Plan, 2007).

Three mainland sites, situated on the western, eastern and southern Loch shore, were chosen based on the following criteria: 1) proximity to the Loch (within 0.5 km); 2) closed, mature forest cover with tree and undergrowth composition similar to that found on islands. Trapping grids were established in representative sections of forest, avoiding particularly open or wet areas that would have been less suitable for woodland rodents.

Table 2-1: Loch Lomond study site characteristics for those sites that were trapped

Mainland/Island	Site Name	Site Code	Grid Reference	Area (hectares)	Vegetation
Mainland	Sallochy	M1	NS 393954	N/A	Oak/managed pine with understorey of bramble, blaeberry and grasses
Mainland	Knockour Wood	KN	NS 390858	N/A	Oak with birch and holly. Understorey relatively open with blaeberry, bramble and grasses.
Mainland	Luss	LU	NS 354908	N/A	Oak with understorey of dense areas of bramble and bracken.
Island	Bucinch	BU	NS 388918	3.1	Scots pine, birch and oak. Understorey of rhododendrons, bracken and grass species.
Island	Inchcailloch	CA	NS 407901	53.0	Oak, birch, holly and alder. Understorey dense with bracken and grass species.
Island	Inchconnachan	CO	NS 373913	41.9	Oak, birch and Scots pine. Understorey consists of blaeberry, bracken and moss species.
Island	Inchcruin	CR	NS 356911	28.3	Birch, oak and alder. Understorey relatively open with blaeberry, bracken and moss species.
Island	Inchmoan	MO	NS 377907	45.6	Birch and alder. Understorey of rhododendrons, blaeberry, gorse and bog myrtle.
Island	Inchtavannach	TA	NS 368915	63.0	Oak and birch. Understorey of blaeberry, bracken and grass species.
Island	Torrinch	TO	NS 403894	7.5	Oak and birch. Understorey dense with blaeberry and bracken.

Table 2-2: Loch Lomond study site characteristics for those sites that were monitored for the presence of rodents. None of these sites yielded signs of rodent activity over the 2-year study period.

Island	Site Name	Site Code	Grid Reference	Area (hectares)	Vegetation
Island	Clarinish	CL	NS 419892	5.6	Oak and holly. An understorey of bracken and blaeberry.
Island	Creinch	CE	NS 397883	5.7	Oak, relatively open understorey with blaeberry and bracken.
Island	Inchfad	FA	NS 396907	45.1	Oak, with a spare understorey of bracken and bramble.
Island	Inchlonaig	LO	NS 375939	75.1	Oak, birch, and yew trees. Understorey dense with bracken.
Island	Inchmurrin	MU	NS 389874	114.7	Oak, birch and Scots pine. An understorey of blaeberry, bracken and bramble.

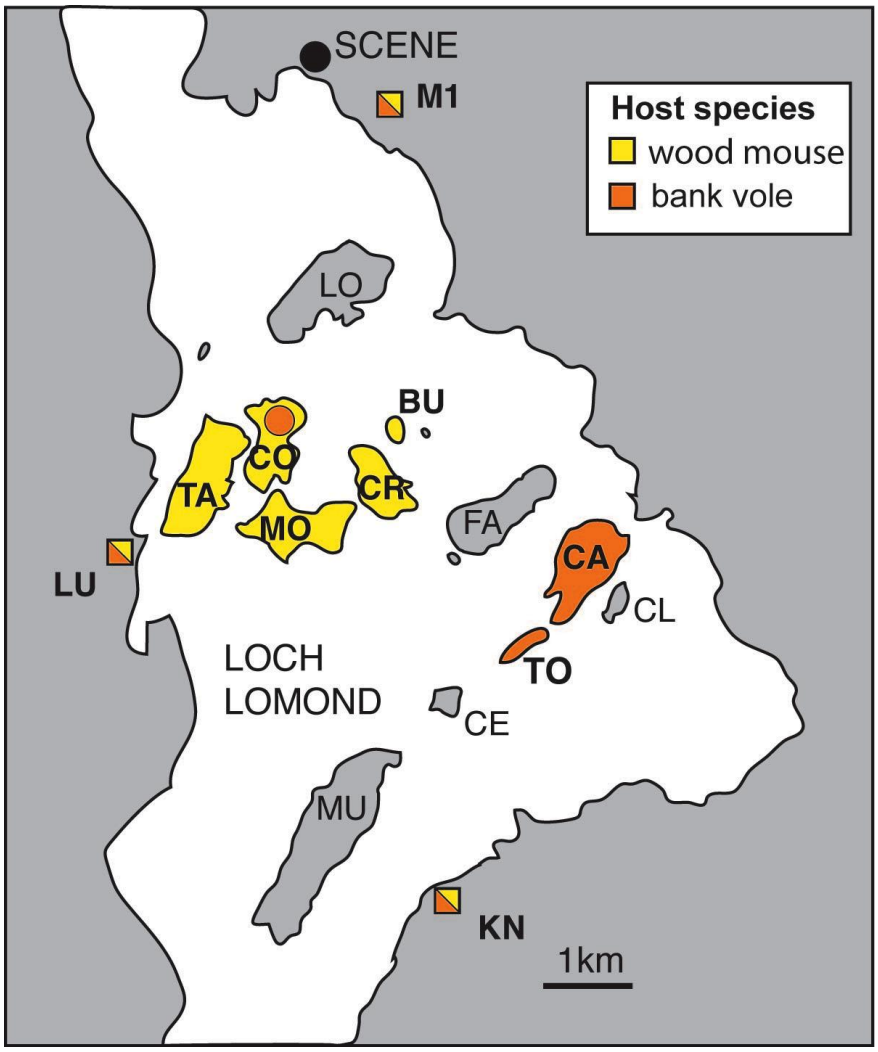


Figure 2-1: Map of study sites. Labels in bold are sites that were trapped continuously over the 2-year study period (Table 2.1). Those sites not in bold were monitored for the presence of rodents during the study period (Table 2.2).

2.2 Study species

Woodland rodents were the target host species, namely wood mice, *Apodemus sylvaticus*, and bank voles, *Myodes glareolus*. Wood mice and bank voles are the most common small mammal species in Europe (Bruhl, Guckenmus et al. 2011).

2.2.1 Wood mouse (*Apodemus sylvaticus*)

Wood mice are light brown in colour with a white to greyish under side, with large prominent ears and a pointy face. The tail is nearly the length of the body. On average males are heavier than females (males: 13-27g and females: 13-24g (>30 if heavily pregnant)). The average lifespan in the wild is 18-20 months (Corbet and Southern 1977; Macdonald and Barrett 1993).

Wood mice are widespread throughout continental Europe, the British Isles and southern Scandinavia; and are found in a variety of habitats. They prefer vegetation cover in some form, and are commonly found in woodlands, hedgerows, fields and gardens. Wood mice are nocturnal and mainly herbivorous feeding on seedlings, buds, fruits, nuts and occasional snails and arthropods. Male home ranges differ between woodland (1800-2500m^2) and farmland (2600-17700$m^{2,}$) (Keene 2009).

Home range size increases in the summer months during the breeding season, and then decreases again in winter. The social organisation of wood mice is based on a group structure with a dominant male, and a few subordinate males and females. Females will defend their breeding ranges (Corbet and Southern 1977). Breeding begins in March and April and reaches a peak in July and August (Baker 1930). The breeding season can extend into winter if food resources are abundant. Gestation lasts around 25 days, with a mean number of five young and a mean number of four pregnancies within a season (Corbet and Southern 1977). Young are born blind and naked weighting 1-2g, and will be weaned by the time they are 18 days. If young are born late in the breeding year, they will not reach maturity until the following breeding season.

In spring, young born in the last breeding season as well as a few adults from the previous year make up the population, those animals that have over-wintered usually don't survive for another year. Populations increase over the summer with a peak seen in September and October. Numbers decline over the winter with population size usually being lowest in March and April (Corbet and

Southern 1977). The main natural enemies of wood mice are birds of prey (e.g. kestrels, owls) and mammalian carnivores (e.g. fox, mustelids).

2.2.2 Bank vole (*Myodes glareolus*)

Bank voles have a blunt muzzle with ears being just visible above their dense fur, which tend to be reddish brown with a white to greyish belly. Bank voles have a short tail that has a slightly bushy tip. Weight ranges from 14-40g in males and 14g-36g in females. The average lifespan in the wild is around 18 months (Corbet and Southern 1977).

Bank voles are found throughout Europe except for the Tundra and Mediterranean lowlands (Corbet and Southern 1977). They are most abundant in deciduous woodland but also found along banks and hedges. They prefer dense ground cover. They are almost exclusively herbivores, and will store food when the day length is reduced (Corbet and Southern 1977). Bank voles are crepuscular species, with an overall increased movement in the summer (Corbet and Southern 1977).

Bank voles are a promiscuous species with a social organisation that differs seasonally, with intersexual groups of close kin forming during winter (Kruczek and Styrna 2009). During spring, the population is characterised by female-defended territories (500 -2000m^2), with large male home ranges that overlap with several female territories, as well as other male home ranges (Korpela, Sundell et al. 2011). Males that have overlapping home ranges will form a dominance hierarchy, with infanticide a common cause of juvenile mortality. Breeding begins in April and is usually finished by September/October. Gestation last around 18 days, but can be prolonged to 22 days if the female is lactating (Corbet and Southern 1977). Mean pup numbers are around four, with young born blind and naked at 2g, and weaned around 18 days. As with wood mice, young born late in the season will not reach sexually maturity until the following year.

Young from the previous season, that have over-wintered will grow rapidly to reach sexually maturity during spring to produce the first generation.

Whilst those that are born late in the year will form the majority of the next over wintering population (Corbet and Southern 1977). The population increases in late summer and autumn, with a decline in winter. They have very similar predators to wood mice in the form of birds of prey (e.g. owls and kestrel) and mammalian carnivores (e.g. fox and mustelids).

2.3 Field methods

Fieldwork was carried out for 2 years (2009-2010), during spring (February- April), summer (June- August) and autumn (October- November). During each season, each site was trapped once using 100 live Sherman traps (Figure 2.2) placed 10 metres apart within a 10 by 10 grid for one week. Traps were pre-baited with a small handful of dry porridge oats mixed with peanut butter for one day, opened the following day and baited with a small piece (~1cm^2) of apple and a small handful of dry porridge oats mixed with peanut butter, and then checked for captures at dawn and dusk for a period of 3 consecutive days. After the last check had been completed on day 5, all traps were removed, cleaned and autoclaved.

Initial opportunistic trapping indicated presence of rodents on those islands that were trapped continuously during the trapping period (Table 2.1). Rodent monitoring was conducted on the other islands (LO, MU, FA, CL and CR) (Table 2.2). Twelve baited open-ended pipes (Figure 2.3) where placed in suitable rodent habitat, and checked weekly during the trapping session. These pipes were used instead of Sherman traps as no rodents had been caught previously at those sites (except from LO). This method allowed monitoring to take place without the regular inspections required when using live trapping. The presence of rodents was not detected on those sites during the 2-year period.

Figure 2-2: Sherman trap on the island of Inchconnachan at base of tree.

Figure 2-3: Baited pipe on the island of Clarinish at base of tree. Metal skewer holds bait of apple and peanut butter *in situ*.

2.3.1 Rodent data collection

Rodents caught were marked subcutaneously with a unique Passive Integrated Transponder (PIT) tag (AVID plc, East Sussex, UK) on the first capture and identified upon recapture using a handheld reader (Minitraker I, AVID plc, East Sussex, UK).

For first captures within each session, species and sex of the animal were recorded together with a set of morphological characteristics (Table 2.3). Coat texture and mass was used to distinguish juveniles from adults, since the coat is fluffy in texture in juveniles compared to a smooth texture in adults. The mass of the rodent was measured using a spring balance and recorded to the nearest gram. Rodents were split into 3 classes based on coat condition and mass (Telfer, Bennett et al. 2002). Wood mice: juvenile (<15g), sub-adult (15-18g), and adult (>18g). Bank voles: juvenile (<14g), sub-adult (14-17g), and adult (>17g). All of the traps were fitted with shrew holes, to allow any shrews caught to escape via a small hole in the back of the trap; this hole would have potentially led to the loss of some juvenile captures.

The reproductive status of males was determined based on the position and size of the testes (abdominal - not fully developed, scrotal - fully developed, and scrotal plus - developed and significantly large). The reproductive status of females was classified based on the following four characteristics: 1) non-gestating or gestating; 2) vulva perforated or non-perforated; 3) cervix closed, partially closed, or open; 4) nipples not visible, small or large (lactating). Body condition was determined by examining the fat deposit along the lower spine and hips through palpation and assigning a score between 1 (low fat deposits) and 5 (high fat deposits).

Wood mice and bank voles were the two focal species caught within this study and represented 99% of captures. Other captured mammalian species include one field vole (*Microtus agrestis)*, two pygmy shrews (*Sorex minutus*) and four common shrews (*Sorex araneus*).

Table 2-3: Data sheet used for recording rodent host characteristics in the field.

Characteristics		Code
Species	Wood mouse	WM
	Bank vole	BV
Sex	Male	M
	Female	F
Recapture? (Based on presence of PIT reading).	New individual	N
	Recapture	R
Coat texture	Adult coat	AD
	Juvenile coat	JC
Hip score (scale 1 to 5)	Very thin	HS1
	Very fat	HS5
Sample label	Parasite sample	P (reference number)
	Blood sample	B (reference number)
	DNA Sample	H or E (reference number)

Ectoparasites	**Code**
Ticks	T (reference number)
Fleas	F (reference number)
Mites	M (reference number)

Sex	**Breeding status**	**Code**
Male	Testes abdominal	TA
	Testes scrotal	TS
	Testes scrotal and large	TS+
Female	Gestating	G
	Non-gestating	NG
	Vulva perforated	PV
	Vulva non-perforated	NV
	Cervix open	OC
	Cervix partially opened	POC
	Cervix closed	CC
	Nipples non-visible	NN
	Nipples visible and small	NS
	Nipples visible and large	NL

2.3.2 Parasite data collection

Rodents were examined for ectoparasites by blowing close to the body against the direction of the hair. The face and ears were examined in particular, as the soft skin provides an ideal site for ticks to attach and obtain a blood meal. The number of visible fleas was recorded and, if possible, specimens were removed with tweezers and stored in 70% ethanol. The number of ticks present and their position on the body was recorded and a sub-sample of ticks were removed from each specimen from every capture with tweezers and stored in 70% ethanol. Not all of the ticks were removed, as the time required for this could have caused undue stress to the rodent. Mites were noted as either present or absent.

A small amount of blood (<20 μL) was taken from the tail vain for serology and parasite detection (see section 2.4.3). This procedure involved trimming the hairs around the tip of the tail before removing ~1mm of the tail by a single cut with sharp scissors. Then the tail vein was bled into an eppendorf tube. During cold conditions, a Gelert hot gel hand-warmer was occasionally used to increase blood circulation. If the rodent was deemed too distressed (low breathing rate, lethargic, too wet and/or cold), no blood was taken. A tissue sample was obtained from a hair plug or by taking a 2mm skin biopsy from the ear as a basis for later genetic analysis.

Rodents were released at the site of capture and a clean trap replaced the used one. All traps that had rodents caught within them were taken back to laboratory in order to process the faeces. Traps were autoclaved before being reused.

2.4 Laboratory techniques and identification of parasites

2.4.1 Ectoparasites

Ectoparasites that were collected from rodents during sampling sessions were brought back to the laboratory for identification down to species level using a binary microscope (Leica DM 1000, 4x/0.10).

2.4.1.1 Ticks

All ticks found on rodents from Loch Lomond were in the family Ixodidae as they all possessed a scutum (shield) indicating they are hard bodied ticks. Males are distinguished by the scutum covering the whole of the dorsal surface, whilst in females the scutum only covers the anterior end of the dorsal surface (Arthur 1963). Ixodes ticks have three life history stages larva, nymph and adult. The larvae are easily distinguished by having 6 legs, whilst nymphs and adults have 8 legs. There is also an increase in overall size from larvae to adult.

Only two species of tick were found on wood mice and bank voles in the study area, I*xodes trianguliceps* (rodent tick) and *I. ricinus* (sheep tick). A third potential species known to infest these species, *Ixodes hexagonus* (hedgehog tick) was not detected.

The two species, *I. ricinus* and *I. trianguliceps*, were distinguished based on morphology. *I. ricinus* has an oval body, compared to *I. trianguliceps* that has a body coming to a point at the front, like a triangle (Figure 2.4). Coxae (Figure 2.4), which act like a hip or shoulder joint, possess spurs at their base for *I. ricinus*; whilst coxae of *I. trianguliceps* do not have spurs, but are covered with a fold of cuticle (Arthur 1963). All 3 life-stages of *Ixodes trianguliceps* (larva, nymph and adult) are found on rodents, whereas with *Ixodes ricinus* only the first two stages of development, larva and nymph, are found on rodents. The

final stage of development takes place on large mammals, namely deer and sheep and larger bird species such as grouse (Perkins, Cattadori et al. 2006).

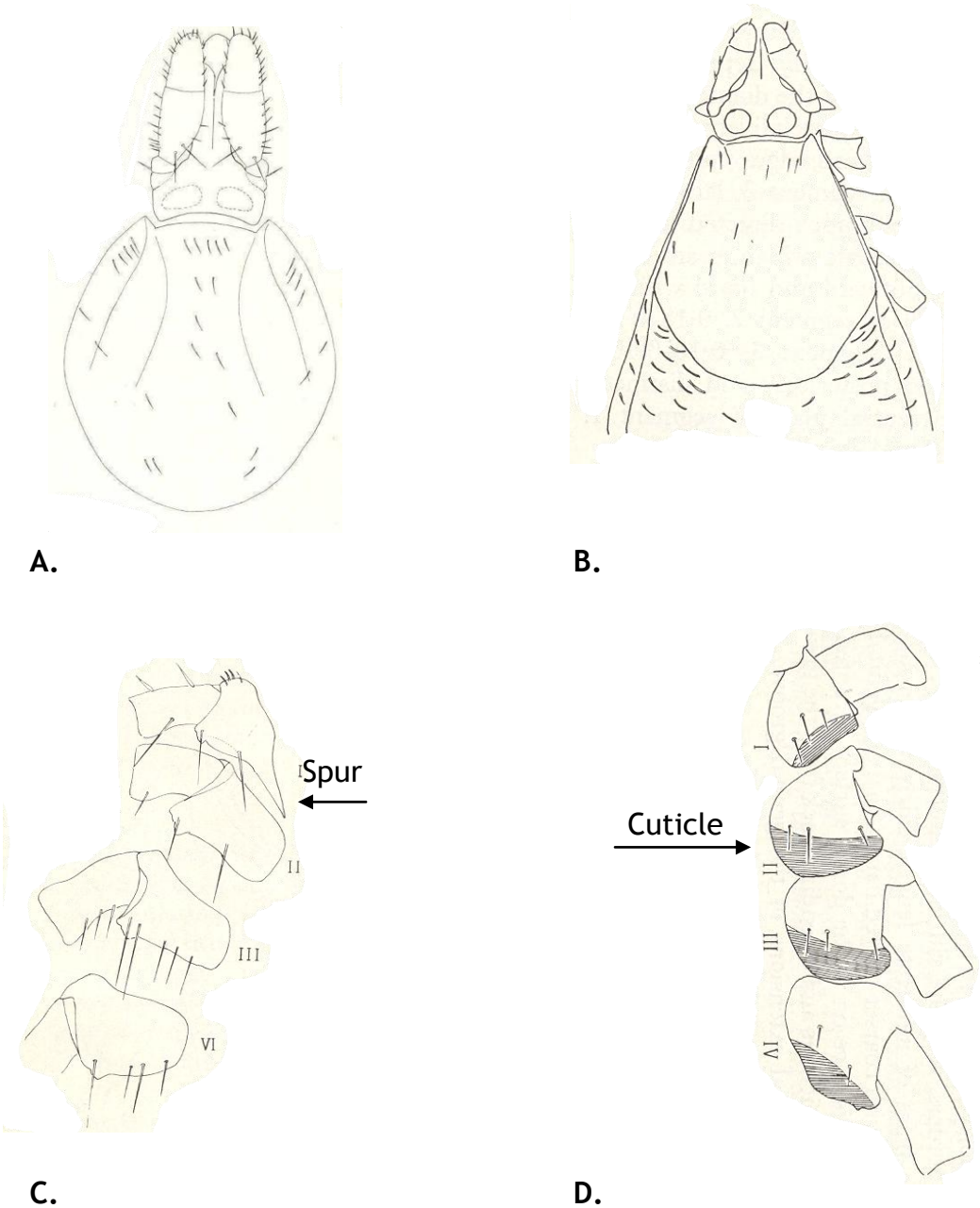


Figure 2-4: Body shape of *Ixodes ricinus* (A) and *Ixodes trianguliceps* (B). Coxae of *Ixodes ricinus* showing spur (C), and coxae of *Ixodes trianguliceps* showing cuticles (D) (Reproduced from Arthur 1963).

2.4.1.2 Fleas

The number of flea (Siphonaptera) species potentially found on UK rodents greatly exceeds that of ticks. Currently in the UK and Ireland, 23 species of flea have been described on wood mice and 27 species on bank voles (George 2008) . During this study, six species of flea were identified from rodents caught at the Loch Lomond sites.

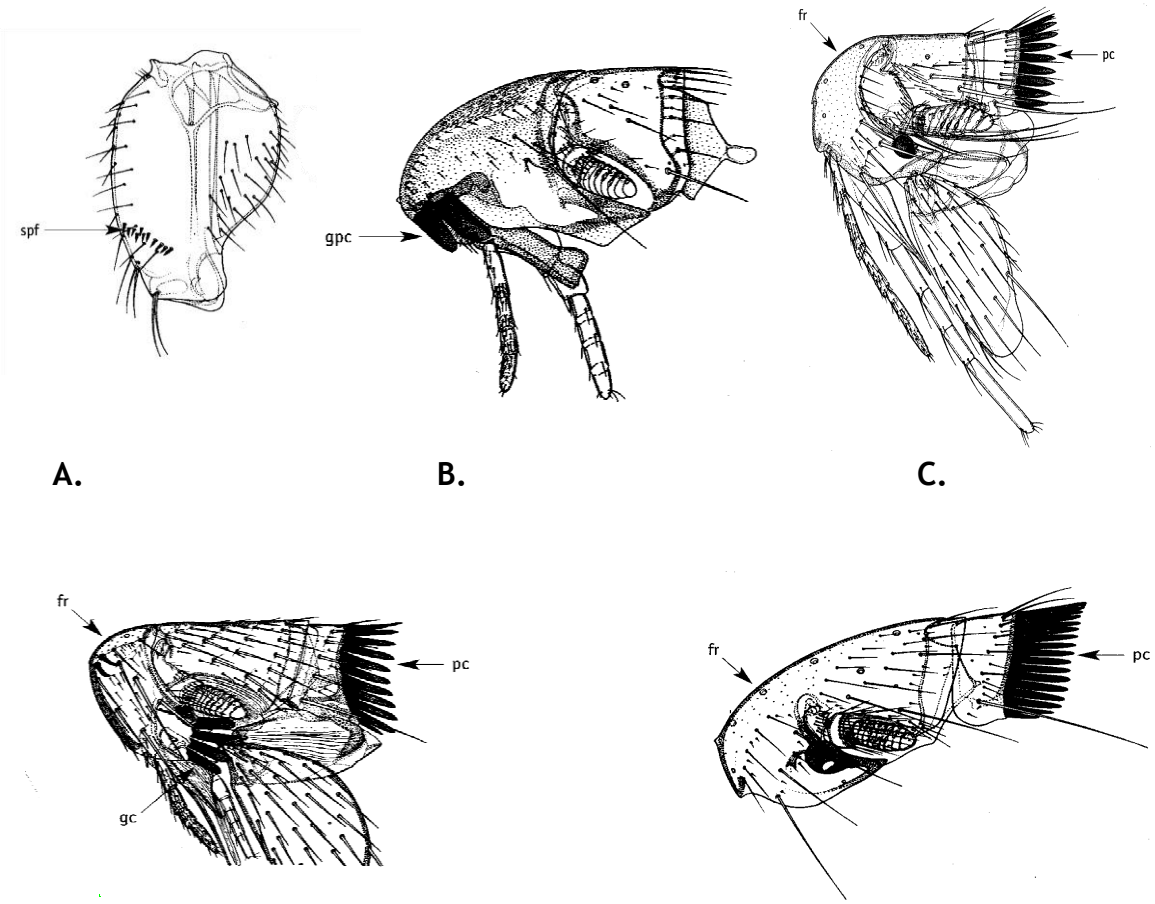
As there is potentially ten times the variety of species of fleas that could be found on rodents compared to the number of tick species, identification of fleas down to species level was more challenging. Based on the following morphological features, flea families can be split into three groups (Whitaker 2007):

1. The terga have rows of small spiniform setae or not (Figure 2.5).
2. The presence of a genal and/or pronotal comb and the number of spines within each comb, if present (Figure 2.5).
3. The shape of the frons; either sharply curved downwards or gently and evenly curved downwards (Figure 2.5).

The families identified according to the criteria above were Ctenophthalmidae, Ceratophyllidae, Hystrichopsylidae and Leptopsyllidae. All four of these families had (1) Terga with rows of small spiniform setae, (2) presence of a genal and pronotal comb (Ctenophthalmidae and Hystrichopsylidae) or only a pronotal comb (Ceratophyllidae and Leptopsyllidae) and (3) frons sharply curved downwards (Ceratophyllidae) or gently curved downwards (Leptopsyllidae). In addition, some specific species could be identified (Table 2.4).

Table 2-4: Flea families and species identified.

