The Social Structure, Ecology and Pathogens of Bats in the UK

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other university.

Signature: ……..

Dedicated to the memory of

Charles William Stewart Hartley

Abstract

This thesis examines the ecology, parasites and pathogens of three insectivorous bat species in Wytham Woods, Oxfordshire; *Myotis nattereri* (Natterer's bat), *M. daubentonii* (Daubenton's bat) and *Plecotus auritus* (Brown long-eared bat).

The population structure was assessed by monitoring associations between ringed individuals, utilising recent advances in social network analysis. Populations of both *M. daubentonii* and *M. nattereri* were found to subdivide into tight-knit social groups roosting within small areas of a continuous woodland (average minimum roost home range of 0.23km^2 and 0.17km^2 respectively). If this population structure is a general attribute of these species it may make them more sensitive to small scale habitat change than previously thought and has implications for how diseases may spread through the population.

M. daubentonii had a strong preference for roosts close to water, away from woodland edge and in areas with an easterly aspect. The factors driving roost choice in *M. nattereri* and *P. auritus* remain elusive. The segregation of *M. daubentonii* into bachelor and nursery colonies was not a result of the exclusion of males from roosts close to water by females, or variation in microclimate preferences between the sexes, as was predicted. Body condition (weight/forearm length) was correlated with host characteristics including age and reproductive status, and weather variables.

Astroviruses and Coronaviruses, which have characteristics typical of zoonotic viruses, were identified in UK bat species for the first time. Coronaviruses identified formed species-specific clades while Astroviruses were highly diverse. Though not closely related to human viruses these are potential zoonotic diseases of the future. Models of Coronavirus and ectoparasite distribution suggest individual attributes (e.g. sex and age) and population structure (e.g. the formation of nursery and bachelor colonies) are important predictors of parasite and pathogen prevalence.

This study characterises a system that offers many opportunities for future research including studies of sociality, disease modelling and conservation management.

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Figure 5.5 – Unrooted, maximum likelihood phylogeny of Astrovirus RdRP sequences (253bp) including novel strains (indicated by bold lettering) found in British bats. Using model comparison the best model of DNA evolution was selected by MEGA5, this was a general time reversible model (GTR), with non-uniformity of evolutionary rates between sites (gamma) and a fraction of sites invariable over time (I). Coloured circles indicate the species from which the virus was detected for samples collected in this study (see colour key above), black circles indicate human Astroviruses. Names in blue represent bat Astroviruses. Two major clades of bat viruses are highlighted. Clade 1 includes a previously identified Rhinolophid clade (Zhu *et al.* 2009). Where more than one identical sequence was obtained from a single individual only one was included in the phylogeny. Boot strap values (1000 replicates) are indicated as percentages where the value was greater than 70%. Scale bar indicates base differences per nucleotide site 210

Abbreviations

^{o}C – Degrees Celcius

µl – Microlitre

ANOVA – Analysis of Variance

AIC – Akaike Information Criterion

BLAST – Basic Local Alignment Search Tool

bp – base pairs

c. – Circa

cf. – Compare to...

CI – Confidence Interval

cm – Centimetre

CoV – Coronavirus

DCHM – Digital Canopy Height Model

DDT - Dichlorodiphenyltrichloroethane

df – Degrees of Freedom

DNA – Deoxyribonucleic acid

DTM – Digital Terrain Model

EBLV – European Bat Lyssavirus

ECN – Environmental Change Network

EGI – Edward Grey Institute of Field Ornithology

EID – Emerging Infectious Disease

EU – European Union

g – Grams

GAM – Generalised Additive Model

GIS – Geographic Information System

GLM – Generalised Linear Model

GLMM – Generalised Linear Mixed Model

GPS – Global Positioning System

Ha – Hectare

HIV – Human Immunodeficiency Virus

I - Importance

Km – Kilometres

LiDAR – Light Detection and Ranging

m – Metres

m/s – Metres per Second

MCP – Minimum Convex Polygon

MLST – Multilocus Sequence Typing

mm – Millimeters

NA – Not Applicable

PBS – Phosphate Buffer Saline

PCA – Principal Component Analysis

PCR – Polymerase Chain reaction

PFU – Plaque Forming Units

PIT tag – Passive Integrated Transponder Tag

RABV – Rabies Virus

RdRP – RNA-dependent RNA-polymerase

RH – Relative Humidity

RNA – Ribonucleic Acid

RT-PCR – Reverse Transcription Polymerase Chain Reaction

SARS – Severe Acute Respiratory Syndrome

s.l. – *Sensu lato*

SNA – Social Network Analysis

ssRNA – Single Stranded Ribonucleic Acid

TOBEC – Total Body Electric Conductivity

UK – United Kingdom

W/m^2 – Watts per metre-squared, a measure of solar radiation.

YPD – Yeast Extract Peptone Dextrose

1 General Introduction

1.1 Introduction

Bats are the most ecologically diverse and second largest order of mammals, accounting for one in every five mammal species. The earliest fossil bat is approximately 53 million years old (Jepsen 1966) and over their long evolutionary history bats have filled a range of ecological niches. For example, the diets of bats include insectivory (insects), fruigivory (fruit), nectivory (nectar), sanguivory (blood), piscivory (fish) and terrestrial and aerial carnivory of non-insect prey. The diversity of bats is not limited to their diets, a similar range of strategies exist for mating, roosting, foraging, and other aspects of their ecology.

Like much wildlife, bats continue to be affected by anthropogenic activities. These include urbanisation, habitat destruction and hunting. The slow reproductive rate of bats, normally giving birth to only one pup a year, means that bat populations typically take a long time to recover from declines.

Over the past 15-25 years bats have been recognised as an important source of disease causing pathogens including Nipah, Hendra, SARS and Ebola. These have caused outbreaks in humans and non-human animals alike, causing many fatalities and large economic losses.

This introduction reviews the past and present threats to the conservation of bats, and the recent emergence of diseases from bats to humans. It puts in context the aims and objectives of this thesis which are presented at the end of this chapter. Broadly, I aim to further our understanding of the ecology, social behaviour and disease dynamics of bats in a British lowland wood.

1.2 Biology of bats

1.2.1 General life history

Bats have long been known to be something of an anomaly amongst small mammals. Whilst mammals of a similar size typically have high mortality and birth rates, bats have low mortality and birth rates and invest in prolonged post-birth care. Bats typically only have one pup a year and are long-lived; of the species present in the UK individuals are known to live for 2-5 years on average but can live for up to 30 years (Schober & Grimmberger 1997).

1.2.2 Taxonomy of bats

The taxonomy of bats has been debated for many years but recent molecular studies have helped to generate consensus (Teeling *et al.* 2005). These studies suggest that flight evolved once amongst bats, all bat species being monophyletic, but that echolocation may have evolved twice. It was originally thought that Pteropid bats (family Pteropodidae: fruit bats), those that do not echolocate, formed a sister group to all other bats, suggesting echolocation evolved once. These two groups were named megachirpotera and microchiroptera respectively. New phylogenies based on sequence data suggest that the megachiroptera clade should also include the echolocating Rhinolophoid bats (super-family Rhinolophoidea). This group (Pteropids and Rhinolophoids) has been termed 'Yinpterochiroptera' and the remaining microchiroptera 'Yangochiroptera'. Thus echolocation either evolved twice, or ancestral Yinpterochiroptera were able to echolocate but this ability was lost by those which evolved into present day pteropid species (Teeling *et al.* 2005).

1.2.3 Reproductive cycle of temperate bats

Temperate bats hibernate during winter months when food is scarce and cold temperatures increase the cost of homeothermy. Bats emerge from hibernation in spring and give birth to young in early summer (Figure 1.1). In most species females come together to form nursery colonies during gestation. These nursery colonies stay together until pups are independent, in the autumn. At birth, pups are approximately 20-30% the weight of adults (Altringham 1996).

Mating occurs in late summer, autumn and during hibernation when males briefly come out of hibernation and mate with torpid females. Sperm is commonly stored until ovulation occurs in late winter or early spring. Mating strategies can vary between species, for example the *Pipistrellus pipistrellus* (Common pipistrelle) is a harem forming bats while others such as *Myotis nattereri* (Natterer's bat) fly great distances in autumn to swarming sites where individuals from a large geographic area congregate to mate (Altringham 2003).

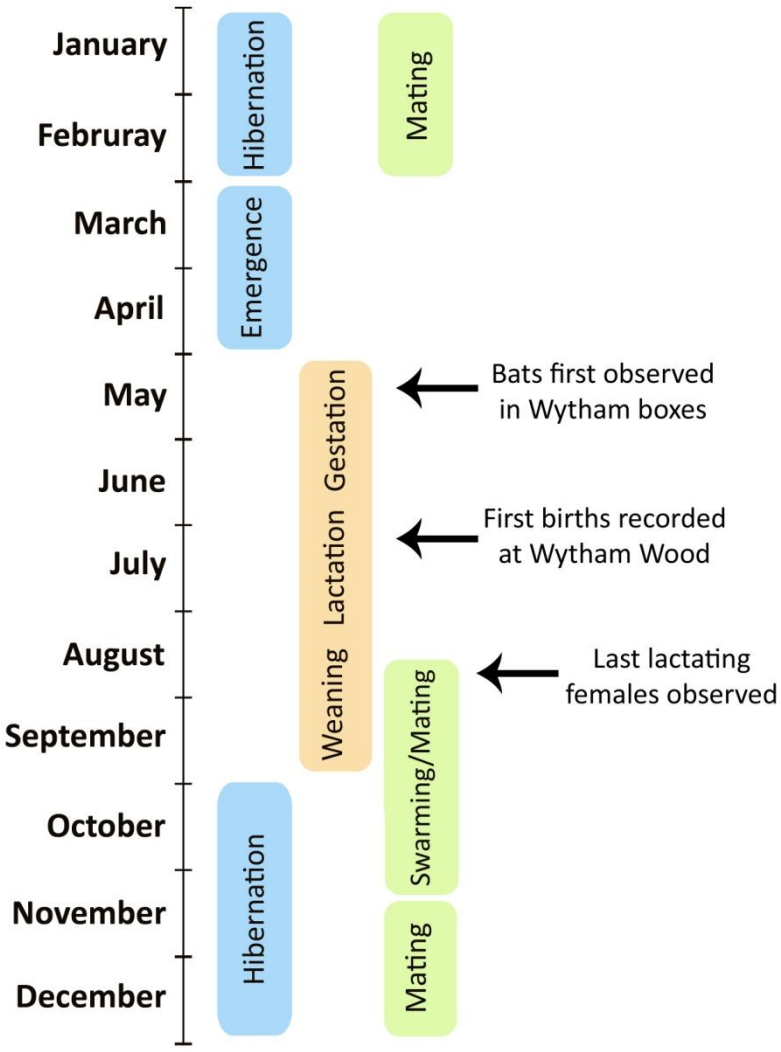

Figure 1.1 – The annual cycle of bat activity in the UK (adapted from Altringham (2003)) with modifications showing details for Wytham Woods (using data from 2009 and 2010).

1.2.4 Life in colonies

Bats are form large aggregations of tens to millions of individuals. There are two principle reasons for colony formation in bats. Firstly, in many landscapes roost sites are limiting. This is particularly true for bats that roost in caves such as the *Tadarida brasiliensis* (Mexican free-

tailed bat) which forms the largest aggregations of any mammal (Davis *et al.* 1962). Secondly, individuals within a colony may gain benefits from roosting with others. These benefits include social thermoregulation, allogrooming, information sharing and predator avoidance (Safi 2008).

As a consequence of their gregarious lifestyle, long lifespan and mobility (allowing individuals to choose where to roost) bats have complex social structures (O'Donnell 2000b; Vonhof *et al.* 2004; Fortuna *et al.* 2009). Evolutionary theory predicts that individual bats will maximise their fitness both in the context of cooperative behaviour and mating opportunity. This behaviour generates the observed social structure. Colonies of temperate bats undergo frequent subdivision and recombination of colonies with varying membership (Kerth & Konig 1999; Willis & Brigham 2004; Popa-Lisseanu *et al.* 2008; Kerth *et al.* 2011), a behaviour shared by a small number of other mammals (Aureli *et al.* 2008). This behaviour is associated with non-random associations, with some pairs of individuals associating more frequently than others (Kerth *et al.* 2011). Another property of some social mammals, such as primates and elephants (Sapolsky 2005; Wittemyer *et al.* 2005), is a dominance hierarchy. There is as yet insufficient evidence to support the presence of hierarchies amongst bats (Kerth *et al.* 2003; Ortega & Maldonado 2006).

1.3 Bats as ecosystem service providers

Bats are often viewed as pests when present in the urban environment. Usual complaints include noise, defacement of property by guano and a perceived risk of disease (Guilliatt 2011). However, bats also provide a number of ecosystem services. Ecosystem services are the elements of ecosystems that are used directly and indirectly to support human wellbeing (Fisher *et al.* 2009). For bats these services include consumption of agricultural pests, pollination and seed dispersal, bush meat, fertiliser (guano) and cultural benefits.

1.3.1 Consumption of agricultural pests

Approximately one third of bats species are insectivores, feeding on a range of insects from midges and mosquitoes to beetles and moths. The high energetic demand of a bat's lifestyle, including flight and high maternal investment, require individuals to eat large quantities of insects each night. Investigation of wild bats suggests that individuals may consume from 50% to over 100% of their body mass in insects in a single night (Kurta *et al.* 1989; Kunz & Stern 1995; Kunz *et al.* 1995; Encarnação 2006). Studies using microscopy and genetic analyses have found the remains of a number of agricultural pests in faeces from numerous bat species (Table 2 in Kunz *et al.* 2011). As many bats are generalists, agricultural pests are likely to be predated by bats all over the world. One well studied example is the *Tadarida brasiliensis* (Brazilian free-tailed bat). This generalist insectivore can form colonies of millions of individuals and is a predator of agricultural pests (Desmarais *et al.* 1980; Mizutani *et al.* 1992). Valuations of the service provided by these bats in terms of damage avoided to cotton crops is estimated at $0.02 per bat per night in the summer, totaling $638,000 annually across a 4000Ha area in Texas (Cleveland *et al.* 2006). The value of bats to American agriculture has been estimated at approximately $22.9 billion per year, with a minimum value of $3.9 billion (Boyles *et al.* 2011). While this study depends on many assumptions, it is clear that even the lower monetary estimate indicates bats are a valuable contributor to natural pest control and reduce agricultural production costs.

1.3.2 Bats as pollinators and seed dispersers

Two families of bats are responsible for pollination and seed dispersal services, the Pteropodidae (fruit bats) and Phyllostomidae (New World leaf-nosed bats) whose members feed on fruit, pollen and nectar. The mobility of bats allows them to spread pollen and seeds over a much larger area then most other plant visitors (de Lacerda *et al.* 2008). While there are no monetary estimates of the value of bats as pollinators and seed dispersers, Kunz *et al* (2011) present a long list of economically and environmentally important plants that are either currently, or were historically, pollinated or dispersed by bats (Table 4 in Kunz *et al*. 2011). These include plants that provide products such as cashew nuts, mangos, dates, tequila, papaya, balsa wood, bananas, passion fruit, coffee and many others. In many cases however, bats are thought to be one of a number of species to pollinate or disperse seeds of a given plant. Furthermore, pollination by bats is often no longer necessary for widely grown, self-fertile, varieties of crops such as banana and mango.

1.3.3 Bats as a resource

Humans use two products of bats as resources, their meat and their guano. Guano is rich in nitrogen and phosphorus, nutrients that are often limiting for plant growth in the environment. It is mined from caves where bats roost, typically in the tropics, and is valued between $1.25 and $12 per pound (Kunz *et al.* 2011). Bats are hunted for meat in many tropical countries both as food for the hunter and to be traded commercially (Mickleburgh *et al.* 2009). The value of a bat as meat varies from $0.50-$1.50 per bat (*Pteropus vampyrus natunae* in Borneo; Struebig *et al.* 2007) to $10 per bat (*P. vampyrus* in Jakarta; Fujita & Tuttle 1991). Exact numbers of bats eaten are hard to obtain, however as an indication Wiles *et al* (1997) report that until 1994 between 10,000-16,000 bats were exported annually from the Palau islands (approximately 460km^2 in area), south east of the Philippines. Additionally, Kamins *et al.* (2011) estimate that a minimum of 128,000 *Eidolon helvum* are sold for meat in Ghana each year.

1.3.4 Cultural services

Perhaps the most difficult service to quantify, the cultural importance of bats, is also the most widely recognized by the public. Bats appear on artifacts from ancient civilizations, from Egypt to China and Japan to Mexico. They appear in paintings, prints and carvings that attract tourists to ancient ruins and museums (Altringham 2003; Kunz *et al.* 2011). In modern day culture bats appear in the media, on products (e.g. Bacardi), in films (e.g. Batman), cartoons (e.g. Batfink), and in nature documentaries. The latter may in part have stimulated the public's interest in bats and as such bat walks, cave tours and educational activities are becoming more commonplace. Indeed tourism generated by bats roosting in Congress Avenue Bridge, Austin, Texas, one of the world's largest colonies of urban bats, is thought to be worth approximately $3 million a year to the local economy (Ryser & Popovici 1999).

1.4 Conservation of bats

The effective conservation of any species requires an understanding of the threats to populations, and the ecology of the species, including their population structure, behavior and habitat requirements. Factors that have caused declines in bats in the past and continue to threaten bat populations today are considered below.

1.4.1 Threats to bat populations

1.4.1.1 Land use change and habitat loss

Land use change and habitat loss are the greatest threats to bat populations worldwide (Mickleburgh *et al.* 2002; Racey & Entwistle 2003). Urbanisation and agricultural expansion have led to the loss of vast areas of natural habitat, the impacts of which are felt across all taxa. For example, in Singapore 95% of natural habitat has been lost due to urbanization resulting in a loss of at least 28% of its biodiversity over 183 years (Brook *et al.* 2003). Conversion of woodlands to agricultural land has contributed to the net loss of 7-11 million km^2 of woodland globally in the past 300 years (Foley *et al.* 2005) resulting in local climate change and biodiversity loss (Soares *et al.* 2006). While bats are extremely diverse, the majority of species are dependent on woodlands for foraging or roosting (Mickleburgh *et al.* 2002) and as a consequence the loss of this habitat has contributed to declines in bat populations across the world (Brosset *et al.* 1996; O'Donnell 2000a; Altringham 2003; Racey & Entwistle 2003; Wiles & Brooke 2010).

1.4.1.2 Hunting

Hunting wildlife as a source of food and income is widespread. In many poor areas wildlife hunting is essential as a source of protein and income, and can be sustainable (Wilkie & Carpenter 1999; Brashares *et al.* 2004; de Merode *et al.* 2004). However, as human populations grow and becomes more effcient (e.g. by the introduction of guns) the pressure on wildlife populations has increased and hunting rates in many areas are now thought to be unsustainable (Milner-Gulland & Bennett 2003).

Bats are a common source of bushmeat in the tropics. Hunting has resulted in the extinction of some species (Cheke & Dahl 1981), and declines in others (Craig *et al.* 1994; Mohd-Azlan *et al.* 2001; Riley 2002). Island populations have been most greatly affected, presumably because there is little immigration into these populations and because large mammals, preferable sources of bush meat in terms of cost benefit for the hunter (Peres 2000; Milner-Gulland & Bennett 2003), are usually absent (Craig *et al.* 1994; Riley 2002). Irrespective of their size, animals like bats that have slow reproductive rates are frequently among the first species to go extinct when overhunted. While hunting bats in small numbers may be sustainable, more research is needed to estimate the sustainable harvest of bats in places were hunting is common (Mickleburgh *et al.* 2009).

Bats have also been the target of culls. In Australia fruit bats have been killed by farmers, who see them as pests. It is thought at least 240,000 fruit bats were culled on the East coast of Australia between 1986 and 1992 (Racey & Entwistle 2003). In South America the rabies vector *Desmodus rotundus* (Common vampire bat) has been persecuted for its role in transmitting rabies to cattle. This persecution included the destruction of 40,000 caves in Venezuela containing *Desmodus rotundus* and many other species (Hutson *et al.* 1993).

1.4.1.3 Exposure to toxins

Pesticides and agrochemicals are thought to have harmed bat populations both directly and indirectly. While little or no quantitative data are available, it is likely that the widespread use of highly effective pesticides in the 20th century negatively impacted bat populations by reducing the abundance of prey species. The presence and toxicity of pesticides and other pollutants in bats has been reported widely. Those chemicals found in bat tissues include organochlorides (Geluso *et al.* 1976; Clark *et al.* 1978; Clawson & Clark 1989; Senthilkumar *et al.* 2001; Stansley *et al.* 2001; Bennett & Thies 2007), organophosphates (Clark 1986; Clark & Rattner 1987; Eidels *et al.* 2007) and heavy metals (Clark 1979; Walker *et al.* 2007; Nam *et al.* 2012). There has also been a recent interest in endocrine disrupters, and though these have not been identified in bats there is evidence that these compounds are present in their insect prey (Park *et al.* 2009). In general bats have not been found to be any more sensitive to organochlorides, organophosphates or heavy metal toxins than other animals, but studies are limited to acute morbidity and mortality. Some of these toxins have been found to reduce bats coordination (Clark 1986; Clark & Rattner 1987) but the effects these toxins have on bats' behaviour, ability to forage or ability to avoid predation is generally poorly understood. Bats

have a number of attributes which make them particularly susceptible to the effects of pollutants. As insectivores they are exposed to higher levels of toxins than herbivores due to biomagnification, the increased concentration of many pollutants along a food chain. Their high energetic demands require increased food intake and their long lifespan allows significant bioaccumulation and increased chances of toxin exposure (Clark 1988). Additionally, mobilisation of fat-reserves after hibernation releases toxins which have been stored in adipose tissue over the winter period, resulting in a spike in their concentration in other tissues (Jefferies 1972). In the 1970's DDT and its breakdown compounds were found at one-third the lethal level in UK bats, and after hibernation this rose close to the lethal level for *Pipistrellus* species tested (Jefferies 1972). Organochlorides used to treat timber in roof spaces against wood-boring beetles and rot-causing fungi have been found to kill bats in the UK and have now been replaced with more suitable chemicals (Racey & Swift 1986). Surveillance for heavy metals in UK bats found low levels of mercury, lead and cadmium (Walker *et al.* 2007). The challenge for future research is to go beyond the current practice of identifying toxins in wild bats, or calculating the mortality rates for a range of doses, and examine the long term effects of specific toxins on fitness.

1.4.1.4 Current threats to bats in the UK

Within the UK bats are protected by law and so threats such as hunting and persecution are not significant. This protection is afforded by the Wildlife and Countryside Act 1981 and the Habitat Regulations, the latter put in place by the UK government as required by the EU Habitat Directive. This legislation prohibits the disturbance of bats or the destruction of their roosts without a license (Mitchell-Jones & McLeish 2004).

Though regulated by UK law, the primary threat to bats in the UK is habitat destruction including the exclusion of bats from human dwelling places. Where roosts need to be destroyed they are replaced with artificial roosts but the effectiveness of this mitigation has been poorly studied. Another area lacking data is the effect of timber harvesting in managed woodlands. Though forestry practice is to leave some mature, or standing dead trees, it is unclear how effective this is at reducing disturbance to bat populations.

A recently identified threat to bats in the UK are wind turbines. This is an area in need of more research, both to assess the numbers of bats killed by wind turbines and how their placement, or the turbines themselves, can be improved to reduce harm to bat populations (Kuvlesky *et al.* 2007).

1.4.2 Understanding species ecology

To conserve wildlife effectively it is necessary to understand what species need to sustain viable populations. For bats this includes an understanding of the type and size of habitat needed to support a population, including both foraging and roosting habitat (Racey & Entwistle 2003).

The general foraging and roosting habitats used by British bats are relatively well understood; that is to say that some information is available on the preferred habitats for roosting and foraging for almost all species (Altringham 2003). Despite this there is little specific, detailed information on roost preference (e.g. height, temperature and tree species) and foraging habitat (e.g. vegetation density and foraging height).

There are also limited data on the requirements of bat populations in terms of land area and number of roosts. In fact, even the concept of a 'population' of bats is poorly defined. It is clear that UK bats form colonies (Altringham 2003), but there are few data on the rate of movements of individuals between colonies or movement of colonies between roosts. Data currently available are based on radio-tracking and so are limited to a small sample size for each study. These studies reveal that bats species including *Myotis daubentonii* (Daubenton's bats) and *M. nattereri* switch roosts every few days and use a number of roosts (Smith 2000; Lucan & Radil 2010). However these studies consider a small number of individuals rather than the colony as a whole. Data are also lacking on the ability of groups to respond to changes such as the loss of individual bats or habitat. Such information would be a useful addition to our current understanding, permitting management plans to take account of the importance of individual roosts, and habitat patches to bats populations.

1.4.3 Social network analysis (SNA): a conservation tool

The current poor understanding of bats' population structure makes it difficult to predict the impacts of anthropogenic activities including habitat alteration or the destruction of roosts.

SNA allows the structure of a population to be identified by constructing a network of individual associations. They are composed of two elements, nodes and associations. Nodes represent individuals within the network, while lines connect nodes (i.e. individuals) that have associated in some way. These networks can be based on a range of association types, for

example individuals may be connected in the network if they interact aggressively, mutualistically or physically, each network providing different information. Networks can also be used to place individuals into social groups, i.e. collections of individuals that associate more frequently with each other than with individuals from other social groups (Figure 1.2). In addition SNA can be used to quantify the position of individuals within a network. For example the number of individuals that a given individual is associated with is defined as its 'degree'. Additionally an individual's 'betweeness' is the number of shortest paths, connecting individuals in the network, which pass through the focal individual, giving an indication of how central to the network an individual is.

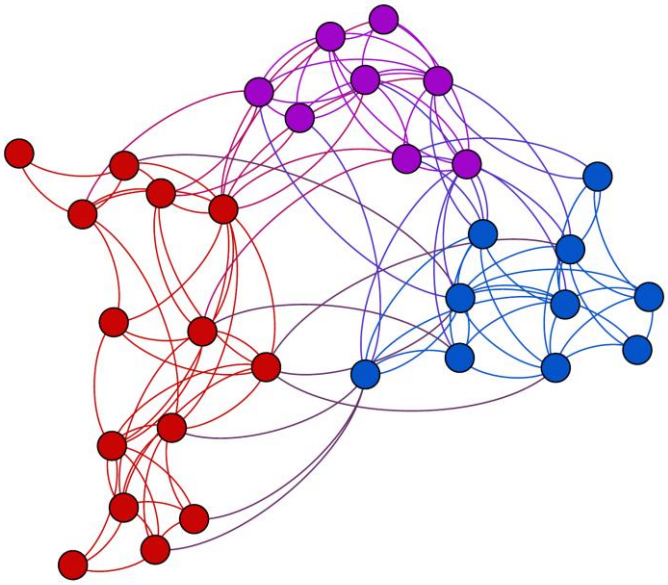

Figure 1.2 – Social networks are composed of individuals (nodes), show here by circles, and associations (lines). This network of students in an Exeter University class assumes individuals are associated if they are friends on Facebook. Individuals can then be assigned to social groups using a quantitative approach, indicated here by the colour of each node.

Network analyses have been used to understand physical systems (e.g. transport connections and data flow through computer networks) for many years. The techniques have also been applied to human social behaviour (e.g. spread of sexually transmitted diseases and rumours). However, SNA has only been applied to the study of wildlife in the past 15 years. The social structure identified in networks can be used to direct the focus of conservation work by identifying functional units of biological significance, such as social groups. SNA can also be used to understand the mechanisms by which diseases may spread through a population, which can be applied to optimize vaccination strategies (Beyer *et al.* 2012). SNA may also

highlight individuals in the population that are disproportionately important for ensuring the reproductive success of a group, for example females within matriarchal societies. Using these methods *Orcinus orca* (Killer whales) have been shown to have a population structure that may increase their susceptibility to disease outbreaks (Guimaraes *et al.* 2007), *Macaca nemestrina* (Pigtailed macaques) suffer a breakdown of social structure if individuals key to policing are removed (Flack *et al.* 2006) and populations of *Chalinolobus tuberculatus* (long-tailed bats) in New Zealand show a high level of social structure suggesting conservation should focus on conserving social groups (O'Donnell 2000b).

At present there have not been any studies of the social structure of bats in the UK using a social networks approach, and only one species (*Myotis bechsteinii,* Bechstein's bat) has been studied elsewhere (Kerth *et al.* 2011). SNA studies of bats not native to the UK have found differences in species' propensity to form social groups, the area used by a social group, and the level of intergroup associations (O'Donnell 2000b; Vonhof *et al.* 2004; Fortuna *et al.* 2009).

Using the results from social network analyses it is possible to explore the spatial structure of the population using geographical information systems (GIS). These analyses reveal the area use by social groups and their habitat preferences. This spatial structure can be important for conservation (O'Donnell 2000b), identifying the area needed to support a social group, and for vaccination campaigns (Haydon *et al.* 2006), predicting the rate of disease spread through a population.

Population structure, which can be identified using SNA, will have a significant impact on the spread of disease. Populations with little structure, in which individuals associate at random with other individuals, allow for diseases to spread rapidly across the network. Populations with discrete subunits (i.e. social groups or cliques) may have high rates of transmission between individuals within the same group but low rates of transmission between groups. One might expect larger social groups, and groups with greater connectivity to others, to have higher prevalence of disease. Among individuals those with many associations with others in the network (i.e. high degree) or those who are members of more than one social group (i.e. high betweeness) might also be expected to have higher disease prevalence.

SNA will be of great importance to the conservation of bats in the future but comes with a number of challenges. The technique requires numerous repeat observations of a large number of marked individuals, however, bats are often hard to observe being highly mobile and roosting in hard to reach locations. As in the current study, these difficulties can be overcome by studying bats that use artificial roosts and are therefore readily accessible.

1.5 Bats and disease

1.5.1 Bats and ectoparasites

The diversity we observe in bats is matched by the diversity of their ectoparasites. Bats are host to a number of different types of ectoparasites the most common being mites, ticks, bat flies, bugs and fleas. In this thesis I examine mites, bat flies and fleas present in the populations under study.

There are 64 species of mite in the UK (Baker 2006), and the most conspicuous of these are members of the Spinturnicidae family (order Mesostigmata). This family is found exclusively on bats, and its members feed predominantly on the wing and tail membranes (Evans 1968). These blood-feeding mites spend their entire lifecycle on the host and are transmitted from host to host via direct contact in roosts. As, like other pathogens, mite transmission rates are likely to be dependent on contact rates it is hypothesised that mites and other directly transmitted pathogens will have a similar distribution within the population. If Spinturnicid mite load can be shown to correlate with pathogen prevalence, future studies may be able to use mite loads as a proxy for the probability of infection by directly transmitted pathogens (e.g. viruses) which may otherwise be difficult to detect.

M. daubentonii is additionally parasitised by bat flies, primarily *Nycteribia kolenatii* (Hurka 1964; Hutson 1984, Gardner & Molyneux 1988). These wingless diptera feed on blood and are thought to transmit *Polychromophilus murinus*, a malaria-like pathogen, and *Bartonella* between bats (Gardner & Molyneux 1988; Billeter *et al.* 2012). Bat flies give birth to terminal (3rd instar) larvae in the roost of their host, which immediately form puparia. Emerging adults locate a host and subsequently only leave the host to deposit larvae (Dick & Patterson 2006).

Bat fleas are commonly found infesting *M. nattereri* (Zahn & Rupp 2004). These fleas spend the first half of their life-cycle in the guano below a bat roost feeding on detritus before they develop into adults and search for a host (Lewis & Lewis 1994). Both bat flies and fleas are found in the fur and not on the wing membranes.

1.5.2 Emerging infectious diseases

The reported incidence of emerging infectious diseases (EIDs) has increased significantly since the 1940s and is thought to have peaked in the 1980's (Jones *et al.* 2008). EIDs are defined as those that have recently increased in incidence or geographic range, recently infected a new host population, recently been discovered or are caused by a newly emerged pathogen (Daszak *et al.* 2001). Some of the best known EIDs include diseases such as AIDS, Ebola and multi-drug resistant tuberculosis.

Of 335 EIDs from the second half of the 20th century studied by Jones et al (2008), 60% were zoonotic i.e. those that can be transmitted between animals and humans. Of those, 72% originated in wildlife. EIDs are twice as likely to be zoonotic than expected, given the proportion of all diseases that are zoonotic (Woolhouse & Gowtage-Sequeria 2005). In addition, the proportion of EIDs attributable to wildlife is thought to be increasing over time (Jones *et al.* 2008). These patterns are likely to be a result of increased contact between humans and animals as a result of agricultural intensification and human encroachment into previously wild habitats.

Viruses account for 37-44% of emerging infectious diseases but only 15% of all human pathogens (Cleaveland *et al.* 2001; Taylor *et al.* 2001; Woolhouse & Gowtage-Sequeria 2005). The high proportion of viruses amongst EIDs is probably due to the high nucleotide substitution rates in many RNA viruses (Woolhouse *et al.* 2001) which allows them to adapt quickly to a new host. RNA viruses account for a number of important zoonotic pathogens including HIV, influenza A virus, SARS coronavirus, and Ebola virus (Woolhouse *et al.* 2005). Movement of humans and animals around the globe in recent times is likely to have resulted in the mixing of viruses that otherwise would have not come into contact. Such mixing can result in recombination among some viruses (e.g. influenza viruses), whereby large parts of the viral genome are swapped between virus strains generating new viruses. As a consequence emergence after recombination events may become more frequent amongst viruses.

1.5.3 Bats as a source of zoonotic EIDS

Bats have become a focus of EID research over recent years. They have been identified as the reservoir host of a number of zoonotic diseases of human health concern, many of them RNA

viruses. Studying these EID events from bats it is possible to identify trends in the conditions surrounding disease spillover that can inform the direction of future research.

1.5.3.1 EID viruses associated with bats around the world

Ebola virus and Marburg virus

The genera *Ebolavirus* and *Marburgvirus* belongs to the family *Filoviridae*. The viruses have enveloped virus particles enclosing non-segmented, negative sense, single stranded RNA (ssRNA) genomes. Both viruses cause viral hemorrhagic fever with very high mortality rates in humans. Since 1976 there have been numerous outbreaks of Ebola virus, thought to total 2317 cases and 1671 deaths (67% mortality) in humans, almost all confined to Africa (Pourrut *et al.* 2005; Leroy *et al.* 2011). Marburg virus outbreaks have been more infrequent and more sporadic – though large outbreaks have also been confined to Africa.

A number of surveillance and experimental infection projects have found that a range of fruit bats and insectivorous bats can support replication and circulation of Ebola virus without succumbing to disease, suggesting they may be reservoir hosts of the virus (Swanepoel *et al.* 1996; Leroy *et al.* 2005; Pourrut *et al.* 2006; Leroy *et al.* 2009; Pourrut *et al.* 2009). Similarly Marburg virus has been associated with *Rousettus aegytiacus* (Egyptian fruit bats) (Towner *et al.* 2009). Studies of the 2007 outbreak of Ebola in the Democratic Republic of Congo have linked the Ebola virus outbreak with annual migrations of fruit bats in the area that coincided with hunting of bats to eat and to sell at markets (Leroy *et al.* 2009).

The more common infection pathway of Ebola virus to humans may be through contact with infected primates, which like humans experience high mortality, however our growing understanding of the role of bats suggests more research in this area is needed.

Hendra virus

Hendra virus is a *Henipavirus* in the family Paramyxoviridae. These viruses have enveloped virus particles enclosing non-segmented, negative sense, ssRNA genomes. Recent work suggests bats are host to many paramyxoviruses including viruses closely related to human mumps virus, mouse pneumonia and canine distemper and may be the ancestral host of these viruses.

It is thought that horses become infected with Hendra virus by consuming fruit dropped by bats and contaminated with their saliva or urine, before passing the infection on to humans (Field *et al.* 2001). Up until 2011 there were 18 fatalities amongst horses and 1 fatality of a man who had cared for infected horses (Plowright *et al.* 2011). In 2011 22 horses died in Queensland and New South Wales. Data collected from the most recent outbreak is yet to be published so this discussion only considers data prior to 2011.

Factors that may have led to the emergence of Hendra include habitat loss, urbanisation, seasonal changes in bat behaviour and climate.

Deforestation has caused Australian fruit bats to move into urban environments where they feed on exotic and native flora (Nobel 1996; Markus & Hall 2004). Colonies numbering up to 50,000 individuals now inhabit urban areas (Parry-Jones & Augee 2001) increasing potential contact rates between bats and humans or domestic animals.

Models of disease transmission (Plowright *et al.* 2011) predict that large urban metapopulations will be persistently infected, triggering waves of outbreaks in rural populations, though the exact patterns depended on the levels of immunity at the outset of the models and the migration distance parameter used. Models also suggest that the seasonality of Hendra virus spillover events (May to October) may correlate to increased prevalence of the virus in bat populations as maternal immunity amongst juveniles wanes (Plowright *et al.* 2011).

These SEIR (susceptible, exposed, infectious, recovered) models of Hendra virus transmission used life history data collected from Australian fruit bats and explored scenarios with different degrees of connectivity between roosts (modelled as the rate at which transmission rate declined with distance) and immunity (modelled as the proportion of individuals immune at the start of the model run), for which data are generally lacking in studies of bats and their pathogens. The connectivity and immunity parameters proved important for predicting the timing of outbreaks, their intensity, and the rate at which they spread through bat populations. The models also found that including heterogeneity in the duration of infections and in transmission rate among individuals, had significant effects on model results. As in other studies of disease (Lloyd-Smith *et al.* 2005; Beldomenico *et al.* 2009), this suggests that super-spreaders, individuals responsible for a higher than expected amount of disease transmission, may be important in Hendra virus dynamics. Additional field studies are now required to test model predictions and more accurately predict transmission rates and heterogeneity.

Previous studies suggest that changes in immunity in bat populations may have been the cause of increased Hendra prevalence. A period of reduced blossom and nectar availability in Australia, thought to be the result of an extended wet season in 2006, resulted in lower body weights and no recorded births in what would normally be the birthing period of *P. scapulatus* (Plowright *et al.* 2008). During the same period 80% seroprevalence was observed, with the odds of an individual being seropositive 14-42 times higher than in any other season. The reduced availability of foraging habitat may have resulted in both reduced immunodefence and dense aggregations of *P. scapulatus* on limited food resources (though neither were tested empirically) which may have contributed to the higher than normal seroprevalence (Plowright *et al.* 2008). While this event was triggered by natural climatic events, habitat destruction and other anthropogenic activities that reduce the availability of foraging habitats may have similar effects. This response to both climate and habitat destruction should be incorporated into future models using information on rates of deforestation and known climate variability.

Nipah virus

Nipah virus is the only other recognised species in the genus *Henipavirus*. It was first recorded during an outbreak of febrile encephalitis in peninsula Malaysia during 1998 and 1999. The outbreak resulted in 265 cases and 106 human fatalities, mostly in pig farmers (Chua *et al.* 2000). Swine were identified as the source of the disease in humans (Goh *et al.* 2000) and 1 million pigs were slaughtered, 60% of farms were closed and 36,000 jobs and $120 million in exports were lost (Daszak *et al.* 2001). Subsequent surveillance of wild animal populations found antibodies to Nipah virus in a number of species of fruit bats in Malaysia (Johara *et al.* 2001; Wacharapluesadee *et al.* 2005).

It has been suggested that regional drought and slash and burn deforestation may have caused an influx of flying foxes to northern peninsula Malaysia (Chua *et al.* 2002). However, there is also evidence to suggest that bats make long distance movements in this region in the absence of such conditions (Breed *et al.* 2006). Fruit bats roost and forage in orchards which are often located close to piggeries in Malaysia (Breed *et al.* 2006), and it is thought that pigs became infected with Nipah virus by consuming fruit dropped by flying foxes and contaminated with their saliva (Chau *et al.* 2002).

Since the original outbreak in Malaysia there has been a series of outbreaks in Bangladesh (Hsu *et al.* 2004; Gurley *et al.* 2007; Luby *et al.* 2009; Homaira *et al.* 2010). Genetic analysis suggested multiple spillover events from bats triggered chains of human-to-human

transmission (Harcourt *et al.* 2005) with date sap consumption a common feature of index cases (Luby *et al.* 2009). Bats have subsequently been shown to lick sap from trees being harvested for their sap and urinate into sap collection pots (Khan *et al.* 2010). Decreased food abundance due to habitat destruction in Bangladesh may have increased opportunistic foraging such as feeding on date palm sap and has been suggested to increase the likelihood of spillovers (Khan *et al.* 2010).

Seasonal changes in the shedding of Hendra virus in colonies of *Pteropus lylei,* a host of Nipah virus, have been detected in Thailand (Wacharapluesadee *et al.* 2010). Higher prevalences were detected in May across all sites, when offspring start to separate from their mothers suggesting maternal immunity may protect very young bats.

Severe acute respiratory syndrome (SARS)

8096 cases of SARS resulted in 774 fatalities between 1st November 2002 and 31st July 2003 (World Health Organisation 2011). Local transmission originated in China but rapidly spread to Mongolia, the Philippines, Singapore, Vietnam and Canada (World Health Organisation 2011). A novel *Betacoronavirus, Severe acute respiratory syndrome-related coronavirus* (SARS-CoV), was found to be associated with patients with SARS (Drosten *et al.* 2003; Ksiazek *et al.* 2003; Peiris *et al.* 2003). Coronavirus species are not uncommon in humans, however this novel virus caused a mortality rate of 9.5%, far greater than other human coronaviruses which are rarely fatal (Evans 1982). The high mortality and rapid spread of this virus made it one of the most important disease outbreaks in recent times.

Coronaviruses (family *Coronaviridae*) have enveloped virus particles enclosing non-segmented, positive sense, ssRNA genomes. They have a high rate of mutation, and recombination, allowing them to evolve rapidly, thereby increasing their chance to infect novel host species (Lai & Cavanagh 1997; Graham & Baric 2010).

The first cases of SARS appeared in restaurant workers handling wild mammals to be sold as food, and so preliminary surveillance work was undertaken at a live-animal market in Shenzhen, China (Guan *et al.* 2003). The study found SARS-CoV in four of five Himalayan palm civets (*Paguma larvata*) by PCR and neutralising antibodies in Himalayan palm civets, a Chinese ferret-badger (*Melogale moschata*) and a raccoon dog (*Nyctereutes procyonoides*). This showed that the markets provided an environment in which SARS-CoV could be transmitted between wild animals and potentially spill over to humans working there. While Palm civets were found to carry the virus, as they showed overt clinical symptoms when infected with

SARS-CoV they were not thought to be the reservoir host of the virus (Li *et al.* 2005). Surveillance of wildlife found SARS-CoV was nested within a group of closely related virues in Chinese horseshoe bats (Lau *et al.* 2005; Li *et al.* 2005). A temporally referenced phylogeny suggests SARS-CoV switched host from bats approximately 4 years prior to the SARS outbreak (Hon *et al.* 2008). Subsequent surveillance efforts identified diverse Coronaviruses from bats in Asia (Tang *et al.* 2006; Woo *et al.* 2006), North America (Dominguez *et al.* 2007), mainland Europe (Gloza-Rausch *et al.* 2008; Drexler *et al.* 2010; Reusken *et al.* 2010; Rihtaric *et al.* 2010; Drexler *et al.* 2011), Africa (Quan *et al.* 2010) and the Americas (Carrington *et al.* 2008; Misra *et al.* 2009; Donaldson *et al.* 2010).

Few studies have looked at the dynamics of Coronaviruses within bat populations, usually going no further than a phylogentic analysis of Coronaviruses detected. However, two studies in Europe suggest that maternal immunity and the introduction of susceptible juveniles in the autumn may be important factors in driving disease dynamics (Gloza-Rausch *et al.* 2008; Drexler *et al.* 2011).

1.5.3.2 EID viruses associated with bats in the UK

Lyssaviruses

Rabies is a disease caused by a number of viruses of the genus *Lyssavirus* within the family *Rhabdoviridae.* These viruses have enveloped virus particles enclosing non-segmented, negative sense, ssRNA genomes. *Rabies virus* (RABV) is responsible for most of the 40,000-50,000 human deaths every year from rabies (Bourhy *et al.* 2005).

The last decade has seen the eradication of RABV from most of Western and Central Europe (Fooks 2005), however, other lyssaviruses including *European bat Lyssavirus 1* (EBLV-1) and *European bat Lyssavirus 2* (EBLV-2) remain present. In Europe there have been approximately 50 recorded cases of rabies in bats every year but only four human cases since 1977 (Bourhy *et al.* 2005). *Eptesicus serotinus* (Serotine bat) is regarded as the main reservoir of EBLV-1 (Vazquez-Moron *et al.* 2008), whilst *Myotis* species host of EBLV-2 (Fooks *et al.* 2003). Unlike RABV which is almost always fatal (Anderson *et al.* 1981), EBLVs do not normally cause death in bats but will often be fatal for other mammals (Stantic-Pavlinic 2005; Amengual *et al.* 2007).

In 2002 a Scottish bat worker died of EBLV-2 (Fooks *et al.* 2003) leading to a range of works on EBLVs in bats in the UK (Fooks *et al.* 2004; Brookes *et al.* 2005; Fooks *et al.* 2006; Harris *et al.* 2006; Smith *et al.* 2006; Banyard *et al.* 2009; Smith *et al.* 2011). These reports have identified

antibodies to EBLV-2 at a low level in *M. daubentonii* (0.7-5.1%, 95% CI) within the UK (Smith *et al.* 2006). Given the low level of infection observed for EBLV-2 it has not been possible to examine the disease dynamics within bat populations. Data needed to allow modelling of lyssaviruses amongst bats includes immunological data on duration of infection and immunity as well as an understanding of transmission rates (Dimitrov *et al.* 2008). While some of this information is available for EBLV-1, little is available for EBLV-2.

Pertinent to this thesis is the recent identification of Bokeloh virus (BBLV), a lyssavirus closely related to EBLV-2 and other bat lyssaviruses (Freuling *et al.* 2011). This virus was isolated from a *M. nattereri* that presented with classic symptoms of rabies (i.e. aggressive behaviour) after 4 months in captivity. The animal died 10 days after the onset of symptoms. Whilst data is currently lacking on the prevalence of this virus amongst *M. nattereri* and other species, should *M. nattereri* prove to be a reservoir host of the virus, my studies of *M. nattereri* presented in this thesis may be a useful resource for disease models.

1.5.4 Can we predict and mitigate zoonotic diseases from bats?

Having reviewed some of the best studied zoonotic disease spillover events from bats to humans it is possible to identify commonalities that may help inform the direction of future work (Table 1.1).

Pathogen	Routes of infection	Drivers of emergence	Dynamics in bat populations	Risk factors for humans	Future research needed
Ebola virus	Human to human or infected mammal to human via contact with bodily fluids	Hunting of bats	Unknown	Contact with infected animals or humans	Empirical data on disease dynamics in bats
Hendra virus	Bats to horses to humans	Habitat loss and urbanisation	Annual cycle, possibly driven by seasonal influx of susceptible juveniles	Contact with infected horses	Empirical data on disease dynamics in fruit bats
Nipah virus	*Malaysia* : Bat to pig to human *Bangladesh* : Bat to human (via contaminated date palm sap) and human to human	*Malaysia* : Deforestation, drought, agricultural intensification *Bangladesh* : Possibly deforestation	*Malaysia* : Annual cycle, possibly driven by seasonal influx of susceptible juveniles *Bangladesh* : Unknown	*Malaysia* : Contact with pigs *Bangladesh* : Consumption of raw date palm sap or contact with an infected human	*Malaysia* : Duration of maternal immunity in bats *Bangladesh* : Empirical data on disease dynamics in fruit bats
SARS coronavirus	Bats to intermediate mammalian host to humans and human to human	High contact rates between animals and humans at wet markets	Maternal immunity may be important	Contact with infected animals or humans	Empirical data on disease dynamics in wild populations and studies of immunity
Lyssaviruses	Bat to human	Human contact	Unknown	Bat bites	Empirical data on disease dynamics in wild populations

Table 1.1 – Summary of previous EIDs thought to originate from bat populations

1.5.4.1 Drivers of disease emergence

Habitat destruction has been suggested as a driver of the emergence of Hendra and Nipah viruses (Chau *et al.* 2002; Markus & Hall 2004). This has caused bats to roost closer to human populations and puts them under greater nutritional stress if foraging habitat is lost (Plowright *et al.* 2008). This may lead to increased pathogen prevalence among bats and contact between bats and humans or domestic animals. Habitat destruction has also been implicated in the emergence of malaria in the Amazon (Vittor *et al.* 2006) and Lyme disease in the United States (LoGiudice *et al.* 2003). Deforestation in both these instances led to favourable conditions for the vector (mosquitoes in the Amazon) or reservoir host (rodents in the United States)

Agricultural intensification has resulted in large, dense populations of domestic animals with which humans have regular contact, making them suitable intermediate hosts of zoonotic diseases. These animals can acquire pathogens through contact with the wild reservoir host, which humans may have little contact with, and then spread the pathogen to other animals in the same herd or market providing multiple opportunities for the pathogen to infect humans (Field *et al.* 2001; Chau *et al.* 2002; Guan *et al.* 2003). In this manner bird flu (H5N1) poses a threat to humans where wild birds may infect domestic flocks (Webster 1997). Additionally pandemic H1N1 2009 (a.k.a. swine flu) spill over to humans can in part be attributable to dense populations of domestic pigs (Gibbs *et al.* 2009).

Hunting brings humans into direct contact with wild animals and can put wildlife populations under increased stress. Contact with animals during hunting, butchering and consumption provides opportunity for the transmission of pathogens and is the route of infection for many cases of Ebola (Leroy *et al.* 2009). Additionally, hunting and culling can increase stress and contact rates in the reservoir host thereby increasing the prevalence of disease and the chance of spillover (Carter *et al.* 2007). Most famously, it is through the hunting of primates in Africa that the HIV is thought to have emerged, resulting in a devastating pandemic (Myers *et al.* 1992).

Host disease dynamics are generally poorly understood (e.g. bat lyssaviruses and Ebola). However, in the case of both Hendra and Nipah, seasonality in disease prevalence in wild bat populations is thought to increase the risk of disease spillover (Plowright *et al.* 2008; Wacharapluesadee *et al.* 2010). Creating models of the disease dynamics in wild bat populations is key to predicting disease emergence in the future. However, models of diseases in bat populations are rare due to a lack of empirical data with which to parameterise models (Dimitrov *et al.* 2008; Plowright *et al.* 2011).

1.5.4.2 Current predictive models of disease emergence

Models of disease dynamics can predict factors that lead to an increase in prevalence within host populations, which in turn is likely to increase the chance of spillover to man and livestock. These models require accurate estimates of transmission and susceptibility. The former is a measure of the rate at which infected individuals expose others to a pathogen and the latter is a measure of the probability that an individual will become infected when they come into contact with an infected host. Recent studies of disease models have revealed that equally as important as the average rates of transmission and susceptibility is the heterogeneity of these values within a population (Lloyd-Smith *et al.* 2005; Beldomenico *et al.* 2009). This is incontrast the mass action models that have been generally used in models of disease. Mass action assumes a constant rate of transmission between individuals and an equal probability of contact between any two individuals in the popualtion (McCallum *et al.* 2001). Variation in transmission and susceptibility is often seen between different sexes, age classes and reproductive stages, and can result from differences in behaviour, immunity and coinfection with other pathogens. This heterogeneity, which is well documented in wildlife populations (Perkins *et al.* 2008; Luong *et al.* 2010), leads some individuals to be disproportionally important for the spread of disease, so called 'super-spreaders'. Models that do not account for this natural variability where it is present cannot accurately model disease (Lloyd-Smith *et al.* 2005).

Models of pathogens in bat populations are crude at present. Even models of Hendra virus and lyssaviruses lack much of the necessary empirical data needed to make accurate predictions (Dimitrov *et al.* 2008, Plowright *et al.* 2011). Future studies should focus on increasing our understanding of the structure of populations so that transmission rates, and heterogeneity in transmission rates can be quantified. Additionally our understanding of bat immunology is extremely poor despite it clearly being an important factor for determining the susceptibility of an individual to infection, and therefore predicting the distribution of pathogens in bat populations.

1.5.4.3 Gaps in our understanding: Contact rates

To estimate transmission rates amongst bats we need to know which bats come into contact with one another and how prolonged these contact events are. This includes question such as; how many bats does an individual come into contact with? And are these contacts with

random individuals or a subset of the population? Studies to date have failed to provide this detailed information.

Current research operates at two scales; at the national scale and at the scale of the roost. Roost scale studies amongst temperate bats focus on the movement of bats between a set of roosts (e.g. Entwhistle *et al.* 2000; Park *et al*. 1998). These studies are limited to known roosts, typically large maternity roosts in human dwelling places. For example Entwhistle *et al.* 2000 studied 30 roosts for 15 years in buildings in Scotland. However, these roosts were over an area of 100,000 hectares, while in the present study we identified 63 roosts in an area of only 415 hectares for the same species. By limiting studies to large, previously known roosts, many other roosts used by individuals within the population remain unobserved. One solution to this problem is to use radio-tracking to locate roosts (e.g. Johnson, 2012; Garroway, 2007). However, not all studies that use this method investigate the occupants of a roost, limiting their study to a description of the roost sites used by bats, their frequency of movement and proximity to concurrently tracked individuals (Johnson, 2012). Some studies go a step further and investigate the occupants of roosts located by radio-tracking (Garroway, 2007). These studies can give an indication of the contact rates between the radio-tracked individuals and others in the population. However, these contact networks are centred on the small number of individuals that are being tracked and may not be representative of the population as a whole. As a research tool on which to base estimates of contact rates, radio-tracking studies are extremely limited. This is because the number of bats tracked is typically small and radio transmitters on temperate bats rarely last more than 2 weeks giving a short observation period.

Studies that are roost-centric provide data on the number of individuals that roost together at any one time. These data tell us about contact rates within the roost. However, if only a small number of roosts are studied then estimates of contact and transmission rates at the population level are likely to be unreliable. To get better results roosts need to be monitored at a higher spatial resolution than has previously been achieved, observing a larger number of roosts used by individuals within a population. This approach will provide better estimates of contact rates between individuals within the population and will identify variability in contact rates within and between different classes of individuals (i.e. males, adults etc).

At the national and continental scale studies of population genetics have been used to infer the connectivity of populations. Such studies of temperate bats are numerous and have typically shown high levels of gene flow at the national scale (e.g. Atterby *et al.* 2010; Bryja *et al.* 2009; Kerth *et al.* 2002). When results from mitochorial DNA (passed from mother to

offspring) are compared to results from nuclear DNA (passed from both parents to offspring) studies have found that for the majority of species, females are more philopatric than males (Castella *et al.* 2001; Kerth & Petit 2005; Pereira *et al.* 2009). Continental scale genetic studies have been used to predictions about the spread of disease through bat populations. Smith *et al.* 2011 studied the genetic structure of populations of *Eptiscus serotinus* and *Myotis daubentonii* in the UK. The authors conclude that the low genetic diversity among *M. daubentonii* suggests greater mixing in the population which would result in the increased likelihood of the maintenance and spread of lyssaviruses arriving from the continent. Conversely the greater genetic isolation by distance observed in *E. serotinus* was taken to suggest that infections would be more likely to die out than for *M. daubentonii*. These assertions assume that mating patterns, which are the direct cause of the observed genetic variation, correlate to contact rates. That is to say that species such as *M. daubentonii* which exhibit high degrees of outbreeding are assumed to be able to more rapidly spread disease through the population. This assumption is untested. *M. daubentonii* are known to undertake autumn mating when contact rates may well be high, however this only occurs for a short period of the year, and it is unclear how many trips an individual makes to swarming sites in a given year. Very little is known about the summer movements of *M. daubentonii* between roosts and populations. Without empirical measurements of this movement it is not possible to reliably predict contact rates and transmission, and therefore the likely spread of disease, from large scale genetic analyses such as these.

Contact rates within bat populations cannot be accurately estimated using current methods such as studies focused on a small number of large roosts, radio-tracking, or studies of population genetics. Future work should study large numbers of roosts at high spatial and temporal resolution. These studies will provide empirical data suitable for estimating contact rates and informing epidemiological models.

Future studies of contact rates amongst bats will benefit from advances made in the field of network analysis described earlier. These analyses quantify contact networks and can be used to assess the heterogeneity of transmission rates (Hamede *et al.* 2009; Perkins *et al.* 2009). These analyses may also help to identify super-spreaders who would be preferential targets of disease control methods such as vaccination or culling. Temporal variation should also be considered. In other species, marked seasonal variation has been noted in contact rates, as in networks of Tasmanian devils (*Sarcophilus harrisii*) (Hamede *et al.* 2009). Given the complex life-history of bats in temperate regions, similar variation in social structure would be expected.

1.5.4.4 Gaps in our understanding: Susceptibility

Susceptibility is a measure of the probability that an individual will become infected when they come into contact with an infective host. This is dictated by the individual's immune system. Many aspects of hosts' immune system are important for estimating host susceptibility including the half-life of antibodies and the effectiveness and duration of maternal immunity. The immunology of temperate bats is poorly understood. The only detailed research has explored the immune response of bats after exposure to lyssaviruses (O'Shea *et al.* 2003; Turmelle *et al.* 2010).

This thesis does not explore the immune system of bats, instead focussing on the contact rate element of transmission, however I make recommendations in the discussion chapter for areas of future research in immunology.

1.5.4.5 Gaps in our understanding: Disease surveillance

Pathogens that spill over to human and domestic animal populations from wildlife are often unknown prior to emergence (e.g. Ebola, SARS, HIV). Identifying pathogens of potential risk to human health prior to their emergence is clearly important and this has spurred a range of disease surveillance projects amongst wild bats and other animal populations (Donaldson *et al.* 2010; Phan *et al.* 2011; Tong *et al.* 2012). Ideally these surveillance studies should also attempt to quantify the disease dynamics within the populations and identify possible pathways of disease emergence to humans or other animals that could act as intermediate hosts. In practise such work is often funded only after a particular pathogen has emerged and caused morbidity or mortality in humans or domestic animals.

Disease surveillance amongst British bats is currently limited. Most work has focussed on Lyssaviruses (Fooks *et al.* 2004; Brookes *et al.* 2005; Fooks *et al.* 2006; Harris *et al.* 2006; Smith *et al.* 2006; Banyard *et al.* 2009; Smith *et al.* 2011), though other work has identified *Babesia* sp., *Bartonella* sp., *Borrelia burgdorferi* sensu lato and trypanosomes in blood samples (Gardner & Molyneux 1987; Concannon *et al.* 2005; Reeves *et al.* 2007; Evans *et al.* 2009; Hamilton *et al.* 2012). Focusing surveillance on diseases of greatest concern, notably viruses (see 1.5.2), is needed in the UK to identify diseases of human health concern in wild bat populations.

1.6 The study system

The primary study system used in this thesis are populations of three insectivorous bats species (*M. daubentonii*, *M. nattereri* and *Plecotus auritus* (Brown long-eared bat)) in Wytham Woods, Oxfordshire. Some data were also used from a few other sites in the South-west of England.

1.6.1 The study site

Wytham Woods (Latitude, 51°77'27"; Longitude, -1°33'41"), is approximately 415 hectares of semi-natural ancient deciduous woodland and 18th-20th century plantations. The woods have been in the ownership of the University of Oxford since 1942, and since then have been used for a wide range of research into plants, animals and climate. Perhaps the most well know research undertaken at Wytham Woods is the Edward Grey Institute's (EGI) work on *Parus major* (Great tits) and *Cyanistes caeruleus* (Blue tits) which has now been running for over 60 years. An integral part of this research is over 1150 georeferenced woodcrete bird boxes distributed throughout the wood. These numbered boxes are used as roost sites by bats from early May to mid-October after the birds have stopped using them. This provides a rare level of access to the bat populations present in the wood as boxes are easily accessible and several colonies can be located in a single day (median = 3). The presence of so many artificial roosts may increase the density of bats if roosts would normally be limiting. It is also unclear what proportion of the population present use the boxes and whether some bats avoid them. While these factors may have some effect on the bat population in Wytham Woods compared to sites with only 'natural' roosts, the benefits the site provides in terms of access and accompanying long term datasets make Wytham Woods a very valuable resource for the study of bats.

Wytham Woods is part of the Environmental Change Network (ECN), a collection of sites where long term data is collected to analyse changes in the environment. ECN climate data used in this thesis include temperature, humidity, rainfall and wind speed.

Remarkably, for this most studied of woods, prior to work published from this thesis, bats in Wytham Woods had not been the subject of any published research.