Family	Subfamily	Species
Ctenophthalmidae	*Rhadinopsyllinae*	*Ctenophthalmus nobilis nobilis*
		Rhadinopsylla pentacantha
Ceratophyllidae	*Ceratophyllinae*	*Megabothris sp.*
		Orchopeas howardi howardi
Hystrichopsylidae	*Hystrichopsyllinae*	*Hystrichopsylla talpae talpae*
Leptopsyllidae	*Leptopsyllinae*	*Leptophsylla segnis*



A. **B.** **C.**

D. **E.**

Figure 2-5: Morphological features of fleas'. A- Terga has rows of spinifrome setae (spf), B- pronotal comb (pc) or C- genal (gpc). The shape of the frons, either D- sharply curved downwards or E- gently and evenly curved downwards (Reproduced from Whitaker 2007).

2.4.2 Gastro-intestinal parasites

2.4.2.1 Salt floatation technique

Rodents that were processed in the field and spent a period of time in the Sherman trap will have deposited faeces on the base of the trap. Once in the laboratory, faeces were removed from the trap and stored in eppendorf tubes, weighed to the nearest milligram, and preserved in 10% formalin solution. This allowed the samples to be preserved until they were processed using a salt floatation technique. The salt floatation technique is a standard procedure for identifying gastro-intestinal parasites from faecal material (Dryden, Payne et al. 2005). Parasites are detected based on expelled oocysts (protozoa) or eggs from the adult worms (helminths). Firstly, half of the faecal sample was transferred from the eppendorf tube into a 15ml centrifuge tube containing a saturated sodium chloride solution. In order to break up the faeces, the test tube was spun on a variable speed shaker (vortex Genie 2) for 2 to 5 minutes depending on the density of the sample.

Once the sample had been sufficiently mixed, the test tube was topped up to the rim with a saturated sodium chloride solution and a microscope cover slip was placed on top and left for ten minutes. This allowed any oocysts or eggs present in the sample to float to the top of the sodium chloride solution and to adhere to the cover slip.

After ten minutes, the cover slip was placed on a clean microscope slide, which was then analysed under a microscope (Leica DM 1000, 10X 0.25). Slides were examined by starting at the bottom right hand corner of the slide and moving from right to left along the slide, then up from left to right once a full length of the cover slide had been performed. Normally, only half of the cover slip was examined and the number of oocysts/eggs recorded was subsequently doubled. If the number of parasite oocysts/eggs counted in half of the slide were less than 20, then the whole of the cover slip was examined.

Any oocysts or eggs seen were recorded and identified using differences in size and morphology. Using a digital camera (Euromexx, Holland 2005) mounted on the light microscope and specialised software (Imagefocus v2.0.0.0), a digital image was taken of each specimen and stored on a laptop computer, along with the power of the magnification to allow for accurate size calculations. Initial weight of the sample was recorded, allowed intensity of infection to be expressed as eggs/oocystes per gram of faecal material.

2.4.2.2 Identification of gastro-intestinal parasites

The gastro-intestinal parasites detected fell into two main groups, nematodes and protozoan, with the exception of one cestode species. Nematodes identified were *Heligmosomoides polygyrus, H. glareolus* (Thomas 1953; Keymer and Dobson 1987); (Montgomery and Montgomery 1990) *Syphacia* species (Abu-Madi, Behnke et al. 2000), several species of *Capillaria* (Meagher 1999), and *Trichuris*. The cestode was identified as *Rodentolepis*. The protozoan detected were coccideans, namely *Eimeria* species (Nowell and Higgs 1989; Higgs and Nowell 2000).

2.4.2.2.1 Nematode identification

Heligmosomoides eggs are large and round in appearance and range in size from 40 – 100 microns (Figure. 2.6). *H. polygyrus* and *H. glareolus* are found within the gastro-intestinal tract of wood mice and bank voles, respectively. *Syphacia* species eggs are oval with slightly pinched ends and flattened on one side and range in size from 80-120 microns. *Capillaria* and *Trichuris* eggs are very similar in shape and size but those of *Trichuris* are more lemon shaped, whilst those of *Capillaria* are round with more pronounced ends. Both range in size from 30- 60 microns (Figure 2.6) (Taylor, Coop et al. 2007).

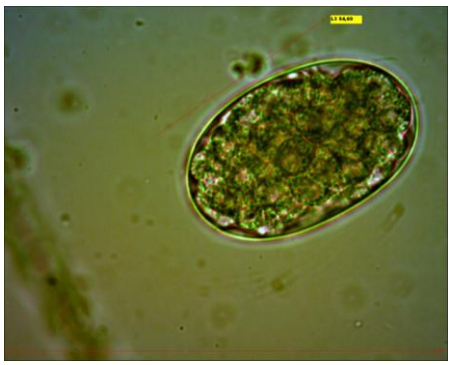
2.4.2.2.2 Cestode identification

Rodentolepis was the only Cestode found in this study. The eggs produced by adult worms are small and round, 40-55 microns in diameter. The internal structure of a lemon shaped embryophore, enables easy identification (Figure 2.6) (Taylor, Coop et al. 2007).

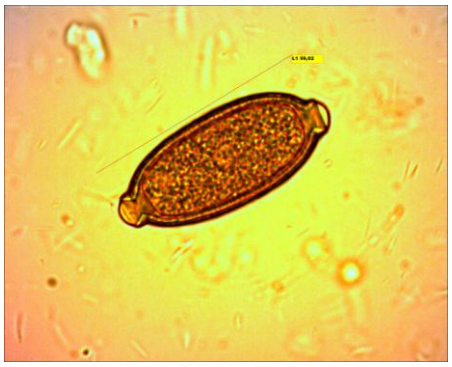
2.4.2.2.3 Protozoan identification

Eimeria oocysts are easily distinguished from nematode eggs' as they are much smaller in size, 10 – 40 microns. Amongst *Eimeria* species the oocysts vary in size but size is an insufficient criterion for species identification. For the purpose of this study, oocysts were therefore classed as either small, 10-20 microns, or large, 21-40 microns (Figure 2.6) (Taylor, Coop et al. 2007).

A.

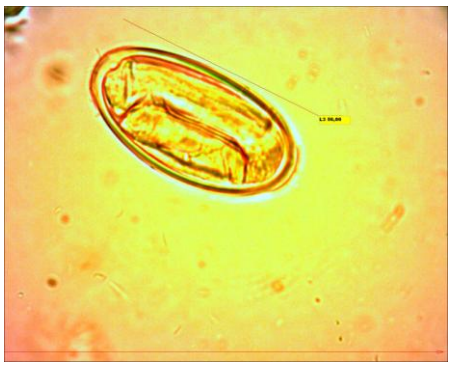


B.



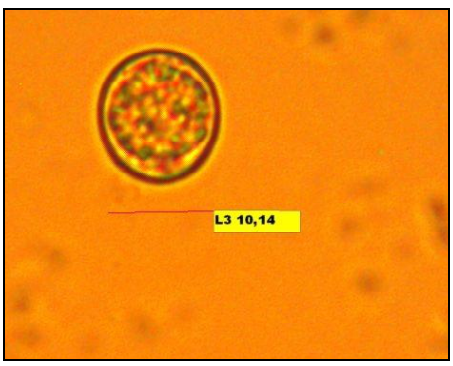
C.

D.

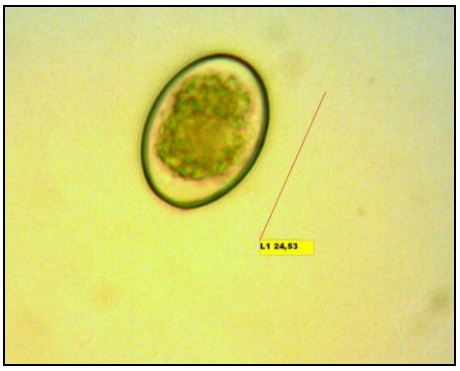


E.

F.



G.



H.

Figure 2-6: Examples of digital images of parasite transmission stages recorded from rodent faecal samples. A - *Heligmosomoides polygyrus* (66.32μm), B- *Heligmosomoides glareolus* (54.69μm), C- *Capillaria sp.* (59.02μm), D- *Trichuris sp.* (54.75μm), E- *Syphacia sp.* (56.80μm), F-

***Rodentolepis sp*. (52.97μm), G- Small *Eimeria sp*. (10.14μm) and H- Large *Eimeria sp*. (24.53μm).**

2.4.3 Blood sample analysis

In the laboratory, the blood samples were spun in a centrifuge (30 minutes at 13 x1000 rpm) to separate the plasma, which was aspirated and stored at -20 °C, as was the remaining cell pellet.

2.4.3.1 Virus detection

Plasma samples were tested by Dr Trevor Jones at The National Centre for Zoonosis Research (University of Liverpool) for the presence of antibodies against murid gamma herpes virus and cowpox virus. An immunofluorescence antibody assay was used with murine alpha-herpes virus (MHV- 68) and cowpox strain L97 infected Vero cells as the antigen. 96-well plates with a monolayer of Vero cells were infected with 5-10 plaque forming units (PFU) of virus. The plates were incubated for 48 hours then fixed with 70% ethanol and stored at 4^{o}C until needed. The cells were rehydrated in phosphate-buffered saline (PBS) then serial incubated with rodent plasma (dilutions 1:20 and 1:40) and rabbit anti-mouse fluorescein isothiocyanate (FITC) (Sigma F0257) and anti-rat IgG FITC (Sigma F6258). Before viewing under UV illumination with an inverted microscope, the cells were washed with PBS. In order to stop cells drying out and lifting, glycerol was added to the cells (Crouch, Baxby et al. 1995; Bennett, Crouch et al. 1997; Telfer, Bennett et al. 2002). A positive result for cowpox virus was recorded if a fluorescent green “Y” was seen within a Vero cell, whilst a florescent green globule indicated a positive result for murid gamma herpes virus.

2.4.3.2 Bacteria detection

Elizabeth Kilbride (University of Glasgow) tested the red blood cells for *Bartonella* infection. DNA was extracted from blood clots using the Qiagen DNA Easy kit according to the manufacturers protocol and tested for *Bartonella* by

amplifying a fragment of the intergenic spacer region (ISR) (Telfer, Bown et al. 2005). While size differences among the amplified products had been used by Telfer et al. (2005) to discriminate between *Bartonella* species, this method was found to produce inconsistent results in our case. Infection was therefore only recorded as *Bartonella* species.

3 Comparative demography and population dynamics of rodents in a fragmented landscape.

3.1 Introduction

Nearly every process in population biology is known to be affected by habitat patchiness (Fahrig and Merriam 1994; Hanski and Gilpin 1997). Current research suggests that low degrees of fragmentation and high connectivity create a more stable environment for species to persist in (Christensen, Ecke et al. 2008; Mortelliti, Amori et al. 2009). Highly connected habitats are important for species dispersal, persistence and for maintaining genetic diversity within a population (Schooley and Branch 2011). Discontinuous habitats can potentially lead to negative genetic consequences such as inbreeding or loss of variation through genetic drift when populations are small and/or isolated (Charlesworth 2003). It is well documented that fragmentation has negative conservation implications, and plays an important role in species extinctions (Ekross, Heliola et al. 2010; Krauss, Bommarco et al. 2010). Where habitats are patchy, habitat quality can further influence population demographics. Small fragments tend to be more vulnerable to disturbance than non-fragmented habitats due to edge effects, and there is often a positive correlation between the size of a habitat fragment and its quality (Holland and Bennett 2010). However, fragmentation does not always result in a negative effect on populations, as species' responses to discontinuous habitats are highly variable. Therefore, there is a need to understand the mechanisms explaining why species are differently affected by fragmented habitats.

Wild rodents are widely used to investigate population-level effects of fragmentation (Krebs 1966; Moretlliti and Boitani 2007; Christensen, Ecke et al. 2008; Suzan, Armien et al. 2008; Holland and Bennett 2010), with different species providing examples of both negative and positive responses. For populations of the native Australian bush rat, *Rattus fuscipes*, fragmentation has a negative effect, since smaller fragments supported lower population densities and a smaller fraction of older individuals where habitat quality was reduced (Holland and Bennett 2010). Conversely for North American deer mice, *Peromyscus maniculatus*, survival and density estimates were higher in patchy forest landscapes (Tallmon, Jules et al. 2003), indicating a potential positive responds to habitat patchiness. Rodents with broader ecological niches are less

likely to be affected by fragmentation than habitat specialists (Puttker, Meyer-Lucht et al. 2008). Such habitat generalists are more likely to be able to move between fragments by using the matrix in between them (Szacki, Babinska-Werka et al. 1993; Andren 1994; Puttker, Meyer-Lucht et al. 2008; Panzacchi, Linnell et al. 2010). In addition, generalist species may be better able to thrive in discontinuous habitats' as there is potentially no deterioration in habitat quality with fragmentation and, unlike specialist species, they do not rely on a specific resources (Marcello, Wilder et al. 2008; Holland and Bennett 2010). Studies have shown that generalist rodents in forest fragments utilize alternative food resources (Tallmon, Jules et al. 2003; Marcello, Wilder et al. 2008), and these populations can show an increase in abundance compared to those inhabiting continuous habitats. Based on the above, it is clear that fragmentation effects may be mediated through mechanisms related to the quality of the habitat patch or of the matrix separating them. However, these effects, which aren't mutually exclusive, are difficult to disentangle in most systems. Islands systems, in which the matrix between patches is completely uninhabitable, therefore provide unique research opportunities in this regard. Here, we are using the natural system of islands located within Loch Lomond to examine how two generalist woodland rodent species respond to being restricted to isolated patches of habitat.

Changes in the demography of one species have the potential to change its ecological interactions with other species. This includes host-parasite interactions, which have received relatively little attention in fragmented landscape research. Population characteristics such as sex ratio, age structure, reproductive activity, size and body condition, dispersal and survival have the potential to affect disease transmission dynamics (Table 3.1). Fragmented populations can reach high population densities, which may result in higher abundance of parasites. For example, Allan, Kessing et al. (2003) showed that forest fragments in the North-eastern US had elevated densities of white-footed mice (*Peromyscus leucopus),* which were more heavily infected with nymphal blacklegged ticks than mice from contiguous forests. Similarly, for forest fragments in Panama rodent density was positively correlated with hantavirus prevalence (Suzan, Armien et al. 2008).

Table 3-1: Population parameters and the potential relationships with diseases within fragmented habitats.

Population parameter	Potential response to fragmentation	Potential effect on parasite infections
Population size	(-) Population size expected to decrease with size of fragment	(-) Parasite extinction below critical community size [1] or stochastic
Population density	(-) Decrease due to reduced habitat quality (e.g. edge effects) [2] (+) Increase due to frustrated dispersal and fence effect [3]	(-) Extinction at low densities [1] (+) Increased transmission at high densities [4]
Sex ratio	(-) Males inhabit poorer fragments than females and take more risks [5]	(+/-) Sex-specific differences in susceptibility [6,7] could result in increased prevalence
Age distribution	(+/-) Younger population due to dispersal from other fragments [2].	(+/-) Age bias in susceptibility [6,8,9] with an increase or decrease in prevalence.
Breeding status	(+) Breeding time and length is similar in small and large fragments [10]	(+) Breeding individuals have an increased chance of becoming infected [7], and therefore increasing prevalence of infection.
Body condition	(-) Poor body condition in fragments due to reduced habitat quality [11]	(+) Poor body condition [12] increases an individual's chance of becoming infected.
Movement	(+/-) Male movement in discontinuous habitats is affected by food distribution and suitable mating opportunities [13]	(+) Increased movement by infected individuals [14, 15] may lead to an increase in prevalence.
Survival	(+/-) Variation in survival in fragmented habitats [2]	(+/-) Infection dynamics can be increased or decreased with changes in individual survival.

[1] Lloyd-Smith et al. 2005; [2] Holland and Bennett 2010; Puttker, Meyer-Lucht et al. 2008 [3] Krebs et al. 1969; Poulin 1996; [4] Anderson and May 1982; [5] Bowers & Smith 1979; [6] Harrison, Scantlebury et al. 2010; [7] Luong, Perkins et al. 2010; [8] Gillespie, Lonsdorf et al. 2010; [9] Liberman, Khokhlova et al. 2011; [10] Adler 1994; [11] Villafuerte et al. 1997; [12] Beldomenico, Telfer et al. 2009; [13] Haapakoski & Ylonen 2010 [14] Brown, Macdonald et al. 1994; [15] Boyer; Reale et al, 2010.

The data and analyses presented within this chapter are part of a broader study addressing how parasites dynamics are affected by the spatial structure of a host population. In order to interpret the parasite data from this system, it is necessary to understand whether host populations on islands exhibit any fundamental differences in terms of their comparative demography and population dynamics compared to those on the mainland. This will be addressed for two woodland rodent species, wood mice, *Apodemus sylvaticus*, and bank voles, *Myodes glareolus*, in the naturally fragmented islands system of Loch Lomond. Pilot studies had shown that these were the two dominant rodent species within this system. The following demographic measures were examined: population size and density, sex ratio, age distribution, breeding status, body condition, movement, and apparent survival. The objectives of this study were (1) to determine whether these population parameters were altered on isolated patches as compared to continuous habitat on the nearby mainland, and (2) to examine whether these changes would have the potential to also alter parasite infection dynamics.

3.2 Methods

A detailed description of the fieldwork carried out is provided in chapter 2 General Methods.

Data was collected from seven islands on Loch Lomond and three nearby mainland sites, over the course of 2009-2010 during spring (February-April), summer (June-August) and autumn (October-November). Woodland rodents were the target species, namely wood mice, *Apodemus sylvaticus*, and bank voles, *Myodes glareolus*.

Each site was trapped using 100 live Sherman traps placed 10 metres apart within a ten by ten metre grid for one week within each trapping session. Rodents caught within each session were marked subcutaneously with a unique Passive Integrated Transponder (PIT) tag (AVID plc, East Sussex, UK), on the first

capture and if re-caught were detected using a handheld reader (Minitraker I, AVID plc, East Sussex, UK).

Rodent morphological characteristics were collected on each capture. Species and sex was recorded, as well as coat texture and mass. A fluffy coat was used to distinguish juveniles from adults in both species. Mass was measured to the nearest gram. Rodents were split into 3 classes based on coat condition and mass (Telfer, Bennett et al. 2002). Wood mice: juvenile (<15g), sub-adult (15-18g), and adult (>18g). Bank voles: juvenile (<14g), sub-adult (14-17g), and adult (>17g), to determine age structure. The reproductive status of males and females were also recorded. Males were classified by the size and the position of the testes as reproductively inactive (testes abdominal) or potentially reproducing (testes scrotal). Female were classified as reproductively active if they met any of the following four characteristics: (1) gestating; (2) vulva perforated; (3) cervix partially open or open; and (4) nipples visible (with large nipples indicating lactation). Body condition was determined by examining the fat deposits along the lower spine and hips and scaled from one (low fat deposits) to five (high fat deposits). The score was measured by palpitating the lumbar spine and pelvic bones of an animal. Rodents were released at the site of capture.

Low capture rates prevented the use of mark-recapture abundance estimators. Abundance for both rodent species was therefore quantified using the minimum number known alive (MNKA) index, defined as the total number of individuals caught within a given trapping session and adding it to those not caught in that session but caught both previously and subsequently (Krebs 1966). Separate generalized linear models using a binomial family was used to test for differences in sex ratios (sex) and proportion of individuals breeding (proportion breeding) between the mainland and islands (m.I), years (year) and seasons (season) using the function *'glm'* in the 'MASS' library of *R* (The R Foundation for Statistical Computing; http://www.r-project.org/). Stepwise removal of the variables was used to produce the final model based on the lowest Aikaike's Information Criterion (AIC). In addition to main effects, potential interactions between patch type (mainland/island) with year and season were considered. If

no explanatory variables were found to be significant then the intercept model is shown.

For recaptures individuals, movement was calculated as the Euclidian distance between trap locations, calculated using a simple distance formula (Equation 3.1). By converting the trap numbers into co-ordinates from the grid, the distance formula could be applied.

$$d = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}$$ **Equation 3-1**

For individuals recaptured multiple times, a single, average maximum distance between trapped locations was calculated to avoid pseudo-replication.

Students T tests and Chi-squared statistics were carried out in a range of the analyses within R (The R Foundation for Statistical Computing; http://www.r-project.org/). For the differences in breeding status the Julian day was used within the analysis and then the day was transferred into a date for ease of recognition. A Wilcoxon's rank test was used for the analysis of body condition since body condition was classed as a rank score when the data was gathered in the field.

3.3 Results

3.3.1 Rodent population size and density through time

During the trapping period from February 2009 to the end of November 2010, a total of 322 wood mice and 345 bank voles were trapped across 10 sites (3 mainland sites and 7 island sites) around Loch Lomond, with substantial variation in numbers trapped across sites (Table 3.2). On average, rodent abundance was higher on mainland sites compared to islands (mean MNKA: mainland= 20.0, ± standard error (SE) 3.9, island= 5.7, ± SE 1.5, $t_{(10)}$= 3.88, p=0.004). When rodents

were analysed separately, both wood mice and bank voles did not show any significant differences between mainland and island sites (**wood mice:** mean MNKA: mainland= 8.7, ± SE 1.8, island= 4.6, ± SE 1.2, $t_{(7)}$=2.01, p= 0.055, **bank voles:** mean MNKA: mainland= 11.1, ± SE 2.6, island= 7.1, ± SE 2.3, $t_{(6)}$=1.159, p=0.255). There was no relationship between total island size and rodent abundance on the trapping grid (r = 0.29, $t_{(9)}$=0.69, p=0.521) (Figure 3.1). An approximate total population size for each island can be deduced from the lowest MNKA index. By using the simple assumption that the trapping grid represents the island as a whole, as trapping grids across mainland and island sites were the same size. It is seen that the larger islands have a larger population size than the mainland sites (Table 3.3). No individuals were found to have moved between sites, thus the islands can be considered distinct populations, at least over the course of this study.

Table 3-2: Mean MKNA across sites.

Site	Mainland or Island	Host species present	**Mean** minimum number known alive ± standard error (range)
Sallochy (M1)	Mainland	Wood mouse	9.2 ± 3.1 (0-17)
		Bank vole	9 ± 3.5 (1-26)
Luss (LU)	Mainland	Wood mouse	9.5 ± 3.9 (0-26)
		Bank vole	17.8 ± 5.7 (5-38)
Knockour wood (KN)	Mainland	Wood mouse	7.8 ± 2.8 (1-17)
		Bank vole	6.5 ±0.9 (0-17)
Bucinch (BU)	Island	Wood mouse	6 ± 1.9 (1-14)
Inchtavannach (TA)	Island	Wood mouse	8.2 ± 2.6 (0-16)
Inchconnachan (CO)	Island	Wood mouse	3.5 ± 1.5 (0-9)
		Bank vole	2 ± 1 (1-4)
Inchmoan (MO)	Island	Wood mouse	4 ± 1.6 (0-9)
Inchcruin (CR)	Island	Wood mouse	0.5 ± 0.4 (0-2)
Inchcailloch (CA)	Island	Bank vole	13.2 ± 4.7 (1-30)
Torrinch (TO)	Island	Bank vole	4.4 ± 1.7 (1-11)

(a) (b)

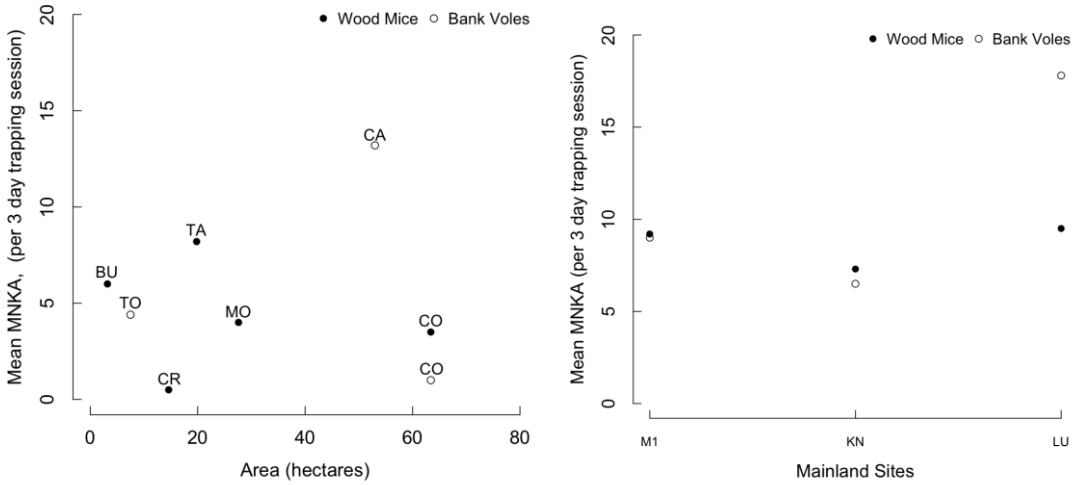


Figure 3-1: Comparing mean MNKA for wood mice (filled circles) and bank voles (open circles) between island (a) and mainland (b) sites. Numbers for island populations (a) are plotted against island size (area in ha).

Table 3-3: An approximate total population size for each island based on the lowest MNKA index for each island.

Island	Area (Hectares)	Lowest MNKA Index per 1ha grid (session observed)	Total population size
Bucinch (BU)	3.1	1 (Spring 2010)	<10
Inchtavannach (TA)	63.0	0 (Autumn 2010)	<100
Inchconnachan (CO)	41.9	0 (Spring 2010)	<50
Inchmoan (MO)	45.6	0 (Spring 2010)	<50
Inchcruin (CR)	28.3	0 (Spring 2009)	<50
Inchcailloch (CA)	53.0	3 (Spring 2010)	<200
Torrinch (TO)	7.5	2 (Spring 2010)	<10

There was variation in the yearly dynamics of rodents caught between 2009 and 2010. Wood mice and bank vole populations seemed to crash in spring 2010 (Figures 3.2 and 3.3), potentially due to the extremely cold temperatures of winter 2009 and spring 2010. Thereafter, populations began to rise throughout all sites during summer 2010. However, numbers did not reach those seen in 2009. Mean MNKA rodent abundance was significantly higher in 2009 than 2010 on the mainland (mean mainland 2009=15.2, mean mainland 2010=5.9, $t_{(5)}$=3.317 p=0.002), with no difference between mean MKNA for rodents in 2009 and 2010

on the islands (mean island 2009=6.7, mean island 2010=3.6, $t_{(13)}$=1.715, p=0.094). However, this relationship is driven by the higher captures of wood mice in summer on the mainland sites 2009. There was no apparent seasonal consistency for wood mice dynamics across sites. In 2009, abundance on all but one site, Luss (LU), decreased from spring to summer then increased in autumn towards the end of the breeding season for wood mice populations. After the spring 2010 crash, numbers increased in summer and autumn, with the exception of Inchtavannach (TA). Bank vole populations showed the seasonal pattern expected in both years, with a rise in captures from spring to summer. Bank vole captures increased faster after spring 2010 than wood mice numbers, suggesting a quicker population recovery.