1.6.2 The study species

M. daubentonii (Daubenton's bat) is a 7-15g insectivorous bat that forages over water where it catches the majority of its prey (Jones & Rayner 1988; Akasaka *et al.* 2009; Langton *et al.* 2010; Lucan & Radil 2010). *M. daubentonii* roost in trees and man-made structures close to water (Boonman 2000; Altringham 2003). In the summer females form nursery colonies in which young are raised. Males are sometimes found in these roosts, though more often they are found in male only bachelor colonies (Altringham 1996). This species attends swarming sites in the autumn where it is thought that mating occurs, though mating also occurs late in the season at summer roosting sites (Senior *et al.* 2005).

M. nattereri (Natterer's bat) is a 6-12g insectivorous bat that catches insects in the air and gleans them from the surface of vegetation. Adapted for this method of hunting, *M. nattereri* has broader wings and a slower flight speed than *M. daubentonii* (Altringham 2003). This species is associated with deciduous woodland (Parsons & Jones 2003; Smith & Racey 2008; Boughey *et al.* 2011) for both foraging and roosting habitat. *M. nattereri* sexes roost apart in the summer when females form nursery colonies, however, *M. nattereri* males do not typically form large bachelor colonies. In the Autumn *M. nattereri* travel to swarming sites where they may account for up to 80% of individuals (Altringham 2003; Rivers *et al.* 2006). It is thought that the majority of mating amongst *M. nattereri* occurs at these sites (Rivers *et al.* 2005).

P. auritus (Brown long-eared bat) is a 6-12g insectivorous bat which specialises in using passive listening to locate and catch insects close to and on vegetation. This species has broad wings and can hover as it gleans prey from vegetation. Moths are known to be an important component of the diet of this species which forages and roosts in deciduous woodland (Entwistle *et al.* 1996, 1997; Boughey *et al.* 2011). During the summer it is suggested that males and females roost together in contrast to the other two main study species (Altringham 2003). *P. auritus* are also found at swarming sites though typically in low numbers, beyond this little is known about their mating habits. A comparison of the three species is shown in Table 1.2.

A small number of samples were collected from bats at sites other than Wytham Woods and from species other than those described above. These sites include Savernake forest in Wiltshire and a number of swarming sites in Devon and Wiltshire. Species sampled at these sites other than those previously described include *Rhinolophus hipposideros* (Lesser Horseshoe), *R. ferrumequinum* (Greater Horseshoe), *Pipistrellus pipistrellus* (Common Pipistrelle) and *Barbastella barbastellus* (Barbastelle).

Species	Weight (g)	Wing area (m^2)	Aspect ratio ($Wingspan^2$/wing area)	Foraging habitat	Roosting habitat	Prey	Foraging strategy
M. daubentonii	7-15g	$0.0098m^2$	6.3	Over calm bodies of water	Trees or man-made structures close to water	Mainly flies hatched from aquatic larvae	Gleans insects from the water's surface and catches insects flying low over water
M. nattereri	6-12g	$0.0113m^2$	6.4	Deciduous woodland	Trees or man-made structures in or close to deciduous woodland	Generalist, including large flies and spiders	Gleans insects from the surface of vegetation and catches insects in mid flight
P. auritus	6-12g	$0.0124m^2$	5.7	Open deciduous woodland	Trees or man-made structures in or close to deciduous woodland	Moths and flies	Gleans insects from the surface of vegetation using passive listening and sight

Table 1.2 – Comparison of the morphology and ecology of the three species studied in detail at Wytham Woods. Data are taken from Altringham (2003) and Norberg and Rayner (1987)

1.6.3 Ethical approval and licensing

All procedures undertaken in the course of this thesis were approved by the Biosciences Ethics Committee, University of Exeter, and carried out under an appropriate Natural England licence (20113601 and previous licences).

1.7 Thesis aims

Having identified conservation threats to bats and information gaps for zoonotic disease prediction amongst bats in the UK, this thesis aims to address the following specific aims:

1. Investigate the social structure of *M. daubentonii* and *M. nattereri* populations by monitoring colony composition and using social networks analysis. This will give a better understanding of contact rates between individuals in a population which can be used to make predictions about the spread of disease.
   This analysis will also explore the area needed to support the roosting requirements of a social group which may have implications for the conservation of these bat species.

2 Assess whether the social structure of bat populations in Wytham woods is a result of roost limitation, or primarily driven by social behaviour. This will have implications for the extrapolation of the results from this thesis to other locations.
   This work will also provide information of interest for conservation, such as habitat preference, and for this reason *P. auritus* will also be included in this analysis.

3 Undertake surveillance of pathogens of potential human health concern in wild bat populations in Britain and place novel pathogens in a phylogeny of their genus.

4 Analyse the distribution of ectoparasites and pathogens to assess whether host ecology and social structure drive parasite and pathogen burden in the study system. Additionally, assess whether climatic variables, ectoparasites and pathogens have a measureable impact on the body condition of individuals.

Collectively these data aim to characterise the population of bats in Wytham Woods for the first time, provide detailed information on bat habitat use and social structure at a high spatial resolution, identify novel pathogens, link ectoparasite and pathogen prevalence to body

condition and establish a basis of understanding the raises new tractable questions for future research.

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2 Woodland bats form tight-knit social groups with exclusive roost home ranges

2.1 Introduction

Bats are a species-rich taxon with over 1100 species globally. They are long lived and have a range of social structures. Although some species are solitary, most are social for at least part of the year: colonies, which can be mixed or single-sex, commonly contain tens to hundreds of individuals (Altringham 1996; Kunz & Fenton 2003). This heterogeneity makes bats an ideal taxon for the study of social structure. The social structure adopted determines, among other factors, mating opportunities, information transfer about food sources and predators, and exposure to parasites and pathogens.

The social structure of a population can be defined by the physical proximity of individuals through time. Social network analysis (SNA) uses observations of interactions between individuals to construct an approximation of the overall population social network, and provide metrics that quantify its structure.

Network analysis originated in the mathematical sciences and has since branched out into economics, politics, human sociology, and more recently animal sociology (Wey *et al.* 2008). Studies have explored social structure in primates (Bezanson *et al.* 2008; Henzi *et al.* 2009; Kasper & Voelkl 2009; Ramos-Fernandez *et al.* 2009) marine mammals (Lusseau 2003; Lusseau *et al.* 2006; Wolf *et al.* 2007; Lusseau & Conradt 2009; Whitehead & Van Parijs 2010), bats (O'Donnell 2000; Vonhof *et al.* 2004; Garroway & Broders 2007; Fortuna *et al.* 2009; Chaverri 2010; Patriquin *et al.* 2010; Kerth *et al.* 2011), reptiles (Godfrey *et al.* 2010), birds (McDonald 2007, 2009), elephants (Wittemyer *et al.* 2005), fish (Croft *et al.* 2004; Croft *et al.* 2006) and others. These studies explore, amongst other things, the effects of removing key individuals, the implications for disease transmission, the influence of individuals in group decision making, the spatial organisation of social groups and the evolution of sociality.

Previous SNAs of bats have considered a single species and most have focused on a single location. These studies have furthered our understanding of fission-fusion dynamics: the frequent subdivision and recombination within social groups that is observed in many bat species (Kerth, 2011). Studies of the spatial arrangement of social groups identified in SNAs have found that whilst some bat species' form social groups occupying exclusive roost home

ranges (O'Donnell 2000; Fortuna *et al.* 2009) others have broadly overlapping roost home ranges (Vonhof *et al.* 2004).

Social networks based on cohabitation, as in the present study, provide quantitative information on contact rates, and their heterogeneity, within a population. These variables have been shown to be important in models of disease transmission (Lloyd-Smith *et al.* 2005; Beldomenico & Begon 2010) where it has frequently been observed that a small number of individuals, with a large number of contacts, account for the majority of disease transmission (Kramer-Schadt *et al.* 2009; Perkins *et al.* 2009; Beldomenico & Begon 2010; Gardy *et al.* 2011). This is different from the, commonly implemented, theory of mass action, whereby contact rate is assumed to be uniform between all individuals (McCallum *et al.* 2001).

In addition to contact rates, disease transmission is influenced by network structure. Populations with strong social structure, in which intergroup contact is low, often have reduced overall prevalence of disease and select for chronic benign infections as herd immunity within a sub-group is quickly achieved (Eames 2007). In these networks the spread of disease can be controlled by targeting intervention at individuals that transmit disease between groups. As outlined in the introduction to this thesis, little is known about contact rates or population structure in bat populations (1.5.4.2), this work therefore provides valuable new information.

Here I use SNA to study *M. daubentonii* and *M. nattereri*, medium sized insectivorous bats weighing 7-15g and 6-12g respectively. *M. daubentonii* typically forages over water whereas *M. nattereri* is a woodland specialist. Both species roost in tree holes and man-made structures close to their foraging sites and form nursery colonies during the summer in which young are born. These colonies form from May to June and split up once the once young are independent, from August to September (Altringham 2003). Both sexes of *M. nattereri* are philopatric, returning from hibernation to spend the summer at the site of their birth (Rivers *et al.* 2006). Studies of *M. daubentonii* population genetics suggest that males are likely to account for most dispersal whilst females are generally philopatric (Ngamprasertwong *et al.* 2008, Smith *et al.* 2011). Ngamprasertwong *et al.* (2008) Compared the variation in mitochondrial DNA (passed to offspring from the mother) and microsatellite DNA (passed to the offspring from both parents) and found much greater variation amongst mitochondrial DNA than microsatellite DNA. *M. daubentonii* adult males form bachelor colonies in the summer numbering up to 60 individuals at some sites in the UK (Altringham 1996). *M. nattereri* also, though less frequently, form bachelor colonies of up to 28 individuals (Swift 1997). Like other *Myotis* bats, both species attend swarming sites, typically cave or mine

entrances, in late summer to early autumn. These sites are thought to be important for mating, though some mating may also occur late in the season at summer roosting sites (Senior *et al.* 2005) and during the winter at hibernacula (Altringham 2003).

Our study was undertaken in Wytham Woods, Oxfordshire. This intensively studied ecosystem has advantages for sampling and recapturing bats, and analysing their social networks, since they utilise many of over 1150 georeferenced bird boxes distributed throughout the wood.

This work reveals contrasting social and spatial structures in the two species with implications for predicting disease transmission within the system. Additionally the results have implications for mating strategies, information transfer, and bat conservation. The study is the first SNA of bats to compare two species in the same location and provides a basis for future comparative analyses of these bats in a tractable study system.

2.2 Methods

2.2.1 Fieldwork

Bats were captured and ringed between May and mid-October annually, from 2006 to 2010, at Wytham Woods (Latitude, 51°77'27"; Longitude, -1°33'41"). This 415 hectare site is composed of semi-natural ancient deciduous woodland and 18th-20th century plantations. Over 1150 woodcrete bird boxes are dispersed through the woods and these are frequently used by bats from early May to mid-October, after which they migrate to unknown hibernation sites. The birds, for whom the boxes are designed, do not occupy the boxes after May. To minimise disturbance boxes were not checked more than once within a two week period and females with attached young were not handled. Areas with higher occupancy rates (pers. obs.) were sampled more frequently to maximise data collection (Figure 2.1).

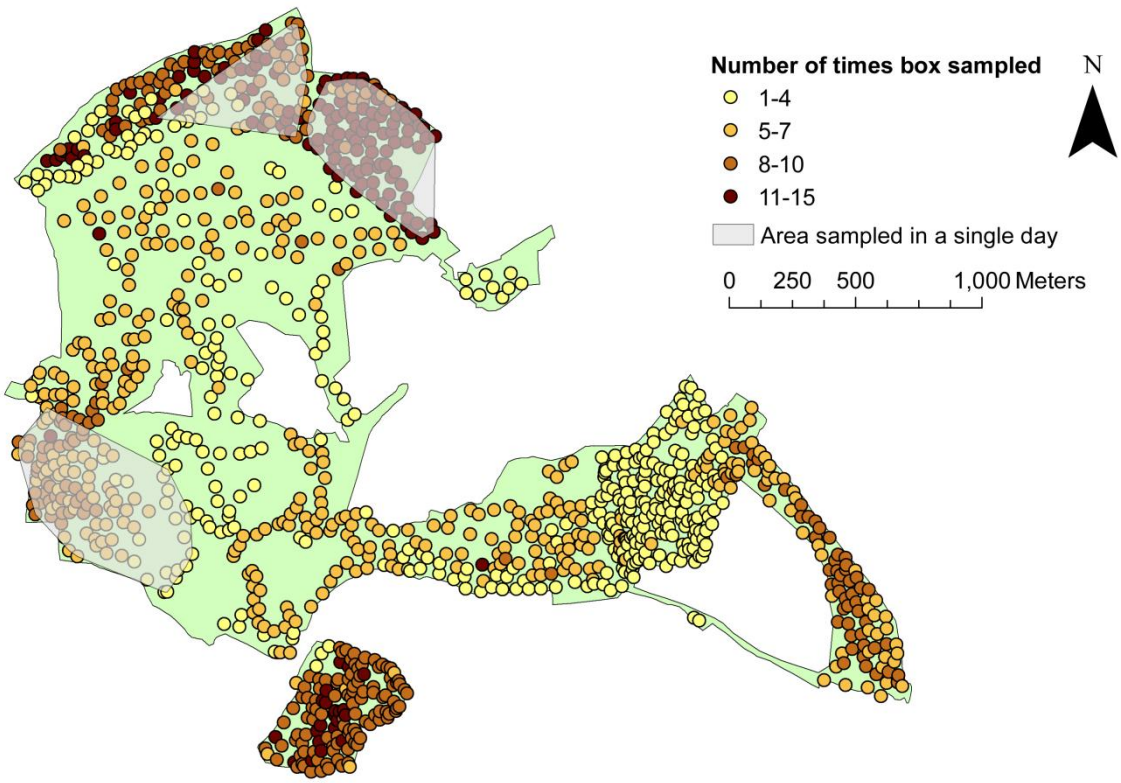

Figure 2.1 – Distribution of sampling effort across bird boxes. Three polygons show examples of the typical area of boxes checked in a day

Bats were ringed with 2.9mm aluminium armbands bearing a unique identification number (The Mammal Society, UK under Natural England license no. 20113601 and previous licences) and classed as juveniles, if the joints between the metacarpals and phalanx were not fully ossified (Racey 1974; Mitchell-Jones & McLeish 2004).

Sex and reproductive status were also recorded. Female reproductive status was divided into 4 categories; pregnant, lactating, post-lactating and non-breeding. Pregnant bats were identified from their weight and gentle palpation of the abdomen. Lactating bats have enlarged nipples and an absence of hair both on the nipple and in a c.3mm circle around the nipple. Post-lactating females' nipples are enlarged, dark in colour and the hairs surrounding the nipple may have begun to grow back but are short. Non-breeding females have no swelling in the nipple and no loss of hair.

Colonies were divided into three types according the composition of adults: those dominated (>66.6% of total) by adult females, those dominated by adult males (>66.6% of total) and those with mixed adult sexes. Bats found roosting on their own were recorded as solitary. Three time periods were defined: pre-nursery, nursery and post-nursery. The nursery period was defined as the time between the first and last recorded colonies of lactating females with juveniles. These dates varied from year to year, since the timing of births is weather dependent.

2.2.2 Social network analysis

A social interaction, or 'association', was said to exist between two individuals if they were found roosting together. The strength of association between two individuals was calculated using the simple ratio equation (Ginsberg & Young 1992). Strength of association (AI_{ij}) was calculated using the number of occasions when both individuals were observed together (x), the number of occasions individual i was observed and j was not (y_i), the number of occasions individual j was observed and i was not (y_j), and the number of occasions both individuals were observed but not together (y_{ij}).

$$AI = \frac{x}{x + y_{ij} + y_i + y_j}$$

2.2.3 Structural analysis

A social network in which individuals associate non-randomly is defined by the presence of preferred and avoided associations. As a consequence the distribution of association strengths is bimodal; association strengths are either small (avoidance) or large (association). The mean association strength in non-random social networks therefore has a higher standard deviation and coefficient of variance (standard deviation/mean) than random networks. Formal tests of non-randomness of the observed networks were conducted using methods described by Bejder *et al* (1998) with modifications from Whitehead (2008). This method has been used previously in the analysis of social networks in bats (Garroway & Broders 2007). Random networks were generated by permuting the composition of colonies sampled on the same day whilst keeping colony sizes the same as those observed. By keeping group sizes the same it was possible to control for the possibility that the structure observed is a result of non-social, random aggregations. As most days' sampling were in a specific area of woodland, permuting roost composition within day accounts for some of the effects spatial sampling may have on the apparent social structure.

Visualisation of the observed networks was undertaken using Netdraw v.2 (Borgatti 2002). Within these visualisations individuals are represented by nodes and an association between two individuals is represented by a line connecting them.

Individuals captured only once (Table 2.1) were excluded from the analysis of social networks and associations were removed if their strength was less than half the mean non-zero association strength of the random networks. This cut-off was selected as the cut-off values produced were similar to those used in previous studies of bats (Vonhof *et al.* 2004; Garroway & Broders 2007; Patriquin *et al.* 2010) and account for variation in structure between random permutations of different networks. By removing weak associations, that could have come about by chance, we can better analyse the core social structure of the network (Croft *et al.* 2008). Individuals captured more than once with no associations (n = 11) were also removed from the analyses.

Species		Number of times captured 1	2	3	4	5	6	7	8	9	10	Total	Proportion captured more than once
M. daubentonii	Male	430	118	59	14	7	0	1	0	0	0	629	0.32
	Female	204	55	33	27	13	11	4	0	2	0	349	0.42
	Total	*634*	*173*	*92*	*41*	*20*	*11*	*5*	*0*	*2*	*0*	*978*	*0.35*
M. nattereri	Male	97	43	18	12	9	2	0	1	0	0	*182*	0.47
	Female	101	71	42	40	25	14	8	7	4	3	315	0.68
	Total	*198*	*114*	*60*	*52*	*34*	*16*	*8*	*8*	*4*	*3*	*497*	*0.60*

Table 2.1 – Summary of the frequency distribution of captured bats by species and sex

Individuals were assigned to social groups using the Girvan-Newman method (Girvan & Newman 2002). This top-down method, successively removes the association with the highest value of 'betweeness'. Betweeness is the number of shortest paths, connecting individuals in the network, which contain a given association. Associations with high values of betweeness are those that connect clusters with otherwise low interconnectivity and by removing them the network is broken down into an increasing number of unconnected components. Each time a new component is created the modularity of the network is calculated (Newman & Girvan 2004). Modularity is calculated using all associations from the original network and is the difference between the observed fraction of associations that are within components and the fraction expected if associations connected individuals at random. Modularity ranges from 0 to 1, with values over 0.3 regarded as evidence of social structure (Newman & Girvan 2004). The division of individuals to components by the Girvan-Newman method that produces the highest modularity value is selected as the best representation of social groups. This method is particularly appropriate for populations with strong social structure such as those in this study.

Evidence of assortment by sex within social networks was examined using join-count in UCInet (Borgatti *et al.* 2002) using 10,000 permutations. This test compares the number of male-male, female-female and intersex associations in the dataset with the number expected by chance.

2.2.4 Spatial Analysis

Roost home ranges of social groups were estimated using minimum convex polygons (MCPs) after the removal of roosts used by a single individual (*M. daubentonii* = 44; *M. nattereri* = 24) and those isolated by over 1km (*M. daubentonii* = 1; *M. nattereri* = 1). Using ArcMap (ESRI v.

9.3, 2008) and Hawth's tools (v. 3.27) MCPs were created and cropped so that habitats such as grassland, which do not provide roosting opportunities, were removed.

Radio-tracking was undertaken in August 2009 and 2010 to compare roost home range estimates produced from the SNA to the roost use of individually tracked bats. Four adult female *M. daubentonii* were fitted with radio transmitters weighing 0.35g or 0.42g (Holohil, Canada, type LB-2N). All tags weighed less than 5% of the body weight of the bat (4.1-4.7%) and were attached by a licensed bat worker using a previously described method (Kelly *et al.* 2008). Bats were located at their day roosts using an Australis receiver (Titley Electronics Ltd, Australia) and a Yagi 3-element directional antennae (Biotrack Ltd, Wareham, UK). Tree roost locations were recorded by GPS and mapped using ArcMap.

2.2.5 Temporal analysis

The temporal structure of associations was examined using the lagged association rate (Vonhof *et al.* 2004; Whitehead 2008). This gives the probability that, after being found together, two individuals will be found together at a set time interval in the future. These trends were calculated for each of the four classes of association within each species (male-male, female-female, male-female and female-male) and compared to the expected trend if individuals were to associate randomly, the null association rate. Standard errors were calculated for these trends by jack-knifing the data over a period of 30 days.

2.2.6 Statistical analysis

All statistical analyses were undertaken in R version 2.11.0 (R Development Core Team 2011). Differences in the relative abundance of colony types between species were analysed using chi-squared tests, comparing proportions within the same season, with Bonferroni corrections. Similarly chi-squared tests were used in the statistical analysis of sex ratio data to test for differences from a 1:1 ratio. When testing for differences in sex ratio between species a generalised linear mixed effects model with binomial error structure was used with year and time period included as random effects using R package 'lme4' (Bates *et al.* 2011). Recapture rates were compared by species and sex using a generalised linear model with a Poisson error structure. There was no improvement when instead using a negative binomial error structure, tested using R package 'pscl' (Jackman 2011). The correlation between the area of a social

group's roost home range and the number of individuals in the social group, species and sampling effort was examined using a multiple regression. Sampling effort was calculated as the average number of recaptures per individual for each social group.

2.3 Results

Over five consecutive summers we performed 7578 box checks, finding bats on 625 occasions. A total of 490 *M. nattereri* and 978 *M. daubentonii* were ringed from 379 colonies (Appendix, Table 7.1). Individuals captured only once were removed prior to SNA leaving 299 *M. nattereri* and 344 *M. daubentonii* captured on average 3.6 (range 2-10) and 2.9 (range 2-9) times respectively (Table 2.1). Due to the limited number of recaptures (Table 2.1) it was not possible to analyse the social structure separately for the nursery and post-nursery period or for each year of the study and so all data were combined for SNA. The two bat species were never found in the same roost at the same time, however, 27 roosts (of 293) were used by both species at different times (Figure 2.2). This is not significantly different from the number of boxes we would expect the species to share if they were roosting randomly within the woods ($\chi^2 = 0.48$, df = 1, $p = 0.49$).

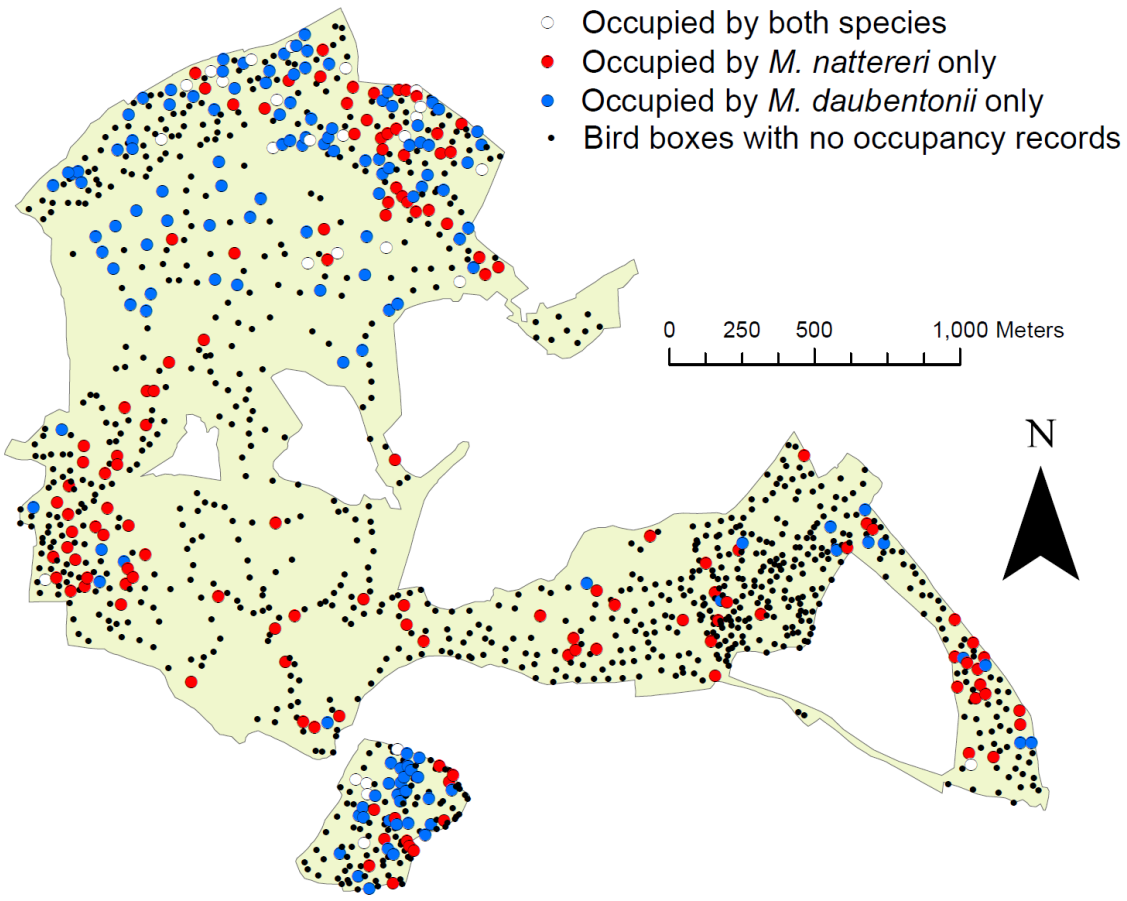

Figure 2.2 – The spatial distribution of roosts used by *M. nattereri* (red) and *M. daubentonii* (blue) and both species (white). Both species have been found in a large number of roosts though occupy few on any given day, suggesting that roosts are not limiting at this site

2.3.1 Species specific differences in sex ratio and recapture rates

Controlling for year and season, a significantly lower proportion of male *M. nattereri* then male *M. daubentonii* were captured (z = 14.83, $p < 0.0001$). Within years (Table 2.2), the average sex ratio (male:female) of *M. nattereri* (0.40:1) and *M. daubentonii* (1.60:1) were significantly different from 1:1 ($\chi^2 = 61.74$, df = 1, $p < 0.001$; $\chi^2 = 121.13$, df = 1, $p < 0.001$, respectively). The juvenile sex ratios for both species did not differ significantly from 1:1 (*M. daubentonii*, n = 133 $\chi^2 = 1.08$, df = 1, $p = 0.30$; *M. nattereri,* n = 154 $\chi^2 = 0.79$, df = 1, $p = 0.38$).

Species	Age	Year	No. male	No. female	Total	Sex ratio (M:F)
M. daubentonii	Adult	2010	188	130	318	1.45:1
		2009	187	108	295	1.73:1
		2008	172	126	298	1.37:1
		2007	164	80	244	2.05:1
		2006	10	7	17	1.43:1
		All years	*721*	*451*	*1172*	*1.60:1*
	Juvenile	2010	30	26	56	1.15:1
		2009	23	15	38	1.53:1
		2008	19	22	41	0.86:1
		2007	10	7	17	1.43:1
		2006	1	1	2	N/A
		All years	*83*	*71*	*154*	*1.17:1*
M. nattereri	Adult	2010	70	144	214	0.49:1
		2009	33	92	125	0.36:1
		2008	34	86	120	0.39:1
		2007	58	159	217	0.36:1
		2006	1	3	4	N/A
		All years	*196*	*484*	*680*	*0.40:1*
	Juvenile	2010	35	20	55	1.75:1
		2009	17	15	32	1.13:1
		2008	3	3	6	1:1
		2007	18	20	38	0.9:1
		2006	0	2	2	N/A
		All years	*73*	*60*	*133*	*1.22:1*

Table 2.2– Summary of the sex ratio by year of *M. daubentonii* and *M. nattereri*, adults and juveniles

Including all bats, the number of times an individual was recaptured was significantly affected by species and sex. *M. nattereri* (z = 8.38, df = 1, $p < 0.001$) and females of both species (z = 8.73, df = 1, $p < 0.001$) had significantly higher numbers of recaptures than *M. daubentonii* and males respectively, whilst the interaction between species and sex was non-significant (z = -

1.53, df = 1, p = 0.13). The recapture probability of juveniles in the years following their birth (Table 2.3) showed the same pattern (species z = 2.43, df = 1, p = 0.02; sex z = 2.62, df = 1, p = 0.01; species*sex z = 1.124, df = 1, p = 0.26).

	M. daubentonii		*M. nattereri*	
	Proportion recaptured (95% CI)	*n*	Proportion recaptured (95% CI)	*n*
Males	0.17 (0.08-0.29)	54	0.23 (0.11-0.39)	39
Females	0.25 (0.13-0.40)	44	0.53 (0.36-0.69)	38

Table 2.3 – Recapture rates of juveniles in the years following their birth

2.3.2 Species specific differences in observed colony types

During the nursery period *M. daubentonii* bachelor colonies were observed more often than for *M. nattereri* (χ^2 = 13.47, df = 1, p < 0.001, Figure 2.3a) and female colonies were recorded significantly less often than for *M. nattereri* (χ^2 = 17.0964, df = 1, p < 0.001). In the post nursery period *M. daubentonii* had a significantly larger proportion of male dominated colonies (χ^2 = 9.76, df = 1, p = 0.002) and a significantly lower proportion of female dominated colonies (χ^2 = 21.07, df = 1, p < 0.001) than *M. nattereri* (Figure 2.3a). The proportion of mixed sex colonies increased for both species in the post-nursery period (Figure 2.3a). Too few mixed sex colonies were observed during the pre-nursery period to allow a comparison of sex ratios (n = 5), however, *M. daubentonii* mixed colonies contained a larger proportion of males than the equivalent *M. nattereri* colonies (Figure 2.3b) during the post-nursery period (χ^2 = 3.83, df = 1, p = 0.05) though not the nursery period (χ^2 = 0.167, df = 1, p = 0.68). There was no significant difference in the proportion of males or females of either species roosting alone (χ^2 = 0.057, df = 1, p = 0.81), however lone roosting *M. nattereri* were more common than lone roosting *M. daubentonii* (χ^2 = 23.2091, df = 1, p < 0.0001, Figure 2.3c).

Male *M. nattereri* who returned to Wytham Woods in the years following their birth (n = 9) were mostly observed in nursery or mixed colonies (n = 7) whilst male *M. daubentonii* (n = 9) were rarely observed in nursery colonies (n = 2) instead roosting in bachelor or mixed colonies (n = 7). Females of both species who returned to Wytham Woods in the years following their birth (n = 32, 20 *M. nattereri*, 12 *M. daubentonii*) were almost all found in female dominated roosts and mixed sex roosts (n = 29), only 3 were found in bachelor roosts. These patterns therefore follow the distribution patterns observed for the adults of each species.

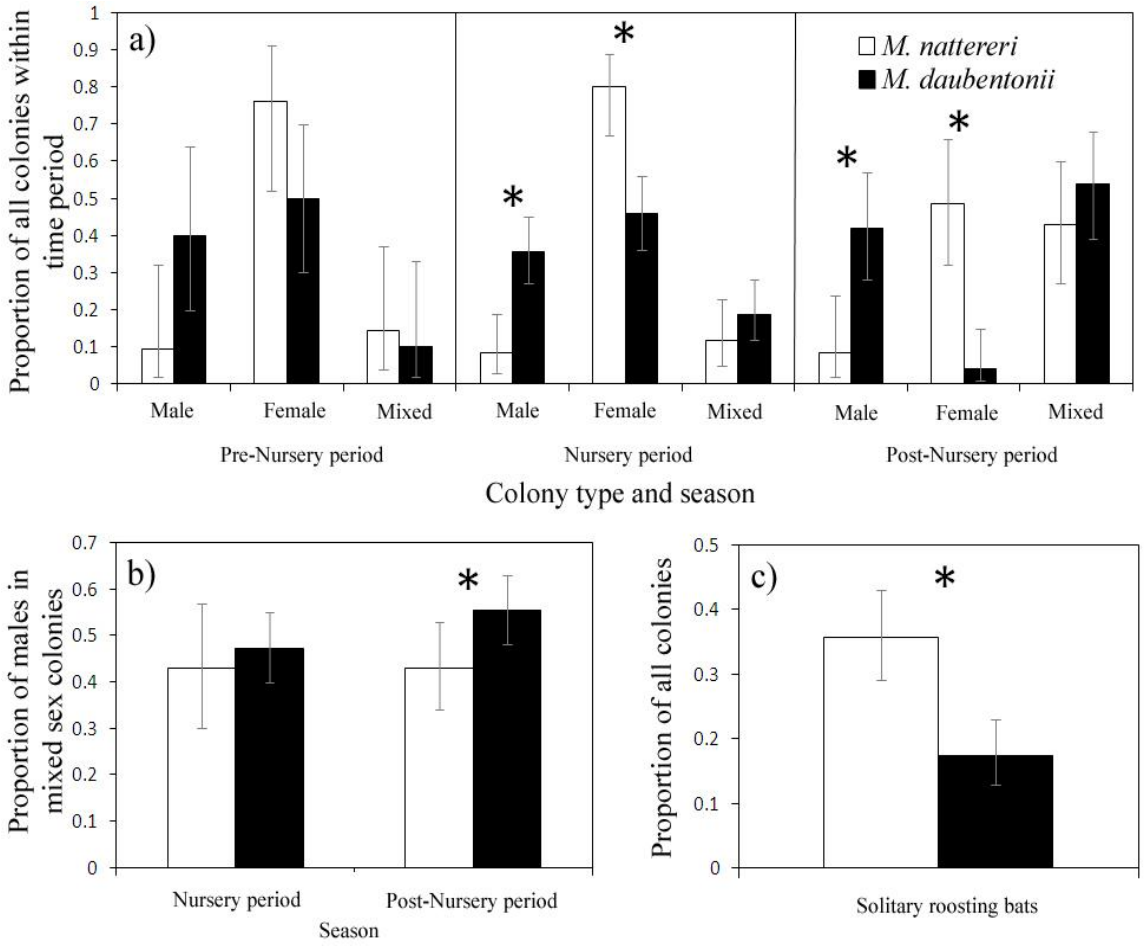

Figure 2.3 – a) The relative abundance of adult male dominated, adult female dominated and adult mixed sex colonies compared within three time periods (pre-nursery, nursery and post-nursery periods). * indicates where there is a significant difference between the species. Error bars indicate 95% confidence intervals. b) The sex ratio of mixed sex groups of adult *M. daubentonii* and *M. nattereri* over the nursery and post-nursery periods. c) The proportion of solitary colonies across all time periods

2.3.3 Identification of multiple social groups within the wood for both species

Associations in all observed networks were significantly non-random since the standard deviation and coefficient of variance of association strengths were higher in observed than permuted networks (20,000 iterations; $p < 0.001$).

Nine *M. nattereri* and two *M. daubentonii* were removed from network analyses as they had no associations to other individuals in the network. Furthermore, 13% of *M. nattereri* associations and 5% of *M. daubentonii* associations were removed as their values were less

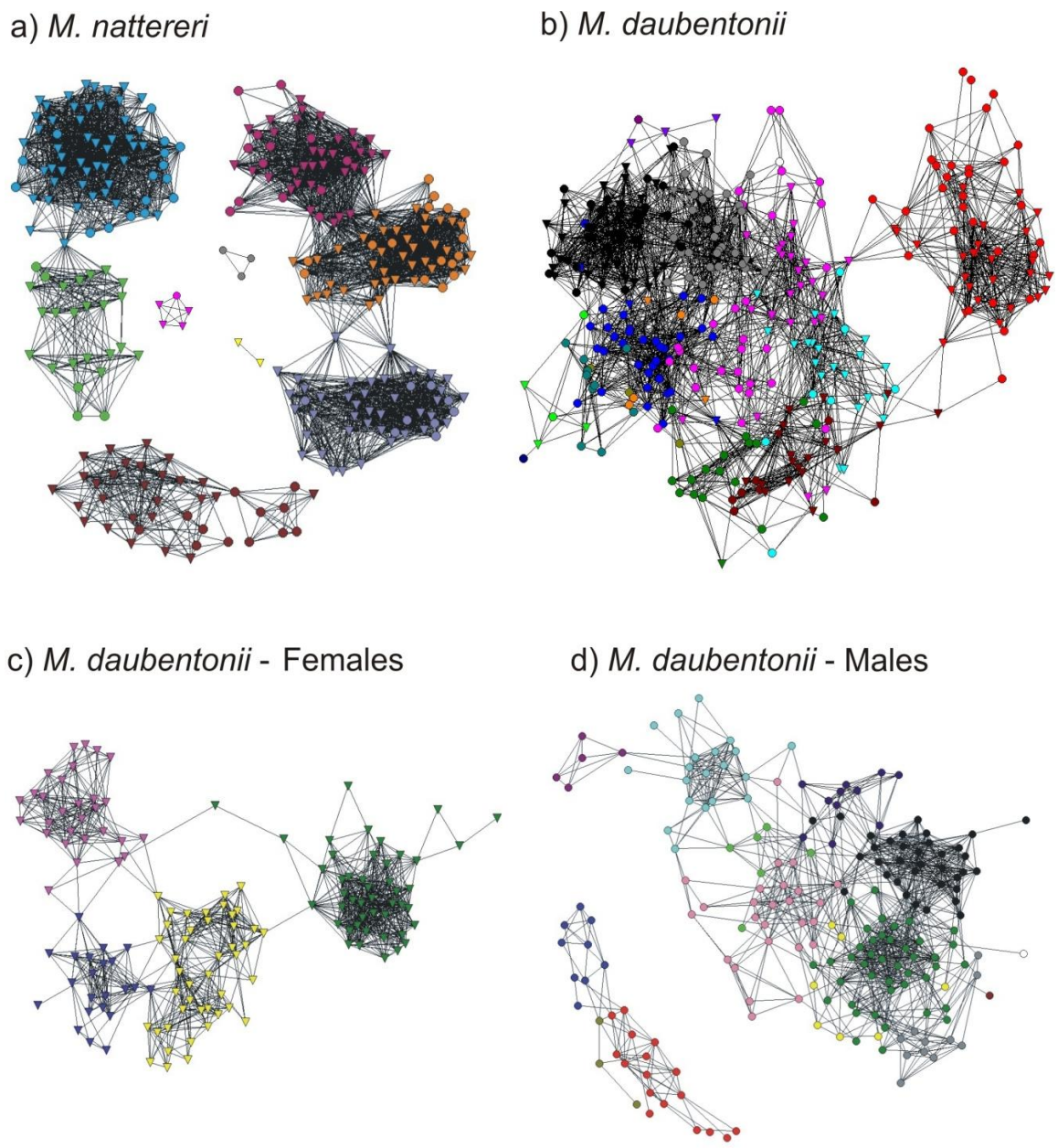

Figure 2.4 – Social network visualisations of a) *M. nattereri* bats (modularity = 0.77), b) *M. daubentonii* (modularity = 0.656), c) female *M. daubentonii* (modularity = 0.68) and d) male *M. daubentonii* (modularity = 0.63). Nodes represent individual bats and associations are represented by the lines that join them. Males are indicated by circles and females by downwards triangles. Associations are filtered at half the mean non-zero association strength of random networks. Colours indicate the assignment of individuals to social groups using the Girvan-Newman algorithm. Colours do not correspond between panels. Colours in a) and c) are comparable to Figure 2.6. The position of individuals within these networks indicates their position in social space and is not an indication of an individual's geographical location

than half the mean non-zero association strength of the corresponding random network (*M. daubentonii* = 0.115; *M. nattereri* = 0.132).

Both species showed significant assortment by sex. Female-female associations were significantly more frequent, and intersex associations significantly less frequent than expected by chance for both species (10,000 permutations, $p < 0.001$). In addition *M. daubentonii* male-male associations were significantly more frequent than would be expected by chance (10,000 permutations, $p < 0.001$).

Our analysis of 214 female and 85 male *M. nattereri* identified 9 social groups (Figure 2.4a). These groups formed 6 unconnected components. Despite evidence of assortment by sex, *M. nattereri* social groups were composed of a mix of males and females suggesting that while they constitute a single social group males and females also form single sex groups at times. Intergroup associations by either sex were rare, making up only 4% of all associations (n = 4258). Social groups were on average 25% males when weighted by social group size.

Our analysis of 145 female and 199 male *M. daubentonii* suggested males and females form discrete social groups, with half of the social groups identified being over 90% male (Figure 2.4b). This sexual segregation was apparent even when the analysis considered only males recaptured in two or three years, so removing 'transient males' who may only have been at the site briefly (Figure 2.5). Males had a significantly weaker social group affiliation compared to *M. nattereri*, with 43% (n = 199) of *M. daubentonii* males associating with females from more than one social group (Figure 2.4b). Consequently *M. daubentonii* were analysed independently for each sex. Individuals in the female network were assigned to 4 social groups (Figure 2.4c) with inter-group associations making up only 2% of all associations. Males were assigned to 11 social groups (Figure 2.4d), however unlike *M. nattereri* and female *M. daubentonii* networks, there was a significant number of inter-group associations between males (16%, n = 1205) (Figure 2.4d). This interconnectivity suggests that social group membership of *M. daubentonii* males is less specific than for the other networks examined here. As a result of this of mixing, spatial analysis was not undertaken for *M. daubentonii* males.

Using the networks created for *M. nattereri* (Figure 2.4a) and *M. daubentonii* females (Figure 2.4c) individuals who moved between social groups were identified (n = 19). These individuals comprised 9 *M. nattereri* (8 females and 1 male) and 10 female *M. daubentonii*. All of these individuals were adults, with the exception of a juvenile female *M. nattereri*. Of those individuals recaptured multiple times after switching social group (n = 11) all but 1 returned to their original social group, again the exception was the single juvenile. Of those juveniles

recaptured in the years after their birth (Table 2.3), only 1 of 47 (8 male, 21 female *M. nattereri*; 6 male, 12 female *M. daubentonii*), the female *M. nattereri* mentioned previously, was identified as having emigrated to another social group whilst all others returned to their natal group.

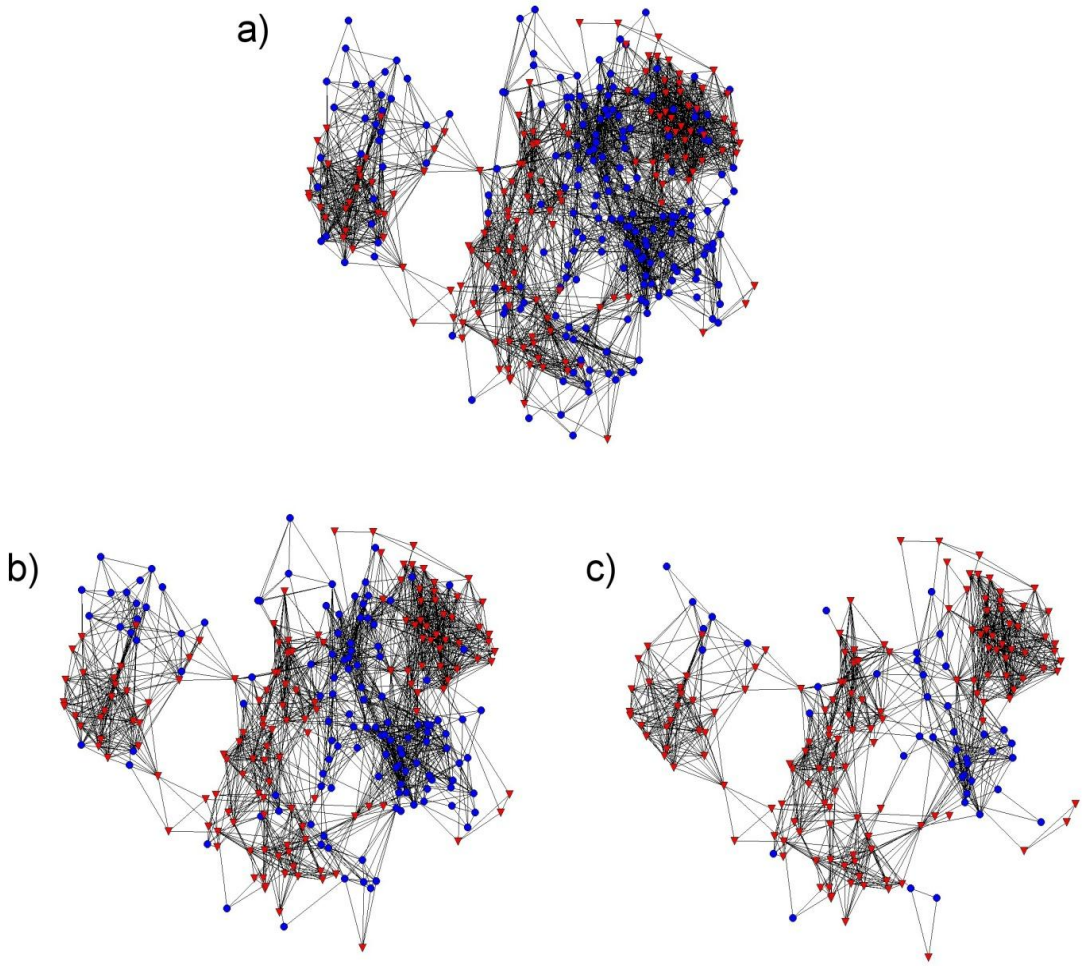

Figure 2.5 – The social network of *M. daubentonii* including all females, and males observed in a) 1 or more b) 2 or more and c) 3 or more years. Males, blue circles; females red triangles. The observed sexual segregation is evident even when only males found in multiple years are considered

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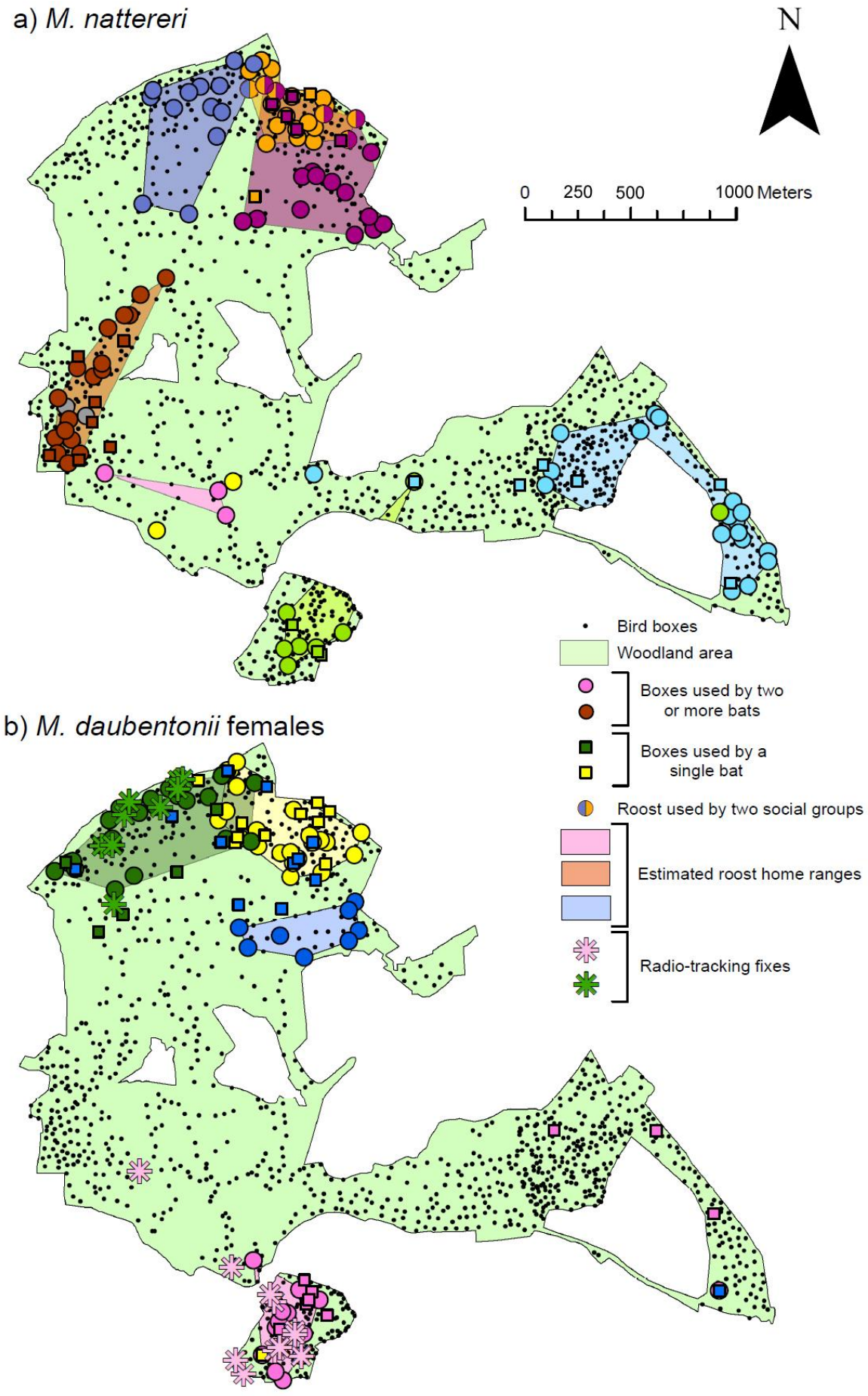
Figure 2.6 (opposite) – Distribution of a) *M. nattereri* both sexes and b) female *M. daubentonii* social groups in Wytham Woods. Roosts used by bats, and home range estimates are coloured according to social group (colours are comparable to Figure 2.4, panels a) and c)). Home ranges are estimated using 100% minimum convex polygons (MCPs). MCPs exclude roosts occupied by a single individual (*M. nattereri*, n = 24; *M. daubentonii*, n = 44) or separated by over 1km from a roost of the same social group (n = 1 for each species). Four adult female *M. daubentonii* were radio-tracked; two from each of two social groups. The daytime roosts (including trees) used by these individuals are indicated by asterisks and are coloured according to the social group to which they belonged


a) M. nattereri
N
0 250 500 1000 Meters
Bird boxes
Woodland area
Boxes used by two or more bats
Boxes used by a single bat
Roost used by two social groups
Estimated roost home ranges
Radio-tracking fixes
b) M. daubentonii females

2.3.4 Spatial distribution of social groups

Social groups showed roost site fidelity, each restricted to a sub-section of the woodland (Figure 2.6). The average minimum roost home range estimates were 0.17km^2 (n = 4, range 0.09 - 0.30km^2) for *M. daubentonii* and 0.23km^2 (n = 6, range 0.12 - 0.44km^2) for *M. nattereri*.

The three *M. nattereri* social groups for which three or fewer roosts were known (Figure 2.6a) were excluded from these calculations as accurate roost home range estimates were not possible. There was little spatial overlap between the estimated roost home ranges within species (*M. daubentonii* = 5%, *M. nattereri* = 6%), and no area was shared by more than two social groups (Figure 2.6). Between species, however, there was significant overlap; 39% of the total area covered by both species was shared. Using a multiple regression, roost home range estimates were shown to positively correlate to the number of individuals assigned to a social group ($F = 9.65$, $df = 1$, $p = 0.02$) but were not correlated to sampling effort ($F = 1.15$, $df = 1$, $p = 0.32$) or species ($F = 1.22$, $df = 1$, $p = 0.31$). The spatial distribution of social groups did not reflect our sampling regime, and areas surveyed in a single day frequently contained more than one social group (representative daily sampling shown in Figure 2.1).

Four female *M. daubentonii*, known to have been present at the site for at least two consecutive summers, were radio-tracked for a total of 51 tag-days (10-15 days per tag). These individuals were located in boxes on 29% (range, 20-55%) of days and in natural tree roosts on all other occasions. The tracked bats changed roosts on average every 2 days (range, 1.1-3.5). Two bats were radio-tracked from each of two social groups and were located inside the roost home range of their group on 49% of occasions (range, 30-71%; Figure 2.6). Of those fixes that were outside the roost home range, 56% were within 15 metres of their respective range. On no occasion was a radio-tracked individual located in the known roost home range of another *M. daubentonii* social group.

M. daubentonii bachelor colonies observed during the nursery period were frequently found within the estimated roost home ranges of female social groups and of these, 5 bachelor colonies were identified in roosts previously used by nursery colonies (Figure 2.7).

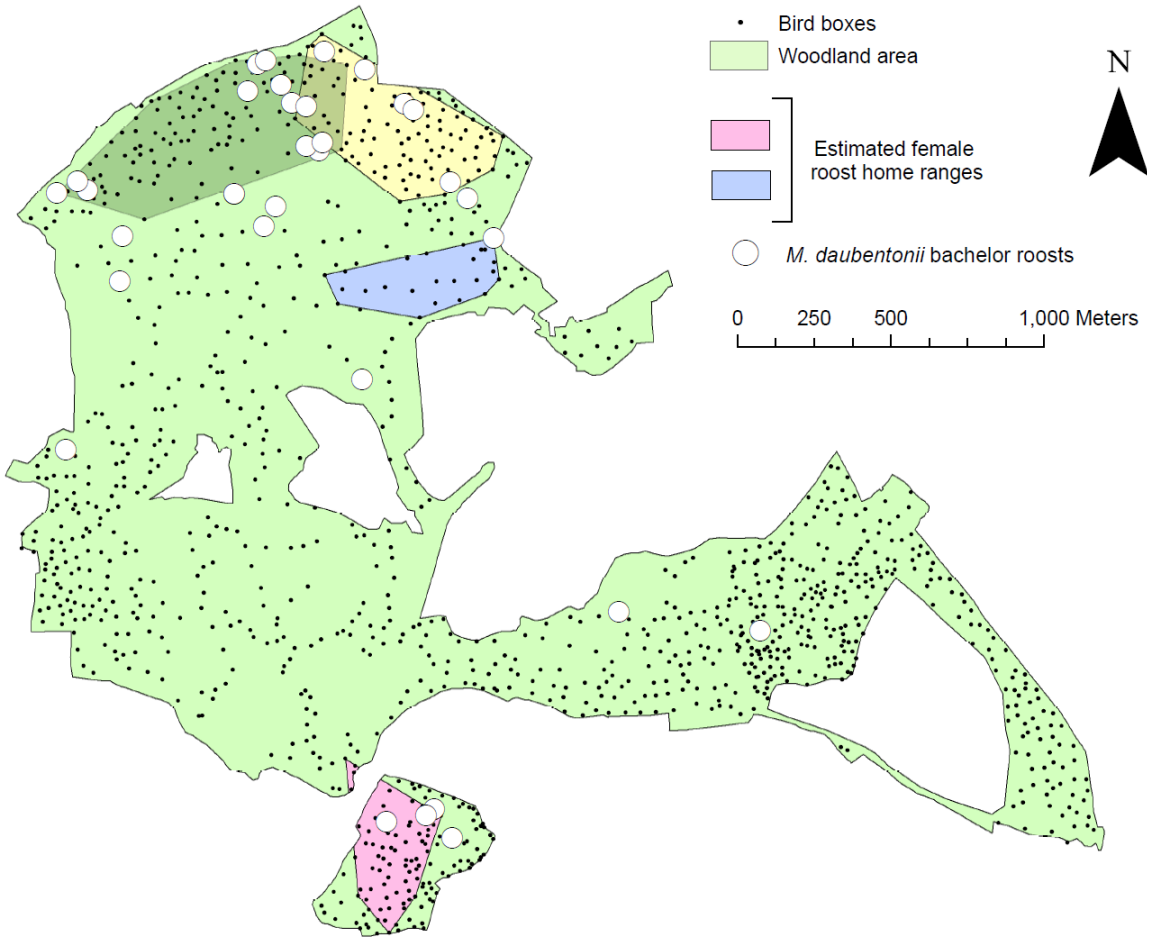
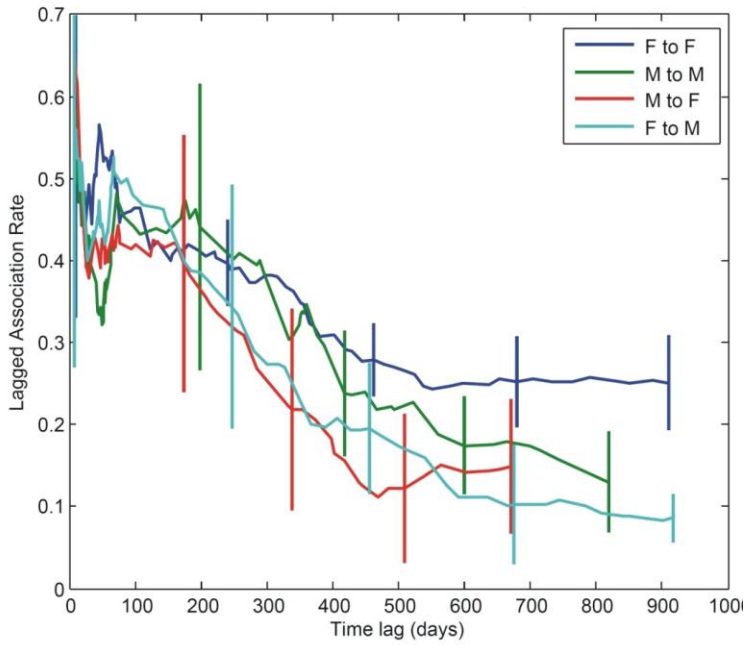


Figure 2.7 – Distribution of *M. daubentonii* bachelor colonies observed during the nursery period compared to the MCPs of female social groups

2.3.5 Duration of association between individuals

Associations within and between sexes were found to differ in their stability over time both within and between species (Figure 2.8). *M. nattereri* show an initial rapid breakdown of associations with a 40-50% chance of individuals reassociating with one another at time lags of only a few days (Figure 2.8a). There is then a gradual decline across all classes of association until day c.550 suggestive of the breakdown of casual acquaintances. From this point until day 920 the lagged association is stable, an indication that associations lasting more than c.550days are constant companionships. Though the lagged association rates of same-sex associations appear higher than those between sexes, this is not statistically significant given the standard error of the trends (Figure 2.8a). Lagged association rates for all classes stayed above the null association rate at all time intervals indicating the presence of preferred association's both within and between sexes.

a) *M. nattereri*



b) *M. daubentonii*

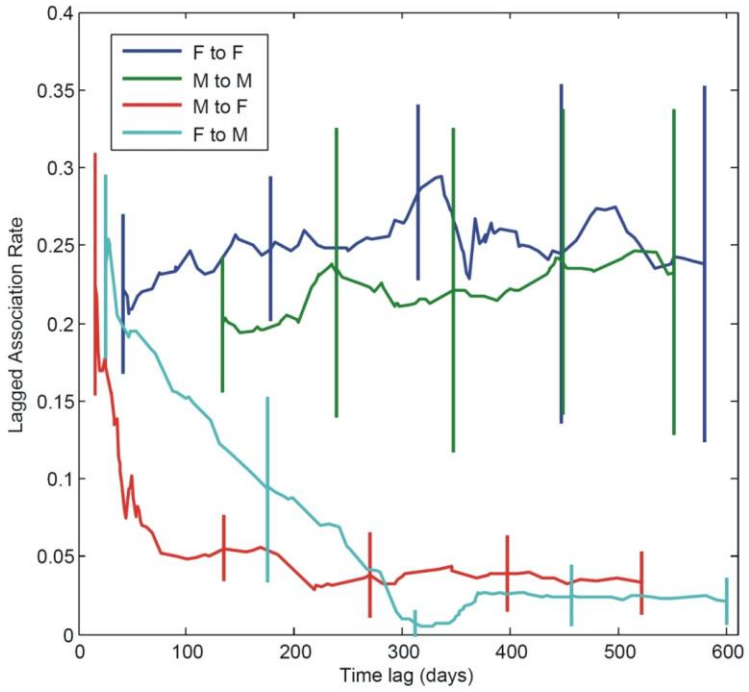


Figure 2.8 – Lagged association rates within and between sexes of a) *M. nattereri* and b) *M. daubentonii*. M = Male, F = Female. Standard error is calculated by jackknifing over a 30-day period

Different trends are seen in the temporal structure of *M. daubentonii* associations (Figure 2.8b). For all classes of association there is an initial rapid decline, which is more pronounced than in *M. nattereri*, with only a 15-25% chance of observing individuals reassociating with one another in the first few days. After this point same-sex associations are constant, indicating

that long-term companionships exist. By contrast between-sex associations show a decline in lagged association rate following the first few days which plateaus at c.300days (Figure 2.8b). After this time there is no difference between the observed level of association and that expected from a random network (data not shown) suggesting that there very few between-sex associations amongst *M. daubentonii* that last more than a year.

2.4 Discussion

This study identified multiple social groups in both *M. nattereri* and *M. daubentonii* populations within a continuous landscape in which roosts are not limiting (Figure 2.2). The social groups formed by *M. daubentonii* females and *M. nattereri* of both sexes show few inter-group interactions and little overlap between roost home ranges (Figure 2.4a, 2.4c and Figure 2.6).

The social structure identified has implications for the potential spread of directly transmitted diseases. The clear separation of *M. nattereri* of both sexes into social groups that show very little interaction over the five years of this study (Figure 2.4a), suggests that contact rates between social groups is very low during the summer months. At the same time, contact rates between individuals within the same social group are high. This structure is likely to select against fast spreading acute infections, since herd immunity will quickly be achieved (Eames 2007, Fine 1993). The social structure of *M. daubentonii* differed significantly from that of *M. nattereri*. While female *M. daubentonii* had few inter-group associations (Figure 2.4c) males had a significant number (Figure 2.4d). As a result the overall network has greater interconnectivity than the *M. nattereri* network (Figure 2.4b). This social structure would allow a disease to spread more rapidly. Interactions between males and females could lead to transmission of diseases from one female social group to another, a process that would be slower in the absence of males, given the females' social structure. It is therefore likely that males play an important role in the spread of disease in summer populations of *M. daubentonii.* This new understanding of contact rates in summer populations of *M. daubentonii* should be incorporated into models of disease transmission and may provide valuable insights into the ecology of diseases of human health concern such as EBLV-2, a lyssavirus, known to be present in *M. daubentonii* in Britain. It is important that this predicted role of male *M. daunbentoi* is tested empirically as studies of both meerkats (*Suricata suricatta*) and lions (*Pantera leo*) have not found roving males to be important in disease transmission (Craft *et al.* 2010, Drewe 2010)

It is important to note the networks presented in this chapter are only an approximation of the true contact network. Since the number of observations per individual is low (Table 2.1) and a proportion of individuals are likely unobserved (Table 2.2). Infrequent, inter-group movements, will almost certainly have been missed (Perkins *et al.* 2009). These lower frequency events are likely to be important for models of disease transmission. An additional study tracing the contacts of a small number of individuals using radio-tracking, could be used

to test whether the networks presented in the chapter reflect the true contact network of individuals.

Members of a social group were often found to be distributed amongst a number of boxes on a given day. Additionally, radio-tracking data from *M. daubentonii* show that individuals switch roosts on average every 2 days. Thus, as suggested by previous studies (Kerth *et al.* 2011), the regular fission and fusion of bat colonies is shown to operate within social groups. That is to say that individuals change who they roost with regularly, but rarely roost with individuals from different social groups.

The bats sampled within Wytham Woods had skewed adult, but not juvenile, sex ratios. Previous research that reported skewed sex ratios in populations of *M. daubentonii* hypothesised females exclude males from lowland areas of high quality foraging habitat (Encarnação *et al.* 2005; Dietz *et al.* 2006). However our work reveals a higher proportion of adult male *M. daubentonii* were captured than adult females (1.6:1) in a lowland woodland. Furthermore, within Wytham Woods *M. daubentonii* bachelor colonies are frequently found within the predicted roost home range of female social groups (Figure 2.7). Though we do not know how the quality of foraging habitat around Wytham Woods compares to previous studies, it is clear that in this woodland males are not excluded by nursery colonies of females. The adult sex ratio of captured *M. nattereri* had a female skew (0.4:1) which has been observed previously in some UK populations (Smith 2000) but not others (Park *et al.* 1998). The low proportion of *M. nattereri* males and *M. daubentonii* females observed in our population may be a result of different habitat or roost preferences between the sexes, for example *M. nattereri* males may have a greater preference for smaller natural roosts or there may be greater emigration of males from the natal range.

The lower recapture rates of adult and juvenile males compared to females, that we observed in both species (Tables 2.1 and 2.3), could have one of three possible causes: i) males are less faithful to specific territories than females (i.e. have higher rates of emigration), (ii) males have higher mortality rates than females, (iii) males prefer to roost in trees rather than bird boxes. Radio-tracking could be used to test the first and last of these hypotheses.

M. nattereri social groups include both sexes with few inter-group associations. By contrast male *M. daubentonii* social structure was shown to be largely independent of female social groups defined by SNA. This manifests as a high proportion of *M. daubentonii* bachelor roosts during the nursery period compared to *M. nattereri*, and significantly more inter-group associations in male *M. daubentonii* than *M. nattereri* (both sexes) or female *M. daubentonii*. The temporal structure of inter-sex associations of *M. nattereri* are comparable in stability to

intra-sex associations, and there was evidence of preferred associations lasting up to 900 days (Figure 2.8). In contrast, inter-sex associations of *M. daubentonii* appeared to last no more than c.300days whilst intra-sex associations appeared long-term (over 600days). Taken together the difference in social structure, duration of associations and the contrasting skewed sex ratios (see above) suggest that the two species may have distinct mating strategies.

Although both species are thought to mate at swarming sites (Altringham 2003), mating may also occur in the autumn at summer roosting sites since in both species adult males occur with females (Park *et al.* 1998; Altringham 2003; Senior *et al.* 2005; Encarnação *et al.* 2006). *M. nattereri* account for up to 80% of captures at swarming sites across the UK (Altringham 2003; Rivers *et al.* 2006) and studies of population genetics suggest that for summer colonies of size 10-30 (similar to those observed in the current study) the majority of mating is likely to occur at swarming sites (Rivers *et al.* 2005). The female-male associations in this study may therefore reflect the benefits to both sexes of natal philopatry, such as information transfer, rather than mate defence.

In contrast, empirical studies of *M. daubentonii* in Yorkshire, UK, show that *M. daubentonii* make up only 6% of bats at swarming sites (Rivers *et al.* 2006) despite being equally, if not more abundant than *M. nattereri* (Altringham 2003). Genetic analysis suggests the majority of successful mating occurs before swarming at summer roost sites (Senior *et al.* 2005). The association of male *M. daubentonii* with females from multiple social groups and the high proportion of males in mixed sex colonies during the post-nursery period (Figure 2.3b, Figure 2.4b) shown in this study are therefore likely to reflect their mating strategy. Unlike *M. nattereri*, male *M. daubentonii* are thought to disperse (Senior *et al.* 2005) which would reduce inbreeding as a result of mating at summer sites. However it is important to note that we observed a small number of juveniles return to their natal colony in the year following their birth.

The observed variation in swarming behaviour, summer social behaviour and dispersal, of these two species suggests differing mating strategies. However, detailed studies into population genetics, social structure and dispersal at Wytham Woods and other sites are needed to test this hypothesis.

Minimum roost home range estimates for *M. daubentonii* (0.09-0.30km^2) and *M. nattereri* (0.12-0.44km^2) were calculated from the location of roosts used by each social group. These data highlight the reliance of bat social groups on a network of roosts in a small area. The results were supported by radio-tracking of 4 female *M. daubentonii* which found bats rarely roosted far from the estimated minimum roost home range of their social group (Figure 2.6).

The overlap of roost home range within species was minimal, 5-6% compared to 39% between species, which suggests active avoidance and perhaps territorial defence between conspecific social groups within the wood. The reliance on a restricted territory may make these bats more susceptible to small scale habitat changes, such as felling an area of wood for timber, than previously suspected. Studies of *Chalinolobus tuberculatus*, a threatened New Zealand bat species, have shown that their social groups have similarly restricted roost home ranges (O'Donnell 2000). Within the year following tree felling, individuals in the area had smaller roosting home ranges and used fewer roosts than individuals in areas away from felling (Borkin *et al.* 2011). A substantial reduction in available roosting habitat within a social group's roost home range may also increase competition between social groups, though the mechanism by which these home ranges are maintained is unclear. It is therefore critical that the needs and locations of bat social groups are considered when undertaking alterations to their habitat to ensure minimal impact.

In summary our study of *M. daubentonii* and *M. nattereri* reveals striking differences in the structure of their populations that would be difficult to visualise without the tools offered by SNA. The social structures observed suggest that contact rates within social groups are much higher than between social groups, and in the case of *M. daubentonii*, transmission of disease between female groups is likely to be facilitated by males. These findings should be taken into account when modelling pathogens in bat populations. Future work should compare the networks presented with those generated from radio-tracking studies to assess their accuracy. Additionally it is hoped that my findings will stimulate further investigations into the evolution of mating strategies and social structure that will have relevance to the conservation of bat populations. Future work should consider the habitat requirements of social groups, the change in population social structure through the year, the role of individuals in maintaining group cohesion, the reproductive success of individuals and the implications of contrasting social structures on information transfer.

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3 Intra- and inter-specific roost preferences in three woodland bat species

3.1 Introduction

Despite their diversity, the majority of bat species are social, coming together to roost (Kerth 2008). The benefits of this behaviour derive both from the roost site, its microclimate and proximity to foraging habitat, and from roost cohabitants, through co-operation and social thermoregulation.

Research examining where, and with whom, bats roost furthers our understanding of the evolution of their social structure, informs models of disease transmission, and aids their conservation by identifying attributes of preferred roosts.

The three sympatric insectivorous bat species studied here are, *Myotis. daubentonii*, *M. nattereri* and *Plecotus auritus*. *M. nattereri* and *P. auritus* predate insects on and around vegetation (Altringham 2003). Roosts of both species are strongly tied to deciduous woodland (Entwistle *et al.* 1996, 1997; Parsons & Jones 2003; Smith & Racey 2008; Boughey *et al.* 2011), however the distribution of their roosts within this habitat has not been explored. *M. daubentonii* forage predominantly over water (Jones & Rayner 1988; Akasaka *et al.* 2009; Langton *et al.* 2010; Lucan & Radil 2010), and roost in trees and man-made structures close to water (Boonman 2000; Altringham 2003).

In many insectivorous bat species the sexes roost apart during the nursery period when females are lactating. Both *M. daubentonii* and *M. nattereri* exhibit this behaviour, however *P. auritus* males and females roost together throughout the nursery period (Altringham 2003). The segregation of male and female *M. daubentonii*, *M. nattereri* and many other species is thought to result from differences in their physiological needs. To maximise milk production females are homoeothermic and may prefer warm roosts, while males may prefer lower temperatures so that they can enter torpor and save energy (Dietz & Kalko 2006). Lower temperatures might be expected in roosts containing small groups or individual bats (Kerth 2008). However, *M. daubentonii* is noted for its formation of male only aggregations of up to 60 individuals during the nursery period (Altringham 1996). Male *Vespertilio murinus* (Particoloured bat), which also form male aggregations, have been suggested to benefit from increased rates of reproductive tissue development when forming large colonies (Safi 2008). This is thought to be a result of either information sharing, whereby individuals may

communicate the location of foraging sites, or social thermoregulation, whereby individuals reduce the energetic cost of homeothermy (Safi 2008). Additionally individuals may choose to leave aggregations once their reproductive tissues are developed (Safi 2008) or at times when roosting alone is more beneficial (e.g. when entering torpor). However, since the benefit of communal roosting would only increase in the presence of females it does not explain why the sexes roost apart. It has been suggested that sexual segregation amongst *M. daubentonii* during the nursery season is a result of females excluding males from good quality foraging and roosting habitat due to their high energy demands during this period (Encarnação *et al.* 2005; Dietz *et al.* 2006). It is unclear why *P. auritus* does not exhibit sexual segregation, however, there is a trend for increased sexual segregation in this species at lower latitudes which suggests social thermoregulation as a possible driver in the UK's cooler climate (Entwistle *et al.* 2000).

The segregation of populations, by whatever means, has implications for the transmission of disease. Populations that consist of groups with little inter-group contact can have reduced overall prevalence of disease and select for chronic diseases (Eames 2007). Understanding why bat species such as *M. daubentonii* exhibit sexual segregation is therefore useful in models of disease. Recent work suggests segregation amongst *M. daubentonii* varies with altitude (Angell, 2013), however there is not a significant enough altitudinal gradient in Wytham woods to explore this further. Instead I will explore other attributes of male and female M. daubentonii roost sites during the summer months.

In chapter 2 I showed that populations of *M. daubentonii* and *M. nattereri* formed social groups and I explored the implications of this population structure for models of disease transmission (2.4). It is possible that the social structure observed could have emerged as a result of limited roost availability in the wood. A better understanding of the roost preferences of these species at the study site will allow this hypothesis to be tested.

This chapter examines the roost preferences of *M. daubentonii*, *M. nattereri* and *P. auritus*, and looks for differences between the roosts used by *M. daubentonii* colony types (bachelor, nursery and mixed) in Wytham Woods, Oxfordshire. This site has many advantages for such a study. GPS data is available for all bird boxes used as roost sites by bats, and as a part of the Environmental Change Network (ECN) detailed weather data, habitat data, and LiDAR (Light Detection And Ranging) data are also available. LiDAR produces detailed attitudinal information which can be used to calculate canopy height and cover, and topological variables such as slope and aspect.

By studying the roost preferences of *M. daubentonii*, *M. nattereri* and *P. auritus* we aim to test whether a) roost preferences of each species are different and can be explained by known differences in species ecology, b) preferred roosts are limiting and can explain the social structure observed in Chapter 1, c) the presence of bats in a roost increases the temperature significantly, d) *M. daubentonii* nursery colonies occupy roosts closer to water and with a warmer microclimate than those used by bachelor colonies and e) bachelor colonies avoid roosts with high numbers of batfly puparia, while maternity colonies, dependent on a small number of high quality roosts (see 'c)'), use roosts with greater number of batfly puparia.

3.2 Methods

3.2.1 Sampling site

Bats were studied at Wytham Woods, Oxfordshire (Latitude, 51°77'27"; Longitude, -1°33'41") where they roost in many (>67%) of over 1150 woodcrete bird boxes spread throughout the wood. For a detailed description of the site see Chapter 1 (1.6.1).

3.2.2 Species' roost preference

3.2.2.1 Occupancy records

The presence of bats was recorded in roost boxes in the summers of 2006 to 2010 (Appendix, table 7.1). Boxes were considered to be used by a species if an individual of that species was found in the box at any time during the study. When modelling the roost preferences for each species, the occupancy records of the other two species were also included. These variables (model variables: 'Co-occurrence of...') allow us to assess whether any of the species avoid or prefer using boxes that are used by one of the other species studied. The relationship between species may be time dependent; for example, a roost recently used by one species may be avoided by another, but may become more likely to be used as the time since occupancy increases. As bats regularly move roosts and the survey method has a low temporal resolution it was not possible to estimate these time intervals and so roosts are categorised in a binary manner, having been used by a species or not. Sampling effort (number of times the box was checked) was recorded for each box and included in data analysis of roost preference (Figure 3.1).

Inevitably, data of this type will underestimate true occupancy as continuous monitoring was impossible. To explore the effects of false-negative results we separately analysed the data including only boxes checked on more than 5 occasions (n = 661 of 1187). One way of inferring roost use without direct observations of bats is through records of faeces. As faeces cannot be reliably identified to the species level without genetic analysis we combined all data on faeces and bats' occupancy to compare roosts used by any bat species, and roosts with no evidence of occupancy.

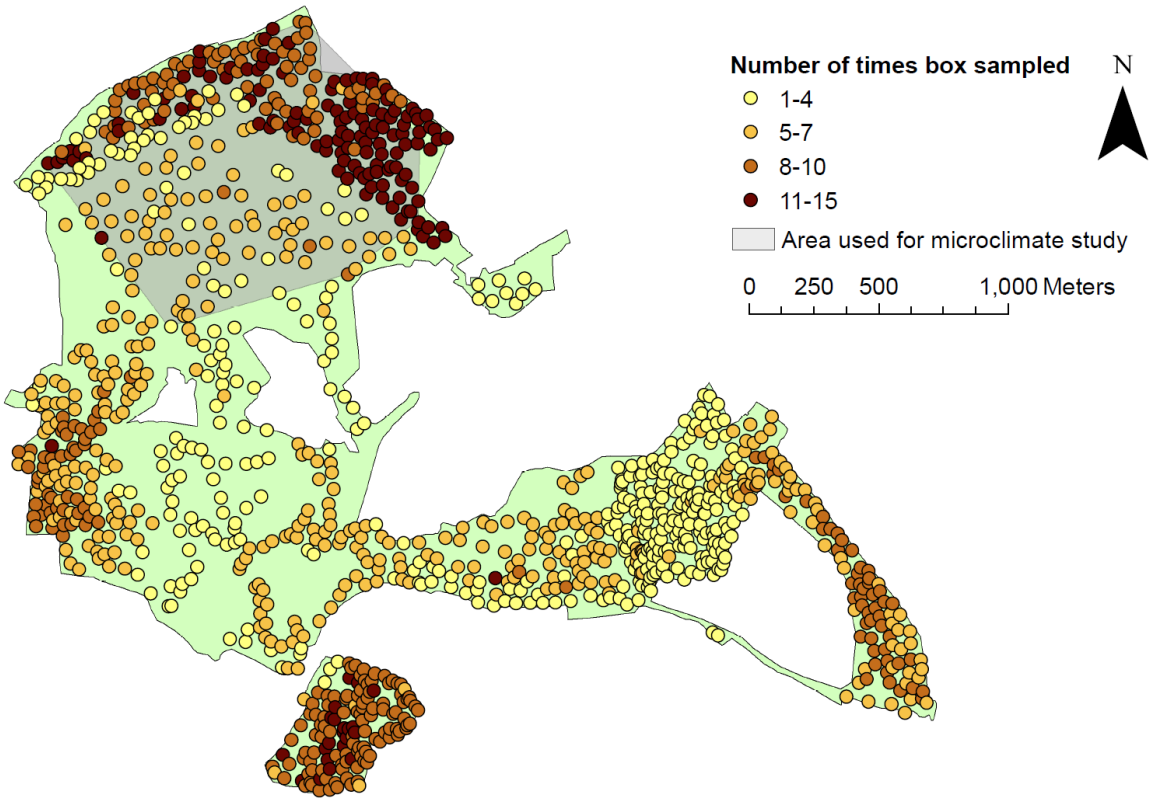

Figure 3.1 – Distribution of sampling effort across roost boxes. Sampling effort was increased in areas known to be frequently used by bats to maximise data collection, particularly for social network analysis. The area used in the study of roost microclimate is highlighted

Colonies of *M. daubentonii* identified in the nursery season were categorised into colony types according the composition of adults: those dominated (>66.6% of total) by adult females, those dominated (>66.6% of total) by adult males (bachelor colonies) and those with mixed adult sexes. The nursery season was defined as the time between the first and last colony of lactating females with juveniles. These dates varied from year to year, since the timing of births is weather dependent. These colony definitions are the same as those used in Chapter 2 and 4.

3.2.2.2 Habitat and box types

The habitat types in Wytham Woods can be defined by their history. Habitat blocks were therefore defined into 3 broad types: semi-natural ancient woodland, secondary woodland and plantation (Figure 3.2). Ancient woodland is characterised by abandoned hazel (*Corylus spp*.) coppice with oak (*Quercus robur*) standards which have not been managed for 40-100

years. Secondary woodland is varied, some is dominated by ash (*Fraxinus excelsior*) and sycamore (*Acer pseudoplatanus*) whilst other areas are dominated by blackthorn (*Prunus spinosa*) and hawthorn (*Crataegus monogyna*) shrubs. Areas classified as plantation were planted in the 1800s and 1900s and are dominated by Beech (*Fagus sylvatica*) and exotic conifers. Habitat type was included in models as a three level factor (semi-natural ancient woodland, secondary woodland and plantation).

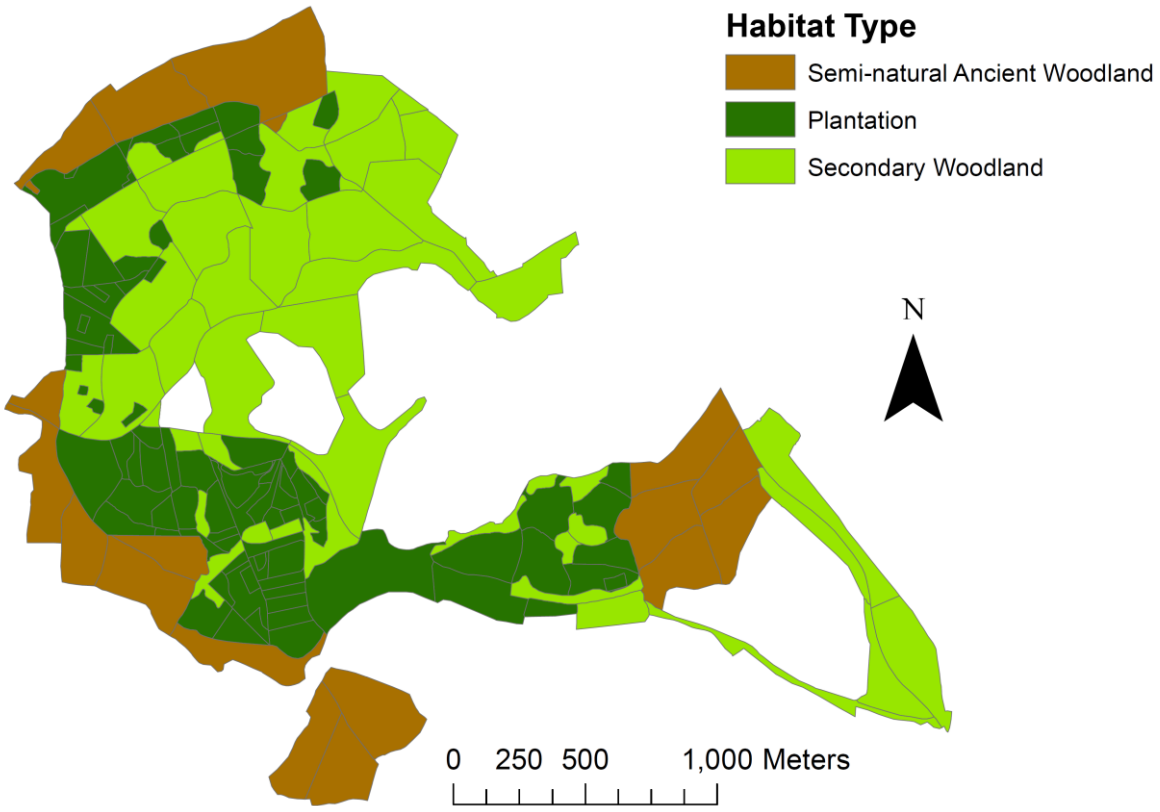

Figure 3.2 – The distribution of the three major habitat types in Wytham Woods. Classifications are made at the scale of the compartments, subdivisions of the wood divided by rides and historic management boundaries

There are differences in the woodcrete bird boxes used as roost sites by bats in Wytham Woods, depending whether they are intended for use by *Cyanistes caeruleus* (Blue tits) or *Parus major* (Great tits). Blue tit boxes have 26mm diameter entrance holes, tend to have flat roofs, and were put up 6 years before the start of this study. Great tit boxes have 32mm diameter entrance holes, tend to have peaked roofs and have been present for more than 50 years (Figure 3.3). Box type was included in models as a two level factor (Blue tit and Great tit).

Figure 3.3 – The design of a) Blue tit and b) Great boxes used as roosting sites by bats

3.2.2.3 LiDAR and derived data

Structural properties of the vegetation surrounding each roost box may influence roost selection by bats. For example, canopy structure may influence exposure to weather and solar radiation (Davies-Colley *et al.* 2000; Shine *et al.* 2002), which may be important drivers of roost selection. This was assessed using a LiDAR dataset.

LiDAR uses light pulses directed at the ground from an aeroplane to gather detailed data on the height of vegetation and land surface in a swath below it. The timings and intensity of the

first and last signals that return to the instrument are recorded. The first return pulse indicates the distance to the closest object, typically the top of the canopy. The last return is of most interest in leaf-off winter conditions when it provides information on the height of the ground. These data were collected twice in 2005**,** once in the summer and once in the winter by the Unit for Landscape Modelling (ULM), Cambridge University.

Using this LiDAR data a digital terrain model (DTM) was created by Dr Ross Hill, Centre for Ecology & Hydrology, Wallingford. This model maps the land surface of the woods by interpolating results from LiDAR ground hits. By subtracting this model from the original digital elevation model it was possible to generate a digital canopy height model (DCHM).

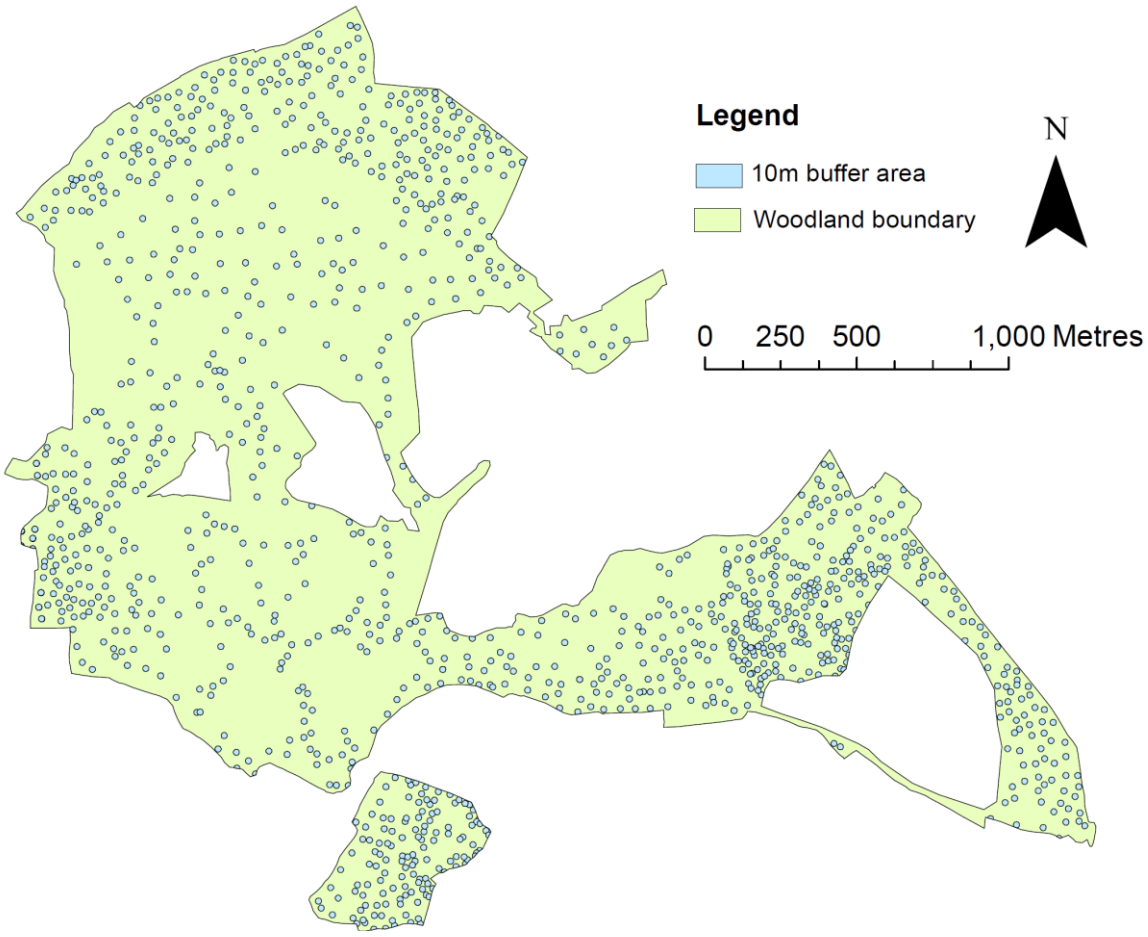

Figure 3.4 – Ten meter buffers surrounding each box were used to calculate canopy variables. Buffers were clipped by the woodland boundary map, marking the extent of the woodland

Canopy attributes, slope and aspect were calculated for the area within 10m radius of each box. This scale was chosen as it is large enough to avoid canopy attributes being disproportionally influenced by the tree to which the box is attached, and small enough to be

indicative of the local canopy structure and not an average of the habitat type. By using a 10m radius buffer it was also possible to avoid pseudo-replication as few buffers (n = 181 out of 1192) overlapped (Figure 3.4). Buffers that went beyond the woodland edge were cropped so that only the area within the woodland was considered. Buffers with more than two thirds of their area outside the woodland boundary or extending into a small area of the wood that was not mapped by LiDAR, were removed from the analysis (n = 12).

The DTM was used to calculate topological variables for Wytham Woods. Altitude, aspect and slope of the terrain were all calculated using ArcGIS (ESRI v. 9.3, 2008) (Figures 3.5-3.7). The altitude of each box was taken from the DTM using the GPS location data available for each box (courtesy of Teddy Wilkin, Oxford University). The mean slope and aspect for a 10m radius buffer around each box was also calculated using data from the DTM (Figures 3.6 and 3.7).

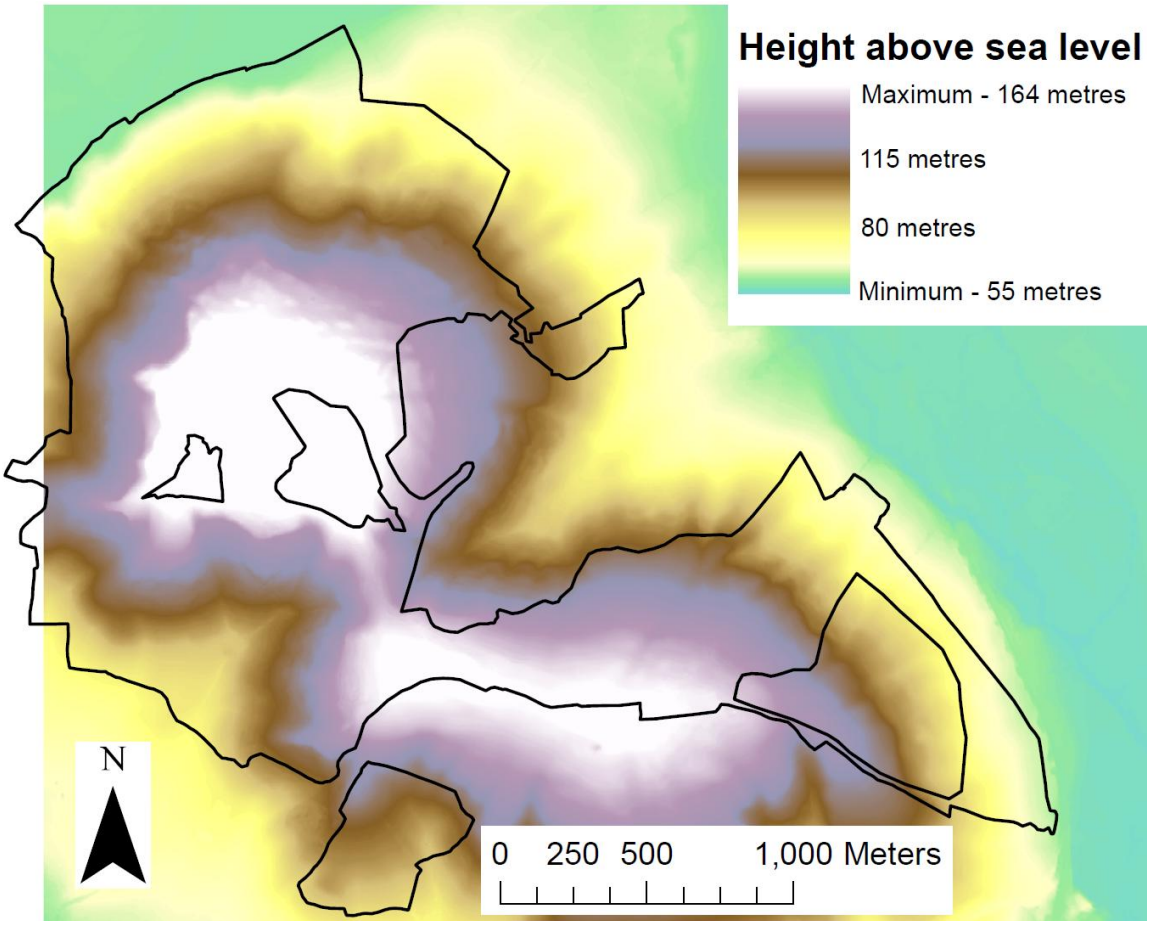

Figure 3.5 – A map of the altitudinal gradient across Wytham Woods. The wood is highest in the middle providing a range of altitudes and aspects

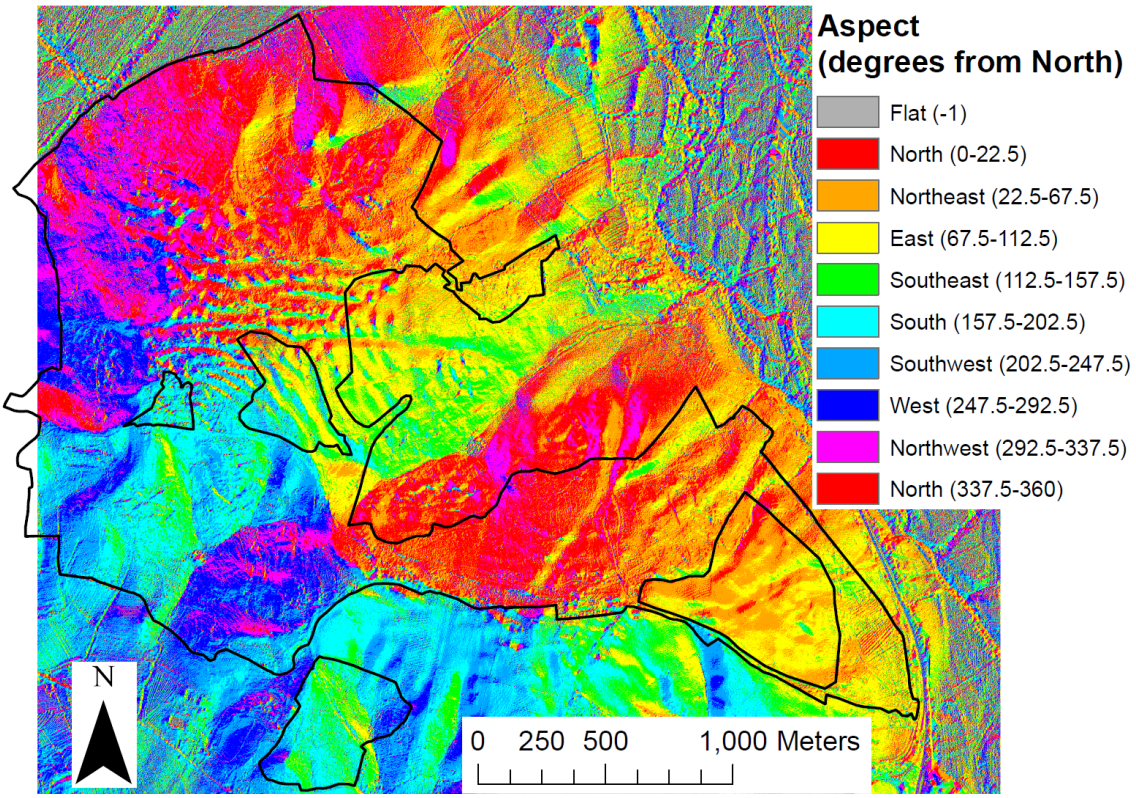

Figure 3.6 – A map of aspect throughout Wytham Woods. The aspect of the terrain around each box was calculated by averaging the aspect within a 10m radius buffer (see Figure 3.4)

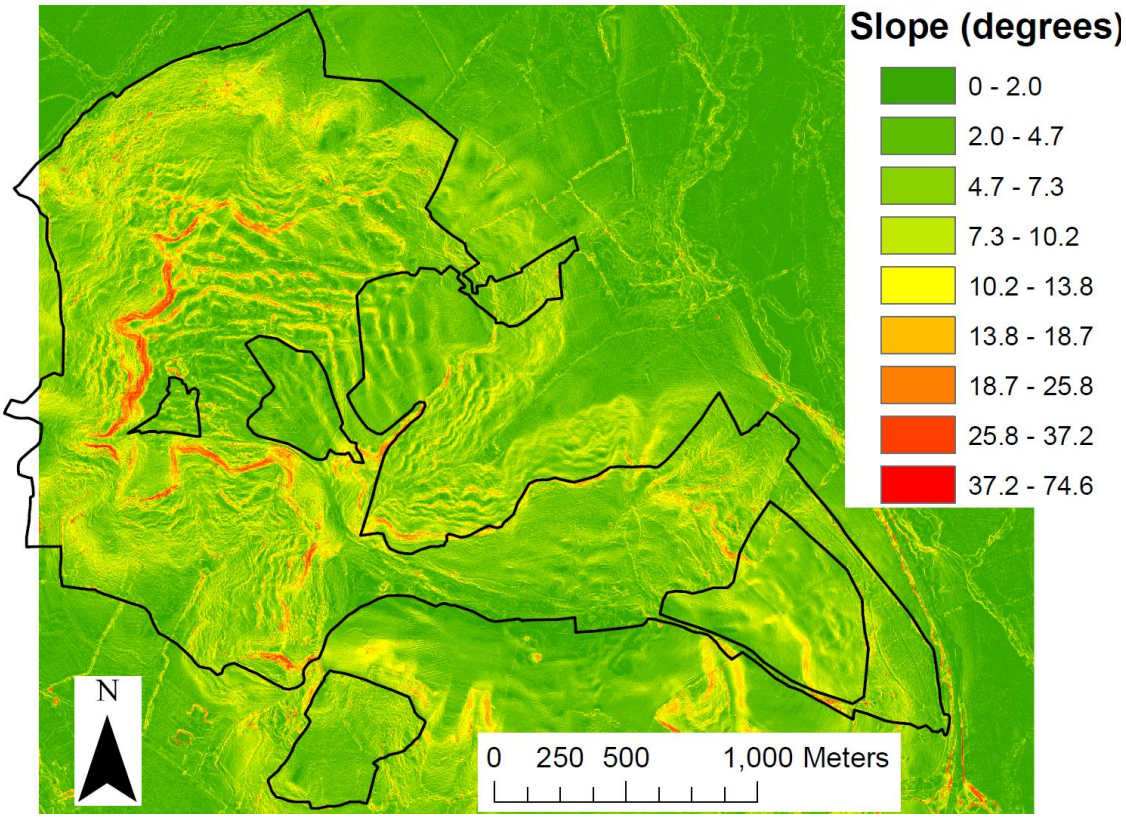

Figure 3.7 – A map of slope throughout Wytham Woods. The slope of the terrain around each box was calculated by averaging the slope within a 10m radius buffer (see Figure 3.4)

Attributes of the canopy surrounding each box was calculated from the DCHM. The minimum cut off height for canopy was taken to be 8m (Figure 3.8). This cut off is from Hill & Broughton (2009) who used a LiDAR dataset to study the canopy of Monks Wood, a woodland comparable in structure to Wytham Woods.

Within each buffer 6 canopy variables were calculated from the LiDAR data. The maximum canopy height, minimum canopy height, average canopy height, the proportion of the area covered by canopy, the standard deviation of canopy height and the proportion of the area covered by vegetation over 3 meters in height. The proportion of each buffer containing vegetation over 3 meters (the maximum height of boxes in the wood) and canopy were included as indicators of the level of shading. These estimates of shading were also calculated for a 3m radius buffer. The ability of these values to accurately predict shading was tested using photography and observer estimates of vegetative cover for a subset of boxes (n = 34). The percentage of sky obscured by vegetation in a 10 metre radius around the box was estimated and at the same visit, a camera fixed with a fish-eye lens was used to take photographs. Vertical images of the canopy at the height of each box (mean = 2m) and Gap light Analyser (v.2) software (http://www.ecostudies.org/gla/) were used to calculate the proportion of each image that represented vegetation. Data from observer estimates and photographs were compared with estimates derived from the LiDAR data.

Whilst the LiDAR data used (from 2005) did not have a measure of accuracy associated with it, a LiDAR dataset of part of the woodland from 2009 collected using the same methodology did. The 2009 dataset was produced by Airborne Research and Survey Facility, NERC and the dataset is known to have a mean error of 4.3cm (standard deviation = 4.8cm). The DTM (from 2005) was compared to the 2009 dataset across 200 randomly selected points in areas of open ground, where ground elevation could be reliably identified. The two datasets were significantly different (paired t-test: $t = 5.553$, $df = 199$, $p < 0.001$), however they had a mean difference of only 4.8cm (standard deviation = 4.8cm). Given the mean error of the 2009 dataset this difference was not large enough to reject the 2005 dataset.

The LiDAR variables calculated for the 10 metre buffers were entered into a principal components analysis (PCA) to consolidate them into variables that described the largest amount of variability in canopy structure. The top two principal components, which captured 91.7% of the observed variance in the data, were used in models (model variables: LiDAR – PC1 and LiDAR – PC2).

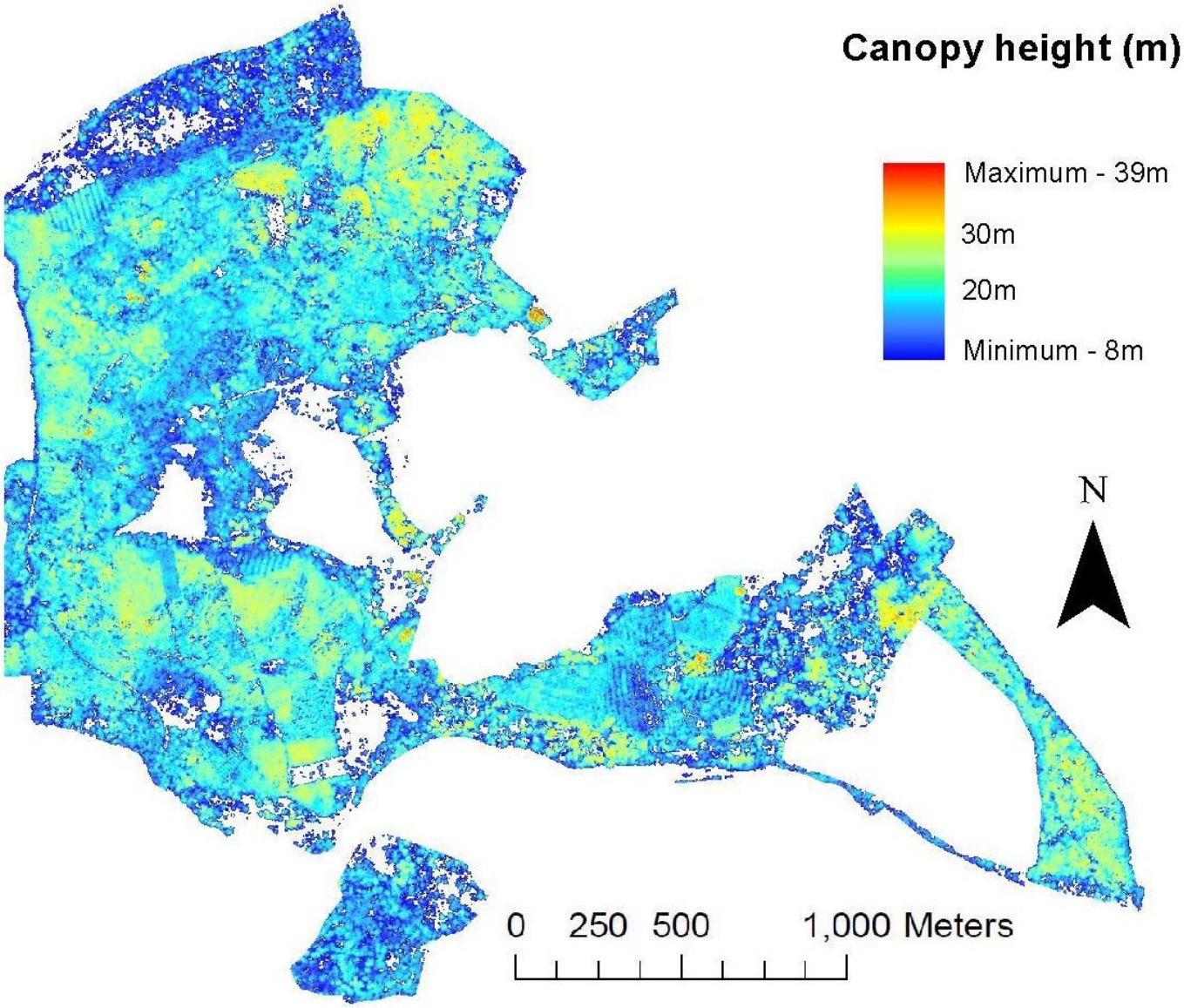

Figure 3.8 – The distribution and height of canopy in Wytham Woods. Canopy was classified as vegetation over 8m

3.2.2.4 Distance to landscape features

The distance from roost boxes to landscape features of potential importance to bats were calculated in ArcGIS.

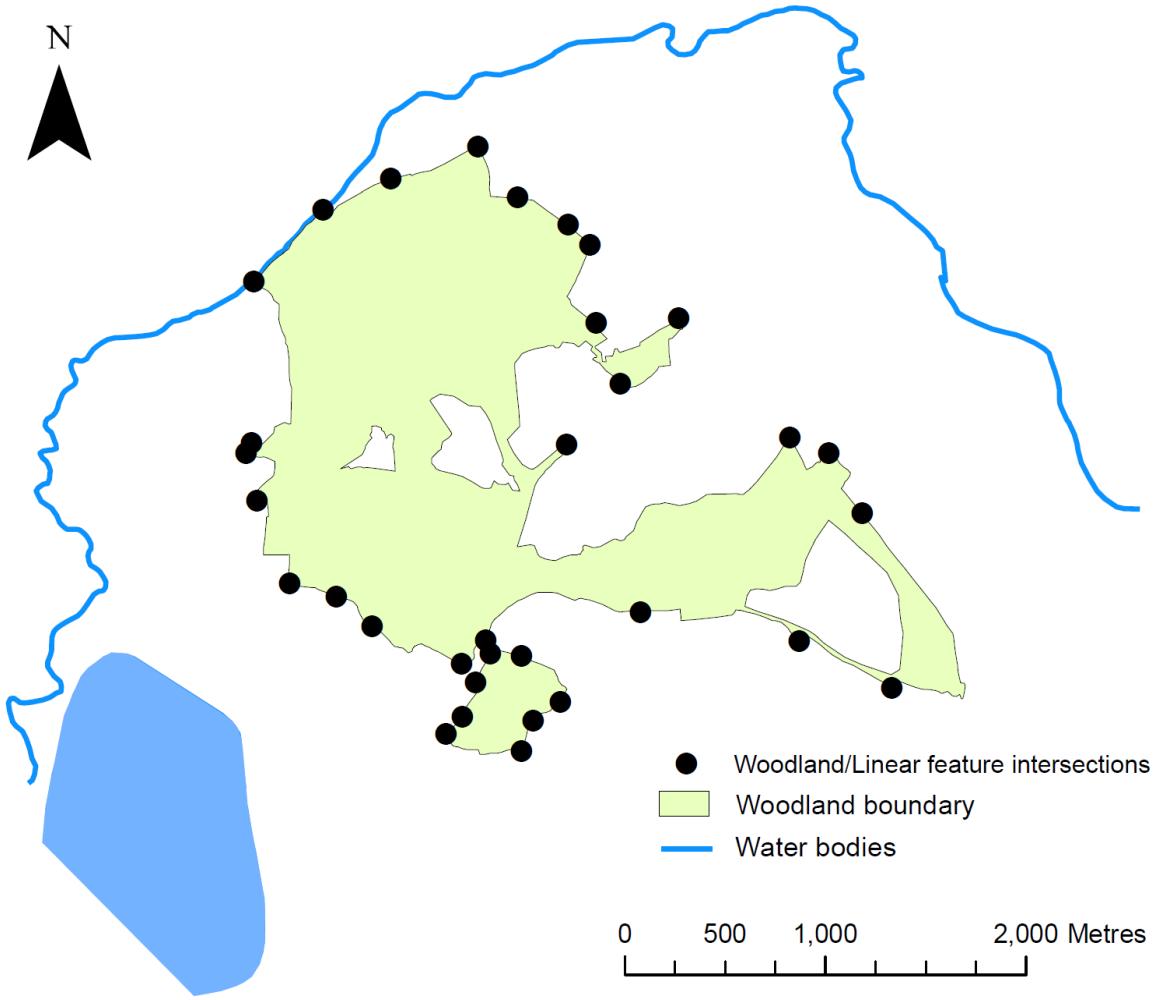

Figure 3.9 – Bodies of water close to Wytham Woods, Farmoor reservoir and the Thames River, were mapped in Arc GIS. Intersections of the woodland edge with linear features including hedgerows and tree lines were also mapped. The distance from each box to the closest water body and woodland/linear feature intersection were calculated

There are two prominent water bodies close to Wytham Woods: Farmoor reservoir, which lies to the South-west, and the River Thames which runs around the eastern, northern, and western sides of the wood (Figure 3.9). The distance from each box to the closest water body was recorded (model variable: 'Distance to water'). The minimum distance to the edge of the woodland (model variable: 'Distance to woodland edge'), including the edges of three large

clearings within the wood (Figure 3.9), was calculated using a woodland boundary map drawn from satellite images (Google Inc., www.maps.google.com, accessed 01/09/11).

Many British bat species are thought to use linear features such as hedgerows and treelines as commuting routes, and in some cases as foraging habitat (Entwistle *et al.* 1996; Walsh & Harris 1996; Lundy & Montgomery 2010). Linear features that connected with the woodland were identified using the DCHM in the first instance. For areas not covered by this dataset satellite images were used (Google Inc.). Points were added where linear features joined Wytham Woods (Figure 3.9) and the distance of roost boxes to the closest linear feature/woodland intersection were then calculated (model variable: 'Distance to linear feature').

Woodland rides, cleared corridors to allow vehicular access, are also commonly perceived to be beneficial for bats, providing corridors for bats to commute along and foraging habitat (Joint Nature Conservation Committee 2001), but there is limited published research (but see Downs & Racey 2006). Wytham Woods contains a network of rides that may be used by bats for commuting and foraging. These rides have been previously mapped by the ECN (Centre for Ecology & Hydrology, Wallingford, UK), and this map was updated using the leaf-off LiDAR data set. The distance from each box to the nearest ride was calculated in ArcGIS (model variable: 'Distance to ride').

3.2.3 Roost preferences in *M. daubentonii* nursery and bachelor roosts

To investigate whether bachelor and nursery roosts of *M. daubentonii* were selected on the basis of microclimate or other roost specific variables we made detailed observations on 34 bird boxes.

3.2.3.1 Sample design

Boxes were selected from the northern region of the wood so that they could be easily checked on each day of observations. The following boxes were selected: 9 nursery roosts, 12 bachelor roosts (3 pre-nursery season colonies, 9 nursery season colonies) and 13 roosts that had no previous records of bats and in most cases (10 of 13) no evidence of occupancy in the form of faeces on the floor of the box. These boxes included both those designed for Great tits

and Blue tits. The number of boxes included in this study was limited by the number of iButton dataloggers available (n = 34).

3.2.3.2 Temperature and humidity recordings

The temperature and humidity of each box was recorded using iButton temperature and humidity data loggers (DS1923: Maxin Integrated Products, CA, USA). Data loggers were set to record the temperature and humidity at 20 minute intervals at an accuracy of 0.0625°C and 0.04% relative humidity (%RH). As the presence of bats was likely to affect the microclimate data recorded (Bartonicka & Rehak 2007) all 34 boxes were checked for the presence of bats each day of deployment. To detect changes in roost microclimate due to the presence of bats dataloggers were placed on the inside of the door where bats would not rest against them. The average temperature and humidity was calculated for each day, from dawn to sunset, and therefore reflected the conditions when bats would be roosting. Temperature and humidity were recorded over 16 days between late July and early August 2010.

3.2.3.3 Roost box variables

Certain physical characteristics of roost sites are thought to make them more or less attractive to bats. Some studies have demonstrated that occupancy rates increase with roost height (Williams & Brittingham 1997; Agnelli *et al.* 2011), perhaps as these roosts afford protection from terrestrial predators. Box height, from the ground to the entrance, was recorded.

It has been suggested that roost switching in bats may result from active avoidance of roost associated parasites such as bat flies and fleas (Reckardt & Kerth 2007). It is also possible that avoidance of parasites plays a part in the sexual segregation seen in many species of bat, including *M. daubentonii*. To investigate whether bat colonies utilised roosts with low parasite burden or whether there is a difference in the parasite burden of roosts used by bachelor and nursery colonies, the number of bat fly puparia attached to the walls of each roost box was recorded. Bat flies are an indirectly transmitted parasite that spend their adult life on their host and only move off-host to deposit puparia in the roost. The presence of bat fly puparia in a roost was not taken to mean that the roost had been occupied by *M. daubentonii* since *M. nattereri* also carry bat flies. As a result, some of the roosts identified as unoccupied by *M.*

daubentonii contained bat fly puparia. A small sample of four pupae were collected and viewed under a microscope. Identification as bat fly pupae was confirmed using a key of pupae (Hurka 1964), and by the identification of bat flies within them.

3.2.3.4 Habitat variables

Canopy cover was recorded for each box as previously described using photography (3.2.2.3), and the distance to water was recorded as for the analysis of species roost preferences (3.2.2.4).

Understory, defined as sub-canopy vegetation over 1.5m, may influence roost choice as a significant amount of clutter may make flight difficult. Understory was recorded in the field and assigned to one of three categories: 0 – no understory, 1 – scattered understory and 2 – dense understory.

3.2.4 Statistical analysis

Roost preference for each species was analysed by comparing occupied and unoccupied roost boxes using logistic regression with model selection following an information theoretic approach (Anderson *et al.* 2000; Anderson *et al.* 2001; Anderson & Burnham 2002; Burnham & Anderson 2002; Burnham *et al.* 2011). All models included sampling effort (Figure 3.1) as an explanatory variable. It is important that sampling effort is included, since the chance of observing bats in a box increases the more times it is checked. When modelling roost preference for each species Akaike's information criterion (AIC) was used to compare models. However when comparing *M. daubentonii* colony types the modified Akaike's information criterion (AICc) was used as sample size was small. This modified criterion accounts for models which include a large number of variables compared to the sample size (Burnham *et al.* 2011). Models with a $\Delta AICc/\Delta AIC < 7$, equivalent to an evidence ratio of 33.1, were selected (Burnham *et al.* 2011). The Akaike weight (w_i) was calculated for each model and can be viewed as the probability that a model is the best of those considered, given the data. Parameter estimates were calculated by averaging across all models in which the parameter appeared and weighting the average according to the Akaike weight of each model. Variables are also given a value of 'importance' (I). This is the sum of the Akaike weights of all the selected models in which it appears and therefore represents the probability that the variable appears in the best model.

All analyses were undertaken in 'R', version 2.13.2 (R Development Core Team 2011) and the package 'MuMIn' (Barton 2011) was used to generate multi-model inferences. Due to the computational intensity of comparing multiple models, and the fact that the number of models to consider doubles with each additional variable, interaction terms were not included in the analyses. Generalised additive models (GAMs) were used for the logistic regression using package 'mgcv' (Wood 2011). GAMs were used so that aspect could be fitted to a smoothing function which limited the variable to a sin wave form with the same value at 0 and 360, thereby accounting for its circular nature. As aspect was modelled using a sin wave, the models produce two coefficients to explain the relationship between aspect and the dependent variable (model variables: 'Aspect – First order' and 'Aspect – Second order'). Pairs plots were used to check for co-correlation between explanatory variables (R Development Core Team 2011).

Differences between roosts occupied with bachelor, nursery and mixed colonies of *M. daubentonii* were compared using ANOVAs and chi-squared tests. Estimates of vegetation cover over bird boxes using LiDAR and visual estimates made by an observer in the field were compared to results from photography using general linear models (GLMs).

The effect of bats, day, and box on the temperature and humidity within boxes were examined using generalised linear mixed effects models (GLMMs) in which the presence of bats was included as a fixed effect and day and box as random effects. Differences between the microclimate and other properties of *M. daubentonii* bachelor roosts, and nursery roosts and unoccupied roosts were analysed by logistic regression analyses.

The variation in the number of bat puparia within bachelor, nursery and mixed roosts was assessed using a GLM with negative-binomial error structure. This error structure was shown to better describe the data than a Poisson distribution ($\chi^2 = 341$, $p < 0.0001$) using the R package 'pcsl' version 1.04.1 (Jackman 2011).

3.3 Results

3.3.1 Assessment of vegetative cover estimates

Comparison of vegetative cover estimates from LiDAR, fish-eye images and observer recordings made on 34 boxes revealed that LiDAR did not give an accurate estimate of cover. There was no significant correlation between LiDAR estimates of cover for either 3m or 10m buffers around boxes and the data from photographs (Table 3.1). However, there was a correlation between observer and photographic data, though I consistently underestimated the proportion of canopy. As canopy cover could not be reliably estimated from LiDAR this was not included in models of roost preference.

Buffer size	Variable	t value	*p*-value
10 metre	Proportion of cover over 8m	0.168	0.87
	Proportion of cover over 3m	0.25	0.80
3 metre	Proportion of cover over 8m	-1.37	0.18
	Proportion of cover over 3m	-1.45	0.16
	Observer estimate	5.94	**<0.0001**

Table 3.1 – Tests for correlations between estimates of vegetative cover using LiDAR and field observations, and estimates from fish-eye photography. Estimates using LiDAR data did not accurately predict canopy cover

3.3.2 Co-correlation of explanatory variables

Pairs plots revealed that altitude was correlated to distance to water and distance to linear features and so it was excluded from the analysis. This correlation exists because Wytham Woods is on a hill with its highest point at the centre (Figure 3.5). No other explanatory variables exhibited significant co-correlation.

3.3.3 Principal components analysis of LiDAR data

LiDAR variables (excluding cover estimates) were entered into a PCA. The first two principal components accounted for 91.7% of the observed variance in the data. PC1, explaining 56.4% of the variance, was correlated to canopy height. PC2, explaining 35.3% of the variance, was representative of the heterogeneity of canopy height, being correlated to the standard deviation and minimum recorded canopy heights within the 10m buffer around each box (Figure 3.10). As described in the methods (3.2.2.5) these two PCs summarising the LiDAR data were used in the GLMs examining roost preferences.

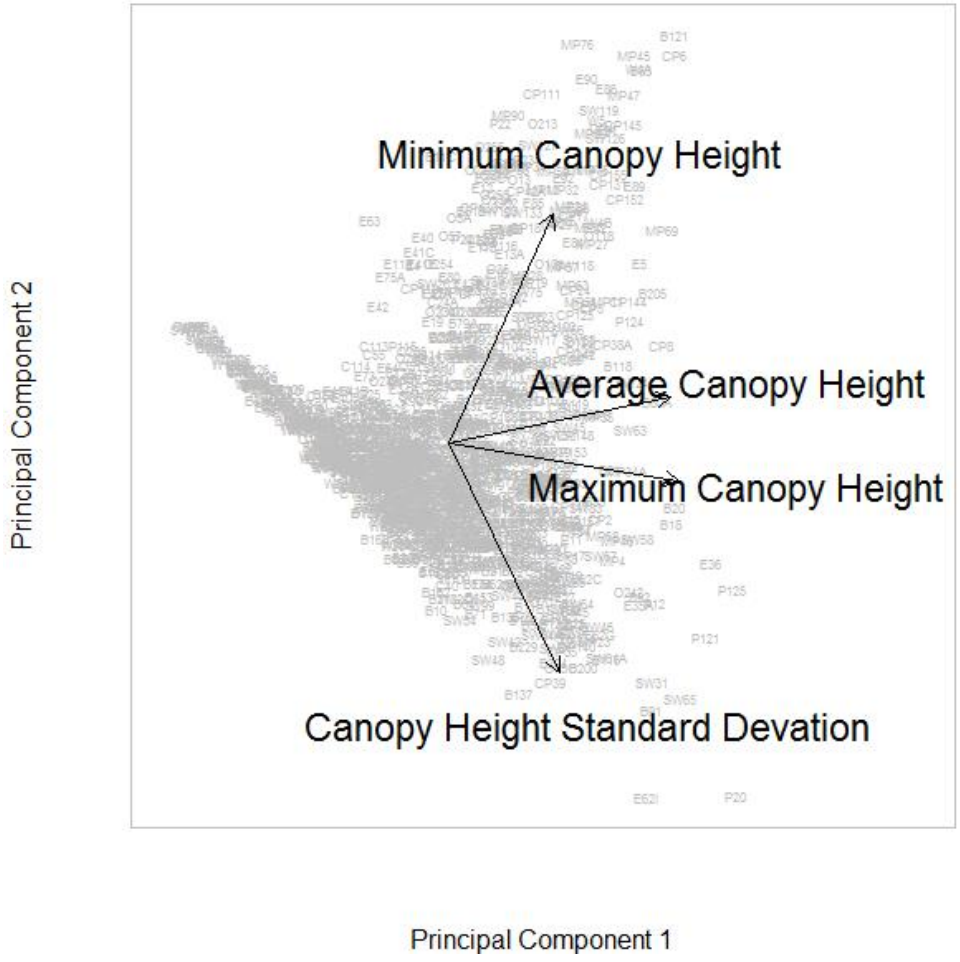

Figure 3.10 – Principal components analysis of canopy attributes for 10m buffers around each roost box. Grey labels represent the data points for each roost box whilst black arrows show the direction of the axes for each variable included in the analysis. Principal component 1 is correlated to canopy height whilst principal component 2 is correlated to canopy height heterogeneity. Together these components account for 91.7% of the observed variation

3.3.4 Roost preference, what is the general trend?

When all roosts containing evidence of occupancy (i.e. observations of bats or bat droppings) were compared to roosts for which there was no record of occupancy, four variables were found to be important. The probability of roost occupancy increased with proximity to water and canopy height heterogeneity, and decreased with proximity to woodland edge (I = 1 in all cases, Table 3.2). Sampling effort was also important, supporting the assumption that boxes checked more frequently are more likely to be recorded as occupied at some point (I = 1). To identify whether these preferences are shared between species, occupancy records were also analysed separately for each species.