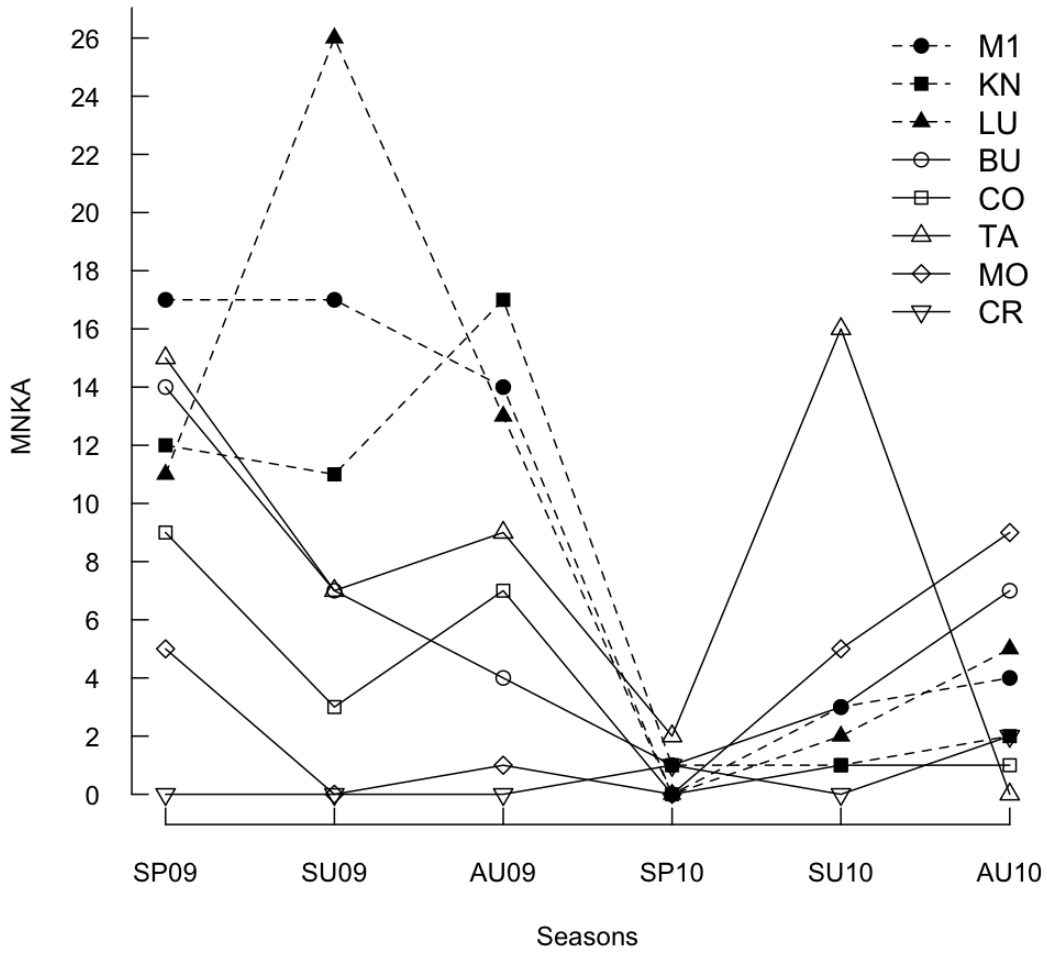


Figure 3-2: Seasonal population dynamics of wood mice across mainland (dashed) and island (solid) sites within Loch Lomond during 2009-2010. Season's represent trapping sessions within spring 2009 (SP09), summer 2009 (SU09), autumn 2009 (AU09), spring 2010 (SP10), summer 2010 (SU10) and autumn 2010 (AU10).

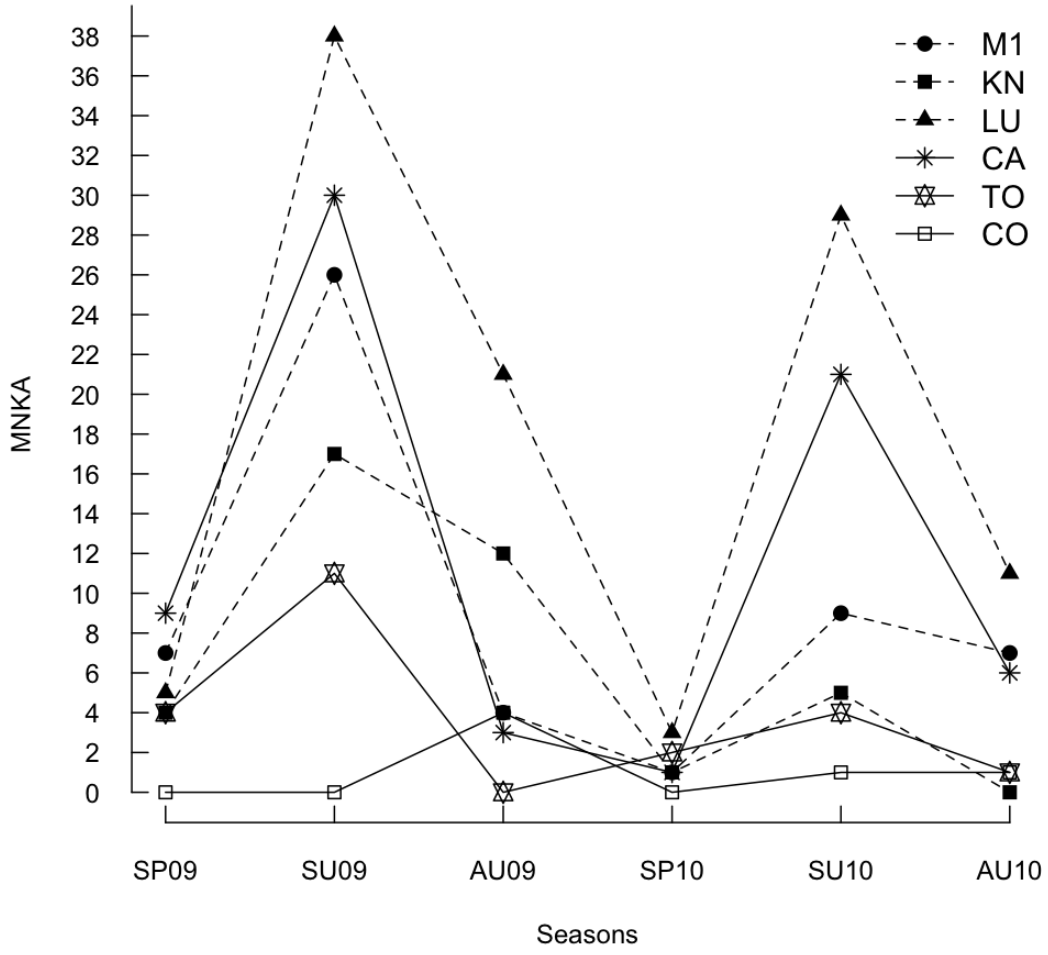


Figure 3-3: Seasonal population dynamics of bank voles across mainland (dashed) and island (solid) sites around Loch Lomond during 2009-2010. Season's represent trapping sessions within spring 2009 (SP09), summer 2009 (SU09), autumn 2009 (AU09), spring 2010 (SP10), summer 2010 (SU10) and autumn 2010 (AU10).

3.3.2 Rodent distribution within trapping grids

Despite overall lower populations sizes seen on island trapping grids, as described in 3.3.1, rodents may in effect experience similar densities if their distribution is clumped within the trapping grid. Indeed, the mean number of individuals per successful trap was not significantly different when comparing mainland and islands for either rodent species (**wood mice**- mainland: mean per trap= 1.5, island: mean per trap= 1.4, $t_{(224)}$=-0.591, p=0.555, **bank voles**: mainland: mean per trap= 2.0 , island: mean per trap= 1.7, $t_{(191)}$=-0.986, p=0.326). Thus, those parts of the island trapping grids that were inhabited by

rodents appeared to have similar numbers of individuals per unit area. By looking at the variance to mean ratio of captures per trap for wood mice (σ^2/μ =1.35 and 1.83 for mainland and islands, respectively) and bank voles (σ^2/μ= 1.94 and 2.39 respectively for mainland and islands) separately, it is seen that within the grid both species are aggregated within the trapping grid (σ^2/μ >1).

3.3.3 Sex ratios

In total, 59.3% of the wood mice caught were identified as male (182 of 307 wood mice) during the 2-year period from February 2009 until November 2010. On the mainland 54.6% were male (89 of 163), compared to 64.6% (93 of 144) on islands. There was no difference seen in the sex ratios between mainland and island populations for wood mice (best model: sex~1, z=(0.13, p=0.896) (Figures 3.2 & 3.3).

For bank voles in total 51.8% were male (171 of 330) caught within the same period as previously stated for wood mice. Bank vole sex ratios on mainland were 52% male (118 of 227) and on islands 51.5% (53 of 103). As before, none of the variables considered were found to cause significant deviations from the overall sex ratio (p>0.05), (Best model: sex~ 1, z=-0.385, p=0.7).

3.3.4 Age distribution

Across the populations of wood mice captured, 72% of individuals were adults, 17.8% were sub-adults and 10.2% were juveniles. Each individual age class was compared between mainland and islands sites The age structure of wood mice did not differ between fragmented and non-fragmented sites for any of the age classes (**adults**: χ^2 (N=387)= 0.002, p= 0.964; **sub-adults:** χ^2(N=265)= 0.221, p= 0.638 0.964; **juveniles**: χ^2(N=248)= 0.026, p= 0.871) (Figure 3.4). The same was true for bank voles (**adults,** 80.3%: χ^2(N=440)= 0.317, p=0.574; **sub-adults,** 12.3%**:** χ^2(N=274)= 2.666, p=0.103; **juveniles,** 7.4%: χ^2(N=262)=0.045, p=0.832) (Figure 3.4).

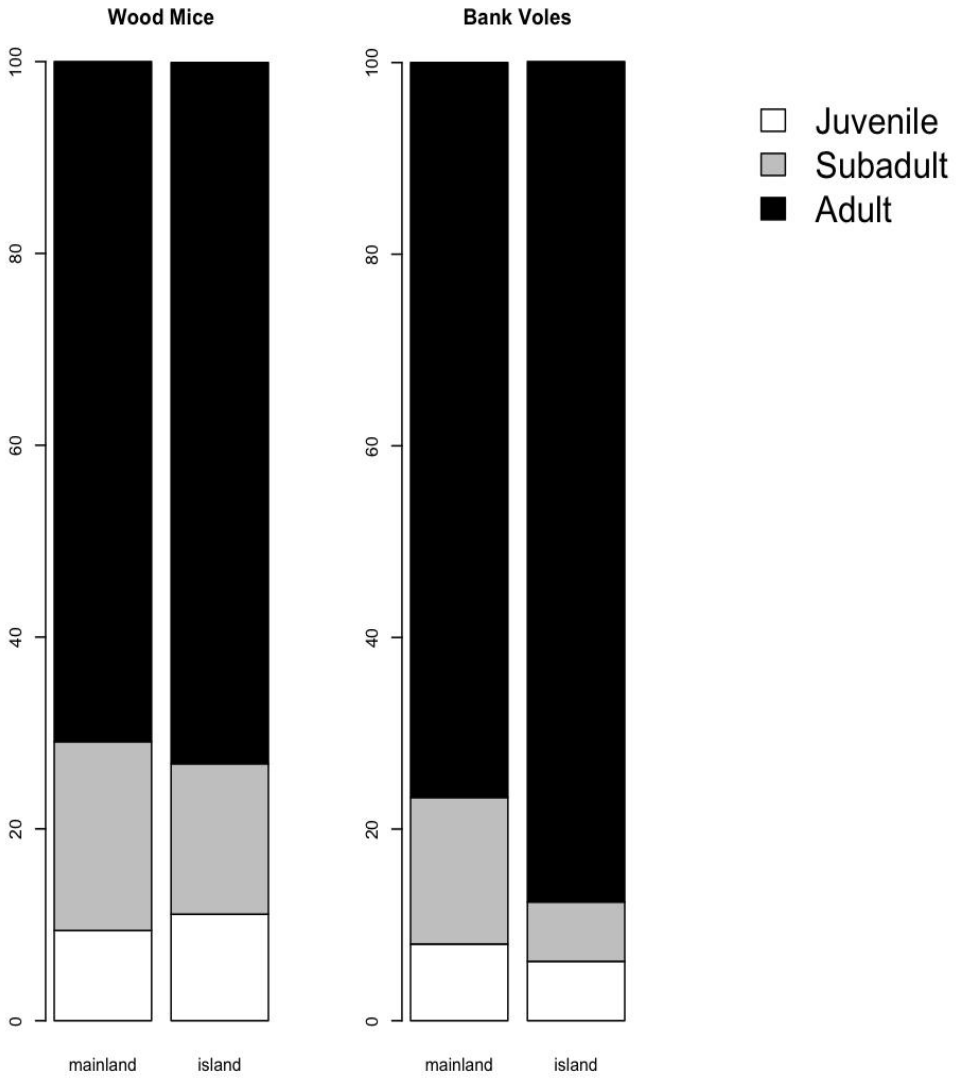


Figure 3-4: Mean proportion of the age structure of wood mice and bank voles, comparing non-fragmented (mainland) and fragmented (island) sites.

3.3.5 Differences in breeding status

Within the trapping sessions the average minimum length of the breeding season can be calculated. This is done by determining the number of days between the average first and average last day of individuals seen in breeding condition across a particular site.

3.3.5.1 Wood mice –males

Wood mice on mainland sites were observed from 9th March 2009 until 16th November 2010 during spring, summer and autumn. On island sites wood mice were observed from the 1st March 2009 until 4th November 2010. Table 3.3 shows the first and last days that potentially reproductively active males were detected within the trapping period. When comparing mainland and islands, breeding male wood mice on islands were detected 14 days earlier than males on the mainland (mean first day of reproductively active male mainland: = 19th March, island: 5th March, $t_{(182)}$=4.83, $p<0.05$). There was no difference between mainland and islands when males were last detected breeding (mainland: mean last day of reproductively active male = 26th October, island: last day of reproductively active male mean= 19th October, $t_{(182)}$= 1.27, p=0.22).

Table 3-4: Potentially reproductively active wood mice males, showing the first and last day of breeding and the average minimum length of the breeding season.

Sites	Potential reproductively active wood mice males		
	First breeding detected	**Last breeding detected**	Average minimum length of breeding season (days)
Mainland	10th March	15th October	225
Island	25th February	04th November	231

3.3.5.2 Wood mice- females

Pregnant wood mice are detected earlier in the breeding season on the mainland compared to those caught on the islands (mainland mean= 9th March, island mean=31st July, $t_{(125)}$=-41.74, $p<0.001$) (Table 3.4). However there was no significant difference seen on the mainland and islands in when the last pregnant female was detected (mainland mean=27th September, island mean=25th October, $t_{(125)}$=0.93, p=0.78).

Table 3-5: Pregnant wood mice, showing the first and last day of breeding and the average minimum length of the breeding season.

Sites	Pregnant wood mice		
	First breeding detected	Last breeding detected	Average minimum length of breeding season
Mainland	4th March	16th November	232
Island	24th August	5th November	86

3.3.5.3 Bank voles- males

Bank voles were observed on island sites from 25th February 2009 until 5th November 2010, whilst on islands bank voles were observed from 1st March 2009 until 27th October 2010. Reproductively active males on islands were detected 24 days earlier than those on the mainland (mainland: mean first day of reproductively active male = 25th March, island: mean first day of reproductively active male =2nd March, $t_{(171)}$= 5.34, p=0.0004), whilst males on the mainland were detected to be breeding significantly later (mainland: mean last day of reproductively active male = 5th August, island: mean last day of reproductively active male =25th July, $t_{(171)}$=2.41, p=0.02). Only one male bank vole was detected to be reproductively active during the summer trapping session (August) and none during the autumn (November) sampling period. Specifically, the last reproductively active male was detected on day 197 of the year.

Table 3-6: Potentially reproductively active bank vole males, showing the first and last day of breeding and the average minimum length of the breeding season.

Sites	Potential reproductively active bank vole males		
	First breeding detected	Last breeding detected	Average minimum length of breeding season
Mainland	10th March	15th November	140
Island	1st March	16th July	206

3.3.5.4 Bank voles –females

Bank vole females showed no significant difference in the first detected pregnant female within the trapping period (mainland mean=29th July, island mean=19th July, $t_{(159)}$= 1.51, p=0.14), or in the last detected pregnant female (mainland mean=19th August, island mean=28th October, $t_{(159)}$=-2.79, p=0.12).

Table 3-7: Pregnant bank voles, showing the first and last day of breeding and the average minimum length of the breeding season.

Sites	Pregnant bank vole		
	First breeding detected	Last breeding detected	Average minimum length of breeding season
Mainland	15th July	15th November	77
Island	29th July	27th October	100

3.3.5.5 Proportion of population breeding

There was no difference in the proportion of breeding males between mainland and island sites for either wood mice (best model: proportion breeding~1, z=-2.75, p= 0.006) or bank voles across the seasons (best model: proportion breeding~1,, z=0.843, p= 0.399) (Figures 3.5 and 3.6). There was also no difference in the proportion of female breeding wood mice (best model: proportion breeding~1, z=-1.96s, p=0.05) or female breeding bank voles (best model: proportion breeding~1,, z=-0.25, p= 0.803) (Figures 3.7 and 3.8).

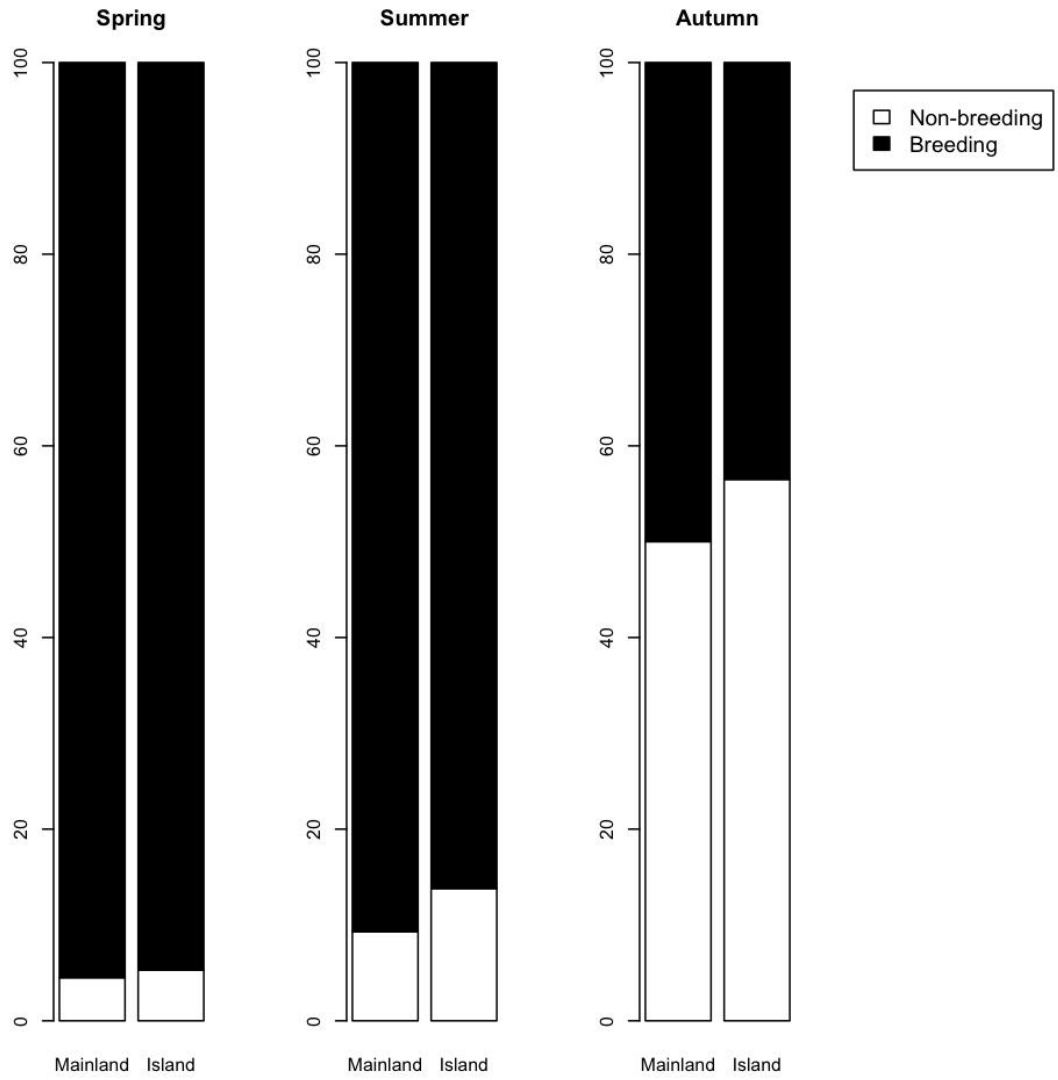


Figure 3-5: Proportion of male wood mice individuals breeding across pooled mainland and island sites for 2009 and 2010 combined. Breeding males (black) were defined by showing descended testes.

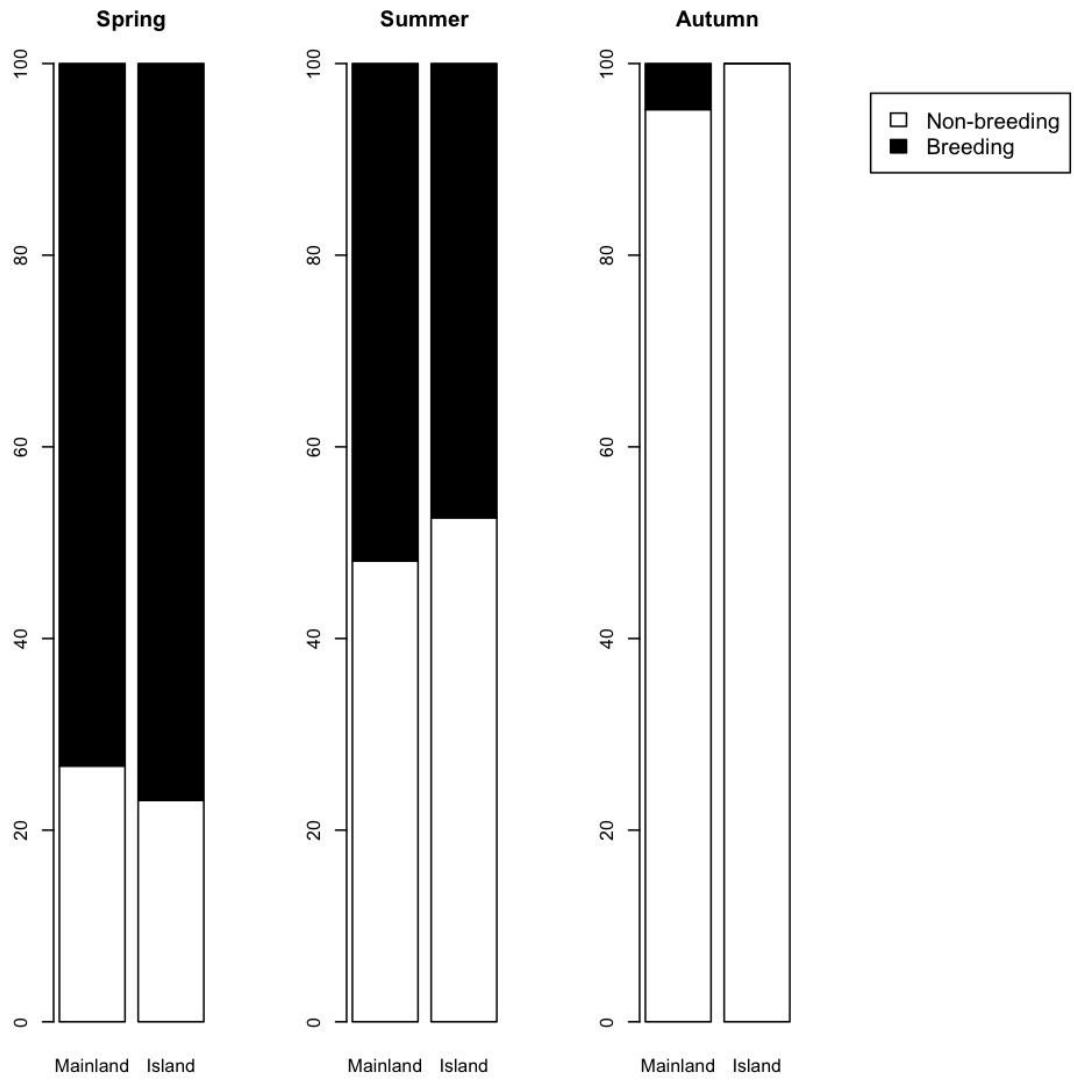


Figure 3-6: Proportion of male bank vole individuals breeding across pooled mainland and island sites for 2009 and 2010 combined. Breeding males (black) were defined by showing descended testes.