Variables	Coefficients (±95% CI)	I
(Intercept)	-2.591 (±1.039)	
Distance to woodland edge (km)	5.727 (±2.31)	**1.00**
Distance to linear feature (km)	1.147 (±1.143)	0.74
Distance to ride (km)	-2.504 (±3.626)	0.47
Distance to water (km)	-0.693 (±0.413)	**1.00**
Box type: Great Tit	0.253 (±0.54)	0.37
Plantation	0.147 (±0.478)	0.17
Secondary Woodland	0.176 (±0.445)	
LiDAR - PC1	0.124 (±0.118)	0.77
LiDAR - PC2	0.304 (±0.162)	**1.00**
Aspect - First order	-0.005 (±0.049)	0.50
Aspect - Second order	0.009 (±0.079)	
Slope	0.036 (±0.064)	0.41
Sampling effort	0.638 (±0.094)	**1.00**
Deviance explained by best model	36%	
Number of models included in inference	116	

Table 3.2 – Results of multimodel inference of variables influencing the probability of roost occupancy by all species. A roost was said to be occupied if bats or faeces were found in it at any time. Variables with an importance greater that 0.9 (i.e. a 90% chance that the variable is present in the best model) are indicated in bold

3.3.5 Species specific roost preferences

3.3.5.1 Roost preference modelling using all roost occupancy records

From 2006 to 2010 we identified 162 *M. daubentonii* roosts, 158 *M. nattereri* roosts and 63 *P. auritus* roosts (Figure 3.11-3.13). Though bats of different species were never found to occupy the same roost at the same time they did use the same roosts at different times. *M. daubentonii* and *M. nattereri* used 27 of the same boxes, *M. daubentonii* and *P. auritus* used 4 of the same boxes and *M. nattereri* and *P. auritus* used 6 of the same boxes. These rates are no different from those expected by chance (χ^2 tests; $p = 0.49$, $p = 0.32$ and $p = 0.74$ respectively).

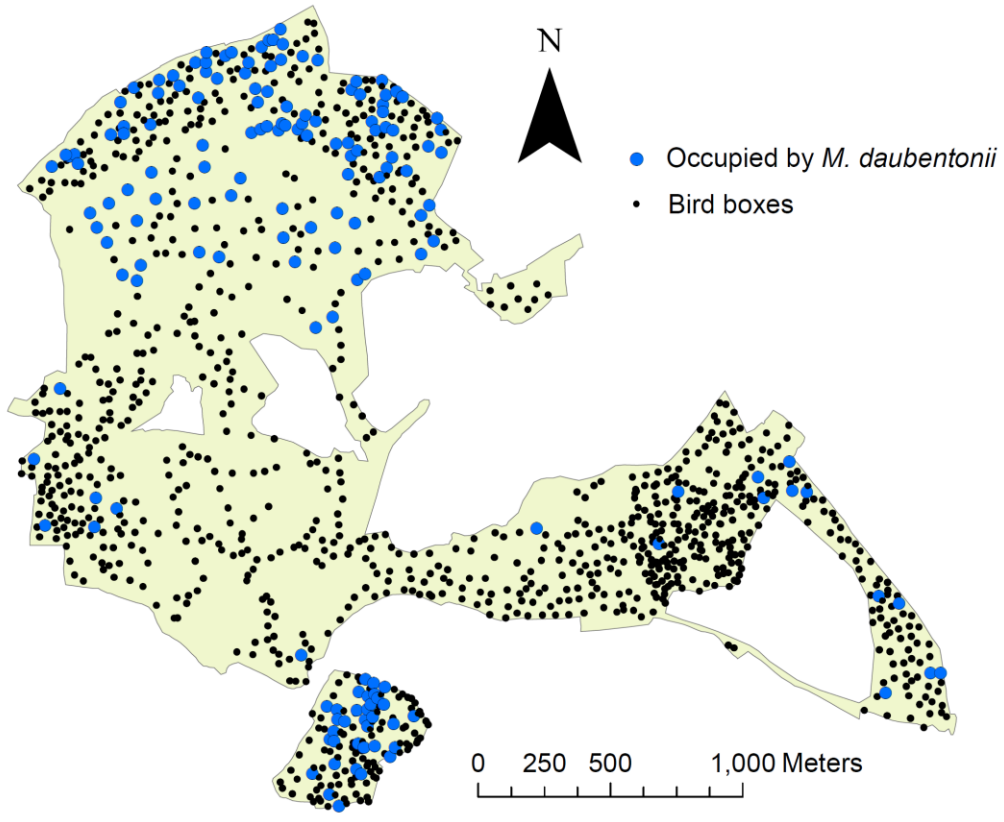

Figure 3.11 – Distribution of *M. daubentonii* summer roosts 2006-2010

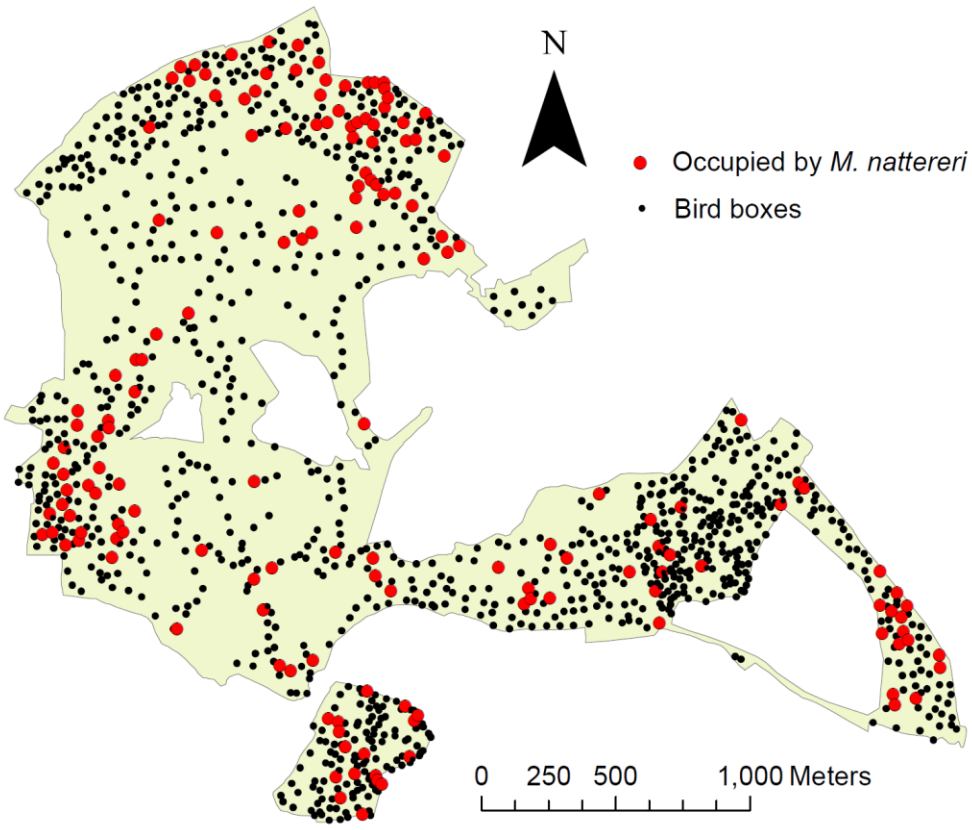

Figure 3.12 – Distribution of *M. nattereri* summer roosts 2006-2010

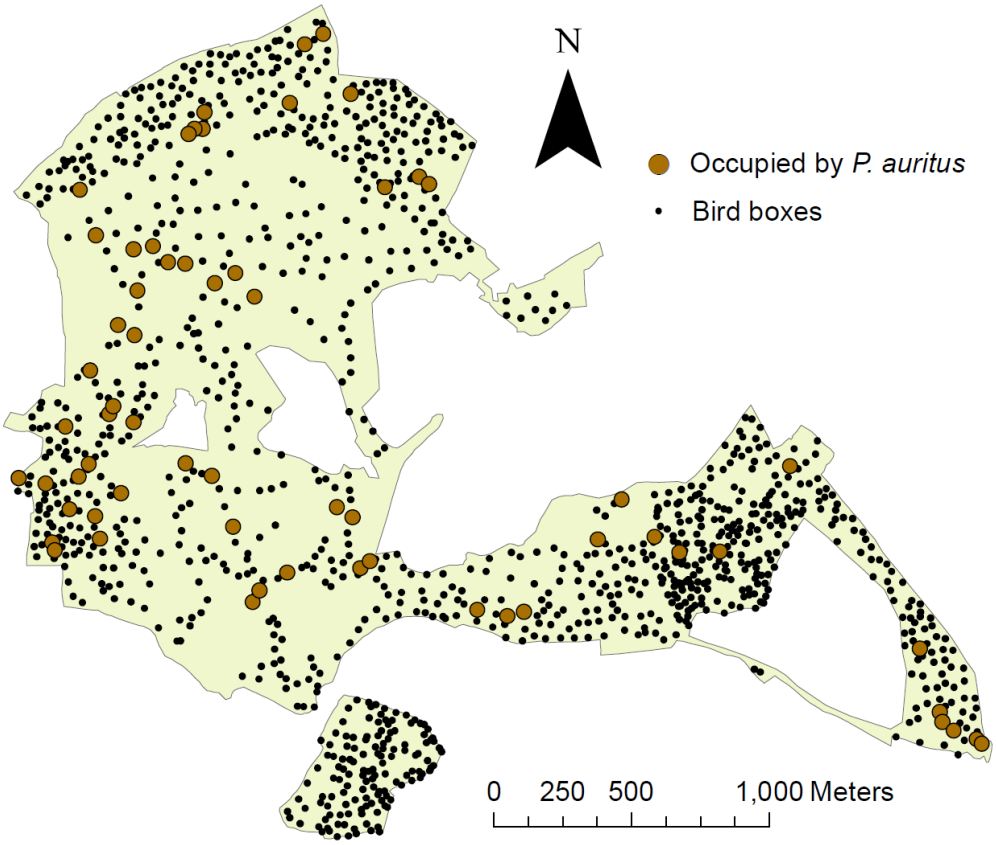

Figure 3.13 – Distribution of *P. auritus* summer roosts 2006-2010

Models of *M. daubentonii* reveal that the species is most common in roosts close to water (*I* = 1) but away from the woodland edge (*I* = 1) (Table 3.3). Additionally there was an observed preference for boxes designed for great tits (*I* = 1) and evidence of higher use of boxes with easterly aspects (*I* = 0.97). Amongst *M. nattereri* there was no observed effects of distance to edge or water but in contrast to *M. daubentonii* there was an increase in the probability of occupancy for roosts designed for blue tits (*I* = 1). *P. auritus* showed a preference for roosts far from linear features (*I* = 0.92) and in areas with a westerly aspect (*I* = 0.91).

Sampling effort was found to be an important factor for predicting whether a box was observed to be occupied by *M. daubentonii* and *M. nattereri* (*I* = 1).

Models of *M. daubentonii* captured a greater amount of the observed deviance than models of *M. nattereri* or *P. auritus* (deviance explained by the best model: *M. daubentonii* = 24%*, M. nattereri* = 8%*, P. auritus* = 10%). The low amount of deviance explained by models of *M. nattereri* and *P. auritus* suggests the roost specific parameters that were measured are poor predictors of the species' distribution.

Variables	*M. daubentonii* Coefficients (±95% CI)	*I*	*M. nattereri* Coefficients (±95% CI)	*I*	*P. auritus* Coefficients (±95% CI)	*I*
(Intercept)	-3.769 (±1.419)		-2.405 (±0.801)		-4.346 (±1.81)	
Distance to woodland edge (km)	4.145 (±2.645)	**1.00**	0.790 (±2.009)	0.30	1.217 (±2.956)	0.30
Distance to linear feature (km)	-1.461 (±1.842)	0.59	0.577 (±1.329)	0.31	2.356 (±1.986)	**0.92**
Distance to ride (km)	2.357 (±3.133)	0.54	0.546 (±3.283)	0.23	-4.214 (±7.385)	0.40
Distance to water (km)	-1.483 (±0.593)	**1.00**	0.087 (±0.428)	0.24	-0.611 (±0.682)	0.58
Co-occurence of *P. auritus*	-1.103 (±1.096)	0.84	-0.495 (±0.891)	0.39	NA	NA
Co-occurence of *M. nattereri*	0.005 (±0.517)	0.23	NA	NA	-0.619 (±0.909)	0.50
Co-occurence of *M. daubentonii*	NA	NA	-0.125 (±0.504)	0.25	-1.096 (±1.107)	0.85
Box type: Great Tit	1.098 (±0.616)	**1.00**	-0.972 (±0.414)	**1.00**	0.158 (±0.798)	0.25
Plantation	-0.242 (±0.840)	0.13	0.169 (±0.584)	0.12	1.159 (±0.9)	0.87
Secondary Woodland	-0.232 (±0.580)		-0.099 (±0.507)		0.917 (±0.864)	
LiDAR - PC 1	0.034 (±0.148)	0.26	0.127 (±0.124)	0.79	0.109 (±0.219)	0.37
LiDAR - PC 2	0.168 (±0.161)	0.77	0.112 (±0.143)	0.55	0.137 (±0.208)	0.44
Aspect - First order	0.438 (±0.333)	**0.97**	0 (±0.006)	0.50	-0.460 (±0.433)	**0.91**
Aspect - Second order	-0.046 (±0.439)		0 (±0.01)		0.4 (±0.527)	
Slope (degrees)	-0.06 (±0.087)	0.46	-0.004 (±0.063)	0.21	-0.022 (±0.094)	0.25
Sampling effort	0.3 (±0.079)	**1.00**	0.17 (±0.062)	**1.00**	0.12 (±0.118)	0.76
Deviance explained by best model	24%		8%		10%	
Number of models included in inference	145		713		1035	

Table 3.3 – Results of multimodel inference of variables influencing the probability of roost occupancy by *M. daubentonii*, *M. nattereri* and *P. auritus*. Variables with an importance greater that 0.9 (i.e. a 90% chance that the variable is present in the best model) are indicated in bold

Variables	*M. daubentonii* Coefficients (±95% CI)	*I*	*M. nattereri* Coefficients (±95% CI)	*I*	*P. auritus* Coefficients (±95% CI)	*I*
(Intercept)	-2.661 (±1.690)		-0.745 (±1.094)		-3.216 (±1.649)	
Distance to woodland edge (km)	4.655 (±3.336)	**1.00**	0.709 (±2.456)	0.25	2.066 (±4.161)	0.35
Distance to linear feature (km)	-2.186 (±2.39)	0.69	0.835 (±1.652)	0.35	3.064 (±2.465)	0.89
Distance to ride (km)	2.098 (±3.285)	0.45	-0.232 (±3.527)	0.21	-5.614 (±8.023)	0.51
Distance to water (km)	-1.659 (±0.742)	**1.00**	0.042 (±0.545)	0.21	-0.584 (±0.986)	0.39
Co-occurence of *P. auritus*	-0.919 (±1.111)	0.64	-0.401 (±0.919)	0.31	NA	NA
Co-occurence of *M. nattereri*	-0.036 (±0.538)	0.23	NA	NA	-0.456 (±0.916)	0.35
Co-occurence of *M. daubentonii*	NA	NA	-0.05 (±0.512)	0.20	-0.998 (±1.107)	0.75
Box type: Great Tit	0.847 (±0.667)	**0.98**	-1.159 (±0.489)	**1.00**	0.196 (±0.969)	0.24
Plantation	-0.451 (±1.076)	0.23	0.422 (±0.768)	0.25	0.907 (±1.315)	0.23
Secondary Woodland	-0.448 (±0.692)		-0.093 (±0.593)		0.685 (±1.11)	
LiDAR - PC1	0.045 (±0.177)	0.28	0.121 (±0.154)	0.56	0.194 (±0.273)	0.50
LiDAR - PC2	0.065 (±0.179)	0.30	0.062 (±0.166)	0.27	0.06 (±0.255)	0.23
Aspect - First order	0.839 (±0.504)	**1.00**	0 (±0.059)	0.50	-0.234 (±0.489)	0.63
Aspect - Second order	-0.319 (±0.487)		-0.007 (±0.076)		0.227 (±0.491)	
Slope	-0.042 (±0.094)	0.32	-0.029 (±0.071)	0.29	-0.009 (±0.11)	0.22
Sampling effort	0.227 (±0.111)	**1.00**	0.053 (±0.103)	0.36	-0.002 (±0.181)	0.23
Deviance explained by best model	15%		4%		8%	
Number of models included in inference	187		1387		1890	

Table 3.4 – Results of multimodel inference of variables influencing the probability of roost occupancy by *M. daubentonii*, *M. nattereri* and *P. auritus*, when only boxes checked on more than five occasions are considered. Variables with an importance greater that 0.9 (i.e. a 90% chance that the variable is present in the best model) are indicated in bold

3.3.5.2 Roost preference modelling using roosts checked on more than five occasions

False negatives, whereby bats are not recorded to occupy a box but in fact do, are known to exist in this dataset as of all boxes checked 35% have records of bats of any species but 67% have records of bat droppings. To reduce the number of false negatives, roost occupancy data were reanalysed including only boxes checked on more than 5 occasions (n = 661 of 1187).

This analysis produced results similar to that with all boxes included (Table 3.4). Increased probability of occupancy by *M. daubentonii* was associated with Great tit boxes (*I* = 0.98), boxes close to water (*I* = 1), away from woodland edge (*I* = 1), increased sampling effort (*I* = 1) and, in this case, in areas with south-easterly aspect (*I* = 1). *M. nattereri* still showed a preference for Blue tit boxes (*I* = 1) however sampling effort was no longer important (*I* = 0.36). *P. auritus* models had no parameters with an importance over 0.9. These models explained less of the observed deviance compared to models run with all available data (deviance explained: *M. daubentonii* = 15%, *M. nattereri* = 4%, *P. auritus* = 8%) and again models of *M. nattereri* and *P. auritus* predicted occupancy poorly.

Variable	df	F-value	*p* -value
Distance to ride (km)	2	0.50	0.61
Distance to woodland edge (km)	2	0.44	0.65
Distance to linear feature (km)	2	0.56	0.57
Distance to water (km)	2	0.40	0.67
LiDAR - PC1	2	0.70	0.50
LiDAR - PC2	2	1.13	0.32
Slope (degrees)	2	0.28	0.76
	df	Chi-squared	*p* -value
Ancient woodland	2	0.24	0.89
Plantation	2	0.87	0.65
Secondary woodland	2	0.02	0.99
Box Type (Blue tit or Great tit)	2	3.26	0.20
Aspect (degrees)	0.3	0.20	0.45

Table 3.5 – ANOVA and Chi-squared tests of variables between *M. daubentonii* bachelor (n = 34), nursery (n = 48) and mixed roosts (n = 20) during the nursery period. There were no significant differences between roost types

3.3.6 Variation in roost preference of *M. daubentonii* bachelor, nursery and mixed colonies using occupancy records

M. daubentonii roosts used by bachelor (n = 34), nursery (n = 48) and mixed (n = 20) colonies within the nursery season were compared. Roosts known to be occupied by more than one colony type (n = 6 of 96) were not removed from the analysis as doing so could artificially increase the differences between groups. There were no conclusive differences between roost types for any of the variables studied (Table 3.5).

3.3.7 Microclimate and field observations of known *M. daubentonii* bachelor and nursery roosts

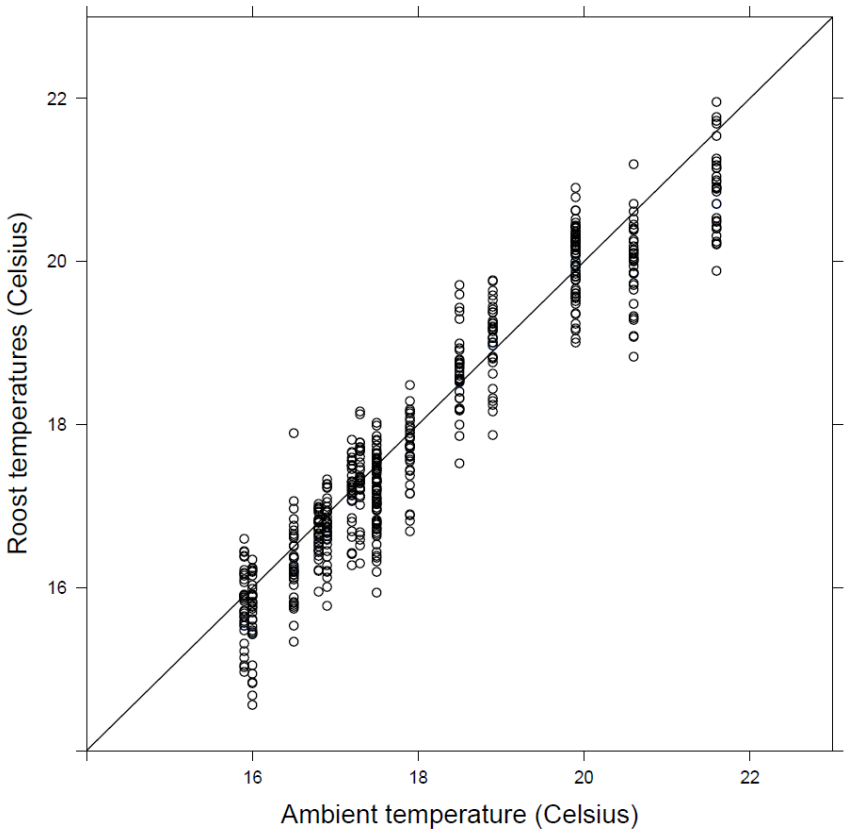

Figure 3.14 – Ambient average daily temperature against roost box temperatures. The solid black line represents the line of equality. The temperature of boxes is closely linked to the ambient temperature

3.3.7.1 The effects of presence of bats on roost temperature and humidity

Temperature in the boxes was closely related to ambient temperature (Figure 3.14), however, humidity in boxes was consistently higher than ambient (Figure 3.15).

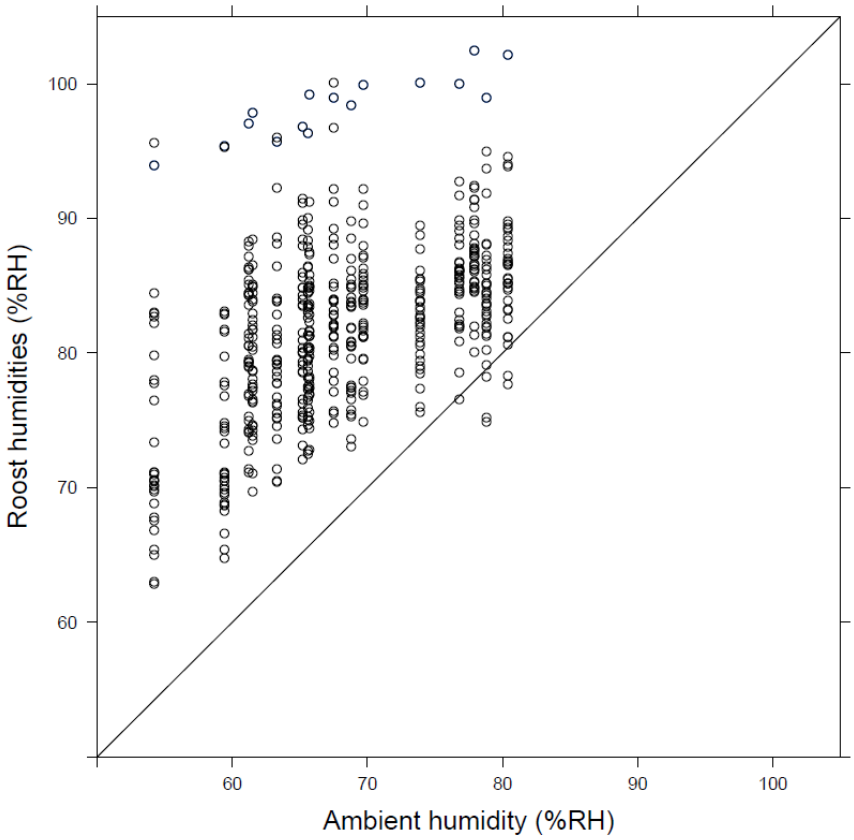

Figure 3.15 – Ambient average daily humidity against roost box humidity. The solid black line represents the line of equality. The humidity recorded in boxes was greater than ambient conditions but increased with increase ambient humidity

Boxes maintained their relative differences in humidity and temperature over time (Figures 3.16 and 3.17). The mean temperature within roosts was significantly correlated with the amount of shade recorded over each box using fisheye images. Boxes with greater amounts of vegetative cover had significantly lower temperatures (df = 1, t = -2.7, p = 0.01) explaining 19% of the observed deviance in average box temperature (Figure 3.18). Thus nest boxes offer bats a choice of microclimates in which to roost.

The effect of bats on the temperature and humidity within roosts was examined using a mixed effects model. Box and day were included as random effects and presence of bats, observed

on only 5 occasions in this study, was added as a fixed effect. Model comparisons were made between models with and without the bat presence variable. The models including bat presence were found to be significantly better at describing the observed temperature ($\chi^2 = 18$, df = 1, $p < 0.001$) and humidity ($\chi^2 = 25$, df = 1, $p < 0.001$) within roosts (Figures 3.16 and 3.17).

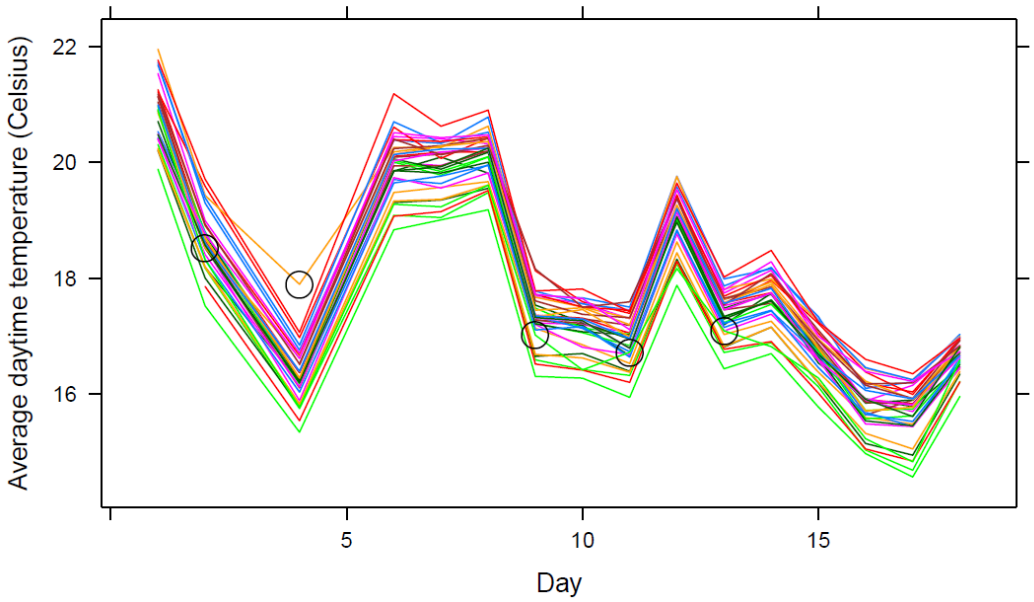

Figure 3.16 – Temperature recordings from 34 roost boxes from late July to early August. Each box is shown by a line of different colour. Records for when bats were present are shown by black circles

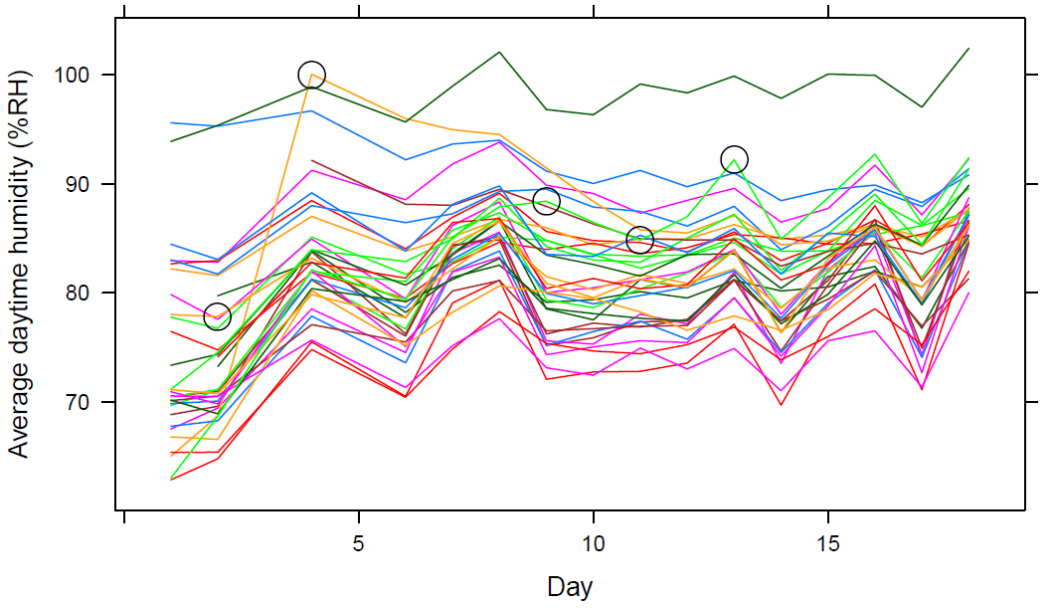

Figure 3.17 – Humidity recordings from 34 roost boxes from late July to early August. Each box is shown by a line of different colour. Records for when bats were present are shown by black circles. The high point on the yellow line was the largest bat colony observed in the microclimate study (estimated at 15-20 bats). From left to right the other bat colonies had approximately 4, 8-10, 3 and 4-5 bats

Model results suggest temperature was an average of 0.31°C higher (95% CI = -0.17-0.46°C) in occupied boxes and humidity on average 6.1%RH higher (95% CI = 3.8-8.6%RH). By comparing the full model with models with either box or day removed it was found that these random effects were also highly significant for explaining variation in roost temperature (Box: $\chi^2 = 947$, df = 1, p <0.001; Day: $\chi^2 = 2282$, df = 1, $p < 0.001$) and humidity (Box: $\chi^2 = 701$, df = 1, $p < 0.001$; Day: $\chi^2 = 504$, df = 1, $p < 0.001$).

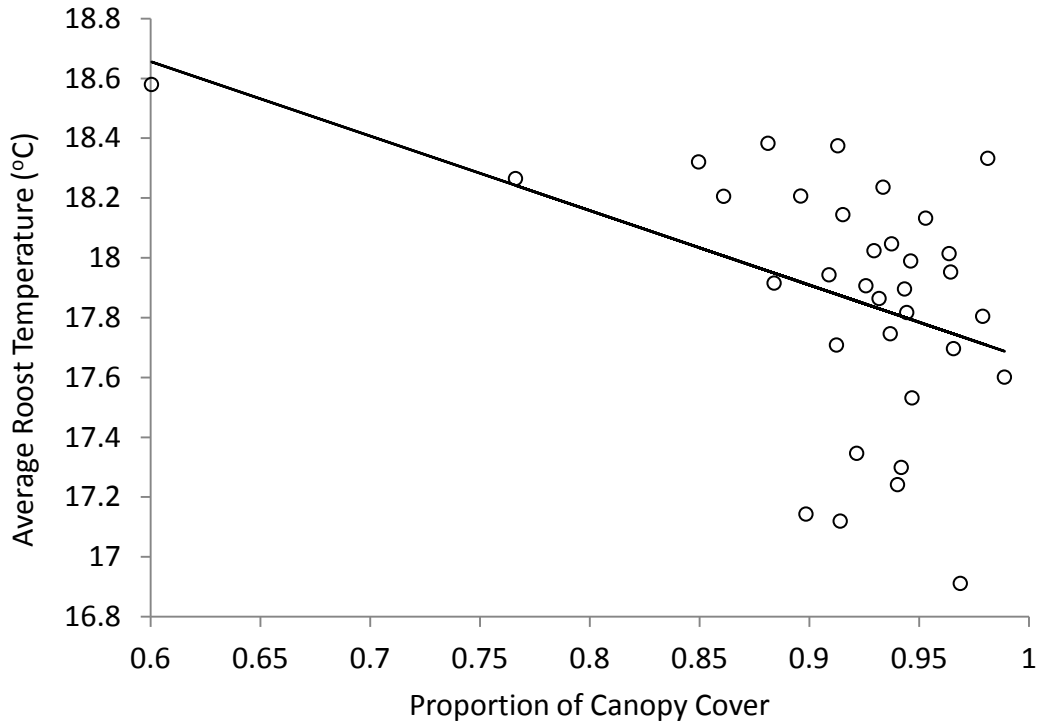

Figure 3.18 – Average roost temperature was shown to decrease with increasing canopy cover as recorded using fish-eye photographs. The regression line is shown (t = -2.7, p = 0.01)

3.3.7.2 Comparison of the properties of nursery, bachelor and random roosts

Using the temperature and humidity data collected using iButtons, and data collected in the field, the differences between nursery and bachelor roosts were assessed. The variables included were the mean temperature and humidity of each box, the canopy cover as calculated from fish-eye photographs, the category of understory, the height of the box entrance, the distance from the box to the closest body of water and the number of bat fly puparia in the box. There were no clear differences between the microclimates of roosts used

by bachelor and nursery colonies, both temperature (*I* = 0.18) and humidity (*I* = 0.19) having low importance (Table 3.6). There was no strong support for any of the other variables (Table 3.6).

Variables	Coefficients (±95% CI)	*I*
(Intercept)	-18.432 (±59.51)	
Average Humidity (%RH)	-0.059 (±0.21)	0.19
Average Temperature (°C)	-0.312 (±5.075)	0.18
Canopy cover (%)	30.754 (±38.97)	0.70
Distance to water (km)	0.41 (±4.647)	0.18
Entrance height (cm)	0.033 (±0.049)	0.44
Bat fly puparia	0.013 (±0.053)	0.18
Understory	1.087 (±1.775)	0.38
Deviance explained by best model	21%	

Table 3.6 – Results of multimodel inference of logistic regression analyses examining variables correlated with nursery roosts (n = 9) compared to bachelor roosts (n = 12). Understory is divided into three categories, 0 – no understory, 1 – scattered understory and 2 – dense understory, and treated as a continuous variable. None of the variables had *I* > 0.9

When using the same explanitory variables to compare roosts known to have been occupied (i.e. bachelor and nursery roosts combined, n = 21) with roosts with no occupancy record (n = 13) the inclusion of bat fly puparia as an explanatory variable leads to over fitting of the data. The number of bat fly in a roost was a significant predictor of occupancy by *M. daubentonii* (z = 2.780, *p* = 0.005) and explained 30% of the observed deviance alone (Figure 3.19). To prevent over fitting and examine other variables bat fly puparia were removed from the models. None of the remaining explanatory variables considered had a high importance (Table 3.7). These results did not change if the 3 roosts classified as 'no recorded occupancy', but which had faeces in them, were removed from the analysis.

Variables	Coefficients (±95% CI)	*I*
(Intercept)	-61.962 (±115.684)	
Average Humidity (%RH)	0.316 (±0.463)	0.66
Average Temperature (°C)	2.756 (±3.89)	0.51
Canopy cover (%)	23.46 (±27.953)	0.83
Distance to water (m)	-0.0031 (±0.004)	0.47
Entrance height (cm)	0.03 (±0.039)	0.55
Understory	0.215 (±2.179)	0.28
Deviance explained by best model	35%	
Number of models included in inference	54	

Table 3.7 – Results of multimodel inference comparing roosts occupied by *M. daubentonii* (i.e. bachelor and nursery roosts, n = 21) with roosts with no recorded occupancy (n = 13). Understory is divided into three categories, 0 – no understory, 1 – scattered understory and 2 – dense understory, and treated as a continuous variable. None of the variables had *I* > 0.9

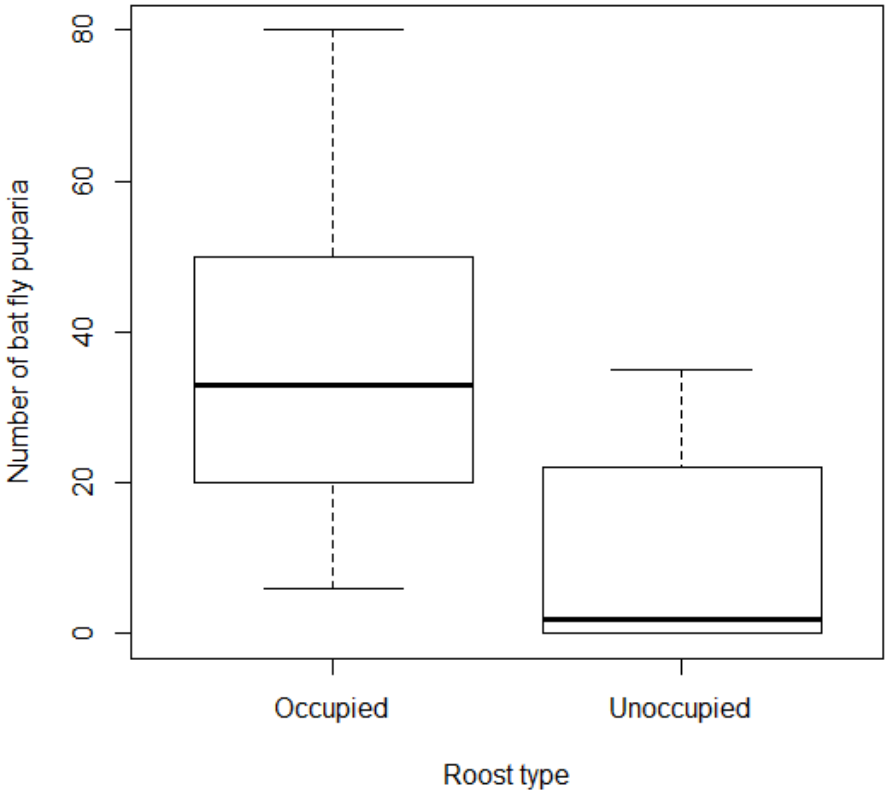

Figure 3.19 – Boxplot of counts of bat fly puparia from occupied (n = 21) and unoccupied roosts (n = 13). The smallest observation, lower quartile, median (bold horizontal bar), upper quartile, and largest observation are shown. Unoccupied roosts have significantly fewer bat fly puparia than recorded in bachelor or nursery roosts

3.4 Discussion

3.4.1 Roost preferences of *M. daubentonii, M. nattereri* and *P. auritus*

There are differences in the roosting preferences of *M. daubentonii*, *M. nattereri* and *P. auritus* within Wytham Woods. Some of these differences can be related to the lifestyle of the different species.

When all occupancy records were considered there were significant differences between boxes occupied by bats and those that were not occupied (Table 3.2). However, species-specific models suggest that much of this explanatory power is accounted for by observations of *M. daubentonii*.

M. daubentonii showed a preference for roost boxes close to water, the primary foraging habitat for this species (Altringham 2003). More surprisingly the probability of box occupancy increased away from the woodland edge. Edge effects have been well documented and include changes in temperature, humidity, plant species composition and growth forms as you approach the edge of a woodland (Murcia 1995). Further studies on roost selection within woodland over small scales are needed to interpret or refute the edge effect we observe for *M. daubentonii*. Roosts used by this species were found more frequently in areas with an easterly aspect. A number of studies have demonstrated a preference for particular aspects amongst a range of bat species (Lausen & Barclay 2002; Neubaum *et al.* 2006; Watrous *et al.* 2006; Chambers *et al.* 2011) though there is no consensus amongst them. Roosts on easterly slopes are likely to warm faster in the morning than those at other aspects (Lausen & Barclay 2002) and may be the characteristic for which they are selected in this study. These variables were also found to be important when only roosts sampled on more than five occasions were analysed.

The results of model comparisons suggest that *M. nattereri* occupancy was not strongly associated with any of the variables examined with the exception of box type and sampling effort. However, support for sampling effort was dropped when only boxes checked more than five times were analysed. Models of *M. nattereri* explained little of the deviance observed. This lack of explanatory power may be a result of the foraging behaviour of *M. nattereri*. This species is a woodland specialist, capturing insect prey close to and on vegetation. It is likely therefore that the majority of Wytham Woods provides good quality foraging habitat for this

species. As a result there may be little difference in the quality of roosts as perceived by *M. nattereri* leading to a fairly homogeneous distribution of this species.

M. daubentonii and *M. nattereri* had a different preference for box type. *M. daubentonii* showed a preference for Great tit boxes, whilst *M. nattereri* preferred Blue tit boxes. There are a number of possible explanations for this effect. The majority of Great tit boxes had peaked roofs, 32mm entrance holes and have been present for 50-60 years, in contrast Blue tit boxes had flat roofs, 26mm entrances and were erected 6 years prior to the start of our study. The observed preferences could therefore result from differences in the thermal properties or entrance size of the two roost types or a preference for novel or long established roosts. Each of these suggestions could be tested by manipulation of boxes in the field.

Like *M. nattereri*, models of *P. auritus* were poor at explaining the observed roost use. While aspect and distance to linear features were suggested as important when all records were included, after excluding roosts checked on five or fewer occasions neither were found to be important. As another woodland generalist it may be the case that, like *M. nattereri*, Wytham Woods offers a homogeneous landscape in terms of foraging habitat. However it could also be the case that the roost preference of *P. auritus* and *M. nattereri* are explained by variables not considered in this analysis, for example the distribution of insect prey species within the woods.

I have shown that each of the species studied uses a large number of boxes within the wood. Furthermore, analysis of roost preference does not suggest that suitable roosts are limiting. This gives strong support to the social network analysis presented in chapter 2, since this discounts the possibility that the observed social structure was the result non-social aggregations in a limited number of suitable roosts. Consequently, the heterogeneity in contact rates (inter- vs. intra-group associations) can be said to be a result of social processes. While I have demonstrated that this is the case in Wytham woods, an environment where roosting sites are plentiful, it is unclear to what degree roost site limitation in other landscapes (e.g. urban areas) shape the social structure of bat populations.

Using the sampling method described, roosts in which bats had not been observed were considered unused by bats. However a larger proportion of boxes (67%) contained bat faeces than had been recorded with bats (35%) indicating that nearly twice as many boxes were occupied by bats than were recorded. Unfortunately faecal morphology cannot be reliably used to identify bat species, preventing the use of these data to infer species-specific occupancy. To address this issue a subsample of boxes, checked on more than five occasions

each, were reanalysed. While this approach reduces the chance of false negatives (i.e. wrongly classifying a box as not used by bats) it results in a non-uniform distribution of boxes through the wood (Figure 3.1) and may lead to other biases in the data. Future work should aim to increase the number of checks per box to a level where false negatives are negligible. This could be measured by assessing the number of boxes recorded with droppings but without occupancy.

3.4.2 Observed variation in roost use by *M. daubentonii* colony types

Sexual segregation of *M. daubentonii* in the summer months has been the subject of a number of studies (Dietz *et al.* 2006; Safi 2008; Encarnação 2011). Possible reasons behind the observed separation include competitive exclusion, differences in microclimate needs and thermoregulation, and parasite avoidance.

3.4.2.1 Competitive exclusion

When comparing *M. daubentonii* roosts known to be occupied by bachelor, nursery and mixed colonies, during the nursery period there was no observable differences in their proximity to landscape features or their surrounding habitat. This contrasts with other studies of *M. daubentonii* which suggest that during the nursery period nursery colonies dominate foraging and roosting habitat forcing males to roost further away from water (Encarnação *et al.* 2005). At Wytham Woods foraging and roosting habitat may not be limiting, allowing nursery and bachelor colonies to exist in the same area without a prohibitive degree of competition.

3.4.2.2 Microclimate and thermoregulation

To test the hypothesis that bachelor and nursery colonies select roosts based on their microclimate we compared the temperature and humidity of these roosts but found no clear differences. This was despite the fact that roosts maintained their relative differences in microclimate over time, providing a range of temperatures and humidity's (Figures 3.16 and 3.17). Having said that, the bird boxes in Wytham Woods are all very similar in design and

construction. They may therefore represent only a small amount of the variation in roosts available to the population under study. Adult female *M. daubentonii* in Wytham Woods are known to occupy natural tree roosts on approximately two thirds of occasions (Chapter 2). As such it may require a more extensive study, including a range of natural tree roosts, to identify differences in the roosts used by bachelor and nursery colonies.

While we found little evidence of differing microclimates in bachelor and nursery roosts, it is known that bats are able to change the temperature of their roost through expelled body heat (Willis & Brigham 2007). We detected a small rise in temperature in roosts that were occupied by bats, supporting these observations. As temperature loggers were at least 10cm away from roosting bats the rise in temperature around them was likely higher than that recorded. It is possible therefore that difference in torpor requirements go some way to explain the observed sexual segregation. As these bats are able to alter the microclimate of their roost the different demands of social thermoregulation between the sexes would predict assortment by sex. Male *M. daubentonii* are known to enter torpor during the nursery period whilst females maintain a constant body temperature to maximise milk production (Dietz & Kalko 2006). However, if this was the only cause of segregation one would expect males to roost individually to reach the deepest possible torpor, since metabolic rate of torpid bats decreased with ambient temperature (Turbill 2009). If another benefit such as information sharing (Safi 2008) accounts for bachelor colony formation we would expect these groups to occupy colder roosts if they are entering deeper torpor, however we detected no difference in the temperature of roosts used by nursery and bachelor colonies. Alternatively if *M. daubentonii* males are not entering topor we would expect them to roost with females, to benefit from social thermoregulation, again, something we did not observe. These results suggest that roost microclimate and torpor requirements may not be driving the sexual segregation observed.

3.4.2.3 Parasite avoidance

We found no difference in the number of bat fly puparia in bachelor and nursery roosts, though there were significantly fewer in boxes thought not to have been occupied by *M. daubentonii* (Figure 3.19). While in Chapter 4 it is suggested that members of *M. daubentonii* bachelor colonies avoid infestation with directly transmitted ectoparasitic mites by isolating themselves from nursery colonies this does not appear to be the case for bat flies. Assuming *M. daubentonii* are able to detect bat fly puparia as *Myotis bechsteinii* (Bechstein's bat) have

been shown to (Reckardt & Kerth 2007), the availability of apparently uninfested roosts suggests that occupied roosts have some benefit that outweighs the cost of bat fly parasitism, despite our inability to clearly identify it.

I have failed to clearly identify why male and females *M. daubentonii* are segregated during the summer at Wytham woods when considering only microclimate and landscape variables. However, I will explore the possibility of segregation as a parasite avoidance strategy by males in chapter 4. The recent discovery of altitudinal variation in sexual segregation of *M. daubentonii* is interesting (Angell, 2013) and further work in that study system may reveal better, why the population is segregated in some areas and not others. This variation in segregation over an altitudinal gradient will likely also have an effect on disease transmission at different altitudes.

3.4.3 Conclusions

This study shows that the roost preference of bats within woodland is species-specific and makes the case that some of the observed differences can be explained by species' foraging behaviour. However, there is currently a lack of detailed descriptions of the foraging behaviour of these species such as their relative ability to navigate through a cluttered environment. Future studies which can describe in detail the foraging behaviour of bats within woodland may further explain the variation we observe in roost preference. These studies could combine radiotracking and light tagging (i.e. attaching a small light source to bats so their movement can be seen) to identify species' foraging preferences, considering properties such as tree species, vegetation density, canopy volume, etc. The distribution of bats within the wood does not suggest that roosts are limiting and so lends support to the analysis presented in chapter 2, showing that *M. daubentonii* and *M. nattereri* have strong social structure that is not the result of limited roost availability.

It was found that the presence of bats significantly increased the temperature within roosts, however there was no difference between *M. daubentonii* bachelor and nursery roosts, both in terms of their proximity to water and their microclimate. This suggests that the segregation of sexes observed in the nursery period cannot be ascribed to either competitive exclusion of males from roosts close to water or microclimate preference, at this site. Instead the segregation of *M. daubentonii* sexes may represent a disease avoidance strategy by a species that carries a higher disease burden than the other species in this study (this hypothesis will be

explored in Chapter 4). While this may be the case for directly transmitted diseases we found no evidence that bachelor colonies avoided roosts with high numbers of bat fly puparia.

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4 Effects of host and ectoparasite ecology on parasite distribution and host body condition.

4.1 Introduction

Closely related bat species exhibit differences in ectoparasite infestation rates. Such differences may be due to relative host abundance, the quality of each species as a nutritional resource, species specific immune responses, host sociality and - by extension - contact rates (Christe *et al.* 2003).

For both *M. daubentonii* and *M. nattereri* at Wytham Woods we have an understanding of where individuals are positioned within their social networks (Chapter 2). It might be expected that individuals with more connections would have a higher probability of being infested by directly transmitted parasites since the networks in Chapter 2 are based on bats occupying the same roost. This would follow observations by Bell *et al.* (1999) that individuals with high values of degree in networks of cocaine injectors are more likely to become infected with HIV. Additionally, individuals who associate with more than one social group might be expected to have an increased probability of infection as they have indirect contact with more individuals than bats which remain within a single social group. In this Chapter we examine to what extent an individual's position within a social network drives parasite load and whether these metrics may be useful for predicting transmission rates in bats populations.

The parasite burden of an individual is also likely to be predicted by its current social environment, the number and type of individuals it is in contact with. Consequently colony type may be a suitable predictor of parasite load. Indeed, previous studies of temperate bat species have found that individuals in nursery colonies typically have the highest loads of bats sampled throughout the year (Lourenco & Palmeirim 2007; Reckardt & Kerth 2009). This may be a result of reduced immune function in reproductive females and juveniles (Christe &Vogel, 2000) or higher contact rates in nursery colonies, which are typically larger than other colony types (Reckardt & Kerth 2009). However, for species previouly studied large bachelor roosts have not been recorded or do not exisit. In this study we record parasite load in a number of different colony types throughout the summer months to assess the impact of both colony type and colony size. This will highlight the importance of variation in contact rates between different demographic groups (e.g. males and females) for disease models.

Studies of the impact of ectoparasites on bats have produced conflicting results. With either negative (Giorgi *et al.* 2001; Lourenco & Palmeirim 2007; Lucan 2006), positive (Christe *et al.* 2003; Lucan 2006; Reckardt & Kerth 2009) or no (Zahn & Rupp 2004) correlation to host condition. In many of these studies body condition index (BCI; weight/forearm) is used as a measure of an individual's health (cf. Giorgi *et al.* 2001). Unfortunately this measure is relatively poorly understood and variations in BCI could be linked to a plethora of explanatory variables including weather, age, sex, reproductive activity and individual body type. While all previous studies of the impacts of ectoparasites on BCI account for age, sex and reproduction in some manner, this study also accounts for the effect of weather. It is surprising that weather is rarely considered in relation to BCI as it may have profound effects on condition particularly in small homeotherms with relatively high energy needs, such as bats.

We studied three sympatric woodland bat species (*M. daubentonii*, *M. nattereri* and *Plecotus auritus*) in order to a) assess similarities and differences in parasite distribution between species in relation to differences in species specific ecology, b) analyse the impacts of an individual's position in a social network on its parasite load c) investigate the effect of colony type (i.e. the sex and reproductive status of cohabitants) and colony size on parasite load and d) investigate the effect of parasitism on BCI, whilst controlling for the effects of weather, age, sex and reproductive status.

4.2 Methods

4.2.1 Sampling site

Bats were studied at Wytham Woods, Oxfordshire (Latitude, 51°77'27"; Longitude, -1°33'41") where they roost in many (>67%) of over 1150 woodcrete bird boxes spread throughout the wood. For a detailed description of the site see Chapter 1.

4.2.2 Recording individual attributes

When bats were captured their ring number was recorded and a range of data were collected. These included species, sex, age and reproductive status.

Age was divided into two categories, adult and juvenile. Juveniles, defined as young of the year, were identified by the lack of ossification in the finger joints between the metacarpals and phalanges and the oval, rather than round, appearance of this joint. This is a reliable identification method for juveniles up until approximately 2 months after birth (Racey 1974; Mitchell-Jones & McLeish 2004). Female reproductive status was divided into 4 categories; pregnant, lactating, post-lactating and non-breeding. Pregnant bats were identified from their weight and gentle palpation of the abdomen. Lactating bats have enlarged nipples and an absence of hair both on the nipple and in a c.3mm circle around the nipple. Post-lactating females' nipples are enlarged, dark in colour and the hairs surrounding the nipple may have begun to grow back but are short. Non-breeding females have no swelling in the nipple and no loss of hair. In previous studies male reproductive status has been categorised by documenting the development of the epididymides, the tissue in which sperm is stored (e.g. Encarnação *et al.* 2004). However, this is a difficult feature to examine and categorise in the field and requires an experienced bat worker. As numerous inexperienced bat workers assisted with this study this measure was deemed too unreliable to be included in the analysis. Pregnant females (n = 178 of 848 female observations) were not included in the analysis of variables influencing BCI as BCI assumes weight is correlated to body fat, whilst in pregnant females weight is also dependent on the developmental stage of the foetus which was not determined in this study.

4.2.3 Body condition index (BCI)

BCI was calculated by dividing weight by forearm length (the 'ratio index'), measured to the nearest 0.1g and 0.1mm. Weight was measured using a 20g spring balance (Pesola, Switzerland) and forearm length was measured with vernier dial callipers (Moore and Wright, UK). While the use of the 'residual index' (the residuals of a regression on body weight on body size) has been suggested as a measure of body condition (Jakob *et al.* 1996) this has not been validated against body fat for bats. The ratio index has been found to accurately predict the body fat of big brown bats (*Eptesicus fuscus*), whilst being more practical than other methodologies including total body electric conductivity (TOBEC) (Pearce *et al.* 2008). It has also produced identical results to the 'residual index' method in studies of parasitism (Lourenco & Palmeirim 2007). The ratio index measure of condition has been used in many other studies of bats, allowing our study to be compared to previous work (Speakman & Racey 1986; Russo 2002; Zahn & Rupp 2004; Senior *et al.* 2005; Lucan 2006; Lourenco & Palmeirim 2007). To allow easier interpretation of model output BCI was multiplied by 100 before analysis, but was back transformed prior to drawing graphs of the observed effects.

4.2.4 Social network attributes

Where data were available (*M. daubentonii* and *M. nattereri* only), information from the social network analysis (Chapter 2), was included in models of ectoparasite abundance and BCI. This included degree (the number of bats an individual associates with), betweeness (the importance of an individual for connecting others in the network), and the social group to which a given individual belonged.

Both parasite counts and social network parameters were available for 457 captures of 197 individual *M. nattereri* (60 males and 137 females from 6 social groups). As *M. daubentonii* males did not form clearly defined social groups (Chapter 2) they were not included in the analysis of the effect of social group on parasite load, restricting this analysis to 214 captures of 107 females (4 social groups). However, as betweeness and degree were calculated from the social network containing both male and female *M. daubentonii*, 453 captures of 247 individuals (140 males and 107 females) were available for this analysis. Since a small number of individuals have a very high value of betweeness this value was log transformed before inclusion in models.

4.2.5 Ectoparasites

M. daubentonii, *M. nattereri* and *P. auritus* are all parasitised by mites (Table 4.1-4.3). The most conspicuous are members of the Spinturnicidae family (order Mesostigmata), a family found exclusively on bats, which feed predominantly on the wing and tail membranes (Evans 1968). They are characterised by their large size and stout legs with many long setae/hairs (Baker 2006 and Figure 4.1). These blood-feeding mites spend their entire lifecycle on the host and are transmitted from host to host via direct contact in roosts.

In Baker and Craven's (2003) checklist of mites found on bats in the UK, both *M. daubentonii* and *M. nattereri* are parasitised by a single species of Spinturnicid mite, *Spinturnix myoti*, whilst *P. auritus* is host to both *S. myoti* and *S. plecotinus*. The taxonomy the Spinturnicidae is still debated, and in some studies the *S. myoti* species complex has been separated into species in accordance to their host (Uchikawa *et al.* 1994). This classification has some support from genetic analysis (Bruyndonckx *et al.* 2009). As a consensus taxonomy for this complex has yet to emerge here we use *S. myoti* sensu lato (*s.l.*) (Rudnick 1960).

All three bat species are also host to a number of other mite species, predominantly belonging to the family Macronyssidae which like Spinturnicids are within the order Mesostigmata. Very little is known about the lifecycle of these mites in the UK, though other members of the family are known to lay eggs off the host (Baker 2006), unlike Spinturnicids. The emerging larvae moult into a protonymph before searching for a host (Baker 2006).

M. daubentonii is also parasitised by bat flies, primarily *Nycteribia kolenatii* (Hurka 1964; Gardner & Molyneux 1988). These wingless diptera feed on blood and are thought to transmit *Polychromophilus murinus*, a malaria-like pathogen, and *Bartonella* between bats (Gardner & Molyneux 1988; Billeter *et al.* 2012). Bat flies give birth to terminal (3rd-instar) larvae in the roost of their host, which immediately form puparia. The adult can emerge after approximately 3-4 weeks, though they can remain inside the pupa for much longer. Emerging adults locate a host and subsequently only leave the host to deposit larvae (Dick & Patterson 2006). Bat flies are highly mobile in the fur and are very difficult to collect, therefore detailed identification of batflies in the present study was not undertaken. It is thought that *M. nattereri* is rarely parasitised by bat flies and is instead parasitised by bat fleas (Zahn & Rupp 2004). These fleas spend the first half of their life-cycle in the guano below roosts, feeding on detritus before they develop into adults and search for a host (Lewis & Lewis 1994). Both bat flies and fleas are found in the fur and not on the wing membranes.

Order	Species	Synonyms	Location of records
Astigmata	*Alabidocarpus intercalatus*	None	East Norfolk
	Notoedres myoticola	None	East Norfolk
	Nycteridocoptes poppei	None	East Norfolk and South Ayrshire
Mesostigmata	*Macronyssus diversipilis*	*Liponyssus granulosus*	East Norfolk, West Sussex, West Suffolk and Surrey
	Macronyssus ellipticus	None	South Wiltshire, Suffolk and West Norfolk
	Euseius finlandicus	None	West Sussex
	Spinturnix myoti	*Spinturnix vespertilionis*	South Hampshire, Surrey and North-East Yorkshire

Table 4.1 – The mites of *M. daubentonii*. Adapted from Baker and Craven (2003)

Order	Species	Synonyms	Location of records
Mesostigmata	*Macronyssus diversipilis*	*Liponyssus granulosus*	Oxfordshire, East Suffolk, West Norfolk, South Wiltshire, West Sussex, Hertfordshire and Fermanagh
	Macronyssus ellipticus	None	South Wiltshire, Surrey and Suffolk
	Ornithonyssus pipistrelli	None	Dorset
	Steatonyssus periblepharus	*Liponyssus chiropteralis, Steatonyssus murinus, Ceratonyssus musculi*	Oxfordshire
	Spinturnix myoti	*Spinturnix vespertilionis*	West Sussex, Surrey, Oxfordshire and Merionethshire
Prostigmata	*Neotrombicula autumnalis*	*Trombicula autumnalis*	North Devon

Table 4.2 – The mites of *M. nattereri*. Adapted from Baker and Craven (2003)

Order	Species	Synonyms	Location of records
Astigmata	*Acarus gracilis* (?) [roost]	*Tyroglyphus sp.*	North Hampshire
	Carpoglyphus munroi (?) [roost]	None	Hampshire
	Glycyphagus domesticus (?) [roost]	None	North Hampshire
	Nycteriglyphus sp. (?) [roost]	None	North Hampshire
Mesostigmata	*Androlaelaps casalis* (?) [roost]	*Hypoaspis casalis*	North Hampshire
	Macronyssus ellipticus	None	West Sussex
	Macronyssus sp. A	None	West Kent
	Ornithonyssus pipistrelli	None	Dorset, Oxfordshire, West Gloucestershire, Kilkenny and Glamorgan
	Steatonyssus murinus (?) [roost]	None	North Hampshire
	Steatonyssus periblepharus	*Liponyssus chiropteralis, Steatonyssus murinus, Ceratonyssus musculi*	Cornwall and West Gloucestershire
	Spinturnix myoti	*Spinturnix vespertilionis*	No sites given
	Spinturnix plecotinus	None	Surrey, South-west Yorkshire, Durham, Merionethshire and Ireland
Oribatida	*Aphelacarus acarinus* (?) [roost]	None	North Hampshire
Prostigmata	*Acaropsellina docta* (?) [roost]	*Acaropsis docta*	North Hampshire
	Cheletonella sp. (?) [roost]	None	North Hampshire
	Cheyletus woodroffei (?) [roost]	*Cheyletus sp.*	North Hampshire
	Demodex chiropteralis	None	British Isles (?)
	Demodex soricinus	None	British Isles (?)
	Neomyobia plecotia	*Myobia chiropteralis, Foliomyobia chiropteralis*	No sites given
	Pteracarus pipistrellius (?)	*Myobia pipistrellia, Neomyobia pispistrellia, Pteracarus pipistrellia*	England
	Leptotrombidium avonense	None	South Wiltshire

Table 4.3 – The mites of *P. auritus*. (?) – indicates that the host species is uncertain. [roost] indicates the mite was collected from the roost and not the host. Adapted from Baker and Craven (2003).

4.2.6 Identification of Spinturnicid mites

For each bat species a small number of Spinturnicid mites (Figure 4.1) were removed for formal identification. Immediately after collection these mites were stored in 70% ethanol or frozen on dry ice. Those stored in ethanol were subsequently stored at 4°C whist those frozen in the field were stored at -80°C. For identification, individual mites were placed onto a drop of 50% lactic acid solution on a microscope slide, covered with a covering slip, and placed on a hot plate at 70°C for 50 minutes. Once cleared, specimens were identified under a microscope using keys for the Spinturnicidae family (Rudnick 1960; Stanyukovich 1997). David Dodds kindly provided training in mite identification.

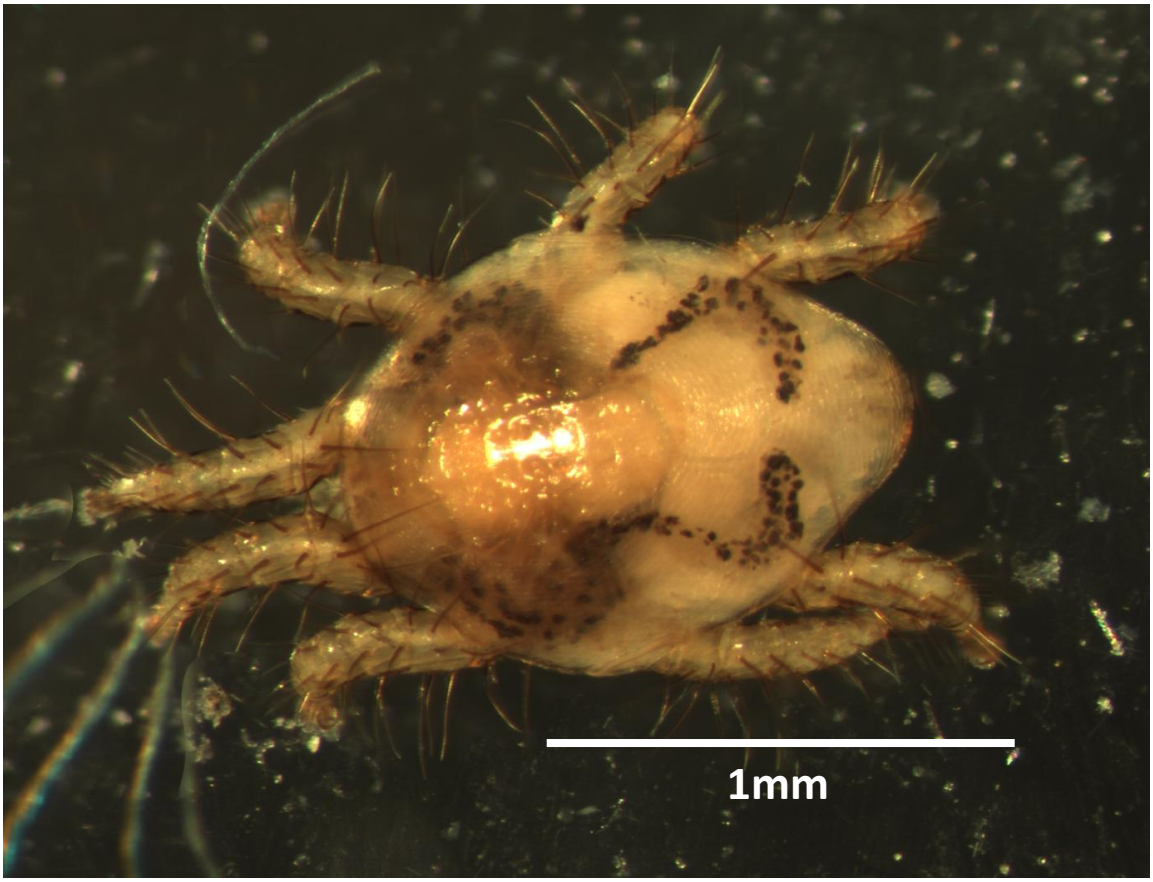

Figure 4.1 – Image of a Spinturnicid mite (*Spinturnix myoti*). This family of mites can be easily identified with the naked eye by their size, colour and position of the legs. Photo credit: Tom August

4.2.7 Parasite abundance estimates

Roost boxes were checked regularly from late-May to early-October in 2009 and 2010, and bats were examined for the presence of ectoparasites.

The total number of mites on both sides of each wing were counted, and the number of these that were Spinturnicid mites was noted. This procedure was most easily done by holding the wing up to a direct light source. It is possible, with the naked-eye, to easily identify mites of the family Spinturnicidae, being larger than all other bat mites with a characteristic orange colour and crab-like appearance (Baker 2006 and Figure 4.1). The dorsal fur was examined for bat flies and fleas by blowing gently through the fur, to part the hairs, for approximately 10-15 seconds. Parasite prevalence was defined as the proportion of individuals hosting parasites while parasite load was defined as the number of parasites infesting an individual.

4.2.8 Weather data collection and transformation

Weather data (obtained from the Environmental Change Network (ECN), Centre for Ecology & Hydrology, Wallingford with special thanks to Michèle Taylor) was collected from a weather station in Wytham Woods (Latitude, 51°77'06"; Longitude -1°33'24", altitude 165m). Data was collected following a previously described protocol (Sykes & Lane 1996) using the equipment listed in the appendix (Table 7.2). Weather data were recorded every hour throughout the year. In this study we used data recorded between 1st May and 31st October in 2009 and 2010.

Some data were transformed into variables more appropriate to the biology of bats. Hours of day and night were calculated from records of solar radiation (W/m^2). Hours in which solar radiation equalled $0W/m^2$ were taken to be hours of night and were used to calculate the average temperature, wind speed and total rainfall for the night prior to each bat's capture. Using this approach hours of night approximate those between sunset and sunrise but are broadened if heavy cloud cover leads to darker conditions, which is known to cause early emergence of bats (Shiel & Fairley 1999).

4.2.9 Colony data

Colony size and type was recorded for inclusion in the analyses. Colonies were divided into three types according the composition of adults: those dominated (>66.6% of total) by adult females, those dominated (>66.6% of total) by adult males (bachelor colonies) and those with mixed adult sexes. These were further divided into three time periods or seasons; the nursery period, and the pre- and post-nursery periods. The nursery period was defined as the time between the first and last colony of lactating females with juveniles. These dates varied from year to year, since the timing of births is weather dependent. These colony definitions are the same as those used in Chapter 2 and 3. Bats found roosting on their own were recorded as solitary. Where fewer than five data points where available for a given colony type it was not included in the analysis (those that were not included are indicated by 'NA' (not applicable) in summary tables).

4.2.10 Statistical analysis

Data were analysed using generalized linear mixed effect models (GLMMs) with individual specified as a random effect to control for repeated captures. Models of parasite abundance included the fixed effects: Age, sex, forearm length, BCI, colony type, colony size, year, and the abundance of parasites other than those being tested. Models of BCI included the fixed effects: Age, sex, year, day of the year, average temperature and wind speed from the previous night, total rainfall from the previous night, and parasite abundances. For each of these models an additional analysis was undertaken including the social network variables – betweeness, degree and social group (see 4.2.4) – on the subset of individuals for which this data was available. Interaction terms were not included in these models as these would have been computationally impractical using model averaging (described below) since the number of models considered increases exponentially with each additional variable.

The distribution of ectoparasites was tested by comparing the log-likelihood of null models using Poisson and negative binomial distributions using the pcsl package, version 1.04.1 in R (Jackman 2011).

Models were selected, and effects estimated, using an information theoretics approach (Anderson *et al.* 2000; Anderson *et al.* 2001; Anderson & Burnham 2002; Burnham & Anderson 2002; Burnham *et al.* 2011). Models were compared using the small sample size corrected

version of Akaike's information criterion (AICc) which scores models on how well they fit observations. AICc accounts for models which include a large number of variables compared to the sample size, by penalising models containing many variables (Burnham *et al.* 2011). Models with a ΔAICc < 7, equivalent to an evidence ratio of 33.1, were selected (Burnham *et al.* 2011). The Akaike weight (w_i) was calculated for each model and can be viewed as the probability that a model is the best of those considered given the data. Parameter estimates were calculated by averaging across all models in which the parameter appeared and weighting the average according to the Akaike weight of each model. Variables are also given a value of 'importance' (*I*). This is the sum of the Akaike weights of all the selected models in which it appears and therefore represents the probability that the variable appears in the best model. All analyses were undertaken in 'R', version 2.13.2 (R Development Core Team 2011), and the package 'MuMIn' version 1.5.2 (Barton 2011) was used for model averaging. Models of the parasite count data were fitted with a Poisson error structure since a method for implementing negative binomial error structure into GLMMs that can be used in model averaging has yet to be developed. Plots of the residuals were used to ensure that the data conformed to the assumption of the models, and to check for over dispersion. Models of bat fly distribution amongst *M. daubentonii* showed evidence of a large amount of over dispersion and so were analysed as a binomial outcome with a log-link function, where individuals were taken to be infested or uninfested. Multiple comparisons of significant factors, where undertaken, use the Tukey method implemented in multcomp version 1.2-12 (Hothorn *et al.* 2008).

4.3 Results

4.3.1 Identification of Spinturnicid mites

Morphological examination of representative mites showed each bat species examined was parasitized by a single species of Spinturnicid mite (Table 4.4). *P. auritus* was parasitised by *Spinturnix plecotinus* and *M. daubentonii* and *M. nattereri* by *Spinturnix myoti s.l.* Macronyssidae mites were not collected for identification and were all classified as non-Spinturnicid mites.

Host species	Number of bats	Number of mites	Mite species identified
M. daubentonii	22	31	*Spinturnix myoti (s.l.)*
M. nattereri	18	27	*Spinturnix myoti (s.l.)*
P. auritus	16	39	*Spinturnix plecotinus*

Table 4.4 – Results of Spinturnicid mite identification by microscopy revealing each host species was parasitised by a single species of mite

4.3.2 Species specific differences in ectoparasite prevalence and load

During 2009 and 2010, 1043 individual bats were captured a total of 1591 times (Table 4.5). The variability in forearm length and weight amongst adults within the population of each species is shown in Figure 4.2.

	Captures (including recaptures)						
	Adult		Juvenile		Year		
Species	Male	Female	Male	Female	2009	2010	Total
M. daubentonii	392	244	44	26	387	319	*706*
M. nattereri	135	362	47	32	155	421	*576*
P. auritus	81	204	14	10	64	245	*309*
Total	*608*	*810*	*105*	*68*	*606*	*985*	*1591*

Table 4.5 – The distribution of capture events in Wytham Woods by species, sex and year.

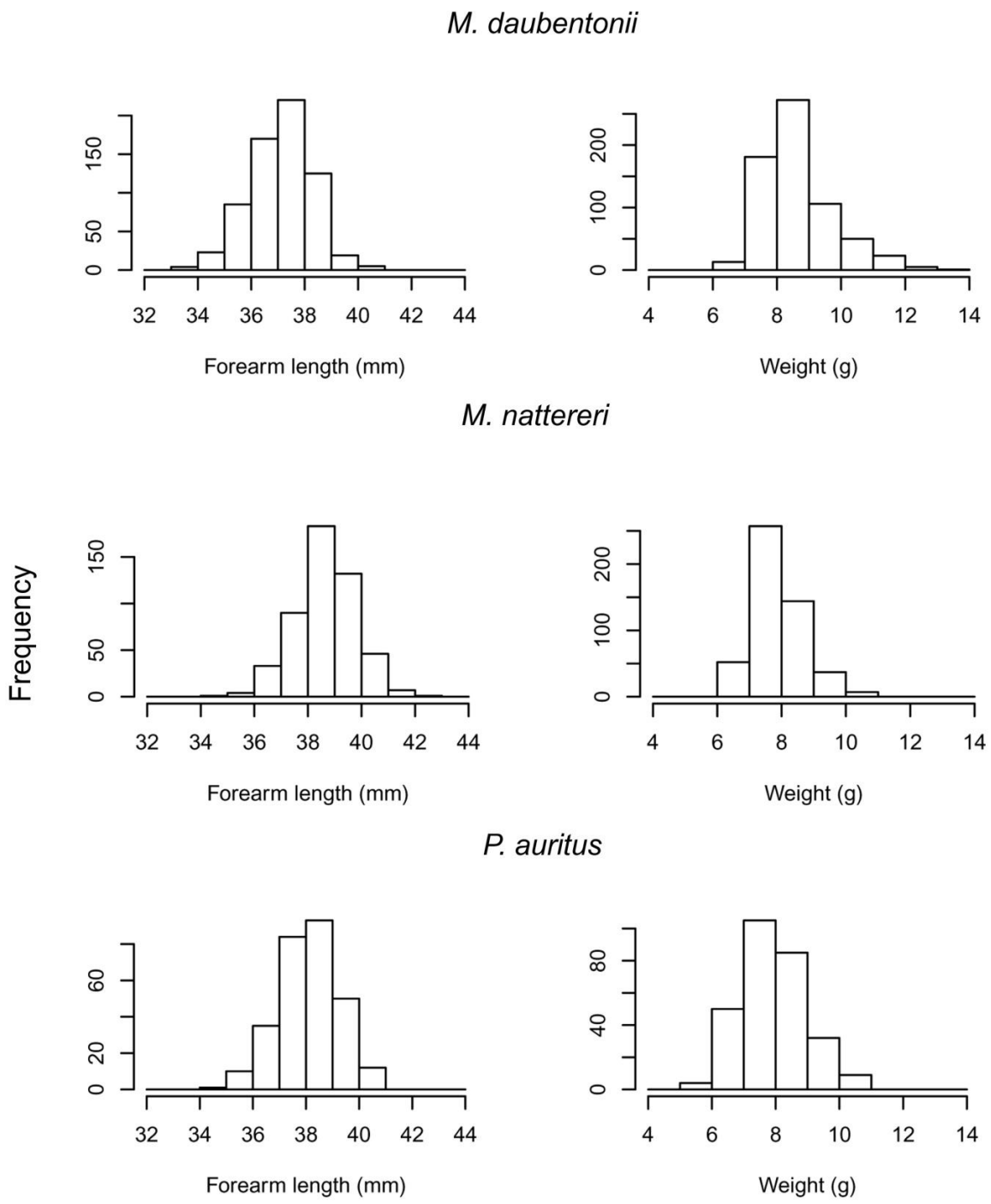

Figure 4.2 – The distribution of adult weight and forearm length for the three bat species studied

The ectoparasite communities differed significantly between the three species (Table 4.6). *M. daubentonii* had a significantly higher prevalence and abundance of Spinturnicid mite, non-Spinturnicid mites and bat flies than both *M. nattereri* and *P. auritus* (multiple pairwise comparisons, $p < 0.02$ in all cases). Bat flies were not observed on *P. auritus* in this study and rarely on *M. nattereri* (n = 7). Bat fleas were observed on *M. nattereri* on 32 occasions, on *M. daubentonii* on just 3 occasions and were not seen on *P. auritus*.

Parasite	Species	Prevalence	Mean non-zero parasite load	Maximum
Spinturnicid mites	*M. daubentonii*	0.86	6.60	46
	M. nattereri	0.61	3.10	19
	P. auritus	0.68	3.17	17
Non-Spinturnicid mites	*M. daubentonii*	0.55	6.21	47
	M. nattereri	0.46	4.48	33
	P. auritus	0.21	2.45	21
Batfly	*M. daubentonii*	0.18	1.32	7
	M. nattereri	0.01	1.00	1
	P. auritus	0.00	-	-
Fleas	*M. daubentonii*	0.004	1.00	1
	M. nattereri	0.06	1.28	3
	P. auritus	0.00	-	-

Table 4.6 – Prevalence and parasite load of ectoparasites observed infesting all *M. daubentonii* (n = 706), *M. nattereri* (n = 576) and *P. auritus* (n = 309). Prevalence is defined as the proportion of individuals carrying a given parasite. Mean non-zero parasite load is calculated from all individuals on which the parasite was present

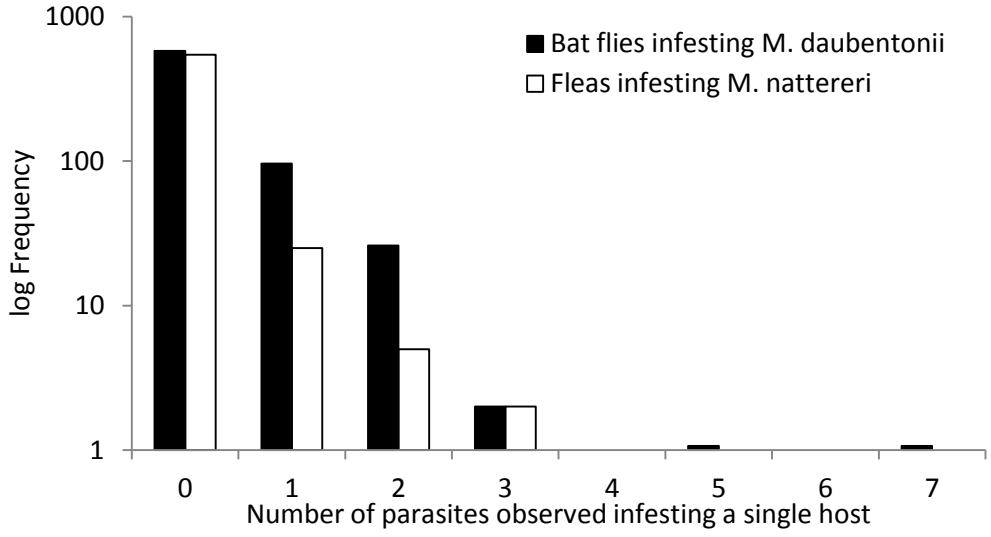

Figure 4.3 – Frequency distribution of bat fly and bat fleas infesting *M. daubentonii* and *M. nattereri* respectively

Models showed the distribution of all ectoparasites were explained best by a negative binomial distribution when compared to a Poisson distribution ($p < 0.001$ in all cases, Figure 4.3 and 4.4). This skew in parasite load was greater for non-Spinturnicid mites than Spinturnicid mites.

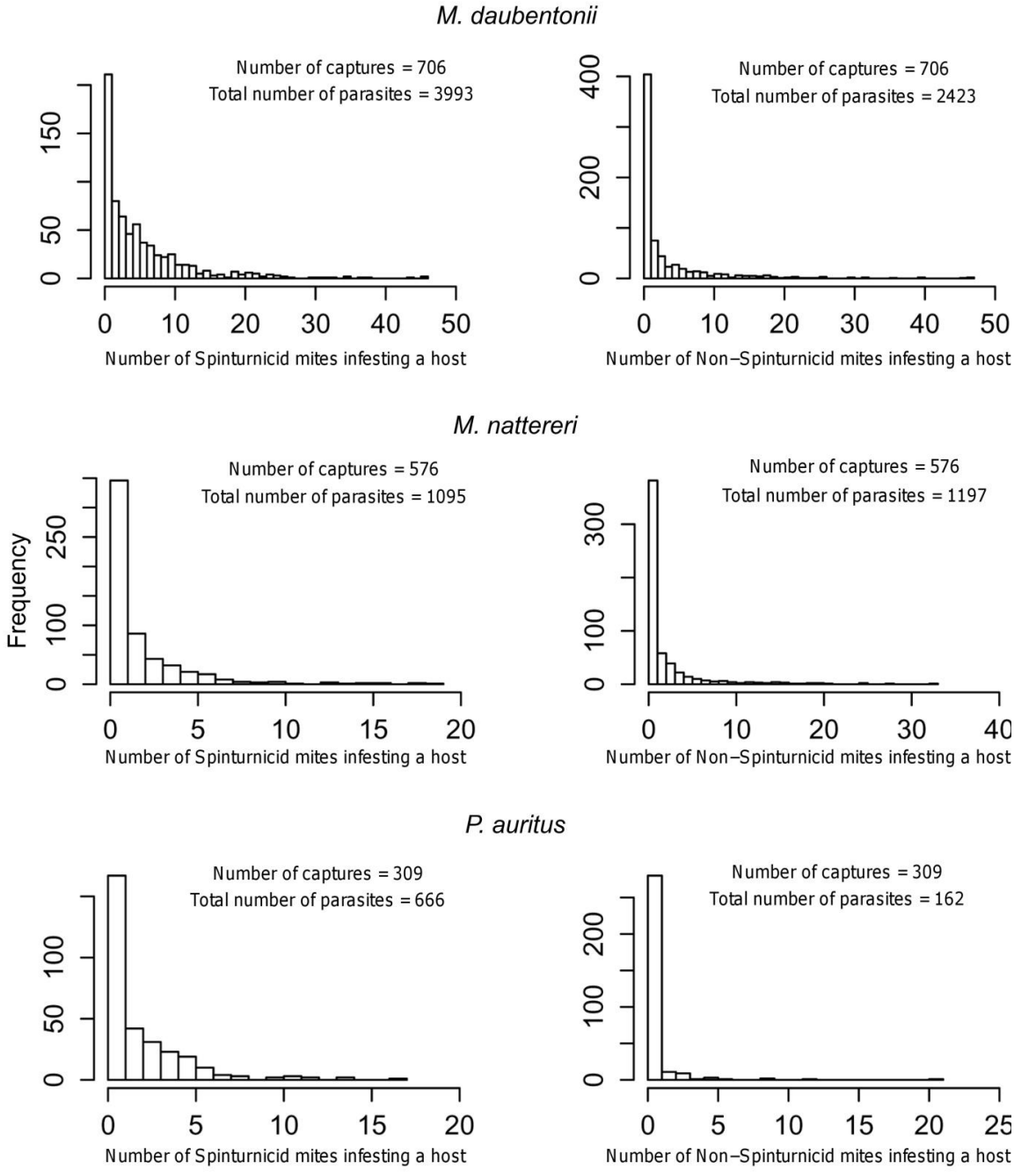

Figure 4.4 – The distribution of Spinturnicid and non-Spinturnicid mites on *M. daubentonii*, *M. nattereri* and *P. auritus*

Parameter		Sample size by species		
		M. daubentonii	*M. nattereri*	*P. auritus*
Adult		636	497	285
Juvenile		70	79	24
Year - 2009		387	155	64
Year - 2010		319	421	245
Male		436	182	95
Female		270	394	214
Pre-nursery season	Bachelor colony	44	9	2
	Mixed sex colony	0	7	25
	Female colony	51	119	96
Nursery season	Bachelor colony	143	14	0
	Mixed sex colony	53	33	11
	Female colony	153	179	69
Post-nursery season	Bachelor colony	96	3	9
	Mixed sex colony	146	80	68
	Female colony	1	106	2
Solitary roosting individuals		19	22	6

Table 4.7 – A summary of the sample size for parameters included in analyses of variables driving parasite abundance. Figures indicate the number of data points (observations of individuals) for each level of categorical variables.

Variables		*M. daubentonii* Coefficients (±95% CI)	*I*	*M. nattereri* Coefficients (±95% CI)	*I*	*P. auritus* Coefficients (±95% CI)	*I*
(Intercept)		1.121 (±1.832)		-2.87 (±3.042)		1.931 (±3.692)	
Age: Juvenile		0.427 (±0.175)	**1.00**	0.502 (±0.289)	**1.00**	0.05 (±0.597)	0.25
Sex: Male		0.001 (±0.163)	0.25	-0.343 (±0.28)	**0.91**	-0.202 (±0.344)	0.39
Forearm length (mm)		0.033 (±0.049)	0.46	0.002 (±0.093)	0.24	0.056 (±0.125)	0.33
Body Condition Index (BCI)		-1.455 (±2.364)	0.43	3.475 (±5.56)	0.44	-9.825 (±6.631)	**1.00**
Bat Fly		0.067 (±0.079)	0.59	NA	NA	NA	NA
Non-Spinturnicid mites		0.009 (±0.007)	**0.91**	0.011 (±0.02)	0.37	-0.032 (±0.084)	0.31
Fleas		NA	NA	0.056 (±0.242)	0.26	NA	NA
Colony size		0.001 (±0.01)	0.25	0.005 (±0.01)	0.33	0.008 (±0.026)	0.28
Pre-nursery season	Bachelor colony	-0.733 (±0.259)		2.729 (±2.492)		*NA*	
	Mixed sex colony	NA		3.024 (±2.4)		-1.007 (±0.464)	
	Female colony	0.686 (±0.243)		1.665 (±2.27)		-0.551 (±0.352)	
Nursery season	Bachelor colony	*0*		*0*		*NA*	
	Mixed sex colony	0.264 (±0.199)	**1.00**	3.114 (±2.265)	**1.00**	1.215 (±0.488)	**1.00**
	Female colony	0.765 (±0.165)		3.308 (±2.259)		-0.12 (±0.391)	
Post-nursery season	Bachelor colony	-0.963 (±0.228)		NA		0.475 (±0.606)	
	Mixed sex colony	-0.555 (±0.177)		2.822 (±2.26)		*0*	
	Female colony	NA		2.887 (±2.259)		*NA*	
Solitary roosting individuals		-0.756 (±0.438)		1.375 (±2.407)		-0.963 (±1.126)	
Year: 2010		-0.568 (±0.105)	**1.00**	-0.038 (±0.203)	0.26	0.14 (±0.342)	0.31
Deviance explained by best model		35%		22%		17%	
Number of models incuded in inference		46		75		57	

Table 4.8 – Results of model averaging, showing associations between Spinturnicid abundance and potential explanatory variables. Variables with an importance (*I*) greater that 0.9 (i.e. a 90% chance that the variable is present in the best model) are indicated in bold. NA (not applicable) indicates where a variable is not included in the analysis as data is lacking. *0* denotes the colony type factor level used as the contrast

4.3.3 Analyses of Spinturnicid mite loads

Models of Spinturnicid mite load explained a reasonably large amount of deviance observed (best model: *M. daubentonii* 35%, *M. nattereri* 22%, *P. auritus* 17%). The sample size for each of the variables used is given in Table 4.7. Spinturnicid loads were higher among juveniles for both *M. nattereri* and *M. daubentonii* (*I* = 1, Table 4.8), and female *M. nattereri* (*I* = 0.91). Colony type had an importance of 1 for all species. For *M. daubentonii,* female colonies in the pre-nursery and nursery periods appeared to have the highest levels of Spinturnicid mites (Table 4.8, Figure 4.5). Estimates for *M. nattereri* had larger confidence intervals indicating increased uncertainty about the effect of each colony type, though during the nursery period bachelor roosts appear to have lower levels of infestation than mixed or female colonies (Table 4.8, Figure 4.6). *P. auritus* had higher levels of infestation in mixed colonies than female colonies in the nursery period and relatively low levels of infestation in the pre-nursery period (Table 4.8, Figure 4.7).

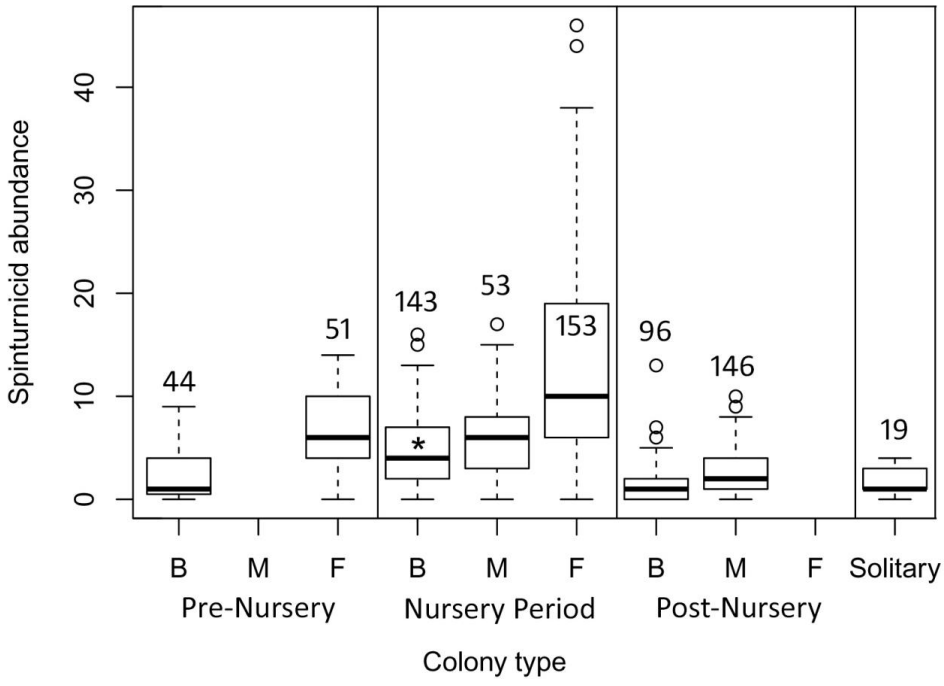

Figure 4.5 - The abundance of Spinturnicid mites on *M. daubentonii* found in different colony types; B – bachelor colonies, M – mixed colonies and F – female colonies. Horizontal black bars indicate the median value and boxes indicate the interquartile range (that which contains 50% of the data). Whiskers indicate the range of the data or 1.5 times the inter quartile range whichever is smaller. Open circles show outliers and labels give the sample size. The asterisk marks the colony type level used as the contrast in models (see Table 4.8)

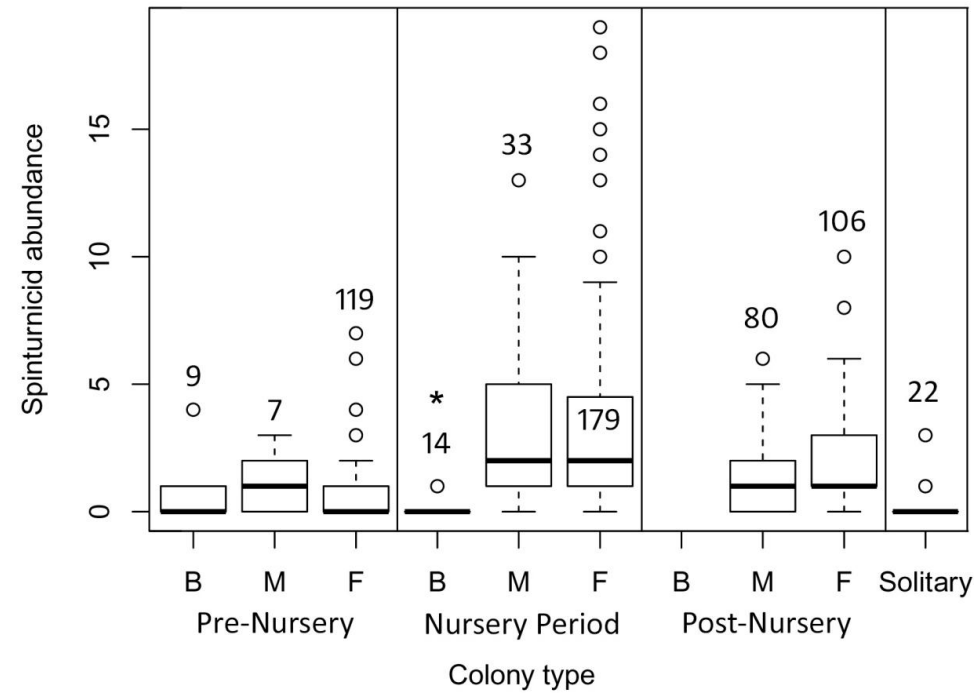

Figure 4.6 - The abundance of Spinturnicid mites on *M. nattereri* found in different colony types; B – bachelor colonies, M – mixed colonies and F – female colonies. Boxes are presented as in Figure 4.5. Labels indicate the sample size. The asterisk marks the colony type level used as the contrast in models (see Table 4.8)

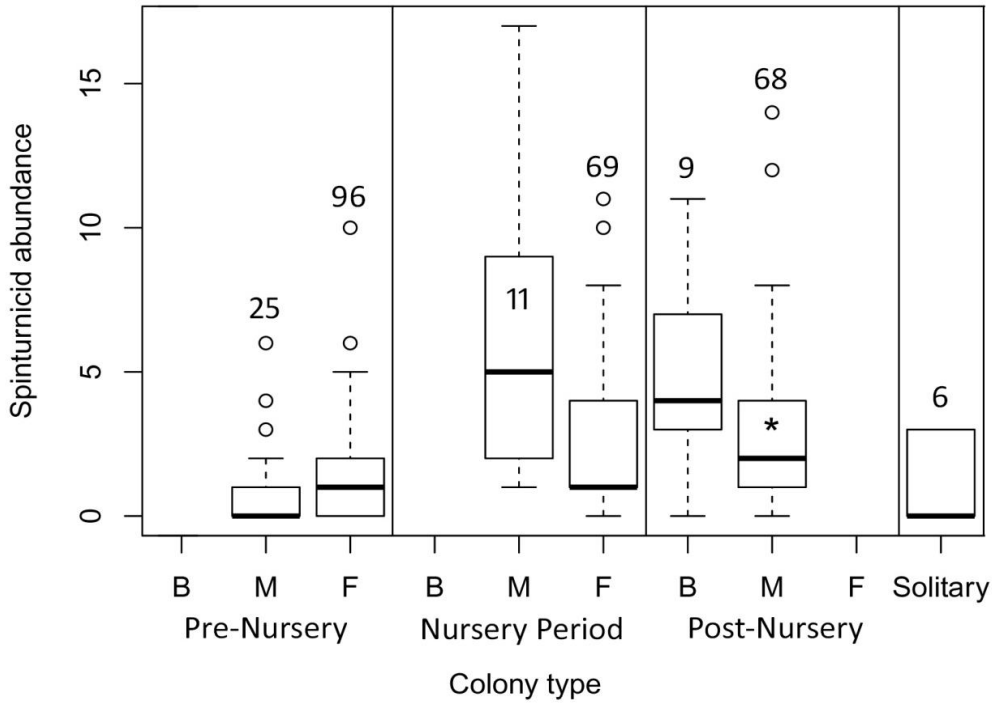

Figure 4.7 – The abundance of Spinturnicid mites on *P. auritus* found in different colony types; B – bachelor colonies, M – mixed colonies and F – female colonies. Boxes are presented as in Figure 4.5. Labels indicate the sample size. The asterisk marks the colony type level used as the contrast in models (see Table 4.8)

Forearm, included in the analyses to account for the size of the host, revealed that larger individuals did not have a different number of Spinturnicid mites than smaller individuals (Table 4.8).

Of all parasites studied only non-Spinturnicid mites infesting *M. daubentonii* were found to be correlated to Spinturnicid load. However, the effect size was small; an increase in non-Spinturnicid abundance from 0 to 47 (the minimum and maximum recorded on this species) predicted an increase of only 0.42 Spinturnicid mites.

BCI was important (I = 1) in models of *P. auritus*, however, this should be interpreted with caution. Pregnant females were included in this analysis as they are important in the parameter colony type where they account for the majority of individuals inhabiting female pre-nursery colonies. If pregnant females had a significantly reduced Spinturnicid load their high average BCI (due to their increased weight) could be the cause of the observed negative correlation observed between BCI and Spinturnicid load. To assess this effect these models were re-run with pregnant females excluded from the analysis. In these models BCI was not found to be important (I = 0.36) suggesting reduced Spinturnicid loads among pregnant females was driving the observed correlation with BCI.

Year was important in models of *M. daubentonii*, with individuals on average having 0.6 fewer Spinturnicid mites in 2010 (Table 4.8).

When social network data was included in the analysis both analyses of *M. daubentonii* and *M. nattereri* found social group to have an importance of 1 (maximum difference in mean Spinturnicid load between different social groups: *M. nattereri* = 1*, M. daubentonii* = 1.1). It was hypothesised that social groups that formed larger colonies may carry higher disease burdens. Female *M. daubentonii* social groups did not form colonies that differed significantly in size (Median: 8-12; ANOVA: df = 3, F-value = 1.164, p = 0.331), however social groups of *M. nattereri* did (Median: 3-14.5; ANOVA: df = 5, F-value = 3.422, p = 0.009). Median colony size was compared with the predicted effect of each social group on Spinturnicid load using a linear regression. No significant correlation was detected (t-value = -1.9, p = 0.13) suggesting that the difference in Spinturnicid load observed between social groups was not a result of their colony size. There was no detectable effect of either betweeness or degree on *M. nattereri* Spinturnicid load (I = 0.22 and 0.3 respectively) or degree on *M. daubentonii* Spinturnicid load (I = 0.33). Betweeness was found to be important for *M. daubentonii* (I = 0.95) however the estimate (0.07, 95% CI 0.02-0.12) predicts a difference of only 0.62 Spinturnicid mites between the highest and lowest values of betweeness.

As reproductive status was only considered for female adults this was analysed separately using a mixed effects model including individual as a random effect to account for repeat captures with reproductive status as the only explanatory variable. For all three species, models including reproductive status were significantly better than null models ($p < 0.0001$ in all cases). The pattern observed was similar across species, with lactating females generally having the highest mean abundance of Spinturnicid mites (significant for *M. daubentonii* and *M. nattereri*, $p < 0.001$) and non-breeding females having a lower mean abundance than either lactating or post-lactating females (Figures 4.8-4.10).

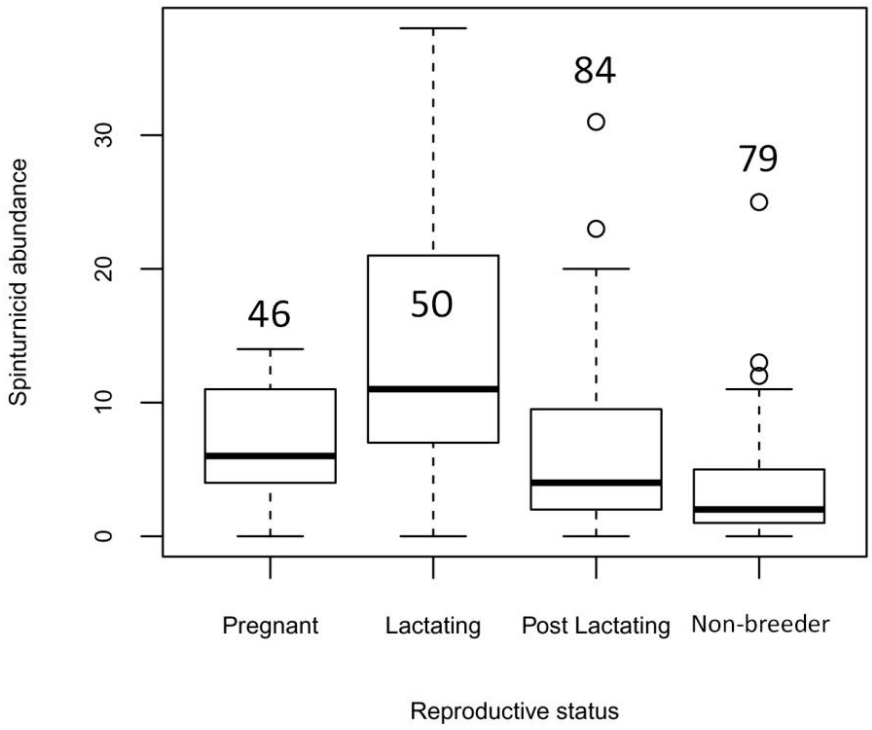

Figure 4.8 – The abundance of Spinturnicid mites observed infesting adult female *M. daubentonii*, grouped by the reproductive status of the host. Pairwise comparisons: Non-breeder<<Post-lactating<Pregnant<<Lactating where '<<' indicates a significant difference and '<' indicates a non significant difference. Boxes are presented as in Figure 4.5

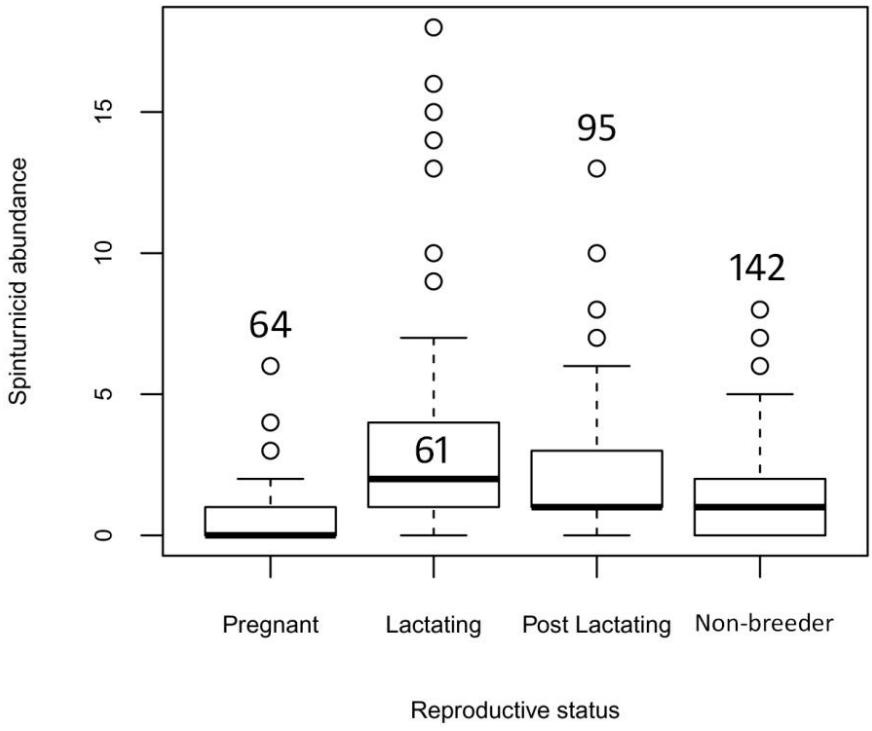

Figure 4.9 – The abundance of Spinturnicid mites observed infesting adult female *M. nattereri*, grouped by the reproductive status of the host. Pairwise comparisons: Pregnant<Non-breeder<<Post-lactating<<Lactating, notation as in Figure 4.8. Boxes are presented as in Figure 4.5

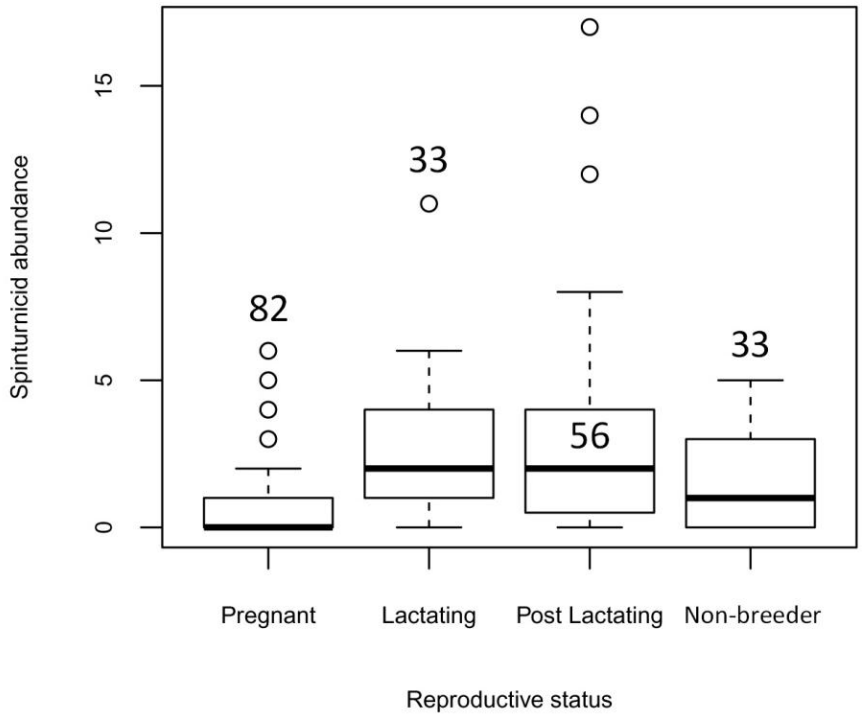

Figure 4.10 – The abundance of Spinturnicid mites observed infesting adult female *P. auritus*, grouped by the reproductive status of the host. Pairwise comparisons: Pregnant<<Non-breeder<Post-lactating<Lactating (Non-breeder<<Lactating), notation as in Figure 4.8. Boxes are presented as in Figure 4.5

4.3.4 Analyses of non-Spinturnicid mite loads

Models of non-Spinturnicid mite load (Table 4.9), like Spinturnicid models, explained a reasonably large amount of the deviance observed (best model: *M. daubentonii* 16%, *M. nattereri* 32%, *P. auritus* 22%). Juvenile *P. auritus* and male *M. nattereri* had reduced abundance of non-Spinturnicid mites (*I* = 0.98 and 1 respectively). Forearm length had a negative effect in non-Spinturnicid abundance for both *P. auritus* and *M. daubentonii* (*I* = 0.95 and *I* = 0.99 respectively). Juveniles were removed from models to account for the effect their smaller size may have had on this result. Whilst forearm was not found to remain important for *M. daubentonii* (*I* = 0.42) with juveniles removed, it was important for *P. auritus* (*I* = -0.96). These results suggest that the largest *P. auritus* individuals will have on average 1.18 fewer non-Spinturnicid mites than the smallest individuals.

Averaged models of both *M. daubentonii* and *M. nattereri* had a high importance of Spinturnicid mites (*I* = 1 and *I* = 0.97 respectively). Both were positively correlated and had similar effect size (Table 4.9). The predicted average increase in non-Spinturnicid mites was 2.02 for *M. daubentonii* and 0.88 for *M. nattereri* across the range of Spinturnicid loads observed for these species in the field. A negative relationship was identified for bat fly infesting *M. daubentonii* (*I* = 1), predicting an average decrease of 1.75 non-Spinturnicid mites between the minimum and maximum number of bat fly observed.

For *M. daubentonii,* colony size had a negative effect on non-Spinturnicid abundance (*I* = 1) predicting individuals in the largest roosts observed would have, on average, 2.1 fewer non-Spinturnicid mites than bats roosting alone (Table 4.9).

Colony type had a high importance for all species (*I* = 1 in all cases) but the error of these estimates was greater than in models of Spinturnicid mites, indicating increased uncertainty in the estimates for each colony type (Table 4.9). To accurately assess the variation between roost types a larger sample size is required.

BCI was reported to have a negative correlation with non-Spinturnicid abundance for *M. daubentonii* and *M. nattereri* (*I* = 1 and *I* = 0.97 respectively, Table 4.9). When pregnant females were removed from the analysis BCI remained important (*I* = 1) for *M. daubentonii*. The effect size predicts a difference in non-Spinturnicid abundance of 2.79 between the highest and lowest BCI recorded, non-Spinturnicid mites being more abundant on individuals with lower condition. This observation is explored further in models of BCI (4.3.7). When pregnant females were excluded from *M. nattereri* models, BCI was no longer important (*I* =

Variables		*M. daubentonii* Coefficients (±95% CI)	*I*	*M. nattereri* Coefficients (±95% CI)	*I*	*P. auritus* Coefficients (±95% CI)	*I*
(Intercept)		10.155 (±4.393)		7.635 (±6.114)		14.66 (±15.703)	
Age: Juvenile		-0.216 (±0.335)	0.43	0.143 (±0.4)	0.32	-2.849 (±3.178)	**0.98**
Sex: Male		0.017 (±0.358)	0.26	-0.585 (±0.382)	**1.00**	-0.764 (±0.974)	0.57
Forearm length (mm)		-0.157 (±0.106)	**0.99**	-0.143 (±0.124)	0.84	-0.448 (±0.363)	**0.95**
Body Condition Index (BCI)		-14.91 (±4.048)	**1.00**	-8.441 (±6.526)	**0.91**	0.713 (±18.063)	0.25
Bat Fly		-0.247 (±0.164)	**1.00**	NA	NA	NA	NA
Spinturnicid mites		0.044 (±0.012)	**1.00**	0.047 (±0.034)	**0.97**	0.034 (±0.139)	0.26
Fleas		NA	NA	-0.22 (±0.351)	0.43	NA	NA
Colony size		-0.081 (±0.017)	**1.00**	0.004 (±0.013)	0.29	0.033 (±0.076)	0.33
Pre-nursery season	Bachelor colony	-1.602 (±0.501)		-1.433 (±1.274)		NA	
	Mixed sex colony	NA		-0.955 (±1.296)		-2.486 (±1.703)	
	Female colony	1.599 (±0.428)		-2.415 (±0.865)		-1.767 (±0.954)	
Nursery season	Bachelor colony	*0*	**1.00**	*0*	**1.00**	NA	**1.00**
	Mixed sex colony	-0.965 (±0.5)		-1.012 (±0.914)		-17.043 (±4049.433)	
	Female colony	0.636 (±0.336)		-0.433 (±0.832)		-0.541 (±1.094)	
Post-nursery season	Bachelor colony	-0.269 (±0.348)		NA		-17.373 (±2788.45)	
	Mixed sex colony	0.235 (±0.308)		0.25 (±0.803)		0	
	Female colony	NA		-0.86 (±0.839)		NA	
Solitary roosting individuals		-0.183 (±0.677)		0.868 (±0.917)		0.534 (±1.977)	
Year: 2010		0.072 (±0.198)	0.31	-1.184 (±0.217)	**1.00**	0.694 (±0.982)	0.53
Deviance explained by best model		16%		32%		22%	
Number of models incuded in inference		9		27		44	

Table 4.9 – Results of model averaging, showing associations between non-Spinturnicid abundance and potential explanatory variables. Variables with an importance greater that 0.9 (i.e. a 90% chance that the variable is present in the best model) are indicated in bold. NA indicates where a variable is not included in the analysis as data is lacking. *0* denotes the colony type factor level used as the contrast

0.32) and so the correlation between BCI and non-Spinturnicid is likely to be driven by the low abundance of mites on pregnant females.

Year was important in models of *M. nattereri,* with individuals predicted to have on average 1.2 fewer non-Spinturnicid mites in 2010 (Table 4.9).

There was no detectable effect of either betweeness or degree on non-Spinturnicid mite load for *M. daubentonii* (I = 0.25 and I = 0.26 respectively) or *M. nattereri* (I = 0.26 and I = 0.79 respectively). *M. daubentonii* also showed little support for an effect of social group (I = 0.71), however social group was found to be important in models of *M. nattereri* (I = 1, maximum difference in mean non-Spinturnicid load between different social groups = 1.96). Using the same approach as described for Spinturnicid mites (4.3.2) there was no correlation between median colony size of each social group and their predicted effect on non-Spinturnicid mite abundance.

Reproductive status of females was analysed as for Spinturnicid mites (4.3.2). For all three species, models including reproductive status were significantly better than null models (p < 0.001 in all cases). The pattern observed was similar across species, though pairwise comparisons showed that only lactating *M. nattereri* had a significantly higher non-Spinturnicid load (Figures 4.11-4.13).

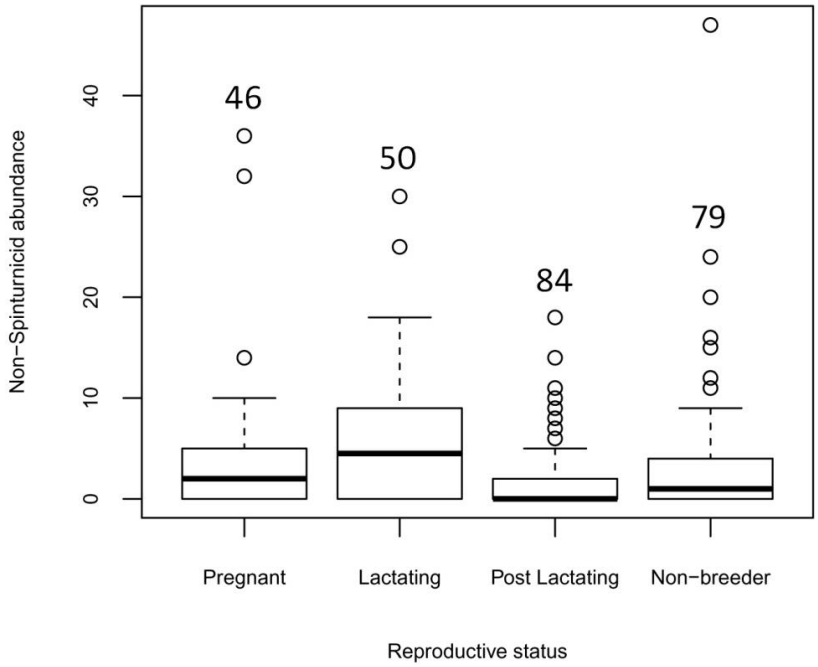

Figure 4.11 – The abundance of non-Spinturnicid mites observed infesting adult female *M. daubentonii*, grouped by the reproductive status of the host. Pairwise comparisons: Post-lactating<Non-breeder<<Pregnant<Lactating, notation as in Figure 4.8. Boxes are presented as in Figure 4.5

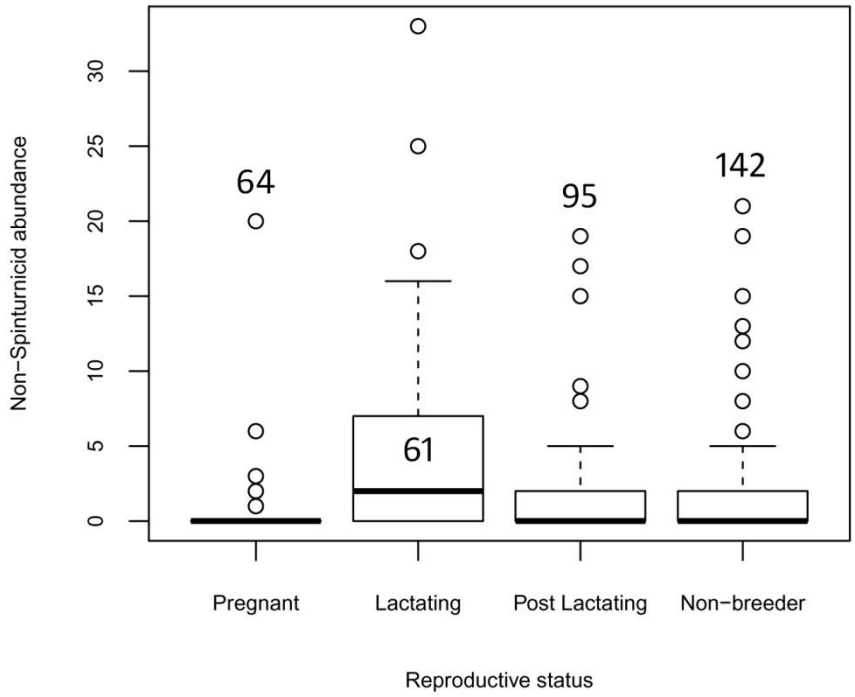

Figure 4.12 – The abundance of non-Spinturnicid mites observed infesting adult female *M. nattereri*, grouped by the reproductive status of the host. Pairwise comparisons: Pregnant<<Non-breeder<<Post-lactating<<Lactating, notation as in Figure 4.8. Boxes are presented as in Figure 4.5

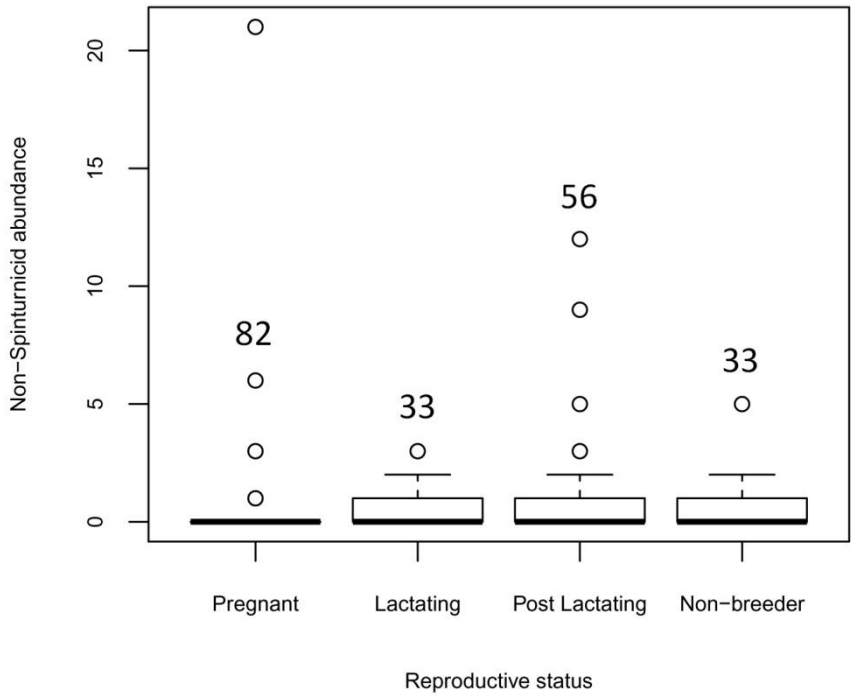

Figure 4.13 – The abundance of non-Spinturnicid mites observed infesting adult female *P. auritus*, grouped by the reproductive status of the host. Pairwise comparisons: Pregnant<<Post-lactating (Non-breeder and Lactating individuals were not significantly different from any other groups), notation as in Figure 4.8. Boxes are presented as in Figure 4.5

4.3.5 Analyses of bat fly loads

Models of bat fly abundance on *M. daubentonii* suggest that year is an important factor, with more infested bats observed in 2010, however, no other variables appeared important and the best model explained only 6% of the deviance observed (Table 4.10). When social variables were considered, none were found to be of high importance (Social group, *I* = 0; Betweeness, *I* = 0.62; Degree, *I* = 0.30).

Variables		*M. daubentonii* Coefficients (±95% CI)	*I*
(Intercept)		-3.529 (±4.971)	
Age: Juvenile		-0.156 (±0.904)	0.26
Sex: Male		-0.521 (±0.54)	0.71
Forearm length		0.025 (±0.199)	0.25
Body Condition Index (BCI)		10.62 (±10.781)	0.72
Non-Spinturnicid mites		-0.011 (±0.039)	0.28
Spinturnicid mites		0.012 (±0.042)	0.29
Fleas		NA	NA
Colony size		-0.028 (±0.038)	0.51
Pre-nursery season	Bachelor colony	-0.901 (±1.39)	
	Mixed sex colony	NA	
	Female colony	-1.815 (±1.296)	
Nursery season	Bachelor colony	*0*	0.72
	Mixed sex colony	0.16 (±0.844)	
	Female colony	-0.136 (±0.764)	
Post-nursery season	Bachelor colony	-0.897 (±0.842)	
	Mixed sex colony	-0.269 (±0.679)	
	Female colony	NA	
Solitory roosting individuals		-0.887 (±1.502)	
Year: 2010		0.902 (±0.47)	**1.00**
Deviance explained by best model		6%	
Number of models incuded in inference		133	

Table 4.10 – Results of model averaging, showing associations between bat fly prevalence and potential explanatory variables. Variables with an importance greater that 0.9 (i.e. a 90% chance that the variable is present in the best model) are indicated in bold. Due to the frequency distribution of bat fly data they were analysed using models with a binomial error structure

4.3.6 Parasite avoidance by non-breeding females

The proportion of non-breeding adult female *M. daubentonii* in nursery colonies (62%, n = 51) was significantly less than the proportion of reproductive females (82% , n = 113; χ^2 = 10.97, df = 1, $p < 0.001$). Since *M. daubentonii* in nursery colonies have the highest loads of mites (Table 4.8 and 4.9) this relatively low occupancy rate of nursery colonies by non-breeding females may explain the lower parasite loads observed on non-breeding when compared to breeding females (Figure 4.8 and 4.11). This difference in the distribution of non-breeding females compared to reproductive females was not observed for *M. nattereri* (χ^2 = 0, df = 1, p = 1) or *P. auritus* (χ^2 = 0, df = 1, p = 1) where the distribution of breeding and non-breeding females were almost identical. However, parasite loads still appear lower on non-breeders suggesting that breeders are either preferentially selected by mites or are less able to fend off infestation either via immune responses or grooming. This may be the case if during pregnancy and lactation resources are diverted away from the immune system and grooming activity and instead invested in offspring.

4.3.7 Analyses of variables predicting body condition index (BCI)

The deviance explained by models of BCI was low (5.0%-10.6%, Table 4.11) demonstrating that a large amount of the variability in BCI remains unexplained, and that these models are fairly poor predictors of BCI.