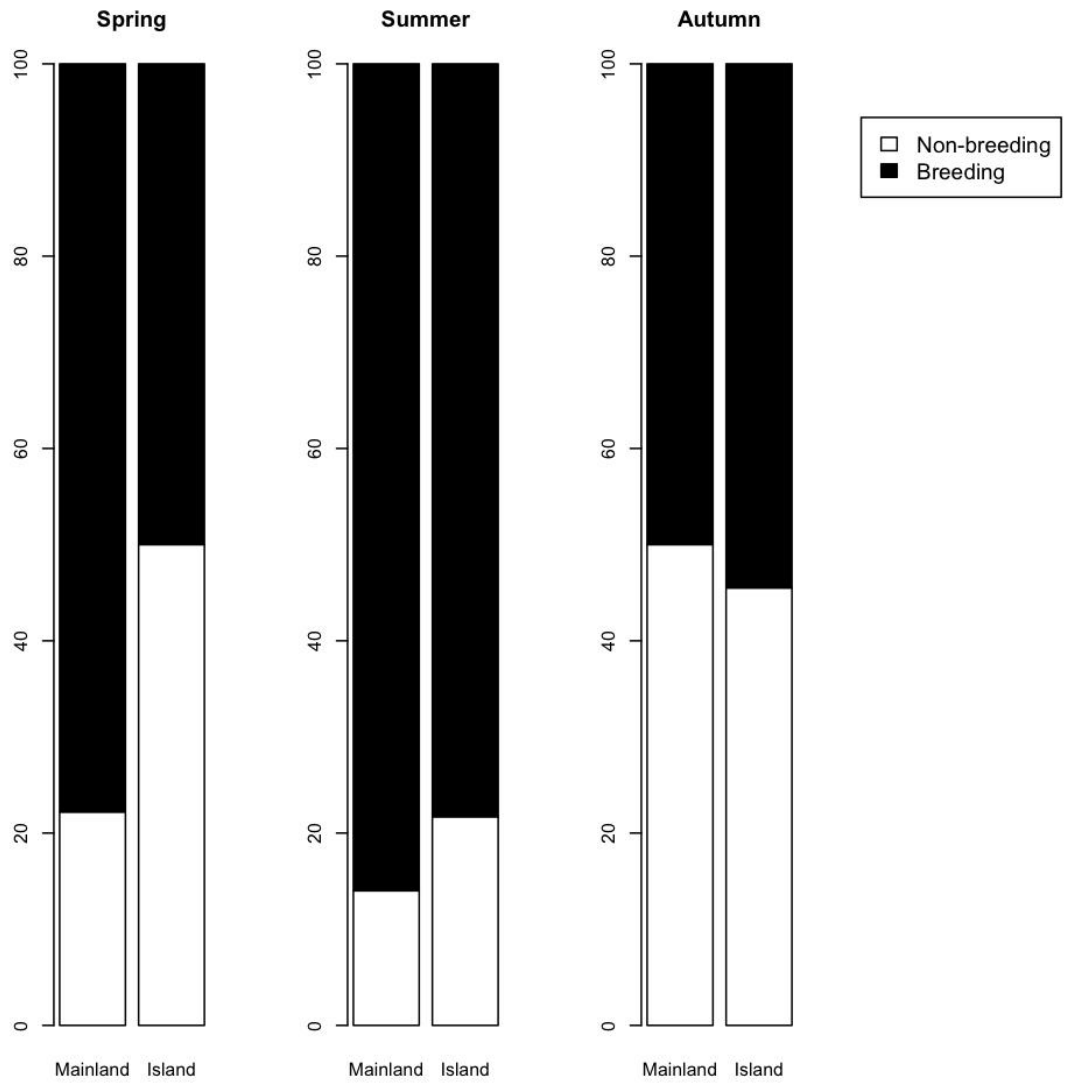


Figure 3-7: Proportion of female wood mice individuals breeding across pooled mainland and island sites for 2009 and 2010 combined. Breeding females (black) were defined by showing one or more of the following characteristics: gestating, perforated vulva, cervix at least partially open, nipples visible.

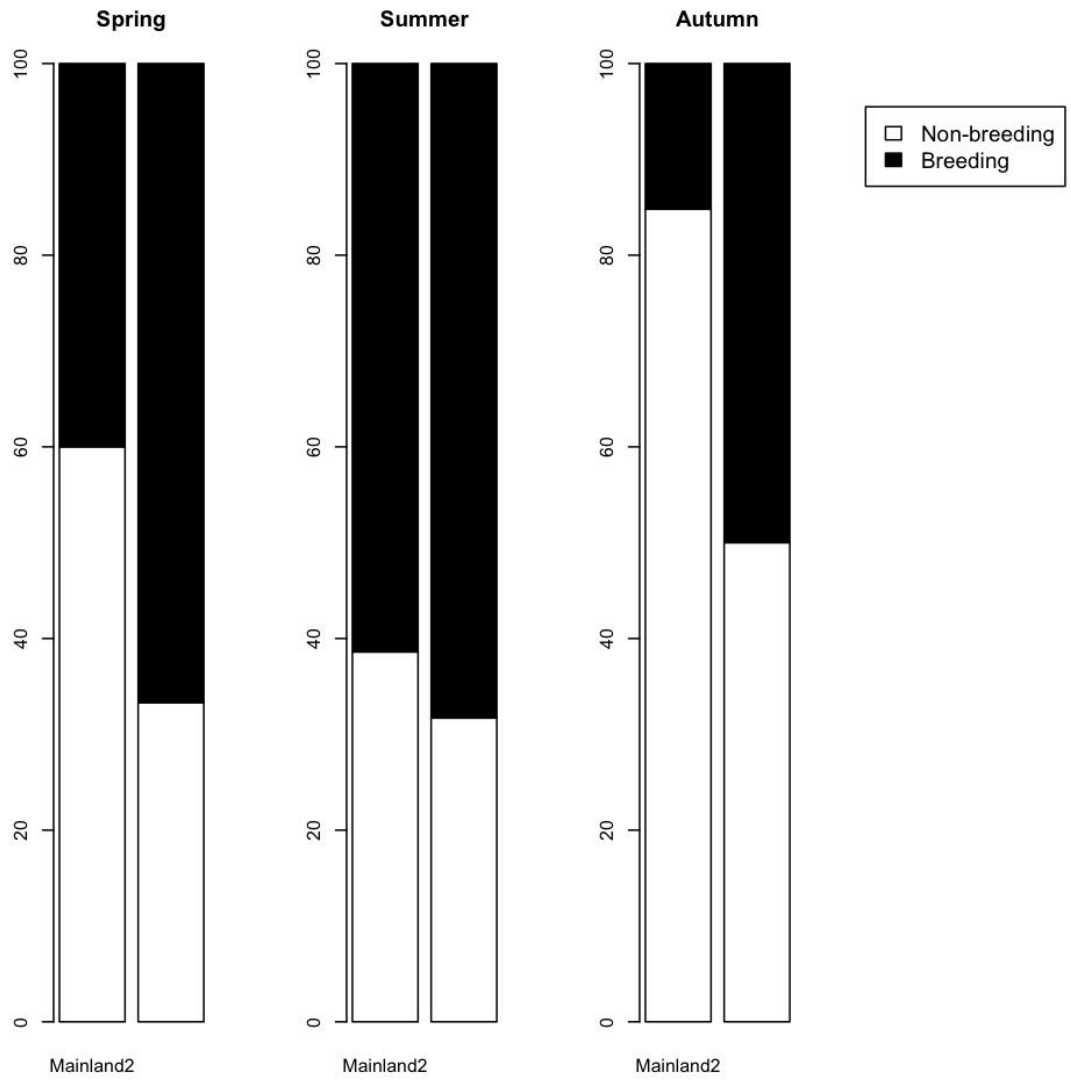


Figure 3-8: Proportion of female bank vole individuals breeding across pooled mainland and island sites for 2009 and 2010 combined. Breeding females (black) were defined by showing one or more of the following characteristics: gestating, perforated vulva, cervix at least partially open, nipples visible.

3.3.6 Body condition

The body condition of rodents was examined separately for wood mice and bank voles. For either species of rodent, there was no difference in the hip scores and therefore the conditioning factor between fragmented (island) and non-fragmented (mainland) populations (Wilcoxon's rank test: wood mice: $w_{(9)}$=225, p=0.827, bank voles: $w_{(9)}$=175.5, p=0.609) (Figure 3.9 and 3.10).

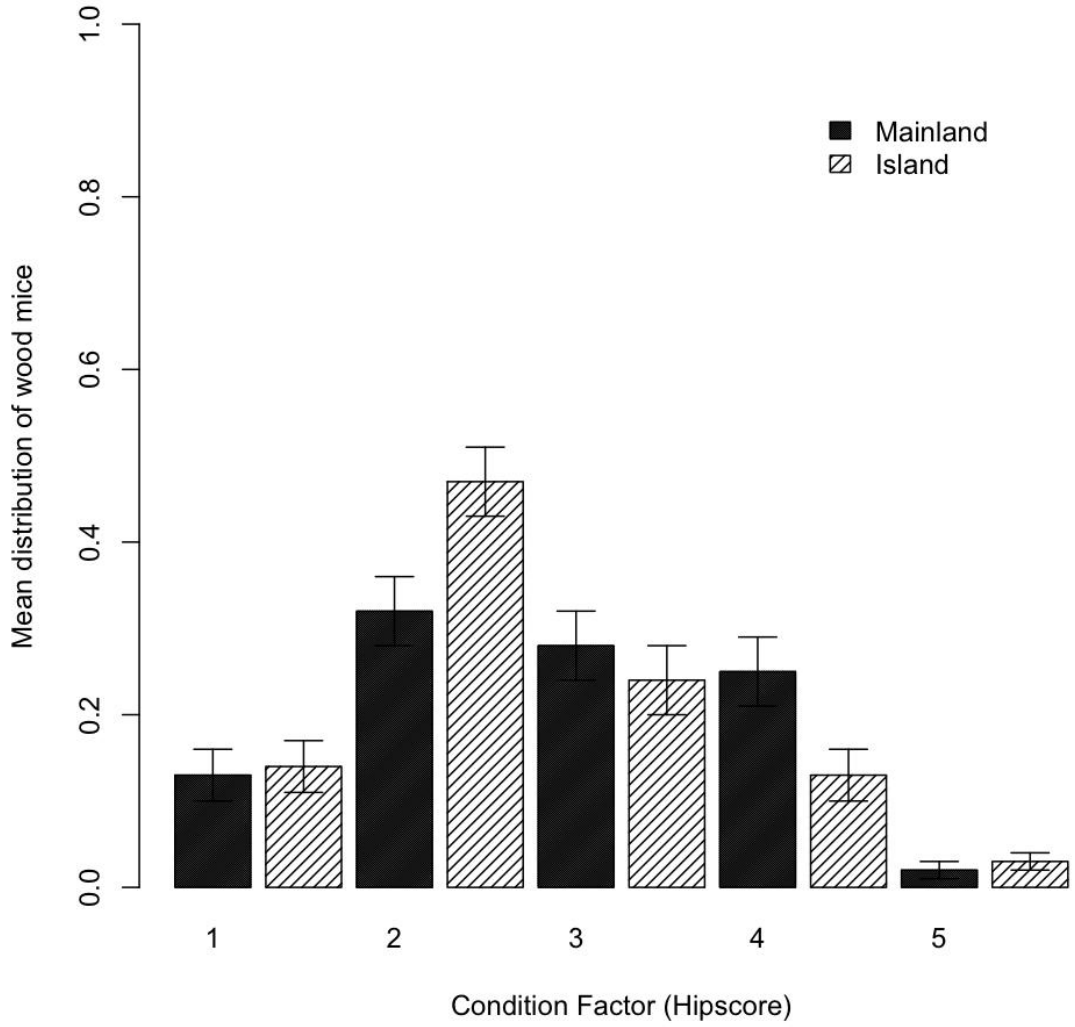


Figure 3-9: The distribution of the five-hipscore categories of wood mice, comparing mainland and island sites.

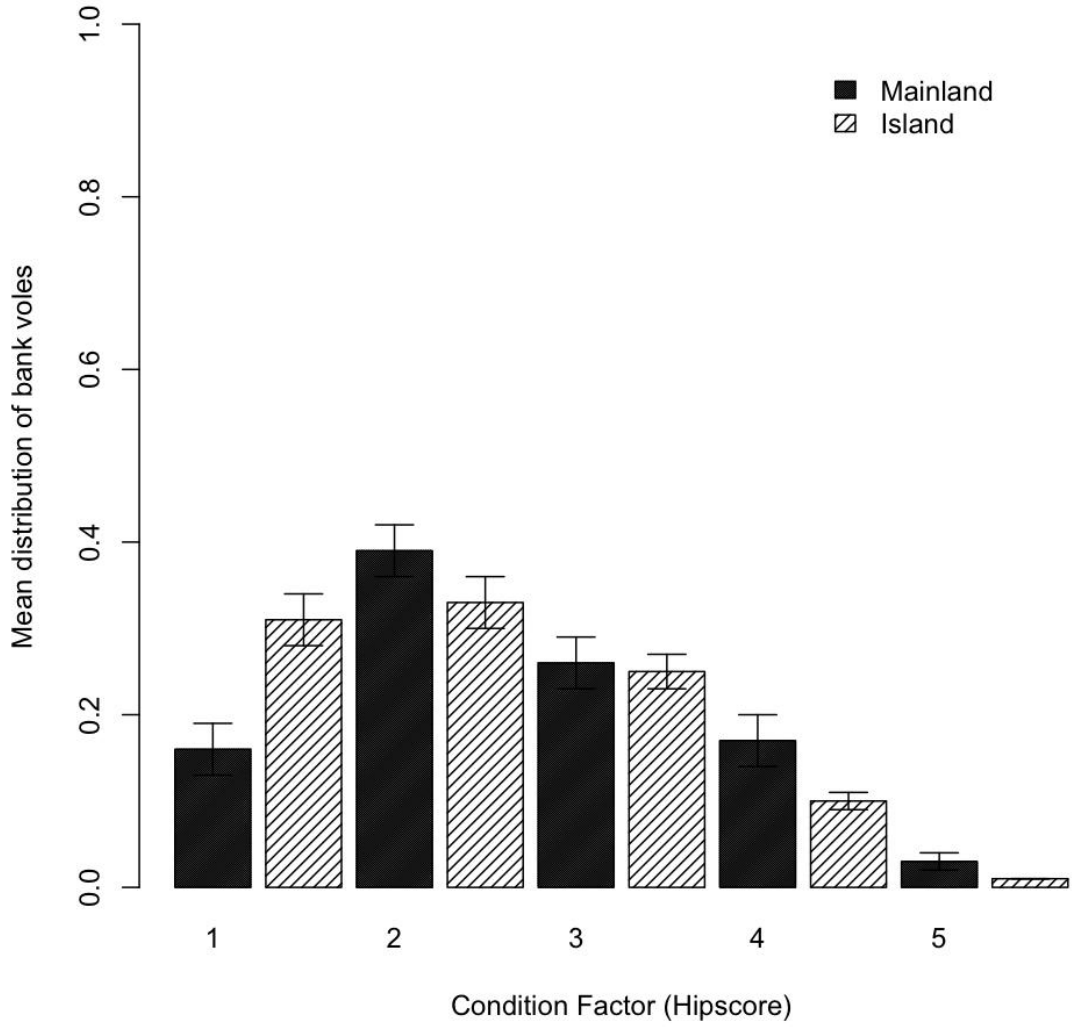


Figure 3-10: The distribution of the five-hipscore categories of bank voles, comparing mainland and island sites.

3.3.7 Movement data

Mark-recapture data from 38 wood mice and 62 bank voles were analysed to investigate distance moved within the 10 by 10-metre trapping grid. The average maximum distance moved by wood mice was more than twice as high on islands compared to mainland grids (mainland: mean=22.1m ± standard error (SE) 5.4, island: mean=50.0,± SE 9.0, $t_{(38)}$=-2.26, p=0.031) (Figure 3.11). In contrast, the average maximum distance moved by bank voles did not differ on mainland grids compared to islands (mainland: mean=27.8m ± SE 4.7, island:

mean=23.7m ± SE 4.6, $t_{(62)}$=0.585 p= 0.561) although higher maximum distances were seen on mainland sites (113.1m) than islands (72.8m) (Figure 3.12).

There was no significant difference in sex ratios of recaptured individuals on mainland and islands in either species, and controlling for sex had no statistical effect on movement characteristics (data not shown).

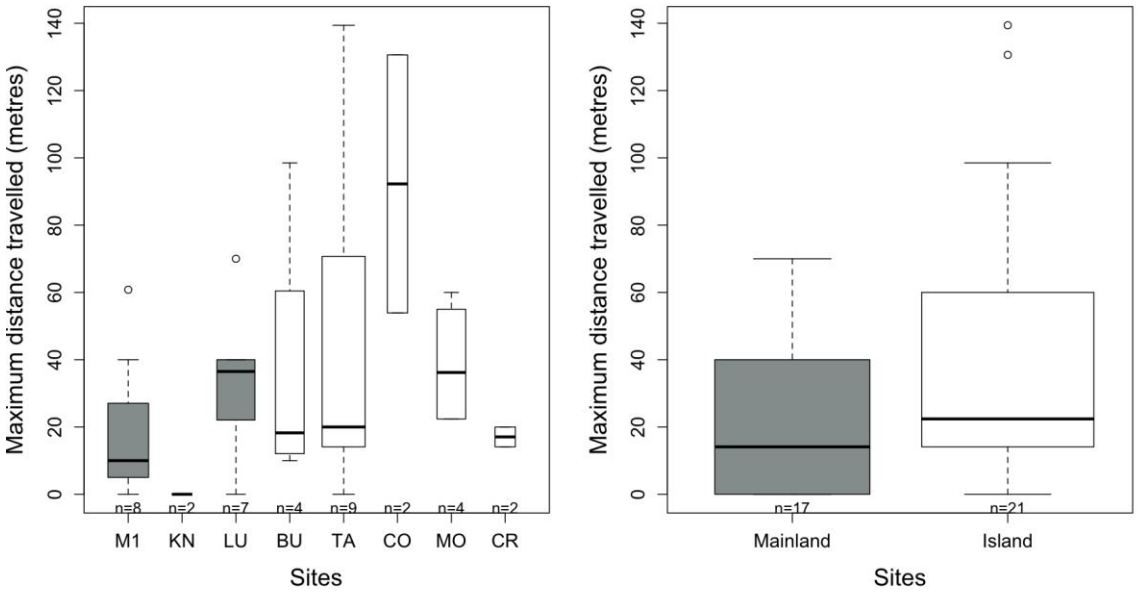


Figure 3-11: The distribution of maximum distances travelled (in metres) within the trapping grid by recaptured wood mice across all sites (left) and comparing mainland and islands (right). The widths of the boxes are proportional to the square root of the sample size. The outliers are 1.5 times the inter quartile range above the upper quartile and below the lower quartile.

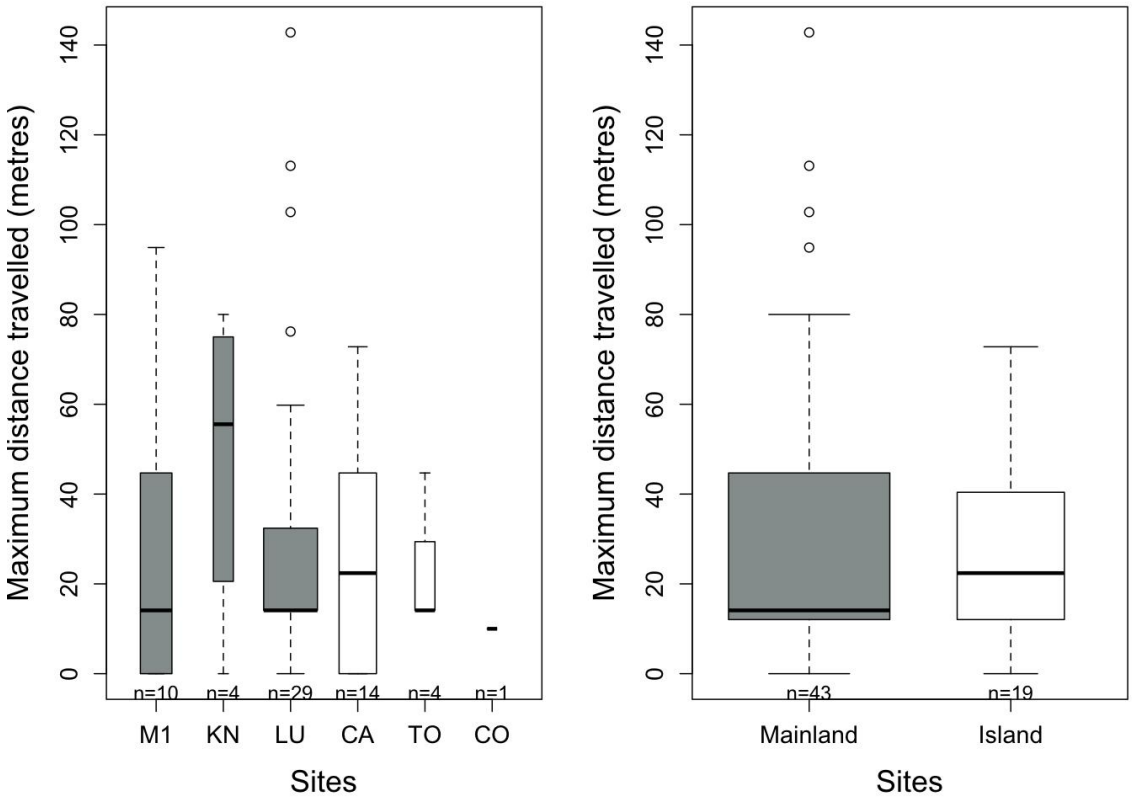


Figure 3-12: The distribution of maximum distances travelled (in metres) within the trapping grid by recaptured bank voles across all sites (left) and comparing mainland and islands (right). The widths of the boxes are proportional to the square root of the sample size. The outliers are 1.5 times the inter quartile range above the upper quartile and below the lower quartile.

3.3.8 Apparent survival

Numbers of recaptured individuals were low throughout this study (wood mice= 15.5%, 50 of 322 captures, bank voles= 24.1%, 83 of 345 captures), with the majority of recaptures occurring within the same trapping session). Therefore I was unable to properly estimate survival rates for the two rodent species across the sites. However, as an index of apparent survival, the proportion of captured individuals that had been previously marked may be used. For wood mice on the mainland, the proportion of captures involving individuals marked at any previous trapping session was 13.2% (23 of 174 captures) and for islands 18.3% (27 of 148 captures). For bank voles, the

proportion of recaptures on the mainland was 26.6% (63 of 237 captures), compared to 18.5% (20 of 108 captures) on islands. The time between recaptured individuals between sessions can also be reported for each rodent species. For wood mice the mean time between recapture events did not differ between fragmented and non-fragmented populations (mainland: mean=28.2 days ± standard error (SE) 21.6, island: mean=62.3 days,± SE 21.2 $W_{(50)}$=158.5, p=0.539). The time between recaptured bank voles did also not differ between fragmented and non-fragmented populations (mainland: mean= 82.1days ± standard error (SE) 17.0, island: mean= 68.9 days,± SE 26.9, $W_{(171)}$=498.5, p=0.386). While not actual estimates of survival, these data do not suggest differences in apparent survival between mainland and islands for either species.

3.4 Discussion

In my study, the fragmented landscape of the islands had little effect on the demographic characteristics of rodent populations in comparison to those on the mainland. This is in contrast to previous studies on fragmented populations (Table 3.1).

For both wood mice and bank voles, I found no difference in age structure between fragmented and non-fragmented sites. Additionally the greatest fraction of rodents caught were adults across all populations examined. The age structure seen on islands is more akin to that expected in well-connected habitats. One reason for this maybe that the fragmented habitats within this study are islands, therefore the chances of new young recruits immigrating is reduced by the nature of sites being separated by unsuitable habitat (water). Previous studies on the effects of fragmentation (Saunders, Hobbs et al. 1991; Alder 1994; Holland and Bennett 2010) have examined populations in forests where recruits may be immigrating from neighbouring fragments or from the sub-optimal matrix habitat surrounding fragments. In my study I found no evidence of any individuals moving between islands, which is not surprising given the nature of the sites.

In small mammal populations, adults, in particular males, are typically the most frequent age classes (Krebs 2003). Sex ratios for both species of rodents showed no significant difference between the mainland and island sites. Previous studies have shown that males may inhabit areas that have poorer habitat quality more readily than females (Bowers and Smith 1979). Many fragmentation studies focus on species for which fragments constitute habitat of lower quality (Puttker, Meyer-Lucht et al. 2008; Haapakoski and Ylonen 2010). I do not have any direct way of assessing habitat quality on my sites but lower rodent densities on islands suggest that some differences may exist (see below). Despite this suggestion, sex ratios on islands were indistinguishable from those on the mainland and thus do not support the idea that islands represent marginal habitat colonised by a higher proportion of males. Mortelliti, Amori et al. (2011) argues for a greater distinction between habitat loss and fragmentation, as the latter may not necessarily reduce habitat quality for some species.

Previous work reported an effect of patch size on rodent survival in fragmented habitats (Holland and Bennett 2010), with adult males surviving better in larger fragments. Estimating survival of the rodent populations examined in my study was difficult due to the low number of recaptured animals. However neither the proportion of recaptured individuals nor the length of time until the same individual was caught again differed between fragmented and non-fragmented sites in the study area. This suggests that apparent survival and longevity were overall similar, even if their determinants, such as resource availability or predation pressure, may be variable among sites. An argument could be made that habitat quality does indeed differ between fragmented and non-fragmented sites, as the population density of rodents is lower on islands. However, no other rodent characteristic infers any such difference. If habitat quality did indeed differ then you would expect body condition of rodents on islands to be lower when compared to those of the mainland, and as already stated this is not the case. A case could be made for space as a limited resource on islands, as by nature they are smaller and not well connected to other fragments unlike the mainland sites.