With pregnant females removed the range of BCI observed in each species was as follows: *M. daubentonii* 0.16-0.33, *M. nattereri* 0.14-0.24 and *P. auritus* 0.16-0.26. Age and sex had high importance (I = 1) across all three species (Table 4.11). Juveniles and males had lower BCI than adults or females respectively. Day of the year was present in all models of *M. daubentonii* BCI (I = 1), with a broad peak in BCI spanning from early July to the end of August.

Wind speed during the night prior to capture was of high importance to models of *M. nattereri* (I = 0.96) and *P. auritus* (I = 1). For both species higher wind speed saw a decrease in BCI (Table 4.11) with the effect greatest for *P. auritus* (Figure 4.14). Additionally average nightly temperature was found to have a positive correlation to *P. auritus* condition (I = 1, Figure 4.15).

	M. daubentonii		*M. nattereri*		*P. auritus*	
	Coefficients (±95% CI)	Importance	Coefficients (±95% CI)	Importance	Coefficients (±95% CI)	Importance
(Intercept)	12.533 (±7.823)		18.681 (±4.75)		17.328 (±5.143)	
Age: Juvenile	-2.888 (±0.605)	**1**	-2.263 (±0.386)	**1**	-1.299 (±0.731)	**1**
Sex:Male	-1.438 (±0.391)	**1**	-1.176 (±0.304)	**1**	-1.239 (±0.437)	**1**
Year: 2010	-0.096 (±0.362)	0.28	-0.273 (±0.298)	0.67	-0.153 (±0.553)	0.27
Day of the year: First order	0.115 (±0.073)	**1**	0.038 (±0.046)	0.73	0.027 (±0.069)	0.46
Day of the year: Second order	-0.0003 (±0.0002)	**1**	-0.0001 (±0.0001)	0.71	-0.0001 (±0.0002)	0.43
Average Temperature	-0.009 (±0.088)	0.26	-0.023 (±0.095)	0.43	0.229 (±0.105)	**1**
Average Windspeed	-0.187 (±0.284)	0.45	-0.309 (±0.199)	**0.96**	-0.769 (±0.347)	**1**
Average Rainfall	-0.301 (±0.649)	0.35	0.227 (±0.197)	0.39	0.191 (±0.222)	0.58
Fleas	NA	NA	0.016 (±0.36)	0.23	NA	NA
BatFly	0.218 (±0.276)	0.54	NA	NA	NA	NA
Spinturnicid mites	-0.019 (±0.031)	0.42	0.02 (±0.046)	0.32	-0.032 (±0.067)	0.34
Non-Spinturnicid mites	-0.036 (±0.027)	**0.95**	-0.018 (±0.032)	0.37	0.19 (±0.139)	**0.95**
Deviance explained by best model	5.0%		10.6%		8.7%	
Number of models icluded in inference	83		144		43	

Table 4.11 – Results of model averaging, showing associations between body condition index (BCI) and potential explanatory variables. BCI was multiplied by 100 before running these models so that output is easier to read. Variables with an importance greater that 0.9 (i.e. a 90% chance that the variable is present in the best model) are indicated in bold. Day of the year was modelled as a polynomial and the first and second order terms that describe the relationship are presented

Non-Spinturnicid mites had high importance for BCI models of both *M. daubentonii* (*I* = 0.95) and *P. auritus* (*I* = 0.95). The effect was negative for *M. daubentonii* and positive for *P. auritus*. (Table 4.11, Figure 4.16). The abundance of bat flies and fleas were not correlated to host condition.

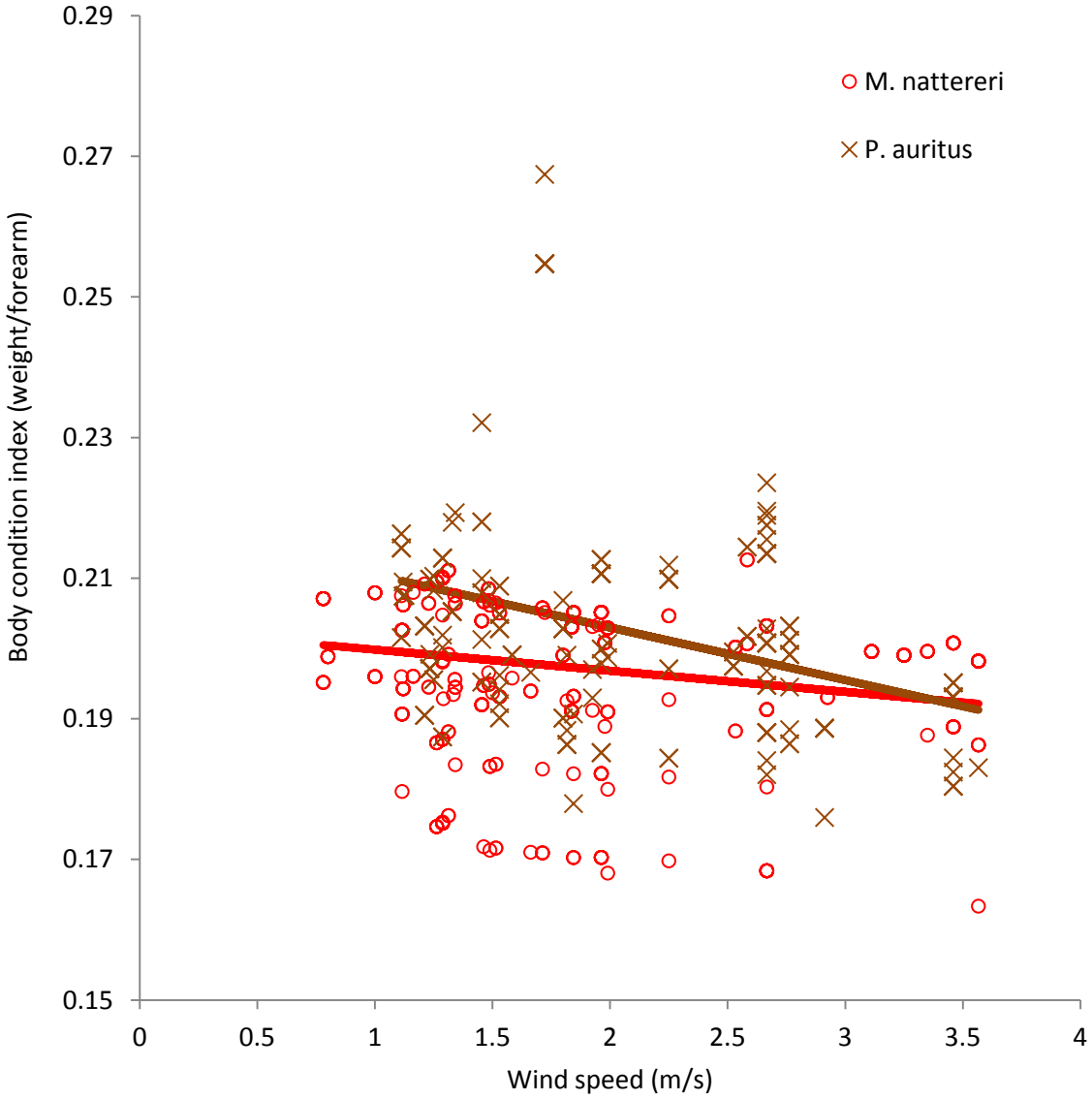

Figure 4.14 – The predicted effect of the previous night's wind speed on body condition index (BCI). Trend lines indicate the predicted effect of wind speed when all other variables are held at their mean. Points indicate the predicted values of BCI from the best model when used with the raw data, this gives an indication of the level of variability in the data

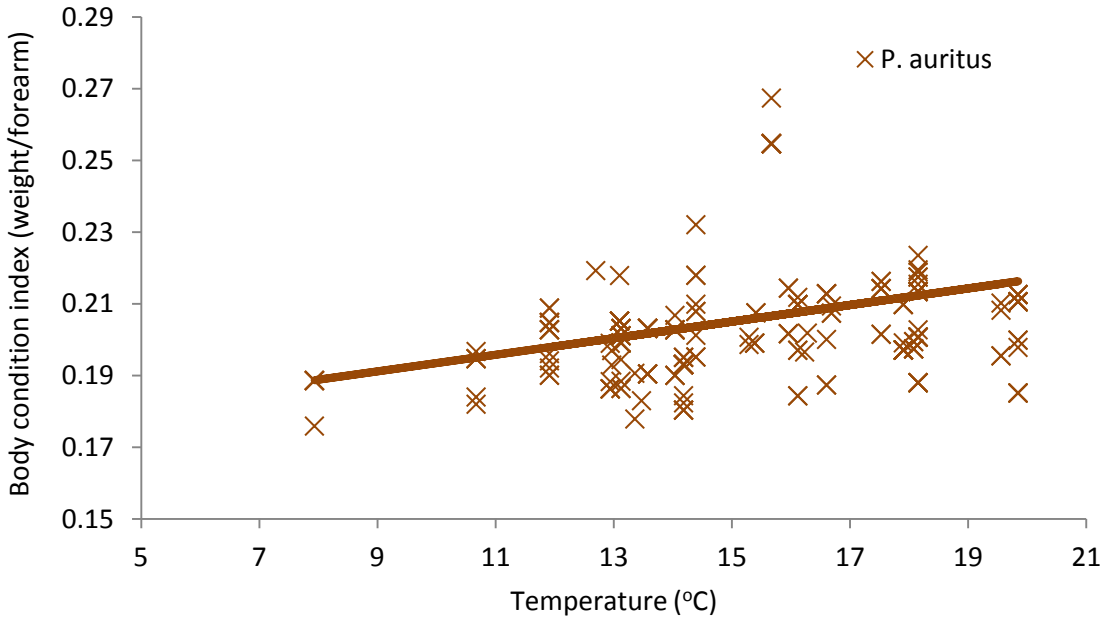

Figure 4.15 - The predicted relationship between mean temperature of the previous night and body condition index (BCI) for *P. auritus*. Trend lines indicate the predicted relationship when all other variables are held at their mean. Points indicate the predicted values of BCI from the best model when used with the raw data, this gives an indication of the level of variability in the data

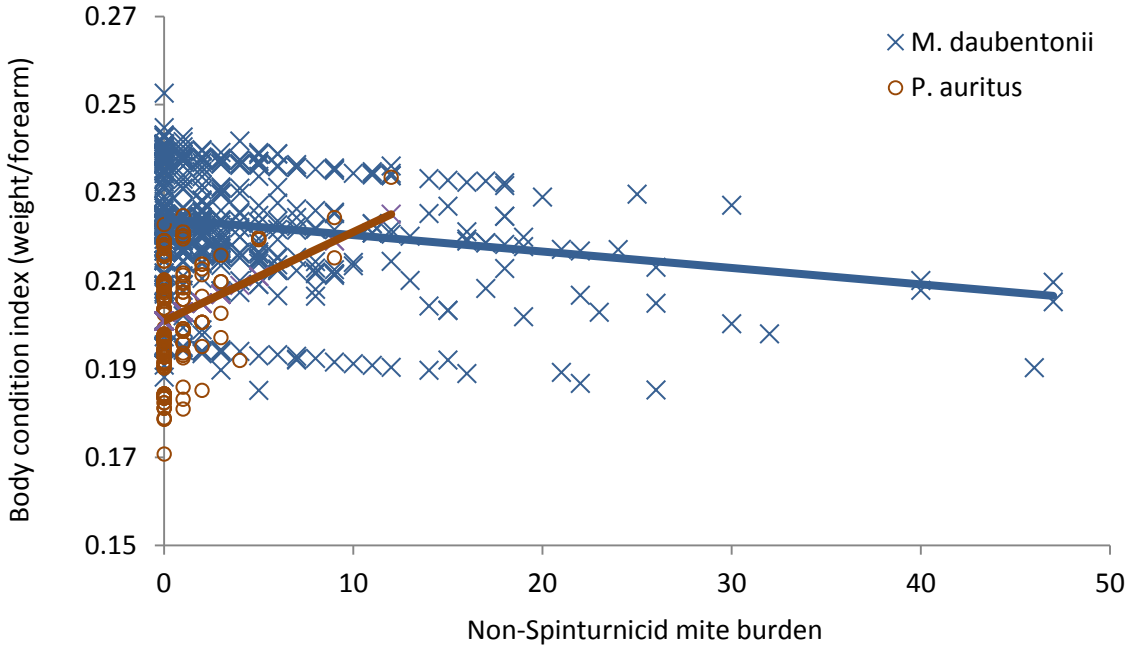

Figure 4.16 – The predicted relationship between non-Spinturnicid mite load and body condition index (BCI). Trend lines indicate the predicted relationship when all other variables are held at their mean. Points indicate the predicted values of BCI using the best model and raw data, this gives an indication of the level of variability in the data

For a subset of 483 captures the time when biometric data was recorded was collected. For this subset the dataset was reanalysed with time since dawn included as an additional variable. Year was not included in these analyses as time was only recorded in 2010. Time since dawn (minutes) had a negative effect on BCI for *M. daubentonii* (I = 1, estimate = -0.007 ± 0.003) but was not found to have a strong effect on *M. nattereri* (I = 0.20, estimate = -0.0002 ± 0.003) or *P. auritus* (I = 0.74, estimate = -0.003 ± 0.003). The negative effect observed for *M. daubentonii* predicts a decline in BCI of 0.03 between the earliest (4.8hours after sunrise) and latest (13.4hours after sunrise) individuals sampled in this study.

For those individuals included in our analysis of social networks (Chapter 2) the data were again reanalysed with social group, degree and betweeness included as variables. None of these variables were found to be important for predicting BCI (I < 0.63).

Reproductive status was analysed as for Spinturnicid mites (4.3.2) with pregnant females removed. Models including reproductive status were significantly better than the null models (p < 0.001 in all cases). Pairwise comparisons found that for all species non-breeding females had significantly lower BCI than both lactating females and post-lactating females (p < 0.0001 in all cases), while lactating females and post-lactating females were not significantly different.

4.4 Discussion

Our study reveals substantial variability in the parasite load and BCI of *M. daubentonii*, *M. nattereri* and *P. auritus*. Some of the observed variation is explained by factors common to all three species whilst other explanatory variables are species-specific.

4.4.1 Patterns of ectoparasite abundance

All species showed a non-random distribution of ectoparasites amongst individuals in the population (Figures 4.3 and 4.4). This is a well know epidemiological phenomenon and has important consequences for the spread of disease within a population (Perkins *et al.* 2003). This non-random distribution means that a small proportion of the population will be responsible for most of the parasite transmission (Beldomenico & Begon 2010).

M. daubentonii had both a higher prevalence and load of mites and bat flies compared to sympatric bat species. The reason for this is unclear at present. The immune response of bats to mites has not been studied but innate differences may explain the differences in prevalences observed. Alternatively, differences in the biology of species-specific parasites, such as cryptic species of Spinturnicid mites infesting *M. daubentonii* and *M. nattereri* (Bruyndonckx *et al.* 2009), might also explain differences in ectoparasite load and prevalence. A third alternative is that the social structure of *M. daubentonii* favours transmission of mites and bat flies.

Amongst both *M. nattereri* and *M. daubentonii*, juveniles, lactating females and individuals in nursery colonies tended to have higher loads of Spinturnicid infestation. Studies of *Miniopterus schreibersii* have shown that ectoparasites reproduce in time with their hosts to take advantage of the arrival of juveniles (Lourenco & Palmeirim 2008). Juvenile *P. auritus* have been shown to take one month to attain adult levels of grooming ability. During this time lactating females groom juveniles, reducing the time spent grooming themselves by 50% compared to non-breeding females (McLean & Speakman 1997). The observed reduction in adult grooming, the inability of juveniles to groom effectively, and the increase in available hosts results in conditions favourable to ectoparasites. The pattern of infestation observed in the present study therefore likely reflects the effects of mites' reproduction and optimal foraging in response to host reproduction and grooming. Interestingly, the same trends for increased abundance on juveniles and in nursery colonies were not observed for *P. auritus*,

though significantly more Spinturnicids were found to infest lactating females than pregnant or non-breeding individuals. This may be a consequence of differences in the immunology and/or population structure of *P. auritus*, or the ecology of its parasites.

The effect of colony type on mite load observed for *M. daubentonii* and *M. nattereri* may reflect an evolutionary strategy for the avoidance of mites by males. For both species bachelor colonies in the nursery period show lower levels of infestation with Spinturnicid mites than female colonies (Figures 4.5 and 4.6). In the case of *M. daubentonii*, males within Wytham Woods routinely form bachelor colonies (Chapter 2) which roost in boxes that do not differ in microclimate or proximity to landscape features compared to roosts used by female colonies (Chapter 3). The segregation of males is therefore not simply an aversion to colony formation, as we find them in large aggregations, nor is it a result of different microclimate requirements as we observed no difference between the climate of roosts used by bachelor and nursery colonies. The increased load of mites in nursery roosts observed here could therefore be an important driver for the segregation of males during this period, especially amongst *M. daubentonii* whose ectoparasite load is higher than other species. This assumes, however, that mites cause decreased fitness. In this study there was no negative association with BCI and Spinturnicid mites, however, these mites could have had effects on other fitness components that we did not measure. Regardless of the cause of the observed sexual segregation it is apparent that females must gain some benefit from nursery colony formation, such as shared body heat for maintenance of homeothermy (Dietz & Kalko 2006) or cooperative care of offspring, which outweighs any costs of increased parasitism.

The lower number of Spinturnicid mites on non-breeding adult female *M. daubentonii* and the higher proportion of these females roosting away from nursery roosts suggests that they may be adapting their roosting behaviour to lower their exposure to mites. However, as non-breeding females enter torpor (Dietz & Kalko 2006), they may choose to avoid the homoeothermic inhabitants of nursery roosts, therefore reducing parasite load as a consequence of their roosting behaviour rather than its cause. Separating the effects of thermoregulation and active avoidance of ectoparasites will require manipulative field experiments.

The patterns of ectoparasite abundance observed for *P. auritus* are difficult to explain. Colony type was an important factor in predicting mite loads, however there was considerable uncertainty in parameter estimates. *P. auritus* males and females roost together throughout the year unlike both other species (Entwistle *et al.* 2000; Altringham 2003). Unfortunately a lack of data did not allow us to analyse their population structure in detail. Such an analysis

may help explain the distribution of mites observed or suggest a better method for classifying colony types in this species.

Forearm length was included in models to control for wing area as it was assumed that bats with a larger wing area would have a larger number of mites. With juveniles removed, to avoid bias due to age, the opposite trend was observed for non-Spinturnicid mites infesting *P. auritus*. Further investigation is needed to interpret this result and should consider possible relationships between forearm length and immune function and grooming rates.

After accounting for the confounding effects of pregnant females, BCI was found to be negatively correlated with non-Spinturnicid mite abundance among *M. daubentonii.* This may be an indication that these mites preferentially infect individuals in poor condition who may be less able to mount an effective immune response or expend energy on grooming. Alternatively, mites may cause their host to lose condition.

Amongst *M. daubentonii* and *M. nattereri* both negative and positive correlations were observed between ectoparasites. This may be due to niche similarity/dissimilarity rather than interactions. For example, Spinturnicid and non-Spinturnicid mites, which in some cases were positively correlated, may have similar host preferences due to similarities in feeding behaviour. In contrast, the difference in life cycle and behaviour of bat flies and non-Spinturnicid mites could explain their negative correlation on *M. daubentonii*.

M. daubentonii bat fly prevalence, in contrast to observed patterns of mite infestation, was not correlated to colony type, reproductive status or age. Instead year was the most important factor and the best model explained only 6% of the observed deviance. As bat flies develop from larva to adult in the roost they have little choice as to whom they infest, being constrained to future occupants of the roost. Bat flies are thought to take between 1 to 2 months (and up to 1 year) to emerge from their puparia (Marshall 1981; Reckardt & Kerth 2006). This off-host life stage may go some way to explain why we do not see similar associations to those of mites. For example, puparia produced by adult bat flies feeding within nursery colonies will not necessarily remain associated with nursing bats as they frequently change roosts. It should also be noted that bat flies were difficult to observe and are certainly under recorded in the present study. As such some significant relationships may have been missed.

4.4.2 Social network variables as predictors of ectoparasite load

Previous studies using social networks to predict the load of ectoparasites on hosts have found a positive correlation between the amount of territory overlap between individuals and tick infestation in tuatara lizards (Godfrey *et al.* 2010). Additionally studies of humans have found that individuals with high values of degree in networks of cocaine injectors are more likely to become infected with HIV (Bell *et al.* 1999). The present study did not find any correlation between the number of associates (degree) an individual had and its parasite load, this is perhaps unsurprising. In bat colonies contact between individuals is intimate and prolonged, providing ideal conditions for the transmission of ectoparasites. Add to this the observed preference of Spinturnicids for reproductive females and juveniles in some species (Christe *et al.* 2007), and it is apparent that infestation risk is not so much linked to how many individuals a bat comes into contact with but rather who they come into contact with. This may also explain the observed positive, though small, effect of betweeness on *M. daubentonii* infested with Spinturnicid mites. Individuals with high betweeness are those that are important for connecting other individuals in the network. This would include, for example, individuals which move between two social groups with otherwise limited connectivity. Such individuals have an increased number of indirect contacts without necessarily having an increased degree. These findings should be interpreted with caution as values of betweeness and degree can be inaccurate in datasets with limited recaptures such as in the present study (Croft *et al.* 2008). Therefore, more work is needed before a conclusive link can be made between an individual's position in a social network and its parasite load in this study system.

Social group was found to be a significant predictor for both *M. daubentonii* and *M. nattereri* Spinturnicid mite abundance, and *M. nattereri* non-Spinturnicid mite abundance. This was not found to be attributable to variation in the typical colony sizes that these social groups formed. Instead, factors such as the social structure within social groups and stochastic events may explain the observed differences between social groups.

Colony size had a negative effect on Spinturnicid mite load amongst *M. daubentonii*. As *M. daubentonii* have the highest mean load of mites amongst the species examined, this may reflect competition, direct or indirect, amongst mites leading to dispersal away from infested hosts. Alternatively this may be a host mediated effect, such as increased allogrooming in larger colonies. The absence of this trend amongst *M. nattereri* and *P. auritus* suggests that amongst these species, which experience lower levels of infestation, competition between mites may be lower or host mediated effects may be reduced or absent.

4.4.3 Patterns of Body Condition Index

Both sex and age had significant impacts on the BCI of individuals across all three species. The lower condition observed in juveniles is likely to be due to the prioritisation of growth, especially in the wings, instead of the generation of fat reserves. Higher BCI among females could be due to the presence of mammary glands which swell up to feed offspring in the summer or more general sexual dimorphism in these species. The peak in BCI in July to September observed for *M. daubentonii* may similarly be driven by this variation in females.

The effect of weather on BCI is almost unexplored in the scientific literature. We found that weather during the night prior to capture had a significant effect on the BCI of bats. Average wind speed had a negative effect on BCI, the strength of this effect being correlated to the morphology of the species (Chapter 1, Table 4.11). *P. auritus*, with the broadest wings (Norberg & Rayner 1987), was most affected by increased wind speed. This species is a slow-flying gleaner and frequently hovers to catch prey. *M. nattereri* is also a slow-flying gleaning species however its wings are not as broad as those of *P. auritus* and the negative effect of wind speed on BCI was less. *M. daubentonii* wings have a similar aspect ratio (breadth:length) to *M. nattereri* but their total area is reduced (Norberg & Rayner 1987), an adaptation to fast low flight over water. *M. daubentonii* have been observed foraging over water in wind speeds up to 7m/s (pers. obs.). Wind speed did not have a detectable effect on the BCI of this species. The robustness of this hypothesised relationship between wind speed, wing morphology, and BCI could easily be tested by studying other bat species.

Prey behaviour may explain the positive effect of temperature on *P. auritus* condition. Moths form a large part of the diet of this species and are known to be more active on nights with an elevated temperature (Anthony *et al.* 1981; McGeachie 1989). Again, further research into the behavioural responses of *P. auritus*'s prey species to nightly temperature could be used to test this hypothesis.

A negative impact of Spinturnicid mites on BCI of bats has been demonstrated in previous studies (Giorgi *et al.* 2001; Lourenco & Palmeirim 2004; Zahn & Rupp 2004; Lucan 2006). However, we observed no such effect, even amongst *M. daubentonii* which carries higher loads of Spinturnicid mites than species where negative impacts have been reported. Non-Spinturnicid mites had contrasting correlations with host BCI. *M. daubentonii* had reduced BCI with high levels of infestation whilst the opposite was true for *P. auritus* (Figure 4.15). The causal link is unclear, and may relate to the behaviour of the mites themselves. For example, non-Spinturnicid mites infesting *M. daubentonii* may be selecting to parasitise bats with poor

condition as they may have reduced immune competence, whereas *P. auritus* mites may preferentially infest individuals with high BCI as they provide a higher quality foraging resource. These hypotheses could be tested with choice experiments in the laboratory and longitudinal studies of the impacts of mites on the BCI of their host.

Since British bats do not feed during hours of sunlight it was predicted that bats would lose condition over the course of the day. We found this prediction was supported by data from *M. daubentonii*. Lamentably this data was not recorded for all individuals throughout the study. Future studies should record the time of day when measurements are taken and account for this in models of BCI.

4.4.4 Implications for models of disease transmission

Results in this chapter highlight the importance of roost type and not social network parameters for explaining the distribution of parasites in populations of bats. Juveniles and lactating females in nursery roosts had the highest levels of infestation with Spinturnicid mites amongst both *M. daubentonii* and *M. nattereri*. Contact rates are high in these colonies but additionally both juveniles and females are thought to be more susceptible at this time due to reduced grooming activity (McLean & Speakman 1997) and reduced immunocompetence (Christe &Vogel, 2000). During the same time period males in bachelor roosts avoided high parasite loads dispite still roosting in groups. Since larger colonies were not found to have larger average parasite loads we show that colony type rather than size is most important for predicting parasite load. Addtitionally social network parameters were poor predictors of parasite burden, perhaps because they consider contacts over the entire duration of the study, or were not based on enough observations to generate accurate parameters. Since colony type sex, reproductive status and age were found to be key variables these must be included in models of disease in bats. Additionally increased susceptibility of reproductive females and juveniles should be considered during the nursery period as well as seasonal variation in the contact rates between males and females (lower in nursery period than post-nursery period, Chapter 2, Figure 2.3a). Models delevoped in the future could be tested against the empirical data used in this chapter to test their ability to produce realistic results.

These analyses have considered factors that predict the abundance of ectoparasites, however, future modelling efforts are likely to focus on pathogens such as viruses, with a human health implication. It is therefore important to note that Spinturnicid mites are known to reproduce in time with their host (Lourenco & Palmeirim 2008) which is likely to explain a large amount of

the variation in mite abundance in this study. However, colony types, age, sex, reproductive status and seasonal changes in contact rates may also be important in transmission rates of pathogens and so we recommend these be included in such models.

4.4.5 Conclusions

Both host and parasite ecology are suggested to be important factors for describing the distribution of parasites within and between bat species. Additionally it is apparent that behavioural adaptations such as sexual segregation in *M. daubentonii* allow a portion of the population to reduce its parasite load. Whether parasite avoidance is the cause or consequence of this segregation is not clear.

Our results suggest that an individual's social group may influence parasite loads, however, more data on network structure, including higher temporal resolution, is needed to test the importance of individuals' position within a social network.

Age, sex, reproductive status and colony type were found to be important parameters for predicting parasite load. These must therefore be included in models of pathogens in bat populations. This suggests state-space models accounting for seasonal changes in contact rate and roosting behaviour would be an appropriate starting point.

We found evidence of both positive and negative correlations between parasite load and BCI. There were also similarities between species in the effect of age, sex and reproductive status, with lactating females and juveniles generally found to have the highest mite load. In contrast, weather was found to have species-specific effects dependant on species' wing morphology and foraging strategy.

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5 Surveillance for pathogens of potential human health concern in British bats

5.1 Introduction

Zoonotic infections originating in wildlife are a significant threat to human health (Daszak *et al.* 2000). Close contact between bats and humans or domestic animals is a principal cause of disease emergence, and such contact has become more frequent (Breed *et al.* 2006; Wong *et al.* 2007) due to increased hunting, habitat loss and agricultural intensification (Daszak *et al.* 2001; Epstein *et al.* 2006; Leroy *et al.* 2009). Add to this the proliferation of modern transport which readily moves zoonoses around the globe, and the chance of zoonotic disease emergence and subsequent spread is perhaps greater than it has ever been.

In addition to their recognised link with rabies transmission, bats have been identified as the reservoir host of several pathogens that have caused disease outbreaks in humans (Wong *et al.* 2007). In some cases the causative agent was not known to infect bats until after the outbreak in humans had occurred (Halpin *et al.* 1998; Johara *et al.* 2001; Poon *et al.* 2005). This highlights the current lack of knowledge of the potentially harmful pathogens hosted by bats. As a result, a number of recent studies have sought to discover the distribution of known zoonoses present in bats (Dominguez *et al.* 2007; Hayman *et al.* 2008; Tong *et al.* 2009; Negredo *et al.* 2011), while others have identified novel pathogens that may be zoonoses of the future (Chu *et al.* 2008).

Disease surveillance amongst British bats is currently limited. Almost all work has focused on European Bat Lyssaviruses (Fooks *et al.* 2004; Brookes *et al.* 2005; Fooks *et al.* 2006; Harris *et al.* 2006; Smith *et al.* 2006; Banyard *et al.* 2009; Smith *et al.* 2011) which caused the death of a bat worker in Scotland in 2002 (Fooks *et al.* 2003). This work identified a low prevalence of seropositive *Myotis daubentonii* (0.7-5.1%, 95% CI) within the UK (Smith *et al.* 2006). Other work has identified *Babesia* sp., *Bartonella* sp., *Borrelia burgdorferi* sensu lato and trypanosomes in blood samples (Gardner & Molyneux 1987; Concannon *et al.* 2005; Reeves *et al.* 2007; Evans *et al.* 2009; Hamilton *et al.* 2012). Focusing surveillance on diseases of greatest concern, notably viruses (see 1.5.2), is needed in the UK to identify diseases of human health concern in wild bat populations. Additionally these surveillance studies should also attempt to quantify the disease dynamics within the populations and identify possible pathways of disease emergence to humans or other animals that could act as intermediate hosts.

In Britain, contact between bats and humans that might lead to disease transmission is rare, although some individuals such as researchers, bat rehabilitators and ecological consultants have regular contact with bats. Despite the generally low encounter rate between bats and humans in the UK, bats commonly roost in buildings occupied by humans and domestic animals (Joint Nature Conservation Committee 2007). This is due to urbanisation and the domination of the landscape by agriculture, which have reduced the availability of natural roosts (Altringham 2003; Simon *et al.* 2004; Zahn *et al.* 2010).

Recent work has highlighted the presence of a number of pathogens in bats or their guano in mainland Europe. These include: Coronaviruses (Gloza-Rausch *et al.* 2008; Reusken *et al.* 2010; Drexler *et al.* 2011), Astroviruses (Drexler *et al.* 2011) and the fungus *Cryptococcus neoformans* (Montagna *et al.* 2003). These, together with *Candida* spp. were the focus of the disease surveillance undertaken for this thesis.

Both Coronaviruses and Astroviruses are single stranded positive sense RNA viruses. They have high mutation rates (Woolhouse *et al.* 2001) and the ability to recombine their genetic material (Pantin-Jackwood *et al.* 2006; Graham & Baric 2010), allowing them to exchange portions of their genome with closely related viruses as is common amongst influenza viruses. This allows them to adapt relatively quickly to novel hosts.

Following the SARS (Severe Acute Respiratory Syndrome) outbreak, caused by the *Betacoronavirus, Severe acute respiratory syndrome-related coronavirus* (SARS-CoV), surveillance efforts detected coronavirus species in bats from every continent they inhabit (GenBank taxonomy data, January 2011). However, the virus has not previously been identified in Britain. Different species of coronaviruses cause gastrointestinal, respiratory and nervous system diseases in a wide variety of host species and are capable of cross-species transmission (Graham & Baric 2010). Coronaviruses known to infect humans include members of the *Alpha*- and *Beta*- but not *Gammacoronavirus* genera (Woo *et al.* 2009).

In humans, Astroviruses commonly cause gastroenteritis in young children, the elderly and the immunocompromised (Kurtz & Lee 1978). Astroviruses have been identified in many domestic species including cows, pigs, sheep, dogs, cats, mice, chickens and turkeys. The diversity of astroviruses in wild animals has not been explored to the same extent, however, Chu *et al* (2008) found a high prevalence and diversity of members of the Mammastrovirus genus in bats from China. A recent study also reported a high prevalence of Mammastroviruses in a colony of *Myotis myotis* (Greater mouse-eared bat) in Germany (Drexler *et al.* 2011).

Bat guano is host to a pathogenic basidiomycete fungus, *C. neoformans* (Kajihiro 1965; Lazera *et al.* 1993). Spores of this fungus can be inhaled when droppings are disturbed leading to the disease cryptococcosis. This disease can become life threatening if the infection spreads, though severe cases are usually limited to those with weakened immune systems such as HIV patients (Levitz 1991). This pathogen has been identified in bat faecal samples from caves in Italy (Montagna *et al.* 2003).

Other important opportunistic fungal pathogens of humans include various members of the *Candida* genus. Most of these yeast are saprophytes, but many also live harmlessly in association with the enteric tract of many animals including mammals, birds and reptiles. However, like *Cryptococcus*, some *Candidia* species can cause severe illness in immunologically compromised individuals. *Candida* spp. have been reported from bats previously (Grose & Marinkelle 1968; Oyeka 1994; Botelho *et al.* 2012) and some of the strains that were isolated may have potential to cause human disease, having been shown to cause mortality in mice (Botelho *et al.* 2012).

A common parasite of the bats in Wytham Woods is the Spinturnicid mite. This mite is directly transmitted, easy to identify in the field and prevalent on *M*. *daubentonii* and *M. nattereri* (Chapter 4). Spinturnicid mites cannot survive off their host for long periods of time and are transmitted between bats by physical contact. Therefore they may be a good model for studying pathogens transmitted in a similar manner. If Spinturnicid mite load can be shown to correlate with pathogen prevalence, future studies may be able to use this as a proxy for the probability of infection by directly transmitted pathogens.

In this chapter we analyse samples for pathogens of potential human health concern. These include Coronaviruses, Astroviruses, *C. neoformans* and *Candida* spp. Pathogens that were detected were compared by sequencing and phylogenetic analysis with those identified outside Britain. In addition we assessed the distribution of coronaviruses amongst individuals to assess how the ecology and social structure of bat populations may drive the observed pattern of infection.

5.2 Methods

5.2.1 Sample collection for Candida, and viral analyses

Samples were obtained from roosting bats at Wytham Woods (415ha) in Oxfordshire, and Savernake Forest (1100ha) in Wiltshire, between July and September 2009 and further samples were collected at Wytham Woods in 2010 between May and October. At these summer sites samples were collected from *M. daubentonii*, *M. nattereri*, *P. auritus*, *Barbastella barbastellus* (Barbastelle bat) and *Pipistrellus pipistrellus* (Common Pipistrelle). Samples were also collected in Devon and Wiltshire from active *Rhinolophus hipposideros* (Lesser Horseshoe bat) in August and September 2009 and hibernating *Rhinolophus ferrumequinum* (Greater Horseshoe bat) in December 2006. Bats were placed into cotton bags prior to being fitted with a permanent arm ring, bearing a unique identification number, and having their breeding status, sex and biometric data recorded. If bats defecated in the holding bags a single faecal pellet was collected into a sterile 1.5ml microcentrifuge tube and either preserved in 250µL RNAlater™ (Applied Biosystems, Warrington, UK) or snap-frozen on dry ice. If bats did not defecate no sample was taken, however, bats defecated on the majority of occasions. Samples were stored at -80°C until analysed. To prevent faecal cross contamination holding bags were sterilised between use by autoclaving followed by soaking in 6% sodium hypochlorite (domestic bleach) and washing. Bags were used once in the field and then kept separately from unused bags until sterilised. Procedures were approved by the Biosciences Ethics Committee, University of Exeter, and carried out under the appropriate Natural England licence.

5.2.2 Detection of Coronaviruses and Astroviruses

Faecal pellets stored in RNAlater™ were homogenised *in situ* whereas samples snap frozen on dry ice were homogenised in 300µL phosphate buffered saline pH 7.2 (PBS) prior to analysis. Positive controls for Coronavirus PCRs were made by spiking selected faecal homogenates with 0.2 plaque forming units (PFU) of *Human coronavirus NL63* stock (HuCoV-NL63) grown and titrated in LLC-MK2 cells. HuCoV-NL63 and cell line LLC-MK2 were generously donated by Dr Christian Drosten and Dr Petra Herzog (Institute of Virology, University of Bonn). PBS or RNAlater™, as appropriate, served as negative controls. For both Astrovirus and Coronavirus analyses RNA was extracted from 100µL of faecal homogenate using a viral RNA Mini Kit

(QIAGEN, Crawley, UK). The eluted RNA (8µL of 60µL) was random primed reverse transcribed following the manufacturer's instructions (SuperScript II™, Invitrogen, Paisley, UK).

Coronaviruses were detected with a semi-nested PCR using ImmoMix™ (Bioline, London, UK) to amplify a conserved ~440bp CoV specific region of the RNA-dependent RNA polymerase (RdRP) gene (de Souza Luna *et al.* 2007). The first round reaction used primers at a concentration of 1µM. Reactions (25µl) were made up of primer PC2S2 (equimolar mixture of TTATGGGTTGGGATTATC and TGATGGGATGGGACTATC), primer PC2As1 (equimolar mixture of TCATCACTCAGAATCATCA, TCATCAGAAAGAATCATCA, and TCGTCGGACAAGATCATCA), 12.5µl of ImmoMix, 2µl of cDNA from the reverse transcription step and water to make up the 25µl reaction. The amplification procedure started with a 7min at 95°C; then 10 cycles of 20 seconds at 94°C, 30 seconds starting at 62°C with a decrease of 1°C per cycle, and 40 seconds at 72°C; then 30 cycles of 20 seconds at 95°C, 30 seconds at 52°C, and 40 seconds at 72°C (de Souza Luna *et al.* 2007). The second round reactions (25µl) consisted of 1µl round 1 product, 80nM of primer PCS (equimolar mixture of CTTATGGGTTGGGATT ATCCTAAGTGTGA and CTTATGGGTTGGGATTATCCCAAATGTGA), 400 nM primer PCNAs (CACACAACACCTTCATCAGATAGAATCATCA), 12.5µl ImmoMix and water to make up the 25µl reaction. The amplification procedure started with 3 min at 94°C and continued with 35 cycles of 20 seconds at 94°C, 30 seconds at 60°C, and 30 seconds at 72°C (minor modification of de Souza Luna *et al.* 2007).

A suitable Astrovirus detection methodology was identified in the literature (Chu *et al.* 2008). This protocol targets a conserved 422bp region of the RNA-dependent RNA polymerase gene and was performed as previously described (Chu *et al.* 2008). First round reactions (50µl) were made up of a 2µM concentration (each) of forward (GARTTYGATTGGRCKCGKTAYGA and GARTTYGATTGGRCKAGGTAYGA) and reverse (GGYTTKACCCACATNCCRAA) primers, and 2µl of cDNA. The amplification procedure started with a 7min at 94°C, and continued with 30 cycles of 30 seconds at 94°C , 30 seconds at 50°C and 30 seconds at 68°C. The second round was carried out as the first which different forward primers (CGKTAYGATGGKACKATHCC and AGGTAYGATGGKACKATHCC), and 40 amplification cycles. Astrovirus positive samples, detected during the testing of the Astrovirus protocol and confirmed by sequencing, were used as positive controls in PCRs.

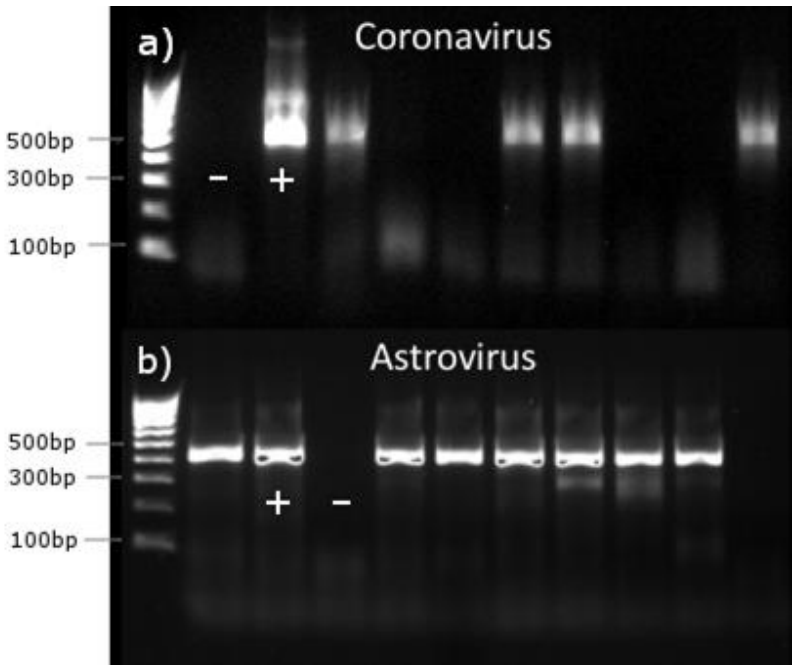

Figure 5.1 – Representative gel electrophoresis RT-PCR results for a) Coronavirus and b) Astrovirus. 2% agarose gels were stained with ethidium bromide and visualised under UV light using a Bio-Rad Gel Doc™ XR+ (Bio-Rad, Hemel Hempstead, UK). Hyper ladder IV (BioLine, London, UK) was used in both assays to evaluate product size. The bands present in this hyperladder increase in 100 base pair (bp) intervals, the 100bp, 300bp and 500bp bands are indicated. '+' indicates positive controls and '–' indicates negative controls. The Coronavirus sequence amplified was c.440bp in length (de Souza Luna *et al.* 2007) and the Astrovirus sequence was 422bp in length (Chu *et al.* 2008)

RT-PCR products were visualised on 2% agarose gels stained with ethidium bromide and photographed under UV. Band size was estimated by comparison to Hyper ladder IV (BioLine, London, UK). Images were recorded on a Bio-Rad Gel Doc™ XR+ (Bio-Rad, Hemel Hempstead, UK). Representative gels are shown in Figure 5.1.

Representative PCR-positive samples of both Coronaviruses and Astroviruses were gel purified (QIAquick kit, QIAGEN), cloned (pGEM-T vector, Promega, Madison, USA) and sequenced using vector-specific T7 and SP6 primers (BigDye® Terminator vs3.1 Cycle Sequencing Kit and ABI 3730 DNA analyser, Applied Biosystems, Carlsbad, USA). Forward and reverse reads were aligned, quality checked and edited using Lasergene 6 software (DNASTAR Inc., Madison, USA). Sequences were trimmed to remove the primers, and aligned with sequences from GenBank using ClustalW in BioEdit 7 (Hall 1999). Phylogenetic analysis was undertaken with MEGA5 (www.megasoftware.net) on a 366bp conserved region of the Coronavirues RdRP, and a 253bp conserved region of the Astrovirus RdRP. These were the longest regions for which matching sequence data was available for the isolates from public databases. MEGA5 was used to statistically compare different models of DNA evolution used to create phylogentic trees from

Coronavirus and Astrovirus data. The best model was chosen using the information criterion AIC. In addition, trees were validated against published trees of these groups.

5.2.3 Sampling and detection methodology for Cryptococcus

Samples of faeces from hibernacula and the floor of Wytham Woods' roost boxes were collected in 2009 and 2010. Samples were collected from 24 summer roosts in Wytham Woods and 21 locations within 5 cave systems known to be used as hibernacula by a range of bat species. Samples were collected in sterile glass vials and stored at room temperature for a maximum of 4 months until analysed. The delay in analysis was as a result of logistical constrains. Since these are habitable conditions for this fungus the delay in culturing was not thought to be significant.

Samples were swabbed and plated onto Staib agar, a media for identification of *C. neoformans*, and the same media as used in a study of *C. neoformans* in bat guano in Italy (Montagna *et al.* 2003). Plates were incubated at 37°C for 26 days and examined regularly. On each occasion plates were examined for the presence of growth similar to that of *C. neoformans* (cream to brown and opaque) (Nardelli *et al.* 2005). The Staib media and a control strain of *Cryptococcus* sp. were donated by Dr Micheal Petrou (Imperial College, London). The control strain grew as expected.

5.2.4 Investigation of putative Candida samples

Samples that were snap frozen in the field were homogenised in 300µL phosphate buffered saline pH 7.2 (PBS), 100µl of which was inoculated onto Sabouraud dextrose agar (Oxoid, Basingstoke, UK) a selective media for fungi. Plates were incubated at both 27°C and 37°C and checked daily for 2 weeks. Putative *Candida* colonies were identified by their appearance, round opaque white or cream in colour, and were picked and replated to ensure a pure colony (i.e. a clone) was obtained. Subsequent identification was undertaken by Dr Mark Ramsdale (University of Exeter). Pure colonies were grown on CHROMagar (Becton, Dickinson and Company, Oxford, UK) and Yeast extract peptone dextrose to aid their identification. Colonies of *Candidia albicans*, *C. tropicalis* and *C. krusei* grown on CHROMagar can be differentiated by their colour while growth on Yeast extract peptone dextrose allowed investigation of the morphology of colony growth. Microscopy was used to rule out the presence of bacteria

where necessary. A germ tube test was conducted on all samples to test for the presence of *C. albicans* or the closely related *C. dubliniensis.* Clonal colonies were homogenised in foetal bovine serum (Sigma, St. Louis, Montana, USA) at a ratio of 1:9. This homogenate was incubated at 37°C for 3 hours and monitored for germ tube formation at 15 minute intervals. The ITS1 and ITS4 regions of a number of samples (11 of 21 isolates) were sequenced using a previously described method (White *et al.* 1990) and compared with sequences from the Genbank database.

Species	Sex	Age	Reproductive status	Nursery period	Post-nursery period
M. daubentonii	Female	Adult	Lactating	20	NA
			Post lactating	NA	18
			Non breeding	10	5
		Juvenile		13	4
	Male	Adult		20	18
		Juvenile		19	13
M. nattereri	Female	Adult	Lactating	17	NA
			Post lactating	NA	20
			Non breeding	15	7
		Juvenile		12	2
	Male	Adult		19	11
		Juvenile		20	7

Table 5.1 – Samples sizes for each combination of factors used in the analysis of Coronavirus prevalence.

5.2.5 Analysis of infection risk factors

There were sufficient data available for the distribution of Coronaviruses in *M. daubentonii* (n = 115) and *M. nattereri* (n = 125) to be analysed for infection risk factors. We used generalised linear models (GLMs) with a binomial error structure to assess for factors associated with increased probability of infection, namely sex, age, season, BCI and year. While there were insufficient samples from the pre-nursery season for analysis, samples from the nursery and post nursery seasons where analysed. There were insufficient samples to permit analysis of the 10 different colony types (see Chapter 4). Backwards stepwise regression was used to select the best model with BCI, year, and a four-way interaction between sex, age, season and species (model formula: Coronavirus presence/absence~ BCI + Year + Species * Sex * Age * Season), until a minimum adequate model was achieved. This analysis identified species-specific effects and so the analysis was repeated for *M. nattereri* and *M. daubentonii*

independently. The sample size for each combination of factors is given in Table 5.1 and a full summary is given in the appendix (Table 7.3).

For all but 21 individuals (2 *M. daubentonii*, 19 *M. nattereri*) data for Spinturnicid mite load was also available. Models were therefore built using all of the original variables and also Spinturnicid load (Coronavirus presence/absence ~ BCI + Year + Spinturnicid load + Species * Sex * Age * Season). The Spinturnicid variable was fitted using a smoothing function in a generalised additive model (GAM) using package R-gam version 1.06.2 (Hastie 2011), and its significance was examined. This was used to test for a non-linear relationship. When this was found to be non-significant the Spinturnicid variable was included as a linear variable and a backwards stepwise regression was carried out as before. Differences in the virus prevalence between females with different reproductive statuses (i.e. pregnant, lactating, post-lactating and non-breeding) were tested separately since this analysis includes only adult females and reproductive status is correlated with season. This test was carried out using a Fisher's exact test.

Where data were available (n = 94 *M. daubentonii*; n = 84 *M. nattereri*), information from the social network analysis (SNA), described in Chapter 2, was used. This includes degree (number of individuals an individual associates with), betweeness (importance of an individual for connecting others in the network), and the social groups to which individuals belong. Again, backwards stepwise regressions using GLMs with binomial error structure were used to select the best model starting with a model containing social group, betweeness and degree (Coronavirus prevalence ~ Social group + Degree + Betweeness). There was no biological reason to include interactions in these models.

5.3 Results

5.3.1 Coronaviruses

Coronavirus was detected for the first time in bats in Britain. The reverse transcription-PCR (RT-PCR) methodology detected coronavirus RdRP RNA in 2 (underlined) of the 7 bat species examined (*B. barbastellus, M. daubentonii, M. nattereri, P. auritus, P. pipistrellus, R. ferrumequinum* and *R. hipposideros*; Table 5.2). This finding was accepted for publication in 2012 (August *et al.* 2012). In subsequent work faecal samples from a total of 290 individual bats were processed. Thirty five percent of *M. daubentonii* (n = 115) and 46% of *M. nattereri* (n = 125) samples were positive by RT-PCR. Identical results were obtained from two independent RT-PCRs of faecal samples and all positive and negative controls gave the expected results.

		Coronavirus		*Astrovirus*	
Species	Location	No. sampled (no. positive)	Prevalence (95% CI)	No. sampled (no. positive)	Prevalence* (95% CI)
M. nattereri	Wytham[1]	109 (49)	45% (36-53)	16 (7)	44% (23-67)
	Savernake[2]	16 (9)	56% (32-81)	16 (12)	75% (51-90)
M. daubentonii	Wytham[1]	115 (55)	35% (28-43)	30 (29)	97% (83-100)
P. auritus	Wytham[1]	26 (0)		26 (12)	46% (29-65)
R. ferrumequinum	South-West England	15 (0)		15 (1)	7% (0-30)
R. hipposideros	South-West England	6 (0)		6 (3)	50% (19-81)
P. pipistrellus	Savernake[2]	2 (0)		2 (1)	50% (3-97)
B. barbastellus	Savernake[2]	1 (0)		1 (0)	
Total		*290 (113)*	*39% (36-45)*	*112 (65)*	*58% (49-67)*

Table 5.2 – Prevalence of Coronaviruses and Astroviruses in seven British bat species by RT-PCR analysis of faecal samples. [1] Wytham Woods (51°77'27"N, -1°33'41"E). [2] Savernake forest (51°39'96"N, -1°67'75"E). *Sequence data showed that the Astrovirus RT-PCR protocol amplified some non-target sequences, therefore these prevalence values are over estimates

The Coronavirus RdRP sequences (from 18 individual bats) fall into a phylogenetic subclade with 99% bootstrap support that lies within the main *Alphacoronavirus* clade defined by the International Committee on Taxonomy of Viruses (ICTV) (Figure 5.2). The members of the

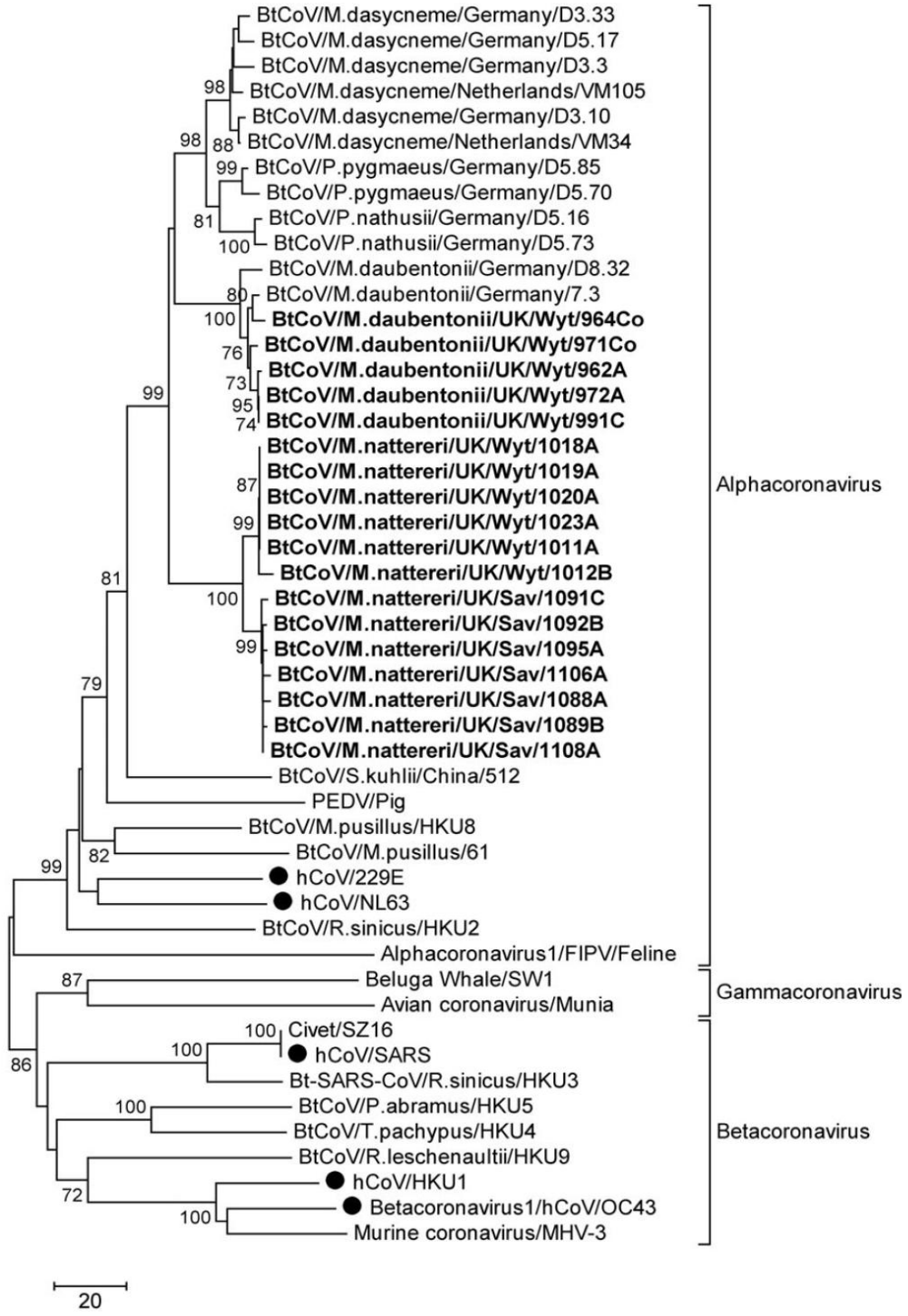

Figure 5.2 – Neighbour joining phylogeny of representative coronavirus RdRP sequences (366bp) including the new strains (bold type) found in British bats. Boot strap values (1000 replicates) are indicated as percentages where the value was greater than 70%. The simplest model of DNA evolution was selected using MEGA5, therefore the tree was built on the number of base differences between sequences. Coronaviruses known to infect humans are indicated by a closed circle (•). The common name of hosts is given for Coronaviruses not derived from bats. Viruses that have been assigned to the *Alpha-*, *Beta-* and *Gammacoronavirus* genera by the International Committee on Taxonomy of Viruses are bracketed. Scale bar indicates base differences per sequence

subclade are all derived from European vespertilionid bats. Maximum likelihood (not shown) and neighbour-joining algorithms produced equivalent trees.

The sequences from British *M. daubentonii* are closely related to sequences obtained from *M. daubentonii* sampled in Germany (Figure 5.2). The sequences from *M. nattereri* represent the first record of a Coronavirus from this bat species and form a distinct, well supported clade (100% bootstrap value). The *M. nattereri* clade divides into two further groups which correspond to the two sites (47km apart) at which the species was sampled in this study. Further sampling and analyses of additional *Alphacoronavirus* loci will be needed to interpret this observation.

Parameters	Estimate	Standard error	z-value	P-value
(Intercept)	-0.772	0.426	-1.812	0.070
M. nattereri	0.788	0.516	1.526	0.127
Male	-0.112	0.607	-0.185	0.853
Juvenile	1.833	0.757	2.421	**0.015**
Post nursery season	1.318	0.587	2.246	**0.025**
Year	-0.835	0.347	-2.404	**0.016**
M. nattereri:Male	-1.219	0.774	-1.574	0.116
M. nattereri:Juvenile	-1.176	1.004	-1.171	0.242
Male:Juvenile	-1.733	0.937	-1.850	0.064
M. nattereri: Post nursery season	-1.375	0.703	-1.955	0.051
Juvenile:Post nursery season	-0.361	0.972	-0.372	0.710
Male:Post nursery season	1.623	0.707	2.295	**0.022**
M. nattereri:Male:Juvenile	4.653	1.475	3.154	**0.002**
M. nattereri:Juvenile:Post nursery season	-3.548	1.592	-2.228	**0.026**

Table 5.3 - Results of a backwards stepwise logistic regression of factors explaining variation in the probability of infection by Coronaviruses. This model, which explains 15% of the observed deviance, demonstrates that species specific effects exist and so the data was subsequently analysed independently for each species (Table 5.4). Parameters with $p < 0.05$ are indicated in bold type

A backwards stepwise logistic regression model was used first to test for species-specific effects of sex, age, season, year and BCI on Coronavirus prevalence. Samples collected from *M. nattereri* in Savernake were included in this analysis as it was assumed that trends in prevalence across sex, age and season would be similar across sites. This model showed there were significant species-specific interaction terms (Table 5.3) and so subsequent models considered species independently for a clearer interpretation of results. For *M. daubentonii* (Table 5.4a) the model showed that males had a significantly increased chance of infection in the post nursery period ($z = 2.219$, $p = 0.027$) and juvenile bats had a higher prevalence than

adults (z = 2.318, p = 0.021) (Figure 5.3). *M. nattereri* (Table 5.4b) juvenile males had a higher prevalence than adult males (z = 2.040, p = 0.041) and the prevalence was higher in 2009 (z = -3.524, p < 0.001) (Figure 5.4).

M. daubentonii

a)

Parameters	Estimate	Standard error	z-value	p-value
(Intercept)	-0.744	0.409	-1.816	0.069
Male	-0.346	0.647	-0.535	0.593
Juvenile	1.564	0.675	2.318	**0.021**
Post nursery season	0.637	0.556	1.147	0.252
Male:Juvenile	-1.585	0.950	-1.668	0.095
Male:Post nursery season	1.964	0.885	2.219	**0.027**

M. nattereri

b)

Parameters	Estimate	Standard error	z-value	p-value
(Intercept)	3.537	2.476	1.428	0.153
Male	-0.489	0.585	-0.836	0.403
Juvenile	0.284	0.784	0.362	0.718
Post nursery season	0.491	0.529	0.928	0.353
Body Condition Index (BCI)	-16.928	11.871	-1.426	0.154
Year (2010)	-1.772	0.503	-3.524	**<0.001**
Male:Juvenile	2.371	1.162	2.040	**0.041**
Juvenile:Post nursery season	-2.210	1.265	-1.752	0.080

Table 5.4 – Results of a backwards stepwise logistic regression of factors explaining variation in the probability of infection by Coronaviruses. a) The *M. daubentonii* model explains 16% of the observed deviance while b) the *M. nattereri* model explains 21% of the observed deviance. Parameters with a p-value less than 0.05 are indicated in bold type.