Whereas the majority of the demographic parameters examined did not differ between continuous and non-continuous habitats, some did differ. Rodent numbers on mainland trapping grids, and thus inferred densities, were higher compared to islands. This could be due to a mainland site being quicker to recover from temporary population size reduction because of higher immigration (Dias 1996). In contrast, isolated islands may stay at lower densities for longer than those on the mainland, because all the recovery has to be internally generated without augmentation of recruits from outside, however this was not evident from the data collected within this study. At the same time, such effects could be countered by a potential fence effect on the islands (Krebs, Keller et al. 1969), which might cause a build up of individuals that are unable to emigrate due to water acting as a strong barrier to dispersal. Overall, lower population densities on islands suggest that these provided large enough suitable habitat for rodents to disperse into when the 'site' becomes too dense. However when looking at the trapping grid specifically, there was no difference observed in the number of individuals caught per successful trap within the grid and that throughout the grids across all sites, rodents showed an aggregated distribution.

Temporal variation in breeding season lengths is a natural occurrence within rodent populations, but in temperate regions even isolated populations tend to be synchronised (Alder 1994), as although isolated they still are experiencing the same environmental conditions (such as extreme weather events) that would affect their demographics, therefore allowing synchronicity in the breeding status. There were differences in breeding parameters between mainland and island sites. Male wood mice and bank voles in breeding condition were detected earlier on islands. This maybe due to male rodents roaming further on island sites and therefore increasing their capture rate, rather than actually reaching a reproductive state earlier. This is plausible as there is a lower population density on island sites and therefore adult dominant males are able to have larger territories compared to those on the mainland. Small sample sizes don't allow for detailed analysis to look for patterns in movement between sexes. However the indication is that male wood mice move further on the islands compared to the mainland sites, whilst the opposite is true for bank voles. In either case, there was no evidence that reproduction itself commenced earlier on islands, since females of both species were not seen to be pregnant

earlier there (female wood mice bred longer and earlier on the mainland) compared to mainland sites. No difference in the proportion of breeding wood mice of either sex was observed.

Movement of rodents within a habitat is often driven by the need to forage for food or for suitable mating opportunities (Burge and Jorgensen 1973; Benhamou 1990). Levels of movement can thus be positively or negatively affected for rodent populations in fragmented habitats, depending on other factors such as habitat quality, food abundance and mating opportunities. Wood mice on islands travelled a greater range of distances than those on the mainland, as well as having a greater average maximum distance travelled (Figure 3.12). A lower population density exists on islands than is seen on the mainland. Wood mice have a social organisation of a group structure with a dominant male, and a few subordinate males and females, with females will defend their breeding ranges (Corbet and Southern 1977). Therefore it maybe that wood mice of islands are less restricted by other wood mice territories allowing them to roam wider. In contrast, bank voles did not differ in the average maximum distances travelled when comparing island and mainland sites. Bank voles have a social organisation that differs seasonally, with intersexual groups of close kin forming during winter (Kruczek and Styrna 2009). They also tend to live more closely (Kruczek and Styrna 2009) than wood mice, which could be why bank voles were not seen to be moving a greater distance of the islands as wood mice did. Nor is the sex ratio significantly different on the islands than on the mainland and therefore the abundance of mating opportunities would not seem to be reduced within island populations, however rodent population densities are lower on islands compared to mainland sites. This does not seem to affect the sex ratio between fragmented and non-fragmented sites.

Any potential changes in the demographics of one species have the potential to alter their interactions with other species in the same system, including host-parasites interactions. The majority of the rodent demographic parameters investigated within this study did not differ between fragmented and non-fragmented sites. This therefore excludes a number of potential drivers for differences in host-parasite dynamics between populations such as sex-

(Harrison, Scantlebury et al. 2010; Luong, Perkins et al. 2010) or age- (Gillespie, Lonsdorf et al. 2010; Harrison, Scantlebury et al. 2010; Liberman, Khokhlova et al. 2011) specific differences in susceptibility which could result in differing prevalence's between connected and non-connected populations. Similarly, we would expect no differences in infection levels due to more individuals breeding (Luong, Perkins et al. 2010) or being in poorer body condition (Beldomenico, Telfer et al. 2009), both of which can increase an individuals susceptibility to infection. However, lower population density on islands has the potential to reduce infection rates for parasites where transmission is dependent on host density, as is generally assumed for non sexually transmitted diseases. This, combined with a more defined upper population size limit on islands could potentially lead to extinction of parasites, consistent with the idea of a critical host community size (Lloyd- Smith, Cross et al. 2005). At the same time, similar numbers of captures per successful trap on the trapping grids suggests that contact rates among rodents may not necessarily be lower on islands.

The data and analysis presented within this chapter will allow us to address the broader question of how parasites dynamics are affected by the spatial structure of a host population, as will be explored in the next chapter.

4 Consequences of natural habitat fragmentation on the dynamics of rodent parasites

4.1 Introduction

Host spatial structure has the potential to change host-parasite interactions in a variety of ways. One general consequence of small isolated habitat fragments tends to be small populations sizes (Kruess and Tscharnke 1994; Pullin 2002; Ameca y Juarez, Mace et al. 2011). Therefore, overall parasite distribution and prevalence may be limited because parasites become locally extinct or even fail to colonize small isolated host populations. Parasites in small habitat fragments may have reduced access to susceptible hosts because the chances of encounter are greatly reduced compared to more connected host populations. Studies have shown that transmission rates are increased when host population densities are high (Morand and Poulin 1998; Puttker, Meyer-Lucht et al. 2008). However other studies have shown that hosts within a fragmented habitat have a higher prevalence of infection, perhaps because hosts respond to fragmentation by increasing home range size (Suzan, Armien et al. 2008).

There are genetic implications of fragmentation that could increase the likelihood of extinction for both host and parasites. Small isolated populations have an increased chance of inbreeding. This can reduce both fitness and genetic variation within the population. Therefore small isolated host populations are at a greater risk from long-term extinction in the face of environmental change, such as climate change or disease (Keyghobadi 2007). However the same genetic effects could apply to the parasites themselves, and parasites could be unable to find and infect a host in discontinuous populations. Specialist parasites that cannot infect their specific hosts are unable to switch to an alternative host and are therefore at a higher risk of extinction than generalist parasites.

There is limited research on how landscape heterogeneity affects diseases or pathogens (Ostfeld, Glass et al. 2005), especially for wildlife systems. Therefore there is a need to carry out field studies in natural populations in order to better understand how relevant landscape structure is in disease ecology. There have however been a few studies of how landscape affects disease dynamics in fragmented populations. One study on large blue butterflies and their parasitoid wasp showed that the parasitoids were more affected by

habitat fragmentation than their hosts, due to the nature of the mode of transmission of the parasitoid (Anton, Zeisset et al. 2007). Alternatively, parasites may become more prevalent in a fragmented population as hosts may increase their home ranges (Gillespie, Chapman et al. 2005; Suzan, Armien et al. 2008), have a higher population density or indeed have a greater number of host species that are generalists (Vaz, D' Andrea et al. 2007; Puttker, Meyer-Lucht et al. 2008). By being a generalist host, this can potentially increase parasite prevalence as hosts are less restricted by habitat and/or food preference than specialist hosts. All of these factors play a potential role in the differences seen between fragmented and non–fragmented landscapes.

Depending on their life history, parasites may be affected by fragmentation differently. Those parasites with a direct lifecycle could be predicted to be less likely to be affected by local extinctions compared to those undergoing an indirect lifecycle (i.e. involving intermediate hosts). Furthermore those parasites that rely on a vector for transmission may be disadvantaged in small host populations due to the chances of local extinction of vector or alternative hosts, therefore leading to a disruption in the mechanisms for transmission. However this will depend on how closely the vector is associated with the host. In our study system, both the tick *Ixodes trianguliceps* and fleas are found within the burrows of their rodent hosts and therefore encounter them on a frequent basis. On the other hand, *Ixodes ricinus* is more opportunistic, and the prevalence of it will depend heavily on the presence of alternative hosts. Some viruses and bacteria rely on such vectors; therefore there is the potentially that parasites will follow the same transmission patterns as their vectors.

In order to understand the relationship between fragmented populations and their parasites you need a study system that is fragmented and is inhabited by a population that can potentially become infected with a variety of parasites. Fragmentation is the break up of continuous habitat into smaller areas, with a reduction in patch size and therefore an increase in distances between these patches (Andren 1994). Although this can be a natural process, there is great current concern about its increase by anthropological factors. A number of studies (Allan, Kessing et al. 2003; Gillespie, Chapman et al. 2005; Puttker, Meyer-Lucht et al. 2008; Puttker, Meyer-Lucht et al. 2008; Suzan, Armien et al.

2008) have focused on the problems of anthropogenic fragmentation on host biodiversity and parasite dynamics. However less research has been conducted on natural fragmentation and the role it has. Natural fragmentation has taken place on a geological time-scale and has occurred naturally mainly due to glacial and volcanic activity. This difference in time-scale compared to human-driven fragmentation may lead to quite different consequences for a range of host-parasite dynamics. The formation of islands is a form of natural fragmentation, providing an ideal situation to study these processes. Most of the studies discussed above have used rodents as a model species. The advantages of using rodents to study parasite dynamics is that they are wide ranging, are relatively easy to trap and to handle and have well-described parasite species; most of which are not fatal to the host. Therefore by using rodents on a naturally fragmented island system we are able to gain a greater understanding of the role natural fragmentation may have on parasites.

Previous studies investigating rodent parasite dynamics in a spatially fragmented system have used small island systems (Begon, Hazel et al. 2003) or forest fragments (Puttker, Meyer-Lucht et al. 2008). However these studies differ from ours as the island system used here involves larger islands and has a greater distance between each of the islands. The second of these factors is particularly important because the chances of rodents moving from one island to another are greatly reduced, compared to the system used by Begon *et al.* (2003), therefore isolation plays a stronger role in our study compared to previous work. Furthermore Puttker, Meyer-Lucht & Sommer (2008) studied three rodent species and two marsupial species. Within our study system we only have two common species of rodents, and even then only one species was present on most of the island sites. Our very low host species diversity should make it a particularly powerful test of the effects of interest, because the chance of parasites being able to survive within our system is greatly reduced due to the possible limiting factor of available hosts. Additionally Begon et al. (2003) only investigated one parasite species (cowpox) whereas our study looks at a broad spectrum of parasite species over three functional groups (ectoparasite, gastro-intestinal parasites and microparasites) that are known to infect wild woodland rodents.

Data is presented here from a naturally occurring island system and surrounding mainland sites that are inhabited by wood mice, *Apodemus sylvaticus*, and bank voles, *Myodes glareolus*. Both species are naturally occurring at these study sites. These species can be host to a number of parasites and I investigated a range of ectoparasites, microparasites and gastro-intestinal parasites. In particular, I addressed the following questions:

1. Do host populations on islands support fewer parasite species than mainland populations?
2. Is the potential absence of parasite species from island populations consistent with predictions based on their life history?
3. Is the prevalence of infection with parasites in island populations different from that on the mainland?

4.2 Methods

A detailed description of the fieldwork carried out is provided in chapter 2 General Methods.

Rodent trapping took place around Loch Lomond over a 2-year period between February 2009 until November 2010, with each mainland (3 sites) and island (7 sites) visited once during spring (February-April), summer (June-August) and autumn (October-November). One hundred live Sherman traps were placed 10 metres apart within a permanent 10 by 10 grid in forested or otherwise suitable rodent habitat. The smallest island Bucinch, had a larger trapping grid of 20 by 20, with 200 traps that encompassed the whole of the island. For each trapping session, traps were pre-baited the first day, set the following evening, and then checked for captures at dawn and dusk for a period of 3 consecutive days. All traps were removed after the last check had been completed on day 5, cleaned and autoclaved before being moved to the next site.

Rodents caught were marked subcutaneously with a unique Passive Integrated Transponder (PIT) tag (AVID plc, East Sussex, UK) on the first capture; recaptures were then detected using a handheld reader (Minitraker I, AVID plc, East Sussex, UK). For first captures, species, sex, mass, reproductive status, age

(based on mass) and body condition of the animal were recorded. These measurements were recorded again if any animal was re-caught in subsequent trapping seasons.

Rodents were examined for ectoparasites around the face and ears and by blowing over the body against the direction of the hair. The number of each group of ectoparasites (ticks, fleas, mites) was recorded. All fleas and a sub-sample of ticks were removed with tweezers and stored in 70% ethanol. A small amount of blood (< 20uL) was taken from the tail vain. Rodents were subsequently released at the site of capture. All used traps were taken back to the laboratory in order to remove the faeces for parasite detection.

Low capture rates prevented the use of mark-recapture abundance estimators. Abundance for both rodent species was therefore quantified using the minimum number known alive (MNKA) index, defined as the total number of individuals caught within a given trapping session plus those not caught in that session but caught both previously and subsequently (Krebs 1966).

Ectoparasites were identified based on morphological characteristics (Arthur 1963; Whitaker 2007; George 2008). Microparasites were identified in one of two ways depending on the group they belonged to. After separating rodent blood samples, sera were tested for cowpox and Murid Gammaherpes 4 viruses using immunofluorescence (IF) assays (Bennett, Crouch et al. 1997). DNA was extracted from blood clots using the Qiagen DNA Easy kit, according to the manufacturers protocol, and tested for *Bartonella* infection using primers and protocols provided by R. Birtles (pers. comm.). Infection with gastro-intestinal parasites was determined from faecal samples by a salt floatation technique (Slater and Keymer 1986; Brown, Macdonald et al. 1994), using morphological features to distinguish species or species groups.

All parasites, were recorded as present, not detected with low confidence ($p>0.05$), or not detected with high confidence ($p<0.05$) Confidence of detection was determined based on the average prevalence of infection. Specifically, if you sample n individuals in a population and all are negative for presence of the parasite then (assuming the individuals are independent in terms of risk of parasitism, and that parasites occur at average prevalence) the probability of

observing that outcome by chance is $(1-p)^n$, where p is the probability of an individual being infected. Therefore you can classify apparent absence as strongly supported if the chance of no detection due to sampling is <0.05.

Mean prevalence of infection (P%) was established for each parasite species across all sites and all seasons. A generalized linear mixed model (GLMM) using a binomial response was used to test for prevalence of infection (prevalence) as a function of mainland/Island, year, mainland/island*year, season, and mainland/island*season, with site as a random factor, using the function *'lmer'* in the 'lme4' library of *R* (The R Foundation for Statistical Computing; http://www.r-project.org/). Based on the global model that contained all the terms, log likelihood ratio test along with Aikaike's Information Criterion (AIC) was used to determine the best model.

4.3 Results

4.3.1 Parasites detected

Table 4.1 provides an overview of the different parasites detected within the study, along with life history information. Seventeen species of parasites were identified that fell into seven groups. Among the ectoparasites, two species of tick and six species of fleas were identified. Evidence for infection of microparasites with both viruses (cowpox, Murid gamma herpes virus -4) and bacteria (*Bartonella*) was found. The majority of gastro-intestinal parasites present were four species of nematodes, with the exception of *Rodentolepis*, a cestode, and *Eimeria* species, protozoan. All of these parasites are known to infect vertebrates, and have a range of host niches; all of these parasites are common to woodland rodent species. They have a range of transmission modes, however contact with the host in some form is the overall connecting factor. There is little documented evidence of how long theses parasites will live outside of their hosts; often the information available only provides the minimum time a parasite has been found to survive out with its host. However

more information is available on seasonal dynamics of these parasites (see Table 4.1 for details), with most tending to have a seasonal peak.

Table 4-1: Parasite species and their characteristics

Parasite Species	Group	Host Range	Host Niche	Evidence	Transmission mode	Documented ability to survive outside of host	Documented seasonality
Ixodes ricinus	Tick	Vertebrate	Ectoparasite	Observations in field	Environmental (Broad[1])	At least 1 year	Summer and autumn peaks [3, 4]
Ixodes trianguliceps	Tick	Small mammals	Ectoparasite	Observations in field	Environmental (Restricted[2])	At least 1 year	Larval – autumn and winter. Nymphs – summer Adults – year round [5, 6].
Fleas	Flea	Small mammals	Ectoparasite	Observations in field	Environmental (Restricted)	At least 1 year	Yes, variable between species [7, 8]
Cowpox	Virus	Vertebrate	Respiratory tract and lymphoid tissue [10]	Serology	Direct contact	Unknown	Peak in autumn during breeding season [9]
Murid gammaherpesvirus 4	Virus	Rodent	Respiratory tract and spleen [11]	Serology	Direct contact	Unknown	No. However lack of studies to support it
Bartonella species	Bacterium	Rodent	Blood-borne	PCR	Vector	Within vector	Variations between species [7, 10]
Heligmosomoides polygyrus (glareolus)	Nematode	Rodent	Gastro-intestinal	Faecal analysis	Direct	Unknown	Spring and summer peaks [12, 13, 14]
Capillaria	Nematode	Vertebrate	Gastro-intestinal	Faecal analysis	Direct	Unknown	Spring and summer peaks [12, 13,14]
Trichuris	Nematode	Vertebrate	Gastro-intestinal	Faecal analysis	Direct	Unknown	Spring peak [12, 13,14]
Eimeria species	Protozoan	Rodent	Gastro-intestinal	Faecal analysis	Direct	Unknown	Spring and summer peaks [12, 13,14]
Rodentolepis spp	Cestode	Rodent	Gastro-intestinal	Faecal analysis	Direct and indirect	Unknown	Unknown
Syphacia spp	Nematode	Rodent	Gastro-intestinal	Faecal analysis	Direct	Unknown	Autumn peak [16]

[1]Environmental (broad) – Vector is found across the environment

[2] Environmental (restricted) – Vector is found within a restricted area in nests and burrows

[3] (Lees and Milne 1951)

[4] (Randolph, Green et al. 2002)

[5] (Bown, Begon et al. 2003)

[6] (Randolph 1975)

[7] (Telfer, Begon et al. 2007)

[8] (Monello and Gompper 2010)

[9] (Hazel, Bennett et al. 2000)

[10] (Telfer, Lambin et al. 2010)

[11] (Telfer, Bennett et al. 2007)

[12] (Gregory, Montgomery et al. 1992)

[13] (Taylor, Coop et al. 2007)

[14] (Montgomery and Montgomery 1988)

[15] (Fuller 1996)

[16] (Behnke, Bajer et al. 2008)

4.3.2 Presence or absence of parasite species across all sites

Island sites did not show an overall reduction in the parasite species present within the rodent populations examined (mean number of species detected per site: mainland= 12.7, island= 11.4, $t_{(17)}$= 1.41, p=0.193) (Table 4.2). Fleas were absent from some of the islands, Bucinch (BU), Inchconnachan (CO), Torrinch (TO), Inchmoan (MO) and Inchcruin (CR), but present on the others. Only *Ixodes ricinus*, *Eimeria* species (large and small), *Heligmosomoides polygyrus/glareolus, Bartonella* and Murid Gammaherpes virus (MHV-4), were found throughout all sites.

Table 4-2: Prevalence of infection of those parasites investigated across all sites.

Parasites		Mean prevalence of infection (P%)	Mainland Sites			Island Sites (decreasing in n)						
			LU n= 194	M1 n= 109	KN n= 51	CA n= 67	TA n= 55	BU n= 36	CO n= 27	TO n= 22	MO n= 20	CR n= 3
Ectoparasite												
Ticks	Ixodes ricinus[1]	78.7										
	Ixodes trianguliceps[1]	41.2										
Fleas	6 species combined	13.4										
Microparasites	Bartonella spp	47.2										
	Cowpox	14.3										
	Murid gammaherpesvirus 4	17.0										
Gastro-intestinal	Heligmosomoides polygyrus (glareolus)	34.3										
	Capillaria	47.0										
	Trichuris	2.6										
	Eimeria (large)	53.1										
	Eimeria (small)	16.5										
	Rodentolepis spp	7.7										
	Syphacia spp	7.8										

■ Detected ■ Not detected low confidence ($p > 0.05$) □ Not detected high confidence ($p < 0.05$)

Table 4-3: Sample sizes of rodents for prevalence of infections. Samples sizes varying due to the differing nature of how samples were collected and analysed.

	Sites with varying sample sizes of rodents analysed									
Parasite	**M1**	**KN**	**LU**	**CA**	**TA**	**BU**	**CO**	**TO**	**MO**	**CR**
Ticks	123	90	199	77	57	38	31	26	25	3
Fleas	123	90	199	77	57	38	31	26	25	3
Bartonella spp	33	15	92	35	3	9	2	13	12	2
Cowpox	51	24	108	48	17	19	7	17	6	2
Murid gammaherpesvirus 4	51	24	108	48	17	19	7	17	6	2
Gastro-Intestinal Parasites	144	80	196	73	54	35	26	25	13	1

4.3.3 Prevalence of Infection

4.3.3.1 Ectoparasites

Ticks had a higher mean prevalence of infection (P%) on islands for both wood mice and bank voles (Figure 4.1, Tables 4.4 & 4.5). There was also a seasonal effect on tick prevalence seen in wood mice, with a higher prevalence observed during summer. Mean P% of fleas for both host species showed no difference between the mainland and island populations (Table 4.4 & 4.5). When comparing the mean tick P% between rodent species combined across sites and seasons, it was higher on wood mice than bank voles (χ^2(N=290) = 21.36, p<0.001). Whilst in contrast, mean flea P% was higher on bank voles (χ^2(N=78) =24.01, p<0.001) than on wood mice.

4.3.3.2 Gastro-intestinal parasites

Mean P% of gastro-intestinal parasites was characteristically different for wood mice than bank voles. Mean P% of *H. polygyrus/glareolus* was higher on islands

than mainland sites for bank voles but not for wood mice (Table 4.4 & 4.5). A seasonal effect was present for both host species, with a higher prevalence of *H.polygyrus/glareolus* in summer. Mean P% of *Capillaria* on wood mice was higher on islands, whilst in contrast mean P% of *Capillaria* in bank vole was higher on the mainland (Figures 4.1& 4.2 Tables 4.4 & 4.5). There was no difference observed between any of the terms investigated for the mean P% of large *Eimeria* species in wood mice. However mean P% was higher on mainland sites for bank voles as well as a between-years effect seen with a higher prevalence of large *Eimeria* in 2009. Lastly for small *Eimeria* species there was no difference between any of the terms investigated for bank voles (Table 4.5). In contrast prevalence of small *Eimeria* species in wood mice is higher on islands, as well as being more prevalent in summer and in the year 2009 (Figure 4.1 & Table 4.4).

4.3.3.3 Microparasites

No serological evidence for cowpox infection was found in wood mice. Cowpox on bank voles was only found on one island site but was present across all mainland sites. Bank voles had a higher prevalence of cowpox infection on the mainland, as well as a year effect, with a higher prevalence seen in 2009 (Table 4.5). There was no difference between prevalence of MHV in either wood mice or bank voles between mainland and islands. Although a seasonal effect was detected, with a higher prevalence of MHV in summer (Tables 4.4 & 4.5). There was no difference in prevalence of *Bartonella* species between mainland and island for both host species (Figures 4.1 & 4.2, Tables 4.4 & 4.5).

Table 4-4: GLMM showing the coefficients (effect of mainland relative to island), standard errors in () and p-values[1] of the variables associated with the prevalence of infection across 2 years (2009 and 2010) and 3 seasons (spring, summer and autumn) between mainland and island sites of wood mice.

Model= Prevalence of infection~ Mainland/Island* Year + Mainland/Island *season+ (1|site)

	Ticks	Fleas	H. polygyrus	Capillaria	Large Eimeria	Small Eimeria	Cowpox	MHV	Bartonella
Intercept	1.43(0.38)***	-3.39 (0.53) ***	-0.33 (0.50)	-0.82 (0.35) *	0.83 (0.29)**	-4193.41 (1068.45) ***	na	-0.13 (0.71)	-18.70 (20.92)
Mainland/Island	- 1.75(0.44)***	0.95 (0.60)	-0.06 (0.68)	-0.83 (0.37) *	-0.38 (0.40)	-4573.67 (1960.78) *	na	-0.20 (0.52)	27.65 (28.48)
Year	-	-	-	-	-	2.09 (0.53) ***	na	-	-
Season	1.70(0.39)***	-	-0.99 (0.33) **	0.97 (0.34) ***	-	1.69 (0.75) *	na	-1.80 (0.86) *	6.30 (5.37)
Mainland/Island*Year	-	-	-	-	-	-2.28 (0.98)*	na	-	-
Mainland/Island*Season	-	-	-	-	-	-2.26 (0.96) *	na	-	-

[1] P-values: ***< 0.001 **<0.01, *<0.05

Table 4-5: GLMM showing the coefficients (effect of mainland relative to island),, standard errors in () and p-values of the variables associated with the prevalence of infection across 2 years (2009 and 2010) and 3 seasons (spring, summer and autumn) between mainland and island sites of bank voles.

Model= Prevalence of infection~ Mainland/Island* Year + Mainland/Island *season+ (1|site)

	Ticks	Fleas	H. glareolus	Capillaria	Large Eimeria	Small Eimeria	Cowpox	MHV	Bartonella
Intercept	-892.59 (567.67)	1140.55 (996.75)	-1965.22 (511.69)***	-2.85 (0.86) ***	3470.67 (982.38) ***	-1.957 (0.30) ***	-6277.09 (968.25)***	-0.92 (0.52)	-0.60 (0.30)*
Mainland/Island	-2.31 (0.84) **	-2458.22 (1285.21)	-0.50 (0.27)	3.78 (1.12) ***	3313.74 (1133.45) **	-0.49 (0.38)	1.33 (0.47)**	-0.52 (0.39)	0.28 (0.36)
Year	0.44 (0.28)	-0.57 (0.50)	0.978 (0.25) ***	-	-1.73 (0.49) ***	-	3.12 (0.48) ***	-	-
Season	-0.79 (0.63)	-	0.83 (0.35)*	-	-	-	-	-2.27 (1.12) *	-
Mainland/Island*Year	-	-	-	-	1.65 (0.56) **	-	-	-	-
Mainland/Island*Season	2.74 (0.76) ***	-	-	-	-	-	-	-	-

[1] P-values: ***<0.001 **<0.01, *<0.05

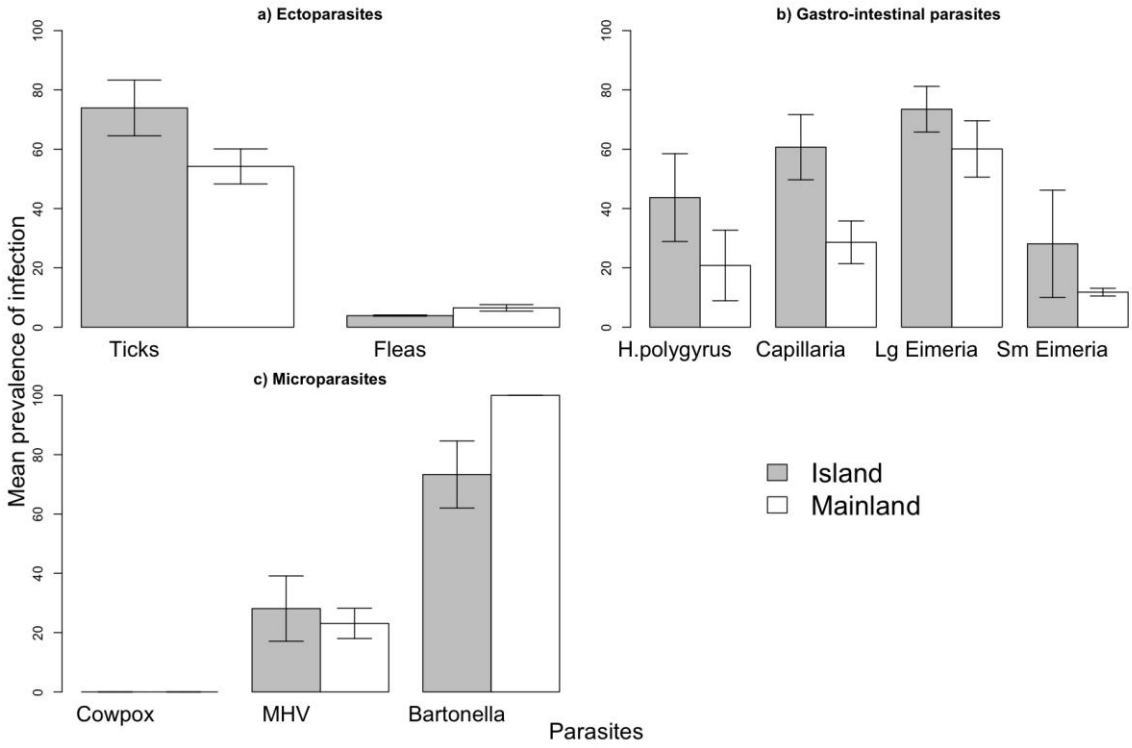


Figure 4-1: Wood mice mean prevalence of infection (P%) for a): ectoparasites, b): gastro-intestinal parasites and c): microparasites, comparing island and mainland (sample sizes are provided in Table 4.4).

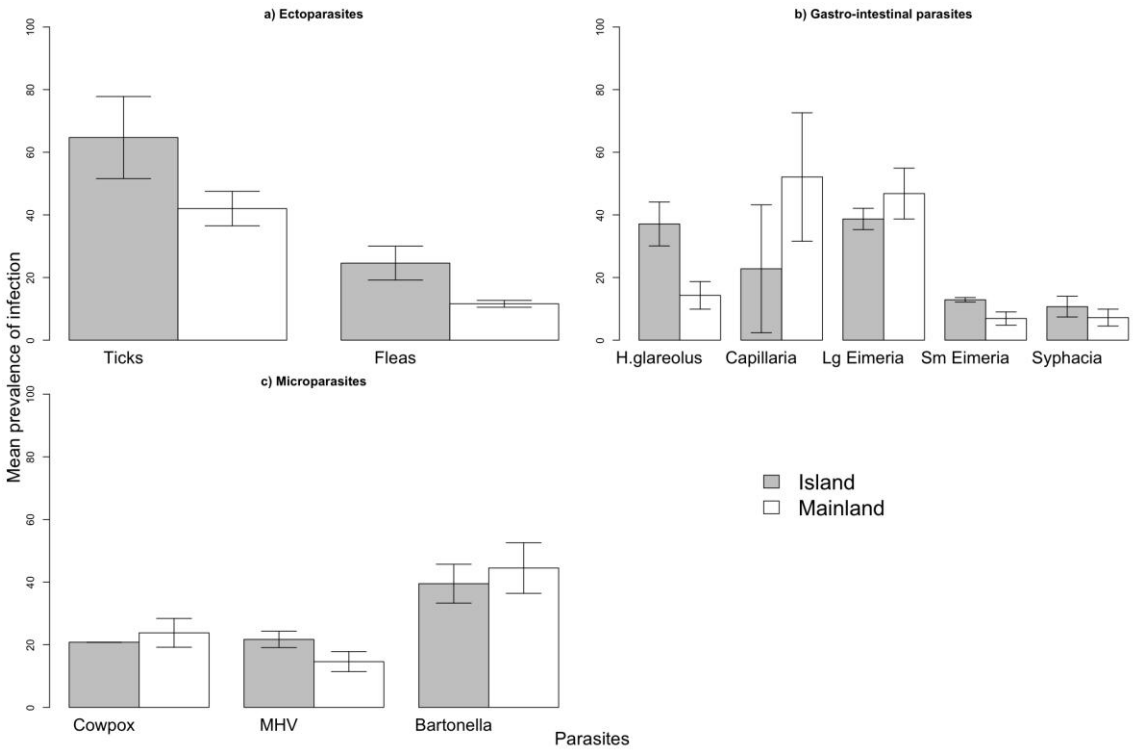


Figure 4-2: Bank vole mean prevalence of infection (P%) for a): ectoparasites, b): gastro-intestinal parasites and c): microparasites, comparing island and mainland (sample sizes are provided in Table 4.4).

4.4 Discussion

4.4.1 Do host populations on island support fewer parasite species than mainland populations?

Overall parasite diversity was not affected by rodent population structure ($t_{(17)}$=1.41, p=0.193, Table 4.2); therefore fragmentation had a limited effect on the range of parasites seen on their hosts. It is important to remember that any parasites that are described as being absent from a site, are absent from the trapping grid. Although in the case of Bucinch (BU) we can say more confidently that is it absent from the island as a whole, due to the small size of the island (3.2 hectares), nearly the whole of the site was trapped.

4.4.1.1 Ectoparasites

Ixodes ricinus and *Ixodes trianguliceps* were observed across all mainland sites sampled, as well as the majority of the islands. *I. trianguliceps* was rarer than *I. ricinus* on the islands and only absent from two of the seven island sites (Table 4.2), this is to be expected as *I.trianguliceps* have a restricted transmission mode as they feed exclusively on small mammals and mainly rodents, whereas *I. ricinus* are not specific to the vertebrate hosts they feed on. Therefore fragmentation does not seem to restrict the distribution of ticks examined in this study.

Fleas were found to be rare on islands, with flea samples only found and collected on two islands (CA and TA). Fragmentation may play a role in the limited distribution observed within this study, due to the potentially restricted movement of fleas between sites. However bank voles on one island (CA) had a higher mean prevalence of flea infection than any mainland site. This may suggest a more stable dynamic than those seen on wood mice. Bank voles tend to live more closely (Kruczek and Styrna 2009) than wood mice and this may therefore increase the chances of fleas infecting individuals more readily than they do in wood mice.

4.4.1.2 Gastro-intestinal parasites

Due to a variety of gastro-intestinal parasites examined within this study, one would expect variation across sites. The majority of those examined were present across nearly all of the sites, even when host population abundance is low, although island sites tended to have a more patchy distribution of parasites (Table 4.2). The more rare parasites, *Rodentolepis*, *Syphacia* and *Trichuris*, although present in small numbers, were still found across the majority of sites sampled. Therefore fragmentation was not seen to reduce the number of gastro-intestinal parasites.

The accuracy of using faecal analysis to determine gastro-intestinal rates within a population requires comment. It has been seen that faecal egg counts do under-estimate the detection rate compared to destructive sampling; seen within a population (Harvey, Paterson et al. 1999). However destructive sampling cannot be used in a longitudinal study such as this.

4.4.1.3 Microparasites

Of the microparasites investigated within this study, only cowpox showed restricted distribution across sites. MHV-4 and *Bartonella* was present in all sites sampled (Table 4.2). Contrary to this, cowpox was present on all three-mainland sites but only one island, Inchcailloch (CA) (Table 4.2). Within CA, only bank voles were trapped, and previous work has found that prevalence of infection of cowpox is higher on bank voles that it is on wood mice (Hazel, Bennett et al. 2000; Begon, Hazel et al. 2003). This may suggest why cowpox infection was absent from the wood mice sampled. Cowpox is a self-limiting virus, where the infection lasts on average for four weeks.

4.4.2 Is the potential absence of parasites from island population consistent with predictions based on life histories?

As previously stated, most parasites were found to persist in island populations. The role that parasite life histories play have contributed to this lack of absence, as most of the life histories of the parasites studied within this investigation have a direct life cycle and are able to persist within the environment (Table 4.1).

4.4.2.1 Ectoparasites

The life history of *Ixodes* ticks lends itself well to host populations that do not have high abundance, since *Ixodes* ticks have a broad vertebrate host range. Although rodent abundance was lower on islands than on the mainland, alternative hosts were present; fallow deer are abundant in the area and frequently move between the mainland and islands. Deer play a vital role in *Ixodes ricinus* life history, as adult ticks feed on deer, with the juvenile and nymph stages preferring rodents. Deer are also important vehicles for moving *I. ricinus* between sites (Ruiz-Fons and Gilbert 2010). *I. trianguliceps* however feeds exclusively on small mammals. Although not targeted for this study, some of the sites had pygmy and common shrews present locally, although their abundance is unknown. As they are competent hosts their presence could provide alternative hosts if wood mice or bank vole numbers were limited in a given fragment.

Fleas were not observed often during sampling, although six species were identified over the course of the study. They have a more restricted transmission mode than ticks as they are mostly found on the hosts themselves or within their burrows, therefore infection is expected to be more localised.

4.4.2.2 Gastro-intestinal parasites

The gastro-intestinal parasites found in this study have a direct life cycle (apart from *Rodentolepis*) (Table 4.1). This reduces the likelihood of extinction in discontinuous habitats' as intermediate hosts are not required. It is unknown how long these parasites are able to persist within the external environment. The survival time away from the host will undoubtedly play a vital role in transmission rates between individual hosts, especially when host population densities are low. Seasonal peaks of gastro-interstitial parasites are seen in spring and summer; this corresponds with the seasonal peak in population densities of their hosts (Montgomery and Montgomery 1988; Gregory, Montgomery et al. 1992; Taylor, Coop et al. 2007; Behnke, Bajer et al. 2008). Rodent population densities showed a reduction in 2010 (Chapter 3). The expected seasonal dynamics of rodents would be an increase in numbers from spring through to the end of the breeding season, then decreasing until the following summer. However this was not found across the study sites, and there was no difference between fragmented and non-fragmented sites (Chapter 3). Even when the unusually harsh winter of 2009-10 is taken into consideration, wood mice do not follow this expected pattern. Bank voles on the other hand did tend to follow the expected seasonal dynamics. Bank vole populations may have been generally higher than those of wood mice, and captures may have reflected that. This will affect the seasonal dynamics of the hosts' corresponding parasites. Therefore if gastro-intestinal parasites prevalence of infection is affected by host abundance it would be expected see a reduction in the prevalence of infection of gastro-intestinal parasites in 2010 (a year effect in the GLMM Tables 4.4 & 4.5). This was not the case for the helminthes investigated within this study (Tables 4.4 & 4.5), as no year effect was seen. This therefore further supports the idea of infectious stages persisting within the environment and thereby providing a constant reservoir of potential infections.

4.4.2.3 Microparasites

Bartonella species that infect rodents are transmitted via vectors, namely fleas and mites (Table 4.1). However fleas were apparently absent from three islands where *Bartonella* infection was detected (Table 4.2). Confidence is particularly high for Bucinch (BU), where both fleas and mites were absent and the whole of the island was subject to trapping. *Bartonella* was present in 22.2% of the individuals tested. This suggests the likelihood of transmission by an alternative vector. *Ixodes* ticks are highly prevalent (97% of captured individuals) within BU; therefore it is plausible that ticks are playing a potential role in the transmission of *Bartonella* in the absence of their known vector. Such an alternate vector may be allowing *Bartonella* to persist within the fragmented landscape. The idea that *Bartonella* could be transmitted via infected tick bites has been debated in recent years (Adelson, Roa et al. 2004; Bown, Bennett et al. 2004; Billeter, Levy et al. 2008; Reis, Cote et al. 2011). However it has been confirmed that *Ixodes ricinus* is competent vector for *Bartonella birtlesii* (known to infect rodents), and that transmission can take place during the moulting stages between larvae and nymphs (Reis, Cote et al. 2011). Our study provides further evidence for the role of *I.ricinus* as a vector for *Bartonella* when fleas are absent.

Unlike *Bartonella*, direct contact between individuals is the assumed method of transmission of MHV-4 (Table 4.1). In populations with reduced host abundance, contact between individuals may be reduced. However this relationship was not supported in our study, since islands had lower host density than the mainland but have a higher mean prevalence of MHV-4 infection. It may be that contact, such as sexual contact and shared nest sites, between individuals is not reduced on smaller fragments if genetic diversity is reduced, since it is known that individuals that are closely related are more tolerant of living together (Kruczek and Styrna 2009). Therefore it could be hypothesised that contact between individuals on islands can occur more frequently than on the mainland. However, to evaluate the importance of this it is important to

explore the ability of individuals detecting kin, and how quickly any tolerance of kin declines with genetic distance between individuals.

As with MHV-4, cowpox virus is thought to be transmitted by direct contact between individuals (Table 4.2); however this has yet to be proven. Bank voles are more competent hosts for cowpox than wood mice (Hazel, Bennett et al. 2000; Begon, Hazel et al. 2003), and cowpox virus could reduce host survival (Feore, Bennett et al. 1997). Therefore as evidence was found for cowpox infection in bank voles and not wood mice, this supports the idea that bank voles and not wood mice drive cowpox persistence. Furthermore once infected with cowpox a host cannot be infected again, so on fragmented sites (islands) there is a higher chance of cowpox running out of susceptible individuals to infect. Therefore the effect of fragmentation cannot be ruled out as a cause of the limited distribution of this virus seen in our study.

4.4.3 Is the prevalence of infection in island populations different from that on the mainland?

There was no consistent pattern for infection prevalence, even among similar parasites in both host species. Some parasites showed elevated prevalence of infection on islands.

4.4.3.1 Ectoparasites

Ectoparasites prevalence of infection was higher in wood mice ($p<0.001$, Figure 4.1). A higher mean prevalence of infection of ticks was observed in wood mice on island sites ($p<0.001$, Table 4.4). The chances of *Ixodes* ticks becoming extinct on fragmented sites are very low, given the high prevalence (~70%) observed. Although distinguishing between *I. ricinus* and *I. trianguliceps* in the field was difficult most of the ticks seen were *I.ricinus*. Since *I. ricinus* is linked to deer abundance, and deer abundance is particularly high on the islands examined (unpublished data) this can account for the high prevalence of infection seen on fragmented sites.

Fleas were found to be rare across all sites and especially on islands, with samples only found on two islands (CA & TA). Bank voles had a higher mean prevalence of infection compared to wood mice ($p<0.001$, Figure 4.2), although no mainland/island effect was observed (Table 4.5). Despite the more limited distribution of fleas, the prevalence of infection was not different between mainland and island sites (Tables 4.4 & 4.5). Prevalence of infection of fleas is driven by the well-connected habitats with close contact between individuals by sharing the same habitat (nests, breeding sites, feeding areas). As the population density of rodents is lower on fragments contact between individuals may occur less frequently than in well connected habitats, therefore reducing the potential overall prevalence of infection within that habitat.

4.4.3.2 Gastro-intestinal parasites

The common wood mice gastro-intestinal parasites had a higher mean prevalence of infection on islands sites than mainland sites. The potential mechanisms for driving this higher prevalence of infection seen in non-continuous habitats could be due to an increase in home range size of wood mice on islands (Chapter 3). Therefore potentially increasing encounter rates, along with gastro-intestinal parasite being able to persist within the environment could account for the higher prevalence of infection seen on wood mice within island sites.

Bank voles followed a similar pattern of infection to wood mice. *H. glareolus* had a higher mean prevalence of infection on islands than on the mainland, although this model did not reach statistical significance. Contrary to wood mice, *Capillaria* had a higher mean prevalence of infection on the mainland. This may allow persistence within the environment, as well as high host abundance, allowing for stable parasite dynamics. We cannot explain the differing prevalence of infection between *H. glareolus* and *Capillaria* on fragmented (island) and non-fragmented (mainland) sites. There is no distinguishing life history difference that would suggest one parasite species doing better on continuous versus non-continuous habitats.

4.4.3.3 Microparasites

No difference in prevalence of infection of *Bartonella* or MHV-4 was seen between continuous (mainland) and non-continuous (island) sites. Variation between species was seen with wood mice having nearly double the mean prevalence of infection than bank voles for *Bartonella*. This does not correspond to higher flea infection patterns that were seen in bank voles. This again poses the question whether fleas are the sole transmitters of *Bartonella*. MHV-4

prevalence was also higher in wood mice than seen in bank voles, which supports earlier studies that suggest wood mice are the major hosts for this pathogen.

4.4.4 Conclusion

In summary there was no reduction in the number of parasite species found within fragmented habitats. The life history of these parasites allows for persistence within fragmented landscapes. The prevalence of infection of parasite infections varied across sites, however the majority of the parasites on islands did not show a reduced prevalence of infection compared to mainland sites.

Most of the parasites investigated in this study have a direct life cycle; this has undoubtedly contributed to the "fitness" of the parasites in the chances of survival, since within a small isolated population a range of parasites seem to thrive when host abundance is low. Infectious stages surviving within the environment is the most obvious life history strategy that allows parasite to persist thereby providing protection from extinction. A high prevalence of infection will further reduce the likelihood of extinction on these isolated sites, as the chances of re-infection remaining high when parasitic egg or oocyst shedding into the environment is also high as higher parasite burdens are likely to indicate a high level of parasite eggs or oocysts. In addition, the example of *Bartonella* shows that parasites may exploit alternative modes of transmission, making them less vulnerable to the changes in vector communities that are found in fragmented populations.

5 Parasite co-infection and co-aggregation and their dependency on host characteristics in woodland rodents

5.1 Introduction

It is well documented that many parasites are over-dispersed within their host population (Shaw and Dobson 1995; Shaw, Grenfell et al. 1998). This has lead to the 20/80 rule, proposing that 20% of the host individuals within a population are responsible for 80% of parasite infections and transmission events, and Taylor's power law of aggregation (Taylor 1961). Both theories basically come down to the same idea that only a few hosts will harbour most of the parasite infections and therefore contribute more to parasite transmission (Taylor 1961; Woolhouse, Dye et al. 1997). Their role as 'super spreaders' suggests that these individuals also play a key role in maintaining disease within a host population. However, while the phenomenon of aggregated parasite distributions is well documented, it is unclear what predisposes certain individuals to high parasite loads. What is even less clear is whether host characteristics can explain aggregation across different kinds of parasites.

Although parasites are normally studied individually, this is not what is seen within natural systems, where most hosts are infected simultaneously with a variety of parasite species. Co-infection is an important concept for free-living populations due to the vast diversity of parasites that occur within natural systems, creating ample opportunity for concurrent infections (Ezenwa and Jolles 2011). This has lead to a proposed community ecology approach, focussing on parasite interactions and their mechanisms within a host as well as the patterns of parasite abundance (Pedersen and Fenton 2007; Telfer, Lambin et al. 2010). Within-host interactions in turn can affect the abundance and distribution of parasites at the host population level (Pedersen and Fenton 2007).

Parasites that co-occur within an individual host are likely to interact in a variety of ways, and by a variety of mechanisms. These interactions are potential drivers in generating the variation seen in the transmission of diseases and in turn disease dynamics (Ezenwa and Jolles 2011). The majority of studied examples on co-infection and parasite interactions within a host have been between helminths (Shaw and Dobson 1995; Poulin 1996; Poulin 1996; Shaw, Grenfell et al. 1998; Grear and Hudson 2011), between microparasites (Telfer,

Lambin et al. 2010; Alizon and Lion 2011) and between helminths and microparasites (Graham 2007; Ezenwa and Jolles 2011). Being infected with one parasite species may strongly influence a host's response to a subsequent infection by another parasite (Ezenwa and Jolles 2011). Where interactions between parasites occur, they may take place as facilitation or inhibition and this can be mediated by resources or the host's immune system (Graham, Cattadori et al. 2007). For example, direct effects imposed by helminths on microparasites can include the reduction of the surface area that allows for microparasite attachment or by reducing the cell type that allows microparasite replication (Graham 2007). Helminths can also induce anaemia by limiting the amount of red blood cells that are available to microparasites and therefore reducing microparasite population size within the host (Graham 2007). Further to this, the immune system plays a role in co-infection mediation. If helminth-induced suppression of inflammatory cytokine interferon is high then this will increase the microparasite density within the host (Graham 2007).

The two phenomena of parasite aggregation and co-infection may be linked in multiple ways. High intensity of infection with one parasite species may accentuate any effects on co-occurring infections. Looking at patterns of co-aggregation could therefore aid in our understanding of parasite interactions within hosts. At the same time, within-host interactions have the potential to affect patterns of parasite aggregation for host populations, either by top-down immune response or bottom-up resource competition (Graham 2007). The nature of these interactions will thereby determine whether aggregation and super-spreading is positively or negatively correlated across different parasites. In the case of facilitation, a small number of individual hosts may be responsible for transmitting most of the diseases. Alternatively, different host individuals may accumulate high levels of infection for different diseases. However, whether patterns of aggregation amongst multiple parasite species are consistent and whether host characteristics can explain parasite community composition is not clear. Very little is known about whether host characteristics explain parasite communities as a whole, however, for individual parasite species, host characteristics that have been found to be informative include (1) sex (Perkins, Cattadori et al. 2003; Ferrari, Cattadori et al. 2004; Ferrari, Rosa et al. 2007), (2) body mass (Gillespie, Lonsdorf et al. 2010; Harrison, Scantlebury et al. 2010),

(3) breeding status (Luong, Perkins et al. 2010), with effects being potentially dependent on sex (Perkins, Cattadori et al. 2003; Ferrari, Cattadori et al. 2004; Ferrari, Rosa et al. 2007),(4) body condition (Beldomenico, Telfer et al. 2008), and (5) increased levels of movement resulting in higher probability of contact with parasites (Brown, Macdonald et al. 1994).

In this study I seek to examine co-infection and co-aggregation and their dependency on host characteristics in two woodland rodent species, *Apodemus sylvaticus* and *Myodes glareolus* across three functional groups of infectious organisms: ectoparasites, gastro-intestinal parasites and microparasites. I ask the questions: (1) what are the patterns of co-infection among rodent parasites, in terms of presence/absence, and are these patterns different from random? (2) Is there any evidence for host aggregation being positively or negatively correlated among different parasite species or parasites functional groups? (3) What host characteristics best explain patterns of co-infection within rodent hosts? (4) To what extent are the identified relationships consistent between two rodent host species? The work carried out within this chapter will add to our understanding of parasite interactions. Additionally, it will also help in explaining parasite distributions and identifying the mechanisms that may limit or indeed facilitate local persistence of parasites.

5.2 Methods

A detailed description of the fieldwork carried out is provided in chapter 2 General Methods. A detailed description of rodent characteristics is found in chapters 3, as well as a detailed description of parasite identification in chapter 4.

Data were collected from wood mice, *Apodemus sylvaticus*, and bank voles, *Myodes glareolus,* across seven islands on Loch Lomond and three mainland sites. Each site was trapped using 100 live Sherman traps, over the course of 2009-2010 during spring (February-April), summer (June-August) and autumn (October-November) (Chapter 2 General Methods).

Rodents caught within each session were marked subcutaneously with a unique Passive Integrated Transponder (PIT) tag (AVID plc, East Sussex, UK), on the first capture and if re-caught were detected using a handheld reader (Minitraker I, AVID plc, East Sussex, UK). Rodent morphological characteristics were collected on each capture. Species and sex was recorded, as well as reproductive status, coat texture, body condition, age (based on coat and weight) and mass (grams) (Chapter 3, 3.2 Methods).

Rodents were examined for ectoparasites and a small amount of blood (< 20uL) was taken from the tail vain (Chapter 4, 4.2 Methods). Rodents were subsequently released at the site of capture. All used traps were taken back to laboratory in order to remove the faeces for parasite detection.

Ectoparasites were identified based on morphological characteristics (Arthur 1963; Whitaker 2007; George 2008). However ectoparasites were later grouped into either ticks or fleas, regardless of species. This was done due to the inability to distinguish different tick species (*Ixodes trianguliceps* vs. *I. ricinus*) in the field and the low numbers detected for each the six different flea species identified within this study (Chapter 2). DNA was extracted from blood clots and tested for *Bartonella* infection using primers and protocols provided by R. Birtles (pers. comm.). Infection with gastro-intestinal parasites was determined from faecal samples by a salt floatation technique (Slater and Keymer 1986; Brown, Macdonald et al. 1994), using morphological features to distinguish species or species groups (Chapter 2 General Methods). Murid gamma herpes and cowpox virus are not included within the analyses of this chapter, as the numbers testing positive were small and serology may reflect past exposure rather than current infection. Because parasites represented a combination of species (e.g. *H. polygyrus*) and higher taxonomic categories (e.g. 'fleas') the term 'parasite taxa' will be used from here on. Only host individuals that had data on all seven-parasite taxa (Table 5.1) were used for analysis (wood mice n=229, bank vole n= 247).