Using the subset of individuals for whom Spinturnicid mite data were available the prevalence of Coronavirus was modelled as before but with the addition of Spinturnicid burden as an explanatory variable. This was first modelled using a GAM, fitting Spinturnicid mite burden with a smoothing function, as the relationship may have been non-linear. Using this method Spinturnicid burden was not found to be significant (*M. daubentonii*: df = 3, χ^2 = 1.47, p = 0.69, *M. nattereri*: df = 3, χ^2 = 2.55, p = 0.47), nor was it found to be significant when assuming a linear response in a backwards stepwise regression as previously described. These results

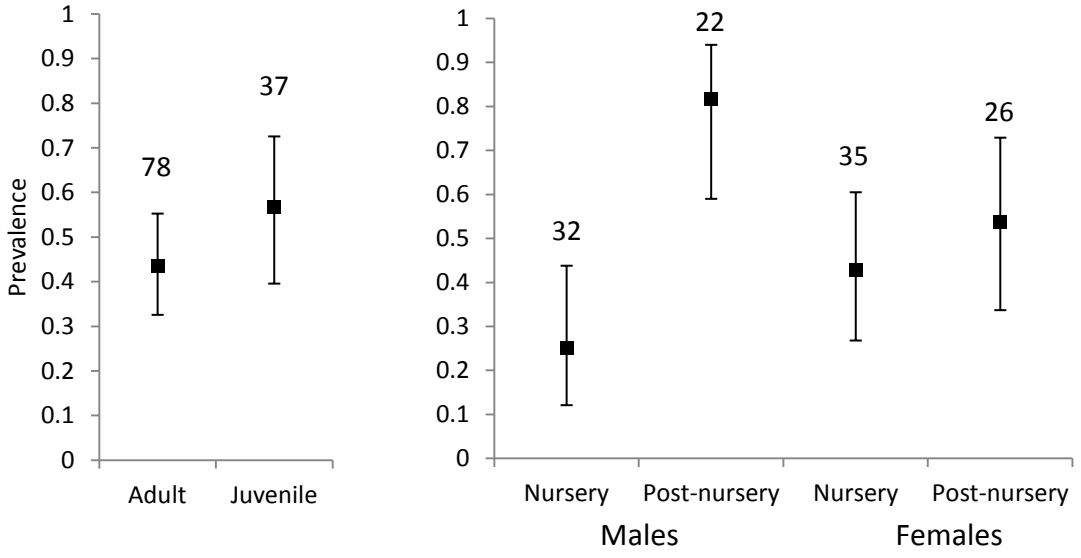

Figure 5.3 – Variation in Coronavirus prevalence amongst *M. daubentonii* for those factors found to be significant in models. Juveniles had a higher prevalence than adults and males had a significantly elevated prevalence in the post-nursery period compared with the nursery period (Table 5.4a). The prevalence was calculated from the raw data and error bars show the 95% confidence intervals. Labels indicate the sample size.

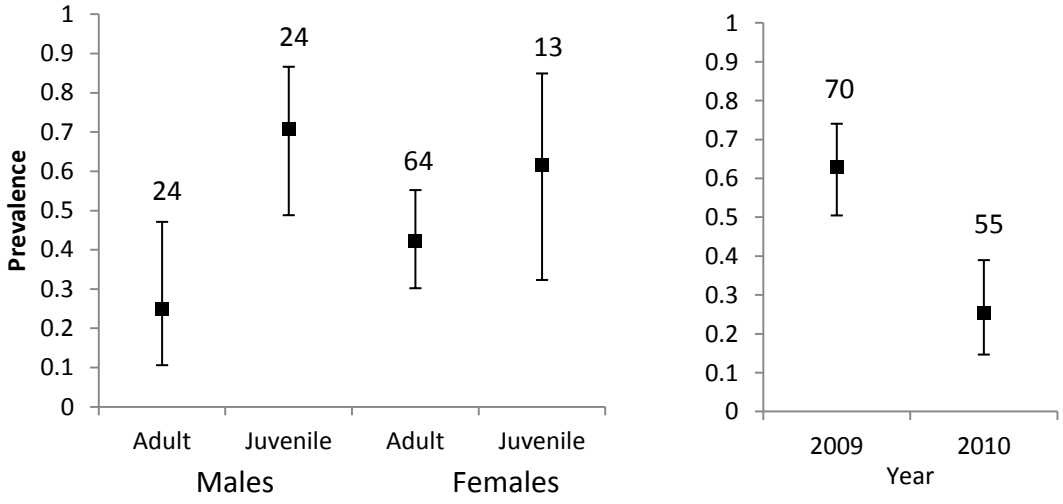

Figure 5.4 – Variation in Coronavirus prevalence amongst *M. nattereri* for those factors found to be significant in models. Juvenile males had a higher prevalence than adults and the recorded prevalence was higher in 2009 than 2010 (Table 5.4b). The prevalence was calculated from the raw data and error bars show the 95% confidence intervals. Labels indicate the sample size.

demonstrate that there is no correlation between Spinturnicid mite abundance and Coronavirus prevalence once sex, age, year and season have been accounted for. Similarly for those individuals with data on BCI (body condition index) this variable was not found to be

significant in explaining which individuals were infected with coronavirus significant (*M. daubentonii*: z-value = 0.56, *p* = 0.58, *M. nattereri*: z-value= -1.426, *p* = 0.15)

There was no significant difference between the prevalence of infection observed in adult lactating, post-lactating and non-breeding females, either for *M. daubentonii* (Fishers exact test, *p* = 0.74) or *M. nattereri* (*p* = 1).

For a subset of individuals, data from the social network analysis was also available (n = 94 *M. daubentonii*; n = 84 *M. nattereri*). However, none of the variables were found to be significantly associated with Coronavirus prevalence (*M. daubentonii*: Social group z = 1.033, *p* = 0.302; Betweeness z = -0.161, *p* = 0.872; Degree z= -0.634 *p* = 0.526. *M. nattereri*: Social group z = -1.192, *p* = 0.233; Betweeness z = 1.196, *p* = 0.232; Degree z = -0.452, *p* = 0.651).

A small sample (n = 10 adults, 3 *M. daubentonii* females and 7 *M. nattereri* (1 male, 6 females)) of faecal samples collected in 2010 from individuals positive for Coronavirus in 2009 were tested. Of these samples one, from a *M. nattereri*, was positive. This indicates that infections with coronavirus are not long lived, or reduce in detectability over time. The prevalence observed is lower than expected given the average prevalence of 39% across both species, but was not statistically significant given the small sample size (Fisher's exact test, *p* = 0.34). It may be that bats can acquire immunity to Coronavirus but this requires a greater understanding of bats' immunology and the Coronavirus strains circulating within the population.

5.3.2 Astroviruses

The presence of Astroviruses was investigated in 112 samples previously tested for Coronaviruses. Of these samples 65 were PCR positive (58%) from 6 (underlined) of 7 species tested (*B. barbastellus, M. daubentonii, M. nattereri, P. auritus, P. pipistrellus, R. ferrumequinum* and *R. hipposideros;* Table 5.2).

A sub-sample of PCR positive isolates from 15 individual bats were cloned, sequenced and phylogenetically compared with previously described Astroviruses and shown to be members of the genus *Mammastrovirus* (Figure 5.5). Of 29 clones successfully sequenced, 5 were not Astroviruses, these included bacteria and a bacteriophage. This amplification of non-specific targets by the generic Astrovirus primers is perhaps unsurprising given their high level of base redundancy (Chu *et al.* 2008). As a result the PCR positive samples cannot all be assumed to be positive for Mammastroviruses. The specificity of the test was approximately 83% (95% CI: 65-92%), therefore statistical analysis of *Mammastrovirus* prevalence was not undertaken.

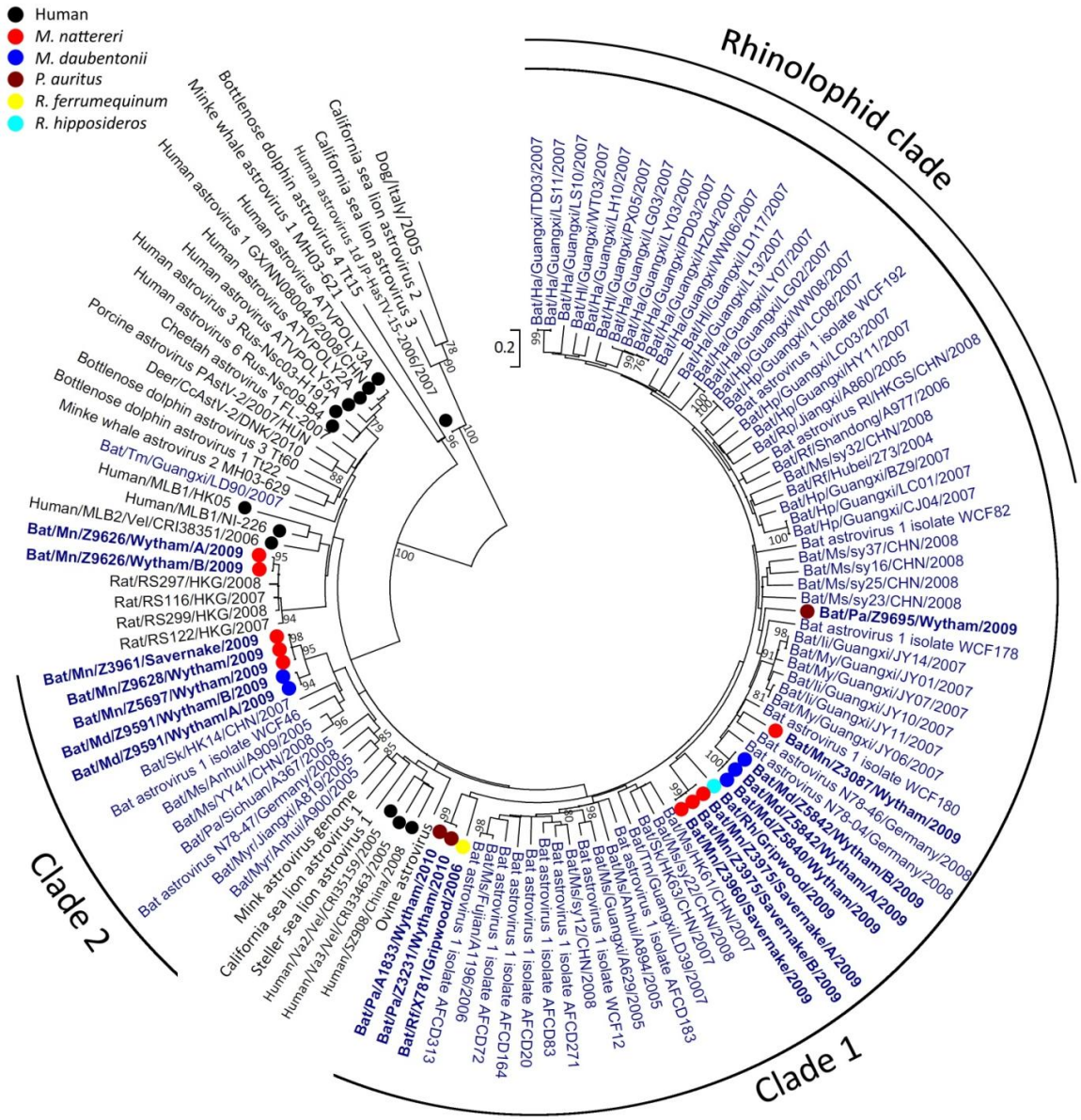

Figure 5.5 – Unrooted, maximum likelihood phylogeny of Astrovirus RdRP sequences (253bp) including novel strains (indicated by bold lettering) found in British bats. Using model comparison the best model of DNA evolution was selected by MEGA5, this was a general time reversible model (GTR), with non-uniformity of evolutionary rates between sites (gamma) and a fraction of sites invariable over time (I). Coloured circles indicate the species from which the virus was detected for samples collected in this study (see colour key above), black circles indicate human Astroviruses. Names in blue represent bat Astroviruses. Two major clades of bat viruses are highlighted. Clade 1 includes a previously identified Rhinolophid clade (Zhu *et al.* 2009). Where more than one identical sequence was obtained from a single individual only one was included in the phylogeny. Boot strap values (1000 replicates) are indicated as percentages where the value was greater than 70%. Scale bar indicates base differences per nucleotide site

The sequencing results show that some bat species host more than one distinct *Mammastrovirus* strain (Figure 5.5). Furthermore both *M. nattereri* and *M. daubentonii* hosted more than one distinct virus at the same sampling point in time and space (i.e. Wytham Woods or Savernake Forest), however, no individual was recorded as hosting more than one virus strain. Four distinct *Mammastrovirus* strains were associated with *M. nattereri*. Three of these strains were found in Wytham Woods and two in Savernake Forest with one strain common to both woodlands. One of the Wytham Woods strains was not nested amongst sequences from bats but had bootstrap support (94%) for membership of a subclade that includes sequences from rats sampled in Hong Kong (Figure 5.5). Two distinct strains were associated with *M. daubentonii*, one of the strains (in bat clade 2, Figure 5.5) was distinct (95% bootstrap support) but closely related to a *Mammastrovirus* strain from *M. nattereri* sampled in Wytham and Savernake. Two distinct strains were identified for *P. auritus*, while *R. ferrumequinum* and *R. hipposideros* each hosted single distinct strains. Of the 15 individuals that were found to be infected with *Mammastrovirus* by sequence analysis, 5 were simultaneously infected with Coronavirus.

5.3.3 Cryptococcus neoformans

All environmental samples plated onto *Cryptococcus* selective media were negative for the presence of *C. neoformans* while the positive control strain showed the expected growth (Table 5.5).

5.3.4 Candida species

Faecal samples from 51 individuals representing five species (*B. barbastellus* (n = 1)*, M. daubentonii* (n = 9)*, M. nattereri* (n = 27)*, P. auritus* (n = 12)*, P. pipistrellus* (n = 2)) were plated onto Sabouraud dextrose agar to culture fungi. Thirteen of these individuals generated a total of 21 fungal isolates whose morphology suggested they might be *Candida* species. Analysis of these isolates by germ-tube test, culture on CHROMagar and YPD, did not provide any evidence of *Candida* species. DNA Sequences were obtained for 11 of the isolates and compared to similar sequences on Genbank using BLAST (Table 5.6). None of the sequences corresponded to *Candida* sp. instead relating to probable plant and insect associated fungi.

Date sampled	Sampling site**	Area‡	Subsample†	Result
29/01/2010	Bath Golf Course	Small seven sisters	1	Negative
29/01/2010	Bath Golf Course	Small seven sisters	2	Negative
29/01/2010	Bath Golf Course	Small seven sisters	3	Negative
29/01/2010	Bath Golf Course	Small seven sisters	4	Negative
29/01/2010	Bath Golf Course	Small seven sisters	5	Negative
29/01/2010	Bath Golf Course	Single Way	1	Negative
29/01/2010	Bath Golf Course	University Quarry	1	Negative
29/01/2010	Bath Golf Course	University Quarry	2	Negative
26/01/2010	Winsley	Lower Rift	1	Negative
26/01/2010	Winsley	Upper Rift	1	Negative
26/01/2010	Winsley	Upper Rift	2	Negative
26/01/2010	Winsley	Upper Rift	3	Negative
26/01/2010	Morton - Bradford-on-Avon	N/A	NA	Negative
30/01/2010	Brown's Folly	N/A	1	Negative
30/01/2010	Brown's Folly	N/A	2	Negative
30/01/2010	Brown's Folly	N/A	3	Negative
30/01/2010	Brown's Folly	N/A	4	Negative
30/01/2010	Brown's Folly	N/A	5	Negative
27/01/2009	Gripwood	Section B	1	Negative
27/01/2009	Gripwood	N/A	1	Negative
27/01/2009	Gripwood	N/A	2	Negative
17/09/2009	Wytham Woods	W95	NA	Negative
17/09/2009	Wytham Woods	W98	NA	Negative
17/09/2009	Wytham Woods	W61	NA	Negative
17/09/2009	Wytham Woods	W93	NA	Negative
17/09/2009	Wytham Woods	W103	NA	Negative
17/09/2009	Wytham Woods	CP11	NA	Negative
17/09/2009	Wytham Woods	CP108	NA	Negative
17/09/2009	Wytham Woods	CP7	NA	Negative
17/09/2009	Wytham Woods	CP107	NA	Negative
17/09/2009	Wytham Woods	CP2	NA	Negative
17/09/2009	Wytham Woods	CP104	NA	Negative
23/09/2009	Wytham Woods	CP3	NA	Negative
23/09/2009	Wytham Woods	CP118	NA	Negative
23/09/2009	Wytham Woods	CP135	NA	Negative
23/09/2009	Wytham Woods	CP100	NA	Negative
23/09/2009	Wytham Woods	CP149	NA	Negative
23/09/2009	Wytham Woods	CP152	NA	Negative
23/09/2009	Wytham Woods	CP108*	NA	Negative
23/09/2009	Wytham Woods	CP134	NA	Negative
23/09/2009	Wytham Woods	CP158	NA	Negative
23/09/2009	Wytham Woods	CP7*	NA	Negative
23/09/2009	Wytham Woods	CP110	NA	Negative
23/09/2009	Wytham Woods	CP30	NA	Negative
23/09/2009	Wytham Woods	CP147	NA	Negative
23/09/2009	Wytham Woods	CP35	NA	Negative
23/09/2009	Wytham Woods	CP36	NA	Negative

Table 5.5 – Summary of the samples screened for the presence of *C. neoformans*. *Boxes CP108 and CP7 were each sampled twice on different dates. **Sampling site indicates the cave system or summer site sampled. ‡Area gives the name of the region of the cave system or the reference for the Wytham Woods roost box from which the sample was collected. †Subsamples were taken from different positions within an area of a cave system. NA = applicable.

Host Species	Closest match on Genbank (% similarity)	Length of match (bp)	Family	Origin of closest match
B. barbastellus	*Zygotorulaspora florentina* (99.6%)	476	Saccharomycetaceae	N/A
M. nattereri	*Kazachstania servazzii* (99.4%)	493	Saccharomycetaceae	N/A
M. nattereri	*uncultured ascomycete* (98.8%)	323	Unknown	House dust
P. auritus	Saccharomycetes sp. HZ184 (100%)	609	Saccharomycetaceae	New Zealand Oak tree
P. auritus	Uncultured *Metschnikowiaceae* (99.6%)	271	Unknown	Caterpillar excrement
P. auritus	Uncultured *Metschnikowiaceae* (97.4%)	311	Unknown	Caterpillar gut
P. auritus	*Saccharomyces sp. HZ184* (99.8%)	483	Unknown	New Zealand Oak tree
P. auritus	*Saccharomyces sp. HZ178* (99.9%)	777	Unknown	New Zealand Oak tree
P. auritus	*Saccharomyces sp. HZ191* (99.8%)	462	Unknown	New Zealand Oak tree
P. auritus	*Lachancea thermotolerans* (99.8%)	572	Saccharomycetaceae	Olive paste
P. pipistrellus	*Saccharomyces sp. HZ184* (100%)	576	Saccharomycetaceae	New Zealand Oak tree

Table 5.6 – Results of sequencing fungal isolates. None were found to be *Candida* species instead representing probable plant and insect associated fungi.

5.4 Discussion

We undertook surveillance for Coronaviruses, Astroviruses, *C. neoformans* and *Candida spp*. associated with bats in Britain. We identified novel Alphacoronaviruses and Mammastroviruses in previously unknown host species but did not detect *C. neoformans* or *Candida* spp.

5.4.1 Coronaviruses

The British bat *Alphacoronavirus* strains we identified are distantly related to the zoonotic pathogen SARS-CoV (Figure 5.2). However, given Coronaviruses recognised ability to switch hosts (Graham & Baric 2010) and evidence suggesting that Alphacoronaviruses from bats have spilled over to humans in the past (Pfefferle *et al.* 2009), bats should be regarded as a possible but unlikely source of zoonotic Coronaviruses in Britain. The use of human dwelling places, cattle sheds and barns as nursery roosting sites by *M. nattereri* (Joint Nature Conservation Committee 2007) make it particularly interesting in this regard. The prevalence of Alphacoronaviruses we identified in *M. nattereri* and *M. daubentonii* were similar to prevalences observed in other species in Europe and Asia (Tang *et al.* 2006; Gloza-Rausch *et al.* 2008; Rihtaric *et al.* 2010). This suggests the virus is endemic in many species of bats throughout their range, and either causes prolonged infection or serially infects the same host. Multi-locus sequence typing of virus isolates combined with longitudinally sampled bats will be needed to address this issue.

In models of coronavirus prevalence, *M. daubentonii* juveniles were found to have higher prevalence of infection than adults across both seasons. This suggests that juveniles did not receive any effective maternal immunity during the nursery season. The lower prevalence in adults would be expected if there is some level of acquired immunity resulting in reduced susceptibility to infection in adults and could be tested with serological surveys for Coronavirus antibodies. Additionally there was a significant interaction between season and sex. Male prevalence, low in the nursery period was significantly increased in the post nursery season. This may be explained by our hypothesis that males mix with a number of female social groups in the post nursery period when mating may be occurring (Chapter 2). This mixing would expose the males to many individuals and therefore significantly increase their chance of exposure to the virus (Chapter 2). However, males may also experience increased energetic

demands in the mating period that could have immunosuppressive effects. The effect of nutritional status and hormones on bat immune systems has not yet been studied in detail, however this understanding will be important for predicting disease dynamics in the future.

It was hypothesised that, as directly transmitted diseases, the prevalence of Spinturnicid mites and Coronaviruses may depend on similar factors such as individuals' contact rates and immunity. However, Spinturnicid mite load was not found to correlate with Coronavirus infection. One possible explanation is that the mode of transmission is different. Mites are able to make an active choice as to whom they parasitise within a colony. Spinturnicid mites infesting *M. daubentonii,* when given a choice, are known to preferentially infest adult females (Christe *et al.* 2007). In contrast, viral transmission is a passive process and relies solely on contact between individuals.

The pattern of Coronavirus infection amongst *M. nattereri* is different from that observed for *M. daubentonii*. Juvenile male *M. nattereri* had an increased prevalence compared to juvenile females. This may be the result of behavioural differences between the juvenile sexes but will require more detailed observations to assess. For example, radio-tracking could be used to compare and contrast the movement of juvenile males and females. Additionally, when accounting for other variables, prevalence was found to be higher in 2009 than 2010. This may indicate that there is significant inter-year variability in prevalence, however, with only two years in this study more data is required to investigate this result.

As for *M. daubentonii,* Spinturnicid mite burden was not found to be related to Coronavirus infection for *M. nattereri.* The reasons for this poor correlation are likely to be the same as those outlined for *M. daubentonii* above. Despite finding no correlation, interactions between co-infecting pathogens due to changes in immune function or competition for resources can explain large amounts of variation in host susceptibility (Telfer 2010). Such interactions are likely to be present in this study system and could be a rich area for further research.

Unlike previous studies we did not observe an overarching effect of season (Gloza-Rausch *et al.* 2008; Drexler *et al.* 2011) in either species. Our data shows that the virus is present within the bat population as a whole through-out the summer. However, a larger longitudinal study is suggested to examine the temporal variation in prevalence in more detail. Neither was there a correlation between coronavirus infection and BCI suggesting that infection with the virus does not cause severe disease in the host.

We did not observe a significant effect of any of the social network variables on the prevalence of Coronaviruses. Degree has been found to be a good predictor of infection in other networks

as it is indicative of the number of contacts an individual has (Bell *et al.* 1999). In a highly social population, such as bats at Wytham Woods, betweeness was also thought to be an important measure. Individuals with high betweeness are those that move between socially isolated groups and would therefore have indirect contact with many more individuals. One possible reason for the non-significance of these variables is their inaccuracy. Values of degree and betweeness can be inaccurate if the number of observations for each individual is low (Croft *et al.* 2008) as was the case for the current study. As a result, the non-significant correlation with Coronavirus prevalence may be a result of an inability to accurately capture social network information rather than the absence of a relationship. Future work should examine how social environment of individuals, in terms of the colonies that they choose to occupy (i.e. colony size and colony type), effects their probability of infection.

These results highlight the importance of age, sex and season for predicting the prevalence of coronavirus in bat populations, and the inability of network parameters to do the same. This echoes the findings in Chapter 4 (4.4.4) where I argue that models of parasites in bat populations must take into account variation observed in the parasite load of different age and sex classes, as well as the seasonal changes in contact rates between males and females. This latter point is apparent in the present analyses where adult *M. daubentonii* have an increased prevalence in the post-nursery period compared to the nursery period, possibly due to increased contact between males and females. The sample size in this study is smaller than that in Chapter 4 and so fewer variables could be examined, however, the results support the suggestion that age, sex and seasonal changes in contact rates are likely to be key parameters in models of pathogens in wild bat populations.

5.4.2 Astroviruses

The prevalence of Mammastroviruses amongst bats was high (58%, 95% CI: 49-67%, n = 112), although an accurate estimate was not possible as the primers used were found to amplify non-target DNA in 17% of cases. Astroviruses are known to occur at high prevalence in Asian bats; a recent study found an average prevalence of 44.8% (n = 500) and up to 93% (n = 172) in some species (Zhu *et al.* 2009).

In contrast to Coronaviruses which often show host species specific clades (Figure 5.2), phylogenetic analysis showed that Mammastroviruses found in bats in Britain are diverse, even within the same host species (Figure 5.5). While I did not identify more than one strain infecting an individual bat I did find multiple strains infecting bats sampled at the same time

and location, including three individual *M. nattereri* sampled on the same day in one roost box. Infection of a local population with more than one strain has also been observed in bats from Asia (Zhu *et al.* 2009). Frequent co-occurrence of multiple strains within colonies will increase the likelihood of co-infection of an individual with more than one strain potentially permitting recombination between strains. Recombination events lead to new stains of virus to which the host may have less immunity than either of the parent strains. The majority of sequences from *M. nattereri*, *M. daubentonii* and *P. auritus* grouped with, or in sister groups to, clades identified as containing viruses from a variety of bat species (Zhu *et al.* 2009). Sequence data from Rhinolophid bats (*R. ferrumequinum* and *R. hipposideros*) did not support a close relationship with a previously identified clade for the rhinolophids (Zhu *et al.* 2009) (Figure 5.5). This may be an indication that European rhinolophid Astroviruses do not group with those from Asia, however more sequence data from bats in Europe is required to resolve this.

One RdRP sequence from *M. nattereri* was found to be closely related to rat Astroviruses from Hong Kong (Figure 5.5). Rodents are occasionally observed in bird boxes in Wytham Woods and yellow necked mice (*Apodemus flavicollis*), wood mice (*Apodemus sylvaticus*) and hazel dormice (*Muscardinus avellanarius*), have also been recorded in other bat box schemes in the South-west of England (Gareth Harris and Steven Laurence, personal communication). It is not possible to say from the current data whether the strain identified was transmissible between bats, or whether the bat was infected by rodent droppings in the box. Further sequencing of samples from this group of bats and rodents within Wytham Woods could give a better picture of how common and widespread this strain is. The presence of a strain closely related to rat viruses in bat faeces highlights the possibility of cross species transmission of Astroviruses. This would not be without precedent as it is thought that a recombination event may have occurred between human and Californian sea lion Astroviruses leading to zoonotic spillover (Rivera *et al.* 2010).

The diversity of astroviruses identified from relatively few bats within a small area of England exemplifies the diversity of this virus family amongst bats and supports the hypothesis presented by Zhu *et al* (2009) that bats may be the ancestral host to many mammalian Astroviruses. However, as this virus group has been the subject of limited study in wildlife, more sequence data from a wide range of mammals is needed to test this hypothesis.

5.4.3 Crypotococcus neoformans and Candida species

Though our study did not find evidence of *C. neoformans* or *Candida* spp., this does not rule out their presence amongst British bats. Our search for *C. neoformans* investigated both summer and winter roost sites. Summer roost sites in Wytham Woods are cleared of all bird nests and faeces in the spring of each year by the EGI ornithological research teams and as such the summer may not provide sufficient time for *C. neoformans* to establish. Samples collected from winter roosts in caves were from scattered droppings rather than large piles of guano that typically collect under large, long-established roosts. We did not have access to such deposits but where they exist in both summer and winter roosts testing for *C. neoformans* may be more successful. While *Candida* spp. were not found, a number of other fungi were identified. There is no evidence to suggest that these were pathogenic but instead are probably associated with plants and insects. Indeed the majority of the fungi sequenced are from the family Saccharomycetaceae, a family that, as its name suggests, is often associated with carbohydrate rich environments such as fruits and flowers. It is therefore likely that these fungi are ingested along with insect prey.

5.4.4 Risk to human health and prevention of transmission

Zoonotic disease spillover events are stochastic and therefore difficult to predict. However, as discussed in Chapter 1, when past events are reviewed there are factors that appear to increase the risk of zoonotic spillover. These include the pathogen being an RNA virus (Cleaveland *et al.* 2001) and high contact rates between wildlife and humans or domestic animals (Chau *et al.* 2002; Breed *et al.* 2006; Bradley & Altizer 2007) caused by hunting, agricultural intensification and urbanisation (Daszak *et al.* 2001).

We have shown that RNA viruses with zoonotic potential are present in bat populations in Britain. If a bat virus infected a human or domestic animal there is a small chance that, as with SARS, an outbreak in humans could ensue. The risk of such a spillover event occurring can be reduced by limiting contact between bats and humans or domestic animals.

Bats commonly use barns as roosts sites in Britain (Joint Nature Conservation Committee 2007), however, these reports typically consider barns that are no longer used to shelter domestic animals. A survey of experienced UK bat surveyors revealed that bats in the UK also roost and feed in stables and barns with domestic animals present (David Dodds, Keith Cohen, Fiona Mathews and Daniel Hargreaves, personal communication). Surveyors reported *M.*

nattereri, *P. auritus*, *P. pygmaeus*, *R. hipposideros* and *R. ferrumequinum* roosting or feeding in stables and cattle sheds with domestic animals present. In mainland Europe *Myotis emarginatus* (Geoffroy's Bat) is known to spend approximately 30% of its foraging time in cattle sheds feeding on flies that thrive on the high density of cattle manure (Zahn *et al.* 2010). Additionally a study in Hesse, Germany found *M. nattereri* frequently roosted in or close to cattle sheds (Simon *et al.* 2004). Using results from temperature loggers the authors suggest bats may be attracted to roost in cattle sheds as a result of the warmth provided by the cohabiting cattle. The use of barns as roost sites by bats may therefore be attributable to agricultural intensification (Simon *et al.* 2004; Zahn *et al.* 2010), but may also be a result of the loss of natural roosting habitat (Altringham 2003).

Contact between bats, their urine and faeces and domestic animals in this setting could be reduced by excluding bats from the inside of sheds, barns and stables. However, given the co-occurrence of bats and domestic animals in these structures that is likely to have occurred over hundreds of years, it could be argued that were these bat viruses to spillover to domestic animals and humans it would already have occurred. The gain from excluding bats from these structures in terms of reduced risk of disease emergence is small and the potential impacts on bat conservation are high and as such this course of action is not recommended. While the exclusion of existing roosts is not worthwhile it should certainly be advised that new roosts, such as bat boxes (erected to house bats displaced by construction or demolition works), not be placed where bats or their droppings would be in prolonged contact with domestic animals.

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6 General discussion

6.1 Introduction

Human activities leading to the loss of natural habitat have caused population declines amongst bats (Altringham 2003) and put them in close contact with humans (Plowright *et al.* 2011). The emergence of Ebola, Nipah, Hendra and SARS can in part also be attributed to human activities including habitat destruction (Chua *et al.* 2002; Plowright *et al.* 2011) and hunting (Leroy *et al.* 2009). Such zoonotic diseases are now capable of spreading rapidly across the globe as demonstrated by SARS in 2002 and more recently by pandemic H1N1 (2009) ('Swine flu') which became a global pandemic within months of being first identified in humans in Mexico.

The cost of emerging infectious diseases originating for wildlife, such as SARS and HIV, both in terms of human lives and economic cost, are great (Dixon *et al.* 2002). However, predicting where and when these diseases will emerge is difficult. Bats have been identified as a common source of emerging infectious diseases and as such, the past 15 years has seen an increase in research into wild bat populations. These studies have sought to identify known and unknown pathogens that may be of human health concern (Johara *et al.* 2001, Leroy *et al.* 2005, Tang *et al.* 2006), and have attempted to predict the drivers of disease emergence (Plowright *et al.* 2011, Dimitrov *et al.* 2008).

It is thought that increased prevalence of a pathogen in wild bat populations has lead to the emergence of diseases in the past (Plowright *et al.* 2008; Wacharapluesadee *et al.* 2010) and so predictive models of pathogen prevalence in wild bat populations may prove a useful tool for predicting the emergence of diseases of the future.

Predicting prevalence is dependent on understanding the drivers of susceptibility and transmission in the population. These parameters are dependent on individuals' attributes (e.g. age, sex, etc.), contact network, the dynamics of these parameters over time (e.g. day, season or life-time), and the heterogeneity amongst individuals within the population (see section 1.5.4). Susceptibility is dependent on an individual's immune system. Immunology is poorly studied amongst temperate bats, with the only detailed studies focussing on lyssaviruses (O'Shea *et al.* 2003; Turmelle *et al.* 2010). Contact rates, key to understanding transmission, are similarly poorly understood. Research to date has been limited by the feasibility of making observations on all individuals in a population. Studies therefore typically

study only a few large roosts in the landscape (Entwhistle *et al.* 2000; Park *et al*. 2000) or track a small number of individuals over a short time period using radio-tracking (Johnson, 2012; Garroway, 2007). Additionally analysis of population genetics at national and continental scale have been used to infer movement of individuals (Atterby *et al.* 2010, Bryja *et al.* 2009) and possible rates of disease transmission (Smith *et al.* 2011) but these tell us little of the heterogeneity of contact rates within populations and how these change over short time periods.

In addition to understanding how bat population structure may affect pathogen prevalence, surveillance of bat populations for pathogens of potential human health concern is important for predicting future spillover events. For example, pathogens closely related to those found in humans, or with characteristics linked to spillover (e.g. generalist viruses with high mutation rates) would warrant greater concern. Additionally these pathogens allow us to test models of disease transmission.

An understanding of pathogens infecting bats, and how they spread through populations is not only important from a human health standpoint. Such information may also be important for their conservation as diseases can cause population declines, as demonstrated by the current spread of white nose syndrome across North America (Blehert *et al.* 2009). Understanding where such diseases originated and how they are spread could help to design strategies to reduce or reverse impacts on bat populations.

Bat populations have been adversely affected by the increase in human population size over the past millennia. No more so than in the past few hundred years in which we have seen a rapid increase in extinctions, loss of biodiversity and anthropogenic climate change (Chapin *et al.* 2000). To conserve bat populations in areas where habitat is being lost or in areas where habitat creation or regeneration is planned it is important to have an understanding of their ecology. For each species of conservation concern this should include details on: life history, social structure, habitat requirements for roosting and foraging, and their response to environmental perturbation such as roost loss. While some of this information is known for many temperate bat species, important details such as the social structure of populations, roost preferences of species, and the area needed to support a population remain poorly understood for many species.

Much of the information required for effective conservation of bats and for accurate modelling of disease in bat populations are the same. Therefore in this thesis I present the conservation implications of the findings alongside the implications for models of disease.

The aim of this thesis was to further our understanding of woodland bat ecology and social structure to better assess how disease may spread through populations. The population structure was investigated over 6 summers providing novel insights into the ecology of bats and how diseases may spread through populations. The impact of the population structure on ectoparasite and pathogen distribution was tested, and at the same time novel viruses of potential human health concern were identified. Additionally, data presented has implications for the conservation of bat populations. Here I discuss my findings in the context of broader research and suggest areas where future work could provide valuable new information.

6.2 The population structure of bats: implications for models of disease

The simplest models of disease use the theory of mass action, where contact rate is assumed to be uniform between individuals, however, recent studies have found this is not an accurate reflection of reality (Bansal *et al.* 2007, McCallum *et al.* 2001). Instead, knowledge of population structure is recognised to be key to predict the spread of pathogens (Perkins *et al.* 2009).

To parameterise epidemiological models of disease parameters must be estimated or derived from empirical data. Chief amongst these are individuals' susceptibility and transmission rates. Susceptibility is the probability that an individual will become infected given appropriate contact with an infected individual. Transmission rate is the rate at which an infected individual exposes others to a pathogen and is dependent on the contact rates between individuals as well as their infectivity. Variability, as well as the absolute values, of susceptibility and transmission rates have been shown to be important in models of disease spread in a number of recent studies (Lloyd-Smith *et al.* 2005; Beldomenico & Begon 2010). Variability in susceptibility can lead to individuals with high disease burden, and variability in transmission rate can result in a small number of individuals with a large number of contacts with others. In both instances this can result in super-spreaders, individuals who contribute disproportionally to the spread of disease within a population (Kramer-Schadt *et al.* 2009; Perkins *et al.* 2009; Beldomenico & Begon 2010; Gardy *et al.* 2011). This is quite different from the theory of mass action (McCallum *et al.* 2001). Targeting super-spreaders during a disease outbreak can help to control its spread (Salathé & Jones, 2010). Social networks constructed from information on contact events, such as in this thesis, can be used to assess rates of transmission amongst individuals.

Amongst bats, very little is known about the heterogeneity of contact rates within a population, in fact, even the definition of a population of bats is poorly understood. In this thesis I have described, in detail, the population structure of two woodland bat species and discussed the implications this had for models of disease.

The population of *M. nattereri* inhabiting Wytham Woods showed a clear separation into social groups with high intra-group connectivity but very low inter-group connectivity (Chapter 2). Furthermore a social group was often found split over a number of roosts on any given day. This observed structure, which is likely to approximate the true contact network, clearly does not fit the assumption of mass action, instead suggesting that contact rates between

individuals, at the scale of a wood such as Wytham, is highly variable. With the observed structure it is likely that pathogens will spread rapidly within social groups but slowly between social groups.

The population structure observed in *M. daubentonii* was different from that of *M. nattereri*. Females had a structure similar to that of *M. nattereri,* however, males showed higher levels of mixing facilitating a higher level of connectivity in the population as a whole. As with *M. nattereri* this social structure demonstrates a high level of variability in the contact rates between individuals in the population. Since males are responsible for connectivity between female groups it could be hypothesised that they would be important for the transmission of disease through the population. However, this has not been supported by some empirical studies of wild mammal populations. A similar structure has been observed in populations of meerkats (*Suricata suricatta*). In meerkat populations males are known to rove between social groups, these males have been found to have an increased risk of infection with TB (*Mycobacterium bovis*) however members of the groups visited were not found to be at increased risk of infection (Drewe 2010). Similarly nomadic lions (*Pantera leo*) that move between prides were not found to be important in the transmission of disease in the population. This was in part because their movements were infrequent and less important than occasional pride-pride interactions (Craft *et al.* 2010). Despite uncertainty in its implications, this new understanding of contact rates in summer populations of *M. daubentonii* may have implications for the spread of diseases, for example EBLV-2, a lyssavirus, known to be present in *M. daubentonii* in Britain, and should be included in future models of this virus.

The strong social structure apparent amongst *M. nattereri* and *M. daubentonii* is similar to the assortative networks discussed by Eames (2007). These networks, in which intergroup contact is low, have reduced overall prevalence and select for chronic benign infections as immunity within a sub-group is quickly achieved. Infections in assortative networks can be best controlled by identifying individuals that may be responsible for transmission between groups such as male *M. daubentonii* in this study. However, this will be moderated by individual behaviour and immune systems. Additionally rare contacts between individuals from different social groups are likely to be important in models of diseases in bats but will only be revealed by social network studies with high temporal resolution

To ensure that the observed population structure was not a result of the aggregation of bats at a limited number of roost sites, the roost preference of the species was investigated (Chapter 3). *M. nattereri* and *P. auritus* showed high variability in the roosts they used when using a

number of local and landscape variables suggesting that roost sites for these species were not limiting. *M. daubentonii* showed lower variability, however, there were many roosts available in the areas preferred by this species (i.e. close to water) and over the course of this study 162 roosts were found occupied by this species. This data supports the conclusion that the population structure observed is a result of behaviour, rather than environmental constraints, and is therefore likely to be common to other populations of the same species occupying similar habitat. Recent work by Angell *et al* (2013) suggests that the population structure of *M. daubentonii* differs over an altitudinal gradient and therefore care must be taken when extrapolating the results presented to dissimilar habitat.

There are a number of limitations when using the social network data presented to predict disease transmission. Firstly, the network is the result of pooling data over 6 years, which is a different time scale to that over which transmission is likely to occur. In addition, since data is pooled over a long time period we do not know how the structure of this network changes over time. The dynamics of population structure are known to be important, but difficult to measure in wildlife populations (Craft & Caillaud 2011, Keeling & Eames 2005).

To better understand the change in social structure over short time periods the composition of colonies was recorded throughout the summer (Chapter 2). Results showed that both species had a greater number of mixed sex colonies in the post-nursery period compared to the nursery period.

The results from chapters 2 and 3 suggest that transmission rates are likely to be higher within social groups than between social groups, and in the nursery period, higher within nursery colonies (those dominated by females) and bachelor colonies than between these colony types. By studying the ectoparasites and pathogens of this population I was able to test these predictions.

Empirical studies of the distribution of ectoparasites and coronavirus found support for the predicted effect of colony type (Chapters 4 and 5). During the nursery period individuals in nursery colonies experience high parasite burdens, perhaps as a result of increased susceptibility of females and juveniles, and Spinturnicid mites preference for females (Christe *et al.* 2007). This high parasitism was not seen in the bachelor colonies whose individuals were segregated from females during this time of the year (Chapter 4). Models of coronavirus found a related trend amongst *M. daubentonii* (Chapter 5). Adult males, isolated from nursery colonies in the nursery period, had a low prevalence of infection, but this increased in the post-nursery period when bachelor colonies were found infrequently and mixed colonies where more commonly observed.

Parameters describing the position of individuals within the social network were poor predictors of disease prevalence. Degree, the number of individuals a target individual has been recorded roosting with, and betweeness, the importance of an individual for connecting others in the networks, where both found to have generally low importance. As suggested previously, the poor ability of these parameters to predict disease prevalence may be a result of the time scale of the study. For example the degree of an individual over the previous week may be a good predictor of whether it is infected with a disease, while the degree over several years, as in this study, may be a relatively poor predictor.

Social group (females only when testing *M. daubentonii*) was found to be an important parameter in models of ectoparasites for both *M. nattereri* and *M. daubentonii*. This was not related to the size of the social group, but might be expected from the social structure we observed. Low connectivity between social groups suggests that stochastic variation in the abundance of parasites or the prevalence of pathogens would not be averaged out across the population. Instead, given the isolation of the social groups from one another, the relative differences in disease prevalence should be approximately maintained throughout the summer.

Future models for disease in bat populations should include colony type, sex, reproductive status and age, since these were all found to be important in our empirical studies. Additionally, increased susceptibility of reproductive females and juveniles during the nursery period, and seasonal variation in social networks, should be the target of future research. Future models could be tested against the empirical data used in this thesis to test their ability to produce realistic results.

Different network measures to those used here may better predict infection risk. Studies have highlighted, for example, the benefits of including network parameters such as path length and clustering coefficient (Ames *et al. 2011*) and using weighted networks in which the direction of behaviour is recorded (Keeling & Eames, 2005). Future analyses which consider these options will undoubtedly make use of rapid technological advances being made in wildlife tracking. For example, the continued miniaturisation of GPS trackers, radio-transmitters and RF-ID tags (Craft & Caillaud, 2011). These technologies will provide wildlife researchers with more accurate data at increased temporal resolution, allowing more accurate quantification of network structure.

6.3 Pathogen surveillance

This work found, for the first time, that Coronaviruses infect bats in the UK (Chapter 5). This finding adds to the consensus that this group of viruses are endemic in many bat species and have a global distribution. We found closely related Alphacoronavirus sequences in *M. nattereri* from Savernake Forest and Wytham Woods, and *M. daubentonii* in Wytham Woods hosted viruses closely related to strains identified in Germany. These and other published data suggest that Coronaviruses are undergoing co-evolution with particular host species.

If directly transmitted viruses followed the same trends that we observed for the directly transmitted mites, the nursery period would be a high risk period for spillover events. However, a seasonal effect on prevalence was not seen in our model of Coronaviruses (Chapter 5). The molecular analysis used for Coronaviruses in this study could be modified to provide data on the amount of virus detected in the faeces. Real-time PCR or quantitative PCR (qPCR), a method that can measure the amount of genetic material present in a sample, could be used to detect peaks in virus shedding. This would provide data better suited to predicting periods when the risk of disease spillover is highest.

An area that has been significantly under researched to date is bat immunology. Laboratory and field studies of rabies in bats show that bats produce antibodies to rabies virus (O'Shea *et al.* 2003; Turmelle *et al.* 2010) but there is little evidence that these antibodies provide protection against subsequent infections. Understanding the mechanisms and dynamics of bat immune systems in response to infection with specific pathogens is important for predicting individuals' susceptibility to disease, and to understand transmission rates among individuals in a social network. For example studies of field voles (*Microtus agrestis*) suggest that individuals with increase susceptibility are not only more likely to become infected but may be the most important source of infection in others (Beldomenico *et al.* 2009). Susceptibility is therefore an important factor for modelling pathogen prevalence in a population and predicting when spillover events may occur. Maternal passive immunity is thought to be an important factor in driving susceptibility in bat populations (Chapter 1). A means to measure antibody responses to a pathogen would help to investigate the length of protection afforded to juveniles by maternal passive immunity and would indicate when juveniles become most susceptible to infection, a time when disease prevalence would be likely to increase (Wacharapluesadee *et al.* 2010).

Our analyses of Astroviruses RdRP sequences present in UK bats revealed a wide range of viruses. This data suggests that unlike Alphacoronaviruses which showed species-specific

associations, a single species of bat may host more than one distantly related Astroviruses. For example we identified Astroviruses infecting *M. nattereri* from 4 different clades (with similarity as low as 55%) and observed three of these strains infecting individuals in the same roost box at the same time. Sequences from one of these clades were closely related to sequences reported from rodents in Hong Kong (Chu *et al.* 2010). If the presence of this virus is taken to be indicative of active infection then this virus may be able to infect both bats and rodents. Coinfection with this virus and other Astrovirus strains may permit recombination which is often presumed to be a precursor to the emergence of infectious diseases. Understanding the potential for recombination between Astroviruses infecting bats and viruses from other species will require a great deal of further work.

Assessing the risk to humans from Astroviruses and Coronaviruses hosted by wild bat populations is very difficult. While these viruses have high prevalence amongst some UK bat species, contact between bats and humans or domestic animals is very infrequent. This gives little opportunity for the virus to infect and replicate in these potential hosts. Therefore the risk of a spillover from bats to humans is extremely low. Nevertheless it is worth knowing what pathogens are present in our wildlife and their biology in their natural hosts so that if a novel pathogen did emerge in man we could rapidly identify and isolate the source. Whilst the risk of disease spill over from bats in the UK has been identified as extremely low, a common route of disease emergence identified in Chapter 1 is from wildlife to domestic animals. It is therefore recommended that artificial bat roosts are not placed in areas where domestic animals will be in prolonged contact with bats, their faeces or urine, such as in and around cattle sheds, stables and piggeries.

6.4 Ecology and conservation of bats

Our study of bats in Wytham Woods shows that bat populations within a contiguous wood from distinct social groups. Although both *M. daubentonii* and *M. nattereri* were studied using the same roost type (bird boxes) the social structure was different for each species. While *M. daubentonii* males were frequently observed in bachelor groups and often associated with more than one female social group, *M. nattereri* males were observed less frequently and only associated with a single, mixed sex, social group. Female *M. daubentonii* and both sexes of *M. nattereri* formed social groups with spatially restricted roost home ranges. Within species there was very little overlap between social group roost home ranges suggesting that there may be competitive exclusion between social groups. This has not been explored previously and is worthy of further investigation. If territoriality does exist it is likely that any major change to habitat, or high mortality within social groups, could lead to increased conflict between groups, analogous to that seen in populations of badgers after culling (Tuyttens *et al.* 2000).

This thesis identifies bat social groups as the functional unit of the population which should be the target of conservation (Chapter 2). At present UK law protects individual bats and roosts from disturbance or destruction (Mitchell-Jones & McLeish 2004) and as such protects social groups. This protection is afforded by the Habitat Regulations put in place by the UK government as required by the EU Habitat Directive. These regulations are currently under review with the aim of reducing 'red-tape' by streamlining guidance. While future research may show that some roosts are of greater importance to social groups than others, such a detailed investigation is beyond everyday bat survey work. As such the current regulations, giving protection to all roosts, are the most practical, and best protect social groups.

When aiming to restore, retain or create habitat for bats the observed population structure should be taken into account. Our study showed social groups used numerous roosts within a small area of woodland. Though more work is needed to investigate if these patterns are seen across other woodlands and habitat types, these results support efforts to increase roost availability in habitats where they are lacking (e.g. immature woodlands) either by introducing artificial roost sites or encouraging the retention of mature trees.

More consideration should also be given to the implications social structure has for bat conservation in woodlands that are being managed for timber. In these woodlands trees are sometimes clear felled which may quickly remove much or all of the roost home range of a given social group, especially since this thesis suggests the roost home ranges of *M.*

daubentonii and *M. nattereri* may be only 42-57 acres. Given the population structure observed, significant disruption or local extinction of social groups is more likely than if they had larger, overlapping roost home ranges. Additionally, felling is likely to directly increase mortality and may increase competition between neighbouring groups for suitable roosts. It is currently recommended practise for woodland managers to leave mature or standing dead trees when felling to encourage wildlife that depend on them. This practice is undoubtedly beneficial to bats and other wildlife. However, since species were found to roost in areas close to favourable foraging habitat, the quality of these roosts to woodland foragers such as *M. nattereri* and *P. auritus* will be diminished when surrounding trees are removed. The present study considers social groups occupying deciduous woodland, while areas managed for timber are primarily coniferous. Caution should be taken when extrapolating our results to the behaviour of species in these woodlands.

The social structure observed will undoubtedly have implications for how populations react to habitat change such as tree felling. Except for male *M. daubentonii*, we observed very few movements of individuals between social groups and so it is unknown whether a decline in one social group could lead to the integration of individuals from another social group as is observed in classic source-sink metapopulation models (Hanski 1999). Though it may appear obvious to think of these social groups in a metapopulation framework this should be done so with caution. Metapopulation theory focuses on the size and connectivity of patches. While it may be true that small social groups are more likely to become extinct than large social groups our understanding of immigration and emigration in social groups is extremely limited. Dispersal between social groups is likely to operate at local and landscape scales and consist of temporary and permanent migrations. The probability of immigration may be related not only to colony size but the probability of acceptance by individuals within the social group. It is also unknown how new social groups may form after local extinctions or die-offs in societies with a high level of structure such as bats. Current studies are limited to either temporary fission-fusion dynamics, as in elephants (Archie *et al.* 2006) or the formation of social groups in captivity (Seres *et al.* 2001). Likely mechanisms of group formation include the splitting or merging of social groups, as has been observed in human populations (Palla *et al.* 2007). The lack of studies among wild animals is likely due to the need for high resolution long-running studies.

Very few similar studies of bat social structure have been undertaken in the UK (Park *et al.* 1998; Entwistle *et al.* 2000; Smith 2000; Rossiter *et al.* 2002) and to date no studies have been published that use social network analysis. Therefore much more research of social structure amongst different species and habitat types is needed. For example, many of the bat-human

conflicts that arise in the UK, such as the presence of bat roosts in buildings needing development, are in urban areas. An understanding of the population structure of specific bat species in this environment using the framework presented here would be invaluable for identifying solutions to such conflicts.

Whether protecting areas of high quality habitat or identifying ways to improve the quality of existing habitat, an understanding of bat roost preferences is important. This thesis suggests roosts are selected for their proximity to preferred foraging habitat. *M. daubentonii* prefer roosts close to water, while *M. nattereri* and *P. auritus,* both woodland foragers, roosted throughout the wood (Chapter 3). Detailed data on foraging behaviour is limited for *M. nattereri* and *P. auritus,* an area where more work is needed. Indeed, as it is suggested bats choose to roost close to their foraging habitat, efforts to identifying the specific foraging habitats preferred by each UK species and the roosting opportunities available in proximity to these areas may be a valuable approach to identify and conserve areas important for bats to prosper.

This study found for the first time that woodland bats BCI (i.e. weight relative to their physical dimensions) was correlated to weather conditions. Notably, BCI was lower in *M. nattereri* and *P. auritus* after nights with high wind speed whereas *M. daubentonii* appeared unaffected. This suggests a relationship between wing shape and the effect of windspeed, with broad winged bats such as *M. nattereri* and *P. auritus* which are adapted to agile, slow flight, more greatly affected than narrow winged species adapted to faster flight such as *M. daubentonii*. Wind speeds may increase with climate change (Pryor *et al.* 2005), though the magnitude may not have a significant effect on bats. *P. auritus* had increased BCI after warm nights, when it is thought moths, a major part of their diet, are in greater abundance (Anthony *et al.* 1981; McGeachie 1989). Insects' abundance and distribution are known to be responding to climate change (Hickling *et al.* 2006) and their predators, including bats, might be expected to alter their distribution with them. It is beyond the scope of this study to predict the impacts climate change may have on bats, however, identifying dominant prey species and the expected shifts in their distribution would be a reasonable first step to identifying those bat species that may be most severely affected in the future.

6.5 Questions raised

Here I propose a series of research programmes that would address some of the issues raised in my thesis. These programmes will improve our understanding of the dynamics of diseases in bats populations and improve bat conservation.

Exploring the dynamics and adaptability of social networks

Studies of wildlife populations using graph theory (i.e. social networks analysis) have only appeared in the past 10 years and are still lagging behind advances made in the social sciences. Studies of wildlife populations, as in this thesis, are typically limited to an analysis of one time period and a simple description of the population's structure. Few studies present data collected in more than one time period, and when this is done information for each period is typically limited preventing an accurate description of the network structure (Perkins *et al.* 2009).

Understanding the changes in social network structure through time and how networks change in response to external effects is important for predicting the effects of human activity on social animal populations. Studies of human networks already explore changes through time (Kossinets & Watts 2006; Palla *et al.* 2007) but few wildlife studies do the same (Cross *et al.* 2004; Perkins *et al.* 2009). The lack of research into temporal dynamics is primarily due to difficulties collecting detailed data at a high temporal resolution, while manipulation experiments in species such as elephants and whales, which are often targets of SNA studies, are impractical. Some studies of wildlife populations suggest that the removal of key individuals could lead to a breakdown of population structure (Wittemyer *et al.* 2005; Williams & Lusseau 2006), but with rare exceptions (Flack *et al.* 2006) few have tested these hypotheses.

A recent study by Kerth, Perony *et al* (2011) has shown that high temporal resolution social networks can be generated for bat populations using passive integrated transponders (PIT) tags and automated readers. This approach can be enhanced by radio-tracking a number of individuals in the population as implemented by Garroway and Broders (2007), allowing colonies to be more easily followed. This method could be applied at Wytham Woods, a study system that offers the possibility for manipulation experiments that are not practical in other populations. The use of roost boxes by bats allows entire colonies to be translocated or for roost boxes to be selectively removed.

High temporal resolution data of bat associations and movement between roosts using PIT tags would provide insights into the sub-structure of social groups, evidence of hierarchy, and how these change over an annual and multi-year time scale. The Wytham Woods study system would allow an assessment of the adaptability of social groups in the face of disturbances. For example, by a) examining the change in social and spatial structure of social groups after the removal of roosts, b) examining the effects of introducing novel radiotracked individuals to explore whether individuals can integrate into foreign social groups, c) removing individuals to assess their role in maintaining group cohesion.

This research would significantly further our understanding of the dynamics and adaptability of wildlife populations and the impacts of roost lost on UK bat populations. As bats and their roosts are protected in the UK this work would need the support of the Statutory Authority, Natural England.

What is the social structure of an urban bat population?

Most conflict between humans and bats occurs in urban settings. Future research should examine whether urban bat populations of the same species studied at Wytham Woods have a significantly different population structure than woodland bats. Since *M. nattereri* is a more frequent inhabitant of urban structures than *M. daubentonii* this would be the recommended study species.

This research would be carried out in an area with abundant *M. nattereri* roosts within a contiguous urban landscape. Such an area could be identified through collaboration with Natural England and bat groups who hold information on known roosts. Initial work would focus on ringing individuals in the population and radio-tracking individuals to other roosts in the area. To get an accurate picture of the social network it is important to capture bats at as many roost sites in the area as possible and it is for this reason that radio-tracking is key. The second phase of the research would focus on capturing and ringing individuals at the roosts identified by radiotracking. The social network could then be characterised using the SNA techniques applied in Chapter 2 of this study and compared to the woodland network. As mentioned previously PIT tagging could also be used to increase the temporal resolution of the data.

This work would allow questions to be answered about urban populations relevant to their conservation. For example the research would highlight the number of roosts and area used by a single social group. Additionally, it may be possible to identify a study population due to

undergo disturbance, such as the exclusion of a colony from a roof space. This would provide the opportunity to assess the effect displacement has on the structure of the bat population as well as the importance of mitigation, such as artificial bat roosts, in maintaining the population after exclusion.

Social structure, heterogeneity and co-infection: Modelling diseases in wildlife populations.

By the virtue of the limited data available, models of diseases in wildlife populations are often over simplified. This includes assumptions of random mixing (McCallum *et al.* 2001, Perkins *et al.* 2009), the use of mean values for transmission and susceptibility parameters (Lloyd-Smith *et al.* 2005) and a focus on a single pathogen (Telfer 2010). Here I suggest a programme of research that will examine the effects of these assumptions on models of bat associated pathogens.

Our analysis of social networks at present is coarse, but by implementing the proposals outlined in the two sections it would be possible to quantify the social network more accurately, including assessing the dynamics of the system. These analyses can be used to estimate contact rates and their heterogeneity between classes of individuals (e.g. juveniles and adults), seasons, and after disturbance events. Models using average data and data inclusive of individual variability would be compared to assess the importance of heterogeneity for model predictions.

To test the ability of models to accurately predict disease within the population will require surveillance of pathogens in greater detail than presented within this thesis. It will be necessary to identify all of the strains of Astroviruses and Coronaviruses present in each individual, as each strain may have different dynamics within the population. This could be achieved through Multi-Locus Sequence Typing (MLST) which uses sequence data from a number of representative regions of the virus genome to assign samples to strains. This method would first require the full genome sequences of the strains identified in this study from which primers could be designed for the MLST. Additionally it may be advisable to undertake a metagenomic analysis of faeces and blood in order to establish which other pathogens of interest are present and worthy of inclusion in this study.

Susceptibility is virtually unexplored amongst bats (but see Christe *et al.* 2000 and Turmelle *et al.* 2010), however, it is important for accurate models of disease (Beldomenico & Begon 2010). General measures of susceptibility could be investigated using haematological indicators such as red blood cell (RBC) and lymphocyte counts. Low counts of red blood cells

can be an indication of malnutrition (Piersma *et al.* 2000) while low lymphocyte counts can be an indication of reduced immune function (Shetty 2001). More specifically susceptibility to known pathogens could be inferred from assays to detect the presence of pathogen specific antibodies. Assays would be developed to assess the presence of antibodies to Astroviruses and Coronavirues amongst bats. This would include a plaque reduction neutralization test (PRNT) assay if it were possible to culture the viruses, or immunoassays using expressed viral proteins or synthesised predicted peptide epitopes should culture prove impossible. Additionally a previously described protocol for the detection of rabies antibodies would be used (Brookes *et al.* 2005).

Collecting blood samples from individuals at Wytham Woods at multiple time points throughout the year it will be possible to assess variation in susceptibility between groups of different age, sex and reproductive status. Additionally multiple samples per individual would permit an assessment of variation within individuals. The assumption that these variables correlate to susceptibility could be tested by using them to predict whether individuals are more or less likely to become infected, and testing predictions against field observations (Beldomenico *et al.* 2009).

The duration of immunity is likely to be an important variable in epidemiological models, especially as bats are long-lived, but would be difficult to study in a field setting where repeated exposure to pathogens would confound results. An investigation in captive individuals would allow the duration of immunity to be examined by exposing individuals to a virus and then challenging them with the same virus at set time intervals, monitoring them for evidence of viral replication. This would also allow the importance of red blood cell counts, lymphocyte counts and pathogen specific antibodies (measures of susceptibility) for predicting whether challenged individuals will become infected.

Results of both field and laboratory work would be used to parameterise an acquired immunity function into disease models. As with estimates of transmission, models would be compared with and without individual variation detected to assess its importance for model predictions.

Infections can increase, decrease or have no effect on the probability that an individual will become infected with a second pathogen. Positive associations between pathogens can result from down regulation of the immune system while negative associations can result from cross-effective immune response (Telfer 2010). Using time-series data it would be possible to test whether infection with one pathogen influenced the probability of co-infection at a future time

point. Additionally ectoparasites could be included in this analysis to study interactions between ectoparasites and pathogens.

Models of disease inherently seek to simplify complex systems into general equations. However, studies are now showing that natural variability is important for accurate predictions. This large research programme would quantify the variables important for population models and assess the importance of variability for generating accurate predictions. More specifically this work would increase our understanding of the immune system of bats, the processes that lead to high disease prevalence including the effects of disturbance and the effect of social structure. This would highlight periods of risk for disease spillover. Surveillance of rabies antibodies in this well characterised system may help explain why the virus is primarily found in *M. daubentonii* and how it is maintained within the UK at a very low prevalence.

6.6 Final remarks

Bats are often perceived as a pest, in part as a result of their association with disease. This is perhaps no more true than in the past 15 years when the media have delighted in publishing alarmist articles about the discoveries of diseases in bats. This has generated tensions between conservationists and disease researchers, the former believing that continued research in to bat diseases harms conservation efforts and reaffirms the image of bats as a pest in the minds of the public.