With respect to parasite species per host individual, the number of parasite species co-infecting each host was determined. To see if the rate of parasite species infecting a host is independent from any other parasite species,

the observed distribution was compared with a Poisson distribution and a chi-squared test was carried out for each rodent species separately. To determine if patterns of co-infection were different from random variance to mean ratio was carried out and this was then compared to a Poisson distribution: n'(i)=Np'(i), where p(i) is the proportion of individuals infected by i parasite species, N= sample size. To look at associations between parasites heat maps were determined by comparing the observed prevalence of infection with the expected prevalence of infection when individuals were infected simultaneously with more than one parasite. This was performed in turn for 2-way associations only. High and low deviations from the expected values are denoted in red and blue respect. Expected values were calculated based on the assumption that if parasite A and parasite B occur more or less frequently than expected, e.g parasite A occurs at 60% and parasite B occurs at 80%, the basis assumption is that 48% (0.6 x 0.8) of individuals should be infected with both simultaneously. Based on this it was asked whether the two parasite species were found together in a smaller or higher proportion of individuals than the expected value.

For intensity of infection data, a principal component analysis (PCA) was performed, to find a small number of linear combinations of parasite loads in order to capture the greatest amount of variation within the data. This was carried out using the *prcomp* function in R (The R Foundation for Statistical Computing; http://www.r-project.org). A generalized linear model (global model= principal component score~ year * Mainland/Island + season * Mainland/Island + sex + hipscore + breeding * age + (1|site) + (1|ID)) using a Poisson family was used to test if host characteristics (hipscore, sex, breeding, and age) explained co-aggregation patterns from the PCA, using the function *'lmer'* in the 'lme4' library of *R* (The R Foundation for Statistical Computing; http://www.r-project.org/). Stepwise removal of the variables was used to produce the final model based on the lowest Aikaike's Information Criterion (AIC). In addition to main effects, potential interactions between patch type (mainland/island) with year and season, as well as an interaction between breeding and age were considered. ID as a random effect allowed multiple observations to be used. A similar generalized linear model (global model= number of parasites per host ~ year * Mainland/Island + season * Mainland/Island

+ sex + hipscore + breeding * age + (1|site) + (1|ID)) using a Poisson family was used to test if host characteristics (hipscore, sex, breeding, and age) explained co-infection patterns. Again using the function '*lmer*' in the 'lme4' library of *R* (The R Foundation for Statistical Computing; http://www.r-project.org/). Removal of variables was done as stated previously.

5.3 Results

5.3.1 Patterns of co-infection among rodent parasites

Wood mice had a lower mean number of parasite taxa per individual (1.75 (± Standard error (SE) 0.06), compared to bank voles (2.05 (± SE 0.07, $t_{(663)}$=3.273, p=0.001). The majority of individual hosts for both species harboured very few parasite species whilst only a few individuals had four or more different parasite species. No individual was co-infected with all seven parasite species for either rodent host; in fact, no individual was simultaneously infected with more than five parasite species.

Table 5-1: Number of potentially co-infecting parasite species for wood mice and bank voles.

Parasite taxa	Host species
Heligmosomoides polygyrus	Wood mice
Heligmosomoides glareolus	Bank voles
Capillaria	Wood mice and bank voles
Small *eimeria*	Wood mice and bank voles
Large *eimeria*	Wood mice and bank voles
Ticks	Wood mice and bank voles
Fleas	Wood mice and bank voles
Bartonella	Wood mice and bank voles

Co-infection patterns were investigated to see if parasite species per individual, infect a host independently from any other parasite (Figure 5.1). The number of co-infecting parasites per individual was significantly different from expected for wood mice ($\chi 2(N=321)= 13.402$, $p=0.04$) but not for bank voles ($\chi 2(N=345)= 10.744$, $p=0.09$).

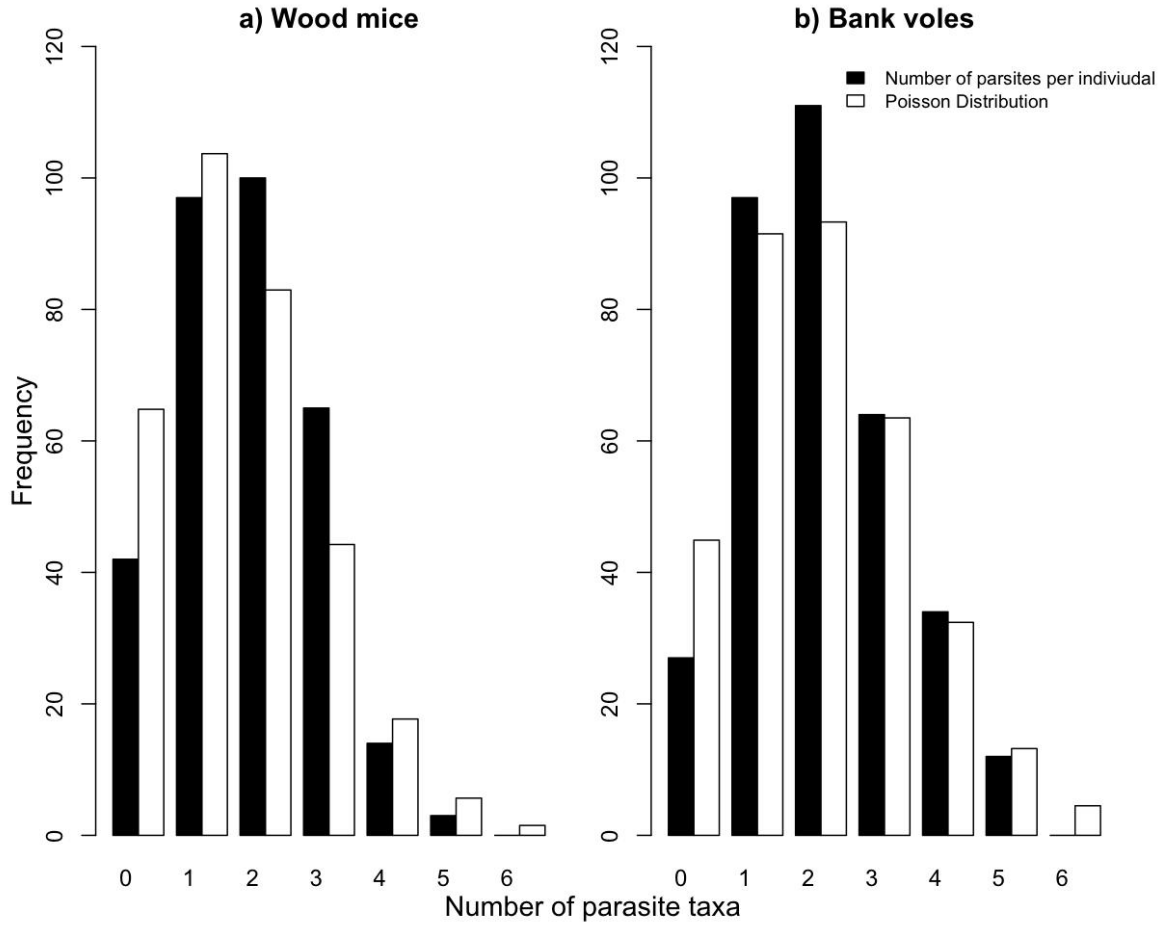


Figure 5-1: Patterns of co-infection based on the number of parasite species simultaneously infecting the same individual for a) wood mice and b) bank voles. The observed patterns (black bars) are compared to those expected of a Poisson distribution (white bars).

Heat maps (Tables 5.2 & 5.3) were constructed using presence/absence data to look at associations between particular parasite taxa. With blue indicating observed values lower than expected and red indicating observed values higher than expected, based on prevalence of infection (P%). For wood mice co-occurrence was much more common than expected when parasites associations were with ticks and *Capillaria*. Prevalence of infection when associated with *Bartonella* was much lower than expected.

Table 5-2: Heat map showing the co-occuring parasites and their prevalence of infection in wood mice. Observed values lower than expected (BLUE) and higher than expected (RED).

Observed / Expected	Ticks (P%= 66.3)	Fleas (P%= 5.7)	*H. polygyrus* (P%= 28.3)	*Capillaria* (P%= 14.6)	Large *Eimeria* (P%= 63.4)	Small *Eimeria* (P%= 13.6)	*Bartonella* (P%= 5.3)
Ticks (P% =66.3)		4.2	19.3	27.7	43.2	8.3	1.5
Fleas (P%= 5.7)	3.8		1.5	2.3	0	4.5	0
H. polygyrus (P%= 28.3)	18.8	1.6		12.7	11.8	3.3	1.1
Capillaria (P%= 14.6)	9.7	0.8	4.1		26.6	3.3	1.1
Large *Eimeria* (P%= 63.4)	27.9	3.6	17.9	1.4		4.8	0.7
Small *Eimeria* (P%= 13.6)	9.0	0.8	3.8	2.0	8.6		0.4
Bartonella (P%= 5.3)	3.5	0.3	1.5	0.8	3.4	0.7	

For bank voles, as for wood mice, a heat map was constructed. Co-occurrence was stronger between the ectoparasites, and between small *Eimeria* with the ectoparasites. Prevalence of infection was lower when parasites were associated with large *Eimeria* and *H. glareolus*.

Table 5-3: Heat map showing the co-occuring parasites and their prevalence of infection in bank voles. Observed values lower than expected (BLUE) and higher than expected (RED).

Observed / Expected	Ticks (P%= 44.5)	Fleas (P%= 20.7)	*H. glareolus* (P%= 31.6)	*Capillaria* (P%= 43.5)	Large *Eimeria* (P%= 28.3)	Small *Eimeria* (P%= 21.9)	*Bartonella* (P%= 13.3)
Ticks (P% = 44.5)		10.0	13.2	18.9	8.9	12.8	7.8
Fleas (P%= 20.7)	9.2		1.8	9.3	4.6	6.4	5.4
H. glareolus (P%= 31.6)	14.1	6.5		13.1	7.0	9.4	4.0
Capillaria (P%= 43.5)	19.4	9.0	13.7		6.1	11.2	6.1
Large *Eimeria* (P%= 28.3)	12.6	5.9	8.9	12.3		3.3	3.6
Small *Eimeria* (P%= 21.9)	9.7	4.5	6.9	9.5	6.2		5.2
Bartonella (P%= 13.3)	5.9	2.8	4.2	5.8	3.8	2.9	

5.3.2 Is host aggregation positively or negatively correlated among different parasite species?

5.3.2.1 Wood mice

Figure 5.2 shows the first two components of a PCA based on co-aggregation for the intensity of infection of six parasite species in wood mice. Principal component 1 (PC1) explained 22.0% of the variation seen, whilst PC2 explained 18.0% of the variation (Table 5.4). The ectoparasites (ticks and fleas) had a positive loading on PC1. The nematodes (*Capillaria* and *H.polygyrus*) had positive loadings for PCs 1 and 2, respectively. Whereas Eimeria species had a positive loading on PC2 (Small *Eimeria*) and a negative loading on PC2 (Large

Eimeria). To investigate if these loadings were positively or negatively correlated with each other, a Pearson's product moment correlation was carried out. A significant positive correlation was found between ticks and *Capillaria* (r = 0.28, $t_{(226)}$=4.46, p<0.001). The remaining four parasites showed no significant correlations among each other.

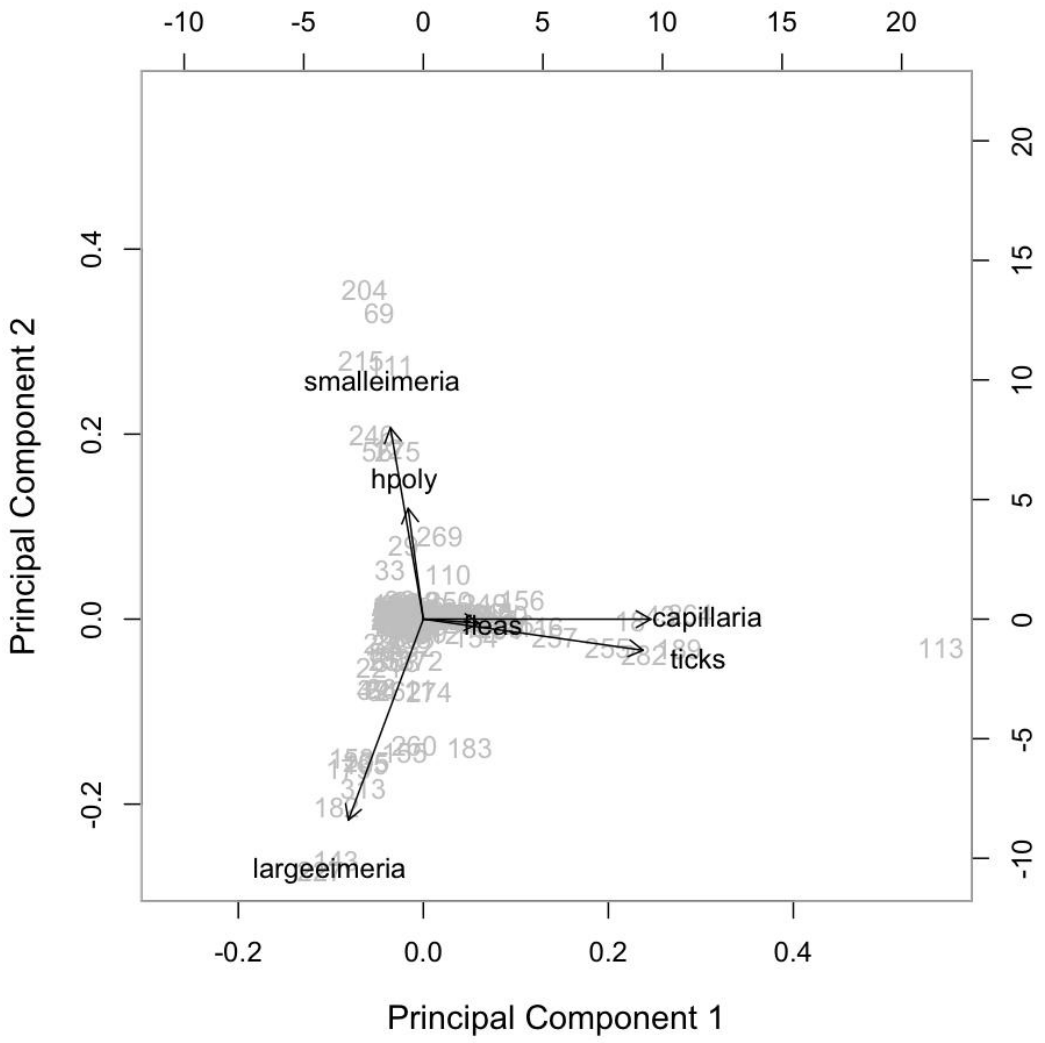


Figure 5-2: First and second components of a principal component analysis of intensity of infection (on a log scale) for six parasite species (black arrows) infecting wood mice. Grey numbers represent individuals.

Table 5-4: PCA results for wood mice

Components	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	1.1485	1.0400	1.0040	0.9931	0.9467	0.8419
Proportion of variance	0.2198	0.1803	0.1680	0.1644	0.1494	0.1181
Cumulative proportion	0.2198	0.4001	0.5681	0.7325	0.8819	1.0000

5.3.2.2 Bank voles

Figure 5.4 shows results of a PCA of the co-aggregation based on the intensity of infection of six parasite species for bank voles. PC1 explained 20.7% of the variation seen, whilst PC2 explained 17.6% of the variation (Table 5.5). Ectoparasites had negative loadings on different principal components (ticks on PC1 and fleas on PC2). This also applied to nematodes with negative loadings on *H. glareolus* on PC1 and negative loadings on PC2 for *Capillaria*. Whereas *Eimeria* species had a positive loading on PC1 and a negative loading on PC2. As with wood mice, I investigated if these loadings were positively or negatively correlated with each other by running a Pearson's product moment correlation test. A significant positive correlation was found between small *Eimeria* and *H. glareolus* ($r = 0.14$, $t_{(244)}=2.20$, $p=0.029$) and *ticks* and *H. glareolus* ($r = 0.14$, $t_{(244)}=2.26$, $p=0.025$). The remaining three parasites showed no significant correlation with any other parasite species.

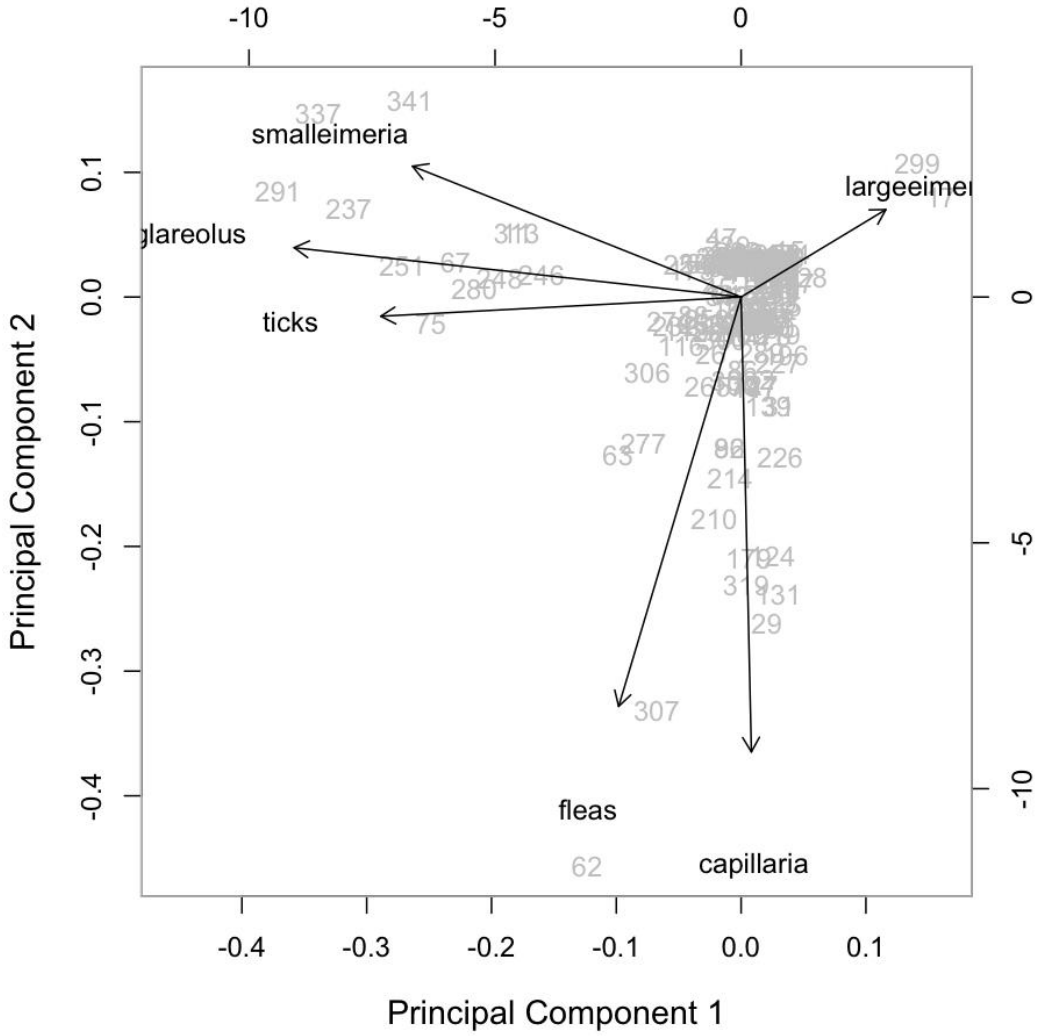


Figure 5-3: Principal component analysis of intensity of infection (on a log-scale) for six parasite species (black arrows) infecting bank vole individuals. Grey numbers represent individuals.

Table 5-5: PCA results for bank voles

Components	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	1.1146	1.0278	1.0172	0.9802	0.9422	0.9044
Proportion of variance	0.2071	0.1761	0.724	0.1601	0.1480	0.1363
Cumulative proportion	0.2071	0.3831	0.5556	0.7157	0.8637	1.0000

5.3.3 What host characteristics best explain patterns of co-infection and co-aggregation within rodent hosts?

5.3.3.1 Wood mice co-infection

A generalised linear mixed model, with Poisson distribution, was used to test whether host characteristics explain patterns of co-infection, based on the number of parasite taxa per individual. None of the host characteristics explained patterns of co-infection within wood mice (Table 5.6). However, season was highly significant with the summer months having more parasite species than in spring or autumn. There were also more parasite taxa co-infecting on the island sites compared to the mainland.

Table 5-6: GLMM showing the coefficients, standard errors in () and p-values[1] of the variables contained in the best model for examining co-infection of wood mice.

Variables	Co-infection
Intercept	0.65 (0.12) ***
Season	0.36(0.13) **
Mainland/Island	-0.29 (0.11)**

P-values: ***<0.001 **<0.01, *<0.05

5.3.3.2 Wood mice co-aggregation

Host characteristics were investigated to see whether they explained patterns of co-aggregation from the principal component scores. None of the variables considered (sex, hipscore, age, breeding, season, year, mainland/island) were found to explain either PC1 or PC2 ($p>0.05$) (best models: PC1~hipscore +(1|site)+(1|ID), p= 0.320, PC2~year +(1|site)+(1|ID), p=0.581).

5.3.3.3 Bank voles co-infection

For bank voles, age was the only host characteristic able to explain patterns of co-infection, with juveniles carrying larger numbers of different parasites (Table 5.7). Additionally to this, year and season also had an effect on patterns of co-infection. With 2010 having higher co-infection rates than in 2009, which was seen also for wood mice. Again season was also an important coefficient in explaining patterns of co-infection, with higher numbers observed in summer compared to spring or autumn.

Table 5-7: GLMM showing the coefficients, standard errors in () and p-values[1] of the variables contained in the best model for examining co-infection of bank voles.

Variables	Co-infection
Intercept	-409.15(171.79)*
Year	0.20 (0.09) *
Season	0.28 (0.11) *
Age	-0.41 (0.19) *

P-values: ***< 0.001 **<0.01, *<0.05

5.3.3.4 Bank vole co-aggregation

Host variables explaining patterns of co-aggregation from principal component scores were also tested. None of the variables considered (sex, hipscore, age, breeding, season, year, mainland/island) were found to explain either PC1 or PC2 ($p>0.05$) (best models: PC1~hipscore +(1|site)+(1|ID), p= 0.310, PC2~breeding +(1|site)+(1|ID), p=0.115).

5.4 Discussion

5.4.1 Patterns of co-infection among rodent parasites

Co-infection is a growing area of interest, however there is still relatively little evidence of how the presence of one parasite species will affect other parasite species within the host (Fenton 2008). Research to date has begun to explore this gap in knowledge. No individual for either host species was found to be co-infected with all seven parasites simultaneously; in fact, the maximum number of co-infecting parasites within any individual was five. The majority of individuals had few parasite taxa co-infections, with the mean number co-infecting wood mice and bank voles at 1.75 and 2.05, respectively. Fewer observations than expected were seen at both ends of the co-infection spectrum (0 and >5 parasites) where seen. Studies have shown that the presence of one parasite species can affect the occurrence of others, even when parasites are within differing functional groups (Lello, Boag et al. 2004; Fenton 2008). Whereby co-infecting parasites can directly influence another parasite via by-products, manipulation of the parasites' location, competition for resources or physical crowding. Furthermore, the hosts' immune system will also play a role in shaping the parasite community seen within a host (Lello, Boag et al. 2004). These influences could either have a positive or negative effect on the co-occurrence of parasites. The lack of individuals within this study with five or more co-infecting parasites could be simply due to a detrimental effect of having too many (>5) co-infecting parasites, with the aforementioned influences being too much across a range of parasite taxa. Either those individuals with such large co-infecting parasite burdens are killed off before they are identified or the resource limitations lead to competitive exclusion among parasites.

Parasite associations are rarely investigated within natural populations (Telfer, Lambin et al. 2010), the heat maps although showing only the simplest 2-way associations between parasite taxa, begin to show that this is a relevant area to explore further. Although a visual representation rather than a statistical representation of the associations, it allows for some inferences to be drawn.

Co-occurrence was more common between parasites in wood mice when parasites were associated with ticks and *Capillaria*. Parasites associations were also more common than expected within the same functional groups (ticks and fleas, *H. polygyrus* and *Capillaria*). This association is possibly due to how the parasites are acquired and transmitted within the environment and between hosts. Ectoparasites can be transmitted between hosts within the nest or during periods of contact, during the breeding season. Helminths acquired infections are also likely to be higher during times of breeding and there is the potential for "hot spots" within the environment when eggs are shed into the environment. As *Bartonella* is transmitted via an ectoparasite vector it is expected that a positive association between *Bartonella* and ectoparasites would be seen. This was not the case, with lower than expected associations between *Bartonella* and ectoparasites seen. *Bartonella* prevalence of infection was low throughout this study; therefore an association might be under-represented. Lower than expected values for *Eimeria* species were seen, competition for resources and/or space within the host might be the potential driver for the lower than expected association. Bank voles also had higher than expected occurrence with ectoparasites, however *Bartonella* had a higher than expected association with ticks and fleas. A higher than expected association with ectoparasites is expected with *Bartonella* as previously stated ectoparasites are the vectors for *Bartonella,* and it is expected that a positive association is seen in order for transmission events to take place.

5.4.2 Evidence for host aggregation being positively or negatively correlated among different parasite taxa

Habitat, seasonality and host characteristics are known to affect hosts' exposure to parasite infections (Ferrari, Cattadori et al. 2009).High intensity of infection may accentuate possible interactions among different parasites within the same host. Host aggregation showed both positive and negative correlations amongst different parasite taxa within this study. For both host species, parasite loads of ticks and certain nematodes were positively correlated (wood mice= *Capillaria* ($p<0.001$), bank voles H. *glareolus* ($p=0.025$) or showed no correlation (*H. polygyrus* in wood mice, *Capillaria* in bank voles). This is in contrast to previous work that found decreasing tick intensity of infection seen with

increasing *H. polygrus* intensity of infection in *Apodemus flavicollis* (Ferrari 2005; Ferrari, Cattadori et al. 2009). For both hosts, *Capillaria* and fleas had a positive association. This could be due to hosts being in poor condition, or that they are more likely to co-occur within nests therefore chances of encounters are higher, than with ticks.