In this thesis I have shown that many of the aims of conservation and disease research are the same, and centre on reducing contact rates between bats and humans. This reduced contact lowers the probability of disease spillover and reduces stress placed on bat populations by activities such as habitat destruction. Both research areas can benefit from collaboration and some research, such as that into Hendra (Plowright *et al.* 2008), is beginning to highlight the importance of reducing anthropogenic pressures on bats to prevent disease spillover. This interdisciplinary research should be encouraged and will benefit both bat conservation and disease spillover prediction and prevention.

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7 Appendix

Date	Box	Species	Date	Box	Species
05/08/2006	C111	*M. daubentonii*	12/09/2006	O218	*M. nattereri*
05/08/2006	C113	*P. pygmaeus*	12/09/2006	O258	*P. pygmaeus*
05/08/2006	C159	*M. daubentonii*	12/09/2006	O30A	*M. nattereri*
05/08/2006	C29	*M. nattereri*	12/09/2006	O211	*P. pygmaeus*
05/08/2006	C31	*P. pygmaeus*	28/09/2006	C101	*M. nattereri*
05/08/2006	C47	*P. pipistrellus*	28/09/2006	C109	*P. pygmaeus*
05/08/2006	C119	*P. pygmaeus*	28/09/2006	C124	*M. daubentonii*
05/08/2006	C49	*M. daubentonii*	28/09/2006	C113	*P. pygmaeus*
05/08/2006	C57	*M. daubentonii*	28/09/2006	C141	*M. daubentonii*
05/08/2006	C14	*M. daubentonii*	28/09/2006	C25	*M. daubentonii*
02/09/2006	C113	*P. pygmaeus*	28/09/2006	C2	*M. daubentonii*
02/09/2006	C119	*P. pygmaeus*	10/10/2006	CP105	*P. pygmaeus*
02/09/2006	C140	*M. daubentonii*	10/10/2006	CP117	*M. daubentonii*
02/09/2006	C45	*M. daubentonii*	10/10/2006	CP134	*P. pygmaeus*
02/09/2006	C9	*M. nattereri*	10/10/2006	W34	*M. daubentonii*
02/09/2006	C151	*M. daubentonii*	10/10/2006	CP113	*M. daubentonii*
02/09/2006	C35	*M. daubentonii*	10/10/2006	W24	*M. daubentonii*
02/09/2006	C110	*M. daubentonii*	10/10/2006	W19	*P. auritus*
02/09/2006	C27	*M. daubentonii*	28/04/2007	O217	*P. auritus*
02/09/2006	C116	*M. nattereri*	23/05/2007	SW11	*M. daubentonii*
02/09/2006	C138	*M. daubentonii*	24/05/2007	W8	*M. daubentonii*
02/09/2006	C137	*M. daubentonii*	26/05/2007	CP11	*M. nattereri*
02/09/2006	C54	*M. nattereri*	01/06/2007	O39	*P. auritus*
02/09/2006	C13	*M. nattereri*	01/06/2007	CP150	*M. nattereri*
02/09/2006	C26	*M. daubentonii*	01/06/2007	CP132	*M. nattereri*
02/09/2006	C159	*M. daubentonii*	05/06/2007	W37	*M. nattereri*
02/09/2006	C46	*M. nattereri*	05/06/2007	W59	*M. daubentonii*
09/09/2006	CP138	*P. pygmaeus*	05/06/2007	W99	*M. daubentonii*
09/09/2006	CP148	*P. pygmaeus*	05/06/2007	W75A	*M. daubentonii*
09/09/2006	CP155	*M. nattereri*	05/06/2007	W47	*M. daubentonii*
09/09/2006	CP20	*P. pipistrellus*	05/06/2007	W100	*M. daubentonii*
09/09/2006	W72	*M. daubentonii*	05/06/2007	W36	*M. daubentonii*
09/09/2006	E16	*M. daubentonii*	05/06/2007	W56	*M. nattereri*
09/09/2006	CP21	*M. daubentonii*	05/06/2007	W64A	*M. daubentonii*
09/09/2006	CP103	*M. daubentonii*	05/06/2007	W68	*M. nattereri*
10/09/2006	B127	*P. auritus*	05/06/2007	W52	*M. daubentonii*
10/09/2006	MP15	*P. pygmaeus*	08/06/2007	E14B	*M. daubentonii*
10/09/2006	MP32	*M. nattereri*	08/06/2007	E9	*M. daubentonii*
10/09/2006	W21	*M. daubentonii*	12/06/2007	O216	*P. pygmaeus*
10/09/2006	W23	*M. daubentonii*	12/06/2007	SW7	*P. auritus*
10/09/2006	W29	*M. daubentonii*	12/06/2007	O51	*M. daubentonii*
10/09/2006	W6	*M. daubentonii*	07/07/2007	CP122	*M. nattereri*
10/09/2006	W11	*M. daubentonii*	15/07/2007	CP101	*P. pygmaeus*
10/09/2006	W59	*M. daubentonii*	15/07/2007	CP103	*M. nattereri*
10/09/2006	W17	*M. daubentonii*	15/07/2007	CP125	*M. nattereri*
10/09/2006	W13	*M. daubentonii*	15/07/2007	CP19	*M. nattereri*
10/09/2006	W61	*M. daubentonii*	15/07/2007	CP100	*M. nattereri*
10/09/2006	W50	*M. daubentonii*	15/07/2007	CP33	*M. nattereri*
12/09/2006	O201	*P. pygmaeus*	15/07/2007	CP153	*M. nattereri*

Table 7.1 (continues over multiple pages) – A summary of all box checks detailing the date, roost box and species found.

Date	Box	Species	Date	Box	Species
15/07/2007	CP32	*M. daubentonii*	12/08/2007	SW31	*M. nattereri*
15/07/2007	CP34	*M. daubentonii*	12/08/2007	SW20	*P. auritus*
15/07/2007	CP16	*M. daubentonii*	16/08/2007	W78	*M. nattereri*
15/07/2007	CP17	*M. daubentonii*	16/08/2007	W93	*M. daubentonii*
15/07/2007	CP3	*M. daubentonii*	16/08/2007	W74	*P. auritus*
15/07/2007	CP20	*M. daubentonii*	16/08/2007	W63	*M. nattereri*
15/07/2007	CP12	*M. daubentonii*	18/08/2007	CP131	*P. pygmaeus*
15/07/2007	CP137	*M. nattereri*	18/08/2007	CP144	*P. pygmaeus*
15/07/2007	CP144	*M. nattereri*	18/08/2007	W42	*Unknown*
18/07/2007	B136	*M. nattereri*	18/08/2007	W52	*M. daubentonii*
18/07/2007	B168	*M. nattereri*	18/08/2007	W44	*M. daubentonii*
18/07/2007	B32	*P. auritus*	18/08/2007	CP21	*M. daubentonii*
18/07/2007	B55	*M. daubentonii*	18/08/2007	W51	*M. daubentonii*
28/07/2007	B225	*P. pygmaeus*	18/08/2007	CP34	*M. daubentonii*
28/07/2007	MP36	*P. pygmaeus*	18/08/2007	W53	*M. daubentonii*
28/07/2007	MP46	*M. nattereri*	18/08/2007	W61	*M. daubentonii*
28/07/2007	MP52	*M. nattereri*	18/08/2007	CP138	*M. nattereri*
28/07/2007	MP64	*P. auritus*	18/08/2007	W68	*M. nattereri*
28/07/2007	MP67	*M. nattereri*	18/08/2007	CP135	*M. nattereri*
28/07/2007	MP92	*M. daubentonii*	22/08/2007	E12	*M. daubentonii*
30/07/2007	O207	*P. pygmaeus*	22/08/2007	E2	*P. pygmaeus*
30/07/2007	O238	*M. nattereri*	22/08/2007	E37	*P. auritus*
30/07/2007	O33	*M. nattereri*	22/08/2007	E14	*M. daubentonii*
30/07/2007	O235	*P. auritus*	22/08/2007	E6	*P. auritus*
30/07/2007	O211	*M. nattereri*	24/08/2007	E42	*P. auritus*
30/07/2007	O217	*M. nattereri*	24/08/2007	E43B	*P. pygmaeus*
04/08/2007	C101	*M. nattereri*	24/08/2007	E49C	*M. nattereri*
04/08/2007	C119	*P. pygmaeus*	24/08/2007	W13	*M. nattereri*
04/08/2007	C13	*P. pygmaeus*	24/08/2007	W21	*M. nattereri*
04/08/2007	C19	*P. pygmaeus*	24/08/2007	W54	*M. nattereri*
04/08/2007	C30	*M. nattereri*	24/08/2007	E49A	*M. daubentonii*
04/08/2007	C31	*P. pygmaeus*	24/08/2007	E47	*M. nattereri*
04/08/2007	C41	*M. daubentonii*	24/08/2007	E19A	*M. daubentonii*
04/08/2007	C50	*M. nattereri*	24/08/2007	E15	*M. daubentonii*
04/08/2007	C136	*M. daubentonii*	24/08/2007	E55	*M. nattereri*
04/08/2007	C35	*M. daubentonii*	24/08/2007	E35A	*M. nattereri*
04/08/2007	C59	*M. daubentonii*	24/08/2007	W26	*M. nattereri*
04/08/2007	C120	*M. daubentonii*	24/08/2007	E43	*P. auritus*
09/08/2007	P109	*P. pygmaeus*	24/08/2007	W15	*M. nattereri*
09/08/2007	P13	*P. auritus*	02/09/2007	B181	*P. pygmaeus*
09/08/2007	P15	*M. nattereri*	02/09/2007	CP101	*M. daubentonii*
09/08/2007	P3	*M. nattereri*	02/09/2007	CP102	*M. nattereri*
09/08/2007	P8	*M. nattereri*	02/09/2007	CP106	*P. pygmaeus*
09/08/2007	SW64	*M. nattereri*	02/09/2007	CP111	*P. pygmaeus*
09/08/2007	P16	*M. daubentonii*	02/09/2007	CP112	*P. pygmaeus*
12/08/2007	P24	*P. auritus*	02/09/2007	B206	*P. auritus*
12/08/2007	SW62	*P. auritus*	02/09/2007	CP127	*M. daubentonii*
12/08/2007	SW109A	*M. nattereri*	02/09/2007	CP3	*M. daubentonii*
12/08/2007	SW28	*M. nattereri*	02/09/2007	CP10	*M. daubentonii*

Date	Box	Species	Date	Box	Species
02/09/2007	CP151	*M. nattereri*	13/10/2007	W54	*M. daubentonii*
02/09/2007	CP117	*M. nattereri*	13/10/2007	W73	*M. nattereri*
02/09/2007	W13	*M. daubentonii*	13/10/2007	W77	*M. daubentonii*
02/09/2007	CP157	*M. nattereri*	13/10/2007	W52	*M. daubentonii*
09/09/2007	O231	*M. nattereri*	13/10/2007	W23	*M. nattereri*
09/09/2007	O249	*P. pygmaeus*	13/10/2007	W3	*M. daubentonii*
09/09/2007	O261	*P. pygmaeus*	13/10/2007	W34	*M. daubentonii*
09/09/2007	O253	*M. nattereri*	13/10/2007	W5	*M. daubentonii*
09/09/2007	O19	*M. nattereri*	11/05/2008	B213	*M. daubentonii*
09/09/2007	O77	*M. nattereri*	15/05/2008	W93	*M. daubentonii*
09/09/2007	O240	*M. daubentonii*	17/05/2008	CP153	*M. daubentonii*
16/09/2007	C103	*M. nattereri*	19/05/2008	O223	*M. nattereri*
16/09/2007	C113	*P. pygmaeus*	20/05/2008	E2	*M. nattereri*
16/09/2007	C37	*M. daubentonii*	07/06/2008	CP110	*M. nattereri*
16/09/2007	C45	*M. nattereri*	13/06/2008	E12	*P. auritus*
16/09/2007	C46	*M. daubentonii*	13/06/2008	W47	*M. daubentonii*
16/09/2007	C102	*M. nattereri*	13/06/2008	W16	*M. nattereri*
16/09/2007	C152	*M. daubentonii*	16/06/2008	MP68	*P. auritus*
16/09/2007	C32	*M. nattereri*	20/06/2008	C59	*M. daubentonii*
16/09/2007	C107	*M. daubentonii*	20/06/2008	C135	*M. daubentonii*
17/09/2007	C24	*M. nattereri*	20/06/2008	C11	*M. daubentonii*
20/09/2007	MP76	*N. noctula*	20/07/2008	E31A	*M. daubentonii*
20/09/2007	MP32	*M. nattereri*	20/07/2008	E59	*M. daubentonii*
20/09/2007	MP67	*M. daubentonii*	24/07/2008	C39	*M. daubentonii*
20/09/2007	MP3	*M. daubentonii*	24/07/2008	C13	*M. daubentonii*
21/09/2007	O34	*P. pygmaeus*	26/07/2008	O228	*P. pygmaeus*
21/09/2007	O227	*M. nattereri*	26/07/2008	O232	*M. nattereri*
21/09/2007	O27	*P. auritus*	26/07/2008	O24	*M. daubentonii*
21/09/2007	O210	*M. nattereri*	26/07/2008	O242	*M. nattereri*
21/09/2007	O213	*P. pygmaeus*	26/07/2008	O82	*M. nattereri*
21/09/2007	O213	*M. nattereri*	26/07/2008	O239	*M. nattereri*
24/09/2007	P103	*M. nattereri*	27/07/2008	W37	*M. nattereri*
24/09/2007	P104	*M. nattereri*	27/07/2008	W15	*M. daubentonii*
24/09/2007	P116	*M. nattereri*	27/07/2008	W41	*M. daubentonii*
24/09/2007	P120	*P. pygmaeus*	27/07/2008	W55	*M. daubentonii*
24/09/2007	P112	*P. pygmaeus*	27/07/2008	W68	*M. daubentonii*
24/09/2007	P23	*M. nattereri*	27/07/2008	W95	*M. daubentonii*
30/09/2007	E4	*N. noctula*	27/07/2008	W103	*M. daubentonii*
30/09/2007	E41A	*M. nattereri*	27/07/2008	W91	*M. daubentonii*
30/09/2007	E62	*M. daubentonii*	27/07/2008	W42	*M. daubentonii*
30/09/2007	E39A	*M. nattereri*	03/08/2008	MP47	*M. nattereri*
30/09/2007	E39C	*M. daubentonii*	03/08/2008	MP76	*N. noctula*
07/10/2007	SW109	*M. nattereri*	03/08/2008	MP82	*M. nattereri*
07/10/2007	SW13	*M. nattereri*	03/08/2008	MP69	*P. auritus*
07/10/2007	SW8	*P. auritus*	03/08/2008	MP38	*M. nattereri*
07/10/2007	SW126	*M. nattereri*	03/08/2008	MP76	*M. daubentonii*
07/10/2007	SW103	*M. daubentonii*	03/08/2008	MP80	*M. nattereri*
07/10/2007	SW19	*P. auritus*	09/08/2008	P117	*P. pygmaeus*
13/10/2007	W105	*P. pygmaeus*	09/08/2008	P16	*M. daubentonii*

Date	Box	Species	Date	Box	Species
23/08/2008	CP108	*M. daubentonii*	21/09/2008	W17	*M. daubentonii*
23/08/2008	CP127	*M. daubentonii*	26/09/2008	MP3	*M. daubentonii*
23/08/2008	CP130	*M. nattereri*	26/09/2008	B122	*M. daubentonii*
23/08/2008	CP142	*P. pygmaeus*	26/09/2008	MP11	*M. daubentonii*
23/08/2008	CP141	*M. nattereri*	09/10/2008	SW104A	*P. pipistrellus*
23/08/2008	CP8	*M. daubentonii*	09/10/2008	SW21	*P. pygmaeus*
23/08/2008	CP112	*M. daubentonii*	09/10/2008	SW31	*M. nattereri*
23/08/2008	CP144	*M. nattereri*	09/10/2008	SW35	*N. noctula*
23/08/2008	CP15	*M. daubentonii*	09/10/2008	SW71	*M. nattereri*
23/08/2008	CP19	*M. daubentonii*	12/10/2008	B156	*M. nattereri*
23/08/2008	CP134	*M. daubentonii*	12/10/2008	B57	*P. auritus*
30/08/2008	E28	*M. nattereri*	15/10/2008	E75	*Pipistrellus sp.*
30/08/2008	E49C	*P. pygmaeus*	15/10/2008	E86	*M. nattereri*
30/08/2008	E50	*P. pygmaeus*	15/10/2008	E88	*P. pygmaeus*
30/08/2008	E51C	*M. nattereri*	15/10/2008	E89	*P. auritus*
30/08/2008	E62G	*M. nattereri*	15/10/2008	E92	*P. auritus*
30/08/2008	SCH	*P. auritus*	19/10/2008	C123	*M. daubentonii*
30/08/2008	E51B	*M. daubentonii*	19/10/2008	C139	*M. daubentonii*
30/08/2008	E63	*M. daubentonii*	19/10/2008	C145	*M. nattereri*
30/08/2008	E31A	*M. daubentonii*	04/05/2009	SW55	*P. auritus*
30/08/2008	E51A	*M. nattereri*	06/05/2009	B91	*M. nattereri*
30/08/2008	E55	*M. daubentonii*	07/05/2009	W64	*M. nattereri*
30/08/2008	E47	*M. daubentonii*	30/05/2009	W33	*M. daubentonii*
31/08/2008	E2	*P. pygmaeus*	30/05/2009	W64A	*M. daubentonii*
31/08/2008	E6	*P. pygmaeus*	06/06/2009	MP5	*M. nattereri*
31/08/2008	E19	*M. daubentonii*	17/06/2009	CP124	*M. nattereri*
31/08/2008	SCH	*P. pygmaeus*	17/06/2009	SCH	*Pipistrellus sp.*
31/08/2008	E14A	*M. daubentonii*	17/06/2009	CP155	*M. daubentonii*
31/08/2008	E8	*M. daubentonii*	17/06/2009	CP32	*P. auritus*
31/08/2008	E8A	*M. daubentonii*	17/06/2009	SCH	*Pipistrellus sp.*
31/08/2008	E19A	*M. daubentonii*	17/06/2009	CP22	*M. nattereri*
31/08/2008	E10A	*P. auritus*	17/06/2009	CP14	*M. daubentonii*
08/09/2008	CP102	*M. nattereri*	18/06/2009	W18	*M. nattereri*
08/09/2008	CP5A	*M. daubentonii*	18/06/2009	W38	*M. nattereri*
08/09/2008	CP148	*M. nattereri*	18/06/2009	W45	*M. daubentonii*
08/09/2008	CP6	*M. nattereri*	18/06/2009	W2	*M. daubentonii*
08/09/2008	W2	*M. daubentonii*	18/06/2009	W39	*M. daubentonii*
11/09/2008	W10	*M. daubentonii*	18/06/2009	W76	*M. daubentonii*
11/09/2008	W12	*M. daubentonii*	18/06/2009	W35	*M. daubentonii*
12/09/2008	C112	*P. pygmaeus*	18/06/2009	W31	*M. daubentonii*
12/09/2008	C116	*M. nattereri*	18/06/2009	W16	*M. nattereri*
12/09/2008	C21	*M. nattereri*	03/07/2009	CP31	*M. nattereri*
12/09/2008	C114	*M. daubentonii*	03/07/2009	CP36	*M. daubentonii*
12/09/2008	C3A	*M. daubentonii*	03/07/2009	E13	*M. daubentonii*
21/09/2008	W74	*P. auritus*	03/07/2009	E21	*M. daubentonii*
21/09/2008	O9	*M. daubentonii*	08/07/2009	W105	*M. daubentonii*
21/09/2008	O261	*P. pygmaeus*	08/07/2009	W102	*M. daubentonii*
21/09/2008	W73	*M. daubentonii*	08/07/2009	W101	*M. daubentonii*
21/09/2008	O256	*M. nattereri*	09/07/2009	C30	*M. nattereri*

Date	Box	Species	Date	Box	Species
09/07/2009	C104	*P. pygmaeus*	22/08/2009	E16	*M. daubentonii*
09/07/2009	C101	*M. daubentonii*	22/08/2009	SCH	*Pipistrellus sp.*
09/07/2009	C13	*M. daubentonii*	22/08/2009	SCH	*Pipistrellus sp.*
09/07/2009	C102	*M. daubentonii*	24/08/2009	W95	*M. daubentonii*
09/07/2009	C122	*M. nattereri*	24/08/2009	W52	*M. daubentonii*
11/07/2009	O204	*P. pygmaeus*	12/09/2009	CP105	*M. nattereri*
11/07/2009	SW109	*M. nattereri*	12/09/2009	CP107	*M. daubentonii*
11/07/2009	SW37	*P. pygmaeus*	12/09/2009	CP157	*M. daubentonii*
11/07/2009	O217	*M. nattereri*	12/09/2009	CP130	*M. nattereri*
11/07/2009	SW124	*M. nattereri*	12/09/2009	CP103	*M. daubentonii*
23/07/2009	W32	*M. daubentonii*	12/09/2009	SCH	*P. pygmaeus*
23/07/2009	W49	*M. daubentonii*	12/09/2009	SCH	*Pipistrellus sp.*
23/07/2009	W52	*M. daubentonii*	16/09/2009	O230	*M. nattereri*
23/07/2009	W68	*M. daubentonii*	16/09/2009	O237	*P. auritus*
23/07/2009	W58	*M. daubentonii*	16/09/2009	O223	*M. nattereri*
26/07/2009	MP82	*M. nattereri*	16/09/2009	O36	*P. auritus*
26/07/2009	MP67	*P. auritus*	21/09/2009	CP103	*M. daubentonii*
26/07/2009	MP29	*M. nattereri*	23/09/2009	CP103	*M. daubentonii*
26/07/2009	MP54	*M. nattereri*	23/09/2009	CP156	*M. nattereri*
30/07/2009	O246	*P. pygmaeus*	23/09/2009	CP154	*M. daubentonii*
30/07/2009	P120	*P. pygmaeus*	23/09/2009	CP133	*M. daubentonii*
30/07/2009	O252	*M. nattereri*	23/09/2009	CP131	*Unknown*
30/07/2009	SW63	*P. auritus*	23/09/2009	CP123	*M. nattereri*
04/08/2009	C119	*M. nattereri*	23/09/2009	W4A	*M. nattereri*
04/08/2009	C43	*M. nattereri*	23/09/2009	W8	*P. pygmaeus*
04/08/2009	C61	*M. nattereri*	24/09/2009	CP103	*M. daubentonii*
04/08/2009	C36	*M. daubentonii*	24/09/2009	W54	*M. daubentonii*
04/08/2009	C26	*M. daubentonii*	25/09/2009	MP37	*P. auritus*
04/08/2009	C58	*M. daubentonii*	25/09/2009	MP55	*M. daubentonii*
04/08/2009	C139	*M. daubentonii*	25/09/2009	MP67	*M. nattereri*
04/08/2009	C124	*M. daubentonii*	25/09/2009	MP27	*M. nattereri*
11/08/2009	CP114	*P. pygmaeus*	25/09/2009	MP33	*M. daubentonii*
11/08/2009	CP115	*P. pygmaeus*	03/10/2009	C113	*P. pygmaeus*
11/08/2009	CP117	*M. nattereri*	03/10/2009	C140	*M. daubentonii*
11/08/2009	CP144	*P. auritus*	03/10/2009	C4	*M. daubentonii*
11/08/2009	CP124	*M. nattereri*	03/10/2009	MP96	*P. auritus*
11/08/2009	CP118	*M. daubentonii*	04/10/2009	W55	*M. daubentonii*
11/08/2009	CP35	*M. daubentonii*	04/10/2009	SCH	*Pipistrellus sp.*
11/08/2009	SCH	*Pipistrellus sp.*	11/06/2010	B45	*M. nattereri*
14/08/2009	W98	*M. daubentonii*	15/06/2010	O20	*M. nattereri*
14/08/2009	W61	*M. daubentonii*	15/06/2010	O45	*P. auritus*
22/08/2009	E31A	*P. pygmaeus*	15/06/2010	O39	*P. auritus*
22/08/2009	E42A	*M. daubentonii*	15/06/2010	O46	*M. nattereri*
22/08/2009	E48A	*M. nattereri*	23/06/2010	O57	*P. auritus*
22/08/2009	E31	*M. daubentonii*	23/06/2010	E8	*P. auritus*
22/08/2009	E33	*M. daubentonii*	23/06/2010	E4	*P. auritus*
22/08/2009	E29	*M. daubentonii*	23/06/2010	E11	*M. daubentonii*
22/08/2009	E22	*M. daubentonii*	02/07/2010	SW124	*P. auritus*
22/08/2009	E40	*P. auritus*	02/07/2010	SW122	*P. auritus*

Date	Box	Species	Date	Box	Species
02/07/2010	SW9	*P. auritus*	16/08/2010	C130	*M. daubentonii*
08/07/2010	O234	*M. nattereri*	16/08/2010	C134	*M. daubentonii*
08/07/2010	O48	*M. nattereri*	24/08/2010	O247	*M. nattereri*
08/07/2010	O235	*M. nattereri*	24/08/2010	O44	*P. auritus*
10/07/2010	CP135	*M. nattereri*	24/08/2010	O245	*P. auritus*
10/07/2010	CP149	*M. nattereri*	01/09/2010	W103	*M. daubentonii*
10/07/2010	CP23	*M. nattereri*	01/09/2010	W65	*M. daubentonii*
10/07/2010	CP29	*P. auritus*	01/09/2010	W72	*P. pygmaeus*
17/07/2010	C43	*M. nattereri*	01/09/2010	W200	*M. daubentonii*
17/07/2010	C54	*M. nattereri*	01/09/2010	W87	*M. daubentonii*
17/07/2010	C134	*M. nattereri*	01/09/2010	W73	*M. nattereri*
17/07/2010	C56	*M. daubentonii*	04/09/2010	ST10	*P. pygmaeus*
17/07/2010	C39	*M. daubentonii*	04/09/2010	ST11	*M. daubentonii*
18/07/2010	W68	*M. daubentonii*	04/09/2010	ST9	*M. daubentonii*
18/07/2010	W101	*M. daubentonii*	08/09/2010	E50	*P. pygmaeus*
18/07/2010	W63	*M. daubentonii*	08/09/2010	E51A	*M. daubentonii*
20/07/2010	E49C	*M. daubentonii*	08/09/2010	E14A	*M. daubentonii*
20/07/2010	E31	*M. daubentonii*	08/09/2010	E21	*M. nattereri*
20/07/2010	E58	*M. daubentonii*	10/09/2010	B192	*M. nattereri*
20/07/2010	E50	*M. daubentonii*	10/09/2010	H26	*P. pygmaeus*
21/07/2010	W22	*P. auritus*	10/09/2010	B92	*M. daubentonii*
21/07/2010	W31	*M. nattereri*	12/09/2010	SW101A	*P. pygmaeus*
23/07/2010	CP124	*M. nattereri*	12/09/2010	SW129	*P. pygmaeus*
23/07/2010	W10	*M. daubentonii*	12/09/2010	SW133	*P. pygmaeus*
23/07/2010	W1	*M. nattereri*	12/09/2010	SW31	*M. nattereri*
26/07/2010	CP102	*M. nattereri*	12/09/2010	SW121	*M. nattereri*
26/07/2010	W67	*M. nattereri*	14/09/2010	W28	*P. auritus*
28/07/2010	CP117	*M. nattereri*	14/09/2010	W29	*M. daubentonii*
28/07/2010	CP104	*M. nattereri*	14/09/2010	W12	*M. daubentonii*
28/07/2010	CP3	*Unknown*	14/09/2010	W45	*M. nattereri*
31/07/2010	CP24	*M. nattereri*	14/09/2010	W42	*M. daubentonii*
31/07/2010	W11	*M. daubentonii*	14/09/2010	W57	*M. nattereri*
31/07/2010	W19	*P. auritus*	15/09/2010	B22	*M. nattereri*
02/08/2010	C151	*M. daubentonii*	15/09/2010	MP77	*M. nattereri*
02/08/2010	C104	*M. daubentonii*	15/09/2010	MP90	*P. pygmaeus*
02/08/2010	C61	*M. daubentonii*	15/09/2010	MP27	*M. nattereri*
05/08/2010	MP66	*P. auritus*	15/09/2010	MP7	*M. daubentonii*
05/08/2010	MP54	*M. nattereri*	16/09/2010	E106	*P. auritus*
05/08/2010	MP48	*M. nattereri*	16/09/2010	W202	*P. pygmaeus*
05/08/2010	MP30	*M. daubentonii*	16/09/2010	W203	*P. pygmaeus*
08/08/2010	O247	*P. pygmaeus*	16/09/2010	E105	*P. auritus*
08/08/2010	O56	*M. nattereri*	16/09/2010	W72	*P. pygmaeus*
08/08/2010	O250	*M. nattereri*	16/09/2010	E120	*P. auritus*
08/08/2010	O13A	*P. auritus*	16/09/2010	W91	*M. daubentonii*
08/08/2010	O9	*M. daubentonii*	16/09/2010	E104	*P. auritus*
15/08/2010	CP13	*M. daubentonii*	16/09/2010	W42	*M. daubentonii*
15/08/2010	W22	*M. nattereri*	16/09/2010	W59	*M. nattereri*
15/08/2010	W64A	*M. nattereri*	18/09/2010	C113	*P. pygmaeus*
16/08/2010	C129	*M. nattereri*	18/09/2010	C145	*M. nattereri*

Date	Box	Species
18/09/2010	C29	*M. daubentonii*
18/09/2010	C24B	*M. daubentonii*
18/09/2010	C58	*M. daubentonii*
18/09/2010	E63	*M. daubentonii*
19/09/2010	CP21	*M. daubentonii*
19/09/2010	CP110	*M. nattereri*
19/09/2010	CP143	*M. nattereri*
19/09/2010	CP147	*M. nattereri*
19/09/2010	CP15	*Unknown*
20/09/2010	E67	*P. pygmaeus*
20/09/2010	E38A	*P. auritus*
21/09/2010	E43C	*P. auritus*
21/09/2010	O9	*M. daubentonii*
22/09/2010	O30	*M. nattereri*
22/09/2010	O39	*M. nattereri*
22/09/2010	O257	*M. nattereri*
22/09/2010	O2	*P. auritus*
22/09/2010	O34	*M. nattereri*
23/09/2010	MP56	*M. daubentonii*
23/09/2010	MP76	*M. nattereri*
23/09/2010	O25	*P. auritus*
24/09/2010	MP12	*P. pygmaeus*
24/09/2010	MP44	*P. pygmaeus*
24/09/2010	MP83	*P. pygmaeus*
24/09/2010	P112	*P. pygmaeus*
24/09/2010	SW102	*M. nattereri*
05/10/2010	W21	*P. pygmaeus*
05/10/2010	W53	*M. daubentonii*
05/10/2010	W211	*M. nattereri*
08/10/2010	E118	*P. pygmaeus*
08/10/2010	W103	*M. daubentonii*
08/10/2010	W6	*M. daubentonii*
08/10/2010	W200	*M. daubentonii*
14/10/2010	CP6	*M. daubentonii*
14/10/2010	CP10	*M. daubentonii*
14/10/2010	CP19	*M. daubentonii*
14/10/2010	CP152	*M. nattereri*

Variable recorded / Purpose	Equipment details
Wind Speed	A100R anemometer
Relative Humidity	HMP45C temperature & relative humidity probe
Air Temperature	107 Termistor probe with 41303-5 Gill radiation shield
Solar Radiation	CM6B Kipp & Zonen pyranometer
Rainfall	ARG100 Raingauge
Data Recorder	CR1000 Campbell datalogger

Table 7.2 – A list of equipment used to record weather variables at Wytham woods. All equipment was supplied by Campbell Scientific, Shepshed, UK.

Species	Age	Sex	Reproductive status	Coronavirus presence	Season	Social group	Betweeness	Degree	BCI	Spinturnicid load	Year
M. daubentonii	Juvenile	M	NA	0	Post-nursery	6	974.051	23	0.185	0	2010
M. daubentonii	Juvenile	F	Non-breeder	0	Post-nursery	6	0	13	0.213	2	2010
M. nattereri	Adult	F	Post-lactating	0	Post-nursery	6	67.899	45	0.180	1	2010
M. nattereri	Adult	F	Non-breeder	0	Post-nursery	6	5.738	24	0.180	2	2010
M. nattereri	Adult	F	Post-lactating	0	Post-nursery	6	67.899	45	0.182	1	2010
M. nattereri	Adult	F	Post-lactating	0	Post-nursery	6	18.019	36	0.199	0	2010
M. nattereri	Adult	F	Post-lactating	1	Post-nursery	1	228.459	39	0.242	1	2010
M. nattereri	Adult	M	NA	0	Post-nursery	1	105.646	38	0.186	2	2010
M. nattereri	Juvenile	M	NA	1	Post-nursery	1	8.5	38	0.184	3	2010
M. nattereri	Juvenile	M	NA	1	Post-nursery	1	8.5	38	0.170	1	2010
M. nattereri	Adult	F	Post-lactating	1	Post-nursery	1	29.675	35	0.211	5	2010
M. nattereri	Juvenile	M	NA	0	Post-nursery	1	6.146	37	0.184	0	2010
M. daubentonii	Adult	M	NA	1	Post-nursery	8	123.188	8	0.237	0	2010
M. daubentonii	Adult	M	NA	1	Post-nursery	7	0	7	0.203	0	2010
M. daubentonii	Adult	M	NA	1	Post-nursery	7	0	7	0.231	1	2010
M. daubentonii	Adult	F	Post-lactating	1	Post-nursery	NA	NA	NA	0.229	1	2010
M. nattereri	Adult	M	NA	0	Post-nursery	5	0.167	7	0.187	0	2010
M. nattereri	Adult	M	NA	0	Post-nursery	5	217.167	12	0.198	0	2010
M. nattereri	Adult	M	NA	0	Post-nursery	5	0	6	0.197	0	2010
M. nattereri	Adult	M	NA	0	Post-nursery	NA	NA	NA	0.199	0	2010
M. nattereri	Adult	F	Non-breeder	0	Post-nursery	3	29.054	33	0.195	2	2010
M. nattereri	Adult	F	Post-lactating	1	Post-nursery	6	22.211	36	0.009	0	2010
M. nattereri	Adult	M	NA	1	Post-nursery	6	2.179	31	0.183	0	2010
M. daubentonii	Juvenile	M	NA	1	Post-nursery	4	153.917	28	0.187	9	2010

Table 7.3 (continues over multiple pages)– A summary of data used in chapter 5 to explore the drivers behind infection with coronavirus. Coronavirus infection is indicated as 1 for positive and 0 for negative.

Species	Age	Sex	Reproductive status	Coronavirus presence	Season	Social group	Betweeness	Degree	BCI	Spinturnicid load	Year
M. daubentonii	Adult	F	Lactating	1	Nursery	4	188.925	39	0.249	13	2009
M. daubentonii	Adult	F	Lactating	0	Nursery	4	26.491	26	0.253	12	2009
M. daubentonii	Adult	F	Lactating	1	Nursery	4	314.188	32	0.237	7	2009
M. daubentonii	Juvenile	M	NA	0	Nursery	4	40.503	27	0.183	21	2009
M. daubentonii	Juvenile	M	NA	1	Nursery	NA	NA	NA	0.189	21	2009
M. daubentonii	Adult	F	Lactating	0	Nursery	4	101.757	35	0.249	6	2009
M. daubentonii	Adult	F	Lactating	1	Nursery	4	179.746	34	0.225	13	2009
M. daubentonii	Juvenile	F	Non-breeder	1	Nursery	4	1777.772	39	0.180	10	2009
M. daubentonii	Adult	F	Lactating	0	Nursery	4	22.604	21	0.241	22	2009
M. daubentonii	Juvenile	M	NA	0	Nursery	NA	NA	NA	0.171	19	2009
M. daubentonii	Adult	F	Lactating	0	Nursery	4	115.338	34	0.234	NA	2009
M. daubentonii	Juvenile	M	NA	0	Nursery	4	626.48	38	0.205	22	2009
M. daubentonii	Adult	F	Lactating	0	Nursery	4	53.131	25	0.245	8	2009
M. daubentonii	Juvenile	F	Non-breeder	0	Nursery	4	250.79	37	0.176	32	2009
M. daubentonii	Juvenile	F	Non-breeder	1	Nursery	4	9.531	21	0.190	17	2009
M. daubentonii	Adult	F	Lactating	1	Nursery	4	113.75	34	0.256	6	2009
M. daubentonii	Adult	F	Lactating	0	Nursery	4	22.284	23	0.249	11	2009
M. daubentonii	Adult	F	Lactating	0	Nursery	4	97.49	30	0.248	38	2009
M. daubentonii	Adult	M	NA	0	Nursery	2	181.263	24	0.251	3	2009
M. daubentonii	Adult	M	NA	0	Nursery	2	301.043	28	0.225	11	2009
M. daubentonii	Adult	M	NA	0	Nursery	NA	NA	NA	0.218	7	2009
M. daubentonii	Adult	M	NA	0	Nursery	2	214.307	27	0.244	3	2009
M. daubentonii	Adult	M	NA	0	Nursery	2	376.731	30	0.235	7	2009
M. daubentonii	Adult	M	NA	0	Nursery	2	181.263	24	0.233	2	2009

Species	Age	Sex	Reproductive status	Coronavirus presence	Season	Social group	Betweeness	Degree	BCI	Spinturnicid load	Year
M. daubentonii	Adult	F	Post-lactating	1	Nursery	2	659.744	24	0.229	9	2009
M. daubentonii	Adult	M	NA	1	Nursery	2	2055.059	27	0.244	7	2009
M. daubentonii	Adult	M	NA	0	Nursery	NA	NA	NA	0.224	2	2009
M. daubentonii	Adult	M	NA	0	Nursery	1	59.149	12	0.223	4	2009
M. daubentonii	Adult	M	NA	0	Nursery	1	110.447	13	0.233	5	2009
M. nattereri	Juvenile	M	NA	1	Nursery	NA	NA	NA	0.178	2	2009
M. nattereri	Juvenile	F	Non-breeder	0	Nursery	2	23.609	14	0.212	2	2009
M. nattereri	Adult	F	Lactating	1	Nursery	2	112.659	20	0.194	1	2009
M. nattereri	Juvenile	M	NA	1	Nursery	NA	NA	NA	0.181	1	2009
M. nattereri	Adult	F	Lactating	1	Nursery	2	112.659	20	0.199	3	2009
M. nattereri	Juvenile	M	NA	1	Nursery	NA	NA	NA	0.167	0	2009
M. nattereri	Adult	F	Lactating	1	Nursery	2	23.609	14	0.208	0	2009
M. nattereri	Adult	F	Lactating	0	Nursery	2	112.659	20	0.196	1	2009
M. nattereri	Juvenile	F	Non-breeder	1	Nursery	NA	NA	NA	0.167	3	2009
M. nattereri	Juvenile	M	NA	1	Nursery	NA	NA	NA	0.178	5	2009
M. nattereri	Adult	F	Lactating	1	Nursery	NA	NA	NA	0.193	4	2009
M. nattereri	Adult	F	Lactating	0	Nursery	2	112.659	20	0.200	4	2009
M. nattereri	Adult	F	Lactating	1	Nursery	2	23.609	14	0.195	2	2009
M. nattereri	Juvenile	M	NA	1	Nursery	NA	NA	NA	0.187	0	2009
M. daubentonii	Juvenile	M	NA	0	Nursery	NA	NA	NA	0.194	26	2009
M. daubentonii	Juvenile	M	NA	0	Nursery	1	85.902	15	0.194	44	2009
M. daubentonii	Juvenile	M	NA	0	Nursery	1	166.434	20	0.182	21	2009
M. daubentonii	Juvenile	F	Non-breeder	0	Nursery	1	381.147	27	0.190	19	2009
M. daubentonii	Adult	F	Non-breeder	0	Nursery	1	4571.703	21	0.246	25	2009

Species	Age	Sex	Reproductive status	Coronavirus presence	Season	Social group	Betweeness	Degree	BCI	Spinturnicid load	Year
M. daubentonii	Adult	F	Lactating	0	Nursery	1	173.303	25	0.235	20	2009
M. daubentonii	Adult	F	Lactating	0	Nursery	1	300.643	31	0.259	26	2009
M. daubentonii	Juvenile	M	NA	1	Nursery	1	4.214	16	0.182	20	2009
M. daubentonii	Adult	F	Lactating	1	Nursery	1	277.528	27	0.260	25	2009
M. daubentonii	Juvenile	F	Non-breeder	1	Nursery	1	164.267	25	0.184	46	2009
M. daubentonii	Adult	F	Lactating	0	Nursery	1	96.337	29	0.267	33	2009
M. daubentonii	Juvenile	M	NA	1	Nursery	1	146.848	16	0.193	20	2009
M. daubentonii	Juvenile	F	Non-breeder	1	Nursery	1	9.679	23	0.193	35	2009
M. nattereri	Juvenile	F	NA	1	Nursery	NA	NA	NA	0.162	NA	2009
M. nattereri	Juvenile	M	NA	1	Nursery	NA	NA	NA	0.161	NA	2009
M. nattereri	Adult	F	NA	0	Nursery	NA	NA	NA	0.202	NA	2009
M. nattereri	Adult	F	NA	1	Nursery	NA	NA	NA	0.202	NA	2009
M. nattereri	Juvenile	F	NA	1	Nursery	NA	NA	NA	0.170	NA	2009
M. nattereri	Adult	F	NA	0	Nursery	NA	NA	NA	0.226	NA	2009
M. nattereri	Adult	M	NA	1	Nursery	NA	NA	NA	0.191	NA	2009
M. nattereri	Adult	F	Non-breeder	0	Nursery	5	0.515	8	0.211	NA	2009
M. nattereri	Adult	F	NA	0	Nursery	NA	NA	NA	0.202	NA	2009
M. nattereri	Adult	M	NA	0	Nursery	NA	NA	NA	0.209	NA	2009
M. nattereri	Juvenile	F	NA	0	Nursery	NA	NA	NA	0.180	NA	2009
M. nattereri	Adult	F	NA	0	Nursery	NA	NA	NA	0.159	NA	2009
M. nattereri	Adult	F	NA	1	Nursery	NA	NA	NA	0.212	NA	2009
M. nattereri	Juvenile	F	NA	1	Nursery	NA	NA	NA	0.174	NA	2009
M. nattereri	Adult	M	NA	1	Nursery	5	0.515	8	0.196	NA	2009
M. nattereri	Adult	F	NA	1	Nursery	NA	NA	NA	0.214	NA	2009

Species	Age	Sex	Reproductive status	Coronavirus presence	Season	Social group	Betweeness	Degree	BCI	Spinturnicid load	Year
M. nattereri	Adult	F	NA	0	Nursery	NA	NA	NA	0.221	NA	2009
M. nattereri	Adult	F	NA	1	Nursery	NA	NA	NA	0.199	NA	2009
M. nattereri	Juvenile	M	NA	1	Nursery	NA	NA	NA	0.197	2	2009
M. nattereri	Adult	F	Non-breeder	1	Nursery	NA	NA	NA	0.210	2	2009
M. nattereri	Juvenile	M	NA	1	Nursery	NA	NA	NA	0.196	1	2009
M. nattereri	Juvenile	F	Non-breeder	1	Nursery	NA	NA	NA	0.197	0	2009
M. daubentonii	Adult	M	NA	0	Nursery	5	152.611	28	0.195	12	2009
M. daubentonii	Adult	M	NA	0	Nursery	5	1352.948	41	0.218	9	2009
M. daubentonii	Adult	F	Non-breeder	0	Nursery	5	932.859	33	0.215	0	2009
M. daubentonii	Adult	M	NA	0	Nursery	5	111.07	25	0.222	16	2009
M. daubentonii	Adult	M	NA	1	Nursery	5	428.632	26	0.208	6	2009
M. nattereri	Adult	F	Non-breeder	1	Nursery	3	1.971	20	0.210	1	2009
M. nattereri	Adult	F	Post-lactating	0	Post-nursery	3	99.685	30	0.215	6	2009
M. nattereri	Adult	F	Non-breeder	1	Nursery	3	8.432	27	0.206	7	2009
M. nattereri	Adult	F	Non-breeder	0	Nursery	3	7.708	21	0.216	4	2009
M. nattereri	Juvenile	M	NA	1	Nursery	NA	NA	NA	0.170	1	2009
M. nattereri	Juvenile	M	NA	1	Nursery	3	14.831	35	0.149	0	2009
M. nattereri	Adult	F	Post-lactating	1	Post-nursery	3	136.78	37	0.216	0	2009
M. nattereri	Juvenile	F	Non-breeder	1	Nursery	NA	NA	NA	0.182	16	2009
M. nattereri	Adult	F	Lactating	0	Nursery	3	9.326	30	0.191	1	2009
M. nattereri	Juvenile	M	NA	0	Nursery	3	2.504	18	0.183	11	2009
M. nattereri	Adult	F	Post-lactating	0	Post-nursery	3	17.274	35	0.208	1	2009
M. nattereri	Juvenile	F	Non-breeder	1	Nursery	NA	NA	NA	0.176	1	2009
M. nattereri	Adult	F	Lactating	0	Nursery	3	7.68	28	0.214	10	2009

Species	Age	Sex	Reproductive status	Coronavirus presence	Season	Social group	Betweeness	Degree	BCI	Spinturnicid load	Year
M. nattereri	Adult	F	Post-lactating	1	Post-nursery	3	26.835	34	0.218	0	2009
M. nattereri	Juvenile	F	Non-breeder	1	Nursery	3	49.289	24	0.145	2	2009
M. nattereri	Adult	F	Non-breeder	0	Nursery	3	12.854	24	0.213	0	2009
M. nattereri	Adult	F	Lactating	0	Nursery	3	39.813	43	0.205	3	2009
M. nattereri	Adult	F	Non-breeder	1	Nursery	3	37.091	40	0.201	8	2009
M. nattereri	Juvenile	M	NA	1	Nursery	NA	NA	NA	0.178	0	2009
M. nattereri	Adult	F	Lactating	0	Nursery	3	18.443	34	0.207	4	2009
M. nattereri	Adult	M	NA	0	Nursery	NA	NA	NA	0.201	1	2009
M. nattereri	Adult	F	Lactating	1	Nursery	3	32.415	36	0.189	0	2009
M. nattereri	Juvenile	M	NA	1	Nursery	NA	NA	NA	0.172	6	2009
M. nattereri	Adult	M	NA	0	Nursery	5	0.167	7	0.207	0	2009
M. nattereri	Adult	M	NA	0	Nursery	2	0	14	0.220	0	2009
M. daubentonii	Adult	F	Non-breeder	1	Nursery	NA	NA	NA	0.242	5	2009
M. daubentonii	Adult	M	NA	0	Nursery	1	31.825	13	0.223	7	2009
M. daubentonii	Adult	F	Non-breeder	0	Nursery	1	3.414	9	0.243	1	2009
M. daubentonii	Juvenile	M	NA	0	Nursery	1	33.752	15	0.178	11	2009
M. daubentonii	Juvenile	M	NA	1	Nursery	1	85.902	15	0.225	19	2009
M. daubentonii	Juvenile	F	Non-breeder	1	Nursery	NA	NA	NA	0.228	4	2009
M. daubentonii	Juvenile	F	Non-breeder	1	Nursery	10	1736.918	19	1.000	19	2009
M. daubentonii	Adult	F	Post-lactating	1	Post-nursery	10	1262.699	27	0.245	16	2009
M. daubentonii	Adult	F	Post-lactating	0	Post-nursery	10	190.078	20	0.249	19	2009
M. daubentonii	Adult	F	Post-lactating	0	Post-nursery	10	61.342	15	0.245	11	2009
M. daubentonii	Adult	F	Post-lactating	0	Post-nursery	10	680.338	26	0.281	9	2009
M. daubentonii	Adult	F	Post-lactating	1	Post-nursery	9	1082.434	28	0.226	7	2009

Species	Age	Sex	Reproductive status	Coronavirus presence	Season	Social group	Betweeness	Degree	BCI	Spinturnicid load	Year
M. daubentonii	Adult	F	Post-lactating	1	Post-nursery	9	336.517	14	0.265	4	2009
M. daubentonii	Adult	F	Post-lactating	0	Post-nursery	9	17.711	8	0.219	5	2009
M. daubentonii	Adult	F	Post-lactating	0	Post-nursery	9	286.339	22	0.245	12	2009
M. nattereri	Adult	F	Post-lactating	1	Post-nursery	1	366.178	42	0.212	13	2009
M. nattereri	Adult	M	NA	0	Nursery	1	0.739	5	0.192	5	2009
M. daubentonii	Adult	M	NA	1	Post-nursery	3	0	8	0.273	1	2009
M. daubentonii	Adult	M	NA	1	Post-nursery	4	36.254	14	0.225	2	2009
M. daubentonii	Adult	M	NA	1	Post-nursery	5	687.645	29	0.328	0	2009
M. daubentonii	Juvenile	M	NA	0	Post-nursery	4	2484.403	40	0.219	7	2009
M. daubentonii	Juvenile	F	Non-breeder	1	Post-nursery	4	938.247	33	0.228	7	2009
M. daubentonii	Juvenile	M	NA	1	Post-nursery	4	72.394	30	0.215	6	2009
M. daubentonii	Adult	F	Post-lactating	0	Post-nursery	4	1258.989	41	0.242	4	2009
M. nattereri	Adult	F	Post-lactating	1	Post-nursery	1	246.124	42	0.216	10	2009
M. nattereri	Adult	M	NA	1	Post-nursery	5	31.671	21	0.185	2	2009
M. nattereri	Adult	F	Post-lactating	1	Post-nursery	5	54.8	25	0.207	3	2009
M. nattereri	Adult	F	Post-lactating	0	Post-nursery	5	26.85	20	0.196	4	2009
M. nattereri	Adult	F	Non-breeder	1	Post-nursery	5	4.892	10	0.198	0	2009
M. nattereri	Adult	F	Non-breeder	0	Post-nursery	5	22.442	9	0.214	1	2009
M. nattereri	Adult	F	Post-lactating	1	Post-nursery	5	12.37	11	0.206	2	2009
M. nattereri	Adult	F	Non-breeder	0	Nursery	3	5.396	25	0.210	0	2010
M. nattereri	Adult	F	Non-breeder	1	Nursery	3	169.031	37	0.195	0	2010
M. nattereri	Adult	F	Non-breeder	0	Nursery	NA	NA	NA	0.203	3	2010
M. nattereri	Adult	M	NA	0	Nursery	NA	NA	NA	0.211	0	2010
M. nattereri	Adult	M	NA	0	Nursery	7	2.152	5	0.200	0	2010

Species	Age	Sex	Reproductive status	Coronavirus presence	Season	Social group	Betweeness	Degree	BCI	Spinturnicid load	Year
M. nattereri	Adult	M	NA	0	Nursery	1	4.854	32	0.191	0	2010
M. nattereri	Juvenile	M	NA	1	Nursery	2	4	10	0.153	2	2010
M. daubentonii	Adult	M	NA	0	Nursery	1	2429.236	16	0.214	13	2010
M. daubentonii	Adult	M	NA	1	Nursery	5	86.606	14	0.211	NA	2010
M. nattereri	Adult	F	Lactating	0	Nursery	1	155.789	37	0.207	0	2010
M. nattereri	Adult	F	Non-breeder	0	Nursery	1	7.272	36	0.216	0	2010
M. nattereri	Juvenile	F	Non-breeder	0	Nursery	1	7.06	37	0.150	18	2010
M. nattereri	Juvenile	F	Non-breeder	0	Nursery	1	8.5	38	0.161	9	2010
M. nattereri	Juvenile	F	Non-breeder	0	Nursery	1	8.5	38	0.157	10	2010
M. daubentonii	Adult	M	NA	1	Nursery	6	1054.109	22	0.227	3	2010
M. daubentonii	Adult	F	Lactating	0	Nursery	4	2216.677	52	0.255	4	2010
M. daubentonii	Adult	F	Lactating	1	Nursery	4	1136.824	37	0.276	10	2010
M. daubentonii	Juvenile	M	NA	0	Nursery	4	98.678	27	0.211	6	2010
M. daubentonii	Juvenile	M	NA	0	Nursery	4	88.804	28	0.218	9	2010
M. nattereri	Adult	M	NA	1	Nursery	NA	NA	NA	0.213	0	2010
M. nattereri	Adult	F	Lactating	0	Nursery	7	82.979	36	0.221	6	2010
M. nattereri	Juvenile	M	NA	0	Nursery	NA	NA	NA	0.185	4	2010
M. nattereri	Adult	M	NA	0	Nursery	7	41.971	20	0.185	0	2010
M. daubentonii	Juvenile	M	NA	0	Nursery	1	18.63	18	0.204	4	2010
M. daubentonii	Adult	F	Non-breeder	0	Nursery	1	309.72	23	0.244	12	2010
M. nattereri	Adult	F	Lactating	0	Nursery	3	153.438	36	0.212	7	2010
M. nattereri	Juvenile	M	NA	1	Nursery	3	5.448	17	0.174	5	2010
M. nattereri	Adult	F	Lactating	0	Nursery	5	15.756	12	0.218	NA	2010
M. daubentonii	Adult	F	Non-breeder	0	Nursery	NA	NA	NA	0.243	5	2010

Species	Age	Sex	Reproductive status	Coronavirus presence	Season	Social group	Betweeness	Degree	BCI	Spinturnicid load	Year
M. nattereri	Adult	F	Non-breeder	0	Nursery	6	22.58	34	0.232	2	2010
M. nattereri	Adult	F	Non-breeder	1	Nursery	6	2.179	31	0.202	2	2010
M. nattereri	Adult	F	Non-breeder	0	Nursery	6	58.927	42	0.210	0	2010
M. nattereri	Adult	M	NA	0	Nursery	NA	NA	NA	0.192	4	2010
M. nattereri	Adult	F	Lactating	1	Nursery	6	68.461	46	0.214	2	2010
M. nattereri	Adult	M	NA	0	Nursery	NA	NA	NA	0.178	0	2010
M. daubentonii	Juvenile	F	Non-breeder	1	Nursery	NA	NA	NA	0.217	9	2010
M. daubentonii	Adult	F	Non-breeder	0	Nursery	10	742.715	24	0.246	5	2010
M. daubentonii	Juvenile	F	Non-breeder	0	Nursery	10	0	6	0.214	2	2010
M. daubentonii	Adult	F	Post-lactating	0	Post-nursery	NA	NA	NA	0.262	1	2010
M. nattereri	Juvenile	M	NA	1	Nursery	NA	NA	NA	0.205	2	2010
M. daubentonii	Adult	F	Non-breeder	0	Post-nursery	NA	NA	NA	0.298	10	2010
M. nattereri	Adult	F	Post-lactating	0	Post-nursery	6	55.573	39	0.213	1	2010
M. nattereri	Adult	M	NA	0	Post-nursery	6	8.021	34	0.181	1	2010
M. nattereri	Adult	M	NA	0	Post-nursery	6	8.021	34	0.193	1	2010
M. nattereri	Juvenile	M	NA	0	Post-nursery	6	5.027	28	0.187	1	2010
M. nattereri	Adult	F	Post-lactating	0	Post-nursery	6	13.63	31	0.197	1	2010
M. daubentonii	Adult	M	NA	0	Post-nursery	2	432.946	21	0.257	2	2010
M. daubentonii	Adult	M	NA	1	Post-nursery	2	495.159	23	0.261	0	2010
M. daubentonii	Juvenile	F	Non-breeder	1	Post-nursery	4	7.538	20	0.232	4	2010
M. daubentonii	Adult	F	Non-breeder	1	Post-nursery	4	215.03	37	0.220	0	2010
M. daubentonii	Juvenile	F	Non-breeder	1	Post-nursery	4	6.617	19	0.219	2	2010
M. daubentonii	Adult	F	Non-breeder	0	Post-nursery	NA	NA	NA	0.223	3	2010
M. daubentonii	Adult	F	Post-lactating	1	Post-nursery	NA	NA	NA	0.254	2	2010

Species	Age	Sex	Reproductive status	Coronavirus presence	Season	Social group	Betweeness	Degree	BCI	Spinturnicid load	Year
M. daubentonii	Adult	F	Post-lactating	1	Post-nursery	NA	NA	NA	0.229	1	2010
M. daubentonii	Juvenile	M	NA	1	Post-nursery	NA	NA	NA	0.211	3	2010
M. daubentonii	Adult	F	Post-lactating	1	Post-nursery	NA	NA	NA	0.229	1	2010
M. daubentonii	Juvenile	M	NA	1	Post-nursery	NA	NA	NA	0.215	1	2010
M. daubentonii	Adult	F	Post-lactating	1	Post-nursery	NA	NA	NA	0.275	1	2010
M. daubentonii	Adult	M	NA	1	Post-nursery	5	29.22	17	0.238	1	2010
M. daubentonii	Juvenile	F	Non-breeder	1	Post-nursery	4	175.011	17	0.207	3	2010
M. daubentonii	Adult	M	NA	1	Post-nursery	5	175.959	30	0.233	1	2010
M. daubentonii	Adult	F	Post-lactating	0	Post-nursery	4	195.345	28	0.243	1	2010
M. nattereri	Adult	F	Post-lactating	0	Post-nursery	6	30.081	38	0.198	0	2010
M. nattereri	Juvenile	M	NA	0	Post-nursery	6	0.753	28	0.186	0	2010
M. nattereri	Adult	M	NA	1	Post-nursery	6	2.179	31	0.182	3	2010
M. daubentonii	Juvenile	M	NA	1	Post-nursery	4	16.874	21	0.196	1	2010
M. daubentonii	Adult	M	NA	0	Post-nursery	4	699.889	23	0.225	0	2010
M. daubentonii	Adult	F	Post-lactating	0	Post-nursery	NA	NA	NA	0.185	1	2010
M. daubentonii	Adult	M	NA	1	Post-nursery	4	67.161	22	0.214	1	2010
M. daubentonii	Juvenile	M	NA	1	Post-nursery	4	124.55	26	0.202	3	2010
M. nattereri	Juvenile	M	NA	0	Post-nursery	3	8.903	25	0.195	1	2010
M. nattereri	Adult	F	Post-lactating	0	Post-nursery	3	39.713	39	0.204	3	2010
M. nattereri	Juvenile	M	NA	0	Post-nursery	3	6.882	18	0.185	1	2010
M. nattereri	Adult	F	Post-lactating	0	Post-nursery	3	30.314	39	0.195	3	2010
M. daubentonii	Adult	F	Post-lactating	1	Post-nursery	NA	NA	NA	0.211	1	2010
M. daubentonii	Adult	M	NA	1	Post-nursery	7	536.737	14	0.199	0	2010
M. daubentonii	Adult	M	NA	1	Post-nursery	6	67.781	10	0.184	1	2010

Glossary

Allogrooming – An activity where individuals groom others in a group (i.e. colony). This may be important for maintaining social bonds and reducing parasite loads.

Bachelor colony – A colony dominated by, or exclusively made up of males. In this thesis a bachelor colony is defined as a colony constituting more than 66.6% adult males.

Biomagnification – The increase in concentration of substances (e.g. pollutants) within organisms as they pass up the food chain.

Body Condition Index (BCI) – A measure used to estimate the amount of fat reserves an individual possesses. This value is calculated by dividing the weight (g) by the forearm length (mm) and is used as an indication of an individual's health.

Clear felling – Also known as clear cutting, a forestry practise where all trees within a given area are cut down.

Clique – A small group of individuals who associate more frequently than others within a social group.

Colony – An aggregation of bats in a roost.

Ecosystem Service – The elements of ecosystems that are used directly and indirectly to support human wellbeing.

Endocrine disruptor – Chemicals that interfere with the hormone system and can lead to developmental defects.

Environmental Change Network (ECN) – A network of study sites and scientists across the UK dedicated to long-term monitoring of changes in the natural environment.

Homeothermy/heterothermy – Homeothermic individuals maintain a stable internal temperature regardless of fluctuations in external temperatures. Heterothermic individuals maintain a stable internal temperature when active, but when inactive this temperature is allowed to drop to the same temperature as the environment as a mechanism to conserve energy.

Minimum Convex Polygon (MCP) – A polygon drawn by connecting the outermost of a set of points in space so as to create the smallest possible convex polygon that encloses all points.

Nursery period – The period between the first and last colony of lactating females with juveniles.

Parasite load – The number of individuals of a given parasite infesting an individual host.

Prevalence – The proportion of a set group of individuals that are infected with a pathogen or parasite.

Protonymph – The developmental stage of a mite after egg and larvae. Spinturnicid mites give birth to young that have already developed to this stage.

Reservoir host –The long-term host species of a pathogen. Typically this species carries the pathogen with limited symptoms.

Roost – The physical space used by bats to rest during the day.

Social group – A group of individuals who associate with each other more than would be expected by chance.

Social group roost home range – A minimum convex polygon encompassing all roosts used by individuals belonging to a specific social group.

Social thermoregulation – A mechanism by which individuals reduce the energetic demands of homeothermy by maintaining close proximity or direct contact with others so that body heat is shared and body surface area in contact with the environment is reduced.

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