5.4.3 Host characteristics explaining patterns of co-infection and co-aggregation within rodent hosts

For both hosts, numbers of co-infecting parasites were higher in the summer months as well as in 2010. The parasites within this study show seasonal variations (chapter 4) and it is known that seasonality plays a role in the increase in infection intensities. Therefore detecting higher intensities of infection within summer is expected. For wood mice it was found that those individuals on island sites have higher levels of co-infecting parasites when compared to those on the mainland. It has been suggested that host populations infected with a range of parasite taxa are actually healthier than those with only a few parasite species. Fenton (2008) has suggested that a population with a variety of parasite taxa is more stable in terms of extinction. This could therefore explain why island host populations show high levels of co-occurrence of parasite taxa and are indeed relatively stable and healthy in terms of host populations, as was previously found in chapters 3 and 4.

No host characteristics (age, sex, breeding, body condition) were found to explain numbers of co-infecting or co-aggregating parasites in wood mice. In contrast, juvenile bank voles were found to be more heavily co-infected than other age groups, although no host characteristic explained patterns of co-aggregation. This is different to what has already been previously been found for age (with body mass being a proxy for age). Heavier (and therefore older) individuals were more heavily parasitized than lighter (therefore younger) individuals (Gillespie, Lonsdorf et al. 2010; Harrison, Scantlebury et al. 2010). Previous studies have found that host characteristics can explain parasite infections. However the main difference between those studies and this one is that previous work has only looked at single parasite species (Perkins, Cattadori et al. 2003; Ferrari, Rosa et al. 2007; Luong, Perkins et al. 2010), whilst here

seven parasite species were investigated. This raises the question as to why within this study similar findings were not shown. This is not what was expected and is not easily explained. However it maybe that the underlying mechanisms that lead to host characteristics explaining patterns of co-infection are lost within the environmental noise of this more-complex study and a much larger data set is needed in order to tease apart any patterns seen.

5.4.4 Consistency of identified relationships between the two rodent host species

Patterns of co-infection and co-aggregation were strikingly different between the two rodent hosts. Bank voles had a higher mean number of co-occurring parasites than wood mice and they differed in the co-occurrence of parasites. This is not what was expected as both wood mice and bank voles are infected with very similar parasites. Furthermore it is not expected that wood mice and bank voles would respond physiologically differently in how the immune system deals with multiple infections. One possible reason for this may be differences in host behaviour and social structure. Bank voles tend to live in intersexual communal groups (Kruczek and Styrna 2009) in contrast to wood mice. This may explain why bank voles have a higher mean number of parasites, and why juvenile bank voles were more frequently co-infected. As living within communal groups allows easier transmission between individuals across parasite taxa, especially when those individuals are juveniles and known to share nests. Bank voles are crepuscular and therefore they are potentially more active overall than wood mice that are nocturnal. There may be differences in the time of day that parasites are more active and therefore more likely to be transmitted. Little is still known about the free-living stages of helminths within the soil, therefore it could be proposed that helminths could potentially be transmitted better during the day than at night; as they may be limited by temperature and/or light intensity, both of which tend to be lower at night than during the day.

6. General discussion

The overall introduction underlined that heterogeneity is important at three levels within disease ecology: (1) the level of different host and parasites species, (2) the level of populations and how they are spatially distributed, and (3) at the hosts' individual level. These three levels have been the over-arching theme within this thesis. Each chapter has addressed these questions within a natural fragmented landscape. This has provided empirical examples across multiple parasites in two wild rodent hosts.

We used island and mainland sites situated around loch Lomond, Scotland's largest loch, to study the rodent dynamics and their parasite communities within a naturally fragmented landscape. The vegetation around loch Lomond provided ideal habitat in order to study woodland rodents. These species prefer relatively dense cover of vegetation, and the majority of the sites had cover in some form, mostly areas of bracken; *Pteridium aquilnum*, blaeberry; *Vaccinium myrtillus* and bramble; *Rubus fruticosus*, and grass species. The variation in island sizes (3-63 hectares) provides an ideal natural setting to study the effects of fragmentation on both rodent and parasite dynamics. With corresponding mainland sites, situated on the western, eastern and southern Loch shore. These sites were chosen based on their proximity to the Loch (within 0.5 km), as well as having a closed, mature forest cover with trees and undergrowth, with vegetation composition similar to that found on islands. Trapping grids were established in representative sections of forest, avoiding particularly open or wet areas that would have been less suitable for woodland rodents.

Woodland rodents were the target host species, namely wood mice, *Apodemus sylvaticus*, and bank voles, *Myodes glareolus*. These species accounted for 99% of captured individuals. Wood mice and bank voles are the most common small mammal species in Europe (Bruhl, Guckenmus et al. 2011). Both species have well described life histories, with similarities and differences between the two species. Both wood mice and bank voles are found within very similar habitats and are both mainly herbivores. However wood mice are nocturnal and bank voles are crespular. The average life span on both species in the wild is 18-20 months (Corbet and Southern 1977; Macdonald and Barrett

1993). Rodent demographics are similar between the two species, which made taking measurements from them very consistent. Both have males that are heavier (13-40g), compared to females (13-35g). Coat texture and mass was used to distinguish juveniles from adults, since the coat is fluffy in texture in juveniles compared to a smooth texture in adults. Rodents were split into 3 classes based on mass (Telfer, Bennett et al. 2002). Wood mice: juvenile (<15g), sub-adult (15-18g), and adult (>18g). Bank voles: juvenile (<14g), sub-adult (14-17g), and adult (>17g). The reproductive status of males was determined based on the position and size of the testes and the reproductive status of females was classified by four characteristics. Body condition was determined from the fat deposit along the lower spine and hips through palpation and assigning a score between 1 (low fat deposits) and 5 (high fat deposits).

Wood mice and bank voles have very well-described parasite species. With similar parasites infecting both hosts. Within this study, parasite species of interest were split into three functional groups: (1) ectoparasites, (2) gastrointestinal parasites and (3) microparasites. Within the ectoparasites two species of Ixodidae ticks were identified (*Ixodes ricinus* and *Ixodes trianguliceps*) and four families of fleas (Ctenophthalmidae, Ceratophyllidae, Hystrichopsylidae and Leptopsyllidae). All of these ectoparasites were found on both wood mice and bank voles. Gastro-intestinal parasites were commonly helminthes (*Heligmosomoides polygyrus, H. glareolus, Syphacia, Capillaria*, *Trichuris* and *Rodentolepis*, but not exclusively as protozoan (*Eimeria* species) were also identified. Microparasites were either viruses (cowpox and murid gamma-herpes virus) or bacteria (*Bartonella* species). Details of the identification methods are found in chapter 2. The study site, species and parasites investigated within this study have allowed us to investigate how natural fragmentation affects demographic and population dynamics of rodents (chapter 3), the consequences of habitat fragmentation on rodent parasite dynamics (chapter 4) and finally parasite co-infection and co-aggregation and their dependency on host characteristics in woodland rodents (chapter 5).

In **chapter 3** I found that, in contrast to previous studies on fragmented populations, the fragmented landscape of the islands had little effect on the demographic characteristics of rodent populations in comparison to those on the

mainland. Overall for both wood mice and bank voles I found no difference in age structure, sex ratios, body condition, survival and longevity. There was however differences seen for some demographics and population structure. Rodent numbers were higher on mainland sites in comparison to those on island sites, with no movement of any individual between mainland and island or island and island sites. Temporal variations in breeding season lengths were observed for both species, with individuals breeding earlier on island sites. However there was no indication that reproduction itself commenced earlier on islands, as females of both species were not pregnant earlier on the island sites. Wood mice on islands were found to travel a greater range of distances than those on the mainland, as well as having a greater average maximum distance travelled. The data and analysis presented within **chapter 3** allowed us to then address the broader question of how parasites dynamics are affected by the spatial structure of a host population.

In **chapter 4** it was found that within our study system there was no reduction in the number of parasite species found within fragmented habitats. Of the ectoparasites *Ixodes ricinus* and *Ixodes trianguliceps* were observed across all mainland sites sampled, as well as the majority of the islands. However fleas were found to be rare on islands, with flea samples only found and collected on two islands. Fragmentation may play a role in the limited distribution observed within this study, due to the potentially restricted movement of fleas between sites. Due to a variety of gastro-intestinal parasites examined within this study, one would expect variation across sites. The majority of those examined were present across nearly all of the sites, although island sites tended to have a more patchy distribution of parasites. The more rare parasites, *Rodentolepis*, *Syphacia* and *Trichuris*, although present in small numbers, were still found across the majority of sites sampled. Therefore fragmentation was not seen to reduce the number of gastro-intestinal parasites. Of the microparasites investigated within this study, only cowpox showed restricted distribution across sites. MHV-4 and *Bartonella* was present across all sites.

Life history strategy of the parasites investigated within this study allowed for their persistence within fragmented landscapes. Most of the life

histories of the parasites studied within this investigation have a direct life cycle and are able to persist within the environment. Ixodes ticks have a broad vertebrate host range, therefore allowing *Ixodes* ticks to feed on other available hosts that were not targeted within this study. Fleas were not observed often during sampling, therefore infection is expected to be more localised to burrows of their hosts. The direct life cycle of the gastro-intestinal parasites within this study has helped reduce the likelihood of extinction in discontinuous habitats, as intermediate hosts are not required. However it is unknown how long these parasites are able to persist within the external environment. If gastro-intestinal parasites prevalence of infection was affected by host abundance we would have expected to see a reduction in the prevalence of infection of gastro-intestinal parasites in 2010 when host populations seemed to crash. However this was not the case as no year effect was seen. This supports the idea of infectious stages persisting within the environment and thereby providing a constant reservoir of potential infections. Interestingly *Bartonella* species that infect rodents are understood to be transmitted via fleas and mites. Fleas were apparently absent from three islands where *Bartonella* infection was detected. This finding suggests that the likelihood of transmission is from an alternative vector. *Ixodes* ticks are highly prevalent within the study; therefore it is plausible that ticks are playing a potential role in the transmission of *Bartonella* in the absence of their known vector. Such an alternate vector may be allowing *Bartonella* to persist within the fragmented landscape. This study has provided further evidence to the debate over the role *I. ricinus* has as a competent vector for *Bartonella* infections when fleas are absent. For MHV-4 and cowpox infections to be transmitted between hosts, direct contact between individuals is needed. For MHV-4 there was a higher prevalence of infection on islands in comparison to mainland sites. Bank voles are more competent hosts for cowpox than wood mice (Hazel, Bennett et al. 2000; Begon, Hazel et al. 2003), and this was supported within our study, as no wood mouse was found to be infected with cowpox. Therefore the effect of fragmentation cannot be ruled out as a cause of the limited distribution of this virus seen in our study.

The prevalence of infection of parasite infections varied across sites, however the majority of the parasites on islands did not show a reduced prevalence of infection compared to mainland sites. Between the host species

there has been very few differences seen. However ectoparasite prevalence of infection was higher in wood mice with a corresponding higher prevalence of infection on islands. In contrast, bank voles had a higher mean prevalence of infection of fleas. Although fleas were rare across all sites, especially on islands, however no mainland/island effect was found. Wood mice and bank voles followed a similar infection pattern for gastro-intestinal parasites. The common wood mouse gastro-intestinal parasites had a higher mean prevalence of infection on islands sites than mainland sites; although visually bank voles also did, however significance was not reached. No difference in the prevalence of infection of *Bartonella* or MHV-4 was seen between continuous (mainland) and non-continuous (island) sites for either species. However wood mice had nearly double the mean prevalence of infection than bank voles for *Bartonella*. This does not correspond to higher flea infection patterns that were seen in bank voles.

The broader question of how parasites dynamics are affected by the spatial structure of a host population was addressed within **chapter 4**. With extinction of the parasites investigated within wood mice and bank voles unlikely due to the direct life cycle of these parasites, the high prevalence of infection of a range of parasites across functional groups and the potential exploitation of alternative vectors, as seen in the case of *Bartonella* and *I. ricinus*. All this combined makes this system less vulnerable to extinction events.

Finally within **chapter 5**, parasite co-infection and co-aggregation and their dependency on host characteristics in woodland rodents were investigated. Of all the parasite species investigated within this study only seven were investigated within this final chapter. This was due to the fact that not all individuals had records for all parasites especially the microparasite species, as blood samples were not easily obtained from rodents. The maximum number of parasites any individual was found to be infected with was five, however the mean for both wood mice and bank voles was around two. Co-occurrence was more common between parasites in wood mice when parasites were associated with ticks and *Capillaria*. Parasites associations were also more common than expected within the same functional groups. There was lower than expected associations between *Bartonella* and ectoparasites seen, as well as lower than

expected values for *Eimeria* species. Competition for resources and/or space within the host might be the potential driver for the lower than expected association seen. Bank voles also had higher than expected occurrence with ectoparasites, however *Bartonella* had a higher than expected association with ectoparasites. This is expected, as ectoparasites are the vectors for *Bartonella,* and it is expected that a positive association is seen in order for transmission events to take place. Within this study, host aggregation was positively correlated with differing parasite taxa. Both rodent hosts showed a significant positive correlation between ticks and nematodes -which contrasts previous work.

When investigating whether host characteristics explain co-infection within rodents, it was found that no host characteristic explained co-infection patterns in wood mice. Only age structure with respect to being juvenile explaining co-infection patterns in bank voles. As previously found within chapter 4, seasonality plays a role in the levels of parasite acquisition. This is not a surprising finding as parasites are known to show seasonal patterns with increased prevalence within the summer months, as was found here.

Throughout this thesis wood mice and bank voles have been analyzed separately. Although both woodland rodents are susceptible to the same parasites there have been differences observed within the study. Differences observed are potentially due to differences is host behaviour. As bank voles are more likely to live in intersexual communal groups, this could therefore facilitate the transmission of parasites more easily within the nest.

6.1 Spatial heterogeneity

Research into spatial heterogeneity suggests that habitats that show a low degree of fragmentation and have a high level of connectivity provide better habitat structure to allow species to persist (Christensen, Ecke et al. 2008; Mortelliti, Amori et al. 2009). Habitats that are highly connected are important for species dispersal, persistence and for maintaining genetic diversity within a

population (Schooley and Branch 2011). Therefore the expectation is that discontinuous habitats can potentially lead to negative genetic consequences such as inbreeding or loss of variation through genetic drift when populations are small and/or isolated (Charlesworth 2003). It has been shown that fragmentation does play a negative role in species (Ekross, Heliola et al. 2010; Krauss, Bommarco et al. 2010), as small fragments tend to be more vulnerable to disturbance than non-fragmented habitats due to edge effects, and there is often a positive correlation between the size of a habitat fragment and its quality (Holland and Bennett 2010). However, fragmentation does not always result in a negative effect on populations, as species' responses to discontinuous habitats are highly variable. Within this study (chapter 3) I was interested to see whether woodland rodents demographics were affected by natural fragmentation.

Wild rodents are have been widely used to investigate population-level effects of fragmentation (Krebs 1966; Moretlliti and Boitani 2007; Christensen, Ecke et al. 2008; Suzan, Armien et al. 2008; Holland and Bennett 2010). Host characteristics are known to vary within fragmented habitats when compared to well-connected habitats. Population size and density is known to decrease in fragmented habitats (Holland and Bennett 2010; Krebs et al. 1969; Poulin 1996). The findings within in this study (chapter 3) also support this conclusion, as mainland populations were larger than those of island populations. The islands have a smaller area of suitable habitat available to them, as well as the area being uninhabitable between sites as there is a body of water separating them. No individual was found to move between sites, so the fragments within this study are not acting as metapopulations within the period of study. Of the host characteristics investigated here there was no difference in age structure, sex ratios, body condition, survival or longevity, which is in contrasts to the findings of others (Bower and Smith 1979; Holland and Bennett 2010; Villafueto 1997). However the majority of these studies have tended towards habitats that are unnaturally fragmented. In contrast, the fragmented habitat within this study has taken place over a geological time scale, with the potential to allow hosts to adapt to their habitat. It can also be suggested that, although fragmented, the habitat quality is not any poorer than that seen on the mainland (although this was not directly measured). The host characteristics examined have not led to

any indication that fragmented populations were any different from those on the mainland. Both wood mice and bank voles are habitat generalists, and it has been found that habitat generalists do better in fragmented populations than specialists (Szacki, Babinska-Werka et al. 1993; Andren 1994; Puttker, Meyer-Lucht et al. 2008; Panzacchi, Linnell et al. 2010).

As any potential changes in the demographics of one species have the potential to alter their interactions with other species in the same system, including host-parasite interactions. This work has allowed us to address the broader question of how parasite dynamics are affected by the spatial structure of a host population. We are able to exclude a number of potential drivers from differences seen between populations in terms of host-parasite dynamics. As no differences were found between population in terms of sex or age, we can rule out any specific differences in susceptibility which could result in differing prevalence's between connected and non-connected populations, as was found for sex - (Harrison, Scantlebury et al. 2010; Luong, Perkins et al. 2010) and age- (Gillespie, Lonsdorf et al. 2010; Harrison, Scantlebury et al. 2010; Liberman, Khokhlova et al. 2011) within other studies. Furthermore, as no measures were found to be different in terms of breeding or that hosts were in poorer body condition, we can rule out an increase in susceptibility to infection, as was found for studies were more individuals were breeding (Luong, Perkins et al. 2010), or being in poorer body condition (Beldomenico, Telfer et al. 2009). Contrary to those demographics that were different, it was found that rodents on islands had a lower population size compared to those on the mainland. Having a lower lower population density on islands has the potential to reduce infection rates for parasites where transmission is dependent on host density, as is generally assumed for non sexually transmitted diseases. This, combined with a more defined upper population size limit on islands could potentially lead to extinction of parasites, consistent with the idea of a critical host community size (Anderson and May 1982; Lloyd- Smith, Cross et al. 2005). However at the same time, similar numbers of captures per successful trap on the trapping grids were found and this suggests that contact rates among rodents may not necessarily be lower on islands.

6.2 Individual heterogeneity

Individual heterogeneity is nested within spatially structured populations. Heterogeneities within natural populations arise from individual differences to environmental variables. When thinking about heterogeneities in parasite infections the variation between individuals is a major feature in the spread of infectious diseases (Cattadori, Albert et al. 2007). Certain individuals have been found to be linked to the spatial persistence of diseases. These individuals can be classed into categories: (1) sex, with males exhibiting higher parasite intensities than females (Perkins, Cattadori et al. 2003; Ferrari, Cattadori et al. 2004; Ferrari, Rosa et al. 2007), (2) body mass, with heavier individuals having higher prevalence and/or intensity of infection (Gillespie, Lonsdorf et al. 2010; Harrison, Scantlebury et al. 2010), (3) breeding status, with individuals in breeding condition increasing the chances of becoming infected (Luong, Perkins et al. 2010),with an effect dependent on sex (Perkins, Cattadori et al. 2003; Ferrari, Cattadori et al. 2004; Ferrari, Rosa et al. 2007), with males more likely than females to have higher prevalence of infection as the chances of infection with a range of parasite species are increased when individuals are interacting and moving around more during the breeding season. (4) Body condition (Beldomenico, Telfer et al. 2008), it is not surprising that those individuals in poor body condition are more likely to be infected with a parasite infection. (5) Finally movement of individuals are likely to have an effect on whether an individual comes into contact with a parasite infection (Brown, Macdonald et al. 1994). However, to date very few studies have involved investigating a range of parasites species and concentrated on single infections, as detailed above. Therefore very little is known about what host characteristics can explain patterns of parasites communities as a whole. From chapter 5, it was found that when looking at a range of parasite taxa only age structure within bank voles explained patterns of co-infection. As weight was used as a proxy as well as coat texture to distinguish rodent age classes (Telfer, Bennett et al. 2002), previous work has suggested that being a larger (and therefore older) individual leads to a higher prevalence of infection, as such individuals have been exposed longer to parasite infections compared to juveniles (Harrison, Scantlebury et al. 2010).

This intuitively makes sense as if you are older (and larger) you are more likely to become infected with a range of parasites across functional groups. However this is the opposite to what was found within this study as juvenile bank voles had a greater number of parasite taxa. However this was not found for wood mice, where previous studies have shown that host characteristics do explain parasites patterns in wood mice specifically (Harrison, Scantlebury et al. 2010).

6.3 Disease persistence

Research into critical community size, as discussed within the overall introduction, is lacking empirical examples in wildlife populations. Even more so, there is a lack of studies that account for parasites communities as a whole rather than investigating only single parasite species. The only study that has managed to demonstrate thresholds was Begon *et al*. 2003 that showed that there was evidence for a threshold population size but only in terms of the numerical size of the population and not in terms of the density of the population, but for only a single parasite infection (cowpox). Their study differs from ours as the island system used here involves larger islands and has a greater distance between each of the islands. As previously stated, no rodent was found to move between island sites or between the mainland and island sites, therefore it can be suggested that isolation plays a stronger role in our study compared to the previous study system. Additionally, the presence of only wood mice and bank voles, and therefore a low species diversity within the sites examined, allowed for a more powerful test, as the chances of parasites being able to survive within our study system, due to the potential lack of hosts was reduced. Furthermore this study has looked at multiple parasite infections across naturally fragmented rodent populations. As fragments tend to be small and isolated (as in the case of the island of Loch Lomond), small populations sizes are predicted (Kruess and Tscharnke 1994; Pullin 2002; Ameca y Juarez, Mace et al. 2011). Which indeed was the case. Therefore, there is an expectation that overall parasite distribution and prevalence of infection may be limited because parasites may become locally extinct or have even failed to colonize these small isolated host populations. This however was not the prediction that was supported (chapter 4).

Previous studies have shown that transmission rates are higher when population densities are high (Morand and Poulin 1998; Puttker, Meyer-Lucht et al. 2008). However, even when host populations on islands were lower than those on the mainland, there was no reduction in the number of parasite species found within fragmented habitats. The likelihood of extinction events for both host and parasites are potentially increased when genetic diversity is low. This is expected in small host populations as small isolated populations have an increased chance of inbreeding, which can reduce both fitness and genetic variation within the population. Extinction events are thought to be unlikely within this system, as the majority of the parasites on islands did not show a reduced prevalence of infection compared to mainland sites.

Furthermore, disease persistence is likely to continue due to the nature of the direct life cycles of these parasites. This matches what is thought, as those parasites that undergo an indirect life cycle are more likely to be affected by local extinction, as intermediate hosts are needed, and could therefore be lacking on island sites. However, parasites that rely on vectors for disease transmission, as in the case of *Bartonella*, within our study system, could potentially be disadvantaged in small host populations due to the chances of local extinction of vector or alternative hosts, therefore leading to a disruption in the mechanisms for transmission. Fleas are the thought to be the primary vector for *Bartonella* transmissions. Fleas are under-represented within our study (although are present on island sites), and absent from three islands were *Bartonella* was found. *Ixodes* ticks are highly prevalent within the study; therefore it is highly plausible that ticks are playing a potential role in the transmission of *Bartonella* in the absence of their known vector. Such an alternate vector may be allowing *Bartonella* to persist within the fragmented landscape. This study has provided further evidence for the putatitive role *I. ricinus* has as a competent vector for *Bartonella* infections when fleas are absent.

Overall it has been found that host-parasites dynamics investigated within this study system, involving a range of parasites species found in two generalists rodents within a naturally fragmented habitat, are not likely to be disturbed due

to the nature of being fragmented. Both rodent host and parasites are able to persist within the island sites, contrary to what has been previously thought.

Suggestions for future research

Building upon the research carried out within this thesis, the study has highlighted some gaps in knowledge that warrant further exploration. It has been shown that parasites on fragmented sites are able to persist within this study system, and that these fragmented sites seen to be relatively stable in terms of host and parasites dynamics. However little is still known about the temporal dynamics of an infectious stage of gastro-intestinal parasites (either helminths or protozoan). Further work is needed in order to understand how long an infectious egg/oocysts can persist within the environment. Within fragmented areas being able to persist within the environment for long periods of time is advantageous when host populations are reduced, therefore reducing the chances of extinction within that system. It was seen within this study that helminths and protozoan were capable of persisting. Therefore further work is needed to understand the mechanisms that allow eggs and oocysts to persist within the environment and the favoured factors that allow them to do so to avoid extinction.

A new area of research is coming to the forefront in disease ecology, the idea of wild immunology. Our understanding and knowledge of the immune system has been gained from laboratory studies on model organisms. However we know that diversity is high in wild population both in terms of genetics and environmental factors. Therefore there needs to be more focused research in wild immunology that links what we know from laboratory studies on the immune system responses to host-parasites dynamics and interaction from empirical wildlife examples (Pedersen & Babayan 2011). From chapter 5, it was seen that there were differences between wood mice and bank voles in terms of the co-infection and co-aggregation of parasites that they harboured. It was suggested that these differences could be explained by differences in host behaviour. However an area that could not be explored within the context of this research was that of differences in the immunity between the two hosts. Further work under the idea of wild immunology could be carried out to see if

there are any underlying differences in how the immune system of wood mice and bank voles deal with multiple parasites infections.

Finally, it is well known that hosts are often infected with multiple parasites simultaneously. More research is being focused on investigating parasites communities rather than single parasites. Field studies are beginning to investigate how parasites are interacting within their hosts (Ferrari, Cattadori et al. 2009; Telfer, Lambin et al. 2011). However further work is needed to understand the mechanisms that allow interactions and associations of parasites across a range of functional groups to co-exist. This needs to be carried out experimentally either in the laboratory or in the field using drugs to experimentally manipulate parasites assemblages in hosts.

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